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VO₂ Suppression During Facial Submersion**ABSTRACT**

The mammalian dive response (DR) is described as oxygen-conserving based on measures of bradycardia, peripheral vasoconstriction and decreased ventilation (V_E). Using a model of simulated diving, this study examined the effect of non-apnoeic facial submersions (NAFS) on oxygen consumption (VO_2). 19 participants performed four, 2 minute NAFS with 8 minutes of rest between each. Two submersions were performed in 5°C water, two in 25°C water. HR was collected using chest strap monitors. A tube connected to the inspired port of a non-rebreathing valve allowed participants to breathe during facial submersion. Expired air was directed to a metabolic cart to determine VO_2 and V_E . Baseline (BL) HR, VO_2 and V_E values were determined by the average during the two minutes prior to facial submersion, cold shock response (CSR) values were the maximum during the first 30 seconds of facial submersion, and NAFS values were the minimum during the last 90 seconds of facial submersion. A two-way repeated measures ANOVA indicated: Both HR and V_E were greater during the CSR (92.5 ± 3.6 bpm, 16.3 ± 0.8 L/min) compared to BL (78.9 ± 3.2 bpm, 8.7 ± 0.4 L/min), while both were decreased from BL during the NAFS (60.0 ± 4.0 bpm, 6.0 ± 0.4 L/min) (all, $p < 0.05$). HR_{CSR} was higher and HR_{NAFS} lower in 5°C versus 25°C water ($p < 0.05$), while V_E was greater in 5°C conditions ($p < 0.05$). VO_2 exceeded BL during the CSR and decreased below BL during the NAFS (BL: 5.3 ± 0.1 , CSR: 9.8 ± 0.4 , NAFS: 3.1 ± 0.2 mL/kg/min, $p < 0.05$). The data illustrate that NAFS alone contributes to the oxygen-conservation associated with the human DR.

Key words: Dive response, cold shock response, oxygen consumption, facial submersion

INTRODUCTION

The dive response (DR) is a survival reflex observed in aquatic mammals and humans (Gooden 1994). The response occurs in humans with apnoea alone and can be amplified by trigeminal nerve stimulation (particularly the ophthalmic branch) (Al-Ani et al. 1995, Alboni et al. 2011, Andersson and Schagatay 1998*a*, Endo et al. 2003, Foster and Sheel 2005, Gagnon et al. 2013, Gooden 1994, Jay et al. 2006, Khurana et al. 1979, Stewart et al. 1998), typically by facial submersion in cold water (Alboni et al. 2011, Schagatay and Holm 1996). The DR involves simultaneous activation of parasympathetic and sympathetic nervous systems resulting in bradycardia, decreased cardiac output (CO), increased total peripheral resistance, increased mean arterial pressure (MAP), in addition to a reduced ventilatory drive (Andersson et al. 2009, Davis 2014, Fagius and Sundlöf 1986, Gooden 1994, Hurwitz and Furedy 1984, Khurana et al. 1979). Together, these respiratory and cardiovascular responses are aimed at the conservation of oxygen (Alboni et al. 2011, Andersson et al. 2004, Ostrowski et al. 2010) by redistributing oxygen from the periphery to oxygen sensitive organs – the brain and heart (Alboni et al. 2011, Andersson et al. 2008, Andersson et al. 2009, Andersson and Schagatay 1988*b*, Foster and Sheel 2005, Hurwitz and Furedy 1984). Blood flow to the periphery decreases 30-50% during the DR due to widespread sympathetic vasoconstriction (Alboni et al. 1995, Hurwitz and Furedy 1984), leading to increased anaerobic metabolism in peripheral tissues (Andersson et al. 2008, Davis 2014). At the same time, blood flow through the carotid artery increases and bradycardia leads to an increase in coronary perfusion by way of increased diastolic duration. These effects delay the onset of hypoxic tissue damage to the brain and heart (Alboni et al. 2011, Gooden 1992, Heusch 2008).

