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Multiparametricendocrine evaluation of healthy subjects undergoing prolonged head down tilt bed rest anti-gravitary posture

Abstract

Great number of space flights demonstrated that human beings can not work and live for a long time in space without effects on their health. Reduced gravity leads to profound changes in neuroendocrine and immune functions. Experimental model systems have been designed to simulate on earth some effects of space flight on immune response. Eight healthy male volunteers have been enrolled and maintained in head down tilt bed rest antigravitary posture (at -10°) for 72 hours. ACTH, cortisol, epinephrine, norepinephrine, growth hormone, prolactin, leptin and adiponectin, TSH, FT3, FT4, LH, FSH and total testosterone have been evaluated. ACTH, LH, epinephrine and prolactin plasma levels significantly increased while FT3, FT4 decreased at the beginning of the bed rest period in subjects maintained in antigravitary posture. Leptin and adiponectin decreased during the bed rest period. Prolonged antigravitary posture may represent a stressing condition leading to an increase of catecolamines and ACTH which can cause decrease of leptin serum levels. Decrease in leptin concentrations can influence many systems including pituitary-gonadal and pituitary-thyroid axis inducing hypogonadism and hypothyroidism. Increase of prolactin could be correlated with reduction of adiponectin concentrations. Prolonged antigravitary posture favors modifications of several endocrine parameters which can produce immunological, cardiovascular and metabolic modifications.

Keywords

Bed rest; Space; Immune response; Hormones; Neuroendocrine system.

INTRODUCTION

The great number of space flights have demonstrated that human beings cannot work and live for a long time in space without effects on their health. In the past, attention was focused on more evident symptoms of exposure to weightlessness like the space adaptation syndrome and cardiovascular effects[1]. Reduced gravity in space as well as psychological and physical stress may also lead to profound changes in a number of neuroendocrine and immune functions[1-3]. Stress represents a condition which modifies organism balance and induces a series of reactions leading to a new balance state. Particularly, stress provokes a neuroendocrine response which results in changes of the blood levels of some hormones and neuropeptides including adrenocorticotropic hormone (ACTH), cortisol, catecholamines, enkephalins and endorphins. Moreover, immune cells express specific receptors for these hormones and neuropeptides and in turn secrete endorphins, enkephalins and lymphokines that may affect the nervous system and other immune cells. Thus, the neuroendocrine and the immune systems seem to be linked by a complete regulatory loop. Furthermore, the headward shift of body fluid and increase in stress-related hormones that occur in hypogravity bring about a number of changes in metabolism and biochemistry of the human body. Indeed, there is a continued loss of protein from muscles and calcium from bones. It has been reported that the neuroendocrine response to stress may result in different types and degrees of immunodepression which are kept in balance by the positive ones exerted by prolactin and growth hormone (GH)[4-6]. If the balance

between these factors favors a depression of immune responses, potential problems over long space flights may arise including development of opportunistic infections, transfer of infections from visiting crews to a permanent crew that may be immunocompromised on a Space Station and development of infections upon return to earth in a crew that has been isolated from terrestrial organisms in a long-lasting space mission[7-10]. Therefore, it has become even more crucial to understand the effect of space flight on immune response and on other physiological conditions which could affect immune system. Since space flight opportunities are rare and expensive, experimental model systems have been designed to simulate on earth some effects of space flight on immune response. These models include rotation of cells in a clinostat[10,11], antiorthostatic, hypokinetic, hypodinamic suspension of rodents[10-14] and chronic bed rest of humans[10,15-22]. Changes in neuro-endocrine and immunological parameters observed utilizing these experimental models are similar to those observed in in-flight studies[10,15,16,19-23].

We have previously reported and hypothesized that the elevated plasma levels of E and NE reached by individuals during antihortostatic posture may justify the concomitant increase in CD4+ T lymphocytes and monocytes, and that the modifications in serum ACTH and cortisol levels could lead to peripheral blood leukocytosis[10]. These data are in keeping with those reported in astronauts whose E and NE levels as well as total leukocytes and T lymphocyte number increase after short space missions[10]. Moreover, cortisol downregulates surface neutrophil CD11b integrin expression and stress hormones decrease free oxygen radicals (e.g. H_2O_2) production by phagocytes[10]. PRL secretion in response to stress may modulate immune responses in two ways. The first one is by directly stimulating immune system and the second one is by reducing cortisol secretion in response to stress. This hyphotesis postulates that PRL secretion is increased by stress in order to counteract the suppressive effects of cortisol on the immune system[10].

