The effects of increased pressure, variation in inspired gases and the use of a mask during dry chamber dives on salivary cortisol in professional divers

Janne Tikkinen, Ari Hirvonen, Kai Parkkola and Martti A Siimes

Abstract

(Tikkinen J, Hirvonen A, Parkkola K, Siimes MA. The effects of increased pressure, variation in inspired gases and the use of a mask during dry chamber dives on salivary cortisol in professional divers. *Diving Hyperb Med.* 2011 December;41(4):211-215.)

Introduction: Stress activates the hypothalamic-pituitary-adrenal axis resulting in measurable changes in hormone levels in blood or saliva in humans. We aimed to find out if professional divers expressed any change in salivary cortisol levels during a simulated dive to 608 kPa (50 meters' sea water) in a hyperbaric chamber. Furthermore, we investigated the effect of wearing a mask or modifying the breathing gas during decompression.

Methods: We investigated 89 Navy and Coast Guard male divers. The divers were randomised into three groups for decompression by inspired gas and the use of a mask. The saliva samples were collected before and approximately 3 minutes after the hyperbaric test.

Results: Salivary cortisol levels decreased from a mean (SD) of 16.0 (8.1) nmol L^{-1} pre-dive to 10.3 (5.0) nmol L^{-1} post-dive ($P < 0.01$). Cortisol values did not relate to the anthropometric and physical fitness characteristics of the divers or to increased pressure, variation in inspired gases or the use of a mask. The individual variation in cortisol values was large.

Conclusions: These findings are in line with previous studies demonstrating large individual variations in salivary cortisol. Our findings suggest that professional divers are well adapted to these hyperbaric conditions. However, there continues to be a need to identify divers sensitive to stress caused by diving and the hyperbaric environment.

Key words

Stress, endocrinology, hyperbaric oxygen, equipment, military diving, occupational diving, hyperbaric research

Introduction

Stress activates the hypothalamic-pituitary-adrenal axis resulting in measurable changes in hormone levels in blood or saliva in humans. Cortisol level in saliva ranges from less than 2 nmol L^{-1} to more than 100 nmol L^{-1}.[1] According to reference ranges by Aardal and Holm, salivary cortisol varies from 3.5 to 27.0 nmol L^{-1} at 0800 h.[2] Further, cortisol levels are age-dependent and vary in circadian fashion after the age of one year. The cortisol levels are typically high in the morning upon waking, increase 50–60 % in the first 30–45 min after awakening then drop over the next few hours and finally decline more slowly to reach the lowest point at 2400 h.[3] Salivary cortisol values vary considerably both within and between individuals and, according to Casals et al., a change greater than 104% between two measurements is needed to be considered as significant.[4] Two- to three-fold elevations above baseline measurement of salivary cortisol were found in men preparing for skydiving.[5] Arithmetic calculations in a noisy environment doubled cortisol values, and the Trier Social Stress Test, which consists of delivering a speech and performing mathematical calculations in front of an audience, led to two- to four-fold increases.[6,7]

Similar salivary cortisol changes have been noted with pharmacological and psychological stimuli.[8] Psychological stressors have been reported to induce cortisol release only if an emotionally negative stressor is employed.[9] In addition, a correlation has been demonstrated between the state of anxiety and cortisol measures in phobic situations.[10] Physical fitness may be important to the cortisol response, since trained men had higher pre-stress cortisol values and a faster fall than untrained.[11] Recent data in soldiers and emergency rescue recruits demonstrated significantly higher cortisol secretion in those suffering from a phobia to a protective mask.[12]

