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THE EFFECTS OF ACUTE HYPERTHERMIA ON THE NEUROVASCULAR UNIT

By

Brooke Renée Shepley

A Thesis
Submitted to the Faculty of Graduate Studies through the Department of Kinesiology in
Partial Fulfillment of the Requirements for the Degree of Master of Human Kinetics
at the University of Windsor

Windsor, Ontario, Canada

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September 17th, 2021

DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

- *The experimental design was part of a larger study with a separate a priori question (Bain et al., 2020). Separate blood samples were acquired specifically for this Thesis.*
- *Analysis of the plasma for biomarker quantification was performed by the co-authors, Zetterberg, H. and Blennow, K.*
- *Further contributions were made by Ainslie, P.N., Hoiland, R.L., Donnelly, J., and Sekhon, M.S.*

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Parts of Chapters 1,2,4 *All of Chapter 3*	*Shepley, B. R., Ainslie, P. N., Hoiland, R. L., Donnelly, J., Sekhon, M. S., Zetterberg, H., Blennow, K., & Bain, A. R. (2021). Negligible influence of moderate to severe hyperthermia on blood-brain barrier permeability and neuronal parenchymal integrity in healthy men. Journal of Applied Physiology, 130(3), 792–800.*	*Published*

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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 adults. Eleven young men (age 23 ± 3 years) 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.

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LIST OF ABBREVIATIONS

BBB	Blood-brain barrier
BP	Blood pressure
BL	Baseline
CBF	Cerebral blood flow
$CMRO_2$	Cerebral metabolic rate of oxygen
CNS	Central nervous system
CPP	Cerebral perfusion pressure
CVD	Cardiovascular disease
HR	Heart rate
HSP	Heat shock protein
HS	Heat stress
ICA	Internal carotid artery
ICP	Intracranial pressure
IL-6	Interleukin-6
MAP	Mean Arterial Pressure
NF-L	Neurofilament-light protein
NO	Nitric oxide
NSE	Neuron-specific enolase
NVU	Neurovascular unit
$PaCO_2$	Partial pressure of arterial carbon dioxide
PAD	Peripheral arterial disease
TBI	Traumatic brain injury
TJ	Tight junction
VA	Vertebral artery

CHAPTER 1: BACKGROUND

INTRODUCTION

Hyperthermia can be defined as an increase in core temperature above the normal level (i.e., >37.5°C), and is on a continuum of mild (≤1.0°C), moderate (1.0 to 1.5°C), and severe (≥1.5°C) increases in core temperature (Bain et al., 2015). Hyperthermia can also be classified into three distinct etiologies: febrile (i.e., a fever), passive (i.e., external heating), or exertional (i.e., exercise-induced) (Bain et al., 2015). Regardless of the etiology, hyperthermia exceeding a core temperature of 40°C is generally associated with a negative sequala of heat-induced cellular damage and a pro-inflammatory state that can result in life-threatening heatstroke (Epstein & Yanovich, 2019). However, in non- pathological conditions, hyperthermia not exceeding 40°C has gained recent attention for its tenable therapeutic properties. The aim of this literature review is to outline the basic origins of heat therapy, with emphasis on the role of hyperthermia for cerebrovascular regulation.

HYPERTHERMIA AS THERAPY

Cardiovascular diseases (CVDs) are a leading cause of death globally, increasing the need for novel therapeutic measures (Cardiovascular diseases (CVDs), 2017). Treatment of various illnesses through heat, in the form of a 'fever', has been utilized for centuries (Bierman,1942). However, owing to recent epidemiology studies (Laukkanen, Khan, Zaccardi, & Laukkanen, 2015; Laukkanen, Laukkanen, Khan, Babar, & Kunutsor, 2018; Laukkanen et al., 2019), the therapeutic use of passive heat stress for CVDs has recently gained attention (Cheng & MacDonald, 2019; Gibbons, Thomas, & Wilson, 2020). It has been well established that endothelial dysfunction precedes the pathogenesis of several CVDs, such as atherosclerosis (Cheng & MacDonald, 2019). Interestingly, passive heat stress has been shown to improve vascular health, specifically through enhanced endothelial function and reductions in arterial stiffness (Cheng & MacDonald, 2019; Bain et al., 2017; Kunutsor et al., 2018b; Heinonen & Laukkanen, 2018; Laukkanen et al., 2017; Brunt, Howard, Francisco, Ely, & Minson, 2016a).

Given these findings, heat therapy has been suggested as a potential alternative to exercising for individuals who are unable to engage in physical activity (Cheng & MacDonald, 2019; Thomas et al., 2017). Accordingly, heat therapy can potentially contribute to reducing the burden of CVDs (Cheng & MacDonald, 2019; Raven & Romero, 2020).

Heat therapy is commonly implemented through the use of Finnish saunas, Waon therapy, and hot-water immersion (Cheng & MacDonald, 2019). Waon therapy is a method of thermal therapy that involves whole-body heating for 15 minutes in a 60°C sauna until an increase in core temperature of 1.0-1.2°C, this temperature is then maintained for 30 minutes via bed rest and a warm blanket (Ohori et al., 2012; Kihara et al., 2009). Waon therapy has been shown to improve symptoms (Kihara et al., 2009), endothelial and cardiac function, and exercise tolerance, in patients with chronic heart failure (Ohori et al., 2012). Additionally, it has been shown to improve myocardial perfusion in patients with chronic total occlusion of the coronary arteries (Sobajima et al., 2013), as well as reduce pain scores and increase walking distance in patients with peripheral arterial disease (PAD) (Shinsato et al., 2010). Similar to Waon therapy, heat therapy in the form of hot-water immersion has also been shown to reduce symptoms and improve lower-limb perfusion (Thomas, van Rij, Lucas, & Cotter, 2017), as well as reduce blood pressure (BP) and walking distance in patients with PAD (Akerman et al., 2019). Moreover, Green et al., (2010) and Chiesa et al., (2016) have demonstrated that hot-water immersion improves vascular function through beneficial changes in shear stress (i.e., a beneficial shear pattern of red blood cells moving across the luminal side of the endothelium). Lastly, hot-water immersion has been shown to improve vascular function through reductions in arterial stiffness and improve endothelial function (Brunt et al., 2016a), and can protect against ischemic- reperfusion injury in the forearm (Brunt et al., 2016b) and lower limbs (Engelland, Hemingway, Tomasco, Olivencia-Yurvati, & Romero, 2020).

Similar to Waon therapy and hot water immersion, regular use of Finnish saunas has been shown to improve cardiovascular and peripheral arterial function through reductions in BP and improved arterial compliance (Lee et al., 2017; Laukkanen et al., 2017; Gravel et al., 2020), and reduced risk of hypertension (Zaccardi et al., 2017). Furthermore, Finnish sauna use is associated with enhanced cardiac autonomic nervous function through favourable adjustments to heart rate (HR) variability

(Laukkanen et al., 2019), reduced risk of sudden cardiac death, fatal cardiovascular diseases, fatal coronary heart disease, and all-cause mortality (Laukkanen et al., 2015). Interestingly, regular sauna bathing has also been associated with a reduced risk of Alzheimer's disease and dementia (Laukkanen, Kunutsor, Kauhanen, & Laukkanen, 2016). Although the exact mechanism is unknown, it is postulated to be related to improvements in cerebrovascular endothelial function and subsequent reductions in inflammation (Laukkanen et al., 2016). However, as discussed below, heat stress is generally associated with acute increases in cerebral inflammation.

