Locomotor muscle fatigue is not critically regulated after prior upper body exercise

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Johnson MA, Sharpe GR, Williams NC, Hannah R. Locomotor muscle fatigue is not critically regulated after prior upper body exercise. J Appl Physiol 119: 840–850, 2015. First published August 13, 2015; doi:10.1152/japplphysiol.00072.2015.—This study examined the effects of prior upper body exercise on subsequent high-intensity cycling exercise tolerance and associated changes in neuromuscular function and perceptual responses. Eight men performed three fixed work-rate (85% peak power) cycling tests: 1) to the limit of tolerance (CYC); 2) to the limit of tolerance after prior high-intensity arm-cranking exercise (ARM-CYC); and 3) without prior exercise and for an equal duration as ARM-CYC (ISOTIME). Peripherally fatigue was assessed via changes in potentiated quadriceps twitch force during supramaximal electrical femoral nerve stimulation. Voluntary activation was assessed using twitch interpolation during maximal voluntary contractions. Cycling time during ARM-CYC and ISOTIME (4.33 ± 1.10 min) was 38% shorter than during CYC (7.46 ± 2.79 min) (P < 0.001). Twitch force decreased more after CYC (−38 ± 13%) than ARM-CYC (−26 ± 10%) (P = 0.004) and ISOTIME (−24 ± 10%) (P = 0.003). Voluntary activation was 94 ± 5% at rest and decreased after CYC (89 ± 9%, P = 0.012) and ARM-CYC (91 ± 8%, P = 0.047). Rating of perceived exertion for limb discomfort increased more quickly during cycling in ARM-CYC [1.83 ± 0.46 arbitrary units (AU)/min] than CYC (1.10 ± 0.38 AU/min, P = 0.003) and ISOTIME (1.05 ± 0.43 AU/min, P = 0.002), and this was correlated with the reduced cycling time in ARM-CYC (r = −0.72, P = 0.045). In conclusion, cycling exercise tolerance after prior upper body exercise is potentially mediated by central fatigue and intolerable levels of sensory perception rather than a critical peripheral fatigue limit.

Exercise tolerance; peripheral fatigue; central fatigue; perceived exertion

A consistent reduction (~35%) in the potentiated quadriceps twitch force is observed after high-intensity cycling (4-6, 66, 73, 79). It is proposed that this reduction represents an “individual critical threshold” of peripheral locomotor muscle fatigue beyond which the degree of associated sensory perception would not be tolerable (3). The observation of similar intramuscular metabolic perturbation at the end of exhaustive exercise, irrespective of the work rate (20, 77), supports the notion that it is probably not peripheral fatigue per se that is monitored/regulated, but the associated fatigue-producing biochemical changes within the muscle (3). The critical limit of peripheral fatigue observed under “normal” conditions is also unchanged when exercise tolerance is reduced (i.e., the critical limit is reached more quickly) due to moderate hypoxia (119: 840–850, 2015. First published August 13, 2015; doi:10.1152/japplphysiol.00072.2015)

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Peripheral fatigue, prior upper body exercise might reduce lower body exercise tolerance by accelerating the attainment of an intolerable level of sensory perception that is mediated, in part, by the ensemble input of group III/IV muscle afferents. Specifically, since group III/IV muscle afferent input may remain elevated for up to 15 min after high-intensity upper body exercise (25, 45), the ensemble group III/IV muscle afferent input would be elevated during subsequent high-intensity lower body exercise. Subsequently, increases in ΔRPE/Δtime and/or Δdyspnea/Δtime may reduce exercise tolerance with less peripheral fatigue incurred. This notion is supported by the observation of increased RPE and reduced exercise tolerance with less peripheral fatigue incurred during single-leg knee extensor exercise, preceded by fatiguing knee extensor exercise using the contralateral leg (10).

Therefore, the present study aimed to elucidate the mechanism(s) by which prior high-intensity upper body exercise reduces subsequent leg cycling exercise tolerance. Specifically, we tested the hypothesis that prior upper body exercise reduces subsequent leg cycling exercise tolerance, and that this is associated with less peripheral fatigue, but an accelerated rise in ΔRPE/Δtime and Δdyspnea/Δtime.

METHODS

Participants

Eight healthy, nonsmoking, moderately trained men (age: 26 ± 4 yr; height: 182 ± 4 cm; body mass: 83 ± 4 kg; peak oxygen uptake: 50 ± 10 ml·kg⁻¹·min⁻¹) provided written, informed consent to participate in the study. Five of the participants had previously taken part in investigations that included assessment of neuromuscular function using the methods described in the present study (38–41). Participants refrained from strenuous exercise and alcohol the day preceding and the day of an exercise test, abstained from caffeine on test days, and reported to the laboratory at least 2 h postprandial. The study was approved by the Nottingham Trent University Human Ethics Committee, and all procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Design

Participants attended the laboratory on five separate occasions, at a similar time of day, separated by at least 48 h. The initial visit comprised a maximal incremental cycling test for the determination of peak oxygen uptake and peak cycling power (W peak). The second visit comprised familiarization with the knee extensor neuromuscular function assessments and arm-cranking protocol. The subsequent three visits comprised the experimental trials. The first two experimental trials were performed in a randomized order and comprised a fixed work-rate cycling test at 85% W peak, and exercise was performed to the limit of tolerance. These two cycling tests were performed without (hereafter termed CYC) and with (hereafter termed ARM-CYC) prior high-intensity arm-cranking exercise. For the third experimental trial, the CYC protocol was repeated, except that the cycling test was terminated after an identical duration to that achieved during ARM-CYC (hereafter termed ISOTIME). Knee extensor force and surface electromyographic (EMG) signals were recorded during a series of electrically evoked and voluntary isometric contractions of the dominant leg to quantify the presence and magnitude of central and peripheral locomotor muscle fatigue. For an illustration of the protocol for the experimental trials, please refer to Fig. 1.

