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Central chemoreflex sensitivity and sympathetic neural outflow in elite breath-hold divers

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1Department of Physiology, University of Split School of Medicine, Split, Croatia; 2Franz-Volhard Clinical Research Center, Max Delbrück Center, Charité Campus Buch, and HELIOS Klinikum Berlin, Berlin, Germany; 3Department of Neurology, Clinical Hospital Split, Split, Croatia; 4Autonomic Dysfunction Service, Vanderbilt University, Nashville, Tennessee; and 5Department of Anesthesiology, Mayo Clinic College of Medicine, Rochester, New York

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Dujic Z, Ivancev V, Heusser K, Dzamonja G, Palada I, Valic Z, Tank J, Obad A, Bakovic D, Diedrich A, Joyner MJ, Jordan J. Central chemoreflex sensitivity and sympathetic neural outflow in elite breath-hold divers. J Appl Physiol 104: 205–211, 2008. First published November 8, 2007; doi:10.1152/japplphysiol.00844.2007.—Repeated hypoxemia in obstructive sleep apnea patients increases sympathetic activity, thereby promoting arterial hypertension. Elite breath-holding divers are exposed to similar apneic episodes and hypoxemia. We hypothesized that trained divers would have increased resting sympathetic activity and blood pressure, as well as an excessive sympathetic nervous system response to hypercapnia. We recruited 11 experienced divers and 9 control subjects. During the diving season preceding the study, divers participated in 7.3 ± 1.2 diving fish-catching competitions and 76.4 ± 14.6 apnea training sessions with the last apnea 3–5 days before testing. We monitored beat-by-beat blood pressure, heart rate, femoral artery blood flow, respiration, end-tidal CO2, and muscle sympathetic nerve activity (MSNA). After a baseline period, subjects began to rebreathe a hyperoxic gas mixture to raise end-tidal CO2 to 60 Torr. Baseline MSNA frequency was 31 ± 11 bursts/min in divers and 33 ± 13 bursts/min in control subjects. Total MSNA activity was 1.8 ± 1.5 AU/min in divers and 1.8 ± 1.3 AU/min in control subjects. Arterial oxygen saturation did not change during rebreathing, whereas end-tidal CO2 increased continuously. The slope of the hypercapnic ventilatory and MSNA response was similar in both groups. We conclude that repeated bouts of hypoxia in elite, healthy breath-holding divers do not lead to sustained sympathetic activation or arterial hypertension. Repeated episodes of hypoxemia may not be sufficient to drive an increase in resting sympathetic activity in the absence of additional comorbidities.

METHODS

Subjects. We included 20 healthy male volunteers in our study. Of those, 11 were experienced elite breath-holding divers. Nine men did not dive regularly and served as control group. The ethical committee of the University of Split School of Medicine approved the study and written informed consent was obtained.

Protocol. We conducted our studies in October 2006 toward the end of the diving season. 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.

Subjects underwent dynamic spirometry (Quark PFT, Cosmed, Rome, Italy) while standing. Then, they were asked to lie down and were instrumented for the rebreathing test. Respiration was measured breath by breath. End-tidal CO2 (PETCO2) was determined using an infrared analyzer (Poet II, Criticare Systems, Waukesha, WI) connected to a mouthpiece. We applied an infrared probe on the middle finger to monitor arterial oxygen saturation (Poet II). Beat-by-beat blood pressure and heart rate were measured using a finger cuff (Finometer, Finapress Medical Systems, Arnhem, The Netherlands) and electrocardiography, respectively. Femoral artery mean blood velocity was determined using pulsed-wave Doppler sonography. The arterial baroreflex to higher blood pressure values together with changes in chemoreflex regulation (18, 21). In animals, intermittent hypoxia augments the sympathetic nervous system response to hypoxia and to hypercapnia (9). A recent study suggested that hypercapnia rather than hypoxia may increase blood pressure and cause baroreflex resetting (2). The state of affairs is disturbing since healthy people, including underwater hockey players, synchronized swimmers, and elite breath-holding divers, practice voluntary apnea on a regular basis. Divers are an extreme example of voluntary apnea. Recently, Tom Sietas set the new world record with more than 9 min of static apnea. After maximal apnea, alveolar partial pressure of oxygen can be as low as 30–40 Torr with an arterial oxygen saturation as low as 50%. Alveolar carbon dioxide partial pressure increases substantially (6). Typically, diving fish-catching competitions last for ~5 h. Cumulative apnea duration during this period is ~1 h. Thus apnea exposures in sleep apnea patients and divers may be similar in severity and duration, at least during the diving season. We hypothesized that trained divers would have increased resting sympathetic activity and blood pressure, as well as an excessive sympathetic nervous system response to hypercapnia.