CEREBROVASCULAR REGULATION IN HEAT STRESS

Although there is an abundance of evidence suggesting that heat therapy is an effective cardiovascular therapeutic, it has yet to be determined if these aforementioned benefits apply to the cerebral vasculature. The derived benefits of passive heat stress to the peripheral vasculature is often assumed to apply to the cerebrovasculature, (Caldwell et al., 2020; Gibbons et al., 2020; Raven & Romero, 2020), however, the peripheral vasculature and cerebral vasculature vary in response to hyperthermic stress, as evidenced by the differing patterns of blood flow. Indeed, heat exposure to the peripheral vasculature induces increased anterograde shear (i.e., forward-moving blood flow) (Green et al., 2010), whereas whole-body hyperthermia greater than 1.0°C results in reduced anterograde shear in the cerebral vasculature (Bain et al., 2013). This is significant considering that anterograde shear stress is understood to be beneficial to endothelial function, whereas retrograde (Cheng et al., 2019) and oscillatory shear stress have been shown to negatively impact the endothelium (Tremblay, Stimpson, & Pyke, 2019; Tremblay, Thom, Yang, & Ainslie, 2016; Jenkins et al., 2013). Indeed, an increase in core body temperature of 1°C will induce a reduction in middle cerebral artery velocity of ~10-15% (Bain et al., 2013). This reduction in cerebral blood flow (CBF) is primarily a result of heat-induced hyperventilation and subsequently decreased partial pressure of arterial carbon dioxide ($PaCO_2$) that causes cerebral vasoconstriction (Bain et al., 2015; Low et al., 2008; Low et al., 2009; Ogoh et al., 2013; Nelson et al., 2011; Sharma & Hoopes, 2013; Caldwell et al., 2020), and secondly a reduced cerebral perfusion pressure from moderate decreases in arterial pressure (Bain et al., 2015). In fact, severe heat stress, in some, can elicit a decrease in $PaCO_2$ below 20mmHg which leads to a drastic decrease in CBF and symptoms such as nausea and tetany (Bain et al., 2015). This response is exacerbated with an increase in core

temperature of >2°C; the extensive reduction in CBF can result in localized cerebral ischemia and subsequent increases in cerebral inflammation — a response associated with heatstroke (Bain et al., 2015). Moreover, there is a concern of severe heat stress as temperatures encroach 40°C. Heatstroke occurs at core temperatures >40°C and is loosely characterized by central nervous system (CNS) damage, cerebral edema, and inflammation (Sharma & Hoopes, 2013; Bain et al., 2015; Bouchama & Knochel, 2002; Sharma, Duncan, & Johanson, 2006). Furthermore, heatstroke can lead to neuronal injury that may not be detectible until a few days after exposure, and can potentially result in irreversible cellular death at core temperatures exceeding 42°C (Bain et al., 2015). Core temperatures >40°C can have a cytotoxic effect on the brain, as the heat exposure can provoke a pro-inflammatory and pro- coagulative environment (Bain et al., 2015). This is especially true for the neurovascular unit (NVU) — collectively made up of the cerebral endothelial, neuronal, and glial cells (Kiyatkin & Sharma, 2009).

NEUROVASCULAR UNIT & BLOOD-BRAIN BARRIER

A particular concern is the implications of passive heat stress to the NVU. The NVU consists of astrocytes, pericytes, a neuron, endothelial cells, and associated blood-brain barrier (BBB) tight junctional proteins (Phillips, Chan, Zheng, Krassioukov, & Ainslie, 2015; Hawkins & Davis, 2005). The cerebrovascular endothelial cells, along with transmembrane proteins in the tight junctions (TJs) between the cells, the pericytes that support the TJs, and surrounding astrocytes form a semipermeable barrier that regulates paracellular diffusion between the cerebral vessels and the brain – termed the BBB (Profaci, Munji, Pulido, & Daneman, 2020; Hawkins & Davis, 2005; Watson et al., 2006; Lu, Chen, Huang, Wang, & Yang, 2004; Sharma & Johanson, 2007; Watson et al., 2004; Sharma & Sharma, 2007). The BBB is integral to the regulation of the brain milieu and protecting neuronal parenchyma (Hawkins & Davis, 2005; Profaci et al., 2020; Lu et al., 2004). This barrier highly restricts the diffusion of large (>400-500 Da) or polar, hydrophilic molecules, whereas passive diffusion of small, lipid-soluble, nonpolar molecules is unrestricted (e.g., CO_2 and O_2) (Profaci et al., 2020; Bain et al., 2015; Lu et al., 2004; Sharma & Johanson, 2007). The essential molecules such as glucose, vitamins, amino acids, peptides, and electrolytes cross the BBB via carrier-mediated transport (Watson et al., 2004).

Although the exact mechanisms are not well understood, it is widely accepted that damage to the BBB is present in several neurological conditions, such as multiple sclerosis, traumatic brain injuries (TBIs), stroke, Alzheimer's disease, bacterial meningitis, and epilepsy (Profaci et al., 2020; Lu et al., 2004; Watson et al., 2004). In these pathological conditions, it is the systemic inflammation that is primarily thought to impact the BBB by compromising the TJs (Hawkins & Davis, 2005), leading to increased BBB permeability and consequent cerebral edema and increased intracranial pressure (ICP) (Chupel et al., 2018; Lu et al., 2004; Bain et al., 2015; Sharma, Zimmermann-Meinzingen, & Johanson, 2010). Importantly, while pro-inflammatory cytokines (e.g., IL-6) are upregulated in heat stress (Bouchama & Knochel, 2002), damage to the endothelium and BBB may also be exacerbated through dehydration (Bain et al., 2015) and increased nitric oxide (NO) production that occurs through hyperthermia (Sharma, Drieu, Alm,& Westman, 2000).

Hyperthermia is associated with poor outcomes when experienced in patients after suffering a TBI (Bain et al., 2020; Thompson et al., 2003). Even a mild increase in core temperature of 1°C is enough to contribute to secondary brain damage and worsened neurological outcomes in patients who have had a TBI (Bonds et al., 2015). This is particularly evident in rats exposed to mild heat stress (≤ 1°C) post-TBI; a 1°C increase in core temperature was associated with BBB disruption, accelerated neurological damage, elevated free radical production, and increased inflammation (Bonds et al., 2015). Interestingly, rats exposed to 4h of heat stress at an ambient temperature of 38°C had a marked increase in BBB permeability, meanwhile, no increase in permeability was detected in rats only exposed to heating for 1 or 2h (Sharma & Hoopes, 2013; Sharma & Johanson, 2007). Moreover, the breakdown of the BBB leads to heat-induced cerebral edema and consequent increased ICP (Sharma & Hoopes, 2013; Bain et al., 2015). However, it is worth noting that rats are not as equipped as humans to handle the physiological stressors of hyperthermia (Bain et al., 2015).

Despite the potential negative impacts of heat stress on the NVU as detailed above, controlled hyperthermia may in fact help to prevent both inflammatory and oxidative stress (Brunt, Wiedenfeld-Needham, Comrada, & Minson, 2018). This may, in part, be related to the upregulation of heat shock proteins (HSPs), as they are involved in the activation of anti- oxidative

and anti-inflammatory proteins (Brunt et al., 2018; Brunt et al., 2016a). Interestingly, Bain et al., (2017) demonstrated that an acute bout of heat stress, until an increase of 2°C in core temperature (~39.5°C), reduced circulating microparticles that are established biomarkers of vascular dysfunction, and is not sufficient to produce an excessive pro-oxidative or pro- inflammatory response (Bain et al., 2020). Thus, indicating a potential benefit to severe passive heating to +2°C core temperature (Bain et al., 2017). Furthermore, HSPs may also be involved in protecting the BBB from damage through the preservation of TJ proteins due to their role in refolding denatured proteins (Lu et al., 2004; Thompson et al., 2003). Thus, suggesting that HSPs could potentially be integral to the maintenance of cerebrovascular functioning during heat stress through BBB integrity (Lu et al., 2004). Potential benefits of heat stress on the cerebrovascular may also result from the transient increases in the cerebral metabolic rate of O2 (CMRO2) which, in turn, can improve immune function (Bain et al., 2020). Indeed, an elevated core temperature of +1.5°C to +2.0°C elicits a ~20% increase in $CMRO_2$, owing to the increased activity metabolic enzymes (namely phosphofructokinase) as a result of the hyperthermia (Bain et al., 2020). However, the change in metabolic rate may be influenced by neural activity (Bain et al., 2020).

