TECHNICAL REPORT

Quadriceps muscles vastus medialis obliques, rectus femoris and vastus lateralis compared via electromyogram bicoherence analysis

R. J. Simeoni and P. M. Mills

School of Physiotherapy and Exercise Science, Griffith University, Gold Coast, Australia

Abstract

Bicoherence analysis is applied to electromyogram (EMG) data for vastus medialis obliques (VM), rectus femoris (RF) and vastus lateralis (VL) quadriceps muscles of 18 adult male subjects for isometric knee extension exercise. Mean average bicoherence for VM, RF and VL is 30.9 ± 5.8, 26.0 ± 1.2 and 25.4 ± 1.4% respectively and repeated measures ANOVA differentiates the muscles on the basis of average bicoherence (F = 16.2 (1, 17), p = 0.0009, VM cf. VL and F = 15.4 (1, 17), p = 0.0011, VM cf. RF). Prominent regions representative of strong second-order phase coupling between constituent EMG frequencies are identified within VM and RF bicoherence spectra. No such prominent regions are identified for VL which is thought to be less activated than VM during the specified task. Hence, the degree of second-order phase coupling may increase as the level of muscle activation increases. The subject group consists of young (24.0 ± 0.9 years) and elderly (68.9 ± 0.9 years) subgroups that cannot be differentiated by standard indices (median and spectral edge frequency) to within $p < 0.05$ using the Mann-Whitney test. Average bicoherence differentiates the subgroups for RF (T = 9 (8,10), $p < 0.005$) but not for VM or VL. The application of a bicoherence threshold that takes harmonic amplitude into account graphically differentiates the subgroups for all muscle types. The findings suggest that nonlinear processes play a role within the EMG generation process and support a mechanomyogram bicoherence analysis study that shows nonlinear processes occur within active muscle fibre twitch summation patterns. A potential exists for bicoherence analysis to complement standard EMG frequency analysis techniques.

Key words bicoherence, bispectrum, electromyogram, EMG, quadriceps

Introduction

Bicoherence analysis provides information on the degree of second-order phase coupling that exists between constituent frequencies of electrocardiogram (ECG), electroencephalogram (EEG) and mechanomyogram (MMG) biosignals[1-8]. Such phase coupling characterises nonlinear biological systems such as the brain and central nervous system and can reflect the clinical state of these biological systems (e.g., the state of consciousness)[1, 3]. Despite the clinical usefulness of bicoherence analysis when applied to the above biosignals and the fact that MMG bicoherence spectra have shown nonlinear processes to be significant within active muscle fibre twitch summation patters, a recent study[9] reports a negative finding for bicoherence analysis applied to vastus lateralis (VL) quadriceps electromyogram (EMG) data during isometric knee extension exercise. Other EMG bicoherence analysis studies are not apparent in the literature.

The aim of the present study is to further investigate whether a potential exists for bicoherence analysis to be used as an assessment tool in the area of EMG analysis. The study is a follow-up to the above-mentioned EMG bicoherence analysis study[9] and compares the responses of vastus medialis obliques (VM), rectus femoris (RF) and VL quadriceps muscles for 18 adult male subjects during isometric knee extension exercise. During this task, there is a likelihood that the levels of activation of VM, RF and VL will differ, and hence the responses of the three quadriceps muscles to bicoherence analysis may also differ. The primary reason why the levels of activation could be expected to differ is that a number of studies[10-14] cited in a comprehensive review [15], report a significantly greater level of activation for VM than for VL during some types of knee extension exercise. However, it should be noted that whether the level of activation of VM differs significantly from that of VL during various knee extension exercises remains a topic of controversy in biomechanics and physiotherapy[15, 16]. The present study therefore contributes to the body of literature that addresses this controversy.

From a physiological perspective, sustained muscle activity is known to produce groupings of motor unit action potentials (MUAPs) into distinct bursts of activity. These groupings have been described as a synchronisation, though technically the term synchronisation does not correctly

Corresponding author: R. Simeoni, School of Physiotherapy and Exercise Science, Griffith University, PMB50 Gold Coast Mail Centre, Gold Coast QLD 9726, Australia, Tel: +61 7 5552 8451, Fax: +61 7 5552 8674, Email: r.simeoni@griffith.edu.au
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describe the effect[17]. However, the onset of synchronous-like behaviour (harmonisation) suggests that phase coupling between constituent EMG frequencies may also increase with sustained muscle activity. Hence, a possible physiological basis exists as to why VM, RF and VL might become differentiable through bicoherence analysis if their levels of activation differ.

