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The effect of acute heat exposure on the determination of exercise thresholds from ramp and step incremental exercise

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79

## ABSTRACT

The aim of this study was to examine how respiratory (RT) and lactate thresholds (LT) are affected by acute heat exposure in the two most commonly used incremental exercise test protocols (RAMP and STEP) for functional evaluation of aerobic fitness, exercise prescription and monitoring training intensities. Eleven physically active male participants performed four incremental exercise tests, two RAMP ( $30 \text{ W} \cdot \text{min}^{-1}$ ) and two STEP ( $40 \text{ W} \cdot 3 \text{ min}^{-1}$ ), both in  $18^{\circ}\text{C}$  (TEMP) and  $36^{\circ}\text{C}$  (HOT) with 40 % relative humidity to determine 2 RT and 16 LT, respectively. Distinction was made within LT, taking into account the individual lactate kinetics ( $\text{LT}_{\text{IND}}$ ) and fixed value lactate concentrations ( $\text{LT}_{\text{FIX}}$ ). A decrease in mean power output (PO) was observed in HOT at LT ( $-6.2 \pm 1.9 \%$ ), more specific  $\text{LT}_{\text{IND}}$  ( $-5.4 \pm 1.4 \%$ ) and  $\text{LT}_{\text{FIX}}$  ( $-7.5 \pm 2.4 \%$ ), compared to TEMP, however not at RT ( $-1.0 \pm 2.7 \%$ ). The individual PO difference in HOT compared to TEMP over all threshold methods ranged from  $-53 \text{ W}$  to  $+26 \text{ W}$ . Mean heart rate (HR) did not differ in LT, while it was increased at RT in HOT ( $+10 \pm 8 \text{ bpm}$ ). This study showed that exercise thresholds were affected when ambient air temperature was increased. However, a considerable degree of variability in the sensitivity of the different threshold concepts to acute heat exposure was found and a large individual variation was noticed. Test design and procedures should be taken into account when interpreting exercise test outcomes.

## KEY WORDS

Heat; incremental exercise test; respiratory threshold; lactate threshold

## 99 INTRODUCTION

100 Incremental exercise tests are widely used to determine exercise thresholds that demarcate the intensity  
101 domains of moderate, heavy and severe exercise (Gaesser & Poole, 1996). By use of these thresholds, aerobic  
102 fitness can be assessed and exercise prescription can be optimized according to the specific profile of the  
103 sports discipline and athlete (Bourgois et al., 2019). It has been shown that incremental exercise tests with  
104 continuous linear (RAMP) or stepwise (STEP) increases in intensity can provide valuable insights into these  
105 thresholds, although they do not represent the gold standard procedures (i.e., multiple constant load  
106 exercise tests) (Keir et al., 2015). Despite a high feasibility (i.e., time-efficiency) of these protocols,  
107 appropriate protocol design and careful data analysis by experienced physiologists or coaches are required  
108 for accurate determination and interpretation of exercise thresholds (Jamnick et al., 2018; Caen et al., 2021).  
109 RAMP tests typically last between 8 and 12 min and are mostly used when thresholds are determined from  
110 pulmonary gas exchange variables (Keir et al., 2022), with maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) and respiratory  
111 thresholds (RT), i.e., gas exchange threshold (GET) and respiratory compensation point (RCP), as key  
112 outcomes. STEP tests are more common when blood lactate measurement is the main parameter for  
113 threshold determination, and a minimum stage length (i.e., 3 min) is proposed (Bentley, 2007). Over the  
114 years, a large variety of lactate thresholds (LT) methods has been suggested and utilized (Faude et al., 2009;  
115 Jamnick et al., 2018). Some of these methods take into account the individual kinetics of the lactate-  
116 performance curve ( $LT_{IND}$ ; e.g.,  $D_{\max}$  method; Cheng et al., 1992), while others rely on fixed blood lactate  
117 concentrations ( $LT_{FIX}$ ; e.g., 4 mmol·L<sup>-1</sup>; Kindermann et al., 1979).

118 Furthermore, even when the testing procedure and analysis are applied adequately, the occurrence of these  
119 exercise thresholds can be influenced by multiple factors, including environmental conditions. In fact, heat  
120 can alter the physiological responses to exercise (Périard et al., 2021). A redistribution of blood to the skin  
121 for heat dissipation leads to a higher cardiovascular strain (Rowell, 1974). Furthermore, there is a shift toward  
122 a greater reliance on the glycolytic metabolism (Febbraio et al., 1994), resulting in a decreased mechanical  
123 efficiency (Hettinga et al., 2007). Subsequently, the impact of heat on parameters used for exercise  
124 prescription and monitoring training intensity, i.e., power output (PO), heart rate (HR), blood lactate

125 concentration ( $[BLa^-]$ ) and oxygen uptake ( $\dot{V}O_2$ ), can result in a modified occurrence of LT from STEP and/or  
126 RT from RAMP during incremental exercise.

127 Several studies reported changes in threshold occurrence when exercise testing is executed in hot  
128 environmental conditions (Tyka et al., 2009; 2010; de Barros et al., 2011; Lorenzo et al., 2011; Maunder et  
129 al., 2021) and made suggestions on how this additional information could be used for training prescription  
130 and monitoring during training camps and tournaments in hot environments. However, these studies do not  
131 compare different incremental exercise tests (RAMP and STEP) and/or only include limited exercise threshold  
132 concepts.

133 Therefore, the purpose of this study is to examine how different exercise thresholds (RT and LT) are affected  
134 by acute heat exposure in the two most commonly used incremental exercise test protocols (RAMP and  
135 STEP) for functional evaluation of aerobic fitness. This will allow us to gain more insight into the physiological  
136 mechanism of exercise threshold determination and provide practical implications for sports scientists and  
137 coaches. We hypothesize that short-term heat exposure (i.e., duration of the test) will have an impact on PO  
138 and HR at all exercise thresholds, although we expect differences related to the protocol of the test (i.e.,  
139 time duration-intensity relationship of RAMP vs. STEP) and the methodology of LT determination (i.e., lactate  
140 kinetics in  $LT_{IND}$  vs.  $LT_{FIX}$ ). Furthermore, we hypothesize that thresholds taking place at a higher intensity will  
141 be affected more as heat exposure time is increased, accelerating cardiorespiratory and metabolic  
142 perturbations.

