Menstrual Cycle Mediates Vastus Medialis and Vastus Medialis Oblique Muscle Activity

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ABSTRACT

TENAN, M. S., Y.-L. PENG, A. C. HACKNEY, AND L. GRIFFIN. Menstrual Cycle Mediates Vastus Medialis and Vastus Medialis Oblique Muscle Activity. Med. Sci. Sports Exerc., Vol. 45, No. 11, pp. 2151–2157, 2013. Purpose: Sports medicine professionals commonly describe two functionally different units of the vastus medialis (VM), the VM, and the vastus medialis oblique (VMO), but the anatomical support is equivocal. The functional difference of the VMO is principle to rehabilitation programs designed to alleviate anterior knee pain, a pathology that is known to have a greater occurrence in women. The purpose of this study was to determine whether the motor units of the VM and VMO are differentially recruited and if this recruitment pattern has an effect of sex or menstrual cycle phase. Methods: Single motor unit recordings from the VM and VMO were obtained for men and women during an isometric ramp knee extension. Eleven men were tested once. Seven women were tested during five different phases of the menstrual cycle, determined by basal body temperature mapping. The recruitment threshold and the initial firing rate at recruitment were determined from 510 motor unit recordings. Results: The initial firing rate was lower in the VMO than that in the VM in women ($P < 0.001$) but not in men. There was no difference in recruitment thresholds for the VM and VMO in either sex or across the menstrual cycle. There was a main effect of menstrual phase on initial firing rate, showing increases from the early follicular to late luteal phase ($P = 0.003$). The initial firing rate in the VMO was lower than that in the VM during ovulatory ($P = 0.009$) and midluteal ($P = 0.009$) phases. Conclusion: The relative control of the VM and VMO changes across the menstrual cycle. This could influence patellar pathologies that have a higher incidence in women. Key Words: ESTRADIOL, KNEE INJURY, MOTOR UNIT, PATELLOFEMORAL SYNDROME, PROGESTERONE, QUADRICEPS

Clinical sports medicine textbooks commonly refer to two anatomically distinct portions of the vastus medialis (VM); the proximal portion is the VM and the distal 10% is termed the vastus medialis oblique (VMO) (22). This distinction is made on the grounds that the VMO pennation runs oblique to the patella and thus has different functional properties than the proximal VM, which has fibers running more longitudinal to the patella (13,22). Anatomical studies have also shown that while the middle VM and the VMO are both innervated by nerve roots originating from L1, L2, and L3, the VMO is innervated by a greater number of terminal nerve branches than the VM (31). Recent cadaveric studies have indicated that after accounting for limb length discrepancies between subjects, the differences in pennation between the VM and the VMO were reduced, and the previously reported fibrofascial plane dividing the muscles is an anatomical rarity (22). There is presently insufficient quality evidence to be certain that the VM and the VMO are anatomically and functionally different muscles, and no study has shown that the two muscles can be differentially recruited for their theorized different actions.

The VM is estimated to generate 25% of the knee extension force (13), whereas the VMO medially vectors the patella (18,24) because of its insertion on the medial border (22). The basic but unsubstantiated functional differences between the VM and the VMO are the crux of rehabilitation protocols for patellofemoral pain syndrome and chondromalacia patella (16). If the VM and the VMO are functionally different muscles, it is vital to understand how their differential activation may contribute to patellar stability because knee pain is the most commonly reported joint pain in the United States (5).

There is a higher incidence of patellofemoral pain in women (3). If the VM and the VMO are controlled independently, the large sex hormone oscillations in women may cause a differential activation of VM and VMO motor units (MU) because progesterone and estradiol are known to affect neurotransmitter function (4,27,28,35). The enigmatic pathophysiology of patellofemoral pain syndrome and the insufficient cadaveric evidence demonstrating functional differences in the VM and VMO have left a need...
for electromyography studies to examine VM and VMO activation patterns (29) and their differential effects of sex. Only the recording of single MU can conclusively demonstrate differences in descending drive and changes in MU recruitment because the surface electromyogram is subject to the superimposition of MU action potentials, individual action potential shape, and signal cross-talk. The goals of this study were twofold: 1) to determine whether the VM and VMO MU can be differentially recruited and 2) to determine whether the MU firing patterns of recruitment are altered as an effect of sex or the menstrual cycle.