The subject group consists of young and elderly subgroups and the present study also aims to investigate the potential for EMG bicoherence analysis to differentiate these subgroups. Standard EMG frequency analysis differentiates the young and elderly[18]. Such age differentiation results from an increase in MUAP amplitude with age due to a loss of motor neurons and a subsequent peripheral reinnervation of muscle fibres by surviving motor neurons, as reported for VL[17, 18]. Also, motor control differs between the young and elderly (e.g., during walking[19]) and VM and VL activations appear to differ in response to a change in motor control[16]. EMG phase coupling differences between the young and elderly might therefore occur for our isometric situation and be detectable through bicoherence analysis.

If the quadriceps muscles or subgroups for the same muscle are differentiable through bicoherence analysis, then a potential would exist for bicoherence analysis to complement standard EMG frequency analysis techniques. The complementary information provided, i.e., the degree of second-order phase coupling between constituent EMG frequencies, would give insight into MUAP harmonisation which is known to be affected by muscle fatigue and changes in neuromuscular function.

Theory

Bicoherence and bispectral analysis investigate the degree of second-order phase coupling between the constituent harmonics, $X_n(\omega_n) = c_n \sin(\omega_n t + \phi_n)$, of a biosignal, where ω_n is angular frequency, c_n is amplitude, ϕ_n is phase and t is time.

The bispectrum, $B(\omega_p, \omega_q)$, is a measure of the degree of second-order phase coupling between $X_p(\omega_p)$, $X_q(\omega_q)$ and $X_{p+q}(\omega_{p+q})$, while also taking into account the amplitudes of these harmonics. $B(\omega_p, \omega_q)$ is calculated by breaking the biosignal into L successive epochs and applying the following equation:

$$B(\omega_p,\omega_q) = \left| \sum_{m=1}^{L} \left(X_p(\omega_p) \cdot X_q(\omega_q) \cdot X^*_{p+q}(\omega_p+\omega_q) \right)_m \right| = \left| \sum_{m=1}^{L} \left(c_p c_q c_{p+q} e^{i\left(\phi_p+\phi_q-\phi_{p+q}\right)} \right)_m \right| \quad (1)$$

where the index, m, is the current epoch and $X^*_{p+q}(\omega_p+\omega_q)$ is the complex conjugate of $X_{p+q}(\omega_p+\omega_q)$.

Bicoherence, $bic(\omega_p, \omega_q)$, is often described as the normalised bispectrum[2, 3, 5], since it is found by dividing $B(\omega_p, \omega_q)$ by the square root of a real (not complex) triple product, $c_p^2 c_q^2 c_{p+q}^2$, that involves the power, c_n^2, of each harmonic:

$$bic(\omega_p,\omega_q) = \frac{\left| \sum_{m=1}^{L} \left(c_p c_q c_{p+q} e^{i\left(\phi_p+\phi_q-\phi_{p+q}\right)} \right)_m \right|}{\sqrt{\sum_{m=1}^{L} \left(c_p^2 c_q^2 c_{p+q}^2 \right)_m}} \times 100\% \quad (2)$$

$bic(\omega_p, \omega_q)$ = 0% indicates that the phases of $X_p(\omega_p)$, $X_q(\omega_q)$ and $X_{p+q}(\omega_{p+q})$ vary independently, while $bic(\omega_p, \omega_q)$ = 100% indicates that a constant phase relationship is maintained between the harmonics. For more detail on and an introduction to bicoherence analysis theory, the reader is referred to references[3, 9].

For the present study, bicoherence and bispectrum information is also combined in a new manner and the theory behind this new analysis method is now explained. A bicoherence spectrum is theoretically independent of harmonic amplitude. That is, $bic(\omega_p, \omega_q)$ may equal 100% for harmonics that only make a small contribution to the EMG frequency spectrum. To give more weighting to harmonics with large amplitudes, we introduce a threshold whereby $bic(\omega_p, \omega_q)$ is set to zero if the $c_p^2 c_q^2 c_{p+q}^2$ sum in Eq.2 is less than an arbitrary threshold that relies on the identification of the maximum $c_p^2 c_q^2 c_{p+q}^2$ for each epoch. The threshold is defined as 0.1 × L × the minimum of the identified maximum $c_p^2 c_q^2 c_{p+q}^2$ values. It should be noted that the resulting bicoherence spectrum differs from the bispectrum. That is, although the bispectrum reflects harmonic amplitude, a relatively large $B(\omega_p, \omega_q)$ value may be obtained if the amplitudes of three harmonics are small but the phase coupling strong. Our proposed spectrum would set $bic(\omega_p, \omega_q)$ to zero in this case. Also, a relatively large $B(\omega_p, \omega_q)$ value may be calculated if the magnitudes of the harmonics are large but the phase coupling weak, if random vectorial cancellation in Eq.1 is non-zero (practically, ideal vectorial cancellation to zero is unlikely). Our proposed spectrum, being bicoherence based, would give a relatively small $bic(\omega_p, \omega_q)$ value that would be set to zero in this second case. Thresholds have been applied to the bicoherence spectrum[3] but not in the manner described above.

