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

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Keiser S, Flück D, Hüppin F, Stravs A, Hilty MP, Lundby C. Heat training increases exercise capacity in hot but not in temperate conditions: a mechanistic counter-balanced cross-over study. *Am J Physiol Heart Circ Physiol* 309: H750–H761, 2015. First published July 1, 2015; doi:10.1152/ajpheart.00138.2015.—The aim was to determine the mechanisms facilitating exercise performance in hot conditions following heat training. In a counter-balanced order, seven males ($\dot{V}O_{2max}$ 61.2 ± 4.4 ml·min^{-1}·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 $\dot{V}O_{2max}$ and 30-min time trial performance in 18 (T18) and 38°C (T38) before and after training. Blood volume parameters, sweat output, cardiac output ($\dot{Q}$), cerebral perfusion (i.e., middle cerebral artery velocity [$MCAv_{mean}$]), and other variables were determined. Before 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 $MCAv_{mean}$ and thereby limit centrally mediated fatigue. $\dot{V}O_{2max}$ and time trial performance were equally reduced in T38 and $T38_A$ (7.2 ± 1.6 and 9.3 ± 2.5% for $\dot{V}O_{2max}$; 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 $\dot{V}O_{2max}$ 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 [Na^+] became reduced by 19 ± 7%. In T38 $\dot{Q}_{max}$ remained unchanged before (21.3 ± 0.6 l/min) to after (21.7 ± 0.5 l/min) training, whereas $MCAv_{mean}$ was increased by 13 ± 10%. However, none of the observed adaptations correlated with the concomitant observed changes in exercise performance.

hyperthermia; blood volume; performance; training; temperature

NEW & 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.

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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 ($\dot{Q}$) (21) and a reduced submaximal heart rate (HR) (24, 40, 49) and submaximal core temperature (T_{core}) (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 T_{core} during submaximal walking (37) and cycling (48) in humans exposed to heat stress. Likewise, PV expansion did not modify T_{core}, 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 $\dot{V}O_{2max}$ improvement despite restoring middle cerebral artery velocity ($MCAv_{mean}$) by administering small volumes of inspired CO_2 during exercise in hot conditions (17). However, the influence of altering cerebral pH via CO_2 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 $MCAv_{mean}$ 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, although it appears of minor relevance for near maximal or maximal intensity exercise challenges such as 5,000 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 $\dot{V}O_{2max}$ 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 with 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 led to no further improvement in normothermic $\dot{V}O_{2max}$ 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) before 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 counterbalanced 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 yr, 74 ± 3 kg, 182 ± 6 cm, $\dot{V}O_{2max}$ 61.2 ± 4.4 ml·min^{-1}·kg^{-1}, BMI 22.3 ± 1.7, means ± SD) who regularly trained >1 h/day for 3–5 days/wk were recruited to participate in the study. Before participation oral and written informed consent was obtained from each participant. All participants refrained from exercise for 24 h and alcohol and caffeine for 12 h before the experimental tests and fulfilled the inclusion criterion of a $\dot{V}O_{2max}$ >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. A randomized and counter-balanced cross-over design was applied, and, therefore, participants completed both the heat and the control trainings (Fig. 1).

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°C 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 3-mo washout period after which the second block started. During this washout period, participants continued with their regular training and avoided any sojourn to

