Effects of three-exercise sessions in the heat on endurance cycling performance.

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Abstract

Purpose: To investigate the effects of a very short-term acclimation protocol (VSTAP) on performance, physiological and perceptual responses to exercise in the heat.
Methods: 12 trained male cyclists (age 31.2±7; weight 71.3±7 kg, VO_{2max}: 58.4±3.7 mL/kg/min) randomly performed two Time to Exhaustion Tests (TTE) at 75% of normothermic peak power output (PPO), one in normothermia (N,18°C-50% RH) and one in the heat (H,35°C-50% RH), before and after a VSTAP intervention, consisting of 3 days- 90 min exercise (10min at 30% of PPO+80 min at 50% of PPO) in H (≈4.5h of heat exposure). Performance time of TTEs and physiological and perceptual variables of both TTEs and training sessions (T1, T2 and T3) were evaluated.
Results: Magnitude Based Inferences (MBI) revealed 92/6/1% and 62/27/11% chances of positive/trivial/negative effects of VSTAP of improving performance in H (+17%) and in N (+9%), respectively. Heart Rate (HR) decreased from T1 to T3 ($p<0.001$) and T2 to T3 ($p<0.001$), whereas Tympanic Temperature (TyT) decreased from T1 to T2 ($p=0.047$) and from T1 to T3 ($p=0.007$). Furthermore, despite the increased tolerance to target Power Output (PO) throughout training sessions, RPE decreased from T1 to T3 ($p=0.032$).
Conclusions: The VSTAP determined meaningful physiological (i.e. decreased HR and TyT) and perceptual (i.e. decreased RPE) adaptations to submaximal exercise. Furthermore, showing good chances to improve performance in the heat, it represents a valid acclimation strategy to be implemented when no longer acclimation period is possible. Finally, no cross-over effect of the VSTAP on performance in temperate conditions was detected.

Keywords (5)
Heat Acclimation, Performance, Time to Exhaustion, Heart rate, Magnitude Based Inferences

Abbreviations
Very Short-Term Heat Acclimation Protocol (VSTAP), Thermal Sensation (TS), Tympanic Temperature (TyT).

1. INTRODUCTION

Endurance performance is impaired by heat stress (Périard et al., 2011), which in the worst situations may result in exertional heat injury (EHI) (Périard et al., 2016). Decreases in endurance performance range from 6 to 16% (Casadio et al., 2017) in trained athletes during endurance and team sport events, while exertional heat injury can range from syncope/collapse to heat stroke (Périard et al., 2016). These impairments are predominantly related to an increased cardiovascular strain when exercising in the heat as a result of the competition for blood flow between metabolic demands in the active musculature and the peripheral microvasculature for heat dissipation at the skin surface (Casadio et al., 2017; Chalmers et al., 2014), which results in accentuated and/or intolerable thermal strain. Also cycling performance is widely influenced by heat stress, with studies showing decrements ranging from 6% (Bradbury et al., 2019) to 16% (Racinais et al., 2014) in average power output during cycling Time Trial tests.

A large number of strategies (Tyler et al., 2016) to overcome heat-induced performance impairments have been proposed, especially considering that nowadays numerous high-profile sporting events are held in hot environments, like some of the most important cycling competitions worldwide (e.g the Tour de France and the Dubai Tour, performed in environmental temperatures that can reach 40 °C, with 20-60% relative humidity(RH)) (James et al., 2017; Moss et al., 2020; Wingfield et al., 2016). These strategies include acute interventions such as pre and/or per-cooling and chronic interventions such as heat acclimation. Heat acclimation (HA) (i.e., repeated training in a hot, artificial environment) or heat acclimatization (i.e., repeated training in a hot, natural environment) results in a range of physiological, metabolic and psychological adaptations, which can ameliorate heat-induced decrements in performance; most of these adaptations occur during the first week of heat exposure, with full acclimation thereafter (Tyler et al., 2016).

Cardiovascular adaptations to HA, including plasma expansion, increased stroke volume (SV) and cardiac output (CO) and decreased heart rate (HR) for the same absolute exercise intensity, and perceptual adaptations (e.g. decreased perception of effort (RPE) and ameliorated thermal sensation) generally occur within 3-6 exposures. Conversely, metabolic (i.e. decreased reliance upon carbohydrates as a fuel source during exercise) and thermoregulatory adaptations require at least 4 to 8 days to start (Chalmers et al., 2014) (i.e., reduced core temperature threshold for the onset of sweating and cutaneous vasodilation), with full adaptation (i.e., increased sweat rate and enhanced skin vasodilation for a given core temperature) (Chalmers et al., 2014) within 14 days. Similarly, endurance performance takes up to 10–14 days of repeated heat exposures to achieve 95% of its maximal response (while performing low intensity exercise at 50–60% of VO_{2max})(Petersen et al., 2010).

Regarding training strategies, HA is habitually classified as either short (STHA, ≤7 days), medium (8–14 days) or long term (LTHA, >15 days) (Tyler et al., 2016), where number of days is referred to actual training days. Unquestionably, LTHA typically allows an individual to achieve his optimal heat acclimation, but the constraints and complexities of an athlete's training and competition schedules often do not permit such a long intervention period (Casadio et al., 2017). In many instances, a STHA strategy is the only suitable option. Most of the previous studies investigated STHA strategies lasting 5 to 7 days (Garrett et al., 2012; James et al., 2017; Moss et al., 2020; Tsai et al., 2013), with few of them considering shorter intervention periods (Petersen et al., 2010; Willmott et al., 2018, 2016). Furthermore, to the best of our knowledge, only two studies (Willmott et al., 2018, 2016) investigated both physiological and perceptual responses and endurance performance outcomes in response to a very STHA intervention. However, a more in-depth understanding of STHA could be of primary importance for elite athletes: in fact, taking into account previously mentioned timeframes of physiological and perceptual adaptations (Chalmers et al., 2014), it is reasonable to think that 3 days of training in a hot environment may induce important adaptations when longer heat acclimatization/acclimation is not logistically possible, but it is still unclear if these adaptations may be useful in improving performance in the heat.