Method

Subjects

10 young (24.0 ± 0.9 years) and 8 elderly (68.9 ± 0.9 years) male subjects participated in the study. All subjects were free from known musculoskeletal and neurological abnormalities. The subjects were considered as 18 adult

males for comparisons between quadriceps muscles and as subgroups for young versus elderly comparisons for the same quadriceps muscle.

EMG experimental procedure

Bipolar configurations of Sentry Ag-AgCl 10 mm pre-gelled, disposable disk electrodes were placed on the skin superficial to VM, RF and VL, with an inter-electrode distance of 20 mm. A reference electrode was placed on the skin superficial to the left anterior superior iliac spine. Electrode impedance did not exceed 2 kΩ for any active-reference electrode pair. A total of 54 EMG data segments were collected for each quadriceps muscle as each subject performed three 6 s isometric knee extension actions with the right leg which was attached to a strain gauge cell. The task goal was to maintain a knee extension torque of 25 $N \cdot m^{-1}$. Subjects received visual feedback on their generated torque in real time. The knee remained in 45 degrees of flexion and the hip in 90 degrees of flexion. An isometric task was chosen to minimise non-stationarity effects[20]. EMG data were sampled at 1000 Hz using a Biopac Data Acquisition system in conjunction with Acknowledge software and raw EMG data were amplified by a factor of 1000. To test for repeatability between EMG channels, measurements were repeated for two subjects with leads and amplifiers swapped between VM, RF and VL.

Bicoherence analysis procedure

The three 6 s segments of EMG data collected from each subject for each quadriceps muscle were adjusted to a mean of zero and filtered with a second-order, zero phase Butterworth filter (12 dB/octave rolloff) with low- and high-pass filter cut-offs set to 450 and 10 Hz respectively. The 10 Hz cut-off and filter order were chosen to allow the detection of nonlinearities associated with motor unit firing rates below 20 Hz.

The EMG data segments were divided into 32 epochs, each consisting of 500 points and of 0.5 s duration. Epoch overlap was 75%. A Blackman window[21] was applied to, and a Fast Fourier Transform (FFT) calculated for, each epoch. FFT resolution was 2 Hz. A bicoherence spectrum was then generated for each data segment by calculating $bic(\omega_p,\omega_q)$ for all linear frequencies, $f_n = \omega_n / 2\pi$, above 10 Hz such that $f_p + f_q \leq 300$ Hz, using a program developed in the Matlab programming language. The 300 Hz maximum was chosen in accordance with the average spectral edge frequency (SEF) for the VL EMG frequency spectra (see Results section), where SEF is defined as the frequency below which 95% of the power exists.

Bicoherence spectra were also calculated with the 10% of maximum real triple product threshold described in the Theory section for the same epoch regime described above.

Statistical analysis procedure

All mean values are reported as mean ± SD. Median frequency (MF) and SEF comparisons between the young and elderly subgroups for each quadriceps muscle were made using the Mann-Whitney test as skew and kurtosis distribution differences between the subgroups were observed in some cases. $\overline{bic(\omega_p,\omega_q)}$ is the average bicoherence of a given bicoherence spectrum and subgroup comparison for each quadriceps muscle, based on this index, was also made using the Mann-Whitney test. The test statistic quoted for the Mann-Whitney test is $T = S - n_e(n_e+1)/2$, where S is the sum of the ranks for the elderly subgroup and n_e is the number of elderly subjects.

