Inter-Gender sEMG Evaluation of Central and Peripheral Fatigue in Biceps Brachii of Young Healthy Subjects

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Abstract

Purpose
The purpose of the present study was to evaluate inter-arm and inter-gender differences in fractal dimension (FD) and conduction velocity (CV) obtained from multichannel surface electromyographic (sEMG) recordings during sustained fatiguing contractions of the biceps brachii.

Methods
A total of 20 recreationally active males (24±6 years) and 18 recreationally active females (22±9 years) performed two isometric contractions at 120 degrees elbow joint angle: (1) at 20% maximal voluntary contraction (MVC) for 90 s, and (2) at 60% MVC until exhaustion the time to perform the task has been measured. Signals from sEMG were detected from the biceps brachii using bidimensional arrays of 64 electrodes and initial values and rate of change of CV and FD of the sEMG signal were calculated.

Results
No difference between left and right sides and no statistically significant interaction effect of sides with gender were found for all parameters measured. A significant inter-gender difference was found for MVC (p<0.0001). Initial values of CV were higher in females than in males at both force levels (20% MVC: p<0.0001; 60% MVC: p<0.05) whereas a lower initial estimate of FD was observed in females compared to males (20% MVC: p<0.05; 60% MVC: p<0.0001). No difference in CV and FD slopes was found at 20% MVC between genders. At 60% MVC significantly lower CV and FD slopes (CV and FD: p<0.05) and a more protracted time to exhaustion were found in females than in males (p<0.0001). When considering time to exhaustion at both levels of contraction no difference in percentage change (Δ%) of CV and FD slopes was found between genders (p>0.05). During the sustained 60% MVC no statistical correlation was found between MVC and CV or FD initial estimates nor between

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MVC and CV or FD slopes both in males and females whereas. A significant positive correlation between CV and FD slopes was found in both genders (males: r = 0.61; females: r = 0.55).

Conclusions
Fatigue determines changes in FD and CV values in biceps brachii during sustained contractions at 60% MVC. In particular males show greater increase in the rate of change of CV and FD than females whereas no difference in percentage change of these sEMG descriptors of fatigue was found. A significant correlation between FD and CV slopes found in both genders highlights that central and peripheral myoelectric components of fatigue may interact during submaximal isometric contractions.

Introduction
Physiological fatigue is an experience of daily life responsible for failure to maintain the required level of force overtime [1]. Central and peripheral components lead to fatigue [2–4] but their relative contribution is not easily suitable for quantification or measurement [5].

Central fatigue originates in structures above the neuromuscular junctions from the central nervous system to the peripheral nerves leading to changes in motor unit (MU) recruitment behavior including decline in the MU recruitment threshold and progressive recruitment of new MU without change to the recruitment order [6]. Peripheral aspects of fatigue include local changes at the skeletal muscle level, namely metabolic acidosis and protons accumulation [7], that may impair sarcolemmal excitability, electro-mechanical coupling, acto-myosin myofibrillar interaction and metabolic fuelling of the myofibers.

Continuous monitoring of local muscle fatigue during a task is possible by measuring the myoelectric activity of a muscle by surface electromyography (sEMG). Biochemical and physiological changes in muscles during fatiguing contractions are, namely, reflected also in properties of myoelectric signals recorded on the surface of the skin above the muscle(s) concerned, leading to what is commonly defined as myoelectric manifestations of muscle fatigue [6].

Myoelectric manifestations of fatigue are mainly related to two physiological phenomena: (1) the slowing of motor unit action potentials (MUAP) during their travelling along muscle fibers, that is the reduction of their conduction velocity (CV) [8,9,10], and (2) the synchronization of MU by the central nervous system, which is defined as a higher occurrence of simultaneous discharge of action potentials from different MU to increase the mechanical output when the whole MU pool is recruited [11], as observed in trained individuals [12] and in presence of central lesions [13]. Therefore, to evaluate peripheral components of muscle fatigue, CV rate of change (i.e. slope) might be estimated during isometric tasks [14–21], whereas to evaluate central components of fatigue sEMG descriptors of MU synchronization may be useful.