Neuromuscular Function

Dynamometer. Participants were seated in a rigid, custom-built dynamometer adapted from Hannah et al. (40), with hip and knee joint angles of 100° and 95° (180° = full extension), respectively. Adjustable strapping across the pelvis and shoulders prevented extraneous movement during muscle activation. A noncompliant strap was attached to the dominant leg of the participant ~2 cm proximal to the medial malleolus and was in series with a linear strain gauge (615, Tedea-Hunteleigh, Herzliya, Israel) oriented perpendicular to the tibia. The dynamometer configuration was established during the familiarization session and replicated thereafter. The force signal was amplified (×1,000) in the frequency range 0–500 Hz, and sampled at 2,000 Hz using an external A/D converter (1401; CED, Cambridge, UK), interfaced with a personal computer using Spike 2 software (CED). Force data were low-pass filtered in both directions at 450 Hz using a fourth-order zero-lag Butterworth filter before analysis. Baseline resting force was subtracted from all force recordings to correct for the effect of gravity.

EMG. EMG signals were recorded from the superficial quadriceps (rectus femoris, vastus medialis, and vastus lateralis) and hamstring (biceps femoris) muscles, as described previously (40). After preparation of the skin by shaving, light abrasion, and cleaning with alcohol, bipolar surface electrodes (2.5-cm interelectrode distance; silver/silver chloride, 95 mm² area, Ambu Blue Sensor; Ambu, Ballerup, Denmark) were attached over each muscle at standardized

![Fig. 1. Experimental protocol. Arrows denote timing of measurement. Note that blood lactate concentration ([Lac] B), heart rate, rating of perceived exertion (RPE), and dyspnea were measured immediately before the start of leg cycling exercise. NMF, neuromuscular function.](http://jap.physiology.org/doi/10.1152/japplphysiol.00072.2015/fig1)
percentages of thigh length measured from the knee joint space to the
greater trochanter (rectus femoris, 55%; vastus medialis, 25%; vastus
lateralis and biceps femoris, 45%). These sites were selected to avoid
the innervation zones of each muscle (65). Electrodes were positioned
parallel to the presumed orientation of the muscle fibers. EMG
signals were preamplified by active EMG leads (input impedance
100 MΩ, common mode rejection ratio > 100 dB, base gain 500,
first-order high-pass filter set to 10 Hz; Noraxon, Scottsdale, AZ)
connected in series to a custom-built junction box and subse-
sequently to the same A/D converter and computer software that
enabled synchronization with the force data. The signals were
sampled at 2,000 Hz. Before analysis, EMG data were band-pass
filtered in both directions between 20 and 450 Hz using a fourth-
order zero-lag Butterworth filter (26, 27, 55).

Electrical stimulation. Equipment and procedures for electrical
stimulation have been described previously (41). A constant-current
variable voltage stimulator (DSTAH; Digitimer, Welwyn Garden
City, UK) was used to assess knee extensor contractile properties
while the participant was voluntarily passive. Square-wave pulses
(0.2-ms duration) were delivered via supramaximal femoral nerve
stimulation to evoke maximal potentiated twitch and triplet (3 pulses
at 300 Hz) contractions (24, 41). Stimulation of the femoral nerve
was achieved via a 1-cm-diameter cathode stimulation probe (Electro
Medical Supplies, Wantage, UK) pressed into the femoral triangle.
The surface of the anode, a 4 × 7-cm carbon rubber electrode (Electro
Medical Supplies), was coated in electrode gel and located over the
greater trochanter. The precise location of the cathode was determined
as the position that evoked the greatest twitch response for a particular
submaximal electrical current (typically 30–50 mA) and was marked
on the skin using indelible ink to ensure accurate repositioning within
each trial.

Procedure. Initially, discrete electrical stimuli were delivered via
percutaneous stimulation of the femoral nerve in the femoral triangle
to elicit twitch contractions of the quadriceps. Stepwise increments in
the current were delivered and separated by 10 s to allow for
neuromuscular recovery, until plateaus were reached in the amplitude
of twitch force and compound muscle action potentials (M-waves).
The stimulus intensity was then increased by 25% above the value
required to elicit a plateau to ensure supramaximal stimulation.
Participants subsequently performed supramaximal warm-up stimu-
lations of the knee extensors, lasting ~3 s and interspersed by ~30-s
rest, at ~50, 75, and 90% of their perceived maximal force. There-
after, and following baseline measurements for heart rate and blood
lactate concentration ([La−]), participants performed four maximum
voluntary contractions (MVCs) lasting 3–4 s and interspersed by
~30-s rest. Participants were instructed to extend the knee “as hard
and as fast as possible.” During and after each contraction, partici-
pants received strong verbal encouragement. Online feedback of the
force signal was provided, and a marker showing maximum force
during that session was displayed onscreen to assist participants in
attempting to maintain a high and stable force level. Each MVC was
followed within 1–2 s by two supramaximal electrical stimuli, sepa-
rated by 1 s, delivered to the femoral nerve to elicit maximal
potentiated twitches (49). Single electrically evoked triplet contrac-
tions (3 supramaximal stimuli delivered at 300 Hz) were superim-
posed on the third and fourth MVC, and at rest ~1–2 s after the two
potentiated twitch contractions (29, 48). Triplets were used in the
calculation of voluntary activation (see below), because the detection
of single superimposed twitches becomes increasingly difficult at high
forces as a result of the decreasing signal-to-noise ratio. This may lead
to the erroneous conclusion that voluntary activation is maximal (i.e.,
100%). Consequently, some studies have suggested the use of multi-
ple stimuli (48, 63, 72). Furthermore, triplets may offer advantages
over potentiated twitches as an indicator of peripheral fatigue, since
pilot data from our laboratory and that of de Ruiter et al. (28, 29) has
found the force evoked by 300-Hz bursts (3–8 pulses) to be insensi-
tive to potentiation, and, because they evoke much greater force than
potentiated twitches, they may better reflect the functional changes
observed during MVCs.