METHODS

Participants and ethical approval. Eleven young men (24.6 ± 5.1 yr) and seven young eumenorrheic women (24.9 ± 4.3 yr) participated in the study. Men participated in one study visit, all of which were conducted at 10:00 am. The women participated in five study visits at defined points in the menstrual cycle: early follicular, late follicular, ovulation, midluteal, and late luteal. All women collected data in the morning. The time of data collection was standardized within each participant. The inclusion criteria for all participants were the absence of neurologic, cardiovascular, endocrine, or metabolic disorders; previous leg surgery; immobilizations; arthritis; or chronic injury to the dominant leg. In addition, the female participants must have been hormonal contraception naive for at least 6 months before testing and have a history of clinically normal menstrual cycles. All participants gave their informed consent in accordance with the Helsinki Declaration and all experimental procedures were approved by the institutional review board of the University of Texas at Austin.

Determination of menstrual cycle phase. Menstrual cycle phase was determined by the basal body temperature mapping technique (9). The cycle was mapped for one cycle before testing, continually monitored during the testing period and then verified upon completion of the study. Participants were instructed to take their temperature with an oral thermometer (BD Basal, Franklin Lakes, NJ) every morning before arising and consuming any beverage. The ovulatory phase was defined as the first day with a sustained temperature rise after basal body temperature nadir (9) (Fig. 1). If an initial temperature map was not clearly defined, the participant performed a second cycle map before admission to the testing portion of the study. If the second map was not well defined, the participant did not enter data collection. The body temperature map was first assessed by a trained investigator (MST) and then confirmed by a senior investigator (ACH) with 25 yr of research experience and 17 peer-reviewed publications using the technique (A. C. Hackney, personal communication). Although the low precision of the basal body temperature technique in predicting exact ovulation day has been noted (11), it is effective in determining the length of the follicular and luteal phases as well as total cycle length and is used extensively in many clinical settings (1). An example of the basal body temperature map from one subject is shown in Figure 1.

Data during each phase were collected in a pseudocounterbalanced design with two women starting data collection in early follicular, two women in late luteal, and one woman in each phase of early late follicular, ovulation, and midluteal. The midluteal phase for one participant was removed from analysis because they exhibited a short luteal defect, and thus progesterone levels were inappropriately low for that phase (8); however, the data from that subject’s other trials were appropriate because the late luteal trial was collected in the preceding cycle. One participant was found to be anovulatory in their last trial of data collection (ovulatory phase); therefore, that participant only had four trials collected because the data collection started at the midluteal phase. Anovulatory status was defined as a lack of biphasic response in basal body temperature and abnormal cycle length.

Experimental protocol. All data collection was performed in the climate-controlled Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were instructed to not perform strenuous physical activity or ingest food containing large amounts of phytoestrogens 48 h before testing. In addition, the participants were instructed to avoid alcohol and caffeine for 8 h before the visit and any food or beverage, except water, 8 h before their study visit.

Participants were seated in an adjustable chair with the dominant hip and knee fixed at 90°. Leg dominance was determined by asking the participant which leg they would typically kick a soccer ball (left, 1; right, 6). The waist and

FIGURE 1—Example of a basal body temperature map (●). Phases are indicated. EF, early follicular; LF, late follicular; OV, ovulatory; ML, midluteal; LL, late luteal.
dominant thigh were immobilized with pads and straps. Next, the individual performed a light warm-up consisting of 12 dynamic submaximal knee extensions without resistance. The dominant ankle was then strapped to a padded restraint attached to the strain gauge (Entran Sensors & Electronics, Fairfield, NJ). The participant was instructed to perform three isometric maximal voluntary contractions (MVC) of the knee extensor muscles for 3 s. All MVCs were separated by at least 60 s of rest. The average of the three MVCs for that trial was used for calculations in the ramp protocol.

