

# The effect of water temperature on cerebral blood flow during aqua cycling

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## Executive summary

Cerebrovascular disease refers to afflictions of the brain's blood vessels, most commonly vascular dementia and stroke (1). Stroke alone is Australia's second deadliest disease, killing more than 10,000 people every year, and greatly impacting the lives of survivors - 75% experiencing decreased employability and 30% dealing with post-stroke depression (13). Economically, cerebrovascular disease cost Australia more than \$5 billion in 2012, with an estimated "burden of disease" cost of more than \$40 billion (12).

Fortunately, a growing body of research suggests that repeated acute increases in cerebrovascular blood flow may significantly reduce the risk of cerebrovascular disease (44-47). Extensive research on peripheral vasculature has demonstrated that vessels undergo beneficial adaptation when repeatedly exposed to increases in mechanical loading from increased blood flow (termed "shear stress") (29, 32-37). Increased shear stress on endothelial cells lining blood vessels upregulates nitric oxide (NO) production, a molecule responsible for vasodilation and shown to have long-term anti-atherogenic effects, decreasing the risk of vascular (and by extension, cerebrovascular) disease (30).

As such, identifying the best modality to acutely increase cerebrovascular blood flow is crucial. Mechanisms responsible for controlling cerebral blood flow include cerebral pressure autoregulation, neurogenic regulation, flow-metabolism coupling and external metabolic agents (19, 23, 24). A promising method of utilising these mechanisms to increase cerebral blood flow is water immersion exercise, which has been shown to augment cerebral blood flow in humans (54, 55). Both moderate-intensity land-based exercise and sedentary water immersion have been shown to increase cerebral blood flow independently, and combining the two has an additive effect, increasing CBF more than either modality individually (51, 53-55). Water immersion exercise provides the additional benefit of reduced joint stress due to buoyancy, potentially allowing for safer and easier exercise for those at higher risk of cerebrovascular disease (e.g. the elderly and obese) (56). As such, identifying optimal conditions for water immersion exercise is an ideal topic for future research.

This study investigated the effect that water temperature has on increases in cerebral blood flow during water immersion cycle ergometer exercise (termed “aqua cycling”). Five healthy young (18-30-year-old) subjects underwent a control land cycling condition, a thermoneutral ( $32^{\circ}\text{C}$ ) aqua cycling condition and a hot ( $38^{\circ}\text{C}$ ) aqua cycling condition (immersed to navel level) in random order. The protocol involved a 10-minute initial rest followed by three ten-minute stages of 60rpm cycling at increasing ergometer resistances (5kg, 10kg, 15kg) before a final 5-minute rest period. Middle ( $\text{MCA}_v$ ) and posterior ( $\text{PCA}_v$ ) cerebral artery velocity, core temperature, end-tidal carbon dioxide ( $\text{P}_{\text{et}}\text{CO}_2$ ), heart rate (HR), mean arterial pressure (MAP), oxygen consumption ( $\text{VO}_2$ ) and perceived exertion (RPE) were measured throughout.

Underpowered results (due to interruptions with COVID-19) showed a non-significant elevation in  $\text{MCA}_v$  during thermoneutral aqua cycling when compared to hot aqua cycling throughout the entire protocol.  $\text{PCA}_v$  was initially similar between thermoneutral and hot aqua cycling conditions, but the thermoneutral condition was non-significantly higher from the 10kg phase onward. MAP was mostly non-significantly higher throughout the thermoneutral condition when compared with the hot condition, while HR was elevated for the duration of the hot condition when compared with the thermoneutral condition. Core temperature was higher in the hot condition during the 10kg and 15kg stages when compared with the thermoneutral condition. There was no difference in  $\text{P}_{\text{et}}\text{CO}_2$ ,  $\text{VO}_2$  or RPE between conditions.

In conclusion, thermoneutral aqua cycling augments  $\text{CBF}_v$  when compared to hot aqua cycling, while reducing likelihood of potential health risks. Relatively reduced  $\text{CBF}_v$  during hot water aqua cycling is likely a result of hyperthermia-induced counterproductive peripheral blood pooling, impeding the core hydrostatic mechanism responsible for elevating  $\text{CBF}_v$  during water immersion exercise. As such, when attempting to maximise increases in  $\text{CBF}_v$  via water immersion exercise, thermoneutral water should be utilised rather than hot water.

## Acknowledgements

To Dr. Howard Carter,

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## List of abbreviations

BP – Blood pressure

CBF – Cerebrovascular blood flow

CBF<sub>v</sub> – Cerebrovascular blood flow velocity

CO<sub>2</sub> – Carbon dioxide

CPP – Cerebral perfusion pressure

CVC – Cerebrovascular conductance

DBP – Diastolic blood pressure

eNOS – Endothelial nitric oxide synthase

HR – Heart rate

ICP – Intracranial pressure

MAP – Mean arterial pressure

MCA – Middle cerebral artery

MCA<sub>v</sub> – Middle cerebral artery velocity

O<sub>2</sub> - Oxygen

PCA – Posterior cerebral artery

PCA<sub>v</sub> – Posterior cerebral artery velocity

P<sub>a</sub>CO<sub>2</sub> – Partial pressure of arterial carbon dioxide

P<sub>et</sub>CO<sub>2</sub> – Partial pressure of end-tidal carbon dioxide

RPE – Rating of perceived exertion

RPM – Revolutions per minute

SBP – Systolic blood pressure

SD – Standard deviation

SEM – Standard error of the mean

VO<sub>2</sub> – Volume of oxygen consumed

## 1.0 Literature Review

### 1.1 Cerebrovascular Disease

#### *1.1.1 What is cerebrovascular disease*

Cerebrovascular disease refers to any disorder in which the cerebrum is impacted due to fault with the cerebral blood vessels, the most significant being vascular dementia and stroke. Stroke is broadly defined as “neurological injury stemming from a vascular cause” and is categorised in three ways – ischaemic stroke (87% of cases), transient ischaemic attack (a subtype of ischaemic stroke) and haemorrhagic stroke (13% of cases) (1-3).

Ischaemic stroke refers to blocking of the cerebral blood vessels by embolism or thrombosis and consequent ischaemia (4). Embolism occurs when a piece of material (embolus) flows through the vessel, while thrombosis is blockage of the artery due to occlusion of the vessel lumen. This results in inadequate blood flow to the affected tissue, consequent ischaemia and potentially cell death (1). If symptoms of an ischaemic stroke dissipate within 24 hours it is classified as a transient ischaemic attack rather than ischaemic stroke (5). Haemorrhagic stroke involves the rupture and consequent bleeding of a cerebral blood vessel (usually due to aneurysm) and is categorised as either subarachnoid or intracerebral, depending on location. Subarachnoid haemorrhage occurs when bleeding from a damaged vessel results in accumulation of blood at the surface of the brain (between the brain and skull), while intracerebral haemorrhage refers to bleeding within the brain (6, 7). In both cases the haemorrhaged blood causes swelling, pressure and consequently damage to cells and tissue (8). If a stroke is not fatal, survivors can experience a range of residual symptoms, depending on the type of stroke, location and area affected. These most common sequelae include weakness or paralysis in the limbs, cognitive impairment, sensory inhibition and more (1).

Vascular dementia refers to dementia (a cognitive disorder) caused by cerebral blood flow impairment. There are three types – multi-infarct dementia, Binswanger’s disease and mixed dementia. Multi-infarct dementia involves damage to the cortex (the area associated with memory and learning) and is caused by multiple strokes, symptoms worsening with successive strokes (9). Binswanger’s disease involves damage to the white matter of the brain

(the area involved with signalling), usually due to hypertension (10). Mixed dementia involves occurrence of two types of dementia simultaneously, usually Alzheimer's disease and vascular dementia (11).

#### *1.1.2 Impact of cerebrovascular disease*

Over 420,000 people in Australia are currently suffering the residual effects of a stroke. In two-thirds of these individuals, stroke resulted in the inability to function independently. Estimates suggest this number will be as high as 700,000 by 2032. Economically, strokes were estimated to cost Australia \$5 billion in 2012, with the total "burden of disease cost" totalling \$49.3 billion (12). Stroke also has immense impact on the personal lives and families of those affected. It causes over 10,000 deaths per year, making it Australia's second most prominent killer following coronary heart disease (13). Those who survive have a 75% chance of experiencing decreased employability, and 30% chance of suffering post-stroke depression (9). Vascular dementia also has serious widespread effects. It is the second most common type of dementia and affects up to 4.2% of those aged 65 or older (14). In the next section, the anatomy and mechanisms of cerebral blood flow regulation will be briefly described.

## **1.2 Cerebral blood flow anatomy**

The brain makes up approximately two percent of the body's mass but receives 15-20% of its blood flow ( $\sim 750 \text{ ml/min}$ ) (15). This occurs via the vertebral and internal carotid arteries (16). The internal carotid arteries (left and right) split into the anterior (ACA) and middle (MCA) cerebral arteries. The anterior cerebral arteries are connected by the anterior communicating artery and supply the medial, frontal and parietal lobes, while the middle cerebral arteries supply the majority of the lateral surface of the cerebrum. The internal carotid arteries also give rise to the anterior choroidal artery and posterior communicating arteries (16). The vertebral arteries join to form the basilar artery, which splits into the two posterior cerebral arteries (PCA). The posterior cerebral arteries supply the inferior and medial surfaces of the temporal and occipital lobes (16).

The ACAs, PCAs and internal carotid arteries are inter-connected via the anterior and posterior communicating arteries to form the Circle of Willis. The Circle of Willis (CoW) allows

for collateral flow between the anterior, middle and posterior circulation to prevent ischaemia in the presence of focal vessel occlusion (16). Notably, less than 20% of the population exhibit a fully formed CoW (17).

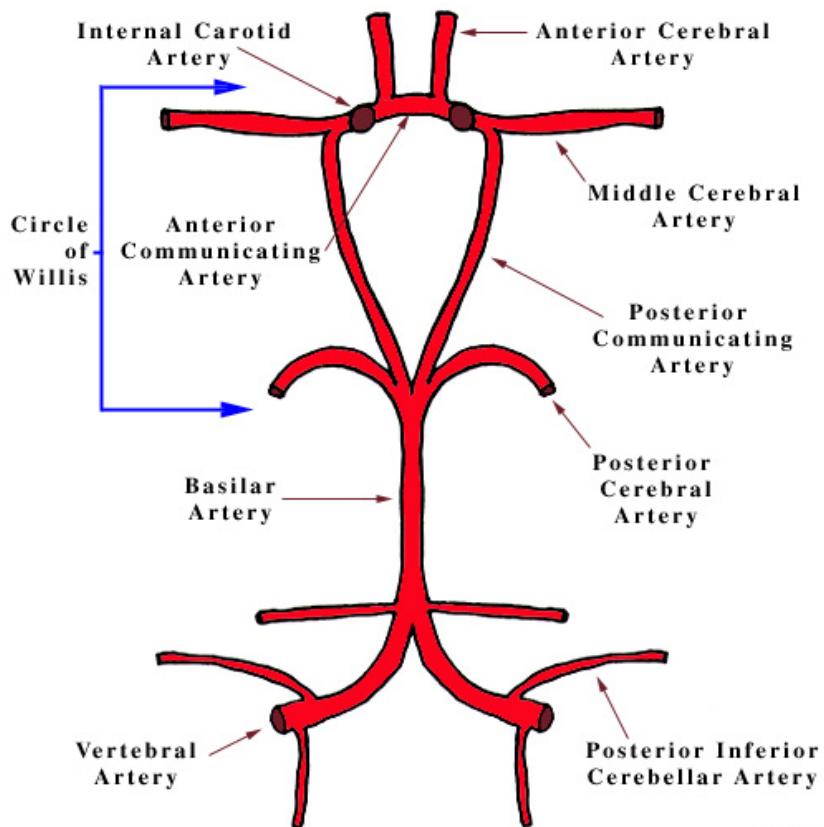


Figure 1.1: Arteries involved in cerebral blood flow, centred around Circle of Willis (18)

### 1.3 Mechanisms of cerebral blood flow regulation

There are four principal regulatory mechanisms controlling cerebral blood flow; cerebral pressure autoregulation, flow-metabolism coupling, neurogenic regulation and effects of external metabolic agents including CO<sub>2</sub> and O<sub>2</sub>. Due to the brain's vital role, high metabolic demands and sensitivity to changes in O<sub>2</sub> and glucose supply, precise control of blood flow is important and tightly regulated (19).