The maximum voluntary force (MVF) of the quadriceps was
defined as the greatest instantaneous force produced during the rele-
vant series of MVCs. The root mean square (RMS) amplitude of the
EMG signal for each agonist muscle (vastus medialis, vastus lateralis,
and rectus femoris) was calculated over a 500-ms epoch surrounding
MVF (250 ms either side) (19). Agonist EMG RMS values were
averaged to calculate a mean quadriceps (QEMGmax) value and
normalized to the peak-to-peak amplitude of the M-wave (see below)
to provide a measure of neuromuscular activation. Potentiated
twitches were measured for peak force and the amplitude of M-wave
response for the three quadriceps electrodes, which were then aver-
ged across the three sites to provide a mean quadriceps value. Mean
quadriceps M-wave amplitude and potentiated peak twitch force were
averaged across the latter four twitch contractions (i.e., after the 3rd
and 4th MVC) within each time period because it typically takes three
MVCs to fully potentiate twitches (49). The mean quadriceps M-wave
amplitude across the four potentiated twitches was defined as the
maximal M-wave amplitude (Mmax) and was used for normalization
of voluntary quadriceps EMG RMS (19). Measures of triplet peak
force were averaged across the two contractions within each time
period. To evaluate the presence and magnitude of central fatigue,
voluntary activation was evaluated for the third and fourth MVC using
the formula for the twitch interpolation technique (56), as described
previously (29, 48):

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\text{Voluntary activation} = 100 - \left[ \text{triplet force increment/resting triplet force} \times 100 \right]
\]

where the triplet force increment refers to that produced by superim-
posed triplet stimulation. The highest voluntary activation of the two
MVCs was retained for analysis. Assessment of neuromuscular func-
tion (from the first MVC to the last triplet) took ~2 min.

Maximal Incremental Cycling Test

Participants initially performed a maximal incremental cycling test
using an electromagnetically braked cycle ergometer (Excalibur
Sport; Lode, Groningen, The Netherlands). Tests began at 0 W, power
was increased by discrete 20-W increments every 60 s, and exercise
was performed to the limit of volitional tolerance or task failure (i.e.,
cycling cadence below 60 rpm) (46). Participants wore a facemask
(model 7940; Hans Rudolph) connected to a flow sensor (ZAN
variable orifice pneumotach; Nspire Health, Oberthulba, Germany)
that was calibrated using a 3-litre syringe. Gas concentrations
were measured using fast-responding laser diode absorption spectroscopy
sensors, which were calibrated using gases of known concentration
(5% CO2, 15% O2, balance N2; BOC, Guilford, UK), and ventilatory
and pulmonary gas exchange variables were measured breath by
breath (ZAN 600USB; Nspire Health), as described previously (46).
Peak oxygen uptake was defined as the highest recorded value over
any 30-s period, and Wpeak was calculated as the sum of the power
output in the last completed stage plus the product of ramp increment
(20 W) and the fraction of the final stage actually completed.

Experimental Trials

During the experimental trials (CYC, ARM-CYC, and ISOTIME),
heart rate was measured using short-range telemetry (Polar S610;
Polar, Kempele, Finland), and fingertip capillary blood samples were
taken and analyzed for [La−], using an automated analyzer (Bösen
C-line Sport; EKF Diagnostics, Barleben, Germany).

An illustration of the timing of measurements taken during the
experimental trials is shown in Fig. 1. Each experimental trial comprised
a fixed work-rate cycling test at 85% W˙ peak, preceded by a
standardized 23.5-min period. During the first 6 min of this period,
baseline measures of heart rate, [La−], and neuromuscular function
were taken.

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After baseline measurements, participants remained seated in the dynamometer and then either rested (CYC and ISOTIME) or performed intense intermittent arm-cranking exercise (ARM-CYC) using an electromagnetically braked arm-cranking ergometer (Angio; Lode). The arm-cranking protocol comprised 8 × 1-min arm-cranking bouts, interspersed with 30-s rest, at a fixed work rate of 1.0–1.5 W/kg body mass (mean: 1.2 ± 0.2 W/kg body mass, 100 ± 15 W) (46). As in our laboratory’s previous study (46), the arm-cranking work rate for each individual was selected based on their habitual upper body exercise regimen. This work rate was trialed during the familiarization session and, based on successful completion by all participants, was deemed suitable for subsequent testing. Cadence was maintained between 90 and 110 rpm. Heart rate was measured at the end of each arm-cranking bout during ARM-CYC, and [La−]b was also measured after the final arm-cranking bout. These measurements were taken at equivalent time points while participants rested during CYC and ISOTIME. Quadriceps and hamstring muscle EMG was recorded throughout the arm-cranking protocol and displayed online with a high gain to aid visual detection of EMG activity. Participants received verbal feedback regarding EMG activity to ensure minimal activation of the leg muscles.

Arm-cranking (ARM-CYC) or seated rest (CYC and ISOTIME) was followed by another 6-min period before the start of the fixed work-rate cycling test. During this period, measures of neuromuscular function were taken, and participants then transferred to the cycle ergometer (positioned ~2 m from the dynamometer). Immediately before the start of the cycling test, heart rate and [La−]b were measured along with dyspnea (defined as breathing “effort”) and RPE for leg discomfort using Borg’s modified CR10 scale (18).

Participants adopted a self-selected cadence between 80 and 100 rpm during the first cycling test, and this was replicated during subsequent tests. Quadriceps and hamstring muscle EMG was synchronized with the cycle ergometer crank position via a reed switch attached to the crank and ergometer. The RMS amplitude of the EMG signal of the quadriceps muscle was measured at the start and end of each minute during each arm-cranking exercise bout and normalized to QEMGmax to quantify quadriceps neuromuscular activation. EMG RMS amplitude of the quadriceps and hamstring muscles was also measured over 10 consecutive pedal revolutions at the end of the first, third, and final minute of cycling exercise and normalized to Mmax (quadriceps only) to quantify changes in neuromuscular activation during cycling. Onsets and offsets of EMG bursts were determined visually by the same investigator, according to a previously published method (22, 23). Threshold methods for determining EMG onsets and offsets are sensitive to changes in background EMG (42) and are unsuitable for this type of analysis, because bursts of EMG activity occur with background activity already present in the muscles, and the amplitude of background activity varies between muscles (22, 23).

