Title: *Negligible influence of moderate to severe hyperthermia on blood-brain barrier permeability and neuronal-parenchymal integrity in healthy men*

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Running Header:

Heat stress and the neurovascular unit

New & Noteworthy:

- The acute effects of passive whole-body hyperthermia on the integrity of the neurovascular unit (NVU) in humans have remained unclear.
- We demonstrate that passive heating for ~one hour until an increase of +2°C esophageal temperature in healthy men does not increase the cerebral release of neuronal parenchymal stress biomarkers, suggesting the NVU integrity is maintained.
- This preliminary study indicates passive heating is safe for the brain, at least in young healthy men.

Abstract:

With growing use for hyperthermia as a cardiovascular therapeutic, there is surprisingly little information regarding the acute effects it may have on the integrity of the neurovascular unit (NVU). Indeed, relying on animal data would suggest hyperthermia comparable to levels attained in thermal therapy will disrupt the blood-brain barrier (BBB) and damage the cerebral parenchymal cells. We sought to address the hypothesis that controlled passive hyperthermia is not sufficient to damage the NVU in healthy humans. Young men (n=11) underwent acute passive heating until +2°C or absolute esophageal temperature of 39.5°C. The presence of BBB opening was determined by trans-cerebral exchange kinetics (radial-arterial and jugular venous cannulation) of S100B. Neuronal parenchymal damage was determined by the trans-cerebral exchange of tau protein, neuron specific enolase (NSE) and neurofilament-light protein (NF-L). Cerebral blood flow to calculate exchange kinetics was measured by duplex ultrasound of the right internal carotid and left vertebral artery. Passive heating was performed via warm-water perfused suit. In hyperthermia, there was no increase in the cerebral exchange of S100B ($p=0.327$), tau protein ($p=0.626$), NF-L ($p=0.447$) or NSE ($p=0.908$) suggesting +2°C core temperature is not sufficient to acutely stress the NVU in healthy men. However, there was a significant condition effect ($p=0.028$) of NSE, corresponding to a significant increase in arterial ($p=0.023$) but not venous ($p=0.173$) concentrations in hyperthermia, potentially indicating extra-cerebral release of NSE. Collectively, results from the present study support the notion that in young men there is little concern for NVU damage with acute hyperthermia of +2°C.

Keywords: Heat stress, Brain, neurovascular unit

Introduction

The use of heat stress, or heat *therapy*, as a remedial and prophylactic tool for cardiovascular health has gained recent attention [reviewed in (15)]. While heat, or fever, as a non-specific therapeutic has been touted since Hippocrates (8), the interest in the potential cardiovascular benefits of heat have largely stemmed from epidemiological studies linking frequent sauna use to a reduced incidence of cardiovascular events and vascular disease (32-35). Supporting the epidemiology data is a growing number of experimental-based studies suggesting a direct benefit of transient heat stress on vascular endothelial function. For example, Brunt et al. (12) reported that eight weeks of passive heat therapy, via hot water immersion, improved vascular function in young, sedentary adults: specifically, increased brachial artery flow-mediated dilation and reduced resting blood pressure. Moreover, in clinical models, heat therapy has been demonstrated to improve cardiac function and increase exercise tolerance in heart failure (27, 39), myocardial perfusion in coronary artery disease (21, 54) and extremity perfusion in peripheral artery disease (1, 53, 55).

What remains to be determined is whether heat therapy can be used as a cerebral vascular therapeutic, particularly of the neurovascular unit (NVU). A major component of the NVU is the cerebral microvasculature, along with the surrounding parenchymal cells. That is, the NVU consists of endothelial cells, associated blood-brain barrier (BBB) tight junctional proteins, pericytes, astrocytes, and neurons, among others (24). It is often assumed that the reported beneficial impacts of heat therapy on the peripheral vasculature also reflect improvements to the cerebral vasculature (14, 19, 43), and improvements of the entire NVU since chronic heat

102 therapy has been associated with a reduced incidence of vascular dementia (33). However, this
103 assumption may be misleading considering the peripheral vs. cerebral vascular beds experience
104 markedly different stressors in acute hyperthermia. For example, during heat stress the peripheral
105 endothelium is exposed to increases in beneficial anterograde shear stress [i.e., increased
106 foreword moving blood flow (22)], while the cerebral endothelium is exposed to reduced
107 anterograde shear (i.e., reduced forward-moving blood flow) secondary to cerebral
108 vasoconstriction as a result of respiratory alkalosis (7). Moreover, hyperthermia which
109 encroaches an absolute core temperature of 40°C may selectively become cytotoxic to the
110 delicate cells of the CNS compared to vascular cells in the periphery (50). Additionally, whole-
111 body hyperthermia exceeding 38.5°C [(a core temperature elevation well within the realm of heat
112 therapy (34)] is recognized to increase the permeability of the BBB in rodent models (52, 28).
113 Importantly, a compromised BBB has the potential to alter the extracellular environment and
114 thereby induce damage to other parts of the NVU by means of neuroinflammation, edema, and
115 ionic imbalances (42).
116
117 Increased permeability of the BBB in humans is often assessed by circulating concentrations of
118 S100B, a ~11 kDa astroglial protein, that has to cross the BBB to enter the bloodstream. Its
119 presence in the peripheral circulation is, in turn, generally reflective of a leaky BBB (62). To
120 date, in humans, there remains little data on the release of S100B in whole-body hyperthermia,
121 with the bulk of data derived from exercise-induced heat stress. For example, Watson et al. (62)
122 demonstrated that peripherally circulating concentrations of S100B is increased during
123 hyperthermic exercise (~2°C increase in core temperature). Conversely, Cheuvront et al. (16)
124 observed no mean increase in circulating S100B during hyperthermic exercise, with temperature