The aim of the present study was to investigate the influence of a 72 hour antihortostatic bed rest on a series of endocrine parameters with the purpose of confirming that endocrine alterations occur during space flight.

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers (mean age: 24 ± 1 years, height: 178–189 cm, weight: 67–83 kg)) have been enrolled and maintained in head down tilt bed rest antigravitary posture (at -10°) for 72 hours. All subjects were normotensive with a normal ECG at rest and had normal values of routine laboratory tests, absence of acute or chronic infections, antibodies directed to HIV, HBV, HCV, CMV, HSV, HZV resulted negative. The detection of autoantibodies resulted also negative and lymphocyte phenotype was normal. They did not use any regular medication and were free of drugs in the previous six months. Finally, all of them signed an informed consent form. Bed rest occurred in the -10° degree head-down tilt position at all times, although subjects were allowed to elevate on one elbow for meals. Fluids were allowed ad libitum during this period. Day-night cycles were strictly monitored and controlled. All volunteers have been also maintained in supine posture at 0° for 72 hours as controls 3 months after the end of bed rest. Subjects were bled before (days –30 and –1), during (days 0, 1, 2 and 3) and after (days 6, 8 and 30) bed rest. All bleedings were performed at 8 a.m. Serum was obtained after centrifugation of coagulated blood and kept frozen at -30° C until use.

Endocrine parameters

Serum or plasma concentrations of ACTH, cortisol, epinephrine (E), norepinephrine (NE), growth hormone (GH), prolactin (PRL), leptin and adiponectin, TSH, FT3, FT4, LH, FSH and total testosterone have been evaluated. ACTH and cortisol were determined by chemiluminescent assay (Byk Gulden, Milano) and results are expressed as ng/mL; E and NE were determined by an enzyme linked immunosorbent assay (ELISA, ALPCO, Solem, NH) and results are expressed as pg/mL; GH and total testosterone were determined by chemiluminescent DPC assay (Medical System, Genova) and results are expressed as ng/mL; PRL, LH and FSH were determined by immunoenzymatic assay (EIA, Radim, Roma) and results are expressed as ng/ml; leptin and adiponectin were determined by an enzyme linked immunosorbent assay (ELISA, R&D System, Milano) and results are expressed as pg/mL and ng/mL, respectively; TSH, FT3 and FT4 were determined by radioimmunoassay (RIA, Radim, Roma) and results are expressed as μUI/L, ng/L and ng/L, respectively.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Bonferroni's post-test. A two-tailed P value less than.05 was considered as significant.

RESULTS

Behavior of ACTH, cortisol, E and NE

ACTH serum levels significantly increased in the antihortostatic bed rest group on days 1 and 3 ($p < 0.05$), while serum cortisol concentration significantly increased

in the antihortostatic bed rest group on the day 0 ($p < 0.05$). In the antihortostatic bed rest group, E showed a significant peak on day 0 ($p < 0.05$) and, in the control group, a significant peak on day 3 ($p < 0.001$). NE significantly increased, in the antihortostatic bed rest group on days 0 and 1 ($p < 0.05$). (Figure 1).

Behavior of GH and PRL

GH serum levels did not show significant modifications in both groups. PRL serum levels significantly increased, in the antihortostatic bed rest group, from the first day of test and during all the test period ($p< 0.001$), whereas, in the control group, a significant peak was observed on day 3 ($p < 0.001$) (Figure 2).

Behavior of leptin and adiponectin

In the antihortostatic bed rest group, both leptin and adiponectin serum levels significantly decreased during the three days of test ($p < 0.001$). In the control group, the serum levels of leptin did not show modifications, whereas the serum levels of adiponectin showed a decrease similar to the one observed in the antihortostatic bed rest group, even if with lower statistical significance ($p< 0.05$) (Figure 3).

