Action potential amplitude as a noninvasive indicator of motor unit-specific hypertrophy

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Pope ZK, Hester GM, Benik FM, DeFreitas JM. Action potential amplitude as a noninvasive indicator of motor unit-specific hypertrophy. J Neurophysiol 115: 2608–2614, 2016. First published March 2, 2016; doi:10.1152/jn.00039.2016.—Skeletal muscle fibers hypertrophy in response to strength training, with type II fibers generally demonstrating the greatest plasticity in regards to cross-sectional area (CSA). However, assessing fiber type-specific CSA in humans requires invasive muscle biopsies. With advancements in the decomposition of surface electromyographic (sEMG) signals recorded using multichannel electrode arrays, the firing properties of individual motor units (MUs) can now be detected noninvasively. Since action potential amplitude (AP\textsubscript{SIZE}) has a documented relationship with muscle fiber size, as well as with its parent MU’s recruitment threshold (RT) force, our purpose was to examine if MU AP\textsubscript{SIZE} as a function of its RT (i.e., the size principle), could potentially be used as a longitudinal indicator of MU-specific hypertrophy. By decomposing the sEMG signals from the vastus lateralis muscle of 10 subjects during maximal voluntary knee extensions, we noninvasively assessed the relationship between MU AP\textsubscript{SIZE} and RT before and immediately after an 8-wk strength training intervention. In addition to significant increases in muscle size and strength (P < 0.02), our data show that training elicited an increase in MU AP\textsubscript{SIZE} of high-threshold MUs. Additionally, a large portion of the variance (83.6%) in the change in each individual’s relationship between MU AP\textsubscript{SIZE} and RT was explained by training-induced changes in whole muscle CSA (obtained via ultrasonography). Our findings suggest that the noninvasive, electrophysiological assessment of longitudinal changes to MU AP\textsubscript{SIZE} appears to reflect hypertrophy specific to MUs across the RT continuum.

Skeletal muscle fibers demonstrate a marked plasticity in response to external stimuli. For instance, evidence from cross-sectional (Schantz et al. 1981) and longitudinal (Hather et al. 1996) investigations suggests that chronic resistance exercise (i.e., strength training) elicits increases in the cross-sectional area (CSA) of muscle fibers. This presumably contributes to the increase in whole muscle CSA (DeFreitas et al. 2011), although training-induced increases in CSA are variable between individuals (Hubal et al. 2005).

To better understand the adaptations to strength training, various approaches involving invasive and painful biopsies have been used to separate muscle fibers into “types” (Scott et al. 2001). However, the tendency of muscle fibers is to display more often a continuum of responses, rather than distinct groupings (Enoka and Duchateau 2015). Although the prevailing finding suggests that type II muscle fibers display a greater capacity for growth than their type I counterparts (Aagaard et al. 2001; Hather et al. 1991; Houston et al. 1983; McCall et al. 1996; Thorstensson et al. 1976), this response does not appear to be uniform within, or across, muscle fiber types (McCall et al. 1996).

In humans, force production is believed to be accomplished through the orderly activation of progressively larger motor units (MUs) in response to a net increase in excitatory synaptic drive to the MU pool (De Luca and Erim 1994). Activated at greater recruitment threshold force (RT) levels, these larger (high-threshold) MUs are also characterized as having greater action potential amplitude (AP\textsubscript{SIZE}), twitch tension, and faster contraction times and are highly fatigable compared with the smaller (low-threshold) MUs (Milner-Brown et al. 1973; Stephens and Usherwood 1977; Tanji and Kato 1973). Although it has not been demonstrated directly in humans, the apparent alignment of functional characteristics regarding voluntary MU recruitment order and muscle fiber type are generally considered as evidence suggesting that low-threshold MUs are associated with type I muscle fibers, while high-threshold MUs are associated with type II muscle fibers.

With the recent technological advancements in the automated algorithms used for MU decomposition, surface electromyographic (sEMG) signals recorded using multichannel electrode arrays can now be used to discriminate MU action potential waveforms and their firing instances from a large population of individual MUs (De Luca et al. 2006; Nawab et al. 2010). Across large force ranges, numerous MU characteristics can now be simultaneously examined. Recently, Hu et al. (2013b) showed that sEMG can be used to demonstrate a strong relationship between a MU’s AP\textsubscript{SIZE} and its RT. In other words, sEMG decomposition can be used to examine the size principle (Henneman et al. 1965), thereby indicating the relative size of MUs across the entire force spectrum. Considering this, in conjunction with the documented relationship between AP\textsubscript{SIZE} and muscle fiber circumference (i.e., larger fibers have larger amplitude action potentials in vitro) (Hakansson 1956), could MU AP\textsubscript{SIZE} be used longitudinally as a noninvasive indicator of hypertrophy specific to MUs of different RT populations?