125 elevations of ~1.5°C while walking in the heat. Apart from the small difference in core
126 temperature, a few alternative explanations may be offered for these data. Most notably, while
127 the majority of S100B is of cerebral origin, it can additionally be released from stressed cardiac
128 and skeletal muscle, which may be exacerbated with hyperthermia (62). The increase in S100B
129 during hyperthermic exercise reported by some (60-62) may therefore, in part, reflect an increase
130 in extra-cerebral sources, rather than BBB opening. Indeed, Watson et al., (62) had participants
131 cycle at 60% of VO_2 peak, whereas participants were walking in Cheuvront et al., (16). Until the
132 present study, there has been a paucity of data on the independent impact of heat stress – i.e.,
133 passive hyperthermia – on BBB leakage and subsequent health of the neuronal parenchyma and
134 entire NVU in humans.
135
136 Given that hyperthermia of up to +2°C core temperature [i.e., during hot yoga, Finnish sauna or
137 Waon therapy (15)] has gained widespread attention as a tool to improve cardiovascular
138 function, there is a need to confirm that this level of heating is safe for the NVU. Accordingly,
139 the purpose of this study was to determine the trans-cerebral arterial-venous kinetics of S100B
140 (an established marker of BBB permeability) in passive hyperthermia, as well as concurrent
141 biochemical metrics of the NVU function as indexed by an established clinical panel for cerebral
142 damage; tau protein, neuron specific enolase (NSE) and neurofilament-light protein (NF-L) (26,
143 57, 59). Based on our previous observation (6) that passive hyperthermia does not evoke a robust
144 increase in the cerebral release of inflammatory and pro-oxidative markers in humans (a tenable
145 mechanism for BBB opening), we hypothesized that acute passive hyperthermia of +2°C core
146 temperature in healthy young men would not elicit marked BBB opening as determined by the

147 trans-cerebral release of S100B, or detectable cerebral parenchymal damage determined by the
148 release of tau protein, NSE, and NF-L.
149

Methods

Subjects and ethical approval:

Eleven healthy young men (age 23 ± 3 years) participated in the study. All subjects were non-obese (body mass index 23.0 ± 2.1 kg/m), normotensive (118/71 ± 6/7 mmHg), normoglycemic (<7.0 mmol/L), non-smoking and free of overt cardiometabolic and respiratory disease (all variables are mean±SD). All experimentation was completed at the Centre for Heart, Lung & Vascular Health, University of British Columbia, Kelowna, BC, Canada. The ethical committee of the University of British Columbia approved the study (H15-00166). The study conformed to the standards set by the *Declaration of Helsinki,* except registry in a database. All subjects provided informed written consent before experimentation. Subset measures from this study have been published elsewhere under separate experimental questions relating to circulating microvesicles (3), and cerebral metabolism (6). The present study encompasses separate *a-priori* hypotheses.

Experimental protocol:

Subjects arrived at the laboratory after a 4 to 12 hr fast and minimum 12 hr abstinence from alcohol and caffeine-containing beverages. Under local anaesthesia (1% lidocaine) and ultrasound guidance, a 20-gauge arterial catheter (Arrow, Markham, ON, Canada) was placed in the right radial artery, and a central venous catheter (Edwards PediaSat Oximetry Catheter, CA, USA) was placed in the right internal jugular vein and advanced towards the jugular bulb. Following cannulation, subjects were fitted into a tube-lined suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body except for the head, feet and hands. The tube-lined suit was

perfused with ~49°C water until an esophageal temperature of +2°C above baseline, an absolute core temperature of 39.5°C, or the subject's volitional thermal tolerance was reached. Core temperature (Teso) was determined by a thermocouple probe (RET-1; Physitemp Instruments, Clifton, NJ, USA) that was inserted 40 cm past the nostril into the esophagus. Blood samples were collected into vacutainers containing ethylenediaminetetraacetic acid (EDTA) for separation of plasma and quantification of tau, as well as tubes containing no anticoagulant for analysis of S100B, NSE and NF-L. Samples were collected simultaneously from the radial artery and jugular bulb immediately before heating (normothermic) and at +2°C core temperature. A time-control group was not incorporated into the experimental design given previous reports demonstrating no time effect of the cross-brain measures (4, 5).

