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## **Cerebral Blood Flow Responses to Aquatic Treadmill Exercise**

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## **Abstract**

**Introduction.** Aquatic treadmills are used as a rehabilitation method for conditions such as spinal cord injury, osteoarthritis and stroke, and can facilitate an earlier return to exercise training for athletes. However, their effect on cerebral blood flow (CBF) responses has not been examined. We tested the hypothesis that aquatic treadmill exercise would augment CBF and lower heart rate compared to land-based treadmill exercise.

**Methods:** Eleven participants completed incremental exercise (crossover design) starting from walking pace [4 km/h, immersed to iliac crest (aquatic), 6 km/h (land)] and increasing 1 km/h every 2 min up to 10 km/h for aquatic (maximum belt speed) or 12 km/h for land. Following this, participants completed two 2-min bouts of exercise immersed to mid-thigh and mid-chest at constant submaximal speed (aquatic), or were ramped to exhaustion (land; increased gradient 2° every min). Middle cerebral flow velocity (MCA<sub>v</sub>) and heart rate (HR) were measured throughout, and the initial 10 min of each protocol and responses at each immersion level were compared.

**Results.** Compared to land-based treadmill, MCA<sub>v<sub>mean</sub></sub> increased more from baseline for aquatic exercise (21 vs. 12%;  $p < 0.001$ ), while being associated with lower overall HR (pooled difference: 11 b/min;  $p < 0.001$ ). MCA<sub>v<sub>mean</sub></sub> increased similarly during aquatic walking compared to land-based moderate intensity running (~10 cm/s;  $p = 0.56$ ). Greater water immersion lowered HR (139 vs. 178 b/min for mid-chest vs. mid-thigh), while MCA<sub>v<sub>mean</sub></sub> remained constant ( $P = 0.37$ ).

**Conclusion.** Findings illustrate the potential for aquatic treadmill exercise to enhance exercise-induced elevations in CBF, and thus optimise shear-stress mediated adaptation of the cerebrovasculature.

**Key words:** Cerebral blood flow, Aquatic treadmill exercise, Deep-water running, neurorehabilitation, brain health

## 1 INTRODUCTION

2 Aquatic treadmill (ATM) exercise allows for lower impact and increased resistance in  
3 comparison to land-based treadmill (LTM) exercise, therefore achieving decreased musculoskeletal  
4 loading of joints and providing potential for enhanced acute and chronic physiological adaptations  
5 (5). The increased hydrostatic pressure applied to the human body upon immersion in water leads to  
6 centralising blood distribution within the body, which enhances cardiac performance and therefore  
7 increases tissue perfusion (32). Furthermore, the mechanical unloading and support of bodyweight  
8 due to buoyancy means that ATMs are a useful tool in gait re-education. Indeed, ATM exercise is  
9 utilised in the rehabilitation of spinal cord injuries (36) and stroke (28) patients. Positive effects of  
10 ATM exercise have also been noted in populations with joint conditions such as osteoarthritis (7),  
11 and those with coronary heart disease (13). At the other end of the spectrum, athletes utilise ATM  
12 exercise to maintain cardiorespiratory fitness while reducing the mechanical load when recovering  
13 from injury (30). Therefore, ATM exercise represents an effective form of therapy and  
14 rehabilitation for a range of healthy and diseased populations. Studies to date comparing  
15 physiological responses between aquatic- and land-based treadmills have mostly focused on  
16 cardiorespiratory responses (e.g., (17, 35)); while no study has examined the effect that ATM  
17 exercise has on cerebrovascular responses, and therefore explored the possibility of how this mode  
18 of therapy may optimise exercise-induced, stimulus-response adaptations leading to improved  
19 cerebrovascular function and ultimately brain health (11).

20 Effective regulation of blood flow to and within the brain is vital for optimal brain function.  
21 Regular exercise and higher cardiorespiratory fitness has been positively linked with CBF and its  
22 regulation (e.g., (3, 6)), shown to offset the natural age-related decline in CBF (1), and reduce risk  
23 of neurodegenerative disease (e.g., dementia (21)). However, the mechanisms that underpin the  
24 neuroprotective benefits of exercise are yet to be established, meaning that the effectiveness of  
25 various exercise parameters such as mode, intensity and duration are not yet understood (22). One

