Relationship between innervation zone width and mean muscle fiber conduction velocity during a sustained isometric contraction

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Abstract

Objectives: To examine the relationship between the biceps brachii muscle innervation zone (IZ) width and the mean muscle fiber conduction velocity (MFCV) during a sustained isometric contraction. Methods: Fifteen healthy men performed a sustained isometric elbow flexion exercise at their 60% maximal voluntary contraction (MVC) until they could not maintain the target force. Mean MFCV was estimated through multichannel surface electromyographic recordings from a linear electrode array. Before exercise, IZ width was quantified. Separate non-parametric one-way analyses of variance (ANOVAs) were used to examine whether there was a difference in each mean MFCV variable among groups with different IZ width. In addition, separate bivariate correlations were also performed to examine the relationships between the IZ width and the mean MFCV variables during the fatiguing exercise. Results: There was a significant difference in the percent decline of mean MFCV (%ΔMFCV) among groups with different IZ width ($\chi^2 (3)=11.571$, $p=0.009$). In addition, there was also a significant positive relationship between the IZ width and the %ΔMFCV (Kendall’s tau= 0.807; $p<0.001$). Conclusions: We believe that such relationship is likely influenced by both muscle fiber size and the muscle fiber type composition.

Keywords: Linear Electrode Array, Surface Electromyography, Fatigue, Innervation Zone, Muscle Fiber Conduction Velocity

Introduction

Muscle fiber conduction velocity (MFCV) is defined as the speed of an action potential that travels along the membrane of a muscle fiber. As an important physiological parameter, MFCV directly reflects the muscle fiber membrane properties. In addition to its intrinsic factors such as intracellular and extracellular ion concentrations, MFCV can also be altered by fatigue, exercise, as well as neuromuscular disorders. Over the last three decades, non-invasive technique such as multichannel surface electromyographic (EMG) recordings from a linear electrode array has been extensively used to estimate MFCV. It is important to mention that, in most situations where the multichannel surface EMG technique is used for the MFCV estimation, MFCV refers to the estimated mean MFCV, because the action potential of each motor unit has a specific conduction velocity of propagation, and the surface EMG signal is comprised of different motor unit action potentials propagating at different velocities. Thus, the estimated mean MFCV reflects the weighted average propagation velocities from all motor unit action potentials detected under the pick-up area of the surface EMG electrodes. Like other surface EMG parameters such as the EMG amplitude and the EMG center frequency (mean frequency or median frequency), mean MFCV can also be influenced by multiple physiological factors such as muscle fiber diameter, muscle fiber length, muscle fiber type, as well as the level of force output. In addition, the spread of the innervation zone (IZ) also plays an important role influencing the mean MFCV estimation.

Specifically, the IZ is a cluster of neuromuscular junctions (NMJs) or motor endplates that occupy a relatively small, localized region on muscle fibers. It is also the origin where...
action potentials are generated and propagate along the active muscle fibers on both directions toward the end of the muscle fibers. Similar to the estimation of MFCV, multichannel surface EMG recordings from one-dimensional or two-dimensional electrode array has been commonly used to examine the IZ location. It is also important to point out that, the location of the IZ is determined by all the motor unit action potentials detected under the pickup area of the surface electrodes. As the connection between nerves and muscle fibers, IZ plays an important role influencing the parameters of the action potentials. For example, the spread of motor endplates (IZ width) can affect variables such as the duration, the amplitude, and the shape of the action potentials. Of these parameters mentioned above, the shape of the action potentials is particularly important for estimating mean MFCV, because the basic requirement to estimate the average MFCV is “…the detection of action potentials along their propagation from the IZ(s) to the tendon regions…” (Farina et al., p. 432). Thus, when examining a muscle in which numerous adjacent motor unit endplates were contained, it is reasonable to hypothesize that the MFCV estimates would be different in situations where the IZ width varies. In fact, a simulation study by Nielsen et al. found that the MFCV estimates could be substantially affected by the distribution of IZs at different excitation levels and/or with different subcutaneous tissue thicknesses. However, less is known regarding the influences of IZ width on mean MFCV during interventions such as fatigue, pain, and exercise.

