

Repeated Apneas and Hypercapnic Ventilatory Response Before and After Apnea Training

Dimitrios I. Bourdas; Theodoros S. Tsakiris; Konstantinos I. Pavlakis; Despoina V. Triantafillou; Nickos D. Geladas

- BACKGROUND:** Habitual exposure to carbon dioxide (CO₂) is expected, but it is not proven, to dull ventilatory sensitivity to CO₂ by reducing hypercapnic ventilatory response (HCVR) as it is expressed by the slope of the derived response curve (CO₂ sensitivity: $\Delta V_E / \Delta P_{et} CO_2$). It was hypothesized that HCVR is decreased by repeated breath hold maximal efforts (RBHE) before and after apnea training in comparison with no training and the control condition.
- METHODS:** Two groups of breath holders, a control (CBH) group and novices to breath hold activities (NBH), visited the laboratory on four different occasions. In the first visit, subjects performed a HCVR test, whereas in the second visit they completed five successive RBHE separated by 2-min intervals. Another HCVR test was performed 2 min after cessation of the last apnea. For the next 14 d, only the NBH group trained by performing daily five RBHE separated by 2-min intervals. Subsequently, in a third and a fourth condition, subjects repeated the experimental protocol of the second and first visit.
- RESULTS:** Although breath hold time (BHT) increased after apnea training in the NBH group by ~46%, CO₂ sensitivity slopes were not different among experimental conditions and groups (2.8 ± 0.3 , $2.9 \pm 0.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ in the CBH and 2.7 ± 0.5 , $2.7 \pm 0.3 \text{ L} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ in the NBH during the second and third visit, respectively).
- CONCLUSION:** HCVR after five RBHE or 14 d of apnea training was not decreased despite the achieved BHT enhancement. Hypercapnic dullness of ventilation is a complex biological process which takes more than 14 d of training to develop.
- KEYWORDS:** breath hold time, CO₂ sensitivity, novice apnea divers.

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Pulmonary ventilation is impressively variable and sensitive to carbon dioxide (CO₂) alterations in order to keep to a minimum any fluctuation of arterial partial pressure of CO₂ (P_aCO₂). Hypercapnic ventilatory response (HCVR) is defined as the ventilatory responsiveness to breathing high concentrations of CO₂. Scuba divers exhibit low ventilatory sensitivity to CO₂ and high whole-body CO₂ retention.^{18,21,39} Trained skin divers, probably due to familiarization and experience, also seem to develop chronic low chemosensitivity to CO₂,^{19,40} which may lead to a reduced need for ventilation.

Apnea, namely breath hold (BH) activity, is usually explored in a repeated form of five to eight successive maneuvers, without or with face immersion (BH_{FI}) in cool water, interspersed with brief intervals among trials. In a study adopting this experimental protocol, Bakovic et al. demonstrated a significant temporary CO₂ retention after the apnea efforts in comparison to the levels of CO₂ before BH;⁵ this phenomenon lasted at least 60 min both in apnea divers and in untrained

subjects. Based on this finding, repeated BH_{FI},⁵ as well as apnea training,¹⁷ have been assumed to reduce chemosensitivity, resulting in a reduced need for breathing. In essence, it is widely accepted by the scientific community that reduction of ventilatory sensitivity to CO₂ during repeated consecutive BH does exist and this may contribute to breath hold time being prolonged as efforts are repeated, ignoring the fact that an old study reported normal ventilatory responses to hypercapnia in elite apnea athletes.⁹

Recently, it has been shown that five repeated apneas do not alter HCVR and, consequently, any gradual improvement

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of breath hold time (BHT) in each consecutive apnea could not be due to HCVR alteration.³ In this study, however, on one hand a control group was not used and, on the other, the sample was heterogeneous since it included experienced and novice apnea subjects of both sexes. Sex, although not having been examined thoroughly, is likely to influence BHT in many ways.^{27,37,38} For instance, traits affecting diving performance, such as regulation of breathing, intensity of diving reflex, pulmonary volume, ability to store and transport oxygen by blood, and hypoxic and hypercapnic ventilatory response, are known to be affected either by sex or by the menstrual cycle in women.^{1,36,41} In addition, subjects who have different experiences with apnea might well have different CO₂ chemosensitivity. It is known from a previous study that chemosensitivity of instructors working at a submarine escape training tank increased after 3 mo absence from training in relation to the baseline measurements.²⁸ There is a great lack of information regarding the factors determining effectiveness of apnea training. In novice skin divers, 2 wk of apnea training appeared to be sufficient to induce bradycardia, which probably contributes to maximal BHT enhancement.³⁵ Therefore, it remains entirely unknown and with paramount importance for a number of apnea activities (i.e., athletics, sleep apnea) whether a small period of apnea exposure (five successive maneuvers) could, before and after moderate training in apnea, reduce ventilatory CO₂ sensitivity. It was hypothesized that five repeated BH efforts, as well as 2 wk of apnea training, would constitute sufficient stimulus to decrease HCVR in novice apnea subjects. Consequently, the main aim of this study was to examine whether HCVR measured at rest is influenced after five repeated BH before and/or after a moderate BH training program using a sound experimental design.