Furthermore, whether HA improves exercise performance in a cool condition is debated: in fact, if some of the previous mentioned adaptations in the heat have been shown to lead to normothermic performance improvements (Corbett et al., 2014; Lorenzo et al., 2010; Minson and Cotter, 2016), others (Keiser et al., 2015; Sotiridis et al., 2019) state no or unclear ergogenic effect of heat acclimation, especially when considering short intervention periods (Neal et al., 2016).
Therefore, the aim of the study is twofold: i) to investigate the effects of a 3-day very short-term heat acclimation protocol (VSTAP) on performance, physiological and perceptual responses to exercise in the heat and ii) to determine whether heat acclimation improves maximal endurance performance in temperate conditions. We hypothesized that a VSTAP would improve cycling endurance performance in the heat (but not in normothermia) as a consequence of cardiovascular, perceptual and partial thermoregulatory adaptations.

2. MATERIALS AND METHODS

2.1 Subjects

12 trained male subjects (De Pauw et al., 2013) (age:31.2±7 years; height:177±6 cm; weight:71.3±7 kg; VO_{2max}:58.4±3.7 mL/kg/min; PPO:5.3±0.4 W/kg) volunteered for this study. Data were collected between October and March (mean outside temperature <20°C) in Trentino (North Italy) to avoid heat acclimatization. None of the participants involved had clinical evidence of cardiovascular, metabolic, or musculoskeletal diseases. Before data collection, all participants were properly informed about the experimental protocol and provided their written consent. They were instructed to avoid caffeine during test and HA days, and to refrain from intense exercise for 48 hours before test days. The protocol was conducted according to the principles of the Declaration of Helsinki.

2.2 Design

Each participant visited the laboratory on 8 different occasions at the same time of the day and completed the experimental protocol within a 3-week period. All tests were conducted in a climatic chamber under two different controlled laboratory conditions: 18°C with 50% RH, referred to as normothermia (N) and 35°C with 50%RH, referred to as heat (H). On visit 1 participants performed a cardiopulmonary exercise test (CPET) to exhaustion in N; on visits 2 and 3 a Time to Exhaustion (i.e. cycling against a constant load until exhaustion) in normothermia (TTEn) and one in the heat (TTEh) were performed in a random order (i.e. 7 subjects started with TTEn, 5 with TTEh); during visits 4,5 and 6 the VSTAP was completed; finally, on visits 7 and 8 TTEn and TTEh were repeated, again in random order and without being matched to TTEs pre-training order (i.e. 5 subjects started with TTEn, 7 with TTEh) (Figure 1). In order to prevent any fatigue effect of the previous visit, each test-session was interspersed by at least 48 hours, so as at least 48 hours have passed between the last training session and the first TTE that followed the VSTAP. Subjects' weight was measured before and after each session, and water intake was measured weighting water bottle at beginning and end of exercise. During no tests/training sessions days, participants followed their normal training routine, with the only recommendation of avoiding intense exercise the day before performance tests.

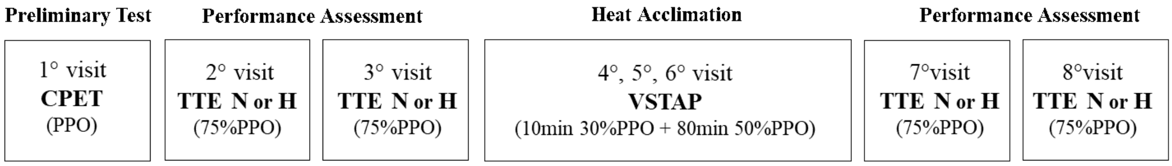

Figure 1 Overview of the experimental design. CPET: cardiopulmonary exercise test; TTE: Time To Exhaustion; PPO: Peak Power Output; VSTAP: Very-Short Term Heat Acclimation Protocol; N: Normothermia (18°C;50%RH); H:Heat (35°C;50%RH).

2.3 Methodology

All exercises were performed on a cyclo-ergometer (Excalibur Sport, Lode BV, Groningen, The Netherland). The CPET protocol started at 75W for 10 min and then increased by 25 W/min. During the test, cardio-respiratory measures were collected continuously with breath-by-breath method using an automated open-circuit gas analysis system (Quark PFT Ergo, Cosmed Srl, Rome, Italy).

TTE cycling intensity was set at 75 % of individual Peak Power Output (PPO) measured during CPET, with pedaling cadence kept constant at 90 revolutions/min (rpm), using a monitor that provided participants with visual feedback. The VSTAP consisted of 3 consecutive days of 90 minutes cycling in H, divided into 10' warm up at 30% PPO and 80' training at 50% PPO. PO was decreased by 5% at a time on subjects' request to ensure completion of sessions. A fixed intensity instead of an iso-thermic heat acclimation strategy has been chosen since it is easier to administrate when considering a real field situation and it has been shown that no differences in the magnitude of adaptation between these two methods after STHA exist (Gibson et al., 2015). Two fan set at their minimal working speed were used, one on the side of the subject at a distance of 1.5m and the other positioned 2m behind the bicycle. The air flow generated was measured with an anemometer (HoldPeak-866B, China) positioned at the level of subjects' trunk and corresponded to 0.83 and 1.49 m/s, respectively. Duration and intensity of training sessions were decided based on the idea that with STHA protocols it is better to spend as much time as possible exposed to the heat rather than shorter periods with higher training intensities. (James et al., 2017; Kirby et al., 2019) Furthermore, a 90-min session is the most used by authors when low-intensity training is proposed (Flouris and Schlader, 2015; Garrett et al., 2012; James et al., 2017; Keiser et al., 2015; Kirby et al., 2019; Willmott et al., 2018; Wingfield et al., 2016).

