

Human body fluid regulation in real and simulated spaceflight

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We investigated cardiovascular regulation during a 14-month spaceflight, and using orthostatic stress on Earth. On ground, we used impedance measurements for thoracic fluid volume monitoring and cardiac output calculations; the mechanical oscillator technique for ultra precise mass densitometry on blood and plasma samples; automated blood pressure monitoring; and drew venous blood samples to investigate endocrine mechanisms of cardiovascular stabilization during various kinds of orthostatic and pseudoorthostatic (lower body ,negative' pressure: LBNP) stimulation in ambulant as well as bed rested healthy people. In space, plasma samples were prepared during LBNP experiments, stored onboard at -20°C or lower, transported to Earth several days later and analyzed for hormones. The results showed that stable cardiovascular regulatory conditions can be maintained during space missions exceeding 1 year in duration. Our ground-based studies shed light on regulatory features during combined stimulation, and demonstrated that the newly discovered hormone adrenomedullin's plasma concentration increases with orthostatic load, thereby counterbalancing the action of ,volume-guarding' hormones like plasma renin activity, aldosterone, or vasopressin that also rise with orthostatic challenge, as head-up tilt or LBNP.

Introduction

Gravity governs the organization and function of all life in the biosphere. From its beginning the human body is continuously under gravitational influence, which shapes its anatomy and physiology. For maintenance of hydrostatic pressure within the cardiovascular system of any larger organism, it is gravitation that presents a formidable challenge to cardiac performance and tissue perfusion. As a result it is necessary that the heart and blood vessels are able to respond immediately, and chronically to adjustments in acceleration stimuli; e.g., from changes in body posture. Such acceleration stimuli can also be induced by reduced or negated gravitational pull over extended time periods, a situation accessible only during actual spaceflight–weightlessness of ballistic trajectories, $1/6\text{ G}$ on the surface of the moon, $3/8\text{ G}$ on Mars; or by simulated weightlessness (head-down tilt immobilization, bed rest); short-term weightlessness of free fall / parabolic flight (,push“ phase); and increased gravity (,pull“ phase, centrifugation).

Similar cardiovascular stimulation can also be provided on Earth by water immersion, lower body compression (e.g., „anti-shock“ trousers, anti-G-suits), simulated orthostatic challenge (lower body negative pressure: LBNP), and positive- and negative-pressure breathing. Several of the above techniques, singularly or in combination, have been employed to study regulatory strategies of hormonal and neural circuits. Short-term stimuli (minutes to hours) typically occur during a circadian cycle (orthostasis of awake-ambulatory activities, bed rest during sleep), to differentiate this from long-term adjustments to a persistently altered acceleration environment; e.g., simulated or real „weightlessness“ (days to months). In the latter case, morphological restructuring complicates the adaptive responses and evokes long-lasting changes that may have functional consequences during recovery (e.g., readaptation to 1 G postflight).

The upright body position usually attenuates venous return of blood to the heart, thereby reducing cardiac preload, arterial pressure, and organ perfusion. One significant question is how the body can resist and counteract the caudal pull from fluid-filled bodily compartments such as in the arterial and venous systems, while in the upright (head-to-foot, +Gz) position? The implications are of both theoretical and practical importance. When a normovolemic person stands up from a supine position in a 1-G environment, arterial pressure in the vicinity of the left ventricle remains unaltered at the arterial hydrostatic indifferent point (HIPa), whereas venous pressure would remain constant near the upper abdominal level at the venous hydrostatic indifferent point (HIPv, 16). Unfortunately, studies on HIP locations under reduced or increased gravity in humans have not yet been performed and are necessary because those data cannot be derived on purely theoretical grounds.

Exposure of mammals to microgravity brings about redistribution of blood throughout the vasculature; vascular mechanoreceptor load is altered during central thoracic engorgement, and associated endocrine systems respond and adapt with accompanying changes of hormone output (4, 5, 15, 17, 19, 22, 23, 28, 29, 37-40, 43, 51, 52, 54, 55, 59, 75). Altered steady-state plasma concentrations of volume sensitive hormones have been observed inflight as well as postflight (28, 37, 39, 40, 43, 47, 54), but endocrine responses, indicative of the adaptive state of cardiovascular-neuroendocrine systems, in conjunction with orthostatic stimulation have rarely been studied in space.

