Peripheral chemoreflex sensitivity and sympathetic nerve activity are normal in apnea divers during training season

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A B S T R A C T
Apnea divers are exposed to repeated massive arterial oxygen desaturation, which could perturb chemoreflexes. An earlier study suggested that peripheral chemoreflex regulation of sympathetic vasomotor tone and ventilation may have recovered 4 or more weeks into the off season. Therefore, we tested the hypothesis that peripheral chemoreflex regulation of ventilation and sympathetic vasomotor tone is present during the training season. We determined ventilation, heart rate, blood pressure, cardiac stroke volume, and muscle sympathetic nerve activity (MSNA) during isocapnic hypoxia in 10 breath hold divers and 11 matched control subjects. The study was carried out at the end of the season of intense apnea trainings.

Baseline MSNA frequency was 30 ± 4 bursts/min in control subjects and 25 ± 4 bursts/min in breath hold divers (P = 0.053). During hypoxia burst frequency and total sympathetic activity increased similarly in both groups. Sympathetic activity normalized during the 30-minute recovery. Hypoxia-induced stimulation of minute ventilation was similar in both groups, although in divers it was maintained by higher tidal volumes and lower breathing frequency compared with control subjects. In both groups, hypoxia increased heart rate and cardiac output whereas total peripheral resistance decreased. Blood pressure remained unchanged.

We conclude that peripheral chemoreflex regulation of ventilation and sympathetic vasomotor tone is paradoxically preserved in apnea divers, both, during the off and during the training season. The observation suggests that repeated arterial oxygen desaturation may not be sufficient explaining sympathetic reflex abnormalities similar to those in obstructive sleep apnea patients.

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1. Introduction
Peripheral and central chemoreceptors are primarily involved in regulation of respiration (O'Donnell et al., 1996), but can also elicit a sympathetically mediated pressor response (Sapru, 1996; Somers et al., 1989). Frequent involuntary apneas in obstructive sleep apnea patients (OSA) may result in augmented peripheral chemoreflex sensitivity (Imadojemu et al., 2007; Narkiewicz et al., 1999), whereas central chemoreflex regulation is unimpaired (Narkiewicz et al., 1999).

Elite breath hold divers are exposed to extreme hypoxia/hypercapnia during maximal apneas lasting for several minutes. After maximal apnea, alveolar oxygen partial pressure can be as low as 20–30 mm Hg with arterial oxygen saturation around 50% (Lindholm and Lundgren, 2006; Overgaard et al., 2006). Breath hold divers compete in different disciplines such as static apnea, dynamic apnea, and constant weight. During static apnea, divers float motionless face down in a pool, while during dynamic apnea, the goal is to attain maximal underwater swimming distances.

Finally, during constant weight, divers swim down as deeply as possible along a vertically suspended rope using fins. With static and dynamic apnea, divers are exposed to progressively increasing hypercapnic hypoxia. During constant weight diving, subjects are exposed during descent and at the bottom to hyperoxic hypercapnia due to hydrostatic pressure-induced compression of the chest wall (Muth et al., 2003).

Voluntary apnea under laboratory conditions and intermittent hypoxia causes acute as well as sustained changes in cardiovascular autonomic regulation (Cutler et al., 2004; Leuenberger et al., 2005; Morgan et al., 1995). Twenty to 30 min of intermittent hypoxia is sufficient to raise muscle sympathetic nerve activity (MSNA) and blood pressure (Leuenberger et al., 2005). Previously, we observed that central chemoreflex control of respiration and sympathetic activity is maintained in breath hold divers (Dujic et al., 2008) and that, after detraining, they have normal peripheral chemoreflex regulation of ventilation and sympathetic vasomotor tone (Breskovic et al., in press), indicating no sustained autonomic impairment. Now, we investigated the hypothesis whether during training season the peripheral chemosensitivity is abnormal in breath hold divers as was previously shown for OSA patients. We measured autonomic, ventilatory, and hemodynamic responses to isocapnic hypoxia in divers and matched control subjects at the end of several months intensive training period.