## METHODS

### Participants

Eleven male participants (age:  $24.9 \pm 1.7$  years, height:  $1.82 \pm 0.06$  m, body mass:  $77.0 \pm 6.6$  kg) volunteered in this study. All participants were physically active, and performed recreational physical exercise on a self-reported basis of  $5.0 \pm 1.4$  hours per week. Participants did not train in hot environments 3 months preceding the study to avoid heat acclimation/acclimatization effects. Participants completed a medical questionnaire and underwent a medical examination. Participants did not report any history of cardiovascular, respiratory or metabolic disease. After receiving a description of the procedure of the experiment, all participants gave their written informed consent. The protocol was in accordance with the Declaration of Helsinki and was approved by the ethical committee of the Ghent University Hospital (Ghent, Belgium).

### Study design

*General procedure.* All participants performed four incremental exercise tests on an electromagnetically braked cycle ergometer (Cyclus 2, RBM Elektronik-Automation, Leipzig, Germany) at the Sport Science Laboratory Jacques Rogge of the Ghent University (Ghent, Belgium, sea level) between 1.00 p.m. and 6.00 p.m. to limit variability due to the circadian rhythm. Each participant performed all tests at the same time of the day ( $\pm 30$  min) with a minimum of 72 hours between two tests. Trials were completed during the spring months. Two ramp and two step incremental exercise tests were executed, one of each in temperate (TEMP:  $18 \pm 1^\circ\text{C}$ ) and one of each in hot environmental conditions (HOT:  $36 \pm 1^\circ\text{C}$ ), with partial counterbalancing to deal with practice effects. Air relative humidity (RH) was kept constant at  $40 \pm 3\%$ . All exercise tests took place in a built-in climatic chamber. Before the start of each exercise test, participants were seated for 10 min to accommodate to the environmental conditions. Participants were asked to maintain the same type of meals at the day of an exercise test and to drink 500 mL of water over 2 hours prior to the beginning of the test. Participants were instructed to abstain from any exhaustive exercise 24 hours leading up to an exercise test and to refrain from consumption of caffeine and alcohol for 24 hours prior to testing. During the first test, participants were instructed to choose their cadence between 70 and 90 revolutions per minute (rpm), and maintain their preferred cadence during all upcoming exercise tests. Strong verbal

encouragement was provided throughout all exercise tests to ensure maximum effort. The protocol was terminated at volitional exhaustion, which was defined as the inability to maintain a minimal cadence of 70 rpm for more than 5 consecutive seconds. HR was monitored on a continuous basis (H7 Sensor; Polar, Kempele, Finland).

*RAMP test.* Warm-up consisted of 6 min cycling at 120 W, 2 min seated rest and 4 min baseline cycling at 70 W. Subsequently, the work rate increased continuously and linear with  $30 \text{ W} \cdot \text{min}^{-1}$ , as this would result in a test duration of 8-12 min in our population based on anthropometrics and reported physical exercise. Pulmonary gas exchanges for the determination of  $\dot{V}\text{O}_{2\text{peak}}$ , GET and RCP were measured on a breath-by-breath basis using a metabolic instrument (Cortex MetaLyzer 3B; Cortex Biophysik, Leipzig, Germany).

*STEP test.* The test started at a work rate of 80 W for 3 min and increased stepwise with 40 W every 3 min to obtain at least five  $[\text{BLa}^-]$  measurements. At the end of each stage, 20  $\mu\text{L}$  of blood from the right middle finger was collected into a capillary tube and analysed for  $[\text{BLa}^-]$  (Biosen C-Line; EKF-diagnostic GmbH, Magdeburg, Germany). Peak  $[\text{BLa}^-]$  ( $[\text{BLa}^-]_{\text{peak}}$ ) was obtained 1 min after cessation of the exercise test.

## Data analysis

For all exercise thresholds, corresponding values of PO and HR were determined and the differences in PO and HR between TEMP and HOT were calculated and expressed as the relative change (%) compared to values of TEMP ( $\Delta$ ). PO at thresholds in TEMP were calculated relative to peak PO ( $\%\text{PO}_{\text{peak}}$ ) within the respective test protocol, to express the relationship between PO- $\Delta$  and (relative) exercise intensity.

*RAMP test.* Breath-by-breath data were averaged into 10 s intervals.  $\dot{V}\text{O}_{2\text{peak}}$  was determined as the average of the highest three consecutive 10 s values. Two respiratory thresholds were determined by four independent researchers using four different criteria for each threshold. The mean of the closest three values was used. GET was defined as [1] the point where  $\dot{V}\text{CO}_2$  increased disproportionately to  $\dot{V}\text{O}_2$  using the V-slope method, [2] the first departure from the linear increase in ventilation ( $\dot{V}_E$ ), [3] an increase in  $\dot{V}_E/\dot{V}\text{O}_2$  without a simultaneous increase in  $\dot{V}_E/\dot{V}\text{CO}_2$  and [4] the first rise in end-tidal oxygen tension ( $P_{\text{ET}}\text{O}_2$ ) (Beaver et al., 1986; Binder et al., 2008). RCP corresponded to [1] the point where  $\dot{V}_E$  increased disproportionately to  $\dot{V}\text{CO}_2$ , [2] the second departure from linearity in  $\dot{V}_E$ , [3] an increase in both  $\dot{V}_E/\dot{V}\text{O}_2$  and  $\dot{V}_E/\dot{V}\text{CO}_2$  and [4] the



195 deflection point of end-tidal carbon dioxide tension ( $P_{ET}CO_2$ ) (Wasserman, 1984; Binder et al., 2008). To  
196 obtain the precise work rate at which GET occurred, an individual correction was made, i.e., to account for  
197 the mean response time (MRT) of the  $\dot{V}O_2$  kinetics. MRT was quantified as the time interval between the  
198 onset of the ramp and the intersection of the forward extrapolation of the baseline  $\dot{V}O_2$  and the backwards  
199 extrapolation of the linear  $\dot{V}O_2$ /time relationship below the GET (Boone & Bourgois, 2012). For RCP, an  
200 additional correction was made to close the gap for the extra dissociation of the  $\dot{V}O_2$ /PO relationship  
201 between ramp incremental exercise and constant work rate exercise at higher intensities (Caen et al., 2020).  
202 Oxygen pulse ( $O_2$  pulse:  $\dot{V}O_2$ /HR) was calculated as an indirect indicator of cardiac stroke volume (SV) at peak  
203 level and the two RT.

204 *STEP test.* Sixteen thresholds were calculated based on  $[BLa^-]$ , using nine threshold methods. A distinction  
205 was made between individual ( $LT_{IND}$ ) and fixed value ( $LT_{FIX}$ ) lactate thresholds.