Limited data based on small numbers of subjects have suggested that although divers may show evidence of a generalised hormonal stress response under some conditions, elevated air pressure itself may not induce this response. For example, Davis et al. found a strong correlation between raised plasma cortisol levels and open-water deep dives.[13] They considered it to be a response to psychological stress. Smith et al. demonstrated that cold-water immersion at 304 kPa elevated plasma cortisol and ACTH levels in sixteen divers.[14] In another study, serum concentrations of cortisol decreased in divers who were exposed to air and oxygen at 254 kPa.[15] Similarly, a Japanese study of six qualified divers who were exposed to heliox at 3.65 MPa showed no changes in salivary cortisol.[16] In another study, hyperoxia did not cause significant changes in cortisol values, whereas using a face mask resulted in a prolonged elevation in cortisol values compared to breathing chamber air.[17] Lower levels of physical fitness have been associated with more pronounced hormonal responses to diving pressure in five professional divers.[18]

In this study we aimed to find out if professional divers expressed changes in salivary cortisol levels during a simulated dry dive to 608 kPa (50 meters' sea water, msw) in a hyperbaric chamber. Furthermore, we investigated the effect of wearing a mask or modifying the breathing gas during decompression.

Methods

SUBJECTS

The study protocol was reviewed and approved by the Ethical Committee of the University Hospital of Helsinki and accepted by the Headquarters of the Finnish Defence Forces. One-hundred-and-five professional Navy and Coast Guard male divers undergoing their annual medical check-up gave their written informed consent to participate following a detailed explanation in writing of the study procedures.

Prior to their medical check-ups, the divers completed a questionnaire seeking information on their diving experience (year of basic training, hours of diving) and the result of the latest Cooper's 12-minute running test. Height and weight were recorded and body mass index (BMI) calculated. Proportional body fat (PBF) was calculated using the Body Impedance Analysis InBody 7200 (Biospace Co, South Korea). Maximal oxygen (O_2) uptake (expressed as ml min^{-1} kg^{-1}) was measured using a treadmill ergometer (Schiller CS-200, Schiller AG, Switzerland) and inspired and expired gas analysis (PowerCube Ergo, Ganshorn Medizin Elektronic GmbH, Germany). The maximum O_2 uptake had to be estimated in six divers instead of direct analysis of the breathing gases because of calibration difficulties.

DIVE PROTOCOL

We exposed two divers at a time to a pressure of 608 kPa (50 msw) in a dry multiplace chamber. The exposure was part of their annual medical check-up. The speed of compression was 9 ± 1 msw min^{-1} and the target pressure was reached in 6 ±1 min. The divers remained at depth for 3 minutes. Decompression was based on a depth of 51 msw and bottom time of 15 min (Finnish Navy, DCAP-FINN), with decompression stops at 9, 6 and 3 msw for 7, 2 and 8 min, respectively. The subjects breathed either the chamber air or from a mask and built-in-breathing-system (BIBS). The breathing gas in the BIBS was changed from air to O_2 for the divers in the O_2 group (see below) at 304 kPa on ascent (15 ±1 min after the onset of the test dive).

RANDOMISATION

We prepared 105 envelopes containing the study design, questionnaire, consent form and the research code forms. When the divers attended their annual medical check-up, each of them picked out one of the shuffled envelopes from the box. The research codes divided the divers initially into three groups for decompression:

- Group 1: the divers breathed chamber air throughout the dive (n = 22 analysed)
- Group 2: the divers breathed air through the mask and BIBS throughout the dive (n = 32 analysed)
- Group 3: the divers breathed air through the mask and BIBS during the dive but the breathing gas was switched to O_2 at 304 kPa during decompression (n = 35 analysed).

Of the 105 divers entered into the study, 89 had a complete set of pre- and post-dive cortisol and VO_2max measurements.

SALIVARY CORTISOL

The saliva samples were collected before and after the hyperbaric chamber test. The tests were performed between 0800 and 1100 h. The divers avoided vigorous physical exercise and intake of any food for two hours prior to the test.[19] To collect a saliva sample, the diver chewed a cotton swab for two min (Salivette, Sarstedt, Numbrecht, Germany). The swabs were collected directly into sampling tubes, which were stored frozen at -21^{O}C prior to analysis. The half-life of salivary cortisol is relatively long (58–113 min) and it peaks 20–30 min after the onset of a stressful stimulus.[19] Therefore, post-dive samples were taken at approximately 3 minutes after reaching the surface. An increase in salivary cortisol of more than 100% between the pre- and post-dive concentrations was considered as a significant change. For technical reasons, not all the cortisol samples could be analysed on five non-mask chamber air subjects.