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2. Methods

2.1. Subjects

We included 21 healthy men (N = 16) and women (N = 5) in our study. Of those, ten were experienced elite breath hold divers (BHD) (3 women) while eleven matched, untrained subjects served as controls (2 women). All divers were competitive in static and/or dynamic apnea. Seven out of ten divers were also non-competitively practicing constant weight apnea discipline but considerably less frequently than their primary competitive discipline(s). Average personal best results for divers were 340 ± 59 s in static apnea and 157 ± 31 m in dynamic apnea. Six out of ten divers previously had episodes associated with hypoxia in range from mild disorientations to blackouts with or without convulsions or loss of motor control. On average, divers had apnea trainings thrice weekly. Apnea training duration ranged between 1 and 1.5 h. We conducted the study in accordance with the Declaration of Helsinki and after approval by the ethical committee of the University of Split School of Medicine. We obtained written consent from each subject.

2.2. Protocol

We conducted our studies in December 2008, at the end of apnea season. The study was carried out within one month after the last major static and dynamic apnea competition of the season. We asked divers to continue their training with the same intensity until they were evaluated in the laboratory. The period between testing in the laboratory and last training was maximally 2 days. All experiments were carried out in a climatized room in the morning hours. Participants were instructed not to eat at least 4 h before the arrival to the laboratory. We studied women during the follicular phase of the menstrual cycle.

Before instrumentation subjects underwent dynamic spirometry (Quark PFT, Cosmed, Rome, Italy) while standing and afterwards their anthropometric measurements were taken. Then, they were asked to lie down and were instrumented for the hypoxic test.

We applied an infrared probe on the middle finger to monitor arterial oxygen saturation (Pois II, Criticare Systems, Waukesha, USA). Beat-by-beat blood pressure and heart rate were measured using a finger cuff (Finometer, Finapress Medical Systems, Arnhem, Netherlands) and electrocardiography, respectively. Multiliint muscle sympathetic nerve activity (MSNA) of postganglionic sympathetic activity was recorded from the right peroneal nerve with a unipolar tungsten electrode as described previously (Ducic et al., 2008). The nerve signal was amplified 100,000 times. Afterwards signal was band-pass filtered (0.7–2.0 kHz), rectified and integrated using 0.1 s time constant (662C-4. Nerve traffic analysis system, Bioengineering, The University of Iowa, USA).

Subjects were breathing from a mouthpiece connected to a non-rebreathing Y-valve (Hans-Rudolph 2730 Series, Large 2-way, NR, Y Shape, K.C., MO, USA) whose inspiratory port was connected to a three-way valve (Hans-Rudolph 4000 Series Large non mixing, 3-way “Y” Stopcock, K.C., MO, USA) allowing switching between room air and gas-reservoir. The spirometer (Harvard apparatus, Student model, Holliston, MA, USA) acted as reservoir for the gas mixture whose composition was regulated by a blender. Blender was attached to three gas cylinders (compressed air, 100% N2 and 100% CO2) thus enabling to produce different hypoxic gas mixtures. The gases were sampled breath-by-breath at the mouth using a respiratory analyzer (Quark b2, Cosmed, Rome, Italy). Before each trial, control data were collected by having subjects breath room air for 3 to 5 min while monitoring the PetCO2 concentration to establish his or her normoxic level. This was followed by progressive normocapnic hypoxia introduced in two steps by increasing the N2 concentraion until the required SaO2 was achieved. Mean SaO2 level of ~0.9 was maintained for 3 min, and ~0.8 for 5 min. Normocapnic PetCO2 was regulated by adding CO2 to the inspired gas, as required, thus minimizing central chemoreceptor engagement. After cessation of hypoxia testing, subjects were switched to breathe room air while measurements were continued for another 30 min.