206 *Individual lactate thresholds:*

- 207 1. Log-log: The lactate curve was divided into two segments and the intersection point of the two lines with  
208 the lowest residuals sum of squares was taken as the lactate threshold (Beaver et al., 1985).
- 209 2. Baseline + absolute value(s) ( $Bsl_n + mmol \cdot L^{-1}$ ): The intensity at which  $[BLa^-]$  increased 0.5 ( $Bsl_n + 0.5$ ), 1.0  
210 ( $Bsl_n + 1.0$ ) or 1.5 ( $Bsl_n + 1.5$ )  $mmol \cdot L^{-1}$  above baseline value (Berg et al., 1990; Zoladz et al., 1995).
- 211 3.  $D_{max}$ : The point on the third-order polynomial regression curve that yielded the maximum perpendicular  
212 distance to the straight line formed by the two end points of the curve (Cheng et al., 1992).
- 213 4. Modified  $D_{max}$  ( $ModD_{max}$ ): The intensity at the point on the third order polynomial regression curve that  
214 yielded the maximal perpendicular distance to the straight line formed by the point preceding the first rise  
215 in  $[BLa^-]$  of  $> 0.4 mmol \cdot L^{-1}$  lactate and the final lactate point (Bishop et al., 1998).
- 216 5. Exponential  $D_{max}$  ( $Exp-D_{max}$ ): The point on the exponential regression curve that yielded the maximum  
217 perpendicular distance to the straight line formed by the two end points of the curve (Hughson et al., 1987;  
218 Machado et al., 2012).

6. Log-log modified  $D_{\max}$  (Log-Poly-Mod $D_{\max}$ ): The intensity at the point on the third order polynomial regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final lactate point (Jamnick et al., 2018).

7. Log-log exponential modified  $D_{\max}$  method (Log-Exp-Mod $D_{\max}$ ): The intensity at the point on the exponential plus-constant regression curve that yielded the maximal perpendicular distance to the straight line formed by the intensity associated with the log-log LT and the final lactate point (Jamnick et al., 2018).

8. First and second lactate turning points: The lactate curvature is divided into three segments. Two double-linear fits are performed, which the intersection points between the lines (segments) are considered as Lactate Threshold 1 (LT<sub>1</sub>) and Lactate Threshold 2 (LT<sub>2</sub>) (Binder et al., 2008).

*Fixed value lactate thresholds:*

9. Fixed lactate thresholds or onset of blood lactate accumulation (OBLA) values of 2.0 (OBLA 2.0), 2.5 (OBLA 2.5), 3.0 (OBLA 3.0), 3.5 (OBLA 3.5), or 4.0 (OBLA 4.0) mmol·L<sup>-1</sup> (Kindermann et al., 1979; Skinner & McLellan, 1980; Heck et al., 1985).

## **Statistical analysis**

All data were expressed as mean values and standard deviations (SD) for  $n = 11$ . A priori sample size calculations have been performed in G\*Power 3.1.9 (University Düsseldorf, Germany) with significance level 0.05 and power 80 %. An estimated effect size of 0.65 results in a total sample size of  $n=11$ . Participants served as their own controls. SPSS statistics 25 (IBM Corp., Armonk, NY) was used for statistical analysis. The Shapiro-Wilk test was used to confirm normal distribution of the data. Repeated-Measures (RM) ANOVA (2 × 2) was performed to investigate differences in time to exhaustion (TTE) and peak performance parameters (PO and HR) between TEMP and HOT in RAMP and STEP. Paired-samples t tests were used to compare  $\dot{V}O_2$  in RAMP and [BLa<sup>-</sup>] in STEP between TEMP and HOT.

RM ANOVA was used to observe a difference how thresholds determined in RAMP (i.e., RT) and STEP (i.e., LT) are affected by heat (design: 18 thresholds × 2 conditions), and this was done for PO and HR corresponding to the threshold. If a significant effect was seen, post hoc paired samples t tests with Bonferroni correction were executed for comparison between TEMP and HOT for one threshold at a time.

245 Significance was set at  $p < 0.05$  and 95 % Confidence Interval (CI<sub>95%</sub>) was given. Cohen's d effect size (ES) was  
246 calculated to standardize mean differences. Pearson correlation coefficient ( $r$ ) was used to mark a linear  
247 relationship between absolute and relative exercise intensity (i.e., PO and %PO<sub>peak</sub>) in TEMP and the size of  
248 difference in PO ( $\Delta$ ) between TEMP and HOT.

## 249 RESULTS

250 At peak level, no interaction effect ( $F = 2.078$ ;  $p = 0.180$ ) was found for  $PO_{peak}$  in TEMP and HOT for RAMP  
251 ( $361 \pm 29$  W vs.  $353 \pm 40$  W) and STEP ( $306 \pm 31$  W vs.  $288 \pm 29$  W); nonetheless, there was a main effect of  
252 temperature ( $F = 24.605$ ;  $p < 0.001$ ), indicating that the  $PO_{peak}$  in HOT was lower compared to TEMP. On the  
253 other hand, significant interaction effects were found for TTE ( $F = 9.718$ ;  $p = 0.011$ ) and HR ( $F = 7.111$ ;  $p =$   
254  $0.024$ ). TTE was reduced in STEP ( $1018 \pm 140$  s vs.  $937 \pm 130$  s;  $p < 0.001$ ) in HOT compared to TEMP, but  
255 not in RAMP ( $581 \pm 59$  s vs.  $566 \pm 80$  s;  $p = 0.212$ ) and HR was higher in RAMP ( $182 \pm 10$  bpm vs.  $187 \pm 9$   
256 bpm;  $p = 0.017$ ) in HOT compared to TEMP, but not in STEP ( $187 \pm 8$  bpm vs.  $185 \pm 7$  bpm;  $p = 0.346$ ).  $[BLa^-]$   
257  $]_{peak}$  did not differ in STEP between TEMP and HOT ( $12.14 \pm 1.65$  mmol·L<sup>-1</sup> vs.  $11.48 \pm 2.17$  mmol·L<sup>-1</sup>;  $p =$   
258  $0.198$ ). Furthermore,  $\dot{V}O_{2peak}$  was higher in HOT in RAMP ( $3.93 \pm 0.46$  L·min<sup>-1</sup> vs.  $4.19 \pm 0.40$  L·min<sup>-1</sup>;  $p <$   
259  $0.001$ ) and  $O_2$  pulse at peak level did not differ in RAMP ( $21.7 \pm 3.0$  ml·bpm<sup>-1</sup> vs.  $22.5 \pm 2.4$  ml·bpm<sup>-1</sup>;  $p =$   
260  $0.053$ ).