Repeated measures ANOVA was performed in SAS software using the Proc Mixed procedure to detect for differences in $\overline{bic(\omega_p,\omega_q)}$ between quadriceps muscles for all subjects. This statistical method was employed because second-order phase coupling independency between muscles for the same subject could not be assumed. The effects of muscle and trial on $\overline{bic(\omega_p,\omega_q)}$ were assessed using a two factor within-subject design. As no significant effect of trial (F = 0.056 (2,17), p = 0.538) or interaction between muscle and trial (F = 2.88 (2,17), p = 0.0548) were identified, the $\overline{bic(\omega_p,\omega_q)}$ values were then averaged across trials for each muscle and subject and the effect of muscle on $\overline{bic(\omega_p,\omega_q)}$ assessed using a single factor within-subject design. The selection of the appropriate covariance matrix was based on evaluation of Akaike's Information Criterion and Schwarz's Bayesian Criterion. Where an effect of muscle was identified, contrasts were used to determine which levels of muscle were significantly different. α was set at 0.05 for all analyses. Repeated measures ANOVA was also used to detect for $\overline{bic(\omega_p,\omega_q)}$ differences between the subgroups for all muscles combined.

Results

MF and SEF of EMG frequency spectra for all subjects and trials are 63 ± 6 and 172 ± 30 Hz (VM), 64 ± 10 and 194 ± 39 Hz (RF) and 91 ± 22 and 282 ± 39 Hz (VL). An example VL biosignal and its corresponding frequency spectrum (MF = 102 Hz, SEF = 288 Hz) are shown in Figs.1(a) and (b) respectively.

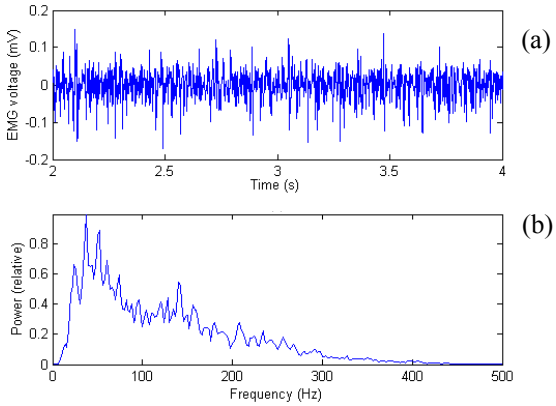

Figure 1. *(a) Example vastus lateralis EMG biosignal and (b) its corresponding frequency spectrum. Median and spectral edge frequencies are 102 and 288 Hz respectively.*

Example bicoherence spectra for VM, RF and VL for simultaneously collected EMG data for the same subject are shown in Figs.2(a), (b) and (c) respectively. $\overline{bic(\omega_p,\omega_q)}$ for each spectrum is 38.8, 28.0 and 25.5% respectively. Mean $\overline{bic(\omega_p,\omega_q)}$ for all subjects and trials is 30.9 ± 5.8% (VM), 26.0 ± 1.2% (RF) and 25.4 ± 1.4% (VL), and repeated measures ANOVA differentiates VM from RF and VL based on $\overline{bic(\omega_p,\omega_q)}$ (F = 16.2 (1, 17), p = 0.0009, VM cf. VL and F = 15.4 (1, 17), p = 0.0011, VM cf. RF). RF is not differentiated from VL (F = 2.96 (1, 17), p = 0.1033, RF cf. VL).

Figs.3(a), (b) and (c) show for VM, RF and VL respectively, bicoherence spectra superimposed to give an average spectrum for all subjects and trials.

MF and SEF Mann-Whitney test comparisons between the young and elderly subgroups do not reveal a significant (T = 21 (8,10), p < 0.05) difference between the subgroups for any of the quadriceps muscles, with T > 21 for all comparisons. Subgroup MFs and SEFs for each muscle are omitted for conciseness.

Mean $\overline{bic(\omega_p,\omega_q)}$ for the young and elderly subgroups are respectively 33.1 ± 6.4 and 28.2 ± 4.0% (VM), 26.7 ± 0.9 and 25.2 ± 0.9% (RF), and 25.7 ± 1.6 and 25.0 ± 0.9% (VL). Differentiation of the subgroups based on $\overline{bic(\omega_p,\omega_q)}$ occurs for RF (T = 9 (8,10), p < 0.005) but not for VM or VL. Repeated measures ANOVA gives a significant age effect for all muscles combined (F = 5.23 (1, 16), p = 0.0362). Figs.4(a) and (b) show for the young and elderly subgroups respectively, VM bicoherence spectra with the 10% of maximum real triple product threshold applied, superimposed for all subjects and trials. Similar spectra are shown for RF in Figs.4(c) and (d) and for VL in Figs.4(e) and (f), while Figs.4(g) and (h) show such spectra for VL without the threshold applied. $\overline{bic(\omega_p,\omega_q)}$ calculated with threshold does not differentiate the subgroups for any of the quadriceps muscles.