2.3. Data acquisition and analysis

All data were acquired using an analog to digital converter (Powerlab/16SP, ADInstruments, Castle Hill, Australia) interfaced with PC. Data were sampled at 1 kHz and stored for subsequent analysis using Chart software (ADInstruments, version 5.5.6.7). MSNA bursts were identified according to the following criteria: (1) signal to noise ratio ≥2; (2) latency limit; (3) burst width limit (short duration = artifact, long duration = skin sympathetic nerve activity or afferent activity; (4) no preceding premature beats (Tank et al., 2001). MSNA activity was expressed as frequency of bursts per minute (burst frequency) and per 100 heart beats (burst incidence). Amplitude and area of each burst was calculated. Total MSNA was calculated as the sum of all burst areas per minute. We obtained good quality MSNA recordings in all subjects. We quantified sympathetic chemoreflex sensitivity and ventilatory responses to incremental hypoxia as change in sympathetic nerve activity or ventilation per change in SaO2 during hypoxic protocol. Changes in left ventricular stroke volume were estimated by pulse wave analysis using an improved method of Wesseling (Modelflow program) (Jellema et al., 1999). The data were analyzed during 3 min period before hypoxia, 3 min during SaO2 = 0.9, last 3 min of hypoxia trial while SaO2 = 0.8, during 3 min upon cessation of hypoxia and during 3 min periods in 10th, 20th and 30th minute of recovery period.

2.4. Statistical analysis

All data were expressed as means ± 95% confidence intervals (95% CI). Baseline values, values at the same time points and chemoreflex sensitivities were compared using unpaired Student t-test. The effects of hypoxia on all measured variables within group were determined using Table 1

<table>
<thead>
<tr>
<th>Anthropometric characteristics of subjects.</th>
<th>Controls (N = 11)</th>
<th>BHD (N = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.5 ± 2.6</td>
<td>27.0 ± 3.4</td>
<td>0.50</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>82.5 ± 8.3</td>
<td>76.0 ± 5.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.83 ± 0.04</td>
<td>1.83 ± 0.04</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI</td>
<td>24.5 ± 2.1</td>
<td>22.6 ± 1.0</td>
<td>0.12</td>
</tr>
<tr>
<td>BFI (%, body fat/kg)</td>
<td>21.1 ± 4.7</td>
<td>22.7 ± 3.9</td>
<td>0.60</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>5.9 ± 0.7</td>
<td>6.9 ± 0.9</td>
<td>0.09</td>
</tr>
<tr>
<td>FEV1 (l)</td>
<td>4.0 ± 0.5</td>
<td>5.5 ± 0.9</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are means ± 95% CI; differences between groups analyzed by unpaired Student t-test; BFI — body mass index; BMI — body fat index (calculated by Jackson and Pollock three site measurement); FVC — forced vital capacity; FEV1 — forced expiratory volume in 1st second.

Table 2

<table>
<thead>
<tr>
<th>Baseline data.</th>
<th>Controls (N = 11)</th>
<th>BHD (N = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNA0 (bursts × min⁻¹)</td>
<td>29.9 ± 3.5</td>
<td>24.5 ± 3.7</td>
<td>0.053</td>
</tr>
<tr>
<td>MSNA1 (bursts × 100h⁻¹)</td>
<td>44.8 ± 5.7</td>
<td>41.5 ± 4.8</td>
<td>0.39</td>
</tr>
<tr>
<td>Burst area (au)</td>
<td>212 ± 36</td>
<td>265 ± 39</td>
<td>0.07</td>
</tr>
<tr>
<td>MSNA0 (au × min⁻¹)</td>
<td>2.0 ± 0.7</td>
<td>1.6 ± 0.7</td>
<td>0.45</td>
</tr>
<tr>
<td>Vb (l × min⁻¹)</td>
<td>7.8 ± 1.2</td>
<td>7.2 ± 0.8</td>
<td>0.49</td>
</tr>
<tr>
<td>HR (beats × min⁻¹)</td>
<td>67.7 ± 6.1</td>
<td>59.1 ± 4.6</td>
<td>0.043</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>95.8 ± 4.4</td>
<td>93.9 ± 5.8</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are means ± 95% CI; differences between groups analyzed by unpaired Student t-test; MSNA0 — burst frequency; MSNA1 — burst incidence; MSNA0 — total MSNA; Vb — minute ventilation; HR — heart rate; MAP — mean arterial pressure.
repeated-measures ANOVA. A Bonferroni test was used as post hoc test. Interactions among responses between the groups were assessed using a general linear model for repeated-measures ANOVA. All analyses were performed with Statistica 7.0 software (Statsoft, Inc., Tulsa, USA).

