



Kent Academic Repository

Iannetta, Danilo, Passfield, Louis, Qahtani, Ahmad, MacInnis, Martin J. and Murias, Juan M. (2019) *Inter-limb differences in parameters of aerobic function and local profiles of deoxygenation during double-leg and counterweighted single-leg cycling*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 317 (6). R840-R851. ISSN 0363-6119.

Downloaded from

<https://kar.kent.ac.uk/77683/> The University of Kent's Academic Repository KAR

The version of record is available from

<https://doi.org/10.1152/ajpregu.00164.2019>

This document version

Author's Accepted Manuscript

DOI for this version

Licence for this version

UNSPECIFIED

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in *Title of Journal*, Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our [Take Down policy](https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies) (available from <https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies>).

1 **Inter-limb differences in parameters of aerobic function and local profiles of deoxygenation**
2 **during double-leg and counterweighted single-leg cycling.**

3 Danilo Iannetta ¹, Louis Passfield ^{1,2}, Ahmad Qahtani ¹, Martin J MacInnis ¹, Juan M Murias ¹

4 ¹ *Faculty of Kinesiology, University of Calgary, Calgary, Canada*

5 ² *School of Sport and Exercise Sciences, University of Kent, Canterbury, United Kingdom*

6

7 **Running head:**

8 Effect of leg-dominance on aerobic exercise capacity.

9

10 **Corresponding authors:**

11 Dr. Juan M Murias

12 Assistant Professor,

13 Faculty of Kinesiology, University of Calgary

14 KNB 434, 2500 University Drive NW, Calgary, Alberta, Canada, T2N 1N4

15 +1 (403) 220-7955

16 jmmurias@ucalgary.ca

17 **ABSTRACT**

18 It is typically assumed that in the context of double-leg cycling, dominant (DOM_{LEG}) and non-
19 dominant ($NDOM_{LEG}$) legs have similar aerobic capacity and that both contribute equally to the whole-
20 body physiological responses. However, there is a paucity of studies that have systematically
21 investigated maximal and submaximal aerobic performance and characterized the profiles of local
22 muscle deoxygenation in relation to leg-dominance. Using counterweighted single-leg cycling, this
23 study explored whether peak O_2 consumption ($\dot{V}O_{2peak}$), maximal lactate steady-state ($MLSS_p$), and
24 profiles of local deoxygenation [HHb] would be different in the DOM_{LEG} compared with the
25 $NDOM_{LEG}$. Twelve participants performed a series of double-leg and counterweighted single-leg
26 (DOM_{LEG} and $NDOM_{LEG}$ *i*) ramp-exercise tests, and *ii*) 30-min constant-load trials. $\dot{V}O_{2peak}$ was greater
27 in the DOM_{LEG} than in the $NDOM_{LEG}$ (2.87 ± 0.42 vs 2.70 ± 0.39 $L\cdot min^{-1}$; $P<0.05$). The difference in
28 $\dot{V}O_{2peak}$ persisted even after accounting for lean mass ($P<0.05$). Similarly, $MLSS_p$ was greater in the
29 DOM_{LEG} than in the $NDOM_{LEG}$ (118 ± 31 vs 109 ± 31 W; $P<0.05$). Furthermore, the amplitude of the
30 [HHb] signal during ramp-exercise was larger in the DOM_{LEG} than in the $NDOM_{LEG}$ during both
31 double-leg (26.0 ± 8.4 vs 20.2 ± 8.8 μM ; $P<0.05$) and counterweighted single-leg cycling (18.5 ± 7.9 vs
32 14.9 ± 7.5 μM ; $P<0.05$). Additionally, the amplitudes of the [HHb] signal were highly-to-moderately
33 correlated with the mode-specific $\dot{V}O_{2peak}$ values (ranging from 0.91 to 0.54). These findings showed,
34 in a group of young men, that maximal and submaximal aerobic capacities were greater in the DOM_{LEG}
35 than in the $NDOM_{LEG}$, and that superior peripheral adaptations of DOM_{LEG} may underpin these
36 differences.

37 **New and Noteworthy**

38 It is typically assumed that the dominant and non-dominant legs contribute equally to the whole-
39 physiological responses. In this study, we found that the dominant leg achieved greater peak O₂ uptake
40 values, sustained greater power output while preserving whole-body metabolic stability, and showed
41 larger amplitudes of deoxygenation responses. These findings highlight heterogeneous aerobic
42 capacities of the lower-limbs which have important implications when examining whole-body
43 physiological responses.

44 **Key words:** dominant; non-dominant; unilateral exercise; muscle deoxygenation; near-infrared
45 spectroscopy.

46

47 **INTRODUCTION**

48 In humans, one side of the body is usually preferred over the other to execute voluntary motor actions.
49 In the context of double-leg cycling, where both legs are simultaneously involved in the motor task,
50 there is evidence that the dominant-leg (DOM_{LEG}) contributes more to the generated power than the
51 non-dominant-leg ($NDOM_{LEG}$) (12, 62). The magnitude of the reported asymmetries can vary (e.g., ~1-
52 40%) and is dependent on the variable of interest (e.g., power, torque, etc.), pedaling phase, intensity,
53 and cadence (66). Musculoskeletal and motor control deficits of the $NDOM_{LEG}$ are typically
54 acknowledged to underpin these differences (66), despite muscle activation patterns during cycling
55 reportedly being unaffected by leg dominance (10).

56 Notwithstanding this evidence, exercise physiology studies generally assume that both legs have
57 similar exercise aerobic capacity and that during cycling they equally contribute to the work that is
58 produced, with the characteristics of whole-body physiological responses (e.g., $\dot{V}O_2$) being the
59 summation of homogenous responses that originate from the DOM_{LEG} and $NDOM_{LEG}$. In support of
60 these assumptions, studies assessing parameters of aerobic function of the right and the left legs have
61 showed no inter-limb differences (45, 60). Additionally, even in studies purposely investigating the
62 effect of leg-dominance, $\dot{V}O_{2peak}$ of the DOM_{LEG} was not different from that of the $NDOM_{LEG}$, with or
63 without normalization for lean mass (9, 42, 63). Similarly, no difference in gross efficiency seemed to
64 exist between the DOM_{LEG} and $NDOM_{LEG}$ when exercising at the same absolute intensity (9).
65 However, a caveat of the studies looking at differences between the DOM_{LEG} and $NDOM_{LEG}$ is that
66 they used “unassisted” single-leg cycling modes, which, due to the accentuated engagement of
67 ipsilateral hip flexor muscles, are less efficient and are associated with greater perception of discomfort
68 (1, 8). Thus, localized pain may lead to exercise failure before the attainment of the “true” maximal
69 aerobic power, regardless of leg-dominance. On the contrary, the use of counterweighted single-leg
70 cycling has been reported to reduce the reliance on the hip flexor muscles (18), which facilitates the

71 tolerability of higher exercise intensity (1, 45). Thus, it is necessary to investigate whether $\dot{V}O_{2\text{peak}}$
72 would differ between the DOM_{LEG} and the NDOM_{LEG} when using counterweighted single-leg cycling
73 exercise.

74 In the context of single-leg exercise, it is well known that peak aerobic capacity is not limited by
75 cardiac output (\dot{Q}), and that there is a greater availability of blood to the exercising (single) leg than
76 during double-leg exercise (16, 36). As a result, the increase in local O_2 delivery (\dot{Q}_m) to utilization
77 ($\dot{V}O_{2m}$) ratio (i.e., $\dot{Q}_m/\dot{V}O_{2m}$) reduces the reliance on O_2 extraction (%) at any given intensity and
78 promotes the achievement of greater maximal O_2 flux rates (36, 52, 57). Moreover, when exercise is
79 performed at a similar relative intensity, the net release of lactate from the exercising leg is lower
80 during single- compared with double-leg cycling (36). While these hemodynamic adjustments during
81 single-leg exercise are well established from a systemic perspective (44), they have not been
82 investigated in conjunction with local indices of muscle deoxygenation nor in relation to leg-
83 dominance. Furthermore, although exercise intensity “thresholds” seemingly occur at the same $\dot{V}O_2$
84 with single- and double-leg cycling (50), it is unknown whether one leg is capable of sustaining greater
85 power outputs than the other while maintaining steady-state metabolic responses during constant-load
86 exercise. Given that mitochondrial capacity exceeds the O_2 delivery capacity during whole-body
87 exercise (7), metabolic stability may be possible at higher relative power outputs with tasks involving a
88 small muscle mass. Collectively, these are important considerations that need to be addressed given
89 that: *i*) local deoxygenation responses (as measured by the near-infrared spectroscopy (NIRS)-derived
90 deoxy-hemoglobin [HHb] signal) have been associated with high local O_2 flux rates (51), and *ii*)
91 whole-body submaximal aerobic performance is a function of the ability of the working muscles to
92 sustain high rates of ATP resynthesis while preserving local metabolic stability (53).

93 Thus, the purpose of this study was to perform a thorough characterization of the physiological
94 responses in the DOM_{LEG} and NDOM_{LEG} during maximal and submaximal double-leg and

95 counterweighted single-leg cycling while also characterizing local deoxygenation responses. Given that
96 muscle activation patterns are similar between the DOM_{LEG} and $NDOM_{LEG}$ during counterweighted
97 single-leg cycling (10), and that cycling knee-joint forces during this exercise mode are similar to
98 double-leg cycling (5, 18), the use of counterweighted single-leg cycling permitted the examination,
99 under relatively constant neuromuscular conditions, of potential differences in maximal and
100 submaximal aerobic capacity as well as deoxygenation responses between the DOM_{LEG} vs $NDOM_{LEG}$
101 and between single-leg vs double-leg cycling.

102 **METHODS**

103 *Participants*

104 A group of recreationally-active men ($n=12$; mean \pm SD values: age 30 ± 8 yr; weight 77 ± 11 kg;
105 height 175 ± 8 cm) voluntarily participated in the study. Participants were aware of the risks and
106 benefits of participating in the study, and all signed an informed consent that was approved by the local
107 research ethics board, in compliance with the latest version of the declaration of Helsinki. All
108 participants were nonsmokers, free of any musculoskeletal condition that could limit their maximal
109 exercise exertion, and not undergoing any medical treatment that could alter their cardiovascular
110 responses to exercise.

111 *Procedures*

112 Each participant visited the laboratory on a minimum of ten occasions to complete the following tests:
113 *i)* two double-leg ramp-incremental tests, *ii)* two counterweighted single-leg ramp-incremental tests
114 (one for each leg), and *iii)* six to eight constant-load trials to determine the power output at maximal-
115 lactate steady state ($MLSS_p$) for double-leg and counterweighted single-leg cycling. Each test was
116 separated by at least 48 hours and performed at a similar time of the day in an environmentally
117 controlled laboratory (temperature: 19-20°C; humidity 50-60%). All participants adhered to the

118 following pre-test instructions: *i*) no vigorous physical activity the day prior to each test, and *ii*) no food
119 or caffeinated beverages for at least 2 and 8 hours, respectively, prior to each test. Participants were
120 blinded to the power output and to the elapsed time during all sessions but received visual feedback on
121 their pedal cadence – which was selected during the first testing session of each condition (i.e., double-
122 leg and counterweighted single-leg) and maintained consistent during the following visits. The position
123 of the handlebar and the seat was recorded during the first visit and kept consistent for the subsequent
124 visits. Additionally, during all experimental conditions participants wore cycling shoes that attached to
125 the pedals.

126 During each counterweighted single-leg test, the electromagnetically-braked cycle ergometer
127 (Velotron; RacerMate, Seattle, Wa) was fitted with a custom-built pedal that held a 6.84 kg
128 counterweight. During these trials the non-exercising leg was kept in a resting position on a stationary
129 platform. Two familiarization trials with this setup were performed after the two double-leg ramp tests.
130 Before each counterweighted single-leg cycling test, a 4-min double-leg cycling baseline was
131 performed to allow the subsequent normalization of the electromyographic (EMG) signal of the vastus
132 lateralis (see *data analysis* section). Lateral preference was assessed by means of the Waterloo
133 Footedness Questionnaire (17).

134 *Ramp-incremental test.* The ramp incremental test consisted of a 4-min baseline cycling stage at 50 W
135 followed by 30 W·min⁻¹ and 10-15 W·min⁻¹ continuous increments in power output for double-leg and
136 counterweighted single-leg cycling exercise, respectively. The ramp-incremental test was stopped when
137 participants failed to maintain the targeted cadence by 10 rpm for more than ten consecutive seconds
138 despite strong verbal encouragement, or when volitional exhaustion ensued.

139 *Constant-load exercise.* A series of constant-load rides were performed to establish MLSS_p (and 10 W
140 above MLSS (MLSS₊₁₀)) for double-leg and for both the DOM_{LEG} and NDOM_{LEG} during

141 counterweighted single-leg cycling. Each ride was performed for 30 min or to exhaustion, which ever
142 occurred earlier. $MLSS_p$ corresponded to the highest power output that elicited a difference in blood
143 lactate concentration ($[La^-]_b$) between the 10th and the 30th min of exercise ≤ 1 mM (4). The power
144 output for the first double-leg constant-load trial was determined from a mathematical equation
145 developed in our laboratory (28). For counterweighted single-leg cycling, the power output of the first
146 constant-load trial was set at 65% of double-leg $MLSS_p$ because this mode of exercise permits the
147 tolerance of greater workloads per leg than what would be predicted by simply dividing the double-leg
148 $MLSS_p$ by two (8). Regardless of the exercise mode, the resistance for the subsequent constant-load
149 rides was either increased or decreased by 10 W depending on $[La^-]_b$ responses. $[La^-]_b$ was measured
150 during baseline and at regular intervals (i.e., every 5 minutes) after the power output was increased to
151 the predetermined value. At 10th and 30th min, measures of $[La^-]_b$ were taken in triplicate and the
152 average of the two closest was used for subsequent analyses. Double-leg $MLSS_p$ was established before
153 the DOM_{LEG} and $NDOM_{LEG}$ single-leg $MLSS_p$. The first DOM_{LEG} and $NDOM_{LEG}$ counterweighted
154 single-leg trial was randomly assigned. Thereafter, these trials were alternately performed during the
155 subsequent visits.