The salivary cortisol levels were measured using a commercially available luminescence immunoassay (Cortisol Saliva LIA, IBL Immuno-Biological Laboratories, Hamburg, Germany). This assay is based on the competition principle and microtitre plate separation. Briefly, an unknown amount of cortisol present in the sample and a fixed amount of enzyme-labelled cortisol compete for the binding sites of the antibodies coated onto the wells. After 3 h incubation, the wells are washed to stop the competition reaction. Once the luminescent substrate solution is added, the relative luminescence units (RLUs) can be read within 10–40 min, the concentration of cortisol being inversely proportional to the measured luminescence. Measuring range of the method is 0.43–110 nmol L^{-1}. The coefficients of variation of intra- and inter-assay of the method are 5% and 8% respectively.

STATISTICS

The data are presented as means (SD). The IBM SPPS 19 computer package was used for all statistical analyses. The Kolmogorov-Smirnov test was used to assess the normality of distribution of the cortisol values. Skewed values were normalised, and the t-tests were performed after logarithmic transformation (natural log). Relationships between pre-dive or post-dive cortisol and various variables were assessed with Pearson correlation analysis. The association of the decompression gas and use of a mask with cortisol levels was determined by an analysis of variance (ANOVA). A pre-study power analysis was not performed.

Results

Of the 105 divers entered into the study, 89 had a complete set of pre- and post-dive cortisol and VO_2max measurements. Demographic data are provided in Table 1. As expected, the individual variation in salivary cortisol values was large. The pre-dive cortisol concentration ranged from 2.6 to 39.6 nmol L^{-1} with a mean (SD) of 16.0 (8.1) nmol L^{-1} and the post-dive cortisol ranged from 3.4 to 29.1 nmol L^{-1} with a mean of 10.3 (5.0) nmol L^{-1}. The decrease in cortisol over the dive was statistically significant ($P < 0.01$). Of the 89 divers, 15 experienced an increase of cortisol concentration during the experiments, only three of these exceeding a rise of more than 100%. On the other hand, 21 divers showed a decrease in cortisol level of more than 50%. The pre- and post-dive salivary cortisol values are plotted against each other in Figure 1.

Using Pearson correlation coefficients, we found no statistically significant associations between the pre- or post-dive cortisol values and the age, weight, BMI, PBF, Cooper's 12 minutes running test, VO_2max or the diving experience of the divers. Neither were the relative or absolute changes in cortisol associated with the age, anthropometric or physical fitness parameters, or the diving experience of the divers.

The findings were similar in all divers within the three groups randomized for decompression gas (Figure 2). The mean pre-dive cortisol values decreased from 16.8 (8.4) nmol L^{-1} (range 2.6–34.0) to 10.7 (5.0) nmol L^{-1} (range 3.8–24.0) ($P < 0.01$) in the divers inhaling O_2 through mask and from 15.3 (8.0) nmol L^{-1} (range 5.0–39.6) to 9.6 (4.7) nmol L^{-1} (range 3.4–26.5) ($P < 0.01$) in the divers inhaling air through mask and from 15.9 ±7.8 nmol L^{-1} (range 4.5–33.1) to 10.8 (5.5) nmol L^{-1} (range 4.8–29.1) ($P < 0.01$) in the divers inhaling chamber air without mask. The ANOVA revealed no differences between the pre-dive and post-dive cortisol values in the three groups.

