Effect of knee joint angle on the neuromuscular activation of the quadriceps femoris during repetitive fatiguing contractions

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ABSTRACT

We assessed the effect of knee joint angle on the EMG amplitude and frequency of the four individual muscles in the quadriceps femoris during repetitive fatiguing maximum voluntary contractions (MVCs). Fifteen healthy men and women performed two fatiguing tasks consisting of 40 MVCs in flexion (80°) and extension (140°) (full extension = 180°). Neuromuscular activation of the vastus intermedius (VI), vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) was recorded using surface electrodes, and median frequency (MF) and root mean square (RMS) of electromyographic (EMG) signals (normalized by pre-test MVCs) were calculated. MVCs significantly decreased from the 10th to the 40th repetition in both flexion and extension. The MFs of VI and VM in flexion and that of RF flexion and extension were significantly decreased after the 10th repetition. There were no significant changes in normalized EMG amplitude in any muscles specific to knee angle. Stepwise regression analysis suggested that predictive synergistic action may occur in RF/VM and RF/VI in flexion and in RF/VM in extension. This suggest that EMG MF of RF/VM is independent, but that of RF/VI and RF/VL is dependent upon knee joint angle, which may, in part, explain joint angle-specific muscle fatigue.

1. Introduction

According to a review by Gandevia (2001), muscle fatigue is defined as any exercise-induced reduction in the ability of a muscle to generate force or power. The muscle groups of the quadriceps femoris (QF) (Akima and Saito, 2013; de Ruiter et al., 2005; Ebenbichler et al., 1998; Ebersole and Malek, 2008; Watanabe and Akima, 2010), triceps surae (Kawakami et al., 2000; Mitsukawa et al., 2009; Pereira et al., 2011) and biceps brachii (Holtermann et al., 2008; Moritani et al., 1986) have been used to assess fatigue based on changes in muscle force and EMG amplitude and/or frequency during force exertion.

In muscle fatigue studies, sustained or repetitive muscle contractions at a submaximal force level (e.g., 50% of maximum voluntary contraction [MVC]) are frequently used as a fatiguing task (Akima and Saito, 2013; Ebenbichler et al., 1998; Ebenbichler et al., 1998; Humphreys and Lind, 1963; Place et al., 2005; Watanabe and Akima, 2010). It is recognized that changes in EMG amplitude generally reflect number and/or size and the firing rate of motor units (MUs). The median frequency (MF) of the surface EMG signal has been well known as an index of muscle fatigue (Farina, 2004; Hägg, 1992). The

conduction velocity of the action potential on muscle fibers is recognized as a main factor, affecting MF during fatiguing contraction (Farina, 2004; Hägg, 1992). The advantage of this type of fatiguing task is expected to induce dramatic changes in electromyographic (EMG) signals (Akima and Saito, 2013; Ebenbichler et al., 1998; Ebersole and Malek, 2008) to maintain the intended force. Contrary to submaximal fatiguing contractions, when 100%MVC is used to induce fatigue, muscle force dramatically decreased in spite of maximal effort, and amplitude and/or frequency of EMG signals also decreased in the triceps surae muscles (Kawakami et al., 2000; Mitsukawa et al., 2009). However, it is unclear how muscle force and EMG signals of the QF will react to the fatiguing task with maximal effort. The advantage of repetitive muscle contractions at 100%MVC task may be that it causes severe force production impairment and changes in EMG amplitude and frequency (Mullany et al., 2002). Therefore, muscle fatigue, i.e., decrease in force production, can be easily monitored along with changes in the amplitude and frequency of EMG signals.

Surface EMG has been conventionally used to assess the fatigue of working muscles such as the vastus lateralis (VL) during knee extension (Behm and St-Pierre, 1997; Ebenbichler et al., 1998; Pincivero et al.,

2006; Rochette et al., 2003). We have succeeded in measuring EMG amplitude and frequency of all the individual muscles comprising the QF, including the vastus intermedius (VI), using surface EMG combined with ultrasonography to detect a superficial region of the VI (Akima and Saito, 2013; Watanabe and Akima, 2009, 2011). Using this technique, it is possible to obtain information on the EMG amplitude and frequency of all individual QF muscles along with exerted muscle force changes during a task. The reason we measured all individual muscles in the QF is that each muscle seems to have different functional characteristics. For example, RF is the most fatigable muscle of the four (Akima et al., 2004; Ebenbichler et al., 1998; Ebenbichler et al., 1998). We have examined the neuromuscular properties of VI, but its functional characteristics during fatiguing contractions have not been fully elucidated. In muscle fatigue studies, the MF of EMG signals has been used as an index of development of muscle fatigue during sustained or intermittent repetitive muscle contractions (Mitsukawa et al., 2009; Moritani et al., 1986; Mullany et al., 2002; Watanabe and Akima, 2009).