HR was recorded continuously using a Polar RS800CX HR monitor (Polar, Kempele, Finland) throughout all sessions. Rating of perceived exertion (RPE) was assessed using the CR100 Scale (Borg and Borg, 2002). Thermal sensation (TS) was assessed using a 9-point scale (from −4 (very cold) to +4 (very hot)) (Arens et al., 2006). Tympanic Temperature (TyT) was measured via Auricular Thermometer with infrared technology (Chicco Comfort Quick, Omron Gentle Temp Technology, Italy). Specifically, measurements were taken every 10 minutes of accumulated work during training sessions and every 2 minutes of accumulated work during TTEs. To measure blood lactate accumulation, a capillary blood sample was collected from the earlobe 3 min after the end of TTE and then analyzed using a lactate analyzer (Biosen C-line, EKF Diagnostics GmbH, Barleben, Germany).

2.4 Data analysis

During CPET, peak power output (PPO) was determined according to the equation: PPO (W) = power output for the last stage completed (W) + [t (s)/stage duration (s) × stage increment (W)], where t is the time of the uncompleted stage. VO_{2peak} and other maximal cardio-respiratory variables were defined as the highest values of a 20-s average. Performance time changes were measured from the absolute differences between PRE and POST tests for both N and H. Performance time was also compared considering % improvement between PRE and POST measurements. HR_{peak} was considered as the highest value registered throughout the test, whereas HR_{mean} is referred to mean HR of the test. RPE and TS were analyzed considering: the first registered value after two minutes of cycling (RPE_{start}; TS_{start}); the median value, correspondent to different time points related to whole test length (RPE_{median}; TS_{median}); the final value, asked at the moment of test interruption (RPE_{end};TS_{end}). Finally, TyT_{TTE} was considered as both the last registered value at the end of TTE (TyT_{end}) and the mean value of all registered temperatures throughout the test (TyT_{mean}). For training data, the first 10 minutes of warm up were excluded from the analysis; $HR_{training}$, $RPE_{training}$, $TS_{training}$ and $TyT_{training}$ were thus compared from minute 20 to the end of the test (t20, t30, t40, t50, t60, t70, t80, t90) among the three training sessions (T1, T2; T3). Moreover, since PO was lowered at subjects' request, the same calculation was computed also for $PO_{training}$ values. Finally, Sweat Rate (SR) during training sessions was calculated according to the equation: SR (L/h) = [weight loss (L) – volume of water consumed (L) -urine loss (L)]/duration of exercise (h) (Baker, 2017).

2.5 Statistical analysis

We used descriptive analysis to report the results (mean and confidence interval Wald 95%). The Generalized Estimating Equations (GEE) was used to test the main effects of VSTAP on TTE measurements, with 'condition'(N and H) and 'time'(PRE and POST) as factors, and on training variables ($HR_{training}$, $RPE_{training}$, $TS_{training}$, $TyT_{training}$ and $PO_{training}$), with 'session' (T1,T2 or T3) and 'time' (t20,t30,t40,t50,t60,t70,t80,t90) as factors. When statistical significance was identified ($p<0.05$), a Sidak post hoc test was used to further delineate differences between conditions, sessions or time. Also, training variables were mediated from t20 to t90 for each subject and GEE was used to identify statistical significant differences between sessions. GEE was implemented to analyze data since 2 subjects did not perform TTEn POST because of the COVID-19 outbreak. All data were analyzed using a standard statistical package (SPSS Inc, Chicago, Illinois, USA), where the level of statistical significance was set at $p<0.05$.
In addition to null hypothesis testing, magnitude based inferences (MBI) were also used for analysis (Mee et al., 2018) in order to determine %chances of positive/trivial/negative effect of HA protocol on performance time and physiological and perceptual variables of both TTE and training sessions, using a dedicated spreadsheet (Hopkins, 2007). The levels of rejection and compatibility are determined as follows: 0-0,5%, strongly reject; 0,5-5%, moderately reject; 5-25%, weakly reject; 25-75%, ambiguous; 75-95%, weakly compatible; 95-99,5%, moderately compatible; 99,5-100%, strongly compatible.

3. RESULTS

3.1 Performance

We found a significant general effect of 'condition', but no effect of 'time' nor 'interaction' of condition*time on TTE performance time, which was higher in N at both PRE and POST measurements. Furthermore, no significant difference in % improvement of TTE performance was detected neither in N(+9%) nor H (+17%) if compared to respective PRE (Fig. 2).
A general effect of 'condition', but no effect of 'time' nor 'interaction' of condition*time was found for RPE_{start}, $TS_{start-median-end}$, TyT_{basal}, TyT_{mean} and TyT_{end}, which were always higher in H, whereas no significant changes were reported for RPE_{median} and RPE_{end}. from PRE to POST neither in N nor H. A significant effect of 'condition' and 'time', with no interaction was found for HR_{peak} and HR_{mean}; they were higher in H at both PRE and POST, and decreased at POST both in N and H. Finally, a significant general effect of 'time' and 'interaction', but no 'condition' effect was found

for blood lactate [La], which slightly decreased in N, and increased in H at POST. Mean data, confidence interval (minimal and maximal values) and statistical significance of performance data are reported in Table 1.
MBI revealed 92.2/6.4/1.4 % chances of positive/trivial/negative effect of the VSTAP on TTE performance time in the heat and 61.9/27/11.1 % chances of positive/trivial/negative effect of the protocol on TTE performance in N. Furthermore, great chances of decreasing HR_{peak} and HR_{mean} both in N and H, and increasing La_{end} only in H were shown (see Table 1).