As a common intervention, sustained isometric exercise has been extensively used for examining neuromuscular responses to exercise-induced fatigue. For example, during sustained isometric fatiguing exercise at relatively high contraction levels (>40% maximal voluntary contraction (MVC)), a decreased mean MFCV has often been reported. This decrease in the mean MFCV, in addition, has been extensively studied, and is believed to be at least partially responsible for the shifts of the frequency content of the surface EMG signals to lower frequencies. As mentioned above, the shapes of action potentials can be potentially affected by the spread of the NMJs or endplates. Thus, during a sustained isometric fatiguing contraction, can IZ width influence the change of the mean MFCV?

To date, limited information is available regarding the relationship between the IZ width and mean MFCV during a sustained isometric fatiguing contraction. Specifically, muscle fibers with different sizes of the spread of endplates may have differential effects on mean MFCV, thereby potentially affecting the central frequency of a surface EMG signal. This information can be useful for researchers such as muscle physiologists and/or exercise physiologists, as it would help to increase knowledge regarding the relationship between IZ width and MFCV during fresh and fatigued statuses. Therefore, the purpose of this study was to examine the influence of the biceps brachii IZ width on the change of mean MFCV during a sustained isometric elbow flexion muscle action at 60% MVC in young men. Considering both IZ width and mean MFCV can serve as important pathophysiological parameters for diagnosing a variety of neuromuscular disorders, the findings of this investigation can eventually be beneficial for clinicians, as they may help to understand underlying pathological mechanisms in some neuromuscular diseases. For example, specific neuromuscular disorders such as muscular dystrophy, myopathies, and myasthenia gravis are characterized by abnormally large IZ width. Thus, the linkage between IZ width and mean MFCV can be potentially used to study the mechanism(s) of fatigability or muscle weakness in these patients.

Material and methods

Participants

Fifteen recreationally active (engaging in 2-8 h of aerobic exercise, 2-10 h of resistance exercise, and/or 2-5 h of recreational sports per week) young men (mean±SD age = 23.2±4.5 years; height= 177.6±8.2 cm; body weight= 83.3±11.0 kg) volunteered to participate in this study. The study was approved by the University Institutional Review Board for Human Subjects. All subjects signed a written informed consent form and completed a health history questionnaire, which indicated that they had no current or recent (at least 6 months prior to the study) neuromuscular or musculoskeletal disorders.

Experimental design

This investigation consisted of two laboratory visits separated by at least 48 hours: (1) familiarization visit, during which the subjects were familiarized with all the testing procedures, and (2) experimental testing visit. The testing was always performed on the dominant arm (based on throwing preference) of the subjects.

Experimental procedures

Isometric testing

The isometric testing was performed on a custom-built isometric strength testing table. Each subject first sat in front of the strength testing apparatus, with the elbow positioned into a U-shaped pad. The investigator then instructed the subjects to grasp a handle that was connected to a load cell (Model SSM-AJ-500; Interface, Scottsdale, AZ, USA) for the measurement of isometric force of the elbow flexors. Adjustments for seat height and the distance between the handle and the load cell were made to ensure that the subject’s arm and forearm were at a 90° elbow joint angle. With the palm supinated, the subjects performed several submaximal isometric elbow flexion muscle actions for the purpose of warming up the elbow flexors. Once the subjects felt comfortable with this type of muscle action, they performed two separate 5-second isometric MVCs, with a 2-minute rest between the contractions. The higher MVC value was then selected as the baseline isometric MVC (MVCb). Five minutes after the isometric strength testing, the subjects were instructed to perform several separate submaximal isometric elbow flexion muscle actions at a target force of 60% MVCb. To help the subjects produce the desired force level, visual feedback was provided on a computer screen to display the target force template and the real-time digitized force sig-
nal. Each submaximal isometric muscle action was 10-second long and used to provide EMG signals for locating the IZ. The location of the IZ was usually identified within two to three trials. In addition, two minutes of rest were provided between consecutive submaximal isometric muscle actions.