The main problem with the existing techniques quantifying MU synchronization using sEMG lies in their dependency on the CV of MU action potentials (MUAPs), since MU synchronization level and CV often change in parallel [6]. Therefore, the use of nonlinear more sensitive parameters to extract information from sEMG signals during isometric and dynamic fatiguing contractions may be preferred over traditional amplitude or spectral parameters (for an exhaustive and comprehensive review, see Gonzalez-Izal et al., 2012) [16]. A sEMG
descriptor of the level MU synchronization is fractal dimension (FD), which shows high
dependency on MU synchronization and, compared FD to other linear and non-linear muscle
fatigue indices, least affected by CV changes and weakly affected by fat layer thickness [22,23].

We recently reported that a decay of FD during sustained fatiguing isometric contrac-
tions of the quadriceps muscles may mirror a progressive MU synchronization due to cen-
tral fatigue [24] in young females [25]. Indeed, Boccia and colleagues (2016) reported a
steeper rate of change of FD in elderly males compared to young males during fatiguing con-
tractions of the knee extensors suggesting a greater increase in motor unit synchronization
with age [26].

In addition to CV and FD rate of change, a different method to assess central and peripheral
fatigue is based on electrical stimulation during MVC combined with sEMG [27,28,29,30].

The sources of inter-individual variability in peripheral and central fatigue, namely inter-
gender differences, are still elusive. Strength-matched females and males show the same time
to failure of a sustained submaximal isometric contraction of the elbow flexor muscles whereas
a significant sex related difference is maintained when an intermittent isometric task is per-
formed until failure [31]. These evidence indicates that a strength-related mechanism may
contribute to the longer time to task failure during isometric sustained contractions [32] and
the shorter time to task exhibited by males during submaximal isometric contractions of the
elbow flexors, unlike lower limb muscles [33,34], has been mainly attributed to peripheral
mechanisms. These mechanisms appear to be mirrored by diverse changes in myoelectric
manifestations of fatigue including higher CV slopes than females [35].

Despite females sustain isometric contractions with the elbow flexor muscles for a longer
duration than males, robust evidences suggest a similar reduction in voluntary activation at
task failure for superimposed stimulations at the motor cortex [36] and skeletal muscle [37].
No data are currently available on whether differences in the initial value and rate of change of
MU synchronization during sustained contractions of the elbow flexors may contribute to dif-
ferent adaptation to muscle fatigue by the central nervous system in males compared to
females. The aim of the study was to use sEMG indexes of fatigue (CV and FD) to compare
healthy young males versus females during prolonged isometric contractions of the biceps bra-
chii. In particular we aimed to test whether a diverse level of initial value and rate of change of
MU synchronization is associated with diverse gender-related resistance to fatigue. The main
conclusions of the study were: in sustained fatiguing task (1) significantly greater decrease in
the rate of change of myoelectric manifestations of fatigue and higher time to task in females
than males; (2) no sex difference in percentage change of CV and FD; (3) a significant correla-
tion between CV and FD slopes at higher contraction level.

Methods

Subjects

A total of 20 recreationally healthy active males (mean±S.D.: age 24.95±6.1 years; height 176±4
cm; weight 73±8 kg) and 18 recreationally healthy active females all within one week after the
menstrual cycle (mean±S.D.: age 22±9.2 years; height 167 ±3 cm; weight 62±9 kg) reporting
no clinical history of previous arms injuries, volunteered to participate. Prior to testing, sub-
jects signed a written informed consent. The study was settled at C.R.I.A.M.S. laboratory in
Voghera. The study was approved by the local ethics committee of the Swiss Italian Health and
Sociality Department, Switzerland. Volunteer subjects were excluded if they had a positive his-
tory of orthopedic disorders affecting neck and shoulder regions and a positive history of neu-
rological disorders.
Experimental procedure

Subjects' arms were positioned correctly and comfortably in an isometric-ergometer (MUC1, OT Bioelettronica, Turin, Italy) equipped with a load cell (CCT Transducer, linear, full scale 100kg). In order to isolate the action of the biceps brachii, the wrist was fastened to the machine. Participants were sat, with the elbow at 120 degrees as described in Fig 1. A 64-channels matrix was placed in parallel with the orientation of the muscle fibers between the distal tendon and the innervation zone of the biceps brachii in order to have a pure propagation of MU action potentials (MUAPs). Subject's position did not provide any support or resistance during contraction.

