Effects of muscle fiber type and size on EMG median frequency and conduction velocity

E. J. KUPA, S. H. ROY, S. C. KANDARIAN, AND C. J. DE LUCA
NeuroMuscular Research Center, Boston University; and Department of Health Sciences, Sargent College of Allied Health Professions, Boston, Massachusetts 02215

Kupa, E. J., S. H. Roy, S. C. Kandarian, and C. J. De Luca. Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *J. Appl. Physiol.* 79(1): 23–32, 1995.—This paper describes an in vitro method for comparing surface-detected electromyographic median frequency (MF) and conduction velocity (CV) parameters with histochemical measurements of muscle fiber type composition and cross-sectional area (CSA). Electromyographic signals were recorded during electrically elicited tetanic contractions from rat soleus, extensor digitorum longus, and diaphragm muscles placed in an oxygenated Krebs bath. Fibers were typed as slow oxidative, fast oxidative glycolytic, and fast glycolytic based on histochemical enzyme stains. Muscles with a greater percentage of fast glycolytic and fast oxidative glycolytic fibers exhibited greater initial values of MF and CV as well as a greater reduction in these variables over the course of the contraction. Regression indicated that fiber type composition could be predicted based on two MF parameters. A weighted measure of muscle fiber CSA was found to be linearly related to both initial MF and CV. The results of this study suggest that MF and CV parameters recorded during a muscular contraction are related to muscle fiber type composition and muscle fiber CSA.

electromyographic; soleus; extensor digitorum longus; diaphragm; M-wave; cross-sectional area

DURING A SUSTAINED CONTRACTION, the depolarization and propagation of muscle fiber action potentials are modified. These modifications produce time-dependent changes to the resultant surface electromyographic (EMG) signal. It is well known, for instance, that the EMG signal undergoes a shift in its power spectrum toward lower frequencies as well as an alteration of its spectral shape during a constant-force isometric contraction (7). The resultant decrease in EMG spectral variables, such as the median or mean frequency, has provided investigators with a noninvasive and localized method of monitoring electrophysiological fatigue processes. Despite the continued interest in this method, the specific relationships between EMG spectral variables and the physiological and morphological properties of the muscle fiber remain elusive.

The few empirical studies that have attempted to identify a relationship between EMG signal variables and muscle fiber characteristics have focused on histochemical measurements of muscle fiber type and muscle fiber size. Previous studies in humans have shown that the classification of muscle fibers into distinct categories of "fast" or "slow" fibers on the basis of myosin adenosinetriphosphatase (mATPase) can be related to specific EMG spectral parameter values. For instance, it has been reported that muscles having a high percentage of fast fibers have a correspondingly greater initial value and rate of decrease of EMG median frequency (MF) than muscles with a low percentage of fast fibers (8, 11, 13, 15, 22, 23, 27, 28, 31). Additionally, human muscles composed primarily of fast fibers have a greater action potential conduction velocity (CV) than muscles composed of mostly slow fibers (15). These findings raise the possibility that the fiber type composition of a muscle can be estimated on the basis of EMG spectral parameters, either singularly or in combination with other parameters (9, 22, 31). This possibility is still highly speculative because previous investigations were limited to in vivo studies in humans during voluntary contractions where confounding physiological and experimental factors could not be controlled. Also, muscle fiber type classification in these studies was derived purely on the basis of mATPase activity without regard to the metabolic profile of the muscle that may also influence the time-dependent behavior of the EMG signal (7). Attempts at relating EMG spectral parameters to the morphological characteristics of muscle fibers have also been inconclusive. The association between EMG spectral variables and muscle fiber size, as measured by fiber cross-sectional area (CSA) or diameter, has not been adequately determined on the basis of empirical measurements. Despite earlier successful investigations in isolated frog muscle (10), which described a linearly increasing relationship between muscle fiber diameter and CV, in vivo studies on whole muscles in humans have not been as successful in confirming this relationship for either CV or EMG spectral parameters (5, 8, 9, 15, 26, 31). Although single-fiber studies have provided the most direct method of relating muscle fiber properties to EMG measurements, they do not provide a realistic simulation of the condition most commonly present in human EMG applications in which surface-detected signals are recorded from whole muscle.

It was therefore the aim of this study to further investigate the relationship between muscle fiber characteristics and EMG signal parameters in whole muscle through the use of an in vitro model in which confounding experimental and physiological factors present during in vivo studies could be more adequately controlled. Specifically, we compared surface EMG measurements of MF and CV with histochemical measurements of muscle fiber type and size in three whole muscles of the rat selected according to distinct differences in fiber type composition and muscle fiber CSA.

METHODS

Animal preparation. A total of 14 female Wistar rats (weight 150–175 g; B. K. Breeding Laboratories) were used. Soleus (SOL) and extensor digitorum longus (EDL) muscles were excised from six animals and the diaphragm (DIA) mus-

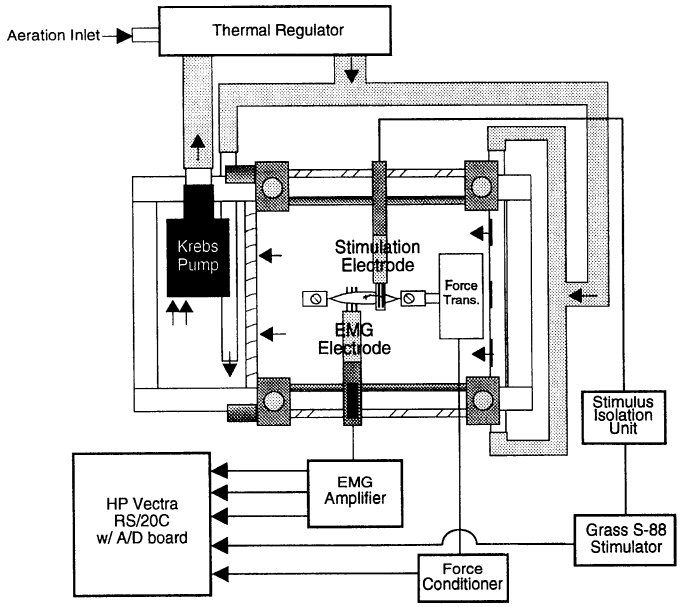

FIG. 1. Schematic representation of in vitro experimental setup for detecting EMG signals from isolated neuromuscular preparations mounted in isothermal bath. A/D, analog to digital. Krebs solution was maintained at constant temperature and circulated through test chamber as indicated by arrows showing direction of flow. Surface EMG electrode was placed on underside of muscle that was secured by its tendons to fixed post and force transducer. Nerve from muscle is shown in figure to be in contact with stimulation electrode located above bath. EMG and force signals were amplified and conditioned before they were digitally sampled using PC workstation.

cle was excised from eight animals (n = 8 for each muscle group). Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium at a dose of 6.25 mg/100 g body wt. EDL and SOL muscles were removed from each hindlimb with their corresponding branches of the sciatic nerve intact. The blood supply to all muscles was maintained until immediately before removal. Before extracting the DIA muscle, a tracheostomy was performed and the animal was connected to a small rodent ventilator. The entire DIA muscle and attached ribs were then removed with the left phrenic nerve intact. A 10-mm-wide muscle strip was cut from the left costal DIA and clamps were attached to the central tendon and ribs.

