

## Human Growth Hormone, Cortisol, and Acid-Base Balance Changes After Hyperventilation and Breath-Holding

T. Djarova, A. Ilkov, A. Varbanova, A. Nikiforova, and G. Mateev

Department of Physiology and Biochemistry, Higher Institute of Physical Education, Sofia, Bulgaria, and Institute of Brain Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

### Abstract

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The purpose of this study was to investigate the effects of hyperventilation and breath-holding on hormonal activity and the acid-base balance in men. Three different experimental procedures were carried out with 11 trained subjects aged 24.5 years. In experiment I, all subjects performed hyperventilation for 3 min maintaining a paced ventilation of  $47 \text{ l} \cdot \text{min}^{-1}$ . In experiment II, they performed a threefold maximal voluntary breath-holding, separated by 1-min periods of normal breathing. Experiment III consisted of a combination of hyperventilation immediately followed by maximal voluntary breath-holding. Capillary blood samples were taken for determination of  $\text{pO}_2$ ,  $\text{pCO}_2$ , and pH. Venous blood samples were drawn before and at the 5th and 30th min after the cessation of the applied procedure for RIA determination of human growth hormone (HGH) and cortisol. During the last 15 s of hyperventilation,  $\text{pO}_2$  increased to  $89.4 \pm 16.2 \text{ mm Hg}$ ,  $\text{pCO}_2$  decreased to  $19.6 \pm 1.6 \text{ mm Hg}$ , and pH increased to  $7.652 \pm 0.041$ . During the last 15 s of the third breath-holding, the results were  $\text{pO}_2 = 58.0 \pm 5.1$ ,  $\text{pCO}_2 = 45.7 \pm 3.7$ , and  $\text{pH} = 7.367 \pm 0.053$ . In experiment III, the mean values were  $\text{pO}_2 = 42.6 \pm 7.9 \text{ mmHg}$ ,  $\text{pCO}_2 = 39.2 \pm 4.6 \text{ mmHg}$ , and  $\text{pH} = 7.320 \pm 0.024$ . A significant hormonal response after the applied experimental procedures was found for HGH (1.5- to 5.56-fold increase) and cortisol (1.5- to 2.2-fold increase). It was concluded that the increased hormonal response of HGH and cortisol is an expression of the stress reaction induced by hyperventilation and breath-holding per se or in combination.

**Key words:** hyperventilation, breath-holding, human growth hormone, cortisol,  $\text{pO}_2$ ,  $\text{pCO}_2$ , pH

### Introduction

The hormonal response to different kinds of stress-like physical exercise, emotional stress, hypoxia, hypo- and hyperthermia, etc., is very similar, but the importance of hormonal changes is not obvious in all these conditions (5). According to Axelrod and Reisine (2), stress stimulates several adaptive hormonal responses. Prominent among these responses are the secretion of catecholamines, corticosteroids, adrenocorticotropin (ACTH), and human growth hormone (HGH). A number of complex interactions are involved in the regulation of these hormones.

The changes of  $\text{pO}_2$ ,  $\text{pCO}_2$ , and pH in blood during hyperventilation and breath-holding were examined in many studies (1, 4, 8, 11, 12, 14, 15), but data about the hormonal responses to them are not available.

The aim of this study was to investigate the effects of hyperventilation and breath-holding (as used in swimming, skindiving, underwater fishing, and other kinds of sport) on hormonal responses of HGH and cortisol in parallel with the changes in  $\text{pO}_2$ ,  $\text{pCO}_2$ , and pH in man.

### Methods

The study was carried out on 11 healthy male volunteers with an average age of 24.5 years who were familiar with laboratory procedures. All subjects initially performed a progressive test on a bicycle ergometer (Medicor KE-Hungary) at  $60 \text{ rev} \cdot \text{min}^{-1}$ . The ergometer was set at 60 W and every 90 s the resistance was increased by 30 W until exhaustion (7). The heart rate was determined from an ECG during the last 10 s of each work step. Oxygen uptake ( $\dot{V}\text{O}_2$ ) was measured continuously during the test with an open circuit system (Spyrolyt-DDR). After the progressive test, three different experimental procedures were carried out in 1 week. In experiment I, all subjects performed hyperventilation (HV) for 3 min maintaining paced ventilation of about  $47 \text{ l} \cdot \text{min}^{-1}$  [ $660 \text{ ml kg}^{-1}$  body weight per minute as used for clinical EEG, after Varbanova et al. (17)].