Antiorthostatic positioning

Antiorthostatic positioning (AOP), i.e., tilting humans head down by 3-10°, (**Fig. 1**) minimizes the effects of +Gz acceleration within the cardiovascular system (7, 8, 14, 36, 38, 50, 65, 71, 76). It also causes chronic loading of cardiopulmonary („central volume“) receptors. One week of AOP seems to be sufficient to reset those receptors (6, 10, 11), implying that their reflex component loses important

function. The bar reflexes adapt rapidly if the daily challenge of +Gz acceleration is removed for several hours as with an overnight rest. The result is reduced stability of arterial blood pressure regulation („bed rest syndrome“) increasing the probability for presyncopal symptoms upon arising (1, 3, 4, 7, 9-12, 14, 15, 30, 31, 33, 36, 42, 46, 57, 58, 62, 66-72, 74, 77).

Antiorthostatic positioning evokes a number of hormonal readjustments which are time-dependent: 60 min of -30° head-down positioning are insufficient to effect plasma renin activity (pRA) or norepinephrine (pNE, 20,21); several hours of -6° produced a clear but non-significant transient depression of plasma vasopressin (pVP), aldosterone (pA), and pRA (48); basal pRA and pA increased after 14 days of -6° head-down position, plasma atrial natriuretic peptide (pANP) and pNE were reduced significantly after 7 days (2, 17), and pVP remained unchanged (17) up to 28 days of AOP (2). Long-term hormonal adaptative mechanisms to the AOP are still unclear.

A recent AOP bed-rest study was performed for 5 days in 10 subjects to investigate pANP and plasma cyclic guanosine monophosphate (pcGMP) responses. There was no significant change in basal thoracic impedance – an index of central blood volume – after 5 days of AOP. Hemodynamic and other hormonal responses (renin activity, aldosterone) increased as expected with no change of pVP. During AOP blood plasma mass density and hematocrit increased significantly in the participants regardless if they exercised during bed rest or not. During recovery, however, these variables decreased significantly below control values only in the non-exercised subjects.

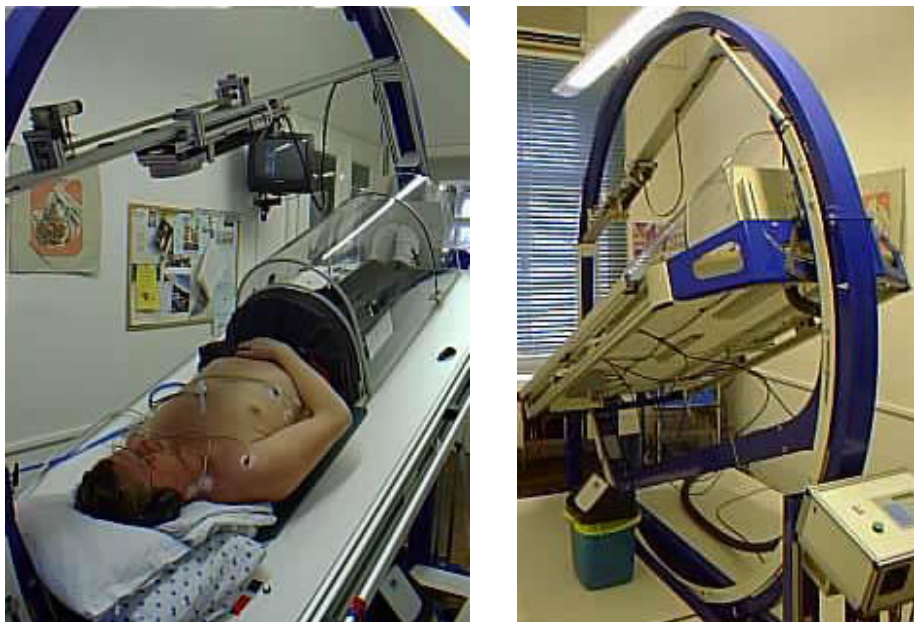


Figure 1. Antiorthostatic positioning combined with LBNP (Institute for Adaptive and Spaceflight Physiology, Graz, Austria)

