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- 1 Accumulated oxygen deficit during exercise to exhaustion determined at different
- 2 supramaximal work-rates.

Abstract

Purpose. The aim of the study was: a) to determine the effect of supramaximal exercise intensity, during constant work-rate cycling to exhaustion, on the accumulated oxygen deficit (AOD); and b) to determine the test-retest reliability of AOD. Methods. Twenty one trained male cyclists and triathletes (means ± standard deviation for age and maximal oxygen uptake ($\dot{V}O_{2max}$) were 41 ± 7 years and 4.53 ± 0.54 L·min⁻¹, respectively) performed initial tests to determine the linear relationship between oxygen uptake ($\dot{V}O_2$) and power output, and $\dot{V}O_{2max}$. In subsequent trials, AOD was determined from exhaustive square-wave cycling trials at 105, 112.5 (in duplicate), 120 and 127.5% $\dot{V}O_{2max}$. Results. Exercise intensity had an effect (P = 0.011) on the AOD (3.84 ± 1.11, 4.23 ± 0.96, 4.09 ± 0.87 and 3.93 ± 0.89 L at 105, 112.5, 120 and 127.5% $\dot{V}O_{2max}$, respectively). Specifically, AOD at 112.5% $\dot{V}O_{2max}$ was greater than at 105% $\dot{V}O_{2max}$ (P = 0.033) and at 127.5% $\dot{V}O_{2max}$ (P = 0.022), but there were no differences between the AOD at 112.5% and 120% $\dot{V}O_{2max}$. In 78% of the participants, the maximal AOD occurred at 112.5 or 120% $\dot{V}O_{2max}$. The reliability statistics of the AOD at 112.5% $\dot{V}O_{2max}$, determined as intraclass correlation coefficient and coefficient of variation, were 0.927 and 8.72% respectively. Conclusion. The AOD, determined from square-wave cycling bouts to exhaustion, peaks at intensities of 112.5-120% $\dot{V}O_{2max}$. Moreover, the AOD at 112.5% $\dot{V}O_{2max}$ exhibits an 8.7% test-retest reliability.

Introduction

During high-intensity exercise, both aerobic and anaerobic energy systems contribute to meet the energy demands. Aerobic energy production is easily quantified as the rate of oxygen uptake (VO₂). However, anaerobic capacity (AnC), defined as the maximum amount ATP resynthesised via anaerobic metabolism during high-intensity whole-body exercise,5 is more difficult to quantify and presents a challenge for exercise physiologists.^{3,4} Since direct methods to quantify AnC are expensive and/or invasive, indirect approaches such as the accumulated oxygen deficit (AOD) have been developed3,6 The AOD is determined as the difference between the sudden increase in oxygen demand and the exponential⁷ increase in $\dot{V}O_2$ at the onset of exercise. The quantification of AnC via the AOD relies on a number of assumptions which might compromise the validity of the test.3

First, determination of AnC requires exercising at intensities that exceed the maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$).^{3,6,8} The oxygen demands at supramaximal intensities need to be estimated, typically from a linear projection of the relationship between steady-state $\dot{V}O_2$ and power output at submaximal intensities. However, the assumption of a linear relationship between $\dot{V}O_2$ and power output, has been challenged due to the emergence of the slow component of $\dot{V}O_2$, which may increase the slope of the $\dot{V}O_2$ -power output relationship at intensities above the gas exchange threshold (GET). Since at intensities greater than $\dot{V}O_{2max}$ there is no slow component of $\dot{V}O_2$ (i.e. $\dot{V}O_2$ increases inexorably towards $\dot{V}O_{2max}$),⁹ Noordhof et al.³ recommended using relatively short exercise bouts to construct the $\dot{V}O_2$ -power output relationship. Secondly, as a measure of AnC, the AOD is assumed to remain constant at any supramaximal intensity lasting 2-5 minutes.^{3,6,10} Whilst consistent AODs have been reported in cycling at 110% and 120% $\dot{V}O_{2max}$,¹¹ whether the AOD remains consistent determined from CWR at intensities outside the range of 110 – 120% $\dot{V}O_{2max}$, but within the range of 2-5 min, remains unknown.