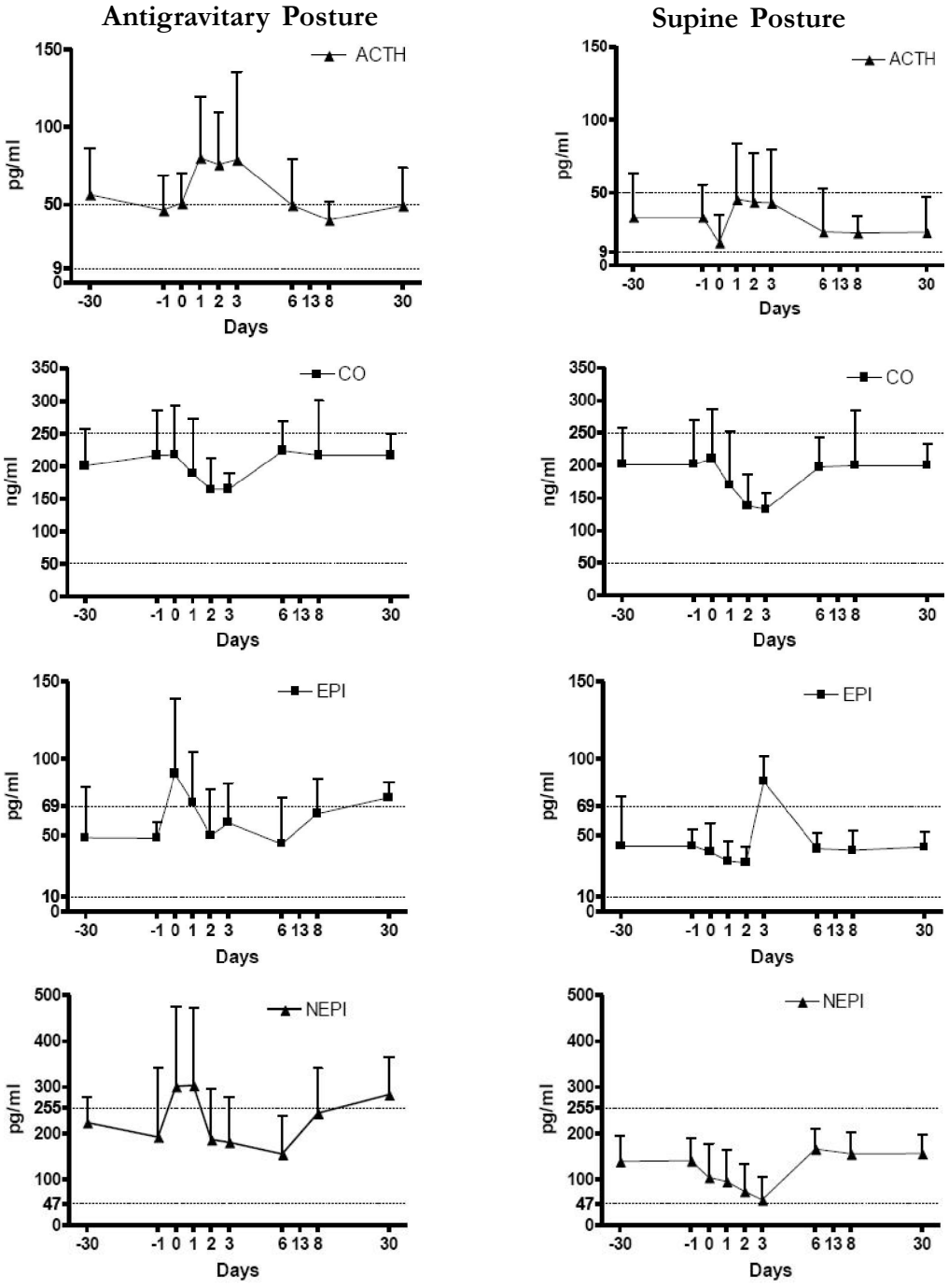

Figure 1

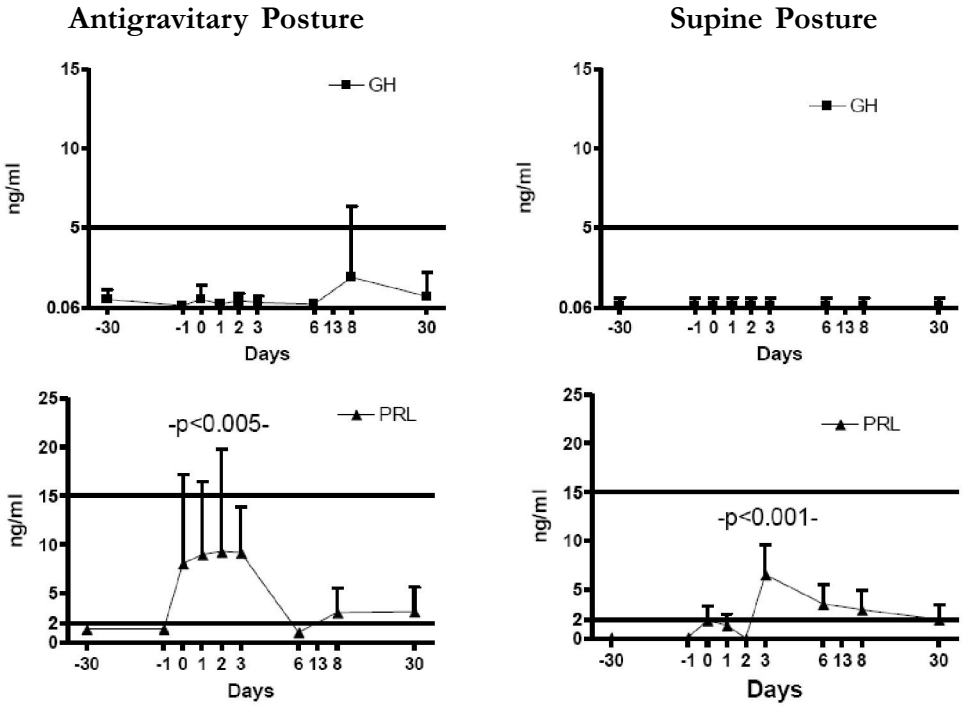

Figure 2

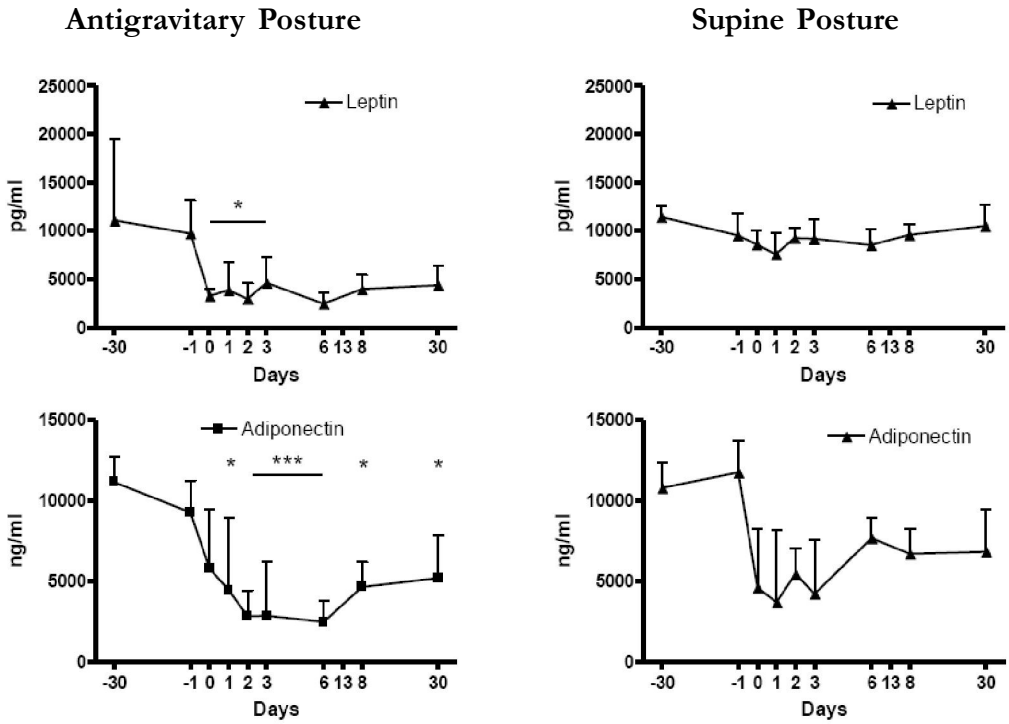

Figure 3

Behavior of TSH, FT3 and FT4

No significant modifications of TSH serum levels were detected in both groups. FT3 serum levels showed a statistically significant decrease in the antihortostatic bed rest group on days 0, 1 and 2 ($p< 0.05$), while no significant modifications were demonstrated in the control group. FT4 decreased in the antihortostatic bed rest group on days 0, 1 and 2, however, the difference did not reach the statistical significance; no significant modifications of FT4 serum levels were detected in the control group (Figure 4).