Extracellular volume

Alteration of fluid balance is a major potentially confounding factor in gravitational studies (as with bed rest) of adaptive changes within the cardiovascular system (34, 49). Extracellular fluid volume, sodium balance, renal function and hydrostatic load are inseparably linked to, and significantly influence cardiovascular function. Extracellular and blood volumes are controlled by factors that regulate sodium excretion under most physiological conditions (18); e.g., renal sympathetic nervous activity and hormones respond to gravitational (+G_z) challenge. A multitude of data revealed general response patterns: Exposure to head-up tilt, centrifugation or LBNP increase pA, pVP, catecholamines, and pRA, and decreases in pANP resulting in decreased sodium and water excretion. On the other hand, simulation of decreased gravitational stress by AOP, lower body compression, or head-out water immersion generally evokes the inverse responses and increased sodium and water excretion.

Bioimpedance spectroscopy can be employed for assessment of fluid volume shifts between intracellular and extracellular fluid compartments. Impedance spectra (5-500 kHz) were recorded for the total body as well as for body segments (leg and arm) during supine rest, after standing, 70°HUT, and supine recovery. Fluid volumes changed significantly during all periods and a steady state could not be reached within 30 min. Further, segmental measurements revealed that leg fluid contributes significantly to the volume changes. The absolute volume changes and the constants differed significantly between leg positioning conditions. It was concluded that the computed volume changes were biased by extracellular fluid redistribution (63, 64).

The newly discovered natriuretic vasodilator 52-amino acid peptide hormone adrenomedullin's role in the adaptation to decreased gravitational stimulation and/or sodium intake has yet to be clarified. We evaluated the effect of orthostasis (12°, 30°, 53° or 70° head up tilt) on the time course of plasma adrenomedullin concentration and found it increased with progressively higher tilting; about half the increase that occurred within 30 min occurred during the first 2 min of upright positioning, and the maximum effect was +70% (61). Thus, adrenomedullin concentration changes quickly during and after passive orthostasis suggesting sensitive baroreceptor-induced secretion. This hormone may play an important role for cardiovascular regulatory stability during gravitational stress.

Central volume expansion accentuates renal fluid and electrolyte excretion, whereas central hypovolemia results in fluid-electrolyte retention. For example, data from positive-pressure breathing studies in humans have suggested that increased sympathetic outflow reduces urinary fluid/sodium excretion during unloading of cardiopulmonary receptors (73). But other factors might be equally important for renal responses to decreased gravitational stress (49); e.g., dietary sodium intake can exert an enormous influence on hormone levels within a few hours.

Regulation of total body water and reduced plasma volume (PV) were studied for 12 hr during acute exposure to 2,800m altitude (24) with consumption of various oral rehydration drink formulae (25). Significant increase in PV occurred after consuming a slightly hypotonic NaCl-NaCitrate beverage, which indicated that drink ionic composition is more important than its osmolality for inducing hypovolemia. Because the 10-hour hypovolemic response was probably due to the confinement rather than reduced ambient pressure, appropriate countermeasures for the hypovolemia could be consumption of sodium-content isotonic beverages, elastic stockings, leg exercise, and leg elevation (24). Similar conclusions emerged from the other study where various carbohydrate (CHO)-electrolyte fluid formulations were tested for consumption by astronauts to maintain or restore their PV and total body water during, after extravehicular activity, and for a few hours before reentry and immediately after landing. Here also the drink sodium content was more important than its total osmotic content for increasing PV at rest. Rest (sitting) data may be applicable for astronauts following reentry from spaceflight, and moderately heavy exercise in the supine position simulated the strain of extravehicular activity. Fluid formulations with greater hypervolemic effect in resting subjects may not be as effective during exercise; therefore, use of different formulations during exercise appears to be necessary (25).

