Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans

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The present study examined the integrative effects of passive heating on cerebral perfusion and alterations in central motor drive. Eight participants underwent progressive hyperthermia (0.5°C increments in core temperature (Tc) from normothermia (37 ± 0.3°C) to their limit of thermal tolerance (T-LIM; 39.0 ± 0.4°C)). Blood flow velocity in the middle cerebral artery (CBFv) and respiratory responses were measured continuously. Arterial blood gases and blood pressure were obtained intermittently. At baseline and each Tc level, supramaximal femoral nerve stimulation and transcranial magnetic stimulation (TMS) were performed to assess neuromuscular and cortical function, respectively. At T-LIM, measures were (in a randomized order) also made during a period of breathing 5% CO2 gas to restore eucapnia (+5% CO2). Mean heating time was 179 ± 51 min, with each 0.5°C increment in Tc taking 40 ± 10 min. CBFv was reduced by ~20% below baseline from +0.5°C until T-LIM. Maximal voluntary contraction (MVC) of the knee extensors was decreased at T-LIM (−9 ± 10%; P < 0.05), and cortical voluntary activation (VA), assessed by TMS, was decreased at +1.5°C and T-LIM by 11 ± 8 and 22 ± 23%, respectively (P < 0.05). Corticospinal excitability (measured as the EMG response produced by TMS) was unaltered. Reductions in cortical VA were related to changes in ventilation (VE; R2 = 0.76; P < 0.05) and partial pressure of end-tidal CO2 (PetCO2; R2 = 0.63; P < 0.05) and to changes in CBFv (R2 = 0.61; P = 0.067). Interestingly, although CBFv was not fully restored, MVC and cortical VA were restored towards baseline values during inhalation of 5% CO2. These results indicate that descending voluntary drive becomes progressively impaired as Tc is increased, presumably due, in part, to reductions in CBFv and to hyperthermia-induced hypocapnia.

transcranial magnetic stimulation; cerebral blood flow; neuromuscular function

EXCESSIVELY ELEVATED BODY temperature (i.e., hyperthermia) is known to impair exercise performance (for review, see Ref. 20) and poses a huge cardiovascular challenge. The additional cardiac output is redistributed to the skin for heat dissipation, while the working muscles require the majority of blood flow to deliver oxygen and metabolites (for review, see Ref. 7). Nevertheless, systemic circulatory failure alone does not appear to be the primary factor influencing exercise tolerance in the heat (although it can also exacerbate hyperthermia). Data indicate that hyperthermia has a direct effect on the ability of the central nervous system (CNS) to activate muscle, independent of the rate of heat storage and initial core, skin, or muscle temperature (13, 18, 31). Exercise-induced hyperthermia elicits near-maximal cardiovascular strain, hypohydration, and exercise-related metabolic changes, factors that confound the ability to examine a direct role of hyperthermia on voluntary control of muscle (e.g., Ref. 21). Consequently, passive heating protocols have been utilized to delineate the effects of hyperthermia on the CNS directly (18, 31, 34).

The thermodynamic response of the brain, and its affect on motor activity, have been investigated using muscle stimulation techniques. Using electrical stimulation of the motor nerve during 3- to 5-s voluntary contractions (MVC), Thomas et al. (31) reported that the ability of the CNS to maximally voluntarily activate the plantar fl exors of one leg was progressively impaired as core body temperature (Tc) was increased during passive heating. In addition, these authors observed a subsequent restoration of voluntary activation (VA) as participants were cooled to normothermic Tc. Furthermore, the pattern of response was similar in the other leg that was held thermoneutral throughout the trial, indicating that these changes occurred independently of local muscle temperature changes. These data illustrate that impairment of force-generating ability during hyperthermia may be due to a failure of the central drive, is progressive, and is capable of being independent of temperature changes within the muscle. While these and other (18) findings identify a “central” impairment of voluntary force production during hyperthermia, the motor nerve twitch interpolation technique only allows this impairment to be located at, or upstream of, the motor nerve. Transcranial magnetic stimulation (TMS) of the motor cortex is a technique that has utility in localizing the site of failure of voluntary drive, since an additional force evoked by TMS during an MVC implies that voluntary output from the motor cortex is insufficient to drive the motoneurone pool optimally. This failure of drive from the motor cortex indicates a supraspinal component to the loss of force-generating capabilities (30). Todd et al. (32) estimated VA using TMS in normothermia and following an increase in Tc by 1.3°C to ~38.5°C. They reported that during sustained MVC motor cortical output was found to be reduced during hyperthermia. In addition, an increased contractile speed (evidenced by a faster peak relaxation rate of muscle) was suggested to contribute to the central fatigue during sustained contractions, such that motor cortical output was unable to...
achieve firing rates fast enough to maximally summate and maintain force generation, given the increased muscle contractility. However, that study used only a one-step increase in Tc; thus the Tc point when changes in cortical output may begin and how such changes may progress with further increases in Tc have yet to be characterized.

Elevated Tc at rest induces hyperventilation and subsequent hypocapnia (37), leading to cerebral vasconstriction and marked reductions in cerebral blood flow (CBF). Indeed, depending on the experimental approach, studies (5, 10) have demonstrated that hyperventilation-induced hypocapnia accounts for 50–100% of the reductions in middle cerebral artery blood flow velocity (CBFv; a surrogate marker of CBF) during progressive passive hyperthermia. The functional consequences of a marked reduction in CBF for voluntary control of muscle during hyperthermia have not been explored. In addition, since measurements of cerebrovascular function and motor cortical output are seldom made concurrently, the relation between them during progressive hyperthermia is not known.