156 *Data collection.* Gas exchange and ventilatory variables were measured using a metabolic cart (Quark
157 CPET, Cosmed, Rome, Italy). The breath-by-breath system was comprised of a low-dead space turbine
158 and gas analyzers that were calibrated as per manufacturer's recommendation.

159 An impedance cardiography system (Physioflow, Enduro, Manatec Biomedical, Macheren, France)
160 was used to measure \dot{Q} during the ramp-exercise tests. Briefly, the system relies on variations in
161 transthoracic impedance occurring due to the changes in aorta blood volume to compute stroke volume.
162 \dot{Q} ($L \cdot min^{-1}$) is then calculated by multiplying stroke volume by body surface area and heart rate (13).
163 Positioning of the electrodes and system calibration were performed according to manufacturer's
164 instructions. \dot{Q} data were acquired every 10 seconds.

165 Capillary blood samples were drawn from the finger and immediately analyzed for $[La]_b$ (Biosen C-
166 Line, EKF Diagnostics, Barleben, Germany) during ramp-exercise and constant-load trials.

167 A frequency-domain NIRS system (Oxiplex TSTM, ISS, Champaign, IL) was used in our study to
168 monitor local [HHb] during ramp-exercise. The total-haemoglobin (*tot*[Hb]) signal was also recorded
169 and subsequently used to correct the [HHb] signal for the adipose tissue thickness (see *Data analysis*
170 section). The NIRS probe was composed of eight laser diodes operating at two wavelengths ($\lambda = 690$
171 and 828 nm, four at each wavelengths), which were pulsed in rapid succession, and a photomultiplier
172 tube. The lightweight plastic NIRS probes consisted of two parallel rows of light-emitting fibers and
173 one detector fibre bundle; the source–detector separations for this probe were 2.0, 2.5, 3.0 and 3.5 cm
174 for both wavelengths. The NIRS probe was placed on the belly of the vastus lateralis muscle of the
175 DOM_{LEG} and NDOM_{LEG} (midpoint between the greater trochanter of the femur and the knee joint). The
176 order during the first two double-leg ramp exercise was randomized. Double-sided tape and an elastic
177 bandage were used to secure the probe in place. An optically dense, black vinyl sheet was used to cover
178 the probe to avoid the intrusion of external light. The apparatus was calibrated on each testing day after
179 a warm-up of at least 30 min, as per the manufacturer recommendations. Data were stored online at an
180 output frequency of 2 Hz, and reduced to 1-s bins for all subsequent analyses within the present study.
181 The area of placement was marked and recorded to ensure consistency for the following visits.

182 A multi-channel surface electromyography system (Delsys Inc, Boston, MA) was used for monitoring
183 EMG at a sampling rate of 1000 Hz. The bipolar surface electrode (41 × 20 × 5 mm) (DE-2.1, Delsys
184 Inc. Boston, MA) was placed on the belly of the vastus lateralis in proximity (longitudinally) of the
185 NIRS probes after the skin area was shaved, abraded, and cleaned to reduce skin impedance. Bi-
186 adhesive and surgical tape were used to secure the electrodes in place. The electrodes were connected
187 to an EMG amplifier which was connected to the acquisition apparatus (Power Lab, ADInstruments,

188 Bella Vista, Australia) linked to a computer software (LabChart 8, ADInstruments, Bella Vista,
189 Australia). Electrodes placement was recorded to ensure consistency between visits.

190 Lower limb lean mass was measured by dual-energy X-ray absorptiometry (Hologic QDR-4500,
191 Hologic, Bedford, MA).

192 *Data analyses*

193 *Ventilatory and gas exchange data.* For each ramp- and constant-power output trial, the breath-by-
194 breath data were edited and aberrant data lying three SD from the local mean were deleted. Thereafter,
195 the $\dot{V}O_2$ data were interpolated on a second-by-second basis. For both double-leg and counterweighted
196 single-leg exercise $\dot{V}O_{2peak}$ corresponded to the highest $\dot{V}O_2$ value computed from a 30-s rolling
197 average. The highest $\dot{V}O_2$ value recorded during the two double-leg ramp-exercise tests corresponded
198 to double-leg $\dot{V}O_{2peak}$. DOM_{LEG} and $NDOM_{LEG}$ $\dot{V}O_{2peak}$ values during counterweighted single-leg
199 cycling were also expressed as ratio of double-leg $\dot{V}O_{2peak}$ (i.e., $\dot{V}O_{2peak}$ ratio) (46). The $\dot{V}O_2$ during the
200 constant-load trials at the 15th and 30th minutes were calculated as the average of 2 min of data
201 surrounding the 15th minute (14th – 16th min) and the last two minutes of the 30-min constant-load
202 exercise. The two minutes average of $\dot{V}O_2$ and respiratory exchange ratio were used to calculate gross
203 efficiency (mechanical work/energy expended per minute) (9).

204 During double-leg ramp-exercise, we used a mono-exponential function and nonlinear least-squares
205 regression (34) to compute the $\dot{V}O_2$ functional gain (G_{ramp}):

$$206 \quad \dot{V}O_2(t) = \dot{V}O_{2BSL} + \Delta\dot{V}O_{2ss} \cdot (t - \tau [1 - e^{-t/\tau}])$$

207 where $\dot{V}O_2(t)$ is the value of $\dot{V}O_2$ at any time during the ramp, $\dot{V}O_{2BSL}$ is the baseline ramp value,
208 $\Delta\dot{V}O_{2ss}$ is the increment above $\dot{V}O_{2BSL}$ required for the power output at time t , and τ is the effective
209 time constant of the response. The fitting window was constrained from the onset ($t = 0$) to the end of

210 the ramp-exercise. The gain of the response was computed in relation to time but converted to power
211 output and expressed as $\Delta\dot{V}O_2/PO$ ($\text{ml}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$).

212 Given the well-documented departure from linearity of the $\dot{V}O_2$ response during single-leg ramp-
213 exercise (40, 45), a piecewise equation with two linear segments was used to fit the $\dot{V}O_2$ data as a
214 function of power output and calculate the $\dot{V}O_2$ functional gain in the two regions of ramp-exercise (G_1
215 and G_2):

$$216 \quad f = \text{if}(PO < TD_{PO} \text{ use } g(t), \text{ else } h(t)); g(t) = i_1 + s_1t; i_2 = i_1 + s_1t; h(t) = i_2 + s_2t - TD_{PO}$$

217 where f is the piecewise function, PO is the power output and g and h are $\dot{V}O_2$, TD_{PO} is the power
218 output corresponding to the intersection of the two regression lines, i_1 and i_2 are the intercepts of the
219 first and second linear function, respectively, and s_1 (i.e., G_1) and s_2 (i.e., G_2) are the slopes with respect
220 to power output ($\Delta\dot{V}O_2/PO$ expressed in $\text{ml}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$).

221 *Cardiac output.* \dot{Q} data were edited and aberrant data lying three SD from the local mean were deleted.
222 Thereafter, the \dot{Q} data were interpolated on a second-by-second basis. Baseline \dot{Q} corresponded to last
223 two minutes of baseline before the ramp-onset, whereas \dot{Q}_{peak} corresponded to the highest \dot{Q} computed
224 from a 30-s rolling average. Baseline \dot{Q} values and \dot{Q}_{peak} were used to compute the functional gain with
225 respect to $\dot{V}O_2$ ($\Delta\dot{Q}/\dot{V}O_2$ expressed in $\text{L}\cdot\text{min}^{-1}\cdot\text{L}^{-1}(\dot{V}O_2)$).

226 *Adipose tissue thickness correction of [HHb] signals.* The [HHb] signal was analyzed after accounting
227 for the adipose tissue thickness under the area of NIRS interrogation (15). Briefly, a Harpenden skin
228 caliper (Baty Int., West Sussex, UK) was used to measure the adipose tissue thickness (mm) in the area
229 of NIRS probe placement. The same investigator took measurements in duplicate and the average of
230 the two was used. Subsequently, a linear regression analysis of the relationship between the adipose
231 tissue thickness and resting tot[Hb] was calculated and the measured [HHb] data were corrected to a
232 common adipose tissue thickness of 0 mm (15).

233 *[HHb]* during ramp incremental test. The *[HHb]* data recorded during the ramp-incremental test on the
234 vastus lateralis muscle were plotted against time and modeled with the following segmented piece-wise
235 linear fit, as previously described (67):

$$236 \quad f = \text{if}(x < BP, g(x), h(x))$$

$$237 \quad g(x) = i_1 + (s_1 \cdot x)$$

$$238 \quad i_2 = i_1 + (s_1 \cdot BP)$$

$$239 \quad h(x) = i_2 + (s_2 \cdot (x - BP))$$

240 fit f to y ,

241 where f is the double-linear function, x is time and y is *[HHb]*, BP is the time coordinate corresponding
242 to the interception of the two regression lines (i.e., *[HHb]* breakpoint), i_1 and i_2 are the intercepts of the
243 first and second linear function, respectively, and s_1 and s_2 are the slopes. Model parameter estimates
244 for each participant were determined by linear least-square regression analysis. A preliminary fit was
245 used to identify and delete aberrant data that were ± 3 SD from the local mean. The model fit was used
246 from the onset of the systematic increase in the *[HHb]* signal until the last data point corresponding to
247 the end of the test. The power output corresponding to the *[HHb]* breakpoint was then determined by
248 linear interpolation. Subsequently, the slope of change in the *[HHb]* signal during ramp-exercise was
249 calculated based on the relative increase in power output (e.g., 0%= baseline; 100= PO_{peak}).

250 *Surface electromyography.* The EMG data recorded during the ramp-exercise were amplified, band-
251 pass filtered (5 – 500 Hz), rectified, and computed as 1-s root mean square (RMS) amplitude.
252 Afterwards, regardless of condition, the edited EMG data were normalized to the average of the last
253 two minutes of the baseline double-leg cycling at 50 W and, thereafter, averaged into 10% of peak
254 power output interval-bins for subsequent statistical analysis. The specific normalization strategy was

255 selected as it is representative of the actual dynamic muscular patterns during cycling. Furthermore, it
256 allowed the comparison of muscle activation between double-leg and counterweighted single-leg
257 cycling exercise.

258 *Statistical Analysis*

259 Data are presented as mean±standard deviation (SD). Repeated-measures ANOVA was performed to
260 detect potential differences in $\dot{V}O_{2\text{peak}}$, PO_{peak} , HR_{max} , \dot{Q}_{peak} , \dot{Q} gain with respect to $\dot{V}O_2$, peak $[La^-]_b$,
261 $[HHb]$ amplitudes, and $[HHb]$ breakpoints between the different exercise modes during ramp-exercise.
262 Furthermore, repeated-measures ANOVA was performed to detect differences in EMG at 10 %
263 intervals during the ramp-exercise across the different exercise-modes. Pearson's coefficients were
264 calculated to evaluate the level of correlation between the amplitudes of the $[HHb]$ signal and $\dot{V}O_{2\text{peak}}$.
265 Student's t-tests were used to compare means values for: *i*) lean mass between DOM_{LEG} and
266 $NDOM_{\text{LEG}}$, *ii*) $\dot{V}O_{2\text{peak}}$ between the DOM_{LEG} and $NDOM_{\text{LEG}}$ normalized for lean mass, *iii*) $\dot{V}O_2$ at the
267 15th and the 30th min during the constant-load trials. Where appropriate a Bonferroni's *post hoc*
268 analysis was performed. Statistical significance was set at a α level of <0.05.

269 **RESULTS**

270 Ramp exercise

271 Peak physiological responses to double-leg and counterweighted single-leg ramp-exercise are displayed
272 in Table 1. PO_{peak} , $\dot{V}O_{2\text{peak}}$, \dot{Q}_{peak} , HR_{max} , and $[La^-]_b$ were higher during double-leg compared with
273 counterweighted single-leg ramp-exercise ($P<0.05$). During counterweighted single-leg ramp exercise,
274 PO_{peak} , $\dot{V}O_{2\text{peak}}$, and \dot{Q}_{peak} were $7.5\pm 5.7\%$, $6.0\pm 5.4\%$, and $6.2\pm 6.5\%$ higher when exercising with the
275 DOM_{LEG} compared with the $NDOM_{\text{LEG}}$, respectively ($P<0.05$). The $\dot{V}O_{2\text{peak}}$ *ratio* values for the
276 DOM_{LEG} and $NDOM_{\text{LEG}}$ were 0.84 ± 0.05 and 0.79 ± 0.05 , respectively. Figure 1 (A,B) depicts the group
277 mean data for $\dot{V}O_2$ and \dot{Q} during ramp-exercise for each exercise mode. There was no difference in the

278 gain of \dot{Q} with respect to $\dot{V}O_2$ between double-leg and counterweighted single-leg DOM_{LEG} and
279 $NDOM_{LEG}$ ramp-exercise (4.9 ± 0.8 , 5.2 ± 1 , 5.0 ± 0.9 $L\cdot min^{-1}\cdot L^{-1}(\dot{V}O_2)$, respectively; $P>0.05$).

280 *Lower limbs lean mass.* No differences in lean mass between the DOM_{LEG} (11.0 ± 1.3 kg) and
281 $NDOM_{LEG}$ (10.8 ± 1.2 kg) were detected ($P>0.05$). There was no significant correlation between lean
282 mass and $\dot{V}O_{2peak}$ of the DOM_{LEG} ($r = -0.06$, $P>0.05$), nor between lean mass and $\dot{V}O_{2peak}$ of the
283 $NDOM_{LEG}$ ($r = 0.32$, $P>0.05$). The difference in $\dot{V}O_{2peak}$ between the DOM_{LEG} and $NDOM_{LEG}$
284 persisted even when $\dot{V}O_{2peak}$ values were normalized by leg-specific lean mass. In this case, the
285 normalized $\dot{V}O_{2peak}$ for the DOM_{LEG} was 0.264 ± 0.052 $mL\cdot g^{-1}\cdot min^{-1}$ whereas for the $NDOM_{LEG}$ was
286 0.250 ± 0.039 $mL\cdot g^{-1}\cdot min^{-1}$ (% difference = $4.57\pm 6.18\%$; $P<0.05$).

