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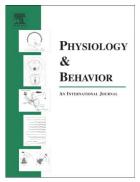
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Integrative physiological and behavioral responses to sudden cold-water immersion are similar in skilled and less-skilled swimmers.

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Abstract

We examined the initial physiological responses and subsequent capacity to swim following cold-water immersion. An ecologically-valid model was used whereby immersion was sudden (<2 s) and participants had to actively remain afloat. Participants (15 skilled swimmers, 17 less-skilled) undertook four experimental test sessions: A physiological test and a swimming test in both cold (10 °C) and temperate (27 °C) water in a swimming flume (temperature order counter-balanced). For physiological testing, measures of brain perfusion [flow velocity (MCAv, Doppler) and oxygenation (NIRS)] and cardiorespiratory function [ventilation parameters and end-tidal PCO₂ (PetCO₂)] were recorded while treading water for 150 s. The swimming test involved treading water (150 s) before swimming at 60% (up to 120 s) and 90% (to intolerance) of pre-determined maximum velocity. Multifactorial analysis revealed swimming duration was influenced most heavily by water temperature, followed by respiratory variables and MCAv in the first 30 s of immersion. The time course and severity of cold shock was similar in both groups (p=0.99), in terms of initial physiological changes (MCAv down ~20 \pm 11%, respiratory frequency increased to 58 \pm 18 breaths.min⁻¹, PETCO₂ dropped to 12 ± 9 mmHg). Treading water following cold-water immersion increased MCAv by 30% above resting values despite maintained cold-shock-induced hyperventilation. In comparison to temperate water, swimming capacity was also reduced similarly between groups in the cold (i.e., distance decreased by $34 \pm 26\%$ skilled; $41 \pm 33\%$ less-skilled, p=0.99). These integrative findings verify that sudden cold-water immersion followed by physical activity leads to similar physiological responses in humans when contrasting between skilled and less-skilled swimmers.

Keywords: cold shock; drowning; hyperventilation; survival; treading water

1. Introduction

Drowning is responsible for at least 388,000 accidental deaths worldwide per year [1], many of which are associated with sudden cold-water immersion (CWI). When humans are immersed suddenly in cold water up to their neck they typically exhibit a set of physiological responses commonly referred to as the cold-shock response [2], characterised by an inspiratory gasp and 1-3 min of hyperventilation and tachycardia. The usual experimental protocol for investigating cold shock involves lowering a seated participant into cold water with a mechanical winch [e.g., 3, 4, 5]. While this immersion technique affords experimental control in a laboratory setting it may not be representative of many aquatic emergency situations - where the immersion is sudden and actively treading water may be the only option to stay afloat (i.e., <2 s vs. controlled immersions in ~28 s [3]). Thus, despite there being a substantial amount of research on CWI, much has utilised a slow, staged immersion protocol, and few studies have included treading water and thereby considered its potential effect on the physiological responses and subsequent behavior. In one exception to this trend, Golden & Tipton [6] found that dynamic immersions over-rode or masked the adaptive benefits gained from repeated static immersions in cold water. Hence, there is some evidence that dynamic immersions have the capacity to alter how humans respond to sudden CWI.

An important, yet less examined element of cold shock is the effect that hyperventilation-induced hypocapnia has on cerebral perfusion and how this may affect behavior. Mantoni *et al.* [5] reported that cerebral blood flow dropped by 43% following a 30s immersion in 0 °C ice water and resulted in symptoms of imminent syncope (i.e., drowsiness, blurred vision, loss of responsiveness) for those with the greatest drop (>60%), likely as a consequence of the severe cerebral hypoperfusion [7]. While Datta and Tipton [8] have also reported reduced cerebral blood flow (CBF) during 12 °C water immersion (CBF down 25%), no study to date has included physical activity (e.g., treading water) during the cold-water immersion or examined the initial response (i.e., <60 s) during realistic sudden

immersion conditions. Given that moderate intensity exercise increases CBF by 10-20% [9], it seems likely that the action of treading water while immersed will influence CBF, via exercise-induced increases in neural activity as well as the concomitant increases in cardiac output and arterial blood pressure. Therefore, the physiological response experienced while actively staying afloat may well differ to that reported to date from passive, slow-onset immersions [6].