Behavior of LH, FSH and testosterone

LH serum levels increased, in the antihortostatic bed rest group, from day 0 to day 3 ($p<0.001$). Moreover, LH serum levels reached a significant peak on day 3 in the control group. No modifications in FSH and total testosterone serum levels were observed both in the

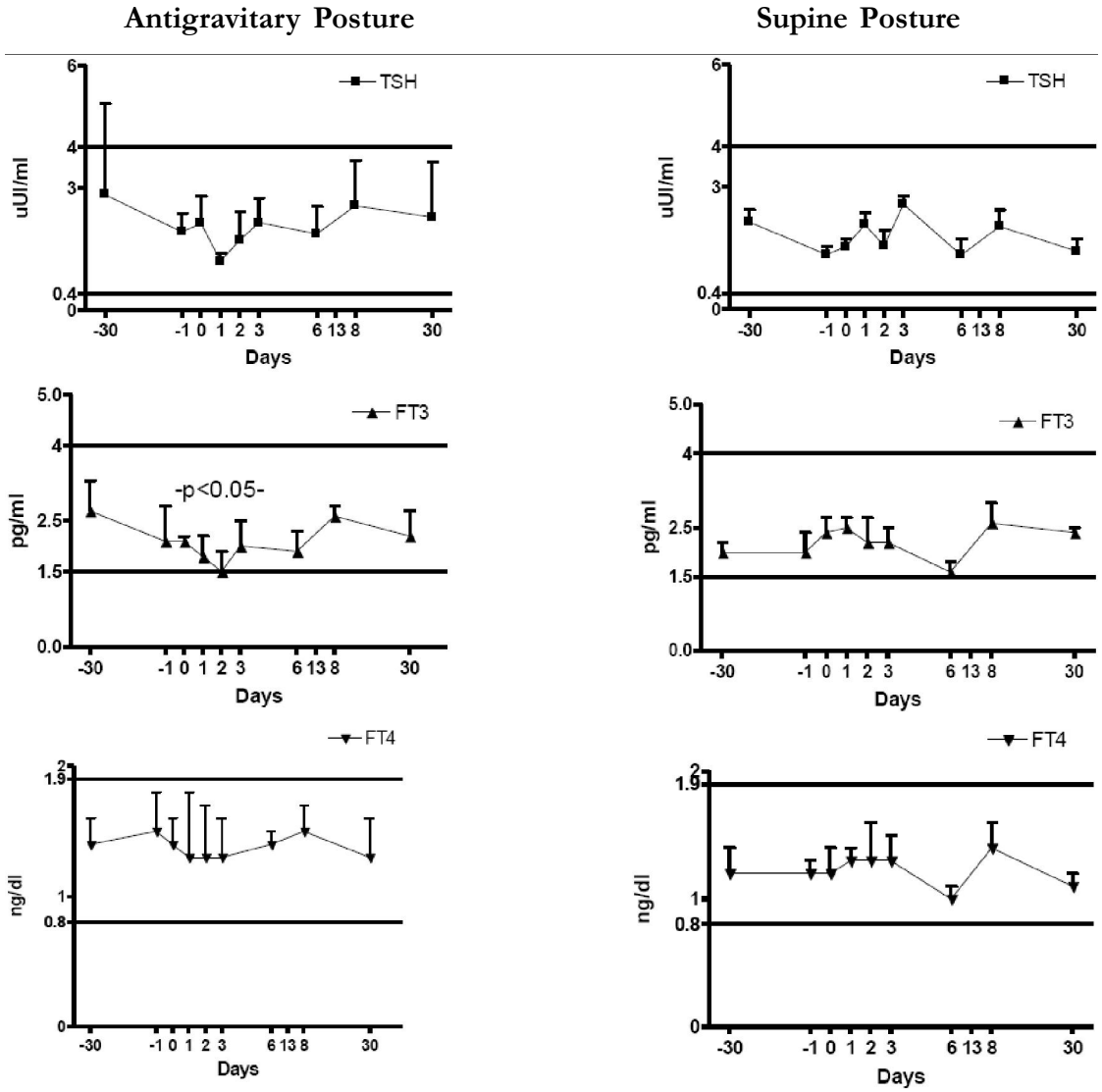

Figure 4

antihortostatic bed rest and in the control group (figure 5).