26 suggested mechanism for exercise-induced improvements in vascular function is via shear-stress  
27 mediated increases in endothelium nitric oxide-dependent vasodilation of blood vessels (18, 39), as  
28 a consequence of the recurrent mechanical force of blood flow on the walls of the arteries (i.e. shear  
29 stress) (27). Numerous studies [e.g., (9, 15, 19)] have reported the functional adaptation and  
30 structural changes to the vasculature that occur as a result of long-term exercise (18), albeit  
31 primarily provided from animal-based and cell-culture studies [see (8)] or within the peripheral  
32 vasculature of humans. Researchers have begun to explore alternative methods [e.g. heat therapy  
33 (12, 38)] that target this mechanism to improve vascular function. Extrapolating this to the brain,  
34 conditioning strategies that increase blood flow, either in combination or independent of exercise,  
35 may enhance shear-stress mediated adaptation of the cerebrovasculature. This has the potential to  
36 directly improve CBF and its regulation and therefore be used in the prevention and treatment of  
37 neurovascular disease.

38         Given the known physiological responses to water immersion, water-based activities may be  
39 one such strategy. Indeed, two studies have explored this possibility, examining CBF responses in  
40 water at rest (12) and during a box-stepping exercise protocol (31). Carter et al. (12) reported a  
41 positive correlation between middle and posterior CBF velocities during resting water immersion,  
42 linking the increase in CBF velocity during immersion to an increase in mean arterial pressure and  
43 arterial carbon dioxide content. From the same group, Pugh et al. (31) compared a 20-min bout of  
44 matched low-intensity stepping exercise ( $HR \leq 100 \text{ b} \cdot \text{min}^{-1}$ ) in a water tank to that on land, finding  
45 CBF velocities to be augmented in water. These studies illustrate the potential for enhanced shear-  
46 stress mediated vascular adaptation by exercising in water, although important questions remain  
47 unanswered relating to the CBF profile during different exercise intensities and at different depths  
48 of water immersion. Such questions can be addressed using an aquatic treadmill, which is already  
49 an established rehabilitation tool for a number of conditions (see above). Therefore, the primary  
50 purpose of this study was to compare changes in CBF (velocity) and heart rate responses during an  
51 incremental exercise test using an aquatic treadmill and a land-based treadmill, and to examine CBF

52 and heart rate responses at different levels of immersion (mid-thigh, iliac crest (hip) and xiphoid  
53 process (mid-chest)) during aquatic treadmill exercise. We hypothesised that: 1) aquatic treadmill  
54 exercise would augment the CBF response to an incremental intensity exercise test compared to  
55 land-based treadmill exercise, and 2) increased water immersion would maintain CBF while  
56 lowering heart rate.

## 57 **METHODS**

### 58 **Participants**

59 Eleven healthy participants (7 females and 4 males; aged  $27 \pm 5$  years) were recruited for  
60 this study, which was approved by the University of Birmingham Science, Technology,  
61 Engineering and Mathematics Ethical Review Committee, and performed in accordance with the  
62 *Declaration of Helsinki*. After providing their written informed consent, all participants completed a  
63 General Health Questionnaire during an initial visit to the laboratory and declared that they were  
64 free of any cardiovascular, cerebrovascular or respiratory disease, were not taking medication (not  
65 including contraceptive medications), or had injuries that would preclude treadmill-based exercise.

### 66 **Study Design and Protocol**

67 Following the initial screening visit, participants completed exercise sessions on both a land-  
68 based (LTM) and an aquatic treadmill (ATM) in a randomised, counterbalanced order. Each session  
69 lasted approximately 1 hour, of which 15-25 minutes were exercise. First, participants completed  
70 incremental exercise starting from walking pace [ $4 \text{ km}\cdot\text{h}^{-1}$ , immersed to iliac crest (aquatic),  $6$   
71  $\text{km}\cdot\text{h}^{-1}$  (land)] and increasing  $1 \text{ km}\cdot\text{h}^{-1}$  every 2 min up to  $10 \text{ km}\cdot\text{h}^{-1}$  for aquatic (maximum belt  
72 speed) or  $12 \text{ km}\cdot\text{h}^{-1}$  for land. On land, participants were then ramped to exhaustion (increased  
73 gradient  $2^\circ$  every min), whereas for aquatic exercise participants completed two 2-min bouts of  
74 exercise immersed to mid-thigh and mid-chest at constant submaximal speed. During exercise at the  
75 different immersion depths on the ATM the speed was held constant; eight participants completed  
76 this protocol at  $10 \text{ km}\cdot\text{h}^{-1}$ , while for 3 participants, who reached near maximal heart rate during the

77 initial incremental intensity protocol, the treadmill speed was lowered (to 8 km·h<sup>-1</sup> for 2 participants  
78 and 5 km·h<sup>-1</sup> for 1 participant) and remained at this speed during the exercise at different immersion  
79 depths. Figure 1 provides a schematic for the ATM and LTM protocols.