*******************************Table 1 about here*****************************

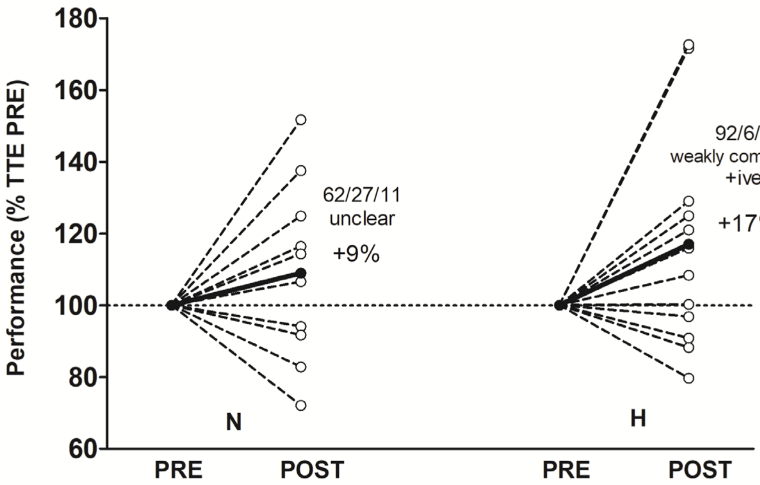

Figure 2 Individual Time to Exhaustion (TTE) performance values (%PRE) in normothermia (N, 18°C;50%RH) and in the Heat (H, 35°C;50%RH). Bold lines represent mean values. %improvement and chances (%) of +ive/trivial/-ive effects from Magnitude Based Inferences with non-clinical decision outcomes are reported.

3.2 Training

Training data has been considered as both mean and single time points changes. Regarding mean data, a general effect of 'session' has been found for HR (T2≠T3; T1≠T3), RPE (T1≠T3) and TyT (T1≠T2; T1≠T3), with strongly compatible effect of training on HR, and moderately compatible effect on RPE and TyT. No other statistical significance was found (see Table 2). Considering single time points**,** general effects of 'session' ($p<0.001$), 'time'($p<0.001$) and 'session*time' interaction ($p<0.030$) were found for $HR_{training}$. HR significantly decreased from T1 to T3 ($p<0.001$), T2 to T3 ($p<0.001$) but not from T1 to T2 ($p=0.060$). On average, HR gradually increased ($p<0.05$) during the first 40 min of accumulated work and remained stable throughout the remaining time of the three training sessions (Fig.3b). Similarly, general effects of 'session' ($p=0.005$), 'time'($p<0.001$) and 'session*time' interaction ($p<0.001$) were seen for $TyT_{training,}$ which significantly decreased from T1 to T2 ($p=0.047$), from T1 to T3 ($p=0.007$), but not from T2 to T3 ($p=0.263$). Similar to HR, TyT gradually increased ($p<0.05$) within the first 40 min of accumulated work and then it slightly increased without statistical significance (Fig.3d). For $TS_{training}$ a general effect of 'time' ($p<0.001$) and 'interaction'($p<0.001$), but no effect of 'session'($p=0.217$) were detected. In general, TS gradually increased ($p<0.05$) from t20 to t40 and then remained constant throughout training sessions. For $RPE_{training}$, a general effect of 'session' ($p=0.037$), 'time' ($p<0.001$) and 'interaction' ($p<0.001$) were found. RPE significantly decreased from T1 to T3 ($p=0.032$), but not from T1 to T2 ($p=0.746$) or T2 to T3 ($p=0.269$). Throughout training sessions, RPE gradually increased ($p<0.05$) during the first hour and then slightly increased without statistical significance until the end of training (Fig. 3c). Finally, a general effect of 'time' ($p<0.001$) and a session*time 'interaction' ($p<0.001$), but not 'session' effect ($p=0.216$), were found for $PO_{training}$. On average, PO was significantly higher ($p<0.05$) during the first 50 minutes of training at T1 and T2 if compared to the last 30 minutes, whereas it was not statistically different throughout training at T3 (even though it slightly decreased from beginning to the end of exercise) (Fig. 3a). Finally, subjects' weight loss was significantly higher at T3 if compared to T1 ($p=0.003$), whereas no statistically significant differences were seen for water intake and SR throughout training sessions ($p=0.053$ and $p=0.352$, respectively).

