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Salivary stress markers and psychological stress in simulated microgravity: 21 days in 6° head-down tilt

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Abstract: Spaceflight occurs in an environment of temperature extremes, microgravity, solar and galactic cosmic radiation, lack of atmospheric pressure, and high-speed micrometeorites. Exposure to microgravity and the space environment during space missions of short and long duration has important medical and health implications for astronauts. Psychological well-being is of increasing importance in planned spaceflights and interplanetary missions of long duration. The 6° head-down tilt (HDT) is an established method of mimicking low gravity on earth. The aim of the present study was to determine the effects of 21 days of HDT on psychological stress in 12 healthy male volunteers. Psychological state was assessed by the current stress test, and chromogranin-A (CgA), cortisol, alpha-amylase, and beta-endorphin were measured in saliva. After one week of HDT, all volunteers developed psychological stress, and secretion of CgA, cortisol, alpha-amylase, and beta-endorphin were all significantly higher. Thus, 6° HDT appears to be a valid model to induce psychological stress changes in the immune system, changes that might also be encountered by astronauts and cosmonauts during both a short stay in space, such as that required while orbiting a space station, and in longer spaceflights. (J Oral Sci 53, 103-107, 2011)

Keywords: salivary stress markers; saliva; cortisol; alpha-amylase; 6° head-down tilt (HDT).

Introduction

Advances in aerospace technology in the 20th century have allowed humans to encounter a microgravitational (i.e., almost 0 g) environment, and the extent of such exposure will increase in the present century. Astronauts experience weightlessness during space flight. Because the human body is designed to live in a 1-g environment, as on Earth, exposure to microgravity causes significant changes in body functions. Thus, to maintain health during space flight, it is important to understand what happens to the human body under microgravity. Exposure to microgravity and the space environment during short- and long-duration space missions has important medical and health implications in astronauts. These include neurovestibular problems involving space motion sickness, disorientation during flight, and impaired balance and neuromuscular co-ordination after landing; cardiovascular and fluid-related problems related to orthostatic hypotension immediately after spaceflight; the possibility of altered cardiac susceptibility to ventricular arrhythmias and reduced cardiac muscle mass and diminished cardiac function; muscle-related problems of atrophy involving loss of muscle mass, strength, and endurance; decreased bone mineral density; circadian rhythm-related problems involving sleep and performance; and immune-related problems involving infections and immunodeficiency (1-8).

It has been reported that in short spaceflights, immune

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response might be affected by psychological stress, radiation, malnutrition, and microgravity (9-12). Two primary neuroendocrine systems are of special interest in the study of human stress: the hypothalamus-pituitary-adrenocortical (HPA) system, which regulates the secretion of cortisol, and the sympathetic adrenomedullary (SM) system, which controls secretion of catecholamine. In the HPA system, cortisol secretion is regulated by adreno-corticotropic hormone (ACTH) from the pituitary gland. Salivary cortisol levels are closely correlated with blood cortisol levels and therefore reliably reflect HPA activity (13-16). It has been reported that various types of psychological stress activate the HPA system and consequently induce substantial increases in salivary cortisol levels. In the SM system, direct measurement of salivary catecholamine does not reflect SM activity (17). Alpha-amylase is a major salivary enzyme in humans and is secreted from salivary glands in response to sympathetic stimuli (18). Recently, it was shown that measurement of this salivary enzyme is useful in evaluating activation of the SM system (19,20). Levels of salivary cortisol, salivary amylase, and chromogranin-A (CgA), which can all be sampled noninvasively, have been used as stress biomarkers (8,11,15-20).

The health of the immune system, however, is of great importance in longer planned spaceflights and inter-planetary missions. Studies of the effects of microgravity on the human immune system were conducted before and after flights and in ground-based studies under conditions of head-down tilt (HDT). It has been shown that a 6° HDT is a reliable model of the effects of microgravity on cerebral and venous hemodynamics, cardiovascular functions and reflexes, thermoregulatory responses, and the hematopoietic system (3,7,12). However, most HDT studies were short and assessed the levels of only 3 hormones (9-12). In addition, there is no published study of the effect of simulated microgravity on salivary stress hormones. In the present study, 12 healthy volunteers maintained a 6° HDT position for 21 days. During this period, we evaluated the effects of long-term hypokinesia and simulated microgravity with respect to psychological stress and the secretion of stress hormones, i.e., the salivary stress markers CgA, alpha-amylase, cortisol, and beta-endorphin.

