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Beltrami, Fernando G., Froyd, Christian, Mauger, Alexis R., Metcalfe, Alan J. and Noakes, Timothy D. (2021) Comparison of Physiological Responses and Muscle Activity During Incremental and Decremental Cycling Exercise. International Journal of Sports Physiology and Performance . pp. 1-8. ISSN 1555-0265.

## DOI

https://doi.org/10.1123/ijspp.2021-0020

## Link to record in KAR

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1	Comparison of physiological responses and muscle activity during incremental
2	and decremental cycling exercise
3	
4 5	Submission type: original investigation
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31 32	Running title: Decremental exercise tests in cyclists
33 34 35 36 37	Abstract word count: 224 Text-only Word Count: 2949 Number of figures and tables: 7
38 39 40 41 42	<b>Funding Disclosure:</b> This work was funded by the funded by Discovery Health (Pty) (Ltd), the Medical Research Council of South Africa, the Harry Crossley and Nellie Atkinson Staff Research Funds of the University of Cape Town, and the National Research Foundation of South Africa through its THRIP initiative.

#### 1 Abstract

- 2 Objective: to investigate whether a cycling test based on decremental loads (DEC) could
- 3 elicit higher  $\dot{VO}_{2max}$  values compared with an incremental test (INC).

4 Design: Nineteen well-trained individuals performed an INC and a DEC test on a single 5 day, in randomized order.

- Methods: During INC the load was increased by 20 W.min<sup>-1</sup> until task failure. During
  DEC the load started at 20 W higher than the peak load achieved during INC
  (familiarization trial) and was progressively decreased. Gas exchange and EMG activity
  (n=11) from four lower limb muscles were monitored throughout the tests. Physiological
- and EMG data measured at  $\dot{VO}_{2max}$  were compared between the two protocols using
- 11 paired t-tests.
- 12 Results:  $VO_{2max}$  during the DEC was 3.0 (5.9) % higher than during INC (range 94 116
- 13 %; p = 0.01), in spite of a lower power output (-21 [20] W, p < 0.001) at  $\dot{V}O_{2max}$ .
- Pulmonary ventilation (p = 0.036) and breathing rate (p = 0.023) were also higher during
- DEC. EMG activity measured at  $\dot{VO}_{2max}$  was not different between tests, despite the lower output during DEC.
- 17 Conclusions: A decremental exercise test produces higher  $\dot{V}O_{2max}$  in cycling compared to
- 18 an incremental test, which was accompanied by higher pulmonary ventilation and similar
- 19 EMG activity. The additional  $O_2$  uptake during DEC might be related to extra work
- 20 performed by the respiratory and the less oxidatively efficient leg muscles.
- 21
- 22 Key-words: maximal oxygen uptake,  $\dot{V}O_{2max}$  plateau, exercise testing, decremental,
- 23 cycling
- 24

#### 1 Introduction

Maximal oxygen uptake ( $\dot{V}O_{2max}$ ) represents the upper limit of cardiorespiratory 2 capacity during exercise. Because of its association with several performance and even 3 4 health indicators, it is important that  $\dot{V}O_{2max}$  is properly assessed during testing, and a substantial body of literature has been dedicated to it <sup>1</sup>. Current standards dictate that 5  $\dot{V}O_{2max}$  should be assessed during incremental exercise tests, in which the workload 6 increases at given time intervals until the participant is exhausted. In these tests, leveling-7 8 off of  $\dot{V}O_2$  with increasing exercise intensities – the  $\dot{V}O_{2max}$  plateau phenomenon – is seen 9 as evidence that  $\dot{V}O_{2max}$  is, indeed, maximal. When evidence of a plateau is not present, 10 a second test at higher workload is recommended to confirm the attainment of  $\dot{V}O_{2max}$ , the so-called verification phase or verification test  $^{1}$ . 11

12 A few studies, however, have shown that alternatives to the incremental exercise test can produce even higher values of  $\dot{V}O_{2max}$ . Some of these tests are based on cycling 13 time-trial efforts <sup>2,3</sup> or self-paced and closed-loop exercise <sup>4</sup>, and even decremental 14 exercise tests in runners <sup>5</sup> and elite runners <sup>6</sup>. While these studies have called into question 15 whether the VO<sub>2max</sub> measured during incremental tests (with or without the verification 16 17 phase) truly represents a cardiorespiratory limitation, later studies have not always corroborated these findings, for instance showing no difference between traditional 18 incremental tests and self-paced tests in cycling <sup>7,8</sup> or decremental tests in runners <sup>9</sup>. 19