In experiment II, the subjects performed a threefold maximal voluntary breath-holding, separated by 1-min periods of normal breathing. The third experimental procedure (experiment III) consisted of a combination of 3 min hyperventilation as described in experiment I, immediately followed by a maximal voluntary breath-holding.

Arterialized capillary blood samples from the earlobe were collected for determination of the parameters of acid-base balance ( $\text{pO}_2$ ,  $\text{pCO}_2$ , pH) by AVL Gas-Check 939 (Austria) at rest, at the last 15 s of the hyperventilation or breath-holding, and at 1 min 30 s, 5 min, and 30 min after the cessation of the applied procedure.

An intravenous cannula (Venflon Viggo AB-Sweden) was inserted in the antecubital vein for blood sampling. The cannula was kept open by continuous slow infusion of 0.9% saline (40 drops per minute). Twenty minutes after the first blood sampling, the subjects began to perform the experimental protocol. After the cessation of the applied experi-

mental procedure, venous blood samples were drawn at the 5th and 30th min. Serum was separated and stored at  $-20^{\circ}\text{C}$  until determination of GHG and cortisol. GHG concentration was measured by MJ 60 GHG RIA kit (Poland) (normal values 0.63–20 ng/ml) and cortisol by CORTCTK-125 RIA kit International CIS (France) with a normal range of 10–85 ng/ml. The hormone assays were carried out in duplicate. All experimental procedures were performed between 8.00–11.00 a.m. on a different day in a randomized manner. The results were treated using nonparametric statistics (Wilcoxon test for paired samples).

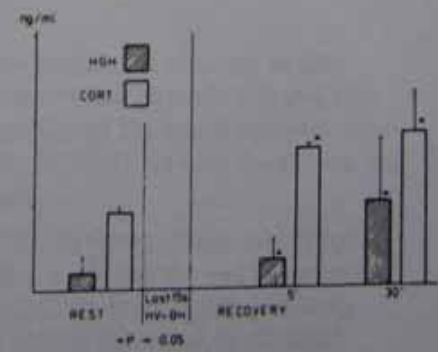
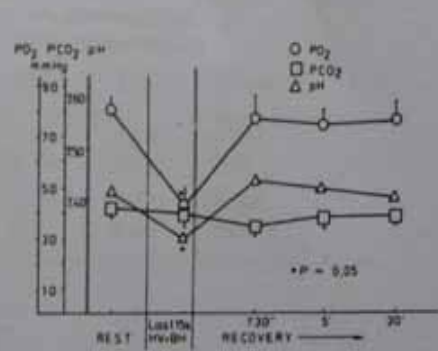
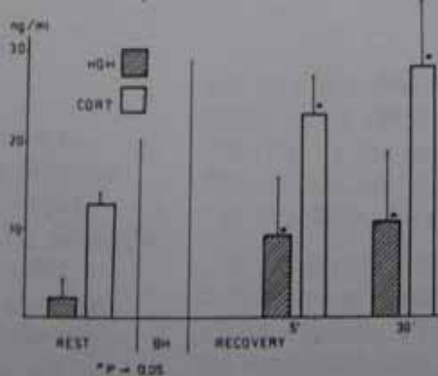
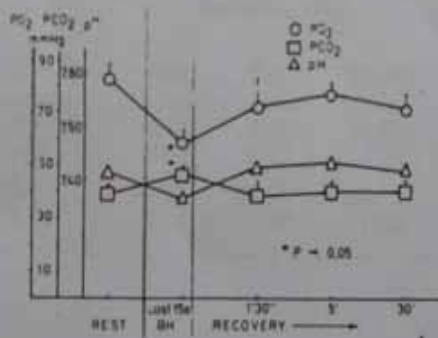
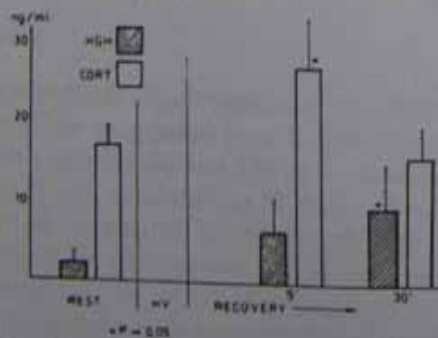
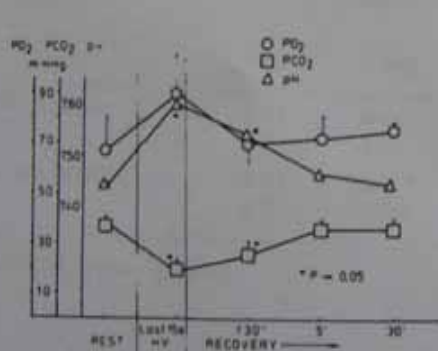
**Table 1** Physical characteristics and results from the progressive bicycle ergometer test.