Therefore, the aim of the present study was to examine the integrative effects of progressive, passive hyperthermia on CBFv and alterations in motor drive. A further aim was to examine whether hyperventilation-induced hypocapnia, as experienced during hyperthermia, has a direct effect on cortical output. We hypothesized that a progressive impairment of cortical output would occur in response to progressive elevations in Tc and that any such changes would be related to reductions in CBFv.

METHODS

Participants

Eight healthy volunteers (6 male, 2 female) participated in the study (means ± SD, age: 27.2 ± 6.3 yr, and body mass: 76.6 ± 9.1 kg). All participants gave written informed consent before commencement of the study once the experimental procedures, associated risks, and potential benefits of participation had been explained. The study was approved by the Institutional Research Ethics Committee and the Declaration of Helsinki.

Experimental Protocol

Following an initial familiarization session, participants arrived in the laboratory having abstained from strenuous exercise and alcohol for 24 h and caffeine for 12 h and not having consumed a heavy meal for 4 h. On arrival, participants voided their bladder and hydration status was estimated from urine specific gravity (Atago Hand Refractometer; Astra Zeneca, Tokyo, Japan).

The experimental protocol is depicted in Fig. 1. During the experimental session, neuromuscular, hemodynamic, and perceptual measurements were made at baseline (normothermia: 37.1 ± 0.3°C) and at 0.5°C increments in Tc until participants reached their limit of thermal tolerance (T-LIM: 39.1 ± 0.4°C). Cardiovascular, cerebrovascular, and thermal measurements were made continuously throughout the protocol. Participants were warned using a water-perfused suit (see below). For the duration of the protocol, participants were seated upright in a high-backed chair. At baseline and at every 0.5°C increment in Tc, six TMS stimuli were delivered at rest. Participants then performed two brief (3–5 s) MVCs of the knee extensor muscles. To determine cortical VA, TMS was then delivered during 5-s voluntary contractions performed at 50, 75, and 100% of MVC, interspersed with a 15-s rest. This contraction set was repeated three times, with a 45-s rest between each set. Participants received visual feedback of the target force on a computer monitor. Finally, participants performed three further MVC efforts, with supramaximal femoral nerve stimulation delivered during each maximal contraction and at rest, 1 s after the MVC to the M point of the quadriceps twitch torque (Qtw,mot; see Data Analysis).

During the protocol, participants were permitted to drink an isotonicsolution (sodium 44 mg/l, 67 kcal/l) ad libitum (mean volume ingested = 858 ± 811 ml) to maintain euhydration. Sweat rate throughout the session was estimated from semi-nude body mass loss (mean sweat rate = 0.59 ± 0.20 l/h).

Effect of hyperventilatory-induced hypocapnia. At T-LIM, the circulation of water through the suit was reduced to prevent further rises in Tc, and measurements were made while participants breathed a humidified 5% CO2 gas mixture through a facemask until end tidal CO2 (PETCO2) reached resting baseline values (~2 min; Ref. 10). The order of measurements made at T-LIM (and associated hypocapnia), and during CO2 breathing (+5% CO2), was randomized. If +5% CO2 measurements were taken before...
those at hypocapnic T-LIM, it was ensured that Pet \(CO_2\), and ventilatory responses returned to pre+5% CO\(_2\) levels before T-LIM measurements were made.

**Thermal Control**

Passive heating was achieved by circulating warm water (50 ± 1°C) through a long-sleeved and -legged, two-piece, tube-lined water-perfusion suit (DTI, Ottawa, Canada). Participants also wore an impermeable rain suit over the top of the water-perfused suit and were wrapped in a foil blanket. At each 0.5°C incremental step in Tc, the circulation of water through the suit was reduced by two-thirds to prevent further rises in Tc; thus all neuromuscular testing was performed at a constant Tc. Measurements at each 0.5°C increment took ~6 min to perform. Since the mean heating time for each 0.5°C rise in Tc took ~40 min, even if water flow had been maintained, Tc would have only risen by an average of 0.08°C during the measurement period. Since we reduced the flow of warm water through the suit and subsequently slowed the heating rate, we can be confident that Tc remained stable to within ~0.05°C during the measurement phase of each increment.

**Thermometry**

Core temperature was recorded as rectal temperature, measured using a sterile, disposable thermistor (Mallinkrodt 400 general purpose; Mallinkrodt Medical, St. Louis, MO; factory calibration ±0.1°C). Skin temperature was measured using insulated surface thermistors (Skin Thermistor EUS-U-V5-V2; Grant Instruments, Cambridge, UK) at four sites: chest, dorsal forearm, front thigh, and posterior calf. Core and skin temperatures were logged at 1-min intervals (Grant Instruments). Mean skin temperature (Tsk) was calculated from standard area weightings: 0.3 (chest + arm) + 0.2 (thigh + calf) (25).

**Torque and EMG Recordings**

Isometric knee extension force during voluntary and evoked contractions was measured by means of a calibrated load cell (model 615; Tedea-Huntleigh). Participants sat upright on a high-backed chair with their hips and legs firmly secured, knee positioned at 90° to the thigh, and arms folded across the chest. A noncompliant cuff was positioned around the lower leg, just proximal to the ankle malleoli, and attached to the load cell. This was connected to an analog-to-digital converter (micro1401; Cambridge Electronic Design, Cambridge, UK), and mechanical responses to voluntary and evoked contraction of the knee extensors were acquired and analyzed on a computer using appropriate software (Spike 2 v4.11; Cambridge Electronic Design).