261 Table 1 gives an overview of all threshold methods (expressed as PO and HR) in TEMP and HOT within their  
262 respective exercise test protocol. When expressed as PO, a significant interaction effect ( $F = 2.038$ ;  $p = 0.012$ )  
263 was seen for the effect of heat on thresholds, determined in RAMP and STEP, meaning that there is a  
264 difference in the way thresholds are impacted by heat exposure. Post hoc analysis shows a significant  
265 decrease in PO in HOT for all thresholds, except GET, RCP and the log-log method. When expressed as HR, a  
266 significant interaction effect ( $F = 4.040$ ;  $p < 0.001$ ) was found for the effect of heat on thresholds, determined  
267 in RAMP and STEP. Post hoc analysis shows a significant increase in HR in HOT at RT, but not LT. Figure 1  
268 provides the representation of individual PO difference in HOT compared to TEMP for all threshold methods,  
269 with a range from -53 W to +26 W.  $\dot{V}O_2$  was higher at GET ( $2.76 \pm 0.30$  L·min<sup>-1</sup> vs.  $2.95 \pm 0.32$  L·min<sup>-1</sup>;  $p =$   
270  $0.006$ ) and RCP ( $3.50 \pm 0.44$  L·min<sup>-1</sup> vs.  $3.74 \pm 0.40$  L·min<sup>-1</sup>;  $p = 0.013$ ) in HOT.

271 A negative correlation was observed between the exercise intensity at which the thresholds occurred and  
272 the change in PO between TEMP and HOT, both for absolute PO (Fig. 2A;  $r = -0.34$ ;  $p < 0.001$ ) as relative to  
273  $PO_{peak}$  (Fig. 2B;  $r = -0.31$ ;  $p < 0.001$ ).

## DISCUSSION

In this study, we made a comparison between 18 exercise thresholds determined from two commonly used incremental test protocols (i.e., RAMP and STEP) in temperate (TEMP: 18°C) and hot (HOT: 36°C) environments with the same relative humidity (RH: 40 %). To our knowledge, this study is the first to comprehensively examine the effect of acute heat exposure on RT and LT in non-acclimatized physically active individuals.

Our first hypothesis was that short-term heat exposure (i.e., only the time duration of an incremental exercise test) would have a negative impact on all exercise thresholds. We found a significant interaction effect of ambient air temperature on the occurrence of the thresholds for PO, meaning that not all thresholds were impacted in the same way. This points at a considerable degree of variability in the sensitivity of the different threshold concepts to acute heat exposure. The PO at some thresholds was highly impacted (e.g., OBLA 2.0) as shown in the medium to large effect sizes (0.50-0.81), whereas in others, PO remained unchanged (e.g., GET). The mean decrease of PO at the different thresholds in our study was less pronounced (RT:  $-1.0 \pm 2.7$  % and LT:  $-6.2 \pm 1.9$  %), as compared to other studies investigating performance decrements in heat. Maunder et al. (2021) reported decreases for OBLA 2.0, OBLA 3.0 and OBLA 4.0, respectively, of 16 %, 13 % and 10 % between 18 and 36°C (60 % RH) in 16 competitive endurance-trained males, and a 17 % and 12 % decrease is noticed, respectively, at first (i.e., GET) and second (i.e., RCP) RT. de Barros et al. (2011) found that PO corresponding to RCP decreased by 18 % in 40°C in comparison to 22°C (50 % RH) for eight healthy young untrained male participants. Furthermore, Tyka et al. (2009; 2010) reported a 13 % and 11 % decrease at, respectively, LT (Exp- $D_{\max}$ ) and GET (V-slope method) in 37°C compared to 23°C (55 % RH). Lorenzo et al. (2011) found an overall decrease of 12 % in power output at several blood- and ventilation-based thresholds in 12 highly trained endurance cyclists (10 men and 2 women) when cycling in 38°C compared to 13°C (30 % RH).

The above variation in results coming from different studies can be explained by differences in test designs and procedures. First, exercise tests took place in various environmental conditions (i.e., ambient air

temperature and RH) using different test populations, so that a direct comparison is difficult. Second, in the present study, participants were exposed to hot environmental conditions for ~30 min (i.e., 10 min rest + exercise test). This exposure time is shorter than in the study of Maunder et al. (2021) and Tyka et al. (2010), where participants rested passively for 20 min and 30 min, respectively. Other studies reported the use of immersion in a hot bath (41°C) for 30 min to induce whole-body hyperthermia before start of incremental exercise (Lorenzo et al., 2011) or do not report the time of exposure before start of the test (de Barros et al., 2011). Furthermore, we speculate that the impact of heat exposure on exercise thresholds, using a protocol that prolongs the duration of the incremental exercise test (e.g., RAMP with a smaller ramp slope or STEP with longer stages), would be more fierce. This could also be the reason why our hypothesis, that thresholds taking place at a higher intensity will be affected more as heat exposure time is increased, was only partially supported by a weak correlation (see Fig. 2). The total duration of our protocol was too short to induce severe cardiorespiratory and metabolic perturbations, even in the thresholds occurring at higher intensities. Finally, it should be pointed out that the exercise test protocol is different. A single RAMP protocol with continuous increase in PO is preferred to determine RT as it is the most appropriate way to detect break point in the slope of the gas exchange and ventilatory response patterns (Keir et al., 2022). This is in contrast to other studies, where they used various STEP protocols to determine RT (Tyka et al., 2009; Tyka et al., 2010; de Barros et al., 2011) or merged STEP and RAMP into one exercise test protocol (Maunder et al., 2021), which will affect threshold determination.

The determination of LT in STEP is based on different underlying methodologies, i.e.,  $LT_{IND}$  or  $LT_{FIX}$ . We observed an alteration in lactate kinetics in HOT ( $LT_{IND}$ :  $-5.4 \pm 1.4$  % and  $LT_{FIX}$ :  $-7.5 \pm 2.4$  %), meaning that all LT methods (except log-log method) are sensitive to heat exposure. Higher  $[BLa^-]$  values were observed at the same absolute intensities in HOT compared to TEMP, resulting in lower PO at OBLA 2.0 – 4.0. This could indicate that there is a more pronounced production rate of  $La^-$  due to a greater reliance on the glycolytic metabolism, possible mediated by a higher thermal strain (i.e., elevated muscle temperature) and an increased sympathoadrenal response (i.e., increased circulating epinephrine) for the same absolute PO (Febbraio et al., 1994, 1996). Although, it should be emphasized that  $BLa^-$  accumulation is the result of a

balance between production by the muscles and clearance from the blood by active and inactive muscle mass, heart, brain, liver and kidneys. A redistribution of the blood flow toward the skin (i.e., vasodilatation for heat dissipation) occurs with heat exposure, suggesting a lower  $La^-$  elimination rate (Brooks, 2018; Rowell et al., 1968). The determination of LT by means of the  $D_{max}$  method ( $D_{max}$ ,  $ModD_{max}$ ,  $Exp-D_{max}$ ,  $Log-Poly-ModD_{max}$ ,  $Log-Exp-ModD_{max}$ ) depends on baseline  $[BLa^-]$ , lactate kinetics and  $[BLa^-]_{peak}$ . An equal mean  $[BLa^-]_{peak}$  was obtained at  $PO_{peak}$  ( $12.14 \pm 1.65 \text{ mmol}\cdot\text{L}^{-1}$  vs.  $11.48 \pm 2.17 \text{ mmol}\cdot\text{L}^{-1}$ ), although  $PO_{peak}$  was lower in HOT ( $306 \pm 31 \text{ W}$  vs.  $288 \pm 29 \text{ W}$ ). As a consequence, the course of the  $[BLa^-]$ -PO curve shifted to the left thus also resulting in a lower PO for the  $LT_{FIX}$  thresholds.

