Heat training increases exercise capacity in hot but not in temperate conditions: a mechanistic counter-balanced cross-over study

Authors: Stefanie Keiser¹, Daniela Flück¹, Fabienne Hüppin², Alexander Stravs², Matthias P. Hilty³ & Carsten Lundby¹, ⁴

Author contributions:
SK: designed the study, performed the experiments, analyzed data and drafted the manuscript.
DF, FH and AS: assisted in performing the experiments and edited the manuscript.
MPH: provided medical support and edited the manuscript.
CL: initiated and designed the study and drafted the manuscript.

Affiliations: ¹Zürich Center for Integrative Human Physiology, Institute of Physiology, University of Zürich, Zürich, Switzerland ²Exercise Physiology, Institute of Human Movement Sciences, ETH Zürich, Zürich, Switzerland, ³Intensive Care Unit, University Hospital of Zürich, Zürich, Switzerland, ⁴Food & Nutrition & Sport Science, Gothenburg University, Gothenburg, Sweden

Running head: Exercise performance and heat training

Corresponding author: Prof. Carsten Lundby, Zürich Center for Integrative Human Physiology, Institute of Physiology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland.
Tel. +41 44 635 50 52; e-mail: carsten.lundby@access.uzh.ch

Copyright © 2015 by the American Physiological Society.
Abstract

The aim was to determine the mechanisms facilitating exercise performance in hot conditions following heat training. In a counter-balanced order seven males (\(\dot{\text{VO}}_2\text{max} 61.2\pm4.4 \text{ ml.min}^{-1}.\text{kg}^{-1}\)) were assigned to either 10 days of 90 min exercise training in 18 or 38 °C ambient temperature (30 % relative humidity) applying a cross-over design. Participants were tested for \(\dot{\text{VO}}_2\text{max}\) and 30 min Time Trial performance in 18 (T18) and 38 (T38) °C before and after training. Blood volume parameters, sweat output, cardiac output (\(\dot{Q}\)), cerebral perfusion (i.e. middle cerebral artery velocity \([\text{MCAvmean}]\)) and other variables were determined. Prior to one set of exercise tests in T38 blood volume was acutely expanded by 538±16 ml with an albumin solution (T38\(_A\)) to determine the role of acclimatization induced hypervolemia on exercise performance. We furthermore hypothesized that heat training would restore MCAv\(_{\text{mean}}\) and thereby limit centrally mediated fatigue. \(\dot{\text{VO}}_2\text{max}\) and Time Trial performance were equally reduced in T38 and T38\(_A\) (7.2±1.6 and 9.3±2.5 % for \(\dot{\text{VO}}_2\text{max}\); 12.8±2.8 and 12.9±2.8 % for Time Trial). Following heat training both were increased in T38 (9.6±2.1 and 10.4±3.1 %, respectively) whereas both \(\dot{\text{VO}}_2\text{max}\) and Time Trial performance remained unchanged in T18. As expected heat training augmented plasma volume (6±2 %) and mean sweat output (26±6 %) whereas sweat [\(\text{Na}^+\)] became reduced by 19±7 %. In T38 \(\dot{Q}_{\text{max}}\) remained unchanged pre (21.3±0.6 l.min\(^{-1}\)) to post (21.7±0.5 l.min\(^{-1}\)) training whereas MCAv\(_{\text{mean}}\) was increased by 13±10 %. However, none of the observed adaptations correlated with the concomitant observed changes in exercise performance.

New and noteworthy

In this study we demonstrate that 10 days of heat training facilitates exercise performance in the heat but not in temperate conditions. Training induced changes in physiological parameters which have previously been suggested to facilitate such responses were however not associated with the observed gains in exercise performance.
Key words: hyperthermia, blood volume, performance, training, temperature
Introduction

Endurance exercise performance is impaired with acute exposure to warm environments but gradually recovers, at least partially, with heat acclimatization (naturally occurring exposures) and/or acclimation (experimentally induced heat adaptation) (21, 23, 38). Physiological adjustments to such heat acclimation have been investigated for centuries (5, 29, 41) and one phenotypic adaptation proposed to facilitate exercise capacity with both acclimatization and acclimation is the hyperthermia-induced expansion of plasma volume (PV) (21, 23, 39). Seemingly, such adaptation could to some extent explain several improvements such as an increased sweat output (24, 40) and maximal cardiac output (Q̇) (21) and a reduced submaximal heart rate (HR) (24, 40, 49) and submaximal core temperature (Tcore) (40) which could potentially all contribute to enhanced exercise performance. However, acute expansion of PV (e.g. with albumin) has failed to reduce HR and Tcore during submaximal walking (37) and cycling (48) in humans exposed to heat stress. Likewise, PV expansion did not modify Tcore, HR and exercise performance in the heat (48). However, due to an increase in PV, heat acclimation might also diminish hyperthermia induced reduction in cerebral perfusion that may accelerate centrally mediated fatigue (25, 35). An increase in cerebral perfusion following heat training could also be speculated to improve cooling of the brain as proposed by Nybo et al (28). Nevertheless, we did not observe any VO₂max improvement despite restoring middle cerebral artery velocity (MCAvmean) by administering small volumes of inspired CO₂ during exercise in hot conditions (17). However, the influence of altering cerebral pH via CO₂ inhalation on exercise performance, independent of cerebral perfusion, remains uncertain. One aim of the current investigation was to determine the relevance of heat acclimation-induced changes in PV and MCAvmean for facilitating exercise performance in the heat.

Further adaptive responses to repeated heat exposure which may favor exercise performance in the heat are suggested to involve a reduced loss of electrolytes in sweat (20, 24). This might be favorable for hour long submaximal exercise tasks, though appears of minor relevance for near maximal or maximal intensity exercise challenges such as 5000 m running.
Heat acclimation has also been reported to augment exercise capacity in conditions comprising normal (21 - 21.5 °C) (22, 38) and cool (13°C) (21) ambient temperatures. Of note only the latter study included a control group. Lorenzo et al (21) showed that 10 days of heat acclimation improves VO2max and Time Trial performance when tested in 13 °C ambient temperature by 5 and 6 %, respectively, in well trained subjects. Yet, the enhanced effect on exercise performance should be taken with caution since the heat training was conducted at a relative higher exercise intensity when compared to control training. Although the difference in exercise intensity is estimated to be within ~20 %, and below the anaerobic threshold in both trials, it cannot be ruled out that at least some of the observed effects were the result of more intense training. Accordingly, it was shown that heat training conducted in competitive cyclists and appropriate control subjects lead to no further improvement in normothermic VO2max or outdoor Time Trial performance (16). One further aim with the current investigation was to test whether heat training facilitates exercise performance in temperate conditions.