Parameter	Mean value	SD
Weight (kg)	72.62	6.21
Age (yrs)	25.4	1.5
$\dot{V}\text{O}_2$ max ( $\text{l}\cdot\text{min}^{-1}$ )	3.621	0.417
$\dot{V}\text{O}_2$ max ( $\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ )	49.29	3.67
Heart rate max ( $\text{bts}\cdot\text{min}^{-1}$ )	189	7
$\dot{V}\text{O}_2$ max/HR max	18.89	1.65

**Table 2**  $\text{pO}_2$ ,  $\text{pCO}_2$ , and pH changes in response to hyperventilation

Parameter		Rest	Hyperventilation			
			Last 15 s	1 min 30 s	5 min	30 min
$\text{pO}_2$ mm Hg	mean	66.48	89.40	69.37	71.17	74.15
	SD	14.4	16.2	8.21	9.16	5.70
$\text{pCO}_2$ mm Hg	mean	36.27	19.60*	25.00*	35.17	35.55
	SD	2.24	1.6	5.5	1.8	2.7
pH	mean	7.425	7.652*	7.533*	7.466	7.441
	SD	0.031	0.041	0.024	0.014	0.036

\* $P < 0.05$  vs resting value



**Fig. 1**  $\text{pO}_2$ ,  $\text{pCO}_2$ , pH, human growth hormone (GHG), and cortisol (CORT) changes (mean  $\pm$  SD) in response to hyperventilation (HV). \* $P < 0.05$  compared with resting values.

**Fig. 2**  $\text{pO}_2$ ,  $\text{pCO}_2$ , human growth hormone (GHG) and cortisol (CORT) changes (mean  $\pm$  SD) in response to a threefold breath-holding (BH). \* $P < 0.05$  compared with resting values.

**Fig. 3**  $\text{pO}_2$ ,  $\text{pCO}_2$ , pH, human growth hormone (GHG), and cortisol (CORT) changes (mean  $\pm$  SD) in response to hyperventilation and subsequent breath-holding (HV+BH). \* $P < 0.05$  compared with resting values.

Table 3 pO<sub>2</sub>, pCO<sub>2</sub>, and pH changes in response to a threefold breath-holding.

Parameter		Rest	last 15 s	Breath-holding		
				1 min 30 s	5 min	30 min
pO <sub>2</sub> mm Hg	mean	81.60	58.00*	72.20	76.90	71.01
	SD	6.63	5.1	11.0	4.7	5.3
pCO <sub>2</sub> mm Hg	mean	39.04	45.7*	38.4	39.6	39.2
	SD	1.76	3.70	2.57	2.01	2.47
pH	mean	7.418	7.367	7.427	7.427	7.418
	SD	0.015	0.053	0.014	0.015	0.012

\*P < 0.05 vs resting value

Table 4 pO<sub>2</sub>, pCO<sub>2</sub>, and pH changes in response to hyperventilation and subsequent breath-holding.

Parameter		Rest	Hyperventilation and breath-holding			
			last 15 s	1 min	5 min	30 min
pO <sub>2</sub> mm Hg	mean	80.30	42.60*	77.68	74.44	75.78
	SD	5.04	7.9	9.3	6.1	7.9
pCO <sub>2</sub> mm Hg	mean	40.78	39.20	35.62	37.32	38.02
	SD	2.1	4.6	4.7	4.1	3.5
pH	mean	7.413	7.320*	7.442	7.419	7.405
	SD	0.030	0.024	0.049	0.042	0.063

\*P < 0.05 vs resting value

Table 5 HGH and cortisol changes in response to hyperventilation, breath-holding, and hyperventilation followed by maximal voluntary breath-holding (means ± SD).