Immediately upon facial submersion in cold water, a transient cold shock response (CSR) is observed prior to the typical physiological changes associated with the DR (Jay et al. 2006, Mantoni et al. 2008). The CSR is due to stimulation of cutaneous cold receptors (Alboni et al. 2011, Datta and Tipton 2006) and is characterized by hyperventilation, increased peripheral resistance, and tachycardia (Jay et al. 2006, Mukhtar and Patrick 1995). The tachycardia is attributed to the dramatic increase in tidal volume associated with the “inspiratory gasp” observed upon submersion (Datta and Tipton 2006, Gagnon et al. 2013). This “inspiratory gasp” associated with the CSR leads to the transient tachycardia by way of two mechanisms: increased venous return stimulating the Bainbridge reflex and increased activity of the lung stretch receptors (Gagnon et al. 2013).

Submersion in colder water (0-10°C) elicits larger increases in both minute ventilation (V_E) and heart rate (HR) during facial submersion compared to warmer water temperatures (33°C) (Datta and Tipton 2006, Gagnon et al. 2013, Jay et al. 2006, Mantoni et al. 2008). Typically, the CSR persists for ~20-30 s before the decrease in HR and V_E below baseline that is normally associated with the DR (Gagnon et al. 2013, Jay et al. 2006, Mukhtar and Patrick 1986). As with the CSR, the magnitude of change in V_E and HR during prolonged facial submersion is dependent on temperature, such that colder temperatures elicit a more pronounced DR (i.e. greater drop in V_E and HR) (Jay et al. 2006). During head-out, whole body submersion in water temperatures of 5 to 35°C, both V_E and oxygen consumption (VO_2) increase linearly due to hypothermic shivering (Newstead 1987), suggesting a direct relationship between V_E and VO_2 .

Although the DR is described as an oxygen-conserving response, this is based largely on the cardiovascular and respiratory responses observed. To our knowledge, only one study has

previously measured whole body VO_2 during facial submersion in humans (Hayashi et al. 1997). While VO_2 was unaltered by facial submersion, there was also no observed change in V_E , so the magnitude of the DR can be questioned and so the VO_2 response during the DR is yet to be elucidated. While non-apnoeic facial submersion (NAFS) can be used as a model for the DR, it is important to note that the mammalian DR proper involves apnoea. The purpose of the current study was to determine if NAFS alone contributes to the oxygen conserving response normally associated with the DR by measuring whole body VO_2 during facial submersion in different water temperatures. It was hypothesized that VO_2 would increase transiently during the CSR, and then decrease below baseline (i.e. resting) similarly to V_E and HR during the remainder of the NAFS. It was further hypothesized that facial submersion in colder water would exacerbate these responses.

METHODS

Twenty-one students at Gonzaga University, age 19 to 22 years, were asked to volunteer for this study and provided both written informed consent and a signed Physical Activity Readiness Questionnaire (PAR-Q). Nineteen subjects, 10 males and 9 females, completed the protocol in its entirety. Gonzaga University's Institutional Review Board approved the study protocol.

This study was a within-subject design involving a single, a one-hour visit to the laboratory for each subject. Physical activity and caffeine consumption were avoided the day of data collection and food consumption was limited 3 hours prior. In addition, alcohol consumption was avoided at least 48 hours prior to data collection. Subjects' stature (cm) and mass (kg) were measured using a Detecto Stadiometer (Detecto Scale Co. Web City, MO). HR

was monitored throughout the experimental protocol using a T31 Polar Heart Rate Monitor (Polar Electro Inc. New York, NY) in communication with a True One 2400 Metabolic Measurement System (Parvo Medics Sandy, Utah). Blood pressure was measured using an Omron 7 series BP652 blood pressure cuff (Omron Healthcare Co. Lake Forrest, IL) during minute 6 of each resting period and during the last minute of each facial submersion. A breathing tube was connected to the inspired port of a watertight Hans Rudolph 2700 series non-rebreathing valve and extended above the surface of the water, allowing subjects to breathe during facial submersion. Oxygen consumption and V_E were determined from expired air from the non-rebreathing valve that was directed to a True One 2400 Metabolic Measurement System that had been calibrated according to the manufacturers specifications after having input environmental conditions (barometric pressure, temperature and relative humidity).