EMG activity was recorded during maximal and submaximal voluntary contractions with surface electrodes (Soft-E H59P: Kendall-LTP, Chicopee, MA) over the vastus lateralis muscle. Preparation included shaving of participants’ skin before application of electrodes, if necessary. The electrodes were used to record the compound muscle action potential (M wave) elicited by electrical stimulation of the femoral nerve, the motor-evoked potential (MEP) elicited by TMS, and the EMG activity of the vastus lateralis during voluntary contraction. EMG signals were amplified (gain × 1000; 1902; Cambridge Electronic Design), band-pass filtered between 20 and 2 kHz and digitized at a sampling rate of 4 kHz using an analog to digital converter (micro1401; Cambridge Electronic Design), and analyzed off line after completion of the experimental session (Spike 2 v4.11; Cambridge Electronic Design).

**Stimulation**

Two forms of stimulation were used 1) electrical stimulation of the femoral nerve (motor nerve stimulation), and 2) TMS over the motor cortex.

**Motor nerve stimulation**. A doublet pulse electrical stimulation (100 Hz, pulse width of 100 μs) was applied to the femoral nerve via a constant voltage stimulator (DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, UK). A 32-mm diameter self-adhesive electrode (cathode; CF3200; Nidd Valley Medical, North Yorkshire, UK) was placed over the femoral nerve, high in the femoral triangle, and a 3” × 5” self-adhesive electrode (anode; Dura-Stick II; Chattanooga Group, Hixson, TN) was placed on the gluteal crease. The site of stimulation that produced the largest twitch amplitude and M wave at rest was first located. Doublet stimuli were then delivered from 20 mA, incrementally increasing in amplitude by 5 mA, until a plateau was observed in the resultant twitch torque. Increasing the final stimulating intensity by a factor of 1.3 ensured supramaximal stimulation throughout the subsequent experimental protocol. Mean stimulating intensity was 85 ± 11 mA.

**TMS over the motor cortex**. TMS was achieved using a Magstim 200 magnetic stimulator and a 90-mm cone-shaped figure of eight coil (Magstim; Whitland, Wales, UK), with a maximum output of 2.5 Tesla. Before the experimental protocol, a mapping procedure was carried out to establish the optimal cortical site for activation of the vastus lateralis. In all cases, this was 0–10 mm contralateral to the vertex. This position was marked with indelible ink to ensure reproducibility of the stimulation conditions for that individual during subsequent testing. Motor threshold (MT) for the vastus lateralis was identified by constructing a stimulus-response curve for each individual. Resting MT was established by decreasing stimulator output from 100% by 5% steps until a vastus lateralis response was visible in less than one-half of eight stimuli. Resting MT occurred at 67 ± 8% of maximum stimulator output. Subsequent experimental TMS were delivered at 1.3 × initial resting MT. Mean TMS intensity was 88 ± 10% of maximum stimulator output. This stimulation intensity elicited a large MEP in the vastus lateralis [area between 52 and 93% of maximum M wave (Mmax) during knee extensor contractions ≥50% MVC; Fig. 2A]. Single participant data are shown in Fig. 3, depicting the evoked responses during voluntary knee extension at 50, 75, and 100% MVC.

**Homodynamic Measurements**

In four participants, a 20-gauge arterial catheter (BD Insyte) was placed, while the patients were under local anesthesia (1% lidocaine), into the right radial artery for the collection of arterial blood. The catheter was kept patent throughout the experiment by being flushed with saline frequently. Arterial blood samples were taken at each 0.5°C increment in Tc. The samples were immediately analyzed for pH, O2 tension (PaO2), CO2 tension (PaCO2), hemoglobin concentration ([Hb]), and O2 saturation (SaO2) using an arterial blood-gas analyzing system (NPT7 series; Radiometer, Copenhagen, Denmark). Arterial O2 content (CaO2) was calculated using the equation: CaO2 (ml/dl) = ([Hb] × 1.39 × SaO2/100) + (PaO2 × 0.003).

**Cerebro- and Cardiovascular Measurements**

CBFv in the right middle cerebral artery was measured using a 2-MHz pulsed Doppler ultrasound system (DWL; Compumedics) using search techniques described elsewhere (2, 40). The Doppler probe was secured with a plastic headband device (Spencer Technologies) to maintain optimal insonation position and angle throughout the protocol. Beat-to-beat mean arterial blood pressure (MAP) was measured using finger photoplethysmography (Finometer PRO; Finapres Medical Systems). Cerebrovascular resistance (CVR) was calculated as MAP/CBFv. Cerebral O\(_2\) delivery was estimated by calculating the change in CBFv from baseline at each 0.5°C increment and multiplying by CaO\(_2\) (in the 4 participants where CaO\(_2\) could be determined) and is expressed as arbitrary units. All data were acquired continuously at 200 Hz (Powerlab 16/30; ADInstruments, Oxfordshire, UK) and processed offline (LabChart v6.0.3; ADInstruments).
Respiratory Measurements

At baseline and at each 0.5°C increment in Tc, participants breathed through a leak-free respiratory mask (Hans-Rudolph 7900 series, Kansas City, MO) attached to a Y-shaped two-way nonre-breathing valve (Hans-Rudolph 7900). Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph 3813). End-tidal fractions of CO2 were sampled from the leak-free mask, measured using gas analyzers (model CD-3A; AEI Technologies, Pittsburgh, PA), and converted to pressures (PETCO2). Ventilation (flow, tidal volume, and frequency) and gas values were displayed in real time during testing (PowerLab; ADI Instruments).