METHODS

Subjects

After approval of the protocol by local the ethical committee, 19 healthy nonsmoking men with moderate aerobic power ($43.9 \pm 1.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, mean \pm SE) gave their informed consent to participate in this study. All subjects were taking no medication and had no previous breath holding experience ($0.5 \text{ min} < \text{BHT} < 3.5 \text{ min}$). Subjects were matched according to their aerobic power and randomly assigned in a double-blind fashion to either a control breath holders (CBH = 9) or novice breath holders (NBH = 10) group. Their age, weight, height, fat mass (FM), and forced vital capacity (FVC) values were $22.9 \pm 2.0 \text{ yr}$, $80.9 \pm 2.5 \text{ kg}$, $180.2 \pm 1.8 \text{ cm}$, $12.2 \pm 1.8\%$, and $6.1 \pm 0.2 \text{ l}$ for the CBH, and $21.7 \pm 0.9 \text{ yr}$, $84.4 \pm 2.2 \text{ kg}$, $180.7 \pm 1.3 \text{ cm}$, $13.5 \pm 1.2\%$, and $5.9 \pm 0.2 \text{ l}$ for the NBH group. Additionally, nine different men from the main study subjects (age: $24.8 \pm 1.5 \text{ yr}$, height: $178.0 \pm 1.5 \text{ cm}$, mass: $82.1 \pm 3.2 \text{ kg}$) were used in order to assess, in an initial pilot study, HCVR reproducibility.

Experimental Procedures

Subjects visited the laboratory on four different occasions, each one representing a different experimental condition (Fig. 1). On the first visit, constituting the baseline measurement (BM) condition, all subjects got familiarized with the methods adopted in the study. Afterwards, on the same day and with the following order, measurements were conducted for weight and height characteristics (scale–stadiometer, Balance Salus S12, Gaggiano, Italy), FVC in a sitting position (MedGraphics CPX/D, St. Paul, MN), HCVR, applying a modified rebreathing Read method,²⁶ and $\dot{V}O_{2\text{max}}$ (MedGraphics CPX/D), adopting a maximal effort graded exercise test starting at 30 W (increments of $30 \text{ W} \cdot \text{min}^{-1}$ to exhaustion) on a cycle ergometer (Lode B.V., Groningen, The Netherlands).

On a second visit 2 to 3 d later which constituted the PRE condition, subjects performed five repeated maximal-effort apneas from a prone position with the face immersed (BH_{FI}) in cold water (12°C), which benefits BHT,^{16,29,32} with an intermediate recovery period of 2 min between efforts. Under a subject's head was a water container covered by a removable wooden pillow layered with a soft hypoallergenic material where the subjects rested their heads. The subjects always kept their arms in a horizontal position beside their heads; they relaxed for 7 min and a stable resting heart rate (HR) was established. The subjects breathed via a mouthpiece and a series of two manual three-way valves (Hans Rudolph, Inc., Kansas City, MO). The first one connected directly to an automated open-circuit spirometer (MedGraphics CPX/D) in order to record breath-by-breath

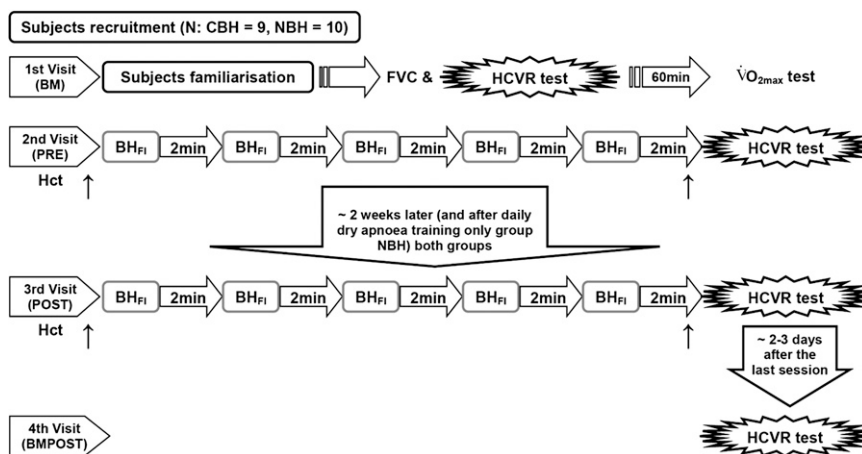


Fig. 1. Schematic depiction of the experimental design. NBH: novice breath holders group subjected to five successive repeated breath hold maximal efforts with face immersion in cold water (BH_{FI}) separated by 2-min intervals (PRE condition) and 2 wk of apnea training (POST condition); CBH: control breath holders group subjected only to five successive repeated BH_{FI} separated by 2-min intervals (PRE and POST). HCVR: hypercapnic ventilatory response, Hct: hematocrit, FVC: forced vital capacity, $\dot{V}O_{2\text{max}}$: maximal oxygen uptake, ↑ indicates the time of blood sampling.