Materials and Methods

Subjects

The participants were 12 men: mean age 24.75 (SD 2.2) years; height 1.98 (0.16) m; weight 78.6 (11.9) kg. Each subject was informed of the aims of the study, and written consent was obtained before the study began. The study was carried out at the J. D. Centre (India) in April 2009, and the study protocol was approved by the Ethics Committee of Jain Diagnostic Centre, New Delhi (India).

Protocol

The volunteers were placed in beds propped up with blocks at the foot to achieve -6° HDT (bed rest condition). During this period, a video camera was used to confirm that the subjects remained in the correct position throughout the experiment. Bed rest, during which subjects rested in a horizontal position wearing loose and comfortable T-shirt and shorts, was maintained for 24 h. The average energy and calcium intake of the volunteers during the microgravity simulation was 2300 kcal/ day (range 2080-3010 kcal/day) and 1200 mg/day, respectively. Dietary sodium and potassium intake were maintained at 90 and 80 mmol/day, respectively. Water intake was ad libitum (mean, 1236 [SD 66] mL/day). Duration of sleep was approximate 8 h, from 10 PM to 6 AM. Baseline measurements were obtained during the hour before initiating HDT. Saliva samples were collected immediately before and immediately after initiating HDT, and at 1, 2, and 3 weeks of HDT. The samples were immediately centrifuged (5,000 g, 20 min, -4°C), and the supernatants were frozen at 60°C until analysis.

Current stress test

The current stress test (CST) was developed and validated by German psychologists and is commercially available as the Actual Strain Short Questionnaire (Kurzfragebogen zur aktuellen Beanspruchung, Beltz Testzentrale European Test Publisher Group, Göttingen, Germany) (3). The CST was designed to repeatedly measure an individual's psychological state under conditions of acute and chronic stress. It is composed of 6 pairs of contradictory feelings of increasing intensity, from which the subject must decide. The final score is calculated by summing the values and may range from 1 (no stress) to 6 (maximal stress). In contrast to other anxiety evaluation questionnaires, e.g., the state-trait anxiety inventory (STAI), the self-estimating CST test is a very short, 1-page paper test that can be performed in 1 min and was, therefore, easy to use in the present study. Because of the structure of the questionnaire, test-takers usually cannot remember their previous responses. Therefore, there is no carryover effect, which is a strict prerequisite for such tests in longitudinal studies. The CST was validated in a comparison with STAI, which is one of the most commonly used scales for measuring state anxiety. The correlation between the CST and STAI ranged from 0.7 to 0.9 (3).

Analytic methods

Salivary CgA, cortisol, alpha-amylase, and beta-endorphin were assayed using kits (YKO70 Human CgA EIA kit, Yanaihara Institute, Shizuoka, Japan; cortisol EIA kit and alpha-amylase assay kit; Salimetrics Inc., State College, PA, USA; beta-endorphin S-1134 kit, Peninsula Laboratories Inc., Belmont, CA, USA).

Statistical analysis

Data were analyzed using SPSS, version 11 (SPSS, Chicago, IL, USA).

Results

Average CST score increased from 3.68 at 1 week after initiation of HDT to 4.02 at 3 weeks. As compared with pre-HDT values, CST score and levels of the salivary stress markers alpha-amylase, cortisol, CgA, and beta-endorphin were all significantly higher at 1 week, although subsequent increases in CST score and stress marker levels were not significant (Table 1). There were significant correlations between the levels of salivary stress markers and CST score (Table 2).

