

# Salivary stress marker in and salivary alpha-amylase levels during a nine hour water immersion in healthy men: Oral based diagnosis

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## Abstract

The objective of this study was to valid a water immersion model for microgravity and to study the effects of simulated condition on salivary cortisol and a-amylase. Increased activity of stress systems is reported during space flight, but unchanged or decreased activity during simulated microgravity. We here investigated the impact of head-out water immersion on the activity of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenal-medullary system. The healthy young men were exposed to a nine -hour water immersion in a thermo neutral bath and a control condition. Saliva samples were taken before, during, and after interventions to assess cortisol as an index for hypothalamus-pituitary-adrenal axis activity, and salivary a amylase as an index for sympathetic-adrenal-medullary system activity. Cortisol and Amylase levels uniformly increased during simulated conditions. In conclusion, Both systems activity shows initial increased during water immersion.

**Key words:** Simulated microgravity, salivary cortisol, salivary a-amylase, water immersion model.

## Introduction

After centuries of wonder, human being finally realized the decisive possibility of going to the moon a little more than 50 years ago, and in 1969 this became a reality, no longer a dream. The human body, however, is designed to live under 1 g as on Earth. Thus, it is important to understand what happens in the human body under microgravity in order to make rapid progress in space development. One of the most serious problems produced by microgravity is a fluid shift from lower to upper body. All life on Earth is accustomed to the presence of gravity. When that presence is removed or altered, biological processes can go awry. While humans have little difficulty surviving in space for short periods of time (with the necessary equipment, oxygen and food of course), long-term exposure to microgravity can trigger detrimental physiological responses in the human body. There is a long list of such effects, ranging from serious medical conditions to less severe side effects. Accordingly, the

developments of biomedical and physiological countermeasures were undertaken in an effort to begin overcoming these stressors. These countermeasures allow us to sustain human presence in flight for increasing periods, as well as to participate in increasingly complex and lengthy missions. Russian experience in long duration spaceflight has revealed that among the most critical problems facing human in long duration spaceflight, after the biomedical, are the psychological and psychosocial 1-4. Physiological stressors inherent in the long-duration space environment pose the greatest challenge to human spaceflight. The human body must physically adapt to the foreign microgravity environment and, in doing so, undergo cardiovascular, muscular, and skeletal deconditioning as well as changes in the immune and nervous systems, and radiation exposure. Regarding the physical effects of adaptation to spaceflight, about 40-50% of flight crews during their first few days of microgravity experience a condition called Space Adaptation Sickness (SAS), which causes symptoms such as nausea, disorientation, headache, and a sea-sick or flu-like feeling. Some of the above named factors can be alleviated by exercise and

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pharmacological interventions, but others remain a significant obstacle to maintaining the health of astronauts during long duration missions. Similarly, crews must undergo the stress associated with re-adapting to the 1-g environment upon return to Earth. These physiological factors are a significant concern for a human mission to Mars. These and other adaptive physiological and physical processes represent change from a normal state of functioning for the astronauts and can thus contribute to increased psychological stress levels. 4-22. It has been reported that in head-out water immersion condition body mimic as microgravity 23-25. Hormonal responses of stress systems to thermo neutral head-out water immersion (HOWI) have only been investigated in studies with rather short interventions. Sramek et al. report a significant decrease of total cortisol during and after a one-hour immersion 23, while a two hour immersion did not change ACTH and cortisol in another study 24. No studies have assessed cortisol responses to longer water immersions, which would be a better simulation of long-term microgravity during space flight. Concerning the response of the sympathetic-adrenal-medullary system to thermo neutral water immersion, some but not all studies report a decrease of heart rate 24, and of epinephrine and norepinephrine 25. The data from space missions would predict an activation of stress systems, while previous data from short-term water immersions found the contrary, i.e. unchanged or slightly decreased HPA axis activity and a decrease of sympathetic-adrenal-medullary system activity. The present study was conducted to assess the effects of a longer (i.e. nine hour) simulation of microgravity through thermo neutral head-out water immersion without the

confounding impact of psychosocial stress on the major stress systems. Stress system activity was measured by the saliva-based parameters salivary cortisol and salivary  $\alpha$ -amylase.

### Materials and methods

After approval by the local ethics committee and receipt of informed consent, ten healthy male volunteers [age 26.8 , 4.6 (SE) yr, weight 68.7, 6.8 kg, height 168.8 , 78.9 cm] were subjected to permanent bed rest for 20 days at 6° HDT were exposed (i) to a six-hour water immersion in a thermo neutral bath and (ii) to a nine hour control condition of sitting on a chair in the same room under thermo neutral conditions, in a within subjects design. Both conditions started at 0700h and ended at 1600h. Saliva samples were taken before, during, and after (up until the following morning) immersion and control conditions. Because glucocorticoids are known to be periodically secreted in response to a variety of environmental and hormonal stimuli (e.g., psychic stress and physical exercise), which alone and/or together with cortisol might affect the immune system, free cortisol was determined in saliva samples collected in the morning (8 AM) and in the evening (7 PM). Saliva was collected by having the subject chew on a cotton swab for 40-45 s; the swab was then stored in a SALIVETTE device tube. Samples were frozen, and free-cortisol concentrations were quantified by a commercially available ELISA according to the instruction of the manufacturer (Orion Diagnostica, Espoo, Finland). Salivary  $\alpha$ -amylase was measured by a modified enzyme kinetic method 26. . Significant differences between mean values were tested with analyses of variance and paired t tests. The Wilcoxon signed-rank test was used for nonparametric tests.

Variables	Microgravity		
	Before ( Mean(SD)	During( Mean(SD)	After ( Mean(SD)
Cortisol nmol/l	15.81 (4.79)	23.67(5.67)	16.67(7.83)
Salivary $\alpha$ -amylase (U/ml)	50 (12)	67(13)	54(12)

## Results

Table-1: Saliva cortisol and salivary  $\alpha$ -amylase levels in before, during and after simulated microgravity.

Salivary cortisol and salivary  $\alpha$ -amylase concentration showed statistically significant increase during simulated microgravity compared to before and after (Table -1,  $P < 0.001$ ).

## Discussion

As reported previously activates cerebral regions, leading to subsequent alterations in the secretion of stress hormones such as cortisol and catecholamines, as observed changes in the diurnal rhythm of cortisol secretion. In this study, cortisol was determined in the saliva specimen for several reasons. First, it represents a noninvasive method. Second, determination of cortisol in saliva allows the detection of the protein-unbound free cortisol, because only this form can enter saliva and is not affected by the saliva flow rate. Thirdly is the unbound, free cortisol that can reach target cells and their receptors and hence reflects the biologically active cortisol that is responsible for the induction of physiological or pathophysiological effects. Salivary amylase in contrast showed higher levels during microgravity compared to the control condition, suggesting an increased activity of the sympathetic-adrenal-medullary system. In summary, we show here that simulation of microgravity through water immersion influences the HPA axis, while the sympathetic-adrenal-medullary system activity seems to be increased during water immersion.

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