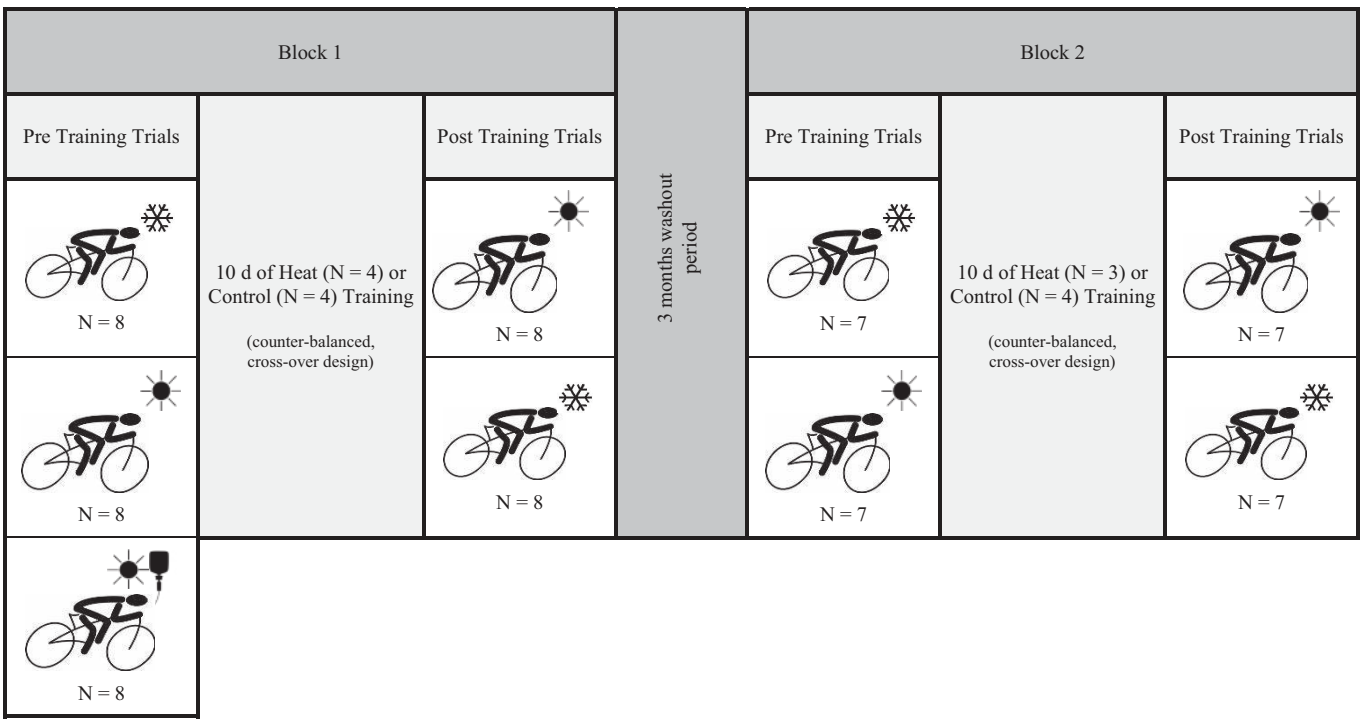

Fig. 1. Schematic illustration of the study design. The albumin trial was performed in the pretraining trials of *Block 1*. The data set for the experimental trial (Heat) consisted of the pre- and postheat training values from *Blocks 1* and *2*, and the data set for the control trial (Control) consisted of the pre- and postcontrol training values also from *Blocks 1* and *2*.

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 ($T38_A$) was conducted in which albumin was infused to expand the PV by 15%. This $T38_A$ trial was conducted once in all participants independently of their subsequent training group assignment and was afterwards compared with the baseline T18 and the T38 trials of the first block.

The performance tests included a maximal incremental exercise test ($\dot{V}O_{2max}$ test) followed by a Time Trial test after a 1-h resting period. To restore fluid compartments and energy stores, ~45 min before the $\dot{V}O_{2max}$ test participants drank 0.4 l of a carbohydrate-electrolyte solution (Isostar) and 0.6 l during the resting period between the $\dot{V}O_{2max}$ 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. After acclimation, studies were completed within 4 to 5 days 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°C to 39°C in the T38 trials and from 17°C to 19.5°C in the cold trials.

Exercise performance tests. Before each exercise test ($\dot{V}O_{2max}$ and Time Trial), participants were immersed into a whole body water bath for 21 ± 5.8 min (means ± SD). The aim herewith was to manipulate rectal (core) temperature without performing exercise. Although before the T18 testing the water temperature was thermoneutral (~34°C), it was ~42°C before the T38 and $T38_A$ trials. This allowed increasing ($P < 0.05$) participants' rectal temperature (T_{rec}) by 0.8 ± 0.5°C (means ± SD) in the T38 and the $T38_A$ trial, whereas in the T18 trial T_{rec} remained unaffected. When target T_{rec} was reached in T38 and $T38_A$, 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°C 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 toward the end of all trials. Maximal workloads reached in the exercise tests were calculated as $W_{max} = W_{compl} + W_{increm} * (t/90)$, with W_{compl} being the last completed workload, W_{increm} 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-gauge catheter placed in an antecubital vein. Preceding the water submersion, a volume corresponding to 15% (538 ± 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 $\dot{V}O_{2max}$ 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 T_{rec} was measured as a surrogate for T_{core}. A flexible rectal probe (YSI M401AC; Advanced Industrial Systems, Louisville, KY) was self-inserted by the participant ~7 cm behind the anal sphincter. Additionally, three skin temperature probes (YSI M409AC; Advanced Industrial Systems, Louisville, KY) were placed on the forehead, the lower back, and the right quadriceps. The average of the three locations was considered as mean skin temperature (T_{skin}). 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 $\dot{V}O_{2max}$ test participants breathed through a mouthpiece (Hans Rudolph, Shawnee, KS) with their noses 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. Before 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 $\dot{V}O_{2max}$.

During the $\dot{V}O_{2max}$ 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 $\dot{Q}$ and SV_{max}, respectively.

$MCAv_{mean}$ 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 $MCAv_{mean}$ 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) on the left forehead. Furthermore, $MCAv_{mean}$ 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 min, 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^+, Ca^{2+}, and Na^+ concentration ([Cl^-], [K^+], [Ca^{2+}], [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 rebreathing 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-gauge 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. Although 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 afterward, participants were switched to a closed rebreathing circuit and breathed 1.5 ml/kg 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 Hb_{mass} and 3.8% for PV.

Statistical analysis. Single differences between the T18, T38, and the $T38_A$ trial were evaluated with a one-way ANOVA for repeated measures, whereas a repeated two-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, $T38_A$) as main effects was applied for the parameters recorded at different intensities. To detect changes from pre to post again, a two-way ANOVA for repeated measures with two times (pre and post) and two interventions (experimental and control) was performed. The parameters recorded at different intensities were analyzed with a three-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 $d \leq 0.2$ 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 are expressed as means ± SE unless otherwise indicated. Statistical analysis was performed using SAS Enterprise Guide (4.3; SAS Institute, Cary, NC).

