Force fluctuations are modulated by alternate muscle activity of knee extensor synergists during low-level sustained contraction

Motoki Kouzaki,1,* Minoru Shinohara,2,* Kei Masani,1 and Tetsuo Fukunaga3

1Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan; 2Department of Integrative Physiology, University of Colorado, Boulder, Colorado 80309-0354; and 3School of Sport Sciences, Waseda University, Saitama 359-1192, Japan

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Kouzaki, Motoki, Minoru Shinohara, Kei Masani, and Tetsuo Fukunaga. Force fluctuations are modulated by alternate muscle activity of knee extensor synergists during low-level sustained contraction. J Appl Physiol 97: 2121–2131, 2004. First published June 18, 2004; doi:10.1152/japplphysiol.00418.2004.—The study examined the hypothesis that altered synergistic activation of the knee extensors leads to cyclic modulation of the force fluctuations. To test this hypothesis, the force fluctuations were investigated during sustained knee extension at 2.5% of maximal voluntary contraction force for 60 min in 11 men. Surface electromyograms (EMG) were recorded from the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM) muscles. The SD of force and average EMG (AEMG) of each muscle were calculated for 30-s periods during alternate muscle activity. Power spectrum of force was calculated for the low- (≤3 Hz), middle- (4–6 Hz), and high-frequency (8–12 Hz) components. Alternate muscle activity was observed between RF and the set of VL and VM muscles. The SD of force was not constant but variable due to the alternate muscle activity. The SD was significantly greater during high RF activity compared with high VL and VM activity (P < 0.05), and the correlation coefficient between the SD and AEMG was significantly greater in RF [0.736 (SD 0.095), P < 0.05] compared with VL and VM. Large changes were found in the high-frequency component. During high RF activity, the correlation coefficient between the SD and AEMG was significantly greater in RF [0.832 (SD 0.087)] compared with VL and VM. Large changes were found in the high-frequency component of the fluctuations.

stediess; synergistic muscles; muscle fatigue; electromyogram

When an individual produces a steady motor output by contracting muscles, the force in steady isometric contractions and acceleration in anisometric contractions fluctuate around the averaged values. The magnitude of fluctuations in motor output is often referred to as “steadiness” (10). The fluctuations in motor output are dependent on force production level, contraction type, muscle used for the task, age of the subjects, and physical activity status (11). Physiological mechanisms underlying the fluctuations in a hand muscle have been confined to motor units: intrinsic properties of motor units, motor unit discharge rate variability, and correlated discharges of motor units (14, 28, 52). In contrast, it is likely that activation patterns of synergistic muscles influence the fluctuations in motor output during contractions that involve multiple muscles. Our recent study showed alterations in the activation pattern within the synergistic muscles after a 20-day bed rest that accompanied increased force fluctuations in knee extension and in ankle extension (45). In addition, Graves et al. (16) have observed differences in the activation patterns among elbow flexor synergistic muscles during isometric contractions in which there was a difference in fluctuations between young and older adults. Thus the activation strategy among synergistic muscles is a possible neural mechanism responsible for fluctuations in motor output during a contraction of multiple muscles.

Phasic changes in muscle activation patterns among synergistic muscles have been demonstrated during low-level sustained contractions [≤10% maximal voluntary contraction (MVC) force, ≥30 min] in a variety of muscle groups. Individual synergistic muscles are not continuously activated, but, rather, they involuntarily alternate between periods of activity and silence (5–10 min for each period) during knee extension (26, 27, 47), ankle extension (37, 46, 51), and elbow flexion (42, 43), despite a task requiring the subject to maintain a constant force. This phenomenon has been called alternate muscle activity of synergistic muscles, and its basic features have been clarified in the knee extensor muscles (26, 27). Alternate muscle activities are observed more often at 2.5% MVC force compared with higher forces. The alternation occurs only between the rectus femoris (RF) muscle and the set of the vastus lateralis (VL) and vastus medialis (VM) muscles. The alternate muscle activity cannot be achieved voluntarily by changing the joint angles or direction of the force. The amplitude of surface electromyogram (EMG) in RF reaches up to 15–20% of maximal amplitude of EMG during a sustained force-matching task at 2.5% MVC force. Therefore, it is expected that the fluctuations in force during low-level sustained contractions are modulated by the alternate muscle activity.

In contractions at higher intensities, where alternate muscle activity does not emerge (26), fluctuations in motor output increase monotonically with development of fatigue. A monotonous increase in the fluctuations in motor output has been repeatedly found in a variety of muscle groups, including knee extensors (7, 41), ankle extensors (6, 31, 32), and elbow flexors (20–22). In addition, most of the increase in force fluctuations is attributed to the high-frequency component (>5 Hz (6, 31, 32), especially around 10 Hz (7). Furthermore, a shift in the tremor frequency from 8–12 Hz to 4–6 Hz has been reported in hand muscles when the contraction is sustained for ≥30 min.