Parameter	Hyperventilation			Breath-holding			Hyperventilation and breath-holding		
	Rest	5 min	30 min	Rest	5 min	30 min	Rest	5 min	30 min
HGH (ng/ml)	1.73	5.71	8.75*	2.13	8.80*	10.35*	1.98	3.15*	9.94*
	1.71	4.16	5.56	2.10	6.67	8.28	2.26	2.67	7.97
Cortisol (ng/ml)	16.63	25.70*	15.70	12.77	22.67*	28.10*	9.17	16.4*	18.20*
	2.39	5.74	3.74	1.36	5.89	7.59	0.57	0.20	5.08

\*P < 0.05 vs resting values.

**Results**

Some physical characteristics of the subjects and the results of the progressive bicycle ergometer test are shown in Table 1. During the last 15 s of hyperventilation (experiment I), pO<sub>2</sub> increased to 89.4±16.2 mm Hg, pCO<sub>2</sub> decreased to 19.6±1.6 mm Hg, and pH increased to 7.652±0.041 (Table 2). In the recovery period 1 min and 30 s after the hyperventilation, pO<sub>2</sub> had mean values similar to the resting values before the experiment, while pCO<sub>2</sub> was still low (25.0±5.51 mm Hg) and pH remained high (7.533±0.024). The latter two normalized at the 5th min of recovery (Fig. 1).

During the last 15 s of the third breath-holding (experiment II), pO<sub>2</sub> decreased to 58.0±5.1 mm Hg, pCO<sub>2</sub> increased to 45.7±3.7 mm Hg, and pH decreased insignificantly, but 1 min

and 30 s thereafter all these parameters returned to the initial resting values and did not change at the 5th and 30th min (Fig. 2). The mean duration of the breath-holdings were 83.22±13.73 s for the first, 95.55±17.84 s for the second, and 114.77±26.99 s for the third.

The results of experiment III (hyperventilation and breath-holding) are presented in Fig. 3. During the last 15 s of the maximal voluntary breath-holding, immediately following the hyperventilation, pO<sub>2</sub> and pH decreased significantly (pO<sub>2</sub> = 42.6±7.9 mm Hg, pH = 7.320±0.024), while pCO<sub>2</sub> did not differ from the initial resting values. The duration of the breath-holding in this experimental procedure was 174.63±36.60 s, and this value was significantly higher (P < 0.05) compared with those established in experiment II.

A significantly enhanced hormonal response ( $P < 0.05$ ) was found for HGH at the 30th min after hyperventilation (5.56-fold increase), at the 5th and 30th min of the recovery after experiment II (4.1 and 4.5-fold increase, respectively), and after experiment III (1.5 and 5.02-fold increase, respectively, Figs. 1–3, Tables 3–5).

An elevated hormonal response for cortisol ( $P < 0.05$ ) was found 5 min after hyperventilation (1.5-fold increase) but could not be detected 30 min thereafter. Cortisol levels were enhanced at the 5th and 30th min after experiment II (1.78 and 2.20-fold increase) and after experiment III (1.78 and 1.98-fold increase, Figs. 1–3).

## Discussion

As a result of hyperventilation, arterial  $p\text{CO}_2$  is reduced significantly below normal levels and the blood becomes more alkaline (12). Arterial  $\text{PO}_2$  may increase insignificantly (6). The changes in these parameters, however, are quite opposite during breath-holding. According to Craig (4), the desire to breathe is mainly due to the stimulating effects of increased arterial  $p\text{CO}_2$  and  $\text{H}^+$  concentration and not to the decreased  $p\text{O}_2$ . The breaking point for breath-holding corresponds to about 50 mm Hg for  $p\text{CO}_2$  in the presence of  $p\text{O}_2$  of the same value. If, however, the  $p\text{O}_2$  is higher (e.g., 150 mm Hg), the interruption of breath-holding can be observed at elevated  $p\text{CO}_2$  values or vice versa. The reduced arterial  $p\text{CO}_2$  after hyperventilation extends the duration of the subsequent breath-holding until the arterial  $p\text{CO}_2$  and/or  $\text{H}^+$  concentration rise to a level to stimulate breathing (12). Under such conditions, arterial  $p\text{O}_2$  may decrease to a greater extent and lower  $p\text{CO}_2$  provokes the interruption of the breath-holding. Our results correspond to the above-mentioned data about changes in  $p\text{O}_2$ ,  $p\text{CO}_2$ , and pH during similar experimental conditions (1, 4, 6, 11).