80         There were at least 48 hours between sessions for each participant, with the majority of  
81 participants completing their second session within 2 weeks of the first. Due to the timing of access  
82 to the ATM facility, the phase of menstrual cycle for female participants was not controlled for  
83 between exercise sessions. Prior to each session participants were asked to refrain from eating a  
84 large meal for 4 hours before arrival, although a light meal was permitted up to 2 hours before  
85 arrival. In order to ensure adequate hydration status, participants were advised to drink 0.5 litres of  
86 water within 4 hours of beginning testing and 0.25 litres of water within 15 minutes of testing, in  
87 accordance with the American College of Sports Medicine Hydration Guidelines (2). Participants  
88 were also asked to refrain from caffeine for 6 hours prior to testing, and refrain from vigorous  
89 exercise and the consumption of alcohol for 24 hours prior to testing.

## 90 **Equipment and Measurements**

91         *Exercise treadmills:* A standard treadmill ergometer (Pulsar, H-P-Cosmos, Germany) was  
92 used for the land-based exercise protocol. The aquatic exercise session was completed on an aquatic  
93 treadmill (FOCUS, HYDRO PHYSIO™, UK) at the Optispine Physiotherapy Clinic in  
94 Birmingham.

95         *Cerebral blood flow velocity (CBFv) and heart rate measures:* Bilateral blood flow velocity  
96 in the left and right middle cerebral arteries (MCAv) was measured using a 2-MHz transcranial  
97 Doppler (TCD) ultrasound system (Dopplerbox, DWL, Compumedics LTD, Germany), in  
98 accordance with search techniques described elsewhere (40). The two ultrasound probes were  
99 placed above each zygomatic arch on the left and right side of the head and secured via an  
100 adjustable headband that maintained a constant insonation angle throughout the testing session. A  
101 small amount of ultrasound gel was placed between the probe and the skin to obtain the highest  
102 quality images. The reliability of measuring CBF using TCD is operator-dependant, thus all

103 measurements were taken by the same experienced sonographer (SJEL), with photographs of probe  
104 placement, and depth and filter settings recorded and kept constant between exercise sessions for  
105 each participant. Cerebrovascular data were acquired continuously via an analogue-to-digital  
106 converter (PowerLab 8/30, ML870, ADInstruments, Dunedin, New Zealand) at 1KHz. Data were  
107 displayed in real time and recorded for off-line analysis using commercially available LabChart Pro  
108 software (v7, ADInstruments).

109 Heart rate (HR) was monitored using telemetry (Polar, Finland) via a belt fitted around the  
110 chest of the participant, as well as derived from the beat-by-beat MCAv waveform. Steady-state  
111 measures for HR were recorded at each stage of the incremental protocols and for the different  
112 immersion depths.

### 113 **Data analysis and statistical approach**

114 Mean values for MCAv and HR at each 2-min stage were determined using an average of  
115 the final 30 seconds of each stage, and used to calculate change from resting (seated) baseline for  
116 each measured time point. Since the aquatic treadmill had a maximum speed of  $10 \text{ km}\cdot\text{h}^{-1}$ , we  
117 intended to use the initial 14 minutes of each protocol to compare the responses between the aquatic  
118 and land treadmill exercise. We also independently compared two exercise intensities; specifically,  
119 walking and the recommended public health guideline of moderate exercise intensity (65%  
120  $\dot{V}O_{2\text{max}}$ ), which was estimated from heart rate measures. Walking pace in the aquatic  
121 treadmill was  $4 \text{ km}\cdot\text{h}^{-1}$ , whereas on the land treadmill it was  $6 \text{ km}\cdot\text{h}^{-1}$ , but both represented the only  
122 speed for which all participants were walking; i.e., participants started jogging in the ATM at  $5$   
123  $\text{km}\cdot\text{h}^{-1}$ . The 65%  $\dot{V}O_{2\text{max}}$  intensity was estimated at 79% HRmax (37), and treadmill speeds that  
124 induced a heart rate response closet to this target were selected (range for land:  $7 - 11 \text{ km}\cdot\text{h}^{-1}$ , range  
125 for aquatic:  $5 - 10 \text{ km}\cdot\text{h}^{-1}$ ).