The experimental protocol consisted of metabolic measurements for 40 minutes with four, 2-minute, seated facial submersions (up to the temples) with 8 minutes of seated rest before each facial submersion. The subjects wore a nose clip while breathing through the non-rebreathing valve for the entire experimental protocol. Two of the four facial submersions were performed in 5°C water and two in 25°C water; the order of submersions was randomized between subjects. Two buckets (46.7cm x 37.8cm x 31.4cm) were filled prior to each subject's visit, one with each water temperature. Water temperature was monitored constantly with a standard thermometer placed in the buckets used for facial submersion. For the colder water conditions, ice was added to room temperature (25°C) water and allowed to cool down to 5°C where it remained quite stable for the duration of the experiment. Additional ice was added if the water rose by 2°C to 7°C, although this was rarely needed through the course of the study. A bucket was placed in

front of the participant at a height that allowed the subject to stay seated during facial submersion.

Heart rate, VO_2 , and V_E data were exported in 5-second averages to Microsoft Excel for initial data analysis, which was performed for each subject. Baseline (BL) values for VO_2 , HR, and V_E were calculated as the average during the two minutes prior to facial submersion. The values for the CSR were determined by using the maximum for each variable during the first 30 seconds of facial submersion. The value used to represent the NAFS was taken as the minimum value during the last 90 seconds of the facial submersion. These values were averaged between the two 5°C and the two 25°C trials to obtain one BL, CSR and NAFS value for each water temperature. Time to $\text{VO}_{2\text{CSR}}$, HR_{CSR} , V_{ECSR} , $\text{VO}_{2\text{NAFS}}$, HR_{NAFS} , and V_{ENAFS} were defined as the time from beginning of facial submersion to their respective values. These were calculated for each temperature. Mean arterial pressure (MAP) was calculated using the following equation: $\text{MAP} = \text{DBP} + 1/3 (\text{SBP} - \text{DBP})$, where DBP (diastolic blood pressure) and SBP (systolic blood pressure) were measured as outlined above. Rate pressure product (RPP) was calculated using the equation $\text{RPP} = \text{SBP} * \text{HR}$.

A two-way repeated measures ANOVA was used to compare $\text{VO}_{2\text{CSR}}$, $\text{VO}_{2\text{NAFS}}$, and baseline VO_2 values in both the 5°C and the 25°C trials. A Bonferroni correction was made for multiple comparisons. For significant F statistics, pair-wise comparisons were analyzed to determine significant differences between the dependent variables. Greenhouse Geisser estimates were used to correct for degrees of freedom when the assumption of sphericity was violated. The same methods were used for analysis of V_E and HR data. Time to CSR and NAFS values for VO_2 , HR, and V_E were compared across the 5°C and 25°C trials using a paired *t*-test with a Bonferroni-Holm correction. A two-way repeated measures ANOVA was used to determine the

effect of facial submersion and water temperature on MAP. All statistical analysis was performed using IBM SPSS Statistics V.22. Significance levels were set at $p < 0.05$.

RESULTS

Due to a main effect of facial submersion, V_E exceeded BL during the CSR and decreased below BL during the NAFS (Table 1, Figure 1, $p < 0.05$). However, cold water led to a V_E that was greater during the CSR and NAFS in 5°C trials compared to 25°C (Table 1, Figure 1, main effect of water temperature, $p < 0.05$). Facial submersion and water temperature did not have a combined effect on V_E (Table 1, Figure 1, interaction effect, $p = 0.132$).

Similar to V_E , HR was elevated above BL during the CSR and was decreased from BL during the NAFS (Table 1, Figure 2, main effect of facial submersion, $p < 0.05$). While water temperature alone did not have a main effect on HR ($p = 0.305$), the combined effect of facial submersion and water temperature augmented the HR response, such that HR_{CSR} was higher and HR_{NAFS} was lower in the 5°C condition (Table 1, Figure 2, interaction effect, $p < 0.05$).

In a similar fashion to HR and V_E , the effect of facial submersion was such that VO_2 exceeded BL during the CSR and decreased below BL during the NAFS (Table 1, Figure 3, $p < 0.05$). This VO_2 response was similar during facial submersion in both 5°C and 25°C water (Table 1, Figure 3, main effect of temperature, $p = 0.068$, interaction effect, $p = 0.175$).

