DEHYDRATION ACCELERATES REDUCTIONS IN CEREBRAL BLOOD FLOW DURING PROLONGED EXERCISE IN THE HEAT WITHOUT COMPROMISING BRAIN METABOLISM

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Abstract
Dehydration hastens the decline in cerebral blood flow (CBF) during incremental exercise, while the cerebral metabolic rate for oxygen (CMRO$_2$) is preserved. It remains unknown whether CMRO$_2$ is also maintained during prolonged exercise in the heat and whether an eventual decline in CBF is coupled to fatigue. Two studies were undertaken. In study 1, ten male cyclists cycled in the heat for ~2 h with (control) and without fluid replacement (dehydration) while internal (ICA) and external (ECA) carotid artery blood flow and core and blood temperature were obtained. Arterial and internal jugular venous blood samples were assessed with dehydration to evaluate the CMRO$_2$. In study 2 (8 males), middle cerebral artery blood velocity (MCA $V_{mean}$) was measured during prolonged exercise to exhaustion in both dehydrated and euhydrated states. After a rise at the onset of exercise, ICA flow declined to baseline with progressive dehydration ($P < 0.05$). However, cerebral metabolism remained stable through enhanced oxygen and glucose extraction ($P < 0.05$). ECA flow increased for one hour but declined prior to exhaustion. Fluid ingestion maintained cerebral and extra-cranial perfusion throughout non-fatiguing exercise. During exhaustive exercise, however, euhydration delayed but did not prevent the decline in cerebral perfusion. In conclusion, during prolonged exercise in the heat dehydration accelerates the decline in CBF without affecting CMRO$_2$ and also restricts extra-cranial perfusion. Thus fatigue is related to reduction in CBF and extra-cranial perfusion rather than in CMRO$_2$. 
**Key words:** Cerebral blood flow, dehydration, extra-cranial blood flow, prolonged exercise
New & Noteworthy (50 words)

Dehydration accrued during prolonged exercise in the heat accelerates the decline in cerebral blood flow and restricts extra-cranial perfusion may be coupled to fatigue. However, cerebral metabolism remains stable through enhanced oxygen and glucose extraction. Thus fatigue developed during prolonged exercise with dehydration is related to reduction in CBF and extra-cranial perfusion rather than to CMRO\textsubscript{2}.
Abbreviations

[A] - Plasma adrenaline

CBF – Cerebral blood flow

CCA – Common carotid artery

CMRO₂ – Cerebral metabolic rate for oxygen:

ECA – External carotid artery

[Hb] – Hemoglobin content

HR – Heart rate

ICA – Internal carotid artery

MAP – Mean arterial pressure

MCA \( V_{\text{mean}} \) – Middle cerebral artery mean blood velocity

[NA] – Plasma noradrenaline

OCI – Oxygen-to-carbohydrate index

OGI – Oxygen-to-glucose index

\( P_a \text{CO}_2 \) – Partial pressure of arterial carbon dioxide

\( P_v \text{CO}_2 \) – Partial pressure of venous carbon dioxide

\( \dot{Q} \) – Cardiac output

\( T_b \) – Blood temperature

\( T_c \) – Core temperature

\( T_m \) – Muscle temperature

\( \overline{T_{sk}} \) – Mean skin temperature
$\dot{V}O_{2max}$ – Maximal oxygen uptake

$W_{max}$ – Maximal work rate
**Introduction**

Dehydration and hyperthermia accrued during prolonged exercise in the heat pose a challenge to cardiovascular control, evidenced by reductions in stroke volume, cardiac output ($\dot{Q}$), skeletal muscle and skin blood flow and to a lesser extent mean arterial pressure (MAP) (12, 16, 29). The cardiovascular strain imposed by combined dehydration and hyperthermia might also encompass cerebral blood flow (CBF) as an orthostatic challenge (35), pharmacological interventions that depress $\dot{Q}$ (19) and also passive heat stress (2, 31), and combined heat stress and exercise (34) compromise cerebral perfusion. However, the role of hydration on the effect of prolonged exercise-induced dehydration on CBF and cerebral metabolism (CMRO$_2$) is unknown.

Reductions in oxygen and substrate supply can compromise organ and tissue metabolism (12) and the circulatory strain induced by dehydration during prolonged exercise in a hot environment may compromise cerebral substrate delivery to the extent that impairs CMRO$_2$ and central nervous system function, ultimately curtailing performance. CMRO$_2$ is maintained during incremental exercise (44, 15), but it remains unknown whether CBF and CMRO$_2$ are preserved during prolonged exercise in the heat or whether eventual deviations relate to fatigue.