Previous work has considered how cold shock can be reduced in humans via staged entry into the water [10], mental preparation [3], and habituation [4]. Whilst it is known that individual variation exists in the extent of cold-shock response [11], the factors influencing this variation are still unclear. For example, it is possible that the cold-shock reflex and swimming skill level are interrelated. Skilled swimmers may have already developed partial habituation to cold-water immersion, thereby resulting in a less severe response than lessexperienced swimmers [12, 13]. Relatedly, experience of cold shock amongst surf swimmers in comparison to swimmers without surf experience contributes to better swimming performance in surf conditions [14]. Furthermore, skilled swimmers should be able to support themselves in the water more effectively (and efficiently) than less-skilled swimmers [15], and hence exhibit a relatively lower ventilation rate during the first few minutes of immersion. Further verification of whether swimming skill can influence the severity and duration of cold shock upon CWI is required.

The present study was developed to extend the ecological validity of previous work concerning cold shock. The testing protocol was devised to examine the initial physiological responses following immersion and subsequent swimming capacity whilst participants were required to actively float (i.e., tread water) and then swim rather than being supported passively in the water. It was of particular interest to determine if the cold-shock response was influenced by swimming skill. We predicted that the severity and duration of cold shock may be less in skilled swimmers compared to less-skilled swimmers, due to prior partial habituation over time. We also predicted that the typical decrease in CBF velocity associated with static cold-water immersion would be less pronounced whilst participants tread water.

This was an exploratory, multidisciplinary study and as such our testing procedure focussed initially upon the physiological responses to sudden cold-water immersion (1st day) followed by subsequent swimming capacity (2nd day).

2. Material and methods

2.1 Participants

Thirty eight participants aged between 18 and 45 years were recruited through advertisements placed on notice boards around the participating institution, at local aquatics/leisure clubs and via a web-based job recruitment facility. Informed consent was obtained prior to any testing. Participants were excluded if they failed a health and fitnessscreening questionnaire (PAR-Q) to demonstrate their competency to carry out the physical tests. Individuals with prior experience of lifesaving or water survival techniques were also excluded. Part of the testing involved wearing a facemask which needed to remain dry, so if participants were unable to tread water or float sufficiently well to keep their head above water for at least 30 s, they were also excluded from the study. Based on these criteria, 6 participants were excluded. The swimming skill of the remaining 32 participants was determined by measuring maximal swimming speed, and the duration of a 200-m swim performed at a self-selected speed (Table 1). If participants completed the 200-m swim in under 300 s they were assigned to the skilled group (N=17, 7 males and 10 females); if they were unable to swim 200 m or they required longer than 300 s to complete the distance they were assigned to the less-skilled group (N=15, 9 males and 6 females). The 300 s time-limit was determined based on pilot work identifying it as a reliable criterion that distinguished between recreational and competitive swimmers. The participants in the two groups were well matched in terms of physical and anthropometric characteristics (Table 1).

	Less-skilled (n=15) Skilled (n=17		Total (n=32)
		7	
Age (y)	22.8 ±5.5	22.7 ±6.9	22.8 ±6.2
		\sim	
Height (m)	171.4 ±6.0	172.6 ±7.1	172.0 ±6.5
Mass (kg)	72.9 ±11.2	70.2 ±9.3	71.5 ±10.2
	C	2	
Skeletal Muscle Mass (kg)	33.6 ±6.8	32.0 ±5.5	32.8 ±6.1
Fat Mass (kg)	14.0 ±7.4	13.0 ±5.8	13.5 ±6.6
Swim duration (s)	227 ±163	255 ±32	242 ±113
Max. swim speed (m.s ⁻¹)	1.0 ±0.3	1.4 ±0.4	1.2 ±0.4

Table 1: Group means and standard deviations of participant characteristics and swimming skill measures for each group. Swim duration refers to the initial 200 m assessment which some of the less-skilled group did not complete (hence the duration is less in this group).