Discussion and conclusions

The example VL biosignal in Fig.1(a) shows the maintenance of consistent muscle activity and is typical for the subjects. The corresponding frequency spectrum in Fig.1(b) is typical of other standard EMG frequency spectra reported in the literature[17] with a peak frequency near 40 Hz and a smaller initial peak at lower frequencies due to the grouping of MUAPs.

Quadriceps muscle comparison

VM bicoherence spectra exhibit comparatively high $\overline{bic(\omega_p,\omega_q)}$ values and repeated measures ANOVA differentiates VM from VL (p = 0.0009) based on this index. Similarly, VM is differentiated from RF (p = 0.0011). VM bicoherence spectra also display regions of pronounced second–order phase coupling, as seen in Fig.2(a), for a number of subjects. If these prominent regions are random in nature, then the superposition of all VM spectra is expected to give a relatively featureless spectrum due to random cancellation. In actuality, when VM spectra are superimposed for all subjects and trials (Fig.3(a)), prominent regions of second-order phase coupling remain, indicating phase coupling commonality within the EMG generation process. These prominent regions in Fig.3(a) are broad with indistinct boundaries and $bic(\omega_p,\omega_q)$ values >35%. The regions indicate that frequencies approximately between 30 and 100 Hz display strong second-order phase coupling with two narrow bands of frequencies near 20 and 40 Hz, while frequencies approximately between 100 and 175 Hz display strong second-order phase coupling with frequencies from 80 to 120 Hz. The differentiation between VM and VL may be directly related to comparatively greater levels of VM activation found during some types of knee extension exercise[10-14]. Hence, the findings suggest that nonlinear processes, in the form of second-order phase coupling, may play an increasingly important role within the EMG generation process as muscle activation level increases. Muscle force and fatigue levels might therefore also influence any nonlinear processes that occur.

A prominent region of second-order phase coupling is also identified for all RF bicoherence spectra superimposed (Fig. 3(b)) and this region generally involves frequencies below 100 Hz. Prominent regions of second-order phase coupling are not apparent for VL in Fig.3(c). Since the specified task was modest and easily maintained by most subjects, it is possible that VL bicoherence spectra would exhibit a positive finding for a more strenuous task. The effect of increasing muscle fatigue on the bicoherence spectrum is the intended topic of a follow-up research project.

It is of interest that $\overline{bic(\omega_p,\omega_q)}$ displays a relatively small deviation between individuals for RF and VL (coefficients of variation = 4.6 and 5.5% respectively), compared to the indices of MF and SEF which display respective coefficients of variation of 16 and 20% (RF) and 24 and 14% (VL). This small deviation is of interest because standard indices such as MF, SEF and average rectified value of an EMG biosignal, can vary markedly between individuals performing the same task or for muscles activated to similar levels, and thus these indices do not provide an absolute measure of muscle activity that allows for direct individual comparison (unless maximum voluntary isometric contraction information is known in the latter case). Based on the small deviation between subjects displayed by $\overline{bic(\omega_p,\omega_q)}$, it cannot be stated that $\overline{bic(\omega_p,\omega_q)}$ will provide the absolute measure described above. However, the ability to provide such a measure should not be excluded and remains an interesting possibility, particularly since $bic(\omega_p,\omega_q)$ is inherently normalised.

Tissue filtering is a common phenomenon in the area of EMG analysis[22] and all EMG frequency spectra are affected