After 5 min rest, two isometric MVCs were performed, separated by 2 min rest. During each contraction, the force trace was displayed on a computer monitor as visual feedback. Participants were instructed to increase the force up to the maximum, and to hold it as steady as possible, for 2–3 s. Participants were given verbal encouragement.

After 2 min rest, a low intensity sustained contraction (20% MVC) was performed for 90s and the Borg scale values (from 6 to 20) [38] were obtained. After 4 min rest, subjects were asked to perform a high level sustained contraction (60% MVC) until exhaustion, during which they were verbally encouraged to keep the force level as long as possible, until the force value decreased to below 90% of the target [39]. The experimental procedure was randomly conducted on both arms (30 min rest between side) in two trials, with an interval of a week.

Fig 1. 64 channels electrode arrays position on biceps brachii muscle with elbow maintained at 120 degrees angle. The electrode array is aligned with respect to the muscle fibers and located between the distal tendon and the innervation zone. On the right side example of recorded signals detected from the biceps brachii muscle of a representative subject during a 60% MVC contraction. The insets show raw surface EMG signals detected during selected epochs at the beginning and at the end of contraction.

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EMG and force measurements
Myoelectric signals were detected through a 64 electrodes matrix (10 mm IED, 8 lines, 8 columns) (Spes Medica, Battipaglia, Italy) used to obtain monopolar sEMG signals. Biceps brachii was chosen primarily to obtain high-quality sEMG signals due to the simplicity of movement, isolation of the muscle contraction, and parallel fiber orientation. The adhesive matrix was applied following muscle fibers leavings in correspondence to the muscle belly previously identified by ultrasound scan (Phillips CX-30). The advantage of multichannel acquisition is the possibility to select the best channels afterwards, reducing the risk of short circuits or bad contacts. The EMG signals were amplified (EMG-USB2; OT Bioelettronica, Turin, Italy), sampled at 2048 Hz, and stored on a computer.

Signal processing
Data were divided in epochs of 0.5 seconds and each variable was computed for each epoch. The exhaustion time was computed as the time instant when the force was below 5% of the target. The regression line was computed for all the values from the beginning of the contraction to the exhaustion time. For each acquisition, we selected the channels for the analysis by means of visual inspection. The column showing the largest portion of propagating channels with biggest amplitude was selected, and the channels between IZ and tendons were selected for CV computation. FD was computed for each of the selected channels and then averaged. FD was estimated using the box-counting method. Briefly, as expressed in [40], a grid of square boxes was used to cover the signal, and the number of boxes that the sEMG waveform passes through was counted. When decreasing the side of the boxes in a dichotomic process, the number of boxes required to cover the signal increased exponentially. However, by plotting the logarithm of the number of boxes counted (log N) vs. the logarithm of the inverse of the box size (log 1/S), the exponential relationship became linear. The slope of the interpolation line (estimated using the least mean squared procedure) is the FD. CV was estimated using a multichannel algorithm on double differential signals, based on the matching between signals filtered in the temporal and in the spatial domains [41].

CV values outside the physiological range (3–6 m/s) [42], were excluded from the analysis.

Statistical analyses
The variables used for the statistics were the initial values and slopes of CV and FD, determined by linear regression overtime.

A Shapiro-Wilk test revealed that the variables were normally distributed. Data were reported as mean ± standard deviation (SD) and statistical significance was set to P<0.05. Paired t-test was used to compare significant differences between arms, unpaired t-test was used to compare significant differences between genders, whereas two-way ANOVA for multiple comparisons was used to find significant interactions between side and gender. Statistical analyses were performed using Prism Graphpad (San Diego, California). Statistical tests were conducted for FD and muscle fiber CV initial values and slopes, considering data from left and right biceps brachii as a single set.