It is known that the endurance capacity of QF is greater with shorter muscle length (extension) than longer length (flexion) during isometric contractions to exhaustion, even at relatively identical contraction levels (Hisaeda et al., 2001; Kooistra et al., 2005; Ng et al., 1994; Place et al., 2005). During fatiguing contractions, the knee joint angle would be assumed to influence muscle force and amplitude and frequency of EMG signals; however, the effects of knee joint angle on fatigue and EMG amplitude and frequency of each individual muscle of the QF during a repetitive fatiguing MVC task have not been examined.

The purpose of this study was to assess muscle force and EMG amplitude and frequency of the individual QF muscles, including VI, during fatiguing isometric MVCs at different knee joint angles. We hypothesized that the muscle fatigability is greater in knee flexion than extension, as it has been suggested that there is a lower energy demand in extension (Kooistra et al., 2005). Thus, decreases in the frequency of EMG signals should be greater in flexion than in extension.

2. Materials and methods

2.1. Subjects

Fifteen healthy subjects (7 men and 8 women) participated in this experiment. Before the experiment, the procedure, purposes, risks and benefits associated with the study were explained and written consent was obtained. The subjects' mean (± standard deviation) physical characteristics were as follows: men; age, 27.1 ± 2.0 yr; height, 174.9 ± 4.1 cm; weight, 64.7 ± 6.5 kg. Women; age, 22.5 ± 3.1 yr; height, 156.1 ± 4.6 cm; weight, 49.4 ± 5.9 kg. Four men and two women among the subjects performed habitual exercise a few times a week, and the others had no regular exercise habits. All experimental protocols were approved by the Ethics Committee of the Research Center of Health, Physical Fitness & Sports at Nagoya University (#28-07), and were in accordance with the Declaration of Helsinki.

2.2. Experimental protocol

The subjects visited our laboratory on three occasions with at least one week in between visits. On the first experimental day, a trial session was conducted; all subjects practiced the MVC force test a few times, followed by fatiguing repetitive isometric knee extensions at 100%MVC approximately fifteen times. On the second experimental day, the subjects performed a fatiguing isometric knee extension task consisting of 40 MVCs with the right knee joint angle at 80° (in flexion) or at 140° (in extension) on their right leg after pre MVC force testing. The third experimental day, they came back to the laboratory and performed the fatiguing task consisting of 40 MVCs at the other knee joint angle with the right leg after pre MVC force testing. The order of the two different knee joint angle fatiguing tasks was randomized. During the task, surface EMG signals of the VI, VL, vastus medialis (VM), and rectus femoris

(RF) were synchronously recorded with MVC force and knee joint angle for later analysis.

2.3. Isometric knee extension test

Subjects performed isometric knee extension MVCs on a custom-designed dynamometer with a mounted force transducer (LTZ-100KA; Kyowa Electronic Instruments, Tokyo, Japan), as previously described (Watanabe and Akima, 2009, 2010). During this task, the pelvis was fixed to the dynamometer by a strap at a hip joint angle of 110° (hip angle at upright position = 180°), the knee joint of the right leg was kept at 80° (in flexion, full extension = 180°) or at 140° (in extension), and the ankle was attached to a pad linked to the force transducer. The MVC test consisted of three phases: the force-rising phase (1–2 s), the sustained phase (≥ 2 s), and the relaxation phase. When the force plateaued during the sustained phase, vigorous encouragement was given by the supervisors. Two MVC trials were performed with ≥ 2 min rest between trials. If the two exerted forces differed by $\geq 5\%$ between trials, an additional trial was performed. During each contraction, the force was sampled and averaged across 1 s in the sustained phase. The MVC force was determined as the highest force in the trials, and it was used to calculate the target force for the fatiguing contraction task (Watanabe and Akima, 2009, 2010). The knee extension force was sampled at 2 kHz using an analog-to-digital converter (PowerLab 16SP; ADInstruments, Melbourne, Australia), stored on a personal computer (s3740jp/CT, Hewlett-Packard Japan, Tokyo, Japan) using commercial software (LabChart, version 7.2.5, ADInstruments), and displayed in real-time on a computer monitor in front of the subject to provide visual feedback of knee extension force.

2.4. Fatiguing task

The fatiguing task was a modified version of task used in a previous study (Mitsukawa et al., 2009). After 10 min of rest following the MVC task, repetitive isometric MVCs of knee extension for 3 s with 3 s of rest between the contractions were performed for 40 repetitions with knee joints in flexion and extension. Note that the 9th, 10th, 19th, 20th, 29th, 30th, 39th, and 40th muscle contractions were performed for 5 s for measurement with ultrasonography, which was used for other purposes. During the fatiguing task, the produced force was shown to the subjects on a personal 17 in. computer monitor placed in front of the subject, and strong encouragement was provided by the investigators.