In addition to the methodological issues described above, the reliability of the AOD remains controversial. It is important for athletes and coaches to know the test-retest reliability of a measurement, 12 but unfortunately only two studies have quantified the test-retest reliability of the AOD. 11,13 Moreover, the results of these studies were inconsistent. Doherty, Smith and Schroder 13 concluded that the AOD determined during running exercise was not a reliable test; whilst Weber and Schneider 11 reported good test-retest reliability of the AOD in cycling tests at both 110 and 120% of $\dot{V}O_{2max}$.

The purpose of this study was to address the above limitations by investigating whether the AOD remains constant during different supramaximal constant work-rate (CWR) cycling bouts to exhaustion, and to determine the test-retest reliability of the AOD. Specifically, the primary aim of the study was to determine whether the AOD remains constant during cycling to exhaustion at four supramaximal CWR intensities. The secondary aim of the study was to determine the test-retest reliability of the AOD during identical supramaximal CWRs tests. It was hypothesized that, as an estimate of AnC, supramaximal exhaustive exercise at different supramaximal intensities would result in similar AODs. It was also hypothesised that the AOD would exhibit acceptable test-retest reliability.

Methods

58 Subjects

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- Twenty-one trained¹⁴ male cyclists and triathletes voluntarily participated in this study. Their mean ±
- standard deviation (SD) for age, height and mass were 41 \pm 7 years, 1.82 \pm 0.08 m and 79.6 \pm 7.5 kg,
- 61 respectively.
- 62 Experimental overview
- 63 Each participant was required to complete seven visits to the physiology laboratory, typically once a
- 64 week (7 ± 2 days between trials), with each trial separated by at least 48 h. All trials were conducted on
- 65 the same individually-adjusted, electromagnetically braked cycle-ergometer (Lode Excalibur Sport,
- 66 Groningen, the Netherlands) at a similar time of the day (±2 h) and under controlled ambient conditions
- 67 (19 \pm 1 °C and 33 \pm 5% humidity). After two preliminary trials to determine GET, $\dot{V}O_{2max}$, and the $\dot{V}O_{2-}$
- power output relationship, participants completed five experimental trials, each consisting of a CWR to
- exhaustion at 105, 112.5, 120 or 127.5% of VO_{2max}. The 112.5% VO_{2max} trial was repeated to determine
- 70 test-retest reliability. The order of the experimental trials was randomised, with the exception of the
- 71 identical trials at 112.5% of $\dot{V}O_{2max}$, which were performed consecutively. Participants were provided
- with a food record diary and instructed to follow a similar diet and to refrain from strenuous exercise in
- the 24 h before each trial. In addition, they were instructed to refrain from caffeine and alcohol ingestion
- 12 h prior to each trial. Figure 1 schematically outlines the protocol.

75 Procedures

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91 92 Initially, participants completed the preliminary trials. First, a ramp test to exhaustion was used to determine the GET. After three minutes of unloaded pedalling, the resistance increased continuously at a rate of 0.5 W·s⁻¹ (i.e. 30 W·min⁻¹) until exhaustion, defined by a decrease >10 rpm for >5 s despite strong verbal encouragement. The cadence for this trial was freely chosen by each participant (87 ± 8 rpm), and remained constant throughout this and subsequent tests. Two researchers independently determined the GET for each participant using the V-slope method. 15 On a separate day, participants performed a submaximal step test to determine the relationship between VO2 and power output followed by a ramp to exhaustion to determine $\dot{V}O_{2max}$. The submaximal step test consisted of 10 × 3 min stages at increasing intensities. The test started at an intensity that corresponded to 50% GET and increased by 10% GET in each subsequent 3 min stage, so that the tenth 3-min stage was completed at 140% GET. There were 30 s of passive recovery between stages to allow a capillary sample to be collected (see below). After completion of the tenth 3 min stage, participants remained seated on the cycle ergometer for five minutes before completing the ramp test to exhaustion. The starting intensity in the ramp test corresponded to 70% GET and increased continuously at a rate of 15% GET min-1 until exhaustion. VO_{2max} was calculated as the highest value derived from a 30-s rolling average; excluding VO₂ values ± 4 SD outside a local 5-breath average. ¹⁶ Approximately 20 min after the completion of the test, participants completed a supramaximal CWR test to exhaustion for familiarization purposes.