Therefore, with the current study we sought to determine physiological mechanisms leading to improved exercise capacity in the heat following heat acclimation. We hypothesized that the enhanced exercise performance would be primarily attributed to the increase in MCAv_mean rather than an expansion of PV. To test this hypothesis, PV was acutely expanded by 15 % (corresponding to the expected heat acclimation increase in PV) prior to exercise conducted with acute exposure to 38 °C and MCAv_mean and cardiovascular parameters were assessed. Furthermore, MCAv_mean and cardiovascular parameters were also assessed after 10 days of heat training. A further aim was to test whether heat acclimation increases exercise capacity when performed in 18 °C ambient temperature. This was tested by a randomized and counter-balanced cross-over design. We hypothesized that heat training, if conducted at a relative similar cardiovascular strain as in temperate conditions, would not enhance exercise performance in 18 °C ambient temperature.
Methods

Participants

Eight well trained males (24 ± 2 yrs, 74 ± 3 kg, 182 ± 6 cm, V̇O₂max 61.2 ± 4.4 ml.min⁻¹.kg⁻¹, BMI 22.3 ± 1.7, mean ± SD) who regularly trained > 1h/d for 3 – 5 d/wk were recruited to participate in the study. Prior to participation oral and written informed consent was obtained from each participant. All participants refrained from exercise for 24 h, alcohol and caffeine for 12 h prior to the experimental tests and fulfilled the inclusion criterion of a V̇O₂max > 55 ml.min⁻¹.kg⁻¹. In addition, they were instructed not to donate blood and to avoid ingestion of nonprescription drugs for the entire duration of the multiple study visits. All experimental protocols and procedures were approved by the ethical committee of the Swiss Federal Institute of Technology Zürich (EK 2013-N-23) and conformed to the Declaration of Helsinki. A limited set of data collected as part of this study has been published elsewhere (17).

Study design (Figure 1)

A randomized and counter-balanced cross-over design was applied and therefore participants completed both the heat and the control trainings. All participants completed a preliminary maximal incremental exercise and 30 min Time Trial test in a temperate environment (18 °C) to become familiar with the experimental set-up. Thereafter, participants completed a battery of physiological and performance tests in two environmental conditions (18 and 38 °C, both at 30 % relative humidity), then completed either a heat (38 °C) or a control training (18 °C ) period of 10 days where after the tests were repeated. The first training block was followed by a three month washout period after which the second block started. During this washout period participants continued with their regular training and avoided any sojourn to conditions above 25 °C. The second block was identical to the first except that participants who first trained in cool conditions now trained in
the heat and vice versa. Additionally, only in the first block and before the training period an additional experimental trial in the heat (T38\textsubscript{A}) was conducted in which albumin was infused to expand the PV by 15\%. This T38\textsubscript{A} trial was conducted once in all participants independently of their subsequent training group assignment and was afterwards compared to the baseline T18 and the T38 trials of the first block.

The performance tests included a maximal incremental exercise test (\(\dot{V}O_2\text{max}\) test) followed by a Time Trial test after a 1 h resting period. To restore fluid compartments and energy stores, approximately 45 min before the \(\dot{V}O_2\text{max}\) test participants drank 0.4 L of a carbohydrate-electrolyte solution (Isostar\textsuperscript{®}) and 0.6 L during the resting period between the \(\dot{V}O_2\text{max}\) and Time Trial test. During the exercise tests participants were not allowed to drink. The order of the training condition was randomized and counter-balanced and all test sessions were separated by at least 48 h. Post acclimation studies were completed within 4-5 d of completion of the training period.

All experiments took place during the cool seasons of fall and winter when participants were naturally unacclimatized to heat. For the entire duration of the study, the temperatures in Switzerland did not exceed 15 °C. For the measurements conducted in the first block the average outdoor air temperature was 14.6 °C and for the second block 4.3 °C. The temperature inside where the experiments were conducted was held as constant as possible ranging from 37 to 39 °C in the T38 trials and from 17 to 19.5 °C in the cold trials.

Exercise performance tests

Prior to each exercise test (\(\dot{V}O_2\text{max}\) and Time Trial) participants were immersed into a whole body water bath for 21 ± 5.8 min (mean ± SD). The aim herewith was to manipulate rectal (core) temperature without performing exercise. While prior to the T18 testing the water temperature was thermoneutral (\(\sim 34\) °C), it was \(\sim 42\) °C prior to the T38 and T38\textsubscript{A} trials. This allowed increasing (P < 0.05) participants’ rectal
temperature ($T_{rec}$) by $0.8 \pm 0.5$ °C (mean ± SD) in the T38 and the T38A trial, whereas in the T18 trial $T_{rec}$ remained unaffected. When target $T_{rec}$ was reached in T38 and T38A, or an equivalent time spent in the water bath in the T18 trial, participants were immediately transferred to the cycle ergometer (Monark Ergomedic 839 E, Vansbro, Sweden) mounted with a triathlon handlebar and a SRM power crank (SRM Science Road, Jülich, Germany) placed in a climatic chamber set to either 18 or 38 °C and 30 % relative humidity.

$\dot{V}O_2max$: After a 3 min resting period, where resting values were obtained, participants exercised for 5 min at 80 and 5 min at 130 W. Thereafter workload was increased by 30 W every 90 s until exhaustion. Verbal encouragement was given towards the end of all trials. Maximal workloads reached in the exercise tests were calculated as $W_{max} = W_{compl} + W_{inrem} \times (t/90)$ with $W_{compl}$ being the last completed workload, $W_{inrem}$ the workload increment per exercise step and t the number of seconds in the not completed workload.

Time Trial: After a 1 h resting period and 20 min water immersion, participants performed a Time Trial test. This recovery time has been demonstrated adequate to prevent any bias in subsequent aerobic performance tests (19). After a 3 min resting period, where again resting values were obtained, and a subsequent 5 min warm-up period at a self-selected workload, participants provided their maximal effort for 30 min. Average power output during 30 min ($P_{avg}$) was the performance measured. During the test, participants were allowed to modify power output as often as needed and were aware of the current power output and total time elapsed.

Plasma volume expansion

Participants were positioned on a bed and prepared with a 18-G catheter placed in an antecubital vein. Preceding the water submersion, a volume corresponding to 15 % ($538 \pm 16$ ml) of the participants’ PV was then infused in form of 20 % human albumin (Albumin CSL 20 %, CSL Behring, Bern, Switzerland).
Such albumin infusion has been reported to expand and maintain an individuals’ PV for > 6 h (9). Blood pressure and HR were continuously monitored to ensure participants’ well-being. After completion of the expansion and as soon as participants felt ready the exercise performance tests were initiated as described above.

Training intervention

The training loads were set corresponding to the HR elicited at 50 % of T18 and T38 VO₂max with the intent to assure the same relative cardiovascular strain in both conditions. Each training session lasted 90 min and was conducted on 10 consecutive days which is considered sufficient to trigger substantial heat acclimation. To minimize dehydration, every 30 min participants were provided with 0.5 L of a carbohydrate and electrolyte enriched drink (Isostar®) independent of whether they trained in hot or temperate conditions.

Experimental measures

Throughout each test Trec was measured as a surrogate for Tcore. A flexible rectal probe (YSI M401AC, Advanced Industrial Systems, Louisville, KY, USA), was self-inserted by the participant approximately 7 cm behind the anal sphincter. Additionally three skin temperature probes (YSI M409AC, Advanced Industrial Systems, Louisville, KY, USA) were placed on the forehead, the lower back and the right quadriceps. The average of the three locations was considered as mean skin temperature (Tskin). HR was assessed using a monitor belt (Cosmed, Rome, Italy) and mean arterial pressure (MAP) was measured noninvasively by means of finger photoplethysmography (Nexfin, BMEYE B.V, Amsterdam, Netherlands).
During the $\dot{V}O_2$max test participants breathed through a mouthpiece (Hans Rudolph, Shawnee, KS, USA) with their nose occluded wearing a nose clip. Ventilatory variables were measured breath-by-breath using an indirect calorimeter (Cosmed Quark CPET, Rome, Italy) consisting of a flow meter and fast responding gas analysers. Prior to each experimental session the system was calibrated using a 3-L calibration syringe (Cosmed, Rome, Italy) and gas mixtures of known concentrations of $O_2$ and $CO_2$. After the test all data points were averaged over the last 30 s of each workload. The highest average value for $\dot{V}O_2$ calculated over 30 s was taken as $\dot{V}O_2$max.