Flow-metabolism coupling refers to the cerebrovascular response to metabolic activity of brain cells. When differing areas of the brain are more active, their metabolic activity will increase, requiring increased 'fuel' from the blood in the form of O<sub>2</sub> and glucose. This increased requirement is likely signalled by metabolic agents such as K<sup>+</sup>, H<sup>+</sup>, nitric oxide and

adenosine which cause vasodilation in the nearby blood vessels and hence increased blood flow (20).

Pressure autoregulation is the mechanism by which cerebral arterioles maintain consistent cerebral blood flow despite inconsistent cerebral perfusion pressure (CPP) (pressure of incoming blood [MAP – ICP]). Pressure is maintained via arteriolar resistance changes in response to CPP fluctuation – when CPP rises, arterioles will constrict appropriately and prevent CBF from rising (and vice versa). The exact mechanism responsible is unknown, but likely involves endothelium and vascular smooth muscle. Historically, pressure autoregulation was thought to be effective between MAP of about 80 to 150 mmHg (19). However, recent literature has challenged this notion and suggests autoregulation acts on a much smaller range and more passively than previously thought (Figure 1.2)(21).

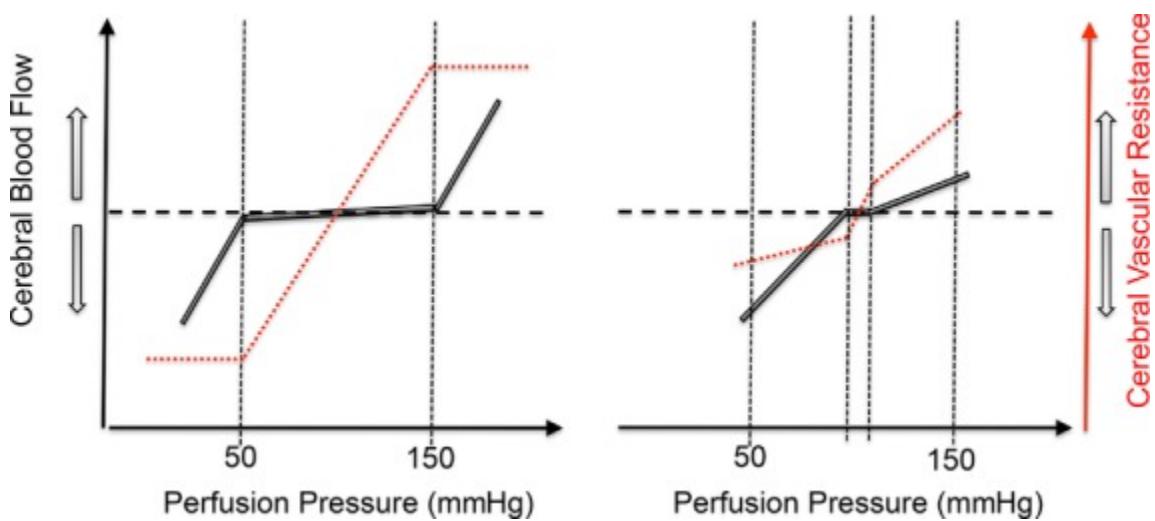


Figure 1.2: Classical (left) and contemporary (right) perspectives on cerebral blood flow autoregulation at varying mean arterial pressures from Willie et al. (2014) (21).

Neurogenic regulation refers to the sympathetic and parasympathetic stimulation of cerebral blood vessels from the extensive perivascular innervation. The innervation is classified into intrinsic and extrinsic, for nerves within and outside of the brain parenchyma, respectively (20). The nerves act via neurotransmitters (e.g. noradrenaline, acetylcholine) causing either contraction (sympathetic) or relaxation (parasympathetic) of the vessel. However, neurogenic

mechanisms are poorly understood and thought to have minimal impact on cerebral blood flow regulation under normal conditions. It is postulated that neurogenic control only plays a significant role during “severely challenging circumstances” such as post-haemorrhagic ischaemia (22).

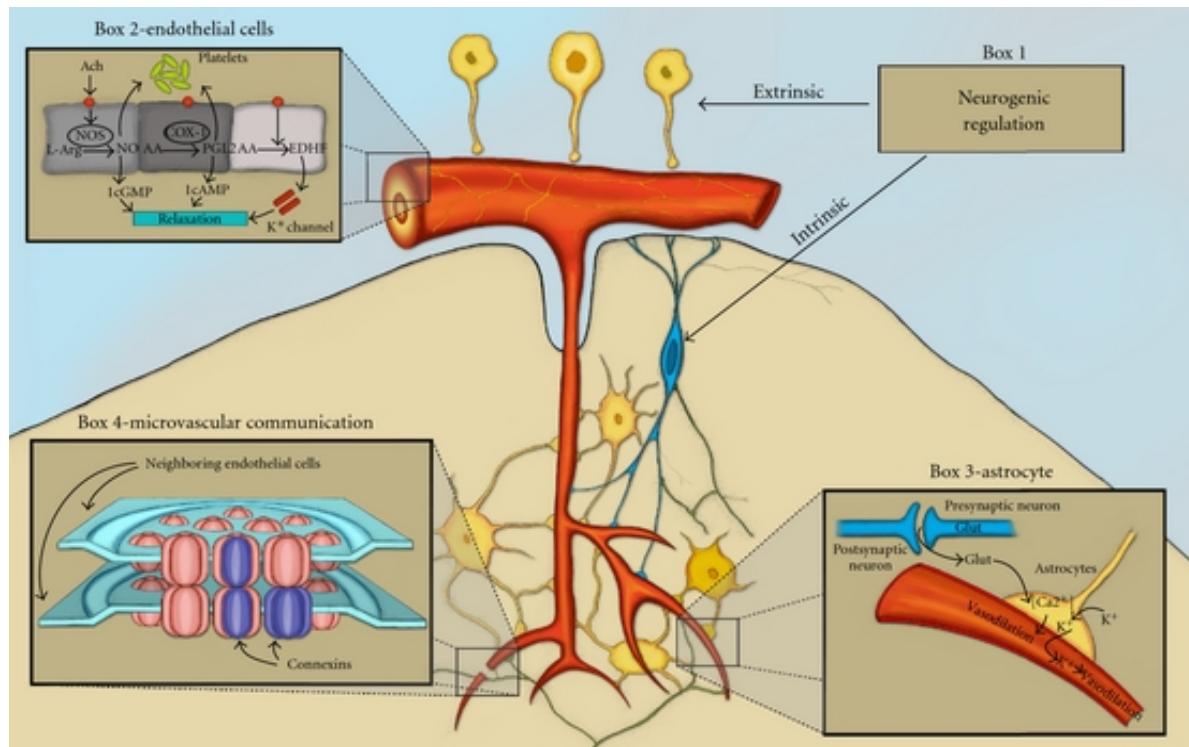


Figure 1.3: Intrinsic and extrinsic innervation of cerebral blood vessels (19)

In contrast, external metabolic agents present within the cerebrospinal fluid (CSF) (fluid surrounding brain) highly affect CBF. Specifically,  $pO_2$  and  $pCO_2$  levels in the CSF have potent effects on vasomotor tone. Hypoxia (low  $pO_2$ ) induces vasodilation when  $pO_2$  falls below ~50 mmHg, with blood flow rising up to 400% of basal levels. This occurs as hypoxia results in lowered ATP, opening smooth muscle  $K_{ATP}$  channels resulting in vasodilation (23-25). Hypercapnia (high  $pCO_2$ ) causes vasodilation so potently that 7%  $CO_2$  inhalation causes a 100% increase in cerebral blood flow. This likely occurs as elevated  $pCO_2$  activates the bicarbonate buffer system, lowering pH which has a direct dilatory effect on vascular smooth muscle (26, 27).

In summary, cerebral pressure autoregulation, flow-metabolism coupling, neurogenic regulation and external metabolic agents are all involved in the exquisite regulation of cerebral blood flow. They are integrated to maintain consistent blood flow to the brain, with

the ability to adjust CBF as metabolic demands vary. However, impairment in the health of cerebral blood vessels can impact the regulatory features of CBF and lead to cerebrovascular disease.

#### **1.4 Protecting against cerebrovascular disease**

Stroke and cerebrovascular diseases, once manifest, have lifelong consequences that place a substantial burden on healthcare systems. This provides a compelling argument for effective cerebrovascular disease prevention. Current guidelines suggest eating well, not smoking, minimising alcohol consumption and staying physically active (28). In the case of the latter risk factor, research is beginning to suggest that exercise is a potent “medicine” for cardiovascular, including cerebrovascular, disorders (29, 30). While some of the benefits of exercise are attributable to improvement in traditional cardiovascular risk factors such as blood pressure, blood glucose and body composition, recent studies in peripheral blood vessels suggest that exercise has a direct and beneficial impact on vascular endothelial health (termed “vascular adaptation”)(29, 31).

##### ***1.4.1 Mechanisms of peripheral vascular adaptation to exercise***

Vascular adaptation occurs as a result of increased shear stress caused by elevated blood flow through the lumen of arteries. When blood flow increases within a vessel, it provides mechanical loading (termed “shear stress”) on the endothelial cells lining the walls of the blood vessel (32). This has a positive effect on the cells’ ability to produce nitric oxide (NO), a molecule that induces relaxation of the vessel’s smooth muscle and, hence, vasodilation. NO has also been shown to have important long-term anti-atherogenic effects; it inhibits vascular smooth muscle proliferation, inflammatory cell activity and platelet aggregation, greatly decreasing the risk of cerebrovascular diseases (33). NO is thought to be so crucial to vascular health that flow-mediated dilation (FMD), a largely NO-mediated test, is a commonly utilised index of arterial function and health *in vivo* (34).

The relationship between exercise and vascular adaptation has been extensively researched. It was first investigated in 1994 by Green *et al.*, who found a significant increase in forearm vasodilator capacity in trained vs. untrained arms after a four-week intervention in young males (35). In a subsequent study (33) Green and colleagues compared the preferred and

non-preferred limbs of elite tennis players and observed that a player's dominant (more heavily trained) forearm exhibited significantly enhanced vasodilator capacity compared to their non-dominant forearm. Maiorana *et al.* (2000, 2001) subsequently found that resistance and aerobic training significantly augmented vascular function and vasodilatory capacity in both chronic heart failure and type two diabetes patients (36, 37). Hambrecht *et al.* (2000, 2003) demonstrated *in vivo* coronary vascular adaptations in patients with coronary artery disease, and showed an association between exercise and endothelial NO synthase (eNOS)(Figure 1.4)(38, 39). The general outcome of these studies was that increased blood flow and shear stress as a result of exercise consistently augmented NO-mediated vasodilator capabilities in both conduit and resistance arteries.

