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1 **Title**

2 Active and inactive leg hemodynamics during sequential single-leg interval cycling

3 **Short Title**

4 Hemodynamics of sequential single-leg cycling

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25

26 **Abstract**

27 **Introduction:** Leg order during sequential single-leg cycling (i.e. exercising both legs
28 independently within a single session) may affect local muscular responses potentially
29 influencing adaptations. This study examined the cardiovascular and skeletal muscle
30 hemodynamic responses during double-leg and sequential single-leg cycling.

31 **Methods:** Ten young healthy adults (28 ± 6 y) completed six 1-min double-leg intervals
32 interspersed with one minute of passive recovery and, on a separate occasion, 12 (six with one
33 leg followed by six with the other leg) 1-min single-leg intervals interspersed with one minute
34 of passive recovery. Oxygen consumption, heart rate, blood pressure, muscle oxygenation,
35 muscle blood volume and power output were measured throughout each session.

36 **Results:** Oxygen consumption, heart rate and power output were not different between sets of
37 single-leg intervals but the average of both sets was lower than the double-leg intervals. Mean
38 arterial pressure was higher during double-leg compared with sequential single-leg intervals
39 (115 ± 9 mmHg vs. 104 ± 9 mmHg; $p < 0.05$) and higher during the initial compared with second
40 set of single-leg intervals (108 ± 10 mmHg vs. 101 ± 10 mmHg; $p < 0.05$). The increase in
41 muscle blood volume from baseline was similar between the active single-leg and double-leg
42 (267 ± 150 $\mu\text{M}\cdot\text{cm}$ vs. 214 ± 169 $\mu\text{M}\cdot\text{cm}$; $p = 0.26$). The pattern of change in muscle blood
43 volume from the initial to second set of intervals was significantly different ($p < 0.05$) when the
44 leg was active in the initial ($-52.3 \pm 111.6\%$) compared with second set ($65.1 \pm 152.9\%$).

45 **Conclusions:** These data indicate that the order in which each leg performs sequential single-
46 leg cycling influences the local hemodynamic responses, with the inactive muscle influencing
47 the stimulus experienced by the contralateral leg.

- 48 **Keywords:** near-infrared spectroscopy; skeletal muscle blood flow; high intensity exercise;
- 49 blood pressure; order effect; active muscle mass

50 **Introduction**

51 When compared with traditional double-leg cycling training, sequential single-leg cycling
52 training (i.e. exercising both legs independently within a single session) is associated with
53 improved maximal cardiac output, leg blood flow and oxygen consumption (1) as well as
54 greater increases in metabolic and oxidative potential of skeletal muscle (2). Mechanistically,
55 single-leg cycling results in greater leg blood flow and oxygen delivery to the active leg (1, 3,
56 4) thus providing the environment to achieve higher individual leg power output (1, 2, 5, 6)
57 and training stimulus (1, 2). Importantly, differences in bulk blood flow and blood flow
58 distribution between active and inactive legs during single-leg cycling have been observed (7-
59 9). It is therefore possible that the order in which each of the limbs perform exercise during
60 sequential single-leg cycling (i.e. active during the initial or second set) could impact the
61 cardiovascular and metabolic response of the modality; however, this is yet to be fully
62 described.

63 There is a growing body of literature that the contralateral leg is not physiologically passive
64 during single leg exercise. For example, femoral blood flow, oxygen consumption and
65 carbohydrate utilization are increased above resting levels in the inactive leg during single-leg
66 cycling (9-11). Additionally, similar increases in muscle blood volume, measured by near-
67 infrared spectroscopy (NIRS), have been observed half way through a single-leg graded
68 exercise test in both the active and inactive legs (8). These acute exercise responses likely
69 contribute to improvements in leg aerobic capacity (12-14), endurance time (15) and femoral
70 vein cross section area (13) of the untrained leg observed in studies using a single-leg cycling
71 exercise model. Indeed, hemodynamic changes within inactive tissue beds has been proposed
72 as an important mechanism underpinning improvements in vascular structure and function
73 observed in untrained tissue beds (16, 17).