We found a higher HR for a given submaximal absolute PO, which can be attributed to the direct temperature effect on intrinsic HR at the sinoatrial node (Jose et al., 1970) and/or indirect effect by a reduced venous return due to increased skin blood flow (Rowell, 1974). In the context of exercise prescription, Maunder et al. (2021) proposed to rely on the HR instead of PO in the early phase of a heat acclimation/acclimatization camp, as HR at the thresholds did not differ between TEMP and HOT. This is in line with what we found in our study in STEP (i.e., equal HR and reduced PO). However, it must be pointed out that they do not take into account the negative heat effects during prolonged exercise at submaximal intensity. Deterioration of cardiac function and more specific reduction cardiac output will be more pronounced with prolonged (intense) exercise in heat as core temperature increases. When prescribing exercise based on HR, it is important that this is in accordance with the correct metabolic intensity (Teso et al., 2022), however, this is complicated in heat as both absolute and relative intensity will change over time.

We found that RT, with PO adjustment for GET (Boone & Bourgois, 2012) and RCP (Caen et al., 2020), in RAMP were less susceptible to heat. This might be due to a difference in duration-intensity ratio between RAMP and STEP where time spent above 50 % $PO_{peak}$  was almost double in STEP compared to RAMP ( $669 \pm 67 \text{ s}$  vs.  $357 \pm 34 \text{ s}$ ). As such, the heat strain, which is a function of absolute intensity and time, could be less pronounced in RAMP vs. STEP. We found that GET and RCP were identified at a higher  $\dot{V}O_2$ , and thus a higher metabolic intensity or internal load. However, converted to PO or external load, the RT did not change. It has been suggested that extra myocardial  $\dot{V}O_2$  in HOT is the reason, at least to a certain degree, for the higher

$\dot{V}O_2$  at a given submaximal power output. Gross efficiency during cycling is impacted by sustaining muscle blood flow in combination with a higher skin blood flow for heat dissipation (Hettinga et al., 2007; Nielsen et al., 1990). The relative intensity (i.e., % $\dot{V}O_{2peak}$ ), however, did not change at GET ( $70 \pm 3$  %) and RCP ( $89 \pm 4$  %) between TEMP and HOT, as also  $\dot{V}O_{2peak}$  reached during RAMP in HOT was higher compared to TEMP. This might be surprising as several other studies (Arngrímsson et al., 2004; James et al., 2017; Lorenzo et al., 2011) found that  $\dot{V}O_{2peak}$  is impaired in the heat, attributed to a lower cardiac output (González-Alonso & Calbet, 2003) and increases in core temperature limiting  $\dot{V}O_{2peak}$  (Arngrímsson et al., 2004). Others, however, observed no reduction in  $\dot{V}O_{2peak}$  (Rowell et al., 1965; Schlader et al., 2011; Tyka et al., 2010) or even an increase (Kuo et al., 2021; Lafrenz et al., 2008), possibly related to the short duration of the test (Rowell, 1974). The characteristics of the participants, who are physically active but not habituated to cycling exercise, must also be taken into account. In this context, it is possible that in TEMP, the exercise tests were terminated as a consequence of fatigue in the locomotor muscles instead of cardiopulmonary exhaustion. In our study, we observed, not only submaximal, but also at peak level a higher HR, so that the  $O_2$  pulse (oxygen consumption per heart beat) is equal at GET, RCP and peak in both environmental conditions. Clearly, further investigation on the effects of acute heat exposure on limitations of exercise performance is required.

In conclusion, acute short-term heat exposure, by means of increased ambient air temperature, does impact RT and LT expressed in PO or HR, determined from a ramp ( $30 \text{ W} \cdot \text{min}^{-1}$ ) or step ( $40 \text{ W} \cdot 3 \text{ min}^{-1}$ ) incremental exercise test. Results regarding the outcomes of exercise tests in heat are still diverse and cannot be generalized without taking into account the underlying components, as consistency in methodology of exercise testing, threshold determination and specific environmental conditions are key. Based on PO and/or physiological values (HR,  $BLa^-$  and  $\dot{V}O_2$ ) obtained from one exercise test in heat, translation to practice remains complicated. However, given the large variation in response to heat exposure (see Fig.1), even a short incremental exercise test with acute heat exposure can give valuable insight on the acute heat response of an athlete. Yet, performing exercise tests in a broad range of environmental conditions provide the opportunity to gather useful information for sports scientists and coaches to optimize exercise prescription



376 and monitoring exercise intensity in moderate physically active individuals. Therefore, every case has to be  
377 (re)viewed individually by an experienced staff, so that optimal training outcomes can be achieved.