2.5. EMG measurements

Surface EMG signals from VI, VL, VM, and RF were recorded during the isometric knee extension task using active electrodes, as previously described (Akima and Saito, 2013; Watanabe and Akima, 2009, 2010). A single differential electrode (4.1 cm long, 2.0 cm wide, 0.5 cm high) with a 1-cm inter-electrode distance, an input impedance of $> 10^{15} \Omega$/ 0.2 pF, and a 90 dB common rejection ratio was used. The sensor pre-amplifier and main amplifier units were set at a gain of 10-fold and 100-fold, respectively. This resulted in a 1000-fold amplification of the original EMG signal and a frequency response of 20 ± 5 to 450 ± 50 Hz (sensor DE-2.1; Main Amplifier Unit: Bagnoli-8, Delsys, Boston, MA, USA). Signals from the EMG system were sampled at 2 kHz using the same analog-to-digital converter and personal computer as used for the force data and were synchronized with force data.

The placement of the electrodes was essentially the same as done previously (Akima and Saito, 2013; Saito and Akima, 2013; Watanabe and Akima, 2011). Prior to attaching the electrodes, the skin was shaved, abraded, and cleaned with alcohol. The electrode for VL were placed at the mid-point between the head of the greater trochanter and the inferior edge of the patella. The electrodes for RF was placed at the mid-point of the line joining the anterior superior iliac spine. The

electrodes for VM was placed at the superior patellar pole, slightly proximal and medial to the patella. Thus, the VM electrodes measured activity of the VM oblique. All electrodes were placed parallel to the estimated fiber direction based on previous studies (Akima and Saito, 2013a, 2013b; Pincivero et al., 2006; Pincivero et al., 2008; Pincivero and Gear, 2000; Watanabe and Akima, 2010).

Using B-mode ultrasonography (Logiq e; GE Healthcare, Wauwatosa, WI, USA), we circled the edge of the superficial region of the VI when the knee joint was at angles of 80° or 140° (Akima and Saito, 2013; Watanabe and Akima, 2011). Ultrasonography was used to determine electrode placement within the superficial region of the VI with attention of volume conductance. It is previously shown that the surface EMG signal recorded using this procedure has a negligible amount of cross-talk from the adjacent VL muscle (Watanabe and Akima, 2009), and is closely associated with the intramuscular EMG signal from the VI (r = 0.926–0.991) (Watanabe and Akima, 2011). The electrodes for VI were placed parallel to the estimated longitudinal axis of the muscle. The reference electrode was attached to the iliac crest.

2.6. Data analysis

For the surface EMG signals, MF and root mean square (RMS) were calculated. For the MF, the frequency that divided the spectrum was calculated into two parts with equal power on the frequency spectrum of EMG (4000 points, Hamming window processing with 50% overlap, fast Fourier transform, LabChart version 7.2.5; ADInstruments) (Basmajian and DeLuca, 1985). The RMS of the EMG signal was calculated based on a previously described equation (Basmajian and DeLuca, 1985). The EMG signal (to calculate MF and RMS) and force were simultaneously sampled over a 1024 ms period in the middle of a 3-sec MVC force production of the 1st, 10th, 20th, 30th and 40th repetitive MVC contractions. MF and RMS were normalized by EMG signals in the pre-fatigue MVC test and expressed as the relative value (%MVC) during the repetitive MVC task.

2.7. Statistical analysis

All data are presented as mean ± standard deviation. A two-way (time × joint angle) analysis of variance (ANOVA) with repeated measures was used to compare MF and RMS across time points and muscles. If a two-factor interaction or a main effect was found, a Tukey's post hoc test was used to identify significant differences. A Mauchly's sphericity test was applied and if violated, the Greenhouse-Geisser correction factor was used to control for Type I errors. The partial eta squared (η^2) statistics was used to evaluate effect size for each ANOVA. Stevens (2007) characterized $\eta^2 = 0.01$ as corresponding to a small effect size, $\eta^2 = 0.06$ to a medium effect size, and $\eta^2 = 0.14$ to a large effect size. Stepwise regression analysis was performed to identify coactivation patterns among individual muscles for each joint angle. The dependent variable was normalized EMG MF of RF at the 40th repetition, and independent variables was that of VI, VL and VM at the 40th repetition. The reason we chose the 40th repetition was because we could expect it to display maximum fatigue. The level of significance was set at P < 0.05. Statistical analysis was performed using commercial software (IBM SPSS statistics version 24.0 J, IBM, Tokyo, Japan).