*******************************Table 2 about here*****************************

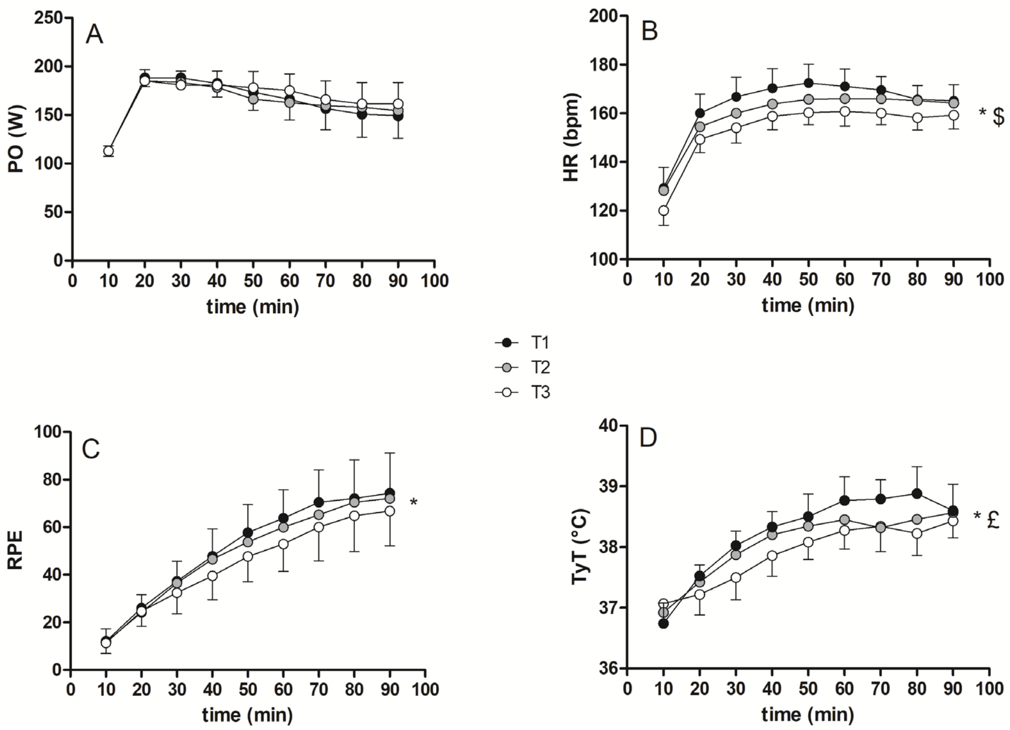

*Figure 3 Physiological and perceptual responses to the three days of training (T1, T2 and T3) during the VSTAP (90 min cycling at 35 °C and 50%RH). (A) Power Output (PO), (B) Heart Rate (HR), (C) Rate of Perceived Exertion (RPE) and (D) Tympanic Temperature (TyT). Data are presented as Mean ±confidence interval. *=p<0.05, *: T1 ≠ T3, £: T1 ≠ T2, $: T2 ≠ T3*

4. DISCUSSION

The aim of this study was to evaluate the effects of a very short-term heat acclimation protocol (VSTAP) on endurance performance and physiological and perceptual responses to exercise in trained male cyclists. The main findings of the study are that such a short intervention period induces meaningful submaximal physiological adaptations and perceptual responses, combined to progressive increases in cycling workload when exercising in the heat. Furthermore, a conventional statistical approach (GEE) did not show relevant significant improvements in high-intensity performance after the VSTAP in hot nor in cool condition; however, Magnitude Based Inferences defined the VSTAP as 'weakly compatible' with performance improvements in the heat.

4.1 Performance

We are aware of few studies investigating heat acclimation performance responses to such a short intervention (≈4.5h of heat exposure in total). In a previous study, Willmott et al. (Willmott et al., 2016) reported no significant improvements in a 3 km Time Trial (TT) in the heat after both 2 days of twice a day or 4 days of once a day training in a hot environment (≈4h of heat exposure at 50% VO_{2peak}). However, Sunderland et al. (Sunderland et al., 2008) reported significant improvements (+33%) in high-intensity intermittent running capacity (i.e., distance covered in the Loughborough Intermittent Shuttle Test) after 4 separated short heat acclimation sessions (≈3h) in well-trained female games players. Regarding STHA (≈5 to 7 days), Kirby et al. (Kirby et al., 2019) found no improvements in 15min self-paced cycling Time Trial in 8 female endurance athletes after 5 days HA (≈6 h), but significant improvements (~6%) in 5km running TT (James et al., 2018, 2017) and 20km cycling TT (Wingfield et al., 2016) performance were reported after 5 days of exercise in the heat characterized by a longer heat exposure per session (≈7.5h in total). However, larger performance improvements can be expected after longer heat acclimation interventions. For instance, the previously mentioned study of Kirby et al. (Kirby et al., 2019) showed a significant improvement in distance cycled, mean PO and cycling speed (+3.2%, +8,1% and +3%, respectively) during a 15-min TT following 9-days of heat-acclimation (≈13.5h); Tyler et al. (Tyler et al., 2016) summarized that the average percentage improvement in endurance performance is greater following LTHA (mean +22 ± 29 %) and MTHA (mean +21 ± 28%) than STHA (mean +7 ± 8 %). In this study, the VSTAP resulted in a non-significant +17% (0.3;33) increase in TTE performance in the heat (Fig.2), despite 8 out of 12 subjects improved TTE time in the heat. Furthermore, a non-significant +9% (-5.3;23.9) increase in TTE performance in normothermia was seen, with 6 out of 10 subjects improving performance time. It might be argued that greater performance improvements were not seen in this study because of the nature of the TTE test; however, TTE has been shown to present the largest magnitude of change (i.e. performance enhancement) after HA induction (Benjamin et al., 2019). Furthermore, Nicolò A. et al. (Nicolò et al., 2019) reported that TTE tests should be preferred when evaluating also the effects of a given experimental intervention on physiological and psychological responses, in order to exclude the potentially confounding factor of varying workload (as it occurs during time trials).