Results
For all parameters measured no statistical difference was found between right and left arm, and no significant two-way interaction effect was found between arm side (left and right) and gender (male and female). Therefore data from left and right biceps brachii were pooled for
subsequent analysis. In females, biceps brachii was characterized by thicker skinfold than in males (females 7.3 ± 2.6 mm; males 4.9 ± 1.8 mm, p < 0.05) detected by a skinfold caliper.

The following Borg scale data were obtained after 20% and 60% MVC contractions respectively: females: 11.6 ± 1, males 11 ± 0.5, p > 0.05 and females 16.45 ± 0.9, males 15.06 ± 1.68, p > 0.05.

A significant inter-gender difference was found for MVC (p < 0.0001, Table 1) and for time of task at 60% MVC p < 0.0001, Table 1).

Initial values of CV were higher in females than in males at both force levels (20% MCV: p < 0.0001; 60% MCV: p < 0.05, Table 2), whereas a lower initial estimate of FD was observed in females compared to males (20% MCV: p < 0.05; 60% MCV: p < 0.0001, Table 2).

No CV and FD slope difference was found at 20% MVC in both genders (CV: males -0.03%/s; females -0.03%/s; FD: male -0.02%/s; females -0.01%/s, p > 0.05 Table 2). A significant slope difference was found at 60% MVC for both parameters and appeared significantly lower in females compared to males (CV: p < 0.05; FD: p < 0.0001, Table 2). No inter-gender difference was found in percentage change of CV and FD slopes at both levels of contraction (Table 2 and Fig 2).

No statistical correlation was found between MVC and CV (males: r = 0.23; p = 0.14; females: r = 0.41; p = 0.011) or FD (males: r = 0.16; p = 0.3; females: r = 0.14; p = 0.39) initial estimates nor between MVC and CV (males: r = 0.01; p = 0.9; females: r = 0.23; p = 0.17) or FD (males: r = 0.01; p = 0.9; females: r = 0.3; p = 0.07) slopes at 60% MVC. No significant correlation was found between MVC and time of task at higher level of contraction for both genders (males: r = 0.17 p = 0.26; females: r = 0.26 p = 0.12; pooled data: r = 0.35, p = 0.001) and for time of task and slopes (FD males: r = 0.30 p = 0.05; females r = 0.25, p = 0.129; CV: males r = 0.03, p = 0.84; females r = 0.47, p = 0.003; pooled data: CV: r = 0.40, p = 0.0002; FD: r = 0.43, p = 0.0001). A significant positive correlation was found in both

<table>
<thead>
<tr>
<th>%MVC</th>
<th>CV</th>
<th>Males</th>
<th>Females</th>
<th>FD</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>Initial value</td>
<td>4.18±0.45</td>
<td>4.79±0.89***</td>
<td>1.59±0.02</td>
<td>1.58±0.02*</td>
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<tr>
<td>60%</td>
<td>Initial value</td>
<td>4.69±0.50</td>
<td>5.41±1.68*</td>
<td>1.62±0.02</td>
<td>1.60±0.02**</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td>Slope</td>
<td>-0.03±0.04</td>
<td>-0.03±0.09</td>
<td>-0.02±0.02</td>
<td>-0.01±0.01</td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>Slope</td>
<td>-0.89±0.29</td>
<td>-0.66±0.41*</td>
<td>-0.22±0.07</td>
<td>-0.14±0.07*</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td>Δ%</td>
<td>-3.74±4.2</td>
<td>-2.75±8.8</td>
<td>Δ%</td>
<td>-1.69±2.1</td>
<td>-1.04±0.9</td>
</tr>
<tr>
<td>60%</td>
<td>Δ%</td>
<td>-3.47±13</td>
<td>-32.15±14</td>
<td>Δ%</td>
<td>-8.4±2.8</td>
<td>-7.2±3.4</td>
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(Asterisk means “significantly different from males”:
* = p < 0.05;
** = p < 0.001;
*** = p < 0.0001)

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genders between CV and FD rate of changes measured during the sustained 60% MVC contraction (males: $r = 0.613$, $p = 3.35e-05$; females: $r = 0.551$, $p = 0.00049$) (Fig 3).