Experimental procedure. The extracted neuromuscular preparations were placed in a bath of Krebs solution (in mM: 137 NaCl, 5 KCl, 1 $MgSO_4$, 1 $NaHCO_3$, 2 $CaCl_2$, and 11 glucose) maintained at a constant temperature of 26°C and continuously aerated with a gas mixture consisting of 80% O_2:5% CO_2:15% N_2. The muscles were attached to a fixed post at one end and to a force transducer at the other using tendon sutures or clamps. The free end of the nerve was placed over a bipolar stimulation electrode positioned just outside the bath to help reduce stimulus artifact. A schematic representation of the setup is shown in Fig. 1. The stimulation was set to a frequency of 40 Hz and a pulse width of 0.2 ms at a supramaximal voltage. Supramaximal stimulation voltage was determined by first increasing the voltage until a peak twitch tension was observed and then setting the voltage to 1.5 times this value. Next, the muscle was adjusted to optimal length according to the position at which a maximum twitch force occurred. The remaining experimental protocol was carried out after allowing 10 min for the muscle to equilibrate in the bath.

The stimulation protocol began with the induction of a 5-s contraction to briefly check for signal quality followed 5 min later by a fatigue-inducing stimulation sustained for 20 s. Bath pH was constantly monitored and maintained at a physiological pH of 7.2 by adding appropriate amounts of 1.0 M HCl during the experiment if necessary. After data collection, the muscle was removed from the bath and frozen at optimal length in isopentane kept at approximately −20°C. Muscles were stored at −70°C before histochemical fiber typing.

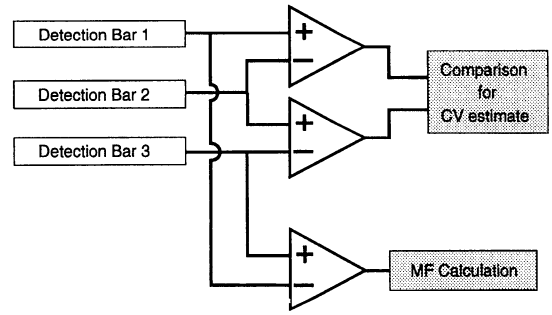

FIG. 2. Schematic representation of EMG signal electrode. Three parallel detection bars of electrode (interelectrode spacing = 2.3 mm) and their differential amplification before calculation of EMG median frequency (MF) and conduction velocity (CV). Differential signals from *detection bars 1* and *3* were used to calculate MF. Time delay between 2 differential signals from *detection bars 1* and *2* and *detection bars 2* and *3* was used to estimate CV.

EMG signal detection. M-waves were detected by using an electrode unit with three detection bars made of gold springs (0.965 mm OD, interelectrode distance 2.3 mm) that was positioned on the underside of the muscle preparation in contact with the muscle fascia. Buffer amplifiers were located close to the electrodes to provide a high-input impedance. The signals from each pair of the three detection bars were differentially amplified and band-pass filtered (20–2,000 Hz) to produce three separate EMG signals from which MF and CV estimates were calculated. MF was determined by using the differential signal of the outer two bars (*bars 1* and *3*), whereas CV was determined by estimating the delay between the two differential signals obtained from *bars 1* and *2* and *bars 2* and *3* (Fig. 2). The position of the EMG electrode was adjusted to ensure that all three detection bars were to one side of the innervation zone and perpendicular to the muscle fibers. Proper electrode placement was determined by using a 1-s train of 40-Hz pulses while observing the M-waves on an oscilloscope and immediate on-line computation of CV and MF parameters.

EMG signal processing. EMG signals from the contractions of 20-s duration were sampled at a rate of 1,024 Hz and divided into 0.50-s epochs. The three differential signals were digitized with a 12-bit analog-to-digital converter connected to a PC workstation. A 20-ms window was digitized after each stimulation pulse, with the first millis of each window eliminated to reduce stimulation artifact. One average 19-ms M-wave was obtained for each epoch by signal averaging. MF and CV were calculated by using a custom software package for EMG data acquisition and processing. MF was calculated by zero padding each average M-wave response to 0.5 s, giving a frequency resolution of 2 Hz. CV was calculated by dividing the delay (defined as the time shift that minimized the mean square error between the Fourier transforms of the two single differential signals) by the interelectode distance (2.3 mm).

Muscle fiber analysis. All muscle samples were analyzed by using three histochemical processes. Frozen muscles were cut into 10-μm serial sections by using a cryostat (−20°C) and were air-dried for 30 min before staining. mATPase activity was determined by using acid (pH 4.5) and alkaline (pH