2.2 Equipment

All testing sessions occurred in a swimming flume (StreamLiNZ, Invercargill, New Zealand). This flume is a 10-m long x 2.5-m wide channel through which the flow and temperature of water can be manipulated. Participants wore a full body harness (Delta[™] Repel[™] Technology Riggers Harness, Capital Safety, Red Wing, MN) so they could be lowered rapidly into the water using a compressed-air powered hydraulic winch. The lightweight harness (<1 kg) did not interfere with arm or leg movements in the water. The rate of descent was fixed for all participants and took 1-2 s from the start of immersion to full immersion up to neck level. Once in the water the winch rope was slack so that they were unsupported. Following each testing session, or in the potential case of an emergency, it was also possible to remove participants rapidly from the water using the winch.

A 2-MHz Doppler ultrasound system (DWL Doppler, Compumedics, Germany) was used to measure blood flow velocity through the middle cerebral artery (MCAv; which supplies ~80% of total brain blood flow). The flow signal was obtained and refined using search techniques described elsewhere [16, 17] before the Doppler probe was maintained in position, at a fixed angle, within a commercially-available fixation headframe (Marc 600; Spencer Technologies, USA). Prefrontal cortical oxygenation was measured noninvasively using near-infrared spectroscopy (NIRS; NIRO-200, Hamamatsu Photonics, Hamamatsu, Japan). A probe holder containing an emission and detection probe 5 cm apart was attached at the right side of the forehead using cloth tape (which also assisted with exclusion of light contamination). The methodology of this system has been described previously [18, 19]. All data from the ultrasound and spectrophotometer were recorded using LabChart®software (Version 7.0, ADInstruments, Dunedin, NZ).

Respiratory flow profiles, breathing rate and minute ventilation, rates of oxygen usage and carbon dioxide (CO₂) production, and end-tidal CO₂ partial pressure ($P_{ET}CO_2$) were measured using a MetaLyzer 3B gas analysis system (Cortex, Leipzig, Germany).

2.3 Procedure

The following procedures were approved by the participating institution's human ethics committee. All participants completed five testing sessions: one familiarization session (27 °C), two physiological tests and two swim capacity tests. One of the physiological tests and one of the swim tests was in cold water (10 °C) while the other two were in temperate water (27 °C). The presentation order was counter-balanced for temperature condition (cold and temperate) amongst participants. The physiology tests were carried out before the swim capacity tests to avoid potential adaptation of the initial cold-shock response in the physiological measures due to prior/recent immersion (and based on our assumption that cardiorespiratory responses to CWI were driving behavioral responses more than vice versa).

Participants' initial visit required them to complete and pass all screening criteria, complete an assessment of their swimming ability, as well as familiarizing them with equipment and testing protocols. During this initial visit, height and body mass were measured and body composition was estimated using multi-frequency electrical bioimpedance (InBody 230, Biospace Ltd, CA). Participants were then required to demonstrate that they could tread water with their head above the surface for at least 30 s whilst watching a video (described below). After a brief rest they were then asked to complete a self-paced 200-m swim or to swim as far as possible for those not able to swim 200 m. After a brief rest, each participant's maximum swimming speed (V_{max}) was estimated by timing a 5-m sprint swim against a $0.5 \text{ m} \cdot \text{s}^{-1}$ current. A 10-s maximal swim at the estimated speed was used to fine-tune V_{max} . Participants then swam at 60% of this maximal speed for 120 s, as was required for the swimming capacity tests. During the course of familiarization testing they also practiced using Borg's 14-point perceived exertion scale (RPE) [20].

2.3.1 Physiological test

Participants sat in a chair wearing their swimming costume and a bathrobe while the ventilatory and brain blood flow equipment was attached and adjusted. They were instructed to remain still while 4 min of baseline measures were recorded. After baseline testing they took off the dressing gown, were fitted with the harness and suspended above the water (~5 s), before being dropped rapidly into the water on the hydraulic winch. They then proceeded to tread water unsupported for 150 s or until they asked to be removed early due to intolerance. Once out of the water they were seated again for resting measurements for 1 min.

