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Five weeks of heat training increases haemoglobin mass in elite cyclists

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Abstract

In this study we tested the hypothesis that performing 1 h of regular light exercise in a heat chamber (HEAT; $37.8 \pm 0.5°C$; $65.4 \pm 1.8\%$ humidity) 5 times $week^{-1}$ for a total of 5 weeks increases haemoglobin mass (Hb_{mass}) and exercise performance in elite cyclists ($\dot{V}_{O_2max} = 76.2 \pm 7.6$ ml min^{-1} kg^{-1}). Twenty-three male volunteers were assigned to HEAT ($n = 11$) or CON ($n = 12$; $15.5 \pm 0.1°C$; $25.1 \pm 0.0\%$ humidity) training groups. Hb_{mass} was determined before and after the intervention period in conjunction with an extensive exercise test protocol (conducted at 16–19°C). HEAT increased ($P < 0.05$) Hb_{mass} by 42 g from 893 ± 78 to 935 ± 108 g whereas Hb_{mass} remained unchanged (+6 g) in CON. Furthermore, statistical analysis revealed a time–group interaction ($P < 0.05$). The greater increase in Hb_{mass} in HEAT, however, did not manifest in a greater increase in $\dot{V}_{O_2max}$ (225 ± 274 ml min^{-1} in HEAT and 161 ± 202 ml min^{-1} in CON). While HEAT reduced ($P < 0.05$) lactate levels during some of the submaximal exercise tests, there was no statistical difference between other performance parameters. There were, however, small to intermediate effect sizes favouring HEAT for lactate threshold power output (2.8 ± 3.9 vs. $−0.4 \pm 5.1\%$ change, effect size (ES) = 0.34), gross economy in the fatigued state (0.19 ± 0.42 vs. $−0.12 \pm 0.49$%-point change, ES = 0.52) and 15 min mean power (6.9 ± 8.4 vs. $3.4 \pm 5.1\%$ increase, ES = 0.22). This study demonstrates an increase in Hb_{mass} and small to intermediate effect sizes on exercise variables in elite cyclists following a 5-week heat training intervention.

KEYWORDS
blood volume, exercise, hot environment

1 | INTRODUCTION

A high maximal oxygen uptake ($\dot{V}_{O_2max}$) is essential for successful exercise performance in endurance sport disciplines. While $\dot{V}_{O_2max}$ is the product of the Fick equation in which both O_2 transport to the exercising skeletal muscles and O_2 extraction by the skeletal muscle contribute, the biggest difference between individuals with a high and low $\dot{V}_{O_2max}$ resides in the capacity for O_2 transport. In the O_2 transport system, the total mass of haemoglobin (Hb_{mass}) plays a decisive role, which is not only related to haemoglobin's ability to bind and transport O_2, but also to the positive impact of red blood cell volume (RBCV) on maximal cardiac output via Frank–Starling mechanisms (Lundby, Montero, & Joyner, 2017).

Hb_{mass} may be increased following exercise training in previously untrained individuals, with improvements averaging 10% (Bonne et al., 2014; Montero et al., 2015, 2017). A detailed review of exercise training-induced erythropoiesis has been published recently (Montero & Lundby, 2018). Hb_{mass} may, however, be augmented by means other than exercise training, and environmental influence seems the most well established. Since chronic exposure to high altitude may augment Hb_{mass} (Siebenmann et al., 2015; Siebenmann, Robach, & Lundby, 2017b), elite athletes who already possess very high Hb_{mass} (Lundby & Robach, 2015) may aim at obtaining even higher values by means of altitude training, although this practice has been questioned in more recent times (Lundby & Robach, 2016). In contrast to chronic altitude exposure, in which the expansion of Hb_{mass} is preceded by a rapid reduction in plasma volume (PV), exposure to a hot environment leads to an expansion of PV of up to 20% within days, which thereafter stabilizes at around +10% (Sawka, Convertino, Eichner, Schneider, & Young, 2000). While such an expansion may not influence exercise capacity despite potentially facilitating a higher maximal cardiac output, due to a concomitant reduction in blood O_2 content, we speculate that if the PV expansion can be sustained for several weeks, this may eventually also lead to an increase in Hb_{mass}, which in turn could facilitate exercise performance.

The underlying rationale for our hypothesis is that the kidney may be seen as a 'critmeter', where both PV and RBCV are proposed to be counter-regulated in response to changes in either, with the aim to keep the haematocrit (Htc) within a normal range (Donnelly, 2001; Montero & Lundby, 2018). In essences, RBCV is regulated through erythropoietin (EPO) synthesized in response to a drop in tissue O_2 pressure by peritubular fibroblast-like cells upon stabilization of hypoxia-inducible factor-2α within the juxtamedullary region. Uniquely for the kidney, tissue O_2 pressure can be modulated by (1) changes in renal O_2 consumption, fundamentally dependent on sodium reabsorption, which in turn is proportional to glomerular filtration rate and renal blood flow, and (2) O_2 delivery to the proximal tubule, which is determined by arterial O_2 content as well as blood flow. Since flow contributes to both sides of the balance governing tissue O_2 pressure, EPO production must be determined by the difference between O_2 consumption and arterial O_2 content (Montero & Lundby, 2018). Thus, O_2 sensors located within the juxtamedullary apparatus are proposed to regulate Htc via modulation of renal EPO production according to arterial O_2 content-dependent changes in tissue O_2 pressure (Donnelly, 2001; Montero & Lundby, 2018). Despite our critmeter focus, other factors also could facilitate, or at least contribute to, a potential increase in RBCV. While the master regulator of the hypoxia inducible factor (HIF) system clearly is oxygen, also heat shock protein (HSP) expression may facility HIF stabilization and hence EPO synthesis. For an extensive account of this topic, the reader is referred to recent reviews (Ely, Lovering, Horowitz, & Minson, 2014; Hawley, Lundby, Cotter, & Burke, 2018).

Based on the above we therefor speculate that a heat training-induced increase in PV, and accordingly a reduction in Htc, may stimulate EPO synthesis and thereby expand Hb_{mass}. Since the increase in Hb_{mass} may take several weeks with exercise training (Montero

> **New Findings**
> - **What is the central question of this study?**
>   Do haemoglobin mass and red blood cell volume increase in elite cyclists training in a hot environment compared to a control group training at normal temperature?
> - **What is the main finding and its importance?**
>   Five weeks of heat training increases haemoglobin mass in elite cyclists. There are small to intermediate effect sizes for exercise parameters favouring heat training.

et al., 2017), 3–4 weeks with altitude exposure (Siebenmann et al., 2015), and 2–3 weeks following injections of supra-physiological levels of recombinant human EPO (Lundby, Achman-Andersen, Thomsen, Norgaard, & Robach, 2008), we have opted for a heat training period lasting 5 weeks. In the present study we applied a heat training strategy which is similar, albeit longer in total length, to a protocol with which we previously have observed an expansion in PV of approximately 200 ml (or 6%) following 10 days' intervention (Keiser et al., 2015). We have furthermore tested the approach within a limited number of active cyclists, where Hb_{mass} became significantly increased by +34 g *vs.* +2 g in control group, although no statistical group differences could be established (Oberholzer et al., 2019). The lack of statistical difference between groups was likely the result of a rather large individual response. Nonetheless, considering the initial promising results, but also realizing that this type of training may have little relevance in amateur sport, and that physiological adaptations may be even more difficult to induce in elite athletes, it is necessary to also test a given training regime in elite athletes before such a training modality is implemented in this population.