After completion of the MVCs, one bipolar intramuscular insulated stainless steel fine-wire electrode (0.002 mm diameter recording area; California Fine Wire Company, Grover Beach, CA) was inserted in the VMO, and a second was inserted into the VM. Electrode placement was performed with a 25-gauge needle, 16 mm in length. The tip of the electrode was placed approximately 5–10 mm below the skin. For the purposes of electrode placement, VMO electrode was inserted immediately medial to the patella, and the VM was defined as the area 7 cm superior to the VMO insertion point. The signals from the fine-wire electrodes were preamplified and band-pass filtered at 8 Hz–3.12 kHz with a gain of 330 (B&L Engineering, Tustin, CA). An adhesive pregelled Ag/AgCl surface electrode of 5 mm diameter was placed over the ipsilateral patella and used as a ground. The participant then practiced performing a stable ramp contraction up to 30% of MVC. The participant was situated with a computer screen facing them with only their target force and force generation provided as visual feedback. They were instructed to trace a line on the screen with a rate of rise of 7.5% up to 30% MVC per second and then hold the 30% MVC force for 3 s. The 7.5% rate of rise and ramp contraction to 30% was chosen based on a pilot study that demonstrated these to have clearly defined MU, low levels of discharge variability, and limited MU superimposition. The participant practiced the ramp task three to six times each study visit before the trial used for data collection to ensure smooth force generation. The data collection trial was separated from the practice ramp contractions by a minimum of 60 s. Data for electromyography (EMG) and force were A/D converted (Micro 1401; Cambridge Electronic Design, Cambridge, England) and collected through Spike2 (version 5.21; Cambridge Electronic Design). Force and intramuscular EMG were sampled at 1 and 30 kHz, respectively.

**Motor unit data reduction.** EMG data were analyzed offline in Matlab (version 2010b; Mathworks, Natick, MA) and Spike2. The data were 100-Hz high-pass filtered using a fourth-order recursive Butterworth filter. MU were visually assessed and identified based upon shape, amplitude and discharge timing. MU recruitment was defined by four consecutive discharges at regular intervals (34). No MU double discharges were detected. The relative force at which that first discharge was recorded was the recruitment threshold. The initial firing rate was the average of the first three interspike intervals converted into hertz.

**Statistical analysis.** All statistical analysis was performed using SAS (version 9.2) with α set at 0.05. When necessary, adjustments for multiplicity were performed using the Bonferroni correction technique. All MU data were considered cross sectional because different MU were presumably sampled on each testing date. MU recruitment threshold and MU initial firing rate were both assessed using a two-step process. The first step pooled all female MU across the menstrual cycle phases for comparison against the male subjects. The pooled data were used to determine whether there was a difference in recruitment threshold and initial firing rate of the VM and VMO muscles between men and eumenorrheic women. The second step eliminated the male data and analyzed only the recruitment threshold and initial firing rate of the VM and VMO across the menstrual cycle.

Because recruitment threshold is a nonnormal bounded measure (0–30) and exhibits a right-skewed distribution, a Mann–Whitney U test was used to test the distribution differences between the VM and the VMO for men and women and at each phase of the menstrual cycle.

The initial MU firing rate during the ramp contraction for the VM and VMO was assessed using 2 × 2 random-effects ANCOVA to determine an effect of sex and a second 2 × 2 random-effects ANCOVA to determine an effect of menstrual phase. The force at which the MU was recruited was used as a covariate because previous literature suggests a correlation between the force output at the time of MU recruitment and the initial firing rate (20). This random-effects approach controls for possible intracorrelation within each subject and is a more conservative approach than using a standard ANCOVA or ANOVA. After determination of omnibus significance, an interaction analysis was performed to assess the differences in VM and VMO initial firing rate at every level of sex or menstrual phase.

**RESULTS**

**Motor unit recordings.** A total of 510 MU were recorded. The mean coefficient of variation for MU recordings was acceptable for initial firing rates (13.6% ± 8.8%). Of the 510 MU, 130 (25.5 %) were collected from male participants. For the MU recorded from women across the menstrual cycle, the breakdown was as follows: 76 (early follicular), 96 (late follicular), 67 (ovulation), 64 (midluteal), and 77 (late luteal).

**Motor unit recruitment threshold.** There was no difference in the recruitment threshold distribution of the VM and VMO for either men (P = 0.411) or women (P = 0.338) (Fig. 2). There were also no differences in the recruitment threshold distributions observed between the VM and the VMO at any of the menstrual phases (Table 1).

**Motor unit firing rate at recruitment.** The two-way ANCOVA for sex and muscle group determined a main effect for VM/VMO muscle group (P = 0.002) but not sex (P = 0.834) for initial MU firing rate. The subject-level random-effects approach was validated with a small but
significant random coefficient (0.369, $P = 0.012$). A post hoc interaction analysis determined that this difference in VM and VMO firing rate was predominately driven by the female MU ($P < 0.001$) (Fig. 3).