**Isometric fatiguing exercise**

Once the IZ was located, each subject was required to rest for 5 minutes for the subsequent isometric fatiguing exercise. This exercise was performed at a target force of 60% MVC. Once the contraction started, the subjects were required to match the target 60% MVC force displayed on the computer screen and sustain the force as long as possible. In addition, strong verbal encouragement was provided by the investigator during the entire contraction. The contraction was terminated when the force production was less than 55% MVC for 3 seconds.

**Measurements**

**Force measurement**

During all isometric MVCs, submaximal isometric contractions, and the fatiguing exercise of the experimental testing visit, force was detected by the tension-compression load cell (Model SSM-AJ-500; Interface, Scottsdale, AZ, USA), digitized at a sampling rate of 20000 samples/second with a 12-bit analog-to-digital converter (National Instruments, Austin, TX, USA), and stored in a personal computer (Dell Optiplex 755, Round Rock, TX, USA) for further analyses.

**Surface EMG acquisition**

During each 10-second submaximal isometric contraction, 15 separate bipolar surface EMG signals were recorded from the biceps brachii with a 16-channel linear electrode array and surface EMG16 data acquisition system (EMG16, LISIN-Prima Biomedical & Sport, Treviso, Italy). Before testing, the skin over the belly of the biceps brachii was prepared by shaving and cleansing with rubbing alcohol. With the conduction gel applied on the muscle belly, a probe of 16 silver bar electrodes (5 × 1 mm contact surface area, 5 mm interelectrode distance, OttinoBioelettronica, Torino, Italy) was placed over the biceps brachii along the muscle fiber direction to examine the general location of the IZ. The raw EMG signals from each electrode of the probe were differentially amplified (gain x1000), analog filtered (4th order Bessel, bandwidth=10-500 Hz) through the surface EMG16 data acquisition system, and displayed on a computer screen for the purpose of locating the IZ. Once the general IZ location was determined, the investigator marked this location, and replaced the probe with another 16-electrode probe, which had smaller contact surfaces and interelectrode distances (1 × 1 mm contact surface area, 2.5 mm interelectrode distance, OttinoBioelettronica, Torino, Italy). Extra care was taken to ensure that the marked IZ was near the middle of the electrode array. This smaller probe allowed us to locate the IZ with greater accuracy, and to determine the IZ width with greater precision when compared with the larger probe. A reference electrode strap (OttinoBioelettronica, Torino, Italy) was moistened and wrapped around the subject’s dominant wrist to reduce electromagnetic noise as much as possible.

During the isometric fatiguing exercise, the 16-electrode probe was placed on the skin surface over the biceps brachii muscle in a direction parallel to the muscle fibers, with the predetermined IZ site near the middle of the electrode array, and fixed with adhesive tape (Figure 2). The raw EMG signals from each electrode of the probe were recorded in bipolar signal acquisition mode (gain x1000) and analog filtered (4th order Bessel, bandwidth=10-500 Hz) with the surface EMG16 data acquisition system. All bipolar EMG signals were digitized with a 12-bit analog-to-digital converter (National Instruments, Austin, TX, USA) at a sampling rate of 2048 Hz and stored in a personal computer (Dell Optiplex 755, Round Rock, TX, USA) for subsequent analyses. Extra care was taken...
to ensure that force and surface EMG recordings were synchronized during the entire isometric fatiguing contraction.

IZ location identification

Based on the 15 separate bipolar surface EMG signals detected from the biceps brachii during each submaximal isometric contraction, the location of the IZ was visually identified by the EMG channel(s) that had minimum amplitude or phase reversal\textsuperscript{22,23}: if there is a reversal in EMG signal polarity in two adjacent channels, the IZ is located between these channels; if there is a single channel exhibiting lowest amplitude, then the IZ is located in this channel. This technique allowed us to define the position of the IZ at a resolution of half of the interelectrode distance (1.25 mm). In addition, the width of IZ was determined by counting the number of EMG channel(s) that demonstrated minimum amplitude, refined to half of the interelectrode distance (1.25 mm)\textsuperscript{13}, and then recorded as 1.25 mm, 2.50 mm, 3.75 mm, and so on. Figure 1 shows an example of IZ location and width detections through the EMG signals recorded from a subject.