CARDIOVASCULAR MORBIDITY AND MORTALITY are profoundly increased in patients with obstructive sleep apnea (26, 27). The increased risk may be explained in part by excessive sympathetic activity, endothelial dysfunction, and arterial hypertension (16, 17, 31). Sympathetic activation occurs during sleep apnea episodes as arterial oxygen saturation decreases (17). Sympathetic activation is sustained during the day when patients are breathing normally (8, 23, 24). Similarly, experimental breath holding and intermittent hypoxemia cause acute as well as sustained changes in cardiovascular autonomic regulation (3, 18, 21, 22). Sympathetic activation in clinical and in experimental apnea may be explained by resetting of the arterioles.

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4-MHz ultrasound probe (Transcranial Doppler, Neurovision System, Multigon, Yonkers, NY) was placed over the left femoral artery and held in place with adhesive tape. Muscle sympathetic nerve activity (MSNA) was recorded from the right peroneal nerve with a unipolar tungsten electrode as described previously (12).

After instrumentation, subjects performed two rebreathing tests with a recovery period of 15 min between tests. The hypercapnic ventilatory response was assessed using a slightly modified rebreathing method (28). Subjects were breathing through a mouthpiece with a pneumatic one-way valve. The valve was connected to breath-by-breath spirometer (Harvard Apparatus, Student model, Holliston, MA). The spirometer was filled with 6 liters of a 5% CO$_2$ gas mixture. Subjects were breathing room air normally and quietly at rest for 2 min through the mouthpiece. Then, they were switched to the spirometer and respiration were defined offline for the complete records using a program written by one of the authors (A. Diedrich) that is based on PV-wave software (Visual Numerics, Houston, TX). MSNA bursts were identified after filtering the integrated signal and defining the baseline according to following criteria: 1) signal-to-noise ratio of >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 (33). To assess chemoreflex regulation of ventilation and sympathetic activity, we plotted minute ventilation or MSNA over ET$_{CO_2}$ during the rebreathing test. The data was analyzed using linear regression analysis. Changes in left ventricular stroke volume were estimated by pulse wave analysis using an improved method of Wesseling (Modelflow program) (14). Femoral artery vascular resistance was calculated as mean arterial pressure divided by femoral artery mean blood velocity. Because the duration of the rebreathing test differed between subjects, data were matched for the relative duration of rebreathing.

### Statistical analysis
All data are expressed as means ± SD. The effects of hypercapnia on all measured variables were assessed using a repeated-measures two-way ANOVA procedure and, if significant, a Bonferroni test was used as post hoc test. To compare ventilatory responses to hypercapnia, an unpaired t-test was applied. The relationships between different variables were examined by using the Pearson correlation test. The level of probability for statistical significance was $P < 0.05$.

### RESULTS
Anthropometric and pulmonary function data are given in Table 1. Age, body mass index, and body fat content determined by skinfold thickness (Harpenden skinfold caliper) measurements were similar in divers and in control subjects. During the diving season that immediately preceded the study, divers participated in 7.3 ± 1.2 diving fish-catching competitions and 76.4 ± 14.6 training sessions. Divers performed four to five training sessions per week in the period of 16–20 wk in row before the study. Personal best apnea time and maximal diving depth were 281.6 ± 34.1 s and 31.6 ± 5.2 m, respectively. All divers went apnea diving 3–5 days before start of the study.

Before the rebreathing test, heart rate, mean arterial pressure, femoral artery blood flow velocity, arterial oxygen saturation, and muscle oxygenation were similar in divers and in control subjects (Table 2). Spontaneous ET$_{CO_2}$ tended to be...
decreased in apnea divers. Baseline MSNA frequency and total MSNA activity were similar in both groups (Table 2).

Original tracings of blood pressure, heart rate, respiration, $P_{ETCO_2}$, and sympathetic activity before and during rebreathing in a diver are illustrated in Fig. 1.

Arterial oxygen saturation did not change during rebreathing, whereas $P_{ETCO_2}$ increased continuously. Increased minute ventilation during rebreathing was primarily explained by an increase in tidal volume (Fig. 2). Breathing frequency increased in divers only.