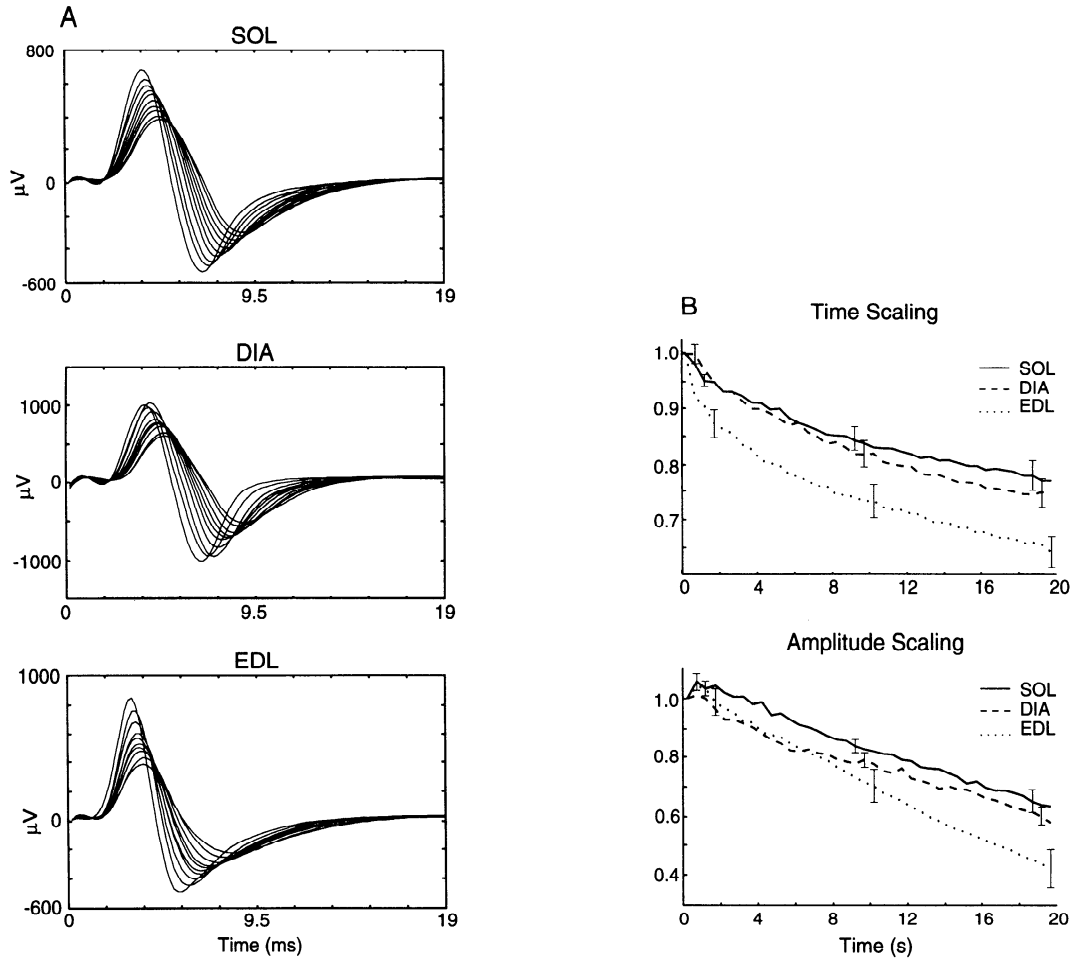

FIG. 3. *A*: changes in shape of sequential M-waves are shown by superimposing M-waves from 20-s duration contraction. Three typical results are compared for soleus (SOL) (*top*), diaphragm (DIA) (*middle*), and extensor digitorum longus (EDL) (*bottom*) muscles. M-waves were selected from every 4th epoch (epoch duration = 0.50 s) and therefore correspond to time intervals of 2 s. M-wave amplitude is in microvolts (μV). *B*: changes in M-wave time duration and amplitude during a 20-s tetanic contraction are depicted by plotting time (*top*) and amplitude (*bottom*) scaling factors from 4th order Hermite functions that were fit to M-waves. Data (means ± SE; n = 8) are presented separately for SOL, DIA, and EDL muscles. Decrease in time and amplitude scaling factors provides quantitative measure of increase in M-wave duration as well as decrease in M-wave amplitude during 20-s contraction.

10.4) preincubations to classify the muscle fibers as type I (slow), type IIa (fast), and type IIb (fast) according to the methods of Brooke and Kaiser (4). Muscle sections were also stained for succinate dehydrogenase, an oxidative enzyme, and α-glucose-6-phosphate dehydrogenase (α-GPDH), a glycolytic enzyme, according to the methods of Wattenberg and Leong (30). Fibers were typed as slow oxidative (SO), fast oxidative, glycolytic (FOG), and fast glycolytic (FG) on the basis of fiber densitometry by using a video analysis system (JAVA, Jandel Scientific). Fiber CSA was measured for each fiber counted. Fiber type percentages by number and by area were computed for each muscle. The mean number of fibers counted per muscle was 421 ± 115 (SD).

Data analysis. An analysis of variance procedure followed by pair-wise comparisons was performed for the EMG and fiber type parameters to determine whether the differences between muscle groups were significant. Multiple-regression procedures were applied to determine whether a predictive model for fiber type composition could be developed by using the EMG parameters. The influence of muscle fiber type properties on the EMG parameters was studied by application of a general linear regression model. All statistical analyses were performed with the assistance of SYSTAT software. Changes in the duration and amplitude of the M-wave during a contraction were analyzed by calculating time and amplitude scaling coefficients from fourth order Associated Hermite functions (16, 17). This family of functions provides an excellent approximation of M-wave shapes and was particularly useful in this application because it resulted in a direct measurement of these scaling coefficients.

RESULTS

EMG parameters. A series of M-waves sampled during the 20-s duration contractions are shown for SOL, DIA, and EDL muscles in Fig. 3*A*. It can be seen that during the course of a contraction the duration of the M-waves increased while the amplitude decreased. Mean values (n = 8) of the time and amplitude scaling coefficients of the Associated Hermite functions are plotted as a function of contraction duration for each muscle group in Fig. 3*B*. The time scaling coefficient of the M-wave decreased during the contraction, indicating an increase in M-wave duration, as observed in Fig. 3*A*. The amplitude scaling coefficient of the M-wave also decreased, which is consistent with the observed decrease in the M-wave amplitude in Fig. 3*A*.

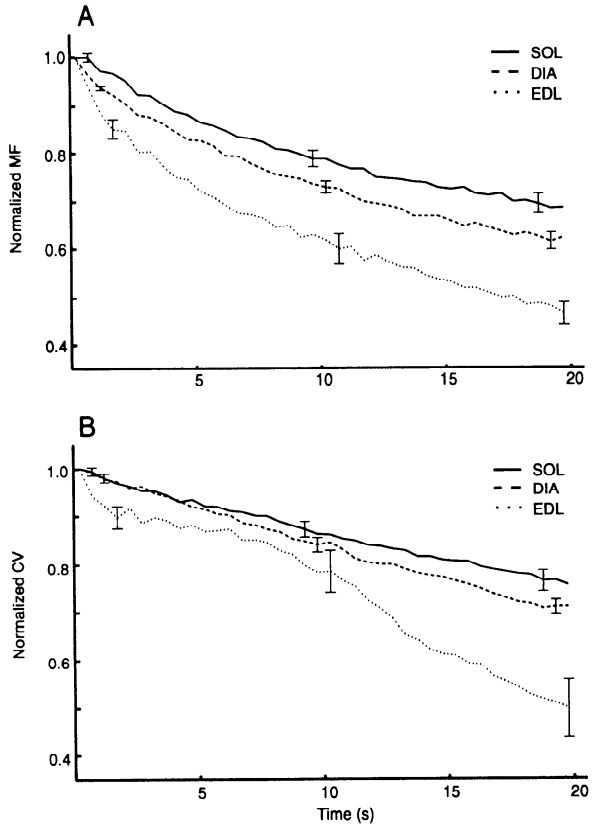

FIG. 4. Normalized values of MF (*A*) and CV (*B*) plotted separately for SOL, DIA, and EDL muscles. Data for each muscle group (means ± SE; $n = 8$) are normalized with respect to their initial values.