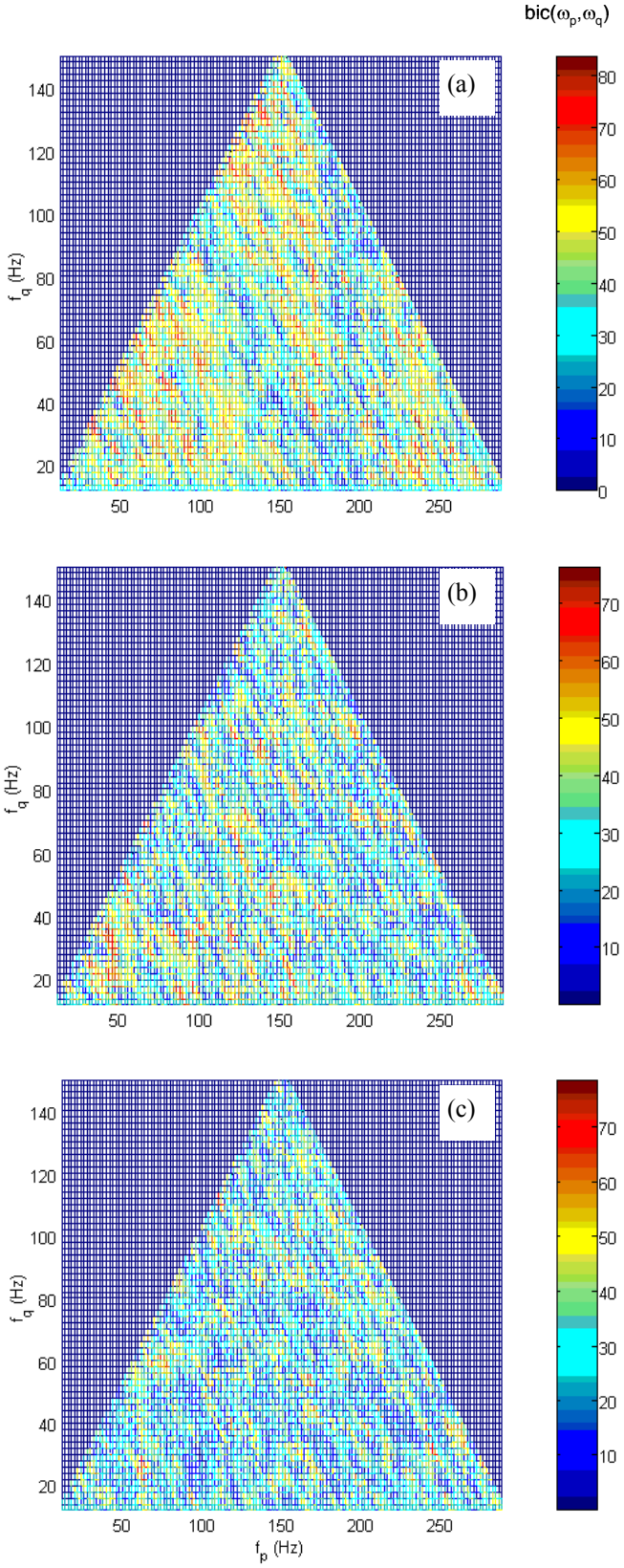

Figure 2. *Bicoherence spectra for (a) vastus medialis,* $\overline{bic(\omega_p,\omega_q)}$ *= 38.8%, (b) rectus femoris,* $\overline{bic(\omega_p,\omega_q)}$ *= 28.0% and (c) vastus lateralis,* $\overline{bic(\omega_p,\omega_q)}$ *=25.5%, for simultaneously collected EMG data for the same subject.*