Cardiovascular and cerebrovascular measures:

Blood flow in the right internal carotid artery (ICA) and left vertebral artery (VA) was simultaneously measured using duplex vascular ultrasound (Terason 3200, Teratech, Burlington, MA), and used to calculated global cerebral blood flow; (ICA x 2) + (VA x 2). The right ICA was on average insonated 2cm from the carotid bifurcation, while the left VA was insonated at the C5–C6 or C4–C5 space depending on the subject’s unique anatomy. The steering angle was fixed to 60 degrees for all measures, and the sample volume was placed in the center of the vessel adjusted to cover the entire vascular lumen. All files were screen-captured and saved as video files for offline analysis at 30Hz using custom-designed software (63). Simultaneous measures of luminal diameter and velocity over a minimum of 12 cardiac cycles were used to calculate blood flow. The within-day coefficient of variation for the ICA and VA blood flow was 7% and 4%, respectively. Heart rate (HR) was obtained from the R-R intervals measured in lead

195 II of the ECG. Mean arterial blood pressure (MAP) was measured with a pressure transducer
196 connected to the radial catheter.

197 *NVU biomarker analysis*:

198 Serum S100B and plasma NSE concentrations were measured using commercially available
199 immunoassays with electrochemiluminescence detection on Cobas according to instructions
200 from the manufacturer (Roche Diagnostics, Penzberg, Germany). Serum NF-L concentration was
201 measured on a Single molecule array (Simoa) HD-1 Analyzer using the commercially available
202 NF-Light kit according to instructions from the manufacturer (Quanterix, Billerica, MA). Plasma
203 tau concentration was measured on a Simoa HD-1 Analyzer using the commercially available
204 Tau Advantage kit according to instructions from the manufacturer (Quanterix, Billerica, MA).
205 All measurements were performed in one round of experiments using one batch of reagents by
206 board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients
207 of variation were 3-5% for S100B and NSE, 7.2% for NF-L and 11% for tau. Cerebral exchange
208 was calculated as the global cerebral blood flow x the arterial-venous difference of each
209 respective biomarker, whereby a negative value denotes cerebral release.

210 NSE, tau protein, and NF-L were used to determine cerebral parenchymal damage as they
211 collectively make up a common clinical panel for the prognosis of brain damage (23, 48, 59).
212 NSE is an enzyme that is typically found in neurons and neuroendocrine cells and has been
213 shown to upregulate following neuronal death [e.g., after a traumatic brain injury (TBI); 23, 59].
214 Similarly, tau protein and neurofilament-light protein (NF-L) are established biomarkers of brain
215 trauma; they both reside in axons that are susceptible to damage (59, 65). NF-L is composed of

polypeptides and provides structural axonal support, whereas tau protein provides stability to the axonal microtubules (59).

Statistical analysis:

Analyses were performed using the statistical software package SPSS (v.22; IBM, Armonk, NY, USA). Cerebral blood flow (to calculate cerebral exchange of brain proteins) was averaged over 20-second bins around the blood draws. Tests for normality were confirmed using repeated Shapiro-Wilks W tests. Statistical analyses for all NVU biomarkers were performed using 2-way [condition (baseline vs. hyperthermia), and site (arterial vs. venous)] repeated-measures ANOVA. After a main effect, post hoc analyses were performed using two tailed repeated-measures Student's t-tests. Effect size was calculated as Hedges' g corrected for a small sample size using the formula: Hedges' g $=\frac{M1-M2}{Pooled\ SD}\ x\ \left(\frac{N-3}{N-2.25}\right) x\ (\sqrt{\frac{N-2}{N}})$, where the mean 1 (M1) was baseline, and mean 2 (M2) was heat stress. Significance was determined at an alpha level of 0.05. All data are presented as means ±SD.

Results

Thermometry and descriptive data:

Absolute esophageal temperature at baseline was 37.3±0.2°C, and at peak heat stress was 39.2±0.2°C. Average heating time (elapsed time between baseline and measures at peak heat stress) was 58±8 min. Participants were kept at peak heat stress for ~five minutes. Individual core temperature for baseline and heat stress, as well as cardiovascular and cerebrovascular descriptive data, is presented in Table 1.

NVU biomarkers:

Mean data are presented in Table 2. Individual data are presented in Figure 1, and individual cerebral exchange data are presented in Figure 2. There were no effects on S100B, tau protein, or NF-L across condition, site, or condition x site (p all >0.05). However, there was a significant main effect of heat stress (condition) on NSE (p=0.028), but no significant main effect of site (p=0.910) or interaction (p=0.908). Post hoc analysis revealed a significant increase in arterial (p=0.023; Hedges' *g*= -0.40) but not venous (p=0.173; Hedges' *g*= -0.43) concentrations of NSE from baseline to heat stress. There were no significant effects of heat stress on the cerebral exchange of S100B (Hedges' *g*= -0.56), NSE (Hedges' *g*= -0.14), tau protein (Hedges *g*= -0.09), or NF-L (Hedges' *g*= -0.12) (p all >0.05).