3. Results

During the repetitive fatiguing MVC tasks, the first contraction was 308.3 ± 106.5 N and 453.4 ± 120.3 N (93.6 ± 8.4% and 95.8 ± 11.5% of pre-fatigue MVC test values, respectively) at 80° and 140° knee joint angles, respectively (Fig. 1 and Table 1). Then, it gradually decreased and reached 184.8 ± 58.9 N and 312.3 ± 85.2 N (56.9 ± 10.7% and 66.7 ± 14.5% of pre-fatigue MVC test values, respectively). There were no significant time-by-joint angle interactions
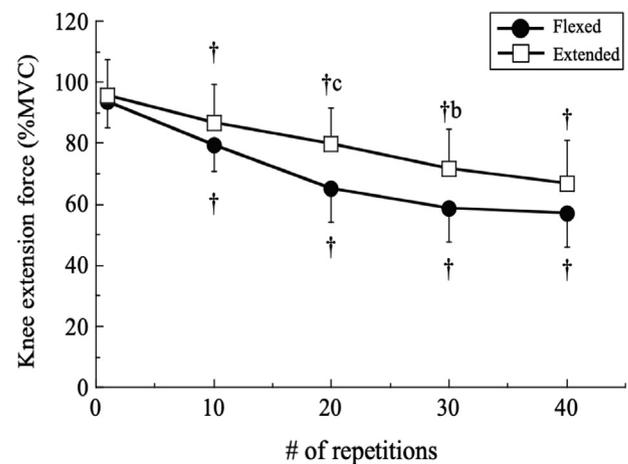

Fig. 1. Time course of knee extension force during isometric repetitive maximum voluntary contractions (MVCs) in flexion and extension. †, P < 0.001 vs. 1st MVC, b, P < 0.01 and c, P < 0.001 vs. in flexion. Knee extension force was expressed as normalized to pre-fatigue MVC (pre-fatigue MVC = 100%).

($F_{2,60} = 0.58$, P = 0.594, $\eta^2 = 0.02$) and significant time effects ($F_{2,67} = 69.12$, P = 0.001, $\eta^2 = 0.71$) on absolute force (Table 1). However, there were significant time-by-joint angle interactions ($F_{3,72} = 3.98$, P = 0.015, $\eta^2 = 0.12$) and significant time effects ($F_{3,72} = 117.96$, P = 0.001, $\eta^2 = 0.81$) in relative force (Fig. 1). As a result of post-hoc test, for both in extension and flexion, absolute and relative MVC values at the 10th, 20th, 30th and 40th contractions were significantly lower than that of the 1st (all P < 0.001) (Fig. 1 and Table 1). Relative MVC forces at the 20th and 30th repetitions in flexion were significantly lower than those in extension (P < 0.01 and P < 0.001, respectively) (Fig. 1).

Fig. 2 shows normalized EMG MF of the VI, VL, VM, and RF during the fatiguing MVC tasks. There were no significant time-by-joint angle interactions in VI ($F_{3,81} = 20.95$, P = 0.059, $\eta^2 = 0.09$), VL ($F_{3,81} = 2.27$, P = 0.084, $\eta^2 = 0.08$), VM ($F_{3,69} = 1.37$, P = 0.262, $\eta^2 = 0.05$), and RF ($F_{2,60} = 0.93$, P = 0.405, $\eta^2 = 0.03$) and significant time effects in all four muscles (VI, $F_{3,81} = 20.95$, P = 0.001, $\eta^2 = 0.43$; VL, $F_{3,86} = 13.30$, P = 0.001, $\eta^2 = 0.32$; VM, $F_{3,69} = 10.13$, P = 0.001, $\eta^2 = 0.69$; RF, $F_{2,60} = 63.65$, P = 0.001, $\eta^2 = 0.69$). Normalized EMG MFs in VI, VL, and RF in flexion and normalized EMG MF in RF in extension at the 10th, 20th, 30th and 40th contractions were significantly lower than those of the 1st contraction (P < 0.05–P < 0.001). Normalized EMG MF in VI in extension at the 40th contraction and VM in flexion at the 30th contraction were significantly lower than that of the pre-fatigue MVC (both P < 0.05).

Fig. 3 shows normalized EMG RMS of VI, VL, VM, and RF during the fatiguing MVC tasks. There was no significant muscle-by-time interaction in any muscles (VI, $F_{3,81} = 0.17$, P = 0.910, $\eta^2 = 0.01$; VL, $F_{3,75} = 0.17$, P = 0.896, $\eta^2 = 0.01$; VM, $F_{3,75} = 0.73$, P = 0.5232, $\eta^2 = 0.03$; RF, $F_{4,112} = 1.28$, P = 0.284, $\eta^2 = 0.04$) and significant effects of time in all four muscles (VI, $F_{3,79} = 3.41$, P = 0.024, $\eta^2 = 0.11$; VL, $F_{3,75} = 3.86$, P = 0.016, $\eta^2 = 0.12$; VM, $F_{3,75} = 5.77$, P = 0.002, $\eta^2 = 0.17$; RF, $F_{4,112} = 5.78$, P = 0.001, $\eta^2 = 0.17$). As a result of post-hoc test, there were no significant differences among muscles and time points.

To assess coactivation patterns among individual muscles at the two different knee joint angles, we performed stepwise regression analysis as a dependent variable of normalized EMG MF of the RF at the 40th repetition and independent variables as that of VI, VL and VM at the 40th repetition (Table 2), because it is the only muscle that shows no angle-specific EMG MF. In flexion, EMG MF of VM and VI were selected as independent variables ($R^2 = 0.670$, P < 0.01), and in extension, EMG MF of VM was solely selected ($R^2 = 0.575$, P < 0.01).