The five experimental trials started with 3 min of unloaded cycling immediately followed by 5 minutes at 70% GET. After a further 5 min of passive rest, participants were instructed to attain their preferred cadence as soon as possible (≤5 s) and to maintain that cadence for as long as possible. The intensity of the trials were 105, 112.5, 120 and 127.5% of $\dot{V}O_{2max}$. This range of supramaximal intensities (105% - 127.5% $\dot{V}O_{2max}$) encompasses the range of typical intensities used during AOD determination, and was intended to cause exhaustion between ~2 and ~5 min.^{6,11,17} Subjects were unaware of the power output (or percentage of $\dot{V}O_{2max}$), elapsed time or expected time to exhaustion (TTE). Capillary blood samples (20 µL) were collected 1, 3 and 5 min after exhaustion. The AOD was determined as the difference between the accumulated oxygen demand and accumulated oxygen uptake.⁶

Measurements

During all trials, participants breathed room air through a facemask (Hans Rudolph, Kansas City, MO, USA). Gas exchange samples were collected and analysed breath-by-breath using an open spirometric system (Oxycon Pro, Jaeger Ltd. Höechberg, Germany). The gas analyser was calibrated before each test accordingly to manufacturer instructions with gases of known concentrations (5% CO2, 16% O2, 79% nitrogen; Carefusion, Höechberg, Germany) and a 3 L syringe (Viasys Healthcare, Höechberg, Germany). Blood samples were analysed for blood lactate concentration (BLa) using the enzymaticamperiometric method (Biosen C-line, EKF Diagnostic, Germany). Heart rate (HR) was measured using a telemetric monitor (Polar S610, Polar Electro, Finland) at 5 s intervals. Breath-by-breath VO2 was filtered (see above) and, subsequently, linearly interpolated to produce second by second data. The accumulated oxygen uptake was determined as the integrated VO₂ values from the onset of exercise until exhaustion (recorded to the nearest second). The accumulated oxygen demand was determined as the product of the oxygen demand and time to exhaustion (TTE). Oxygen demand, in turn, was determined as a linear projection of the $\dot{V}O_2$ -power output relationship. In the experimental trials, peak HR and peak BLa were determined as the highest value recorded during exercise, and the highest postexercise BLa concentration, respectively. End-exercise VO₂ corresponded to the average VO₂ during the last 10 s of exercise before exhaustion.

Statistical Analysis

Data were analysed using IBM SPSS 21 (IBM Corp, Armonk, NY) and presented as mean \pm *SD*. Differences between AOD at 105% $\dot{V}O_{2max}$ (AOD₁₀₅), AOD_{112.5}, AOD₁₂₀ and AOD_{127.5}, alongside other physiological variables (power output, TTE, accumulated oxygen demand and oxygen uptake, peak BLa, peak HR and end-exercise $\dot{V}O_2$), were determined using repeated measures ANOVA. The presence of a training or learning effect in the AOD was evaluated by studying the difference between AOD in consecutive trials using repeated measures ANOVA. A *post hoc* Bonferroni t-test was conducted to locate differences between trials if a significant *F* value was detected. The test-retest reliability of the AOD was determined as coefficient of variation (CV) and intraclass correlation coefficient (ICC). The CV was determined from the typical error expressed as percentage of the mean;¹² whilst the ICC was calculated from the standard error of measurement derived from the ANOVA using

- the 3,1 ICC.¹⁸ 95% confidence limits (CL) were determined for both measures of reliability. Significance
- 131 was accepted at P < 0.05.