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(15, 49). Even during a low-level sustained contraction at 2.5% MVC, blood circulation is locally occluded by the effect of alternate muscle activity (27), and the MVC force is reduced by ~15% after 60 min of sustained contraction at 2.5% MVC force (26, 27). These results indicate that the task induces muscle fatigue. However, it is not known how fluctuations in force change during alternate muscle activity in low-level sustained contraction involving muscle fatigue.

Hence, activation strategy among synergistic muscles may influence the fluctuations in force, and nonmonotonic, repeated changes in the activation strategy are expected due to muscle fatigue during low-level sustained contractions. We hypothesized, therefore, that fluctuations in force do not increase simply during low-level sustained contraction of knee extensor muscles but are strongly modulated by the emergence of alternate muscle activity. To test this hypothesis, fluctuations in force were compared based on the period of alternate muscle activity during low-level sustained knee extension.

METHODS

Subjects. Eleven healthy men volunteered to participate in the study. The age, height, and body mass of the subjects (means with SD) were 25.9 (SD 3.0) yr, 171.4 (SD 6.7) cm, and 68.0 (SD 6.3) kg, respectively. They had no significant medical history or signs of neurological disorders and had not participated in any programs of regular exercise. All subjects gave their written, informed consent for the study after receiving a detailed explanation of the purposes, potential benefits, and risks associated with participation in the study. All procedures used in this study were in accordance with the Declaration of Helsinki and were approved by the ethical standards of the Committee for Human Experimentation at the Department of Life Sciences, The University of Tokyo.

Recording techniques. The basic setup for the knee extension procedure has been described in our previous study (26, 27). Each subject performed a static unilateral knee extension exercise by the right leg in a seated position with the hip and knee joint angles of 100 and 90° flexed (full extension = 0°), respectively. Throughout the experiment, the subject’s upper body was firmly fixed on a chair by a seat belt. No feedback or encouragement was provided by the investigators during performance of the tasks to prevent intentional changes in muscle activation strategy. The force of isometric contraction of the knee extensor muscles was measured by a strain gauge force transducer (model 274H, Minebea, Tokyo, Japan), which was coupled with a strain amplifier and attached by a strap to the subject’s ankle just above the malleolus. The sensitivity of the force transducer was 10.22 N/V. The produced force and the target were displayed as horizontal lines on a storage oscilloscope in front of the subject to provide visual feedback. Bipolar surface EMG was recorded from RF, VL, VM, and biceps femoris long head (BF) using Ag-AgCl electrode diameter of 5 mm and an interelectrode distance (center to center) of 20 mm. After careful abrasion of the skin, the electrodes were placed on the skin over the muscle belly of the respective muscles. The reference electrode was placed on the iliac crest. The electrodes were connected to a preamplifier and a differential amplifier having a bandwidth of 5 Hz to 1 kHz (1253A, NEC Medical Systems, Tokyo, Japan). All electric signals were stored with a sampling frequency of 1 kHz on the hard disk of a personal computer using a 16-bit analog-to-digital converter with a recording range of ±5 to ±5 V (PowerLab/16SP, ADInstruments, Sydney, Australia). The resulting resolution of the measuring force was 1.56 × 10⁻³ N/bin.

Experimental protocol. The MVC task involved a gradual increase in knee extension force exerted by the quadriceps muscle from baseline to maximum in 3–4 s and then sustained at the maximum for 2 s. The knee extension force was displayed in real time on the oscilloscope. The timing of the task was based on a verbal count given at 1-s intervals, with vigorous encouragement from the investigator when the force began to plateau. Each subject performed at least three MVC trials, with subsequent trials performed if the differences in the peak force of two MVCs were >5%. Subjects were allowed to reject any effort that they did not regard as “maximal.” The trial with the highest peak force was chosen for analysis. After a sufficient rest period (~10 min) following the MVC measurement, the subject performed a sustained knee extensor muscle contraction at 2.5% of subject’s MVC force for 60 min. MVC measurement was also performed immediately after the sustained contraction. Furthermore, six of the subjects performed an additional brief contraction to match the target force at 5, 10, 15, 20, and 30% MVC force for 40 s on a separate day. Experimental setup and measurements were identical to that of sustained knee extension at 2.5% MVC force. The order of the target was pseudorandomized across subjects.