Understanding the physiological consequence of leg order during sequential single-leg cycling would provide valuable information for the prescription of aerobic exercise completed with one muscle group followed by another. Indeed, order effects when comparing aerobic and resistance exercise (18) or multiple resistance exercises (19) within a single exercise session have been observed. In contrast, no studies have investigated the hemodynamic and metabolic responses of aerobic exercise performed with different muscle groups within a single exercise session. This information is important in informing the exercise prescription using this training modality as well as training of multiple muscle groups (e.g. training lower before upper body) since exercise order can influence acute performance, neuromuscular activity, oxygen consumption and ratings of perceived exertion (19) which will likely influence chronic adaptations. For example, if local muscle responses during sequential single-leg cycling demonstrate a leg order effect and training is conducted as per Klausen et al. (1) (i.e. same leg first in all sessions), it is possible that each leg may experience different muscular adaptations. As such, alternating the starting leg could mitigate this discrepancy, ensuring each leg receives the same stimulus within a training program. Therefore, this study will, for the first time, investigate the local muscular responses of each leg using NIRS during a session of sequential single-leg cycling intervals. Additionally, we will compare the mean hemodynamic response (i.e. right and left leg) during double-leg cycling with the active and inactive legs during single-leg cycling to ascertain the potential contribution of the inactive leg during single-leg cycling to the overall increased peripheral stimulus.

Methods

Participants

Ten young healthy individuals (7 males and 3 females, age: 28 ± 6 y, body mass index: 22.7 ± 2.2 kg.m², VO_{2max}: 53 ± 11 mL.kg⁻¹.min⁻¹, peak power output: 314 ± 96 W) volunteered to

participate in this study. At the time of this study, all participants were considered to be physically active and no individuals were excluded on the basis of their current exercise habits (aerobic or resistance based training). Participants attended a laboratory setting on four separate occasions to perform a graded exercise test, familiarization session and two experimental sessions with no less than five and no greater than ten days between testing sessions. Participants were asked to avoid strenuous physical activity for at least 24 h prior to the day of testing and all tests were completed at a similar time of day. Written informed consent was obtained prior to data collection. This study received ethical clearance from Murdoch University Human Research Ethics Committee (2012/157) prior to the start of this study and conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Procedures

During the initial testing session, participants completed a graded exercise test using an electronically braked Velotron cycle ergometer (RacerMate; USA). Male participants started at a power output of 70 W increasing 25 W·min⁻¹ until volitional fatigue, while females started at 50 W increasing 20 W·min⁻¹ until volitional fatigue. Expired ventilation was collected at a frequency of one Hz and analyzed for the volume of oxygen consumed and carbon dioxide produced using a Parvo TrueOne metabolic analysis system (ParvoMedics; USA). From this data, 15 sec mean values were calculated for the determination of maximal oxygen consumption, highest 30-s average during exercise, and the first ventilator threshold (20).

During the familiarization session, participants completed six one-min cycling intervals on a Velotron cycle ergometer. The session started with a standardized 15 min double-leg warm-up with participants cycling at 30% and 40% (5 min at 30% and 10 min at 40%) of the maximal power output achieved during the graded exercise test. Participants then cycled for a further five min at 50% of the power output at the first ventilator threshold (88 ± 16 W) immediately followed by two 1-min double-leg cycling intervals completed at the participant's highest

maintainable power output with one min of passive recovery between intervals. Following the initial two double-leg intervals, participants were familiarized with single leg cycling. Participants performed four single-leg cycling intervals (two with each leg). Each set of the two single-leg intervals started with five min of cycling at half the power output recorded at ventilator threshold during the double-leg graded exercise test (44 ± 8 W) followed immediately by two one-min maximal intervals with one min passive recovery between intervals. After completing the intervals using one leg, the ergometer was adjusted to allow the participant to complete the identical procedure with the opposite leg. During the single-leg intervals a specially designed counterweight (10 kg) was attached to the opposite pedal to allow a fluid pedaling motion. Previous research has successfully used this method in both healthy and clinical populations (3, 5, 21-24) and has shown that counterweighted single-leg cycling is more similar to double-leg cycling when compared with unassisted single-leg cycling (3, 21). During single-leg cycling, participants rested their inactive leg on a chair placed directly next to the unoccupied crank arm.