During the $\dot{V}O_2$max test $\dot{Q}$ and stroke volume (SV) were assessed at 80 and 130 W and then every 3 min with the Innocor M400 device (Innovision, Glamsbjerg, Denmark), which is based on an inert gas rebreathing technique previously described elsewhere (42). When a measurement is initiated participants are switched from breathing room air to the closed circuit and breathe a known gas mixture for 3 breaths which concentration allows making predictions about $\dot{Q}$. Measurements are based on the assumption that pulmonary uptake of blood soluble testing gas is proportional to pulmonary blood flow which in turn can be considered equal to cardiac output. The highest obtained value was defined as $\dot{Q}_{\text{max}}$ and $SV_{\text{max}}$, respectively.

MCAv$\text{mean}$ was assessed as an estimate of cerebral blood flow (CBF) using transcranial Doppler ultrasonography (Doppler Box, DWL, Singen, Germany). A 2 MHz probe prepared with ultrasound gel was adjusted over the temporal window to insonate the right MCA and was held in place with a snug-fitting headgear. Great care was taken that MCAv$\text{mean}$ was always assessed at the same angle, position (pictures were taken for this) and depth. Cerebral tissue oxygenation (ScO$2$) was monitored by near infra-red spectroscopy (NIRS; INVOS-5100c, Covidien, Minneapolis, MN, USA) on the left forehead. Furthermore, MCAv$\text{mean}$ and ScO$2$ are always presented as delta values (% baseline) to draw conclusions independently of different resting values.
Dry, nude body weight was determined at the beginning and at the end of each exercise test and training session by a precision weighing balance to the nearest 0.1 kg (Kern MPB300K100, Balingen, Germany). After correcting for fluid intake and time, the weight difference before and after the tests or trainings was considered as sweat output.

Sweat samples were obtained during the training sessions on days 1 and 10 after 30, 60 and 90 min of exercise. First the skin area between the scapulae was cleared with ultra-pure water (Milli Q, 18.2 MΩ ionic purity). A cotton tissue was then placed on the same area and fixed with tape. After 5 minutes the cotton tissue was removed and squeezed and transferred into a syringe (Pico 50, Radiometer, Brønshøj, Denmark). The obtained sweat was then analyzed in duplicate for Cl⁻, K⁺, Ca²⁺ and Na⁺ concentration ([Cl⁻], [K⁺], [Ca²⁺], [Na⁺]) by an automated hemoximeter (ABL800, Radiometer, Copenhagen, Denmark).

PV, red blood cell volume (RBCV) and total blood volume (BV) was estimated with a CO re-breathing method introduced by Burge and Skinner (4) but included small modifications. Briefly, participants were positioned on a bed with elevated legs to facilitate venous return (18). Then, a 18-G catheter was placed in an antecubital vein and participants were asked to drink 0.5 l of water. This was followed by a 4 min period were participants breathed 100 % oxygen. While participants were still connected to the oxygen a blood sample was taken and analyzed in quadruplicate for the fraction of carboxyhemoglobin (HbCO) and hemoglobin concentration ([Hb]) on a hemoximeter (ABL800, Radiometer, Copenhagen, Denmark) and for hematocrit (Hct) by the micro-method (4 min at 13,500 rpm). Immediately afterwards participants were switched to a closed re-breathing circuit and breathed 1.5 ml.kg⁻¹ of 99.997 % chemically pure CO (CO N47, Air Liquide, Pullach, Germany) for 10 min. After these 10 min another blood sample was taken and analyzed the same way as the first one. Finally, BV parameters could be derived from these variables (4). In this study the coefficient of variance, expressed as percent typical error (15), was 1.8 % for Hbmass and 3.8 % for PV.
**Statistical analysis**

Single differences between the T18, T38 and the T38A trial were evaluated with a 1-way ANOVA for repeated-measures whereas a repeated 2-way ANOVA design with exercise intensity (Rest, 80 W, 130 W, 160 W, 190 W, 220 W, 250 W, 280 W, Max) and condition (T18, T3, T38A) as main effects was applied for the parameters recorded at different intensities. To detect changes from pre to post again a 2-way ANOVA for repeated-measures with 2 times (pre and post) and 2 interventions (experimental and control) was performed. The parameters recorded at different intensities were analyzed with a 3-way ANOVA with the additional main effect of intensity (Rest, 80 W, 130 W, 160 W, 190 W, 220 W, 250 W, 280 W, Max). Tukey’s range test was applied for post hoc analysis after a significant F main effect and interaction. Where appropriate, single comparisons were made using a paired t-test. Bivariate associations were determined by Pearson’s Correlation Coefficients where r = 0.1 represents a small, r = 0.3 a moderate and r = 0.5 a large correlation. A P-value < 0.05 was considered statistically significant. Effect-sizes were described using Cohen’s d (with d ≤ 0.2 representing a trivial difference; 0.2 – 0.5, a small difference; 0.5 – 0.8 a moderate difference; and > 0.8 a large difference). Data is expressed as mean ± SEM unless otherwise indicated. Statistical analysis was performed using SAS Enterprise Guide (4.3, SAS Institute, Inc., Cary, NC, USA).
Results

One volunteer withdrew from the study after completing the first block due to personal reasons. Therefore, the experimental group consisted of seven participants. The T38_A trial (conducted prior to the exercise training intervention) however was completed by all eight volunteers.

Exercise performance with acute heat exposure following plasma volume expansion

W_{max} (Figure 2A), \dot{V}O_{2max} (Figure 2B) and P_{avg} (Figure 2C) were highest when conducted during the T18 trial. Compared to T18, W_{max} was decreased by 7.6 ± 1.5 and 9.0 ± 2.1 % (both P < 0.01) in T38 and T38_A. The corresponding reduction in \dot{V}O_{2max} was 7.2 ± 1.6 and 9.3 ± 2.5 % (P < 0.05), respectively. P_{avg} was reduced by 12.8 ± 2.8 and 12.9 ± 2.8 % (both P < 0.05) in T38 and T38_A. W_{max}, \dot{V}O_{2max} and P_{avg} during the T38 and T38_A trials were similar (P > 0.99, P = 0.9 and P > 0.99, respectively).

Figure 3 illustrates HR, SV and Q assessed during the \dot{V}O_{2max} test. Overall HR was higher (P < 0.01) during the T38 compared to the T18 trial. HR was decreased (P < 0.01) in the T38_A trial compared to the T38 trial but still elevated (P < 0.01) compared to the T18 trial. Overall, albumin infusion led to a higher (P < 0.01) SV and Q compared to the T18 and T38 trial. Furthermore, overall SV but not Q was decreased (P < 0.01 and P = 0.97, respectively) when conducted during the T38 trial compared to the T18 trial. The same applied for SV_{max} and Q_{max} which were enhanced (P < 0.01) with albumin infusion compared to the T18 and T38 trial but did not differ (P = 0.53, P = 0.78) between T18 and T38.