ventilation (V_E) and partial pressure of end tidal CO_2 ($P_{et}CO_2$). The subjects always wore a nose clip (Paradis, Villefranche-sur-mer, France). The second one was connected with an elastic vinyl bag of 5-L maximum capacity (Vacumed, Ventura, CA) filled with atmospheric air, which was inhaled by the subjects just one moment before BH_{FI} . Subjects were verbally given temporal information at 60 s, 30 s, and 15 s prior to each BH_{FI} . At 10 s prior to each BH_{FI} , a verbal signal reminded the subjects to raise their heads while the pillow was removed. At that time subjects fully exhaled to their residual volume. Then, they inhaled 85% of their FVC and, while the mouthpiece with the attached bag was removed by a researcher, subjects immersed their entire face, including chin and forehead, in the water (12°C). No hyperventilation, swallowing, or any kind of maneuvers, such as Valsava or Mueller, were allowed.⁷ As soon as subjects raised their head above the water's surface upon termination of each BH_{FI} , they immediately repositioned their head on the cover pillow to rest and a standby researcher inserted the mouthpiece into their mouth for exhalation to the metabolic cart. Breath hold time and the intermediate intervals between BH_{FI} were recorded using two stopwatches (Casio HS30W, Shibuya, Tokyo, Japan) without giving the subjects any feedback about running time. Hemodynamics and arterial hemoglobin oxygen saturation were also continuously recorded with a cuff attached on the right middle finger (Finometer, Finapres Medical Systems BV, Amsterdam Zuidoost, The Netherlands) and a pulse oximeter (Nellcor Symphony N-3000, Covidien, Mansfield, MA) on the left pointer finger, respectively, only for safety reasons.

Subjects carried out a HCVR test 2 min after the last BH_{FI} . The starting time of the second lab visit was about 30 min earlier than the HCVR test of the first visit, so both HCVR tests were performed in approximately the same time frame. The next day, for 14 consecutive days, the NBH group trained under supervision, performing every day a series of five dry, maximal-effort apneas without hyperventilation separated with 2-min intervals at rest in a sitting or a supine position. In a third lab visit 2 wk later, constituting the POST condition, subjects repeated the experimental protocol of the second lab visit at the same time of the day. In the fourth lab visit 2-3 d after the last session, constituting the BMPOST condition, all subjects repeated the HCVR as in the first visit at the same time of the day. The HCVR test was always conducted in a sitting position 10 min after the FVC test [first and fourth visit] or 2 min after the five repeated BH_{FI} [second and third visit]. Briefly, at the beginning of the HCVR test, subjects fully exhaled through the spirometer mouthpiece and then were connected with a bag containing hyperoxic-capnic gas (94% of O_2 enriched with ~6% CO_2), where they rebreathed until $P_{et}CO_2$ reached 65 mmHg for a 4-min minimum to 6-min maximum. Upon initiation of the HCVR test, the plot of V_E vs. $P_{et}CO_2$ showed an initial plateau, followed at a threshold point by a steep linear increase. With respect to $P_{et}CO_2$, the threshold (HCVRT) was determined at the point where V_E responsiveness started increasing in an obvious way, confirmed by linear fitting analysis ($y = ax + b$). Initial

or final data which fell outside the line were excluded from the analysis based on the criterion that correlation value r had to be greater than 0.95. Derived slope of the response curve corresponded to $\Delta V_E / \Delta P_{et}CO_2$, indicating ventilation sensitivity to CO_2 [for details and validity of the HCVR test see Read and Leigh²⁵ and Read²⁶]. Finger blood samples were taken at rest and immediately after the end of the five repeated BH_{FI} in order to measure hematocrit (Hct) level (Miniphotometer plus LP20, Dr. Lange, Hamburg, Germany). All instruments that were used were calibrated according to manufacturers' manuals prior to each laboratory test. Subjects abstained from heavy exercise for 3 d before each experimental condition and fasted for 3 h before arrival at the laboratory. Prior to each condition, subjects were asked to consume the same meals so as to best duplicate pretrial macronutrient intake. Each experimental condition was conducted at the same standard laboratory condition [24–25°C and $50 \pm 5\%$ relative humidity].

Statistical Analysis

All variables are presented as mean \pm SE unless otherwise stated. Shapiro-Wilks tests and Q-Q graphs of dependent variables indicated that data were normally distributed ($p > 0.05$). Independent t -tests were used for comparing anthropometric and physiological traits between groups and dependent t -tests were used for comparing one variable in the same group in different conditions. ANOVA with two factors was also used for analyzing average BHT, HCVR, and respiratory data. One factor entailed two independent levels (control and training group; different subjects in each group, CBH vs. NBH). The other factor entailed four dependent levels (repeated measures of the same subjects) during visits 1, 2, 3, and 4. Data collected during repeated breath hold maximal efforts (RBHE) were analyzed with a 2 (NBH, CBH) \times 2 (Pre, Post training) \times 5 (BH efforts) ANOVA design with repeated measures on the second and third level, and Bonferroni post hoc tests were used whenever a significant main effect or interaction was found. The statistical level of significance was set at $p \leq 0.05$. SPSS 20.0 (IBM) was used for statistical analysis and GPower 3.1 (HHU, Düsseldorf, Germany) was used for power analysis.