During the experimental sessions (Figure 1), using a cross-over design, participants completed one session of double-leg and one session of sequential single-leg intervals in a randomized and counterbalanced order. At the start of each session, participants rested in a supine position for 10 min during which time expired ventilation was collected via the metabolic analysis system. Participants then, irrespective of condition, completed a 15-min standardized double-leg cycling warm-up identical to the familiarization trial. In the double-leg session, participants completed six maximal one-min intervals with one min passive recovery between intervals. In the sequential single-leg session, participants completed twelve (six with each leg in a sequential order as depicted in Figure 1) maximal one-min intervals with one min passive recovery between each interval. During the sequential single-leg session, the order of legs (i.e. right first then left or left first then right) were randomized and counterbalanced. During all

intervals, participants were instructed to produce the highest maintainable power output possible. Throughout each interval session, power output (Velotron cycle ergometer software), oxygen consumption (metabolic analysis system) and heart rate (810i, Polar; Finland) were measured at one Hz and mean values over the one-min intervals were calculated. Muscle blood volume and oxygenation in the rectus femoris muscle were measured during the 10-min supine rest and throughout the interval sessions using NIRS. Blood pressure was measured manually using an aneroid sphygmomanometer immediately after each interval.

Near-infrared spectroscopy measures

Muscle blood volume and oxygenation during the experimental trials were monitored using the NIRO-200 oximeter (Niromonitor NIRO-200, Hamamatsu Photonics; Japan). This system simultaneously uses the modified Beer-Lambert and spatially-resolved spectroscopy methods to measure changes in oxygenated hemoglobin/myoglobin (ΔHbO_2), deoxygenated hemoglobin/myoglobin (ΔHHb) and total hemoglobin/myoglobin (ΔtHb); $\Delta\text{tHb} = \Delta\text{HbO}_2 + \Delta\text{HHb}$, expressed in micromoles x centimeter ($\mu\text{M}\cdot\text{cm}$). The contribution of myoglobin to the NIRS signal cannot be differentiated by near-infrared light, but is believed to be minimal (< 20%) (25). For simplicity, the abbreviations $\Delta\text{O}_2\text{Hb}$, ΔHHb and ΔtHb refer to the combined signal of hemoglobin and myoglobin. This system also provides a measure of HbO_2 saturation, indicated by the tissue oxygenation index [$\text{TOI} (\%) = \Delta\text{HbO}_2 / \Delta\text{tHb} \times 100$]. TOI reflects the dynamic balance of O_2 supply and demand within the muscle microcirculation (26), while the changes in tHb can be considered an indirect measure of changes in muscle blood volume (25).

Each NIRS probe unit consisted of a detector (includes two silicon photodiodes) and an emitter probe (includes three laser-emitting diodes of 775, 810 and 850 nm), supported 4 cm apart by a rubberized shell casing. Given the penetration depth of the NIRS signal is almost half the emitter-detector distance (i.e. 2 cm), it is reasonable that changes in NIRS-derived variables