Thermoregulatory processes increase and modify the distribution of skin and deep tissue blood flow (6, 7) to regulate body temperature during exercise, particularly during heat stress (14). Across the head circulation, increasing skin and body temperatures
lead to progressive elevations in external-caroid artery (ECA) blood flow as evidenced during incremental exercise in normothermia (40) and with passive heat stress (2, 36). The ECA serves the cutaneous circulation of the neck and face which is important for heat liberation. In contrast, CBF progressively declines at high exercise intensities and with high body temperature (36, 41). Altered perfusion pressure, blood gas tensions (particularly the partial pressure of arterial CO$_2$; $P_a$CO$_2$) (48) and sympathetic activity (28) have been implicated in the control of CBF whereas body temperature is known to influence ECA blood flow (36, 40, 41). No study has characterized the regional distribution of blood flow across the head during prolonged exercise, with and without progressive reductions in total body water.

The aim of the present study was to assess the effect of dehydration on cerebral and extra-cranial hemodynamics and CMRO$_2$ during prolonged exhaustive exercise in the heat. A second aim was to gain insight into potential mechanisms underlying the CBF responses to dehydration and prolonged exercise during non-exhaustive and exhaustive exercise. We hypothesized that dehydration accrued during prolonged exercise in the heat would reduce CBF but that CMRO$_2$ would be maintained. A second hypothesis was that ECA blood flow would increase in relation to an increasing core temperature. Finally, we hypothesized that the cerebral circulatory strain occurring during strenuous exercise in the heat is an epiphenomenon of fatigue, rather than the direct consequence of dehydration.
Methods

Fully informed, written consent was obtained from the participants prior to the studies. All procedures were approved by the Brunel University London Research Ethics Committee (RE07-11 and RE20-09) and conformed to the guidelines of the World Medical Association (Declaration of Helsinki).

Ten healthy endurance-trained males (mean ± SD; age of 29 ± 5 yrs, stature of 183 ± 5 cm, mass of 78 ± 9 kg, and aerobic capacity (\(\dot{V}O_2\)max) of 59 ± 6 ml·kg·min\(^{-1}\)) participated in study 1. In study 2, the age, stature, body mass, and \(\dot{V}O_2\)max of the eight endurance trained males were 33 ± 4 yrs., 173 ± 4 cm, 75 ± 11 kg, and 56 ± 7 ml·kg·min\(^{-1}\), respectively. All participants arrived at the laboratory with a normal hydration status and were required to have abstained from alcohol intake for 24 h and caffeine consumption for 12 h.

Experimental protocols

The experimental design of study 1 is described (44). The participants visited the laboratory for 3 preliminary days followed by 2 experimental days, each separated by at least one week. During the preliminary days the participants were familiarized with the methodology before completing incremental exercise on a semi-recumbent cycle ergometer (Lode Angio, Groningen, Netherlands), with a backrest inclination of 45 °, to establish the maximal work rate (\(W_{max}\)), maximal heart rate, and \(\dot{V}O_2\)max. Participants were then familiarized to the heat and experimental protocol by cycling in an environmental chamber set at 35 °C (relative humidity 50%) in the semi-recumbent
position for 2 h at 55% $W_{\text{max}}$ with heart rate and intestinal temperature recorded. No fluid consumption was permitted during exercise. To determine hydration status body mass was recorded before and immediately post exercise in all trials.

On the two experimental days (visits 4 and 5), the participants performed prolonged (~2 h) continuous cycling exercise, following an initial incremental exercise test and 1 h of rest. In the first experimental trial, participants were not permitted to consume fluid during prolonged exercise whereas, on the second experimental trial (i.e., control trial), participants completed the same exercise protocol, but hydration was maintained through fluid ingestions according to the participants’ body mass loss during the previous visit. Fluid was provided in aliquots of ~200 ml every 10 min during exercise. Both experimental trials were performed in the heat (same conditions as in the familiarization sessions) and pedal cadence was maintained at 70-90 r.p.m.

In the dehydration trial, CBF and blood samples from the brachial artery and left internal jugular vein were obtained simultaneously at rest and every 30 min during exercise. Core, skin and jugular venous temperatures and arterial and jugular venous pressures were recorded continuously. The same measures were collected in the control trial, except for the arterial and internal jugular venous blood sampling and intra-arterial/venous blood pressures.

A similar experimental design including 5 laboratory visits separated by a week was
used in Study 2. During the experimental trials, however, participants cycled in the heat (35 °C, 50% relative humidity and fan cooling) at 60% $V_{O2\text{max}}$ until volitional exhaustion in the dehydration and euhydration conditions while heart rate, core, skin temperature and middle cerebral artery blood velocity ($MCA \ V_{\text{mean}}$) were monitored.