**Discussion**

In the present study FD and CV measurements were used as myoelectric manifestations of muscle fatigue (central and peripheral) and were recorded by multichannel surface EMG.
during fatiguing isometric contractions of the biceps brachii. The most significant conclusion was that males showed a greater increase in the rate of change but no difference in the percentage change of CV and FD in comparison with females. Moreover, our results showed that gender and arm side do not have any significant interaction effect in the sEMG parameters measured.

These results show that gender slightly accounts for differences in myoelectric manifestations of peripheral fatigue, as mirrored by time changes in CV, and central fatigue, as mirrored by changes in FD/MU synchronization.

**MVC**

Males muscles are usually stronger than females [43]. This difference might be due to the presence of larger fibers, as observed both in the upper and lower limbs muscles, and not to differences in mode of fibers recruitment [44]. However, the mechanisms responsible for sex differences in fatigability are not dependent on MVC as no difference in time to failure has been observed in strength-matched males and females during sustained isometric submaximal contractions [37]. On account of this, we found that MVC was significantly higher in males than in females and no significant correlation was found between maximal torque and initial values and rate of change of measured myoelectric descriptors of fatigue.

**Initial values and of CV and FD**

Based on Rainoldi’s observations, CV would be a good indicator of motor unit recruitment [45]. Initial values of CV are known to depend on muscle fibers recruitment, type of contraction [46], and fibers diameters [47]. Accordingly, our results showed that mean initial values of CV were higher during isometric contractions at 60% than at 20% MVC. This might be explained by the recruitment of progressively larger MU with increasing force output, and, therefore, with progressively higher CV values during fatiguing contractions, according to the Henneman size principle [48].

We observed with great interest that, at both force levels, initial values of CV were higher in females than in males with no interaction with arm side. These data, although in contrast with the presence of larger sized fibers in males than in females, agree with previous results obtained in our laboratory in vastus lateralis muscle [25] and may be explained considering that initial values of EMG variable estimates are not only related to the recruited MU pool, but are also affected by factors such as subcutaneous tissue thickness, skinfold thickness and fiber end effects [49,50]. Confirming to previously published data [51], we found that female biceps brachii was surmounted by a thicker skinfold than male one, probably causing overestimation of CV. In contrast, males reported higher FD initial values than females at both force levels analyzed. Although this difference may justify inter-gender differences in MU synchronization, caution should be used as it has been suggested that subject-dependent factors (e.g. fat layers and skin properties) may influence FD but not its rate of change [22]. Therefore initial values of FD not necessarily reflect the actual MU synchronization level whereas FD slope provides a reliable estimate of the actual change in MU synchronization.

**CV and FD slopes**

**CV**: at 20% of MVC males and females presented an equal slope (around -0.03%/s, Table 2), consequently no statistically significant difference between genders was found, probably due to the low intensity level of contraction. At 60% MVC females displayed slower slope (-0.66±0.41%/s, Table 2) than males (-0.89±0.29%/s, Table 2) and the difference between genders appeared statistically significant (p<0.05). Differences in fibers size [52] and muscle
composition [53,54], unlike subcutaneous thickness [50], may account for the observed inter-
gender differences in peripheral sEMG descriptors of fatigue. Accordingly, it has been shown
that a lower proportion of type II muscle fibers in females and a higher proportion of type I
fibers in upper and lower limb muscles [44] may contribute to a lower decrease of CV over
time compared to males [55,56,57]. Another aspect may concern blood flow because during
low to moderate intensity sustained isometric contractions, when the muscle is not fully
occluded, blood flow might be more restricted in males than in females at the same contraction
intensity [58,59] due to their higher force generation [53,60].

**FD:** As previously reported, an increase in MU synchronization may be observed during
fatiguing isometric contractions at 25% MVC or higher [11,25,26,61–65].