Heart rate, RPE, and dyspnea were measured after 3 min of cycling. During CYC and ARM-CYC, cycling exercise was performed to the limit of volitional tolerance. An additional criterion for terminating a cycling test was a fall in cadence below 60 rpm. During ISOTIME, cycling exercise was terminated by the investigators after an identical duration to that achieved during ARM-CYC.

On cessation of cycling exercise heart rate, [La−]b, RPE, and dyspnea were measured immediately, and participants were assisted to the dynamometer for neuromuscular function evaluation with the first MVC initiated after 2 min (±9 s).

**Statistical Analyses**

Data were analyzed using SPSS for Windows (IBM, Chicago, IL). Trial-to-trial variation in baseline neuromuscular function was calculated as the within-participant coefficient of variation. Measurement error and reproducibility of baseline neuromuscular function were calculated, and the smallest meaningful change was subsequently determined (16, 43). A one-way repeated-measures ANOVA followed by Tukey’s post hoc test was used to analyze differences between trials for cycling exercise duration and rates of change in perceptual responses expressed relative to absolute exercise time (ΔRPE/Δtime and Δdyspnea/Δtime) and when normalized to total cycling exercise duration (ΔRPE/Δtime and Δdyspnea/Δtime). All other data were analyzed using a two-way (trial × time) repeated-measures ANOVA. Significant interactions were further explored by performing one-way repeated-measures ANOVA: 1) within each trial and 2) across trials at individual time points, followed by Tukey’s post hoc test. When differences were observed within or between trials, 95% confidence intervals (CI) for the difference were calculated (2). Pearson’s correlation coefficient was used to determine the relationship between selected variables. Statistical significance was set at P < 0.05. Results are presented as means ± SD.

**RESULTS**

**Cycling Exercise Tolerance**

There was an effect of trial on cycling exercise duration at 85% Wpeak (273 ± 26 W) [F(2,14) = 16.8, P < 0.001], which was, as expected, identical (4.33 ± 1.10 min) during ARM-CYC and ISOTIME and 38 ± 17% shorter than CYC (7.46 ± 2.79 min) (mean difference = 3.13 ± 2.15 min, 95% CI = 1.50–4.75 min, P < 0.001). Cycling cadence at the termination of cycling exercise in CYC (68 ± 6 rpm, range: 61–78 rpm), ARM-CYC (66 ± 5 rpm, range: 60–74 rpm), and ISOTIME (92 ± 10 rpm, range: 85–104 rpm) was always ≥60 rpm. Therefore, cycling exercise during CYC and ARM-CYC was always performed to the limit of volitional tolerance rather than being terminated by the investigators.

**Neuromuscular Function**

Baseline measures of neuromuscular function are shown in Table 1, and these were highly reproducible between trials. Raw traces of force from a representative participant at baseline performing a MVC with superimposed triplet, followed by twitch and triplet contractions, are shown in Fig. 2. In all trials, measures of neuromuscular function were unchanged from

<table>
<thead>
<tr>
<th>Cytoplasm</th>
<th>ARM-CYC</th>
<th>ISOTIME</th>
<th>Within-participant CV, %</th>
<th>Measurement Error</th>
<th>Reproducibility</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVF, N</td>
<td>616 ± 75</td>
<td>602 ± 84</td>
<td>616 ± 73</td>
<td>4</td>
<td>19</td>
<td>53</td>
</tr>
<tr>
<td>Potentiated twitch force, N</td>
<td>201 ± 33</td>
<td>200 ± 20</td>
<td>203 ± 22</td>
<td>3</td>
<td>11</td>
<td>29</td>
</tr>
<tr>
<td>Potentiated triplet force, N</td>
<td>339 ± 35</td>
<td>328 ± 37</td>
<td>337 ± 33</td>
<td>3</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Voluntary activation, %</td>
<td>94 ± 5</td>
<td>95 ± 6</td>
<td>94 ± 6</td>
<td>2</td>
<td>1.8</td>
<td>5.1</td>
</tr>
<tr>
<td>Quadriceps M-wave amplitude, mV</td>
<td>6.3 ± 2.1</td>
<td>6.1 ± 1.9</td>
<td>5.8 ± 2.5</td>
<td>10</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Quadriceps EMG RMS at MVF, %Mmax amplitude</td>
<td>8.8 ± 2.9</td>
<td>8.5 ± 2.9</td>
<td>8.9 ± 2.5</td>
<td>11</td>
<td>1.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Measured variables are shown as means ± SD. CYC, cycling test to the limit of tolerance; ARM-CYC, cycling test to the limit of tolerance after prior high-intensity arm-cranking exercise; ISOTIME, cycling test without prior exercise and for an equal duration as ARM-CYC; CV, coefficient of variation; SMC, smallest meaningful change; MVF, maximal voluntary force; EMG, electromyography; RMS, root mean square; Mmax, maximal M-wave amplitude.
baseline to precycling (data not shown). Thus arm-cranking per se did not result in central or peripheral locomotor muscle fatigue.

For MVF, there was a trial × time interaction \(F(4,28) = 6.2, P < 0.001\) and an effect of time in CYC \(F(2,14) = 14.3, P < 0.001\), ARM-CYC \(F(2,14) = 11.5, P = 0.001\), and ISOTIME \(F(2,14) = 8.5, P = 0.003\). MVF decreased from baseline to postcycling in CYC (mean difference = 95 ± 70 N, 95% CI = 45–145 N, \(P < 0.001\)), ARM-CYC (mean difference = 56 ± 39 N, 95% CI = 22–89 N, \(P = 0.002\)), and ISOTIME (mean difference = 49 ± 48 N, 95% CI = 13–85 N, \(P = 0.008\)). Furthermore, there was an effect of trial on the decrease in MVF \(F(2,14) = 8.3, P = 0.004\), which was greater in CYC than ARM-CYC (mean difference = 39 ± 38 N, 95% CI = 7–71 N, \(P = 0.02\)) and ISOTIME (mean difference = 46 ± 43 N, 95% CI = 14–78 N, \(P = 0.005\)) (Fig. 3A).