With the aim to test whether heat training can increase Hb_{mass} in elite endurance athletes, we enrolled a group of elite cyclists to a heat training group and a control group. Both groups underwent an extensive test battery before and after the 5-week intervention period including blood volume and exercise performance assessments.

2 | METHODS

2.1 | Ethical approval

The study was performed according to the ethical standards established by the *Declaration of Helsinki* 2013 and was approved by the local ethical committee at Lillehammer University College (MR241018). The study was not registered in a database. All cyclists signed an informed consent form prior to participation.

2.2 | Participant characteristics

The number of subjects to be included was based on experience from a previous study with a similar heat exposure profile (Oberholzer et al., 2019). Twenty-three male cyclists ($\dot{V}_{O_2max}$ = 76.2 ± 7.6 ml min^{-1} kg^{-1}) expressed an interest in volunteering for the study. They were matched in pairs based on $\dot{V}_{O_2max}$ and randomly assigned to either control or intervention group, and none of them dropped out. Based on the peak aerobic power output (W_{max}), $\dot{V}_{O_2max}$ and training characteristics, the cyclists were regarded elite (Jeukendrup, Craig, & Hawley, 2000). The cyclists were matched to create two homogeneous groups based on $\dot{V}_{O_2max}$: a heat training group (HEAT; n = 11, age = 19 ± 2 years, body height = 178 ± 8 cm, body mass = 68.6 ± 6.9 kg) and a control group (CON; n = 12, age = 19 ± 3 years, body height = 179 ± 5 cm, body mass = 70.8 ± 5.6 kg). Two of the cyclists in CON did not perform the exercise testing due to logistic problems with weekend scheduling and hence for these measurements n = 10 in CON while for the remaining measurements n = 12.

2.3 | Experimental design

Participants were tested (test procedures described later in this section) before and after a 5-week intervention period. All testing was performed on one day and started with an incremental cycle test for determination of cycling economy, power output and fractional utilization of $\dot{V}_{O_2max}$ at a blood lactate concentration ([La$^-$]) of 4 mmol l^{-1}. After a 5 min recovery period, a $\dot{V}_{O_2max}$ test was performed before a new 5 min recovery period was given. Thereafter 30 min cycling at the power output at 2 mmol l^{-1} [La$^-$] was performed before the third and second last 5-min step from the blood lactate profile test was repeated and directly thereafter a 15 min cycling performance test was performed. The intervention was completed during the cyclists' preparatory period.

2.4 | Training intervention

The HEAT group performed the heat session as an afternoon session 5 times a week for the first 4 weeks and 4 times during the fifth week using their own bicycle connected to electromagnetically braked rollers (Computrainer Lab, Racer Mate Inc., Seattle, WA, USA). The session started with 5 min easy spinning before the rollers were calibrated to quantify and adjust wheel-ergometer rolling resistance to 1.0–1.4 kg, as prescribed by the manufacturer. Thereafter the participants aimed at performing 50 min cycling at 45% of power output at 4 mmol l^{-1} [La$^-$]. However, due to individual differences, the power output was upregulated with 25 W for the next session if rating of perceived exertion (RPE) on the 6–20 scale (Borg, 1982) was ≤11 and downregulated with 20 W for the next session if RPE was ≥15. During all heat sessions, participants consumed 0.5 litre water (at no specific time point or rate). The mean temperature and humidity during the first 2.5 weeks was 37.5 ± 0.3°C and 66 ± 2%, respectively, while

it was 38.5 ± 0.2°C and 64 ± 1%, respectively, for the last 2.5 weeks. The participants were encouraged to rehydrate after the heat session and to consume extra fluid until their urine had a normal pale yellow or straw colour (Armstrong et al., 1994). The CON group also performed a supervised afternoon session 5 times per week for the first 4 weeks and 4 times during the fifth week with the same duration and RPE target and measurements as HEAT using their own bicycle connected to electromagnetically braked rollers (Computrainer Lab). In addition to the afternoon sessions, both HEAT and CON perform normal training during the morning session.

During the training intervention there were no differences between HEAT and CON in mean weekly duration of the endurance training and the distribution of this training into low intensity training (zone 1, <55% of functional threshold power (FTP); 6.52 ± 1.75 *vs.* 7.90 ± 2.52 h, respectively), endurance training (zone 2, 55–75% of FTP; 1.22 ± 1.05 *vs.* 1.47 ± 1.07 h, respectively), threshold training (zone 3, 76–105% of FTP; 1.70 ± 0.18 *vs.* 1.88 ± 1.55 h, respectively) and high intensity training (HIT) (zone 4, 106–120% of FTP; 0.23 ± 0.27 *vs.* 0.10 ± 0.10 h, respectively). There were no differences between HEAT and CON in mean weekly number of heavy strength training sessions (0.7 ± 0.9 *vs.* 0.8 ± 0.8 sessions, respectively).