It took longer for V_E to reach its maximum value during the CSR in 5°C water compared to 25°C water (Table 2, Figure 1, $p < 0.05$). It also took longer for VO_2 to reach its minimum during the DR in 5°C water compared to 25°C water (Table 2, Figure 3, $p < 0.05$). All remaining measures of VO_2 , V_E , and HR reached their maximum (i.e. CSR) and minimum (i.e. NAFS) values on similar time courses between temperatures (Table 2, Figures 1-3, $p > 0.05$).

MAP exceeded BL during the NAFS (Table 1, main effect of facial submersion, $p < 0.05$) and facial submersion in colder temperature water augmented the increase in MAP above BL (Table 1, interaction effect, $p < 0.05$). Because of transient nature of the change in MAP after initiating the facial submersion and the method of measurement, MAP was not measured during the CSR. Rate pressure product was similar between rest and facial submersion (Table 1, main effect of facial submersion, $p = 0.243$), as well as between temperatures (Table 1, main effect of temperature, $p = 0.146$). Facial submersion and temperature did not have a combined effect on RPP (Table 1, interaction effect, $p = 0.488$).

DISCUSSION

The present study characterized changes in whole body VO_2 during non-apnoeic facial submersion in humans in different water temperatures. The primary finding was that facial submersion elicited an immediate increase in VO_2 above baseline during the cold shock response followed by a depressed VO_2 below baseline during the remainder of the NAFS. Previous reports have described the physiological responses during the dive response as an oxygen-conserving mechanism (Alboni et al. 2011, Andersson et al. 2004, Ostrowski et al. 2010). This has often been based primarily on the cardiovascular and ventilatory responses to facial submersion (Andersson et al. 2004, Gooden et al. 1994, Jay et al. 2006), however none of these studies have measured VO_2 using direct or indirect calorimetry to verify the change in oxygen consumption. To the best of our knowledge, only one study has previously measured whole body VO_2 during NAFS as a model of the dive response. Hayashi et al. (2007) reported no change in VO_2 with 2-minutes of NAFS in 8-10°C water. However, they also reported no change in V_E in response to NAFS. As mentioned earlier, a reduced ventilatory drive has been considered one of the key

features of the dive response in previous literature (Andersson et al. 2009, Davis 2014, Fagius and Sundlöf 1986, Gooden 1994, Hurwitz and Furedy 1984, Khurana et al. 1979). Hayashi et al. (2007) also reported no decrease in HR during NAFS, although they did observe an increase in heart rate variability, which indicates increased vagal activity. Given the lack of a significant V_E or HR response, which are hallmarks of the dive response, one can question whether they appropriately modeled the dive response using NAFS. Therefore, their results indicating VO_2 remained unchanged during NAFS should be verified. The model used in the current study elicited a decreased V_E in response to both 5°C and 25°C NAFS, along with decreased HR and increased MAP. These results are more consistent with previous studies detailing the dive response which suggests that current model of NAFS may have been a more appropriate model to simulate the dive response. The current study provides evidence that facial submersion alone contributes to the oxygen-conservation associated with the human dive response.

Oxygen consumption increased above baseline immediately upon facial submersion during the cold shock response (Figure 3). This increase is likely due to an increase in cardiac output and arteriovenous oxygen difference ($a-vO_2$ difference) leading to an increased VO_2 in accordance with the Fick principle. During the cold shock response, the participants in this study exhibited increases in heart rate above baseline (Figure 2). This finding is consistent with previous studies determining cardiovascular responses to facial submersion in water temperatures ranging from 0°C to 34°C (Gagnon et al. 2013, Jay et al. 2006, Mukhtar and Patrick). Furthermore, Wittmers et al. (1987) reported increases in stroke volume during the first 35 seconds of apnoeic facial submersion in 4°C water. The increase in stroke volume during the cold shock response can occur by way of multiple mechanisms, all of which are related to the increased sympathetic nervous activity associated with facial submersion (Datta and Tipton