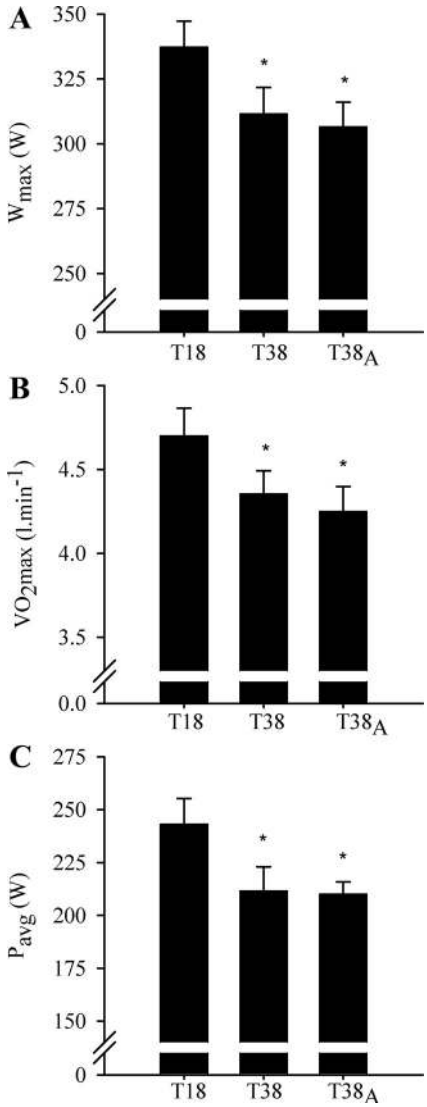

Fig. 2. Maximal achieved workload (W_{max}; in W) (*A*), oxygen uptake ($\dot{V}O_{2max}$; in l/min) (*B*), and average power output (P_{avg}; in W) (*C*) in temperate conditions (30-min time trial performance in 18°C, or T18), hot conditions (30-min time trial performance in 38°C, or T38), and hot conditions with prior albumin infusion (30-min time trial performance in 38°C with an albumin solution, or $T38_A$) (before the training intervention). Values are means ± SE. *$P < 0.05$ vs. T18; $N = 8$.

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 before the exercise training intervention), however, was completed by all eight volunteers.

Exercise performance with acute heat exposure following plasma volume expansion. W_{max} (Fig. 2*A*), $\dot{V}O_{2max}$ (Fig. 2*B*), and P_{avg} (Fig. 2*C*) were highest when conducted during the T18 trial. When compared with 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 $\dot{Q}$ assessed during the $\dot{V}O_{2max}$ test. Overall HR was higher ($P < 0.01$) during the T38 compared with the T18 trial. HR was decreased ($P < 0.01$) in the $T38_A$ trial compared with the T38 trial but still elevated ($P < 0.01$) compared with the T18 trial. Overall, albumin infusion led to a higher ($P < 0.01$) SV and $\dot{Q}$ compared with the T18 and T38 trial. Furthermore, overall SV but not $\dot{Q}$ was decreased ($P < 0.01$ and $P = 0.97$, respectively) when conducted during the T38 trial compared with the T18 trial. The same applied for SV_{max} and $\dot{Q}_{max}$, which were enhanced ($P < 0.01$) with albumin infusion compared with 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 with the T38 trial. MAP was

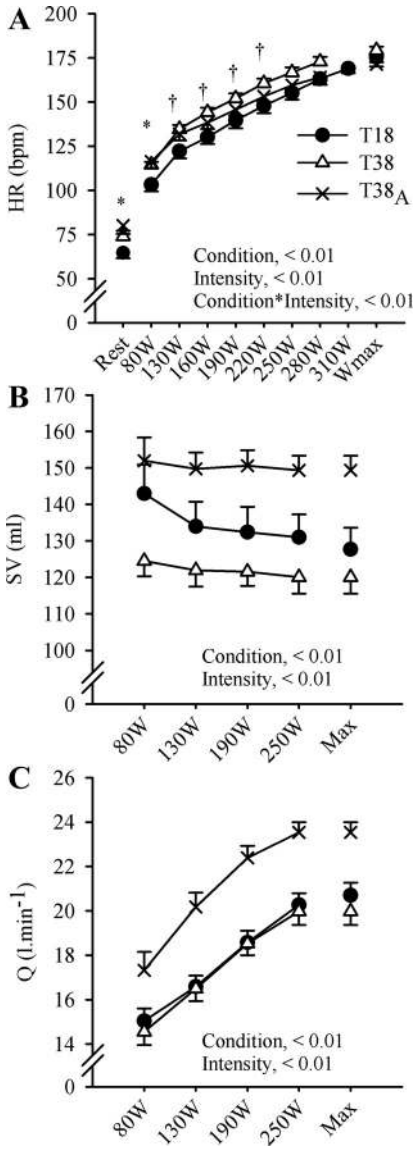

Fig. 3. Heart rate [HR; in beats/min (bpm)] (*A*), stroke volume (SV; in ml) (*B*), and cardiac output ($\dot{Q}$; in l/min) (*C*) in temperate conditions (T18; ●), hot conditions (T38; △), and hot conditions with prior albumin infusion ($T38_A$; ×) (before the training intervention). Values are means ± SE. *$P < 0.05$, T18 vs. $T38_A$; †$P < 0.05$, T18 vs. T38; $N = 8$.

