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1 **Abstract**

2 Objective: to investigate whether a cycling test based on decremental loads (DEC) could
3 elicit higher $\dot{V}O_{2max}$ 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.

6 Methods: During INC the load was increased by 20 W.min⁻¹ until task failure. During
7 DEC the load started at 20 W higher than the peak load achieved during INC
8 (familiarization trial) and was progressively decreased. Gas exchange and EMG activity
9 (n=11) from four lower limb muscles were monitored throughout the tests. Physiological
10 and EMG data measured at $\dot{V}O_{2max}$ were compared between the two protocols using
11 paired t-tests.

12 Results: $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$.
14 Pulmonary ventilation (p = 0.036) and breathing rate (p = 0.023) were also higher during
15 DEC. EMG activity measured at $\dot{V}O_{2max}$ was not different between tests, despite the lower
16 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 O₂ 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

2 Maximal oxygen uptake ($\dot{V}O_{2\max}$) represents the upper limit of cardiorespiratory
3 capacity during exercise. Because of its association with several performance and even
4 health indicators, it is important that $\dot{V}O_{2\max}$ is properly assessed during testing, and a
5 substantial body of literature has been dedicated to it ¹. Current standards dictate that
6 $\dot{V}O_{2\max}$ should be assessed during incremental exercise tests, in which the workload
7 increases at given time intervals until the participant is exhausted. In these tests, leveling-
8 off of $\dot{V}O_2$ with increasing exercise intensities – the $\dot{V}O_{2\max}$ plateau phenomenon – is seen
9 as evidence that $\dot{V}O_{2\max}$ 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 $\dot{V}O_{2\max}$,
11 the so-called verification phase or verification test ¹.

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

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

33 Methods

34 *Subjects*

35 The study was approved by the Ethics and Research Committee of the Faculty of
36 Health Sciences of the University of Cape Town (253/2009). Participants were clearly
37 informed about the risks and procedures involved in this investigation and gave their
38 written consent for participation. The study was divided in two parts (see figure 1). In
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,
41 visited the laboratory on two occasions. In part B, eight male participants (age 23.8 [5.5]
42 years, body mass 69.8 [8.1] kg, height 179 [4] cm), all accustomed to high-intensity
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 ⁵
9 and elite runners ⁶ showed a higher $\dot{V}O_{2max}$ during an incremental test following two
10 exposures to the decremental test (in contrast to a control group with stable $\dot{V}O_{2max}$ over
11 five consecutive incremental test trials), we sub-divided the present study into two parts
12 (Figure 1). Part A was designed to ensure no adaptative effect from the decremental test
13 could take place (i.e. no familiarization with the protocol was allowed prior to the main
14 comparison), whereas in Part B an additional visit was included where participants were
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
18 a cycle ergometer (INC_{fam}), followed 15 minutes later by a verification test to confirm the
19 attainment of $\dot{V}O_{2max}$. On visit two, the participants performed three exercise tests, each
20 separated by approximately one hour. First, they performed an incremental test (INC_1),
21 following which a decremental (DEC) and another incremental test (INC) were
22 performed in randomized, counter-balanced order. During the trials on visit two, gas
23 exchange data, heart rate and EMG activity of four leg muscles (*vastus lateralis*, *biceps*
24 *femoris*, *vastus medialis* and *gastrocnemius medialis*) were continuously monitored.
25 Prior to the first incremental test, a 10-s sprint was performed, with the ergometer set in
26 linear (cadence-dependent) mode. This sprint served as a reference for the EMG analysis
27 in the time domain, as it has been recommended ¹².

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

34 *Cycling tests*

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

1 of each individual's time to task failure recorded during the verification phase test. The
2 average duration for the first stage of DEC was 90 s (range 75-105 s). Following the first
3 stage, the load was decreased by 20 W and maintained for 30 s. Subsequent decrements
4 of 10 W were maintained for 45, 60, 90 and 120 s, thus totaling ~7 min 15 s of test time.
5 If this protocol proved too demanding for the subject (time to fatigue < 3 minutes during
6 DEC_{fam}, part B only), on the second occasion that they performed the decremental
7 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
11 used (AEI Technologies, IL, USA) paired with a Polar S410 (Polar Electro OY, Kempele,
12 Finland). In Part B, a Cortex Metalyser was used (3B, Biophysik, Leipzig, Germany)
13 paired with a Polar T31. All gas exchange and heart rate data exported as consecutive 5 s
14 means. $\dot{V}O_{2max}$ was defined as the highest 60 s moving average measured during a test
15 and all other physiological variables were recorded at the same time as $\dot{V}O_{2max}$. For
16 comparison of $\dot{V}O_{2max}$ values on day 1, between the incremental and verification tests,
17 30-s moving averages were used in order to compensate for the much shorter duration of
18 the verification tests, where $\dot{V}O_{2max}$ was not necessarily constant for a full minute.