20 Some these contradicting results might be specific to the conditions of each particular study. For example, Jenkins and colleagues found that a self-paced protocol 21 produced higher  $\dot{V}O_{2max}$  values in younger, but not in older individuals <sup>10</sup>. Furthermore, 22  $\dot{V}O_{2max}$  not only varies according to one's training background <sup>11</sup>, but is also specific to 23 a given exercise modality<sup>8</sup>. In this context, to date only one independent attempt has been 24 made to replicate the finding of a higher  $\dot{V}O_{2max}$  during a decremental test, also in runners 25 <sup>9</sup>. Therefore, the aim of this investigation was to test whether the findings of a higher 26 VO<sub>2max</sub> during decremental running exercise tests originally reported in well-trained 27 28 individuals <sup>5</sup> would also be present in a group of well-trained cyclists. In addition, we wished to determine whether muscle activity of the lower limbs would be different at the 29 time that  $\dot{V}O_{2max}$  was achieved in both tests. We postulated that a higher  $\dot{V}O_{2max}$  would 30 be present during the decremental exercise test and that in spite of a lower power output 31 at VO<sub>2max</sub> the EMG activity would be higher, indicating a greater muscle activation. 32

#### 33 Methods

#### 34 Subjects

35 The study was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town (253/2009). Participants were clearly 36 37 informed about the risks and procedures involved in this investigation and gave their written consent for participation. The study was divided in two parts (see figure 1). In 38 39 part A, eleven participants (ten male and one female, (mean [SD]) age 29.0 [7.4] years, 40 body mass 75.2 [7.8] kg, height 181 [9] cm), all accustomed to high-intensity cycling, visited the laboratory on two occasions. In part B, eight male participants (age 23.8 [5.5] 41 years, body mass 69.8 [8.1] kg, height 179 [4] cm), all accustomed to high-intensity 42 43 cycling, visited the laboratory on three occasions, each separated by 2-3 days.

44

45 Design

1 This investigation was set-up as repeated measures design where  $\dot{V}O_{2max}$  was 2 defined as the dependent variable and primary outcome and the exercise test (i.e. 3 incremental or decremental exercise test) as the independent variable. Both exercise tests 4 were performed in the same day (see below) and order in which the tests were performed 5 was randomized and balanced between participants, so that an equal number of 6 participants performed each of the two test orders. Other outcomes of interest were gas 7 exchange, heart rate and workload at the time of  $\dot{V}O_{2max}$ .

8 Furthermore, based on prior data from our group that showed that both trained <sup>5</sup> and elite runners <sup>6</sup> showed a higher  $\dot{V}O_{2max}$  during an incremental test following two 9 exposures to the decremental test (in contrast to a control group with stable  $\dot{V}O_{2max}$  over 10 five consecutive incremental test trials), we sub-divided the present study into two parts 11 (Figure 1). Part A was designed to ensure no adaptative effect from the decremental test 12 could take place (i.e. no familiarization with the protocol was allowed prior to the main 13 comparison), whereas in Part B an additional visit was included where participants were 14 15 familiarized with the decremental test.

16 In part A, the participants visited the laboratory on two occasions. On their first 17 visit (familiarization), the participants performed a maximal incremental exercise test on a cycle ergometer (INC<sub>fam</sub>), followed 15 minutes later by a verification test to confirm the 18 attainment of  $\dot{V}O_{2max}$ . On visit two, the participants performed three exercise tests, each 19 separated by approximately one hour. First, they performed an incremental test (INC<sub>1</sub>), 20 21 following which a decremental (DEC) and another incremental test (INC) were performed in randomized, counter-balanced order. During the trials on visit two, gas 22 23 exchange data, heart rate and EMG activity of four leg muscles (vastus lateralis, biceps femoris, vastus medialis and gastrocnemius medialis) were continuously monitored. 24 Prior to the first incremental test, a 10-s sprint was performed, with the ergometer set in 25 26 linear (cadence-dependent) mode. This sprint served as a reference for the EMG analysis in the time domain, as it has been recommended <sup>12</sup>. 27

In part B, the participants visited the laboratory on three occasions, each separated by 2-3 days. The first visit was similar to that described in part A, and on the second visit a familiarization with the decremental exercise test (DEC<sub>fam</sub>) was performed. On their third visit, the participants repeated the INC and DEC exercise tests in randomized, counter-balanced order, separated by at least 60 minutes of rest. Gas exchange data, heart rate and power output were monitored throughout the tests.

#### 34 Cycling tests

35 All exercise trials were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, Netherlands). Prior to each exercise test, the participants 36 37 warmed-up for 10 minutes at a self-selected cadence and power output. The INC tests started at 200 W for men and 160 W for women, with increments of 20 W applied every 38 60 seconds thereafter, until cadence fell below 60 revolutions per minute (rpm). Strong 39 verbal encouragement was given whenever the pedaling cadence approached the 60-rpm 40 mark. The verification test consisted of a square-wave test to exhaustion performed at one 41 stage higher (20 W) than the last completed stage during INC<sub>fam</sub>. Immediately prior to the 42 test, the participants cycled for three minutes at 150 W (120 W for women). The load was 43 then increased to the desired power within 20 seconds, and participants were instructed 44 to cycle for as long as they could. The DEC test started with three minutes of light cycling 45 (150 W for men, 120 W for women), following which the load was raised within 20 46 seconds to the equivalent used during the verification test (that is, 20 W higher than the 47 load of the last completed stage during INC<sub>fam</sub>). The initial load was maintained for 60% 48