Perceptual Responses

Perceived body temperature and ratings of thermal comfort were ascertained from 13- and 5-point scales, respectively (11), at baseline (normothermia) and at each 0.5°C increment in Tc.

Data Analyses

Torque was recorded during each voluntary effort, and during MVCs the highest value was recorded as the maximal value. The EMG signal was analysed in the time domain, as root mean square (RMS) amplitude with a time constant of 25 ms. Computer-aided analysis was performed over a 0.5-s window initiated at the point of peak force during the MVC effort or during a plateau in force in submaximal contractions.

Peripheral VA was quantified by measurement of the torque responses to motor nerve stimulation. VA was calculated using a standard twitch interpolation equation: \[1 - (\text{SIT}/\text{POT}) \times 100\], where SIT is the superimposed twitch, i.e., any increment in torque elicited by the stimulation during a maximal knee extension, and POT is the potentiated twitch elicited at rest 1 s after the MVC.

Cortical VA was quantified by measurement of the torque responses to TMS delivered during submaximal and maximal voluntary contractions. In this technique, the amplitude of the “resting twitch” evoked by TMS must be estimated rather than measured directly, since corticospinal excitability increases during voluntary contraction (26). Thus the amplitude of a resting twitch with a comparable background excitability to twitches elicited during contraction is derived. For each participant, a linear regression of the mean amplitude of three SITs evoked by TMS during voluntary contractions of 100, 75, and 50% MVC was performed (Fig. 3A), and the y-intercept was taken as the amplitude of the resting twitch evoked by TMS [estimated resting twitch (ERT); Refs. 14, 33]. Cortical VA was subsequently quantified using the equation: \[1 - (\text{SIT}/\text{ERT}) \times 100\].

Peak-to-peak amplitude of evoked MEP and M\text{max} were measured offline. The amplitude of the MEPs was normalized to M\text{max} amplituder.
tude elicited during a “nearby” MVC (12). Muscle contractility was assessed by measurement of the amplitude and the maximum relaxation rate of the potentiated muscle twitch evoked by motor nerve stimulation (\(Q_{\text{tw,pot}}\)). In addition, the relaxation rate of muscle during each MVC was calculated using methods previously described by Todd et al. (32). Briefly, the steepest rate of decline of torque immediately following cortical stimulation during an MVC was normalized to total torque (the product of voluntary and evoked torque during that MVC). This method allows the relative relaxation rate of all the muscle fibers active in the knee extension contraction or activated by cortical stimulation to be determined (32).

**Statistical Analyses**

A repeated-measures ANOVA (v15; SPSS, Chicago, IL) was used to assess whether increasing Tc influenced neuromuscular, cerebrovascular, and hemodynamic responses. Given the variable limit of thermal tolerance between participants, the ANOVA was performed across the six levels of Tc that all participants achieved (baseline, +0.5°C, +1.0°C, +1.5°C, +2.0°C, and T-LIM), with pair-wise comparisons (Bonferroni corrected) used to identify main effects. Pearsons correlations were used to examine the relationships between the change in VA with middle cerebral artery velocity (%change from baseline), ventilation (\(\dot{V}_E\)), and \(P_{\text{ETCO}_2}\). Data are presented as means ± SD within the text and as ± SE in Figs. 1–6. Statistical significance was established at an α-level of 0.05.

**RESULTS**

All participants tolerated passive heating to at least +2°C from baseline. Data were collected at +2.5°C in three participants and at +3°C from one participant. Typical symptoms for withdrawal at T-LIM were lightheadedness and severe nausea. All participants presented to the laboratory in a euhydrated state (baseline USG, 1.014 ± 0.006). The body mass loss across the session was small (1%; from 76.6 ± 9.1 to 75.8 ± 8.9 kg; \(P < 0.01\)), and end-trial USG showed a small elevation (to 1.018 ± 0.008; \(P < 0.05\)).

**Thermal and Perceptual Responses**

Mean heating time was 179 ± 51 min, with each 0.5°C increment in Tc taking 40 ± 10 min to achieve. During the passive heating protocol, thermal sensation and thermal comfort ratings increased from the onset of heating, to values corresponding to “unbearably hot” and “extremely uncomfortable,” respectively (Table 1).

**Cerebrovascular and Cardiorespiratory Responses to Passive Heating**

**Cerebrovascular variables.** CBFV decreased progressively during heating, being significantly different from baseline by +1.0°C (Fig. 4B). Regardless of the restoration of \(P_{\text{ETCO}_2}\), during +5% \(CO_2\), CBFV remained ~20% lower than baseline (\(P < 0.05\); Fig. 4B). Changes in CBFV and \(P_{\text{ETCO}_2}\) were significantly correlated during poikilocapnia (\(R^2 = 0.91; P = 0.01\)), but this relationship was uncoupled during the period of breathing the hypercapnic gas mixture (\(R^2 = 0.08; P = 0.58\)). The calculated CVR remained unchanged throughout the heating session, except during +5% \(CO_2\), where CVR was significantly elevated above baseline (by 23.6 ± 42.0%; \(P < 0.05\)).

Cerebral oxygen delivery, as calculated from the arterial blood samples obtained from four participants, was significantly decreased from baseline after +2.0°C (\(P < 0.05\); Table 2).

**Cardiovascular variables.** Heart rate was elevated at all stages of the heating protocol (\(P < 0.05\); Table 1), although estimated stroke volume was not lowered significantly until +1.5°C. Estimated cardiac output was elevated at all increments in Tc from +1°C through to T-LIM (\(P < 0.05\); Table 1). Despite the increases in cardiac output during passive heating, MAP was significantly reduced at all Tc elevations, except for during +5% \(CO_2\), when MAP was restored to baseline values (Table 1; Fig. 4D).