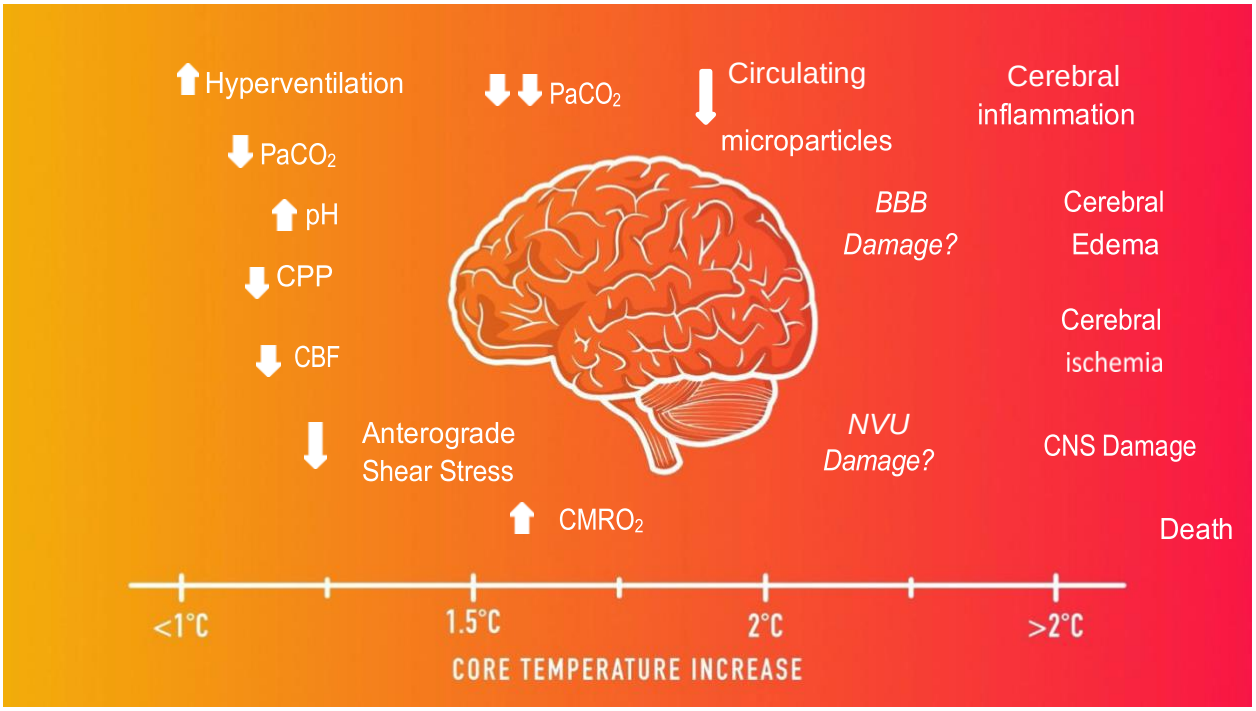

Figure 1. *Continuum of major cerebrovascular impacts with progressive heat stress. An increase in core temperature exceeding 1°C is known to cause progressive reductions in cerebral blood*

flow (CBF) and anterograde shear via hyperventilation induced hypocapnia and secondly reduced cerebral perfusion pressure (CPP). The cerebral metabolic rate of oxygen ($CMRO_2$) is progressively increased related to the Q10 effect for biological tissue. The temperature at which extensive blood-brain barrier (BBB) opening and damage to the neurovascular unit (NVU) occurs, thus leading to pathological damage, is unknown. See text for details.

STUDY RATIONALE AND HYPOTHESIS

Given that hyperthermia of up to +2°C core temperature [i.e., during hot yoga, Finnish sauna or Waon therapy (Cheng & MacDonald, 2019)] has gained widespread attention as a tool to improve cardiovascular function, there is a need to confirm that this level of heating is safe for the NVU in humans. Accordingly, the purpose of this study was to determine the trans- cerebral arterial-venous kinetics of S100B (an established marker of BBB permeability) in passive hyperthermia, as well as concurrent biochemical metrics of the NVU function as indexed by an established clinical panel for cerebral damage; tau protein, neuron-specific enolase (NSE) and neurofilament-light protein (NF-L) (Kanner et al., 2003; Topolovec-Vranic et al., 2011; Wang et al., 2018).

Based on previous observations by Bain text al., (2020) that passive hyperthermia does not evoke a robust increase in the cerebral release of inflammatory and pro-oxidative markers in humans (a tenable mechanism for BBB opening), ***we hypothesized that acute passive hyperthermia of +2°C core temperature in healthy young men would not elicit marked BBB opening as determined by the trans-cerebral release of S100B, or detectable cerebral parenchymal damage determined by the release of tau protein, NSE, and NF-L.***

CHAPTER 2: METHODOLOGY

SUBJECTS AND ETHICAL APPROVAL

Eleven healthy young men (age 23 ± 3 years) participated in the study. As a result of the experimental setup, study requirements, and optimal neck anatomy for ultrasound measures, the participants chosen were all males. Additionally, it is known that the menstrual cycle can impact thermoregulatory responses, and we were limited to a short physician schedule to insert the catheters. Thus, controlling for menstrual cycle was not possible. All subjects were non-obese (body mass index 23.0 ± 2.1 kg/m^2), 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 (Bain et al., 2017), and cerebral metabolism (Bain et al., 2018). 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. Upon arrival, participants were immediately assessed for adequate (≤1.020) hydration, via urine specific gravity (model TS 400, Reichert Analytical Instruments, Depew, NY). Under local anaesthesia (1% lidocaine) and ultrasound guidance, a 20-gauge arterial catheter (Arrow, Markham, ON, Canada) was placed inthe 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 (Bain et al., 2016; Bain et al., 2018).

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 (Woodman et al., 2001). 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 (sonographer: co-author R.L.H.) and VA (sonographer: co-author A.R.B.) blood flow was 7% and 4%, respectively. Heart rate (HR) was obtained from the R-R intervals measured in lead II of the ECG. Mean arterial blood pressure (MAP) was measured with a pressure transducer connected to the radial catheter.

NVU BIOMARKER ANALYSIS

Serum S100B and plasma NSE concentrations were measured using commercially available immunoassays with electrochemiluminescence detection on Cobas according to instructions from the manufacturer (Roche Diagnostics, Penzberg, Germany). Serum NF-L concentration was measured on a Single molecule array (Simoa) HD-1 Analyzer using the commercially available

NF-Light kit according to instructions from the manufacturer (Quanterix, Billerica, MA). Plasma tau concentration was measured on a Simoa HD-1 Analyzer using the commercially available Tau Advantage kit according to instructions from the manufacturer (Quanterix, Billerica, MA).

All measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients of variation were 3-5% for S100B and NSE, 7.2% for NF-L and 11% for tau. Cerebral exchange was calculated as the global cerebral blood flow x the arterial-venous difference of each respective biomarker, whereby a negative value denotes cerebral release.

RATIONALE FOR BIOMARKERS USED

S100B

S100B is a calcium-binding protein that has a low molecular weight and is predominantly expressed in glial cells in the CNS, with the majority located in the end-feet of astrocytes (Watson et al., 2004; Bain et al., 2015; Topolovec-Vranic et al., 2011; Neselius et al., 2012; Wang et al., 2018; Schulpis et al., 2007; Kanner et al., 2003; Chupel et al., 2018; Koh & Lee, 2014; Tubaro, Arcuri, Giambanco, & Donato, 2010). Due to the close proximity of the astrocyte end-feet to the cerebral blood vessels, S100B is freely released into the cerebral circulation if the permeability of the BBB is increased (Bain et al., 2015; Cheuvront et al., 2008) and, for this reason, it is the most commonly used biomarker for BBB damage (Watson et al., 2004; Koh & Lee, 2014). Granted, small amounts of S100B can also be found in extra-cerebral locations, including the heart, muscle, adipose tissue, GI tract, and bone (Watson et al., 2006; Buonora et al., 2015; Wang et al., 2018; Cheuvront et al., 2008; Schulpis et al., 2007; Koh & Lee, 2014), and serum S100B concentrations have been shown to moderately increase following acute bouts of exercise (Cheuvront et al., 2008; Schulpis et al., 2007), potentially indicating a transient release from contracting skeletal muscle. However, the serum concentration of this protein in healthy individuals is typically minuscule and is therefore considered a reliable indicator of compromised BBB integrity (Watson et al., 2004; Cheuvront et al., 2008; Kanner et al., 2003; Chupel et al., 2018). Interestingly, prolonged exercise in a warm environment may increase BBB permeability and can be detected by measuring concentrations of serum S100B, comparable to passive heat stress (Watson, Shirreffs, & Maughan, 2005). This may be due to the stress placed on the BBB during prolonged exercise, as there may

be transient increases in permeability as a result of endothelial cell shrinkage and therefore widened gaps between the cells, allowing an influx and efflux of substances that normally would be restricted from paracellular diffusion (Watson, Black, & Maughan, 2006). This reduced BBB integrity has also been shown to occur in rats subjected to exercise in a hyperthermic environment (Watson et al., 2005).