of each individual's time to task failure recorded during the verification phase test. The
average duration for the first stage of DEC was 90 s (range 75-105 s). Following the first
stage, the load was decreased by 20 W and maintained for 30 s. Subsequent decrements
of 10 W were maintained for 45, 60, 90 and 120 s, thus totaling ~7 min 15 s of test time.
If this protocol proved too demanding for the subject (time to fatigue < 3 minutes during</li>
DEC<sub>fam</sub>, part B only), on the second occasion that they performed the decremental
protocol (DEC, Part B) the first decrement in workload was increased from 20 to 30 W.

#### 8 Cardiorespiratory variables

9 Gas exchange and heart rate were continuously monitored during the exercise tests 10 using calibrated metabolic carts. In Part A, a MOXUS Modular Metabolic System was used (AEI Technologies, IL, USA) paired with a Polar S410 (Polar Electro OY, Kempele, 11 Finland). In Part B, a Cortex Metalyser was used (3B, Biophysik, Leipzig, Germany) 12 13 paired with a Polar T31. All gas exchange and heart rate data exported as consecutive 5 s means. VO<sub>2max</sub> was defined as the highest 60 s moving average measured during a test 14 and all other physiological variables were recorded at the same time as  $\dot{V}O_{2max}$ . For 15 comparison of  $\dot{VO}_{2max}$  values on day 1, between the incremental and verification tests, 16 30-s moving averages were used in order to compensate for the much shorter duration of 17 the verification tests, where  $\dot{V}O_{2max}$  was not necessarily constant for a full minute. 18

19 *Muscle electrical activity* 

In Part A, EMG signals from the vastus lateralis, vastus medialis, gastrocnemius 20 medialis and biceps femoris of the right leg were continuously recorded (Part A) using 21 DE-2.1 single differential surface sensors with 10 mm inter-electrode distance (Delsys 22 Inc., Boston, MA, USA). SENIAM<sup>13</sup> and Delsys recommendations for the placement of 23 the sensors on the skin were followed. Before electrode placement, the skin was shaved 24 25 to remove excessive hair and wiped with isopropyl alcohol. A reference electrode was applied over the patella. The EMG signals were sampled at 2000 Hz and amplified using 26 27 Bagnoli-8 (Delsys Inc). The EMG signals were transferred into Power Lab 28 (ADInstruments, Colorado Springs, CO, US) and filtered using a 15-500 Hz band pass filter in Lab Chart Pro software (ADInstruments). Root mean square EMG activity was 29 calculated for every 20 ms and averaged over the minute in which  $\dot{V}O_{2max}$  was measured. 30 Only the periods of active contraction were used for analysis, which were determined as 31 32 root mean square EMG values > 10% above baseline (quiet sitting on ergometer). EMG 33 activity was expressed as a percentage of the root mean square activity recorded during the 10-s sprint which was performed immediately prior to the first exercise trial (but after 34 the 10 min warm-up period). The % activation was also multiplied by the fraction of time 35 in which the muscle was active to obtain an estimate of total activation, akin to the 36 integrated EMG signal <sup>14</sup>. The frequency contents of the EMG signal were analyzed using 37 fast Fourier transformation, and the mean and median frequency from the EMG signal 38 during the period in which VO<sub>2max</sub> was measured were determined. Prior to the sprint, the 39 participants cycled at a low power output for two minutes. EMG data is presented as n = 40 41 7 for the vastus lateralis, gastrocnemius medialis and biceps femoris and n = 9 for the vastus medialis due to technically inadequate data for at least one test on some 42 43 participants, usually the result of electrode detachment/excessive noise mid-test.

#### 44 Statistics

All data are presented as mean (standard deviation), unless stated otherwise. The reproducibility of  $\dot{VO}_{2max}$  and EMG variables between INC<sub>1</sub> (performed before the experimental trials) and INC (performed in randomized order with DEC) was analyzed by calculating the intraclass correlation coefficient (ICC), the typical error of

measurement (the standard deviation of the difference between measurements divided by 1 the square root of two) and the individual coefficient of variation <sup>15</sup>. The physiological 2 responses from the participants in both parts of the study were pooled together and the 3 physiological responses between the DEC and INC analyzed used Student's paired t-tests, 4 5 with the normality of residuals analyzed using Q-Q plots. Comparisons are presented with 6 the t-statistic, 95% confidence interval of the differences (95% CI) and the effect size as Cohen's d for independent samples (mean difference divided by the pooled standard 7 8 deviation of both groups) or Cohen d<sub>z</sub> for dependent samples (mean of differences divided 9 by the standard deviation of the difference scores). The effect of adding a specific familiarization trial for the decremental test (part B vs. part A) was analyzed by 10 comparing the DEC/INC VO<sub>2max</sub> ratio in parts A and B of the study, using Student's 11 independent t-test. Significance level was set at p < 0.05, and all statistical procedures 12 13 were calculated using Prism 8.3 (GraphPad, La Jolla, CA) or dedicated Excel spreadsheets. 14