Data analyses. Alternate muscle activity was detected and counted according to the previously established method (26), based on the abrupt decrease and increase in the EMG of RF (15, 49). This is because the alternate muscle activity of the knee extensor synergists is limited to the alternation between the activity of RF and the set of VL and VM muscles, and abrupt change is more distinct in the EMG of RF compared with VL or VM (26, 27) (Fig. 1). The full-wave-rectified EMG of RF was low-pass filtered (cutoff frequency = 0.01 Hz) by a fourth-order Butterworth filter employing a zero-phase lag (59). EMGdiff/dt was then given as a result of time differentiation of the filtered EMG sequence. To characterize force fluctuations associated with the emergence of alternate muscle activity, data were analyzed at the start (S), middle (M), and end (E) of the alternate muscle activity, in which activity of RF was high with low activity of vasti muscles (Rf, RfM, and Re, respectively) in which activity of vasti muscles was high with low activity of RF (Vs, Vm, and Ve, respectively). The period between two negative peaks of EMGdiff/dt in RF was defined as a cycle. Two cycles were used for analyses because the maximal number of complete cycles in all subjects was two. A 30-s data set for each of these epochs was extracted in the following manner: Vs was extracted at 10 s after the negative peak of EMGdiff/dt of RF, whereas Ve was extracted at 10 s before the positive peak of EMGdiff/dt of RF. Vm was extracted as the middle portion between Vs and Ve in each cycle. Similarly, Rf was extracted at 10 s after the positive peak of EMGdiff/dt of RF, whereas Re was extracted at 10 s before the negative peak of EMGdiff/dt of RF. RfM was extracted as the middle portion between Rf and Re in each cycle. In addition, a 30-s force and EMG data set was extracted at 10 s after the onset of sustained contraction (onset).

The force data and EMG signals were analyzed for 30 s in each period. After confirmation that there was little power for force >30 Hz, the signal was passed through a low-pass filter of 30 Hz by using a fourth-order Butterworth filter to remove the high-frequency noise components (3, 6, 9, 31, 32). The mean value and SD of force were calculated for each entire period. The SD of force was used as a measure of the force fluctuations because the target force was constant across time. For further analysis of the power spectrum of the force, the 30-s data were first divided into 13 segments that were 212 points across time. For further analysis of the power spectrum of the force, the order of the segments overlapped with the adjacent segments (4). A 12-bit fast Fourier transform algorithm was then applied to these segments to yield the segments’ power spectrum (N²). Consequently, frequency resolution of the power spectrum was 0.244 Hz. An ensemble-averaged power spectrum across these segments was calculated as a power spectrum of the force for each period. The power spectrum of force greater than direct current and <3 Hz was integrated and defined as the low-frequency component of the force fluctuations. This is because force fluctuations in the fatigued muscle state have been shown to be predominantly in the low-frequency range (~3 Hz (54) (Fig. 2, onset). In contrast, fluctuations of force or acceleration around 8–12 Hz,
referred to as “tremor,” have been observed in fatigued muscle (13, 29, 35). Therefore, the power spectrum of force from 8 to 12 Hz was integrated and defined as the high-frequency component of the force fluctuations. In addition, the power spectrum from 4 to 6 Hz was integrated and defined as the middle-frequency component of the force fluctuations based on literature that suggests a shift from 8–12 Hz to 4–6 Hz during a prolonged contraction sustained for 30 min (15, 49). Furthermore, the rectified EMG of each muscle was averaged over the period (30 s) to yield the average amplitude of EMG (AEMG). The AEMG in absolute units (V) was further expressed in normalized values as a percentage of the corresponding value during MVC measurement. To examine the degree of local muscle fatigue, the median frequency of EMG was calculated from raw EMG in the same process applied to force measurements (38). Finally, the variables were averaged across cycles.

In the additional brief contractions at 5, 10, 15, 20, and 30% MVC force in a fresh muscle state, force and EMG signals over the middle 30 s of the contraction were used to calculate the SD of force and AEMG.

Statistical analysis. One-way ANOVA with repeated measures with a Tukey post hoc test was used to determine significant differences in the SD; the low-, middle-, and high-frequency components of force; and the AEMG across 12 periods. A two-way ANOVA with repeated measures with Tukey’s post hoc test was used to compare the SD; the low-, middle-, and high-frequency components of force; and the AEMG over three periods (start, middle, and end) and two EMG activity levels (high RF and high VL and VM) as factors. To compare the SD of force and AEMG between the two constant-force tasks (sustained contraction and brief contraction), one-way ANOVA with repeated measures with a Tukey post hoc test was used. Linear regression analysis was conducted between the SD and AEMG of each muscle head, and between the SD and low-, middle-, and high-frequency components during two EMG levels (high RF and high VL and VM) in each subject to calculate the correlation coefficient between the variables. One-way ANOVA with repeated measures with Tukey’s post hoc test was used to find a significant difference in correlation coefficient for these variables. Linear regression analysis was also conducted between the SD and low-, middle-, and high-frequency components during brief contractions. The level of significance for all comparisons was set at $P = 0.05$. Values are given as means and SD in the text and means ± SE in Figures 3, 4, 6, and 7.