2.5 | Exercise tests

An overview of the exercise test protocol is given in Figure 1. The training during the 2 days preceding pre- and post-test were standardized and similar in both groups. Participants were also instructed to consume the same type of meal before each test and were not allowed to eat during the hour preceding a test or to consume coffee or other products containing caffeine during the 5 h preceding the tests. All tests were performed under similar environmental conditions (16–19°C) with airflow of 2–3 m s^{-1} towards the participants' frontal surface. Strong verbal encouragement was given during all tests to ensure maximal effort. All tests for the individual cyclists were conducted at the same time of day (±1 h) to avoid influence of circadian rhythm. The individual amount of water and sports drink consumed during the entire test session was noted during the pre-test and replicated during the post-test. All testing was performed on the same electromagnetically braked cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, The Netherlands), which was adjusted according to each cyclist's preference for seat height, horizontal distance between tip of seat and bottom bracket, and handlebar position. Identical seating positions were used during all tests. After a 5-min warm up on an ergometer cycle at a RPE of 11–12, the participants performed a one-repetition maximum leg press test (Keiser AIR300 Leg Press, Keiser Corp., Fresno, CA, USA). This test was performed to rule out that potential performance gains were associated to changes in strength, since previous studies have demonstrated that strength training can improve cycling performance (Rønnestad, Hansen, & Raastad, 2011; Rønnestad, Hansen, Hollan, & Ellefsen, 2015). The participants sat with knees flexed at 90 to
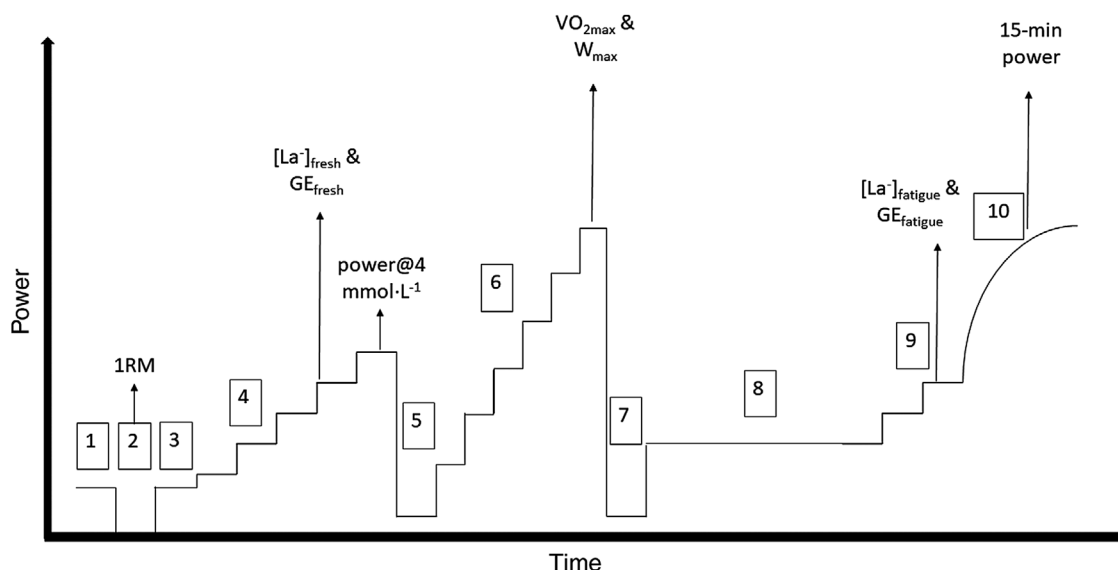
FIGURE 1 Study exercise test flow chart. The exercise tests consisted of a number of different tests all conducted in continuum and within the same day. (1) 5 min warm-up, (2) 1RM leg press, (3) 5 min easy cycling, (4) lactate profile test, (5) 5 min rest/easy cycling, (6) $\dot{V}_{O_2max}$ test, (7) 5 min rest/easy cycling, (8) 30 min at power output equal to 2.0 mmol l^{-1} [La$^-$], (9) 5 min bouts at power output equal to 3rd and 2nd last workload from lactate profile test, and (10) 15 min all-out. The overall aim with the applied test battery was to evaluate exercise performance in a realistic manner while at the same time taking advantage of the controlled settings provided by an exercise laboratory

96 deg and the hips flexed at approximately 45 deg with the individual seating position being identical for pre- and post-tests. Submaximal lifts at 50, 70 and 90% of self-estimated 1RM (8, 5 and 1 repetitions, respectively) with 1 min rest between sets were performed before the 1RM assessment. The smallest increase in 1RM load allowed was 5 kg and the test was terminated after failure of two lifts in a row. After a 5 min recovery the blood lactate profile test was started.

The cycling testing started with a blood lactate profile initiated with 5 min cycling at 125 W followed by 50 W increases every 5 min. Blood was sampled from a fingertip at the end of each 5-min bout and analysed for whole blood [La$^-$] using a Biosen C-line lactate analyser (EKF Diagnostic GmbH, Barlebe, Germany). When reaching a [La$^-$] of 2 mmol l^{-1} every 5 min bout increased by 25 W and the test was terminated when a [La$^-$] of 4 mmol l^{-1} or higher was measured. $\dot{V}_{O_2}$, respiratory exchange ratio (RER) and heart rate (HR) were measured during the last 3 min of each bout. Metabolic strain in the fresh state was measured as gross economy (GE$_{fresh}$) and [La$^-$]$_{fresh}$ at the second last bout of the blood lactate profile. HR was measured using a Polar S610i heart rate monitor (Polar, Kempele, Finland). $\dot{V}_{O_2}$ was measured (30 s sampling time) using a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany). The gas analysers were calibrated with certified calibration gases of known concentrations before every test. The flow turbine (Triple V, Erich Jaeger) was calibrated before every test with a 3 litre, 5530 series, calibration syringe (Hans Rudolph, Kansas City, USA). The same metabolic system with identical calibration routines was used on all subsequent tests. From this cycling test, power output and fractional utilization of $\dot{V}_{O_2max}$ at 4 mmol l^{-1} [La$^-$], a common measure for lactate threshold power, was calculated. GE

was calculated by the oxygen equivalent (Peronnet & Massicotte, 1991) and the matching RER values to establish the energy expended ($\dot{V}_{O_2}$ (l s^{-1}) × (4840 J l^{-1} × RER + 16,890 J l^{-1})), (Noordhof, Skiba, & de Koning, 2013), and this was divided by the power output and multiplied by 100. After termination of the blood lactate profile test, the cyclists had 5 min of recovery before completing another incremental cycling test for determination of $\dot{V}_{O_2max}$. The test was initiated with 1 min of cycling at a power output corresponding to 3 W kg^{-1} (rounded down to the nearest 50 W). Power output was subsequently increased by 25 W every minute until exhaustion, defined as a cadence below 60 r.p.m. $\dot{V}_{O_2max}$ was calculated as the average of the two highest subsequent 30 s $\dot{V}_{O_2}$ measurements. W_{max} was calculated as the mean power output during the last minute of the incremental $\dot{V}_{O_2max}$ test. Following the $\dot{V}_{O_2max}$ test, the cyclists had another 5 min recovery period before they started on 30 min cycling at the power at 2 mmol l^{-1} [La$^-$] calculated from the blood lactate profile. Thereafter, the third and second last 5 min step from the blood lactate profile test was repeated. During the second last 5 min step, [La$^-$] and GE were measured to assess metabolic strain in a more fatigued state ([La$^-$]$_{fatigue}$ and GE$_{fatigue}$, respectively). The 30 min at 2 mmol l^{-1} [La$^-$] followed by measurement of [La$^-$] and GE was performed since we previously have observed different training adaptations in GE between fresh and more fatigued states (Rønnestad et al., 2011). A 15 min cycling performance test followed directly after the measurement of GE (Figure 1). The participants were allowed to adjust the power output throughout the 15 min cycling test using an external control unit placed next to the handlebar of the Lode Excalibur Sport cycle ergometer. HR and $\dot{V}_{O_2}$ were measured continuously during test, and performance was measured as the average power output.

TABLE 1 Blood variables measured in Control and HEAT participants before (pre) and after (post) the intervention period

| | | | | ANOVA | | |
	Pre	Post	Effect size (d)	Time	Group	Interaction
[Hb] (g dl^{-1})			0.25	0.801	0.146	0.622
Control	15.3 ± 0.6	15.2 ± 0.7				
Heat	14.7 ± 0.9	14.8 ± 1.0				
Htc (%)			0.09	0.029	0.170	0.766
Control	44.5 ± 1.9	43.5 ± 1.8				
Heat	43.2 ± 2.5	42.4 ± 2.4				
Osmolality (mosmol (kg H$_2$O)$^{-1}$)			1.27	0.197	0.354	0.004
Control	297.0 ± 2.5	294.8 ± 3.0				
Heat	296.3 ± 2.2	297.2 ± 2.1				
Total protein (g l^{-1})			0.25	0.001	0.948	0.295
Control	71.9 ± 3.6	70.0 ± 2.8				
Heat	71.4 ± 2.3	70.3 ± 2.6				
Albumin (g l^{-1})			0.38	0.002	0.267	0.397
Control	49.9 ± 1.8	48.4 ± 1.7				
Heat	50.2 ± 1.1	49.3 ± 1.6				

Data are shown for haemoglobin concentration ([Hb]), haematocrit (Htc), osmolality, total protein and albumin. Data are expressed as means $\pm$ SD.