2.3.2 Swimming capacity test

On another day, at the same time of day as the physiological test, participants returned to the flume for the swimming capacity test. After the baseline measures were obtained, participants were lowered rapidly into the water 2 m in front of a large screen which hung just above the water surface. While treading water they watched a 150 s video of being swept down a river from a first-person perspective, ensuring they maintained their position in the water with their head above the water line. After the video, participants then swam unaided for 120 s or as long as they could against a current of 60% of their pre-determined V_{max} towards an expanding object (i.e., boat) projected onto the large screen. Finally, they swam for as long as they could manage at 90% of V_{max}. The flume was set at these relative speed values to control for likely differences in fitness between the groups [21], however we acknowledge that prescribing swimming speed in this manner (akin to running on a treadmill) is not representative of most survival situations. Overall, the duration of the swimming test lasted up to approximately 6 mins if participants did not ask to be removed from the water earlier due to intolerance.

2.4 Data Analysis

All data were checked manually by four investigators independently, for problems due to equipment malfunction or erroneous signals, and such sections were removed following discussion and comparison with related data. For example, due to likely leakages during gas expiration the VO₂ data were not considered sufficiently valid for further analysis. A 5-s moving average was applied to the remaining data and various discrete values were identified (unless noted we were interested only in values during the immersion): Maximum respiratory frequency (R_Fmax) during the first 30 s; Minimum end-tidal $P_{ET}CO_2$ during the first 30 s; Minimum MCAv during the first 30 s relative to resting baseline; Mean prefrontal cerebral total oxygenation index (BrTOI) during the last 30 s relative to resting baseline; Ratings of perceived exertion during treading water; Total distance and duration of swims in swimming capacity tests.

High correlation between dependent variables, which was likely in the current data set, creates problems with a traditional MANOVA. A statistical technique that is robust to colinearity is the ANOVA-simultaneous component analysis (ASCA; [22]) in which the data are decomposed into effect matrices containing the level averages for the experimental factors and an unexplained residual matrix. The effect matrix is then analyzed using Principal Components Analysis (PCA) to extract the systematic variation of the measured variables. The following predictor variables were entered into the ASCA: P_{ET}CO₂, R_Fmax, MCAv, BrTOI, RPE, and Duration. Using the method described by Zwanenburg et al. [23], individual observations were projected on the principal component subspace to graphically show the variation at levels of each experimental factor (Skill Level and Water Temperature). The significance of the effects due to the experimental factors was estimated using p-values derived from permutation testing (10,000 permutations). All data were processed using custom written scripts in Matlab (Mathworks, Natick, Mass, USA) followed by the ASCA Matlab script [24]. On the basis of the multivariate analysis, the time series of selected variables (R_Fmax, PETCO₂ and MCAv) were used to compare between groups in eight 30 s bins using 2-way GLM ANOVAs.

Results

Discrete values of the dependent variables differed by temperature (p < 0.0001) and by skill level (p < 0.0001) but there was no interaction between temperature and skill level (p =0.85). Figure 1 shows the score plot (upper) and loadings (lower) for each factor: skill level (left) and water temperature (right). ASCA also calculates the contribution of each main effect and interaction to total sum of squares variance. Overall, water temperature contributed 31% to total variance, skill level contributed 9%, and the remaining variance was unexplained by these independent factors. The score plots are a graphical representation of the loadings of each variable on the first principal component of the effect matrices. They

indicate the relative contribution of each variable in explaining the observed outcome. Hence

the separation between the water temperature conditions occurred largely through the

contributions of R_F max and RPE (positive) and $P_{ET}CO_2$, Duration, and MCAv (negative).