19 *Muscle electrical activity*

20 In Part A, EMG signals from the *vastus lateralis*, *vastus medialis*, *gastrocnemius*
21 *medialis* and *biceps femoris* of the right leg were continuously recorded (Part A) using
22 DE-2.1 single differential surface sensors with 10 mm inter-electrode distance (Delsys
23 Inc., Boston, MA, USA). SENIAM¹³ and Delsys recommendations for the placement of
24 the sensors on the skin were followed. Before electrode placement, the skin was shaved
25 to remove excessive hair and wiped with isopropyl alcohol. A reference electrode was
26 applied over the patella. The EMG signals were sampled at 2000 Hz and amplified using
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
29 filter in Lab Chart Pro software (ADInstruments). Root mean square EMG activity was
30 calculated for every 20 ms and averaged over the minute in which $\dot{V}O_{2max}$ was measured.
31 Only the periods of active contraction were used for analysis, which were determined as
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
34 the 10-s sprint which was performed immediately prior to the first exercise trial (but after
35 the 10 min warm-up period). The % activation was also multiplied by the fraction of time
36 in which the muscle was active to obtain an estimate of total activation, akin to the
37 integrated EMG signal¹⁴. The frequency contents of the EMG signal were analyzed using
38 fast Fourier transformation, and the mean and median frequency from the EMG signal
39 during the period in which $\dot{V}O_{2max}$ was measured were determined. Prior to the sprint, the
40 participants cycled at a low power output for two minutes. EMG data is presented as n =
41 7 for the *vastus lateralis*, *gastrocnemius medialis* and *biceps femoris* and n = 9 for the
42 *vastus medialis* due to technically inadequate data for at least one test on some
43 participants, usually the result of electrode detachment/excessive noise mid-test.

44 *Statistics*

45 All data are presented as mean (standard deviation), unless stated otherwise. The
46 reproducibility of $\dot{V}O_{2max}$ and EMG variables between INC₁ (performed before the
47 experimental trials) and INC (performed in randomized order with DEC) was analyzed
48 by calculating the intraclass correlation coefficient (ICC), the typical error of

1 measurement (the standard deviation of the difference between measurements divided by
2 the square root of two) and the individual coefficient of variation¹⁵. The physiological
3 responses from the participants in both parts of the study were pooled together and the
4 physiological responses between the DEC and INC analyzed used Student's paired t-tests,
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
7 Cohen's d for independent samples (mean difference divided by the pooled standard
8 deviation of both groups) or Cohen d_z for dependent samples (mean of differences divided
9 by the standard deviation of the difference scores). The effect of adding a specific
10 familiarization trial for the decremental test (part B vs. part A) was analyzed by
11 comparing the DEC/INC $\dot{V}O_{2max}$ ratio in parts A and B of the study, using Student's
12 independent t-test. Significance level was set at $p < 0.05$, and all statistical procedures
13 were calculated using Prism 8.3 (GraphPad, La Jolla, CA) or dedicated Excel
14 spreadsheets.

15 **Results**

16 *$\dot{V}O_{2max}$ during the incremental and verification tests*

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

27 On the experimental day of part A of the study, when two incremental tests were
28 performed (INC₁ and INC), $\dot{V}O_{2max}$ values were very similar between them (4,366 [464]
29 vs. 4,354 [499] mL.min⁻¹ for INC₁ and INC respectively, 95% CI -80 – 57 mL.min⁻¹, $p =$
30 0.716). Intra-class coefficient for $\dot{V}O_{2max}$ (INC₁ vs INC) was 0.98, and the typical error
31 of measurement was 72 mL.min⁻¹ (1.7%).

32

33 *$\dot{V}O_{2max}$ during the decremental test*

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

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 $\dot{V}O_{2\max}$ (Table 2).

5 *Effects of familiarization on the decremental test*

6 In Part B, there were no differences in $\dot{V}O_{2\max}$ between DEC_{fam} and DEC (3,904 [718] vs.
7 3,953 [758] mL.min⁻¹ respectively, 95% CI -17 – 71 mL.min⁻¹, $d_z = 0.34$, $n = 8$, $p = 0.371$).
8 Likewise, when parts A and B were compared with regards to the ratio of DEC/INC
9 $\dot{V}O_{2\max}$ 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, $d = 0.54$, $p = 0.258$).