**Respiratory variables.** \(\dot{V}_E\) was elevated at +1.0°C and thereafter (\(P < 0.05\); Fig. 4A) but returned to baseline values during the +5% \(CO_2\) protocol. As expected, \(P_{\text{ETCO}_2}\) decreased gradually throughout the heating protocol with increasing \(P_{\text{ETCO}_2}\) (\(P < 0.05\); Fig. 4C) and returned to baseline during +5% \(CO_2\), as intended.

**Arterial blood variables.** Hemoglobin concentration and consequently \(CaO_2\), were elevated significantly above baseline throughout heating (\(P < 0.05\); Table 2), while \(PaO_2\) was not increased significantly until participants reached +2°C above baseline (\(P < 0.05\); Table 2). Consistent with the \(P_{\text{ETCO}_2}\) measures, hyperthermia-induced hyperventilation resulted in a lowering of \(PaCO_2\), and pH was elevated. These values became significantly different from baseline at +2°C and T-LIM (\(P < 0.05\); Table 2). During +5% \(CO_2\), \(PaCO_2\) and pH returned towards baseline values.

**Neuromuscular Function**

\(MVC\). Force production started to be impaired after Tc was increased above 1°C from baseline, although a significant impairment in MVC was only observed at T-LIM (~9 ± 11% change from baseline; Fig. 5A; \(P < 0.05\)). During +5% \(CO_2\), MVC was restored to baseline levels. Voluntary RMS EMG amplitude of the vastus lateralis during maximal efforts was decreased significantly at all increments in Tc >0.5°C above

| Table 1. Thermal and cardiovascular responses at baseline and 0.5°C increments to the limit of thermal tolerance |
|------------------------------|--------|--------|--------|--------|--------|
|                             | Baseline | +0.5°C | +1.0°C | +1.5°C | +2.0°C |
| Thermal                     |         |        |        |        |        |
| Mean skin temperature, °C   | 28.1 ± 2.0 | 33.5 ± 0.9† | 34.4 ± 0.8† | 34.8 ± 0.7† | 34.9 ± 0.6† |
| Thermal sensation (1-13)    | 6.6 ± 0.7 | 9.9 ± 0.8† | 10.3 ± 1.1† | 11.4 ± 1.1† | 12.3 ± 1.6† |
| Thermal comfort (1-5)       | 1.0 ± 0.0 | 3.1 ± 1.7* | 4.5 ± 2.5† | 4.8 ± 1.8† | 4.9 ± 0.5† |
| Cardiovacular               |         |        |        |        |        |
| Heart rate, beats/min       | 66 ± 8  | 90 ± 20† | 102 ± 17† | 113 ± 20† | 122 ± 19† |
| Stroke volume, ml           | 98.5 ± 13.7 | 90.1 ± 20.0 | 87.4 ± 20.5 | 78.4 ± 15.8* | 75.2 ± 13.9† |
| Mean arterial pressure, mmHg| 76.3 ± 10.2 | 66.3 ± 6.1* | 67.3 ± 4.4* | 69.2 ± 4.8* | 67.0 ± 5.4* |
| Cardiac output, l/min       | 6.7 ± 1.1 | 8.2 ± 0.9 | 8.8 ± 1.3† | 8.6 ± 1.2* | 9.2 ± 0.8** |

Values are means ± SD (\(n = 7\)). Thermal sensation scale range: 1, unbearably cold; 7, neutral; 13, unbearably hot. Thermal comfort: 1, comfortable; 5, extremely uncomfortable. T-LIM, limit of thermal tolerance. *\(P < 0.05\), †\(P < 0.01\), significant difference from baseline.
baseline, falling 27 ± 23% below baseline at T-LIM (P < 0.01; Fig. 5E).

**Responses to electrical stimulation.** $M_{\text{max}}$ amplitude did not change throughout the passive heating protocol (Fig. 5D). However, contractility of the muscle was enhanced by the heating, evidenced by a significant increase from baseline in $Q_{\text{m,post}}$ at +1.5°C (+21 ± 27%) and thereafter (Fig. 5C). The maximal relaxation rate of the resting twitch evoked by motor nerve stimulation became faster throughout the passive heating protocol and was significantly so at T-LIM ($P < 0.05$; Fig. 5G). VA measured via electrical stimulation of the motor nerve was 88 ± 8% at baseline. VA was reduced at +1.5°C (82 ± 9%; $P < 0.05$) and remained decreased, although not significantly, at +2.0°C (82 ± 8%; $P = 0.06$), at T-LIM (82 ± 9%; $P = 0.07$), and during +5% CO$_2$ (78 ± 15%; $P = 0.08$; Fig. 5F).

**Responses to TMS.** Motor nerve estimates of VA were comparable to those derived from TMS at baseline (~88% vs. 85%). Cortical VA was decreased significantly at +1.5°C and T-LIM by 11 ± 8 and 22 ± 23%, respectively ($P < 0.05$; Fig. 5B); yet during +5% CO$_2$, cortical VA was not significantly below baseline ($P = 0.30$). The amplitude of evoked MEPs (relative to $M_{\text{max}}$) in the knee extensors during each contraction intensity (100, 75, and 50% MVC) was unaffected by the passive heating protocol (Fig. 2B). The normalized peak relaxation rate of the superimposed twitch following cortical stimulation was increased at +1.5°C, +2.0°C, T-LIM, and +5% CO$_2$, reaching its peak rate at T-LIM (~7.9 ± 2.9 s$^{-1}$), a 54 ± 28% increase from baseline values.