Means ± SE ($n = 8$) of normalized MF and CV are plotted with respect to the 20-s duration of the contraction in Fig. 4. Values were normalized to the first epoch. During the course of the contraction, MF and CV decreased at different rates for each muscle group.

Table 1 summarizes the mean values for the EMG parameters calculated in this study. Initial MF (IMF) and CV (ICV) are the initial values of MF and CV, respectively, calculated from the first epoch. ΔMF and ΔCV are the differences in MF and CV, respectively, for the first and last epochs. SLPMF and SLPCV are the coefficient of a linear regression that was fit to the first 5 s of MF and CV data, respectively, from the 20-s contractions and SLP is slope.

The difference between the initial and final values of MF and CV was significant for all muscles ($P < 0.001$). Pair-wise differences between muscle groups for each of the EMG parameters were significant ($P < 0.01$), with the following exceptions: all pair-wise differences between muscle groups for the SLPCV parameter were nonsignificant ($P < 0.05$), and differences between DIA and SOL muscles for ICV and ΔCV parameters were not significant ($P = 0.25$ and $P = 0.07$, respectively). MF parameters were in general less variable than CV parameters. There was a greater variability in CV measurements for SOL and EDL muscles compared with the DIA muscle.

Muscle morphology and fiber type percentages. Mean fiber type percentage by number and by area were calculated for each muscle and are summarized in Table 2. Fiber type percentages by area were calculated according to

$$\%\text{Fiber type}_f = \frac{A_f\%_f}{\Sigma_f(A_f\%_f)} \times 100$$

where f is SO, FOG, or FG; A_f is mean CSA of fibers of a particular type; and $\%_f$ is the percentage of fibers of a particular type. In all cases the fibers that were typed as type IIa fibers corresponded to FOG fibers and those typed as IIb fibers corresponded to FG fibers. All pairwise differences between muscle groups for mean fiber type percentages were significant ($P < 0.001$) except for the difference between DIA and EDL muscles for the %FOG measurement (not significant, $P = 0.18$). Mean CSA for each of the fiber types was different across muscle groups, with the SOL muscle having the largest CSA for both SO and FOG fibers and the EDL muscle having the smallest CSA. The DIA muscle contained slightly larger FG fibers on average than the EDL muscle. The percentage of fibers staining positively for α-GPDH was equivalent to the percentage of fast fibers in each muscle, since all fast fibers stained positive for α-GPDH. For each muscle ($n = 24$), an average CSA was calculated from the total number of fibers measured.

Relationships between EMG and fiber type. A Pearson correlation matrix that summarizes the relationships between fiber type percentages by area and the EMG parameters is presented in Table 3. The strongest correlations were between the MF parameters and fiber type percentages. Muscles with a greater percentages of glycolytic enzymes (greater %fast) had greater values of IMF ($r = 0.92$) and ΔMF ($r = 0.85$) and more negative values of SLPMF ($r = -0.80$). Muscles with a greater percentage of fibers staining positively for succinate dehydrogenase had lower values of ΔMF ($r = -0.91$) and less negative values of SLPMF ($r = 0.85$). The relatively high correlations between ΔMF and IMF ($r = 0.81$) as well as between ΔCV and ICV ($r = 0.82$) indicated that muscles with high values of IMF or ICV had greater changes in these parameters over the

TABLE 1. *EMG parameters*

	IMF, Hz	ΔMF, Hz	SLPMF	ICV, m/s	ΔCV, m/s	SLPCV
SOL	134.3±5.6	42.1±7.6	−3.8±0.88	1.70±0.31	0.42±0.12	−0.034±0.030
DIA	158.3±6.3	59.6±6.0	−5.6±0.97	1.89±0.14	0.55±0.09	−0.033±0.009
EDL	167.4±8.1	89.6±11.6	−8.8±1.80	3.02±0.50	1.50±0.48	−0.058±0.054

Values are means ± SD. MF, median frequency; CV, conduction velocity; IMF, initial MF; ΔMF, change in MF; ICV, initial CV; ΔCV, change in CV; SLPMF and SLPCV, coefficient of linear regression fit to 1st 5 s of MF and CV data, respectively, from 20-s contractions; SOL, DIA, and EDL, soleus, diaphragm, and extensor digitorum longus muscles, respectively.

course of a sustained contraction. The high correlation between ΔMF and SLPMF ($r = -0.94$) indicated that the parameters were essentially interchangeable. The interrelationships between the percentage fiber type by area, IMF, and ΔMF are shown in the 3-dimensional plots shown in Figs. 5 and 6.

Step-wise multiple regressions were performed to determine how well the percentage of fiber type by area could be predicted on the basis of the six EMG parameters measured. The results are shown in *Eqs. 1–3* and include r^2 values and the corresponding standard error of estimate (SEE). SEE provides an overall indication of the accuracy with which the fitted regression function performs as a predictive model given values for the independent variables.

$$\%SO = -1.55(IMF) - 0.55(\Delta MF) + 310 \quad r^2 = 0.88 \quad SEE = 13.3\% \tag{1}$$

$$\%FOG = 0.75(IMF) - 6.35(ICV) - 75.1 \quad r^2 = 0.78 \quad SEE = 5.1\% \tag{2}$$

$$\%FG = 0.85(IMF) + 0.68(\Delta MF) - 139 \quad r^2 = 0.91 \quad SEE = 8.9\% \tag{3}$$

A second regression equation for estimating %FOG was calculated to determine whether the number of independent variables could be limited to just two for estimating any of the three fiber type percentages. IMF and ΔMF were forced into the %FOG regression equation because the stepwise regression procedure had selected these parameters as the best predictors for %SO and %FG. The resulting regression equation for %FOG is described in *Eq. 4*, and the values of r^2 and SEE are only slightly changed compared with *Eq. 2*:

$$\%FOG = 0.70(IMF) - 0.13(\Delta MF) - 71.9 \quad r^2 = 0.72 \quad SEE = 5.7\% \tag{4}$$