126 Two-way repeated measures ANOVA were used to compare changes in  $\text{MCAv}_{\text{mean}}$  and HR  
127 across the incremental protocol (time \* treadmill) and at walking and 65%  $\dot{V}O_{2\text{max}}$  intensity



128 (intensity \* treadmill), while a one-way ANOVA compared changes in  $MCA_{v_{mean}}$  and HR across  
129 the three different immersion levels while running at the same speed in the aquatic treadmill. Post-  
130 hoc comparisons were done using pairwise comparisons (Bonferroni corrected) to show where  
131 effects occurred. Paired t-tests were used to determine whether significant differences existed  
132 between comparable data sets of interest (e.g., resting and peak HR/ $MCA_v$ ,  $MCA_v$  during walking  
133 vs. 65%  $\dot{V}O_{2max}$ ). All statistical analysis was carried out using SPSS statistical software (v22,  
134 Chicago, USA), with *a priori* statistical significance set at  $P \leq 0.05$ . Data are presented as mean  $\pm$   
135 SD.

136

## 137 RESULTS

138 All 11 participants who began the exercise sessions completed both protocols. All eleven  
139 participants reached the maximum belt speed in the aquatic treadmill ( $10 \text{ km}\cdot\text{h}^{-1}$ ), therefore  
140 completing all 14 min of incremental aquatic exercise. For the land-based treadmill protocol, one  
141 participant stopped at the completion of the  $10 \text{ km}\cdot\text{h}^{-1}$  stage (at 10 min) and two participants  
142 stopped after the  $11 \text{ km}\cdot\text{h}^{-1}$  stage (at 12 min) due to reaching voluntary exhaustion. Consequentially,  
143 the two-way ANOVA for the comparison of incremental exercise protocols used the first 5 stages of  
144 exercise for which all 11 participants had paired data sets for (as indicated in Figure 1).

145 There was no significant difference ( $P=0.79$ ) between left and right  $MCA_{v_{mean}}$  in  
146 participants that had a TCD signal on both sides throughout testing in both sessions ( $n=6$ ), therefore  
147 data were pooled and presented as a combined mean value. In 4 aquatic sessions and one land-based  
148 session, the TCD signal on one side was either lost during exercise or not found initially; for these  
149 trials the remaining side was used as the mean value.

150 There was a small, but significant, difference for baseline resting (seated)  $MCA_{v_{mean}}$   
151 between land and aquatic testing sessions ( $70 \pm 9 \text{ cm}\cdot\text{s}^{-1}$  vs.  $66 \pm 9 \text{ cm}\cdot\text{s}^{-1}$ , respectively;  $p=0.023$ ),  
152 while resting HR was similar ( $70 \pm 13 \text{ b}\cdot\text{min}^{-1}$  vs.  $69 \pm 14 \text{ b}\cdot\text{min}^{-1}$ ;  $p=0.738$ ).

### 153 **Effects of increased exercise intensity on cerebrovascular and heart rate responses**

154 As illustrated in Figure 2A, there was a significant main effect of time ( $p=0.004$ ) and  
155 treadmill type ( $p=0.003$ ) on the change in  $MCAv_{\text{mean}}$  from baseline over the initial 10 minutes of  
156 each protocol. The pooled difference across the 10 minutes for  $MCAv_{\text{mean}}$  between the treadmill  
157 protocols was  $\sim 6 \text{ cm}\cdot\text{s}^{-1}$ , with the largest difference occurring at the 4-min stage ( $\sim 11 \text{ cm}\cdot\text{s}^{-1}$ ). The  
158 4-min stage also represented the peak change in  $MCAv_{\text{mean}}$  from baseline in the water ( $\sim 16 \text{ cm}\cdot\text{s}^{-1}$ ),  
159 which was maintained to within  $3 \text{ cm}\cdot\text{s}^{-1}$  for the remainder of the protocol. On land, however, the  
160 peak change in  $MCAv_{\text{mean}}$  ( $\sim 12 \text{ cm}\cdot\text{s}^{-1}$ ) did not occur until the 10<sup>th</sup> minute. Nevertheless, this  
161 difference in the pattern of increase did not reach statistical significance (interaction effect:  
162  $p=0.073$ ).

163 For HR, there was an interaction effect for the change in HR across time ( $p=0.020$ ). Post-  
164 hoc analysis revealed that HR increased for each incremental stage except for the transition between  
165 5 and 6  $\text{km}\cdot\text{h}^{-1}$  (mins 4 and 6) on the ATM, and HR was significantly higher on the LTM than the  
166 ATM except for at 4 minutes (7 and 5  $\text{km}\cdot\text{h}^{-1}$ , respectively, see Figure 2B). Overall, and in contrast  
167 to the  $MCAv_{\text{mean}}$  observations, HR was higher with land-based exercise compared to aquatic  
168 exercise (pooled difference:  $\sim 11 \text{ b}\cdot\text{min}^{-1}$  greater for land; main effect:  $p=0.028$ ). Further, the peak  
169  $MCAv_{\text{mean}}$  during the aquatic incremental protocol tended to be at a lower percentage of  $HR_{\text{max}}$   
170 (determined during the land-based protocol) compared to land-based incremental exercise ( $75 \pm$   
171  $12\%$  vs.  $84 \pm 15\%$  of  $HR_{\text{max}}$  for aquatic and land, respectively;  $p=0.069$ ).