2.6 | Haematology

Hb$_{mass}$ and intravascular volumes were assessed on four occasions: two pre-intervention (Pre-1 and Pre-2) and again upon termination of the intervention period (Post-1, obtained 1–2 days after intervention; and Post-2, obtained 3–4 days after last intervention). Pre-1 and Pre-2 were averaged into one single point, Pre, and also Post-1 and Post-2 were averaged into a single point, Post. All measurements were completed with an automated blood volume analyser based on CO rebreathing (Detalo Performance, Detalo Health, Birkerod, Denmark). All procedures have been described in detail elsewhere (Siebenmann, Keiser, Robach, & Lundby, 2017a).

After arrival at the laboratory, the participant was placed in supine position for 20 min and capillary blood was sampled from a fingertip and venous blood from a forearm vein. Blood was analysed immediately in triplicates for (1) percentage carboxyhaemoglobin (%HbCO) (ABL80 CO-OX; Radiometer, Copenhagen, Denmark), (2) Htc and [Hb], by means of a Cell-Dyn Sapphire Hematology Analyzer (Abbott Laboratories Diagnostics Division, Irving, TX, USA), (3) osmolality (Fiske Model 210 Osmometer, Fiske Associates, Norwood, MA, USA), and (4) total protein and albumin concentration (Cobas c 501 Instrument, Roche Diagnostics, Indianapolis, Indiana, USA). The participant was then connected to a breathing circuit and breathed 100% O$_2$ for 4 min. A bolus of 1.5 ml (kg body weight)$^{-1}$ of 99.997% chemically pure CO (AGA, Oslo, Norway) was thereafter administrated and this O$_2$–CO gas mixture was rebreathed for 10 min. A second capillary blood sample was obtained at 10 min of CO rebreathing

and analysed in quadruplicate for %HbCO. Total RBCV, PV and blood volume were then derived from Hb$_{mass}$, [Hb] and Htc. In the second blood sample, %HbCO never exceeded 10% in any of the participants. The percentage typical error (%TE) was calculated for Pre and Post Hb$_{mass}$ and was 1.9 and 2.1%, respectively, which is in line with previous reported values (Siebenmann et al., 2017a).

2.7 | Statistical analysis

Statistical analyses were performed using SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). Data were tested for normal distribution with the Kolmogorov–Smirnov test and for homogeneity of variances with Levene's test. To assess the effect of HEAT vs. CON on study variables, two-way ANOVA with repeated measures was performed. Main effects for 'group' and 'time', as between- and within-subject factors, respectively, were determined along with interaction among these factors. Pairwise specific comparisons were carried out by Student's t test. In addition, the effect of HEAT vs. CON on study variables was analysed using ANCOVA, with post-intervention outcomes as dependent variables and baseline values as covariates. Linear regression analyses were performed to assess correlates of changes in Hb$_{mass}$. Furthermore, the effect size (ES) was calculated as Cohen's d by using the mean pre–post change in HEAT minus the mean pre–post change in CON, divided by the pooled pre-test standard deviation (Morris, 2008). A two-tailed P-value less than 0.05 was considered significant.
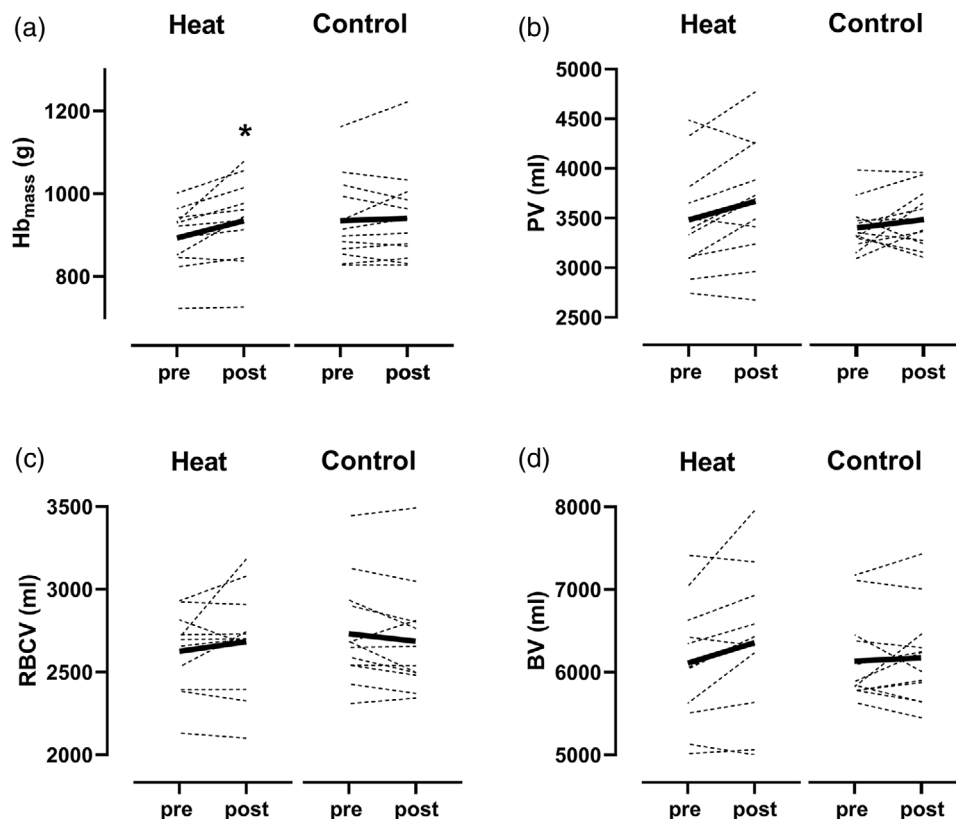
FIGURE 2 Hb_{mass} and blood volumes. (a) Hb_{mass} (g), (b) plasma volume (PV, mL), (c) red blood cell volume (RBCV, mL), and (d) total blood volume (BV, mL) in Heat and Control participants before (pre) and after (post) the 5-week intervention period. Each dashed line represents one study participant and the thick line represents the group average. $^*P < 0.05$ for time and group interaction

3 | RESULTS

3.1 | Haematological adaptations

The main haematological parameters determined in this study are presented in Table 1 and Figure 2. There were no differences between groups in any of these variables prior to the start of the intervention. While the determinations of [Hb], Htc, total protein and albumin revealed no changes pre- vs. post-intervention, blood osmolality analysis revealed a time–group interaction ($P = 0.005$). Following the 5-week intervention period, Hb_{mass} was +4.6% higher in the HEAT group but remained unchanged (+0.5%) in the CON group, and statistical analysis revealed a time–group interaction (Figure 2, $P < 0.05$). A time effect was observed for plasma volume, which was altered by 4.8% (188 ml ± 246) and 2.1% (84 ml ± 2.1) in HEAT and CON, respectively ($P = 0.034$). A group effect could, however, not be found. There was a positive correlation between changes in plasma volume and changes in Hb_{mass} across the two groups ($r = 0.54$, $P = 0.01$). Red blood cell volume and blood volume remained unaffected by the intervention. Similar results were found using ANCOVA with baseline values as covariates (see Table 3).