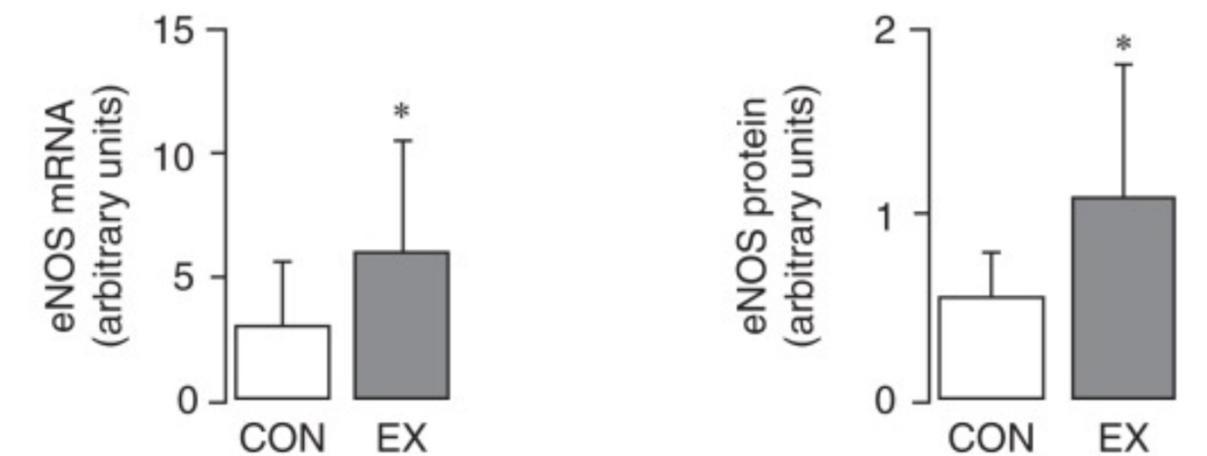


Figure 1.4: Difference in endothelial nitric oxide synthase (eNOS) mRNA and protein between control and exercise groups from Hambrecht *et al.* (2003)(39)

Important follow up studies confirmed that increased blood flow and consequent shear stress can induce vascular adaptation, independent of exercise *per se*. Naylor *et al.* (40) and Carter *et al.* (41) utilised bilateral forearm heating to induce exercise-like increases in blood flow and hence shear stress, and cuffed one arm to limit increases in blood flow. They found that vascular adaptation occurred exclusively in the uncuffed arm experiencing augmented blood flow.

The studies above were undertaken in peripheral arteries. However, the cerebral circulation is controlled by different regulatory mechanisms, as explained above, and may not respond

to vascular stimuli in the same manner as peripheral blood vessels. This raises the question of whether cerebral blood vessels are sensitive to increases in blood flow and shear stress, and whether these stimuli induce cerebrovascular adaptation.

#### *1.4.2 Mechanisms of cerebrovascular adaptation to exercise*

Due to the difficulties in imaging cerebral blood vessels in humans and manipulating haemodynamic stimuli, the majority of literature on this topic has been performed in animals. Acute studies have demonstrated the ability of cerebral arteries to dilate in response to shear stress. Dilation has been shown to be mostly NO-mediated, but is also partially dictated by endothelium-independent pathways (42-45).

A longitudinal study by Gertz *et al.* (2006) strongly suggested that the previously mentioned mechanisms of vascular adaptation resulting from exercise are integral for long-term cerebrovascular protection (46). Gertz *et al.* induced stroke-like ischaemia in mice and had them undergo either an exercise or sedentary protocol for three weeks. When compared with sedentary mice four weeks post-injury, exercised mice exhibited increased density of microvessels, more newly generated cells in vascular sites, increased eNOS levels and continued elevated CBF at the site of ischaemia (Figure 1.5). This finding is corroborated by a similar study on mice from Endres *et al.*, who found congruent positive vascular changes post exercise intervention in wild-type mice but no such changes in eNOS-deficient mice, highlighting the NO system's involvement in post-stroke vascular adaptation (47).

Similar to the above animal studies, human extracranial cerebral blood vessels display flow-mediated dilation via shear stress (48). A human study on stroke survivors (Ivey *et al.*, 2011) had subjects undergo three 40-minute sessions per week of either aerobic exercise or non-aerobic stretching for six months and examined basal cerebral blood flow via transcranial doppler ultrasonography before and after the six-month intervention (49). Exercised subjects were found to have significantly augmented post-intervention basal cerebral blood flow when compared with non-aerobic stretching subjects.

Ivey *et al.* did not speculate about mechanisms of vascular adaption, but given prior research it is reasonable to assume involvement of the NO dilator system, in accordance with findings

in mice. As such, the common outcome from these studies is that long-term repeated exercise-induced episodic increases in cerebral blood flow, and hence shear stress, seem to represent a physiological stimulus for improvement in cerebrovascular health in stroke survivors. Furthermore, cognitive function has been found to be augmented long-term by exercise-induced increases in blood flow, and diminished by low long-term blood flow (50-52).

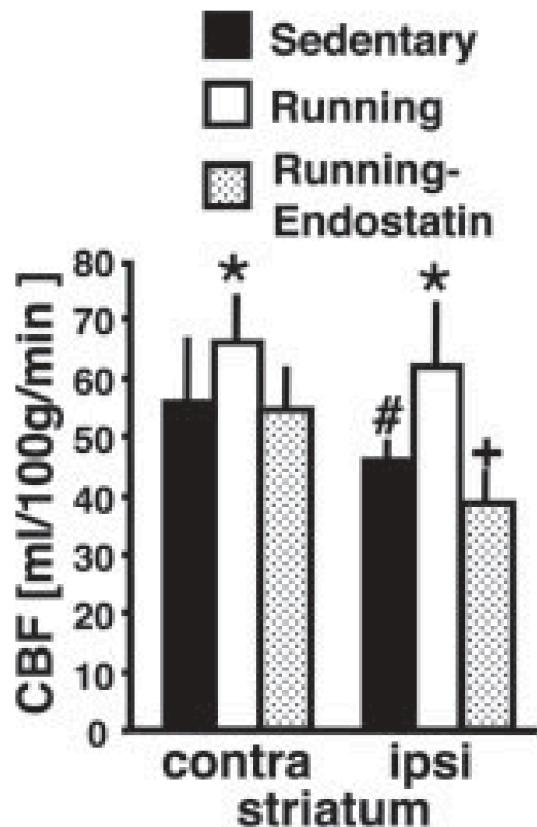


Figure 1.5: Differences in cerebral blood flow (ml/100g/min) in contralateral and ipsilateral hemispheres (relative to injured hemisphere) between running, running/endostatin and sedentary mice groups one week after conclusion of three-week post-stroke intervention  
 (adapted from Gertz *et al.*, 2006)(46)

### 1.5 How much does acute exercise increase cerebral blood flow in humans?

Due to recent studies suggesting that habitual exercise seems improves cerebrovascular health and prevents disease, there has been a surge in research investigating the relationship between exercise and cerebral blood flow, and optimal exercise strategies to enhance cerebrovascular health.

In general terms, research suggests that exercising at a “moderate” intensity (up to ~60%  $\text{VO}_{2\text{max}}$ ) increases CBF (53). However, more intense exercise seems to decrease CBF to basal levels as the resulting hyperventilation lowers  $\text{pCO}_2$ , causing vasoconstriction (Figure 1.6) (54). More recently, CBF has been investigated in the context of water immersion. One study demonstrated that immersion in 30°C water, to the level of the heart, increases CBF at rest. It was postulated that this is due to the hydrostatic pressure of water compressing superficial veins, thereby increasing venous return, cardiac output, stroke volume and mean arterial pressure (55).

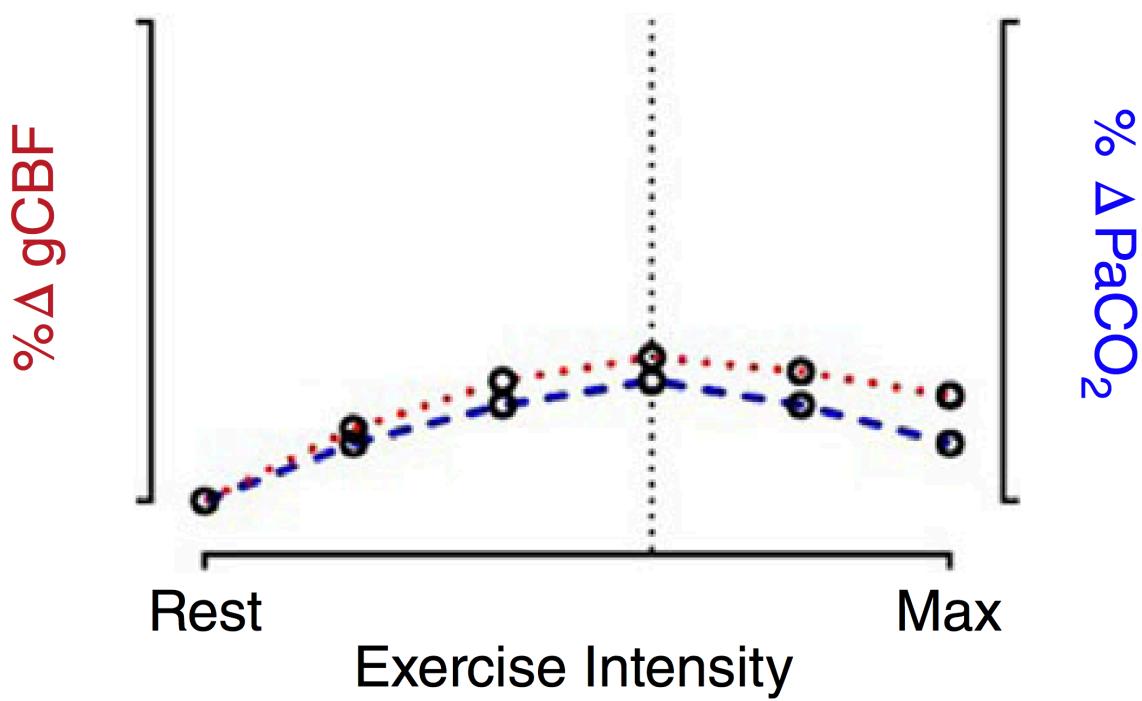


Figure 1.6: Relative change in global cerebral blood flow (%  $\Delta\text{gCBF}$ ) and partial pressure of carbon dioxide (%  $\Delta\text{PaCO}_2$ ) at varying exercise intensities (adapted from Smith *et al.*, 2017)(56)

Two further studies assessed the impact of water immersion during exercise on CBF. Pugh *et al.* (2014) had subjects perform stepping exercise both on land and in water and examined differences in mean arterial pressure (MAP), heart rate (HR), oxygen consumption ( $\text{VO}_2$ ), end-tidal carbon dioxide ( $\text{P}_{\text{et}}\text{CO}_2$ ) and cerebral blood flow velocities ( $\text{MCA}_v$  and  $\text{PCA}_v$ ). The

conditions were matched for intensity via  $\text{VO}_2$  and HR. Water-based exercise was found to increase  $\text{MCA}_v$  and  $\text{PCA}_v$ , MAP and  $\text{P}_{\text{et}}\text{CO}_2$  more than land-based exercise. Pugh *et al.* suggested that, whilst precisely identifying all factors involved in the observed increase in CBF is difficult, a causal relationship exists between MAP,  $\text{P}_{\text{et}}\text{CO}_2$  and CBF (57). Parfitt *et al.* (2017 subsequently investigated CBF differences between land- and aquatic-based treadmill exercise, focusing on  $\text{MCA}_v$  and HR. The aquatic-based exercise was found to elicit both increased  $\text{MCA}_v$  and decreased HR in comparison to land-based exercise. Parfitt *et al.* did not measure  $\text{P}_{\text{et}}\text{CO}_2$ , however speculated on a relationship between brain neuronal and metabolic activity, arterial carbon dioxide, cardiac output, blood pressure and CBF (58). Both studies noted the potential of water immersion exercise as an optimal method for inducing shear stress and hence endothelial and vascular adaptation of the cerebrovasculature (57, 58).