**Cerebral blood flow**

Vessel blood flow was obtained sequentially at rest and every 30 min from the right internal (ICA), external (ECA) and common carotid arteries (CCA) using an ultrasound system (Vivid 7 Dimension, GE Healthcare, UK) equipped with a 10 MHz linear array transducer. ICA, ECA, and CCA measurements were typically obtained ~1.0-1.5 cm above and ~1.5 cm below the carotid bifurcation, respectively, and the coefficient of variations for measurements of ICA, ECA and CCA vessel diameter and volume flow was considered within an acceptable range both at rest (2.8 ± 0.9%, 2.1 ± 1.1% and 4.3 ± 1.0%) and during exercise (5.3 ± 1.6%, 5.1 ± 1.4% and 5.0 ± 1.6%). For the calculation of blood flow, two-dimensional brightness mode images for vessel diameter were obtained and the mean diameter calculated as systolic diameter $\times$ 1/3 + diastolic diameter $\times$ 2/3. Time-averaged mean flow velocity (TAM $V_{\text{mean}}$; cm/s) was measured continuously in pulse-wave mode over 60 s. Throughout blood flow measurements care was made to ensure a consistent insonation angle below 60° and the sample volume was maintained at the center of the vessel lumen and adjusted to cover its width. Mean flow velocity profiles were traced automatically and analyzed offline for determination of TAM $V$ (EchoPAC BT12, Version: 112 GE Healthcare, Norway). Blood flow (ml/min)
was then calculated by mean flow velocity times cross sectional area \[CSA = \pi \times \left(\frac{\text{mean diameter}}{2}\right)^2\]; Blood flow = TAM \(V\times CSA \times 60\). MCA \(V_{\text{mean}}\) was measured using a 2 MHz pulsed transcranial Doppler ultrasound system (DWL, Sipplingen, Germany). The right MCA was insonated through the temporal ultrasound window, distal to the MCA-anterior cerebral artery bifurcation, at a depth of 45-60 mm. Signal quality was optimized according to Aaslid et al. (1).

**Catheter placement and blood sampling**

Catheters for blood sampling, arterial pressure, internal jugular venous pressure, and blood temperature were inserted into the brachial artery of the non-dominant arm and, after local anesthesia (2% lidocaine), in the left internal jugular vein (Double Lumen Catheter, 16 gauge, 2.3 mm; Multi-Med M2716HE, Edwards Lifesciences, USA) using Seldinger technique and the catheter was advanced to the jugular bulb. For measurement of jugular venous blood temperature, a thermistor (T204-D, PhysiTemp, Clifton, New Jersey, USA) was inserted through the catheter and connected to a thermocouple (TC-2000, Sable Systems, NV, USA). The internal jugular catheter was inserted under ultrasound guidance and catheters were flushed with 0.9% saline to maintain patency. A ~1 h period of rest was observed between catheterization and the commencement of resting measurements.

**Blood variables**

Arterial and jugular venous blood samples were drawn into pre-heparinized syringes
and analyzed immediately for blood gas variables (ABL 800 FLEX, Radiometer, Copenhagen, Denmark), corrected for blood temperature in the internal jugular vein. The analyzer was calibrated at regular intervals in accordance with manufacturer guidelines. Additional arterial and jugular venous blood were collected in 2 ml syringes and transferred to EDTA tubes, centrifuged and separated. Plasma adrenaline (A) and noradrenaline (NA) were subsequently determined using an enzyme-linked immunoassay kit (DEE6500 2-CAT, Demeditec Diagnostics GmbH, Germany).

**Heart rate, blood pressure and temperature**

Heart rate was obtained by telemetry (Polar Electro, Kempele, Finland). Arterial and internal jugular venous pressure waveforms were recorded using transducers (Pressure Monitoring Kit, TruWave, Edwards Lifesciences, Germany) zeroed at the level of the right atrium in the midaxillary line (arterial) and at the level of the tip of the catheter (jugular venous). During the control trial, reconstructed brachial artery pressure waveforms were obtained non-invasively (Finometer® Pro, Finapress Medical Systems, The Netherlands). Arterial pressure waveforms were sampled at 1000 Hz, amplified (BP Amp, ADInstruments, Oxfordshire, UK) and connected to a data acquisition unit (Powerlab 16/30, ADInstruments) for offline analysis. Intestinal temperature was measured using an ingestible telemetry pill (HQInc, Palmetto, Florida, USA) and mean skin temperature ($\overline{T}_{sk}$) was obtained using a wired thermocouple system (TC-2000, Sable Systems, NV, USA) from the standard weightings of chest, abdomen, thigh and
calf temperatures (38).

**Calculations**
Cerebral vascular conductance (CVC) indices were calculated by dividing blood flow in the ICA, ECA, CCA, and MCA by mean arterial pressure (MAP). Arterial oxygen content was used to quantify O\textsubscript{2} delivery through the ICA and MCA, respectively. The CMRO\textsubscript{2} and cerebral glucose and lactate uptake was calculated as ICA flow x 2, multiplied by the corresponding arterial to venous difference. The molar ratio of oxygen to glucose (O\textsubscript{2}/glucose index: OGI) and the oxygen to carbohydrate (O\textsubscript{2}/glucose + ½lactate index: OCI) were also calculated.