2006, Gagnon et al. 2013, Gooden 1994, Jay et al. 2006, Paton et al. 2005). The tachycardia that occurs during the cold shock response, which has been observed in previous literature and in the present study, are evidence of increased sympathetic input to the heart at the onset of facial submersion (Gagnon et al. 2013, Jay et al. 2006, Paton et al. 2005). Increased sympathetic activity to the heart leads to increased myocardial contractility both directly, by way of adrenergic effects, and indirectly, by way of the Bowditch effect (Gagnon et al. 2013, Jay et al. 2006, Paton et al. 2005). Additionally, increased sympathetic nervous activity leads to peripheral vascular constriction, which, along with increased resistance, also leads to greater venous return by way of venous constriction. In turn, greater venous return takes advantage of the Frank-Starling mechanism to increase stroke volume (Bjertnaes et al. 1984). Further, the increased V_E during the cold shock response (Figure 1) likely increased venous return by way of the respiratory pump. Although not measured directly, it is likely that cardiac output was elevated during the cold shock response in the current study due to the observed increase in heart rate and probable increase in stroke volume. Given that, it is plausible myocardial oxygen consumption (MVO_2) also increased during the first 30 seconds of facial submersion (i.e. the CSR), contributing to the increase whole body VO_2 observed.

In addition to the cardiovascular response, the results also showed that subjects hyperventilated during the cold shock response (Figure 1). This rise in V_E has been attributed to stimulation of cutaneous pressure and cold receptors (Alboni et al. 2011, Datta and Tipton 2006). Excitation of cutaneous pressure receptors is due to the hydrostatic pressure of water, while the excitation of cutaneous cold receptors is due to the rate of change of skin temperature (Spray 1986). As a result of increased V_E , it is likely that muscles involved in ventilation experienced an increase in oxygen consumption (Mekjavic and Bligh 1989). Because peripheral vasoconstriction

increases as submersion progresses (Andersson et al. 2004, Jay et al. 2006), during the first 30 seconds of facial submersion (i.e. the CSR), peripheral tissues should still have adequate O₂ delivery. Thus, during the cold shock response, peripheral oxygen conserving effects due to vasoconstriction are yet to be observed. Therefore, the increased cardiac output and associated increase in MVO₂ in combination with increased oxygen consumption in muscles of ventilation are the likely causes of the increase in whole body VO₂ during the observed cold shock response.

Following the cold shock response, VO₂ decreased below baseline during the remainder of the NAFS (Figure 3). It is proposed that this is due to a decrease in cardiac output along with a decrease in the a-vO₂ difference. Previous studies detailing cold-water submersion have reported decreases in cardiac output during the dive response (Foster and Sheel 2005, Gooden 1994, Kjeld et al. 2009, Marabotti et al. 2008, Wittmers et al. 1987, Kawakami et al. 1967). In the present study, the bradycardia observed during NAFS (Figure 2) likely caused a reduction in cardiac output. Certainly, previous studies have attributed reductions in cardiac output largely to bradycardia characteristic of the dive response (Andersson et al. 2004, Gooden 1994, Kjeld et al. 2009). However, whether cardiac output decreased or not would be dependent on changes in stroke volume along with the decreased heart rate observed. The effect of facial submersion on stroke volume, however, is not well documented.

Decreases in stroke volume have been observed under apnoeic conditions, and that has been explained largely by the reduction in preload that occurs as a result of increased intrathoracic pressure during breath-holding (Bjertnaes et al. 1984, Marabotti et al. 2008). It is plausible stroke volume decreased during NAFS in the present study due to similar (although not as dramatic) reductions in preload. Consistent with previous literature, the present findings show that V_E was depressed during NAFS. This change in ventilation, attributed largely to trigeminal

nerve stimulation (Gagnon et al. 2013), leads to decreased venous return by way of decreased respiratory pump activity during the dive response. Simultaneously, the bradycardia observed during the dive response promotes decreased force of myocardial contraction via the Bowditch effect (Paton et al. 2005). Lastly, the current study found MAP to be approximately 15 mmHg greater during NAFS than during rest (Figure 4), which could cause decreased stroke volume by way of increased afterload. Taken together, decreased venous return and myocardial force of contraction combined with increased afterload would promote decreased stroke volume during NAFS.

A decreased a-vO₂ difference may have also contributed to the depressed VO₂ observed during the NAFS. The decreased V_E that was observed during the dive response indicates less inspiratory muscle activity, which would partly account for the decreased a-vO₂ difference (Andersson et al. 2004, Andersson and Schagatay 1998*a*, Andersson and Schagatay 1998*b*). It has also been proposed that reductions in heart rate and stroke volume, and thus cardiac output, lead to decreased MVO₂ during the dive response (Andersson et al. 2004, Andersson and Schagatay 1998*a*, Gooden 1994), contributing to the decreased a-vO₂ difference. Overall, the decreases in VO₂ observed during NAFS in the present study are likely due to decreases in both the cardiac output and the a-vO₂ difference.