3. Results

Anthropometric and pulmonary function data of the subjects are presented in Table 1. Participants in both groups were acceptably
matched. Even though divers had higher pulmonary function parameters, absolute values didn't reach statistical significance, however, relative predicted values did. Forced vital capacity in controls was 105.8 ± 8.9 vs. 131.9 ± 7.1% predicted in divers (P < 0.001), and forced expiratory volume in 1st second in controls was 105.2 ± 6.0 vs. 123.7 ± 11.0% predicted in divers (P = 0.013).

Divers had slightly lower baseline burst frequency (MSNAi) and higher burst area, although the difference was not statistically significant. Total sympathetic activity (MSNAi) and burst incidence (MSNAi) was comparable among the groups. Divers had lower heart rate (HR), Baseline minute ventilation (VE) and mean arterial pressure (MAP) were similar (Table 2).

![Fig. 1](image1.png)

**Fig. 1.** Ventilatory responses in two groups during all phases of hypoxic protocol. Circles represent means, error bars denote 95% confidence intervals. Significant changes within single group were determined using repeated-measures ANOVA with Bonferroni test as post hoc test (†P < 0.05). Interactions among responses of the two groups were tested using general linear model for repeated-measures ANOVA. Differences between groups in the same time point were identified with unpaired Student t-test (⁎P < 0.05).

During hypoxia, ventilation increased 0.35 ± 0.16 l/min per 1% change in SaO₂ in the control group and BHD 0.27 ± 0.16 l/min per 1% change in SaO₂ in divers (P = 0.48).

With hypoxia, MAP and stroke volume (SV) did not change in either group. Heart rate (HR) and cardiac output (CO) increased as hypoxia progressed. Total peripheral resistance (TPR) decreased during hypoxia trial in both groups, although in divers the reduction reached statistical significance. The hemodynamic response to hypoxia was similar in both groups (Fig. 4).

**4. Discussion**

The main finding of our study is that autonomic, ventilatory, and cardiovascular responses to hypoxia are normal in elite divers during intensive apnea trainings. Divers showed normal baseline blood pressure and sympathetic activity. The observation that peripheral and central chemosensitivity are preserved is reassuring (Breskovic et al., in press; Dujic et al., 2008). Normal peripheral and central chemosensitivity in elite breath hold divers may serve as a protective mechanism for maintaining cerebral perfusion during extreme asphyxia associated with long breath holds, since increased CO₂ and decreased O₂ have major vasodilatory effect on this vascular bed, whereas increased peripheral sympathetic nerve traffic during hypoxia in divers counteracts direct vasodilatory effects.

We studied divers competing in static and dynamic apnea since they are most intensively exposed to hypoxia. Despite extreme conditions of the sport, world records are constantly improved. For example current world record in static apnea is 11.6 min in men and 8.4 min in women. Elite breath hold divers are adapted to extreme hypoxia/hypercapnia due to ventilatory, cardiovascular, and cerebrovascular adjustments, such as decreased ventilatory sensitivity to CO₂, increased lung volume, enhanced peripheral sympathetic and parasympathetic activation, and increased lactate production among others (Bakovic et al., 2003; Heussner et al., 2009; Palada et al., 2007). Furthermore, they show reduced post-apnea as well as post-exercise blood acidosis and oxidative stress, mimicking the responses of diving animals (Jouila et al., 2002). We tested our divers at the end of the competitive apnea season after divers underwent numerous intensive apnea trainings and competitions lasting for more than half of a year. Sixty percent of our subjects had during their career hypoxia related loss of consciousness. Moreover, two of them had multiple occurrences of such episodes, suggesting that our subjects have been regularly exposed to severe hypoxia.