Table 1
Demographics of 89 professional divers studied

Parameter	Mean	SD	Range
Age (years)	36.0	(8.5)	20–50
Body weight (kg)	84.2	(8.2)	66–110
Body mass index (kg m^{-2})	25.9	(2.2)	21.7–30.9
Proportional body fat (%)	16.0	(5.1)	7.3–29.1
12-min run distance (m)	2900	(240)	2200–3550
VO_2max (ml min^{-1} kg^{-1})	54.8	(8.1)	34.4–69.4
Diving experience			
Years	12.0	(8.1)	1–26
Hours	521	(603)	14–3300

Discussion

The study aimed to find out if professional divers expressed any change in salivary cortisol values as a marker of stress during a simulated dive to 608 kPa in a hyperbaric

Figure 1
The pre- and post-dive salivary cortisol values (nmol L^{-1}) in 89 professional divers; the line of identity is drawn to separate those with increased/decreased values

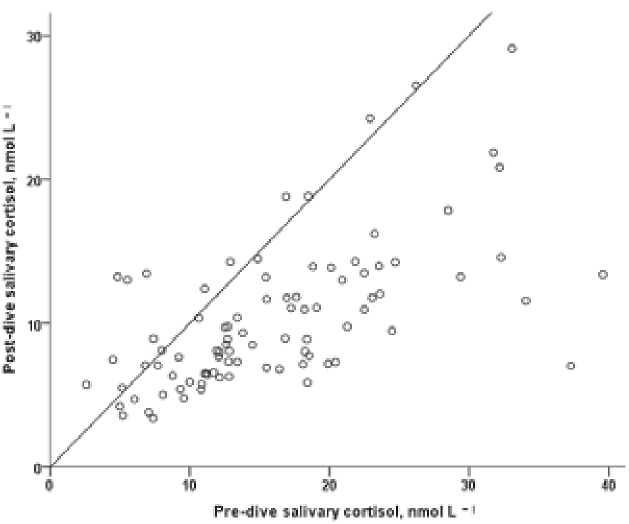
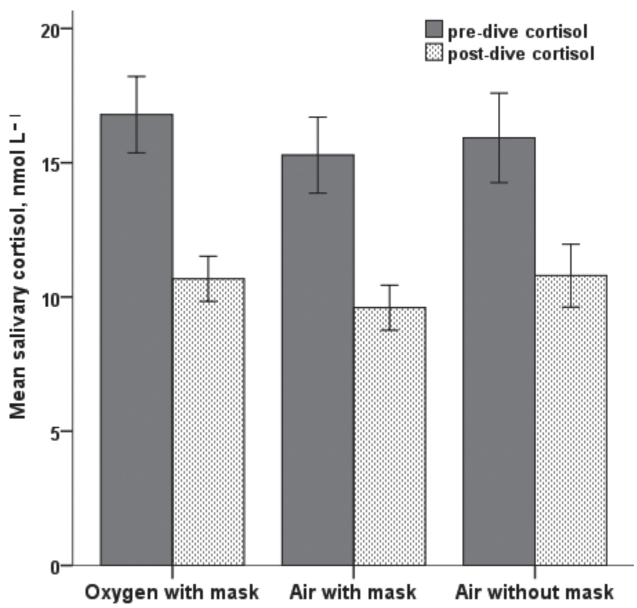


Figure 2
The mean pre- and post-dive salivary cortisol values (± SEM) of the 89 divers divided into three groups according to the breathing gas used and its mode of delivery during decompression



chamber. We used salivary cortisol because the method is non-invasive and does not itself activate the hypothalamic-pituitary-adrenal axis. Salivary cortisol is often used as a marker for stress but has a large variation both biologically and for methodological reasons.[3,19] We aimed to reduce the variability by utilising the same commercial preparation and laboratory for collecting and analysing the saliva samples. Further, the samples were collected at the same time of the day and year. The divers also received the same instructions prior to their tests. The individual variation of cortisol values was large, but they were within the normal range in the majority of individuals and in line with previous studies.[2,12,18]

We observed no evidence that absolute values or relative changes in salivary cortisol levels correlated with the physical fitness of the divers as in a previous study.[11] However, these Navy and Coast Guard divers were a selected, homogeneous group with good to excellent physical fitness compared to the general population. This may explain the lack of an association.