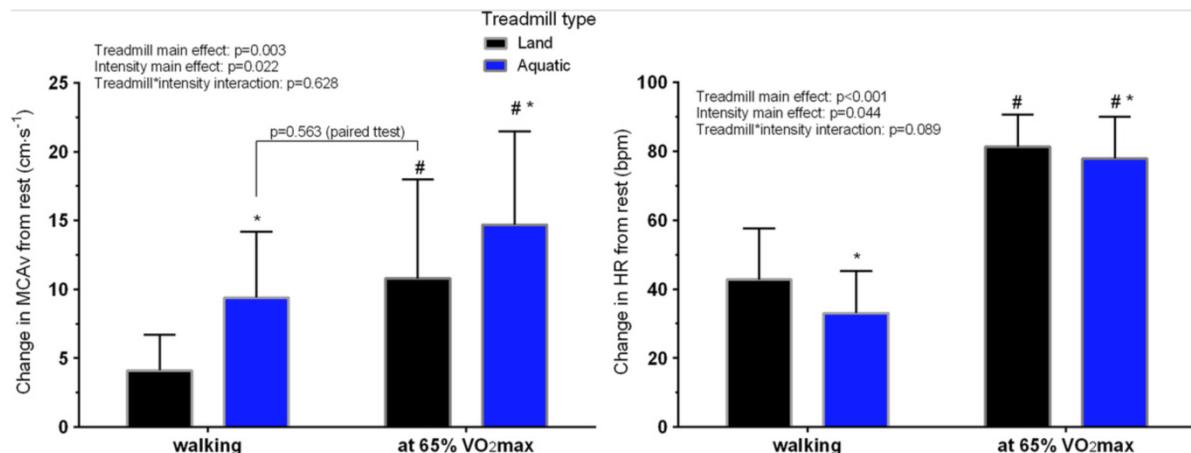


Figure 1.7: Mean change in mean middle cerebral artery velocity ( $\text{MCA}_{\text{mean}}$ ) (left panel) and heart rate (HR) (right panel) from resting baseline for walking and moderate intensity (65% of max oxygen consumption ( $\text{VO}_{2\text{max}}$ )) running exercise using land treadmill and aquatic treadmill. Data are means +/- SD.  $N = 11$ . \*Significant difference between treadmills. #Significant difference between preceding stage (i.e., walking)(Adapted from Parfitt et al.)(58)

These studies have potential real-world implications. Many individuals, especially those likely at higher risk of cerebrovascular diseases (obese, aged), can struggle to exercise on land due to injury, frailty and risk of falls. Water immersion exercise can greatly alleviate joint stress via buoyancy, providing a double benefit of safer exercise, and improved CBF (59). As such,

more research should be conducted to identify optimal conditions for water immersion exercise.

No studies have directly investigated the effect temperature has on cerebral blood flow during water immersion exercise. This is particularly important in the context of a recent study (60), where the authors increased core temperature in healthy subjects by 2°C via passive heating and reported a ~20% increase in cerebral metabolism. Increased cerebral metabolism evokes neurovascular coupling and localised increases in blood flow. Both hydrostatic pressure and temperature can increase CBF, however the impact of exercise in different water temperatures has never been investigated.

### 1.6 Hypotheses

We hypothesise that:

- Cerebral blood flow will be higher during both thermoneutral (32°C) and hot (40°C) water aqua cycling, compared to land-based cycling.
- Cerebral blood flow will be higher during thermoneutral (32°C) aqua cycling than during hot water (40°C) aqua cycling.

## 1.7 References

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## 2.0 The effect of water temperature on cerebral blood flow during aqua cycling

# The effect of water temperature on cerebral blood flow during aqua cycling

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## 2.1 Abstract

Introduction: Water immersion exercise has been shown to acutely increase cerebral blood flow. Repeated acute increases in blood flow have been shown to improve long-term peripheral vascular health. This study examines the effect of varying water temperature during aqua cycling immersion exercise on cerebral blood flow.

Methods: Eight young healthy participants rested for 10 minutes, then cycled either on land or immersed to navel level in 32°C or 38°C water at 60rpm for three immediately sequential 10-minute stages of increasing resistance (5kg, 10kg and 15kg) before resting for five minutes. Middle (MCA<sub>v</sub>) and posterior (PCA<sub>v</sub>) cerebral artery velocity, mean arterial pressure (MAP), heart rate (HR), core temperature, end-tidal carbon dioxide (P<sub>et</sub>CO<sub>2</sub>), oxygen consumption (VO<sub>2</sub>) and perceived exertion (RPE) were recorded throughout.

Results: MCA<sub>v</sub> increased with exercise across all conditions and differed non-significantly between conditions. MCA<sub>v</sub> was non-significantly higher during thermoneutral aqua cycling when compared to both hot aqua cycling and land cycling at every stage of the protocol, while MCA<sub>v</sub> during hot aqua cycling was similar when compared to land at rest and after five minutes of low intensity exercise, but non-significantly lower through the remainder of the protocol.

Conclusion: Thermoneutral aqua cycling augments cerebral blood flow more than hot water aqua cycling at low to medium exercise intensity, while also being safer. Hyperthermic-related changes in centralisation of blood volume are likely the primary mechanism involved in varying the cerebral blood flow response between hot and thermoneutral water immersion exercise. As such, thermoneutral water should be utilised rather than hot water when attempting to induce acute increases in CBF via water immersion exercise.

## 2.2 Introduction

Cerebrovascular disease is an umbrella term referring to affliction of brain blood vessels, the most prominent being stroke and vascular dementia. It is Australia's second deadliest disease, after coronary heart disease, ending the lives of more than 10,000 people every year.

Economically, stroke alone was estimated to cost Australia \$5 billion in 2012, with the total “burden of disease” totalling \$49.3 billion. More than 400,000 stroke survivors experience lifelong residual effects, with 75% experiencing decreased employability and 30% suffering from post-stroke depression (1).

Extensive research has been undertaken to combat cerebrovascular diseases. A principal finding has been that repeated acute increases in cerebrovascular blood flow can significantly enhance cerebrovascular health (2). Increased blood flow induces mechanical loading, or “shear stress”, on endothelial cells lining the walls of all blood vessels. Shear stress induces the production of nitric oxide (NO), a molecule that causes vasodilation by relaxing smooth muscle surrounding the vessels (3). Elevated endothelium-derived NO has been shown to have long-term anti-atherogenic effects, including inhibiting inflammatory cell activity, platelet aggregation and smooth muscle proliferation. Enhanced endothelial function as a consequence of repetitive increases in brain blood flow and shear stress also maintains the integrity of the blood brain barrier and decrease the risk of ischaemic and non-ischaemic cerebrovascular disease (2).

There are multiple mechanisms responsible for regulating cerebrovascular blood flow, including flow-metabolism (neurovascular) coupling, cerebral pressure autoregulation and metabolic control via agents such as arterial blood gases (4-6). Exercise is a potent stimulus to all of these mechanisms and exercise at moderate intensity (up to ~60% VO<sub>2max</sub>) has been shown to increase CBF (7). Recently, it was reported that immersion of the body in 30°C water to the level of the heart also increase CBF at rest, due to hydrostatic pressure compressing superficial veins and increasing venous return, stroke volume, cardiac output and mean arterial pressure (8). Furthermore, combining exercise and water immersion appears to have an additive effect, increasing CBF more than either intervention alone (9, 10).

Optimising brain blood flow through immersion exercise has potential real-world benefits. Many individuals, especially those at higher risk of cerebrovascular diseases (e.g. the elderly and obese), struggle with land-based exercise due to frailty, debility and risk of falls. The buoyancy effect present during water immersion can alleviate the risks associated with exercise, with the potential dual benefit of also improving CBF (11). Identifying optimal

conditions for immersion exercise should therefore be a research focus. However, no studies have directly investigated the relationship between water temperature and cerebral blood flow during water immersion exercise, leading to the aim of this study: *to investigate the effect water temperature has on cerebral blood flow during aqua cycling tasks*. We hypothesized that aqua cycling in thermoneutral water (32°C) would induce higher cerebrovascular blood flows than exercise during hot water (38°C) aqua cycling and a land-based control cycling condition.

## 2.3 Methods

### 2.3.1 Ethics

Subjects were provided a document outlining the experiment and all procedures involved. All provided written consent. The study conformed to the Declaration of Helsinki and was approved by the University of Western Australia's Human Research Ethics Committee (Ref: RA/4/1/5642).

### 2.3.2 Participants

Eight healthy young normotensive participants ( $23.3 \pm 3.6$  yr,  $23.5 \pm 3.1$  kg/m<sup>2</sup>, 7 ♂) were recruited (Table 2.1). All subjects were healthy with no injuries impeding cycling exercise, and no evidence of cerebrovascular, cardiovascular, metabolic or respiratory disorders. The sole female subject was tested during the early follicular phase of the menstrual cycle (days 1-7 of the cycle).

### 2.3.3 Experimental Design

In random order, each subject performed a land (control), thermoneutral water immersion (32°C) and hot water immersion (38°C) cycling condition, at the same time every day ( $\pm$  one hour) and with a minimum of 47 hours between conditions. All were fasted for a minimum of 8 hours, abstained from caffeine for a minimum of 12 hours and abstained from alcohol and vigorous physical exercise for a minimum of 24 hours prior to testing. Middle (MCA<sub>v</sub>) and posterior (PCA<sub>v</sub>) cerebral artery velocity, mean arterial pressure (MAP), heart rate (HR), core temperature, end-tidal carbon dioxide (P<sub>et</sub>CO<sub>2</sub>), oxygen consumption (VO<sub>2</sub>) and perceived exertion (RPE) were recorded throughout each session.

Table 2.1 Subject characteristics

Variable	Mean ± SD
N	8
Gender	7 male ; 1 female
Age (years)	23.3 ± 3.6
Height (m)	1.80 ± 0.12
Weight (kg)	76.98 ± 16.27
BMI ( $\text{kg}\cdot\text{m}^{-2}$ )	23.5 ± 3.1

#### 2.3.4 Experimental Procedures

Upon arrival for each session, height, weight, resting blood pressure and core temperature were measured, after which subjects sat and rested for a minimum of 20 mins. Subjects were then placed on a cycle ergometer (Figure 2.2 and Figure 2.3, arrow D) either on land (no water immersion) or in a water tank (immersion) filled to navel level with either 32°C (thermoneutral) or 38°C (hot) water. Instruments were attached and calibrated, at which point an initial 10-minute rest period began. After the 10-minute rest period, subjects underwent three 10-minute stages of 60 rpm ergometer cycling at increasing resistances: 5kg, 10kg and 15kg, before a final rest period of five minutes (Figure 2.1). Core temperature and RPE were recorded every five minutes while respiratory and haemodynamic data were recorded continuously throughout and averaged (mean) over the five minutes prior to each time point.

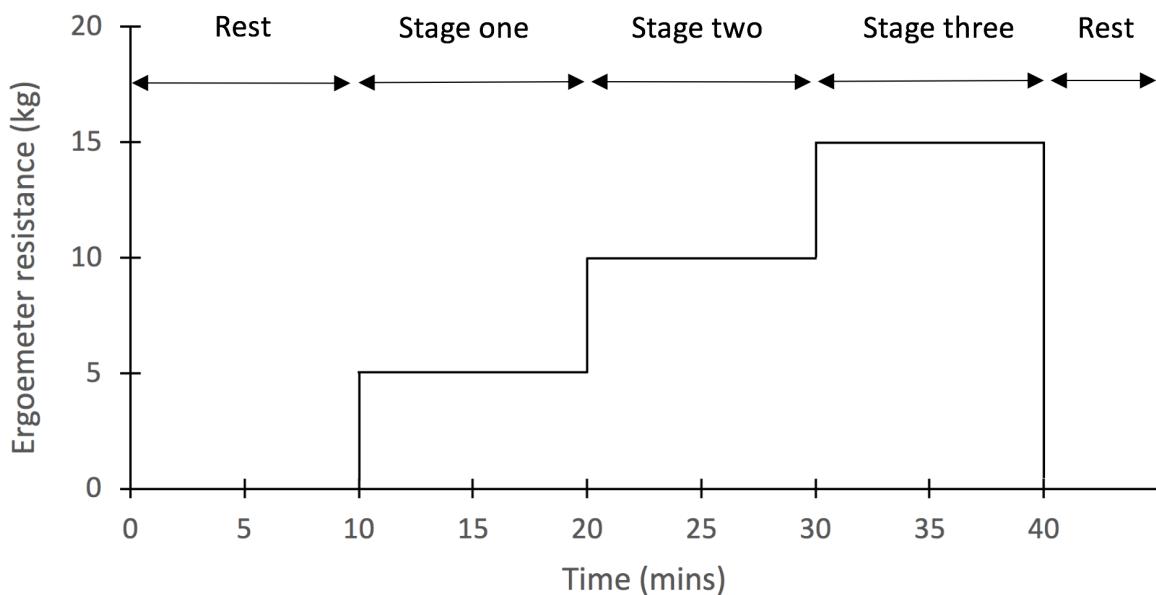


Figure 2.1 Experimental protocol of cycling sessions showing ergometer resistance (kg) over time (mins) during each stage. Resistance during initial rest, stage one, stage two, stage three and final rest were 0kg, 5kg, 10kg, 15kg and 0kg respectively.