Rebreathing duration until subjects reached a $P_{ETCO_2}$ of 60 Torr was on average 76 s longer in divers than in control subjects. The slope of the hypercapnic ventilatory response was similar in both groups (Table 3). All subjects remained normoxic throughout the rebreathing test.

With hypercapnia, blood pressure increased from 89/110 to 97/110 mmHg in divers and from 92/110 to 98/110 mmHg in control subjects ($P < 0.001$ for both; Fig. 3).

Total peripheral resistance and femoral vascular resistance did not increase during rebreathing. During the recovery period, blood pressure rapidly returned to the baseline value. After rebreathing, total peripheral resistance and femoral vascular resistance decreased below the baseline value. Increased cardiac output maintained blood pressure during this period. During rebreathing, MSNA frequency increased in divers only. However, total MSNA activity increased similarly in both groups (Fig. 4).

We plotted individual sympathetic chemoreflex curves in divers and in control subjects as illustrated in Fig. 5. The slope at the linear portion of these curves was identical in both groups (Fig. 6).

### DISCUSSION

Elite divers regularly explore the limits of human physiology in terms of hypoxia and hypercapnia. Therefore, we reasoned that breath-hold diving could serve as a human model of intermittent asphyxia, similar to the situation that occurs in sleep apnea. The model is appealing because confounding co-morbid conditions are absent in divers. In the present study, we explored cardiovascular sympathetic mechanisms in divers using microneurography. Resting sympathetic activity and blood pressure were similar in breath-hold divers and in control subjects. This observation suggests that the set point of the sympathetic baroreflex was within the normal range in divers. In contrast, intermittent asphyxia during obstructive sleep apnea episodes results in chronically increased MSNA during wakefulness, thus promoting systemic arterial hypertension (8, 23, 24). The observation suggests that intermittent asphyxia in the absence of additional risk factors is not sufficient to drive a sustained increase in sympathetic vasomotor tone and blood pressure. We cannot exclude the possibility that the slope of

### Table 3. Hypercapnic ventilatory response

<table>
<thead>
<tr>
<th></th>
<th>Breath-Hold Divers</th>
<th>Nondivers</th>
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</thead>
<tbody>
<tr>
<td>$SHCVR$, liter/min/mMg</td>
<td>1.7 ± 1.0</td>
<td>2.1 ± 0.76</td>
</tr>
<tr>
<td>$IHCVR$, mMg</td>
<td>30 ± 6.7</td>
<td>37 ± 2.9*</td>
</tr>
</tbody>
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Values are means ± SD. SHCVR, slope of the hypercapnic ventilatory response; IHCVR, intercept of the hypercapnic ventilatory response. *$P < 0.05$. 

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Fig. 2. Minute ventilation ($V_E$), tidal volume ($V_T$), respiratory frequency (RF), and $P_{ETCO_2}$ at baseline, during rebreathing, and during recovery. Significant within-group difference ($P < 0.05$); *when baseline value is compared with rebreathing data points; #when baseline is compared with recovery data points; +when end of rebreathing is compared with recovery data points; and #when between-groups values by repeated-measures ANOVA are compared with Bonferroni post hoc test.
the baroreflex curve was altered as it is in patients with obstructive sleep apnea (23).

Hypercapnia elicits ventilatory, cardiovascular, and autonomic responses mainly through central chemoreceptors located at the ventral surface of the medulla (7). We applied a rebreathing protocol to increase PETCO₂ by ~20 Torr. We added oxygen to achieve selective central chemoreceptor stimulation. With this method, inspired CO₂ is a function of previously expired Pco₂, leading to equilibration of the venous, arterial, and tissue CO₂ (20). Central chemoreflex regulation elicits a profound increase in ventilation. The slope of the hypercapnic ventilatory response was similar in breath-holding divers and in control subjects. However, basal PETCO₂ was slightly reduced in divers. Previously, the ventilatory response to hypercapnia was shown to be blunted in persons engaging in various underwater sports in some (4, 5, 19) but not in all studies (1). Similarly, studies in obstructive sleep apnea patients showed variable hypercapnic ventilatory responses (24, 30, 34).