Insert Figure 1 about here

Table 2: Means \pm SD for each of the two groups (Less-skilled and Skilled) at each of two water temperatures (10 °C and 27 °C). Where, R_Fmax is the maximum respiratory frequency in the first 30 s after immersion; Min P_{ET}CO₂ is the nadir pressure of expired end-tidal carbon dioxide; Min MCAv is the nadir blood flow velocity in the middle cerebral artery during the first 30 s (as a percentage change relative to baseline, % Δ BL); Mean Br TOI is the change in cerebral total oxygenation index (%) in the prefrontal cortex during the last 30 s of the immersion from baseline resting measures. Swim duration was the total duration of swimming in the behavioral session following 150 s of treading water.

Group	Less-skilled	Less-skilled	Skilled	Skilled
Water Temp	10 °C	27 °C	10 °C	27 °C
R _F max (breaths.min ⁻¹)	68 ±18	45 ±12	49 ±15	36 ±7
Min P _{ET} CO ₂ (mm Hg)	11 ±9	32 ±5	14 ±9	32 ±4
Min MCAv (%∆ BL)	-20 ±11	-6 ±10	-18 ±10	-3 ±7
Mean Br TOI (%)	-2.7±3.0	-2.9 ±1.9	-2.4 ±1.9	-1.5 ±2.2
Swim distance (m)	61 ±28	104 ±38	75 ±29	116 ±22
Perceived exertion (scale: 6-20)	16 ±2.0	14 ±1.7	14 ±2.5	11 ±2.8

The cardiorespiratory and cerebrovascular responses (i.e., R_Fmax , $P_{ET}CO_2$ and MCAv) to the sudden water immersion were more pronounced in the cold compared to the temperate water (Figure 2 and Table 2). The time course comparisons are presented in Figure 3. Although, at first glance, the less-skilled group appear to exhibit a stronger and more persistent cold shock response, there was no main effect of group or interaction between

group and time (p's > 0.05). While the initial response of MCAv to cold-water immersion was similar in both groups, by 60 s MCAv had increased above resting levels and was sustained 15-30% above resting for the duration of treading water (Figure 3).

Insert Figures 2 and 3 about here

4. Discussion

The main findings were: 1) the cold-shock response was a universal feature of sudden coldwater immersion for skilled and less-skilled swimmers; 2) multifactorial analysis revealed that $P_{ET}CO_2$, R_Fmax , and swimming duration were influenced most heavily by water temperature, and; 3) after an initial drop in MCAv within the first 30 s of the immersion, MCAv increased ~30% above resting levels despite maintained hyperventilation-induced hypocapnia.

4.1 Physiological responses to cold-water immersion while treading water

When suddenly immersed in cold water all participants experienced the cold shock physiological response. For example, maximum breathing frequency increased by more (approximately 50%) in cold compared to temperate water, and ~400% above resting level. This finding confirms indications from earlier research that competent swimmers are also susceptible to cold shock [25]. It should be acknowledged that the severity of cold shock varied considerably from participant to participant. Hyperventilation was typically most pronounced within the first minute of immersion, which would coincide with the fastest rate of skin temperature reduction. Directly indicative of hyperventilation, end-tidal PCO₂ was reduced with initial immersion in water and notably more in the cold water (Figure 2). Both groups experienced similar initial responses in the first 30 s of cold-water immersion and this was matched with concomitant responses in CBF velocity; specifically, MCAv decreased more in the cold water (~20% below baseline) compared to temperate water (~5% reduction). This was expected given previous observations of cerebral blood flow velocity

during passive cold-water immersion [8] and the strong relation between brain blood flow and arterial carbon dioxide pressure [26].

In contrast to the findings reported to date following passive and seated immersion, MCAv increased above baseline levels following the initial drop in MCAv upon immersion. Interestingly, this was despite the maintained hypocapnia (see Figure 2) and thus illustrates an uncoupling of the normally tight relation between $P_{ET}CO_2$ and MCAv [26]. The increased MCAv is likely explained by the combined effects of increased cardiac output and mean arterial pressure associated with exercise when treading water, since the hydrostatic and cold-shock effects would be expected to be the same as previous reports. Thus, engaging in some level of physical activity (i.e., treading water) rather than passively floating as recommended currently in the HELP posture, may serve to maintain or even elevate cerebral blood flow whilst in cold water (thereby potentially preventing a reduction in vigilance and eventual loss of consciousness). However, the benefits of such a survival strategy must be considered against potentially prolonging the disruption to baseline breathing rates and increased convective heat loss in the cold water [27]. Further research directly comparing passive and active floatation strategies in cold water is necessary to explore whether benefits of maintaining brain blood flow during cold-water immersion applies more generally.