Increased serum levels of S100B are associated with poor patient outcomes and neurological damage when serum concentrations are greatly elevated (Watson et al., 2004; Wang et al., 2018; Kanner et al., 2003). Specifically, elevated levels of this protein have been correlated with increased cerebral damage in post-stroke patients (Anderson et al., 2001), after subarachnoid hemorrhaging, and TBIs (Kanner et al., 2003; Wang et al., 2018). Interestingly, heightened serum S100B is also associated with neurodegenerative diseases, such as Alzheimer's disease and Parkinson's Disease (Chupel et al., 2018). S100B has also been shown to increase following TBIs and is associated with poor clinical outcomes, specifically in boxers (Shahim et al., 2016; Neselius et al., 2012), hockey players (Shahim et al., 2014), and soldiers (Topolovec-Vranic et al., 2011). Furthermore, in rats subjected to TBIs through blast trauma, serum S100B was significantly elevated, indicating a compromised BBB (Liu et al., 2015).

NEURON-SPECIFIC ENOLASE (NSE)

NSE is a glycolytic enzyme in neurons and neuroendocrine cells with typically low baseline expression that is upregulated only during neuronal stress. An increase in circulating NSE is, in turn, a recognized indicator of neuronal damage (Topolovec-Vranic et al., 2011; Isgrò, Bottoni, & Scatena, 2015; Chupel et al., 2018; Haque, Polcyn, Matzelle, & Banik, 2018; Schmechel, Marangos, & Brightman, 1978; Wang et al., 2018; Polcyn et al., 2017). Although the exact role requires further investigation, NSE is involved in the neuroinflammatory response following neuronal injury and is thus considered a highly specific biomarker (Haque et al., 2018; Jouffroy et al., 2019). Similar to S100B, upregulation of NSE is also associated with TBIs, stroke, neurodegenerative diseases, in addition to cardiac arrest, lung cancer, seizures, neuroblastoma, spinal cord injuries, and ischemia-reperfusion injury (Polcyn et al., 2017; Isgrò et al.,2015). Moreover, NSE is clinically used as a quantitative measure of the severity of brain damage, and to determine prognosis in patients who have suffered from an ischemic stroke, seizures, cardiac

arrest, TBI, or intracerebral hemorrhage (Isgrò et al., 2015; Haque et al., 2018). Specific to neurodegenerative disorders, elevated NSE is correlated with the level of disability and severity of neurological deficits (Haque et al., 2018).

NEUROFILAMENT-LIGHT PROTEIN (NF-L)

NF-L is a scaffolding protein that is entirely specific to neurons and is primarily expressed in axonal white matter (Wang et al., 2018; Shahim et al., 2016). Due to its location, elevated concentrations of NF-L are used as a biomarker of axon degeneration (Neselius et al., 2012; Wang et al., 2012; Shahim et al., 2016; Zetterberg et al., 2006). In fact, NF-L is most commonly used as a clinical marker for the diagnosis and prognosis of TBIs (Wang et al., 2018; Shahim et al., 2016; Neselius et al., 2012), as several studies have identified the correlation with upregulated NF-L and the presence of a cerebral insult, particularly in boxers (Neselius et al., 2012; Zetterberg et al., 2006).

TAU PROTEIN

Similar to NF-L, tau is a protein that is predominantly located in neuronal axons and provides stability to microtubules (Neselius et al., 2012; Mandelkow & Mandelkow, 2012; Barbier et al., 2019; Pîrscoveanu et al., 2017; Rubenstein et al., 2017). Modifications to tau protein structure and function are characteristic of many neurodegenerative diseases, termed tauopathies (Barbier et al., 2019; Pîrscoveanu et al., 2017). Moreover, studies investigating the role of tau in brain injuries and neurodegenerative diseases have shown that concentrations of tau are remarkably increased following TBIs (Shahim et al., 2016; Wang et al., 2016), specifically in boxers (Neselius et al., 2012; Zetterberg et al., 2006), hockey players (Shahim et al., 2014), and soldiers (Olivera et al., 2015). As such, tau is a widely used biomarker of neuronal damage and is frequently used in the assessment of brain injuries (Neselius et al., 2012; Mandelkow & Mandelkow, 2012; Wang et al., 2018; Olivera et al., 2015).

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, whereby concentration differences of all four markers at baseline and heat stress were non-significant ($p > 0.05$). 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:

$$\text{Hedges'}\, g = \frac{\text{M1} - \text{M2}}{\text{Pooled SD}} \times \left(\frac{N-3}{N-2.25}\right) \times \left(\sqrt{\frac{N-2}{N}}\right)$$

Where; mean 1 (M1) is baseline, and mean 2 (M2) is heat stress. Significance was determined at an alpha level of 0.05. All data are presented as means ± SD.

CHAPTER 3: RESULTS

THERMOMETRY AND DESCRIPTIVE DATA

Absolute esophageal temperature at baseline was 37.3±0.2ºC, and at peak heat stress was 39.2±0.2ºC. 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).

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 vertebral carotid artery. HR; heart rate from lead II. The participant symbols are consistent with Figure 2.

		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 s calculated from the global cerebral blood flow x 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	

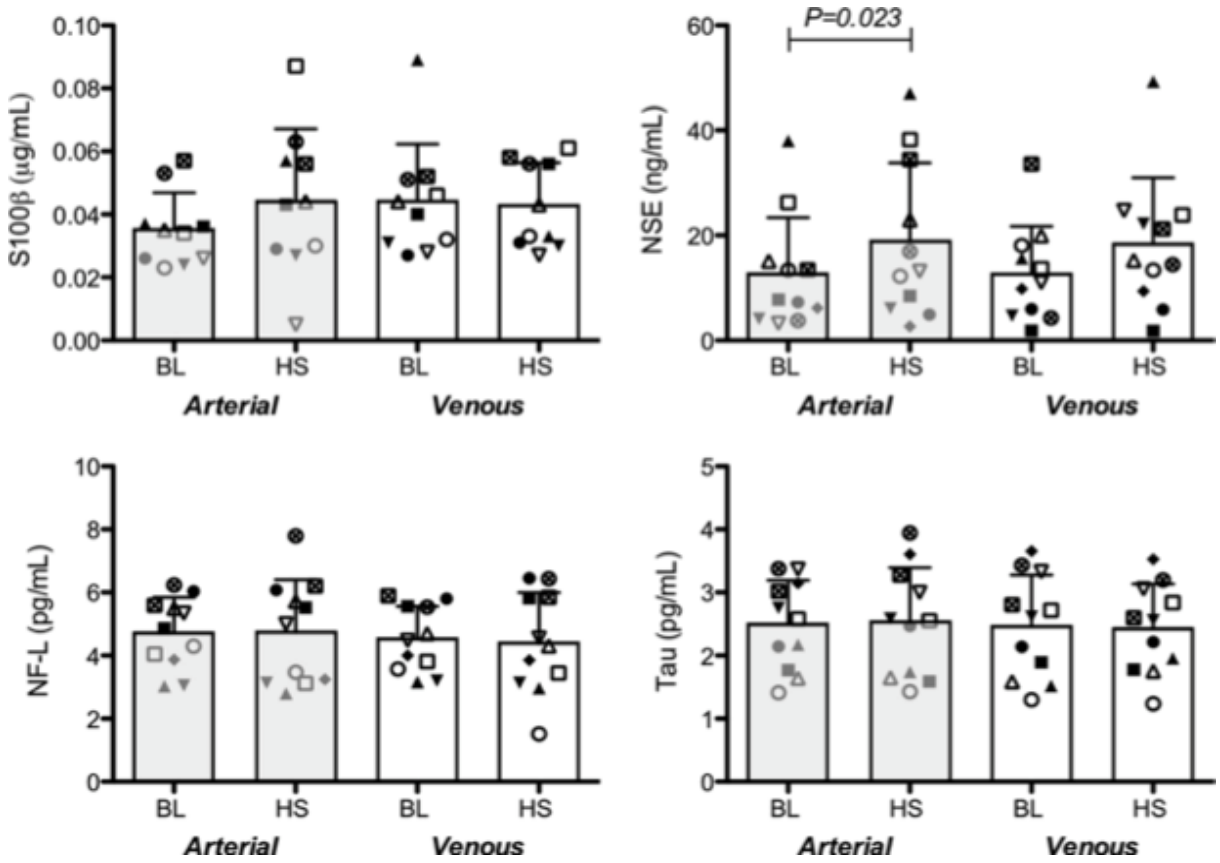

Figure 2. *Individual values for arterial (gray filled bars) and venous (open bars) S100B (top left), NSE (top right), NF-L (bottom left), and tau protein (bottom right), at normothermic baseline (BL) and hyperthermic heat stress (HS). No significant interaction was observed in any variable. However, there was a significant condition effect (P = 0.028) of NSE, corresponding to a significant increase in arterial (P = 0.023) but not venous NSE (P = 0.173) from BL to HS. Sample size = 11 except for S100B where n = 10. Statistical analysis performed by 2-way ANOVA.*