However, when applying performance improvements seen in this study to real field situations, a 17% improvement in performance time, despite not significant, could be determinant for high level athletes. Thus, we implemented MBI analysis in order to more objectively define the VSTAP chances of improving performance, and it revealed that a 3-day VSTAP results in 92.2% chances of improving performance in the heat, with 6.4% and 1.4% chances of trivial and negative effects, respectively. When considering this non clinical study, the VSTAP intervention is referred to be effective when the effect is possibly 'beneficial' (>25%) and very unlikely harmful (<5%) (Batterham and Hopkins, 2006). Accordingly, when no longer acclimation period is possible, a VSTAP could represent a time-efficient and effective means to increase individual's chance of a better performance in the heat. On the contrary, the 61.9% chances to improve and the 11.1% chances to worsen in TTE time in normothermia demonstrates scarce beneficial cross-over effect of the VSTAP on performance in temperate conditions. These findings agree with the study of Neal et al. (Neal et al., 2016), who reported that a STHA protocol (≈7.5 h of training in the heat) reduced thermal and cardiovascular strain in the heat but it did not improve TT performance in normothermia. Furthermore, Keiser et al. (Keiser et al., 2015) showed that 10 days of 90 minutes heat training facilitated exercise performance in hot but not in temperate conditions. Despite improvements in Wpeak(+8%) (Sotiridis et al., 2019), VO_{2max}(+5%) and Time Trial Performance(+6%) (Lorenzo et al., 2010) in normothermia have been reported after MTHA, most of the findings have been confounded by several factors such as the absence of a control group, the inclusion of untrained subjects, suboptimal acclimation programs, or the application of an unclear study design(Keiser et al., 2015). Thus, our results confirm that a VSTAP does not improve performance in normothermia.

Regarding physiological measurements, no significant modifications have been reported, except for Post exercise [La] in the heat, which also presented strongly compatible chances of increasing following MBI. In their meta-analyses, Tyler et al. (Tyler et al., 2016) reported an effect of heat acclimatization on substrate metabolism, that led to an increased lactate threshold and reduced lactate concentrations during exercise. It has been suggested that HA may change the lactate kinetics by reducing the aerobic metabolic rate or the rate of glycogenolysis (Sawka et al., 1983); however, others proposed that the HA-induced increase in central blood volume may increase lactate removal (Febbraio et al., 1994). By contrast, Rahimi et al. (Rahimi et al., 2019) did not find significant changes in blood lactate after HA when considering 4 studies proposing strategies of short-term heat acclimatization (maximum 5 days of training). Our findings are in line with these latest results, showing no effects on [La] production after exercise in normothermic TTE, and a slight increase in TTEh at POST, which could be easily related to the tendency to increase in performance time.

Finally, RPE and TS during TTE tests did not change after the VSTAP, and MBI analysis confirmed this trend, with very low chances of improving these aspects. We expected improvements in perceptual measurements, since previous studies (Artrong and Maresh, 1991; Petersen et al., 2010) have seen changes in just 3 days of heat acclimation. However, such a short intervention period could also have had little influence on maximal perceptual responses, not detectable with validated scales.

4.2 Physiological and perceptual responses

Submaximal responses to the VSTAP must be considered taking into account the confounding factor of changes in mean power output; this is due to the fact that some of the participants (n=9) were not able to complete the entire sessions at the pre-determined workload (i.e. 50%PPO), and thus PO was decreased by 5% at a time on subjects' request to ensure completion of sessions (see method section) . However, it has to be noticed that during T1 and T2, PO was significantly higher at the beginning if compared to the end of exercise, wherease at T3 no statistical significance was seen throughout the session, suggesting an increased tolerance of the subjects to target training intensity.

$HR_{training}$ decreased throughout training sessions (Fig.3b), confirmed also by a strongly compatible effect of the VSTAP in decreasing mean exercising HR from T2 to T3 and from T1 to T3 (see Table 2) . This is an important finding, which reflects our expectations, e.g., partial heat acclimation linked to cardiovascular benefits (Chalmers et al., 2014; Tsai et al., 2013) expected after only 3 days of heat exposure. HR decrements may be related to different aspects: Senay et al. (Senay et al., 1976) found a relationship between plasma volume expansion and reduced heart rate during exercise in the early stages (days 1-4) of heat acclimatization. Similarly, Convertino et al. (Convertino, 1983) demonstrated that a 1% increase in plasma volume resulted in a 1% reduction in exercising HR of healthy males. This adjustment is essential to guarantee CO maintenance without excessive reliance on HR to overcome the 'cardiovascular challenge' between skin and muscles in the heat (Casadio et al., 2017; Convertino, 1983). Moreover, plasma norepinephrine (NE) concentration during exercise has been shown to be reduced within three HA sessions, suggesting that reduced sympathetic activation may play a role in attenuating HR response to exercise following HA (Hodge et al., 2013). Accordingly, these adaptations could also account for mean and maximal HR reductions in both TTEn and TTEh at POST (Tab.1) of the present study, as previously observed (Convertino, 1983) following 8 days of exercise training in normothermia.

Some thermoregulatory adjustments were noted throughout the training sessions, with $TyT_{training}$ decreasing from day 1 to day 3 (Fig. 3d), with great chances of decreasing already from T1 to T2 (Table 2). Previous studies (Sunderland et al., 2008) showed that thermoregulatory adaptations depend on different mechanisms that take from 4 to more than 8 days to occur. However, our results show that some of these adaptations could be achieved after only 3 days of HA. Likely, the aspect that mostly influenced TyT changes throughout training sessions was a reduced core temperature threshold for the onset of sweating and cutaneous vasodilation (generally occurring within 4 to 8 days), facilitating heat dissipation and leading to a reduced core temperature for a given absolute exercise intensity. However, since we just measured tympanic temperature, we can't directly apply these outcomes to rectal temperature variations, which still remains the considered reference method for temperature measurement in hyperthermic athletes (Ganio et al., 2009; Towey et al., 2017). Moreover, we could just speculate on the mechanisms behind TyT modifications and thus further studies including direct measurements of thermoregulatory processes (i.e sweat and skin vasodilation rates, so as core temperature threshold for the onset of these two mechanisms) are needed to better adress thermoregulatory changes after such a short intervention period.