The two-way ANCOVA for menstrual phase and muscle group determined a main effect for VM/VMO muscle group ($P < 0.001$) and menstrual phase ($P = 0.003$). The subject-level random-effects approach was validated with a small but significant random coefficient (0.579, $P = 0.046$). The post hoc interaction analysis determined that the difference between VM and VMO firing rate was significantly different in the ovulation ($P = 0.009$) and midluteal menstrual phases ($P = 0.009$) (Fig. 4).

**DISCUSSION**

This study demonstrates that there are differences in the rate-coding strategy of MU recruitment between the VM and the VMO. The higher initial MU firing rate in the VM versus the VMO was statistically significant in women, but not in men. This may be due to a smaller relative sample size in men, although greater differences in VM-to-VMO firing rates were observed in the women compared with the men (0.66 vs 0.47 Hz). To our knowledge, this is the first study to show that the VM and the VMO are differentially activated during simple knee extension and are thus muscles that receive different neurological drive. Furthermore, the hormones that oscillate across the menstrual cycle seem to promote the differential activation of the two muscles. The menstrual cycle timing of the VM/VMO differentiation, in the ovulatory and midluteal phases, suggests that progesterone is the strongest mediator of this differentiation with a possible secondary action of estradiol.

The findings of this study in combination with the different neurological innervation (31) and difference in pennation angles (13) support a functional difference between the VM and the VMO despite a lack of a distinct fibrofascial division between the muscles (22). Specifically, the present study answers a recent call from the anatomical community for electromyography studies to determine whether *in vivo* functional differences exist between the VM and the VMO (29). Our study also supports sports medicine and orthopedic textbooks that differentiate the muscles into different functional units and leaves open the possibility that the two muscles may have different biomechanical purposes (18,24).

The approximately 1-Hz difference in MU firing rates observed in the ovulatory and luteal phases may seem small when viewed in isolation, but this change can substantially affect muscular force generation. In the soleus, a 1-Hz decrease in MU discharge at recruitment can cause a 10% decrease in muscular force generation (36). The finding that the VM and VMO are differentially activated in women but not men may explain why women outperform men in sports that require knee extension, such as tennis.

**TABLE 1.** The median recruitment thresholds (% MVC) for MU in the VM and VMO during the five phases of the menstrual cycle.

<table>
<thead>
<tr>
<th>Phase</th>
<th>VM (% MVC)</th>
<th>VMO (% MVC)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early follicular</td>
<td>8.7</td>
<td>8.7</td>
<td>0.72</td>
</tr>
<tr>
<td>Late follicular</td>
<td>10.9</td>
<td>8.6</td>
<td>0.38</td>
</tr>
<tr>
<td>Ovulatory phase</td>
<td>12.4</td>
<td>8.5</td>
<td>0.26</td>
</tr>
<tr>
<td>Midluteal phase</td>
<td>10.7</td>
<td>10.7</td>
<td>0.84</td>
</tr>
<tr>
<td>Late luteal phase</td>
<td>10.4</td>
<td>7.1</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**FIGURE 2**—The recruitment threshold histogram distributions for the VM and VMO for both sexes. No differences were observed between the muscle groups.

**FIGURE 3**—Motor unit firing rates at recruitment assessed by sex, after controlling for recruitment threshold force. The VM firing rate is significantly higher than VMO for female subjects ($*P < 0.05$).
In addition to decreasing GABA release, estradiol acts to result in decreased GABA transmission (25) and enhanced sites on \( F \) level affect the central nervous system because steroids lipid solubility (30). Estradiol binds to estrogen receptor easily traverse the blood–brain barrier due to their high nature, progesterone metabolites can directly activate GABAA pyramidal cells (35). Although estradiol is excitatory in in force generation (21). The changes in MU discharge in the current study are greater than the decrease in MU firing observed after 6–8 wk of immobilization (10). The approximately 1-Hz change is also commensurate with the change in MU discharge from the VMO and the vastus lateralis when knee pain is induced (33). Therefore, we can surmise that our data indicate a possible change in the relative force output of these muscles as a result alterations in MU discharge across the menstrual cycle.