Our findings of lower post-dive cortisol values are in line with the results of the previous study at 254 kPa.[15] Only a few of the 89 divers had an increment of more than 100 % in their salivary cortisol concentrations and in most cases the elevation, if present, was marginal (see Figure 1). On the other hand, we observed a decrease greater than 50% in 21 divers. The decrease might be in part due to normal circadian variation.[3] Nevertheless, it is known that anticipation of an emotionally stressful situation can induce cortisol secretion.[10] We speculate that, at least in some of the divers, anticipation of the forthcoming dry dive and the various tests may have been sufficient to stimulate an increase in cortisol release. Some of the pre-dive cortisol values were higher than the upper limit of the Aardal and Holm reference range for morning salivary cortisol.[2] Studies in athletes such as paragliders, tennis players, wrestlers, judo wrestlers and swimmers have demonstrated increases in pre-competition plasma or salivary cortisol concentrations.[20–24]

The use of a mask during the chamber test or the nature of the inhaled gas during decompression (air or O_2) did not have any influence on the cortisol levels. The subjects were professional divers who are accustomed to heavy masks which would normally be uncomfortable and cause stress. Our observation that hyperoxia was not related to the change in the cortisol values supports the findings of two previous studies.[15,16]

The main limitations of our study are the lack of a control group, utilisation of only one marker for stress and use of only single pre- and post-dive samples. However, we were unable to expand the study design since the experiments were performed in combination with the clinical medical check-ups of the divers during a working day.

Our earlier experiences with measuring subjective psychological stress responses by a self-reporting method (unpublished observations) in professional divers have been discouraging. Over the last 10 years, none of the divers reported any sensations of anxiety, nervousness, anger, palpitations, etc., during their annual dry chamber dives to 608 kPa. There continues to be a need to identify divers sensitive to stress caused by diving and the hyperbaric environment. In future studies, a combination of markers of activation of the hypothalamic-pituitary-adrenal axis and autonomic nervous system responses would be useful.

Acknowledgements

We thank Harri Mäkitalo RN, Antti Jarho RN and Pia Sopanen RN for their valuable help in gathering and storing the data and Sirpa Hyttinen for the salivary cortisol measurements.

Conflict of interest: none

References

1 Kiess W, Meidert A, Dressendörfer RA, Schriever K, Kessler U, König A, et al. Salivary cortisol levels throughout childhood and adolescence: relation with age, pubertal stage and weight. *Pediatr Res*. 1995;37:502-6.
2 Aardal E, Holm AC. Cortisol in saliva – reference ranges and relation to cortisol in serum. *Eur Clin Chem Clin Biochem*. 1995;33:927-32.
3 Kirchbaum C, Hellmanner DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology*. 1989;22:150-69.
4 Casals G, Foj L, de Osaba MJ. Day-to-day variation of late-night salivary cortisol in healthy voluntaries. *Clin Biochem*. 2011;44:665-8.
5 Chatterton RT, Vogelsong KM, Lu Y-C, Hudgens GA. Hormonal responses to psychological stress in men prepairing for skydiving. *J Clin Endocrinol Metab*. 1997;82:2503-9.
6 Miki K, Kawamorita K, Araga Y, Musha T, Sudo A. Urinary and salivary stress hormone levels while performing arithmetic calculation in a noisy environment. *Ind Health*. 1998;36:66-9.
7 Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychology*. 1993;28:76-81.
8 Schlotz W, Kumsta R, Layes I, Entringer S, Jones A, Wust S. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. *Psychosom Med*. 2008;70:787-96.
9 Nejtek VA. High and low emotion events influence emoitional stress perceptions and are associated with salivary cortisol response changes in a consecutive stress paradigm. *Psychoneuroendocrinology*. 2002;27:337-52.
10 Alpers GW, Abelson JL, Wilhelm FH, Roth MT. Salivary cortisol response during exposure treatment in driving phobics. *Psychosom Med*. 2003;65:679-87.
11 Sinyor D, Schwartz SG, Peronnet F, Brisson G, Seraganian P. Aerobic fitness level and reactivity to psychosocial