primarily reflect that of the muscle tissue (26, 27). Probe units were positioned on both limbs during double-leg and sequential single-leg cycling, on the quadriceps rectus femoris muscle, mid-way between the anterior superior iliac spine and the base of the patella. The distance from the patella was recorded and used for accurate repositioning during subsequent experimental trials. The probes were affixed using double-sided adhesive tape and covered with a soft black cloth to prevent movement and signal contamination from external light sources. Wire regions immediate to the probe were neatly secured along the participants' thighs to minimize movement during cycling. Following instrumentation, the zero set procedure was applied to reset O₂Hb, HHb and tHb values to an arbitrary zero value. TOI values are not affected by the zeroing procedure as it is measured in absolute values. During the 10 min of supine rest prior to exercise, baseline NIRS parameters were established. Changes in NIRS-derived tHb and TOI were then normalized to mean baseline value (i.e., average of last min during rest). All NIRS data were sampled at 6 Hz and collected on data acquisition software (Powerlab, 16/30, AD Instruments, Bella Vista; Australia). Values were then converted to 1-min averages for statistical analysis. Time-averaging NIRS-derived signals has been shown to result in better reliability, with coefficient of variation values of 4.6 % and 13.7 % reported for TOI and tHb, respectively, during interval exercise (27).

Data Processing

Similar to previous research (2), double-leg power output was halved for comparison with single-leg cycling to give an indication of power output produced per leg. Baseline oxygen consumption measured during the final two minutes of the 10-minute resting period of each session was subtracted from the values obtained during each interval to represent only the oxygen consumption related to the work bout. Work completed during the double-leg and sequential single-leg interval sessions were calculated accordingly to the formula: work (J) = $P * t$, where P is the average power output (W) produced during the intervals (i.e. six 1-min

double-leg intervals and each of the six 1-min single-leg intervals) and t is the total time (s) performing intervals. Total work completed during the single-leg session was calculated as the sum of the initial and second active leg.

Sample Size Calculation

Sample size calculation was based on differences in per leg power output achieved during high intensity intervals completed with double-leg (198 ± 29 W) and single-leg cycling modalities (172 ± 19 W) (2). Given the limited research on local hemodynamic differences between single-leg and double-leg cycling, we powered this study to ensure that differences in power output could be observed; thus allowing comment on the hemodynamic changes associated with such differences in power output. We recruited 10 participants to ensure we had the power to observed a moderate to large effect (~ 0.7 , $\alpha = 0.05$ and power = 0.8).

Statistical Analysis

A single mean value for power output, work, oxygen consumption, heart rate and MAP were calculated from the six 1-min double-leg intervals and 12 1-min single-leg intervals to represent the overall session. Paired t tests were used to compare the double-leg and sequential single-leg sessions.

Power output, work, oxygen consumption, heart rate and MAP were calculated separately for the initial six 1-min single-leg intervals and for the second six single-leg intervals and compared using paired t tests.

Local muscular data (ΔtHb , ΔTOI) collected on both legs during the six 1-min double-leg intervals and during the first six single-leg 1-min intervals were collated to provide one mean value to represent the mean of the double-leg, the active single-leg and the inactive single-leg.

Differences in ΔtHb and ΔTOI between the mean double-leg, active single-leg and inactive single-leg were analyzed using a one-way repeated measures analysis of variance.

To further characterize the influence of the active leg order (i.e. active during the initial or second set) during the sequential single-leg interval session, local muscular data collected on each leg during the 12 single-leg intervals were first collated into one mean value to represent the active and inactive legs during the initial six and second six intervals. Leg One denotes the leg which was active in the initial six single-leg intervals and inactive in the second six intervals. Conversely, Leg Two denotes the leg which was inactive in the initial six intervals and active in the second six intervals. The percent change in ΔtHb and ΔTOI from the initial to second six intervals in Leg One and Leg Two were analyzed using paired t tests.

Significant main effects or interactions were analyzed using a Fisher's LSD post-hoc analysis. Effect size estimates (ES; Cohen's d) were calculated to confirm the meaningfulness of the difference. Statistical analyses were conducted using SPSS (version 24; IBM; USA) and variables were deemed significant when $p \leq 0.05$. All data are presented as mean \pm standard deviation.