172 Figure 3 shows the comparison between walking and moderate intensity running (at 65%  
173  $\dot{V}O_{2\text{max}}$ ) on each treadmill for  $MCAv_{\text{mean}}$  and HR responses. Both walking and running at 65%  
174  $\dot{V}O_{2\text{max}}$  elicited a greater increase in  $MCAv_{\text{mean}}$  during ATM as compared to LTM (main effect:  
175  $p=0.003$ ). Interestingly, while there was a main effect of intensity ( $p=0.022$ ) for  $MCAv_{\text{mean}}$ ,  
176 subsequent analysis revealed that while  $MCAv_{\text{mean}}$  increased similarly within each treadmill  
177 modality (interaction effect:  $p=0.628$ ), there was no difference between  $MCAv_{\text{mean}}$  for ATM  
178 walking and LTM running at 65%  $\dot{V}O_{2\text{max}}$  (paired ttest:  $p=0.563$ ).

179 Collectively, these data indicate that water-based exercise across a range of intensities  
180 stimulates greater increases in MCAv for a relatively lower heart rate response compared to land-  
181 based exercise, and that water-based walking elicits a similar increase in blood flow (velocity) as  
182 running on land at 65%  $\dot{V}O_2\text{max}$ .

183

#### 184 **Effect of immersion level on cerebrovascular and heart rate responses**

185 Figure 4 illustrates that HR decreased with greater levels of water immersion on the aquatic  
186 treadmill while the treadmill belt speed remained constant. Post-hoc analysis showed that the mean  
187 decrease in HR from the water level at mid-thigh to iliac crest was  $\sim 18 \text{ b}\cdot\text{min}^{-1}$  ( $p=0.001$ ), and from  
188 iliac crest to xiphoid process was  $\sim 21 \text{ b}\cdot\text{min}^{-1}$  ( $p=0.002$ ). This was in contrast to  $\text{MCAv}_{\text{mean}}$ , with  
189 the change from resting baseline not different between immersion levels ( $p=0.371$ ). Finally, the 2-  
190 min exercise bout at mid-thigh water depth elicited near maximal heart rates ( $95 \pm 5\%$  of  $\text{HR}_{\text{max}}$ ; see  
191 figure 4).

192

## 193 **DISCUSSION**

194 The aim of this study was to examine CBF responses during incremental exercise on an  
195 aquatic treadmill as compared to a land-based treadmill, and while exercising at different levels of  
196 water immersion on the aquatic treadmill. Our main novel findings were that: 1)  $\text{MCAv}_{\text{mean}}$  was  
197 augmented during aquatic treadmill exercise compared to land-based treadmill exercise across the  
198 range of exercise intensities tested, and this augmented  $\text{MCAv}_{\text{mean}}$  was associated with a relatively  
199 lower heart rate response; 2) walking on an aquatic treadmill elicited a similar increase in  
200  $\text{MCAv}_{\text{mean}}$  to that of running at moderate intensity (65%  $\dot{V}O_2\text{max}$ ) on land, and 3) immersion depth  
201 altered heart rate while maintaining  $\text{MCAv}_{\text{mean}}$  during exercise at a constant aquatic treadmill speed.  
202 Collectively, these data indicate that aquatic treadmill exercise augments CBF. **Further, although we**

203 have not quantified differences in shear stress *per se*, the elevated flow velocity demonstrates the  
204 potential for aquatic treadmill exercise to enhance shear-stress mediated cerebrovascular adaptation  
205 and thus optimise exercise-induced adaptations linked with improved brain health.

206 The findings of the current study are consistent with previous research reporting elevated  
207 MCAv during exercise (e.g., (10, 23), and an augmented MCAv response when in water [observed  
208 at rest and during light intensity exercise (12, 31)]. Here, we show for the first time that this  
209 augmented MCAv response occurs across a range of exercise intensities and that MCAv can be  
210 maintained while exercising at lower intensities with greater depths of water immersion. One  
211 notable observation was that walking on an aquatic treadmill elicited a similar increase in MCAv  
212 ( $\sim 10 \text{ cm}\cdot\text{s}^{-1}$ ) to that of running on land at the exercise intensity promoted by current public health  
213 guidelines (i.e., 65% of aerobic capacity for 150 min / week). Furthermore, the profile of exercise-  
214 induced changes in MCAv was different between the protocols, with ATM exercise producing  
215 maximal gains in MCAv within 4 minutes of starting the protocol, a time point that also represented  
216 the greatest difference between treadmill protocols, while MCAv during LTM exercise increased  
217 linearly across the incremental protocol yet remained lower (see Figure 2).