Table 1
Pre-fatiguing maximum voluntary contraction (MVC) force and MVCs during repetitive fatiguing task.

	Pre-test MVC	Number of fatiguing MVCs				
		1	10	20	30	40
Flexed angle	330.5 ± 106.5	308.3 ± 97.6	263.2 ± 89.5[†]	217.1 ± 82.2[†]	194.0 ± 71.7[†]	184.8 ± 58.9[†]
Extended angle[§]	473.9 ± 131.6	453.4 ± 120.3	412.8 ± 125.7[**]	380.0 ± 105.7[†]	337.5 ± 87.1[†]	312.3 ± 85.2[†]

Values are means and SD. Unit = N.
[**] $P < 0.01$.
[†] $P < 0.001$ vs 1st contraction of each joint angle.
[§] Represents all values in extension were significantly higher than that of flexion.

4. Discussion

The purpose of this study was to assess the effect of two different knee joint angles on the EMG amplitude and frequency of the four individual muscles in QF during repetitive fatiguing MVCs. The main finding of this study was a significant decrease in MVCs in flexion and extension, and that significant decreases in normalized EMG MFs in VI and VL in flexion, and in RF in both flexion and extension.

In this study, we assessed changes in muscle force, EMG amplitude, and EMG MF during fatiguing contractions. This study is valuable for the following two reasons: first, we can recognize how muscle function and electrophysiological parameters change with repetitive muscle contractions with maximum efforts, to provide the neuromuscular adaptations needed during extremely strenuous repetitive muscle contractions. Second, we were able to obtain electrophysiological information on all four individual muscles of the QF in this study.

Relative MVC force finally reached $56.9 \pm 10.7\%$ and $66.6 \pm 14.5\%$ of pre-fatigue MVC in flexion and extension, respectively, and there was no significant difference in the final %MVC force between the two joint angles. A similar result was reported by Place et al. (2005), who demonstrated that force loss after sustained fatiguing contraction of QF at 20%MVC was 28% in both flexion (105°) and extension (145°). However, in this study, the normalized MVC forces at the 20th and 30th repetitions were significantly lower in flexion compared with extension. Ando et al. (2018) showed that isometric continuous contraction forces of QF evoked by femoral nerve stimulation significantly decreased after 30 s in flexion compared with extension. However, it should be noted that our experimental setting was considerably different from these previous studies, thus caution is advised when comparing differences in our MVC force results with those of the previous studies.

It is known that MF determined based on surface EMG signals of working muscles decreases during fatiguing tasks (Beck et al., 2014; Ebenbichler et al., 1998; Masuda et al., 1999; Moritani et al., 1986). The MF of EMG signals primarily reflects the conduction velocity of the action potential in muscle fibers and is a representative index of muscle
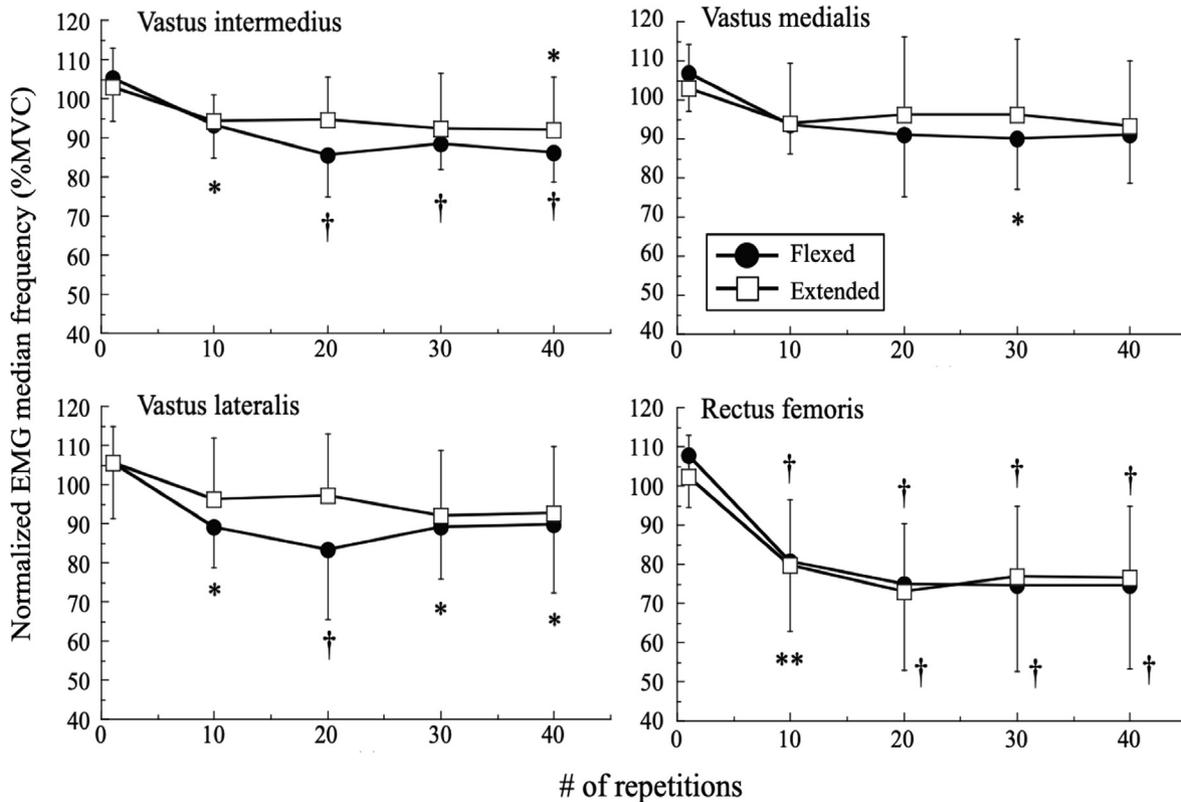