RESULTS

Age, weight, height, FM, and FVC values were not different between subjects in the CBH and NBH groups ($p = 0.617$, $p = 0.304$, $p = 0.822$, $p = 0.508$, $p = 0.410$, respectively). Similarly, no differences were found in subjects' Hct (%) at rest (43.8 ± 1.0 and 43.2 ± 0.8 for the CBH in the PRE and POST conditions, $p = 0.154$; 44.0 ± 0.5 and 43.5 ± 0.6 for the NBH group in the PRE and POST conditions, $p = 0.343$). After the five repeated BH_{FI} efforts, the mean delta (post-pre BH effort) values of Hct (ΔHct) were increased above rest values by 2.4% for the NBH in the POST condition, which was significantly different from the PRE condition ($t(9) = -2.866$, $p \leq 0.019$; Fig. 2).

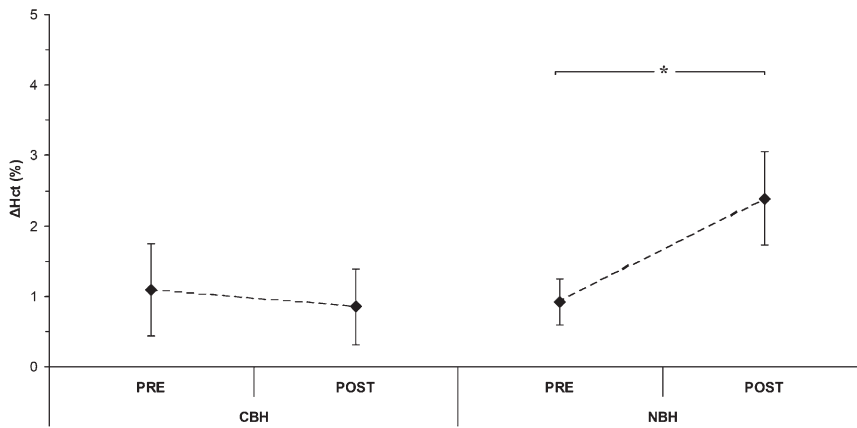


Fig. 2. Mean values (\pm SE) of the average delta (post-pre BH effort) of Hct (Δ Hct) after five repeated apneas in the pre-training condition (PRE) and after the apnea training intervention (POST) in the control (CBH) and training groups (NBH). * Indicates significant difference between conditions, $p \leq 0.05$.

Subjects' BHT values ranged from 82.4 to 180.0 s and 75.2 to 166.5 s for the CBH group in the PRE and POST conditions, and 61.0 to 196.0 s and 124.0 to 258.0 s for the NBH group in the PRE and POST conditions. A repeated measures ANOVA revealed that from the third effort onwards, BHT values were significantly higher than the values of the initial three BH_{F1} trials in the NBH in both conditions [PRE: $F(3.2, 29.0) = 13.7$, $p \leq 0.001$; POST: $F(2.9, 26.5) = 10.2$, $p \leq 0.001$] and in CBH, in the PRE [$F(2.7, 21.5) = 9.6$, $p \leq 0.001$], but marginally not in the POST condition ($p = 0.096$). On the average, BHT of the NBH group in the POST after-training condition (147.1 ± 5.9 s) was significantly higher than the BHT of the same group in the PRE condition (100.7 ± 5.6 s), as well as of the CBH group in the PRE (105.5 ± 5.0 s) and POST (107.1 ± 5.0 s) conditions [$F(1.0, 34.0) = 4.3$, $p = 0.045$].

Hypercapnic ventilatory response data (Fig. 3) showed that the slope curve of the relationship between ΔV_E and ΔP_{etCO_2} did not differ between groups ($p = 0.760$) or conditions ($p = 0.974$; Table I, power: 0.097). With regard to the

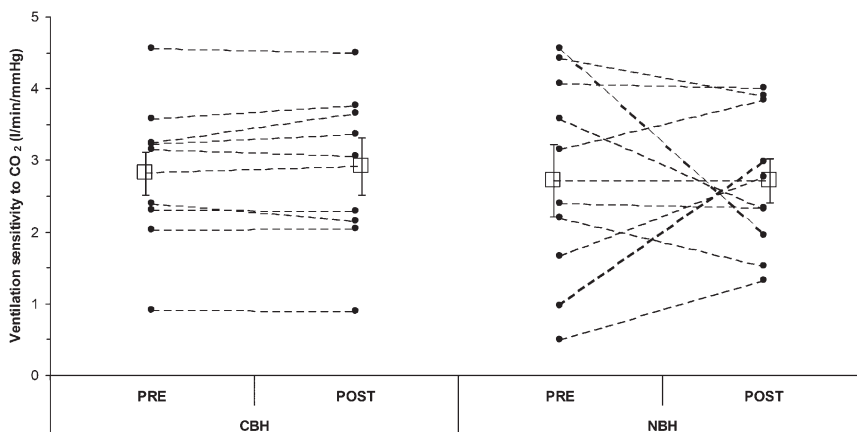


Fig. 3. Individual values (filled circles) and groups mean (white squares, \pm SE) for the slope curve of the hypercapnic ventilatory response test (CO_2 sensitivity: $\Delta V_E / \Delta P_{etCO_2}$) after five repeated apneas in the pre-training condition (PRE) or after the apnea training intervention (POST) in the control (CBH) and apnea training groups (NBH).