287 *[HHb] signal.* One individual was excluded from the analysis as the quality of his [HHb] signal during
288 ramp-exercise was not satisfactory. Table 2 displays the values for baseline, amplitude and slope of
289 increase of the [HHb] signal. Figure 2 shows the dynamic profiles of [HHb] during ramp-exercise as a
290 function of relative (panels A and B) and absolute (panel C) changes in power output. There was no
291 difference at baseline in the [HHb] signal across the exercise modes ($P>0.05$). However, the [HHb]
292 amplitudes during double-leg and counterweighted single-leg cycling were greater in the DOM_{LEG}
293 compared with the $NDOM_{LEG}$ ($P<0.05$). $S1$ of the [HHb] response was similar between legs across the
294 exercise modes when calculated against relative power output ($P>0.05$). However, when calculated
295 against absolute power output (W), $S1$ was greater during single-leg compared to double-leg ($P<0.05$).
296 There was no difference in $S2$ amongst all conditions ($P>0.05$). The [HHb] breakpoints in the DOM_{LEG}
297 and the $NDOM_{LEG}$ during double-leg cycling were not different in terms of $\%PO_{peak}$ (75 ± 7 vs 70 ± 10
298 %; $P>0.05$), nor in terms of $\%\dot{V}O_{2peak}$ (83 ± 8 vs 80 ± 9 %; $P>0.05$). Similarly, the [HHb] breakpoints in
299 the DOM_{LEG} and the $NDOM_{LEG}$ during single-leg cycling were not different in terms of $\%PO_{peak}$
300 (63 ± 10 vs $63\pm 9\%$; $P>0.05$), nor in terms of $\%\dot{V}O_{2peak}$ (68 ± 10 vs $64\pm 9\%$; $P>0.05$). However, the [HHb]

301 breakpoints during counterweighted single-leg cycling occurred at lower fractions of $\dot{V}O_{2\text{peak}}$ and
302 PO_{peak} compared with double-leg cycling ($P < 0.05$).

303 Figure 3 (panels A-D) displays the correlation plots between the [HHb] amplitudes and the $\dot{V}O_{2\text{peak}}$
304 among legs and exercise modes. There was a strong correlation between the amplitude of the [HHb]
305 signals of both the DOM_{LEG} and the $NDOM_{\text{LEG}}$ during double-leg cycling with double-leg $\dot{V}O_{2\text{peak}}$
306 (DOM_{LEG} : $r = 0.86$, $P < 0.05$; $NDOM_{\text{LEG}}$: $r = 0.91$, $P < 0.05$). A significant correlation was also detected
307 between the [HHb] amplitude during counterweighted single-leg cycling of the DOM_{LEG} and the leg-
308 specific $\dot{V}O_{2\text{peak}}$ ($r = 0.64$, $P < 0.05$) but not for the [HHb] amplitude during counterweighted single-leg
309 cycling of the $NDOM_{\text{LEG}}$ and the leg-specific $\dot{V}O_{2\text{peak}}$ ($r = 0.54$, $P > 0.05$).

310 *EMG*. The peak RMS at the end of double-leg ramp-exercise was $393 \pm 150\%$ for the DOM_{LEG} and
311 $355 \pm 161\%$ for the $NDOM_{\text{LEG}}$ ($P > 0.05$); during counterweighted single-leg cycling the peak RMS were
312 $391 \pm 129\%$ and $406 \pm 150\%$ for DOM_{LEG} and $NDOM_{\text{LEG}}$, respectively ($P > 0.05$). There was no
313 difference in EMG between the DOM_{LEG} and the $NDOM_{\text{LEG}}$ at peak ramp-exercise ($P > 0.05$).
314 Throughout the ramp-exercise, the EMG signal was greater during single- compared with double-leg
315 cycling only within the first 10% of the ramp-exercise (irrespective of leg dominance) ($P < 0.05$).
316 Thereafter, no differences were detected between exercise modes nor between legs ($P > 0.05$). Figure 4
317 displays the dynamic profiles of EMG during ramp-exercise between legs and exercise modes.

318 Constant-load exercise

319 $\dot{V}O_2$ responses to double-leg and counterweighted single-leg constant-load cycling at $MLSS_p$ and
320 $MLSS_{+10}$ are displayed in Table 3. Figure 5 (panels A-D) displays the group mean data for $\dot{V}O_2$, and
321 $[La^-]_b$ at $MLSS_p$ and $MLSS_{+10}$ for double-leg and counterweighted single-leg cycling.

322 *Double-leg*. During double-leg constant-load cycling, time-to-exhaustion at $MLSS_{+10}$ during double-leg
323 was 28.6 ± 4.0 min. $\dot{V}O_2$ stabilized at $MLSS_p$ within the first 15 min and was stable until the end of the

324 trial (15th min = 2.68±0.25 L·min⁻¹; end-trial = 2.72±0.24 L·min⁻¹; $P>0.05$) but progressively increased
325 at MLSS₊₁₀ (15th min = 2.78±0.29 L·min⁻¹; end-trial = 2.87±0.28 L·min⁻¹; $P<0.05$). Delta [La⁻]_b
326 between 10th and 30th min during MLSS_p and MLSS₊₁₀ were 0.4±0.5 and 1.5±0.6 mM ($P<0.05$),
327 respectively.

328 *Counterweighted single-leg constant-load exercise.* During counterweighted single-leg cycling, MLSS_p
329 (W) of the DOM_{LEG} was greater than MLSS_p of the NDOM_{LEG} (Table 3). MLSS_p (W) of the DOM_{LEG}
330 and NDOM_{LEG} during counterweighted single-leg cycling were highly correlated to double-leg MLSS_p
331 ($r = 0.80$ and 0.81 , respectively; $P<0.05$). Time-to-exhaustions at MLSS₊₁₀ during the DOM_{LEG} and the
332 NDOM_{LEG} counterweighted single-leg cycling were 26.8±6.0 and 26.0±7.4 min, respectively. There
333 was no difference in gross efficiency between the DOM_{LEG} (20.0±2.3%) and the NDOM_{LEG}
334 (19.5±1.9%) during their respective MLSS_p ($P>0.05$). $\dot{V}O_2$ of the DOM_{LEG} was stable at MLSS_p (15th
335 min = 2.16±0.25 L·min⁻¹; end-trial = 2.18±0.24 L·min⁻¹; $P>0.05$) but progressively increased at
336 MLSS₊₁₀ (15th min = 2.29±0.28 L·min⁻¹; end-trial = 2.38±0.32 L·min⁻¹; $P<0.05$). Similarly, $\dot{V}O_2$ of the
337 NDOM_{LEG} was stable at MLSS_p (15th min = 2.07±0.29 L·min⁻¹; end-trial = 2.08±0.31 L·min⁻¹; $P>0.05$)
338 but progressively increased at MLSS₊₁₀ (15th min = 2.24±0.32 L·min⁻¹; end-trial = 2.33±0.32 L·min⁻¹;
339 $P<0.05$). Delta [La⁻]_b of the DOM_{LEG} at MLSS_p and MLSS₊₁₀ were -0.2±0.5 and 1.3±0.2 mM,
340 respectively. Delta [La⁻]_b of the NDOM_{LEG} at MLSS_p and MLSS₊₁₀ were -0.2±0.4 and 1.7±0.9 mM,
341 respectively.

342 **DISCUSSION**

343 The aim of this study was to characterize the physiological responses in the DOM_{LEG} and the
344 NDOM_{LEG} double-leg and counterweighted single-leg cycling in order to gain further insights on the
345 potential mechanisms that determine central and peripheral responses to maximal and submaximal
346 exercise. The main findings were as follows: *i*) during counterweighted single-leg cycling, the DOM_{LEG}

347 achieved greater $\dot{V}O_{2\text{peak}}$ values during ramp-exercise compared with the $NDOM_{\text{LEG}}$; *ii*) the DOM_{LEG}
348 was able to sustain greater power outputs compared with the $NDOM_{\text{LEG}}$ at an intensity that reflected
349 the critical intensity for counterweighted single-leg exercise; *iii*) during double-leg cycling, the
350 amplitudes of the [HHb] signal for each leg were highly correlated with $\dot{V}O_{2\text{peak}}$ and were greater in the
351 DOM_{LEG} compared with the $NDOM_{\text{LEG}}$; *iv*) the pattern of increase of the [HHb] signal during
352 counterweighted single-leg resembled that typically observed during double-leg cycling, although the
353 onset of the characteristic plateau in the [HHb] signal occurred at a lower leg-specific percent of
354 $\dot{V}O_{2\text{peak}}$ during single-leg compared with double-leg cycling.

355 *DOM_{LEG} vs NDOM_{LEG} during double-leg and counterweighted single-leg cycling.*

356 In contrast to previous observations (9, 42, 63), the present study found that during single-leg cycling
357 the DOM_{LEG} achieved greater PO_{peak} and $\dot{V}O_{2\text{peak}}$ values compared with the $NDOM_{\text{LEG}}$. In absolute
358 terms, the inter-limb difference in $\dot{V}O_{2\text{peak}}$ was ~6% and persisted (~5%) even after the $\dot{V}O_{2\text{peak}}$ values
359 were normalized by leg-specific lean mass. This observation is in contrast to previously reported data
360 showing that inter-limb discrepancies in absolute $\dot{V}O_{2\text{peak}}$ between the DOM_{LEG} and the $NDOM_{\text{LEG}}$
361 during single-leg cycling were due to differences in lean mass (63). The authors indicated that, in a
362 scenario where \dot{Q}_m is not a limiting factor (36), a greater muscle mass can achieve greater power
363 outputs and, thus, higher absolute metabolic rates (46, 63). However, in the present study, given that
364 differences in $\dot{V}O_{2\text{peak}}$ persisted even after normalization for lean mass of the DOM_{LEG} and the
365 $NDOM_{\text{LEG}}$, it is likely that other peripheral factors contributed to the observed differences in PO_{peak} and
366 $\dot{V}O_{2\text{peak}}$.

367 From this perspective, we characterized the profiles of the [HHb] signal during double-leg and single-
368 leg ramp exercise in the DOM_{LEG} and the $NDOM_{\text{LEG}}$ (Figure 2, A-C). The [HHb] signal represents an
369 index of local fractional O_2 extraction (21), and its amplitude during double-leg incremental-exercise

370 has been suggested to relate to the capacity of the active muscle fibers to extract O_2 from the
371 surrounding microcirculation and has been found to be positively correlated to $\dot{V}O_{2peak}$ (51). This latter
372 speculation agrees with our findings (Figure 3). Furthermore, we found that the [HHb] amplitudes were
373 greater in the DOM_{LEG} compared with the $NDOM_{LEG}$ during both double- and counterweighted single-
374 leg cycling. In addition to this, we found that the power output and the $\dot{V}O_2$ at MLSS during single-leg
375 cycling were greater in the DOM_{LEG} than in the $NDOM_{LEG}$ (~ 10 W and ~ 100 mL \cdot min $^{-1}$, respectively)
376 (Table 3, Figure 5, A and B); interestingly, despite this increased power output and metabolic rate, $[La^-$
377 $]_b$ values at the respective $MLSS_p$ were similar between the two legs (Figure 5, C and D).

378 Collectively, these observations support the idea that dissimilar peripheral adaptations may play an
379 important role in the differences in maximal and submaximal aerobic capacity between the DOM_{LEG}
380 and the $NDOM_{LEG}$. Indeed, superior capacity for fractional O_2 extraction and higher metabolic rates at
381 $MLSS_p$ (or similar “thresholds”) are both associated with enhanced oxidative capacity (24, 33, 48).
382 However, whether these superior peripheral adaptations in the DOM_{LEG} stem from functional or
383 structural differences is presently unknown. In this perspective, inter-limb “asymmetries” in functional
384 hemodynamics responses, potentially leading to a more efficient diffusion of O_2 at the capillary-to-
385 muscle interface (19, 36), are possible when one limb is regularly exposed to a greater metabolic stress
386 compared with the other limb (59, 64, 65). However, this was likely not the case in the present study, as
387 none of our participants was engaged in unilateral-type activities that would be expected to cause
388 enhanced functional adaptations of the DOM_{LEG} . Interestingly, a recent investigation observed, in a
389 large group of resistance-trained men, that type I fibers were more abundant in the DOM_{LEG} compared
390 with the $NDOM_{LEG}$ (3). Although the biological reasons underpinning these asymmetries in fiber type
391 distribution are elusive at this moment, these observations may support the interpretation of a greater
392 oxidative potential of the DOM_{LEG} . Indeed, type I fibers have a greater oxidative capacity, an increased
393 number of capillaries perfusing each fiber, and a greater $\dot{Q}_m/\dot{V}O_{2m}$ ratio (47, 61), all of which are

394 important features for the achievement of high O_2 flux rates. This interpretation, however, must be
395 taken with caution as in previous studies fiber type distribution between legs was not different (43, 60).
396 Alternatively, it could be hypothesized that a superior neuromuscular control of the DOM_{LEG} (e.g., a
397 smaller amount of muscle fibers needed to be recruited to sustain a given power output) would result in
398 a lower ATP requirement to support a given metabolic rate (i.e., improved efficiency). However, we
399 found no difference in the pattern of activation of the vastus lateralis muscle in the $NDOM_{LEG}$
400 compared to the DOM_{LEG} throughout the counterweighted single-leg (nor double-leg) ramp-exercise
401 (Figure 4). Additionally, no difference in gross efficiency and $\dot{V}O_2$ functional gain (i.e., G_1 and G_2)
402 were found between the DOM_{LEG} and the $NDOM_{LEG}$ when exercising at $MLSS_p$ and during the ramp-
403 exercise, respectively. Thus, considering our findings and those of a previous study which also showed
404 no difference in efficiency between the DOM_{LEG} and the $NDOM_{LEG}$ (9), it is unlikely that a potential
405 enhanced neuromuscular control of the DOM_{LEG} played a major role.