**Statistical analysis**
All analyses were made using IBM SPSS Statistics (Version 20, IBM Corporation, Armonk, NY, USA). Variables were assessed using two-way repeated measures ANOVA in which condition (dehydration and control) and exercise phase (rest, 30, 60, 90 and 120 min) were the main factors, with the Dunn-Sidak correction used for multiple comparisons. Multiple regression for within-subject repeated measures was used for analysis of the relationship between blood flow and blood gas variables and temperature (3). Data are presented as mean ± SEM and the alpha level for significance was set at \( P < 0.05 \).
Results

Hydration and temperature

Exercise without supplementation of fluid resulted in a 3% body mass reduction (78.2 ± 2.7 to 75.8 ± 2.7 kg) and a 10 min reduction in exercise duration (110 ± 2 vs. 120 min in the control trial; both \( P < 0.001 \)). In the control trial, body mass was maintained at the pre-exercise level (79 ± 3 kg) through the consumption of fluid (~1.2 l·h\(^{-1}\)). The decline in body mass with dehydration was accompanied by increases in arterial and venous [Hb] \( (P < 0.05) \), indicative of plasma volume reductions. Intestinal temperature increased progressively in both trials, but was higher at end-exercise in dehydration compared to control (38.7 ± 0.1 vs. 38.2 ± 0.2 °C; \( P < 0.05 \); Table 1). Internal jugular venous blood temperature mirrored the rise in intestinal temperature in the dehydration trial (Fig. 4B). \( \overline{T}_{sk} \) was maintained throughout exercise in both the dehydration and control trials (~32.8 and ~32.6 °C; Table 1). Heart rate was similar at rest but during the 2\(^{nd}\) h of exercise was maintained ~14 beats·min\(^{-1}\) higher in the dehydration trial, compared to the control trial \( (P < 0.05) \).

Brain and extra-cranial hemodynamics and metabolism

In the dehydration trial, ICA blood flow and MCA \( V_{mean} \) increased by ~12% at 30 min \( (P < 0.05) \), before declining progressively to baseline values at the end of exercise \( (P < 0.05) \); Fig. 1A and D). The decline in ICA blood flow was associated with a reduction in blood flow velocity \( (P < 0.05) \), but not in vessel diameter. On the other hand, in the
control trial, ICA blood flow and MCA $V_{\text{mean}}$ increased and remained stable throughout exercise. During the dehydration trial, ECA (and CCA) blood flow almost doubled from rest to 90 min ($0.42 \pm 0.03$ to $0.74 \pm 0.04 \, \text{l} \cdot \text{min}^{-1}$), but then declined at the end of exercise ($P < 0.05$; Fig. 1B and C). In contrast during the control trial, ECA flow increased similarly up to 90 min of exercise and then plateaued.

The decline in ICA blood flow at the end of dehydration exercise was accompanied by increased a-$vO_2$ ($P < 0.05$), but no changes in a-$vCO_2$ or brain $\dot{V}CO_2$ index. Thus, CMRO$_2$ was stable throughout exercise. Both arterial and jugular venous plasma glucose gradually declined throughout prolonged exercise ($5.4 \pm 0.2$ to $5.1 \pm 0.2$ and $5.4 \pm 0.2$ to $4.4 \pm 0.2 \, \text{mmol} \cdot \text{l}^{-1}$, respectively; $P < 0.05$). However, brain a-$v$ glucose was stable during the early stages of exercise, before increasing prior to the end of exercise (peak value of $0.7 \, \text{mmol} \cdot \text{l}^{-1}$; $P < 0.05$: Fig. 2A), while the brain glucose uptake increased initially ($0.33$ to $0.43 \, \text{mmol} \cdot \text{min}^{-1}$; $P < 0.05$: Fig. 2B) and then remained stable.

At rest the brain released a small amount of lactate ($0.2 \pm 0.05 \, \text{mmol} \cdot \text{l}^{-1}$) and during prolonged exercise with dehydration, arterial and jugular venous lactate gradually declined ($3.4 \pm 2.4 \pm 0.3$ and $3.6 \pm 0.5$ to $2.4 \pm 0.3 \, \text{mmol} \cdot \text{l}^{-1}$; $P < 0.05$). Brain a-$v$ lactate was maintained throughout exercise, as was lactate exchange (Fig. 2D and E). The molar ratio of O$_2$ to glucose declined at the onset of exercise ($6.1 \pm 0.5$ vs. $4.5 \pm 0.3$; $P < 0.05$: Fig. 2C) and thereafter remained stable, with a similar response when lactate metabolism was accounted for (Fig. 2F).
Brain and extra-cranial vascular conductance

In the dehydration trial, MAP increased ~18% from rest to 30 min (105 ± 3 to 124 ± 4 mmHg; \( P < 0.05 \)), before declining progressively until exercise termination (\( P < 0.05 \)). In the control trial MAP increased by 11% from rest to 30 min and then remained stable. During prolonged exercise, ICA and MCA \( V_{\text{mean}} \) vascular conductance were lower in the dehydration compared to the control trial (\( P < 0.05 \)). At the end of exercise in dehydration, both ICA and MCA \( V_{\text{mean}} \) vascular conductance indices were reduced (\( P < 0.05 \); Fig. 3) whereas in control, they remained stable. CCA and ECA vascular conductance were similar between trials during early exercise, but were reduced at the end of exercise in dehydration compared to control (\( P < 0.05 \)).