Oscillations of estradiol and progesterone at the plasma level affect the central nervous system because steroids easily traverse the blood–brain barrier due to their high lipid solubility (30). Estradiol binds to estrogen receptor \( \alpha \) sites on \( \gamma \)-aminobutyric acid (GABA) releasing neurons resulting in decreased GABA transmission (25) and enhanced cerebellar neuron discharge during rodent locomotion (28). In addition to decreasing GABA release, estradiol acts to sensitize N-methyl D-aspartate receptors to glutamate in pyramidal cells (35). Although estradiol is excitatory in nature, progesterone metabolites can directly activate GABA\( _{\alpha} \) receptors (4,27). However, progesterone has also been shown to decrease cerebellum Purkinje cell discharge rates in rats during locomotion (28). Because Purkinje cells inhibit the cerebellar nuclei, progesterone could also have a net excitatory effect on motor output. The first-order metabolite of progesterone, pregnenolone, enhances motor activity in various maze and open field–type challenges compared with control mice (14). These hormones could also affect the basal ganglia–ventral anterior/ventrolateral thalamic pathway, which is known to initiate and modulate voluntarily generated and visually guided movements (17), similar to the visually guided ramp contraction performed in the present study. This pathway is a hybrid neurotransmitter pathway (23), affected by changes in glutaminergic and GABAergic activity, both of which are modified by progesterone and estradiol (4,25,27,35).

The current study is the first to demonstrate that voluntary MU activation in any muscle can be modulated by the menstrual cycle. Transcranial magnetic stimulation can stimulate the orderly recruitment of MU via the corticospinal tract (2), but it does not account for changes in subcortical or cerebellar brain areas. Transcranial magnetic stimulation studies have shown an initial depression in corticospinal excitability in the early follicular phase (26). This is also the time when MU firing rates were observed to be the lowest in the present study. There is also lower corticospinal tract excitability at the midluteal phase (26). We did not find this to be the case for the VM firing rates. It is apparent that menstrual cycle sex hormones profoundly affect excitability of the central nervous system. Using H-reflexes as a metric for excitability at the spinal level, Hoffman et al. (15) demonstrated that there was no change across the menstrual cycle. This indicates that changes in central nervous system excitability likely occur at the cortical and/or subcortical levels. These changes may manifest themselves to a different degree in the VM and VMO muscles that extend the knee and stabilize the patella.

It should be noted that muscle force is produced by both the MU firing rate and the total number of MU recruited. We did not measure the total number of MU recruited in each muscle; thus, we are unable to estimate the relative forces produced by these muscles. If the primary function of the VMO is to stabilize the patella and the primary function of the VM is to assist with leg extension, presumably more force would be required by the VM than the VMO. It is possible that the overall firing rates were higher in the VM because higher forces were required to produce leg extension than patellar stabilization. The differences in firing rate between the two muscles could also be indicative of differences in fiber type. The VM tends to have higher proportions of Type I and Type IIa muscle fibers compared with the VMO, although there is substantial intersubject variability (32) and no differences in MU recruitment thresholds were observed in the present study. Nevertheless, if the VMO is composed of a greater proportion of the larger Type II muscle fibers, lower firing rates would be required to reach twitch fusion. It is also possible that there are differences in MU rate-coding and recruitment strategies between the two muscles.

We did not find statistically significant differences in MU firing rates between the sexes. Although we are not aware of research demonstrating significant differences in VM/VMO muscle fiber types between the sexes, previous studies in other muscles have indicated that women may have a lower proportion of Type II fibers compared with men (19). Previous
work in the animal model has indicated that MU recorded in men fire more rapidly than those in women (12), a finding our study fails to confirm. Miller et al. (19) also found no difference between the sexes when examining total number of MU, MU size, or MU activity in the VM. Some differentiation between the sexes has been noted in the architecture of the soleus muscle (6), and although we are unable to discount this possibility within the VM and the VMO of our sample population, there is no reason to expect that any change in muscle architecture occurred across the menstrual cycle.

Future studies are needed to determine whether the differences in VM/VMO activation across the menstrual cycle could affect patellofemoral pain.

Moreover, the effects of hormonal contraception on muscle activation and recruitment patterns are unknown and in need of future research. Hormonal contraception decreases circulating estradiol and progesterone (7); however, it is unknown if the synthetic sex hormone analogs have physiological actions on the neurotransmitter system similar to their endogenous counterparts. Furthermore, the effects of amenorrhea, oligomenorrhea, pregnancy, and menopause on MU activation and recruitment patterns are also potential areas of future research.

In summary, our study shows that MU activation differs between the VM and the VMO and across the menstrual cycle. The difference in activation is highest during the ovulatory and midluteal phases, and the general activation of both the VM and the VMO are increased in the late luteal phase of the menstrual cycle.

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The authors have no conflicts of interest to declare.

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REFERENCES