The finding that oxygen consumption was increased during the cold shock response and decreased during the remainder of the NAFS was consistent between water temperatures of 5°C and 25°C, contrary to the initial hypothesis. It is well documented that the cardiovascular responses during the cold shock response and dive response are augmented by submersion in colder water (Foster and Sheel 2005, Gagnon et al. 2013, Gooden 1994, Gooden 1992, Jay et al. 2006, Mekjavic and Bligh 1989, Schagatay and Holm 1996). As originally hypothesized, the

data from the current study are in agreement with these previous studies showing a greater tachycardia during the cold shock response in colder water, as well as a greater degree of bradycardia during the remainder of the NAFS in the colder water (i.e. 5°C vs. 25°C, Figure 2). It was hypothesized that the VO_2 response would mirror the cardiovascular response; that it did not, is likely the effect of facial submersion in the colder water on the ventilatory response. In looking closely at the data of the present study, the effect of water temperature during the cold shock response is consistent between HR and V_E (Figures 1 and 2). That is to say, facial submersion in 5°C water lead to augmented responses in HR, V_E . As the facial submersion progressed, V_E remained elevated in the 5°C condition, while the bradycardia was augmented (i.e. HR was lower in colder water). Applying the same rationale as earlier in the discussion, a lower VO_2 would be expected in colder water due to the enhanced bradycardia, however the increased V_E and associated oxygen requirement for increased ventilatory muscle activation during NAFS in colder water may have eliminated any further conservation of oxygen versus facial submersion in warmer water. Ultimately, the overall effect was that VO_2 was reduced to a similar extent during non-apnoeic facial submersion in cold vs. warm water.

Though previous literature has suggested the cardiovascular response to facial submersion causes decreased MVO_2 , (Andersson et al. 2004, Andersson and Schagatay 1998a, Foster and Sheel 2005, Kawakami et al. 1967) few studies have data supporting this theory. In order to more adequately evaluate the role for MVO_2 in altering whole body VO_2 during facial submersion, future studies should incorporate beat-by-beat blood pressure measurements to better determine changes in blood pressure and thereby changes in RPP, during the cold shock and dive responses. The use of the wrist cuff blood pressure monitor in the current study did not provide sufficiently accurate measures since blood pressures were changing rapidly and the

monitors needed approximately 20-30 seconds to record a measurement. The current measures of RPP showed no changes throughout the protocol (Figure 5), however a more precise measure of RPP could shed more light on the myocardial oxygen consumption as RPP and MVO_2 are highly related. Further, the effects of facial submersion on stroke volume need to be properly elucidated. This is especially a concern for non-apnoeic models of facial submersion, as the ventilatory response will affect stroke volume by way of venous return.

In conclusion, non-apnoeic facial submersion in 5°C and 25°C water resulted in increased VO_2 during the cold shock response and suppressed VO_2 during the dive response. Previously, the oxygen conserving effects of the dive response have been largely attributed to cardiorespiratory measures (i.e. HR and V_E). To the best of our knowledge, this is the first study to report whole body VO_2 decreases during non-apnoeic facial submersion in resting humans. Thus the present findings provide a novel contribution to the characterization of the dive response in humans and provide evidence that non-apnoeic facial submersion alone contributes to the oxygen-conservation associated with the human dive response. The authors declare that there are no conflicts of interest.

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Table 1. Group means $\pm$ SD for HR (bpm), VO_2 (mL/kg/min), V_E (L/min), MAP (mmHg), and RPP (mmHg*bpm) for BL, CSR, and NAFS values during two-minute facial submersion in 5°C and 25°C water. (N=19).