12 *Muscle electrical activity*

13 For the EMG variables, reliability data for each muscle between INC₁ and INC
14 are shown in Table 1. Overall, all four analyzed muscles showed a good degree of
15 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 $\dot{V}O_{2\max}$ 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).

22 **Discussion**

23 The main findings from this study are that: i) trained subjects achieved higher
24 $\dot{V}O_{2\max}$ values during a cycling DEC in comparison to INC; ii) the higher $\dot{V}O_{2\max}$ during
25 DEC was accompanied by higher \dot{V}_E in comparison to INC; iii) EMG activity for four
26 lower limb muscles was similar at the time of $\dot{V}O_{2\max}$ in both testing protocols; and iv)
27 the order of the tests or previous familiarization with DEC did not affect the $\dot{V}O_{2\max}$
28 results.

29 The ideal protocol for the assessment $\dot{V}O_{2\max}$ has been the topic of research for
30 years. Under the current paradigm of incremental tests, it has become clear that $\dot{V}O_{2\max}$ is
31 robust over a wide range of protocols and test durations¹⁶. Furthermore, it has also been
32 shown that simply requiring higher energetic output does not result in higher $\dot{V}O_{2\max}$,
33 whether the additional output comes from the exercising limbs, as in the case of the
34 verification test¹⁷, or the upper arms¹⁸. Our data confirms these findings, as the values
35 obtained during the verification tests were if anything lower (although the difference did
36 not reach statistical significance) than those measured during the preceding incremental
37 test. Thus, the $\dot{V}O_{2\max}$ determined during the incremental test in our study is within what
38 is currently considered best practice in terms of protocol and analysis.

39 In this context, the decremental exercise actually operates within a similar range
40 of workloads, as the test starts at the same workload as the verification test and only goes
41 down. This eliminates the possibility that any differences between protocols would be the
42 result of a poor choice of work rate increase from the incremental tests. Based on our
43 previous research and pilot testing, however, it became evident that a perfect mirror-
44 image of an incremental test would lead to a too rapid decrement in workload, and
45 eventually all cardiorespiratory responses would turn downwards before $\dot{V}O_{2\max}$ could be

1 reached. Exercise intensities within the so-called “severe” domain are known to
2 eventually lead to $\dot{V}O_{2\max}$ ¹⁹, and in trying to balance a prolongation of the protocol
3 towards more than 2-3 min with eliciting the highest possible physiological response a
4 protocol with decreasing workloads of increased durations was envisioned. Although the
5 decremental and incremental protocol differ in terms of time taken to reach $\dot{V}O_{2\max}$ as
6 well as work performed, , this should not prevent a comparison between the two, much
7 like different incremental tests with slightly different ramps are routinely compared and
8 also do not show differences in $\dot{V}O_{2\max}$ ¹⁶, or even the comparison between all-out
9 exercise and incremental tests²⁰.

10 In spite of the lower power output at $\dot{V}O_{2\max}$ during DEC, EMG activity was
11 similar between DEC and INC at the time of $\dot{V}O_{2\max}$. The increase in $\dot{V}O_2$ during all-out
12 exercise tests (where $\dot{V}O_2$ increases up to $\dot{V}O_{2\max}$ despite decreasing power output and
13 EMG activity) has been proposed to be a “slow-component”-like phenomenon²¹,
14 resulting from the loss of efficiency in the exercising muscles. $\dot{V}O_2$ then increases during
15 exercise because of progressive recruitment of less efficient, type IIx fiber, which
16 increases the O₂ cost for force production per fiber. An alternative explanation is that
17 while an initial bout of very intense exercise creates the demand for O₂, a subsequent
18 decrease in power output might improve limb blood flow, as muscle contraction intensity
19 and vasoconstriction would be likely reduced, thereby improving O₂ extraction. The
20 similar EMG activity coupled with lower power output found in the present study likely
21 facilitated higher blood flow, which could indeed have facilitated higher O₂ extraction.