406 *DOM_{LEG} vs $NDOM_{LEG}$: implications for double-leg cycling*

407 There is evidence of marked heterogeneity in the way O_2 is delivered and utilized within the same
408 muscle or muscular groups (30, 41). The present study provides novel information showing not only
409 that the DOM_{LEG} and the $NDOM_{LEG}$ may have different capacities to deliver and utilize O_2 but also
410 that, when tested separately using counterweighted single-leg cycling, they differ in terms of maximal
411 and submaximal aerobic capacity. The question that arises from these observations is, how do these
412 inter-limb differences affect double-leg cycling aerobic performance? In the context of maximal
413 aerobic exercise, given that mitochondrial potential “exceeds” O_2 delivery capacity within the active
414 muscles (7), one possibility is that, even when marked inter-limb differences exist, the “weaker” leg –
415 from an oxidative capacity perspective – may not be a factor limiting whole-body $\dot{V}O_{2peak}$. However,
416 given that O_2 diffusive limitations may exist even in the presence of a reserve in mitochondrial capacity

417 (56), it could still be possible that the “weakest link” (i.e., the $NDOM_{LEG}$ in the present study) may set
418 the peripheral upper limit for whole body $\dot{V}O_{2peak}$. Additionally, assuming a perfect symmetry in the
419 generated power output between legs, the finding of a lower $MLSS_p$ in the $NDOM_{LEG}$ compared to
420 DOM_{LEG} may imply that during double-leg cycling, the $NDOM_{LEG}$ might contribute more to the
421 progressive loss of whole-body metabolic stability. From this perspective, given that fatigue-sensitive
422 afferent feedback (i.e. group III/IV) from exercising muscles is an important modulator of
423 compensatory (e.g., increase in ventilation (2)) and perceptual responses (26), it is possible that
424 increased feedback from the $NDOM_{LEG}$ may trigger and/or alter these responses earlier or to a greater
425 extent compared to those from the DOM_{LEG} during the task. It is important to acknowledge, however,
426 that the generation of power output during double-leg cycling in “*real life*” scenarios may not be
427 symmetric between legs (11). In this circumstance, a neural strategy that promotes a higher
428 contribution to the generated power output of the leg with the greatest oxidative capacity (e.g., the
429 DOM_{LEG} in the context of the present study) could be hypothesized; this strategy, in line with the
430 *optimal control theory* for motor control (69), could be adopted to *i*) optimize metabolic efficiency, and
431 *ii*) minimize neural drive and perceptual responses (25). However, future studies will be required to test
432 this hypothesis.

433 *Single-leg vs Double-leg; implications for $\dot{V}O_2$ and [HHb] responses*

434 In this study, the $\dot{V}O_2$ response during counterweighted single-leg cycling was consistent with the
435 notion that above the critical intensity of exercise (in this case represented by $MLSS$), attainment of
436 $\dot{V}O_2$ steady-state is no longer feasible (55). It is interesting to note, however, that during
437 counterweighted single-leg cycling, the “upper limit” at which $\dot{V}O_2$ steady-state was attainable
438 represented ~80% of the $\dot{V}O_2$ corresponding to the double-leg $MLSS_p$. The augmented capacity of the
439 (single) exercising leg to sustain work in steady-state condition at a greater metabolic rate compared to
440 double-leg is likely due to the increase in O_2 availability during single-leg exercise. Indeed, an

441 increased O₂ availability enhances the “critical metabolic rate” at which oxidative phosphorylation is
442 able to provide all the ATP required by the task (70). In the context of double-leg cycling, this implies
443 that, at any submaximal power output, increasing local O₂ delivery (by convection or diffusion) will
444 reduce the reliance on substrate level phosphorylation and the magnitude of the $\dot{V}O_2$ slow component,
445 with this mechanisms having important implications for the etiology of fatigue and exercise tolerance
446 (22, 35).

447 In agreement with previous reports using single-leg models (either knee-extension (57) or cycling
448 ergometers (38, 45)), the slope of the $\dot{V}O_2$ -to-power output relationship during ramp-exercise was
449 greater and “upwardly-curvilinear” during single-leg compared to double-leg cycling (Figure 1, A). In
450 the context of the present study, there are several putative reasons that might have contributed to the
451 greater and progressively increasing $\dot{V}O_2$ cost for a given change in power output during
452 counterweighted single-leg compared to double-leg ramp-exercise: *i*) earlier/greater activation of type
453 II fibers (36) which might necessitate a greater O₂ cost of contraction; *ii*) disproportional increase of
454 $\dot{V}O_2$ associated with ventilatory and postural muscle activity (16, 54); *iii*) slower rate of increase in
455 power output during single-leg (15-20 W·min⁻¹) vs double-leg ramp-exercise, which allowed more time
456 for muscle $\dot{V}O_2$ kinetics to be developed and expressed at the level of the mouth (27, 71); *iv*) greater
457 and progressively increasing external forces associated with the counterweight load applied on the
458 contralateral crank, which might increase the O₂ cost of pedaling at a given power output. Although
459 discriminating among these factors would require uniform exercise protocols between double- and
460 single-leg exercise (i.e., similar ramp-rate) as well as continuous measurements of leg blood flow, $\dot{V}O_2$,
461 and EMG, the analysis of the [HHb] patterns from the present study offers some insights. We found
462 that the *slope 1* of the [HHb] signal during ramp-exercise was unchanged between counterweighted
463 single-leg and double-leg cycling when normalized to the relative power output (Figure 2; Table 2).
464 This observation could imply that the balance between O₂ delivery and utilization remained unaltered

465 between the two exercise modes, which is partly confirmed by the similar patterns of increase in EMG
466 between single- vs double-leg cycling (Figure 4). It must be acknowledged, however, that a greater
467 mass-specific blood flow during counterweighted single-leg exercise might have promoted a greater
468 $\dot{Q}_m/\dot{V}O_{2m}$ ratio (39), confounding the interpretation of the dynamic changes of the [HHb] signal across
469 different exercise modes. However, the relationship between \dot{Q}_m and $\dot{V}O_{2m}$ during single-leg exercise
470 could be have been preserved considering that the greater mass-specific blood flow could be matched
471 with the greater mass-specific metabolic rate associated with single-leg exercise (38). Overall, these
472 adjustments may have preserved the same dynamics between O_2 delivery and utilization during single-
473 leg exercise. This suggestion finds support in the observation that, similarly to the $\dot{V}O_2$ response, at a
474 given power output there was a greater [HHb] signal during single-leg cycling compared to double-leg
475 cycling (Figure 2, C). Collectively, these observations may justify the hypothesis that the greater O_2
476 cost of counterweighted single-leg cycling may primarily originate within the working musculature of
477 the exercising leg, although some contribution of areas outside of the exercising muscles cannot be
478 excluded (54).

479 The observation of a plateau in the [HHb] signal during counterweighted single-leg exercise is
480 interesting and may help shed light on the debated physiological mechanisms underpinning this
481 phenomenon (6, 20, 29, 32). In this regard, it has been suggested that the plateau in the [HHb] signal
482 during ramp-incremental cycling is explained by a greater $Q_m/\dot{V}O_{2m}$ in the region of NIRS
483 interrogation driven by locally-released vasodilators at metabolic rates similar to, or above, the
484 maximal lactate steady state (49). This redistribution of blood flow would happen at the expenses of
485 less metabolically challenged areas of the quadriceps muscles, and be dictated by the fiber type
486 characteristics of the region investigated (14, 68). Contrarily, it was recently suggested that the
487 levelling-off of the [HHb] signal during double-leg ramp-exercise is caused by the lower O_2 diffusion
488 gradient due to the near-equilibrium between the microvascular and intramyocyte O_2 pressures that is

489 achieved at near-maximal exercise intensities (20). However, if this suggestion were true, a plateau in
490 the [HHb] response should have not occurred during single-leg cycling, as the greater microvascular O₂
491 pressure resulting from the greater mass-specific blood flow (37, 57) should have preserved the O₂
492 diffusion gradient up to near-maximal intensities, thus allowing the [HHb] to continue its increase until
493 exercise termination (i.e., $\dot{V}O_{2\text{peak}}$). Yet, the [HHb] signal during counterweighted single-leg plateaued
494 at even slightly lower percentages of leg-specific $\dot{V}O_{2\text{peak}}$ compared to double-leg cycling (Figure 2).
495 Therefore, while recognizing that a reduced O₂ diffusion gradient will eventually limit the achievement
496 of higher O₂ flux rates at maximal exercise intensity (particularly during double-leg exercise) (58), the
497 present data question whether this mechanism would underpin the [HHb] plateau.

498 *Methodological considerations*

499 An important methodological difference compared with previous studies examining maximal aerobic
500 capacity of the DOM_{LEG} and the NDOM_{LEG} (9, 42, 63) is that in the present study the exercising leg
501 during single-leg cycling was assisted by a weight applied to the contralateral crank. This setup, by
502 reducing the discomfort associated with the excessive engagement of the ipsilateral hip flexor muscles
503 during the upstroke phase (8), might have facilitated the achievement of leg-specific aerobic
504 performance that was closer to the “true” maximum for the limb under investigation. This suggestion is
505 supported by the fact that the average $\dot{V}O_{2\text{peak}}$ ratio (i.e., the ratio between single-leg and double-leg
506 $\dot{V}O_{2\text{peak}}$) was 0.84 for the DOM_{LEG}, while in a previous investigation using “unassisted” single-leg
507 cycling this ratio was 0.76 (46). Therefore, recognizing that inter-limb asymmetries in maximal and
508 submaximal aerobic capacity might be subtle (63), the use of a counterweight may be important for
509 their detection.

510 Furthermore, it is important to consider that the application of the counterweight reduces but does not
511 abolish biomechanical differences between single- vs double-leg cycling (18). Therefore, although we

512 assume similar neuromuscular dynamics between these two exercise modes, potential differences in
513 joint kinematics (which could also be expressed differently in relation to limb dominance) could have
514 played a role in our findings. This is an important methodological consideration for the interpretation of
515 our results, where the [HHb] signal is tightly matched to the level of muscle activity and resultant
516 dynamics of local blood flow (31, 39).

517 Finally, in this study the [HHb] response of the vastus lateralis of the quadriceps was monitored, thus
518 our interpretations related to the amplitudes of this signal are specific to that muscle area. However,
519 given that this muscle is the prime mover (23) during cycling and that the relationship between the
520 [HHb] amplitudes and $\dot{V}O_{2\text{peak}}$ was observed in other muscle areas of the same muscle group (such as
521 the rectus femoris) (51), it can be suggested that the amplitudes of the [HHb] signal in the vastus
522 lateralis may well reflect the “whole-quadriceps” fractional O_2 extraction capacity.

523 **CONCLUSIONS**

524 To summarize, findings from the present study showed that, during single-leg exercise, the DOM_{LEG}
525 achieved greater $\dot{V}O_{2\text{peak}}$ values and was able to sustain greater power outputs with stable metabolic
526 responses compared with the $NDOM_{\text{LEG}}$. While the exact physiological reasons of these differences are
527 difficult to establish, the facts that the [HHb] amplitudes and the $MLSS_p$ were greater in the DOM_{LEG}
528 may suggest the presence of superior peripheral adaptations in this leg compared with the $NDOM_{\text{LEG}}$.
529 These findings have important implications for the design of future studies using counterweighted
530 single-leg cycling. In addition to this, the present study observed that the patterns of increase of the
531 [HHb] signal during counterweighted single-leg were similar to double-leg cycling during the ramp-
532 exercise. This is indicative of similar dynamics during counterweight single-leg and double-leg cycling
533 in the balance between O_2 delivery and utilization.

534 **ACKNOWLEDGMENTS**

535 We express our gratitude to the participants in this study. We also extend our gratitude to Rafael
536 Azevedo for his help during data collection.

537 **GRANTS**

538 This study was supported by National Science and Engineering Research Council of Canada (RGPIN-
539 2016-03698) and the Heart & Stroke Foundation of Canada (1047725).

540 **DISCLOSURES**

541 The authors declare no conflict of interest

542 **AUTHOR CONTRIBUTIONS**

543 DI, LP, AQ, MJM and JMM conceived and designed research; DI and AQ performed experiments; DI
544 analyzed data; DI, LP, MJM, and JMM interpreted results of experiments; DI prepared figures and
545 drafted the manuscript; DI, LP, AQ, MJM, and JMM edited, revised, and approved final version of
546 manuscript.

547 **REFERENCES**

- 548 1. **Abbiss CR, Karagounis LG, Laursen PB, Peiffer JJ, Martin DT, Hawley JA, Fatehee NN,**
549 **Martin JC.** Single-leg cycle training is superior to double-leg cycling in improving the
550 oxidative potential and metabolic profile of trained skeletal muscle. *J Appl Physiol* 110: 1248–
551 1255, 2011.
- 552 2. **Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA.** Group III and
553 IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in
554 humans. *J Appl Physiol* 109: 966–76, 2010.
- 555 3. **Arevalo JA, Lynn SK, Bagley JR, Brown LE, Costa PB, Galpin AJ.** Lower-Limb
556 Dominance, Performance, and Fiber Type in Resistance-trained Men. *Med Sci Sports Exerc* 50:
557 1054–1060, 2018.
- 558 4. **Beneke R.** Maximal lactate steady state concentration (MLSS): experimental and modelling
559 approaches. *Eur J Appl Physiol* 88: 361–9, 2003.
- 560 5. **Bini RR, Jacques TC, Vaz MA.** Joint Torques and Patellofemoral Force during Single-Leg
561 Assisted and Unassisted Cycling. *J Sport Rehabil* 25: 40–47, 2014.
- 562 6. **Boone J, Vandekerckhove K, Coomans I, Prieur F, Bourgois JG.** An integrated view on the
563 oxygenation responses to incremental exercise at the brain, the locomotor and respiratory
564 muscles. *Eur J Appl Physiol* 116: 2085–2102, 2016.
- 565 7. **Boushel R, Gnaiger E, Larsen FJ, Helge JW, González-Alonso J, Ara I, Munch-Andersen**
566 **T, van Hall G, Søndergaard H, Saltin B, Calbet JAL.** Maintained peak leg and pulmonary
567 VO₂ despite substantial reduction in muscle mitochondrial capacity. *Scand J Med Sci Sport* 25:
568 135–143, 2015.