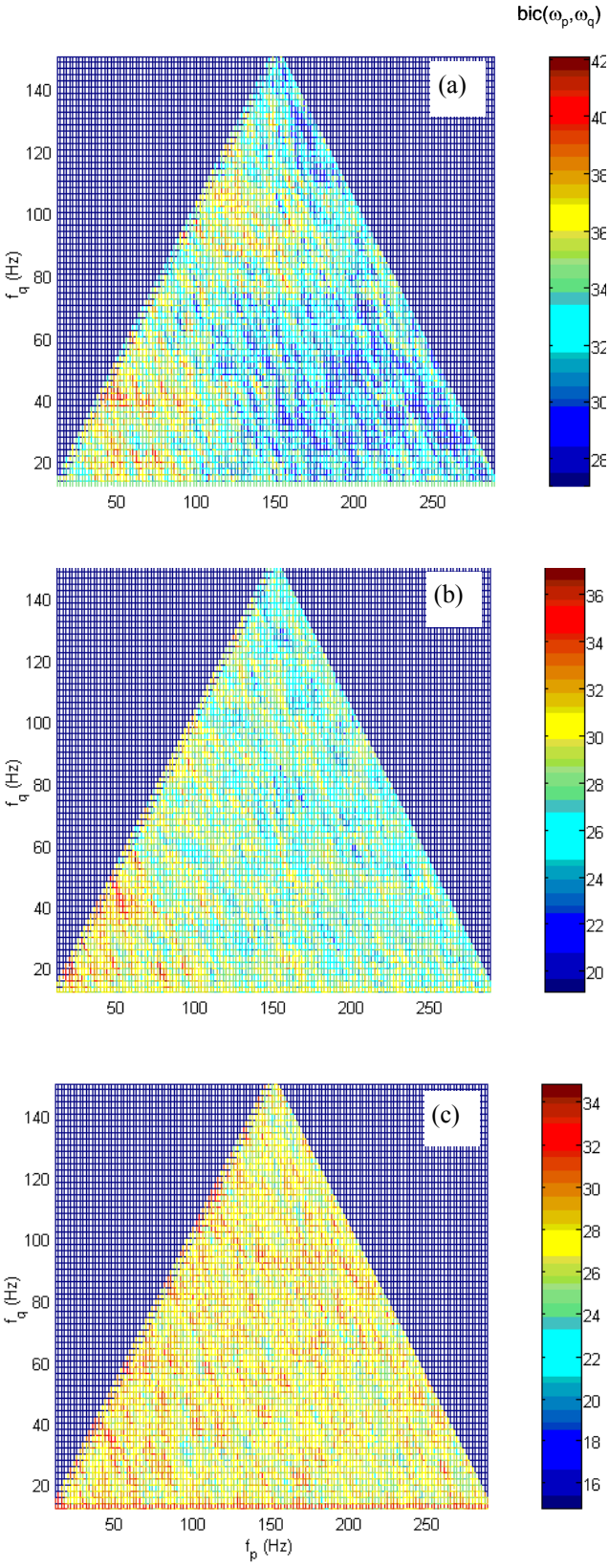

Figure 3. *Bicoherence spectra superimposed for all subjects and trials for (a) vastus medialis and (b) rectus femoris, showing regions of prominent second-order phase coupling between constituent EMG frequencies. The comparable spectrum for vastus lateralis (c) is relatively featureless.*

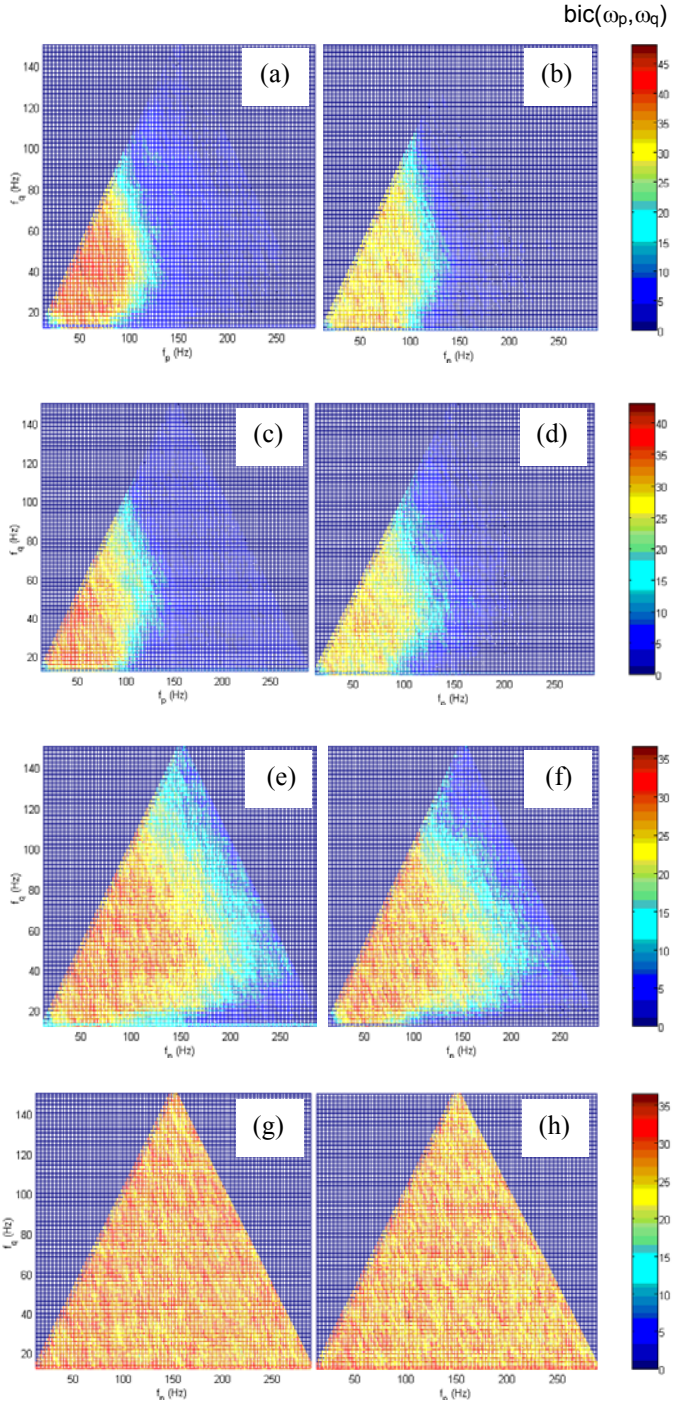

Figure 4. *Bicoherence spectra superimposed with 10% of maximum real triple product threshold applied for all young (left) and elderly (right) subjects and trials. (a) and (b) are for vastus medialis, (c) and (d) for rectus femoris, and (e) and (f) for vastus lateralis. (g) and (h) are for vastus lateralis without the threshold applied.*