Albumin infusion did not alter VE (P = 0.38) in the T38_A \dot{V}O_{2max} test when compared to the T38 trial. MAP was increased (P < 0.01) in the T38_A trial compared to the T38 trial but was still reduced (P < 0.01) compared to the T18 trial. MCA_{vmean} was also reduced in the T38 trial compared to the T18 trial. With albumin infusion, however, MCA_{vmean} was restored to the same level as in the T18 trials throughout the entire exercise test (P = 0.96).
Detailed thermoregulatory parameters of all three trials are displayed in table 1. Briefly, due to the passively induced hyperthermia prior to exercise, resting T_{rec} and T_{skin} reached higher (P < 0.05 for T_{rec} and P < 0.01 for T_{skin}) levels during the T38 and T38\textsubscript{A} trial than in the T18 trial. Furthermore, during the T38 and T38\textsubscript{A}, trial maximal T_{rec} and T_{skin} were higher (P < 0.05) compared to the T18 trial in the Time Trial but not in the \textit{VO}_{2}\textsubscript{max} test for T_{rec} (P = 0.53 and P = 0.37, respectively). Albumin infusion did not lead to a reduction (P > 0.99, P = 0.15) in maximal T_{rec} and T_{skin} compared to the T38 trial.

Effects of heat training on exercise performance when conducted in 18 and 38 °C

Figure 4 summarizes the mean performance responses for each test and for both groups. Before heat training, a hyperthermia induced reduction in W\textsubscript{max} (8.5 ± 2.2 %, P < 0.01), \textit{VO}_{2}\textsubscript{max} (4.1 ± 1.4 %, P < 0.05) and P\textsubscript{avg} (13.1 ± 2.9 %, P < 0.01) was apparent in the T38 trial compared to the T18 trial. There was a significant main effect of time and time*intervention interaction from pre to post when conducted at 38 °C for W\textsubscript{max}, \textit{VO}_{2}\textsubscript{max} and Time Trial P\textsubscript{avg}. In the experimental group W\textsubscript{max} increased by 7.9 ± 1.7 % (311 ± 13 vs. 335 ± 12 W, P < 0.01, d = 0.74) and \textit{VO}_{2}\textsubscript{max} by 9.6 ± 2.1 % (4.3 ± 0.2 vs. 4.8 ± 9.2 l.min\textsuperscript{-1}, P < 0.05, d > 0.80) and P\textsubscript{avg} by 10.4 ± 3.1 % (208 ± 12 vs. 228 ± 11 W, P < 0.01, d = 0.63). In contrast neither were elevated compared to pre heat training when tested in T18, as there was no main effect of time (P = 0.19 for W\textsubscript{max}, P = 0.11 for \textit{VO}_{2}\textsubscript{max}, and P = for 0.32 for P\textsubscript{avg}) or intervention (P = 0.61 for W\textsubscript{max}, P = 0.22 for \textit{VO}_{2}\textsubscript{max}, and P = 0.27 for P\textsubscript{avg}), and no interaction of the two (P = 0.52 for W\textsubscript{max}, P = 0.57 for \textit{VO}_{2}\textsubscript{max} and P = 0.64 for P\textsubscript{avg}) from pre to post (340 ± 13 vs. 350 ± 10 W, d = 0.32 for W\textsubscript{max}, 4.5 ± 0.2 vs. 4.7 ± 0.1 l.min\textsuperscript{-1}, d = 0.43 for \textit{VO}_{2}\textsubscript{max} and 240 ± 15 vs. 246 ± 14 W, d = 0.16 for P\textsubscript{avg}).

Blood volume parameters
The expansion of PV with albumin by 15 % corresponded to an increase (P < 0.01) in PV of 538 ± 16 ml. This was more (P < 0.05) than the corresponding increase induced by heat training (6 ± 2 %, 201 ± 88 ml). Nonetheless, the results of the CO re-breathing revealed a significant time*intervention interaction (P < 0.05), due to an increase in PV for the experimental group (+ 6 ± 2 % from 3370 ± 115 to 3571 ± 169 ml, P < 0.05, d = 0.53) but an unaltered PV for the control group (3524 ± 128 vs. 3467 ± 135 ml, P = 0.80, d = 0.15).

Heat training did not affect RBCV (2563 ± 113 vs. 2523 ± 111 ml, d = 0.19) and also in the control group no differences in RBCV (2546 ± 98 vs. 2511 ± 95 ml, d = 0.13) were observed, as there was no main effect of time (P = 0.22) or intervention (P = 0.93), and no interaction (P = 0.95) of the two from pre to post.

Although there was a significant time*intervention interaction (P < 0.05) with no main effect for time (P = 0.59) and intervention (P = 0.68) for total BV, the post hoc analysis for the experimental group revealed no significantly increased total BV after heat training (5916 ± 210 vs. 6111 ± 257 ml, P = 0.12, d = 0.31). Also in the control group BV remained unchanged from pre to post training (6089 ± 200 vs. 5949 ± 187 ml, P = 0.25, d = 0.26).

**Effects of heat training on cardiovascular and thermoregulatory parameters**

Detailed cardiovascular and thermoregulatory responses of all exercise tests are provided in table 2. Briefly, in both, the experimental and the control group, training did not lead to an altered \( \dot{Q}_{\text{max}} \) or \( SV_{\text{max}} \). \( T_{\text{rec}} \) in the T38 trials was always elevated to the same initial temperature by water immersion and therefore resting \( T_{\text{rec}} \) did not differ from pre to post training. In contrast, we did not control for \( T_{\text{rec}} \) in the T18 trials and therefore participants’ resting \( T_{\text{rec}} \) varied by approximately 0.25 ± 0.04 °C (main effect of time P < 0.05). However, no significant time*intervention interaction could be found due to similar
changes in the exercise and the control group. The same was true for maximal T_{rec} but only in the Time
Trial test. Statistical analysis revealed no other significant training induced changes in any of the
measured parameters.

Effects of heat training on ventilation, mean arterial pressure and cerebral perfusion

Exposure to heat (T38) increased (P < 0.05) ventilation (VE) compared to the T18 trial (Figure 5A). For
both the T18 and the T38 trial a significant main effect for time and intensity was found, however, VE
response to training was not different between the control and the experimental group since no significant
interaction between time, intensity and intervention was observed for T18 and T38.

MAP was reduced (P < 0.05) in the T38 compared to T18 trials (Figure 5B). In the T38 trial, after a
significant time*intervention interaction, post-hoc analysis revealed a significantly increased (P < 0.05)
MAP after training for the experimental but not for the control group (P = 0.98). For the T18 trial no such
differences between the experimental and the control group could be demonstrated.

Delta MCA_{v,mean} (% baseline) for both groups are displayed in Figure 5C. Compared to the control group,
in the experimental group % baseline MCA_{v,mean} was significantly enhanced (P < 0.05) after heat training
in the T38 trial but not in the T18 trial. In absolute terms, resting and maximal MCA_{v,mean} was reduced (P
< 0.05) from 55.5 ± 3.0 to 42.3 ± 2.9 and from 69.6 ± 5.7 to 52.5 ± 3.4 cm.s^{-1}, respectively in T38
compared to T18. In the experimental group exercise training increased (P < 0.05) MCA_{v,mean} in T38 and
reached similar absolute resting and maximal values as in the T18 trial.