#### 15 **Results**

#### 16 VO<sub>2max</sub> during the incremental and verification tests

During INC, participants were able to exercise for 8.3 (1.7) minutes reaching a 17  $\dot{V}O_{2max}$  of 55.8 (8.2) mL.kg<sup>-1</sup>.min<sup>-1</sup>(parts A and B pooled). In the familiarization visits, 18  $\dot{V}O_{2max}$  obtained from the incremental test was not different from that measured during 19 the verification tests (30-s moving averages, 4,139 [743] vs. 3,989 [717] mL.min<sup>-1</sup> 20 respectively, 95% CI -345 – 46 mL.min<sup>-1</sup>, p = 0.125). 30-s moving averages of  $\dot{V}O_2$  were 21 used in both tests due to the much shorter nature of the verification test. When the  $\dot{V}O_{2max}$ 22 of the familiarization session as compared with that from the main experimental session, 23 there was no statistical difference between the two (mean difference 0.0 mL.min<sup>-1</sup>, 95%) 24 CI -195 – 196 mL.min<sup>-1</sup>, p = 0.99). This is in spite of a significant difference increase in 25 exercise tolerance on the second attempt (+0.6 min, 95% CI 0.2 - 1.0 min, p = 0.008). 26

On the experimental day of part A of the study, when two incremental tests were performed (INC<sub>1</sub> and INC),  $\dot{V}O_{2max}$  values were very similar between them (4,366 [464] vs. 4,354 [499] mL.min<sup>-1</sup> for INC<sub>1</sub> and INC respectively, 95% CI -80 – 57 mL.min<sup>-1</sup>, p = 0.716). Intra-class coefficient for  $\dot{V}O_{2max}$  (INC<sub>1</sub> vs INC) was 0.98, and the typical error of measurement was 72 mL.min<sup>-1</sup> (1.7%).

32

#### 33 $\dot{VO}_{2max}$ during the decremental test

During DEC (parts A and B pooled), VO<sub>2max</sub> values were on average 3.0% higher 34 than during INC (4,259 [695] vs. 4,139 [657] mL.min<sup>-1</sup> respectively, 95% CI 16 - 224 35 ml.min<sup>-1</sup>,  $d_z = 0.56$ , p = 0.025, Figure 2), although they were exercising at a significantly 36 lower power output at the time that  $\dot{V}O_{2max}$  was achieved (312 [43] vs. 333 [47] W for 37 DEC and INC respectively, 95% CI 10.8 – 31.1 W,  $d_z = 1.03$ , p < 0.001). Individual data 38 for 60-s moving averages in both tests are provided in supplemental figure 1. An 39 additional comparison was performed between DEC and INC using 30-s moving averages 40 and yielded similar results (data not shown). Test termination during DEC occurred at 5.7 41 (1.6) min, (range 2.5 - 9.5 min, not considering the preceding 3 min lead-in pahse at 42 moderate power) which was significantly shorter than the INC test (-2.9 min, 95% CI -43 4.3 - 1.6 min, p = 0.0002). Important, two decremental tests were interrupted by the 44 45 experimenter as participants reached the end of the protocol. All physiological data measured during the exercise tests are shown in table 2. During DEC, participants also 46 reached 4.5% higher pulmonary ventilation ( $V_E$ ) in comparison to INC (p = 0.036), due 47

1 to an increased breathing rate (BR) (Table 2, p = 0.023). Respiratory exchange ratio,

2 carbon dioxide output and heart rate were not different between the two exercise tests at 3  $\dot{VO}_{2max}$  (Table 2).

4

#### 5 Effects of familiarization on the decremental test

6 In Part B, there were no differences in  $\dot{VO}_{2max}$  between DEC<sub>fam</sub> and DEC (3,904 [718] vs.

7 3,953 [758] mL.min<sup>-1</sup> respectively, 95% CI -17 – 71 ml.min<sup>-1</sup>,  $d_z = 0.34$ , n = 8, p = 0.371).

8 Likewise, when parts A and B were compared with regards to the ratio of DEC/INC

9  $\dot{V}O_{2max}$  elicited there were no significant differences (1.017 [0.042] vs. 1.047 [0.069] for

10 parts A and B respectively, 95% CI -0.02 – 0.08, d = 0.54, p = 0.258).

11

#### 12 Muscle electrical activity

For the EMG variables, reliability data for each muscle between  $INC_1$  and INCare shown in Table 1. Overall, all four analyzed muscles showed a good degree of reliability (ICC 0.85 – 0.98).

16 Individual data for EMG activity of the four evaluated muscles is presented in 17 Figure 3. There were no significant differences in mean EMG activity or total activation 18 measured at  $\dot{V}O_{2max}$  between INC and DEC for any of the analyzed muscle groups. There 19 were also no significant differences between INC and DEC for any of the muscles for 20 mean or median frequency (Table 3).