Discussion

The primary finding of this study is that marked passive hyperthermia of ~2.0ºC by means of passive heating is not sufficient to acutely open the BBB or provoke any discernable cerebral neuronal parenchymal damage in young healthy males. This finding was evidenced by an unaltered cerebral exchange of S100B, NSE, tau protein, and NF-L. However, heat stress increased circulating NSE in the arterial circulation, perhaps indicating contribution from non-cerebral sources.

Is +2℃ Core Temperature Safe for the NVU?

Results of the present study are timely given the recent surge in employing passive heat stress as a cardiovascular therapeutic (12, 21, 27, 29, 32-36, 39, 54). In experimental use of thermal therapy (e.g., sauna use), core temperature elevations of up to 2°C are often reported (34). While it is generally accepted that induced heat stress should not exceed an absolute core temperature of ~40°C [to avoid life-threatening complications of heat illness (10)], impetus for the present study relates to the notion that the cerebral tissue may become damaged at a much lower threshold temperature. For example, with progressive continuous heating, as employed in the current study, BBB leakage in rats (assessed by stained albumin and astrocytic activation) begins to occur at 38.5°C (28). Moreover, even a 1.0°C increase in core temperature is problematic for cerebral outcomes in human conditions of traumatic brain injury (9, 56), likely in part related to increased pro-inflammatory responses. That is, passive heat stress ubiquitously increases IL-6, a known cytokine that stimulates BBB opening (60), which can induce or exacerbate damage to

278 the NVU (in the setting of TBI) by means of neuroinflammation, edema, and ionic imbalances
279 (42). Importantly, we have previously demonstrated that with +2.0°C core temperature the
280 cerebral exchange of pro-oxidative and inflammatory markers (oxidative-low density lipoprotein,
281 myeloperoxidase, and IL-6) are not increased, however, the increase in IL-6 in a sample of six
282 participants trended to selectively increase more in the cerebral tissue (6). At least in healthy
283 young males, results from the present study reassure that this trend for increases in cerebral IL-6
284 does not lend to BBB opening. (There is an absence of a relationship between jugular venous IL-
285 6 and S100B in heat stress; r=0.454, p=0.219, unpublished data, n=10.)
286
287 *Biomarkers for NVU Damage; Impact of Extracerebral Sources?*
288
289 The primarily astroglial protein S100B was used to quantify BBB leakage (16, 26, 60), while
290 concentrations of the CNS dominant tau protein, NF-L, and NSE were measured to provide
291 insight on neuronal parenchymal damage. While the lack of cerebral release or increase in
292 jugular venous concentrations of S100B, tau protein, NF-L and NSE collectively suggests that
293 passive heat stress up to 39.5°C core temperature in healthy young men is not sufficient to
294 acutely increase BBB permeability or damage the cerebral neuronal parenchyma; the increase in
295 NSE from BL to HS is notable. Because of the lack of cerebral exchange, this net increase in
296 NSE from baseline to heat stress may be attributed to release from non-cerebral sources, which is
297 consistent with systemic release of NSE driving a similar average but highly variable (and
298 therefore non-significant) increase in venous NSE (Figure 1). That is, the arterial increase in
299 NSE may have carried over to the cerebral venous side in some. For example, although NSE is
300 most abundant in neurons located in the brain, it is also located in neuroendocrine tissues

301 throughout the body, specifically the adrenal glands (23, 46). Hyperthermia activates the
302 hypothalamic-pituitary-adrenal axis through feedforward mechanisms (31, 41), contributing to
303 the heat-induced hyperadrenergic state (44). In turn, it has been demonstrated in rats that heat
304 stress acutely decreases adrenal cortex volume and mass with concomitant increases in
305 circulating corticotrophin and corticosterone (31). Concentrations of circulating cortisol are also
306 significantly elevated in heat-stressed humans (11, 17, 18). It is, therefore, reasonable to suggest
307 that the increase in systemic concentrations of NSE was from adrenal sources consequent to the
308 profound heat-induced excitation. Regardless of its source, however, it remains to be determined
309 whether the increase in NSE is an inert bi-product of hyperthermia, or a marker of important
310 physiologic function / malfunction. Furthermore, an important consideration is whether the
311 average increase in NSE of only ~6ng/mL (from ~13 to 19 ng/mL) has physiologic relevance.
312 For comparison, a two-fold increase in NSE (compared to controls) has been reported in humans
313 less than 48 hrs following mild traumatic brain injury (13).
314
315 *Exercise, Temperature & NVU Biomarkers:*
316
317 Exercise has been shown to increase the circulating concentration of some CNS-targeted
318 biomarkers, particularly S100B [reviewed in (30)]. It is often assumed that the exercise-induced
319 increase in S100B is indicative of BBB opening and, in part, as a consequence of the increases in
320 cerebral temperature (62) in the absence of physical head trauma. Results from the present study,
321 however, suggest that temperature alone may have a negligible impact on circulating S100B
322 from cerebral sources, at least when core temperature does not exceed ~39.5°C, and in the
323 absence of head trauma. Several alternative mechanisms may explain increased circulating