In line with a reduced MCA_{v,mean} cerebral oxygenation was also decreased (P < 0.05) in the T38 compared
to the T18 trials in both the experimental and the control group (Figure 5D). However, the enhanced
MCA_{v,mean} after heat training did not improve cerebral oxygenation and also in the control group cerebral
oxygenation remained unchanged in the T18 and the T38 trial since neither the main effects for time and intervention nor any interaction was significant.

Cerebrovascular conductance (CVC; \( \text{MCAv}_{\text{mean}}/\text{MAP} \)) demonstrated no significant change from pre to post heat acclimation in the T38 trial (\( P = 0.08 \) for time, \( P = 0.27 \) for intervention, \( P = 0.85 \) for time*intervention). In the T18 trial a main effect of time could be observed (\( P < 0.05 \)), however no significant interaction between time and intervention was demonstrated (\( P = 0.26 \)).

Sweat output and electrolyte concentration during training

Sweat output and sweat electrolyte concentration on training days 1 and 10 in both environmental conditions are presented in table 3. In the experimental group \([\text{Na}^+]\) decreased by \( 19 \pm 7 \% \) (\( P < 0.05, d > 0.80 \)) from day 1 to day 10 while no other parameter was changed as a result of either training intervention.

Sweat output ANOVA results revealed a significant main effect of time and intervention as well as a significant interaction of these two. Mean sweat output in the experimental group was higher (\( P < 0.05, d > 0.80 \)) compared to the control group during trainings on days 1 and 10. Furthermore, a \( 26 \pm 6 \% \) increase (\( P < 0.05, d > 0.80 \)) in sweat output from day 1 to 10 was found in the experimental group. In contrast, in the control group an unaltered (\( P = 0.43, d = 0.12 \)) water loss was observed from day 1 to day 10.

Correlations

Training induced relative percent changes in PV, sweat output and electrolyte concentration, \( \text{MCAv}_{\text{mean}} \), \( \dot{Q} \) and \( T_{\text{rec}} \) were analyzed for correlation with the concomitant occurring gain in exercise...
performance. However, no correlation reached significance (P value ranging from 0.18 for PV to 0.82 for sweat output) and Pearson Correlation Coefficient ranging from 0.1 for $Q_{max}$ to 0.3 for PV which can be considered as small to moderate correlations.
Discussion

The main findings in the present study are: (i) heat training facilitated $\overline{V}O_2\text{max}$ and Time Trial exercise performance in the heat (38 °C) but not in normal (18 °C) thermal conditions, (ii) improved exercise performance did not correlate with adaptations in MCAvmean (i.e. cerebral perfusion) as well as PV, sweat output and sweat [Na⁺] following heat training, and finally (iii) acute expansion of PV with albumin infusion did not facilitate exercise performance in 38 °C.

Level of heat acclimation

Heat acclimation largely depends on the magnitude of heat stress, duration of exposures, frequency and total number of stimuli but it is also well accepted that most adaptations to heat stress are completed after 7-10 days of daily exposure (46). Typical indicators that sufficient heat acclimation has occurred include a reduced HR, $T_{\text{core}}$ and sweat electrolyte concentration but also an increased PV and sweat output. In the present study we decided to passively pre-heat participants before the maximal incremental exercise and the Time Trial tests to always have the same initial $T_{\text{rec}}$. This experimental approach allowed us to examine the impact of heat acclimation at standardized heat strain conditions. This approach furthermore assured that participants initiated all exercise tests in a hyperthermic condition since $\overline{V}O_2\text{max}$ might be maintained with heat exposure if initiated with a ‘normothermic’ body temperature and also Time Trial performance in the heat might remain unaltered for the first minutes (30). As will be discussed later we also applied this approach to have a similar study design to that of Lorenzo and co-workers (21). In contrast, choosing such study design will most likely have blunted the potential acclimation induced reductions in submaximal $T_{\text{rec}}$ and HR. However, since we observed an increase in PV and sweat output and a concomitant reduction in sweat [Na⁺] together with an improved exercise performance at 38 °C we are confident that significant heat acclimation has taken place in the study participants.
As expected (21, 45), in passively pre-heated participants, acute heat exposure led to a 4.1 ± 1.4 and 13.1 ± 2.9 % reduction in \( \dot{V}O_2 \text{max} \) and Time Trial performance, respectively. Also, the observed increase in \( \dot{V}O_2 \text{max} \) and Time Trial performance corresponding to 9.6 ± 2.1 and 10.4 ± 3.1 % following heat training concurs with previous findings (21, 22). Of note is that the increase in \( \dot{V}O_2 \text{max} \) fully compensated the initial heat induced decrement and also \( W_{\text{max}} \) and \( P_{\text{avg}} \) after heat training were almost completely restored to levels initially obtained at 18 °C. So far this has only been observed in the field (34). Collectively, the accumulated evidence emphasizes the need for heat acclimation to maximize performance in a hot environment and furthermore, suggests that acclimation can at least be acquired partially without traveling to the site of competition. However, heat training in the present study did not facilitate exercise performance when tested in 18 °C ambient conditions. In a recent review Corbett et al. (6) analyzed several studies investigating the effects of acclimation on exercise performance in temperate and cold conditions. Although some studies have reported an ergogenic effect of acclimation, most of the findings have been confounded by several factors such as the absence of a control group, the inclusion of untrained subjects, sub-optimal acclimation programs or the application of an unclear study design. The most convincing study mentioned in this review was conducted by Lorenzo et al. (21). Although we applied a similar study design, they found heat training induced gains of 5 and 6 % for \( \dot{V}O_2 \text{max} \) and Time Trial performance, respectively, when tested at 13 °C (21) which contrasts our study. In this regard, it seems unlikely that such divergence in performance is related to the temperature difference (18 °C vs 13 °C) in which the participants were tested (16). In addition, the degree of adaptations to heat exposure has previously been linked to training status (41). Nonetheless, the participants in the latter study were well trained (\( \dot{V}O_2 \text{max} \approx 67 \text{ ml.min}^{-1}.\text{kg}^{-1} \)) and hence of similar fitness levels to the volunteers included in the present study (\( \dot{V}O_2 \text{max} \approx 61 \text{ ml.min}^{-1}.\text{kg}^{-1} \)). Otherwise, it is noteworthy that in the aforementioned study (21) the training sessions in the heat were conducted at a relatively higher exercise intensity when compared to the training conducted in the control trials. In the present study all trainings were matched to
elicit the same relative cardiovascular strain. Although the difference was rather small (~ 20 %) this may explain, at least in part, the difference in study outcomes. The finding that exercise performance following heat acclimatization is not enhanced in a temperate environment is in agreement with a controlled study performed in competitive cyclists (\(\dot{V}O_2\text{max} \approx 63 \text{ ml.min}^{-1}\cdot\text{kg}^{-1}\)) (16). Here it was demonstrated that compared to control training no additional benefit of two weeks of heat training was apparent when tested in 5 - 13 °C whereas an expected effect was observed when the exercise tests were conducted at 35 °C.