We applied isocapnic hypoxia testing. In the event, end tidal CO₂ was well maintained throughout the test. We are confident that we minimized influences of CO₂ changes on central chemoreceptors and cerebral vasculature. Stimulation of peripheral chemoreflex elicited a moderate increase in ventilation. The slope of the hypoxic ventilatory response was similar in divers and in control subjects, suggesting unchanged ventilatory drive to hypoxia. Previously, the ventilatory response to hypoxia was shown to be blunted in persons engaging in underwater sports such as synchronized swimmers (Bjurstrom and Schoene, 1987) and in the Japanese Ama (Masuda et al., 1981). The observation suggests adaptations that may conserve oxygen, thus, prolonging maximal asphyxia during long breath holds.

The overall cardiovascular response to hypoxia may result from direct influences on vascular tone and cardiac contractility together with centrally and reflex mediated changes in autonomic nervous system activity. Several previous studies investigated the influence of hypoxia on peripheral vasodilatation. In subjects exposed to lower body negative pressure during hypoxia, norepinephrine and angiotensin doses had to be doubled to restore vascular tone to levels occurring during normoxia (Heistad and Wheeler, 1970). Furthermore, during pharmacological α-adrenoreceptor blockade, peripheral
vasodilation was massively prolonged after cessation of hypoxia (Tamisier et al., 2004). Both studies suggest that hypoxia-induced vasodilation is masked by chemoreflex mediated sympathetic activation. Additionally, hypoxia also exerts a direct negative inotropic effect on the myocardium (Henderson and Brutsaert, 1973). In our study, sympathetic vasomotor tone started to normalize after termination of hypoxia and returned to baseline 20 to 30 min after the end of the hypoxic protocol. Xie et al. (Xie et al., 2001) observed increased sympathetic activity which outlasted hypoxia up to 60 min. Differences between studies may be due to various duration and magnitude of hypoxia. The increase in sympathetic vasomotor tone was not sufficient to completely abrogate the vasodilator response to hypoxia. Arterial pressure was maintained through augmented cardiac output. More marked increase in total sympathetic activity than in burst frequency suggests increased recruitment of efferent sympathetic neurons (Elam et al., 2003).

Contrary to obstructive sleep apnea patients, breath hold divers are not exposed to excessive sympathetic activation. One possible explanation for the discrepancy could be a difference in the time course of hypoxic episodes between groups. Breath hold divers are exposed to hypoxia during voluntary end-inspiratory apnea while in OSA patients the apneas are involuntary and end-expiratory. Additionally, divers usually train 3 or 4 times per week for 1–1.5 h while OSA patients have repetitive apneic episodes almost every time when they are asleep. Therefore, total apnea time and exposure to hypoxia may be greater in OSA patients than in elite apnea divers. Age may be another variable explaining the discrepancy between divers and OSA patients in terms of chemoreflex regulation. Our divers were relatively young. In contrast, most OSA patients are older people. Finally, OSA patients often have multiple comorbidities like obesity, heart disease and diabetes, among others which can all potentiate increased sympathetic activation in this population. On contrary, apnea divers are, usually, group of healthy, young individuals.

One potential limitation of our study is that ventilatory, cardiovascular, and autonomic measurements to isocapnic hypoxia were only obtained during wakefulness, and not during apnea. Apnea per se may

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**Fig. 4.** Hemodynamic responses in two groups during all phases of hypoxic protocol. Circles represent means, error bars denote 95% confidence intervals. Significant changes within single group were determined using repeated-measures ANOVA with Bonferroni test as post hoc test ($^{*}P<0.05$). Interactions among responses of the two groups were tested using general linear model for repeated-measures ANOVA. Differences between groups in the same time point were identified with unpaired Student t-test ($^{†}P<0.05$).
potentiate the sympathetic response to hypoxia, since stimulation of pulmonary stretch receptors and baroreceptors affects sympathetic activity (Somers et al., 1995).

In summary, our observations suggest that repeated voluntary exposure to intermittent hypoxia/hypercapnia in the absence of additional risk factors like hypertension, glucose intolerance or hyperlipidemia may not have a negative impact on autonomic, cardiovascular and ventilatory regulation. Although elite divers are not exposed to sustained changes in sympathetic nervous system regulation, we cannot exclude that acute sympathetic activation during prolonged breath hold has harmful effects on cardiovascular health.

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References