Results

Whole body responses to the overall session: double-leg versus sequential single-leg cycling

Power output, work, oxygen consumption, heart rate and MAP during sequential single-leg and double-leg high intensity interval cycling are presented in Table 1. Power output ($p < 0.01$; ES = 1.77), oxygen consumption ($p < 0.01$; ES = 1.13), heart rate ($p < 0.01$; ES = 0.59) and MAP ($p < 0.01$; ES = 1.22) were lower during sequential single-leg cycling when compared with double-leg cycling. Power output produced per leg ($p < 0.01$; ES = 0.69) and work completed

($p < 0.01$; ES = 0.71) were greater during sequential single-leg compared with double-leg cycling.

Whole body responses to the sequential single-leg cycling session: initial versus second set of intervals

Power output, oxygen consumption, heart rate and MAP during the initial and second sets of single-leg cycling intervals are shown in Table 2. Power output ($p = 0.51$; ES = 0.09), work ($p = 0.51$; ES = 0.05), oxygen consumption ($p = 0.19$; ES = 0.17) and heart rate ($p = 0.29$; ES = 0.00) during the intervals were not different between sets. Mean arterial pressure ($p < 0.05$; ES = 0.70) was lower during the second set compared with the initial set of intervals.

Local muscular responses to double-leg and single-leg cycling: mean double-leg versus active single-leg and inactive single-leg

The responses of ΔtHb and ΔTOI in the mean double-leg, active single-leg and inactive single-leg during high intensity interval cycling are presented in Figure 2. The ΔtHb in the inactive single-leg was smaller than the active single-leg ($p < 0.01$; ES = 1.17). No differences in ΔtHb were observed between the mean double-leg and active single-leg ($p = 0.26$; ES = 0.33) or inactive single-leg ($p = 0.13$; ES = 0.76). The ΔTOI was not different between the mean double-leg, active single-leg and inactive single-leg.

Local muscular responses to sequential single-leg interval cycling: Leg One versus Leg Two

To visually represent the local muscular responses of each leg during the sequential single-leg interval cycling session, one sec averages of a representative participant were plotted for Leg One and Leg Two from supine rest to the end of the intervals (Figure 3). No analyses were performed on the 1-sec averages.

The change in ΔtHb from the initial to second six intervals demonstrated significantly different ($p < 0.05$; 0.89) patterns in Leg One ($-52.3 \pm 111.6 \%$) when compared with Leg Two ($65.1 \pm 152.9 \%$). Specifically, when the leg is active during the initial six intervals (i.e. Leg One), muscle blood volume was reduced by $52.3 \pm 111.6 \%$ during the second six intervals when the leg is now inactive. Conversely, when the leg is active during the second six intervals (i.e. Leg Two), muscle blood volume was increased $65.1 \pm 152.9 \%$ from the initial six intervals when it was inactive. The change in ΔTOI from the initial to second six intervals was not different ($p = 0.18$; 0.92) between Leg One ($-25.0 \pm 77.1 \%$) and Leg Two ($45.5 \pm 76.2 \%$).

Discussion

This study investigated the cardiovascular and skeletal muscle hemodynamic responses to a single session of high intensity interval cycling using either sequential single-leg or double-leg cycling in young healthy adults. The main findings from this study were; 1) a higher individual leg power output was produced during single-leg compared with double-leg cycling, 2) a reduction in mean arterial pressure was observed during the second set of single-leg intervals, 3) skeletal muscle blood volume (ΔtHb) and oxygenation (ΔTOI) were similar between the active single-leg and mean double-leg, and 4) the pattern of change in muscle blood volume was significantly different between Leg One (active in initial set of single-leg intervals) and Leg Two (active in second set of single-leg intervals).

Whole body responses to the overall session: double-leg versus sequential single-leg cycling

Absolute power output during the single-leg intervals was lower compared with the double-leg intervals; however, the power output produced by the active single-leg was $12 \pm 2\%$ higher than that calculated for each leg during the double-leg condition (Table 1). This finding is consistent with previous research from our laboratory which demonstrated a $\sim 15\%$ higher per leg power output during single-leg compared with double-leg intervals in trained cyclists (2).