Data analyses

All EMG signals were automatically broken into 0.25-second length epochs. The mean MFCV was calculated for each epoch through the entire fatiguing contraction using an EMG acquisition software package (EMGACQ 1.6, LISiN-Prima Biomedical & Sport, Treviso, Italy). Figure 2 shows a schematic diagram of the three bipolar EMG signals (triplet) that were used to estimate the mean MFCV. Since the 16-electrode probe produced 16 single differential bipolar EMG signals, 13 triplets were obtained (i.e. signals of triplet 1 are obtained from electrodes 1-2-3-4, triplet 2 from electrodes 2-3-4-5 and so on). The selected triplet for the mean MFCV estimate was the one showing the highest maximal cross-correlation coefficient between its two adjacent double differential signals (greater than 0.80 in all cases of this study)\textsuperscript{8,24}. In addition, extra care was taken to ensure that the selected triplet was at least 2 channels (10 mm) away from the IZ\textsuperscript{22}.

With the epoch set at 0.25 second, a 50-s fatiguing contraction can produce a total of 200 data points (epochs). Thus, in order to perform subsequent statistical analyses, these data series were normalized to 10 data points (epochs). Thus, in order to perform subsequent statistical analyses, these data series were normalized to 10 data points, which was done by splitting the data into 10 equal blocks and averaging over each block. The averaged MFCV (FreshMFCV) during the fresh state were calculated from the data in the first block of the fatiguing contraction, and the averaged MFCV (FatiguedMFCV) during the fatigued state were calculated from the data of the last block. In addition, the percent decline in MFCV (%ΔMFCV) during the isometric fatiguing exercise was calculated as (FreshMFCV-FatiguedMFCV) ÷ FreshMFCV×100%.

Statistical analyses

Three separate non-parametric Kruskal-Wallis one-way analyses of variance (ANOVA\textsuperscript{s}) were used to examine whether there was a difference in each mean MFCV variable (FreshMFCV, FatiguedMFCV, and %ΔMFCV) among groups with different IZ width. When appropriate, follow-up tests included non-parametric Mann-Whitney U-tests. In addition, Separate non-parametric bivariate correlation analyses were used to examine the relationships between the IZ width and the FreshMFCV, the IZ width and the FatiguedMFCV, and the IZ width and the %ΔMFCV. All statistical analyses were conducted using statistical software IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA) with alpha set at p≤0.05.

Results

The IZ widths of the biceps brachii muscle in the current investigation varied in the range of 1.25 to 5.0 mm, with 1.25 mm for 4 subjects (mean±SD age = 24.5±6.0 years), 2.5 mm for 6 subjects (mean±SD age= 22.9±5.7 years), 3.75 mm for 2 subjects (mean±SD age= 23.0±1.4 years), and 5.0 mm for 3 subjects (mean±SD age= 22.7±1.5 years). In addition, the FreshMFCVs ranged from 3.13 to 5.94 m/s, with Mean ± SD= 4.47±0.81 m/s; and the FatiguedMFCVs ranged from 2.53 to 4.87 m/s, with Mean ± SD= 3.36±0.75 m/s.

The results from the Kruskal-Wallis tests indicated that there was a statistically significant difference for %ΔMFCV among groups with different IZ width (χ² (3)=11.571,
Table 1. Kendall’s tau as well as p-values for the relationships between the IZ width and the FreshMFCV, the IZ width and the FatiguedMFCV, and the IZ width and the %ΔMFCV.*

| Bivariate Correlation         | Kendall's tau | p-value  |
|-------------------------------|---------------|----------|
| IZ width & FreshMFCV          | 0.153         | 0.232    |
| IZ width & FatiguedMFCV       | -0.284        | 0.087    |
| IZ width & %ΔMFCV             | 0.807         | <0.001   |

* IZ: innervation zone; FreshMFCV: muscle fiber conduction velocity during the fresh status; FatiguedMFCV: muscle fiber conduction velocity during the fatigued status; %ΔMFCV: percent decline of the muscle fiber conduction velocity during isometric fatiguing exercise.