Carbon dioxide is one of the most important factors which govern the activity of the nervous tissue so that changes in blood  $\text{CO}_2$  are quickly followed by changes within nerve cells. Consequently, when arterial  $\text{CO}_2$  drops, carbon dioxide rapidly leaves nerve cells, they become more alkaline, and their activity increases in both sensory and motor neurons. When  $\text{CO}_2$  falls, cerebral blood flow decreases. In addition, the hemoglobin dissociation curve shifts to the left (Bohr effect). The combined effect is that of cerebral hypoxia (3).

Hypoxia is a potent stressful stimulus for hormonal release of HGH and ACTH (16). The latter can be assumed to be one of the factors explaining the increased hormonal response of cortisol to the applied experimental procedures in our study.

According to Galbo (5), afferent nerve pathways originating in peripheral mechanoreceptors may directly activate the hypothalamus and, this in turn, elicits ACTH release. These data also have to be considered in the explanation of the increased cortisol level in our experiments involving hyperventilation.

Stress-induced activation of the pituitary-adrenal system appears to be mediated predominantly by hypothalamic neurons containing corticotropin-releasing factor (CRF) and vasopressin. Other mediators, such as catecholamines, serotonin, angiotensin, and "tissue CRF," may also play a certain role under certain conditions (9). Nahas (13) established that the increased  $p\text{CO}_2$  and/or acidosis can influence both catecholamine production and activity. Probably this mechanism acts mainly in our experimental procedure with a threefold breath-holding.

Galbo (5) reported that individual changes in HGH concentration have a rather irregular time course due to the pulsatile secretion which is characteristic for HGH (10). Our data for HGH, both at rest and after the different experimental procedures, agree with such a hypothesis, and the established wide range of HGH concentration (0.67–25.9 ng/ml) in our opinion exemplifies the normal oscillation in HGH release.

Our data provide evidence that hyperventilation or breath-holding per se, or the combination of both, act as stressful stimuli which induce hormonal response. This fact has to be considered in the evaluation of the hormonal responses to those kinds of physical exercise that involve hyperventilation and breath-holding (swimming, skin diving, spear fishing). The described hormonal response with increased HGH and cortisol is an expression of the respiratory stress reaction induced by hyperventilation and breath-holding probably via humoral and mechanical interoceptive stimuli.

## References

- 1 Albano G.: *Principles and Observations on the Physiology of the Scuba Diver*. Washington, U.S. Government Printing Office, 1970, p 312.
- 2 Axelrod J., Reisine T.D.: Stress hormones: their interaction and regulation. *Science* 224: 452–459, 1984.
- 3 Cluff R.A.: Chronic hyperventilation and its treatment by physiotherapy: discussion paper. *J R Soc Med* 77: 855–862, 1984.
- 4 Craig A.B., Jr.: Principles and problems of underwater diving. *Physician Sports Med* 8: 72, 1980.
- 5 Galbo H.: *Hormonal and Metabolic Adaptation to Exercise*. Stuttgart, New York, Georg Thieme Verlag, 1983, pp 46–63.
- 6 Green M.F.: *Resuscitation and First Aid*. Royal Life Saving Society, London, Devonshire Press Limited, 1978.
- 7 Iliev I.: Applied value of  $\dot{V}\text{O}_2$  max in the functional diagnosis of top athletes, thesis, Sofia, 1981.
- 8 Lanphier E.H., Rahn H.: Alveolar gas exchange during breath-hold diving. *J Appl Physiol* 18: 471–475, 1963.
- 9 Makara G.B.: Mechanisms by which stressful stimuli activate the pituitary adrenal system. *Fed Proc* 44: 149–153, 1985.
- 10 Martin J.B.: Functions of central nervous system neurotransmitters in regulation of growth hormone secretion. *Fed Proc* 39: 29P2–29P6, 1980.
- 11 Mateev G., Djarova T.: Physiological problems during life saving actions in drowning accidents. Proceedings of the First National Symposium on Life Saving, October 5th, Sozopol, Bulgaria, 1984, in press.
- 12 McArdle W.D., Katch F.I., Katch V.L.: *Exercise Physiology*. Philadelphia, Lea & Febiger, 1981, p 182.
- 13 Nahas G.C.: Mechanism of carbon dioxide and pH effects on metabolism, in Nahas G., Schaefer K.E. (eds): *Carbon Dioxide and Metabolic Regulation*. New York, Springer Verlag, 1974, pp 107–117.