3.2 | Exercise parameters

Both HEAT and CON increased absolute $\dot{V}_{O_2max}$ (4.6 ± 5.6 and 3.2 ± 3.9%, respectively, $P = 0.002$), with no group differences (Table 2). There were no changes in W_{max} in any of the groups. HEAT increased power output at 4 mmol l^{-1} [La$^-$] ($P = 0.035$), while no changes occurred in CON (9.1 ± 12.4 vs. −0.4 ± 5.1%, respectively), but there were no group differences. However, the effect size showed a small benefit of HEAT vs. CON (ES = 0.34; Table 2). There were no changes or group differences in GE_{fresh} and [La$^-$]$_{fresh}$, but HEAT achieved a larger reduction in [La$^-$]$_{fatigue}$ than CON (−15.7 ± 24.9 vs. 15.9 ± 26.7%, respectively, $P = 0.038$), which was accompanied by a large effect size favouring HEAT (ES = 1.04; Table 2). There were no changes or group differences in $GE_{fatigue}$ from pre to post, but there was an intermediate effect size in favour of HEAT (ES = 0.52; Table 2). Both HEAT and CON increased mean power output during the 15 min cycling trial (6.9 ± 8.4 vs. 3.4 ± 5.1%, respectively), with no differences between groups (Table 2). The effect size revealed a small effect on mean power output favouring HEAT (ES = 0.22). Fractional utilization of $\dot{V}_{O_2max}$ at 4 mmol l^{-1} [La$^-$] and during the 15 min cycling trial did not change in any of the groups and there were no group differences (Table 2).

TABLE 2 Data from the submaximal-, maximal incremental exercise and 15 min cycling tests before (Pre) and after the intervention period (Post) in the heat training group (HEAT) and the control group (CON); the magnitude of improvements of HEAT vs. CON is also shown

| | HEAT (n = 11) | | CON (n = 10) | | HEAT vs. | ANOVA | | |
	Pre	Post	Pre	Post	CON (ES)	Time	Group	Interaction
Body mass (kg)	67.6 ± 6.9	67.7 ± 6.8	70.8 ± 5.6	70.8 ± 5.1	0.015	0.756	0.262	0.756
Submaximal exercise								
Power at 4 mmol l^{-1} (W)	304 ± 31	$314 \pm 34^{*}$	304 ± 32	303 ± 31	0.335	0.195	0.670	0.110
%$\dot{V}_{O_2max}$ at 4 mmol l^{-1} (%)	80.6 ± 3.6	80.4 ± 2.2	79.6 ± 3.1	78.6 ± 2.8	0.228	0.382	0.245	0.543
GE$_{fresh}$ (%)	20.22 ± 0.70	20.03 ± 0.95	19.76 ± 0.66	19.59 ± 0.72	−0.028	0.173	0.181	0.954
GE$_{fatigue}$ (%)	19.27 ± 0.57	19.46 ± 0.82	19.00 ± 0.57	18.88 ± 0.83	0.517	0.718	0.160	0.133
[La$^-$]$_{fresh}$ (mmol l^{-1})	2.41 ± 0.43	2.29 ± 0.89	2.78 ± 0.60	3.06 ± 1.19	−0.742	0.656	0.095	0.270
[La$^-$]$_{fatigue}$ (mmol l^{-1})	2.49 ± 0.57	2.04 ± 0.62	2.78 ± 1.06	3.26 ± 1.72	−1.036	0.949	0.129	0.038
$\dot{V}_{O_2max}$ test								
$\dot{V}_{O_2max}$ (l min^{-1})	5.15 ± 0.51	$5.38 \pm 0.45^{*}$	5.33 ± 0.40	$5.49 \pm 0.29^{*}$	0.146	0.002	0.431	0.552
$\dot{V}_{O_2max}$ (ml min^{-1} kg^{-1})	76.6 ± 6.9	$79.6 \pm 3.8^{*}$	75.7 ± 7.8	77.8 ± 5.2	0.131	0.007	0.601	0.606
RER ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	1.14 ± 0.04	1.13 ± 0.03	1.13 ± 0.03	1.12 ± 0.04	0.000	0.283	0.518	0.812
Ventilation$_{peak}$ (l min^{-1})	188 ± 26	193 ± 22	195 ± 18	199 ± 14	0.043	0.046	0.452	0.822
HR$_{peak}$ (beats min^{-1})	194 ± 8	192 ± 10	192 ± 5	194 ± 5	−0.569	0.555	0.970	0.079
[La$^-$]$_{end}$ (mmol l^{-1})	12.6 ± 2.8	12.7 ± 1.1	12.6 ± 2.9	13.7 ± 2.2	−0.337	0.234	0.608	0.311
RPE (6–20)	19.5 ± 0.5	19.5 ± 0.5	19.3 ± 0.5	19.3 ± 0.7	0.000	0.798	0.376	0.798
W_{max} (W)	442 ± 44	452 ± 42	439 ± 41	445 ± 37	0.090	0.035	0.794	0.624
15 min cycling test								
Power$_{15min}$ (W)	311 ± 46	$330 \pm 38^{*}$	309 ± 30	319 ± 30	0.223	<0.001	0.698	0.243
$\dot{V}_{O_2 mean}$ (l min^{-1})	4.42 ± 0.55	$4.62 \pm 0.46^{*}$	4.49 ± 0.32	$4.66 \pm 0.30^{*}$	0.063	<0.001	0.776	0.796
%$\dot{V}_{O_2max}$ at 15 min test	85.7 ± 5.3	85.9 ± 3.7	84.2 ± 2.8	84.9 ± 2.4	−0.112	0.629	0.373	0.799
HR$_{peak}$ (beats min^{-1})	188 ± 8	189 ± 8	190 ± 4	191 ± 5	0.000	0.298	0.547	0.856
[La$^-$]$_{end}$ (mmol l^{-1})	8.1 ± 2.5	8.8 ± 2.2	7.5 ± 2.5	$9.6 \pm 2.4^{*}$	−0.461	0.005	0.901	0.110
RPE (6–20)	19.1 ± 0.9	19.6 ± 0.5	19.3 ± 0.5	18.9 ± 0.6	1.171	0.693	0.252	0.017