increased ($P < 0.01$) in the $T38_A$ trial compared with the T38 trial but was still reduced ($P < 0.01$) compared with the T18 trial. $MCAv_{mean}$ was also reduced in the T38 trial compared with the T18 trial. With albumin infusion, however, $MCAv_{mean}$ 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 before 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 $T38_A$ trial than in the T18 trial. Furthermore, during the T38 and $T38_A$, trial maximal T_{rec} and T_{skin} were higher ($P < 0.05$) compared with the T18 trial in the Time Trial but not in the $\dot{V}o_{2max}$ 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 with 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_{max} (8.5 ± 2.2%, $P < 0.01$), $\dot{V}o_{2max}$ (4.1 ± 1.4%, $P < 0.05$), and P_{avg} (13.1 ± 2.9%, $P < 0.01$) was apparent in the T38 trial compared with 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_{max}, $\dot{V}o_{2max}$, and Time Trial P_{avg}. In the experimental group, W_{max} increased by 7.9 ± 1.7% (311 ± 13 vs. 335 ± 12 W, $P < 0.01$, d = 0.74), $\dot{V}o_{2max}$ by 9.6 ± 2.1% (4.3 ± 0.2 vs. 4.8 ± 9.2 l/min, $P < 0.05$, d > 0.80), and P_{avg} by 10.4 ± 3.1% (208 ± 12 vs. 228 ± 11 W, $P < 0.01$, d = 0.63). In contrast neither were elevated compared with preheat training when tested in T18, since there was no main effect of time ($P = 0.19$ for W_{max}, $P = 0.11$ for $\dot{V}o_{2max}$, and $P =$ for 0.32 for P_{avg}) or intervention ($P = 0.61$ for W_{max}, $P = 0.22$ for $\dot{V}o_{2max}$, and $P = 0.27$ for P_{avg}), and no interaction of the two ($P = 0.52$ for W_{max}, $P = 0.57$ for $\dot{V}o_{2max}$, and $P = 0.64$ for P_{avg}) from pre to post (340 ± 13 vs. 350 ± 10 W, d = 0.32 for W_{max}, 4.5 ± 0.2 vs. 4.7 ± 0.1 l/min, d = 0.43 for $\dot{V}o_{2max}$ and 240 ± 15 vs. 246 ± 14 W, d = 0.16 for P_{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 rebreathing revealed a significant time*intervention interaction ($P < 0.05$), due to an increase in PV for the experimental group (+6 ± 2% from 3,370 ± 115 to 3,571 ± 169 ml; $P < 0.05$; d = 0.53) but an unaltered PV for the control group (3,524 ± 128 vs. 3,467 ± 135 ml; $P = 0.80$; d = 0.15).

Heat training did not affect RBCV (2,563 ± 113 vs. 2,523 ± 111 ml; d = 0.19), and also in the control group no differences in RBCV (2,546 ± 98 vs. 2,511 ± 95 ml; d = 0.13) were observed, since 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 (5,916 ± 210 vs. 6,111 ± 257 ml; $P = 0.12$; d = 0.31). Also in the control group, BV remained unchanged from pre- to posttraining (6,089 ± 200 vs. 5,949 ± 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 $\dot{Q}_{max}$ or SV_{max}. T_{rec} in the T38 trials was always elevated to the same initial temperature by water immersion, and therefore resting T_{rec} did not differ from pre- to posttraining. In contrast, we did not control for T_{rec} in the T18 trials, and therefore participants' resting T_{rec} varied by ~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

Table 1. *Thermoregulatory responses to exercise ($\dot{V}O_{2max}$ and time trial tests) in temperate conditions (T18), hot conditions (T38), and hot conditions with prior albumin infusion ($T38_A$)*

	$\dot{V}O_{2max}$ Test			Time Trial Test		
	T18	T38	$T38_A$	T18	T38	$T38_A$
Resting T_{rec}, °C	38.0 ± 0.1†	38.8 ± 0.2*	38.9 ± 0.1*	37.9 ± 0.1††	38.8 ± 0.2**	39.0 ± 0.1**
Maximal T_{rec}, °C	38.5 ± 0.2	38.8 ± 0.1	38.8 ± 0.1	39.2 ± 0.1††	39.6 ± 0.2*	39.6 ± 0.1*
Resting T_{skin}, °C	34.0 ± 0.2††	37.0 ± 0.3†*	37.8 ± 0.2**	32.6 ± 0.5††	36.9 ± 0.4**	37.4 ± 0.8**
Maximal T_{skin}, °C	36.8 ± 0.5†	37.7 ± 0.3*	38.2 ± 0.3*	36.4 ± 0.4†	38.1 ± 0.3*	38.2 ± 0.3*

Values are means ± SE; $n = 8$. Resting T_{rec}, resting rectal temperature; maximal T_{rec}, maximal rectal temperature; resting T_{skin}, resting mean skin temperature; maximal T_{skin}, maximal mean skin temperature; T18, $\dot{V}O_{2max}$ or 30-min time trial performance in 18°C; T38, $\dot{V}O_{2max}$ or 30-min time trial performance in 38°C; $T38_A$, $\dot{V}O_{2max}$ or 30-min time trial performance in 38°C with an albumin solution. *$P < 0.05$ and **$P < 0.01$ vs. T18; †$P < 0.05$ and ††$P < 0.01$ vs. $T38_A$.

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 with the T18 trial (Fig. 5*A*). 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 with T18 trials (Fig. 5*B*). 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.