### 2.3.5 Experimental measures

#### *Cerebral blood flow*

As shown in Figures 2.3 (arrow A) and 2.4, a pulsed 2-MHz ST3 Transcranial Doppler (TCD) ultrasound system (Spencer Technologies, Seattle, WA) was used in combination with a Marc 600 head frame (Spencer, USA) to measure middle ( $MCA_v$ ) and posterior ( $PCA_v$ ) cerebral artery velocities. Insonation techniques utilised to locate and identify MCA and PCA involved examination of velocity, waveform and depth (see Figure 2.4) as comprehensively described elsewhere (14). A probe was secured at each temporal window and adjusted appropriately until an ideal m-mode image was found. Probe configuration for each subject was consistent between conditions.  $MCA_v$  and  $PCA_v$  were collated via PowerLab exported in raw analog form to LabChart (LabChart 8; ADInstruments, Sydney, Australia). Mean values of five-minute interval prior to each time point were subsequently calculated.



Figure 2.2 Cycle ergometer in unfilled tank



Figure 2.3 Equipment configuration. Arrow A: Headframe with attached TCD ultrasound probe; Arrow B: Mouthpiece with expired gas tube; Arrow C: Finometer armband with finger cuff; Arrow D: Immersed cycle ergometer

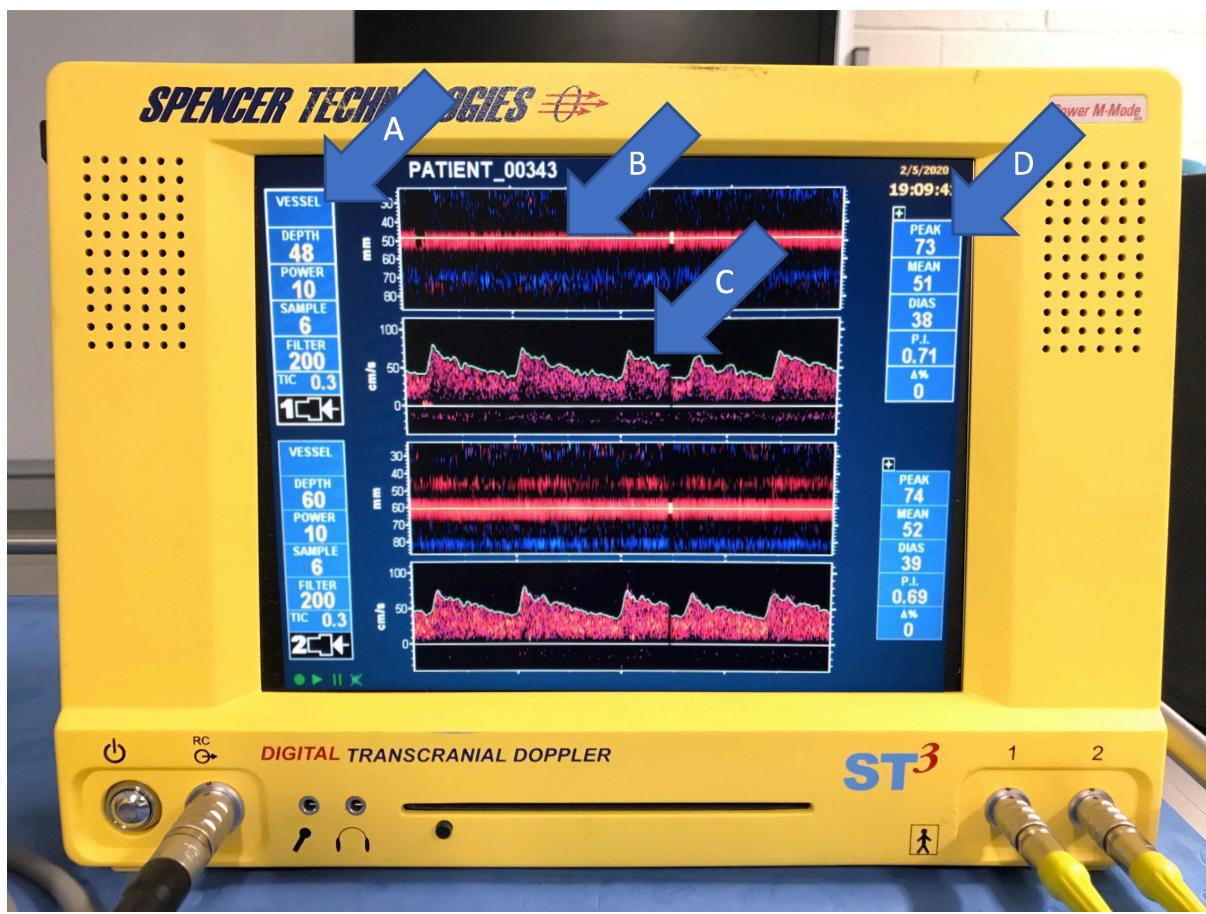


Figure 2.4 TCD interface. Arrow A: Probe settings; Arrow B: Depth (mm), blood flow direction indicated by colour (red/blue); Arrow C: Velocity trace (cm/s); Arrow D: Velocity characteristics



Figure 2.5 Headframe with attached TCD ultrasound probe (arrow A)

#### *Core temperature*

Core temperature was collected using wireless CorTemp core body temperature monitoring system (CorTemp, HQInq, Palmetto, FL, USA). Subjects ingested a CorTemp temperature sensor telemetry capsule 6-7 hours prior to experiment onset to ensure the sensor was at an ideal point in digestive tract during data collection. Readings were taken every 5 minutes with hand-held CorTemp data monitor (Figure 2.6).



Figure 2.6 Hand-held CorTemp data monitor

#### *Ventilation*

Oxygen consumption ( $\text{VO}_2$ ) and end-tidal carbon dioxide ( $\text{P}_{\text{ET}}\text{CO}_2$ ) were recorded via Parvo Medics TrueOne® metabolic cart (Parvo Medics, Salt Lake City, UT, USA) with associated

mouthpiece and tubing (Figure 2.3, arrow B). One-way valves and a nose peg were utilised to ensure end-tidal gases were accurately analysed. End-tidal CO<sub>2</sub> was secondarily measured using a sampling tube attached to the mouthpiece, feeding into PowerLab and exported in real time to LabChart. Mean values of five-minute period prior to time points were calculated.

#### *Systemic haemodynamics*

Blood pressure was recorded via photo plethysmography using a Finometer finger cuff (Finometer Pro, Finapres Medical systems, The Netherlands) (Figure 2.3 arrow C, Figure 2.8) and exported continuously to Powerlab throughout the experiment. Subjects placed their left arm on a platform at approximately heart level while a height sensor was taped to the torso at atrium level to automatically account for elevation changes of the finger cuff (which impact recorded pressure). Mean arterial pressure (MAP) and heart rate (HR) were calculated in real time by PowerLab cyclically using the formula (1/3 SBP + 2/3 DBP) and measuring systolic peak rate respectively.

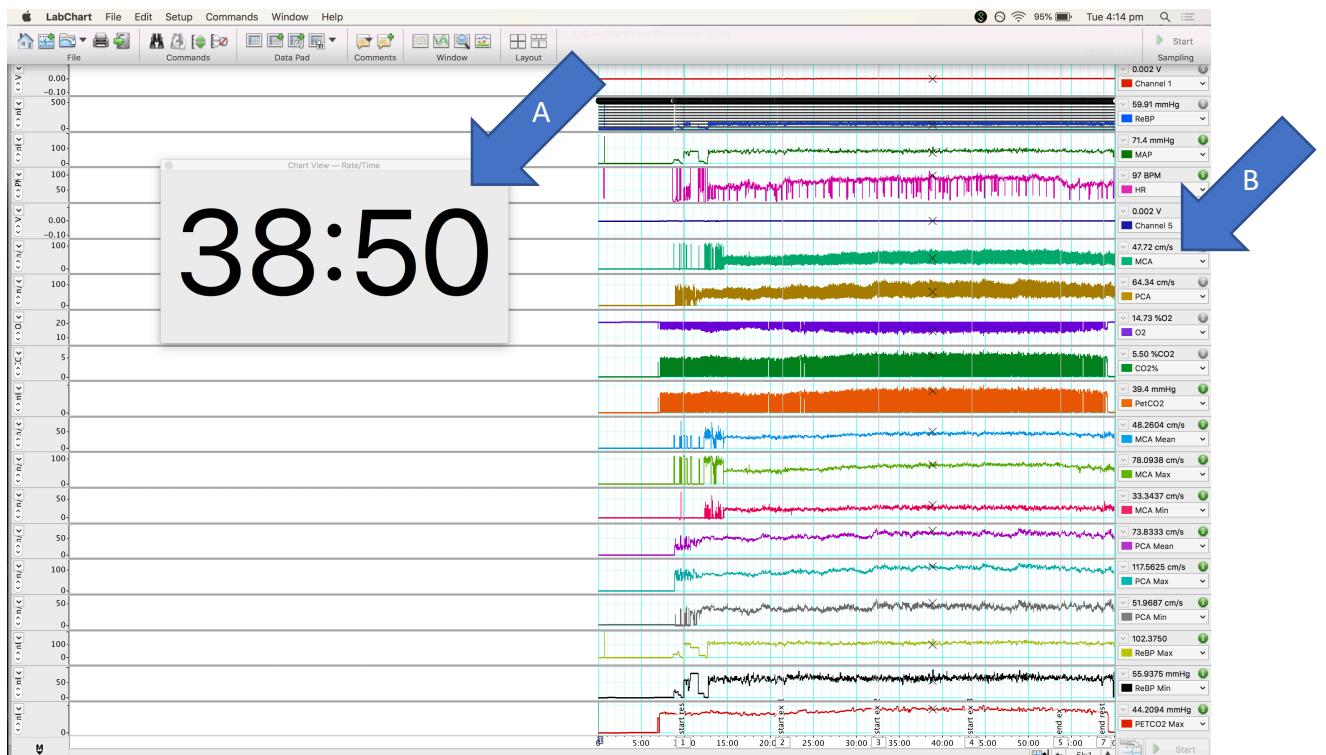


Figure 2.7 Labchart software interface with timer (arrow A) and live sampling channels (arrow B)



Figure 2.8 Finometer armband with attached finger cuff

#### *Perceived exertion*

Perceived exertion was reported every 5 minutes by subjects pointing at a Borg RPE chart (Figure 2.9) with their free hand.

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Figure 2.9 Borg rating of perceived exertion (RPE) scale

#### *Ergometer resistance*

Cycle ergometer resistance was standardised between subjects (5kg, 10kg and 15kg stages) rather than weighted relative to estimated individual performance. While individually-tailored resistances may have more accurately controlled for exercise intensity and exertion, standardised resistance provides a more practical guide for weight selection to lay people who may benefit from the research. In addition, the primary statistical comparison was to assess within-subject differences between conditions, rather than analysis of between-subject differences in response.