A recent study looked into factors that may account for the dissociation between beverage-induced plasma sodium and osmotic concentrations that appear to refute the high theoretical correlation between those two variables. This is important because it has been shown that plasma volume decreases on a short-term basis after high altitude, physical exertion, and cold; whereas chronic exercise increases plasma volume on a longer-term basis. Interactive factors are, i.a., osmolality, plasma protein concentration, and endocrine variables like renin activity and vasopressin concentration. We therefore investigated such interactions together with urinary parameters and concluded that maintenance of drink-induced hypervolemia requires near-isotonic sodium rather than increased non-ionic osmols (26a).

The body's fluid balance is influenced significantly by extracellular macromolecules; e.g., hyaluronic acid exerts large oncotic pressure effects. These complex molecules are, to some extent, subject to lymphatic transport from the interstitial compartment, partly escape degradation within lymph nodes, and emerge in the bloodstream only to be rapidly degraded in the liver and other organs. Physiological responses of plasma hyaluronic acid concentration (pHA) were measured to determine if a circadian rhythm exists in the absence of physical activity (6° head-down bed rest); and if pHA is associated with a quasi-continuous ingestion of calories compared to a normal, three-portion daily diet of equivalent energy content versus fasting (60). Without physical activity and without food ingestion the pHA was unchanged and displayed no diurnal rhythm, but pHA increased after the first food intake peaking after 60 min; further intake of food on the same day produced little effect. Thus, without

ingestion of a larger meal it was concluded that no circadian plasma hyaluronane rhythm exists. Changes in pHA therefore indicate the effect of physiological stimuli and are not influenced by time of day per se.

Cardiac preload and arterial pressure regulation

Altered arterial pressure at heart level influences cardiac afterload; changed filling (central venous) pressure shifts cardiac preload and diastolic blood supply to the ventricles. Head-up tilting reduces cardiac output immediately and significantly by about 30%. Thus, postural changes induce orthostatic „training“ (conditioning) for cardiovascular regulation (13, 17, 50). In fact, absence of Gz stimuli brings about rapid „deconditioning“ as evidenced by decreased baroreceptor reflex efficiency after a few days of spaceflight or bed rest (6, 56, 71).

Short (30 min) cardiovascular stimulation of moderate LBNP creates transient cardiovascular effects: Hemodynamic and thoracic impedance (TI) changes were measured during LBNP levels of 15 to 65 mmHg. LBNP >15mmHg increased TI by almost 10%, indicating decreased fluid volume (41). Mean arterial pressure remained stable whereas heart rate increased up to 55% and then fell to values below pre-LBNP control (“post-LBNP bradycardia”). It was concluded that various levels of LBNP produced different time courses and dose-response patterns, and the subjects remained non-hypotensive to 65-mmHg suction, indicating an altered cardiovascular state after 30 min of LBNP. It is probable that altered hormone concentrations; e.g., attenuated pRA and angiotensin II levels may contribute to this escape-like phenomenon (26). Repeated orthostatic challenge may produce some effects, which may persist even after an overnight supine rest period. Conversely, microgravity exposure during prolonged spaceflight should reduce orthostatic competence.

Local tissue composition

Astronauts can experience changes in body composition during / after their missions. The problem of monitoring such changes is pertinent not only to spaceflight physiology but to medicine in general: Mass and distribution of body fat is relevant as a possible cardiovascular risk factor as well. Prospective studies have demonstrated associations between body mass and shape, and the occurrence of stroke, myocardial infarction, angina pectoris, and death from coronary heart disease. We decided to find a novel, direct and sensitive method to assess central (abdominal) fat mass since established methods all suffer from drawbacks. To do so, we estimated subcutaneous fat layer thickness (SFL) by transimpedance measurement between two pairs of electrodes attached directly above the area of interest (64a) – **Fig. 2**.