- 569 8. **Burns KJ, Pollock BS, Lascola P, McDaniel J.** Cardiovascular responses to counterweighted
570 single-leg cycling: Implications for rehabilitation. *Eur J Appl Physiol* 114: 961–968, 2014.
- 571 9. **Carpes FP, Diefenthaler F, Bini RR, Stefanyshyn D, Faria IE, Mota CB.** Does leg
572 preference affect muscle activation and efficiency? *J Electromyogr Kinesiol* 20: 1230–1236,
573 2010.
- 574 10. **Carpes FP, Diefenthaler F, Bini RR, Stefanyshyn DJ, Faria IE, Mota CB.** Influence of leg
575 preference on bilateral muscle activation during cycling. *J Sports Sci* 29: 151–159, 2011.
- 576 11. **Carpes FP, Mota CB, Faria IE.** On the bilateral asymmetry during running and cycling - A
577 review considering leg preference. *Phys Ther Sport* 11: 136–142, 2010.
- 578 12. **Carpes FP, Rossato M, Faria IE, Mota CB.** Bilateral pedaling asymmetry during a simulated
579 40-km cycling time-trial. *J Sports Med Phys Fitness* 47: 51–57, 2007.
- 580 13. **Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald-Mammosser M,**
581 **Mettauer B, Geny B, Lonsdorfer J.** A new impedance cardiograph device for the non-invasive
582 evaluation of cardiac output at rest and during exercise: Comparison with the "direct" Fick
583 method. *Eur J Appl Physiol* 82: 313–320, 2000.
- 584 14. **Copp SW, Hirai DM, Musch TI, Poole DC.** Critical speed in the rat: implications for hindlimb
585 muscle blood flow distribution and fibre recruitment. *J Physiol* 588: 5077–87, 2010.
- 586 15. **Craig JC, Broxterman RM, Wilcox SL, Chen C, Barstow TJ.** Effect of adipose tissue
587 thickness, muscle site, and sex on near-infrared spectroscopy derived total-[hemoglobin +
588 myoglobin]. *J. Appl. Physiol.* .
- 589 16. **Davies CT, Sargeant AJ.** Physiological responses to one- and two-leg exercise breathing air
590 and 45 percent oxygen. *J Appl Physiol* 36: 142–148, 1974.

- 591 17. **Elias LJ, Bryden MP, Bulman-Fleming MB.** Footedness is a better predictor than is
592 handedness of emotional lateralization. *Neuropsychologia* 36: 37–43, 1998.
- 593 18. **Elmer SJ, McDaniel J, Martin JC.** Biomechanics of counterweighted one-legged cycling. *J*
594 *Appl Biomech* 32: 78–85, 2016.
- 595 19. **Esposito F, Reese V, Shabetai R, Wagner PD, Richardson RS.** Isolated quadriceps training
596 increases maximal exercise capacity in chronic heart failure: The role of skeletal muscle
597 convective and diffusive oxygen transport. *J Am Coll Cardiol* 58: 1353–1362, 2011.
- 598 20. **Grassi B.** New data and well-established concepts. *J Appl Physiol* 125: 1354–1355, 2018.
- 599 21. **Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli P.**
600 Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions
601 in humans. *J Appl Physiol* 95: 149–158, 2015.
- 602 22. **Grassi B, Rossiter HB, Zoladz J a.** Skeletal Muscle Fatigue and Decreased Efficiency. *Exerc*
603 *Sport Sci Rev* 43: 75–83, 2015.
- 604 23. **Hautier CA, Arsac LM, Deghdegh K, Souquet J, Belli A, Lacour JR.** Influence of fatigue on
605 EMG/force ratio and cocontraction in cycling. *Med Sci Sports Exerc* 32: 839–43, 2000.
- 606 24. **Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, Weibel ER.**
607 Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol*
608 59: 320–327, 1985.
- 609 25. **Hug F, Goupille C, Baum D, Raiteri BJ, Hodges PW, Tucker K.** Nature of the coupling
610 between neural drive and force-generating capacity in the human quadriceps muscle. *Proc R Soc*
611 *B Biol Sci* 282, 2015.
- 612 26. **Hureau TJ, Romer LM, Amann M.** The “sensory tolerance limit”: A hypothetical construct

- 613 determining exercise performance? *Eur J Sport Sci* 18: 13–24, 2018.
- 614 27. **Iannetta D, Azevedo R de A, Keir DA, Murias JM.** Establishing the $\dot{V}O_2$ versus constant-
615 work rate relationship from ramp-incremental exercise: Simple strategies for an unsolved
616 problem. *J Appl Physiol* 1: japplphysiol.00508.2019, 2019.
- 617 28. **Iannetta D, Fontana FY, Maturana FM, Inglis EC, Pogliaghi S, Keir DA, Murias JM.** An
618 equation to predict the maximal lactate steady state from ramp-incremental exercise test data in
619 cycling. *J Sci Med Sport* 21: 1274–1280, 2018.
- 620 29. **Iannetta D, Okushima D, Inglis EC, Kondo N, Murias JM, Koga S.** Blood flow occlusion-
621 related O_2 extraction “reserve” is present in different muscles of the quadriceps but greater in
622 deeper regions after ramp-incremental test. *J Appl Physiol* 125: 313–319, 2018.
- 623 30. **Iannetta D, Qahtani A, Mattioni Maturana F, Murias JM.** The near-infrared spectroscopy-
624 derived deoxygenated haemoglobin breaking-point is a repeatable measure that demarcates
625 exercise intensity domains. *J Sci Med Sport* 20: 873–877, 2017.
- 626 31. **Iannetta D, Qahtani A, Millet GY, Murias JM.** Quadriceps Muscles O_2 Extraction and EMG
627 Breakpoints during a Ramp Incremental Test. *Front Physiol* 8: 1–9, 2017.
- 628 32. **Inglis EC, Iannetta D, Murias JM.** The plateau in the NIRS-derived [HHb] signal near the end
629 of a ramp incremental test does not indicate the upper limit of O_2 extraction in the vastus
630 lateralis. *Am J Physiol Integr Comp Physiol* 313: R723–R729, 2017.
- 631 33. **Ivy JL, Withers RT, Van Handel PJ, Elger DH, Costill DL.** Muscle respiratory capacity and
632 fiber type as determinants of the lactate threshold. *J Appl Physiol* 48: 523–527, 2017.
- 633 34. **Keir DA, Benson AP, Love LK, Robertson TC, Rossiter HB, Kowalchuk JM.** Influence of
634 muscle metabolic heterogeneity in determining the $\dot{V}O_{2p}$ kinetic response to ramp-incremental

- 635 exercise. *J Appl Physiol* 120: 503–13, 2016.
- 636 35. **Keir DA, Copithorne DB, Hodgson MD, Pogliaghi S, Rice CL, Kowalchuk JM.** The slow
637 component of pulmonary O₂ uptake accompanies peripheral muscle fatigue during high-
638 intensity exercise. *J Appl Physiol* 121: 493–502, 2016.
- 639 36. **Klausen K, Secher NH, Clausen JP, Hartling O, Trap-Jensen J.** Central and regional
640 circulatory adaptations to one-leg training. *J Appl Physiol* 52: 976–983, 1982.
- 641 37. **Knight DR, Schaffartzik W, Poole DC, Hogan MC, Bebout DE, Wagner PD.** Effects of
642 hyperoxia on maximal leg O₂ supply and utilization in men. *J Appl Physiol* 75: 2586–2594,
643 1993.
- 644 38. **Koga S, Barstow TJ, Shiojiri T, Takaishi T, Fukuba Y, Kondo N, Shibasaki M, Poole DC.**
645 Effect of muscle mass on VO₂ kinetics at the onset of work. *J Appl Physiol* 90: 461–8, 2001.
- 646 39. **Koga S, Okushima D, Poole DC, Rossiter HB, Kondo N, Barstow TJ.** Unaltered $\dot{V}o_2$ kinetics
647 despite greater muscle oxygenation during heavy-intensity two-legged knee extension versus
648 cycle exercise in humans. *Am J Physiol Regul Integr Comp Physiol* 317: R203–R213, 2019.
- 649 40. **Koga S, Poole DC, Shiojiri T, Kondo N, Fukuba Y, Miura A, Barstow TJ.** Comparison of
650 oxygen uptake kinetics during knee extension and cycle exercise. *Am J Physiol Integr Comp*
651 *Physiol* 288: R212–R220, 2004.
- 652 41. **Koga S, Rossiter HB, Heinonen I, Musch TI, Poole DC.** Dynamic heterogeneity of exercising
653 muscle blood flow and O₂ utilization. *Med Sci Sports Exerc* 46: 860–876, 2014.
- 654 42. **Larson RD, McCully KK, Larson DJ, Pryor WM, White LJ.** Bilateral differences in lower-
655 limb performance in individuals with multiple sclerosis. *J Rehabil Res Dev* 50: 215–221, 2013.
- 656 43. **Lexell J, Taylor CC.** A morphometrical comparison of right and left whole human vastus

- 657 lateralis muscle: how to reduce sampling errors in biopsy techniques. *Clin Physiol* 11: 271–276,
658 1991.
- 659 44. **MacInnis MJ, McGlory C, Gibala MJ, Phillips SM.** Investigating human skeletal muscle
660 physiology with unilateral exercise models: when one limb is more powerful than two. *Appl*
661 *Physiol Nutr Metab* 42: 563–570, 2017.
- 662 45. **MacInnis MJ, Morris N, Sonne MW, Zuniga AF, Keir PJ, Potvin JR, Gibala MJ.**
663 Physiological responses to incremental, interval, and continuous counterweighted single-leg and
664 double-leg cycling at the same relative intensities. *Eur J Appl Physiol* 117: 1423–1435, 2017.
- 665 46. **McPhee JS, Williams AG, Stewart C, Baar K, Schindler JP, Aldred S, Maffulli N, Sargeant**
666 **AJ, Jones DA.** The training stimulus experienced by the leg muscles during cycling in humans.
667 *Exp Physiol* 94: 684–694, 2009.
- 668 47. **Mitchell EA, Martin NRW, Bailey SJ, Ferguson RA.** Critical power is positively related to
669 skeletal muscle capillarity and type I muscle fibers in endurance-trained individuals. *J Appl*
670 *Physiol* 125: 737–745, 2018.
- 671 48. **Murias JM, Kowalchuk JM, Paterson DH.** Time course and mechanisms of adaptations in
672 cardiorespiratory fitness with endurance training in older and young men. *J Appl Physiol*
673 *(Bethesda, Md 1985)* 108: 621–627, 2010.
- 674 49. **Murias JM, Spencer MD, Keir D a, Paterson DH.** Systemic and vastus lateralis muscle blood
675 flow and O₂ extraction during ramp incremental cycle exercise. *AJP Regul Integr Comp Physiol*
676 304: 720–725, 2013.
- 677 50. **Neary PJ, Wenger HA.** The effects of one- and two-legged exercise on the lactate and
678 ventilatory threshold. *Eur J Appl Physiol Occup Physiol* 54: 591–595, 2005.

- 679 51. **Okushima D, Poole DC, Barstow TJ, Rossiter HB, Kondo N, Bowen TS, Amano T, Koga S.**
680 Greater VO₂peak is correlated with greater skeletal muscle deoxygenation amplitude and
681 hemoglobin concentration within individual muscles during ramp-incremental cycle exercise.
682 *Physiol Rep* 4: 1–12, 2016.
- 683 52. **Pernow B, Saltin B.** Availability of substrates and capacity for prolonged heavy exercise in
684 man. *J Appl Physiol* 31: 416–422, 1971.
- 685 53. **Poole DC, Burnley M, Vanhatalo A, Rossiter HB, Jones AM.** Critical power: An important
686 fatigue threshold in exercise physiology. *Med Sci Sports Exerc* 48: 2320–2334, 2016.
- 687 54. **Poole DC, Richardson RS.** Determinants of oxygen uptake: Implications for exercise testing.
688 *Sport Med* 24: 308–320, 1997.
- 689 55. **Poole DC, Ward SA, Gardner GW, Whipp BJ.** Metabolic and respiratory profile of the upper
690 limit for prolonged exercise in man. *Ergonomics* 31: 1265–79, 1988.
- 691 56. **Richardson RS, Knight DR, Poole DC, Kurdak SS, Hogan MC, Grassi B, Wagner PD.**
692 Determinants of maximal exercise VO₂ during single leg knee-extensor exercise in humans. *Am*
693 *J Physiol* 268: H1453-61, 1995.
- 694 57. **Richardson RS, Poole DC, Knight DR, Kurdak SS, Hogan MC, Grassi B, Johnson EC,**
695 **Kendrick KF, Erickson BK, Wagner PD.** High muscle blood flow in man: is maximal O₂
696 extraction compromised? *J Appl Physiol* 75: 1911–1916, 1993.
- 697 58. **Roca J, Hogan MC, Story D, Bebout DE, Haab P, Gonzalez R, Ueno O, Wagner PD.**
698 Evidence for tissue diffusion limitation of VO₂max in normal humans. *J Appl Physiol* 67: 291–
699 299, 1989.
- 700 59. **Rowley NJ, Dawson EA, Birk GK, Cable NT, George K, Whyte G, Thijssen DHJ, Green**