For voluntary activation, there was a trial × time interaction \(F(4,28) = 3.8, P = 0.013\) and an effect of time in CYC \(F(2,14) = 8.0, P = 0.005\) and ARM-CYC \(F(2,14) = 4.7, P = 0.027\), but not ISOTIME \(F(2,14) = 0.8, P = 0.46\). Voluntary activation decreased from baseline (see Table 1) to postcycling in CYC (89 ± 9%, mean difference = 5.0 ± 4.8%, 95% CI = 1.4–8.7%, \(P = 0.012\)) and ARM-CYC (91 ± 8%, mean difference = 3.8 ± 4.7%, 95% CI = 0.1–7.4%, \(P = 0.047\)). Furthermore, there was an effect of trial on the decrease in voluntary activation \(F(2,14) = 5.2, P = 0.021\), which was greater in CYC than ISOTIME (mean difference = 4.4 ± 4.9%, 95% CI = 0.7–8.0%, \(P = 0.019\)) (Fig. 3B).

For potentiated twitch force, there was a trial × time interaction \(F(4,28) = 8.8, P < 0.001\) and an effect of time in CYC \(F(2,14) = 49.4, P < 0.001\), ARM-CYC \(F(2,14) = 48.3, P < 0.001\), and ISOTIME \(F(2,14) = 22.7, P < 0.001\).
Potentiated twitch force decreased from baseline to postcycling in CYC (mean difference = 77 ± 30 N, 95% CI = 55–98 N, P < 0.001), ARM-CYC (mean difference = 52 ± 21 N, 95% CI = 38–66 N, P < 0.001), and ISOTIME (mean difference = 50 ± 24 N, 95% CI = 30–70 N, P < 0.001). Furthermore, there was an effect of trial on the decrease in potentiated twitch force [F(2,14) = 10.9, P = 0.001], which was greater in CYC than ARM-CYC (mean difference = 25 ± 17 N, 95% CI = 8–41 N, P = 0.004) and ISOTIME (mean difference = 27 ± 22 N, 95% CI = 10–43 N, P = 0.003) (Fig. 3C).

For potentiated triplet force, there was a trial × time interaction [F(4,28) = 9.1, P < 0.001] and an effect of time in CYC [F(2,14) = 11.1, P = 0.001], ARM-CYC [F(2,14) = 5.4, P = 0.018], and ISOTIME [F(2,14) = 7.2, P = 0.007]. Potentiated triplet force decreased from baseline to postcycling in CYC (mean difference = 63 ± 50 N, 95% CI = 26–100 N, P = 0.001), ARM-CYC (mean difference = 37 ± 40 N, 95% CI = 7–66 N, P = 0.014) and ISOTIME (mean difference = 31 ± 26 N, 95% CI = 9–52 N, P < 0.001). Furthermore, there was an effect of trial on the decrease in potentiated triplet force [F(2,14) = 9.0, P = 0.003], which was greater in CYC than ARM-CYC (mean difference = 27 ± 18 N, 95% CI = 5–48 N, P = 0.015) and ISOTIME (mean difference = 33 ± 28 N, 95% CI = 11–54 N, P = 0.004) (Fig. 3D).

Quadriceps M_{max} and neuromuscular activation (i.e., RMS EMG normalized to M_{max}) at MVF remained unchanged in all trials.

**Leg Muscle EMG During Cycling**

Quadriceps EMG RMS during arm-cranking was ≤3% of the QEMG_{max} during an MVC (data not shown), thus demonstrating minimal leg activation. For quadriceps neuromuscular activation (EMG RMS normalized to M_{max}) during cycling, there was a trial × time interaction [F(4,28) = 6.1, P = 0.001] and an effect of time in CYC [F(2,14) = 36.3, P < 0.001], ARM-CYC [F(2,14) = 11.6, P = 0.001], and ISOTIME [F(2,14) = 36.3, P = 0.012]. There was also an effect of trial on neuromuscular activation in the final minute of cycling [F(2,14) = 6.2, P = 0.012], which was greater in CYC than ARM-CYC (mean difference = 0.76 ± 0.84% M_{max}, 95% CI = 0.03–1.5% M_{max}, P = 0.040) and ISOTIME (mean difference = 0.91 ± 0.87% M_{max}, 95% CI = 0.18–1.64% M_{max}, P = 0.014) (Fig. 4). The absolute hamstrings EMG RMS remained constant during cycling and was not different between trials (pooled data: 0.08 ± 0.05 mV).

**Heart Rate and [La\textsuperscript{-}\textsubscript{1}B]**

For heart rate, there was a trial × time interaction [F(24,168) = 81.7, P < 0.001] and an effect of trial on the mean of the eight heart rate measurements taken during the 11.5-min period of arm-cranking in ARM-CYC or seated rest in CYC and ISOTIME (see Fig. 1) [F(2,24) = 144.3, P < 0.001]. The mean heart rate during this period was higher in ARM-CYC (153 ± 19 beats/min) than CYC (72 ± 14 beats/min) (mean difference = 81 ± 20 beats/min, 95% CI = 67–96 beats/min, P < 0.001) and ISOTIME (73 ± 11 beats/min) (mean difference = 80 ± 16 beats/min, 95% CI = 66–95 beats/min, P < 0.001). There was also an effect of trial on heart rate measured precycling [F(2,14) = 21.7, P < 0.001], after 3 min of cycling [F(2,14) = 17.8, P < 0.001], and postcycling [F(2,14) = 12.3, P < 0.001]. Precycling, heart rate was higher in ARM-CYC than CYC (mean difference = 34 ± 21 beats/min, 95% CI = 18–49 beats/min, P < 0.001) and ISOTIME (mean difference = 34 ± 15 beats/min, 95% CI = 18–49 beats/min, P < 0.001). After 3 min of cycling, heart rate was higher in ARM-CYC than CYC and ISOTIME (mean difference from both trials = 10 ± 6 beats/min, 95% CI = 5–15 beats/min, P < 0.001). Postcycling, heart rate was lower in ISOTIME than CYC (mean difference = 10 ± 6 beats/min, 95% CI = 3–16 beats/min, P = 0.005) and ARM-CYC (mean difference = 12 ± 8 beats/min, 95% CI = 5–18 beats/min, P = 0.001) (Fig. 5A).