concentrations of S100B during exercise (30). For example, although S100B is primarily located in the brain, it is also in the skeletal myofibrils and adipocytes (2, 20, 58). Indeed, S100B has been shown to positively correlate with increases in creatine kinase after exercise which is indicative of muscular degradation (47). However, Watson et al. (62) demonstrated that peripherally circulating concentrations of S100B is higher during exercise in warm versus cold conditions at the same workload. While these data certainly indicate hyperthermia as a variable for the additional release of S100B, it still does not provide insight into its source. That is, hyperthermic muscle may release more S100B. This notion is consistent with the present study whereby no increase in S100B is observed in passive hyperthermia, notably with identical elevations in core temperature to Watson et al., (62). A similar assumption may be held with reported increases in NSE during long-distance running (25) – that is, increased circulating concentrations from extra-cerebral sources, which is likely in part temperature-dependent.

Limitations & Future Research:

The data herein must be interpreted solely within the context of the study – an acute setting with an average heating duration of ~1hr in young healthy adult men. Although these initial results corroborate the safety of passive heating for the brain, there remain many important areas for future research. Foremost, future studies should consider differences in sex, age, and in people with co-morbidities. This latter group is especially important given the target population for heat therapy [e.g., heart failure (21, 27, 39) and peripheral arterial disease (1, 53, 55)]. Another important consideration is the acute heating stimulus and timing of measurements. In this respect, concentrations of NF-L should be interpreted with the most caution. Neurofilament

proteins are found exclusively in neurons, which make them ideal markers for CNS injury; however, their release to the circulation can be delayed by days following the initial injury (38, 48, 59, 65). NF-L was included in the present analysis given the unique setting to address cross-brain kinetics with the potential to observe a snapshot of increased cerebral release, as opposed to the conventional measures limited to the peripheral venous circulation. Nonetheless, NF-L is generally classified as a 'delayed' axonal injury marker. On the other hand, both NSE (13, 37, 57) and tau protein (40, 45, 49, 64) are elevated in the acute setting of cerebral injury. We are therefore confident that, collectively, our measures had the sensitivity to demonstrate cerebral injury in the present study setting, had it occurred. Still, future studies should consider tracking (at least in the peripheral venous system) CNS biomarkers over days following the hyperthermic stress. The duration and rate of the heating stimulus should also be considered, under the premise that longer heat stress durations (>1 hour) may be necessary for disruption of the BBB (51, 52). Additionally, future studies should consider cross-brain measures of S100B during steady-state exercise in cold or warm environments, to establish contribution from extra-cerebral sources. Lastly, but importantly, future studies should consider a timed control group, especially with heating conditions of longer durations. A normothermic time control group was not attainable in the present study given the invasive experimental setup. However, in our previous studies (4, 5), participants were cannulated for well over six hours (under varying apneic conditions), and an increase in NSE was not observed. We are confident that the increase in NSE is therefore related to the hyperthermia, and not a time effect.

Conclusion:

In summary, passive acute heating that approaches +2°C core temperature, or absolute core temperature of ~39.2°C, is not sufficient to increase the cerebral release of S100B, NSE, tau protein, and NF-L. We interpret these data to indicate that, in contrast to the prevailing data in rodents, this level of hyperthermia does not open the BBB and elicit damage to the neurovascular unit in healthy young adult males. These preliminary data are encouraging for subsequent studies aiming to extend the utility of heat therapy for improvements in cerebrovascular function.

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Conflicts of interest

392 HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed,
393 Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia
394 sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions
395 in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside
396 submitted work). KB has served as a consultant, at advisory boards, or at data monitoring
397 committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche
398 Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in
399 Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside
400 submitted work).