RESULTS

The MVC force was 735.3 (SD 138.3) N, and the target force (2.5% MVC) was 18.4 (SD 3.5) N. All subjects could maintain the target force for 60 min. The MVC force fell to 84.6 (SD 5.4)% of the initial MVC measurement after the sustained contraction. Alternate muscle activity was observed between RF and both VL and VM in all subjects. Total counts of alternate muscle activity in 60 min were 6.5 (SD 1.6)
between RF and VL, 6.7 (SD 2.0) between RF and VM, and 0.4 (SD 0.7) between VL and VM, findings that were similar to those in our previous studies (26, 27).

As mentioned in METHODS, two or more cycles of alternate muscle activity were observed across subjects. Data on time, duration, average force, and the AEMG in absolute units for the extracted data periods are shown in Table 1. Durations for VS-VE and RS-RE were around 5–8 min and showed no significant differences across periods in both cycles. Average force was constant around 18.4 N (as described in Table 1), which was equivalent to the target force throughout the task. The AEMG of BF was constant at a much lower level compared with agonists, as found in previous studies (26, 27).

A representative example of fluctuations and power spectrum of force at each period is shown for two cycles in Fig. 2. Clearly, the fluctuations in force were greater during high RF

Table 1. *Time, duration, average force, and average amplitude of electromyogram of rectus femoris, vastus lateralis, vastus medialis, and biceps femoris long head at each period during sustained contraction*

<table>
<thead>
<tr>
<th></th>
<th>Onset</th>
<th>Duration, min</th>
<th>Force, N</th>
<th>AEMG of RF, μV</th>
<th>AEMG of VL, μV</th>
<th>AEMG of VM, μV</th>
<th>AEMG of BF, μV</th>
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<tbody>
<tr>
<td>First cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS</td>
<td>0.2 (0.0)</td>
<td>18.6 (3.6)</td>
<td>4.1 (1.6)</td>
<td>16.1 (6.0)</td>
<td>11.3 (5.4)</td>
<td>2.2 (1.0)</td>
<td></td>
</tr>
<tr>
<td>V_S</td>
<td>22.1 (6.7)</td>
<td>18.5 (3.4)</td>
<td>6.6 (1.7)</td>
<td>38.5 (13.7)</td>
<td>36.3 (16.7)</td>
<td>2.7 (1.2)</td>
<td></td>
</tr>
<tr>
<td>V_M</td>
<td>24.9 (6.0)</td>
<td>18.6 (3.4)</td>
<td>6.6 (1.3)</td>
<td>40.4 (13.5)</td>
<td>36.6 (17.4)</td>
<td>2.8 (1.2)</td>
<td></td>
</tr>
<tr>
<td>V_E</td>
<td>27.7 (5.6)</td>
<td>18.5 (3.4)</td>
<td>6.2 (1.3)</td>
<td>38.4 (16.0)</td>
<td>36.2 (18.5)</td>
<td>2.6 (1.3)</td>
<td></td>
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<tr>
<td>R_S</td>
<td>28.4 (5.6)</td>
<td>18.5 (3.6)</td>
<td>25.9 (11.4)</td>
<td>28.5 (9.8)</td>
<td>24.7 (10.6)</td>
<td>2.7 (1.4)</td>
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</tr>
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<td>R_M</td>
<td>32.5 (5.1)</td>
<td>18.6 (3.6)</td>
<td>34.2 (9.2)</td>
<td>25.5 (12.4)</td>
<td>21.1 (9.9)</td>
<td>2.6 (1.3)</td>
<td></td>
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<tr>
<td>R_E</td>
<td>36.6 (4.9)</td>
<td>18.6 (3.7)</td>
<td>349.1 (10.8)</td>
<td>271.1 (11.6)</td>
<td>21.8 (10.3)</td>
<td>2.6 (1.3)</td>
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<tr>
<td>Second cycle</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>VS</td>
<td>37.5 (5.0)</td>
<td>18.5 (3.7)</td>
<td>6.2 (1.5)</td>
<td>39.3 (16.2)</td>
<td>35.4 (17.8)</td>
<td>2.8 (1.4)</td>
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</tr>
<tr>
<td>V_S</td>
<td>40.4 (4.7)</td>
<td>18.5 (3.9)</td>
<td>6.4 (1.7)</td>
<td>39.7 (16.0)</td>
<td>39.3 (17.7)</td>
<td>2.7 (1.3)</td>
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<td>V_M</td>
<td>43.3 (4.6)</td>
<td>18.6 (4.0)</td>
<td>6.8 (2.1)</td>
<td>40.5 (16.4)</td>
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<td>V_E</td>
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<td>18.6 (3.9)</td>
<td>34.1 (9.2)</td>
<td>28.2 (13.5)</td>
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<td>38.4 (8.8)</td>
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<tr>
<td>R_M</td>
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<td>18.7 (3.8)</td>
<td>39.2 (9.4)</td>
<td>26.7 (13.7)</td>
<td>19.9 (9.8)</td>
<td>2.6 (1.3)</td>
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</table>