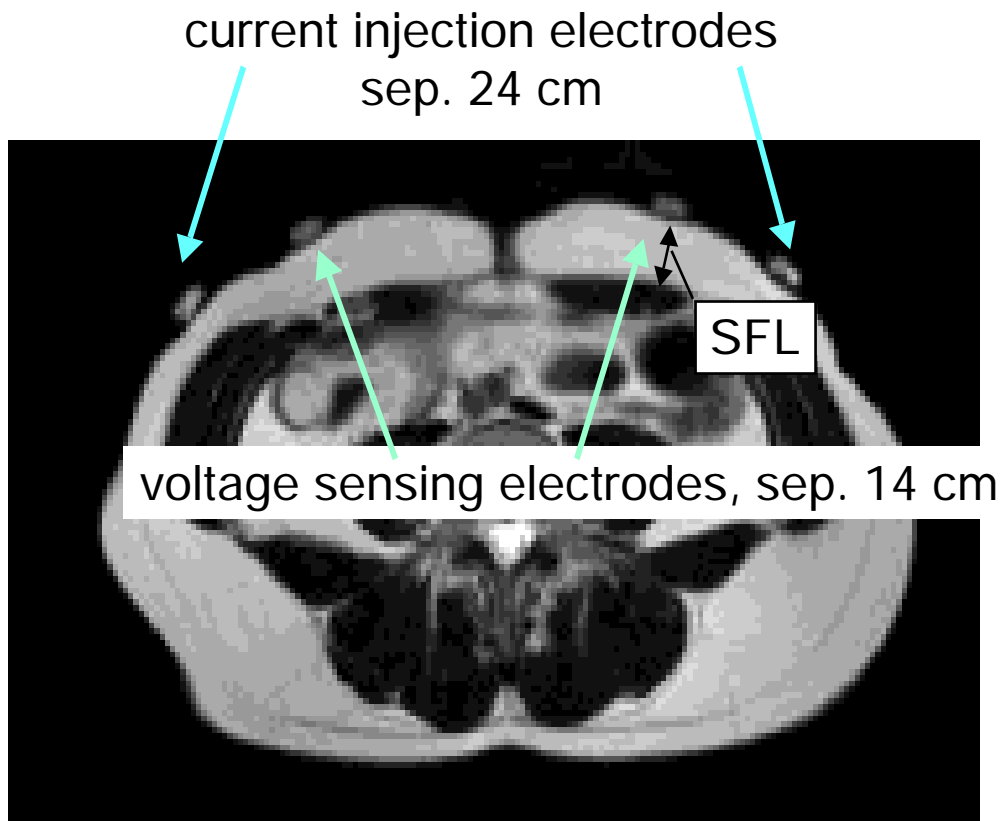


Fig. 2: Transversal abdominal cross section at umbilical level.

It was not expected to find a linear relationship between abdominal transimpedance and SFL, but this is exactly how it turned out to be (**Fig. 3**). The theory would predict a non-linear relationship between impedance and fat layer thickness; in our setup, obviously the postulated non-linear correlation is linearized due to contributions from deeper adipose structures, like the mesentery. This renders our electrode arrangement particularly attractive for simple data analysis. Further, it can be seen from fig. 2 that there is just a minor difference between standing (upper panel) and supine position (lower panel), indicating that this approach would probably yield rather robust data during microgravitational conditions and reduced gravity (as on Mars) as well.