Fig. 2. Time course of normalized electromyographic (EMG) median frequency during isometric repetitive maximum voluntary contractions (MVCs) in flexion and extension. *, $P < 0.05$, **, $P < 0.01$, †, $P < 0.001$ vs. 1st MVC, a, $P < 0.05$, b, $P < 0.01$ vs. flexion. Normalized EMG median frequency was expressed as normalized to pre-fatigue MVC test (normalized EMG median frequency pre-fatigue MVC test = 100%).
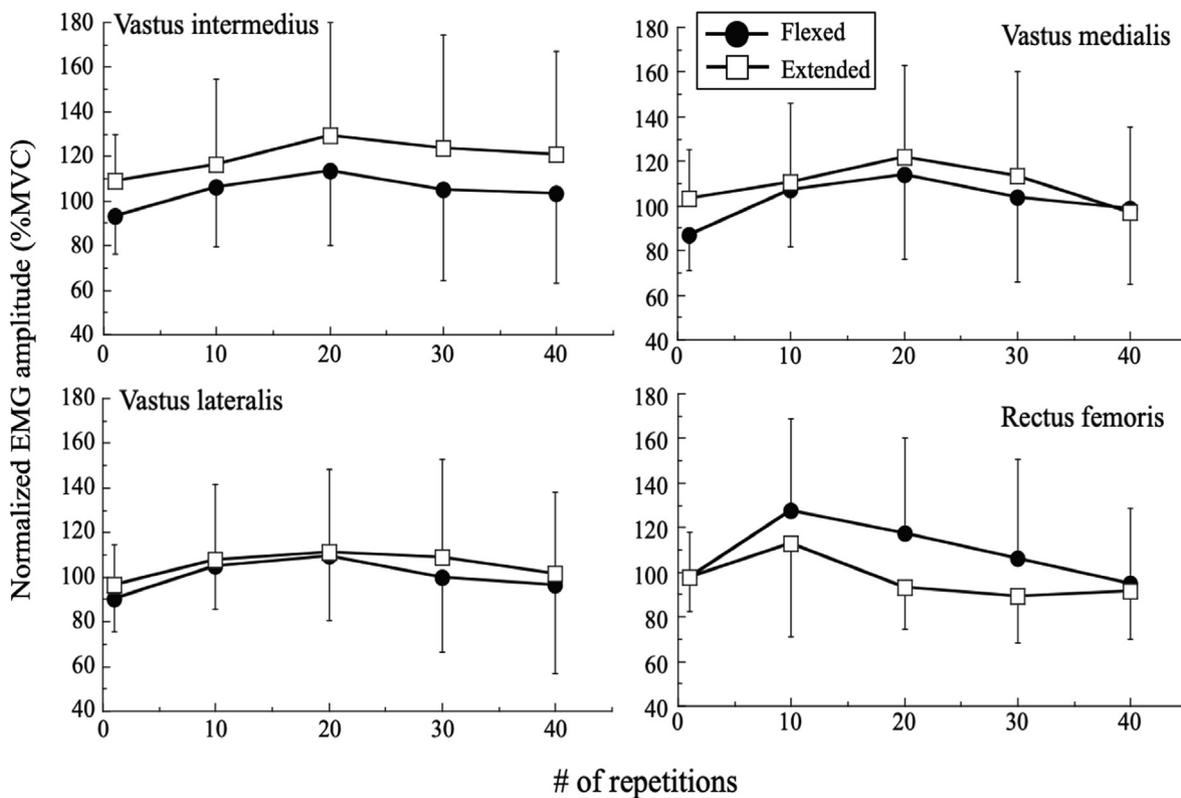
Fig. 3. Time course of normalized electromyographic (EMG) amplitude during isometric repetitive maximum voluntary contractions (MVCs) a in flexion and extension. Normalized EMG amplitude is expressed as normalized to pre-fatigue MVC test (normalized EMG amplitude pre-fatigue MVC test = 100%).