In attempt to answer this, our investigation provided 8 wk of strength training intended to induce muscle hypertrophy and increase strength. Both before and following the training intervention, we assessed MU AP\textsubscript{SIZE} as a function of RT (MU AP\textsubscript{SIZE} vs. RT) on the individual and group levels. The purpose of this study was twofold: 1) to examine if this noninvasive measure is longitudinally responsive to training, and 2) to evaluate if each individual’s change in MU AP\textsubscript{SIZE} was due to a respective change in whole muscle CSA. Evidence suggests
that the plasticity of type II muscle fibers is greater than type I (Häkkinen et al. 1981a), which may explain why short-term (8–10 wk) strength training research studies observed hypertrophy of only type II muscle fibers (Houston et al. 1983; Thorstensson et al. 1976). Presuming that type II muscle fibers are likely constituents of high-threshold MUs, we hypothesized that the slope of the MU AP$_{\text{SIZE}}$ vs. RT relationship would become steeper due to a increase in the AP$_{\text{SIZE}}$ of the high-threshold MUs (compared to low-threshold MUs). Additionally, we hypothesized that the extent of change to an individual’s MU AP$_{\text{SIZE}}$ vs. RT relationship would be dependent on the extent of their training-induced muscle hypertrophy.

**METHODS**

*Study design and experimental procedures.* Twenty young, recreationally-trained males (mean ± SD: age = 22.2 ± 2.6 yr; body mass = 85.3 ± 14.8 kg; height = 1.8 ± 0.1 m; and resistance training experience ≥ 6 mo) performed 8 wk of high-intensity strength training (3 training sessions/wk). The training sessions consisted of 3 sets of 10 repetitions for 7 dynamic, resistance exercises (3 of which targeted the knee extensors) with a load roughly corresponding to each participant’s 10 repetition maximum. Ultrasound images (US) and MU recordings were obtained from the vastus lateralis (VL) muscle of the dominant leg before (PRE) and after (POST) the training intervention. All participants were informed of the experimental procedures, risks, and their ability to withdraw from the study without penalty before signing consent. This investigation was conducted in accordance with the Declaration of Helsinki and was approved by the Oklahoma State University Institutional Review Board.

*MU recordings and processing.* Following a brief warm up, three separate isometric maximal voluntary contractions (MVC) were performed using a force transducer attached to a cuff around the ankle (Model SSM-AJ-500; Interface, Scottsdale, AZ) with the participants seated upright and restrained (the hip and knee angles were ~90°, respectively). Following the three MVC trials, each participant performed a MVC ramp contraction (MVC$_{\text{Ramp}}$) using a trapezoidal-shaped force trajectory. Illustrated in Fig. 1A, the trajectory increased linearly at a rate of 10% of MVC/s, was held at the maximal sustainable force level for 5 s, and followed by a linear return to zero (−20% MVC/s). Each participant was familiarized and practiced several submaximal versions of the trapezoidal-shaped ramp contractions during the warm-up using real-time visual feedback for accurate force trajectory replication. Sufficient rest was allotted between all trials to avoid fatigue. sEMG was recorded during the MVC$_{\text{Ramp}}$ contractions using a five-pin surface electrode array and a 16-channel Bagnoli acquisition system (Delsys, Boston, MA). Following thorough, recommended skin preparation procedures, the specialized sensor was securely fastened over the VL muscle with hypoallergenic tape at approximately two-thirds the distance between the center of the muscle belly toward the distal tendon (Zaheer et al. 2012) and a reference electrode (Dermatrode; American Imex, Irvine, CA) was placed on the spinous process of the C7 vertebrae. The four channels of raw sEMG from the 5-pin sensor were decomposed into their constituent MU action potential trains (see Fig. 1B) using the Precision Decomposition III algorithm described by De Luca et al. (2006) and improved upon by Nawab et al. (2010). This algorithm has been shown to reliably discriminate the discharge characteristics of a large number of individual MUs up to maximal force levels (Nawab et al. 2010). The accuracy and validity of the decomposition process, as described by De Luca and Contessa (2012), have recently been independently validated using both simulated and naturally occurring signals (Hu et al. 2013a,b, 2014). Furthermore, the decomposition output provides four unique action potential template waveforms (1 from each sEMG channel) for each individual MU (see Fig. 1C, for example). The shape and size of the action potential waveforms have been shown to compare favorably with those derived using spike-triggered averaging (Hu et al. 2013b). The decomposition algorithm involves separate complex stages to identify, match, and update each MU’s action potential waveform template. Figure 1C shows the resulting four MU action potentials templates from a single MU. These four waveforms represent a weighted average action potential shape and amplitude throughout the contraction from each of the four different sEMG channels. From each these four waveforms, the peak-to-peak amplitude (mV) was determined before averaging the four peak-to-peak amplitude values to calculate a single AP$_{\text{SIZE}}$ representing that individual MU.