#### *Statistics*

SPSS 23.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. The average (mean) of the prior five minutes was calculated at each time point for continuous variables. Two-way repeated-measures ANOVAs were performed to compare conditions with time. Individual

pairs of data points were compared using paired t-tests with appropriate Tukey's HSD corrections. Statistical significance was set at  $p < 0.05$ .

## 2.4 Results

### 2.4.1 Cerebrovascular variables

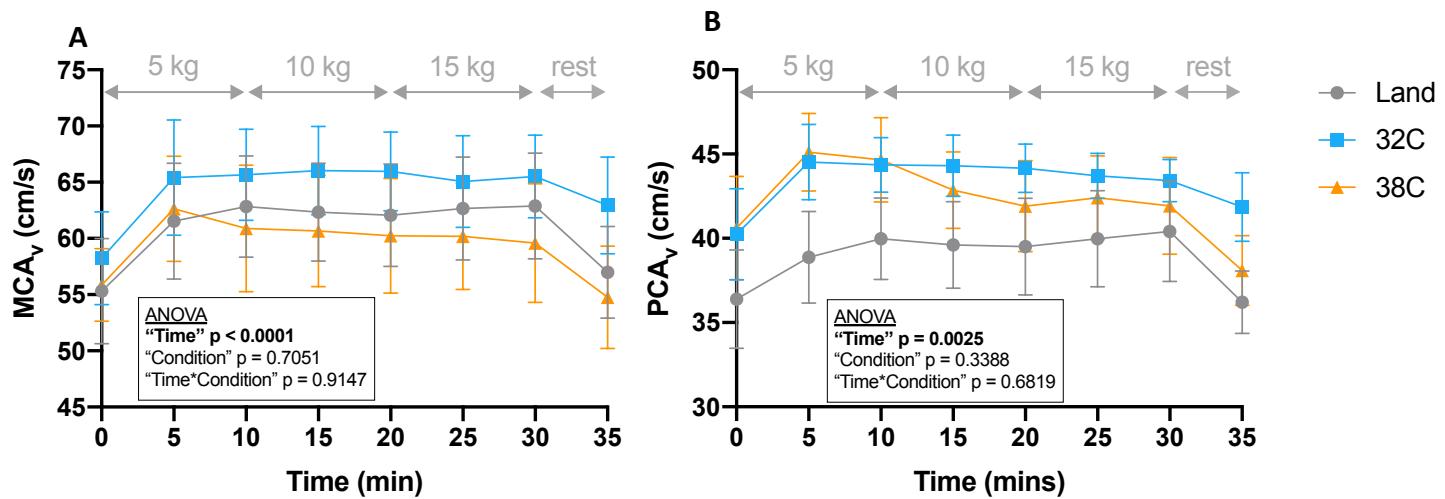


Figure 2.10 Mean middle (MCA<sub>v</sub>) (panel A) and posterior (PCA<sub>v</sub>) (panel B) cerebral artery blood flow velocities (cm/s) over time (mins) during land (grey circles), 32°C (blue squares) and 38°C (orange triangles) aqua cycling conditions in seven healthy subjects. Error bars show SEM. After 10 minutes of rest, subjects cycled either on land or immersed in 32°C or 38°C water at 60rpm with 5kg (0-10 mins), 10kg (10-20 mins) and 15kg (20-30 mins) resistance before resting for five minutes (30-35 mins). MCA<sub>v</sub> and PCA<sub>v</sub> were recorded via transcranial doppler. Two-way repeated measures ANOVA p values are stated.

Mean MCA<sub>v</sub> and PCA<sub>v</sub> (Figure 2.10) increased (mostly non-significantly) from rest ( $t = 0$ ) across all exercise time points in all conditions and decreased following exercise ( $t = 35$ ). Mean MCA<sub>v</sub> (Figure 2.10, panel A) was (non-significantly) higher at all time points during the 32°C condition (peak  $66.1 \pm 3.9$  cm/s) when compared to both the land (peak  $62.9 \pm 4.7$  cm/s) and 38°C conditions (peak  $62.6 \pm 4.7$  cm/s) and the 38°C and land conditions were similar at  $t=0$  and  $t=5$ , while land was (non-significantly) higher from  $t=10$  onwards. Mean PCA<sub>v</sub> (Figure 2.10, panel B) was (non-significantly) higher at all time points during the 32°C (peak  $44.5 \pm 2.2$  cm/s)

and 38°C (peak  $45.1 \pm 2.3$  cm/s) conditions when compared to land (peak  $40.4 \pm 3.0$  mmHg), while the 38°C and 32°C conditions were similar from t=0 to t=10, after which 38°C was (non-significantly) lower.

#### 2.4.2 Cardiovascular variables

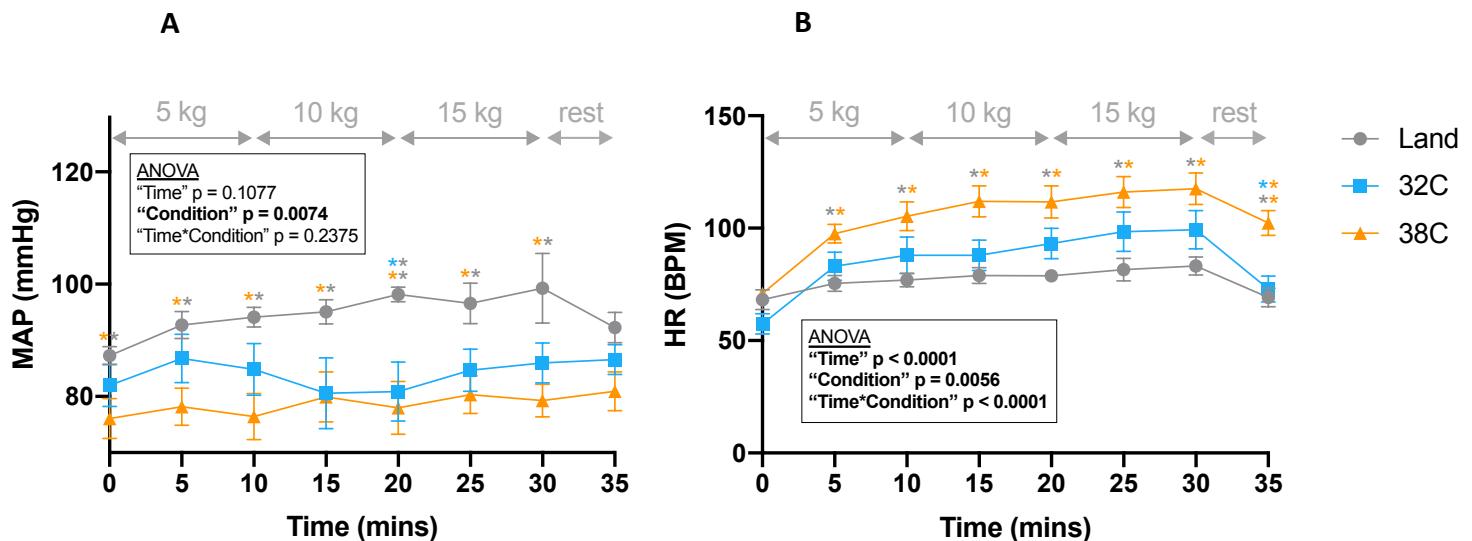


Figure 2.11 Mean arterial pressure (MAP) (mmHg) (A) and heart rate (HR) (bpm) (B) over time (mins) during land (grey circles), 32°C (blue squares) and 38°C (orange triangles) aqua cycling conditions in seven healthy subjects. Error bars show SEM. After 10 minutes of rest, subjects cycled either on land or immersed in 32°C or 38°C water at 60rpm with 5kg (0-10 mins), 10kg (10-20 mins) and 15kg (20-30 mins) resistance before resting for five minutes (30-35 mins). MAP and HR were measured via photoplethysmography finger cuff. Two-way repeated measures ANOVA p values are stated. Asterix (\*) indicates significant difference ( $p < 0.05$ ) following post-hoc analysis.

MAP (Figure 2.11, panel A) was (mostly significantly) higher at all time points during land (peak  $99.3 \pm 6.2$  mmHg) when compared to both 32°C (peak  $86.8 \pm 4.3$  mmHg) and 38°C (peak  $80.9 \pm 3.5$  mmHg) conditions. It was (non-significantly) higher during the 32°C condition when compared to the 38°C condition at all time points. There was a significant main effect between conditions ( $p < 0.01$ ). MAP was significantly higher in the land condition when

compared to the 32°C condition at t=20 ( $p < 0.05$ ) and the 38°C condition from t=0 to t=30 ( $p < 0.05$ ).

Mean HR (Figure 2.11, panel B) was (partially significantly) higher during the 38°C condition (peak  $117.6 \pm 7.0$  bpm) when compared to the 32°C ( $99.3 \pm 8.5$  bpm) and land ( $83.2 \pm 4.1$  bpm) conditions at all time points, and the 32°C condition was (non-significantly) higher when compared to the land condition at all time points aside from t=0. There was a significant main effect between conditions for HR ( $p < 0.01$ ) and a significant interaction effect between condition and time ( $p < 0.0001$ ). HR was significantly higher during the 38°C condition when compared to the land condition at t=5, 10, 15, 20, 25, 30 and 35 ( $P < 0.05$ ), and 32C at t=35 ( $p < 0.05$ ).

### 2.4.3 End-tidal carbon dioxide

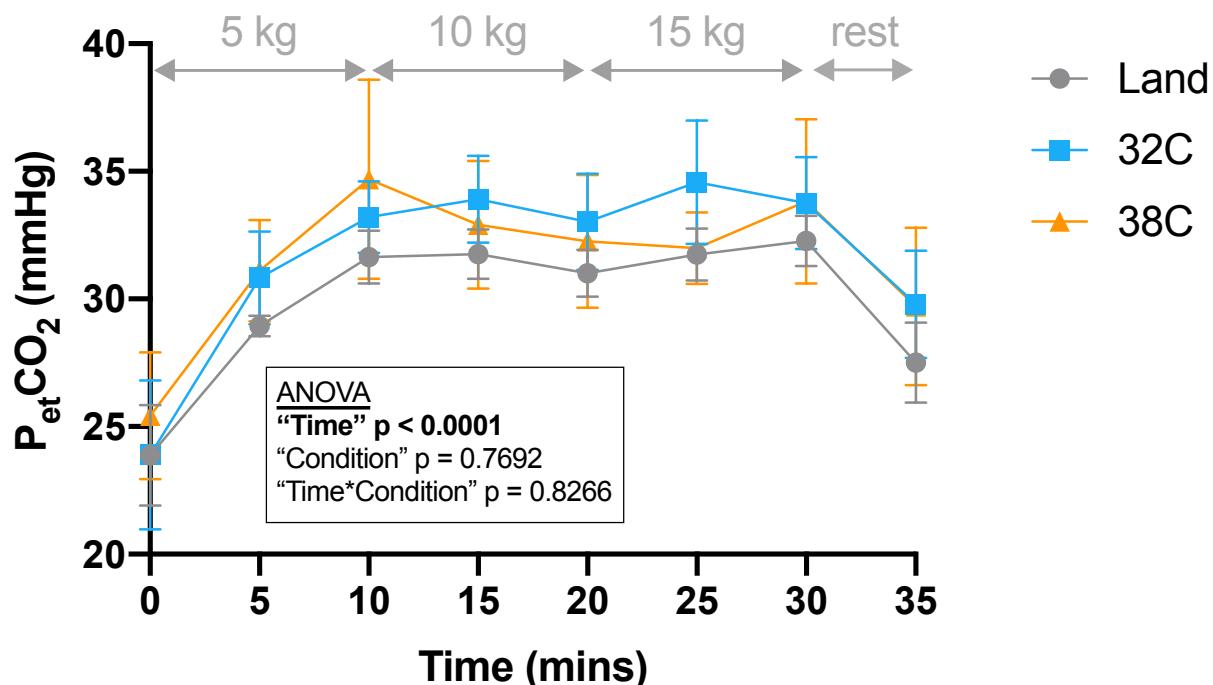


Figure 2.12 Partial pressure of end-tidal carbon dioxide ( $P_{et}CO_2$ ) over time (mins) during land (grey circles),  $32^{\circ}C$  (blue squares) and  $38^{\circ}C$  (orange triangles) aqua cycling conditions in six healthy subjects. Error bars show SEM. After 10 minutes of rest, subjects cycled at 60rpm either on land or immersed in  $32^{\circ}C$  or  $38^{\circ}C$  water, with 5kg (0-10 mins), 10kg (10-20 mins) and 15kg (20-30 mins) resistance before resting for five minutes (30-35 mins).  $P_{et}CO_2$  was measured via metabolic cart. Two-way repeated measures ANOVA p values are stated.