Accordingly, during 90s 20% MVC contraction no change in MU synchronization was
observed in males and females, which showed similar FD rate of changes (-0.02%/s and -0.015/
s, respectively, p>0.05, Table 2). We reckon that the level of contraction was insufficient to
highlight gender differences due to task intensity. These conclusions are in concordance with
the observation by Fallentin et al. [66] demonstrating that the patterns of motor unit recruit-
ment during sub-maximal isometric contractions can be influenced by the level of contraction.
The same authors highlighted the existence of a close relationship between intrinsic properties
of the muscle and recruitment strategies of the central nervous system, which appear entirely
different during static contractions at high or low intensities. Accordingly we found that at
60% MVC, FD slopes were lower in females than in males (-0.22%/s and -0.14%/s, respectively; p< 0.05, Table 2) probably suggesting a diverse level of MU synchronization at this contrac-
tion level.

**Time to task and percentage change in CV and FD**

In agreement with previous investigations [31,60,67], we observed that at higher contraction
percentage males had briefer endurance time than females. This difference may be attributed
to the higher absolute target force in males compared to females.

In fact, a strong association between elbow flexors absolute force and time to task has been
found and this relation appears to be exponential and independent from gender [67]. The
shorter endurance time in stronger individuals has been attributed to their higher reflex-medi-
ated adjustment of the mean arterial pressure during isometric contractions. This response,
namely pressor response, is finalized to adjust the mismatch between muscular metabolic
request and blood perfusion. Consistently a lower pressure response was found in females
than males during submaximal contractions [67].

There was no sex difference in percentage change of CV and FD. Considering the difference
in endurance time between males and females, our finding suggests that differences in the CV
and FD rate of change probably occur as a result of differing times to task failure. These data
reinforce the role of the absolute contraction intensity in determining the percentage change
of myoelectric manifestations of fatigue at least at higher contraction levels [67]. However,
electrophysiological adaptations of the skeletal muscle fibers leading to changes in myoelectric
manifestations of fatigue may arise independently from changes in absolute torque have been
reported following endurance training [68].

**Correlations:** No significant correlation was found between MVC or time to task and any
initial values or slopes of CV and FD. Considering that the maximal voluntary strength that
subjects can express may influence the time to task failure, these findings confirm the not so
close relationship between time to task failure and sEMG descriptors of fatigue [67].

In agreement with previous findings from our laboratory [25,26], a significant positive cor-
relation was found between FD and CV normalized slopes at 60% MVC in both genders.
Considering that FD is less sensitive to changes in CV than other indices derived from the sEMG [22], we can assume that at higher level of contraction muscular fatigue may result of mutual interactions between central and peripheral mechanisms as previously hypothesized [69]. Moreover, a lower level of correlation found in females compared to males that may be explained by sex related differences in fatiguing threshold [70,71].

Limitations of the study
The fatiguing isometric task used in the present study, although highly reliable for sEMG measurements, is far from real-life movement and everyday physical demands. A second limitation of the present study is that only one low intensity and one high intensity isometric sustained contraction was analyzed but, in our experimental conditions, a protocol setup consisting of contractions from 10 to 100% MVC, although much more exhaustive, would have been too long and unsustainable.

Finally Salomoni et al. recently evidenced the need to consider the effects of hormonal fluctuations in females when observing the effects of gender on muscle fatigue [72]. Therefore, in the present study females during menstrual cycle were excluded.

Conclusions
This study investigated inter-gender differences in CV and, for the first time, FD slopes as indexes of peripheral and central myoelectric manifestations of fatigue during isometric contractions in healthy young humans. Results indicate that gender-related differences in fibers conduction velocity and MU synchronization rate of change may lie in disparities in strength. The correlation between FD and CV found at 60% MVC suggests that, during submaximal isometric contractions, central and peripheral myoelectric manifestation of fatigue mutually adapt to muscle fatigue in both genders.

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Author Contributions

Conceptualization: GD.
Data curation: CC FM.
Formal analysis: CC.
Funding acquisition: GD.
Investigation: FM LC MB VS MBP GG.
Methodology: CC GD MB.
Project administration: MBP LC FM GG.
Resources: GD.
Software: CC.
Supervision: GD.
Validation: CC.
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