Finally, $RPE_{training}$ showed a significant reduction from T1 to T3. Interestingly, some of the participants expressed lower RPE for an increased external workload during the latter days of the VSTAP. In line with this, Wingfield et al. (Wingfield et al., 2016) reported improved RPE after a high intensity-short duration (5 sessions of training) heat acclimation protocol. Accordingly, the progressive reduction in thermoregulatory and cardiovascular strain observed in this study may have played an important role in influencing RPE during the VSTAP.

The first limitation of this study is the absence of a control group, which prevents discrimination between a possible training effect of the VSTAP protocol and effective HA adaptations. However, our subjects were classified as trained cyclists (De Pauw et al., 2013) and they reported to train at least 3 days/week: therefore, the proposed protocol (i.e. 10 min cycling at 30%PPO + 80 min cycling at 50% PPO) should not have been determinant in terms of improving aerobic fitness or cycling economy if compared to pre-test conditions. Moreover, other studies (Keiser et al., 2015; Willmott et al., 2016) developed heat acclimation strategies including also a control group that exercised in normothermia, and no training effect was seen in both well trained and moderately trained subjects, even when considering longer intervention periods. (e.g. 10 days of 90 min cycling at a training load correspondent to the HR elicited at 50% VO2 max in the Heat and 4 days of 45 min cycling at 50% VO2peak, respectively.) However, having a control group would be the only solution to definitely state weather or not a possible training effect influenced the data, thus we advice future researches on Very Short Term Heat Acclimation Strategies to include a control group in the study design. The second limitation is the use of Tympanic Temperature to estimate Core Temperature of the subjects: compared to other type of measurements (i.e. telemetry pills), TyT has been shown to present a mean bias of ≈0.5 °C if compared to rectal temperature (Towey et al., 2017). However, a previous study (Morán-Navarro et al., 2019) revealed that wind and skin sweat invalidate the use of skin infrared thermometry to estimate Tcore during exercise in the heat, whereas tympanic temperature measurements based on infrared technology seem to be less affected by these aspects, also with a frontal fan-generated airflow higher than that provided for our subjects (i.e. 2.55 m/s vs 0.8 m/s, respectively).

5. CONCLUSIONS

Overall, a VSTAP represents a time-saving heat acclimation strategy, which allows athletes to reach partial heat acclimation with low risks of detrimental effects, reducing costs of travel and avoiding excessive impact on training schedule. In this study, we did not find any correlation between % improvement in performance and changes in physiological (i.e. $HR_{training}$ and $TyT_{training}$) or perceptual (i.e. $TS_{training}$ and $RPE_{training}$) responses throughout training sessions or subjects' characteristics. For these reasons, further research is needed to better explain the thermoregulatory and cardiovascular adjustments to VSTAP and their relations with performance improvements, especially measuring also plasma volume expansion and looking into thermoregulatory responses through rectal thermometers or telemetry pills, but also considering sweat rate and skin vasodilation mechanisms. Moreover, future strategies include the possibility of testing the effects of combined VSTAPs (divided by a few days of normal training) on performance, physiological and perceptual exercise responses in the heat, as previously investigated for other extreme environmental conditions (i.e. hypoxia (Mujika et al., 2019)), with the aim of understanding whether it could lead to similar responses as more traditional medium and long-term acclimation strategies, but being easier to match with an athlete's training program.

In conclusion, the VSTAP program induced beneficial physiological (i.e. decreased $HR_{training}$), thermoregulatory (i.e. decreased $TyT_{training}$) and perceptual (i.e. decreased $RPE_{training}$) adaptations to submaximal exercise. Furthermore, it showed a good probability to improve high-intensity exercise performance (i.e. TTE) in the heat, with very-low risks of detrimental effects. Finally, low probability of a cross-over effect of the VSTAP on performance in temperate conditions was detected.

6. Sample CRediT author statement

Alexa Callovini: Conceptualization, methodology, formal analysis, investigation, writing-original draft, writing- review and editing. Alessandro Fornasiero: Conceptualization, methodology, formal analysis, writing- review and editing. Aldo Savoldelli: Conceptualization, methodology, writing-review and editing. Federico Stella: Conceptualization, methodology, writing- review and editing. David A.Low: Writing-review and editing. Barbara Pellegrini: Writing-review and editing. Federico Schena: Conceptualization, Supervision. Lorenzo Bortolan: Conceptualization, Supervision

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Table 1 Physiological and perceptual responses during time to exhaustion tests in normothermia (N, 18 °C - 50% RH) and in the heat (H 35 °C - 50% RH).