Mean  $P_{et}CO_2$  (Figure 2.12) increased significantly ( $p < 0.05$ ) from rest ( $t = 0$ ) across all exercise time points in all conditions and decreased significantly ( $P < 0.05$ ) following exercise ( $t = 35$ ). Peak ( $\pm$  SEM) mean  $P_{et}CO_2$  during land,  $32^{\circ}C$  and  $38^{\circ}C$  conditions were  $32.2 (\pm 1.0)$  mmHg,  $34.6 (\pm 2.4)$  mmHg and  $34.7 (\pm 3.9)$  mmHg respectively. There was no significant main effect between conditions ( $p > 0.05$ ).

#### 2.4.4 Core temperature

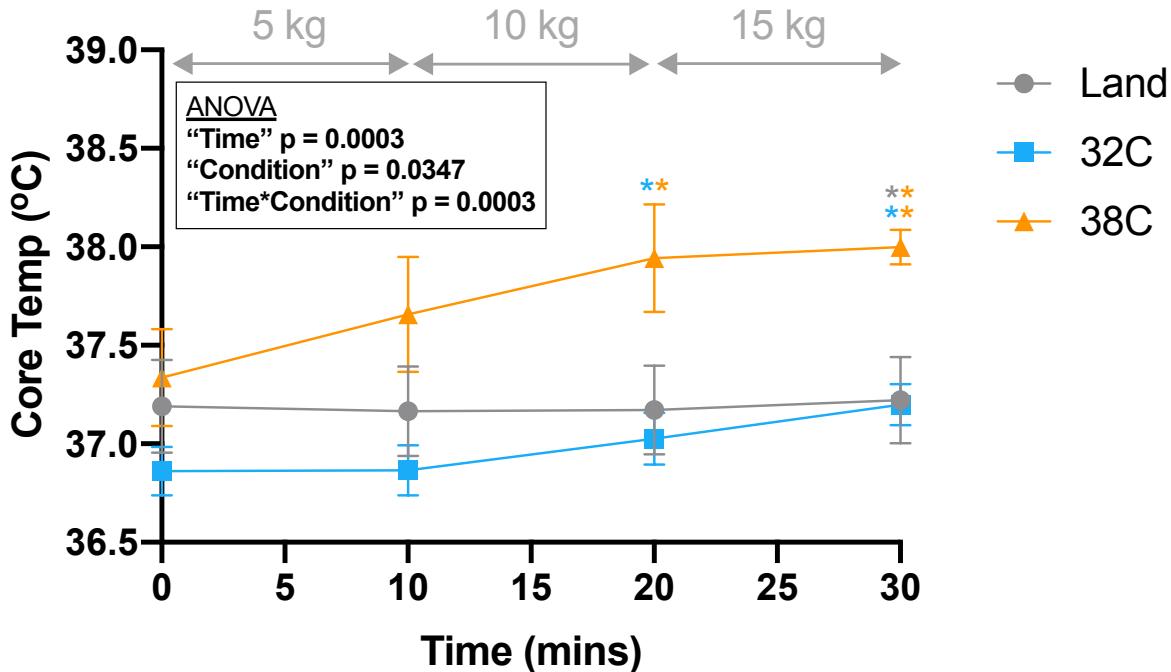


Figure 2.13 Core temperature (Core Temp) ( $^{\circ}\text{C}$ ) over time (mins) during land (grey circles),  $32^{\circ}\text{C}$  (blue squares) and  $38^{\circ}\text{C}$  (orange triangles) aqua cycling conditions in eight healthy subjects. Error bars show SEM. After 10 minutes of rest, subjects cycled at 60rpm either on land or immersed in  $32^{\circ}\text{C}$  or  $38^{\circ}\text{C}$  water, with 5kg (0-10 mins), 10kg (10-20 mins) and 15kg (20-30 mins) resistance before resting for five minutes (30-35 mins). Core temperature was measured using ingested sensor and wireless handheld receiver. Two-way repeated measures ANOVA p values are stated. Asterix (\*) indicates significant difference ( $p < 0.05$ ) following post-hoc analysis.

During the  $38^{\circ}\text{C}$  condition, core temperature (Figure 2.13) rose gradually from  $37.34^{\circ}\text{C}$  ( $\pm 0.25^{\circ}\text{C}$ ) at rest ( $t=0$ ) to a peak of  $38.00^{\circ}\text{C}$  ( $\pm 0.09^{\circ}\text{C}$ ) at  $t=30$ . The  $32^{\circ}\text{C}$  condition saw a steady increase from  $36.86^{\circ}\text{C}$  ( $\pm 0.12^{\circ}\text{C}$ ) at  $t=0$  to  $37.20^{\circ}\text{C}$  ( $\pm 0.10^{\circ}\text{C}$ ) at  $t=30$ , while core temperature during the land condition remained fairly constant from  $37.19^{\circ}\text{C}$  ( $\pm 0.24^{\circ}\text{C}$ ) at  $t=0$  through to  $37.22^{\circ}\text{C}$  ( $\pm 0.22^{\circ}\text{C}$ ) at  $t=30$ . There was a significant main effect between conditions ( $p < 0.05$ ), and a significant interaction effect between condition and time ( $p < 0.0005$ ). Core

temperature was significantly higher during the 38°C condition when compared with the land condition at t=30 ( $p < 0.05$ ), and the 32°C condition at t=20 and t=30 ( $p < 0.05$ ).

#### 2.4.5 Exertion variables

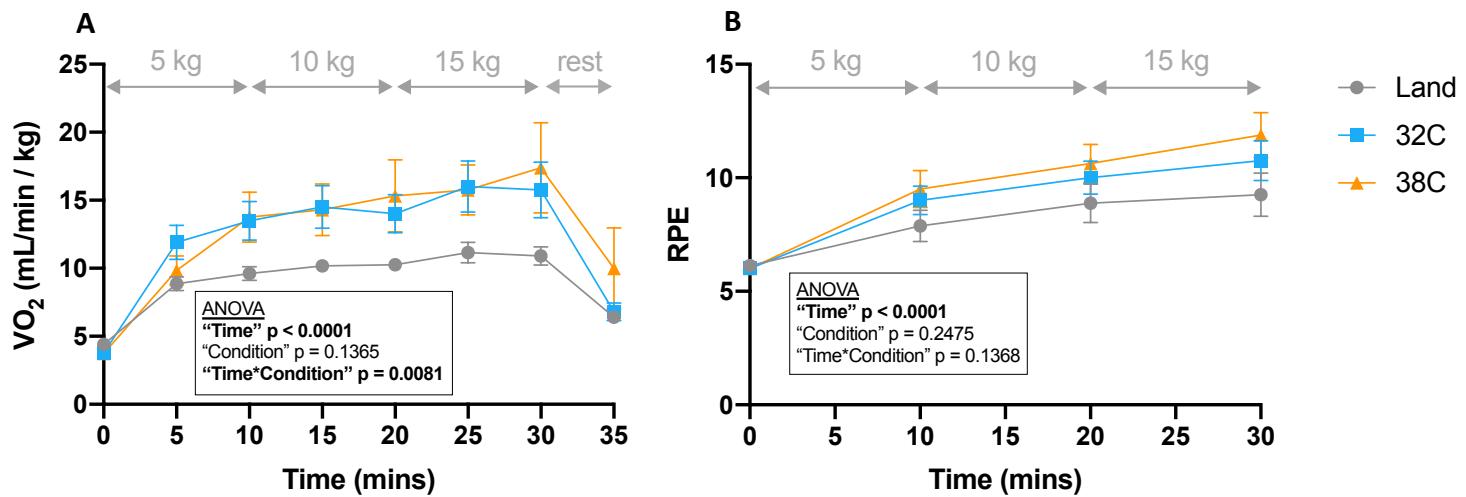


Figure 2.14 Mean relative oxygen consumption ( $\dot{V}O_2$ ) (mL/min / kg) and perceived exertion (RPE) over time (mins) during land (grey circles), 32°C (blue squares) and 38°C (orange triangles) aqua cycling conditions in six ( $\dot{V}O_2$ ) and eight (RPE) healthy subjects. Error bars show SEM. After 10 minutes of rest, subjects cycled at 60rpm either on land or immersed in 32°C or 38°C water, with 5kg (0-10 mins), 10kg (10-20 mins) and 15kg (20-30 mins) resistance before resting for five minutes (30-35 mins).  $\dot{V}O_2$  was measured via metabolic cart while RPE was reported through non-verbal gesturing at a chart. Two-way repeated measures ANOVA p values are stated. Asterix (\*) indicates significant difference ( $p < 0.05$ ) following post-hoc analysis.

$\dot{V}O_2$  (Figure 2.14, panel A) increased significantly from rest during all exercise conditions and decreased significantly after the conclusion of exercise ( $p < 0.05$ ).  $\dot{V}O_2$  was similar during the 38°C condition (peak  $17.4 \pm 3.3$  ml/min / kg) when compared to the 32°C condition (peak  $16.0 \pm 1.9$  ml/min / kg) at all points. Both the 32°C and 38°C conditions were non-significantly higher during exercise when compared to the land condition (peak  $11.1 \pm 0.8$  ml/min / kg),

but similar during both rest periods. There was no significant main effect between conditions ( $p > 0.05$ ), although there was a significant interaction effect ( $p < 0.05$ ).

## 2.5 Discussion

### 2.5.1 Discussion

Due to COVID-19 interruptions and the Honours submission deadline, the results presented in this paper are thoroughly underpowered and not necessarily indicative of true physiological phenomena. Additional data collection is currently underway (August 2020) to reach appropriate sample size for publication. As such, following expert advice some conclusions have been speculatively drawn based on non-significant but clinically meaningful trends in data, such as the consistent 2-10 cm/s difference present between conditions in MCA<sub>v</sub> – notably large, yet statistically insignificant (61).

This study aimed to examine the effect that water temperature had on CBF during aqua cycling tasks at varying matched exercise intensities, and how this compared with equivalent land-based cycling. MCA<sub>v</sub> was utilised as the main indicator of CBF, as studies have shown the MCA to be the artery most representative of global CBF, but PCA<sub>v</sub> was also used as a supplementary measure (13-15). Hot (38°C) and thermoneutral (32°C) aqua cycling conditions were utilised. Our key findings were A) Thermoneutral water aqua cycling increased MCA<sub>v</sub> during pre- and post-exercise rest and throughout all exercise intensities when compared to both hot water aqua cycling and land-based cycling, B) The hot water condition induced the same MCA<sub>v</sub> as the land-based condition during the initial rest period, but lower MCA<sub>v</sub> throughout the remainder of the protocol C) Thermoneutral aqua cycling and land-based cycling maintained relatively constant MCA<sub>v</sub> and PCA<sub>v</sub> throughout the increasingly intense exercise protocol while hot aqua cycling saw a constant decline in MCA<sub>v</sub> and PCA<sub>v</sub> after the initial peak (at the onset of exercise). As a result, thermoneutral water is likely a better choice than hot water for aqua cycling tasks aimed to augment CBF<sub>v</sub>.