**Relationship Between Cerebrovascular and Respiratory Responses and Cortical VA**

The reduction in cortical VA was correlated with the decreases in PETCO$_2$ for data pooled across participants ($R^2 = 0.63; P = 0.03$; Fig. 6B). Individual regression analysis ranged between $R^2$ 0.38 and 0.93. Reductions in cortical VA also correlated with

### Table 2. Hemodynamic data collected from the arterial blood samples of 4 participants who were catheterized for the duration of the experimental protocol

<table>
<thead>
<tr>
<th>Baseline</th>
<th>+0.5°C</th>
<th>+1.0°C</th>
<th>+1.5°C</th>
<th>+2.0°C</th>
<th>T-LIM</th>
<th>EUCAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaO$_2$, ml-O$_2$·dl</td>
<td>19.5 ± 1.3</td>
<td>21.2 ± 0.2†</td>
<td>20.9 ± 1.5*</td>
<td>21.7 ± 0.7†</td>
<td>21.8 ± 0.8†</td>
<td>21.9 ± 0.8†</td>
</tr>
<tr>
<td>PaO$_2$, mmHg</td>
<td>101.3 ± 4.0</td>
<td>100.7 ± 8.1</td>
<td>106.0 ± 0.2</td>
<td>105.7 ± 8.1</td>
<td>115.7 ± 0.6†</td>
<td>116.4 ± 0.6†</td>
</tr>
<tr>
<td>PaCO$_2$, mmHg</td>
<td>41.0 ± 1.7</td>
<td>40.7 ± 1.2</td>
<td>35.3 ± 1.2</td>
<td>31.6 ± 7.5</td>
<td>26.0 ± 1.7*</td>
<td>25.6 ± 2.3*</td>
</tr>
<tr>
<td>COD, AU</td>
<td>—</td>
<td>-83 ± 72</td>
<td>-131 ± 200</td>
<td>-253 ± 205</td>
<td>-277 ± 202*</td>
<td>-317 ± 150*</td>
</tr>
<tr>
<td>[Hb], g·dl</td>
<td>15.1 ± 1.1</td>
<td>16.4 ± 0.2†</td>
<td>16.0 ± 1.2†</td>
<td>16.6 ± 0.6†</td>
<td>16.7 ± 0.6†</td>
<td>16.9 ± 0.8**</td>
</tr>
<tr>
<td>pH</td>
<td>7.42 ± 0.01</td>
<td>7.44 ± 0.01</td>
<td>7.48 ± 0.05</td>
<td>7.51 ± 0.11</td>
<td>7.55 ± 0.04*</td>
<td>7.56 ± 0.07*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Cerebral oxygen delivery (COD) is expressed arbitrary units (AU) in terms of a change in middle cerebral artery blood flow velocity from baseline. CaO$_2$, arterial O$_2$ content; PaO$_2$, O$_2$ tension; PaCO$_2$, CO$_2$ tension; [Hb], hemoglobin concentration; EUCAP, eucapnia. *$P < 0.05$, **$P < 0.01$, significant difference from baseline.
increases in $\dot{V}E$ whether using pooled data ($R^2 = 0.76; P = 0.01$; Fig. 6C) or individual data (0.52–0.81). Decreases in cortical VA also tended to correlate with reductions in CBFv for pooled ($R^2 = 0.61; P = 0.07$; Fig. 6A) and individual regressions (0.13–0.86).

**DISCUSSION**

This study is the first to assess both cerebrovascular and cortical function during progressive, passive hyperthermia in humans. The novel findings were that 1) a reduction in CBFv from the onset of heating is subsequently accompanied by a progressive impairment of motor cortical output and neural drive, which appears to contribute to a loss of force generating capacity during maximal contraction of the knee extensors; 2) during the period of breathing the hypercapnic gas mixture at the limit of heating tolerance, MVC and cortical VA were restored towards baseline values despite CBFv remaining reduced; and 3) changes in CBFv, PETCO2, and ventilation during poikilocapnia were related to cortical VA across levels of heat strain. Collectively, these findings indicate that rather than hyperthermia per se, heat-induced hyperventilation, and the subsequent changes in $PaCO_2$ and CBF, are associated with decreased voluntary drive. The discussion below focuses on $Tc$ and not $Tsk$ as the functional component of hyperthermia, despite elevated $Tsk$ having modulatory roles in cardiovascular, respiratory (15), and motor activation (13) during heat stress. Rather, the focus on $Tc$ is because it was the controlled variable in...
this study and is more instrumental in modulating cardiovascular, respiratory and motor responses.

Cerebrovascular and Hemodynamic Changes During Passive Heating

Consistent with previous reports (10) during passive hyperthermia, an increase in Tc of approximately +0.5°C initiated an increase in VE and thus constituted a heat-induced hyperventilation threshold. Consistent with previous studies (e.g., Refs. 10, 21), we found that decreases in PETCO2 were highly correlated with decreases in CBFv during passive heating. Also consistent with a recent study (5), a period of breathing a 5% CO2 gas mixture, which restored PETCO2 to resting values, did not elicit concomitant increases in CBFv. Collectively, these findings are coherent with the view that during passive heat stress, other factors (e.g., cardiac output redistribution and sympathetic nerve activity-induced cerebral vasoconstriction) may play a contributory role in changes in CBFv.