Blood flow and \( P_a\text{CO}_2 \), plasma catecholamines and temperature

In the dehydration trial, \( P_a\text{CO}_2 \) declined to below baseline at the end of exercise (~6% reduction from peak value; \( P < 0.05 \); Fig. 4A) whereas the partial pressure of venous \( \text{CO}_2 \) (\( P_v\text{CO}_2 \)) remained unchanged throughout exercise. During exercise, the decline in both ICA blood flow (\( R^2 = 0.44 \); Fig. 4C) and MCA \( V_{\text{mean}} \) (\( R^2 = 0.5 \); data not shown) were correlated to reduced \( P_a\text{CO}_2 \) (both, \( P < 0.01 \)) and to a weaker extent also to arterial [NA] (\( R^2 = 0.15 \); \( P < 0.05 \)), but not to jugular venous [NA] (\( R^2 = 0.02 \); \( P = 0.58 \)).

In the dehydration trial, arterial [A] increased from rest to 30 min (0.7 ± 0.2 to 1.1 ± 0.2 nmol·l\(^{-1}\); \( P < 0.05 \)), but remained stable throughout exercise (range 1.1-2.7 nmol·l\(^{-1}\)) whereas jugular venous [A] did not increase until after 90 min of exercise (110 ± 2 min =
2.3 ± 0.7 nmol·l⁻¹; \( P < 0.05 \) vs. rest). Arterial [NA] increased to a peak of 33 ± 8.2 nmol·l⁻¹ \( (P < 0.05 \) vs. rest), whereas jugular venous [NA] increased to 90 min (3.6 ± 1.2 to 31.0 ± 5.2 nmol l⁻¹; \( P < 0.01 \)), before declining prior to exhaustion (14.1 ± 1.2 nmol l⁻¹), indication of a cerebral uptake of catecholamines at exhaustion.

The alterations in ECA blood flow and vascular conductance were associated with increases in internal jugular venous blood temperature (\( R^2 = 0.48; \ P < 0.01 \): Fig. 4B and D, respectively) and jugular venous [NA] (\( R^2 = 0.48; \ P < 0.01 \)). However, a consistent relationship with blood temperature was not observed beyond 90 min where the 7% decline in ECA blood flow (and conductance) was matched with an increased blood temperature (Fig. 4B).

**Hydration, core temperature and cerebral circulation during exhaustive exercise**

Hydration status was the same prior to exercise as indicated by the same body mass in both trials (75.2 ± 4.2 and 75.3 ± 4.2 kg). When participants did not ingest fluids during exercise, they became progressively dehydrated to a 3.2 ± 0.3% reduction in body mass, whereas they maintained body mass in the euhydration trial (+0.3 ± 0.2% body mass). In addition, they became exhausted sooner in the dehydrated state than when euhydrated (123 ± 7 vs. 145 ± 9 min; \( P < 0.05 \)) with higher core temperature and heart rate at exhaustion (38.7 ± 0.2 vs 38.3 ± 0.1 °C and 156 ± 4 vs 148 ± 5 beats·min⁻¹; both \( P < 0.05 \)). Core and skin temperatures and heart rate, however, were similar between trials during the first 40-50 min of exercise. MCA \( V_{\text{mean}} \) increased during the first 30 min
of the exercise and thereafter declined in both trials ($P < 0.05$ vs. 30 min value).

Discussion
The main finding of the present study is that dehydration impaired prolonged exercise capacity in the heat in association with an accelerated reduction in CBF. However, in spite of the reduced CBF, cerebral metabolism was preserved by elevated oxygen and glucose extraction from blood. A second novel finding was that in both experimental trials the rise in ECA blood flow was attenuated prior to volitional exhaustion. The reduction in CBF was related to a reduced $P_a\text{CO}_2$, whereas increased ECA vascular conductance was associated with increasing internal temperature. Although maintenance of euhydration by fluid ingestion preserved cerebral and extra-cranial blood flow throughout non-fatiguing exercise, it only delayed the decline in CBF during exhaustive exercise. These findings suggest the CBF decline with dehydration is coupled to factors associated with the fatigue processes, rather than dehydration *per se*.