Importantly, in our previous study greater per leg power output was hypothesized as the stimuli promoting enhanced cellular glucose transport and mitochondrial enzyme capacities following three weeks of single-leg high intensity interval cycling (2). We have previously hypothesized that these higher power outputs observed during single-leg cycling were achieved due to a greater leg blood flow and oxygen extraction during single-leg compared with double-leg cycling (1, 3, 9, 11). However, contradictory to this hypothesis, we observed similar increases in quadriceps muscle blood volume (Figure 2) (discussed below).

Whole body responses to the sequential single-leg cycling session: initial versus second set of intervals

There were no differences in power output, work, oxygen consumption or heart rate between the initial and second sets of single-leg intervals (Table 2) indicating similar exercise intensities. However, during the second set of intervals, MAP was significantly lower (Table 2). It is possible that the decrease in MAP was a result of reduced systemic vascular resistance by way of alterations in shear stress throughout the vasculature (16, 17) as a result of exercise and thermoregulatory mechanisms (28, 29). Additionally, blood pooling in the initially active leg (discussed below) may also have contributed to the decreased MAP during the second set of intervals due to a reduction in venous return of this localized blood volume. Importantly, despite this reduced perfusion pressure, the vasculature was able to appropriately redistribute the available blood to maintain performance (i.e. power output) during the second set of intervals.

Local muscular responses to double-leg and single-leg cycling: mean double-leg versus active single-leg and inactive single-leg

The ΔtHb and ΔTOI were not different between the active single-leg and mean double-leg intervals (Figure 2) indicating similar quadriceps muscle blood volume and oxygenation. These

findings are consistent with previous studies demonstrating no differences in the quadriceps muscle activation, measured by surface electromyography, between single-leg and double-leg interval cycling despite the per leg power output being ~13% higher during the single-leg intervals (30). Greater semitendinosus muscle activation has been observed during single-leg cycling (30); thus, greater knee flexion work (31) could have contributed to the greater per leg power output observed during single-leg cycling (1, 2, 5, 6). Alternatively, it is possible that additional areas of the quadriceps muscle were perfused and/or additional motor units were recruited which were not detected by the non-invasive methods used in the present study and that of MacInnis et al. (30). Previous findings of greater improvements in quadriceps metabolic and oxidative potential following single-leg compared with double-leg cycling support this hypothesis (2), specifically as biochemical adaptations do not appear to transfer from active to inactive legs during single-leg cycling (32, 33). However, as mentioned, the inactive leg is not hemodynamically nor metabolically dormant and thus will likely contribute to the peripheral stimulus experienced during single-leg cycling.

In addition to the increase in muscle blood volume in the active single-leg, there was also an increase, albeit smaller, in the inactive leg (Figure 2A). Cooper et al. (8) observed similar increases in muscle blood volume between the active and inactive leg half way through a single-leg graded exercise test; however, at peak exercise, the active leg demonstrated a significantly higher muscle blood volume. Additionally, increases in femoral blood flow, oxygen consumption and carbohydrate utilization in the inactive leg were observed at submaximal exercise intensities (9-11). Since the active single-leg and mean double-leg local muscular responses were similar (Figure 2), the hemodynamic responses observed within the inactive leg would likely increase the peripheral stimulus associated with single-leg cycling. This has important implications since previous research has identified that altered hemodynamic responses in the inactive musculature contribute to the vascular adaptations

observed in muscle beds not directly related to the activity (17). These data suggest that single-leg high intensity interval cycling could provide additional vascular and muscular adaptations beyond that which is achievable with double-leg cycling.