respective threshold analysis, no acute or moderate BH training effects were found in either group ($p = 0.454$; Table II). Similarly, the average values of resting P_{etCO_2} and partial pressure of end tidal O_2 (P_{etO_2}) after repeated BH_{F1} efforts in both the CBH and NBH groups were not different between the PRE and POST conditions ($p = 0.401$ and $p = 0.084$, respectively; Table II). During RBHE maneuvers throughout all conditions P_{etCO_2} returned to pre-apneic values prior to the next breath hold effort. Furthermore, no significant difference was observed in post breath hold V_E values between the PRE and POST conditions ($p = 0.503$; Table II), since in both conditions and groups hyperventilation was not allowed.

Reproducibility of the HCVR was tested in an initial pilot study where subjects were evaluated twice. A high Pearson-product moment correlation coefficient between the slope curves of the HCVR data of the two different measurements was provided ($r = 0.99$, $p \leq 0.001$). Mean differences (Δ) between the two trials were at an acceptable level of -0.02 ± 0.05 according to the Bland Altman plot (Fig. 4).¹⁰ Moreover, the coefficient of variation of Δ between the two series was $\sim 1.6\%$.

DISCUSSION

The purpose of this study was to examine, in novice apnea subjects, to what extent BH_{F1} maneuvers are capable of changing the hypercapnic ventilatory response either in acute, five static breath hold efforts, or in a prolonged, 2-wk daily moderate apnea training. The experimental protocol of five repeated static BH_{F1} with a 2-min interval in between has been widely used.^{3,6,34} The present study tested 2 equivalent groups (control and training) randomly derived from a pool of 19 subjects. There were no differences between the groups in any variable in the PRE condition and the CBH group did not show any change in any of the variables tested between PRE and POST; the NBH group showed a significant increase in BHT between the PRE and POST conditions. Namely, five repeated static BH_{F1} efforts with a 2-min interval were capable of increasing BHT compared to the values attained in the first effort before and after a 2-wk dry apnea training. Contrary to our assumptions, it was found that HCVR remained unaltered in both the acute (five BH_{F1} efforts) and

Table I. Mean Values (\pm SE) for the Slope Curve of the Hypercapnic Ventilatory Response Test (CO_2 Sensitivity: $\Delta V_E/\Delta P_{\text{etCO}_2}$) at Initial Baseline (BM), After Five Repeated Apneas in The Pre-Training Condition (PRE), After Five Repeated Apneas in the Post Training Intervention (POST), and Final Baseline (BMPOST) in the Control (CBH) and the Training Group (NBH).

	VENTILATORY SENSITIVITY TO CO_2 ($\text{L} \cdot \text{MIN}^{-1} \cdot \text{MMHG}^{-1}$)							
	CBH				NBH			
	BM	PRE	POST	BMPOST	BM	PRE	POST	BMPOST
Mean	2.6	2.8	2.9	2.9	2.4	2.7	2.7	2.3
SE	0.3	0.3	0.4	0.4	0.6	0.5	0.3	0.5

moderate training (2-wk apnea training) interventions. That means that HCVR is not a contributing factor to BHT prolongation observed after five repeated dives or after 2 wk of dry apnea training. It appears that a more extensive, longer than 2-wk training period is probably needed in order for HCVR to be altered. The exact minimum training period which would be sufficient to decrease HCVR remains a subject of further research.

In the present study, values of ventilatory sensitivity to CO_2 either after five repeated BH_{FI} or after BH dry training for 2 wk were within the range of normal values.^{12,20,39} Carbon dioxide chemosensitivity, as indicated by the slope curve of the $\Delta V_E/\Delta P_{\text{etCO}_2}$ ratio, remained unaltered after five BH efforts, as Andersson and Schagatay have also shown.³ However, for the first time the present study proved, unexpectedly, that ventilatory sensitivity to CO_2 was not altered after 14 d of dry apnea training. The classic notion that apnea reduces respiratory chemosensitivity to carbon dioxide, and, in extension, on the one hand diminishes the need for inspiration and on the other extends breath hold time, was not verified. This notion has probably originated from old papers, which had used different, questionable methodologies for testing ventilatory responses to high CO_2 .^{18,40} For instance, in 1963,⁴⁰ 20 female Korean ama divers and a control group were initially connected to a bag containing 3% CO_2 in O_2 for 15 min while breath frequency was counted; afterwards, they repeated an identical protocol, but inspiring a mixture of 5.5% O_2 diluted in nitrogen. At rest and at the end of each ventilatory test an alveolar gas sample was obtained for analysis of CO_2 in a Scholander microgas analyzer. By comparing the hypercapnic ventilatory responses to the rest values of each group, they concluded that ama divers had ventilatory carbon dioxide hyposensitivity. In another old study,¹⁸ 10 experienced scuba divers were compared with 10 nondivers of similar age and physique. Ventilatory response to CO_2 was tested under a

steady-state condition.¹³ The divers exhibited lower respiratory response to CO_2 , but the sensitivity method which was used¹³ has been estimated to be, on average, as half that obtained by the classical Read rebreathing method.⁸ Although we cannot rule out methodological differences to explain incompatible results between old and new studies examining ventilatory sensitivity to carbon dioxide, it is noteworthy that both of the previously mentioned old studies used experienced skin and scuba divers.