The regression equations composed of only IMF and ΔMF for estimating percentage fiber type by area can be visualized as a line providing the best fit to the data in Fig. 5. Regressions were also performed with SLPMF in place of ΔMF to demonstrate the effect of using only MF parameters calculated from the first 5 s of a contraction to predict the percentage of fiber type by area. The resulting regression equations are shown in *Eqs. 5–7* and demonstrate little change to the values of r^2 and SEE:

$$\%SO = -1.69(IMF) + 4.05(SLPMF) + 323 \quad r^2 = 0.88 \quad SEE = 13.3\% \tag{5}$$

$$\%FOG = 0.62(IMF) + 0.67(SLPMF) - 65.4 \quad r^2 = 0.71 \quad SEE = 5.8\% \tag{6}$$

$$\%FG = 1.07(IMF) - 4.71(SLPMF) - 158 \quad r^2 = 0.89 \quad SEE = 9.5\% \tag{7}$$

To examine more closely the relative influences of the fiber type percentages by area on the EMG parameters,

TABLE 2. *Fiber type proportions and areas*

	SO	FOG	FG
SOL			
% by number	80.3±4.8	19.7±4.8	0±0
Area, μm^2	3690±1232	2405±563	0±0
% by area	87.2±4.3	12.8±4.3	0±0
DIA			
% by number	35.2±4.0	43.0±3.6	21.8±1.4
Area, μm^2	1189±374	1183±453	2603±903
% by area	28.0±2.6	34.0±3.1	38.0±2.4
EDL			
% by number	2.7±0.5	51.2±4.0	46.1±4.3
Area, μm^2	930±215	1033±386	2400±718
% by area	1.5±0.4	31.9±2.5	66.6±2.6

Values are means ± SD. SO, FOG, and FG, slow oxidative, fast oxidative glycolytic, and fast glycolytic fibers, respectively.

the following general linear regression models were calculated.

$$IMF = 1.28(\%SO) + 1.76(\%FOG) + 1.65(\%FG) \quad r^2 = 0.85 \quad SEE = 6.4 \text{ Hz} \tag{8}$$

$$\Delta MF = 0.54(\%SO) - 0.39(\%FOG) + 1.52(\%FG) \quad r^2 = 0.90 \quad SEE = 7.3 \text{ Hz} \tag{9}$$

$$ICV = 0.021(\%SO) - 0.015(\%FOG) + 0.051(\%FG) \quad r^2 = 0.71 \quad SEE = 0.4 \text{ m/s} \tag{10}$$

$$\Delta CV = 0.008(\%SO) - 0.023(\%FOG) + 0.032(\%FG) \quad r^2 = 0.70 \quad SEE = 0.3 \text{ m/s} \tag{11}$$

In calculating these regression equations, it was assumed that the independent variables summed to a constant because the percentages of the three fiber types must always sum to 100%. Equations 8–11 demonstrate that the fiber type percentages can account for much of the variance in the EMG parameters, particularly for MF parameters as indicated by the relatively high values of r^2 for each regression equation. The regression equations were recalculated by using standard coefficients to indicate the relative importance of each of the independent variables in accounting for the variance in the dependent variable (Table 4). The parameter %FG consistently had the highest standard coefficient and therefore accounted for most of the variance in the EMG signal parameters. This finding indicates that for this study the FG fibers had the greatest influence on the EMG parameters. Standard coefficients of the regression for estimating %SO and %FOG indicated that %SO was more influential than %FOG in accounting for the variance in the IMF, ΔMF, and ICV, whereas %FOG accounted for more of the variance in ΔCV.

EMG parameters and muscle morphology. The relationship between average fiber CSA and IMF is shown in Fig. 7 ($n = 24$). There are no similar trends in the data for the three different muscles studied. Analysis of the data revealed one significant regression: an increasing linear relationship ($r = 0.72$, $P < 0.05$, $n = 8$) between the IMF and average fiber CSA for the EDL muscle (Fig. 8). This finding indicated that there may have been an underlying characteristic of the EDL

TABLE 3. *Pearson correlation matrix*

	%SO	%FOG	%FG	%Oxid	%Glyc	IMF	ΔMF	SLPMF	ICV	ΔCV	SLPCV
%SO	1.00										
%FOG	−0.89	1.00									
%FG	−0.99	0.81	1.00								
%Oxid	0.99	−0.81	−1.00	1.00							
%Glyc	−1.00	0.89	0.99	−0.99	1.00						
IMF	−0.92	0.83	0.90	−0.90	0.92	1.00					
ΔMF	−0.85	0.57*	0.91	−0.91	0.85	0.81	1.00				
SLPMF	0.80	−0.53*	−0.85	0.85	−0.80	−0.73	−0.94	1.00			
ICV	−0.71	0.43*	0.78	−0.78	0.71	0.74	0.81	−0.75	1.00		
ΔCV	−0.70	0.41*	0.77	−0.77	0.70	0.65	0.87	−0.84	0.82	1.00	
SLPCV	0.32‡	−0.08‡	−0.40†	0.40†	−0.32‡	−0.29‡	−0.54*	0.55*	−0.46*	−0.63	1.0

%Oxid and %Glyc, percentages by area of oxidative and glycolytic enzymes, respectively. All correlations were significant at $P < 0.001$ except: * significant at $P < 0.05$; † borderline significant at $P = 0.06$; ‡ not significant.

muscle that distinguished it from the other muscles studied and therefore contributed to this significant relationship. The most obvious characteristic of the EDL muscle that we considered was that its composition was almost entirely (98.5%) fast fibers. This fast fiber dominance and its apparent relationship with CSA led us to evaluate the possibility that the CSA and percentage of fast fibers play a role in determining IMF. To explore this possibility further, we defined a measure that accounted for the proportional differences of fast fiber type (FG and FOG) among the three different whole muscles. This measure is referred to as the "weighted" average fiber CSA (wAREA) and was calculated for each whole muscle ($n = 24$) based on

$$\text{wAREA} = (\%\text{ by area of fast fibers}) \times (\text{average muscle fiber CSA})$$

where it can be seen that wAREA is the average muscle fiber CSA weighted according to the percentage (by area) of fibers classified as FG or FOG. The association between IMF and wAREA was highly correlated and well represented by a positive linearly increasing relationship ($r = 0.92$, $P < 0.001$), as shown in Fig. 9. We conducted the same analysis for ICV and found that the association between ICV and wAREA was also linearly increasing ($r = 0.81$, $P < 0.001$), although the degree of correlation with wAREA was less than that observed for IMF (Fig. 9).