Local muscular responses to sequential single-leg interval cycling: Leg One versus Leg Two

The present study is the first to demonstrate a leg order effect on blood volume during sequential single-leg cycling. Specifically, the increase in blood volume for Leg Two (active in the second set) was not consistent with the decrease in blood volume for Leg One (active in the initial set) indicating possible blood pooling. This finding has important implications for the interpretation of previous research and future prescription of sequential single-leg cycling training. For instance, the majority of previous studies utilizing sequential single-leg cycling training either did not specify leg order (34-37) or always exercised the right leg before the left leg (1). Based on our findings, it is possible that differences in acute cardiovascular and metabolic response between the initial and second sets of sequential single-leg cycling could have influenced the outcome of these studies. These findings are also important in the prescription of aerobic exercise using multiple modalities, and thus different muscle groups, within a single exercise session. While this study indicates that the acute metabolic and cardiovascular response may be influenced by the order of exercising muscle groups, further research is needed to examine training adaptations associated with high intensity aerobic exercise using multiple muscle groups within a single exercise session.

The findings from this study provide important information about the leg order effect associated with sequential single-leg cycling. However, this study is not without limitations. Performing twice as many single-leg compared with double-leg intervals could have influenced the comparison of the hemodynamic measures, specifically when comparing double-leg to the second set of single-leg intervals. Nevertheless, this methodology was chosen as it is consistent

with previous training studies that have used sequential single-leg interval cycling (2, 38) and this methodology ensured that the total time exercising each leg was consistent between sessions (i.e. similar number of muscular contractions). The use of a double-leg warm up prior to single-leg intervals, while consistent with methods used in previous sequential single-leg training studies (38), could have influenced the local hemodynamics of both the active and inactive legs (39). Nevertheless, the intensity of the warm up was relatively low when compared to the intervals and performed with both legs. As such, any change in hemodynamics would have influenced both legs equally. Finally, tHb measures were obtained in an upright cycling posture; yet, normalised to mean resting value obtained in a supine position. It is possible that postural changes may have altered fluid shifts influencing tHb measures in this study. However, NIRS in the supine position was only used for normalisation and was consistent across conditions.

Conclusions

The present study provides insight into the potential mechanisms responsible for the benefits of sequential single-leg cycling. In particular, the hemodynamic responses of the inactive single-leg will likely contribute to the overall increased peripheral stimulus typically observed with single-leg cycling since the active single-leg and mean double-leg responses were similar. Additionally, differences in MAP and blood volume distribution during sequential single-leg cycling indicates attention should be given to active leg order since it has the potential to alter the acute cardiovascular and metabolic responses. Future research should examine the leg vascular and muscular responses of sequential single-leg compared with double-leg cycle training to provide further insight into the mechanisms behind the established benefits of sequential single-leg cycling training.

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386 **Conflicts of Interest**

387 The authors declare no conflicts of interest, financial or otherwise. The authors declare that the

388 results of the study are presented clearly, honestly, and without fabrication, falsification, or

389 inappropriate data manipulation and do not constitute endorsement by the American College

390 of Sports Medicine.