218 It is widely reported that the greatest exercise-induced elevation in CBF is achieved at  
219 moderate exercise intensity ( $\sim 65\% \dot{V}O_2\text{max}$ ), as above this threshold CBF will decrease back  
220 towards resting values as a result of hyperventilation-induced cerebral vasoconstriction due to lower  
221  $\text{PaCO}_2$  (24). Our data indicate that aquatic treadmill exercise may have a different exercise-induced  
222 CBF profile to this commonly reported profile, most of which come from cycling-based exercise  
223 protocols. The findings of the current study indicate that optimal CBF gains may be achieved at  
224 lower exercise intensities in water than on land (see Figure 2A), and even at higher exercise  
225 intensities (induced via less water immersion) MCAv is consistently elevated above resting  
226 measures (see Figure 4). Based on these findings it could be suggested that changes in arterial  
227 carbon dioxide above anaerobic threshold during aquatic treadmill exercise has less influence on  
228 CBF relative to other factors involved in CBF regulation (e.g., blood pressure, cardiac output).

229 Unfortunately, we were unable to measure end-tidal  $\text{PCO}_2$  during our study due to equipment  
230 unavailability, thus we can only speculate about the relation between  $\text{PCO}_2$  and CBF during aquatic  
231 treadmill exercise. Further research is needed in order to determine the influence of this key  
232 regulator of CBF during aquatic treadmill exercise of increasing exercise intensity.

233 The regulation of CBF during exercise is multifactorial and complex (25), with an  
234 integrative combination of exercise-induced changes in brain metabolic and neuronal activity, blood  
235 pressure, cardiac output and arterial  $\text{PCO}_2$  all likely to contribute to changes in cerebral perfusion  
236 during any exercise paradigm. Based on previous water immersion studies (12, 31), an elevated  
237  $\text{PCO}_2$  likely explains some of the difference in  $\text{MCAv}_{\text{mean}}$  with aquatic exercise as compared to  
238 land-based exercise. Further, given the linear relation between cardiac output and CBF (26), another  
239 likely contributor to the augmented blood flow velocity is related to the well-documented increases  
240 in cardiac output during water immersion (29), due to the effects of increased hydrostatic pressure  
241 centralising blood within the trunk and increasing stroke volume (5). Interestingly, an elevated  
242 stroke volume would appear to be the key mediator of this increased cardiac output, since a  
243 reduction in HR (as we observed) during water immersion is also well documented (4, 16, 20). In  
244 contrast, Pugh et al. (31) reported no significant difference in HR between water and land during  
245 their low-intensity exercise. However, it is worth noting that the box-stepping exercises in their  
246 study were matched for HR between land and water protocols in order to compare similar  
247 intensities. Importantly, regardless of the mechanisms regulating CBF during this modality of  
248 exercise, our observed differences in exercising  $\text{MCAv}$  between our treadmill protocols  
249 demonstrates that aquatic treadmill exercise produces higher blood flow velocity across a range of  
250 intensities, and particularly so at lower exercise intensities (i.e., walking / light jogging); thus  
251 illustrating **the potential** for an enhanced shear-stress mediated pathway for cerebrovascular  
252 adaptation following repeated exposure (i.e., training).

253 The decrease in HR associated with increasing immersion levels noted in this study is  
254 supported by previous studies that have reported a continuous decrease in HR from hip level up to

255 head-out immersion in water at rest (4, 20). Our findings illustrate that a similar elevation in  
256  $MCA_{V_{mean}}$  can be achieved with greater water immersion for a comparatively lower heart rate. As  
257 such, aquatic treadmill exercise training at higher levels of water immersion **could optimise** shear-  
258 stress mediated vasculature adaptations, while lowering the risk of a cardiac event in populations  
259 with elevated risk.