Lorenzo and co-workers (21) speculated that the observed increase in PV (estimated from changes in Htc) of \(\approx 200 \text{ ml}\) could have facilitated exercise capacity in 13 °C. Herein, the heat training induced increase in PV of 201 ± 88 ml did not facilitate exercise performance in 18 °C, which adds to the controversy on whether PV expansion facilitates \(\dot{V}O_2\text{max}\) or Time Trial performance in temperate conditions (7, 47). While there is little doubt that PV expansion can enhance \(\dot{Q}\text{max}\) (3), the concomitant induced hemodilution has to be counterbalanced by an even greater increase in \(\dot{Q}\text{max}\) in order to increase exercise performance. In the present study the increase in PV of 15.7 ± 0.6 % led to an 18.2 ± 2.1 % increase in \(\dot{Q}\text{max}\) and a concomitant 8.2 ± 0.1 % reduction in Hct. Furthermore, as will be discussed below, the expansion of PV did not enhance exercise performance in a hot environment (48).

Therefore, if applying an appropriate study design and controlling for training load, we suggest that heat training does not facilitate exercise performance in a temperate environment any more than ordinary exercise training does.

Heat training-induced increase in plasma volume and exercise performance in the heat

The first speculations that BV might increase in response to a rise in ambient temperature is believed to date back to 1923 (2). Since then, numerous studies have confirmed that exposure to warm environments increases PV after a few days of acclimation, and even so if the length of heat exposure is as little as 1
Hypervolemia is often suggested to be the most important feature of heat acclimation (24, 40). In the current study, heat training augmented PV by 6 ± 2% which is in agreement with previous findings (21, 23). PV was not increased in the control group. In previously untrained individuals, PV usually increases within a few days of exercise training regardless of the thermal environment (1, 14). The reason for the control training not to expand PV is likely related to the already relative fit status of the included participants. Moreover, in the current study, the heat training-induced increase in resting PV was not statistically associated with the concomitantly occurring improvements in $W_{\text{max}}$, $\dot{V}O_{2\text{max}}$ or Time Trial performance which is in line with previous findings (32, 33). However, these studies also reported significant correlations between acclimatization-induced changes in exercise performance and dynamic changes in PV while exercising in the heat, suggesting that PV retention during exercise is more important than absolute resting PV. Of note is that whereas $\dot{V}O_{2\text{max}}$ when tested in 38 °C was increased by 9% following heat training, $\dot{Q}_{\text{max}}$ remained nearly unchanged (21.3 to 21.7 l.min⁻¹).

Since maximal O₂ extraction across the exercising skeletal muscle is reported unchanged following heat training (23, 24) we assume that the ≈ 400 ml numerical higher $\dot{Q}_{\text{max}}$ may at least be partially responsible for the increase in $\dot{V}O_{2\text{max}}$, but that statistical power may have lacked to establish this association. We acknowledge, that 400 ml of extra $\dot{Q}_{\text{max}}$ can not explain the entire 400 ml elevation in $\dot{V}O_{2\text{max}}$, we can however not account for the remaining part. The modest increase in $\dot{Q}_{\text{max}}$ was likely the result of the also modest increase in PV (≈ 200 ml) whereas in a previous study the removal of 382 ml of whole blood reduced $\dot{Q}_{\text{max}}$ by 2.3 l.min⁻¹ (3). In the present study, the acute expansion of PV by 538 ± 16 ml (15%) of albumin solution did not facilitate exercise performance with acute heat exposure, which is in agreement with the only previous study that applied a similar experimental approach (48). Nevertheless, in the present study PV expansion by albumin led to an increase in submaximal and maximal SV and $\dot{Q}$. We speculate that $\dot{V}O_{2\text{max}}$ and Time Trial performance were not enhanced despite these improvements since PV expansion also prompted a 8% reduction of Htc, and thereby likely offset some of the potential blood flow-dependent improvements in O₂ transport to the exercising skeletal muscles. An interesting point
would be to see whether artificial PV expansion might have a different influence on highly dehydrated subjects, where hemoconcentration has occurred. Indication therefore is given by the fact that fluid ingestion during exercise in the heat decelerates fatigue. Dehydration induced reduction in skin and locomotor muscle blood flow rapidly lowers O$_2$ delivery to the exercising legs and the capacity of heat dissipation (11). Therefore, a higher PV might have a beneficial effect on exercise capacity by increasing blood flow to the important organs without lowering Hct. However, this remains to be established.

Acclimation induced normalization of cerebral perfusion (i.e. MCA$_{v_{mean}}$) and its relation to exercise performance

As expected (26), acute exposure to 38 °C reduced MCA$_{v_{mean}}$ during exercise, whereas heat training facilitated MCA$_{v_{mean}}$ during exercise at 38 °C to such an extent that it became restored to levels observed with exercise at 18 °C. This has not been demonstrated previously. MCA$_{v_{mean}}$ is regulated by various factors where, PaCO$_2$ (ventilation) and MAP are the most important ones (31). VE was not reduced with heat training and was hence not associated with the reduction in MCA$_{v_{mean}}$ (R = 0.23) which is in line with previous findings (10). On the other hand we found MAP to be increased with heat training. With exercise in the heat, blood flow to the skin is facilitated to favor cooling (12, 36), and this will inevitably lead to a reduction in blood availability for other organs such as the brain (26). It could hence be speculated that the increase in plasma volume observed with heat training could lead to increased MAP, restored cerebral perfusion and preserved skin perfusion, although this depends on the interplay between the degree of vasodilation and the volume increase in plasma. In the present study, the association between increases in PV and MCA$_{v_{mean}}$ and PV and MAP was R = 0.26 and R = 0.20 (P = 0.57 and P = 0.43), respectively, with a tendency for a moderate association between MCA$_{v_{mean}}$ and MAP (R = 0.41, P = 0.07). Therefore, the mechanisms facilitating MCA$_{v_{mean}}$ with heat acclimation could not be determined. Nonetheless, it should be noted that CVC was not different between pre and post heat acclimation.
measurements. Hence, when accounting for MAP, heat acclimation does not alter MCA\textsubscript{v,mean} which suggests that changes in MCA\textsubscript{v,mean} can be mainly be attributed to changes in MAP.

An augmented MCA\textsubscript{v,mean} could facilitate exercise capacity by several mechanisms. Cerebral hypoxemia has been suggested to facilitate centrally mediated fatigue (35), and a restoration of cerebral oxygenation secondary to enhancing brain blood flow has hence been proposed as a candidate to facilitate exercise performance. However, although the proposed mechanism has received much attention within the last decade (26, 27, 35), in studies in which MCA\textsubscript{v,mean} and cerebral oxygenation have been increased by the administration of small volumes of CO\textsubscript{2} to the inspiration in hypoxia, this has not improved exercise performance (8, 43), also with additional heat exposure (17). It has to be mentioned that when artificially manipulating cerebral blood flow (e.g. with CO\textsubscript{2}), simultaneously other factors such as pH, ventilation and breathing resistance will be affected which all could negatively influence exercise performance. Alternatively an improved cerebral perfusion has been suggested to favor cooling of the brain (28) and thereby improve exercise capacity in the heat but this cannot be addressed in the current study.

As mentioned, MAP was reduced at 38 °C in the study participants. MAP is generally reported being maintained with heat exposure until dehydration occurs (13). Although we cannot exclude that dehydration may have occurred this seems unlikely since the duration of the VO\textsubscript{2,max} test was less than 20 minutes. One possible explanation for the reduced MAP could be related to our pre-heating protocol where participants intentionally initiated the exercise tests hyperthermic which in turn could have decreased MAP. However, since this observation was similarly present before and after the training and in the experimental and the control group it should not have influenced our finding that MAP was increased by heat training.