*MU exclusion criteria and analysis.* All MUs that did not demonstrate at least 90.0% accuracy assessed using the Decompose-Synthesize-Decompose-Compare test (De Luca and Contessa 2012) were eliminated from all subsequent analyses. For the remaining MUs, custom-written software (using LabVIEW 2012; National Instru-
The effect of our strength training intervention on the MU APSIZE vs. RT, defined as the relative force level (%MVC) at the instance of the MU’s first discharge; and 2) MU APSIZE, calculated by taking the average of the peak-to-peak amplitudes (mV) from each of the four action potential waveforms. Considering the regression analyses required to establish a sufficient relationship (see Statistical analysis) for MU APSIZE vs. RT, a RT range ≥25% of MVC was required (for both PRE and POST) for each participant to be included in any analysis (i.e., the MUs detected could not be clustered together across a small RT range). In such a case, the regression equation could be strongly affected by minimal changes to the limited range of data.

Ultrasonography. Transverse US images were obtained of each participant’s VL muscle using procedures similar to those described previously (Ahtiainen et al. 2010). As demonstrated in Fig. 2A, real-time panoramic cross-sectional images were generated using B-mode US imaging with LogicView software (GE Logiq S8) and a linear-array probe (Model ML-615; 12 MHz). Equipment settings were optimized for image quality by a trained sonographer and held constant across visits. For each laboratory visit, whole muscle CSA (cm²) and subcutaneous fat thickness were measured for each of the US images using Adobe Photoshop (Version 12.1 × 32; Adobe Systems, San Jose, CA). CSA was measured by carefully selecting as much of the muscle as possible while avoiding the outer fascia. Subcutaneous fat thickness was measured as the linear distance (mm) between the VL’s outer fascia and the dermis where the sEMG sensor array was to be attached to confirm that changes in MU APSIZE were not attributable to altered subcutaneous fat thickness. From the multiple US images obtained during each individual visit, the closest two were averaged to obtain a single CSA and subcutaneous fat thickness for PRE and POST, respectively.

Statistical analysis. All statistical analyses were administered using SPSS software (version 21, Chicago, IL). Paired-samples t-tests were conducted to compare the group means between PRE to POST using an α-level of P < 0.05 for statistical significance. Regression analyses were performed separately for PRE and POST MU data to determine the effect of our strength training intervention on the MU APSIZE vs. RT relationship. Recruitment threshold bin widths of 10% (e.g., 0–10, 10–20%, etc.) were utilized to condense the data. The average for each bin was used in the regression analyses. The group’s PRE and POST data were assessed using polynomial regression analysis to determine the best fit model for the relationship (i.e., linear vs. quadratic vs. cubic). The statistical significance for the increment in the proportion of the variance that would be accounted for by a higher degree polynomial (i.e., F-test for R²-change) was determined using the F-test described by Pedhazur (1997a).

To test the hypothesis that high-threshold MUs would predominantly be affected by strength training, linear regression was applied separately to the low- and high-threshold MUs for a comparison between PRE and POST. MUs activated at <30% of MVC were classified as low threshold, while ≥30% of MVC was considered high threshold in the VL muscle as described previously (DeFreitas et al. 2014). Two tests for differences in linear slope coefficients (as described by Pedhazur 1997b) for the MU APSIZE vs. RT relationships were performed to separately compare the PRE and POST slopes of the low- and high-threshold MUs.

Linear regression was also used for each individual’s PRE and POST training MU data to examine the change in slope coefficient for their MU APSIZE vs. RT relationship (AP-RTSLOPE; expressed as mV/%MVC). Lastly, each individual’s change (PRE vs. POST) in AP-RTSLOPE and change in VL CSA were used in a linear regression model to determine what percentage of the variance in AP-RTSLOPE could be accounted for by the variance in whole muscle CSA, as assessed by the coefficient of determination (R²).

RESULTS

MU exclusion analysis. Of the original 20 participants, 10 did not meet the established MU criteria (i.e., a RT range < 25.0% of MVC for either PRE or POST) and were eliminated from all further analyses. From the remaining 10 participants, 632 MUs were initially detected (PRE = 299/POST = 327). Of these MUs, 81 were discarded due to poor decomposition accuracy (i.e., < 90.0%). The final number of MUs used for statistical analyses (PRE = 270 / POST = 281), as well as their distribution across RTs, can be seen in Fig. 3B. The MU numbers used and discarded (in parentheses) for each individual are shown in Table 1.