Discussion

During spaceflight, astronauts and cosmonauts encounter various unusual conditions, including microgravity, radiation, malnutrition, and alterations of the day-night cycle. From a phylogenetic point of view, changes in response to adverse environmental conditions are not restricted to spaceflight. Indeed, such changes have been the driving force in the development of the human body's ability to mount an adaptive stress response. Although adaptive stress responses are mainly directed at enabling an individual to survive, the mechanisms involved might have adverse effects, such as immune suppression. In the case of spaceflight, the immune effect of adaptive stress responses might be of great importance during long missions, e.g., a mission to Mars. Using a ground-based model that simulated microgravity by maintaining subjects in a 6° HDT position for 21 days, we explored the development of psychological stress and alterations in the regulation of stress hormones in saliva.

Psychological stress due to prolonged 6° HDT was measured by using CST score, which was developed to repeatedly assess psychological well-being during longitudinal studies (9). Average CST score significantly increased from 3.68 at 1 week after initiation of HDT to 4.02 at 3 weeks, although subsequent increases in CST score were not significant. These findings are similar to those of previous studies (9,10). One hypothesis maintains that stress activates cerebral regions, which alters the secretion of stress hormones.

In the present study, the salivary stress markers alpha-amylase, cortisol, CgA, and beta-endorphin were also

Table 1 CST (current stress test) score and levels of salivary alpha-amylase, cortisol, CgA (Chromogranin), and beta-endorphin immediately before and after initiating HDT (-6 degree bed rest condition), and at 1, 2, and 3 weeks of HDT in 12 healthy men

Parameters	Immediately before HDT	After initiation of HDT	At week 1	At week 2	At week 3
CST Score	2.45 (0.12)	3.01 (0.13)	3.68 (0.15)*	3.98 (0.14)*	4.02 (0.18)*
Alpha-amylase (U/mL)	59.4 (24.1)	62.3 (22.1)	85.3 (23.2)*	92.1 (23.6)*	99.3 (0.25)*
Cortisol (μg/dL)	0.267 (0.113)	0.289 (0.124)	0.307 (0.145)*	0.345 (0.167)*	0.389 (0.46)*
CgA (pmol/mg protein)	2.78 (1.34)	2.89 (1.31)	3.12 (1.65)*	3.56 (1.82)*	3.89 (1.45)*
Beta-endorphin (pg/mL)	1278.6 (412.7)	1302.5 (508.7)	1412.6 (456.9)*	1543.7 (435.8)*	1678.9 (543.9)*

* $P < 0.01$ vs. pre-HDT values

Table 2 Bivariate correlations between CST (current stress test) score and salivary stress markers immediately before and after initiating HDT (-6 degree bed rest condition), and at 1, 2, and 3 weeks of HDT in 12 healthy men (ANCOVA)

CST Score	Alpha-amylase	Cortisol	Chromogranin	Beta-endorphin
Before initiation of HDT	0.56	0.67	0.58	0.54
After initiation of HDT	0.57	0.66	0.56	0.54
Week 1	0.56	0.67	0.56	0.55
Week 2	0.57	0.67	0.57	0.56
Week 3	0.55	0.68	0.56	0.55

significantly higher after 1 week of HDT, although subsequent increases in these markers and were not significant. Stress due to microgravity activates the HPA system and consequently induces significant increases in salivary cortisol and beta-endorphin levels (9,13). In this study, salivary CgA levels were higher after 1 week of microgravity stress. Direct measurements of salivary catecholamine do not reflect SM activity (14,16). Alpha-amylase is an important salivary enzyme in humans and is secreted from salivary glands in response to sympathetic stimuli. It is believed to be a useful marker for evaluating activation of the SM system (15). Hence, increased salivary alpha-amylase during simulated microgravity might be due to activation of the SM system, although after 1 week, the SM system might adapt to conditions of simulated microgravity.

The present results suggest that prolonged 6° HDT is a reliable method of inducing psychological stress and is thus useful for studies that aim to evaluate stress by measuring salivary stress markers. Because of the projected increase in the number and duration of spaceflights, including those to orbiting space stations and Mars, there is a need for additional investigations of the biological significance of spaceflight-induced changes in stress parameters. HDT appears to be a helpful ground-based model of such changes.

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