fatigue (Basmajian and DeLuca, 1985; Farina, 2004; Hägg, 1992; Vøllestad, 1997). It has been suggested that fatigue-induced decreases in MF of EMG signals are associated with increased intramuscular pressure, reduced blood flow, and/or chemical changes in working muscles (Humphreys and Lind, 1963; Moritani et al., 1986; Sadamoto et al., 1983; Sjøgaard et al., 1986). In this study, we found a significant decrease in normalized EMG MF of VI and VL from the 10th repetition to the final repetition of a fatiguing task in flexion only, not extension, but it was found at only the 30th repetition in VM. This suggests that knee joint flexion induces EMG MF changes sooner compared with extension. This result partially supports our previous study (Ando et al., 2018). We demonstrated that the M-wave amplitude and duration, indices of muscle fatigue, in VI, but not in VL and VM, significantly changes in flexion (70°) compared with extension (120°) in fatiguing contractions evoked by tetanic femoral nerve stimulation (Ando et al., 2018). Additionally, Beck et al. (2014) showed that EMG frequency shifts from high to low during fatiguing dynamic contractions. Also, the spectral shifts were most pronounced for the RF, followed by the VL, then the VM, which is very similar to this study. In light of these results, we suggest that muscle fatigue-induced EMG MF changes in VI and VL correlated with the knee joint angle, in particular, in flexion, which is partially supported by our previous studies (Akima and Saito, 2013a, 2013b). Furthermore, de Ruiter et al. (2005) showed muscle oxygen consumption of the VL in extension (150°) is significantly lower

compared with flexion, suggesting less oxygen uptake is needed to exert the same muscle force production. In our experimental conditions, we used a repetitive MVCs task. Thus, lower levels of metabolites such as lactic acid would be accumulated in muscle cells, which may affect knee joint-dependent EMG MF changes between flexion and extension.

The VL has been frequently assessed as a representative muscle of the QF to record fatigue-related EMG MF in many previous studies, because it is the largest of the individual muscles in the QF. According to the current study, EMG MF in the VL in extension did not vary significantly throughout the task, except for during flexion, where it decreased significantly. This result clearly demonstrates that EMG MF in VL response is knee joint angle-specific. Therefore, we need to be very careful in interpreting the results reported in many previous studies of EMG MF of VL with respect to the knee joint angle.

MF is also known to be affected by several parameters such as temperature, additional recruitment of MUs, and other factors during fatiguing contractions (Hägg, 1992). Here, the effect of additional recruitment of MUs was eliminated as a potential contributor to affect fatigue-related MF change, because it is assumed that almost all MUs would be recruited during the preceding repetitive MVCs task. From a muscle fiber characteristics point of view, Johnson et al. (1973) and Edgerton et al. (1975) reported that the %type II fibers of VI and VL are 53% and 67%, respectively. We assessed whether this 14% of type II fibers difference between the two muscles might affect metabolic

Table 2
Stepwise linear regression analysis as a dependent variable of normalized EMG median frequency of RF.

Knee joint angle	Independent variables	Regression coefficient	SE	Standardized regression coefficient	P	R	Adjusted R^2
Flexed position	MF of VM	0.947	0.295	0.545	0.001	0.819	0.670
	MF of VI	1.458	0.489	0.506			
Extended position	MF of VM	0.831	0.198	0.758	0.001	0.758	0.575

Dependent variables, normalized EMG median frequency of VI, VL and VM. SE, standard error.
MF, median frequency; RF, rectus femoris; VI, vastus intermedius; VM, vastus medialis.

properties. In our previous study, we measured muscle tissue saturation (StO_2) using near infrared spectroscopy and EMG MF of the QF during sustained fatiguing contraction at 50%MVC, and found there were no significant differences in StO_2 between VI and VL (Akima and Ando, 2017), but EMG MF in VL was significantly lower than that of VI at task failure, suggesting that different fatigue responses between VI and VL are specific to parameters.

A notable finding in this study was that there were significant decreases in normalized EMG MF in RF regardless of knee joint angle, which was clearly in contrast to the other three muscles. It has been frequently found that RF is more fatigable than VL and VM during isometric or dynamic knee extensions (Akima et al., 2004; Kooistra et al., 2005; Mullany et al., 2002). For example, Mullany et al. (2002) showed the EMG frequency spectrum in RF significantly decreased compared with VL and VM during isometric knee extension with MVC effort. Ebenbichler et al. (1998) also showed that muscle fatigue developed more in RF compared with VL and VM during fatiguing isometric knee extension tasks. This result is consistent with the result that RF is the most fatigable muscle of the individual muscles in the QF (Akima and Saito, 2013; Kooistra et al., 2005). The physiological reason for this is unknown.