$\Delta MCAv_{mean}$ (%baseline) for both groups are displayed in Fig. 5*C*. When compared with the control group, in the experimental group percent baseline $MCAv_{mean}$ 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 $MCAv_{mean}$ 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, respectively, in T38 compared with T18. In the experimental group, exercise training increased ($P < 0.05$) $MCAv_{mean}$ in T38 and reached similar absolute resting and maximal values as in the T18 trial.

In line with a reduced $MCAv_{mean}$, cerebral oxygenation was also decreased ($P < 0.05$) in the T38 compared with the T18 trials in both the experimental and the control group (Fig. 5*D*). However, the enhanced $MCAv_{mean}$ 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; $MCAv_{mean}$/MAP) demonstrated no significant change from pre- to postheat 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 [Na^+] decreased by 19 ± 7% ($P < 0.05$, d > 0.80) from *day 1* to *day 10*, whereas 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 with that of the control group during trainings on *days 1* and *10*. Furthermore, a 26 ± 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, $MCAv_{mean}$, HR, $\dot{Q}$ and T_{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 $\dot{Q}_{max}$ to 0.3 for PV, which can be considered as small to moderate correlations.

DISCUSSION

The main findings in the present study are *1*) heat training facilitated $\dot{V}O_{2max}$ and Time Trial exercise performance in the heat (38°C) but not in normal (18°C) thermal conditions; *2*) improved exercise performance did not correlate with adaptations in $MCAv_{mean}$ (i.e., cerebral perfusion) as well as PV, sweat output, and sweat [Na^+] following heat training; and finally *3*) 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_{core}, and sweat electrolyte concentration but also an increased PV and sweat output. In the present study, we decided to passively preheat participants before the maximal incremental exercise and the Time Trial tests to always have the same initial T_{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 $\dot{V}O_{2max}$ 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

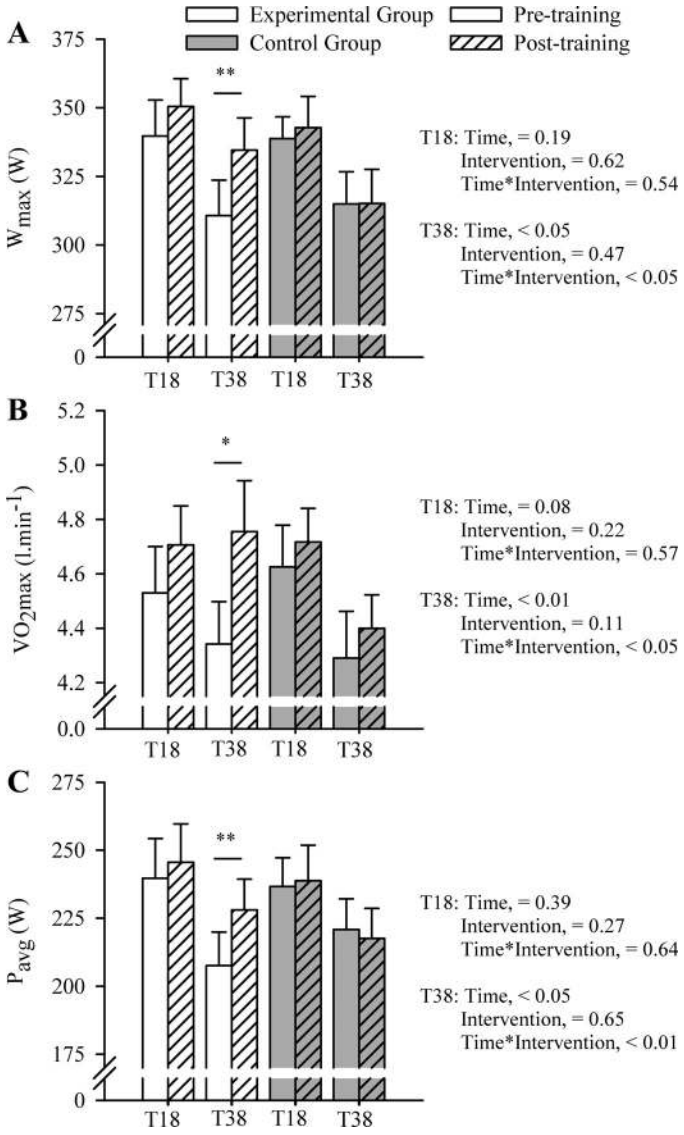

Fig. 4. Absolute pre- and posttraining responses for the experimental and the control group in maximal achieved. W_{max} (in W; *A*), $\dot{V}O_{2max}$ (in l/min; *B*), and P_{avg} (in W; *C*) in temperate conditions (T18) and hot conditions (T38) are shown. Time refers to pre- vs. posttraining, and intervention refers to experimental vs. control group. Values are means ± SE. $*P < 0.05$ and $**P < 0.01$, pre- vs. posttraining within environmental condition; $N = 7$ for the experimental group and $N = 8$ for the control group.

will be discussed later, we also applied this approach to have a similar study design to that of Lorenzo and coworkers (21). In contrast, choosing such study design will most likely have blunted the potential acclimation induced reductions in submaximal T_{rec} and HR. However, because 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.