For [La\textsuperscript{-}\textsubscript{1}B], there was a trial × time interaction [F(6,42) = 79.7, P < 0.001] and an effect of trial on [La\textsuperscript{-}\textsubscript{1}B] measured immediately after the period of arm-cranking in ARM-CYC or seated rest in CYC and ISOTIME [F(2,14) = 167.2, P < 0.001]. Immediately after this period, [La\textsuperscript{-}\textsubscript{1}B] was higher in ARM-CYC than CYC and ISOTIME (mean difference from both trials = 10.3 ± 2.2 mmol/l, 95% CI = 8.6–12.0 mmol/l, P < 0.001). There was also an effect of trial on [La\textsuperscript{-}\textsubscript{1}B] measured precycling [F(2,14) = 158.2, P < 0.001], which was higher in ARM-CYC than CYC (mean difference = 8.5 ± 2.0 mmol/l, 95% CI = 7.0–10.0 mmol/l, P < 0.001) and ISOTIME (mean difference = 8.6 ± 1.8 mmol/l, 95% CI = 7.2–10.1 mmol/l, P < 0.001). Furthermore, there was an effect of trial on [La\textsuperscript{-}\textsubscript{1}B] measured postcycling [F(2,14) = 31.9, P < 0.001], which was higher in ARM-CYC than CYC (mean difference = 2.3 ± 1.0 mmol/l, 95% CI = 0.8–3.9 mmol/l, P = 0.003) and ISOTIME (mean difference = 4.6 ± 1.8 mmol/l, 95% CI = 3.1–6.1 mmol/l, P < 0.001). Postcycling, [La\textsuperscript{-}\textsubscript{1}B] was also higher in CYC than ISOTIME (mean difference = 2.3 ± 2.0 mmol/l, 95% CI = 0.8–3.8 mmol/l, P = 0.004) (Fig. 5B).

**RPE and Dyspnea**

There was a trial × time interaction for RPE [F(4,28) = 14.7, P < 0.001] and an effect of trial on RPE measured after 3 min of cycling [F(2,14) = 11.7, P = 0.001], which was
higher in ARM-CYC than CYC [mean difference = 2.4 ± 1.7 arbitrary units (AU), 95% CI = 1.0–3.9 AU, P = 0.002] and ISOTIME (mean difference = 2.3 ± 1.9 AU, 95% CI = 0.8–3.8 AU, P = 0.003). There was also an effect of trial on RPE measured postcycling [F(2,14) = 18.4, P < 0.001], which was lower in ISOTIME than CYC (mean difference = 3.3 ± 1.9 AU, 95% CI = 1.4–5.2 AU, P = 0.001) and ARM-CYC (mean difference = 4.1 ± 2.6 AU, 95% CI = 2.2–5.9 AU, P < 0.001) (Fig. 6A).

There was a trial × time interaction for dyspnea [F(4,28) = 5.8, P < 0.001] and an effect of trial on dyspnea measured after 3 min of cycling [F(2,14) = 16.3, P < 0.001], which was lower in ISOTIME than CYC (mean difference = 3.3 ± 1.9 AU, 95% CI = 1.4–5.2 AU, P = 0.001) and ARM-CYC (mean difference = 4.1 ± 2.6 AU, 95% CI = 2.2–5.9 AU, P < 0.001) (Fig. 6A).
higher in ARM-CYC than CYC (mean difference = 1.9 ± 1.4 AU, 95% CI = 0.9–3.0 AU, \(P < 0.001\)) and ISOTIME (mean difference = 2.1 ± 1.1 AU, 95% CI = 1.0–3.2 AU, \(P < 0.001\)). There was also a main effect of trial on dyspnea measured postcycling [\(F(2,14) = 11.8, P = 0.001\)], which was lower in ISOTIME than CYC (mean difference = 2.8 ± 2.4 AU, 95% CI = 0.9–4.6 AU, \(P = 0.004\)) and ARM-CYC (mean difference = 3.1 ± 2.6 AU, 95% CI = 1.3–5.0 AU, \(P = 0.002\)) (Fig. 6B).

There was an effect of trial on \(\Delta\text{RPE}/\Delta\text{time}\) [\(F(2,14) = 11.7, P = 0.001\], which was higher in ARM-CYC than CYC (mean difference = 0.72 ± 0.63 AU/min, 95% CI = 0.25–1.21 AU/min, \(P = 0.003\)) and ISOTIME (mean difference = 0.79 ± 0.55 AU/min, 95% CI = 0.31–1.27 AU/min, \(P = 0.002\)) (Table 2). There was also an effect of trial on \(\Delta\text{dyspnea}/\Delta\text{time}\) [\(F(2,14) = 4.5, P = 0.031\)], which was higher in ARM-CYC than ISOTIME (mean difference = 0.46 ± 0.58 AU/min, 95% CI = 0.02–0.90 AU/min, \(P = 0.038\)) (Table 2).