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

37 The results from the present study extend the findings originally reported during
38 uphill running⁵, showing ~3% higher $\dot{V}O_{2\max}$ during DEC in comparison to INC when
39 exercise is performed on a cycle ergometer. These results were seen even though
40 participants had performed a verification test, current seen as the best standard to ensure
41 the attainment of “true” maximal oxygen uptake. The additional O₂ uptake requires either
42 additional blood supply and/or increased oxygen extraction to take place during exercise,
43 be it in the respiratory muscles, exercising muscles or elsewhere. Mauger et al.⁴
44 hypothesized that a better distribution of blood flow in the active limbs due to a decrease
45 in power output^{22,23} could explain the higher $\dot{V}O_{2\max}$ measured during a self-paced test,
46 but this proposition remains purely speculative. In muscle preparations $\dot{V}O_{2\max}$ can be
47 altered by increasing O₂ extraction while O₂ delivery is kept constant when independently
48 manipulating SaO₂ and PaO₂, although to our knowledge this not been demonstrated in
49 vivo²⁴.

1 In the present study, both \dot{V}_E and BR were higher during DEC at the time of
2 $\dot{V}O_{2max}$, different to the findings of Beltrami⁵ and Taylor⁹. Thus, at least in cycling part
3 of the difference in $\dot{V}O_{2max}$ measured between tests might be related to the additional O₂
4 cost of breathing. The 7.0 L.min⁻¹ difference in \dot{V}_E measured between DEC and INC could
5 raise $\dot{V}O_2$ by approximately 20-30 mL.min⁻¹²⁵, or explain 15-25% of the difference in
6 $\dot{V}O_{2max}$ measured between DEC and INC (120 mL.min⁻¹). It must be noted, however, that
7 differences in \dot{V}_E do not always result in measurable differences in pulmonary $\dot{V}O_2$ ²⁶.
8 Moreover, simply forcing the respiratory muscles to perform more work does not increase
9 whole-body $\dot{V}O_{2max}$ or cardiac output²⁷, similar to what happens when an additional
10 (supra-maximal) load is imposed to the muscles of the exercising limbs¹⁸. Therefore,
11 while the respiratory muscles can be seen as the receiver of the additional $\dot{V}O_2$, it does
12 not serve a mechanical explanation of how $\dot{V}O_2$ was increased.

13 The differences in $\dot{V}O_{2max}$ between DEC and INC in the present study were
14 independent of whether participants were allowed a specific familiarization trial for DEC
15 or not and were also independent of the order in which INC and DEC were performed.
16 This contrasts with previous findings, in which following DEC participants retained the
17 higher $\dot{V}O_{2max}$ on a subsequent INC despite an unchanged performance in the final
18 incremental test⁵. Should the same had happened in the present study, $\dot{V}O_{2max}$ would have
19 increased from INC_{fam} to INC (Part B), since participants performed the DEC_{fam} trial
20 between these two tests. Also, if participants had retained the higher $\dot{V}O_{2max}$ values
21 following DEC, the $\dot{V}O_{2max}$ differences between INC and DEC would have been blunted
22 for all participants who performed INC after DEC, which did not happen. Although these
23 findings do not help to explain why the running studies showed a persistent increased
24 $\dot{V}O_{2max}$ following the DEC protocol, they show that future research using cycling exercise
25 can be performed using a familiarization trial for both INC and DEC.

26 This investigation is not without its limitations. First, the decremental test per se
27 could be criticized for the tailored approach and the duration of the first stage (60% of the
28 time to exhaustion during the verification test). From our experience, this proved to be a
29 good compromise between sufficient intensity to drive O₂ uptake upwards and preventing
30 premature exhaustion. In contrast to our running experiments, anecdotally the cyclists
31 often reported not even feeling the first two (20 W each) drops in workload, but merely a
32 non-worsening of the associated pain and discomfort. While it is entirely possible that a
33 different duration of the first stage – or indeed a different rate of load decrement – would
34 have led to different results, this remains speculative. Another possibility would be the
35 use of a V-shaped protocol as recently proposed⁶, however it remains to be seen how
36 tolerable such protocol would be in cycling. Second, participants in part A performed an
37 additional incremental test (always first of the day). The reason for this test was that as
38 participants were unfamiliarized with the decremental protocol, the initial incremental
39 test provided an updated value of peak power output, from which the starting load of the
40 decremental protocol could be more accurately calculated. Although this additional test
41 might have caused some level of fatigue (despite over 60 min of recovery), we note that
42 $\dot{V}O_{2max}$ from this test was not different from the one performed in randomized order with
43 the decremental test. Furthermore, it has been demonstrated that trained individuals can
44 perform up to four incremental tests in a day (with 1.5h passive rest) without losses to
45 $\dot{V}O_{2max}$ or peak power output²⁸. Finally, even if some fatigue was present, this would
46 have affected both the decremental and incremental tests, as the order of the two was
47 balanced. At present we see no reason to believe that increased fatigue should lead to
48 higher $\dot{V}O_{2max}$, as if this was the case the verification tests – performed within minutes of
49 an incremental test – should have shown this effect. Lastly, while the decremental