21

#### 22 **Discussion**

The main findings from this study are that: i) trained subjects achieved higher VO<sub>2max</sub> values during a cycling DEC in comparison to INC; ii) the higher  $\dot{VO}_{2max}$  during DEC was accompanied by higher  $\dot{V}_E$  in comparison to INC; iii) EMG activity for four lower limb muscles was similar at the time of  $\dot{VO}_{2max}$  in both testing protocols; and iv) the order of the tests or previous familiarization with DEC did not affect the  $\dot{VO}_{2max}$ results.

The ideal protocol for the assessment  $\dot{V}O_{2max}$  has been the topic of research for 29 vears. Under the current paradigm of incremental tests, it has become clear that  $\dot{V}O_{2max}$  is 30 robust over a wide range of protocols and test durations <sup>16</sup>. Furthermore, it has also been 31 shown that simply requiring higher energetic output does not result in higher  $\dot{V}O_{2max}$ . 32 whether the additional output comes from the exercising limbs, as in the case of the 33 verification test <sup>17</sup>, or the upper arms <sup>18</sup>. Our data confirms these findings, as the values 34 obtained during the verification tests were if anything lower (although the difference did 35 not reach statistical significance) than those measured during the preceding incremental 36 test. Thus, the  $\dot{VO}_{2max}$  determined during the incremental test in our study is within what 37 is currently considered best practice in terms of protocol and analysis. 38

In this context, the decremental exercise actually operates within a similar range of workloads, as the test starts at the same workload as the verification test and only goes down. This eliminates the possibility that any differences between protocols would the result of a poor choice of work rate increase from the incremental tests. Based on our previous research and pilot testing, however, it became evident that a perfect mirrorimage of an incremental test would lead to a too rapid decrement in workload, and eventually all cardiorespiratory responses would turn downwards before VO<sub>2max</sub> could be

reached. Exercise intensities within the so-called "severe" domain are known to 1 eventually lead to  $\dot{V}O_{2max}$  <sup>19</sup>, and in trying to balance a prolongation of the protocol 2 towards more than 2-3 min with eliciting the highest possible physiological response a 3 protocol with decreasing workloads of increased durations was envisioned. Although the 4 decremental and incremental protocol differ in terms of time taken to reach VO<sub>2max</sub> as 5 well as work performed, , this should not prevent a comparison between the two, much 6 like different incremental tests with slightly different ramps are routinely compared and 7 also do not show differences in  $\dot{VO}_{2max}^{16}$ , or even the comparison between all-out 8 exercise and incremental tests <sup>20</sup>. 9

In spite of the lower power output at VO<sub>2max</sub> during DEC, EMG activity was 10 similar between DEC and INC at the time of  $\dot{V}O_{2max}$ . The increase in  $\dot{V}O_2$  during all-out 11 exercise tests (where  $\dot{V}O_2$  increases up to  $\dot{V}O_{2max}$  despite decreasing power output and 12 EMG activity) has been proposed to be a "slow-component"-like phenomenon<sup>21</sup>, 13 resulting from the loss of efficiency in the exercising muscles. VO<sub>2</sub> then increases during 14 15 exercise because of progressive recruitment of less efficient, type IIx fiber, which increases the  $O_2$  cost for force production per fiber. An alternative explanation is that 16 while an initial bout of very intense exercise creates the demand for  $O_2$ , a subsequent 17 18 decrease in power output might improve limb blood flow, as muscle contraction intensity 19 and vasoconstriction would be likely reduced, thereby improving  $O_2$  extraction. The similar EMG activity coupled with lower power output found in the present study likely 20 21 facilitated higher blood flow, which could indeed have facilitated higher O<sub>2</sub> extraction.

To test whether our EMG measures were sensitive enough to detect differences 22 23 caused by a 20 W change in power output (i.e. the difference between INC and DEC at  $\dot{VO}_{2max}$ ), we compared the root mean square and total activation of the analyzed muscles 24 prior to VO<sub>2max</sub> during the incremental test, since our incremental protocol had a load rate 25 26 of 20 W.min<sup>-1</sup>. For both the *vastus lateralis* and *vastus medialis*, root mean square and/or total activation were sufficiently sensitive to have detected changes equivalent to a 20 W 27 change in power output, at least during a ramp-up protocol (Table 4). We did not find 28 29 differences in the mean or median frequency of the EMG signal of any of the analyzed 30 muscles, which precludes any suggestion of change in recruitment pattern. Furthermore, the total EMG activity was similar between INC and DEC, suggesting indeed lower 31 efficiency of the muscle contraction rather than exclusively higher oxidative metabolism 32 33 per se. Although our results suggest a loss of efficiency in producing mechanical work, as shown by a higher  $\dot{V}O_2$  for a similar EMG activity, it is not possible to argue 34 conclusively that the measured difference in  $\dot{VO}_2$  (~125 mL.min<sup>-1</sup>) is related to changes 35 36 in muscle efficiency.