There was an effect of trial on \(\Delta\text{RPE}/\Delta\text{time}\) [\(F(2,14) = 19.1, P < 0.001\)] (Fig. 6C and Table 2), which was lower in ISOTIME than CYC (mean difference = 0.03 ± 0.01 AU/min, 95% CI = 0.01–0.05 AU/min, \(P < 0.001\)) and ARM-CYC (mean difference = 0.04 ± 0.02 AU/min, 95% CI = 0.02–0.05 AU/min, \(P < 0.001\)). There was also an effect of trial on \(\Delta\text{dyspnea}/\Delta\text{time}\) [\(F(2,14) = 7.5, P = 0.006\)] (Fig. 6D and Table 2), which was lower in ISOTIME than CYC (mean difference = 0.03 ± 0.01 AU/min, 95% CI = 0.01–0.05 AU/min, \(P = 0.006\)) and ARM-CYC (mean difference = 0.02 ± 0.01 AU/min, 95% CI = 0.001–0.04 AU/min, \(P = 0.036\)).

When data from CYC and ARM-CYC were pooled, \(\Delta\text{RPE}/\Delta\text{time}\) was negatively correlated with the time to the limit of cycling exercise tolerance (\(r = -0.74, P = 0.001\)). Furthermore, the reduction in cycling exercise tolerance during ARM-CYC compared with CYC was negatively correlated with the increases in \(\Delta\text{RPE}/\Delta\text{time}\) (\(r = -0.72, P = 0.045\)) and \(\Delta\text{dyspnea}/\Delta\text{time}\) (\(r = -0.80, P = 0.018\)).

### DISCUSSION

The present study examined the effects of prior high-intensity upper body exercise on subsequent high-intensity leg cycling exercise tolerance and associated changes in neuro muscular and perceptual responses. Our main findings were threefold: 1) prior upper body exercise in ARM-CYC reduced subsequent cycling exercise tolerance by 38%; 2) the reduced cycling exercise tolerance in ARM-CYC was associated with less peripheral muscle fatigue incurred but a similar reduction in voluntary activation compared with CYC; and 3) the reduced cycling exercise tolerance in ARM-CYC was related to increases in \(\Delta\text{RPE}/\Delta\text{time}\) and \(\Delta\text{dyspnea}/\Delta\text{time}\). These findings suggest that exercise tolerance is not regulated by a critical level of peripheral fatigue. Instead, central fatigue and an exacerbation of perceptual responses are the potential mechanisms underlying the reduced cycling exercise tolerance after prior upper body exercise.

Our laboratory recently showed that high-intensity cycling exercise tolerance was reduced by a strikingly similar extent after an identical upper body exercise protocol (46). Several authors suggest that reduced lower limb exercise tolerance after prior upper body exercise occurs because of accelerated development of peripheral fatigue caused by greater intramuscular metabolic perturbation (12, 17, 36, 44, 46, 61). This notion is supported, indirectly, by the observation that prior high-intensity upper body exercise elevated leg muscle [La] and [H+] at the onset of isolated knee extensor exercise (11, 12), accelerated the exercise-induced increase in interstitial [K+] (61), and reduced exercise tolerance (12, 61). However, although such metabolite accumulation has been implicated in the etiology of peripheral fatigue (21, 33), previous prior upper body exercise studies did not measure peripheral fatigue or neuromuscular activation. Comparisons of our work with isolated knee extensor exercise studies are also complicated by the task-specificity of fatigue etiology (13, 74). Two observations from the present study suggest that peripheral fatigue during cycling exercise was not accelerated by prior upper body exercise. First, the extent of peripheral fatigue in ARM-CYC and ISOTIME was the same, even though there was considerable systemic metabolic perturbation in ARM-CYC. Indeed, in our previous study, the same upper body exercise protocol reduced the strong ion difference by 15%, increased plasma [H+] by 33%, reduced \(W_{\text{peak}}\) by 29%, and accelerated the increase in plasma [K+] during subsequent cycling exercise by 56% (46). Second, if peripheral fatigue during cycling exercise was accelerated, this would be expected to result in greater neuromuscular activation (i.e., increased motor unit recruitment and/or firing frequency) to compensate for the reduced force-generating capacity (14, 30); however, this was not observed. Collectively, these observations, therefore, suggest that systemic metabolite perturbation plays a minor role in peripheral fatigue generation.

Our findings contrast previous cycling exercise studies in which moderate hypoxia (7, 66), superimposed inspiratory muscle loading (67), volitionally induced inspiratory or expiratory muscle fatigue (73, 80), prior high-intensity cycling exercise (5), and prior electrically induced quadriceps muscle fatigue (34) reduced exercise tolerance but resulted in the same degree of peripheral fatigue incurred compared with control conditions. These observations are taken as evidence for inhibitory group III/IV muscle afferent feedback to the central nervous system regulating central motor drive to confine the development of peripheral fatigue to a critical threshold (3, 31). However, although the 38% reduction in twitch force after CYC is comparable to the proposed critical threshold of pe-
Peripheral fatigue previously reported after high-intensity fixed work-rate cycling exercise (4, 5, 66, 73, 80), this degree of peripheral fatigue was not reached during ARM-CYC (26% reduction in twitch force). This finding is similar to the observation of less peripheral fatigue incurred after high-intensity cycling exercise in severe hypoxia ($F_{O2}$, 0.10) compared with normoxia (8). The notion that peripheral fatigue is not critically regulated is also supported by two recent isolated muscle studies: Rossman et al. (68) observed greater quadriceps muscle fatigue during single-leg compared with double-leg knee extensor exercise, whereas Amann et al. (10) observed less quadriceps muscle fatigue during single-leg knee extensor exercise after fatiguing knee extensor exercise with the contralateral leg. The present study thus extends these observations to whole body exercise by providing novel evidence that peripheral fatigue is not independently regulated during high-intensity fixed work-rate cycling exercise to volitional tolerance.