Values are means with SD in parentheses. Onset indicates data immediately after sustained contraction commencement. Duration indicates the time at which the data were extracted for calculation. See text for further explanation.
activity (R_S-R_E) compared with high vasti muscle activity (V_S-V_E). In addition, the amplitude of the fluctuations tended to increase with time during high RF activity. Furthermore, the majority of the power spectrum was within the range ≤3 Hz during high vasti muscle activity, whereas a power spectrum peak around 8 Hz was developed during high RF activity.

Changes in the AEMG and SD of force averaged across subjects are presented in Fig. 3A. Two distinct cycles of alternate muscle activity are observed in the AEMG (top panel). In other words, the AEMG of RF increased as the AEMG of VL and VM decreased and vice versa over both cycles. The SD of force at the onset of sustained contraction was 0.40 (SD 0.16) N (bottom panel). It showed substantial changes throughout the period at significantly higher levels compared with the onset of sustained contraction (P < 0.05). Significant differences were observed between V_S and R_E in the first cycle (P < 0.05), between R_E in the first cycle and V_S in the second cycle (P < 0.01), and between V_S and all of R_S,E in the second cycle (P < 0.05). In particular, the difference between the periods around the abrupt decrease in the AEMG of RF (R_E-V_S) was prominent. The AEMG and SD of force were further averaged across cycles for the start, middle, and end periods of high RF activity (R_S,E, open symbols) and for those of high vasti muscle activity (V_S,E, solid symbols) (Fig. 3B). The AEMG of RF amounted to 15–20% during R_S,E (open circles), which was significantly higher compared with V_S,E (P < 0.01, solid circles; top panel). The AEMG of VL and VM in V_S,E (solid triangles and squares) were greater compared with R_S,E (P < 0.05). The SD of force was significantly greater in R_S,E (open diamonds) compared with V_S,E (P < 0.05, solid diamonds; bottom panel). Furthermore, the SD of force increased with time during high RF activity (open diamonds), resulting in a significantly greater value at R_E compared with R_S (P < 0.05).

To investigate the association between the SD of force and the AEMG of each muscle head, the correlation coefficient between the SD of force and the AEMG in individual heads of the knee extensors was assessed by using the correlation coefficient of each individual. During high RF activity, the correlation coefficient between the SD and the AEMG in RF [0.736 (SD 0.095), n = 11] was significantly (P < 0.05) greater compared with the correlation coefficient between the SD and the AEMG in either of the vasti muscles [VL: −0.041 (SD 0.566), VM: −0.134 (SD 0.570)]. When muscle activities of VL and VM were high, the correlation coefficient was not significantly different across muscle heads [RF: 0.035 (SD 0.437), VL: 0.169 (SD 0.095), VM: −0.001 (SD 0.397)]. These results indicate the strong association between the SD of force and RF activity.

As illustrated in Fig. 2, throughout the task, the majority of the power in the force fluctuations fell in the ranges of ≤3 Hz (low-frequency component) and 8–12 Hz (high-frequency component). To examine the frequency range that contributed to changes in the SD of force, the mean spectrum of each frequency component was calculated at each period (Fig. 4A). On average, the low-frequency component was around 5–10 times as high as the high-frequency component across periods. The low- and middle-frequency components were fairly constant during alternate muscle activity throughout the task (top and middle panels). In contrast, the high-frequency component showed cyclical changes for two cycles (bottom panel). It was small during high vasti muscle activity at V_S, with a tendency of gradual increase toward R_E as the activity of RF gradually increased. The unique feature of the change in force fluctuations has been confirmed by the significant difference between R_E in the first cycle and V_S in the second cycle (P < 0.01). Each variable was then averaged across two cycles for the start,
middle, and end periods for each alternate muscle activity (V_{S,E} in solid symbols and R_{S,E} in open symbols) as in Fig. 3B (Fig. 4B). Clearly, there was no change in the low- and middle-frequency components (top and middle panels). There was no change in the high-frequency component during high vasti muscle activity (V_{S,E}) either (solid squares, bottom panel). However, the high-frequency component in R_{S,E} increased with time during high RF activity (open squares), resulting in a significantly greater value at RE compared with RS and VE (P < 0.05).