Exercise performance in 18° and 38°C following heat training. As expected (21, 45), in passively preheated participants, acute heat exposure led to a 4.1 ± 1.4% and 13.1 ± 2.9% reduction in $\dot{V}O_{2max}$ and Time Trial performance, respectively. Also, the observed increase in $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ fully compensated the initial heat-induced decrement, and also W_{max} and P_{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, suboptimal 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 $\dot{V}O_{2max}$ 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 ($\dot{V}O_{2max} \approx 67$ ml·min^{-1}·kg^{-1}) and hence of similar fitness levels to the volunteers included in the present study ($\dot{V}O_{2max} \approx 61$ ml·min^{-1}·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 with 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 ($\dot{V}O_{2max} \approx 63$ ml·min^{-1}·kg^{-1}) (16). Here it was demonstrated that when compared with control training, no additional benefit of 2 wk 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 coworkers (21) speculated that the observed increase in PV (estimated from changes in Htc) of ≈200 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 $\dot{V}O_{2max}$ or Time Trial performance in temperate conditions (7, 47). Although there is little doubt that PV expansion can enhance $\dot{Q}_{max}$ (3), the concomitant-induced hemodilution has to be counterbalanced by an even greater increase in $\dot{Q}_{max}$ 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 $\dot{Q}_{max}$ and a concomitant 8.2 ± 0.1% reduction in

Table 2. *Cardiovascular and thermoregulatory responses to pre- and posttraining $\dot{V}O_{2max}$ and 30-min time trial tests in temperate conditions (T18) and hot conditions (T38) in the EG and CG*

	$\dot{V}O_{2max}$ Test				Time Trial Test						
	EG		CG		EG		CG		*P* Value		
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Time	Intervention	Interaction
$\dot{Q}_{80W}$, l/min											
18°C	14.0 ± 0.9	13.3 ± 0.7	14.0 ± 0.4	13.3 ± 0.6	—	—	—	—	0.29/−	0.39/−	0.72/−
38°C	15.3 ± 0.6	15.3 ± 0.6	14.2 ± 0.6	15.1 ± 0.7	—	—	—	—	0.67/−	0.06/−	0.88/−
$\dot{Q}_{peak}$, l/min											
18°C	20.6 ± 0.8	20.3 ± 1.0	20.8 ± 0.7	19.9 ± 0.9	—	—	—	—	0.15/−	0.77/−	0.57/−
38°C	21.3 ± 0.6	21.7 ± 0.5	20.0 ± 1.1	20.1 ± 0.8	—	—	—	—	0.29/−	0.25/−	0.22/−
SV_{80W}, ml											
18°C	146 ± 8	139 ± 8	133 ± 6	135 ± 7	—	—	—	—	0.62/−	<0.05/−	0.60/−
38°C	135 ± 5	138 ± 9	127 ± 9	130 ± 6	—	—	—	—	0.87/−	0.05/−	0.81/−
SV_{peak}, ml											
18°C	131 ± 5	129 ± 5	122 ± 5	121 ± 5	—	—	—	—	0.79/−	0.06/−	0.08/−
38°C	129 ± 7	131 ± 6	118 ± 7	117 ± 5	—	—	—	—	0.63/−	0.34/−	0.96/−
Resting T_{rec}, °C											
18°C	38.0 ± 0.0	37.8 ± 0.0	37.9 ± 0.2	37.7 ± 0.2	37.9 ± 0.0	37.7 ± 0.1	37.9 ± 0.1	37.6 ± 0.1	<0.05/<0.05	0.31/0.29	0.97/0.49
38°C	38.7 ± 0.1	38.6 ± 0.1	38.6 ± 0.1	38.7 ± 0.1	38.5 ± 0.2	38.7 ± 0.1	38.6 ± 0.2	38.7 ± 0.0	0.94/0.22	0.63/0.63	0.19/0.98
Maximal T_{rec}, °C											
18°C	38.7 ± 0.1	38.5 ± 0.1	38.4 ± 0.2	38.4 ± 0.1	39.2 ± 0.1	38.8 ± 0.2	39.2 ± 0.1	38.6 ± 0.3	0.57/<0.05	0.27/0.31	0.45/0.34
38°C	38.8 ± 0.1	38.8 ± 0.1	38.7 ± 0.1	38.7 ± 0.1	39.7 ± 0.2	39.6 ± 0.2	39.5 ± 0.1	39.6 ± 0.1	0.53/0.71	0.31/0.59	0.86/0.51
Resting T_{skin}, °C											
18°C	34.2 ± 0.2	32.5 ± 0.7	34.0 ± 0.2	33.0 ± 0.6	33.3 ± 0.6	32.6 ± 0.4	32.6 ± 0.4	32.7 ± 0.7	0.06/0.53	0.50/0.52	0.26/0.46
38°C	36.5 ± 0.5	36.4 ± 0.4	36.2 ± 0.3	36.7 ± 0.4	36.0 ± 0.6	35.9 ± 0.4	36.0 ± 0.6	36.6 ± 0.4	0.59/0.30	0.97/0.71	0.46/0.68
Maximal T_{skin}, °C											
18°C	35.5 ± 0.6	34.9 ± 1.0	35.9 ± 0.4	34.6 ± 1.1	36.2 ± 0.6	35.6 ± 0.8	36.2 ± 0.3	35.4 ± 1.1	0.25/0.47	0.92/0.80	0.69/0.86
38°C	37.2 ± 0.3	36.8 ± 0.3	37.5 ± 0.3	37.9 ± 0.3	37.7 ± 0.5	37.3 ± 0.1	37.8 ± 0.4	38.0 ± 0.2	0.99/0.75	<0.05/0.47	0.19/0.61
Resting HR, beats/min											
18°C	68 ± 4	65 ± 4	66 ± 5	65 ± 6	73 ± 10	67 ± 4	68 ± 5	70 ± 6	0.36/0.52	0.64/0.96	0.87/0.45
38°C	75 ± 3	75 ± 4	70 ± 4	73 ± 4	79 ± 3	80 ± 3	79 ± 7	83 ± 4	0.51/0.91	0.29/0.40	0.70/0.60
Maximal HR, beats/min											
18°C	180 ± 2	176 ± 2	177 ± 3	178 ± 1	182 ± 4	177 ± 3	180 ± 1	180 ± 2	0.40/0.20	0.82/0.84	0.25/0.16
38°C	179 ± 2	181 ± 2	180 ± 2	180 ± 1	183 ± 4	186 ± 2	181 ± 2	179 ± 4	0.32/0.96	0.99/0.05	0.60/0.23