The results from the present study extend the findings originally reported during 37 uphill running <sup>5</sup>, showing ~3% higher  $\dot{VO}_{2max}$  during DEC in comparison to INC when 38 exercise is performed on a cycle ergometer. These results were seen even though 39 participants had performed a verification test, current seen as the best standard to ensure 40 41 the attainment of "true" maximal oxygen uptake. The additional O<sub>2</sub> uptake requires either additional blood supply and/or increased oxygen extraction to take place during exercise, 42 be it in the respiratory muscles, exercising muscles or elsewhere. Mauger et al.<sup>4</sup> 43 hypothesized that a better distribution of blood flow in the active limbs due to a decrease 44 in power output  $^{22,23}$  could explain the higher  $\dot{V}O_{2max}$  measured during a self-paced test, 45 but this proposition remains purely speculative. In muscle preparations  $\dot{V}O_{2max}$  can be 46 altered by increasing O<sub>2</sub> extraction while O<sub>2</sub> delivery is kept constant when independently 47 manipulating SaO<sub>2</sub> and PaO<sub>2</sub>, although to our knowledge this not been demonstrated in 48 vivo  $^{24}$ . 49

In the present study, both  $\dot{V}_E$  and BR were higher during DEC at the time of 1 VO<sub>2max</sub>, different to the findings of Beltrami <sup>5</sup> and Taylor <sup>9</sup>. Thus, at least in cycling part 2 of the difference in  $\dot{V}O_{2max}$  measured between tests might be related to the additional  $O_2$ 3 cost of breathing. The 7.0 L.min<sup>-1</sup> difference in  $\dot{V}_E$  measured between DEC and INC could 4 raise  $\dot{V}O_2$  by approximately 20-30 mL.min<sup>-1 25</sup>, or explain 15-25% of the difference in 5 6  $\dot{V}O_{2max}$  measured between DEC and INC (120 mL.min<sup>-1</sup>). It must be noted, however, that differences in  $\dot{V}_E$  do not always result in measurable differences in pulmonary  $\dot{V}O_2$ <sup>26</sup>. 7 Moreover, simply forcing the respiratory muscles to perform more work does not increase whole-body  $\dot{V}O_{2max}$  or cardiac output <sup>27</sup>, similar to what happens when an additional 8 9 (supra-maximal) load is imposed to the muscles of the exercising limbs <sup>18</sup>. Therefore, 10 while the respiratory muscles can be seen as the receiver of the additional  $\dot{V}O_2$ , it does 11 not serve a mechanistical explanation of how  $\dot{V}O_2$  was increased. 12

The differences in  $\dot{V}O_{2max}$  between DEC and INC in the present study were 13 independent of whether participants were allowed a specific familiarization trial for DEC 14 15 or not and were also independent of the order in which INC and DEC were performed. This contrasts with previous findings, in which following DEC participants retained the 16 higher VO<sub>2max</sub> on a subsequent INC despite an unchanged performance in the final 17 incremental test <sup>5</sup>. Should the same had happened in the present study,  $\dot{V}O_{2max}$  would have 18 increased from INC<sub>fam</sub> to INC (Part B), since participants performed the DEC<sub>fam</sub> trial 19 between these two tests. Also, if participants had retained the higher VO<sub>2max</sub> values 20 21 following DEC, the VO<sub>2max</sub> differences between INC and DEC would have been blunted for all participants who performed INC after DEC, which did not happen. Although these 22 23 findings do not help to explain why the running studies showed a persistent increased 24  $\dot{\rm VO}_{2\rm max}$  following the DEC protocol, they show that future research using cycling exercise can be performed using a familiarization trial for both INC and DEC. 25

26 This investigation is not without its limitations. First, the decremental test per se could be criticized for the tailored approach and the duration of the first stage (60% of the 27 time to exhaustion during the verification test). From our experience, this proved to be a 28 29 good compromise between sufficient intensity to drive O<sub>2</sub> uptake upwards and preventing premature exhaustion. In contrast to our running experiments, anecdotally the cyclists 30 often reported not even feeling the first two (20 W each) drops in workload, but merely a 31 non-worsening of the associated pain and discomfort. While it is entirely possible that a 32 different duration of the first stage - or indeed a different rate of load decrement - would 33 have led to different results, this remains speculative. Another possibility would be the 34 use of a V-shaped protocol as recently proposed <sup>6</sup>, however it remains to be seen how 35 tolerable such protocol would be in cycling. Second, participants in part A performed an 36 37 additional incremental test (always first of the day). The reason for this test was that as participants were unfamiliarized with the decremental protocol, the initial incremental 38 39 test provided an updated value of peak power output, from which the starting load of the decremental protocol could be more acurately calculated. Although this additional test 40 might have caused some level of fatigue (despite over 60 min of recovery), we note that 41  $\dot{V}O_{2max}$  from this test was not different from the one performed in randomized order with 42 the decremental test. Furthermore, it has been demonstrated that trained individuals can 43 perform up to four incremental tests in a day (with 1.5h passive rest) without losses to 44  $\dot{V}O_{2max}$  or peak power output <sup>28</sup>. Finally, even if some fatigue was present, this would 45 have affected both the decremental and incremental tests, as the order of the two was 46 balanced. At present we see no reason to believe that increased fatigue should lead to 47 48 higher  $\dot{V}O_{2max}$ , as if this was the case the verification tests – performed within minutes of an incremental test - should have shown this effect. Lastly, while the decremental 49