RF is unique among the individual muscles in QF, because it is the only two-joint muscle; however, muscle fiber type in RF (71% type II fibers) is similar to VL (67% type II fibers) and VM (56% type II fibers) (Johnson et al., 1973). This suggests that the metabolic capacity of RF, VL and VM would be similar. In some studies, there were region-specific differences in RF between proximal and distal regions (Akima et al., 2004; Watanabe et al., 2012). Akima et al. (2004) showed that muscle activity of the RF was significantly higher proximally than distally during repetitive isokinetic knee extensions using muscle functional magnetic resonance imaging (MRI). However, we placed our RF EMG electrode in the middle of the thigh in this study; therefore, the effect of region-specific neuromuscular activity, if any, could affect the results to a lesser degree, because normalized EMG MF and the numbers of repetitions were likely very similar between the two knee joint positions (Fig. 2). In addition, RF is a biarticular muscle across the hip and knee joints, therefore the effect of the hip joint might affect the EMG amplitude of the QF (Carr et al., 2018).

We found no significant differences in intermuscle and time-associated comparisons among normalized EMG amplitudes in any muscles (Fig. 3). We hypothesized that muscle force contribution affects the changes in EMG amplitude of any individual muscles of the QF when there are difference in knee joint angle, because muscle size (Akima et al., 2004) and force-length relationship in sarcomeres (Cutts, 1988) are different among individual muscles. The results did not support our hypothesis, but are consistent with Mullany et al. (2002), who demonstrated normalized EMG amplitude of VL, VM, and RF was constant during sustained MVC effort until task failure. These results clearly represent that EMG amplitude is constant when muscle contractions are performed with maximal effort. While EMG amplitude responses were force level dependent. Mullany et al. (2002) showed EMG amplitude was gradually increased when sustained fatiguing contraction was performed at 25–75% MVC force levels. In addition, Moritani et al. (1986) reported that EMG amplitude slightly decreased with time in the biceps brachii during a sustained MVC task. Because we used "repetitive" MVC task in this study, but Mullany et al. and Moritani et al. used "sustained" MVC tasks, we should pay attention to the type of fatiguing tasks when comparing studies. The "repetitive" MVC task has a rest period for a few seconds between each contraction, thus the progression of fatigue, which affects EMG amplitude, is expected to be less than that of the "sustained" MVC task.

We assessed the relationship of normalized EMG MF among RF and the three vasti muscles to estimate the coactivation pattern during fatiguing tasks. This kind of analysis has not been performed in previous studies. The advantage of this result is useful to estimate the coactivation pattern of synergistic muscles among the working muscles,

which will give us useful information regarding delaying of muscle fatigue in any specific condition. We found that RF was free from angle-specific muscle fatigue and it may be coactivated with VI and VM in flexion and with VM in extension. Interestingly, VI was selected as an independent variable to explain MF of RF only in flexion as a result of stepwise regression analysis. We have shown that there is an advantage to force production based on the EMG amplitude in VI in flexion during both static and dynamic contractions (Akima and Saito, 2013; Watanabe and Akima, 2011). This would be a reason to select a flexed position and eliminate an extended position as an independent variable. In addition, VM was selected as an independent variable in both flexion and extension. This result supports de Ruiter et al. (2008), who showed that VM contributes equal force for production of knee extension during fatiguing tasks from 90–170°. We also confirmed that VM greatly contributes to force production the extended range of motion during a fatiguing dynamic knee extension task (Akima and Saito, 2013).

Finally, the time course changes in normalized EMG of amplitude and MF were assessed using surface EMG technique, which has excellent time resolution but poor spatial resolution. In particular, it is known that there is an evidence of spatial difference in activation patterns along the proximal-to-distal direction of the RF using multiple-channel surface EMG technique (Watanabe et al., 2012) as well as muscle functional MRI (Akima et al., 2000). There is a possibility that the activation pattern was heterogeneous within a single muscle. However, our result was consistent with those of previous studies with regard to changes in EMG MF pattern (Ebenbichler et al., 1998; Mullany et al., 2002). In the light of these previous studies, we need to be mindful of the limitations of spatial resolution of surface EMG technique when interpreting the results of this study.

In conclusion, normalized EMG MF in VI and VL in flexion significantly decreased with increasing number of repetitions compared with pre-fatigued MVCs; however, that of VI, VL, and VM in extension were overall constant throughout the fatiguing contractions. Of particular note is that the normalized EMG MF in RF was significantly decreased starting at the 10th repetition to the end of the task regardless of knee joint angle. The coactivation pattern estimated based on EMG MF among QF synergists suggests that RF/VM is independent, but RF/VI is dependent upon knee joint angle, which may, in part, explain joint angle-specific muscle fatigue. The results of the present study will be useful to understand the redundancy of the human neuromuscular system during fatiguing heavy loaded conditions with difference joint angles.

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Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelekin.2019.102356.

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