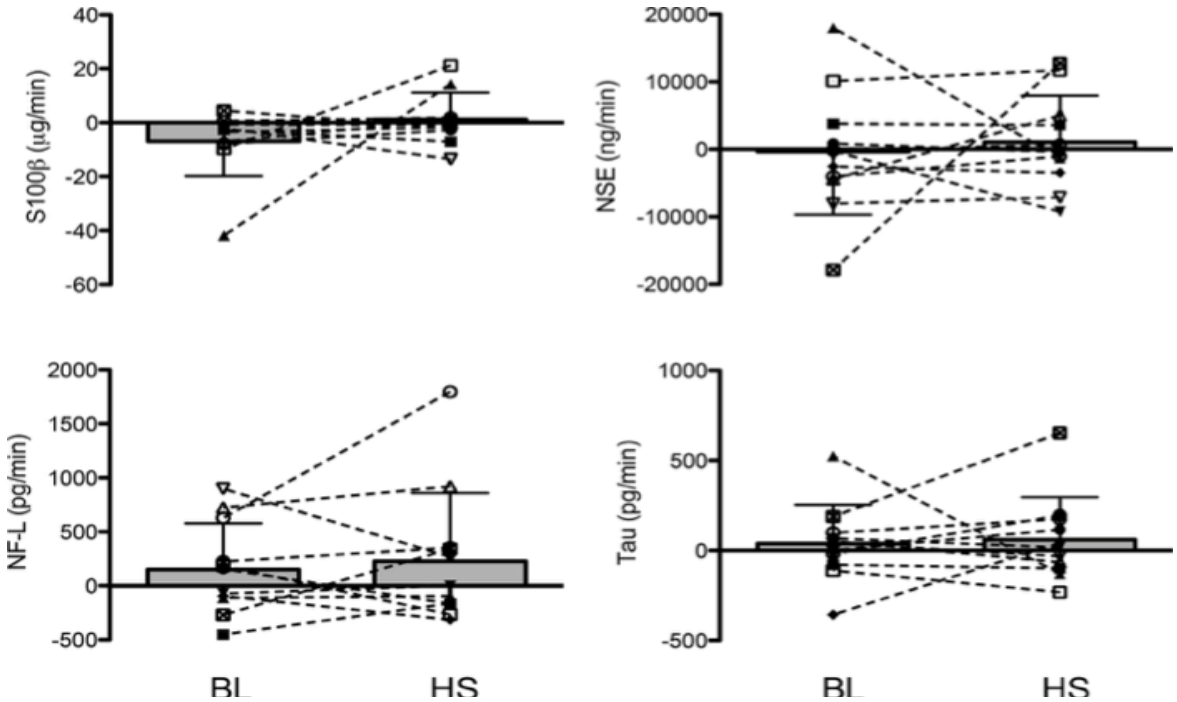

Figure 3. *Individual values for cerebral exchange of S100B (top left), NSE (top right), NF-L (bottom left), and tau protein (bottom right), at normothermic baseline (BL) and hyperthermic heat stress (HS). Gray filled bars with error bars denoted means ± SD. Negative values denote net cerebral release; positive values denote uptake. No significant difference between (P > 0.05) was observed between BL and HS in any measure. Sample size = 11 except for S100B where n = 10. Statistical analysis performed by 2-way ANOVA and repeated-measures Student's t-tests when appropriate.*

CHAPTER 4: 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 discernible 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 +2ºC 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 (Brunt et al., 2016a; Gravel et al., 2020; Kihara et al., 2009; Kunutsor et al., 2018; Laukkanen et al., 2015; Laukkanen et al., 2016; Laukkanen et al., 2017; Laukkanen et al., 2018; Lee et al., 2017; Ohori et al., 2012; Sobajima et al., 2013). In experimental use of thermal therapy (e.g., sauna use), core temperature elevations of up to 2ºC are often reported (Laukkanen et al., 2017). While it is generally accepted that induced heat stress should not exceed an absolute core temperature of ~40ºC [to avoid life-threatening complications of heat illness (Bouchama et al., 2002)], 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 38.5ºC (Kiyatkin et al., 2009). Moreover, even a 1.0ºC increase in core temperature is problematic for cerebral outcomes in human conditions of traumatic brain injury (Bonds et al., 2015; Thompson et al., 2003), likely in part related to increased pro-inflammatory responses. That is, passive heat stress invariably increases IL-6, a known cytokine that stimulates BBB opening (Brunt et al., 2018; Watson et al., 2006; Bain et al., 2015), which can induce or exacerbate damage to the NVU (in the setting of TBI) by means of neuroinflammation, edema, and ionic imbalances (Profaci et al., 2020). Importantly, we have previously demonstrated that with +2.0ºC core temperature the cerebral exchange of pro-oxidative and inflammatory markers (oxidative-low density lipoprotein, myeloperoxidase, and IL-6) are not increased, however, the increase in IL-6 in a sample of six participants trended to selectively

increase more in the cerebral tissue (Bain et al., 2020). At least in healthy young males, results from the present study reassure that this trend for increases in cerebral IL-6 does not lend to BBB opening. (There is an absence of a relationship between jugular venous IL-6 and S100B in heat stress; r=0.454, p=0.219, unpublished data, n=10). Furthermore, elevated concentrations of IL-6 during the acute-phase immune response may be beneficial due to the upregulation of antioxidant pathways (Bouchama & Knochel, 2002).

BIOMARKERS FOR NVU DAMAGE; IMPACT OF EXTRACEREBRAL SOURCES?

The primarily astroglial protein S100B was used to quantify BBB leakage (Cheuvront et al., 2008; Kanner et al., 2003; Watson et al., 2006), while concentrations of the CNS dominant tau protein, NF-L, and NSE were measured to provide insight on neuronal parenchymal damage. While the lack of cerebral release or increase in jugular venous concentrations of S100B, tau protein, NF-L and NSE collectively suggests that passive heat stress up to 39.5ºC core temperature in healthy young men is not sufficient to acutely increase BBB permeability or damage the cerebral neuronal parenchyma; the increase in NSE from BL to HS is notable. Because of the lack of cerebral exchange, this net increase in NSE from baseline to heat stress may be attributed to release from non-cerebral sources, which is consistent with systemic release of NSE driving a similar average but highly variable (and therefore non-significant) increase in venous NSE (Figure 2). That is,the arterial increase in NSE may have carried over to the cerebral venous side in some. For example, although NSE is most abundant in neurons located in the brain, it is also located in neuroendocrine tissues throughout the body, specifically the adrenal glands (Haque et al., 2018; Schmechel et al., 1978). Hyperthermia activates the hypothalamic-pituitary-adrenal axis through feedforward mechanisms (Koko, 2004; Path et al., 2000), contributing to the heat-induced hyperadrenergic state (Rowell, 1990). In turn, it has been demonstrated in rats that heat stress acutely decreases adrenal cortex volume and mass with concomitant increases in circulating corticotrophin and corticosterone (Koko, 2004). Concentrations of circulating cortisol are also significantly elevated in heat-stressed humans (Brenner et al., 1998; Collins et al., 1969; Follenius et al., 1982). It is, therefore, reasonable to suggest that the increase in systemic concentrations of NSE was from adrenal sources consequent to the profound heat-induced excitation. Regardless of its source, however, it remains to be determined whether the increase in NSE is an inert bi-product of

hyperthermia, or a marker of important physiologic function / malfunction. Furthermore, an important consideration is whether the average increase in NSE of only ~6ng/mL (from ~13 to 19 ng/mL)has physiologic relevance. For comparison, a two-fold increase in NSE (compared to controls) has been reported in humans less than 48 hrs following mild traumatic brain injury (Buonora et al., 2015).