	V_E (L/min)		HR (bpm)		VO_2 (mL/kg/min)		MAP (mmHg)		RPP (mmHg*bpm)	
Temperature	5°C	25°C	5°C	25°C	5°C	25°C	5°C	25°C	5°C	25°C
Baseline	8.9 $\pm$ 2.1	8.5 $\pm$ 1.7	79.0 $\pm$ 14.7	78.8 $\pm$ 13.9	5.4 $\pm$ 0.6	5.1 $\pm$ 0.7	91.5 $\pm$ 10.7	92.4 $\pm$ 9.5	9320.5 $\pm$ 2462.9	9542.3 $\pm$ 2268.6
CSR (max)	17.5 $\pm$ 5.5 ^{*β}	15.2 $\pm$ 3.8 [*]	94.7 $\pm$ 18.1 ^{*β}	90.4 $\pm$ 14.8 [*]	10.4 $\pm$ 2.3 [*]	9.3 $\pm$ 2.1 [*]	-	-	-	-
NAFS (min)	6.9 $\pm$ 3.0 ^{#β}	5.1 $\pm$ 1.6 [#]	55.4 $\pm$ 17.6 [#]	17.9 ^{#α}	3.0 $\pm$ 0.9 [#]	3.1 $\pm$ 0.8 [#]	109.5 $\pm$ 10.5 ^{φβ}	99.7 $\pm$ 11.3 ^φ	8813.8 $\pm$ 2015.8	9391.0 $\pm$ 2540.0

Significant differences (p<0.05) denoted by: ^{*} CSR>BL, [#] NAFS<BL, ^φ NAFS>BL, ^β 5°C>25°C, ^α 25°C>5°C

Table 2. Mean time ± SD to maximum and minimum measures during the CSR and NAFS respectively for HR (bpm), VO₂ (mL/kg/min), and V_E (L/min) during two-minute facial submersion in 5°C and 25°C water. (N=19).

	V _E		HR		VO ₂	
Temperature	5°C	25°C	5°C	25°C	5°C	25°C
Time to CSR (s)	14.8 ± 7.6 ^θ	11.3 ± 4.5	9.3 ± 2.6	12.6 ± 12.1	13.7 ± 7.2	12.4 ± 5.2
Time to NAFS (s)	93.5 ± 19.1	83.2 ± 17.8	59.3 ± 20.6	61.9 ± 21.3	91.8 ± 16.0 ^θ	77.8 ± 20.3

Significant differences (p<0.05) denoted by: ^θ 5°C>25°C (i.e. 5°C slower than 25°C)

Figure 1. The effect of facial submersion and temperature on V_E during non-apnoeic facial submersion for 2 minutes in 5°C and 25°C water. Vertical error bars indicate standard deviation of mean V_E . Significant differences ($p<0.05$) for effect of facial submersion and water temperature on V_E denoted on vertical error bars by: *CSR>BL, #NAFS<BL, $\beta 5^\circ\text{C}>25^\circ\text{C}$. Horizontal error bars indicate standard deviation of the mean time to V_{ECSR} and time to V_{ENAFS} . Significant differences ($p<0.05$) for effect of water temperature on time denoted on horizontal error bars by: $\theta 5^\circ\text{C}>25^\circ\text{C}$ (i.e. 5°C slower than 25°C).

Figure 2. The effect of facial submersion and temperature on HR during non-apnoeic facial submersion for 2 minutes in 5°C and 25°C water. Vertical error bars indicate standard deviation of mean HR. Significant differences ($p<0.05$) for effect of facial submersion and water temperature on HR denoted on vertical error bars by: *CSR>BL, #NAFS<BL, $\beta 5^\circ\text{C}>25^\circ\text{C}$, $\alpha 25^\circ\text{C}>5^\circ\text{C}$. Horizontal error bars indicate standard deviation of the mean time to HR_{CSR} and time to HR_{NAFS} .

Figure 3. The effect of facial submersion and temperature on VO_2 during non-apnoeic facial submersion for 2 minutes in 5°C and 25°C water. Vertical error bars indicate standard deviation of mean VO_2 . Significant differences ($p<0.05$) for effect of facial submersion and on VO_2 denoted on vertical error bars by: *CSR>BL, #NAFS<BL. There was no significant effect of water temperature on VO_2 . Horizontal error bars indicate standard deviation of the mean time to VO_{2CSR} and time to VO_{2NAFS} . Significant differences ($p<0.05$) for effect of water temperature on time denoted on horizontal error bars by: $\theta 5^\circ\text{C}>25^\circ\text{C}$ (i.e. 5°C slower than 25°C).