DISCUSSION

Antihortostatic bed rest has been identified as an appropriate ground-based model to mimic the main conditions that contribute to the imbalance of immune homeostasis during space-flight including microgravity, weightlessness and prolonged stay in compulsory posture. Moreover, it also represents a psychological stress condition on the ground. The aim of the present study was to investigate the influence of a 72 hour antihortostatic bed rest on a series of endocrine parameters. The most interesting results refer to the modifications of E, NE and PRL. In fact, a significant increase in serum E, NE and PRL levels occurred in subjects maintained in antigravitary posture at the beginning of the bed rest period. These data may suggest that the bed rest itself represents a stressing condition. However, the stressing event seems to occur earlier in subjects resting in antigravitary posture than in those resting in supine posture. We hypothesize that antigravitary posture may induce a perturbation in homeostasis because of the loss of hydrostatic pressure, conflicting inputs into the neurovestibular system, and lack of physical tension on the musculoskeletal system. The fluid shifts and the neurovestibular disorientation generally resolve within the first two days, explaining why the increase in the stress hormone levels occur at the beginning of the antigravitary posture. On the contrary, supine posture does not perturb homeostasis and, thus, the change in the serum stress hormones levels may be linked to the psychological stress induced by the prolonged supine posture for 72 hours.

Of interest, the results of this study also indicate that modifications of adipokine serum levels occur during a prolonged bed rest. Leptin levels significantly decreased during the bed rest period in the antihorthostatic bed rest group while no significant modifications were observed in subjects maintained in supine posture. It is known that leptin levels decrease after long-term exercise or exercise