Respiratory Changes During Passive Heating

Ventilation rose linearly with increasing Tc during passive heating, such that it had doubled from baseline values at T-LIM. This increased respiratory drive has been consistently reported during hyperthermia, and although its causation or function is not fully understood, it has been suggested to be integral to the thermoregulatory response (1, 6, 38). An elevated Tc is seemingly a more potent stimulus to ventilation than the inhibition brought about by the ensuing hypocapnia, and this increased ventilation may play a role in “selective brain cooling” through respiratory evaporative heat loss and countercurrent heat exchange in the cranial vessels (38). Because of the profound effects of PETCO2 on CBFv (3) as well as on cortical excitability (28), we also made measurements whilst participants breathed a hypercapnic gas mixture at a high Tc. PETCO2 (and in the 4 subjects, Pao2) was increased during this period, and VE was reduced back towards baseline values yet Pao2 remained elevated. Upon examination of the individual VE traces, each subject showed the reduction with 5% CO2 breathing when at thermal tolerance. The reason for this paradoxical reduction in ventilation in the face of an increasing ventilatory stimulant (i.e., 5% CO2) is unclear and may warrant further study. However, because Paco2 is more tightly influenced by alveolar ventilation (i.e., Paco2 = VCO2/alveolar ventilation), caution should be expressed in interpreting the relationship between PETCO2 and pulmonary ventilation.

CNS function during passive heating. In conjunction with the decreased CBFv and PETCO2, the first statistically significant impairment in CNS function was observed at +1.0°C Tc. EMG amplitude during a maximal effort was decreased in the absence of any changes in the amplitude of the M wave. Since temperature-induced changes in the muscle might affect sarcolemmal excitability, the changes in RMS EMG have to be considered in light of changes in the muscle action potential (17). That the EMG amplitude was decreased but the M-wave amplitude remained unchanged is evidence of a reduction in the number of motoneurons voluntarily recruited during maximal knee extension. EMG remained decreased to a similar extent for the remainder of the heating protocol. As Tc increased to +1.5°C, cortical VA was reduced by ~11%. This reduction demonstrates supraspinal involvement in the failure of central drive during passive heating. In the only other study (32) to examine cortical VA in hyperthermia, a one-step heating protocol was utilized, such that measurements of cortical drive to the elbow flexors were made in normothermia and at one elevated Tc (+1.3°C). In that study, passive hyperthermia did not reduce cortical VA during brief (3 s) contractions; however, during longer, sustained MVCs, VA was reduced. Our observed decreases in output from the motor cortex during brief (3–5 s) contractions during hyperthermia are likely due to a number of factors. Due to measurements being made at each 0.5°C increment in Tc, and a

Fig. 6. Relationships between changes in CBFv (A), PETCO2 (B), and VE (C) with changes in cortical voluntary activation (VA) during passive heating. Values are expressed as group means (n = 8). ○, Data points during the passive heating protocol; ◦, data collected during a period of breathing a 5% CO2 gas mixture (+5% CO2) at T-LIM.
different heating method being utilized in the current study (water perfused suit vs. water immersion), the heating time was 177 ± 5 vs. 19 ± 7 min in the study of Todd et al. (31), thus exposing participants to a higher Tc and hyperthermic state for a longer duration. In addition, the hyperthermic measurements in the study of Todd et al. were made after participants had been removed from hot water immersion, and no data were presented as to how well hyperthermia was “maintained” during the subsequent data collection period. Our measurements were made while participants were held at a constant Tc at each 0.5°C increment by the manipulation of the flow rate through the water-perfused suit.

VA derived from electrical stimulation of the motor nerve was also decreased (by 9%) as Tc was elevated +1.5°C above baseline. Such decreases are consistent with previous reported reductions in peripherally derived VA of 5–14% following passive heating of +1.5–2.0°C (18, 19, 31). Moreover, since it has been shown that during cooling from hyperthermia, force and VA measurements return to baseline values (18), such findings confirm that neuromuscular function was influenced directly by Tc.

At the limit of thermal tolerance, cortical VA, maximal EMG, and MVC were all significantly decreased from baseline. Thus the failure in voluntary drive, evidenced by a decreased EMG and loss of maximal volitional force can be attributed, in part, to a reduction in output from the motor cortex, due to impairments within the motor cortex itself or in upstream brain regions. These changes in cortical drive cannot be explained by changes in motor cortex excitability, since the MEP amplitude was unchanged throughout the protocol. Severe hypocapnia (PETCO2 of <15 Torr) has been shown to increase motor cortex excitability (29), but those authors utilized a voluntary hyperventilation protocol of 10-min duration. The levels of PETCO2 experienced by our participants would seem too high to have an effect on cortical excitability, and the more prolonged heat-induced hyperventilation is likely an incomparable phenomenon to acute voluntary hyperventilation.

Notably, during the period when participants breathed the hypercapnic gas mixture, MVC and cortical VA returned towards baseline values. Since these measurements were always made at an elevated core body temperature (i.e., at the limit of thermal tolerance), our findings demonstrate that the reduction in voluntary drive cannot be explained by increased core temperature per se. The two significant physiological changes that accompanied the partial restoration of MVC and VA were the increase in PaCO2 and a decrease in Ve.

Relationships Between Cerebrovascular and Corticomotor Responses to Passive Heating

Changes in CBFv during the heating protocol tended to correlate with changes in cortical VA. The cerebral circulation is known to remove heat from the brain during periods of elevated Tc (22). It is tempting to suggest that the reduction in CBFv may have augmented any increases in brain temperature due to raised Tc and had direct effects on motor cortical output. For example, while speculative, a “hot brain” might regulate corticomotor output, such that premotor cortical regions receive hypothalamic input during hyperthermia and as such modulate output from the motor cortex, to reduce muscular work and subsequent metabolic heat production, which would contribute to further increases in Tc.