- 701 **DJ.** Exercise and arterial adaptation in humans: Uncoupling localized and systemic effects. *J*
702 *Appl Physiol* 110: 1190–1195, 2011.
- 703 60. **Saltin B, Costill DL, Essén B, Jansson E, Gollnick PD, Nazar K, Stein E.** The Nature of the
704 Training Response; Peripheral and Central Adaptations to One-Legged Exercise. *Acta Physiol*
705 *Scand* 96: 289–305, 2008.
- 706 61. **Saltin B, Henriksson J, Nygaard E, Andersen P, Jansson E.** Fiber Types and Metabolic
707 Potentials of Skeletal Muscles in Sedentary Man and Endurance Runners. *Ann N Y Acad Sci* 301:
708 3–29, 1977.
- 709 62. **Sargeant AJ, Davies CT.** Forces applied to cranks of a bicycle ergometer during one- and two-
710 leg cycling. *J Appl Physiol* 42: 514–518, 1977.
- 711 63. **Sargeant AJ, Davies CTM.** Limb volume, composition, and maximum aerobic power output in
712 relation to habitual “preference” in young male subjects. *Ann Hum Biol* 4: 49–55, 1977.
- 713 64. **Sinoway LI, Musch TI, Minotti JR, Zelis R.** Enhanced maximal metabolic vasodilatation in
714 the dominant forearms of tennis players. *J Appl Physiol* 61: 673–678, 1986.
- 715 65. **Sinoway LI, Shenberger J, Wilson J, McLaughlin D, Musch T, Zelis R.** A 30-day forearm
716 work protocol increases maximal forearm blood flow. *J Appl Physiol* 62: 1063–1067, 2017.
- 717 66. **Smak W, Neptune RR, Hull ML.** The influence of pedaling rate on bilateral asymmetry in
718 cycling. *J Biomech* 32: 899–906, 1999.
- 719 67. **Spencer MD, Murias JM, Paterson DH.** Characterizing the profile of muscle deoxygenation
720 during ramp incremental exercise in young men. *Eur J Appl Physiol* 112: 3349–3360, 2012.
- 721 68. **Thomas GD, Hansen J, Victor RG.** Inhibition of alpha 2-adrenergic vasoconstriction during
722 contraction of glycolytic, not oxidative, rat hindlimb muscle. *Am J Physiol Circ Physiol* 266:

- 723 H920–H929, 1994.
- 724 69. **Todorov E.** Optimality principles in sensorimotor control. *Nat Neurosci* 7: 907–915, 2004.
- 725 70. **Vanhatalo A, Fulford J, Dimenna FJ, Jones AM.** Influence of hyperoxia on muscle metabolic
726 responses and the power-duration relationship during severe-intensity exercise in humans: A 31P
727 magnetic resonance spectroscopy study. *Exp Physiol* 95: 528–540, 2010.
- 728 71. **Wilcox SL, Broxterman RM, Barstow TJ.** Constructing quasi-linear $\dot{V}O_2$ responses from
729 nonlinear parameters. *J Appl Physiol* 120: 121–9, 2016.
- 730

731 **FIGURE CAPTIONS**

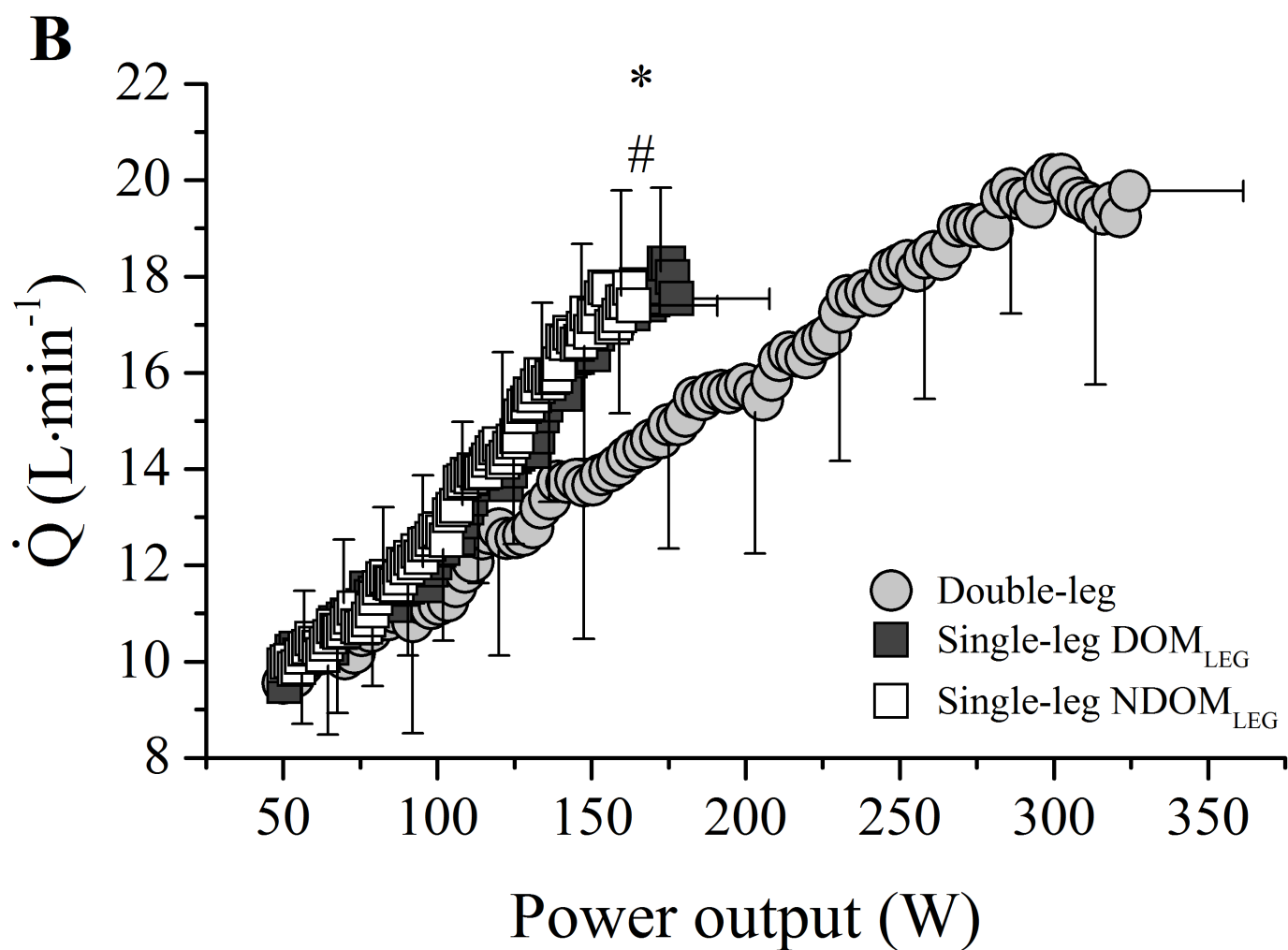
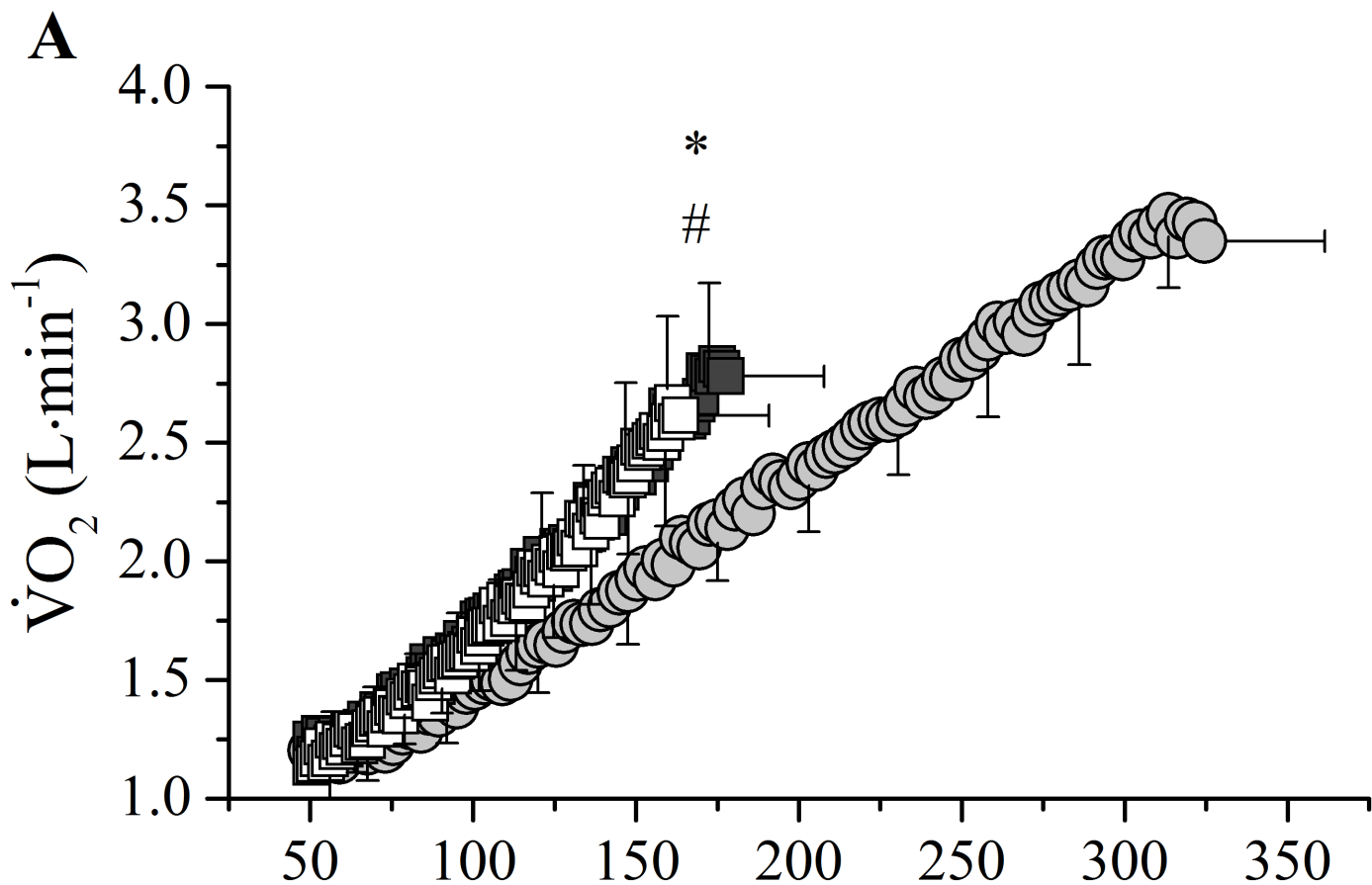
732 **Figure 1.** Group mean data of $\dot{V}O_2$ ($L \cdot \text{min}^{-1}$) and \dot{Q} ($L \cdot \text{min}^{-1}$) with respect to absolute power output
733 during double-leg and counterweighted single-leg cycling. * Denotes significance between
734 counterweighted single-leg and double-leg cycling. # Denotes significance between dominant
735 (DOM_{LEG}) and non-dominant (NDOM_{LEG}).