Whether peripheral fatigue plays an important role in governing exercise tolerance remains controversial (9, 53, 54, 60). Consistent with previous observations (5, 7, 54, 71, 75), submaximal quadriceps muscle recruitment was observed at the limit of exercise tolerance in CYC and ARM-CYC (55 and 50%, respectively, of the QEMGmax), and Noakes (58) argues that this negates peripheral fatigue as the single limiting factor to exercise tolerance. Furthermore, Decorte et al. (30) have shown that peripheral fatigue during cycling exercise at 80% $W_{peak}$ develops mostly during the first half of the test, such that the limit of tolerance approaches without further peripheral fatigue, but with a significant reduction in voluntary activation. The similar reduction in voluntary activation after CYC and ARM-CYC indicates that central fatigue developed more quickly in ARM-CYC, possibly due to a “spill-over” of central fatigue from the exercised upper body muscles to the leg locomotor muscles. In support, it was recently demonstrated that fatiguing leg cycling exercise resulted in a “spill-over” of central fatigue (i.e., reduced voluntary activation) to the remote unexercised elbow flexors (69). This effect was attributed to inhibitory group III/IV muscle afferent feedback originating in fatigued leg muscle, since attenuating this feedback using intrathecal fentanyl abolished the decline in voluntary activation of the elbow flexors. Whether a fall in voluntary activation limits cycling exercise that is characterized by submaximal muscle contractions remains uncertain (74). However, it is also recognized that a limiting influence of central fatigue on exercise tolerance may be manifest by changes in sensory perception (57, 74).

The conscious perception of fatigue is thought to reflect the complex integration and interpretation of central motor drive and an associated corollary discharge, somatosensory feedback (4, 50, 70, 78), and cognitive functions, such as motivation and emotional state (70). After 3 min of cycling, RPE for leg discomfort was greater in ARM-CYC compared with CYC and ISOTIME, despite similar levels of quadriceps neuromuscular activation, which supports observations made during single-leg knee extensor exercise after fatiguing knee extensor exercise with the contralateral leg (10). Furthermore, at the end of cycling, RPE was greater in ARM-CYC compared with ISOTIME, despite similar levels of peripheral fatigue incurred, whereas RPE was similar at the end of cycling in CYC and ARM-CYC, despite less peripheral fatigue incurred during ARM-CYC. Collectively, our findings suggest that the perception of leg discomfort during cycling exercise does not exclusively reflect the extent of quadriceps neuromuscular activation or degree of peripheral fatigue incurred. Similar observations have been made in COPD patients, who sometimes stop exercise because of leg discomfort, and in the absence of quadriceps muscle fatigue (52). These observations suggest that the conscious perception of leg discomfort likely reflects a complex interplay between multiple factors other than peripheral fatigue and neuromuscular activation (70). Minute ventilation was not measured in the present study, and thus it cannot be ruled out that the greater $\Delta$RPE/$\Delta$time during ARM-CYC resulted, in part, from a greater ventilatory response (10). However, afferents involved in the perception of dyspnea and limb discomfort project to the same sensorimotor brain areas (62), and, therefore, a heightened level of one perception may potentiate the other. In support, quadriceps fatigue induced by sustained contractions increased dyspnea during a subsequent inspiratory loaded breathing challenge without affecting breathing pattern or pleural pressure swings (37). Thus, although we could not elucidate the precise causative mechanism(s), we propose that the greater $\Delta$RPE/$\Delta$time and $\Delta$dyspnea/$\Delta$time during ARM-CYC reflects, in part, greater ensemble group III/IV afferent projections to integrated sensorimotor brain structures due to cycling, commencing with preexisting afferent input originating from the previously exercised respiratory (50) and upper body musculature (10, 25, 45), lungs (50), and heart (78). During cycling exercise, the preexisting afferent input would have been added to the prevailing inputs related to central motor drive, and locomotor muscle (10) and cardiorespiratory (50, 78) activity, thereby accelerating the increase in perceptual responses and reducing exercise tolerance. The correlation between increased $\Delta$RPE/$\Delta$time and $\Delta$dyspnea/$\Delta$time and reduced cycling exercise tolerance in ARM-CYC also supports sensory perception as an important mediator of exercise tolerance. Our findings are, therefore, consistent with the “flush model” proposed by Millet (57), which suggests that exercise tolerance is mediated primarily by $\Delta$RPE/$\Delta$time, which, in turn, depends mainly on feedback (i.e., peripheral) and feed-forward (i.e., central) mechanisms.

The greater $\Delta$RPE/$\Delta$time and $\Delta$dyspnea/$\Delta$time during ARM-CYC compared with CYC, but similar $\Delta$RPE/$\Delta$time and $\Delta$dyspnea/$\Delta$time, suggests that the preexisting afferent input at the onset of cycling in ARM-CYC affected perceptual responses by increasing their gain. Similar effects on the absolute and normalized RPE are observed when exercise tolerance is reduced by muscle glycogen depletion (59), warm and cold ambient temperatures (24), and prior fatiguing activity using the same muscle groups (32). These observations underpin the notion that perceptual responses are set in anticipation, otherwise known as teleoanticipation (76), so that exercise terminates at a critical sensory tolerance limit (32, 58, 60, 75). By limiting exercise tolerance, the sensory tolerance limit will, therefore, also mediate the degree of peripheral fatigue incurred, which is consistent with the findings of recent studies using the isolated knee extensor exercise model (10, 68). We note, however, that the limit of cycling exercise tolerance during CYC and ARM-CYC was sometimes associated with submaximal RPE and dyspnea, suggesting that ad-

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ditional influences, such as psychological factors (15, 54), were also mediating the limit of exercise tolerance.

In conclusion, reductions in cycling exercise tolerance due to prior upper body exercise are associated with an acceleration of central fatigue and greater perceptual responses rather than an accelerated development of peripheral fatigue. These findings suggest that peripheral fatigue is not independently regulated during high-intensity fixed work-rate cycling exercise to volitional tolerance, and that exercise tolerance, and thus the degree of peripheral fatigue incurred, is potentially determined by intolerable levels of sensory perception.

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

AUTHOR CONTRIBUTIONS

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