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Cerebral Blood Flow Responses to Aquatic Treadmill Exercise

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Running Head:

Cerebral blood flow and aquatic exercise

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_v) 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_{v_{mean}} 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_{v_{mean}} 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_{v_{mean}} 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 ± 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° 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⁻¹ for 2 participants
78 and 5 km·h⁻¹ 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 10th 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_{max}
170 (determined during the land-based protocol) compared to land-based incremental exercise ($75 \pm$
171 12% vs. $84 \pm 15\%$ of HR_{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 HR_{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 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 PCO_2 during our study due to equipment
230 unavailability, thus we can only speculate about the relation between 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 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 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 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 PCO₂ 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 PCO₂ associated with exercise. Nevertheless, given the differential pattern of MCA_v changes
312 between the ATM and LTM protocols while changes in P_{ET}CO₂ 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° 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°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 ($p < 0.05$).