protocol might be able to elicit higher VO<sub>2max</sub> values, an important pitfall of the protocol
is that it does not allow for the determination of commonly assessed variables such as
ventilatory or lactate thresholds or cycling efficiency, or even peak power output, as this
is a function of the starting load and thus imposed by the experimenter.

#### 5 **Practical Applications**

6 Decremental exercise tests open a new paradigm for exercise testing, possibly 7 leading to higher  $\dot{V}O_{2max}$  than incremental tests. Muscle activity at  $\dot{V}O_{2max}$  suggests that, during cycling tests, decremental protocols lead to greater physiological strain, with loss 8 of metabolic efficiency. While a more optimal protocol might emerge, at present a 9 10 successful alternative is a starting load equal to VER with 60% of time to exhaustion and two subsequent decreases of 20 W within 30 s in order to prevent exhaustion, after which 11 decreases can be attenuated and stages elongated. It is possible that the VO<sub>2max</sub> measured 12 during a decremental exercise tests presents a different relationship with markers of health 13 and performance, but this remains to be established. Due to the nature of the workload 14 and physiological response, however, the decremental test paradigm is unable to produce 15 other markers that could be of interest in athletic settings, such as lactate or ventilatory 16 17 thresholds.

#### 18 Conclusion

19 Trained individuals achieved higher  $\dot{V}O_{2max}$  during a decremental cycling 20 protocol in comparison to a traditional incremental exercise test. The additional  $\dot{V}O_2$  may 21 partly be attributed to the extra work performed by the respiratory muscles and less 22 efficient lower limbs, but it remains unclear whether it originates from higher  $O_2$  delivery 23 and/or extraction. These results argue against the notion that  $\dot{V}O_{2max}$  as determined from 24 an incremental test represent an absolute ceiling in cardiorespiratory capacity.

- 25
- 26

#### 27 Acknowledgements

The authors are grateful to all participants for volunteering their time to this investigation. No specific financial support was used in this investigation.

30

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#### 1 **TABLES**

Table 1: Reproducibility of normalized root mean square activity (%RMS<sub>max</sub>) for four 2

lower limbs muscles during a maximal incremental cycling test. 3

	ICC	CV (%)	Typical error (percentage points)
VL (%RMS <sub>max</sub> )	0.98	9.8	5.8
VM (%RMS <sub>max</sub> )	0.85	23.5*	15.0*
BF (%RMS <sub>max</sub> )	0.96	5.7	3.2
GM (%RMS <sub>max</sub> )	0.97	6.6	4.8

ICC, Intraclass correlation coefficient; CV, coefficient of variation; VL, vastus lateralis; 4

5 VM vastus medialis; BF, biceps femoris; GM, gastrocnemius medialis. \* Removal of one

extreme participant would reduce CV to 7.6 and the typical error to 8.1; ICC would 6 increase to 0.98.

7

Table 2: Physiological data at  $\dot{V}O_{2max}$  and P value for Student's *t-tests* between an 

2	incremental	(INC)	and a	decremental	(DEC)	exercise	tests.
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	INC	DEC	t-test	95% CI	dz
<sup>V</sup> O <sub>2max</sub> (mL.min <sup>-1</sup> )	4,139 (657)	4,259 (695)	$t_{(18)} = 2.433, p = 0.026$	16 - 224	0.56
HR (beats.min <sup>-1</sup> )	180 (9)	179 (11)	$t_{(18)} = 0.691, p = 0.495$	-3 - 1.6	0.16
$\dot{V}_E$ (L.min <sup>-1</sup> )	153.7 (26.7)	160.7 (29.5)	$t_{(18)} = 2.263, p = 0.036$	0.5 - 13.5	0.52
BR (breaths.min <sup>-1</sup> )	49.0 (4.8)	54.2 (6.6)	$t_{(10)} = 2.670,  p = 0.023$	0.8 - 9.5	0.80
V <sub>T</sub> (mL)	3.30 (0.53)	3.05 (0.40)	$t_{(10)} = 2.073,  p = 0.065$	-0.51 - 0.02	0.62
RER	1.13 (0.18)	1.11 (0.14)	$t_{(17)}=0.945,p=0.357$	-0.05 - 0.02	0.22
VCO₂ (mL.min <sup>-1</sup> )	4,770 (852)	4,817 (826)	$t_{(18)} = 0.738,  p = 0.470$	-80 - 177	0.17

 $\dot{VO}_{2max}$ , maximal oxygen consumption; HR, heart rate;  $\dot{V}_E$ , ventilation (BTPS); VT, tidal volume; BR, breathing rate; RER, respiratory exchange ratio;  $\dot{VCO}_2$ , carbon dioxide 

output.