DISCUSSION

The progressive alteration of the EMG frequency spectrum during a contraction has been well documented for a variety of human muscles as well as for in vitro animal preparations (3, 11, 13–15, 23, 28). With the exception of the current study, no other reports have established a quantitative relationship between EMG spectral parameters and percentages of SO, FOG, and FG muscle fibers under in vitro conditions. We demonstrated in this study that the use of isolated whole muscle preparations tested under conditions in which the entire population of muscle fibers could be activated was advantageous in that EMG signal parameters representative of the whole muscle specimen were related to muscle fiber characteristics derived from the entire muscle cross section. Previous attempts by others to quantitatively relate these parameters in humans relied on biopsy samples for histochemical analysis that represented only a portion of the fibers activated during a contraction (9, 22, 31). Also, the muscle biopsies in these other studies did not include specific metabolic analyses. Instead, they were histochemically analyzed based solely on mATPase, an indicator of the velocity of shortening of the muscle fibers. Because the decrease of MF is related to the accumulation of metabolites (7), fiber classification for this study also included metabolic analysis.

Changes in the EMG signal during a sustained voluntary contraction can be influenced by a number of confounding factors that may detract from establishing a direct relationship between the fiber type characteristics of a muscle and its EMG signal parameters. For instance, changes in extracellular pH, blood flow, temperature, and muscle fiber recruitment have been shown to influence EMG spectral parameters during fatiguing contractions (1, 3, 7, 16, 22, 23, 25, 27). Differences in muscle CSA, skin thickness beneath the EMG electrode, and cross talk from neighboring muscles can also modify some of these same EMG parameters (7, 24). We were able to minimize or, in some cases, eliminate these confounding factors through the use of stimulated contractions of an isolated muscle preparation in a controlled in vitro environment. The controlled experimental conditions may account for the relatively low SD values of the EMG parameters seen in Table 1. Another advantage is that stronger relationships can be formulated between muscle fiber type percentages and EMG signal parameters than previously described for in vivo studies. For instance, in a study by Wretling et al. (31) in which human subjects performed maximal knee extension, MF of the surface

TABLE 4. *Standard coefficients of regression equations*

Variable	%SO	%FOG	%FG
IMF	4.43	3.22	4.76
ΔMF	1.34	0.51	3.17
ICV	1.69	−0.64	3.38
ΔCV	0.74	−1.18	2.59

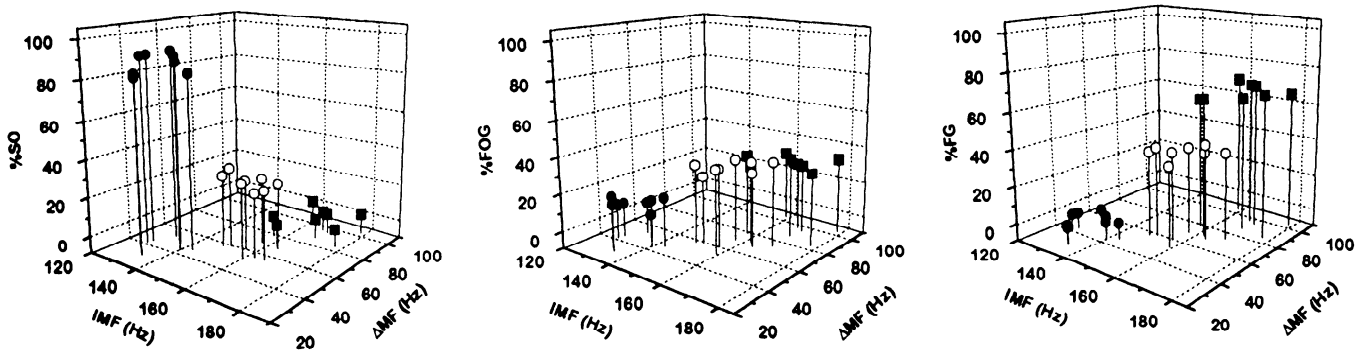

FIG. 5. Three-dimensional scatter plots of initial MF (IMF), difference between IMF and final MF (ΔMF), and percentage by area of slow oxidative (%SO), fast oxidative glycolytic (%FOG), and fast glycolytic (%FG) fiber types.

EMG signal from the vastus lateralis muscle predicted the percentage of type I fibers from biopsied muscle with an r^2 value of 0.75, which was lower than the value we report in *Eq. 1* of this study for predicting %SO (type I). Moritani et al. (22) also calculated regression equations for estimating muscle fiber type percentages (in human gastrocnemius muscles), but their equations included measurements of blood pressure and amplitude of the EMG signal in addition to MF parameters. Although no r^2 values for their regression equations were reported, they described SEE values of 4.89 and 5.11 for %type I and %type IIb fibers, respectively. In contrast to these studies, our multiple regression equations predicted the percentage of fiber type based on as few as two parameters: IMF and ΔMF of the EMG signal (*Eqs. 1*, *3*, and *4*). The inclusion of other EMG signal parameters did not improve the prediction of fiber type percentages. Even when the regressions were forced to consider parameters derived from only the first 5 s of the contraction (i.e., IMF and SLPMF), the resultant r^2 values for the multiple regressions remained relatively unchanged (see *Eqs. 5–7*). This finding suggests that in future applications, muscle stimulation may only need to be sustained for a 5-s duration to discern the muscle fiber type composition based on MF parameters.

Our finding that muscles with a greater percentage of FG and FOG fibers had greater IMF values agrees with several previous studies in human muscles (9, 11, 22, 23, 31). The effect of different fiber type proportions on the EMG signal can be explained if we consider that fast- and slow-twitch fibers have different action potential shapes. Fast fibers have a greater maximum rate of depolarization and repolarization than slow fibers and therefore produce an action potential that has a shorter duration (29). Shorter duration action potentials contribute high-frequency components to the EMG spectrum that result in a greater value of IMF. Because all fibers were activated simultaneously in this study as a result of supramaximal stimulation to the nerve, the M-wave was the synchronous sum of all the individual motor unit action potentials of that muscle. Therefore, if there is a greater proportion by area of FG and FOG fibers in a muscle, then the resulting M-wave will have a greater spectral content than a muscle that is composed of a smaller proportional area of these fibers.