To further examine the relation between the SD of force and the frequency components of the force, the association between the SD of force and each frequency component was assessed by using the correlation coefficient of each individual. During high RF activity, the correlation coefficient between the SD and high-frequency component [0.832 (SD 0.087), n = 11] was significantly (P < 0.05) greater compared with the correlation coefficients between the SD and low- [0.241 (SD 0.520)] and middle-frequency components [0.097 (SD 0.422)]. During high activities of both VL and VM, the correlation coefficient was not significantly different across the frequency components [low-frequency: 0.374 (SD 0.410), middle-frequency: 0.241 (SD 0.520), high frequency: 0.423 (SD 0.387)]. In addition, mean data for the SD of force were plotted against mean values of low-, middle-, and high-frequency components (Fig. 5). A significantly linear relation between the SD of force and the high-frequency component (r = 0.922, P < 0.05) during high RF activity (open squares, right panel) confirmed the strong association between the high-frequency component and the SD of force.

**Fig. 5.** SD of force as a function of the low-, middle-, and high-frequency components of the force. Open and solid symbols represent the data during high RF activity and high vasti muscle activity, respectively. The superimposed line indicates the linear regression line with statistical significance between the measures (P < 0.05).
To quantify the degree of muscle fatigue, the median frequency of raw EMG was calculated when each muscle had the larger share of muscle activity. With respect to the RF (open circles), median frequency of EMG was calculated for the period from RS to RE. Median frequency of EMG of VL and VM was calculated from VS to VE. The obtained values were then averaged across two cycles (Fig. 6A). There was no change in the median frequency of EMG in VL or VM during high vasti muscle activity. In contrast, the median frequency of EMG in RF decreased with time during high RF activity, leading to a significantly lower value at the end period ($P < 0.05$). The decrease in the median frequency of RF was more obvious when the relative change in the median frequency was compared (Fig. 6B). The relative change at the middle and end periods was significantly larger in RF compared with that in the vasti muscles ($P < 0.05$).

The greatest fluctuations in force were found when the activity of RF was highest (RE) during alternate muscle activity (Fig. 3A). To compare the force fluctuations and AEMGs at these periods with the values during contractions in unfatigued muscle state, six of the subjects also performed brief contractions at 5–30% MVC force. As the level of force increased, the SD of force increased monotonically with similar monotonically increases in the AEMGs across muscles during brief contractions. The SDs of force at RE were significantly greater compared with a brief contraction at 5–10% MVC force ($P < 0.05$) and were equal to the value during a brief contraction at 15% MVC force (Fig. 7A). The AEMGs of VL and VM at RE were smaller compared with a brief contraction at 10% MVC force ($P < 0.05$, Fig. 7B). In contrast, the AEMGs of RF at RE were significantly greater compared with a brief contraction ±15% MVC force ($P < 0.05$) and were equal to the value during a brief contraction at 20% MVC force. In addition, the association between the SD of force and each frequency component was assessed by using the correlation coefficient of each individual. In contrast to the alternate muscle activity, the correlation coefficient during brief contractions was high in all of the frequency components [low-frequency: 0.990 (SD 0.005), middle-frequency: 0.970 (SD 0.012), high frequency: 0.974 (SD 0.013)]. These results indicate that the increase in force fluctuations during high RF activity is different from that due to the change in the level of muscle activity during brief contractions.
DISCUSSION

Consistent with our previous reports (26, 27), alternate muscle activity of the knee extensor synergists emerged in all subjects, especially between RF and vasti muscles, during sustained (60-min) contraction at 2.5% MVC force. In support of our hypothesis, fluctuations in force were modulated during alternate muscle activity. Furthermore, their increase was associated with an increase in rhythmic oscillation around 8–12 Hz during high RF activity. Fluctuations in force during alternate muscle activity at 2.5% MVC force were equal to those during a brief contraction at 15% MVC force.

Force fluctuations during alternate muscle activity compared with brief contractions. Despite constant mean force output at 2.5% MVC force, fluctuations in force were not constant or did not change monotonically but varied widely across the task. The fluctuations of force at the onset of the task (0.40 N in SD) corresponded to a coefficient of variation for force of 2.0%. This value in the coefficient of variation was similar to the previous finding in young adults (45). Substantially larger fluctuations in force were observed during high RF activity. The force fluctuations during high RF activity were equivalent to a coefficient of variation at 2% MVC in elderly men (3.8%) (53) and that at 2.5% MVC in young adults after prolonged bed rest (2.2%) (45). A comparison to observations during brief contractions at different force levels in the same subjects provides further insight into the uniqueness of the fluctuations during alternate muscle activity (Fig. 7). It is known that fluctuations in force increase linearly with the level of force when they are expressed in absolute units (SD) (14, 19, 28, 53). The SD of force during high RF activity was similar to that seen during a brief contraction at 15% MVC force. These comparisons demonstrate the significance of the large fluctuations in force during high RF activity in the present study.