\textit{Heat training facilitated sweat rate and lowered sweat electrolyte concentration and their effect on exercise performance in the heat}
With heat training a substantial increase in sweat output (+26 ± 6 %) and decrease in sweat [Na+] (-19 ± 7 %) occurred. This is in line with previous research (16, 24), and indicates that substantial heat acclimation had occurred. Despite that these have previously been suggested to facilitate exercise performance in the heat, no such correlations could be established in the current study. It could be speculated however that at least the more diluted sweat could become advantageous especially for longer lasting submaximal events. It also needs to be mentioned that the increased sweat output likely minimally increased evaporative heat loss since sweat rate was certainly beyond the maximal evaporative capacity of a 38 °C environment (i.e. low sweating efficiency).

Limitations to the study

Due to the limited sample size, it cannot be ruled out that a statistical type II error prevented the correlational analysis to reveal associations between the observed performance gains and the measured physiological variables which also applies to the ANOVA testing. As already mentioned, since we raised the participants’ Trec to always the same initial value, part of the potential adaptations, such as submaximal Tcore and HR, may have become blunted. Furthermore, a possible acclimation decay has to be considered. Although the T38 trial was deliberately conducted before the T18 trial to have an additional heat stimulus during the 4 – 5 d post-testing period it can not be excluded that partial de-acclimation in the T18 trial has occurred and therefore influenced our results. Other limitations to the study are that the included study volunteers were not blinded towards the treatment. It is however virtually impossible to blind humans when it comes to heat exposure, and the use of a cross-over study design was judged the best solution. It would have been advantageous to blind the investigators towards the treatment also, but due to staff limitations this was unfortunately impossible. Furthermore, it has to be mentioned that the artificial PV increase was higher (15 %) than the actual heat training induced increase (6 %). The aim was to expand PV equal to or more than what normally occurs with heat training since if we were to expand
by too little a negative result could be argued to derive from too little of an expansion. Some of our study participants displayed an increase in PV of up to 13.5 % which further strengthens this assumption. However, we acknowledge that the higher PV infusion might have led to such hemodilution which might have offset a potential beneficial effect of an elevated Q̇. Besides, it needs to be acknowledged that MCAvmean is only a surrogate measure of cerebral perfusion and that hyperthermia induced vasconstriction may have influenced those results. Also the recording of cerebral oxygenation (NIRS) in the heat proves to be challenging and has to be interpreted with caution. Interfering sweat can cause missing values and also skin blood flow may have introduced bias as this greatly influences cerebral NIRS signal (44). Furthermore, it needs to be acknowledged that also the measurement of Q̇max with the Innocor device has its limitations since it highly depends on the participants’ ability to follow the given breathing pattern. Recently however, we demonstrated the devices ability for repeated measures (42). Lastly, as already mentioned, it has to be acknowledged that in some participants not allowing drinking might have led to hypohydration even though exercise tests were always initiated well hydrated.

The novel findings of the present study are that 10 days of heat training facilitated exercise performance in the heat but not in temperate conditions. Furthermore, adaptations in PV, sweat output and sweat [Na⁺] and cerebral perfusion which have previously been proposed to facilitate exercise performance in the heat were not associated with the observed gains in performance. Lastly, in well trained subjects acute expansion of plasma volume with albumin infusion did not facilitate exercise performance in 38 °C.
References


<table>
<thead>
<tr>
<th></th>
<th>VO₂max test</th>
<th></th>
<th>Time Trial test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T18</td>
<td>T38</td>
<td>T38 A</td>
<td>T18</td>
</tr>
<tr>
<td>Resting Trec, °C</td>
<td>38.0 ± 0.1†</td>
<td>38.8 ± 0.2*</td>
<td>38.9 ± 0.1††</td>
<td>37.9 ± 0.1††</td>
</tr>
<tr>
<td>Maximal Trec, °C</td>
<td>38.5 ± 0.2</td>
<td>38.8 ± 0.1</td>
<td>38.8 ± 0.1</td>
<td>39.2 ± 0.1††</td>
</tr>
<tr>
<td>Resting Tskin, °C</td>
<td>34.0 ± 0.2††</td>
<td>37.0 ± 0.3†*</td>
<td>37.8 ± 0.2**</td>
<td>32.6 ± 0.5††</td>
</tr>
<tr>
<td>Maximal Tskin, °C</td>
<td>36.8 ± 0.5††</td>
<td>37.7 ± 0.3†*</td>
<td>38.2 ± 0.3*</td>
<td>36.4 ± 0.4††</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>T18</th>
<th>T38</th>
<th>T38 A</th>
<th>T18</th>
<th>T38</th>
<th>T38 A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Trec, °C</td>
<td>38.0 ± 0.1†</td>
<td>38.8 ± 0.2*</td>
<td>38.9 ± 0.1††</td>
<td>37.9 ± 0.1††</td>
<td>38.8 ± 0.2**</td>
<td>39.0 ± 0.1**</td>
</tr>
<tr>
<td>Maximal Trec, °C</td>
<td>38.5 ± 0.2</td>
<td>38.8 ± 0.1</td>
<td>38.8 ± 0.1</td>
<td>39.2 ± 0.1††</td>
<td>39.6 ± 0.2†</td>
<td>39.6 ± 0.1†</td>
</tr>
<tr>
<td>Resting Tskin, °C</td>
<td>34.0 ± 0.2††</td>
<td>37.0 ± 0.3†*</td>
<td>37.8 ± 0.2**</td>
<td>32.6 ± 0.5††</td>
<td>36.9 ± 0.4**</td>
<td>37.4 ± 0.8**</td>
</tr>
<tr>
<td>Maximal Tskin, °C</td>
<td>36.8 ± 0.5††</td>
<td>37.7 ± 0.3†*</td>
<td>38.2 ± 0.3*</td>
<td>36.4 ± 0.4††</td>
<td>38.1 ± 0.3*</td>
<td>38.2 ± 0.3*</td>
</tr>
</tbody>
</table>

Resting Trec, resting rectal temperature; Maximal Trec, maximal rectal temperature; Resting Tskin, resting mean skin temperature; Maximal Tskin, maximal mean skin temperature. Values are mean ± SEM. *P < 0.05 and **P < 0.01 vs. T18, †P < 0.05 and †† P < 0.01 vs. T38A. N = 8.
Table 2: Cardiovascular and thermoregulatory responses to pre (Pre)- and post-training (Post) VO₂max (VO₂max test) and 30 min Time Trial (Time Trial test) tests in temperate conditions (T18) and hot conditions (T38) in the experimental (EG) and control group (CG).