stress: physiological, biohemical and subjective measures. *Psychosom Med*. 1983;45:205-17.
12 Brand S, Annen K, Holsboer-Trachsler E, Blaser A. Intensive two-day cognitive-behavioral intervention decreases cortisol secretion in soldiers suffering from phobia to wear protective mask. *J Psychiatr Res*. 2011;18 (Epub ahead of print).
13 Davis FM, Charlier R, Saumarez R, Muller V. Some physiological responses to the stress of aqualung diving. *Aerosp Med.* 1972;43:1083-8.
14 Smith DJ, Deuster PA, Ryan CJ, Doubt TJ. Prolonged whole body immersion in cold water: hormonal and metabolic changes. *Undersea Biomed Res*. 1990;17:139-47.
15 Lund V, Kentala E, Scheinin H, Klossner J, Koskinen P, Jalonen J. Effect of hyperbaric conditions on plasma stress hormone levels and endothelin-1. *Undersea Hyperb Med.* 1999;26:87-92.
16 Hirayanagi K, Nakabayashi K, Okonogi K, Ohiwa H. Autonomic nervous activity and stress hormones induced by hyperbaric saturation diving. *Undersea Hyperb Med.* 2003;30:47-55.
17 Kawada S, Fukusaki C, Ohtani M, Kobayashi K. Effects of hyperoxic inhalation on psychological stress-induced salivary biomarkers. *Biomed Res*. 2009;30:245-9.
18 Mateev G, Djarova T, Ilkov A, Sachanska T, Klissurov L. Hormonal and cardiorespiratory changes following simulated saturation dives to 4 and 11 ATA. *Undersea Biomed Res*. 1990;17:1-11.
19 Hansen AM, Garde AH, Persson R. Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: a review. *Scand J Clin Lab Invest*. 2008;68:448-58.
20 Filaire E, Alix D, Rouveix M, Le Scanff C. Motivation, stress, anxiety and cortisol responses in elite paragliders. *Percept Mot Skills*. 2007;104:1271-81.
21 Filiare E, Alix D, Rerrand C, Verger M. Psychophysiological stress in tennis players during the first singles match of a tournament. *Psychoneuroendocrinology*. 2009;34:150-7.
22 Passelergue P, Lac G. Saliva cortisol, testosterone and T/C ratio variations during a wrestling competition and during the post-competitive recovery period. *Int J Sports Med*. 1999;20:109-13.
23 Salvador A, Suay F, Gonzáles-Bono E, Serrano MA. Anticipatory cortisol, testosterone and psychological responses to judo competition in young men. *Psychoneuroendocrinology*. 2003;28:364-75.
24 Bonifazi M, Sardella F, Lupo C. Preparatory versus main competitions: differences in performance, lactate responses and pre-competition plasma cortisol concentrations in elite male swimmers. *Eur J Appl Physiol*. 2000;82:368-73.

Submitted: 04 April 2011
Accepted: 08 September 2011

Janne Tikkinen, MD, Diving Medical Centre, Centre for Military Medicine, Kirkkonummi, Finland and Department of Physiology, University of Helsinki, Finland
Ari Hirvonen, PhD, Finnish Institute of Occupational Health, Helsinki, Finland
Kai Parkkola, MD, Navy Command, Turku, Finland
Martti A Siimes, MD, Diving Medical Centre, Centre for Military Medicine, Kirkkonummi, Finland

Address for correspondence:
Janne Tikkinen
SLK, PL5
FIN-02471 Upinniemi,
Finland
Phone: *+358-(0)299-581-551*
E-mail: *<janne.tikkinen@mil.fi>*