Values are means ± SD. *Different from Pre ($P < 0.05$). The relative change from Pre is larger than in CON ($P < 0.05$). Power at 4 mmol l^{-1}: power output at a blood lactate concentration of 4 mmol l^{-1}; %$\dot{V}_{O_2max}$ at 4 mmol l^{-1}: fractional utilization of $\dot{V}_{O_2max}$ at the power output at 4 mmol l^{-1}; GE$_{fresh}$ and [La$^-$]$_{fresh}$: gross economy and blood lactate concentration at the second last bout of the blood lactate profile; GE$_{fatigue}$ and [La$^-$]$_{fatigue}$: gross economy and blood lactate concentration at the power output from second last bout of the blood lactate profile after the prolonged cycling. $\dot{V}_{O_2max}$: maximal oxygen consumption; RER: respiratory exchange ratio; HR$_{peak}$: peak heart rate; [La$^-$]$_{end}$: blood lactate concentration 1 min after exercise; RPE: rate of perceived exertion; W_{max}: peak aerobic power output; Power$_{15min}$: mean power output during 15 min cycling test; $\dot{V}_{O_2 mean}$: mean oxygen consumption during the 15 min cycling test; %$\dot{V}_{O_2max}$ at 15 min test: fractional utilization of $\dot{V}_{O_2max}$ during the 15 min cycling test.

Both HEAT and CON increased 1RM leg press (from 315 ± 39 to 327 ± 39 kg and from 296 ± 46 to 309 ± 42 kg, all $P > 0.05$), with no group differences. Similar results were found using ANCOVA with baseline values as covariates (Table 3).

4 | DISCUSSION

In the present study elite cyclists were exposed to 5 weeks of heat training (37.8 ± 0.5°C; 65.4 ± 1.8% humidity) consisting of five weekly sessions with each session lasting 1 h. During each heat session, the participants conducted light exercise. The main finding of the present study is that Hb$_{mass}$ in the HEAT group increased by 42 g (4.6%), whereas no changes were observed in CON. Despite the increase in Hb$_{mass}$, $\dot{V}_{O_2max}$ did not become

concomitantly elevated. This type of heat training may nonetheless be of relevance for elite cyclists as exercise performance tests revealed a reduced blood lactate concentration towards the end of a prolonged test battery. Furthermore, there were also small to intermediate effect sizes favouring HEAT for lactate threshold power output (2.8 ± 3.9 vs. −0.4 ± 5.1% change, ES = 0.34), gross economy in the fatigued state (0.19 ± 0.42 vs. −0.12 ± 0.49%-point change, ES = 0.52) and 15 min mean power (6.9 ± 8.4 vs. 3.4 ± 5.1% change, ES = 0.22).

4.1 | Heat training and blood volume expansion

Acclimatization to a hot environment leads to hypervolaemia, which for a long time was thought exclusively to be the response of an

TABLE 3 Analysis of covariance for post intervention haematological, submaximal exercise, maximal incremental exercise and 15 min cycling test outcomes in the heat training (HEAT) vs. control (CON) group

	ANCOVA		
	Mean effect[*]	**95% CI**	**P**
Body mass (kg)	−0.02	1.27, −1.31	0.974
Haematology			
[Hb] (g dl^{-1})	0.05	−0.41, 0.51	0.812
Htc (%)	−0.26	−1.75, 1.23	0.722
Hb$_{mass}$ (g)	40.66	5.62, 75.69	0.025
PV (ml)	107.52	−111.14, 326.18	0.317
RBCV (ml)	105.54	−16.25, 227.34	0.086
BV (ml)	207.43	−65.74, 480.59	0.129
Submaximal exercise			
Power at 4 mmol l^{-1} (W)	10.14	−2.75, 23.03	0.116
%$\dot{V}_{O_2max}$ at 4 mmol l^{-1} (%)	1.40	−0.69, 3.49	0.175
GE$_{fresh}$ (%)	0.01	−0.57, 0.58	0.985
GE$_{fatigue}$ (%)	0.25	−0.18, 0.68	0.231
[La$^-$]$_{fresh}$ (mmol l^{-1})	−0.25	−1.04, 0.53	0.506
[La$^-$]$_{fatigue}$ (mmol l^{-1})	−0.89	−1.79, 0.02	0.055
$\dot{V}_{O_2max}$ test			
$\dot{V}_{O_2max}$ (l min^{-1})	12.22	−179.86, 204.30	0.895
$\dot{V}_{O_2max}$ (ml min^{-1} kg^{-1})	1.29	−0.58, 3.16	0.165
RER ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	0.00	−0.03, 0.03	0.932
Ventilation$_{peak}$ (l min^{-1})	−0.81	−8.32, 6.70	0.823
HR$_{peak}$ (beats min^{-1})	−4.14	−9.14, 0.87	0.099
[La$^-$]$_{end}$ (mmol l^{-1})	−0.98	−2.19, 0.23	0.105
RPE	0.11	−0.50, 0.72	0.711
W_{max} (W)	4.11	−10.65, 18.87	0.566
15 min cycling test			
Power$_{15min}$ (W)	9.34	−4.79, 23.46	0.182
$\dot{V}_{O_2mean}$ (l min^{-1})	9.49	−164.60, 183.58	0.910
%$\dot{V}_{O_2max}$ at 15 min test	0.58	−2.19, 3.35	0.666
HR$_{peak}$ (beats min^{-1})	0.04	−4.26, 4.34	0.985
[La$^-$]$_{end}$ (mmol l^{-1})	−1.24	−2.88, 0.41	0.132
RPE (6–20)	0.77	0.27, 1.27	0.005

[*]Mean difference in post-intervention outcomes in HEAT vs. CON, including baseline values as covariates. BV: blood volume; GE$_{fatigue}$ and [La$^-$]$_{fatigue}$: gross economy and blood lactate concentration at the power output from second last bout of the blood lactate profile after the prolonged cycling; GE$_{fresh}$ and [La$^-$]$_{fresh}$: gross economy and blood lactate concentration at the second last bout of the blood lactate profile; [Hb]: haemoglobin concentration; Hb$_{mass}$: haemoglobin mass; HR$_{peak}$: peak heart rate; Htc: haematocrit; Power at 4 mmol l^{-1}: power output at a blood lactate concentration of 4 mmol l^{-1}; [La$^-$]$_{end}$: blood lactate concentration one min after exercise; PV: plasma volume; RBCV: red blood cell volume; RER: respiratory exchange ratio; RPE: rate of perceived exertion; $\dot{V}_{O_2mean}$: mean oxygen consumption during the 15 min cycling test; %$\dot{V}_{O_2max}$ at 15 min test: fractional utilization of $\dot{V}_{O_2max}$ during the 15 min cycling test; %$\dot{V}_{O_2max}$ at 4 mmol l^{-1}: fractional utilization of $\dot{V}_{O_2max}$ at the power output at 4 mmol l^{-1}; W_{max}: peak aerobic power output; Power$_{15 min}$: mean power output during 15 min cycling test.

increase in PV (Sawka et al., 2000). Recent studies, however, suggest that also RBCV becomes elevated (Karlsen et al., 2015a; Oberholzer et al., 2019).