by this phenomenon to various degrees. Noting that the average SEFs for VM and RF are lower than that for VL due to tissue filtering, it must be established that the above findings are not a consequence of this filtering. This possibility, and the possibility of the findings being due to different sensitivities between sets of EMG channels, are excluded as follows:

- Prominent regions within Figs.3(a) and (b) involve frequencies much lower than those affected by tissue filtering.
- Unfiltered components of EMG frequency spectra preserve phase relationships.
- Despite the fact that average MF and SEF are similar for VM and RF, $\overline{bic(\omega_p,\omega_q)}$ for RF is significantly less than that for VM and corresponds more closely with the value for VL.
- For a given subject $\overline{bic(\omega_p,\omega_q)}$ for VM does not significantly change when the maximum value of $f_p + f_q$ within bicoherence spectra is reduced to 200 Hz, a value closer to the average SEF for VM.
- Repeatability measurements with leads and amplifiers swapped between VM, RF and VL show that the findings are not due to differences between channel sensitivity or signal-to-noise ratio.

Subgroup comparison

The young and elderly subgroups are not differentiated on the basis of MF or SEF using the Mann-Whitney test for any of the muscles. The findings that the Mann-Whitney test differentiates the subgroups on the basis of $\overline{bic(\omega_p,\omega_q)}$ for RF ($p < 0.005$) and that repeated measures ANOVA of $\overline{bic(\omega_p,\omega_q)}$ gives a significant age effect for all muscles combined ($p = 0.0362$), raise the possibility that known motor neuron population or motor control-associated changes in the elderly[17-19] might indirectly affect second-order phase coupling between constituent EMG frequencies during certain tasks.

Since the superposition of all spectra yields no distinctive features for VL, it is of interest that for VL the application of the 10% of maximum real triple product threshold graphically differentiates the young and elderly subgroups (see Figs.4(e) and (f) respectively). Here, differentiation is on the basis of a more intense region of prominence existing within the spectrum for the young. Graphical differentiation is not apparent without the threshold applied as shown by comparative Figs.4(g) and (h). Spectra, with threshold applied, for VM (Figs.4(a) and (b)) and RF (Figs.4(c) and (d)) graphically differentiate the subgroups in a similar manner to that of VL. Differentiation, based on $\overline{bic(\omega_p,\omega_q)}$, of the subgroups is not significant for Figs.4(a) to (f), indicating that a more specific index based on defined regions within bicoherence spectra[6] is required to match the graphical differentiation result. That is for example, Fig.4(a) contains higher $bic(\omega_p,\omega_q)$ values than Fig.4(b), while Fig.4(b) displays a broader region of prominence, resulting in similar $\overline{bic(\omega_p,\omega_q)}$ values for the two spectra. Hence, the calculation of $\overline{bic(\omega_p,\omega_q)}$ over a confined range of frequencies is required for differentiation.

In conclusion, bicoherence analysis clearly differentiates the VM quadriceps muscle from the RF and VL quadriceps muscles and this differentiation may be directly related to greater VM activation levels compared to VL reported in the literature. Hence, second-order phase coupling may increase with the level of muscle activation. A separate study is needed to further explore the physiological basis of this differentiation and thus establish the manner in which bicoherence analysis might be used as a quantitative EMG assessment tool. It cannot be concluded that $\overline{bic(\omega_p,\omega_q)}$ provides an absolute measurement that allows for direct comparison of muscle activity level between individuals. However, the ability to provide such as absolute measure remains a possibility based on the small deviation in $\overline{bic(\omega_p,\omega_q)}$ between individuals performing the same modest task for RF and VL and the normalised nature of the bicoherence calculation. The graphical differentiation of the young and elderly subgroups by the application of a real triple product threshold is an interesting finding perhaps indirectly related to known motor neuron population or motor control-associated differences between the subgroups.

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