Muscles with a greater IMF and ICV also exhibited a greater decrease in the magnitude of the MF and CV variables over the duration of the contraction, a result consistent with previous studies in humans (11, 13, 15, 22, 23, 25, 28). Changes in these EMG variables during a contraction were not the result of alterations in motor unit firing rates because the contractions in our experiments were induced by stimulation at a fixed rate. It is more likely that the different rates of decay for the EMG spectral variables were related to the differences in metabolic activity and membrane properties of the muscle fibers. Although specific muscle membrane properties were not measured directly in this study, there is evidence from other studies that fibers of different histochemical types have different membrane properties that might influence the M-wave. It is known, for instance, that the accumulation of extracellular K^+

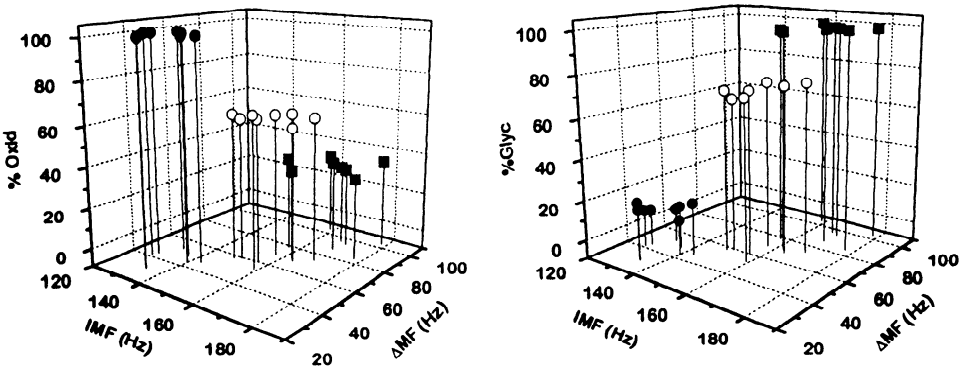

FIG. 6. Three-dimensional scatter plots of IMF, ΔMF, and percentages by area of oxidative (%Oxid) and glycolytic (%Glyc) enzymes.

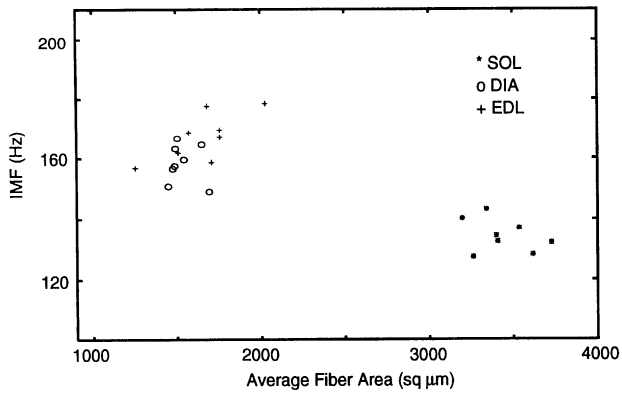

FIG. 7. IMF plotted with respect to average muscle fiber cross-sectional area (CSA) for each muscle specimen (n = 24).

can contribute to the slowing of the membrane action potential as well as to a decrease in action potential amplitude (21). If we consider the findings of Juel (12), where a greater change in extracellular K^+ was reported in muscles composed primarily of fast fibers, we can infer that in fast muscles there will be a greater change in the muscle fiber action potential CV and amplitude. Our findings of different rates of amplitude and duration scaling for muscles of different fiber composition (Fig. 3*B*) support this argument. Metabolically, fast fibers also have a greater potential for lactate accumulation and, therefore, a greater potential for the intramuscular pH to decrease during a contraction (27). This change in pH toward acidity should lead to a larger decline in CV for fast acid-labile fibers and, therefore, a greater decline in the MF of these fibers (27). We found that muscles with a greater percentage of glycolytic fibers and, therefore, a greater potential for lactate accumulation do indeed have a larger decline in MF (Fig. 6).

The influence of percentage fiber type on the MF and CV parameters was significant, as described in *Eqs. 8–11*. Standard coefficients for the multiple regression equations demonstrated that the EMG parameters were most strongly influenced by the percentage of FG fibers. The standard coefficients for the regression of ΔCV, in *Eq. 11*, further indicated that the fast (glycolytic) fibers have more influence on this parameter than

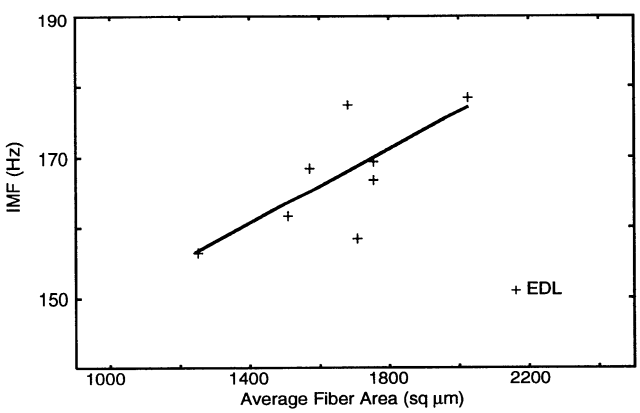

FIG. 8. Scatter plot showing significant linearly increasing relationship between IMF and average muscle fiber CSA for EDL muscle (r = 0.72, $P < 0.05$; n = 8).

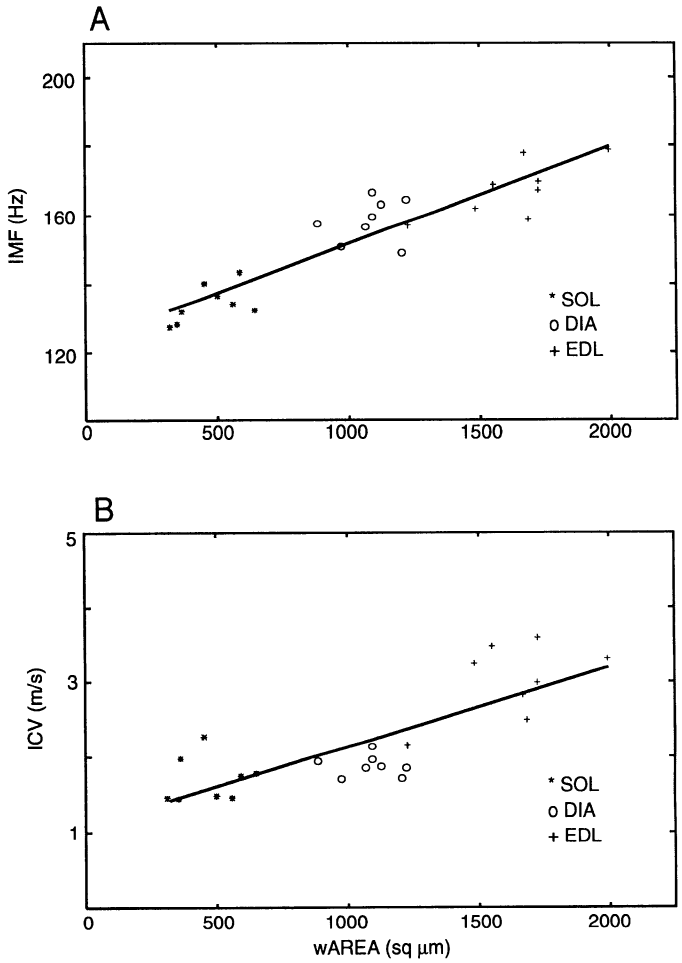

FIG. 9. *A*: scatter plot showing significant linearly increasing relationship between IMF and weighted muscle fiber area (wAREA) for all muscle specimens from SOL, DIA, and EDL muscles (r = 0.92, $P < 0.001$; n = 24). wAREA parameter was calculated according to wAREA = (% by area of fast fibers) × (average muscle fiber CSA). *B*: scatter plot showing significant linearly increasing relationship between initial CV (ICV) and wAREA for all muscle specimens studied (r = 0.81, $P < 0.05$; n = 8).