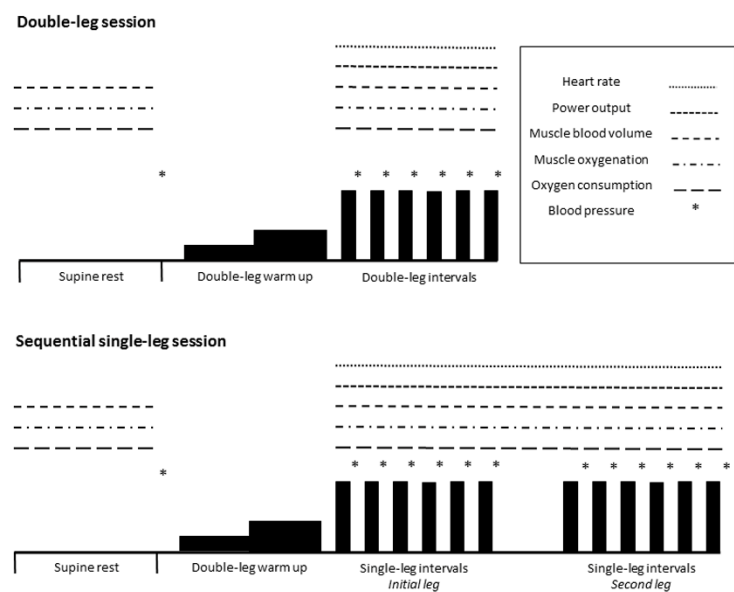
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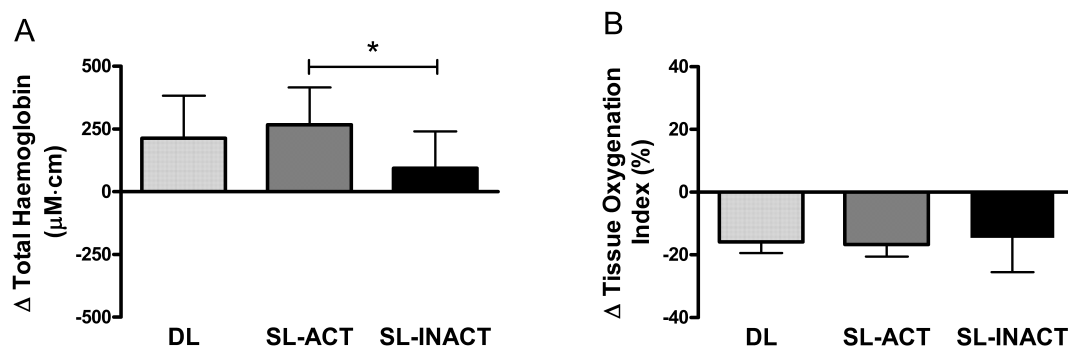
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492
493 Figure 1. Schematic of experimental sessions.



494
495 Figure 2. Change in total hemoglobin (A) and tissue oxygenation index (B) in the mean double-
496 leg (DL), active single-leg (SL-ACT) and inactive single-leg (SL-INACT) during high
497 intensity interval cycling. *Inactive single-leg less than active single-leg ($p<0.01$).

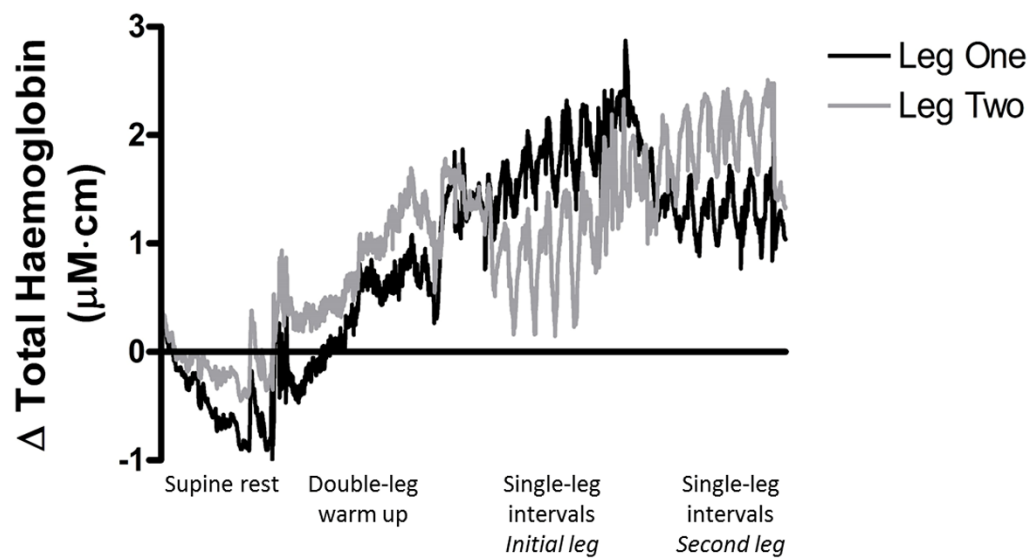


Figure 3. Raw values for Δ tHb of a representative participant during sequential single-leg cycling. Leg One was active during the initial set of intervals and inactive during the second set. Conversely, Leg Two was inactive during the initial set of intervals and active during the second set.