In the literature, there is a great paucity of data concerning novice divers and HCVR and a sparse and conflicting coverage regarding experienced free divers.^{9,19,28} Ventilatory response to CO_2 was similar in 10 elite women performing synchronized swims and 10 age-matched controls when a modified rebreathing Read method was applied.⁹ Contrariwise, when a steady-state ventilatory response to CO_2 test was applied on three members of the same family, who were elite breath hold divers, a blunted ventilatory response to acute hypercapnia was found compared to nine healthy untrained control subjects.¹⁹

In the only relevant study, Bakovic et al.⁵ showed that five repeated BH efforts with face immersion in 12°C water and a 2-min interval in between is associated with a significant temporary retention of subcutaneous CO_2 that lasts at least 60 min, in apnea divers as well as in untrained subjects, in relation to the levels of CO_2 before the BH.⁵ It is noteworthy that the statistically significant postdive subcutaneous CO_2 found in this study was only slightly elevated and it does not necessarily imply central CO_2 alterations. It could only be a reflection of CO_2 unloading from the tissues to the blood, with the respiratory response remaining regular. Similarly with subcutaneous CO_2 baseline values in Bakovic's study, baseline P_{etCO_2} values in the present study ranged within a normal spectrum of values, arguing against a permanent CO_2 retention after five BH efforts in novice apnea divers.

Table II. Mean Values (\pm SE) of Post-Apneic Ventilation (V_E), Partial Pressure of End Tidal CO_2 (P_{etCO_2}) and O_2 (P_{etO_2}), and Hypercapnic Ventilatory Response Threshold (HCVRT) After Five Repeated Apneas in the Pre-Training Condition (PRE) and After Five Repeated Apneas in the Post-Training Intervention (POST) in the Control (CBH) and Training Groups (NBH).

	CBH		NBH	
	PRE	POST	PRE	POST
V_E ($\text{L} \cdot \text{min}^{-1}$)	10.28 \pm 0.80	9.78 \pm 0.68	9.93 \pm 0.76	11.82 \pm 1.53
P_{etCO_2} (mmHg)	37.85 \pm 1.83	40.61 \pm 0.97	39.77 \pm 2.05	39.59 \pm 0.81
P_{etO_2} (mmHg)	100.54 \pm 3.16	101.50 \pm 1.66	96.28 \pm 1.96	102.69 \pm 1.12
HCVRT (mmHg)	49.33 \pm 1.43	49.23 \pm 1.48	51.40 \pm 1.66	49.30 \pm 1.17

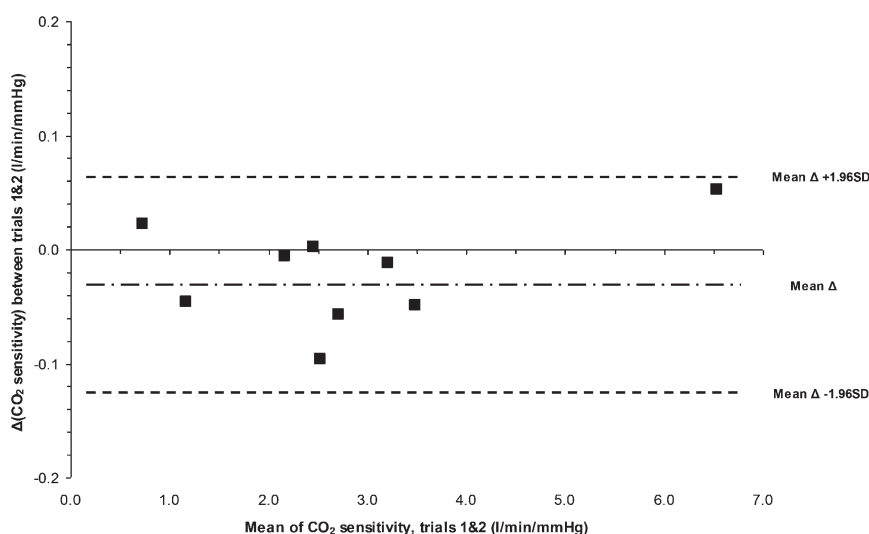


Fig. 4. Difference between values of the first and second measurement of hypercapnic ventilatory response against their mean in nine subjects; data refer to the slope curve (CO_2 sensitivity: $\Delta V_E/\Delta P_{\text{et,CO}_2}$). The mean difference is -0.02 with a 95% confidence interval; the limits of agreement ($0.07 - -0.11 = \pm 1.96 \text{ SD}$) are small enough to consider the test reliable.