EXERCISE, TEMPERATURE & NVU BIOMARKERS

Exercise has been shown to increase the circulating concentration of some CNS-targeted biomarkers, particularly S100B [reviewed in (Koh & Lee, 2014)]. It is often assumed that the exercise- induced increase in S100B is indicative of BBB opening and, in part, as a consequence of the increases in cerebral temperature (Watson et al., 2005) in the absence of physical head trauma. Results from the present study, however, suggest that temperature alone may have a negligible impact on circulating S100B from cerebral sources, at least when core temperature does not exceed ~39.5ºC, and in the absence of head trauma. Several alternative mechanisms may explain increased circulating concentrations of S100B during exercise (Koh & Lee, 2014). For example, although S100B is primarily located in the brain, it is also in the skeletal myofibrils, cardiac muscles, chondrocytes, melanocytes, and adipocytes (Anderson et al., 2001; Gonçalves et al., 2010; Tubaro et al., 2010; Wang et al., 2018). Indeed, S100B has been shown to positively correlate with increases in creatine kinase after exercise which is indicative of muscular degradation (Schulpis et al., 2007). However, Watson et al. (Watson et al., 2005) 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., (2005). A similar assumption may be held with reported increases in NSE during long-distance running (Jouffroy et al., 2019) – that is, increased circulating concentrations from extra-cerebral sources, which is likely in part temperature-dependent. However, this may also be time- dependent as S100B have been shown to markedly increase in long-distance runners (Jouffroy et al., 2019). Indeed, it is worth noting that this

elevation of S100B may be related to the level of clearance from the kidneys (reviewed in Bain et al., 2015).

CONSIDERATIONS & 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 (Gravel et al., 2020; Kihara et al., 2009; Ohori et al., 2012) and peripheral arterial disease (Akerman et al., 2019; Shinsato et al., 2010; Thomas et al., 2017)]. 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 (Neselius et al., 2012; Shahim et al., 2016; Wang et al., 2018; Zetterberg et al., 2006). 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 (Buonora et al., 2015; Liu et al., 2015; Topolovec-Vranic et al., 2011) and tau protein (Olivera et al., 2015; Rubenstein et al., 2017; Shahim et al., 2014; Yang et al., 2014) 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 (Sharma & Hoopes, 2003; Sharma & Johanson, 2007). 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. 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 previous studies by Bain et al., (2016; 2018), 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.

Lastly, future research should consider heat shock protein (HSP) analysis. HSPs are involved in the stabilization and activation of additional proteins that are vital to the cardiovascular system – namely anti-oxidative and anti-inflammatory proteins, in addition to proteins that improve NO signaling which, collectively, influence vascular function (Brunt et al., 2016a; Brunt et al., 2018). Additionally, HSPs are considered to be chaperone molecules that are involved in the refolding or folding of denatured or misfolded proteins (Lu et al., 2004; Thompson et al., 2003), and are expressed upon heat exposure (Brunt et al., 2016a). Specifically, the upregulation of HSPs in response to severe heat stress has been shown to protect against cerebral damage, whereas low levels of HSPs have been associated with conditions such as aging and lack of heat acclimatization (Bouchama & Knochel, 2002). This may be due to the involvement of HSPs in the structural protection of the cytoskeleton and TJ proteins, and thus, maintenance of endothelial cell integrity (Lu et al., 2004). Accordingly, the analysis of a potential HSP response may have provided insight into the beneficial role of an acute bout of severe, passive heat stress.

REFERENCES

Akerman, A., Thomas, K., van Rij, A., Body, D., Alfadhel, M., & Cotter, J. (2019). Heat therapy vs. supervised exercise therapy for peripheral arterial disease: a 12-week randomized, controlled trial. The American Journal of Physiology-Heart and Circulatory Physiology, 316(6), H1495-H1506.

Anderson, R., Hansson, L., Nilsson, O., Liska, J., Settergren, G., & Vaage, J. (2001). Increase in Serum S100A1-B and S100BB During Cardiac Surgery Arises From Extracerebral Sources. The Annals of Thoracic Surgery, 71(5), 1512-1517.

Bain, A., Ainslie, P., Bammert, T., Hijmans, J., Sekhon, M., Hoiland, R., . . . DeSouza, C. (2017). Passive heat stress reduces circulating endothelial and platelet microparticles. Experimental Physiology, 102(6), 663-669.

Bain, A., Hoiland, R., Donnelly, J., Nowak-Flück, D., Sekhon, M., Tymko, M., . . .Ainslie, P. (2020). Cerebral metabolism, oxidation, and inflammation in severe passive hyperthermia with and without respiratory alkalosis. The Journal of Physiology, 598(5), 943-954.

Bain, A., Nybo, L., & Ainslie, P. (2015). Cerebral Vascular Control and Metabolism in Heat Stress. Comprehensive Physiology, 5, 1345-1380.

Bain, A., Smith, K., Lewis, N., Foster, G., Wildfong, K., Willie, C., . . . Ainslie, P. (2013). Regional changes in brain blood flow during severe passive hyperthermia: effects of PaCO2 and extracranial blood flow. Journal of Applied Physiology, 115, 653-659.

Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., . . .Landrieu, I. (2019). Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects. Frontiers in Aging Neuroscience, 11, 204.

Bell, A., Millre, S., Castillo-Melendez, M., & Malhotra, A. (2020). The Neurovascular Unit: Effects of Brain Insults During the Perinatal Period. Frontiers in Neuroscience, 13, 1452.

Bierman, B. (1942). The History of Fever Therapy in the Treatment of Disease*. Bulletin of the New York Academy of Medicine, 18, 65-75.

Bonds, B., Hu, P., Li, Y., Yang, S., Colton, K., Gonchigar, A., . . . Stein, D. (2015). Predictive value of hyperthermia and intracranial hypertension on neurological outcomes in patients with severe traumatic brain injury. Brain Injury, 29(13-14), 1642-1647.

Bouchama, A., & Knochel, J. (2002). Heat Stroke. The New England Journal of Medicine, 346(25), 1978-1988.

Brunt, V., Howard, M., Francisco, M., Ely, B., & Minson, C. (2016). Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. The Journal of Physiology, 594(18), 5329-5342.

Brunt, V., Jeckell, A., Ely, B., Howard, M., Thijssen, D., & Minson, C. (2016). Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans. The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 311, R1060- R1067.

Brunt, V., Wiedenfeld-Needham, K., Comrada, L., & Minson, C. (2018). Passive heat therapy protects against endothelial cell hypoxia-reoxygenation via effects of elevations in temperature and circulating factors. The Journal of Physiology, 596(20), 4831-4845.

Buonora, J., Yarnell, A., Lazarus, R., Mousseau, M., Latour, L., Rizoli, S., . . . Mueller, G. (2015). Multivariate analysis of traumatic brain injury: development of an assessment score. Frontiers in Neurology, 6, 68.

Caldwell, H., Coombs, G., Howe, C., Hoiland, R., Patrician, A., Lucas, S., & Ainslie, P. (2020). Evidence for Temperature-Mediated Regional Increases in Cerebral Blood Flow during Exercise. The Journal of Physiology, 598(8), 1459-1473.

Cardiovascular diseases (CVDs). (2017, 05 17). Retrieved from World Health Organization. Cheng, J., & MacDonald, M. (2019). Effect of heat stress on vascular outcomes in humans. Journal of Applied Physiology, 126, 771-781.

Cheuvront, S., Chinevere, T., Ely, B., Kenefick, R., Goodman, D., McClung, J., & Sawka, M. (2008). Serum S-100B Response to Exercise–Heat Strain before and after Acclimation. Medicine & Science in Sports & Exercise, 40(8), 1477-1482.

Chiesa, S., Trangmar, S., & González-Alonso, J. (2016). Temperature and blood flow distribution in the human leg during passive heat stress. Journal of Applied Physiology, 120, 1047-1058.