Water immersion has been shown to increase CBF<sub>v</sub>, presumably by inducing hydrostatic pressure on superficial veins, increasing centralisation of blood volume and hence venous return, cardiac output and blood pressure, and also by increasing P<sub>a</sub>CO<sub>2</sub> (8, 10, 16). Research

has also indicated that land-based exercise increases CBF<sub>v</sub>, most reporting maximal CBF<sub>v</sub> during moderate intensity exercise, as higher-intensity exercise induces hyperventilation, decreasing P<sub>a</sub>CO<sub>2</sub>, a key mechanism in cerebral blood flow regulation (7, 17). As such, past studies that have investigated the effect of combining (30-32°C) water immersion and low-moderate intensity exercise found a summative effect on CBF<sub>v</sub>, it being higher during immersion exercise than during either intervention alone (9, 10). Our study corroborated these expected augmentations in CBF<sub>v</sub> (MCA<sub>v</sub> and PCA<sub>v</sub>) during the thermoneutral immersion condition when compared to land at rest (effect of immersion), during all conditions from rest to exercise (effect of exercise) and between the thermoneutral and land conditions throughout the exercise protocol (combined effect of exercise and immersion)(Figure 2.10).

However, the main novel component of this study was the comparison of thermoneutral and hot water temperatures during aqua cycling. Thermoneutral aqua cycling was found to augment MCA<sub>v</sub> at every time point when compared to hot aqua cycling, the effect being less prominent in PCA<sub>v</sub> but nonetheless present at the 10kg, 15kg and final rest stages (Figure 2.10). MCA<sub>v</sub> also declined steadily throughout the exercise protocol during hot aqua cycling, while maintaining a relatively constant elevated level during the thermoneutral condition. The mechanisms involved in this difference in CBF<sub>v</sub> are unclear - due to the complex nature of cerebral blood flow regulation, no specific combination of factors responsible for augmentation of CBF<sub>v</sub> during immersion exercise has been defined, but P<sub>a</sub>CO<sub>2</sub>, blood pressure, cardiac output and brain metabolic activity have all been theorised to contribute (9, 10).

Firstly, exercise intensity has been speculated to impact CBF<sub>v</sub> during immersion exercise in a similar manner to land-based exercise, although this is unconfirmed (10). However, VO<sub>2</sub> (a reasonable indicator of exercise intensity) was no different between thermoneutral and hot conditions throughout the exercise protocol, despite a minor difference in RPE (Figure 2.14) (18).

The first main hypothesised causative mechanism of increases in CBF<sub>v</sub> is P<sub>a</sub>CO<sub>2</sub>. As mentioned, CO<sub>2</sub> is a powerful vasodilator and elevated P<sub>a</sub>CO<sub>2</sub> has been shown to increase CBF<sub>v</sub> in isolation (16, 19). Pugh et al.'s 2014 water immersion stepping-based exercise study found that P<sub>et</sub>CO<sub>2</sub>

(commonly used as a surrogate measure of  $P_aCO_2$ ) correlated with increases in CBF<sub>v</sub> and hypothesised a causative connection (9). While the current study corroborated the increase in  $P_{et}CO_2$  with exercise, no difference in  $P_{et}CO_2$  was found between the thermoneutral and hot conditions throughout the protocol, suggesting  $P_aCO_2$  is unlikely to play a significant role in the hot condition's relatively reduced CBF<sub>v</sub> (Figure 2.12).

A more recently hypothesised factor involved in CBF<sub>v</sub> fluctuation is changes in core temperature - a novel component of this study hence not considered in past immersion exercise studies. A recent non-immersion/exercise study found that increases in core temperature via passive heating decrease  $P_aCO_2$  due to heat-stress-induced ventilation augmentation causing respiratory alkalosis, which consequently decreases CBF<sub>v</sub> (20, 21). However, when  $P_aCO_2$  was restored to normothermic levels during passive heating, CBF<sub>v</sub> was shown to increase above basal levels, probably due to increases in cerebral metabolic rate associated with passive heating (22). Hyperthermia-induced decreases in  $P_aCO_2$  and CBF<sub>v</sub> have also been noted during land-based exercise (23). In the current study, core body temperature increased throughout the hot water aqua cycling protocol, and by the midpoint of exercise was greatly elevated ( $\sim 0.8^\circ\text{C}$ ) over the thermoneutral condition (Figure 2.13). Interestingly, as mentioned previously, there was no difference in  $P_aCO_2$  between the thermoneutral and hot conditions, suggesting that CBF<sub>v</sub> should have been relatively elevated in the hot condition as a result of a hyperthermia-based increase in cerebral metabolic rate. Given CBF<sub>v</sub> was lower during the hot condition than the thermoneutral condition, the effect of increased cerebral metabolic rate must be either absent or outweighed by another mechanism.

The likely mechanism is a drop in cardiac output and blood pressure resulting from thermoregulatory redistribution of blood volume in response to rising core temperature. During heat stress, peripheral cutaneous vasculature dilates to allow increased blood flow to the skin and hence heat loss to the environment via convection (24). In doing so, it seems to counteract the central redistribution of blood induced by hydrostatic pressure – the main beneficial mechanism of water immersion exercise – opposing the augmentation of cerebrovascular blood flow. This phenomenon can be observed in the current study's subjects' changes in MAP and HR. In previous immersion exercise CBF studies, greatly increased venous return due to blood centralisation has allowed for elevated cardiac output

at a lower or similar heart rate when compared to equivalent exercise on land as a result of the Frank-Starling law (9, 10). In the current study, the hot condition induced a greatly elevated heart rate (~18 – 25%) when compared to the thermoneutral and land conditions, while exhibiting lower MAP, indicating a drop in stroke volume and/or total peripheral resistance (Figure 2.11). This is owing to the fact that, as shown in the equation

$$\downarrow \text{MAP} = \uparrow \text{HR} * ? \text{SV} * ? \text{TPR}$$

where SV is stroke volume and TPR is total peripheral resistance, if there was no drop in stroke volume and/or TPR, the elevated heart rate would cause MAP to increase – yet a reduction in MAP was observed, meaning stroke volume and/or TPR must have lowered, likely a result of redistribution of blood to the peripheries.

This blood redistribution idea is reinforced by the trends in MCA<sub>v</sub>, PCA<sub>v</sub> and core temperature over time during the hot condition. MCA<sub>v</sub> and PCA<sub>v</sub> peak at the onset of exercise and fall continually throughout the protocol (Figure 2.10), while core temperature rises throughout and peaks at the conclusion (Figure 2.13). The increase in core temperature presumably corresponds with an increasingly pronounced heat-stress response, further minimising the blood centralisation effect of immersion and lowering MCA<sub>v</sub> and PCA<sub>v</sub>. As a result, a minor conclusion could be drawn that if hot water immersion exercise is being conducted with the goal of acutely maximising CBF<sub>v</sub>, intensity should be minimised.

As such, as TPR is reduced due to peripheral vascular dilation, venous return and stroke volume would fall, reducing cardiac output and MAP and hence CBF<sub>v</sub>. In summary, the thermoregulatory peripheral blood redistribution response resulting from heat stress in the hot condition is likely the main causative mechanism involved in lowering CBF<sub>v</sub>, counteracting the centralisation of blood and associated CBF<sub>v</sub> benefits found in thermoneutral water immersion exercise.

Interestingly, augmentation of peripheral blood flow is well known to have its own set of cardiovascular (rather than cerebrovascular) benefits, so hot immersion exercise may be a useful modality for those less interested in CBF<sub>v</sub> augmentation.

Another novel element of the study was use of a cycle ergometer during immersion exercise. Use of an ergometer allows for a higher level of water immersion in a tank of equivalent depth than comparable upright exercise modalities. However, it should be noted that cycling in water was found to be marginally more strenuous when compared to equivalent resistance on land, so differing exercise intensity may have contributed to the differences in CBF<sub>v</sub> between the aqua cycling and land conditions.

Finally, from a clinical perspective, a consideration when comparing thermoneutral and hot water immersion as a modality of treating or preventing cerebrovascular disease, especially in higher-risk populations (elderly, obese), is the risk of acute health complications. Cerebrovascular disease predominantly affects the elderly and physically incapable and/or inactive populations, who are more susceptible to ailments such as sudden cardiac death (25-27). Risk of sudden cardiac death during exercise increases with level of exertion and cardiovascular stress (28, 29). In the current study, during the low-moderate intensity level (the suggested level for maximal CBF<sub>v</sub> augmentation) hot water aqua cycling increased heart rate almost 20% (118 bpm vs 99 bpm) over thermoneutral aqua cycling (Figure 2.11, panel B) (10). While measured absolute heart rates (up to  $118 \pm 7$  bpm during hot aqua cycling) are considered within safe ranges during exercise, it should be noted that this study was conducted predominantly in healthy young males, and elderly and/or unhealthy individuals may respond differently to strenuous heating exercise (thermoregulatory ability has been shown to decline with age) so minimising cardiac stress may be beneficial (30, 31). Health risks aside, most exercise-based benefits (cerebrovascular and otherwise) occur over time as a result of habitual/repeated exercise, so selecting the modality preferred by an individual may augment long-term health benefits more effectively than an equivalently potent but less enjoyable modality due to improved adherence (32, 33).

#### 2.5.2 Limitations:

The study was underpowered due to equipment failure and unplanned discontinuation of data collection due to world-wide pandemic (COVID-19). The problem was further

exacerbated by data storage errors which prevented inclusion of two subjects' respiratory data (PetCO<sub>2</sub> and VO<sub>2</sub>). This will be amended via continued data collection.

The choice to standardise cycle ergometer resistance was justified as it allows for more generalised results for the lay person to interpret, and the within-subject design allowed for valid comparison between conditions. However, differences in difficulty between cycling during land and immersion were not examined and controlled for, meaning stages of equivalent resistance during land and immersion were not necessarily matched for exertion. This may have an impact on the validity of comparisons between land and immersion conditions but likely has minimal impact on the key component of this study, comparing immersion conditions. This could be amended by adjusting ergometer resistance to the relative difficulty of the cycling modality.

Another potentially confounding variable is ambient air temperature. Land conditions were performed in an air-conditioned lab while immersion conditions were performed in a semi-open outdoor environment somewhat susceptible to variations in weather. Data collection also occurred during both summer and winter months. Respiratory gas measurements were appropriately calibrated but changes in ambient air conditions may have influenced physiological responses. Conducting all trials in the same, air-temperature-controlled environment could resolve this issue.

Finally, participant age and physiology were unindicative/representative of the target demographic of this study. Populations at high risk of cerebrovascular disease such as the elderly or obese are most likely to benefit from improvements in vascular health associated with repeated augmentation of cerebral blood flow as investigated by this study. Performing a similar study on subjects from high-risk populations will improve external validity and allow for better guidelines on safety and prevention of health risks.

### 2.5.3 Conclusions:

In summary, thermoneutral aqua cycling augments acute cerebral blood flow responses when compared with hot water aqua cycling, whilst also improving safety. Thermoneutral water

immersion exercise should therefore be considered over hot water immersion exercise as a modality of inducing repeated increases in cerebral blood flow and shear stress for long-term cerebrovascular benefits.

#### 2.5.4 Future research directions:

A study similar to ours with improved statistical power would likely allow for better understanding/statistical confirmation of the mechanisms responsible for differences in cerebral blood flow between conditions. Examination of cutaneous blood flow during immersion exercise could allow for better understanding of blood redistribution mechanisms with differing water temperature. An immediate extension of our study would be to examine the effect of cold water on cerebral blood flow during water immersion exercise. A longitudinal study examining the long-term effects of repeated acute increases in cerebral blood flow and shear stress on cerebral vessel health and physiology could highlight the importance of optimising acute cerebral blood flow responses and lead to more widespread usage in treating or preventing cerebrovascular disease.

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