Due to technical difficulties, HCVR was measured 2 min after terminating the breath hold maneuvers, which might have contaminated the recorded CO_2 sensitivity. It appears that the elimination of excess CO_2 developed during apneas can be eliminated within 2 min.^{22,31} However, it is a common practice for HCVR to be measured either 2 min after the last apnea³ or at rest without any apnea preceding.^{9,14}

Despite HCVR not being altered, 2 wk of daily apnea training resulted in higher BHT in relation to pre-training values. This could be attributed to a number of factors, such as individual differences in vital capacity,^{11,17,33} enhanced tolerance to unpleasant hypoxic/hypercapnic stimuli,^{15,34} a high ability to overcome involuntary movements of respiratory muscles,²⁴ and a different hematological profile.⁴ In the present study, not only both groups had, on average, similar vital capacity values, but all apnea maneuvers were performed at 85% of the individual vital capacity as is indicated by the literature.^{2,23} In addition, both groups tested started repeated BH_{FI} with no differences in Hct levels. However, after 2 wk of dry BH training, the NBH group's Hct increased more compared to PRE condition values. This is an original finding, probably associated with the increase in BHT in the respective condition. Theoretically, higher values of Hct are desirable, particularly when it is caused by an active contraction of the spleen, resulting in systemic circulation enrichment with red blood cells;^{6,30} this phenomenon may contribute not only to a better oxygen delivery, but also to an improvement of acid-base balance by buffering CO_2 accumulation.

In conclusion, the present study showed that ventilatory chemosensitivity to carbon dioxide is not reduced after 5 repeated apneas before or after 14 daily sessions of dry apnea training. Therefore, the prolongation of breath hold time

observed in earlier studies should probably be attributed to other factors such as improvements in blood profile and enhancement in psychological stamina to unpleasant stimuli.

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REFERENCES

1. Aitken ML, Franklin JL, Pierson DJ, Schoene RB. Influence of body size and gender on control of ventilation. *J Appl Physiol* (1985). 1986; 60(6):1894–1899.
2. Andersson J, Schagatay E. Effects of lung volume and involuntary breathing movements on the human diving response. *Eur J Appl Physiol Occup Physiol*. 1998; 77(1-2):19–24.
3. Andersson JP, Schagatay E. Repeated apneas do not affect the hypercapnic ventilatory response in the short term. *Eur J Appl Physiol*. 2009; 105(4): 569–574.
4. Baković D, Eterović D, Saratlija-Novaković Z, Palada I, Valic Z, et al. Effect of human splenic contraction on variation in circulating blood cell counts. *Clin Exp Pharmacol Physiol*. 2005; 32(11):944–951.
5. Baković D, Eterović D, Valic Z, Saratlija-Novaković Z, Palada I, et al. Increased pulmonary vascular resistance and reduced stroke volume in association with CO_2 retention and inferior vena cava dilatation. *J Appl Physiol* (1985). 2006; 101(3):866–872.
6. Baković D, Valic Z, Eterović D, Vuković I, Obad A, et al. Spleen volume and blood flow response to repeated breath-hold apneas. *J Appl Physiol* (1985). 2003; 95(4):1460–1466.
7. Bartlett D Jr. Effects of Valsalva and Mueller maneuvers on breath-holding time. *J Appl Physiol Respir Environ Exerc Physiol*. 1977; 42(5):717–21.
8. Berkenbosch A, Bovill JG, Dahan A, DeGoede J, Olievier IC. The ventilatory CO_2 sensitivities from Read's rebreathing method and the steady-state method are not equal in man. *J Physiol*. 1989; 411:367–377.
9. Bjurström RL, Schoene RB. Control of ventilation in elite synchronized swimmers. *J Appl Physiol* (1985). 1987; 63(3):1019–1024.
10. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986; 1(8476): 307–310.
11. Carey CR, Schaefer KE, Alvis HJ. Effect of skin diving on lung volumes. *J Appl Physiol*. 1956; 8(5):519–523.
12. Chang LP, Lundgren CE. Maximal breath-holding time and immediate tissue CO_2 storage capacity during head-out immersion in humans. *Eur J Appl Physiol Occup Physiol*. 1996; 73(3-4):210–218.
13. Cunningham DJ, Cormack RS, O'Riordan JL, Jukes MG, Lloyd BB. An arrangement for studying the respiratory effects in man of various factors. *Q J Exp Physiol Cogn Med Sci*. 1957; 42(3):294–303.