Values are means ± SE; $N = 7$ for the experimental group (EG) and $N = 8$ for the control group (CG). $\dot{Q}_{80W}$, cardiac output at 80 W; $\dot{Q}_{max}$, maximal cardiac output; SV_{80W}, stroke volume at 80 W; SV_{max}, maximal stroke volume; HR, heart rate; Pre, pretraining; post, posttraining. *P* values for $\dot{V}O_{2max}$ test/time trial test are shown.

Hct. Furthermore, as will be discussed below, the expansion of PV did not enhance exercise performance in a hot environment (48).

Therefore, if an appropriate study design and controlling for training load is applied, 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 h/day (23, 24, 40, 49). 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_{max}, $\dot{V}O_{2max}$, 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 $\dot{V}O_{2max}$ when tested in 38°C was increased by 9% following heat training, $\dot{Q}_{max}$ remained nearly unchanged (21.3 to 21.7 l/min). Because maximal O_2 extraction across the exercising skeletal muscle is reported unchanged following heat training (23, 24), we assume that the ≈400 ml numerical higher $\dot{Q}_{max}$ may at least be partially responsible for the increase in $\dot{V}O_{2max}$, but that statistical power may have lacked to establish this association. We acknowledge that 400 ml of extra $\dot{Q}_{max}$ cannot explain the entire 400 ml elevation in $\dot{V}O_{2max}$; we cannot, however, account for the remaining part. The modest increase in $\dot{Q}_{max}$ 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