Cerebral and extra-cranial hemodynamics
A first aim of the study was to characterize the hemodynamic responses of the cerebral and extra-cranial circulations to dehydration during prolonged exercise in the heat. We found that following the well-established increase upon the onset of exercise, CBF and MCA $V_{\text{mean}}$ declined gradually to resting values at volitional exhaustion concomitantly with the development of dehydration. Conversely, when hydration was maintained
during similar duration exercise, CBF did not decline. Similarly, CBF remains stable during prolonged exercise in a thermo-neutral environment when the degree of dehydration is negligible (33, 34). However, when exercise in a warm environment causes hyperthermia and cardiovascular strain, CBF is suppressed (37) before declining to resting values (34). These studies, however, did not establish whether hyperthermia, dehydration, or other factors underpinning volitional exhaustion are responsible for the fall in CBF. An important observation from a parallel study (study 2) was that cerebral perfusion gauged by MCA $V_{\text{mean}}$ declined when control (euhydration) exercise was continued to exhaustion and core temperature remained at normothermic levels (Fig. 5). Together, the findings from both studies indicate that CBF declines during exhaustive exercise, regardless of the hydration status and the level of core temperature, yet maintenance of euhydration and stable core temperature slows the rate of CBF decline late in fatiguing exercise. The CBF decline with dehydration is therefore closely coupled to factors associated with the fatigue processes, rather than reduced body fluids or core hyperthermia *per se*.

Another pertinent finding was that blood flow to the extra-cranial tissues displayed a distinct temporal dynamic response to that of the cerebral circulation, but was blunted late in exercise in the dehydrated condition. Yet these findings agree with reports of a 2-3 fold increases in ECA blood flow in response to passive heat stress (2, 36) and incremental exercise (40). The ECA supplies blood to the skin circulation of the face and neck. In this light, the enhanced ECA perfusion may be part of the circulatory
adjustments required to meet the thermoregulatory demands for heat transfer to the environment surrounding the head (36). Collectively, these data suggest that dehydration accentuates the rise in internal temperature, reduces extra-cranial blood flow at point of exhaustion, accelerates the decline in cerebral perfusion, and leads to early exhaustion during prolonged exercise in the heat.

**Regional head blood flow regulation**

Both local and systemic factors are implicated in regulation of CBF through modulation of vascular conductance (or resistance) and cerebral perfusion pressure. The decline CBF in the dehydration trial was accompanied by a falling cerebrovascular conductance, indicative of augmented vasoconstriction. Changes in blood gas variables (48) and sympathetic activity (28) may play a role in control of CBF during conditions including exercise. In particular, CO$_2$ is a potent vasoactive substance within the cerebral vasculature with reductions in $P_a$CO$_2$ inducing cerebral vasoconstriction and increases leading to vasodilation (2, 25, 48). The decline in $P_a$CO$_2$ is attributed to hyperventilation with increasing exercise intensity and a rising core body temperature (46, 47). Thus, the decline in vascular conductance with dehydration was associated with reductions in $P_a$CO$_2$ ($R^2 = 0.44; P < 0.01$; Fig. 4C) and cerebral perfusion pressure, but unrelated to the stable arterial and internal jugular venous oxygen variables (data not shown). $P_a$CO$_2$, however, accounted for only half of the variance in vascular conductance, while there was only a modest relationship between CBF and cerebral perfusion pressure ($R^2 = 0.18, P < 0.05$). Another potential contributing factor is enhanced sympathetic activity.
The cerebral vasculature is richly innervated with sympathetic nerves and observations of NA spillover into the internal jugular venous outflow, as seen here with dehydration, may reflect sympathetic-mediated vasoconstriction of the cerebral vessels (28, 50). However, despite increases in arterial and jugular venous [NA], the relationships between cerebrovascular tone and plasma NA were weak (26, 43, 49).

The distinct dynamics of the extra-cranial circulation might involve different regulatory mechanisms. There was a close coupling between the increase in ECA blood flow/conductance and the rise in internal jugular blood temperature ($R^2 = 0.64$, $P < 0.01$; Fig. 4D). We did not investigate the control of skin blood flow, but cutaneous blood flow is enhanced by elevated local and core temperature (5, 21, 39). The role of sympathetic activity in the response to local and internal temperature changes is substantiated by an elevation in skin sympathetic nerve activity (7, 32), promoting cutaneous blood distribution and sudomotor function (5, 24). With a similar ECA profile in both trials, it is more likely that exercise per se attenuates cutaneous perfusion as rising internal temperature (above 38 °C) is not matched by further increases in skin perfusion as measured in the forearm (4, 17). Dehydration also limits maximal skin perfusion during exercise (30) and enhances systemic vascular resistance (16), leading to attenuated cutaneous blood flow (23). Taken together, these findings support that blood flow to the extra-cranial vascular bed is influenced by local and internal temperature and enhanced sympathetic nerve activity.
Impact of dehydration on cerebral metabolism