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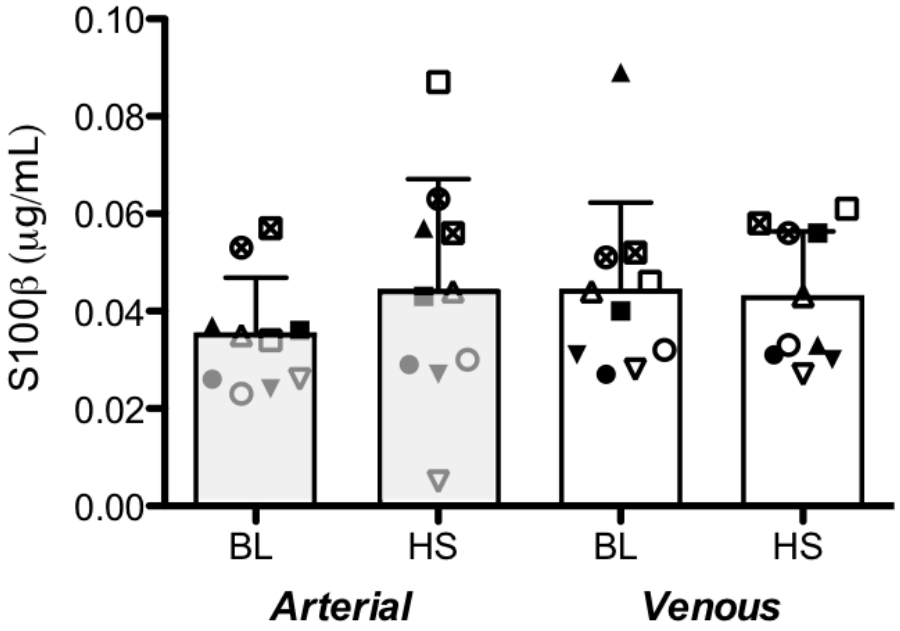
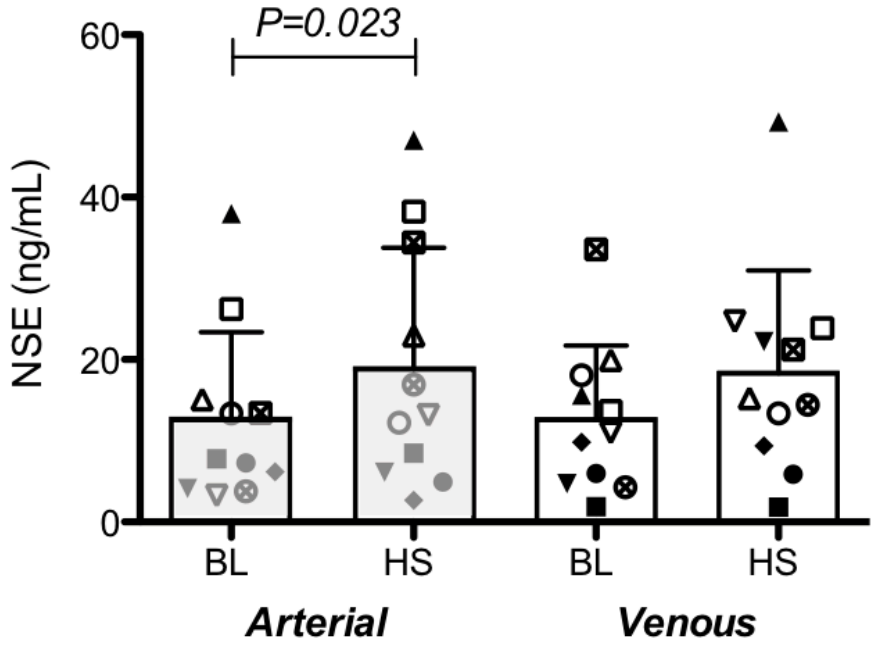
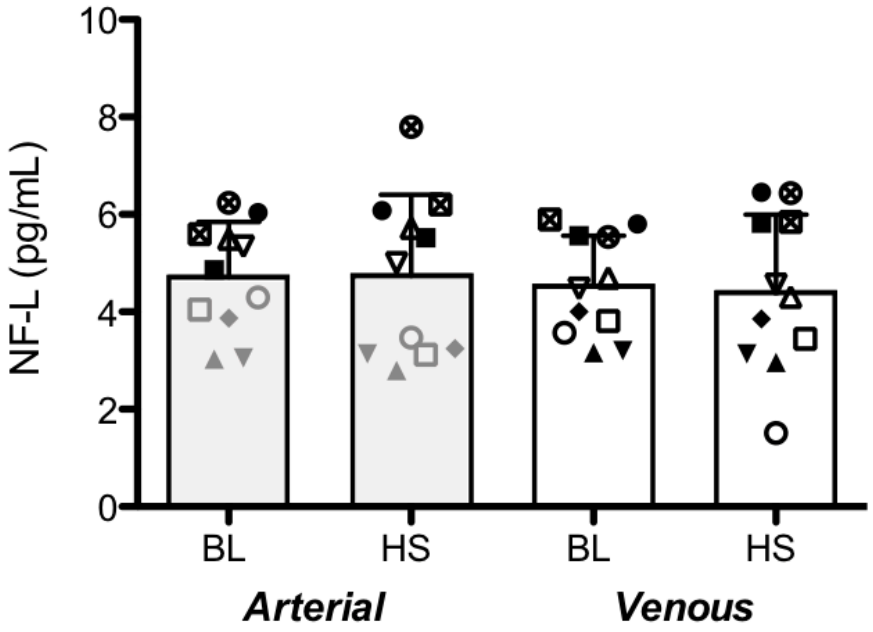
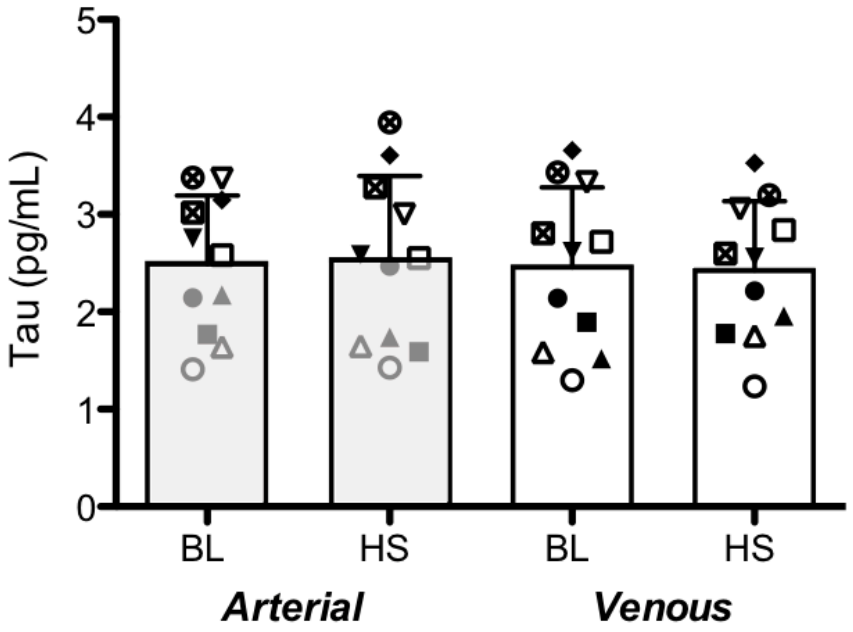
658 **Figure legend**
659
660 **Figure 1**. Individual values for arterial (gray filled bars) and venous (open bars) S100B (top
661 left), NSE (top right), NF-L (bottom left), and tau protein (bottom right), at normothermic
662 baseline (BL) and hyperthermic heat stress (HS). No significant interaction was observed in any
663 variable. However, there was a significant condition effect (P=0.028) of NSE, corresponding to a
664 significant increase in arterial (P=0.023) but not venous NSE (P=0.173) from BL to HS.
665
666 **Figure 2**. Individual values for cerebral exchange of S100B (top left), NSE (top right), NF-L
667 (bottom left), and tau protein (bottom right), at normothermic baseline (BL) and hyperthermic
668 heat stress (HS). Gray filled bars with error bars denote mean ±SD. Negative values denote net
669 cerebral release, positive values denote uptake. No significant difference (P>0.05) was observed
670 between BL and HS in any measure.