4.2 Swimming capacity in cold water

Regardless of swimming skill level, participants could not swim for as long in the cold water condition (Table 2). On average, swimming distance in the cold water was reduced by approximately one third compared to the temperate water. With regards to the less-skilled group, some participants in this `novice-like' group were capable of swimming the equivalent of at least 2 to 3 lengths of a 25-m pool (at a comfortable self-selected speed in temperate water). Perhaps using true novices that were unable to swim a single length would have resulted in a bigger and 'clearer' effect of swimming proficiency on subsequent capacity;

however, we found that some level of swimming competency was necessary for participants to satisfy the treading water requirements of testing.

Participants reported higher perceived exertion in the cold condition (Table 2). It is possible that amongst other signs (e.g., increased heart rate, blood pressure) participants perceived their rapid breathing as a sign of increased physical demand.

4.3 Limitations

Past research concerning the risk of drowning in cold water has been largely epidemiological or restricted to relatively slow-onset and passive tank immersions due to the obvious logistical and ethical barriers that exist, and different research questions. We set out to create a controlled testing environment in which participants could be suddenly immersed safely. Hence an air-pressurised hydraulic winch and safety harness worn by the participants in combination with an aquatic flume was employed. This laboratory environment enabled us to more closely simulate sudden CWI, to control water temperature, and also to modify water flow appropriately for the survival swim test. While such features are generally positive aspects of the study, participants were fully aware of the various safety precautions taken and also partially aware of the likely water temperature before immersion (based upon ambient temperature). Given such precautionary measures, one can be reasonably confident that the levels of anxiety that we and others [12] have reported in laboratory testing were much less than might be expected in actual CWI incidents. Furthermore the swimming capacity test represented a tightly constrained simulation in which swimming speed was predetermined and imposed upon the participants. In real survival scenarios such restrictions on behaviour would not exist and therefore the generality of these results must be considered with caution. It should also be acknowledged that the water conditions in our testing environment were relatively calm and therefore less problematic for keeping the airway clear than might be encountered in open water conditions (e.g., "choppy" ocean or river). However

in terms of receiving ethical approval for such a study these were deemed necessary limitations in the study.

Finally, we used transcranial Doppler ultrasound as a surrogate for cerebral blood flow. This holds true as long as the insonated artery does not change diameter, which has been shown under various stimuli for the MCA [28]. We acknowledge the two (opposing) possible stimuli that may have changed the diameter of the MCA in this study; namely, potential dilation due to large increases in blood pressure associated with the exercise pressor effect of treading water, and a possible constriction due to the direct effect of increased sympathetic activation associated with, particularly, the cold-water immersion. While modest increases in MAP (up to ~30 mm Hg) result in minimal (<4%) changes in MCA diameter (Ref), the latter effect, while still a controversial issue within the literature [29, 30], could explain some of the increased *velocity* we observed without necessarily reflecting an increase in flow.

4.4. Conclusions

In conclusion, this study supports previous evidence that the cold-shock response appears to be a universal feature of sudden CWI (regardless of swimming skill). Hyperventilation and reduced brain blood flow characterises the first minute of CWI but there appeared to be individual variation in the subsequent duration of cold shock. A strong predictor of altered swimming capacity (i.e., duration in water tolerated) was heavily influenced by water temperature. Moreover, physiological responses obviously linked with cold shock (e.g., hyperventilation and thus reduced brain blood flow) were also predictors of behavior. Finally, treading water following cold-water immersion increases brain blood flow velocity, despite maintained cold-shock-induced hyperventilation.