Muscle activation strategy. In a steady motor output task, the magnitude of fluctuations in force can be associated with alterations in the distribution of muscle activity across synergistic muscles (16, 45). In a previous study, an increase in force fluctuations accompanied increased activity of VL relative to RF after bed rest (45). In the present study, however, temporal variation in the SD of force seems to be associated with temporal changes in the AEMG of RF. In particular, the SD of force decreased from 1.5–1.6 to −0.9 N when the AEMG of RF abruptly decreased from 17 to 3% (Fig. 3A). Furthermore, the SD of force was significantly greater during high RF activity compared with high vasti muscle activity (Fig. 3B). In addition, a high correlation coefficient between the SD of force and the AEMG was found only in RF during high RF activity (r = 0.736). During brief contractions, the AEMG of each muscle increased monotonically with force, indicating that there was little change in the distribution of muscle activity across forces. In contrast, during high RF activity in sustained contractions, the AEMGs of VL and VM were equal to that during a brief contraction at 5% MVC force, whereas the AEMG of RF was equal to that during a brief contraction at 20% MVC force. These findings imply that pronounced RF muscle activity may be the primary source of the fluctuations in force in the present study. Although the change in the distribution of EMG across knee extensor muscles was not the same as the change after bed rest (45), which usually accompanies atrophy, the results are consistent with the idea that fluctuations in force are influenced by the distribution of muscle activity across synergistic muscles.

Fatigability of RF muscle. It has been indicated that enhanced fluctuations of force are accompanied by development of muscle fatigue during isometric contraction of knee extensors (7), ankle extensors (6, 31, 32), elbow flexors (20–22), and hand muscles (33). The SD of force during alternate muscle activity was greater compared with the onset across periods. This implies that muscle fatigue was induced across all heads of the quadriceps muscle during the sustained contraction, which partly contributed to the increase in fluctuations in force. The increase in the SD of force from the start to end of high RF activity accompanied relatively high AEMG (15–17%) and a decrease in the median frequency of EMG in RF, suggesting that the increase in the SD during these periods was due to the additional development of fatigue in RF. In contrast, during high vasti muscle activity, the level of AEMG was moderate (6–9%), and the median frequency of EMG in vasti muscles did not change. It seems, therefore, that RF is more susceptible to fatigue and produces greater fluctuations in force compared with VL and VM during alternate muscle activity.

There are other studies that support greater fatigability of RF compared with VL and VM. Ebenbichler et al. (8) showed a greater rate of decline in median frequency of EMG in RF compared with VL and VM during submaximal fatiguing contraction. One limitation of examining the frequency characteristics of the surface EMG signal during muscle fatigue is that the signal is influenced by other factors than conduction velocity (12). However, studies that used other techniques, such as mechanomyogram (24, 44) and magnetic resonance technique (56), have all shown that RF is more susceptible to fatigue compared with VL and VM during a fatiguing task. The underlying mechanism of this general finding is yet to be elucidated, but the greater fatigability in RF during alternate muscle activity could be explained, at least in part, by a high mechanical stress on RF. Despite the low level of the contraction at 2.5% MVC force, the AEMG of RF amounted to 15–20% maximum, whereas the AEMG of VL and VM were 4–7% maximum (Fig. 3A). The physiological cross-sectional area (PCSA) of RF is no more than 15% of the total knee extensor muscles, whereas the PCSA of VL and VM is 60% of the total knee extensor muscles (1, 2). A detailed account of muscle force per PCSA of individual heads of knee extensor muscles during alternate muscle activity has been given by Kouzaki et al. (27). Hence, the majority of the knee extension force must be produced by the activity of this smaller muscle during high RF activity, leading to the mechanical stress of RF being equivalent to 15% maximum, even though the level of force is low for the knee extensor muscle group as a whole. It is known that the blood supply to the knee extensor muscles is impaired at 15% of MVC during isometric contraction (48). In fact, we have demonstrated this impairment of local blood circulation in RF during high RF activity in alternate muscle activity (27). Hence, greater fluctuations of force during high RF activity seem to be associated with the greater fatigability of RF due to its relatively high mechanical stress in alternate muscle activity compared with VL and VM.