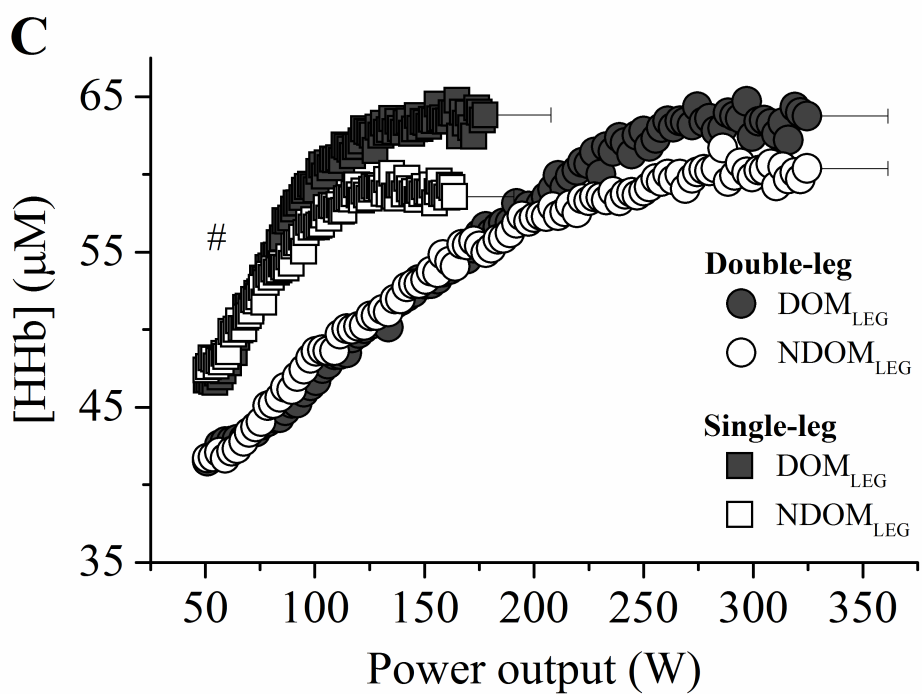
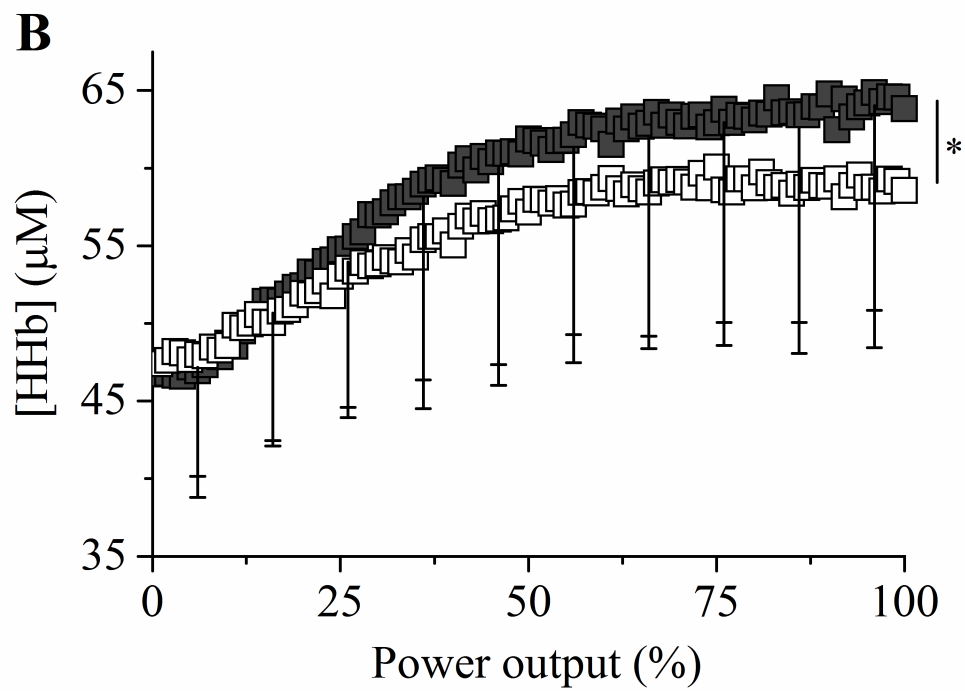
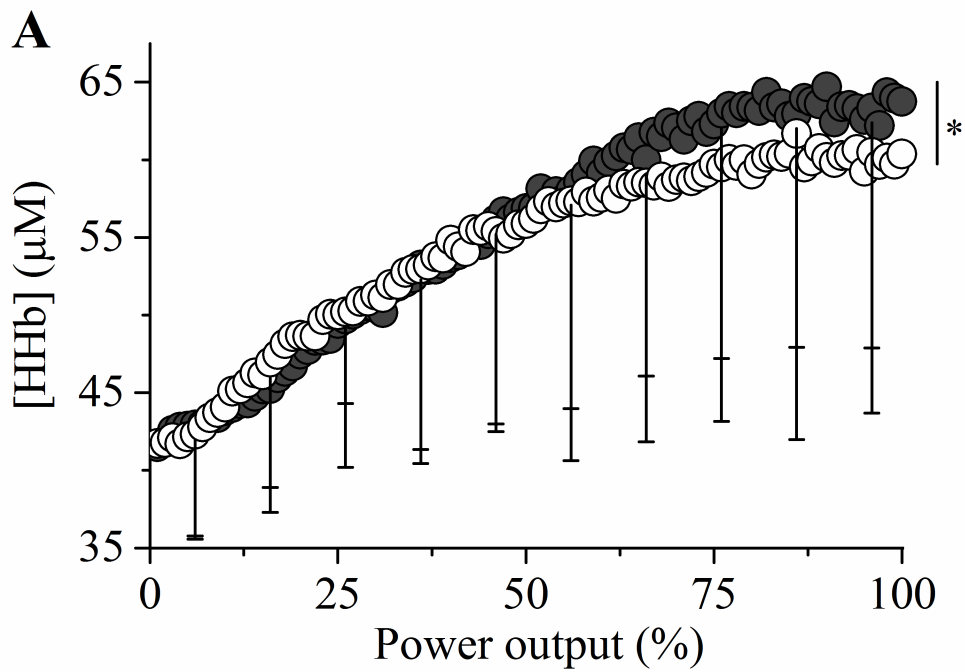
736 **Figure 2.** Group mean [HHb] (μM) profiles with respect to relative (A,B) and absolute (C) power
737 output during double-leg and counterweighted single-leg cycling. * Denotes significance in relation to
738 [HHb] signal amplitude between dominant (DOM_{LEG}) and non-dominant (NDOM_{LEG}). # Denotes
739 significance in relation to *slope 1* of the [HHb] signal between counterweighted single- vs double-leg
740 cycling (irrespective of leg-dominance). For clarity, y-axis error bars on panel C are not displayed.

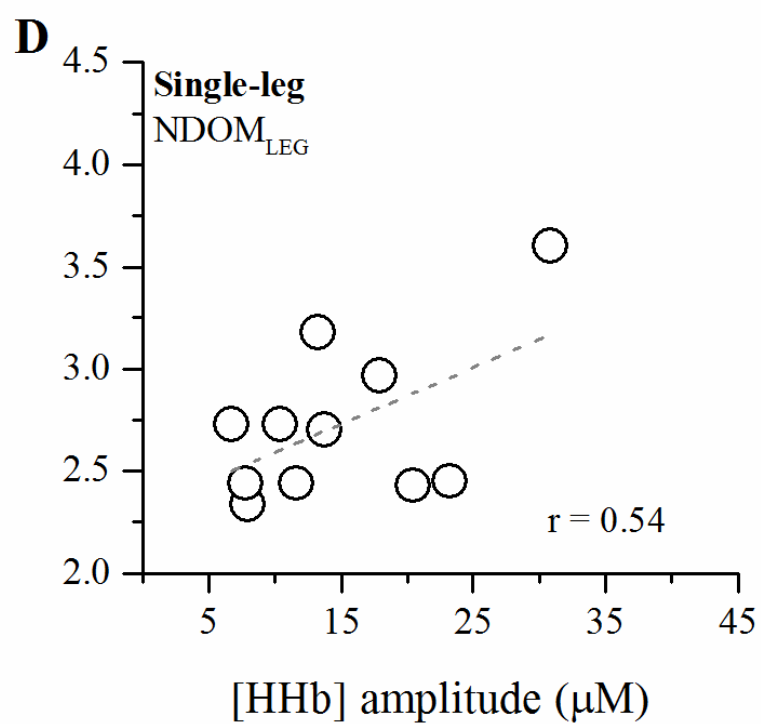
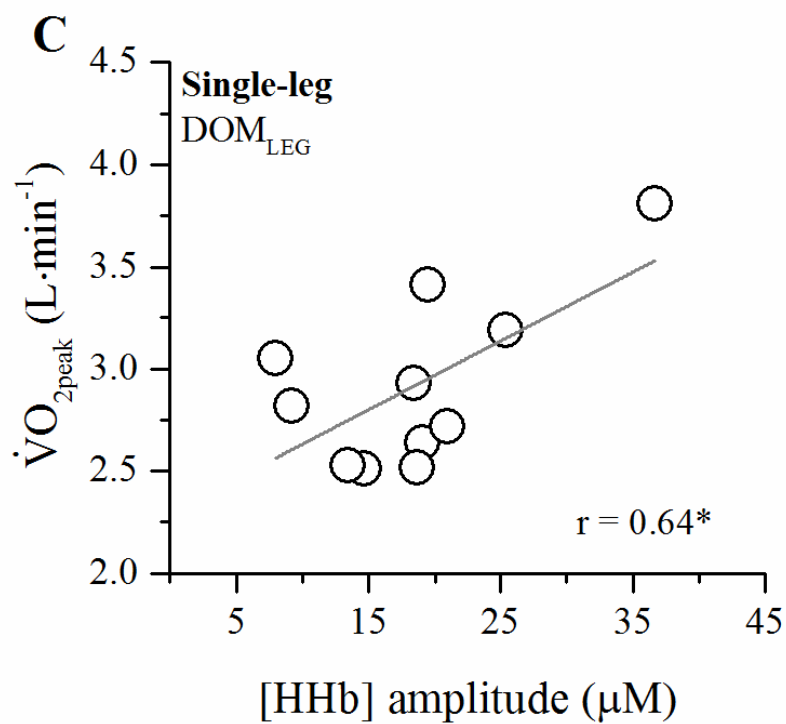
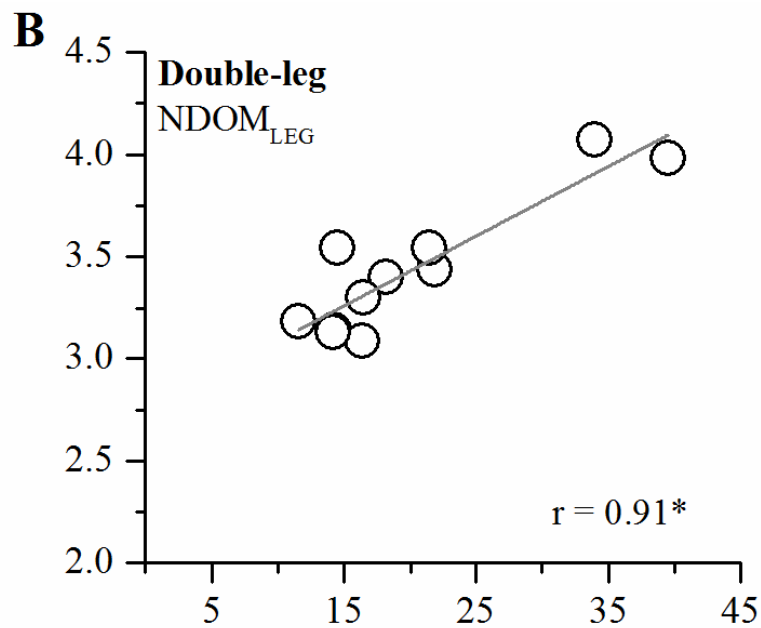
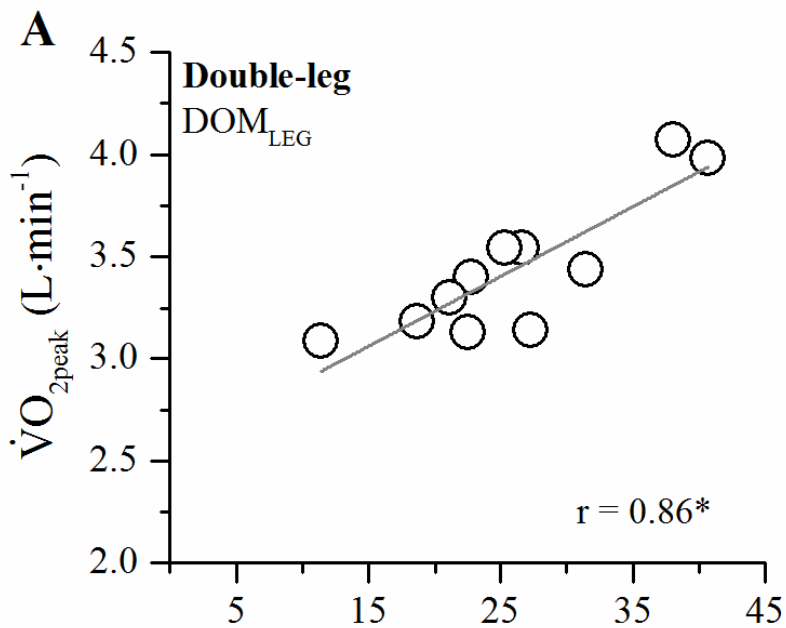
741 **Figure 3.** Relationship between the amplitude of the [HHb] (μM) signal and $\dot{V}O_{2\text{max}}$ ($L \cdot \text{min}^{-1}$) recorder
742 at the end of double-leg and counterweighted single-leg ramp-exercise in the DOM_{LEG} and NDOM_{LEG} .
743 * <0.05 .

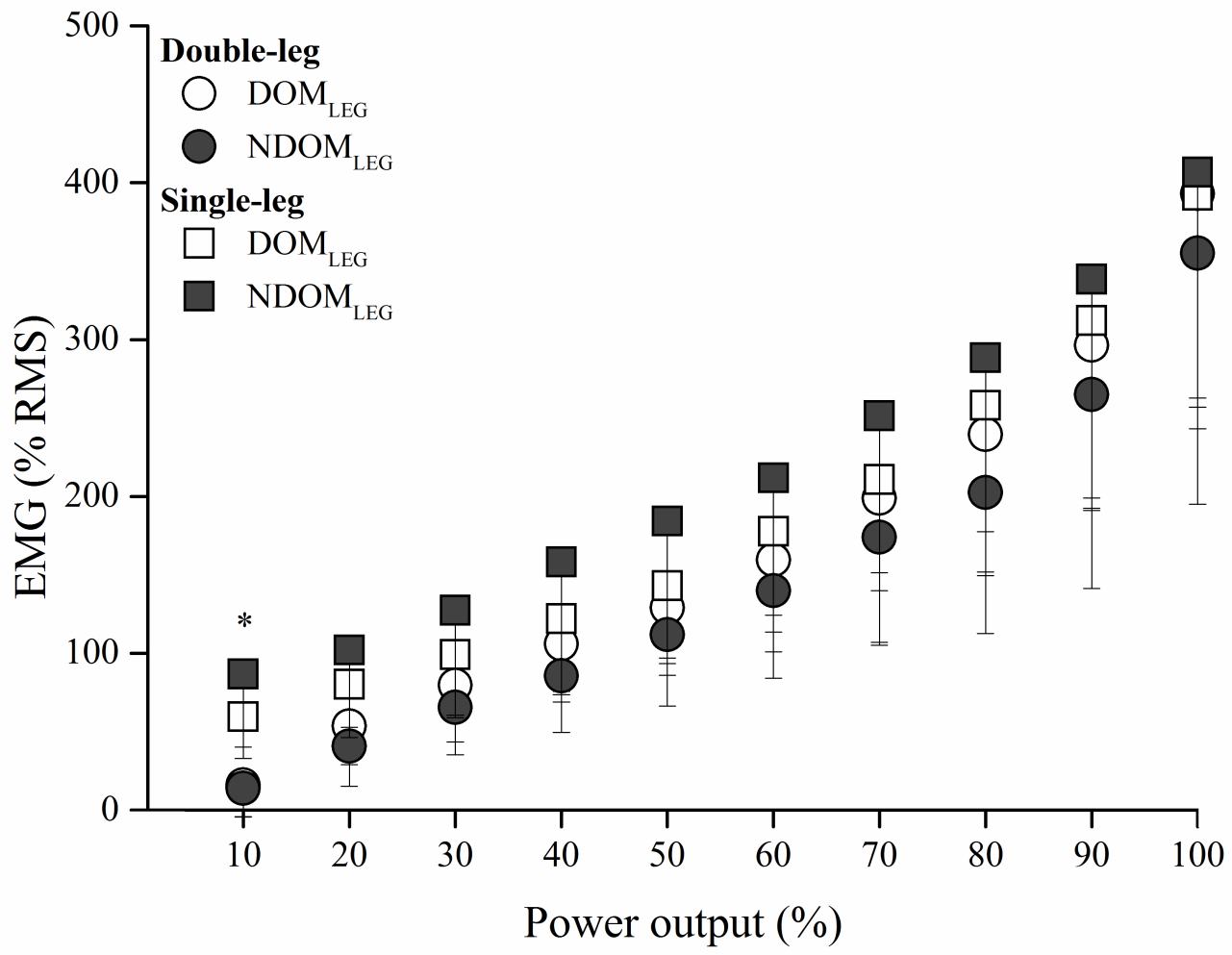
744 **Figure 4.** Group mean EMG profiles (%RMS) with respect to relative power output during double-leg
745 and counterweighted single-leg ramp-exercise in the DOM_{LEG} and NDOM_{LEG} . * Denotes significance
746 at the corresponding time-point between counterweighted single- vs double-leg cycling (irrespective of
747 leg-dominance).