the other fiber type percentages. This finding is consistent with the proposition that specific membrane and metabolic properties of FG fibers have greater influence on ΔCV than SO fibers. Gerdle et al. (9) also developed regression equations that describe the influence of fiber type percentage on EMG spectral parameters. Their regression equation related initial mean frequency to the composition of types IIa, IIb, and IIc (r^2 = 0.86). However, the applicability of their results to other muscle groups is limited because they reported that the percentage of type IIc fibers had the greatest influence on estimating initial mean frequency. The presence of type IIc fibers is highly variable across muscles, as well as between subjects, and in fact was reportedly absent in some of the subjects tested during their study. Our findings, however, agree with them in that the percentage of type IIb (%FG) fibers in a muscle had the greatest influence in determining the EMG spectral parameters compared with the percentage of type IIa (%FOG) fibers of a muscle.

The results of this study also demonstrated that CV

parameters were more variable and not as strongly associated with percentage of fiber type as MF parameters. The weaker association may indicate that MF is more strongly influenced by both the membrane characteristics of the muscle fibers and its metabolic capacity than CV parameters. The greater variability of CV parameters may be explained by the greater sensitivity of this measurement to EMG electrode placement and differences in muscle geometry. It has been shown in human muscles that the placement of the electrode can effect the EMG spectral parameters and CV such that an electrode position close to either the innervation zone or termination zone (muscle-tendon junction) will result in an increase in the high-frequency component of the EMG signal as well as a poorer estimate of CV (24, 25). Because CV is calculated by cross correlating the EMG signal spectrums from two adjacent electrode pairs, if one electrode pair is affected by the termination zone and the other by the innervation zone, CV estimates may be unreliable. This argument is supported by our finding that CV measurements were less variable in the DIA muscle strip than in either of the hindlimb muscles tested. The relatively simple anatomic construction of the DIA muscle strip, which contains relatively few layers of parallel fibers, favors a more consistent placement of the EMG electrode with respect to the innervation and termination zones of the muscle fibers than the pennated geometry of EDL and SOL muscle fibers.

The results of this study, which were limited to whole muscle preparations, suggest that unlike single-fiber studies, IMF and ICV of the EMG signal did not increase linearly with average muscle fiber CSA. Instead, we found that IMF and ICV increased linearly with the proportion (by area) of the whole muscle that was composed of FG and FOG fibers. The common belief that muscles with a relatively large fiber CSA have high ICV values (and therefore, according to previous studies, correspondingly high IMF values; Refs. 1, 25) was clearly not supported by data in the present study. Our results are in agreement with the work of Linssen et al. (15) on human quadriceps muscle, which demonstrated that ICV was dissociated from muscle fiber diameter. Other studies on human quadriceps muscles have also reported nonsignificant relationships between muscle fiber size and either ICV or EMG mean frequency (26, 31).

For the EDL muscle, it was evident that IMF increased with increasing average muscle fiber CSA (Fig. 8, $r = 0.72$, $P < 0.05$). The question that arises is why this relationship did not exist in the other two muscles. The answer, we believe, can be found by looking more closely at the fiber composition of the different muscle groups. The EDL muscle was the only muscle in this study that was almost entirely composed of fast fibers (98.5%). It was therefore possible that the relationship between IMF and the average fiber CSA was present due to the dominant proportion of fast fibers in the muscle. The effects of proportion and area were accounted for in the measure designated as wAREA, which provided a representation of the contribution of fast fiber area to the EMG signal independent of the muscle being studied. The scatter plot in Fig. 9 demonstrates that the IMF increased in relation to wAREA. A similar relationship can be seen for ICV. The combined effect of fiber type proportions and CSA on the EMG signal can be explained if we consider the different action potential shapes of fast and slow fibers that were mentioned previously in this discussion. If CSA of the fast fibers is increased, the power density spectrum of the resulting M-wave will have more power in the high-frequency band of the spectrum, which will in turn be measured as a greater IMF. This increase in MF can be directly attributed to the fact that the signals from the larger fibers will have a greater amplitude and therefore contribute more of an influence to the spatial summation process that determines the final M-wave shape. Fiber type specific differences in membrane properties are the most likely source of the different frequency characteristics of their respective M-waves. If there is a greater number of fast fibers, or the fast fibers are larger, the effects due to differences in membrane properties will manifest themselves more readily in the M-wave that is evoked. One such difference in membrane properties was described by Luff and Atwood (18). They found that the mouse SOL muscle, a mostly slow-twitch muscle, had a greater membrane resistance than the EDL muscle, which was primarily composed of fast-twitch fibers. This difference in muscle membrane resistance can be a result of the more developed T-system and sarcoplasmic reticulum that fast-twitch fiber membranes have compared with slow-twitch fiber membranes (6). Differences in membrane resistance associated with fiber type may have contributed to the differences in ICV and IMF that were reported in this study.

In summary, the results of this study suggest that the modification of the M-wave and resultant changes in MF and CV during a sustained contraction were related to the muscle fiber composition. We have shown that it is possible to predict muscle fiber composition for three different whole rat muscles based on only two MF parameters. A significant linear relationship was described for both the initial MF and CV when plotted with respect to a measure of muscle fiber CSA that accounted for the proportion FG and FOG fibers in the whole muscle. The findings of this study and the work of others, that MF and CV are significantly associated with fiber type content, raise the question of which mechanisms are responsible for this association. Is it contractile speed, metabolic capacity, or some other fiber type related characteristic of the muscle that determines the EMG characteristics? The question is still unresolved because this and other studies were not designed to isolate specific physiological or morphological mechanisms to establish causality. Although no causal relationships were identified between EMG signal parameters and mATPase, metabolic capacity, or CSA of the muscle, possible mechanisms accounting for their significant associations were discussed. These findings support the possibility of utilizing surface EMG techniques to obtain a noninvasive electrophysiological "muscle biopsy" for estimating muscle fiber composition.

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Address for reprint requests: E. Kupa, NeuroMuscular Research Center, 44 Cummington St., Boston, MA 02215

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