133 Results

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Preliminary trials

- The GET and $\dot{V}O_{2max}$ corresponded to $2.60 \pm 0.33 \, \text{L} \cdot \text{min}^{-1}$ (189 ± 25 W) and $4.53 \pm 0.54 \, \text{L} \cdot \text{min}^{-1}$ (57 ± 6 mL·kg⁻¹·min⁻¹), respectively. The power output for the initial 3 min stage in the step test was 95 ± 13 W, and increased by 19 ± 3 W in each subsequent stage until the tenth stage, which was completed at 265 ± 36 W. These workloads represent intensities from 42 ± 4% to 85 ± 6% $\dot{V}O_{2max}$ and were accompanied by increases in BLa from $0.97 \pm 0.22 \, \text{mmol·L}^{-1}$ at the end of the first stage to $4.01 \pm 1.73 \, \text{mmol·L}^{-1}$ at the end of the tenth stage. There was a strong linear relationship between $\dot{V}O_2$ and power output (P < 0.001 for all the subjects; $r = 0.995 \pm 0.005$).
- 143 Experimental trials
 - One participant experienced technical problems during the supramaximal test at 105% $\dot{V}O_{2max}$, and his data were removed from the analysis. Data presented in Table 1, therefore, summarises the result for the rest of participants (n=20). The intensity of the supramaximal CWR tests had a significant effect on TTE, accumulated oxygen demand and accumulated oxygen uptake (all P < 0.001; Table 1). Post-hoc tests confirmed that, as expected, TTE, accumulated oxygen demand and accumulated oxygen uptake decreased with each increase in oxygen demand (all P < 0.001; Table 1). There was no training effect on AOD, as no differences were observed between the AOD during consecutive supramaximal trials (P = 0.563). The AOD, however, was affected by the intensity of the supramaximal exercise (P = 0.011). Post-hoc tests revealed that AOD_{112.5} was significantly greater than AOD₁₀₅ (P = 0.033) and AOD_{127.5} (P = 0.022). There were no differences ($P \ge 0.05$) between AOD₁₀₅, AOD₁₂₀ and AOD_{127.5}. The maximal AOD (MAOD) corresponded to 4.46 ± 0.96 L (or 56.1 ± 11.1 mL·kg⁻¹). Ten percent of the participants achieved their MAOD at 105% $\dot{V}O_{2max}$, 48% at 112.5% $\dot{V}O_{2max}$, 28% at 120% $\dot{V}O_{2max}$ and 14% at 127.5% $\dot{V}O_{2max}$. The determination of the AOD for a representative subject at each supramaximal intensity is presented in Figure 1.
- 158 *** Table 1 near here ***
- 159 *** Figure 1 near here ***
- 160 Test-retest reliability
- One participant did not perform the retest trial at 112,5% $\dot{V}O_{2max}$, due to training commitments, and retest data from another subject could not be used due to technical problems during data collection. Therefore, results presented in Table 2 correspond to test-retest bouts to exhaustion of the remaining participants (n = 19). The test-retest ICC and CV of the AOD were 0.869 [0.691, 0.947] and 8.72% [6.52, 13.16], respectively.
- 166 ***Table 2 near here***

Discussion

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The main aim of this study was to determine whether AOD, as a means of quantifying AnC, remains constant during exercise to exhaustion at supramaximal intensities that ranged from 105 to 127.5% $\dot{V}O_{2max}$. The secondary aim of the study was to determine the test-retest reliability of AOD. The main original finding of the study is that, contrary to the hypothesis, cycling AOD determined from exhaustive CWR supramaximal exercise is affected by the intensity of exercise. Specifically, the AOD at supramaximal intensities followed an inverted U-shape with highest values attained at 112.5% and 120% $\dot{V}O_{2max}$. Moreover, at 112.5% $\dot{V}O_{2max}$, the AOD has acceptable test-retest reliability. These results suggest that, for endurance-trained athletes, such as those in the current study, AnC should be determined from a supramaximal CWR to exhaustion at 112.5-120% $\dot{V}O_{2max}$. In addition, athletes and coaches need to consider the test-retest reliability of the AOD when using the AOD as a means of quantifying AnC.