260 The water temperature used in this study ( $32^{\circ}C$ ) is representative of conditions regularly  
261 used in rehabilitative therapy, and is within  $3^{\circ}C$  of the temperatures used in previous studies. While  
262 changes in water temperature have been reported to translate into changes in the cardiac response  
263 (5), the relatively small variation ( $<2^{\circ}C$ ) in temperature between this study and recent research (12,  
264 31) is unlikely to impact on the relative changes in  $MCA_{V_{mean}}$  and HR observed here. **Another**  
265 **consideration is the different heat conduction capacities of water versus air, which may**  
266 **differentially alter exercise-induced changes in body core temperature. As such, measures of body**  
267 **core temperature would be of value in future studies to assess differences between modalities and**  
268 **potential effects of thermal stress related adaptations during the aquatic treadmill exercise. Indeed,**  
269 one further possibility for this form of exercise therapy is to alter the water temperature to  
270 investigate the potential additive therapeutic impact of thermal stress, which may further optimise  
271 the stimulus-response interaction and promote greater neuroprotection against neurodegenerative  
272 diseases (11).

### 273 *Limitations*

274 Speed limitations of the aquatic treadmill prevented a full comparison between treadmill  
275 modalities for an incremental test to exhaustion. Nevertheless, both protocols started at a walking  
276 pace and increased at the same rate ( $1 \text{ km}\cdot\text{h}^{-1}$  every 2 minutes), which resulted in a similar rate of  
277 increase in HR and therefore allowed for a meaningful comparison between the aquatic- and land-  
278 based treadmill exercise across a range of exercise intensities. **This study design meant we were**  
279 **unable to compare matched HR responses across all intensities. We acknowledge that the**  
280 **differences in cardiorespiratory responses may influence the absolute values we show here, but**

281 ultimately will not affect the pattern of MCAv that we observed across the range of exercise  
282 intensities we tested. Measurements of  $\dot{V}O_2$  were originally planned in addition to HR to further  
283 quantify the cardiorespiratory strain and energy expenditure during both protocols, but this was not  
284 possible due to equipment unavailability. However, similar decreases in HR and  $\dot{V}O_2$  between land  
285 running and deep and shallow water running have previously been noted (16), indicating that heart  
286 rate alone can adequately reflect measures of exercise intensity on land as compared to in water. It  
287 is also acknowledged that the reduction in heart rate alone does not necessarily reflect a reduction in  
288 cardiac work (as reflected by myocardial  $\dot{V}O_2$ ). Given the linear relationship that  $\dot{V}O_2$  and cardiac  
289 output share (14), cardiac work can be indexed via the combined measures of heart rate, stroke  
290 volume and blood pressure (i.e., HR x SV x systolic BP (or MAP); (33)). However, we chose not to  
291 fit a blood pressure measuring device (e.g., finometer) so that participants could perform the  
292 exercise in the water as naturally as possible (i.e., fitting this device would have required them to  
293 hold their arm up out of the water). In addition to providing a measure of stroke volume (e.g., via  
294 Beatscope software) to determine cardiac work, measures of BP would have also provided  
295 insightful data regarding the influence of blood pressure on CBF for these different exercise  
296 modalities. Nevertheless, our primary question was to examine the CBF (velocity) differences  
297 between these modalities across a range of exercise intensities and at different immersion depths.  
298 The mechanism(s) that underpin these differences were not the primary focus of our study, but  
299 based on previous studies (12, 32), differences in BP (and  $P_{ET}CO_2$ ) would likely be involved.

300 As mentioned above, we were also unable to measure end-tidal gas content and therefore  
301 assess the influence of  $P_{ET}CO_2$  on MCAv during our exercise protocols. Nevertheless, given the  
302 earlier peak in  $MCAv_{mean}$  during the initial incremental protocol on the aquatic treadmill and the  
303 consistently elevated  $MCAv_{mean}$  during near maximal exercise in water (see Figures 2 and 4), the  
304 typical influence of changes in  $PaCO_2$  on CBF during exercise would appear to be different for  
305 aquatic treadmill exercise. Future studies that include measures of both  $\dot{V}O_2$  and  $P_{ET}CO_2$  are needed

306 to confirm the hypothesis that there may be an altered relation between CBF and PCO<sub>2</sub> during  
307 aquatic treadmill exercise.

308 We measured blood flow velocity using transcranial Doppler as an index of CBF. The  
309 validity of this approach and the likelihood of vessel diameter changes affecting interpretations of  
310 these measures should be considered, especially given the likely changes in blood pressure and  
311 PCO<sub>2</sub> associated with exercise. Nevertheless, given the differential pattern of MCA<sub>v</sub> changes  
312 between the ATM and LTM protocols while changes in PETCO<sub>2</sub> and BP were likely similar [albeit  
313 elevated in water (12, 31)], it seems unlikely that changes in MCA diameter would affect the  
314 interpretation of the findings here. Further, TCD is the ideal brain imaging tool to use in this setting,  
315 while other approaches are not feasible or realistic (e.g., MRI or Duplex Doppler).