Reduction in oxygen supply can compromise organ metabolism, as shown in contracting skeletal muscle with significant hyperthermia during maximal exercise (13). Here, we asked whether prolonged fatiguing exercise and dehydration is compromises cerebral metabolism. Similar to findings during maximal exercise (44), the decline in CBF was met by an equal increase in oxygen extraction such that CMRO$_2$ was maintained during prolonged exercise in the dehydrated state in agreement with other independent metabolic measures obtained in this study. Also glucose uptake, cerebral lactate exchange, molar oxygen/glucose ratio (OGI), and the oxygen/carbohydrate index (OCI) remained unchanged, and the cerebral respiratory quotient (~1.03) was stable (8). There is contrasting evidence that CMRO$_2$ might be elevated during strenuous exercise combined with severe hyperthermia compared to control exercise (33), thereby arguing that the metabolic demand of the brain increases during exhaustive exercise. This conclusion, however, is based on two data points. To provide a more comprehensive account of the cerebral metabolic responses to exercise and establish whether CMRO$_2$ is altered during fatiguing exercise, we plotted the anterior cerebral blood flow and a-vo$_2$ difference data from the current prolonged exercise protocol together with the reported baseline and incremental exercise data obtained in the same individuals (Fig. 6; (44). This analysis shows that CMRO$_2$ remained stable across a variety of exercise intensities, exercise durations, hydration conditions and rest-to-exercise transitions, as variations in CBF were met by proportional changes in oxygen extraction. The unchanged global cerebral metabolism may reflect a balance of
regional alterations in metabolic (and thus flow) demand. In this construct, exercise of increasing intensity both augments, and down-regulates, regional blood flow across the brain (9). It remains to be clarified whether regional cerebral hypoperfusion, particularly that which is assessed in the frontal cortex, reflects an important process leading to fatigue, or a selective down-regulation in neuronal activity in regions of the brain that are less important during physical exertion (10). Restoring CBF and cerebral oxygenation during strenuous exercise in the heat does not affect the development of fatigue (22). Therefore, although regional differences might exist, our data suggest that the metabolic activity of the brain as a whole is neither enhanced nor compromised during exhaustive exercise in trained healthy individuals.

**Experimental considerations**

Ultrasound imaging of the extra-cranial vessels and MCA $V_{\text{mean}}$ are appropriate for assessing CBF during dynamic exercise in an upright position (18, 40, 45). Notwithstanding, some flow is directed to branch vessels (e.g. the choroidal artery), but this is unlikely to impact on the measured CBF during exercise (27). Further, changes in MCA $V_{\text{mean}}$ may underestimate cerebral perfusion as vessel diameter changes are unknown. However, during exercise, changes in MCA $V_{\text{mean}}$ are similar to absolute blood flow measured in the ICA (18). Blood samples were obtained from the left internal jugular vein and asymmetry may be present in the venous drainage of the brain (42). Yet, blood gas variables obtained from the left and right internal jugular vein are similar both at rest and during exercise (11, 20). We were unable to obtain internal jugular
venous blood samples in the control trial but it has been demonstrated that leg and systemic a-v $O_2$ differences are stable when euhydration is maintained during prolonged exercise in the heat (12). We therefore expect that a-v $O_2$ differences across the brain would also be unchanged when CBF is not compromised. Finally, the present studies utilized only male participants but. Previous studies using both male and female participants have not demonstrated any sex differences in the CBF response to exercise (40).

Conclusion
Dehydration leads to augmented cerebrovascular strain, as indicated by blunted cerebral and extra-cranial perfusion during prolonged exercise in the heat. However, despite the circulatory strain associated with fatigue secondary to dehydration and hyperthermia, cerebral metabolism is not impaired due to compensatory increases in oxygen and substrate extraction across the brain. The findings imply that reduced cerebral metabolism is unlikely to explain the reduced exercise capacity with dehydration during prolonged exercise in the heat. In contrast, both reduction in CBF and extra-cranial perfusion may influence fatigue.
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Disclosures

The authors declare no conflict of interest.

Author contributions

Experiments were performed at the Centre for Sports Medicine and Human Performance, Brunel University London. S.J.T. & J.G.A. were involved in the conception and design of the invasive experiment, as well as the analysis and interpretation of the data. S.J.T., S.T.C., K.K.K., N.H.S., and J.G.A. were involved in data collection of the invasive study, whereas I.L., B.G. and J.G.A. conducted the parallel non-invasive study. The article was drafted by S.J.T and critically revised for important intellectual content by S.J.T., S.T.C., K.K.K., I.L., B.G., N.H.S. and J.G.A. All authors approved the final version of the manuscript.
References


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Table 1. Temperature and heart rate responses to dehydration and euhydration (control) during prolonged exercise