TIME TO EXHAUSTION RESULTS										
		N_PRE	H_PRE	N_POST	H_POST	p value			N	H
		Mean (min;max)	Mean (min;max)	Mean (min;max)	Mean (min;max)	cond	time	cond*time	+ive/trivial/-ive(%)	+ive/trivial/-ive(%)
TTE_time	(s)	1205 (911 ; 1498)	638 (517 ; 759)	1238 (947 ; 1529)	728 (598 ; 858)	**<0.001**	0,456	0,661	62,27,11	**92,6,1**
HRpeak	(bpm)	186 (179 ; 192)	190 (184 ; 196)	182 (175 ; 189)	189 (183 ; 194)	**0,011**	**<0.001**	0,056	**0,0,100**	**2,7,92**
HRmean	(bpm)	174 (167 ; 181)	178 (172 ; 185)	170 (163 ; 177)	176 (170 ; 181)	**0,013**	**<0.001**	0,181	**0,1,99**	**1,3,96**
La_end	(mMOL)	9,6 (8,3 ; 10,8)	10,5 (9,4 ; 11,5)	9,3 (7,8 ; 10,8)	11,7* (10,4 ; 13,0)	**<0.001**	0,296	**0,001**	6,18,76	**97,3,1**
TyT_basal	(°C)	35,7 (35,3 ; 36,1)	36,8 (36,7 ; 36,9)	35,5 (35,1 ; 35,8)	36,7 (36,6 ; 36,9)	**<0.001**	0,19	0,483	6,18,77	11,28,61
TyT_end	(°C)	36,9 (36,2 ; 37,5)	38,2 (37,7 ; 38,7)	37,0 (36,1 ; 37,9)	38,3 (38,0 ; 38,6)	**<0.001**	0,737	0,888	30,37,33	12,28,60
TyT_mean	(°C)	36,4 (36,1 ; 36,8)	37,8 (37,6 ; 38,0)	36,2 (35,7 ; 36,7)	37,7 (37,5 ; 37,9)	**<0.001**	0,155	0,462	9,24,67	28,28,61
RPE_start		34 (25 ; 43)	49 (38 ; 60)	36 (25 ; 47)	44 (34 ; 53)	**<0.001**	0,646	0,187	16,32,52	8,23,69
RPE_median		74 (69 ; 79)	78 (73 ; 83)	73 (67 ; 79)	77 (72 ; 83)	0,107	0,837	0,998	6,18,76	26,37,36
RPE_end		100 (93 ; 107)	100 (94 ; 106)	100 (91 ; 109)	101 (95 ; 107)	0,882	0,817	0,912	17,33,50	40,36,24
TS_start		0,9 (0,5 ; 1,3)	2,7 (2,3 ; 3,0)	0,8 (0,3 ; 1,3)	2,6 (2,3 ; 3,0)	**<0.001**	0,598	0,782	21,35,44	23,36,40
TS_median		2,4 (2,1 ; 2,8)	3,5 (3,2 ; 3,7)	2,2 (1,7 ; 2,6)	3,4 (3,2 ; 3,7)	**<0.001**	0,122	0,320	5,17,77	21,36,43
TS_end		2,9 (2,5 ; 3,4)	3,9 (3,8 ; 4,0)	2,9 (2,5 ; 3,3)	3,7 (3,5 ; 3,9)	**<0.001**	0,364	0,549	45,35,20	5,17,78

Data are presented as mean with 95% Confidence Interval (CI). Bold characters denote statistical significance p<0.05. GEE: generalized estimating equations; TTE: Time to Exhaustion; HR: heart rate; [La]: blood lactate accumulation; TyT: tympanic temperature; RPE: rate of perceived exertion; TS: Thermal Sensation; *: POST ≠ PRE. The levels of rejection and compatibility are determined as follows: 0-0,5%, strongly reject; 0,5-5%, moderately reject; 5-25%, weakly reject; 25-75%, ambiguous; 75-95%, weakly compatible; 95-99,5%, moderately compatible; 99,5-100%, strongly compatible. +ive: positive; -ive: negative

Table 2 Mean physiological and perceptual responses throughout training sessions (T1, T2, T3) in the heat (H 35 °C - 50% RH).

		TRAINING								
		T1	**T2**	**T3**	**p value**			**+ive/trivial/-ive(%)**		
		Mean 1 (min;max)	**Mean 2 (min;max)**	**Mean 3 (min;max)**	**T1 vs T2**	**T2 vs T3**	**T1 vs T3**	**T1 vs T2**	**T2 vs T3**	**T1 vs T3**
HR	(bpm)	168 (162 ; 175)	163[£] (160 ; 167)	158[*$] (153 ; 162)	**0,027**	**<0,001**	**<0,001**	**1,6,93**	**0,0,100**	**0,0,100**
TS		3,4 (3,1 ; 3,6)	3,3 (3,0 ; 3,5)	3,2 (2,9 ; 3,5)	0,483	0,089	0,207	13,30,57	3,11,86	6,19,75
RPE		56 (46 ; 66)	54 (43 ; 64)	49[*] (38 ; 59)	0,406	0,092	**<0,01**	11,28,61	3,12,86	**1,3,97**
TyT	(°C)	38,4 (38,2 ; 38,7)	38,2[£] (37,9 ; 38,5)	38,0[*] (37,7 ; 38,3)	**0,012**	0,11	**0,002**	**1,3,96**	3,13,84	**0,1,98**

Data are presented as mean with 95% Confidence Interval (CI). Bold characters denote statistical significance (p<0.05), *: T1 ≠ T3, £: T1 ≠ T2, $: T2 ≠ T3. GEE: generalized estimating equations; HR: heart rate; TS: Thermal Sensation; RPE: rate of perceived exertion; TyT: tympanic temperature. The levels of rejection and compatibility are determined as follows: 0-0,5%, strongly reject; 0,5-5%, moderately reject; 5-25%, weakly reject; 25-75%, ambiguous; 75-95%, weakly compatible; 95-99,5%, moderately compatible; 99,5-100%, strongly compatible. +ive: positive; -ive: negative