Figure 3. The relationship between IZ width and percent decline of the mean MFCV (%ΔMFCV).

Discussion

The primary finding from this study is that the percent decline of the mean MFCV during high intensity (60% MVC) isometric fatiguing exercise is positively related to the width of the IZ. In order to examine whether this relationship was due to a greater FreshMFCV, and/or a less FatiguedMFCV, we performed two separate bivariate correlations to examine the relationships between the width of the IZ and the FreshMFCV and the IZ width and the %ΔMFCV. Specifically, only the IZ width and the %ΔMFCV were positively and significantly correlated (Kendall’s tau= 0.807; p<0.001; Figure 3).

Although this number is quite small, comparing to motor units or muscle fibers that have much smaller size, it is possible that the wide IZs observed from the current study were simply from relatively large muscle fibers. However, this hypothesis does not fully explain our finding on the weak and non-significant relationship between the IZ width and the FreshMFCV. Therefore, not only the muscle fiber size, but other factor(s)/mechanism(s) might have also influenced the mean MFCV in the current investigation. For example, based on the core conductor model, MFCV is proportional to the square root of the muscle fiber radius. Therefore, a dramatic difference between the radiiuses of muscle fibers may only result in a small or moderate difference between the MFCVs, which might have contributed to the weak and non-significant relationship between the FreshMFCV and the IZ width. In addition, another explanation for this weak and non-significant relationship is possibly due to the measurement technique we have used in this investigation. As we mentioned, 1.25 mm was the finest resolution for IZ width determination. Although this number is quite small, comparing to motor units or muscle fibers that have much smaller size, it is possible that the resolution of the current measurement technique was still not small enough to detect the differences (in both MFCV and IZ width) that may exist between motor units of different type.

Another very important factor that can also affect the mean MFCV is the muscle fiber type composition. Sadoyama et al. were one of the first few researchers to examine the relationship between MFCV and muscle fiber type composition. Specifically, the authors reported a significant and positive relationship between the percentage area of FT muscle fibers and the MFCV in human vastus lateralis muscle after they cor-
related these two variables. A similar study conducted by Kupa et al.\textsuperscript{29} examined three different rat muscles (soleus, extensor digitorum longus, and diaphragm) \textit{in vitro}, and the authors\textsuperscript{29} found that muscles with a greater percentage of FT fibers demonstrated a greater value for the initial MFCV, as well as a greater percent decline for this variable during a sustained contraction. However, an important note should be taken: the initial MFCV was only proportional to the percentage area of the whole muscle that was composed of FT fibers, rather than the percentage of FT fibers in total number of muscle fibers being examined\textsuperscript{29}. Therefore, mean MFCV is influenced by the combined effect from both muscle fiber type composition and muscle fiber size.

Generally speaking, FT muscle fibers tend to have larger cross-sectional area (CSA) when compared to ST muscle fibers\textsuperscript{30}. However, muscle fibers with large CSA are not necessarily composed of high percentage area of FT fibers. For example, endurance trained individuals tend to have higher percent area of ST muscle fibers and muscle fiber CSA in general when compared with sedentary individuals\textsuperscript{31}. Thus, to explain our finding on the non-significant correlation between the IZ width and the FreshMFCV, it is possible that a wider IZ (e.g., 5 mm in the current study) was indeed detected from the muscle fibers that have larger CSA with slightly high percentage of FT fibers (thus making a greater percent decline of mean MFCV during fatiguing exercise, with a trend of slightly lowered FatiguedMFCV (Kendall’s tau = -0.284, \(p = 0.087\)); however, a high percentage FT fiber of all fibers examined does not necessarily indicate a relatively high percentage area of the FT fibers. Instead, a relatively higher percentage area of ST fibers might exist possibly due to endurance training, considering some of our subjects in the current study might be slightly endurance-trained. In this case, a relatively low initial MFCV (FreshMFCV) can be expected, even for muscle fibers with relatively wide IZs. A biopsy study on muscle fiber type composition\textsuperscript{32} suggested that the mean fast-twitch fiber type percentage for the biceps brachii was 57.7\%, with a 95\% confidence interval between 49.3\% and 66.2\%. Thus, it is possible that the subjects in the current study had large variations in both fiber composition and fiber CSA. Therefore, the combination of both factors (muscle fiber type and size) might have a contradicting effect on the initial MFCV, making its correlation to IZ width weak and non-significant. This hypothesis is supported by the concern mentioned in Farina et al.\textsuperscript{33}, where the authors questioned the rationale of “…analyzing MFCV from the entire motor unit pool with high-force contractions and attributing lower average MFCV values simply to larger proportion of type I fibers…” (Farina et al.\textsuperscript{33}, p. 398).