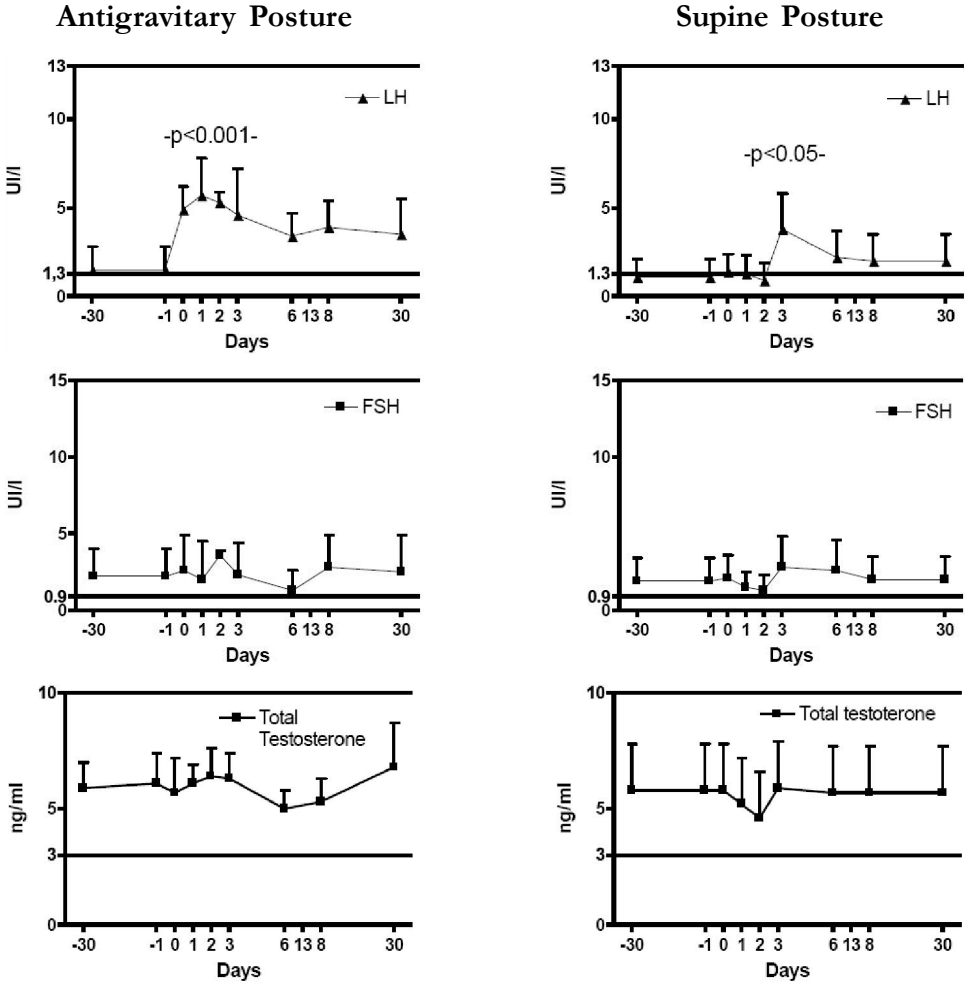

Figure 5

that generates high energy expenditure as well as during an hyperadrenergic state[24-27]. Stress is defined as a constellation of events, which begins with a stimulus (stressor) that precipitates a reaction in the brain (stress perception), which subsequently activates physiologic systems in the body (stress response). The physiologic stress response results in the release of neurotransmitters and hormones such as E and NE, that serve as the brains' messengers to the rest of the body. Therefore, it may be proposed that a prolonged bed rest in antihorthostatic posture represents a stressing event, as suggested by the increase in E levels found in this condition. Notably, adiponectin levels significantly decreased in both groups. It is known that adiponectin plays an important role as endothelial anti-inflammatory molecule and cardio-vascular protector agent and that its decrease is associated with insulin resistance, glucose intolerance, dyslipidemia, atherosclerosis and ischemic cardiomiopathy[24,25,27,28]. Our data, showing a decrease of adiponectin levels during a prolonged bed rest posture, indicate that this condition might predispose to an increased cardiovascular risk. Both leptin and adiponectin have shown an increase after the end of the bed rest period that, however, did not reach the pre-test level suggesting that more than 30 days are required for these parameters to return to baseline level. To the best of our present knowledge, no data have been published on the behavior of leptin and adiponectin during space flights and, thus, non comparisons can be made with our data obtained on ground.

Conflicting results have been found on the behavior of stress hormones studied in animal models and in humans during space flight or in experimental model systems designed to simulate on earth some effects of space flight. In fact, some authors have found a significant increase of these hormones suggesting that space flight may represent a highly stressing condition. By contrast, other authors have found only a little increase or a reduction of these hormones stating that space flight is not correlated with a stressing condition[29-35]. Our data on stress hormones seem to confirm the first hypothesis[9,34-37]. Many studies have been performed on the alterations of thyroid hormones during space life. All studies in mouse agree showing that space flight induces a hypothyroid state[31,32,39] whereas studies in men are in disagreement[31,32,37,39]. Our data reporting that space flight induces a transient hypothyroid state are in keeping with studies in

animal models. Of note, we did not detect significant modification of TSH levels. This finding can be explained by the long time required by TSH to respond to FT3 and FT4 modifications. Our data about testosterone modifications are in agreement with the published literature, whereas our data about LH modifications are in disagreement with some authors and in agreement with others[9,28-30,32,38,40]. Collectively, these data suggest that space life induces a primary hypogonadic state. As only few studies have been published on FSH modifications during space life or during head down tilt bed rest no correlations with our data can be reported[39,40,32].

A definitive explanation of this complex array of findings is difficult. However, a tentative interpretation can be proposed. First, the prolonged antigravitary posture may represent a stressing condition leading to an increase of catecolamines, cortisol and ACTH which can cause the decrease of leptin serum levels. Second, the decrease in leptin concentrations can influence many systems including the pituitary-gonadal and pituitary-thyroid axis inducing hypogonadism and ipothyroidism. Lastly, the increase of PRL serum levels could be correlated with the reduction of adiponectin concentrations. Taken together, these results suggest that a prolonged bed rest and, in particular, the antigravitary posture, can be considered as an acute stressing condition inducing modifications of several endocrine parameters which can, in turn, produce the immunological, cardiovascular and metabolic modifications reported in humans during and after space life because of, as reported, immune cells express specific receptors for these stress hormones.

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