Low-frequency component of force fluctuations. At the onset of sustained contraction, the low-frequency component was
~20 times greater than the high-frequency component and accounted for most of the force fluctuations. Although the low-frequency component had increased by the time that alternate muscle activity emerged, it remained almost constant throughout sustained contraction (Fig. 4). The low-frequency component (≤3 Hz) in force fluctuation has been known to be associated with the slow changes in net activity of the whole motoneuron pool, including changes in the discharge frequency and the number of active motor units (3). Because the level of force is dependent on these two motor unit strategies (13), the low-frequency component (≤3 Hz) would be related to the produced level of force. In line with this, the low-frequency component of force fluctuations linearly increased with the SDs in force during brief contractions in knee extension. We may, therefore, reasonably conclude that the low-frequency component (≤3 Hz) in force fluctuations in the knee extension is influenced by the level of force and a modest muscle fatigue but not by cyclical alternate muscle activity.

**High- and middle-frequency components of force fluctuations.** The study found a lack of changes in the low- and middle-frequency components of force during alternate muscle activity (Fig. 4) and an increase in the high-frequency component of force (Fig. 4) that was associated with the increase in the SD during high RF activity (Fig. 3). A high correlation coefficient between the SD and the frequency component was found only for the high-frequency component (r = 0.832). These findings indicate that modulation of the force fluctuations in alternate muscle activity is attributable to changes in the high-frequency component of force during high-RF activity.

The dominant frequency of 8–12 Hz in limb or force fluctuations has been widely termed "physiological tremor" (13, 35). It had been suggested that physiological tremor is partly due to rhythmic modulation of the activity of several motor units caused by a servo-loop oscillation in the stretch reflex arc as a result of muscle spindle activity (13, 23, 29, 30). In isometric contraction, the muscle spindles have been reported to respond to small force fluctuations (55), and the discharges from muscle spindles were demonstrated in relation to contraction strength during very low-level contractions of 1–10% MVC (58). RF muscle spindles were the most likely to discharge in the present study, which might have led to the production of the high-frequency component. In contrast, there are some studies that argue against the importance of peripheral mechanisms for physiological tremor. Marsden et al. (34) found that physiological tremor was preserved but less tuned in deafferentated patients. More recently, Wessberg and Vallbo (57) demonstrated the lack of temporal relation between Ia afferent discharges and 8- to 10-Hz oscillation for acceleration and muscle activity during a slow finger movement. These studies argue for the importance of oscillations in the central nervous system, which could be cortical (9, 36) or subcortical (39). There are, however, no available data from which we can speculate on the contribution of oscillations in the central nervous system during alternate muscle activity. Currently, there seems to be no definite consensus about the origins of physiological tremor; it seems that both central and peripheral mechanisms contribute to the generation of physiological tremor and that the contribution of each factor could be task dependent (35).

The literature also implies that amplitude of physiological tremor is closely related to the degree of muscle fatigue. Lösch et al. (32) reported that ankle extensor tremor increased throughout a fatiguing contraction at 30% MVC. Furthermore, the increase in the amplitude of the tremor was inversely correlated with the mean power frequency of EMG (31). Indeed, in the present study, the high-frequency component (8–12 Hz) increased in parallel with a decrease in the median frequency of EMG in RF during high RF activity. Cresswell and Lösch (6) have shown an increase in physiological tremor during a fatiguing ankle extension at 30% MVC. They further demonstrated an attenuation of this increase by applying prolonged vibration (5, 25) or partial ischemic nerve block (40), both of which are known to depress Ia afferent input. It seems, therefore, that rhythmic oscillation in the stretch reflex arc (13, 18, 29, 30) is involved in the increase in physiological tremor, which is known to increase with muscle fatigue (6, 31). Furthermore, a gradual shift from the high to middle frequency during a prolonged contraction has been observed in some studies (15, 49). The potential mechanisms for the middle-frequency component seem to include viscoelastic properties of the limb, a long delay developing in the muscle, and an oscillation in a long loop within the spinal cord (17, 50). In the present study, however, there was no increase in the middle-frequency component during the alternate activity. Taken together, the fatigue-induced increase in the RF activity seems to increase the high-frequency component of the force fluctuations during the alternate muscle activity.

**Conclusion.** In conclusion, fluctuations in force were modulated by the alternate muscle activity of knee extensor synergists during low-level sustained contraction. The modulation of the fluctuations was primarily due to changes in the high-frequency component (8–12 Hz), which were associated with the high activity of RF and low activity of VL and VM. The findings suggest that the patterns of activity between synergistic muscles influence fluctuations in force, especially when the muscle most susceptible to fatigue is activated during alternate muscle activity. Although further studies are necessary to determine whether the current findings hold true in other synergistic muscles, alternate muscle activity could be used in studies that examine mechanisms of fluctuations in force or muscle fatigue in synergistic muscles as a model to induce large changes in activation strategy of synergistic muscles.

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