Ultrasonography. Following our strength training intervention, our results revealed no significant change (P = 0.12) in subcutaneous fat thickness (PRE = 77.8 ± 18.5 mm; POST = 79.3 ± 19.7 mm). However, a significant (P < 0.01) increase of 13.7% was observed for VL CSA. The PRE and POST group means are illustrated in Fig. 2B. Each individual’s PRE and POST CSA values are shown in Table 1.

MU APSIZE vs. recruitment threshold. Following training, knee extension MVC force significantly increased (P < 0.02) by 9.6%. The PRE and POST group means are illustrated in Fig. 2C. The RT range and APSIZE range of the MUs analyzed for each participant (PRE and POST) can be seen in Table 1. The pooled PRE and POST group MU APSIZE vs. RT data were assessed separately using polynomial regression analysis to determine the best fit model for the relationship (Pedhazur 1997a). Figure 3A illustrates that the PRE data were best fit with a linear function (y = 0.0025x + 0.0692; r = 0.882; P < 0.01). However, the POST data were best fit with a quadratic function (y = 0.0001x² −0.0023x + 0.1048; r = 0.985; P < 0.01). A qualitative examination of Fig. 3A seemingly indicates that the APSIZE of MUs with higher RTs experienced the...
DISCUSSION

The purpose of this study was twofold: 1) to examine if a noninvasive measure of MU AP_SIZE is longitudinally responsive to 8 wk of strength training, and 2) to evaluate if each individual’s change in MU AP_SIZE was due to a respective change in whole muscle CSA (obtained via ultrasonography). Despite some anticipated variability between participants (Hu-bal et al. 2005), there was a significant increase in knee extension MVC force and VL CSA with training. The change we observed in the MU AP_SIZE vs. RT relationship indicates an increase in the MU AP_SIZE of the higher threshold MUs following the training intervention. Furthermore, the change in this relationship was highly correlated with changes occurring in whole muscle CSA. Following a discussion of some potential physiological mechanisms, we will summarize some unique aspects of our approach, potential applications, as well as the current limitations that require methodological refinement before any widespread use.

During our investigation, MUs were recruited in accord with Henneman’s “size principle” (i.e., increasing AP_SIZE with increasing excitation/force suggests that the smaller MUs were recruited before the larger ones) (Henneman and Olson 1965; Hu et al. 2013b; Olson et al. 1968; Tanji and Kato 1973) throughout the entire force spectrum. As previously demonstrated, this orderly recruitment was evident before and was not affected by training (Van Cutsem et al. 1998). The change we observed in the MU AP_SIZE vs. RT relationship indicates that training elicited an increase in MU AP_SIZE of predominantly the high-threshold MUs. Speculatively, this observation offers support to the presumed relationship between high-threshold MUs and type II muscle fibers in human muscles with mixed fiber composition. It is important to note, however, that fiber type distribution is variable between individuals (Staron et al. 2000) and the direct relationship with MU distribution across RTs requires further investigation. Häkkinen et al. (1998b) reported that the percentage values for type I muscle fibers were inversely correlated ($r = -0.56$) with training-induced whole muscle hypertrophy. Perhaps the ratio of muscle fiber types may have provided some explanation for the susceptibility for whole muscle hypertrophy reported in the current and previous research investigations (Hubal et al. 2005).

Our regression analysis revealed a strong correlation ($R^2 = 0.836$) between each individuals’ change in the slope coefficient of the MU AP_SIZE vs. RT relationship (AP-RT_SLOPE) and change in muscle CSA (see Fig. 3D). Previous investigations have used muscle biopsies with traditional fiber typing techniques to investigate how the extent of change in fiber CSA contributes to change in muscle CSA, but these results have been incongruent. For example, McCall et al. (1996) found...
little correlation when comparing change in muscle CSA: vs. mean fiber CSA ($R^2 = 0.037$), vs. type I area ($R^2 = 0.039$), or vs. type II area ($R^2 = 0.125$). Yet, Aagaard et al. (2001) found a considerably different relationship between the training-induced increase in muscle CSA vs. mean fiber CSA ($R^2 = 0.336$). Conversely, Narici et al. (1996) found no change in mean fiber CSA, despite a muscle CSA increase of 19%. The substantial improvement in the variance explained by our approach may be the added benefit of expressing RT as a continuous variable across the continuum from 0 to 100% of MVC.