1. Arnggrimsson, S. A., Petitt, D., Borrani, F., Skinner, K., & Cureton, K. (2004). Hyperthermia and maximal oxygen uptake in men and women. *European Journal of Applied Physiology*, 92(4–5), 524–532. <https://doi.org/10.1007/s00421-004-1053-1>
2. Beaver, W. L., Wasserman, K., & Whipp, B. (1985). Improved detection of lactate threshold during exercise using a log-log transformation. *J Appl Physiol*, 59(6), 1936–1940. <https://doi.org/10.1152/jappl.1985.59.6.1936>
3. Beaver, W. L., Wasserman, K., & Whipp, B. J. (1986). A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol*, 60(6), 2020–2027. <https://doi.org/10.1152/jappl.1986.60.6.2020>
4. Berg, A., Jakob, E., Lehmann, M., Dickhuth, H., Huber, G., & Keul, J. (1990). Current aspects of modern ergometry. *Pneumologie (Stuttgart, Germany)*, 44(1), 2–13.
5. Binder, R., Wonisch, M., Corra, U., Cohen-Solal, A., Vanhees, L., Saner, H., & Schmid, J. P. (2008). Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. *European Journal of Cardiovascular Prevention & Rehabilitation*, 15(6), 726–734. <https://doi.org/10.1097/HJR.0b013e328304fed4>
6. Bishop, D., Jenkins, D. G., & Mackinnon, L. T. (1998). The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. *Med Sci Sports Exerc*, 30(8), 1270–1275. <https://doi.org/10.1097/00005768-199808000-00014>
7. Boone, J., & Bourgois, J. (2012). The Oxygen Uptake Response to Incremental Ramp Exercise Methodological and Physiological Issues. *Sports Med*, 42(6), 511–526.
8. Bourgois, J. G., Bourgois, G., & Boone, J. (2019). Perspectives and Determinants for Training-Intensity Distribution in Elite Endurance Athletes. *International Journal of Sports Physiology and Performance*, 14(8), 1151–1156. <https://doi.org/10.1123/ijsp.2018-0722>
9. Brooks, G. A. (2018). The Science and Translation of Lactate Shuttle Theory. *Cell Metabolism*, 27(4), 757–785. <https://doi.org/10.1016/j.cmet.2018.03.008>
10. Caen, K., Boone, J., Bourgois, J. G., Colosio, A. L., & Pogliaghi, S. (2020). Translating Ramp VO2 into constant power output: a novel strategy that minds the gap. *Med Sci Sports Exerc*, 52(9), 2020–2028. <https://doi.org/10.1249/MSS.0000000000002328>
11. Caen, K., Pogliaghi, S., Lievens, M., Vermeire, K., Bourgois, J. G., & Boone, J. (2021). Ramp vs. step tests: valid alternatives to determine the maximal lactate steady-state intensity? *European Journal of Applied Physiology*, 121(7), 1899–1907. <https://doi.org/10.1007/s00421-021-04620-9>
12. Cheng, B., Kuipers, H., Snyder, A. C., Keizer, H. A., Jeukendrup, A., & Hesselink, M. (1992). A new approach for the determination of ventilatory and lactate thresholds. *International Journal of Sports Medicine*, 13(7), 518–522. <https://doi.org/10.1055/s-2007-1021309>

13. de Barros, C. L. M., Mendes, T. T., Mortimer, L. Á. C. F., Simões, H. G., Prado, L. S., Wisloff, U., & Silami-Garcia, E. (2011). Maximal Lactate Steady State is Altered in the Heat. *International Journal of Sports Medicine*, 32(10), 749–753. <https://doi.org/10.1055/s-0031-1277191>
14. Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate threshold concepts: How valid are they? *Sports Medicine*, 39(6), 469–490. <https://doi.org/10.2165/00007256-200939060-00003>
15. Febbraio, M. A., Carey, M. F., Snow, R. J., Stathis, C. G., & Hargreaves, M. (1996). Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 271(5), R1251–R1255. <https://doi.org/10.1152/ajpregu.1996.271.5.r1251>
16. Febbraio, M. A., Snow, R. J., Stathis, C. G., Hargreaves, M., & Carey, M. F. (1994). Effect of heat stress on muscle energy metabolism during exercise. *J. Appl. Physiol*, 77(6), 2827–2831. <https://doi.org/10.1152/jappl.1994.77.6.2827>
17. Gaesser, G. A., & Poole, D. C. (1996). The slow component of oxygen uptake kinetics in humans. *Exercise & Sport Science Reviews*, 24(1), 35–70.
18. González-Alonso, J., & Calbet, J. A. L. (2003). Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. *Circulation*, 107(6), 824–830. <https://doi.org/10.1161/01.CIR.0000049746.29175.3F>
19. Heck, H., Mader, A., Hess, G., Mücke, S., Muller, R., & Hollmann, W. (1985). Justification of the 4-mmol/l lactate threshold. *Int J Sports Med*, 6(3), 117–130. <https://doi.org/10.1055/s-2008-1025824>
20. Hettinga, F. J., De Koning, J. J., de Vrijer, A., Wüst, R. C. I., Daanen, H. A. M., & Foster, C. (2007). The effect of ambient temperature on gross-efficiency in cycling. *European Journal of Applied Physiology*, 101(4), 465–471. <https://doi.org/10.1007/s00421-007-0519-3>
21. Hughson, R. L., Weisiger, K. H., & Swanson, G. D. (1987). Blood lactate concentration increases as a continuous function in progressive exercise. *Journal of Applied Physiology*, 62(5), 1975–1981. <https://doi.org/10.1152/jappl.1987.62.5.1975>
22. James, C. A., Hayes, M., Willmott, A. G. B., Gibson, O. R., Flouris, A. D., Schlader, Z. J., & Maxwell, N. S. (2017). Defining the determinants of endurance running performance in the heat. *Temperature*, 4(3), 314–329. <https://doi.org/10.1080/23328940.2017.1333189>
23. Jamnick, N. A., Botella, J., Pyne, D. B., & Bishop, D. J. (2018). Manipulating graded exercise test variables affects the validity of the lactate threshold and VO<sub>2</sub>peak. *PLoS ONE*, 13(7), e0199794. <https://doi.org/10.1371/journal.pone.0199794>
24. Jose, A. D., Stitt, F., & Collison, D. (1970). The effects of exercise and changes in body temperature on the intrinsic heart rate in man. *American Heart Journal*, 79(4), 488–498. [https://doi.org/10.1016/0002-8703\(70\)90254-1](https://doi.org/10.1016/0002-8703(70)90254-1)
25. Keir, D. A., Fontana, F. Y., Robertson, T. C., Murias, J. M., Paterson, D. H., Kowalchuk, J. M., & Pogliaghi,