With chronic heat exposure albumin moves from the intervascular to the extravascular space and as a consequence water follows and PV is increased (Sawka et al., 2000). However, other mechanisms may also be involved and chronic heat exposure is not necessarily needed to expand PV as PV also becomes increased when exercise training is conducted in a hot environment while at the same time residing in a cool environment – i.e. repeated, intermittent short duration heat exposure combined with exercise training. Accordingly, Nielsen et al. (1993) found PV to increase by 13% following 9–12 days with exhausting exercise training conducted at 40°C. Similarly, Lorenzo and co-workers (2010) found PV to increase by 6.5% following 10 days with light exercise training conducted at 40°C. In a similar manner, we have previously found PV to be elevated by 6% following 10 days of similar heat training as in the present study (Keiser et al., 2015). The magnitude of acute increase in PV has been suggested to be related to the magnitude of fluid loss during heat exercise (Akerman, Lucas, Katare, & Cotter, 2017). Despite the increase in PV being a robust observation following heat training, we have recently conducted a 5-week heat training study (i.e. the longest heat training intervention in duration to date) in which both control and heat group experienced a PV increase (188 and 304 ml, respectively) with no statistical differences between groups (Oberholzer et al., 2019). In line with the latter, but at odds with most previous acclimatization studies, we observed a main effect of time for PV increases, but no differences between groups. It should also be noted that we previously have validated the CO rebreathing approach for the determination of PV (Keiser et al., 2017) and that we have used this approach in both studies (Keiser et al., 2015; Oberholzer et al., 2019). The lack of measurable effects on PV with these long duration studies could be related to the fact that with chronic heat exposure a peak in PV is typically reached after about 5–10 days (20% increase) whereafter PV declines again (∼10% increase) (Sawka et al., 2000). If this were the case in the present study and if at the same time accepting the critmeter theory, it must also be assumed that the erythropoietic stimulus is greatest in the initial phase of heat acclimatization. One other obvious difference between previous studies (Karlsen et al., 2015b; Oberholzer et al., 2019) and the current study is that the participants in the present study were much fitter, with an average $\dot{V}_{O_2max}$ of 76.2 ± 7.6 ml min^{-1} kg^{-1}. In this regard, we have previously reported that the expansion of red blood cell volume at altitude is less in already fit individuals with high blood volumes (Robach & Lundby, 2012), but whether this observation can be transferred to PV and heat exposure remains speculative.

Instead of exposing study volunteers to heat in a climatic chamber as done in the current and above-mentioned studies, Karlsen and coworkers (Karlsen et al., 2015b) exposed non-heat acclimated Danish cyclists to outdoor exercise training in Qatar (36°C) while for the reminder of the day the volunteers where kept indoors and under air-conditioned conditions. Interestingly, after the 2-week intervention period they reported an increase in PV of 13.5% and a concomitant increase in Hb$_{mass}$ of nearly 60 g. The latter is to the best of our

knowledge the first study demonstrating an increase in Hb_{mass} (or red blood cell volume) with heat acclimatization. In a similar manner, we have recently observed a tendency (time × group interaction: $P = 0.061$) for a larger increase in Hb_{mass} following 5 weeks of heat training (5 sessions per week for 1 h at 40°C) compared to a control group (Oberholzer et al., 2019). While the average response tended to be higher in the intervention group, it should be noted that individual responses varied markedly (+34 ± 36 g in HEAT and +2 ± 33 g in CON).

In the current study, we found Hb_{mass} to increase by 42 g (4.6%) after 5 weeks of heat acclimatization (RBCV $P = 0.081$ for it to increase in HEAT). Intriguingly, the observed increase in Hb_{mass} in the present study is similar to the 5% improvement we have observed after 4 weeks' acclimatization to 3450 m altitude in healthy but untrained study volunteers (Siebenmann et al., 2015), but far greater than what we have observed in endurance athletes with normobaric hypoxic live high–train low (LHTL) (Siebenmann et al., 2012) or LHTL at natural altitude where we have observed no changes (Robach et al., 2018).

4.2 | What could be the mechanisms behind the increase in Hb_{mass}?

Our hypothesis was that the increase in Hb_{mass} would be preceded by an increase in PV and hence reduction in Htc and that this would facilitate the synthesis of EPO according to the kidneys 'critmeter' function (Montero & Lundby, 2018). In line with our hypothesis, there was a positive correlation between changes in plasma volume and changes in Hb_{mass} across the two groups ($r = 0.54$, $P = 0.01$). In a previous heat study, we have also observed a positive correlation ($r = 0.493$, $P = 0.023$) between changes in PV and Hb_{mass} when pooling the heat and the control group (Oberholzer et al., 2019). In line with the latter, we have also in a previous exercise training study in untrained individuals observed that the increase in Hb_{mass} was preceded by decreases in Htc (Montero et al., 2017). While our hypothesis was based on the critmeter function of the kidney, other mechanisms may be involved of course. The rate of EPO synthesis is mainly regulated by the HIF system that is globally known to be ubiquitinated in normoxic conditions but stabilized in hypoxic conditions. However, the HIF system has also been shown to stabilize with increased HSP expression (Maloyan et al., 2005) and it may hence be speculated that the augmented Hb_{mass} occurred secondary to a heat exposure-induced increase in HSP. For a detailed discussion on HSP expression with heat exposure and its relation to exercise performance, the reader is referred to two recent reviews on that matter (Ely et al., 2014; Hawley et al., 2018).

Since the current study was conducted in elite athletes, blood sampling was kept at a strict minimum which circumscribed the determination of EPO or other candidate erythropoietic hormones such as angiotensin-2, which could have shed light on the underlying mechanisms for the expansion in Hb_{mass}. It has also been suggested that noradrenaline has erythropoietic capabilities (Schrier, 1974), and although we did not determine noradrenaline levels, it is well established that hyperthermia increases sympathetic nervous activity (Nielsen et al., 1993).

4.3 | Heat acclimatization and exercise performance in normal temperature conditions

The larger increase in Hb_{mass} in HEAT in the present study did not lead to a greater increase in $\dot{V}_{O_2max}$ or W_{max}. Considering that for each gram of haemoglobin $\dot{V}_{O_2max}$ may increase about 4 ml, it would be expected that exercise performance would become increased in those studies in which an increase in Hb_{mass} is reported (Prommer & Schmidt, 2010). Thus the 60 g increase observed by Karlsen et al. (2015b) could theoretically increase $\dot{V}_{O_2max}$ by a substantial 240 ml min^{-1}, but did not. Similarly, the 34 g increase observed by Oberholzer et al. (2019) did not result in a concomitant increase in $\dot{V}_{O_2max}$ (Mikkelsen et al., 2019). However, in the present study the change in $\dot{V}_{O_2max}$ was in the same direction as the development of Hb_{mass}, i.e. the 42 g increase in HEAT was accompanied by a 225 ml min^{-1} increase in $\dot{V}_{O_2max}$, while the 6 g difference in CON was accompanied by 161 ml min^{-1} increase in $\dot{V}_{O_2max}$ (non-statistical differences between groups). The lack of difference between groups in $\dot{V}_{O_2max}$ improvements despite differences in augmented Hb_{mass} is at odds with exercise training studies in previously untrained individuals conducted in a thermoneutral environment in which most of the improvement in $\dot{V}_{O_2max}$ can be ascribed to concomitant improvements in blood volume/RBCV (Bonne et al., 2014; Montero et al., 2015).