Part of the variation observed in AOD can be explained by the range of times to exhaustion. Medbø et al.6,10 reported increases in the AOD concurrent with increases in TTE during CWR to exhaustion shorter than 2 min, likely because shorter bouts did not allow a full depletion of AnC. Since the CWR test at 127.5% VO_{2max} lasted ~1.5 min, it is possible that AnC was not fully depleted at the time of exhaustion. The finding of a lowered AOD₁₀₅ compared to AOD₁₁₂ was, however, somewhat unexpected. There are various plausible reasons to explain the reduced AOD observed at the lowest supramaximal intensity. First, exhaustion in the AOD₁₀₅ trial occurred in ~4.44 min. Early studies reported a constant AOD during square-wave-exercise bouts lasting up to 15 min^{6,19}, although neither of these studies^{6,19} reported the actual intensity as a percentage of VO_{2max}. Besides, the chosen exercise modality was running in the study of Medbo et al.6 instead of cycling in the current study and only three subjects participated in the study of Karlsson and Saltin¹⁹. Secondly, it has been suggested that the MAOD is reached during an exercise protocol that best simulates the athlete's actual competitive event.^{3,20} Using time-trials to determine AOD, however, might be affected by pacing strategies.²¹ Moreover, the AOD cannot be determined during long events because they are performed at submaximal intensities just above the critical power,8 despite an increased contribution from anaerobic energy sources. Thirdly, we assumed a linear relationship between VO₂ and power output, which implies that efficiency is not affected by intensity. However, there is evidence that gross efficiency decreases as the intensity of exercise increases.²² Assuming a constant efficiency has been shown decrease the AOD during time-trials of increasing duration.²³ Nevertheless, the relationship between VO₂ and power output in the present study was very strong for all participants. Whilst unfortunately the data presented herein cannot explain the lowered AOD observed at 105% VO_{2max}, the present study suggests that supramaximal intensities of 110 to 120% VO_{2max} should be used in order to estimate AnC by means of the AOD method.

The second aim of the present study was to determine the test-retest reliability of AOD at 112.5% $\dot{V}O_{2max}$. Weber and Schneider¹¹ reported high correlation coefficients (\geq 0.95) and low CVs (\leq 7%) for

AOD determined at both 110% and 120% $\dot{V}O_{2max}$. Doherty et al.¹³ concluded that the AOD determined from three running tests to exhaustion at 125% $\dot{V}O_{2max}$ was unreliable; despite an ICC and CV of 0.91 and 6.8% respectively, because of large 95% limits of agreement. The limits of agreement, in turn, have been disregarded by some authors because they are too stringent.^{12,18} It is important to note that the variability in the measurement of AOD reported in the present and previous studies^{11,13} is still greater than the ~5% test-retest variability typically observed in other physiological parameters such as VO_{2max} or lactate threshold.²⁴

The large variability in AOD compared with other physiological measures can be explained by the protocol employed in the current study to quantify AOD. Open-loop tests have more variation than closed-loop tests (i.e. tests where the duration, distance or work to be completed is known), even at high exercise intensities, which have a lower TTE. ²⁵. The variability in 1.5 km and 5 km running time trials (2.0% and 3.3%, respectively), for instance, is smaller than that of tests at constant speed to exhaustion of similar durations (15.1% and 13.2%, respectively). ²⁶ The latter values approximate the test-retest variability in TTE reported in the present study, despite different modes of exercise (cycling vs. running). Moreover, in cycling, there is a 6–10% variability during exercise at intensities at or close to $\dot{V}O_{2max}$. ^{24,27} Interestingly, the curvature constant of the power-duration relationship, which can be considered as a means at estimating anaerobic work capacity, ²⁸ also presents high test-retest variability. ^{29,30} It is therefore plausible that the large test-retest variability of the measurement in the AOD represents the large variability of AnC itself.

Practical Applications

Athletes wishing to determine their AnC by means of the AOD method typically use a single supramaximal exercise bout to exhaustion at constant intensity. The present study demonstrates that the intensity of the supramaximal exercise does affect AOD. It is suggested, therefore, that the determination of AnC using the AOD method is performed from a CWR to exhaustion at 112.5-120% $\dot{V}O_{2max}$, where it peaks for 77% of the participants. Moreover, athletes and coaches using the AOD to evaluate AnC should consider that the test-retest reliability is 8.72%.

Conclusion

- This study demonstrates that the AOD determined from cycling CWR to exhaustion is affected by the intensity of the exercise (and, consequently, TTE). The AOD followed an inverted U-shape, with 77% of subjects reaching its peak (i.e. MAOD) at either 112.5% or 120% VO_{2max}. The AOD can be used to estimate AnC during a CWR test to exhaustion at 112.5-120% VO_{2max}. At supramaximal intensities, the
- 236 test has a test-retest reliability of 8.72%.