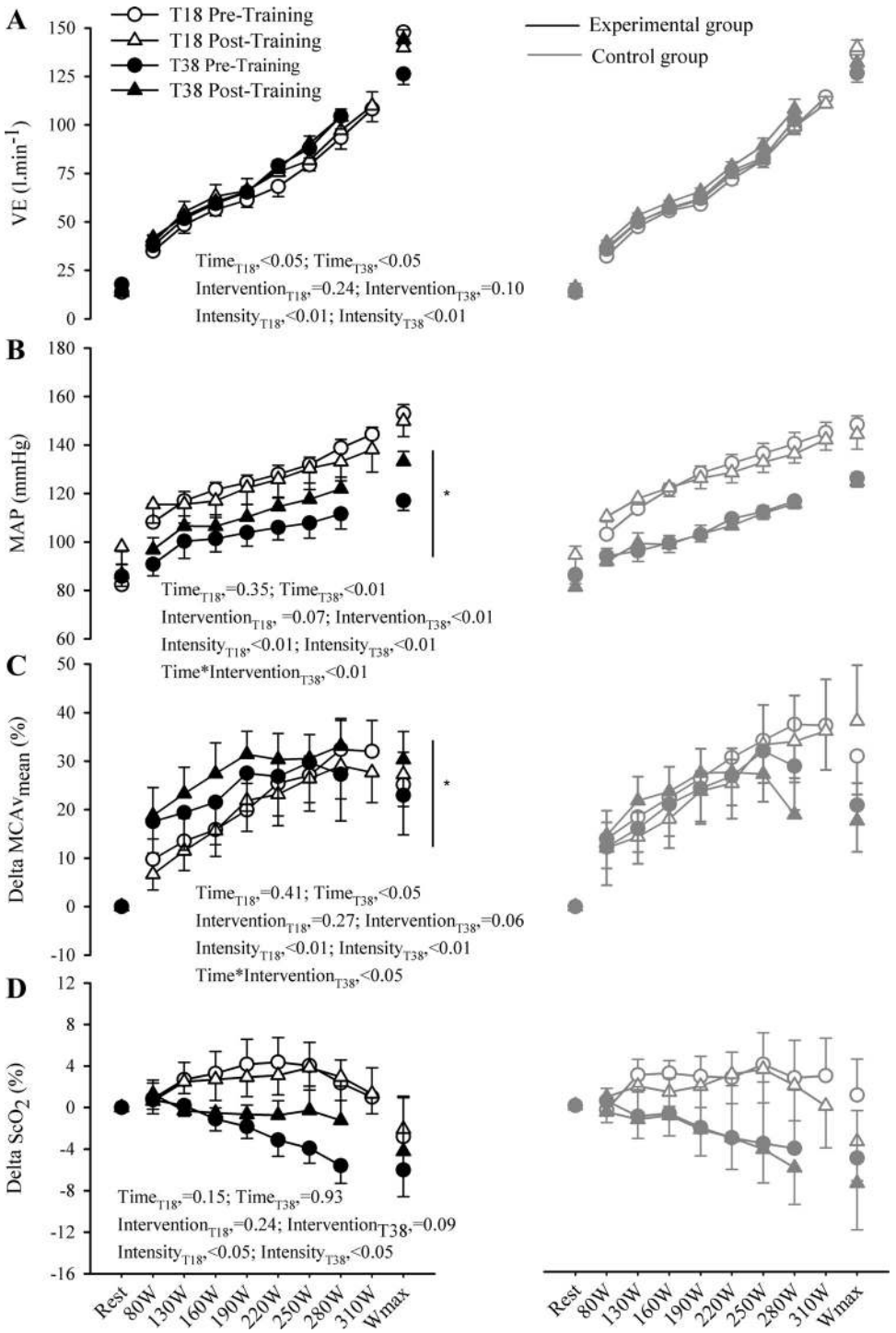

Fig. 5. Ventilation (VE; in l/min) (*A*), mean arterial pressure (MAP; in mmHg) (*B*), changes in middle cerebral artery velocity ($\Delta MCAv_{mean}$; in percentage) (*C*), and changes in cerebral oxygenation (ΔSco_2; in percentage) (*D*) in temperate conditions (T18) and hot conditions (T38) for the experimental group (black lines) and the control group (gray lines). Values are means ± SE. $*P < 0.05$, overall pre- vs. posttraining 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.

$\dot{Q}_{max}$ by 2.3 l/min (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 $\dot{V}O_{2max}$ and Time Trial performance were not enhanced despite these improvements, since PV expansion also prompted an 8% reduction of Htc, and thereby likely offset some of the potential blood flow-dependent improvements in O_2 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

Table 3. *Sweat composition and sweat rate collected during the first training day (Day 1) and the last training day (Day 10) in the experimental and the control groups*

	Experimental Group		Control Group		*P* Value		
	Day 1	*Day 10*	*Day 1*	*Day 10*	Time	Intervention	Interaction
Mean concentration, mmol/l							
Sodium	167 ± 22	127 ± 10*	119 ± 16	123 ± 14	<0.05	0.16	<0.05
Calcium	0.7 ± 0.2	0.9 ± 0.2	1.1 ± 0.3	0.9 ± 0.1	0.85	0.39	0.31
Potassium	8.1 ± 2.2	8.3 ± 2.3	12.8 ± 3.0	11.2 ± 1.9	0.80	0.13	0.60
Chloride	159 ± 23	136 ± 20	112 ± 13	113 ± 14	0.60	0.10	0.55
Sweat rate, kg/h	1.44 ± 0.10	1.74 ± 0.11*	0.82 ± 0.09†	0.83 ± 0.23†	<0.05	<0.01	<0.05

Values are means ± SE; $N = 7$ for the experimental group and $N = 8$ for the control group. *$P < 0.05$ vs. *Day 1*; †$P < 0.05$ vs. experimental group.

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., $MCAv_{mean}$) and its relation to exercise performance. As expected (26), acute exposure to 38°C reduced $MCAv_{mean}$ during exercise, whereas heat training facilitated $MCAv_{mean}$ 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. $MCAv_{mean}$ 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 $MCAv_{mean}$ ($R = 0.23$), 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 $MCAv_{mean}$ 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 $MCAv_{mean}$ and MAP ($R = 0.41$; $P = 0.07$). Therefore, the mechanisms facilitating $MCAv_{mean}$ with heat acclimation could not be determined. Nonetheless, it should be noted that CVC was not different between pre- and postheat acclimation measurements. Hence, when MAP was accounted for, heat acclimation does not alter $MCAv_{mean}$, which suggests that changes in $MCAv_{mean}$ can be mainly be attributed to changes in MAP.

An augmented $MCAv_{mean}$ 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 $MCAv_{mean}$ and cerebral oxygenation have been increased by the administration of small volumes of CO_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 cerebral blood flow (e.g., with CO_2) was artificially manipulated, 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 $\dot{V}O_{2max}$ test was less than 20 min. One possible explanation for the reduced MAP could be related to our preheating protocol where participants intentionally initiated the exercise tests hyperthermic, which in turn could have decreased MAP. However, because 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.

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, because we raised the participants' T_{rec} to always the same initial value, part of the potential adaptations, such as submaximal T_{core} 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- to 5-day posttesting period, it cannot 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 toward 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 toward 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 $\dot{Q}$. Besides, it needs to be acknowledged that $MCAv_{mean}$ 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 since this greatly influences cerebral NIRS signal (44). Furthermore, it needs to be acknowledged that also the measurement of $\dot{Q}_{max}$ 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). Finally, 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. Finally, in well-trained subjects acute expansion of plasma volume with albumin infusion did not facilitate exercise performance in 38°C.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.K. and C.L. conception and design of research; S.K., D.F., F.H., A.S., and M.P.H. performed experiments; S.K. analyzed data; S.K. and C.L. interpreted results of experiments; S.K. prepared figures; S.K. and C.L. drafted manuscript; S.K., D.F., F.H., A.S., M.P.H., and C.L. approved final version of manuscript; D.F., F.H., A.S., and M.P.H. edited and revised manuscript.

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