14. Dujic Z, Ivancev V, Heusser K, Dzamonja G, Palada I, et al. Central chemoreflex sensitivity and sympathetic neural outflow in elite breath-hold divers. *J Appl Physiol* (1985). 2008; 104(1):205–211.
15. Elsner R, Gooden B. *Diving and asphyxia: a comparative study of animals and man*. Cambridge (MA): Cambridge University Press; 1983.
16. Espersen K, Frandsen H, Lorentzen T, Kanstrup IL, Christensen NJ. The human spleen as an erythrocyte reservoir in diving-related interventions. *J Appl Physiol* (1985). 2002; 92(5):2071–2079.
17. Ferretti G, Costa M. Diversity in and adaptation to breath-hold diving in humans. *Comp Biochem Physiol A Mol Integr Physiol*. 2003; 136(1):205–213.
18. Florio JT, Morrison JB, Butt WS. Breathing pattern and ventilatory response to carbon dioxide in divers. *J Appl Physiol Respir Environ Exerc Physiol*. 1979; 46(6):1076–1080.
19. Grassi B, Ferretti G, Costa M, Ferrigno M, Panzacchi A, et al. Ventilatory responses to hypercapnia and hypoxia in elite breath-hold divers. *Respir Physiol*. 1994; 97(3):323–332.
20. Hirshman CA, McCullough RE, Weil JV. Normal values for hypoxic and hypercapnic ventilatory drives in man. *J Appl Physiol*. 1975; 38(6):1095–1098.
21. Kerem D, Melamed Y, Moran A. Alveolar PCO₂ during rest and exercise in divers and non-divers breathing O₂ at 1 ATA. *Undersea Biomed Res*. 1980; 7:117–26.
22. Linér MH, Linnarsson D. Tissue oxygen and carbon dioxide stores and breath-hold diving in humans. *J Appl Physiol* (1985). 1994; 77(2):542–547.
23. Overgaard K, Friis S, Pedersen RB, Lykkeboe G. Influence of lung volume, glossopharyngeal inhalation and P(ET) O₂ and P(ET) CO₂ on apnea performance in trained breath-hold divers. *Eur J Appl Physiol*. 2006; 97(2):158–164.
24. Parkes MJ. Breath-holding and its breakpoint. *Exp Physiol*. 2006; 91(1):1–15.
25. Read DJ, Leigh J. Blood-brain tissue PCO₂ relationships and ventilation during rebreathing. *J Appl Physiol*. 1967; 23(1):53–70.
26. Read DJ. A clinical method for assessing the ventilatory response to carbon dioxide. *Australas Ann Med*. 1967; 16(1):20–32.
27. Sánchez J, Sébert P. Sex differences in cardiac responses to breath holding during dynamic and isometric exercises. *Eur J Appl Physiol Occup Physiol*. 1983; 50(3):429–444.
28. Schaefer KE. Adaptation to breath hold diving. In: Rahn H, Yokoama T, editors. *Physiology of breath hold diving and the ama of Japan*. Washington (DC): National Academy of Sciences, National Research Council; 1965:237–252. Publication 1341.
29. Schagatay E, Andersson J. Diving response and apneic time in humans. *Undersea Hyperb Med*. 1998; 25(1):13–9.
30. Schagatay E, Andersson JP, Hallen M, Palsson B. Selected contribution: role of spleen emptying in prolonging apneas in humans. *J Appl Physiol* (1985). 2001; 90(4):1623–1629.
31. Schagatay E, Andersson JP, Nielsen B. Hematological response and diving response during apnea and apnea with face immersion. *Eur J Appl Physiol*. 2007; 101(1):125–132.
32. Schagatay E, Holm B. Effects of water and ambient air temperatures on human diving bradycardia. *Eur J Appl Physiol Occup Physiol*. 1996; 73(1-2): 1–6.
33. Schagatay E, Lodin A, Richardson M. Lung volume and diving performance in elite apnoeists. [Abstract.] Proceedings of the 33rd Annual Scientific Meeting of the European Underwater and Baromedical Society; September 8-15, 2007; Sharm el Sheikh, Egypt. Essex, UK: EUBS; 2007.
34. Schagatay E, van Kampen M, Andersson J. Effects of repeated apneas on apneic time and diving response in non-divers. *Undersea Hyperb Med*. 1999; 26(3):143–149.
35. Schagatay E, van Kampen M, Emanuelsson S, Holm B. Effects of physical and apnea training on apneic time and the diving re-sponse in humans. *Eur J Appl Physiol*. 2000; 82(3):161–169.
36. Sebert P, Barthelemy L, Mialon P. CO₂ chemoreflex drive of ventilation in man: effects of hyperoxia and sex differences. *Respiration*. 1990; 57(4):264–267.
37. Sebert P, Sanchez J, Monod H. Sex differences in cardiac responses to successive apnea periods. *Aviat Space Environ Med*. 1982; 53(5):485–488.
38. Sébert P, Sanchez J. Sexual and postural differences in cardioventilatory responses during and after breath holding at rest. *Eur J Appl Physiol Occup Physiol*. 1981; 47(3):209–22.
39. Sherman D, Eilender E, Shefer A, Kerem D. Ventilatory and occlusion-pressure responses to hypercapnia in divers and non-divers. *Undersea Biomed Res*. 1980; 7(1):61–74.
40. Song SH, Kang DH, Kang BS, Hong SK. Lung volumes and ventilatory responses to high CO₂ and low O₂ in the ama. *J Appl Physiol*. 1963; 18(3):466–470.
41. White DP, Douglas NJ, Pickett CK, Weil JV, Zwillich CW. Sexual influence on the control of breathing. *J Appl Physiol Respir Environ Exerc Physiol*. 1983; 54(4):874–879.