**Table 3:** Frequency domain variables of EMG during the INC and DEC tests (at

**VO**<sub>2max</sub>).

	INC	DEC	t-statistic	95% CI	$d_z$
Vastus Lateralis					
Median Frequency (Hz)	44.6 (3.8)	44.2 (4.1)	$t_{(7)} = 1.206, p = 0.267$	-1.3 - 0.4	0.43
Mean frequency (Hz)	46.3 (3.6)	45.8 (3.8)	$t_{(7)} = 1.042, p = 0.332$	-1.6 - 0.6	0.37
Vastus Medialis					
Median Frequency (Hz)	41.1 (3.1)	41.1 (2.5)	$t_{(8)} = 0.056,  p = 0.957$	-1.6 - 1.7	0.0
Mean frequency (Hz)	43.2 (3.2)	43.3 (2.4)	$t_{(8)} = 0.104,  p = 0.919$	-1.3 - 1.5	0.0
<b>Biceps Femoris</b>					
Median Frequency (Hz)	81.9 (16.9)	84.1 (16.5)	$t_{(8)} = 1.935, p = 0.101$	-0.6 - 4.8	0.73
Mean frequency (Hz)	100.8 (19.3)	103.3 (19.9)	$t_{(6)} = 1.606, p = 0.159$	-1.3 - 6.3	0.60
Gastrocnemius Medialis	S				
Median Frequency (Hz)	43 (2.5)	42.7 (2.4)	$t_{(6)} = 0.515,  p = 0.625$	-1.6 - 1.0	0.19
Mean frequency (Hz)	45 (1.8)	45 (2.2)	$t_{(6)} = 0.175, p = 0.866$	-0.7 - 0.8	0.00

 $\dot{VO}_{2max}$ , maximal oxygen consumption.

	@ VO <sub>2max</sub>	$@ \dot{V}O_{2max} - 20 W$	t-statistic	95% CI	dz
Vastus Lateralis	Vastus Lateralis				
RMS (% Sprint)	100.1 (32.4)	92.8 (28.6)	$t_{(6)} = 2.441, p = 0.050$	-14.6 - 0.02	0.92
TA (%)	44.3 (17.2)	40 (15.5)	$t_{(6)} = 2.930, p = 0.026$	-7.80.7	1.10
Vastus Medialis					
RMS (% Sprint)	103.3 (40.4)	99.8 (32.1)	$t_{(8)} = 0.906, p = 0.391$	-12.4 - 5.4	0.30
TA (%)	49.1 (28.5)	45.5 (27.4)	$t_{(8)} = 3.384, p = 0.010$	-6.01.1	1.06
Biceps Femoris					
RMS (% Sprint)	85.7 (20.5)	76.5 (31.1)	$t_{(6)} = 0.747,  p = 0.483$	-39.2 - 20.8	0.28
TA (%)	44.0 (5.5)	38.5 (14.2)	$t_{(6)} = 0.993, p = 0.359$	-18.9 - 8.0	0.37
Gastrocnemius Medialis					
RMS (% Sprint)	76.5 (24.7)	77.7 (24.4)	$t_{(5)} = 0.232, p = 0.825$	-12.4 - 14.8	0.09
TA (%)	31.8 (19.2)	34.1 (19.0)	$t_{(5)} = 0.426, p = 0.688$	-11.3 - 15.9	0.17

**Table 4:** Changes in EMG activity prior to  $\dot{V}O_{2max}$  of the incremental test

2 RMS, root mean square; TA, total activation. All comparisons performed using

3 Student's T-test for paired samples.

#### 1 FIGURE LEGENDS

- 2 Figure 1: Study design diagram. Visits were separated by 2-3 days. See text for detailed
- 3 description of each testing protocol. Part A tested the  $\dot{VO}_{2max}$  between protocols when
- 4 subjects had never been exposed to a DEC test before. In Part B, one familiarization

5 procedure had been performed on a previous session.

6 **Figure 2:** Individual and mean (SD)  $\dot{V}O_{2max}$  responses during an incremental and a decremental exercise test. \* p < 0.05.

8 Figure 3: Individual and mean (SD) root mean square (RMS) of EMG activity (as a

9 percentage of sprint RMS) and total activation (TA, RMS % multiplied by the fraction of

10 active time within a minute) at  $\dot{V}O_{2max}$  during an incremental exercise test and a

11 decremental exercise test. VL, vastus lateralis (n = 7); GM, gastrocnemius medialis (n = 7)

12 7); VM, vastus medialis (n = 9); BF, *biceps femoris* (n = 7).