Chupel, M., Minuzzi, L., Furtado, G., Santos, M., Hogervorst, E., Filaire, E., & Teixeira, A. (2018). Exercise and taurine in inflammation, cognition, and peripheral markers of blood-brain barrier integrity in older women. Applied Physiology, Nutrition, and Metabolism, 43, 733-741.

Engelland, R., Hemingway, H., Tomasco, O., Olivencia-Yurvati, A., & Romero, S. (2020). Acute Lower Leg Hot Water Immersion Protects Macrovascular Dilator Function Following Ischaemia-Reperfusion Injury in Humans. Experimental Physiology, 105(2), 302-311.

Epstein, Y., & Yanovich, R. (2019). Heatstroke. The New England Journal of Medicine, 380, 2449-2459.

Gibbons, T., Thomas, K., & Wilson, L. (2020). Is all heat equal? Implications for the stimulus for adaptation in the brain. The Journal of Physiology, 598(11), 2051- 2052.

Gravel, H., Behzadi, P., Cardinal, S., Barry, H., Neagoe, P., Juneau, M., . . . Gagnon, D. (2020). Acute vascular benefits of Finnish sauna bathing in patients with stable coronary artery disease. Canadian Journal of Cardiology, S0828-282X(20), 30580-30588.

Green, D., Carter, H., Fitzsimons, M., Cable, T., Thijssen, D., & Naylor, L. (2010). Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. The Journal of Physiology, 588(9), 1571-1577.

Haque, A., Polcyn, R., Matzelle, D., & Banik, N. (2018). New Insights into the Role of Neuron-Specific Enolase in Neuro-Inflammation, Neurodegeneration, and Neuroprotection. Brain Sciences , 8(2), 33.

Hawkins, B., & Davis, T. (2005). The Blood-Brain Barrier/Neurovascular Unit in Health and Disease. Pharmacological Reviews, 57, 173-185.

Heinonen, I., & Laukkanen, J. (2018). Effects of heat and cold on health, with special reference to Finnish sauna bathing. The American Journal of Physiology- Regulatory, Integrative and Comparative Physiology, 314, R629-R639.

Isgrò, M., Bottoni, P., & Scatena, R. (2015). Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. Advances in Experimental Medicine and Biology, 867, 125-143.

Jenkins, N., Padilla, J., Boyle, L., Credeur, D., Laughlin, M., & Fadel, P. (2013). Disturbed Blood Flow Acutely Induces Activation and Apoptosis of the Human Vascular Endothelium. Hypertension, 61(3), 615-621.

Jouffroy, R., Alves, B., Mauvieux, B., Mallet, L., Beaudeux, J., & Cottart, C. (2019). NSE & S100B protein blood level assessment during a long-distance trail race. Annales De Biologie Clinique, 77(5), 532-536.

Kanner, A., Marchi, N., Fazio, V., Mayberg, M., Koltz, M., Siomin, V., . . . Janigro, D. (2003). Serum S100B: A Noninvasive Marker of Blood-Brain Barrier Function and Brain Lesions. Cancer, 97(11), 2806-2813.

Kihara, T., Miyata, M., Fukudome, T., Ikeda, Y., Shinsato, T., Kubozono, T., . . . Tei, C. (2009). Waon therapy improves the prognosis of patients with chronic heart failure. Journal of Cardiology, 53, 214-218.

Kiyatkin, E., & Sharma, H. (2009). Permeability of the blood-brain barrier depends on brain temperature. Neuroscience, 161(3), 926-939.

Koh, S., & Lee, J. (2014). S100B as a Marker for Brain Damage and Blood–Brain Barrier Disruption Following Exercise. Sports Medicine, 44, 369-385.

Kunutsor, S., Häkkinen, A., Zaccardi, F., Laukkanen, T., Lee, E., Willeit, P., . . . Laukkanen, J. (2018b). Short-term effects of Finnish sauna bathing on blood- based markers of cardiovascular function in non-naive sauna users. Heart and Vessels, 33, 1515-1524.

Kunutsor, S., Khan, H., Zaccardi, F., Laukkanen, T., Willeit, P., & Laukkanen, J. (2018a). Sauna bathing reduces the risk of stroke in Finnish men and women. Neurology, e1-e8.

Laukkanen, J., Laukkanen, T., Khan, H., Babar, M., & Kunutsor, S. (2018). Combined Effect of Sauna Bathing and Cardiorespiratory Fitness on the Risk of Sudden Cardiac Deaths in Caucasian Men: A Long-term Prospective Cohort Study. Progress in Cardiovascular Diseases, 60, 635-641.

Laukkanen, T., Khan, H., Zaccardi, F., & Laukkanen, J. (2015). Association Between Sauna Bathing and Fatal Cardiovascular and All-Cause Mortality Events. JAMA Internal Medicine, 175(4), 542-548.

Laukkanen, T., Kunutsor, S., Kauhanen, J., & Laukkanen, J. (2016). Sauna bathing is inversely associated with dementia and Alzheimer's disease in middle-aged Finnish men. Age and Ageing, 46, 245-249.

Laukkanen, T., Kunutsor, S., Zaccardi, F., Lee, E., Willeit, P., Khan, H., & Laukkanen, J. (2017). Acute effects of sauna bathing on cardiovascular function. Journal of Human Hypertension, 32, 129-138.

Laukkanen, T., Lipponen, J., Kunutsor, S., Zaccardi, F., Araújo, C., Mäkikallio, T., . . . Laukkanen, J. (2019). Recovery from sauna bathing favorably modulates cardiac autonomic T nervous system. Complementary Therapies in Medicine, 45, 190- 197.

Lee, E., Laukkanen, T., Kunutsor, S., Khan, H., Willeit, P., Zaccardi, F., & Laukkanen, J. (2017). Sauna exposure leads to improved arterial compliance: Findings from a non-randomised experimental study. European Journal of Preventative Cardiology, 1-9.

Liu, M., Zhang, C., Liu, W., Luo, P., Zhang, L., Wang, Y., . . . Fei, Z. (2015). A novel rat model of blast-induced traumatic brain injury simulating different damagedegree: implications for morphological, neurological, and biomarker changes. Frontiers in Cellular Neuroscience, 9, 168.

Low, D., Wingo, J., Keller, D., Davis, S., Cui, J., Zhang, R., & Crandall, C. (2009). Dynamic cerebral autoregulation during passive heat stress in humans. The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology , 296(5), R1598-R1605.

Low, D., Wingo, J., Keller, D., Davis, S., Zhang, R., & Crandall, C. (2008). Cerebrovascular responsiveness to steady-state changes in end-tidal CO2 during passive heat stress. Journal of Applied Physiology, 104, 976-981.

Lu, T., Chen, H., Huang, M., Wang, S., & Yang, R. (2004). Heat shock treatment protects osmotic stress–induced dysfunction of the blood-brain barrier through preservation of tight junction proteins. Cell Stress & Chaperones, 9(4), 369-377.

Mandelkow, E., & Mandelkow, E. (2012). Biochemistry and Cell Biology of Tau Protein in Neurofibrillary Degeneration. Cold Spring Harbor Laboratory Press, 2(7), a006247.

McConnell, H., Kersch, C., Woltjer, R., & Neuwelt, E. (2017). The Translational Significance of the Neurovascular Unit*. The Journal of Biological Chemistry, 292(3), 762-770.

Nelson, M., Haykowsky, M., Stickland, M., Altamirano-Diaz, L., Willie, C., Smith, K., . .. Ainslie, P. (2011). Reductions in cerebral blood flow during passive heat stress in humans: partitioning the mechanisms. The Journal of Physiology, 589(16), 4053-4064.

Neselius, S., Brisby, H., Theodorsson, A., Blennow, K., Zetterberg, H., & Marcusson, J. (2012). CSF-Biomarkers in Olympic Boxing: Diagnosis and Effects of Repetitive Head Trauma. PLoS One, 7(4), e33606.

Nielsen, B., & Nybo, L. (2003). Cerebral Changes During Exercise in the Heat. Sports Medicine, 33(1), 1-11.

Nybo, L., Møller, K., Volianitis, S., Nielsen, B., & Secher, N. (2002). Effects of hyperthermia on cerebral blood flow and metabolism during prolonged exercise in humans. Journal of Applied Physiology, 93, 58-64.