1 protocol might be able to elicit higher $\dot{V}O_{2\max}$ values, an important pitfall of the protocol
2 is that it does not allow for the determination of commonly assessed variables such as
3 ventilatory or lactate thresholds or cycling efficiency, or even peak power output, as this
4 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 $\dot{V}O_{2\max}$ than incremental tests. Muscle activity at $\dot{V}O_{2\max}$ suggests that,
8 during cycling tests, decremental protocols lead to greater physiological strain, with loss
9 of metabolic efficiency. While a more optimal protocol might emerge, at present a
10 successful alternative is a starting load equal to VER with 60% of time to exhaustion and
11 two subsequent decreases of 20 W within 30 s in order to prevent exhaustion, after which
12 decreases can be attenuated and stages elongated. It is possible that the $\dot{V}O_{2\max}$ measured
13 during a decremental exercise tests presents a different relationship with markers of health
14 and performance, but this remains to be established. Due to the nature of the workload
15 and physiological response, however, the decremental test paradigm is unable to produce
16 other markers that could be of interest in athletic settings, such as lactate or ventilatory
17 thresholds.

18 **Conclusion**

19 Trained individuals achieved higher $\dot{V}O_{2\max}$ 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 $\dot{V}O_{2\max}$ as determined from
24 an incremental test represent an absolute ceiling in cardiorespiratory capacity.

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1 **TABLES**

2 **Table 1:** Reproducibility of normalized root mean square activity (%RMS_{max}) for four
3 lower limbs muscles during a maximal incremental cycling test.

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

4 ICC, Intraclass correlation coefficient; CV, coefficient of variation; VL, *vastus lateralis*;
5 VM *vastus medialis*; BF, *biceps femoris*; GM, *gastrocnemius medialis*. * Removal of one
6 extreme participant would reduce CV to 7.6 and the typical error to 8.1; ICC would
7 increase to 0.98.

8

1 **Table 2:** Physiological data at $\dot{V}O_{2\max}$ and P value for Student's *t*-tests between an
 2 incremental (INC) and a decremental (DEC) exercise tests.

	INC	DEC	t-test	95% CI	d_z
$\dot{V}O_{2\max}$ (mL.min ⁻¹)	4,139 (657)	4,259 (695)	$t_{(18)} = 2.433, p = 0.026$	16 – 224	0.56
HR (beats.min ⁻¹)	180 (9)	179 (11)	$t_{(18)} = 0.691, p = 0.495$	-3 – 1.6	0.16
\dot{V}_E (L.min ⁻¹)	153.7 (26.7)	160.7 (29.5)	$t_{(18)} = 2.263, p = 0.036$	0.5 – 13.5	0.52
BR (breaths.min ⁻¹)	49.0 (4.8)	54.2 (6.6)	$t_{(10)} = 2.670, p = 0.023$	0.8 – 9.5	0.80
V_T (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
$\dot{V}CO_2$ (mL.min ⁻¹)	4,770 (852)	4,817 (826)	$t_{(18)} = 0.738, p = 0.470$	-80 – 177	0.17

3 $\dot{V}O_{2\max}$, maximal oxygen consumption; HR, heart rate; \dot{V}_E , ventilation (BTPS); V_T , tidal
 4 volume; BR, breathing rate; RER, respiratory exchange ratio; $\dot{V}CO_2$, carbon dioxide
 5 output.

6

1 **Table 3:** Frequency domain variables of EMG during the INC and DEC tests (at
 2 $\dot{V}O_{2max}$).

	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	0.01
Mean frequency (Hz)	43.2 (3.2)	43.3 (2.4)	$t_{(8)} = 0.104, p = 0.919$	-1.3 – 1.5	0.03
Biceps Femoris					
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					
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	0.06

3 $\dot{V}O_{2max}$, maximal oxygen consumption.

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5

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

	@ $\dot{V}O_{2max}$	@ $\dot{V}O_{2max} - 20$ W	t-statistic	95% CI	d_z
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)	$t_{(6)} = 2.930, p = 0.026$	-7.8 – -0.7	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)	$t_{(8)} = 3.384, p = 0.010$	-6.0 – -1.1	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

2 RMS, root mean square; TA, total activation. All comparisons performed using
 3 Student's T-test for paired samples.

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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 $\dot{V}O_{2max}$ 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
7 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* ($n =$
12 7); VM, *vastus medialis* ($n = 9$); BF, *biceps femoris* ($n = 7$).