748 **Figure 5.** Group mean data of $\dot{V}O_2$ ($L \cdot \text{min}^{-1}$) and $[\text{La}^-]_b$ (μM) during double-leg and DOM_{LEG} and
749 NDOM_{LEG} counterweighted single-leg cycling at MLSS_p and MLSS_{+10} . Refer to the Results section for
750 loci of significance.









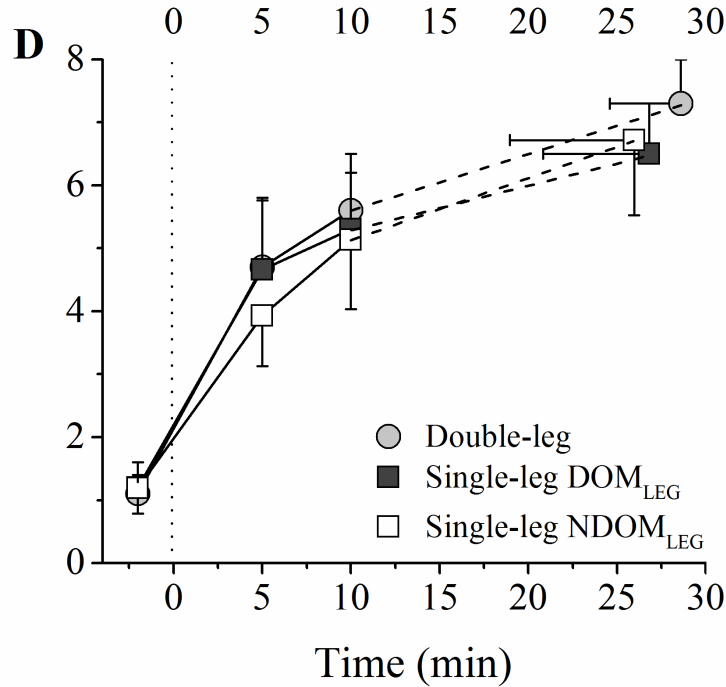
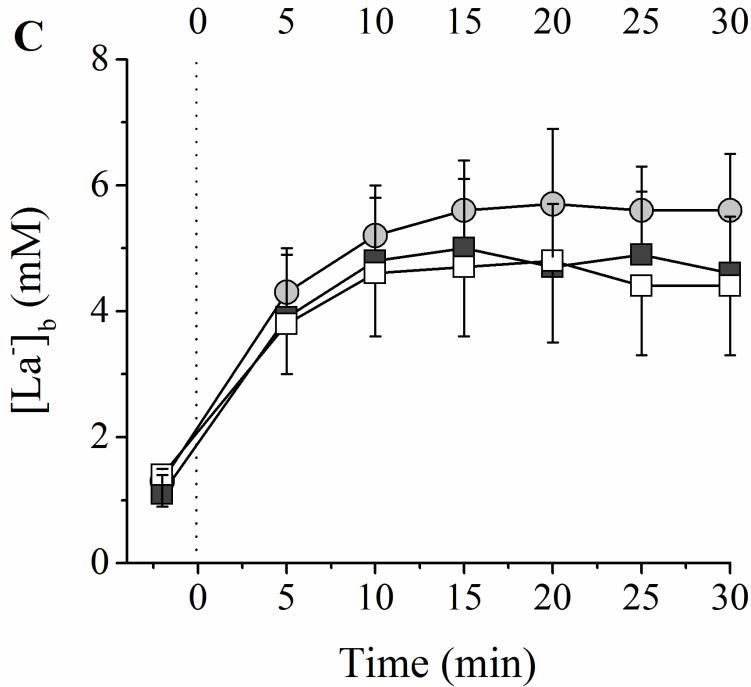
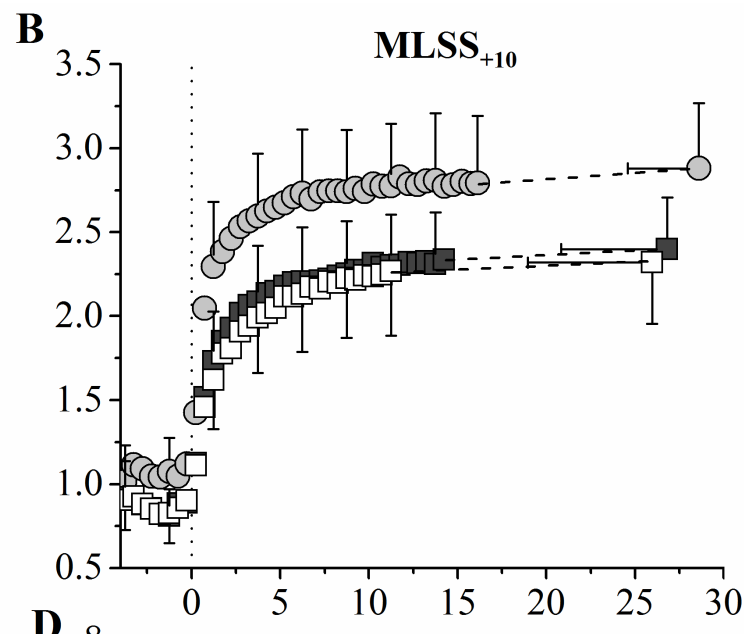
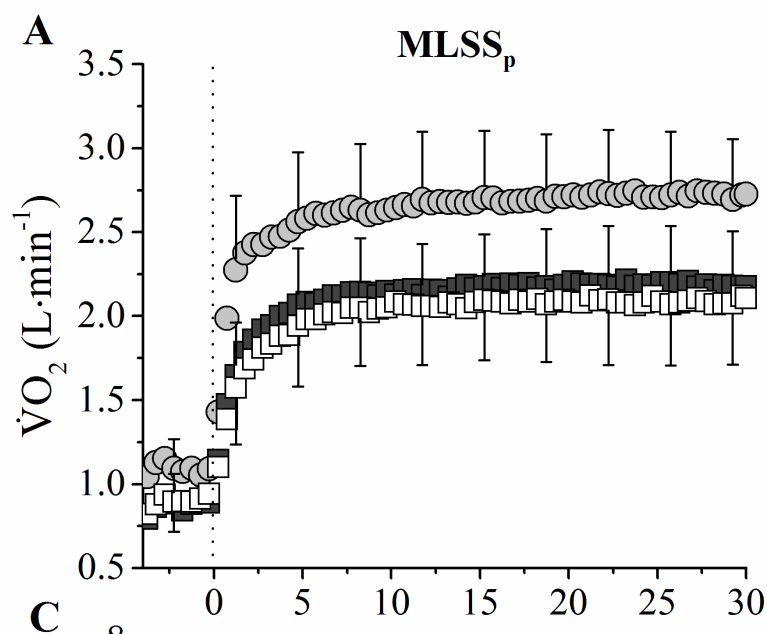


Table 1. Peak physiological responses during double-leg, and dominant (DOM_{LEG}) and non-dominant (NDOM_{LEG}) counterweighted single-leg cycling ramp-exercise.

<i>Exercise mode</i>	Double-leg	Counterweighted single-leg	
		DOM _{LEG}	NDOM _{LEG}
PO _{peak} (W)	327±37	179±30 *	165±27 *,#
$\dot{V}O_{2\text{bsln}}$ (L·min ⁻¹)	1.16±0.12	1.14±0.10	1.12±0.14
$\dot{V}O_{2\text{peak}}$ (L·min ⁻¹)	3.43±0.33	2.87±0.42 *	2.70±0.39 *,#
$\dot{V}O_{2\text{peak}}$ (mL·kg ⁻¹ ·min ⁻¹)	45.1±6.1	-	-
G _{ramp} (mL·W ⁻¹ ·min ⁻¹)	9.2±1.0	-	-
G ₁ (mL·W ⁻¹ ·min ⁻¹)	-	12.1±2.5 §	12.3±2.0 §
G ₂ (mL·W ⁻¹ ·min ⁻¹)	-	17.9±7.3 §	19.4±7.0 §
HR _{max} (bpm)	180±12	164±10 *	165±27 *
\dot{Q}_{peak} (L·min ⁻¹)	20.7±2.9	19.0±2.3 *	17.8±2.4 *,#
[La ⁻] _b (mM)	12.4±1.7	8.2±1.6 *	8.0±1.6 *

Data are presented as mean±SD; PO_{peak}: peak power output. $\dot{V}O_{2\text{bsln}}$: baseline rate of O₂ uptake at 50 W. $\dot{V}O_{2\text{peak}}$: peak rate of O₂ uptake; G_{ramp}: $\Delta\dot{V}O_2/\text{PO}$ during double-leg ramp-exercise; G₁ and G₂: $\Delta\dot{V}O_2/\text{PO}$ during single-leg ramp-exercise within the first and second portion of the ramp-exercise, respectively; HR_{max}: maximal heart rate. \dot{Q}_{peak} : peak cardiac output. [La⁻]_b: blood lactate concentration immediately after the ramp-exercise.

* Denotes significance from double-leg.

Denotes significance from DOM_{LEG}.

§ Denotes significance from G_{ramp} of double leg

Table 2. Baseline, amplitude, and slope of increase in the [HHb] signal of the vastus lateralis during double-leg, and dominant (DOM_{LEG}) and non-dominant (NDOM_{LEG}) counterweighted single-leg cycling ramp-exercise.

<i>Exercise mode</i>	Double-leg		Counterweighted single-leg	
	DOM _{LEG}	NDOM _{LEG}	DOM _{LEG}	NDOM _{LEG}
Baseline (μM)	41.2 ±8.6	41.1±9.0	45.9±7.3	46.8±7.3
Amplitude (μM)	26.0±8.4	20.2± 8.8 *	18.5±7.9 *	14.9±7.5 *,#,§
<i>S</i> _{1(%PO)}	0.41±0.22	0.42±0.26	0.43±0.36	0.42±0.36
<i>S</i> _{2(%PO)}	0.00±0.02	0.00±0.02	0.01±0.02	0.01±0.02
<i>S</i> _{1(W)}	0.10±0.06	0.10±0.07	0.16±0.06 *	0.18±0.08 #
<i>S</i> _{2(W)}	0.00±0.02	0.00±0.02	0.01±0.02	0.01±0.02

Data are presented as mean ± SD. *S*₁ and *S*₂ are slope 1 and 2 of the [HHb] signal calculated against relative (%PO) and absolute (W) power output.

* Denotes significance from double-leg DOM_{LEG}.

Denotes significance from double-leg NDOM_{LEG}.

§ Denotes significance from counterweighted single-leg DOM_{LEG}.

Table 3. Power output (W) and $\dot{V}O_2$ ($L \cdot \text{min}^{-1}$) data at MLSS_p and MLSS₊₁₀.

<i>Exercise mode</i>	Double-leg		Counterweighted single-leg			
			DOM _{LEG}		NDOM _{LEG}	
<i>Condition</i>	MLSS _p	MLSS ₊₁₀	MLSS _p	MLSS ₊₁₀	MLSS _p	MLSS ₊₁₀
Power output (W)	183±31	193±31 *	118±24 #	128±24 *,#	109±23	119±23 *
Power output (% of double-leg)	-	-	65.5±8.8 #	66.4±8.3 *	60.0±8.4	62.1±8.0 *
$\dot{V}O_{2\text{bsln}}$	1.11±0.09	1.06±0.16	1.19±0.10	1.14±0.10	1.19±0.12	1.15±0.11
$\dot{V}O_{2\text{end}}$ ($L \cdot \text{min}^{-1}$)	2.73±0.32	2.87±0.28 *	2.18±0.25 #,§	2.39±0.31 *	2.09±0.29	2.33±0.31 *
$\dot{V}O_{2\text{gain}}$ ($\text{ml} \cdot \text{min}^{-1} \cdot \text{W}^{-1}$)	12.3±1.1	12.8±1.6	15.4±3.4 ^	16.6±3.24	15.6±3.3 ^	17.9±3.3
$\dot{V}O_2$ (% of double-leg)	-	-	79.9±7.3 #	87.8±9.3 *	76.5±7.8	85.7±10.1 *

Data are presented as mean±SD. $\dot{V}O_{2\text{bsln}}$: baseline rate of O₂ uptake at 50 W; $\dot{V}O_{2\text{end}}$: rate of O₂ uptake during the last two minutes of the constant-load trials.

Percent values of power output and $\dot{V}O_2$ are calculated based on the double-leg MLSS_p.

* Denotes significance from MLSS_p of same exercise mode.

Denotes significance from NDOM_{LEG} of same condition.

§ Denotes significance from NDOM_{LEG} of different condition.

^ Denotes significance from double-leg of same condition.