We observed a similar increase in mean arterial blood pressure in divers and in control subjects during rebreathing. The pressor response was not mediated through systemic vasoconstriction. Hyperoxic hypercapnia increased mean arterial blood pressure and cardiac output in a previous study (29). The hemodynamic response after discontinuation of the rebreathing test differed between divers and control subjects. In both groups, mean arterial blood pressure rapidly returned to the baseline values while heart rate increased. Divers exhibited a marked increase in cardiac output and reduction in systemic vascular resistance after discontinuation. The response was less pronounced or absent in control subjects. The cardiovascular response after discontinuation is somewhat unexpected because PETCO₂ rapidly returned to baseline.

The overall cardiovascular response to hypercapnia may result from direct influences on vascular tone and cardiac contractility together with reflex-mediated changes in sympathetic activity. The microneurography data are useful in dissecting central nervous and peripheral mechanisms. We observed a marked increase in sympathetic nerve traffic during rebreathing with a rapid return to the baseline value after discontinuation. Previous studies showed a similar response (29, 32). Total sympathetic activity increased more markedly than burst frequency. This observation suggests increased recruitment of efferent sympathetic neurons during hypercapnia. Individual relationships between PETCO₂ and sympathetic nerve traffic and sympathetic activity at 60-Torr PETCO₂ were similar in divers and in control subjects.

With an increase in sympathetic vasomotor tone during rebreathing, one would expect to observe systemic vasoconstriction. Instead, vascular resistance remained unchanged. Apparently, hypercapnia interfered with coupling between sympathetic nerve traffic and vascular contraction. Our finding that vascular resistance was decreased in the recovery period supports the idea. We propose that influences of hypercapnia on peripheral vascular regulation were unmasked after discontinuation of the rebreathing protocol. Previous studies assessed peripheral hemodynamic responses to hypercapnia in patients with severe autonomic failure (25) or in healthy subjects.

Fig. 3. Changes in MAP, HR, stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR) are presented as relative values between baseline, five rebreathing data points (%), and six points during recovery (each 20 s). Significant within-group difference (P < 0.05) by repeated-measures ANOVA with Bonferroni post hoc test: *when baseline value is compared with rebreathing data points; #when baseline is compared with recovery data points; and + when end of rebreathing is compared with recovery data points.
during near complete pharmacological ganglionic blockade (15). In the absence of sympathetic nervous system input, hypercapnia elicited a pressor response. Thus a direct influence of CO$_2$ on vascular tone may not explain our findings. Possibly, hypercapnia altered norepinephrine and/or epinephrine release through presynaptic mechanisms. Acidosis has been shown to attenuate electrically evoked norepinephrine release (11).

The main limitation of our study is that the divers did not undergo standardized diving protocols before the study. Even when all precautions are taken into account, breath-hold diving is not without risk. Deadly accidents can and do occur. We therefore did not assign our subjects additional dives for the
purpose of standardization. In previous studies, combined hypoxia with or without hypercapnia evoked sympathetic activation in human subjects that was sustained for more than an hour when subjects returned to room air breathing (3, 22, 35). Because we did not study our subjects immediately after diving, we cannot exclude a short-lived increase in sympathetic activity and blood pressure. Indeed, in obstructive sleep apnea patients, treatment with a continuous positive airway pressure apparatus appears to attenuate sympathetic activity within 2 wk (13). In another study, exposure to hypobaric hypoxia resulted in sympathetic activation in healthy subjects. Sympathetic activity was still increased 3 days after return to sea level (10). Our subjects performed their last dives 3–5 days before start of the study. The observation that hypobaric hypoxia but not apnea diving elicits sustained sympathetic activation over several days is surprising. One possible explanation is a difference in the physiological stimulus elicited by high altitude and repeated breath holding. For example, in high altitude, hypoxia occurs together with hyperventilation-induced hypocapnia. Breath holding causes hypercapnia and hypocapnia.

**Perspective**

Repeated bouts of hypoxemia in elite divers do not lead to sustained sympathetic activation or arterial hypertension. Moreover, central chemoreflex control of respiration and sympathetic activity is maintained in these unique individuals. Preserved central chemoreflex control of sympathetic activity may serve as a protective mechanism by stabilizing blood pressure during hypercapnia. Sympathetic responses differ markedly between apnea divers and patients with obstructive sleep apnea. Our findings suggest that repeated episodes of hypoxemia alone are not sufficient to drive an increase in resting sympathetic activity in the absence of additional comorbidities.

**ACKNOWLEDGMENTS**

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**GRANTS**

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