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Figure captions

Figure 1: Principal Component (PC) scores plot (upper) and loadings (lower) for factors (*Skill Level*, left and *Water Temperature*, right). The different symbols indicate the different levels of factors (i.e *Skill Level*, left: downward triangle = skilled; circle = less-skilled; *Water Temperature*, right: upward triangle = cold; square = temperate). Level averages are indicated by filled symbols. The ellipses indicate the three standard deviation contours for the observations in each level. Loadings of each variable on the first principal component of the effect matrices are shown by the bars in the lower plots. The higher the loading score, the greater the amount of variance between factors explained by the dependent variable.

Figure 2: Time series of physiological measures whilst treading water for each group (Lessskilled – left; Skilled– right). Lines indicate mean values, with the dashed line for 27 °C water and the solid line for the 10 °C water. The duration that each participant remained in the water differed so the number of participants used to calculate the mean at each time is indicated in the plots labeled 'n'. Immersion occurred at time 0 s and the trial terminated at time 150 s (events marked by vertical lines) unless the participant requested an earlier termination. All post-test recovery periods are aligned to start at the 150 s line.

Figure 3: Bar charts of selected physiological measures in the cold water condition by group (Less-skilled – open bar; Skilled - shaded bar). Immersion occurred at time 0 s and the trial terminated at time 150 s (events marked by vertical lines).

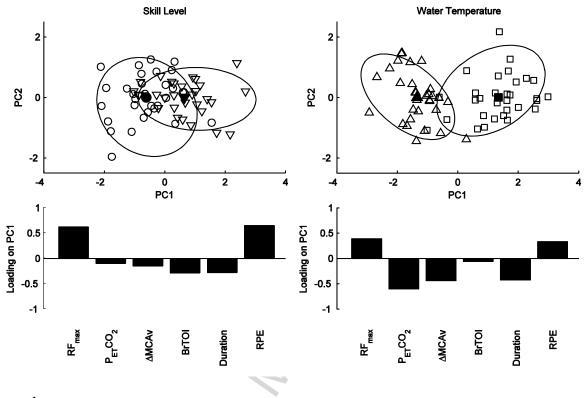


Figure 1

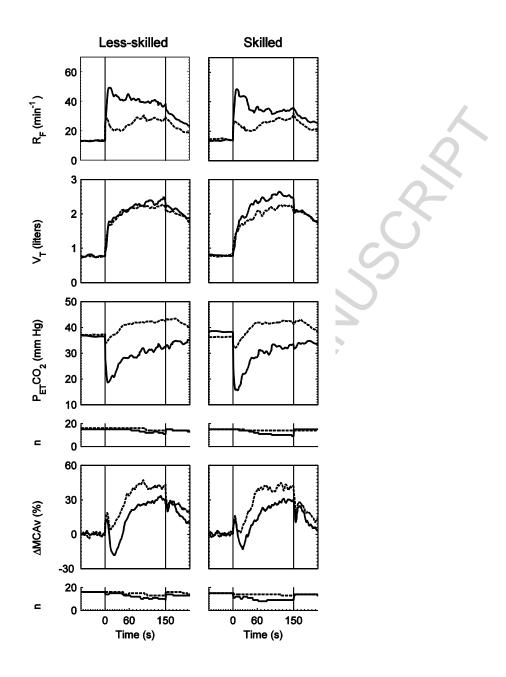


Figure 2

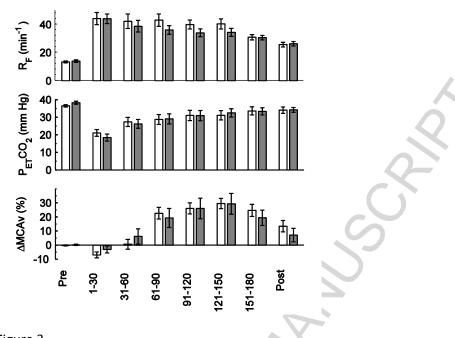


Figure 3

Highlights

- Severity of cold shock while treading water not influenced by swimming skill.
- Treading water increased brain blood flow despite cold shock-induced hypocapnia.
- Cold-water swimming capacity was at least one third lower than in temperate water.

A CERTING