Aside from any presumed relationship regarding muscle fiber type, the adaptation we observed in the high-threshold MU types may plausibly be improved by including an additional contraction force (MVC). MU action potential amplitude (MU APSIZE), range (expressed in mV), slope coefficient (AP-RT SLOPE) from the MU APSIZE vs. recruitment threshold force (RT) linear regression (expressed as mV/percent MVC), regression $r$ value, MVC (expressed as N), and cross-sectional area (CSA) of the vastus lateralis (expressed as cm$^2$) of each participant from before (PRE) and following (POST) 8 wk of strength training. The group means, as well as the mean change from PRE to POST, are shown in the bottom row.

### Table 1. Individual responses and grouped means for each variable from before and following 8 wk of strength training

<table>
<thead>
<tr>
<th>Subject</th>
<th>MUs</th>
<th>RT Range</th>
<th>MU APSIZE Range</th>
<th>AP-RT SLOPE</th>
<th>$r$</th>
<th>MVC</th>
<th>CSA</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>28 (2)</td>
<td>11.4–83.8</td>
<td>0.059–0.228</td>
<td>0.0018</td>
<td>0.965</td>
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<td>2</td>
<td>28 (0)</td>
<td>13.0–59.9</td>
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<td>0.0067</td>
<td>0.973</td>
<td>553.8</td>
<td>25.39</td>
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<tr>
<td>3</td>
<td>40 (2)</td>
<td>10.2–46.6</td>
<td>0.087–0.320</td>
<td>0.0077</td>
<td>0.991</td>
<td>827.2</td>
<td>37.97</td>
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<tr>
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<td>38 (0)</td>
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<td>598.7</td>
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</tr>
<tr>
<td>5</td>
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<td>24.7–58.2</td>
<td>0.086–0.252</td>
<td>0.0030</td>
<td>0.949</td>
<td>578.9</td>
<td>30.83</td>
</tr>
<tr>
<td>6</td>
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<td>16.7–42.1</td>
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<td>0.0040</td>
<td>0.923</td>
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<td>19 (3)</td>
<td>3.2–47.9</td>
<td>0.086–0.252</td>
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<td>0.949</td>
<td>876.1</td>
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<td>23.3–70.7</td>
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<td>855.7</td>
<td>36.05</td>
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<td>0.064–0.224</td>
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<td>796.4</td>
<td>21.96</td>
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<td>796.4</td>
<td>21.96</td>
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<td>Mean</td>
<td>27 (3.1)</td>
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<td>0.087–0.316</td>
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<tr>
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<td>970.0</td>
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<td>0.986</td>
<td>798.6</td>
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Mean change from PRE to POST

-0.0013

+0.0004

3.41

70.6
tion(s) (i.e., pooling MUs from multiple contractions for each participant). However, this would first require a critical evaluation. Additionally, longitudinal investigations should use intermediate time points and control groups for validation. Furthermore, while the individuals that showed the largest increases in CSA also showed the largest increases in AP-RT 
SLOPE, the individuals that showed small increases in CSA did not necessarily exhibit small increases in AP-RT 
SLOPE (see Fig. 3D). This suggests our current approach may not be sensitive enough for detecting very small hypertrophic changes. More research is required for a more comprehensive understanding of the utility of this measure as a tool (e.g., reliability, sensitivity to change, additional potential applications, and threats to its validity).

Conclusions. Our findings suggest that the noninvasive, electrophysiologically assessed size of the size principle (i.e., MU AP 
SIZE as a function of RT) is longitudinally sensitive to change and may be a useful indicator of MU-specific hypertrophy. Using this approach, we demonstrate that 8 wk of strength training elicited an increase in the MU AP 
SIZE of high-threshold MUs. There was also a strong positive relationship ($R^2 = 0.836$) between the subject’s change in the slope of MU AP 
SIZE vs. RT relationship and their change in whole muscle CSA. This new assessment also explains a substantially larger percentage of the variance (83.6%) in whole muscle hypertrophy compared with the invasive biopsy studies that examined fiber size by type (only 3.7–33.6% of the variance explained, respectively). An important advantage to our approach is that RT is a continuous variable that can fall anywhere on a continuum from 0 to 100% of MVC.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Z.K.P. and J.M.D. conception and design of research; Z.K.P., G.M.H., and F.M.B. performed experiments; Z.K.P., G.M.H., and F.M.B. analyzed data; Z.K.P. and J.M.D. interpreted results of experiments; Z.K.P. prepared figures; Z.K.P. drafted manuscript; Z.K.P., G.M.H., F.M.B., and J.M.D. approved final version of manuscript; J.M.D. edited and revised manuscript.

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