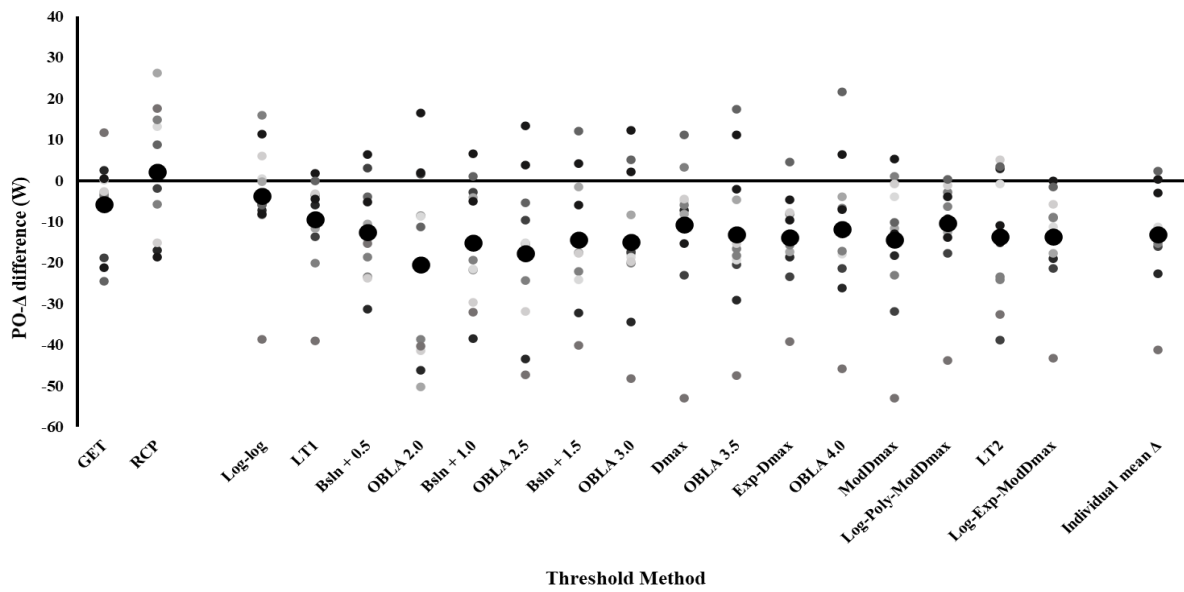
- S. (2015). Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable Performance. *Medicine and Science in Sports and Exercise*, 47(9), 1932–1940. <https://doi.org/10.1249/MSS.0000000000000613>
26. Keir, D. A., Iannetta, D., Mattioni Maturana, F., Kowalchuk, J. M., & Murias, J. M. (2022). Identification of Non-Invasive Exercise Thresholds: Methods, Strategies, and an Online App. *Sports Medicine*, 52(2), 237–255. <https://doi.org/10.1007/s40279-021-01581-z>
27. Kindermann, W., Simon, G., & Keul, J. (1979). The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *European Journal of Applied Physiology and Occupational Physiology*, 42(1), 25–34. <https://doi.org/10.1007/BF00421101>
28. Kuo, Y., Cheng, C., & Kuo, Y. (2021). Determining Validity of Critical Power Estimated Using a Three-Minute All-Out Test in Hot Environments. *International Journal of Environmental Research and Public Health*, 18(17), 9193. <https://doi.org/10.3390/ijerph18179193>
29. Lafrenz, A. J., Wingo, J. E., Ganio, M. S., & Cureton, K. J. (2008). Effect of Ambient Temperature on Cardiovascular Drift and Maximal Oxygen Uptake. *Med Sci Sports Exerc*, 40(6), 1065–1071. <https://doi.org/10.1249/MSS.0b013e3181666ed7>
30. Lorenzo, S., Minson, C. T., Babb, T. G., & Halliwill, J. R. (2011). Lactate threshold predicting time-trial performance: impact of heat and acclimation. *J. Appl. Physiol*, 111(1), 221–227. <https://doi.org/10.1152/jappphysiol.00334.2011>
31. Machado, F. A., Nakamura, F. Y., & de Moraes, S. M. F. (2012). Influence of regression model and incremental test protocol on the relationship between lactate threshold using the maximal-deviation method and performance in female runners. *Journal of Sports Sciences*, 30(12), 1267–1274. <https://doi.org/10.1080/02640414.2012.702424>
32. Maunder, E., Plews, D. J., Merien, F., & Kilding, A. E. (2021). Stability of heart rate at physiological thresholds between temperate and heat stress environments in endurance-trained males. *International Journal of Sports Physiology and Performance*, 16(8), 1204–1207. <https://doi.org/10.1123/IJSP.2020-0351>
33. Nielsen, B., Savard, G., Richter, E. A., Hargreaves, M., & Saltin, B. (1990). Muscle blood flow and muscle metabolism during exercise and heat stress. *Journal of Applied Physiology*, 69(3), 1040–1046. <https://doi.org/10.1152/jappl.1990.69.3.1040>
34. Périard, J. D., Eijssvogels, T. M. H., & Daanen, H. A. M. (2021). Exercise under heat stress: thermoregulation, hydration, performance implications, and mitigation strategies. *Physiological Reviews*, 101(4), 1873–1979. <https://doi.org/10.1152/physrev.00038.2020>
35. Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiological Reviews*, 54(1), 75–159. <https://doi.org/10.1152/physrev.1974.54.1.75>
36. Rowell, L. B., Blackmon, J. R., Martin, R. H., Mazzarella, J. A., & Bruce, R. A. (1965). Hepatic clearance

- of indocyanine green in man under thermal and exercise stresses. *J. Appl. Physiol.*, 20(3), 384–394.  
<https://doi.org/10.1152/jappl.1965.20.3.384>
37. Rowell, Loring B, Brengelmann, G. L., Blackmon, J. R., Twiss, R. D., & Kusumi, F. (1968). Splanchnic blood flow and metabolism in heat-stressed man. *Journal of Applied Physiology*, 24(4), 475–484.
  38. Schlader, Z. J., Stannard, S. R., & Mündel, T. (2011). Is peak oxygen uptake a determinant of moderate duration self-paced exercise performance in the heat? *Applied Physiology, Nutrition and Metabolism*, 36(6), 863–872. <https://doi.org/10.1139/H11-111>
  39. Skinner, J. S., & McLellan, T. H. (1980). The Transition from Aerobic to Anaerobic Metabolism. *Research Quarterly for Exercise and Sport*, 51(1), 234–248. <https://doi.org/10.1080/02701367.1980.10609285>
  40. Teso, M., Colosio, A. L., & Pogliaghi, S. (2022). An Intensity-dependent Slow Component of HR Interferes with Accurate Exercise Implementation in Postmenopausal Women. *Medicine and Science in Sports and Exercise*, 54(4), 655–664. <https://doi.org/10.1249/MSS.0000000000002835>
  41. Tyka, A. A., Palka, T., Tyka, A. A., Cisoń, T., & Szygula, Z. (2009). The influence of ambient temperature on power at anaerobic threshold determined based on blood lactate concentration and myoelectric signals. *International Journal of Occupational Medicine and Environmental Health*, 22(1), 1–6. <https://doi.org/10.2478/v10001-009-0005-8>
  42. Tyka, A., Wiecha, S., Palka, T., Szygula, Z., Tyka, A., & Cison, T. (2010). Effects of Ambient Temperature on Physiological Responses to Incremental Exercise Test. *Journal of Human Kinetics*, 26, 57–64. <https://doi.org/10.2478/v10078-010-0049-7>
  43. Wasserman, K. (1984). The anaerobic threshold measurement to evaluate exercise performance. *American Review of Respiratory Disease*, 129(2P2), S35–S40. <https://doi.org/10.1164/arrd.1984.129.2p2.s35>
  44. Zoladz, J. A., Rademaker, A. C., & Sargeant, A. J. (1995). Non-linear relationship between O<sub>2</sub> uptake and power output at high intensities of exercise in humans. *Journal of Physiology*, 488(1), 211–217. <https://doi.org/10.1113/jphysiol.1995.sp020959>