While there were no differences between groups in [La$^-$]$_{fresh}$, HEAT reduced [La$^-$]$_{fatigue}$ to a larger extent than CON. The latter observations are in line with previous findings in well-trained endurance athletes, indicating that favourable adaptations at submaximal exercise intensities are more challenging to induce in the fresh state than after prolonged exercise (Øfsteng et al., 2018; Rønnestad et al., 2011; Vikmoen, Rønnestad, Ellefsen, & Raastad, 2017). The lack of an effect of HEAT on GE in the fresh state is in accordance with heat training studies lasting 2–5 weeks performed on trained cyclists (Karlsen et al., 2015b; Mikkelsen et al., 2019). Despite minor statistical differences between groups, the numerical differences observed at submaximal exercise favours HEAT, with ∼3% increase in power output at 4 mmol l^{-1} [La$^-$] (vs. ∼0% in CON), ∼16% reduction of [La$^-$]$_{fatigue}$ and 0.2 percentage-point increase in GE$_{fatigue}$ (vs. ∼16% increase in [La$^-$]$_{fatigue}$, and −0.1 percentage-point decrease in GE$_{fatigue}$ in CON). Taking the high training status of the cyclists and the relatively short intervention period into account, these numerical differences can be viewed as relevant for elite exercise performance and this is supported by the effect size which elucidates a small to intermediate effect of HEAT vs. CON. In accordance with the current study, previous studies on endurance-trained participants have observed improved power output at lactate threshold in normal temperature after heat training (Lorenzo, Halliwill, Sawka, & Minson, 2010), although two of these did not include a control group (Neal, Corbett, Massey, & Tipton, 2016; Rendell et al., 2017). Similarly, a reduction in aerobic metabolism, glycogen utilization and [La$^-$] in normal temperature

has been observed after a period of heat training (Young, Sawka, Levine, Cadarette, & Pandolf, 1985). However, due to lack of control group and the rather poor training status of the included participants, care should be taken in the interpretation of these results and the relevance for elite exercise performance may be questioned. The present observation of a small effect size of HEAT on power output at 4 mmol I^{-1} [La^-] can be speculated to be related to the increase in Hb_{mass}, which increases the oxygen availability to the exercising skeletal muscles. Furthermore, it has been observed that repeated exposure to passive heat stress increases skeletal muscle mitochondrial function (Hafen, Preece, Sorensen, Hancock, & Hyldahl, 2018) and capillarization (Hesketh et al., 2019). Even though the latter studies were performed on sedentary participants, they nonetheless indicate that HEAT may have induced peripheral adaptations that may have contributed to the small beneficial effect of HEAT. Lastly, it has been suggested that increased blood volume, as observed in HEAT (with a small ES), may lead to increased blood flow through the splanchnic circulation and thereby increasing lactate removal (Lorenzo et al., 2010) or may also simply be related to a dilution of lactate from blood volume expansion, both with a consequence of delayed blood lactate accumulation.

Power output at lactate threshold has been shown to be a good predictor of performance in endurance events (Coyle et al., 1991; Jacobs et al., 2011) and is sensitive to changes in performance (Jones, 1998). The observed small effect size of HEAT on mean power output during the final 15 min all-out test was therefore expected. The larger oxygen delivery capacity due to the ~5% increase in Hb_{mass} in HEAT is an obvious candidate to partly explain these improvements. In support thereof, we observed a positive correlation between increases in mean power output during the 15 min cycling test and increases in Hb_{mass} ($r = 0.47$, $P = 0.03$). Although differences in 15 min test between groups did not reach statistical significance, it can be speculated that the numerical increase observed in HEAT of ~7% has performance relevance compared to the ~3% increase in CON in this group of highly trained cyclists. In line with the latter, it has previously been documented that heat training induces larger improvements in endurance performance than in a control setting (Lorenzo et al., 2010). In support, studies have also observed ergogenic effects of heat training on endurance performance in trained cyclists (Neal et al., 2016; Rendell et al., 2017), although the already mentioned lack of control groups requires careful interpretations. To the contrary, other studies on trained cyclists have not found any difference in endurance exercise capacity between a heat-trained group and a control group (Karlsen et al., 2015b; Keiser et al., 2015; Mikkelsen et al., 2019). The study by Mikkelsen et al. (2019) applied a similar intervention duration as the present study, but did not find any advantage of heat training compared to their control group. However, in the present study we observe a small effect size of HEAT on certain physiological determinants of endurance performance (mean power during 15 min trial) which were not tested in the previous study. The reason(s) for this discrepancy remains unknown, but we can point at some small differences that might contribute to explaining it. The present participants had a higher fitness level ($\dot{V}_{O_2max}$: ~76 vs. ~60 ml min^{-1} kg^{-1}; W_{max}: ~6.3 vs. ~5.1 W kg^{-1}),

and were younger (~19 vs. ~39 years). During the heat sessions, the participants in the present study were limited to drinking 0.5 litres of water (to ensure dehydration), while they were allowed to consume water ad libitum in the Mikkelsen et al. (2019) study. The latter could have induced a smaller degree of dehydration with a potentially smaller stimulus to increase Hb_{mass} and potentially contribute to explaining the slightly larger Hb_{mass} increase in the present study (~5 vs. ~3%). Lastly, endurance performance in the present study was measured during slightly warmer conditions than in the Mikkelsen et al. (2019) study (~18 vs. ~14°C) and was performed at the end of a prolonged exercise protocol, while it was performed on a separate day in Mikkelsen et al. (2019).

Finally, since it is established that both normal endurance training, especially high intensity aerobic training (Laursen, 2010), and heavy strength training (Rønnestad & Mujika, 2014) can improve endurance performance, it is important to stress that there were no difference between HEAT and CON in conducted training volume, intensity distribution or amount of heavy strength training. Accordingly, there were no group differences in 1RM leg press. Therefore, is it likely that the numerical advantage and small to intermediate effect size of HEAT is due the heat training.

4.4 | Perspective

The usual pitfall with studies on interventions that may or may not favour elite exercise performance is that these are not always conducted in elite individuals. In the current study we argue that this limitation was overcome by selecting participants with an already high $\dot{V}_{O_2max}$ of 76.2 ml min^{-1} kg^{-1}. Albeit this is the first study to demonstrate increases in Hb_{mass} following laboratory-based heat training in such athletes, it is tempting to advocate this strategy to Olympic calibre/champion athletes also. However, as with other new/unfamiliar training strategies for elite performance, this should be adequately tested by the given athletes before it is implemented before important sporting events.

COMPETING INTERESTS
None declared.

AUTHOR CONTRIBUTIONS
B.R.R.: planned the study, conducted research and drafted the manuscript; H.H., J.H., E.H. and J.E.W.: conducted research and revised the manuscript; D.M.: statistical analysis and revised the manuscript; C.L.: conceived the study and drafted the manuscript. All authors have read and approved the final version of this manuscript and agree to

be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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