Ogoh, S., Sato, K., Okazaki, K., Miyamoto, T., Hirasawa, A., Morimoto, K., & Shibasaki, M. (2013). Blood flow distribution during heat stress: cerebral and systemic blood flow. Journal of Cerebral Blood Flow & Metabolism, 33, 1915- 1920.

Ohori, T., Nozawa, T., Ihori, H., Shida, T., Sobajima, M., Matsuki, A., . . . Inoue, H. (2012). Effect of Repeated Sauna Treatment on Exercise Tolerance and Endothelial Function in Patients With Chronic Heart Failure. The American Journal of Cardiology, 109, 100-104.

Olivera, A., Lejbman, N., Jeromin, A., French, L., Kim, H., Cashion, A., . . . Gill, J. (2015). Peripheral Total Tau in Military Personnel Who Sustain Traumatic Brain Injuries During Deployment. JAMA Neurology, 72(10), 1109-1116.

Phillips, A., Chan, F., Zheng, M., Krassioukov, A., & Ainslie, P. (2015). Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. Journal of Cerebral Blood Flow & Metabolism, 36(4), 647-664.

Polcyn, R., Capone, M., Hossain, A., Matzelle, D., Banik, N., & Haque, A. (2017). Neuron specific enolase is a potential target for regulating neuronal cell survival and death: implications in neurodegeneration and regeneration. Neuroimmunology and Neuroinflammation, 4, 254-257.

Pîrscoveanu, D., Pirici, I., Tudoricâ, B. T., Albu, V., Bondari, S., Bumbea, A., & Pirscoveanu, M. (2017). Tau protein in neurodegenerative diseases – a review. Romanian Journal of Morphology & Embryology, 58(4), 1141-1150.

Profaci, C., Munji, R., Pulido, R., & Daneman, R. (2020). The blood–brain barrier in health and disease: Important unanswered questions. Journal of Experimental Medicine, 217(4), e20190062.
Raven, P., & Romero, S. (2020). Increasing body temperature with dynamic exercise and/or by wallowing/bathing in hot water or saunas: effects on cerebral blood flow. The Journal of Physiology, 598(8), 1421-1422.

Rubenstein, R., Chang, B., Yue, J., Chiu, A., Winkler, E., Puccio, A., . . . Wang, K. T.-T. (2017). Comparing Plasma Phospho Tau, Total Tau, and Phospho Tau–Total Tau Ratio as Acute and Chronic Traumatic Brain Injury Biomarkers. JAMA Neurology, 74(9), 1063-1072.

Schmechel, D., Marangos, P., & Brightman, M. (1978). Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature, 276(5690), 834-836.

Schulpis, K., Moukas, M., Parthimos, T., Tsakiris, T., Parthimos, N., & Tsakiris, S. (2007). The effect of α-Tocopherol supplementation on training-induced elevation of S100B protein in sera of basketball players. Clinical Biochemistry, 40(12), 900-906.

Shahim, P., Gren, M., Liman, V., Andreasson, U., Norgren, N., Tegner, Y., . . . Blennow, K. (2016). Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Scientific Reports, 6, 36791.

Shahim, P., Tegner, Y., Wilson, D., Randall, J., Skillbäck, T., Pazooki, D., . . . Zetterberg, H. (2014). Blood Biomarkers for Brain Injury in Concussed Professional Ice Hockey Players. JAMA Neurology, 71(6), 684-692.

Sharma, H., & Hoopes, P. (2003). Hyperthermia induced pathophysiology of the central nervous system. International Journal of Hyperthermia, 19(3), 325-354.

Sharma, H., & Johanson, C. (2007). Blood–cerebrospinal fluid barrier in hyperthermia. Progress in Brain Research, 162, 459-478.

Sharma, H., & Sharma, A. (2007). Nanoparticles aggravate heat stress induced cognitive deficits, blood–brain barrier disruption, edema formation and brain pathology. Progress in Brain Research, 162, 245-273.

Sharma, H., Drieu, K., Alm, P., & Westman, J. (2000). Role of Nitric Oxide in Blood- Brain Barrier Permeability, Brain Edema and Cell Damage Following Hyperthermic Brain Injury. An Experimental Study Using EGB-761 and Gingkolide B Pretreatment in the Rat. Acta Neurochirurgica, 76, 81-86.

Sharma, H., Duncan, J., & Johanson, C. (2006). Whole-body hyperthermia in the rat disrupts the blood-cerebrospinal fluid barrier and induces brain edema. Acta Neurochirurgica Supplement, 96, 426-431.

Sharma, H., Zimmermann-Meinzingen, S., & Johanson, C. (2010). Cerebrolysin reduces blood-cerebrospinal fluid barrier permeability change, brain pathology, and functional deficits following traumatic brain injury in the rat. Annals of the New York Academy of Sciences, 125-137.

Shinsato, T., Miyata, M., Kubozono, T., Ikeda, Y., Fujita, S., Kuwahata, S., . . . Tei, C. (2010). Waon therapy mobilizes CD34+ cells and improves peripheral arterial disease. Journal of Cardiology, 56, 361-366.

Sobajima, M., Nozawa, T., Ihori, H., Shida, T., Ohori, T., Suzuki, T., . . . Inoue, H. (2013). Repeated sauna therapy improves myocardial perfusion in patients with chronically occluded coronary artery-related ischemia. International Journal of Cardiology, 167, 237-243.

Thomas, K., van Rij, A., Lucas, S., & Cotter, J. (2017). Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls. The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 312, R281- R291.

Thompson, H., Tkacs, N., Saatman, K., Raghupathi, R., & McIntosh, T. (2003). Hyperthermia following traumatic brain injury: a critical evaluation. Neurobiology of Disease, 12, 163-173.

Topolovec-Vranic, J., Pollmann-Mudryj, M., Ouchterlony, D., Klein, D., Spence, J., Romaschin, A., . . . Baker, A. (2011). The Value of Serum Biomarkers in Prediction Models of Outcome After Mild Traumatic Brain Injury. The Journal of Trauma® Injury, Infection, and Critical Care, 71(5), S478-86.

Tremblay, J., Stimpson, T., & Pyke, K. (2019). Evidence of sex differences in the acute impact of oscillatory shear stress on endothelial function. Journal of Applied Physiology, 126(2), 314-321.

Tremblay, J., Thom, S., Yang, M., & Ainslie, P. (2016). Oscillatory shear stress, flow- mediated dilatation, and circulating microparticles at sea level and high altitude. Atherosclerosis, 256, 115-122.

Tubaro, C., Arcuri, C., Giambanco, I., & Donato, R. (2010). S100B Protein in Myoblasts Modulates Myogenic Differentiation Via NF-kB-Dependent Inhibition of MyoD Expression. Journal of Cellular Physiology, 270-282.

Wang, K., Yang, Z., Zhu, T., Shi, T., Rubenstein, R., Tyndall, J., & Manley, G. (2018). An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Review of Molecular Diagnostics, 18(2), 165-180.

Waston, P., Black, K. C., & Maughan, R. (2006). Exercise in the Heat: Effect of Fluid Ingestion on Blood–Brain Barrier Permeability. Medicine & Science in Sports & Exercise, 38(12), 2118-2124.

Watson, P., Head, K., Pitiot, A., Morris, P., & Maughan, R. (2010). Effect of Exercise and Heat-Induced Hypohydration on Brain Volume. Medicine and Science in Sports and Exercise, 42(12), 2197-2204.

Watson, P., Shirreffs, S., & Maughan, R. (2005). Blood-brain barrier integrity may be threatened by exercise in a warm environment. The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 288, R1689- R1694.

Wilson, T., & Crandall, C. (2011). Effect of Thermal Stress on Cardiac Function. Exercise and Sport Sciences Reviews, 39(1), 12-17.

Zaccardi, F., Laukkanen, T., Willeit, P., Kunutsor, S., Kauhanen, J., & Laukkanen, J. (2017). Sauna Bathing and Incident Hypertension: A Prospective Cohort Study. American Journal of Hypertension, 30(11), 1120-1125.

Zetterberg, H., Hietala, M., Jonsson, M., Andreasen, N., Styrud, E., Karlsson, I., . . . Wallin, A. (2006). Neurochemical Aftermath of Amateur Boxing. Archives of Neurology, 63, 1277-1280.

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