<table>
<thead>
<tr>
<th></th>
<th>VO₂max test</th>
<th></th>
<th>Time Trial test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EG</td>
<td>CG</td>
<td>EG</td>
<td>CG</td>
</tr>
<tr>
<td>Q̇sw, l/min¹</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>14.0±0.9</td>
<td>13.3±0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>38 °C</td>
<td>15.3±0.6</td>
<td>15.3±0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q̇peak, l/min¹</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>20.6±0.8</td>
<td>20.3±1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>38 °C</td>
<td>21.3±0.6</td>
<td>21.7±0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SVsw, ml</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>146±8</td>
<td>139±8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>38 °C</td>
<td>135±5</td>
<td>138±9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SVpeak, ml</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>131±5</td>
<td>129±5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>38 °C</td>
<td>129±7</td>
<td>131±6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resting Trec, °C</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>38.0±0.0</td>
<td>37.8±0.0</td>
<td>37.9±0.0</td>
<td>37.7±0.1</td>
</tr>
<tr>
<td>38 °C</td>
<td>38.7±0.1</td>
<td>38.6±0.1</td>
<td>38.6±0.2</td>
<td>38.7±0.1</td>
</tr>
<tr>
<td>Maximal Trec, °C</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>38.7±0.1</td>
<td>38.5±0.1</td>
<td>38.4±0.2</td>
<td>38.4±0.1</td>
</tr>
<tr>
<td>38 °C</td>
<td>38.8±0.1</td>
<td>38.8±0.1</td>
<td>38.7±0.1</td>
<td>38.7±0.1</td>
</tr>
<tr>
<td>Resting Tskin, °C</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>34.2±0.2</td>
<td>32.5±0.7</td>
<td>34.0±0.2</td>
<td>33.0±0.6</td>
</tr>
<tr>
<td>38 °C</td>
<td>36.5±0.5</td>
<td>36.4±0.4</td>
<td>36.2±0.3</td>
<td>36.7±0.4</td>
</tr>
<tr>
<td>Maximal Tskin, °C</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>35.5±0.6</td>
<td>34.9±1.0</td>
<td>35.9±0.4</td>
<td>34.6±1.1</td>
</tr>
<tr>
<td>38 °C</td>
<td>37.2±0.3</td>
<td>36.8±0.3</td>
<td>37.5±0.3</td>
<td>37.9±0.3</td>
</tr>
<tr>
<td>Resting HR, bpm</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>68±4</td>
<td>65±4</td>
<td>66±5</td>
<td>65±6</td>
</tr>
<tr>
<td>38 °C</td>
<td>75±3</td>
<td>75±4</td>
<td>70±4</td>
<td>73±4</td>
</tr>
<tr>
<td>Maximal HR, bpm</td>
<td></td>
<td></td>
<td>Time</td>
<td>Intervention</td>
</tr>
<tr>
<td>18 °C</td>
<td>180±2</td>
<td>176±2</td>
<td>177±3</td>
<td>178±1</td>
</tr>
<tr>
<td>38 °C</td>
<td>179±2</td>
<td>181±2</td>
<td>180±2</td>
<td>180±2</td>
</tr>
</tbody>
</table>

Q̇sw, cardiac output at 80 W; Q̇max, maximal cardiac output; SVsw, stroke volume at 80 W; SVmax, maximal stroke volume; Resting Trec, resting rectal temperature; Maximal Trec, maximal rectal temperature; Resting Tskin, resting mean skin temperature; Maximal Tskin, maximal mean skin temperature; Resting HR, resting heart rate; Maximal HR, maximal heart rate. Values are mean ± SEM. P-values for VO₂max test/Time Trial test. *P < 0.05 and **P < 0.01 vs. pre training. N = 7 for EG, N = 8 for CG.
Table 3: Sweat composition and sweat rate collected during the first (Day 1) and the last (Day 10) training day in the experimental and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 10</td>
<td>Day 1</td>
</tr>
<tr>
<td>[Na⁺] (mmol.L⁻¹)</td>
<td>167 ± 22</td>
<td>127 ± 10*</td>
<td>119 ± 16</td>
</tr>
<tr>
<td>[Ca²⁺] (mmol.L⁻¹)</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>[K⁺] (mmol.L⁻¹)</td>
<td>8.1 ± 2.2</td>
<td>8.3 ± 2.3</td>
<td>12.8 ± 3.0</td>
</tr>
<tr>
<td>[Cl⁻] (mmol.L⁻¹)</td>
<td>159 ± 23</td>
<td>136 ± 20</td>
<td>112 ± 13</td>
</tr>
<tr>
<td>Sweat rate (kg.h⁻¹)</td>
<td>1.44 ± 0.10</td>
<td>1.74 ± 0.11*</td>
<td>0.82 ± 0.09†</td>
</tr>
</tbody>
</table>

[Na⁺], mean sodium concentration; [Ca²⁺], mean calcium concentration; [K⁺], mean potassium concentration; [Cl⁻], mean chloride concentration. Values are mean ± SEM. *P < 0.05 vs. Day 1, †P < 0.05 vs. experimental group. N = 7 for the experimental group, N = 8 for the control group.
Figure legends

Figure 1. Schematic illustration of the study design. The albumin trial was performed in the pre training trials of block 1. The data-set for the experimental trial (Heat) consisted of the pre and post heat training values from block 1 and 2 and the data-set for the control trial (Control) consisted of the pre and post control training values also from block 1 and 2.

Figure 2. Maximal achieved A: Workload (W_{max}; W), B: Oxygen uptake (\bar{V}O_2_{max}; l.min^{-1}) and C: Average power output (P_{avg}; W) in temperate conditions (T18), hot conditions (T38) and hot conditions with prior albumin infusion (T38_A) (before the training intervention). Values are mean ± SEM. *P < 0.05 vs. T18. N = 8.

Figure 3. A: Heart rate (HR; bpm), B: Stroke volume (SV, ml) and C: Cardiac output (Q; l.min^{-1}) in temperate conditions (T18, filled circles), hot conditions (T38, open triangles) and hot conditions with prior albumin infusion (T38_A, crosses) (before the training intervention). Values are mean ± SEM. *P < 0.05 T18 vs. T38A, †P < 0.05 T18 vs. T38. N = 8.

Figure 4. Absolute pre- and post-training responses for the experimental and the control group in maximal achieved A: Workload (W_{max}; W), B: Oxygen uptake (\bar{V}O_2_{max}; l.min^{-1}) and C: Average power output (P_{avg}, W) in temperate conditions (T18) and hot conditions (T38). ‘Time’ refers to pre vs. post training and ‘intervention’ refers to experimental vs. control group. Values are mean ± SEM. *P < 0.05 and **P < 0.01 pre vs. post training within environmental condition. N = 7 for the experimental group and N = 8 for the control group.

Figure 5. A: Ventilation (VE; l.min^{-1}), B: Mean arterial pressure (MAP; mmHg), C: Changes in middle cerebral artery velocity (Delta MCA{\nu}_{mean}; %) and D: Changes in cerebral oxygenation (Delta ScO_2; %) in temperate conditions (T18) and hot conditions (T38) for the experimental group (black lines) and the control group (grey lines). Values are mean ± SEM. *P < 0.05 overall pre vs. post training intervention for T38. N = 7 for the experimental group and N = 8 for the control group for A-C. For D, N = 4 (experimental and control group) for the T38 trial and N = 6 (experimental group) and N = 7 (control group) for the T18 trial.
<table>
<thead>
<tr>
<th>Block 1</th>
<th>3 months washout</th>
<th>Block 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Training Trials</td>
<td>Post Training Trials</td>
<td>Pre Training Trials</td>
</tr>
</tbody>
</table>
| ![Bike](image1)  
N = 8 | 10 d of Heat (N = 4) or Control (N = 4) Training  
(counter-balanced, cross-over design) | 10 d of Heat (N = 3) or Control (N = 4) Training  
(counter-balanced, cross-over design) |
| ![Bike](image2)  
N = 8 | ![Bike](image3)  
N = 8 | ![Bike](image4)  
N = 7 |
| ![Bike](image5)  
N = 8 | ![Bike](image6)  
N = 8 | ![Bike](image7)  
N = 7 |