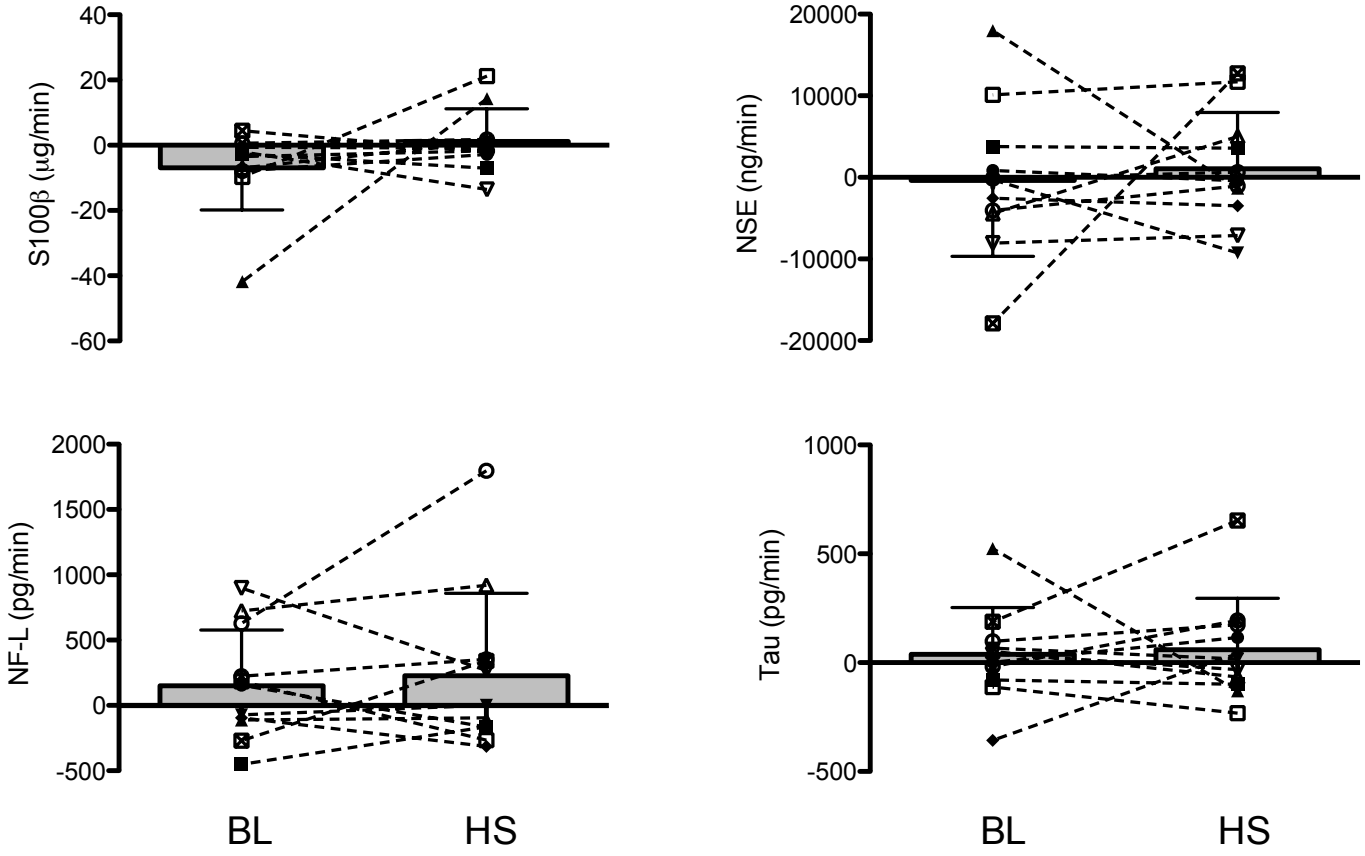


Table 1. Baseline and heat stress participant descriptives. Tcore; esophageal temperature. MAP; mean arterial blood pressure, intra-radial. CBF; cerebral blood flow (duplex ultrasound of the internal and vertebral carotid artery. HR; heart rate from lead II. The participant symbols are consistent with Figure 1.

		Baseline				Heat Stress			
Subject	**Symbol**	**Temp °C**	**MAP (mmHg)**	**CBF (ml/min)**	**HR (BPM)**	**Temp °C**	**MAP (mmHg)**	**CBF (ml/min)**	**HR (HR)**
1	●	37.5	97	664	74	39.6	79	449	124
2	⊠	37.4	95	887	63	39.5	79	966	134
3	○	37.4	97	871	48	39.3	88	917	92
4	▽	37.0	88	1038	69	39.0	86	617	126
5	▼	37.0	89	494	53	39.2	90	574	114
6	⊗	37.3	80	618	80	38.9	57	461	144
7	□	37.1	99	807	62	39.2	73	815	100
8	▲	37.0	95	804	79	39.0	82	596	131
9	■	37.4	92	646	65	39.1	73	540	112
10	△	37.2	88	906	62	38.8	77	651	114
11	◆	37.4	78	704	64	39.4	69	517	124
Mean		**37.3**	**91**	**767**	**65**	**39.2**	**77**	**646**	**119**
±SD		**0.2**	**7**	**157**	**10**	**0.2**	**9**	**177**	**15**

Table 2. Mean values ±SD of arterial and venous S100B, NSE, tau protein, and NF-L at baseline (normothermia) and heat stress (+2°C esophageal temperature). Net exchange is calculated from the global cerebral blood flow x the arterial-venous difference. Condition = baseline vs. heat stress; Site = arterial vs. venous; Condition x Site = interaction.

Condition:	**Baseline**		**Heat stress**	
Site:	**Arterial**	**Venous**	**Arterial**	**Venous**
S100B (μg/mL) *n=10*	0.035 ± 0.012	0.044 ± 0.019	0.044 ± 0.024	0.043 ± 0.014
Condition (P = 0.445); Site (P = 0.156); Condition × Site (P = 0.327)				
a-v_D (μg/mL)	-0.009 ± 0.017		0.001 ± 0.016	
Net exchange (μg/min)	-7.003 ± 13.715		1.050 ± 10.583	
NSE (ng/mL) *n=11*	12.6 ± 10.8	12.6 ± 9.1	18.8 ± 15.0	18.3 ± 12.7
Condition (P = 0.028); Site (P = 0.910); Condition × Site (P = 0.908)				
a-v_D (ng/mL)	-0.007 ± 11.0		0.52 ± 9.7	
Net exchange (ng/min)	-422.5 ± 9301.8		1006.3 ± 6913.9	
Tau (pg/mL) *n=11*	2.5 ± 0.7	2.5 ± 0.8	2.5 ± 0.9	2.4 ± 0.9
Condition (P = 0.877); Site (P = 0.315); Condition × Site (P = 0.626)				
a-v_D (pg/mL)	0.04 ± 0.3		0.1 ± 0.3	
Net exchange (pg/min)	36.8 ± 214.8		58 ± 236.7	
NF-L (pg/mL) *n=11*	4.7 ± 1.1	4.5 ± 1.0	4.7 ± 1.7	4.4 ± 1.6
Condition (P = 0.800); Site (P = 0.198); Condition × Site (P = 0.447)				
a-v_D (pg/mL)	0.2 ± 0.5		0.3 ± 0.9	
Net exchange (pg/min)	165.2 ± 428.5		243.3 ± 630.0	