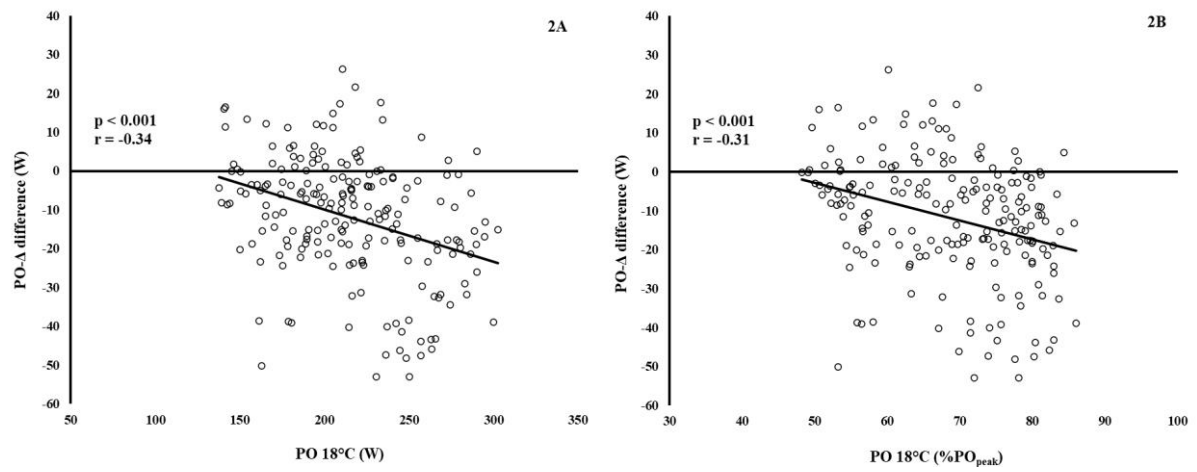
**Table 1** Power output (PO) and heart rate (HR) at which the exercise thresholds occurred in RAMP and STEP for TEMP and HOT in order of appearance

Threshold method	Power output				Heart rate			
	18°C	36°C	Δ	ES	18°C	36°C	Δ	ES
	PO (W)	PO (W)	%		HR (bpm)	HR (bpm)	%	
<b>RAMP</b>								
<i>GET</i>	198 ± 26	193 ± 27	-2.9	0.31	142 ± 9	154 ± 9 *	8.6	1.95
<i>RCP</i>	240 ± 33	242 ± 27	0.9	0.10	163 ± 10	171 ± 8 *	5.1	1.26
<b>STEP</b>								
<i>Log-log</i> <sup>IND</sup>	159 ± 20	155 ± 19	-2.3	0.27	130 ± 9	136 ± 7	4.7	1.04
<i>LT<sub>1</sub></i> <sup>IND</sup>	166 ± 22	156 ± 21 *	-5.7	0.62	132 ± 8	136 ± 8	2.8	0.66
<i>Bsln + 0.5</i> <sup>IND</sup>	179 ± 24	166 ± 21 *	-7.0	0.79	137 ± 8	139 ± 8	1.6	0.38
<i>OBLA 2.0</i> <sup>FIX</sup>	185 ± 38	164 ± 33 *	-11.1	0.81	139 ± 11	139 ± 11	-0.4	0.07
<i>Bsln + 1.0</i> <sup>IND</sup>	201 ± 31	186 ± 25 *	-7.6	0.76	146 ± 7	147 ± 8	0.8	0.22
<i>OBLA 2.5</i> <sup>FIX</sup>	204 ± 40	186 ± 30 *	-8.7	0.71	147 ± 10	147 ± 9	0.2	0.04
<i>Bsln + 1.5</i> <sup>IND</sup>	216 ± 33	202 ± 29 *	-6.7	0.66	152 ± 10	153 ± 9	0.8	0.19
<i>OBLA 3.0</i> <sup>FIX</sup>	218 ± 40	203 ± 32 *	-6.9	0.59	152 ± 8	153 ± 9	0.9	0.24
<i>D<sub>max</sub></i> <sup>IND</sup>	218 ± 23	207 ± 23 *	-4.9	0.66	152 ± 9	155 ± 7	2.0	0.54
<i>OBLA 3.5</i> <sup>FIX</sup>	229 ± 39	215 ± 32 *	-5.8	0.53	156 ± 9	158 ± 8	1.3	0.32
<i>Exp-D<sub>max</sub></i> <sup>IND</sup>	231 ± 25	217 ± 23 *	-6.0	0.81	157 ± 8	159 ± 7	1.0	0.30
<i>OBLA 4.0</i> <sup>FIX</sup>	238 ± 37	226 ± 30 *	-5.0	0.50	160 ± 9	162 ± 8	1.4	0.37
<i>ModD<sub>max</sub></i> <sup>IND</sup>	240 ± 30	226 ± 28 *	-6.0	0.71	161 ± 9	162 ± 7	0.8	0.23
<i>Log-Poly-ModD<sub>max</sub></i> <sup>IND</sup>	241 ± 26	231 ± 25 *	-4.3	0.57	161 ± 9	164 ± 7	1.9	0.51
<i>LT<sub>2</sub></i> <sup>IND</sup>	246 ± 31	232 ± 33 *	-5.5	0.60	162 ± 8	164 ± 7	0.7	0.21
<i>Log-Exp-ModD<sub>max</sub></i> <sup>IND</sup>	250 ± 28	236 ± 26 *	-5.5	0.73	164 ± 9	166 ± 7	1.1	0.30

Values are mean ± standard deviation (SD) for *n* = 11 participants. Effect size (ES) is calculated and significant differences are marked (\*) for *p* < 0.05. In STEP, a distinction has been made between individual (<sup>IND</sup>) an fixed value (<sup>FIX</sup>) lactate threshold methods



**Fig. 1** Mean (big dots) and individual (small dots) difference in PO between 18°C and 36°C for all determined exercise thresholds; two respiratory thresholds, GET and RCP, in RAMP and 16 lactate thresholds in STEP. Mean difference of all exercise thresholds within the individual is displayed as Individual mean Δ.



**Fig. 2** Scatterplots showing the relationship between all 198 (11 participants x 18 exercise thresholds) unique thresholds determined in 18°C for (A) absolute and (B) relative PO and the PO difference between 18°C and 36°C for the determined threshold.