Although the impairments in voluntary drive were related to CBFv during the progressive hyperthermia, the restoration of PETCO2 did not affect CBFv values (remained ~20% below baseline). Conversely, there was a recovery of cortical VA and MVC with this restoration of PETCO2 during +5% CO2. Thus it is unlikely that CBFv is the determining factor causing impairments in central drive during hyperthermia. However, the strong relations between changes in both PETCO2 and Ve and cortical VA, indicate that this hyperventilation response may have been integrated into the regulation of voluntary drive.

Muscle Function During Passive Heating

Increases in muscle temperature via passive heating have been shown to increase the speed of contraction and relaxation of muscle twitches evoked by electrical stimulation (18, 31) and by tMS (32). Our observations were consistent with these previous findings, as relaxation rate of the potentiated muscle twitch and the force response to TMS during an MVC were increased. This faster relaxation rate has been associated with a decrease in isometric force production, since higher motor unit firing rates are required to fuse force and maintain a contraction over longer durations (32). However, in the present study, the brief duration of the MVCS (3–5 s) was probably not long enough for the temperature-induced changes in muscle contractility to have an influence on isometric force production. Todd et al. (32) suggested that during short contractions motor unit discharge may be sufficiently increased to maintain maximal force.

In contrast to the findings of others (18, 31, 32), who did not observe an effect of heating on the peak peripherally evoked twitch tension, we observed an increase in Qtw,pot amplitude during the passive heating protocol. Potentially, this may be related to changes in pH. The data from arterial blood samples showed that pH during hyperthermia increased to 7.56 ± 0.07, which is indicative of alkalaemia and is consistent with findings from exercise-induced hyperthermia (1). Alkaline pH has been reported to cause increased tetanic force in mouse skeletal muscle, possibly due to improved Ca2+ dynamics within the muscle (38). Thus although muscle contractility was affected by temperature-induced changes in relaxation rates, it may also have been augmented by the increasing alkaline environment during the heating protocol. The increase in Qtw,pot might also have helped to maintain force generating capacity (MVC) in spite of decreasing voluntary drive at the elevations in Tc before T-LIM.

Technological Considerations

In the present study, we used middle cerebral artery velocity as an index of CBF. This relation is valid so long as the sonicated vessel diameter does not change during the intervention. The diameter of the middle cerebral artery has been shown to be unaffected by hyperventilation (36) or changes in PETCO2 (27). In addition, CBFv has been shown to provide a reproducible and quantifiable index of changes in cerebral blood flow when validated against other techniques (e.g., 8, 23). However, it is important to consider that changes in CBFv measured in the MCA do not proportionally reflect regional changes in cerebral blood flow (16). Thus it is unknown
whether, despite a decrease in measured CBFv, there might have been regional increases in cerebral blood flow to motor regions of the respiratory or lower limb muscles due to their increased activation during the experiment (39).

The fact that CBFv was not restored towards baseline values during +5% CO₂ when PETCO₂ was increased was unexpected since CBF is highly sensitive to direct changes in PaCO₂. However, our experimental measurements were made shortly after PETCO₂ reached eucapnic levels for each individual (after 1–2 min), and this may not have been enough time to achieve a steady-state change in CBFv. It has been shown that the time constant of the CBF response to a step increase in CO₂ is slower than that in response to a decrease (24), and it seems plausible that this response may be further attenuated by the marked alkalosis. Thus, during the reversal of hypocapnia, while PETCO₂ increased quickly, the CBF response may have needed longer to stabilize. In addition, during conditions of CO₂ change, the extent to which PaCO₂ increases may be less than that of PETCO₂ (35). As such, we may have overestimated the extent of hypocapnia “reversal” from PETCO₂ values. Thus a greater and more sustained increase in PaCO₂ back to actual baseline levels (rather than within ~8 Torr as observed in the present study) may have been required to elicit more substantial changes in CBFv. It therefore seems that PETCO₂ may overestimate PaCO₂ during CO₂ change in the severely hyperthermic state. Importantly, however, although CBFv was not fully restored, MVC and cortical VA were restored towards baseline values during inhalation of 5% CO₂. Thus, if anything, it would seem likely that we have underestimated the effect of full PaCO₂ restoration.

Activation of the motor cortex by magnetic stimulation could have confounding effects on our other measurements of cerebral function, particularly CBFv. Single pulse TMS has been shown to increase the blood-oxygenation-level-dependent-functional MRI response, presumably correlated with increased blood flow (4). However, these changes were transient (~12 s), and since our measurements of CBFv were always made before TMS at each incremental Tc, ~40 min would have elapsed since the last TMS measurements were made at the preceding Tc.

It was not deemed necessary for the current experimental design to employ a normothermic control group/condition with measurements made at corresponding time points to the hyperthermic trial, since the stability of the measured dependent variables is well established. We have demonstrated that within day measurement of cortical VA has minimal systematic bias (0.23%) and a coefficient of variation of 3.7% (14). In addition, CBFv of the MCA has been shown to remain stable over a 12-h period as long as measurements are taken with subjects in the same postural position (9).

In summary, this study is the first to examine both cerebrovascular and corticomotor function during passive, progressive hyperthermia. We have shown that the central neural drive is impaired at temperature increments >0.5°C above normothermia and that failure of voluntary drive at or above the level of the motor cortex is evident after a 1.0°C rise in Tc. These impairments may be due, in part, to reductions in CBFv, which may impair the ability of the brain to dissipate heat. The hyperventilatory response to increasing Tc and ensuing hypocapnia appear to play an important contributory role in modulating cortical output.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