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307 Tables and figures legends 308 Table 1. Characteristics and physiological responses for cycling bouts to exhaustion at A: 105; B: 309 310 112.5; C: 120; and C: 127.5% of $\dot{V}O_{2max}$ (n = 20). 311 Table 2. Characteristics and physiological responses to two identical cycling trials to exhaustion at 312 112.5% $\dot{V}O_{2max}$ (n = 19). 313 314 Figure 1. Outline of the experimental approach. Figure 2. Determination of the AOD in a representative subject during cycling exercise to exhaustion 315 316 at 105 (Panel A), 112.5 (Panel B), 120 (Panel C) and 127.5% $\dot{V}O_{2max}$ (Panel D). Dotted lines represent oxygen demand and open circles VO₂. 317 318

319 **Tables**

320

321 Table 1

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	105%	105% 112.5%		127.5%	
Power output (W)#	341 ± 48	370 ± 52	399 ± 56	428 ± 59	
TTE (s)#	267 ± 78	173 ± 48	123 ± 31	91 ± 20	
Acc O ₂ demand (L)#	21.28 ± 6.69	14.81 ± 4.37	11.15 ± 2.95	8.83 ± 2.10	
Acc O ₂ uptake (L)#	17.40 ± 6.02	10.55 ± 3.62	7.03 ± 2.21	4.88 ± 1.33	
End-exercise VO2 (L·min ⁻¹)¥	4.50 ± 0.53	4.30 ± 0.63	4.20 ± 0.56	4.12 ± 0.55	
AOD (mL·kg ⁻¹) ^{\$}	48.52 ± 12.83	53.65 ± 11.86	51.90 ± 11.14	49.74 ± 10.82	
Anaerobic contribution (%)#	19.1 ± 5.0	29.9 ± 6.0	37.8 ± 5.0	45.1 ± 4.6	
Peak BLa (mmol·L-1)\$	11.67 ± 2.58	10.92 ± 2.48	10.24 ± 2.38	9.56 ± 2.58	
Peak HR (beats·min⁻¹)	169 ± 13	168 ± 11	166 ± 12	162 ± 11	

TTE: time to exhaustion; Acc O2 demand/uptake: accumulated oxygen demand/uptake; EE: end-exercise; AOD: accumulated oxygen deficit.

#: Denotes significant differences between all trials.

*: Trial at 105% VO_{2max} was greater than all others.

\$: Trial at 105% VO_{2max} significantly different than at 120 and 127.5; and 112.5% was different than the 127.5%

trial.

Table 2.

	Trial 1	Trial 2	Trial 1 – Trial 2 [95% CL]	ICC [95% CL]	CV [95% CL]
TTE (s)	168 ± 44	160 ± 49	-7 [-22, 7]	0.792 [0.537, 0.914]	14.31 [10.63, 21.87]
Acc VO ₂ (L)	10.11 ± 3.37	9.56 ± 3.47	-0.55 [-1.93, 0.45]	0.735 [0.433, 0.889]	18.79 [13.90, 29.00]
End-exercise VO ₂ (L·min ⁻¹)	4.27 ± 0.66	4.27 ± 0.55	0.00 [-0.11, 0.11]	0.927 [0.822, 0.971]	3.78 [2.84, 5.64]
AOD (L)	4.19 ± 0.99	4.09 ± 0.98	-0.10 [-0.56, 0.38]	0.869 [0.691, 0.947]	8.72 [6.52, 13.16]
AOD (mL·kg ⁻¹)	52.3 ± 11.7	51.1 ± 11.8	-1.2 [-6.5, 4.8]	0.866 [0.685, 0.946]	8.72 [6.52, 13.16]
Anaerobic contribution (%)	30.3 ± 6.1	31.0 ± 5.1	0.7 [-1.3, 5.1]	0.669 [0.320, 0.858]	10.68 [7.97, 16.19]
Peak BLa (mmol·L ⁻¹)	10.88 ± 2.60	10.41 ± 2.75	-0.37 [-1.16, 0.42]	0.818 [0.587, 0.926]	14.16 [10.45, 21.97]
Peak HR (beats·min ⁻¹)	167 ± 11	165 ± 11	-2 [-5, 1]	0.896 [0.751, 0.959]	2.26 [1.66, 3.51]

ICC: intraclass correlation coefficient; CV: coefficient of variation. 95% CL: 95% confidence limits.

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