<table>
<thead>
<tr>
<th>Cycling time (min)</th>
<th>Rest</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>End exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$T_i$ (°C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>37.4 ± 0.1</td>
<td>38.0 ± 0.1*</td>
<td>38.4 ± 0.1*#</td>
<td>38.6 ± 0.1*#</td>
<td>38.7 ± 0.1*#</td>
</tr>
<tr>
<td>Control</td>
<td>37.3 ± 0.1</td>
<td>37.9 ± 0.1*</td>
<td>38.1 ± 0.1*</td>
<td>38.2 ± 0.1*</td>
<td>38.2 ± 0.2*</td>
</tr>
<tr>
<td><strong>$\overline{T}_{sk}$ (°C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>34.0 ± 0.3</td>
<td>33.1 ± 0.4</td>
<td>32.7 ± 0.4</td>
<td>32.8 ± 0.4</td>
<td>32.6 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>33.5 ± 0.3</td>
<td>32.6 ± 0.3</td>
<td>32.6 ± 0.3</td>
<td>32.8 ± 0.3</td>
<td>32.3 ± 0.3</td>
</tr>
<tr>
<td><strong>HR (beats min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>80 ± 3</td>
<td>148 ± 2*#</td>
<td>157 ± 2*†#</td>
<td>163 ± 2*†#</td>
<td>166 ± 3*†#</td>
</tr>
<tr>
<td>Control</td>
<td>77 ± 2</td>
<td>142 ± 3*</td>
<td>145 ± 3*</td>
<td>149 ± 3*†</td>
<td>149 ± 3*†</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 10 subjects. $T_i$, intestinal temperature; $\overline{T}_{sk}$, mean skin temperature; HR, heart rate. Data are from the dehydration trial only. * $P < 0.05$ vs. rest, † $P < 0.05$ vs. 30 min, # $P < 0.05$ vs. control.
Figure legends

Figure 1. Cerebral and extra-cerebral hemodynamics and oxygen parameters during prolonged exercise. Values are mean ± SEM for 10 subjects. Cerebral hemodynamics were obtained in both the dehydration and control exercise trials (left panel) but not for O₂/CO₂ parameters (right panel). * P < 0.05 vs. rest, † P < 0.05 vs. 30 min value.

Figure 2. Cerebral lactate and glucose exchange during prolonged exercise. Values are mean ± SEM for 10 subjects. Data presented from the dehydration trial. * P < 0.05 vs. rest, † P < 0.05 vs. 30 min value.

Figure 3. Blood pressure and cerebral and extra-cerebral vascular conductance during prolonged exercise. Values are mean ± SEM for 10 subjects. * P < 0.05 vs. rest, † P < 0.05 vs. 30 min value.

Figure 4. Blood temperature, PₐCO₂ and relationships with blood flow during prolonged exercise. Values are mean ± SEM for 10 subjects. Data presented are from the dehydration trial. Relationships were obtained using multiple regression for within-subject repeated measures. * P < 0.05 vs. rest, † P < 0.05 vs. 30 min value.

Figure 5. MCA Vₘean with and without dehydration during prolonged exercise to exhaustion. Values are mean ± SEM for 8 subjects. MCA Vₘean declined after 30 min of
exercise in both trials. † $P < 0.05$ vs. 30 min value.

Figure 6. Anterior arterial-to-internal jugular venous oxygen difference, anterior cerebral blood flow and the cerebral metabolic rate for oxygen during prolonged and incremental exercise. Values are mean ± SEM during prolonged (n=9) and incremental (n=8) exercise. Baseline and incremental exercise to exhaustion data in the control and dehydrated conditions are reported (38). An inverse relationship between changes in blood flow and a-vO$_2$ differences is shown (all points; $R^2$ = -0.29; $P = 0.01$). Data are from a variety of exercise intensities, exercise durations, hydration status, and rest-to-exercise transitions where the cerebral metabolic rate for oxygen remained stable at ~ 45 ml·min$^{-1}$. 
Figure 2

**A**

\( a-v \) (Glu) Difference (mmol l\(^{-1}\))

**B**

Brain glucose uptake (mmol min\(^{-1}\))

**C**

Oxygen-to-glucose index (\(\text{O}_2\)/glucose)

**D**

\( a-v \) [La] Difference (mmol l\(^{-1}\))

**E**

Brain lactate exchange (mmol min\(^{-1}\))

**F**

Oxygen-to-carbohydrate index (\(\text{O}_2\)/glucose + lactate)

* denotes significant difference.
Figure 3

A. Blood pressure (mmHg)
- Mean Arterial Pressure
- Jugular Venous Pressure

B. ICA vascular conductance (ml min⁻¹ mmHg⁻¹)

C. MCA vascular conductance (cm² s⁻¹ mmHg⁻¹)

D. CCA vascular conductance (ml min⁻¹ mmHg⁻¹)

E. ECA vascular conductance (ml min⁻¹ mmHg⁻¹)

Legend:
- Control
- Dehydration

* p < 0.05
** p < 0.01
† p < 0.001
Figure 4

(A) PaCO₂ (mmHg) over time (min).
(B) Jugular Venous Temperature (°C) with time.
(C) Graph showing ICA blood flow x 2 vs. PaCO₂ (mmHg) with a regression line and R² = 0.44; P < 0.01.
(D) ECA blood flow x 2 vs. Jugular Venous Temperature (°C) with a regression line and R² = 0.64; P < 0.01.
Figure 5

![Graph showing MCA V\textsubscript{mean} (cm s\textsuperscript{-1}) over time (min) with Control and Dehydration lines.](Image)