316 Finally, the relatively small and demographically limited sample population (mostly young  
317 university students) should be taken into account. As such, whether similar responses occur in older  
318 and clinical populations remain to be determined.

### 319 *Implications*

320 Based on the findings of the current study, aquatic-based treadmill exercise **could provide** an  
321 ideal exercise modality to maximise the stimulus-response for shear-stress mediated adaptation of  
322 the cerebrovasculature in clinical and non-clinical populations. Research is now needed to establish  
323 whether this augmented acute response translates into permanent adaptation of the  
324 cerebrovasculature, and how such training may improve other aspects of brain structure and  
325 function.

326 Exercise training is recommended in clinical populations with elevated risk of  
327 neurodegenerative disease to aid rehabilitation (e.g., stroke (34)), however physical disability may  
328 impact on the effectiveness of traditional exercise programmes to improve vascular health via  
329 shear-stress mediated adaptation. **Our findings demonstrate the potential for aquatic treadmill**  
330 **exercise to optimise this stimulus** for vascular adaptation at exercise intensities (e.g., walking) that



331 are feasible for clinical populations with impaired physical function (e.g., stroke survivors). As  
332 such, the utility of aquatic treadmills for brain-targeted exercise training may be another important  
333 reason to promote such a rehabilitation approach.

#### 334 *Conclusion*

335 Aquatic treadmill exercise augments cerebral blood flow velocity across a range of  
336 intensities, and particularly so at lower exercise intensities (i.e., walking / light jogging). This  
337 elevated blood flow **has the potential** to enhance shear-stress mediated cerebrovascular adaptation  
338 and thus optimise exercise-induced adaptations linked with improved brain health. Research is now  
339 needed to establish whether this augmented acute response translates into permanent adaptation of  
340 the cerebrovasculature, and how such training may improve other aspects of brain structure and  
341 function.

342

#### 343 **AUTHOR CONTRIBUTIONS**

344 RP, MH, SJEL were involved in conception and design of research. RP and SJEL conducted  
345 experiment, performed data analysis and interpretation, and contributed to the drafting the  
346 manuscript. MH provided critical review of the manuscript. All authors approved the final version  
347 of this manuscript.

348

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356

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360

### 361 Disclosure

362 There were no conflicts of interest

363

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458 **Figure 1:** Schematic outlining treadmill belt speed, land treadmill gradient and aquatic treadmill  
459 water depth for the aquatic- and land-based treadmill exercise protocols. Participants completed  
460 seven 2-min stages of incremental exercise intensity, induced via  $1 \text{ km}\cdot\text{h}^{-1}$  increases in treadmill belt  
461 speed. Participants were then ramped to exhaustion ( $2^\circ$  every minute) for the land-based protocol or  
462 completed two 2-min stages of exercise at two alternative water depths on the aquatic treadmill.  
463 Numbers of participants completing each stage of the land-based protocol through to exhaustion are  
464 shown, along with the numbers of participants at each submaximal aquatic belt speed for the 2-min  
465 stages of different immersion depths. Room temperature was maintained at  $\sim 21^\circ\text{C}$  for both, while  
466 water temperature was  $32^\circ\text{C}$ . Abbreviations: ATM, aquatic treadmill; LTM, land-based treadmill.

467

468 **Figure 2:** Changes in middle cerebral artery blood flow velocity ( $\text{MCAv}_{\text{mean}}$ , A) and heart rate (B)  
469 from resting (seated) baseline values over the initial 10 minutes of aquatic- and land-based exercise  
470 protocols. Data are means  $\pm$  SD. Symbols: \* significant difference between treadmills; # significant  
471 difference between preceding stage.

472

473 **Figure 3:** Mean change in  $\text{MCAv}_{\text{mean}}$  (left panel) and HR (right panel) from resting baseline for  
474 walking and moderate intensity ( $65\% \text{VO}_2\text{max}$ ) running exercise using land- and aquatic-based  
475 treadmills. Data are means  $\pm$ SD.  $N=11$ . \* significant difference between treadmills; # significant  
476 difference between preceding stage.

477

478 **Figure 4:** Changes in middle cerebral blood flow velocity ( $\text{MCAv}_{\text{mean}}$ ) and heart rate from resting  
479 (seated) baseline values during constant speed aquatic treadmill exercise immersed to mid-thigh,  
480 iliac crest and xiphoid process. Data are means  $\pm$ SD for 11 participants. \* different from mid-thigh  
481 ( $p<0.05$ ); † different from iliac crest ( $p<0.05$ ).