**Technical concerns and limitations**

Our study showed a novel finding regarding the relationship between the width of IZ and the change of MFCV. However, this study is not without limitations. First and foremost, we have noticed through the current study, that with the majority of the subjects having IZ width of 1.25 and 2.5 mm, only a smaller portion (33\%) of the subjects had relatively wider IZs (3.75 and 5.0 mm). Obviously, this limited sample size in the wider IZ groups could have affected the results from the bivariate correlation analyses. Although it might be difficult to have subjects with relatively wide IZ, to establish more accurate relationship between MFCV variables and IZ width, we suggest future research using much larger sample size with similar number of subjects among groups with different IZ widths. Second, the linear electrode array used in this investigation only provided one-dimensional (muscle fiber direction) IZ and MFCV estimations, but not for the information in the medial-lateral (circumference) direction. Based on the description from Masuda et al.\textsuperscript{34}, it is possible that the IZ distributions differ at medial-lateral direction in the biceps brachii muscle. Thus, results could be different if applying the linear electrode array at different medial-lateral locations. Therefore, when interpreting the results, it is important to mention that the results of this study may not be generalized to the entire muscle. In fact, we are aware that such limitation can be overcome by using high-density two-dimensional surface EMG recordings, which would allow researchers to determine the topographic map of IZ in both muscle fiber and circumference directions\textsuperscript{34,36}. Third, caution should also be taken when interpreting the results from this study, as the examined muscle biceps brachii is a fusiform muscle with neuromuscular junctions being distributed within a relatively small region, and specifically we used 60\% MVC as the exercise intensity. Results can potentially be different if a muscle with pennation angles (e.g. vastus lateralis) is examined with slightly different exercise intervention (e.g. different contraction intensity) applied. Specifically, although a large portion of the biceps brachii motor units are recruited at 60\% MVC\textsuperscript{37}, a small number of high-threshold motor units (recruitment threshold greater than 60\% MVC) are recruited during the isometric fatiguing contraction to compensate for fatigue. In this case, the estimations of both IZ width and MFCV could have been altered due to the recruitment of new motor units, which might have influenced the results of the current investigation.

In conclusion, the current investigation suggests that the width of the IZ is positively correlated to the percent decline of the mean MFCV during sustained isometric fatiguing contraction at 60\% MVC. This relationship is likely influenced by the combined effect of both muscle fiber size and the muscle fiber type composition. We believe that the finding of this investigation is very important for understanding the underlying mechanism(s) of the decline of MFCV during isometric fatiguing exercise. However, due to the potential technical errors (e.g. recording electrodes, signal analysis, etc…) and limited sample size in the current investigation, it could be possible that the changes in MFCV observed in the current study were not physiologically meaningful. Thus, it may be still too early to use the current findings to assess clinical changes in special populations. Future research should use more advanced technique (e.g. high-density two-dimensional surface EMG recordings) to examine the relationship between MFCV variables and IZ width in other muscles with different functions and structures, under the exercise intervention with different contraction intensities. In addition, emphasis should also be placed on ex-
amining individuals who have specific neuromuscular disorders that are characterized by abnormal IZ width and/or mean MFCV in the future, as this type of research can potentially help clinicians and researchers understand the mechanism(s) of fatigability and/or muscle weakness in this population.

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