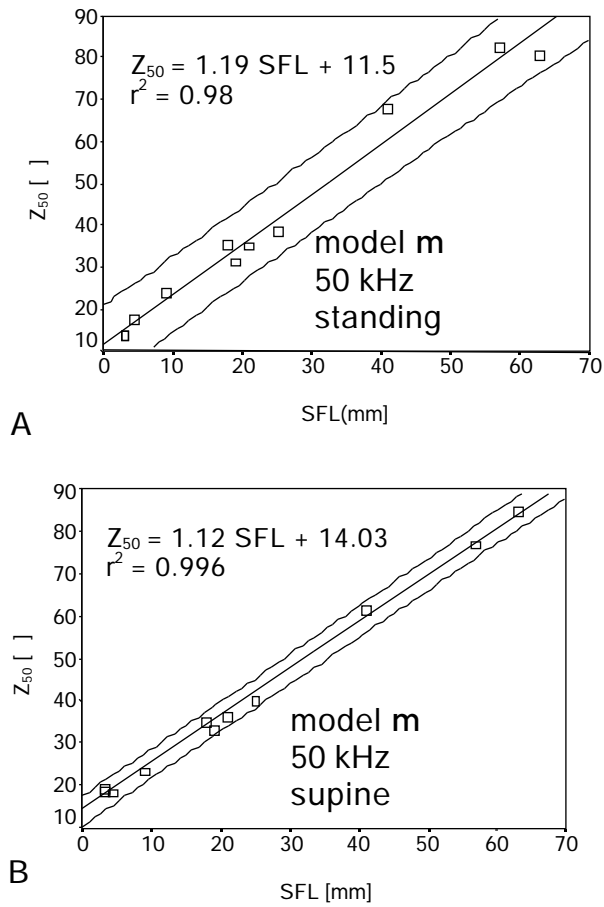


Figure 3: Correlation between abdominal subcutaneous fat layer thickness (SFL) as validated by NMR imaging, and impedance (Z , in Ohms) at 50 kHz with upright (A) and lying (supine) position (B). Regression lines with 95% prediction confidence bands.

Inflight experiments

On the basis of ground-based simulation data, it is logical to ask whether hormonal systems respond similarly in astronauts during an extended stay in microgravity. After preliminary experiments during a 9-day space mission (35), our hypothesis was that endocrine stress-responses would deviate from ground-based patterns during extended flight. Of particular interest and importance were pANP and pcGMP, hormones related directly to cardiac distension (17,35,37,44), because transmural central venous pressure (TCVP) which influences ANP output from the heart and is elevated despite decreased CVP in parabolic flight (78) might be downregulated on a longer duration flight.

Basal levels and LBNP-induced changes of volume regulating and stress-sensitive hormones were measured in venous plasma of one cosmonaut (52 yr, 174 cm, 88.3 kg preflight) before (-45 days: supine), during (3, 170, 287, 430 days) and after (+4, +90 days: supine) this 438-day flight. Blood was taken at the beginning and immediately after LBNP (-15/-30/-35 mmHg for 15/15/10 min). There was persistent lowering of pANP inflight along with extremely low pcGMP levels; however, transient hormonal changes after LBNP (as % of pre-LBNP values) were not different from ground-control findings; and other hormone levels did not deviate consistently from ground control values (27,32,53). Based on findings from prolonged head-down tilt (HDT) experiments (17), we hypothesized that ANP would be decreased similarly during the mission (confinement per se does not change pANP: 44). This assumption was validated by a 70 to >95% reduction of pANP inflight. Since the preflight pANP values were rather high, flight levels stayed within the normal range except on day 430 where they fell below the lower limit assigned as 20 pg.ml⁻¹. After landing pANP returned to 100 pg.ml⁻¹, presumably due to readjustment to 1-G cardiac preload and atrial distension.

It is not clear why central venous pressure is not altered during early exposure to microgravity (37) when, at the same time, cardiac filling is increased. Recent data from parabolic flight experiments have demonstrated an increased transmural atrial pressure and diameter vis-à-vis decreased CVP (78). Transmural CVP, rather than CVP per se, stimulates ANP secretion from the atria; however, TCVP has not yet been determined during prolonged flight. A lasting suppression of pANP, as observed in our study, could be the result of at least two mechanisms with continued stay in microgravity, e.g., Hemodynamic adaptation to lowered TCVP, or decreased ANP secretion despite high TCVP.

The pattern of low inflight pANP was underlined by the pcGMP data in which inflight levels were well below the 3-7 nM normal range on Earth. A 70-90% fall in pcGMP concentration occurred on day 6 of the previous short-term mission (35); now pcGMP remained depressed during the long-term flight as well, and also on the 4th day postflight; but returned to preflight levels by postflight day 90 (**Fig. 4**). It seems that the cGMP system responds to spaceflight and post-landing conditions with delayed adaptation / readaptation.

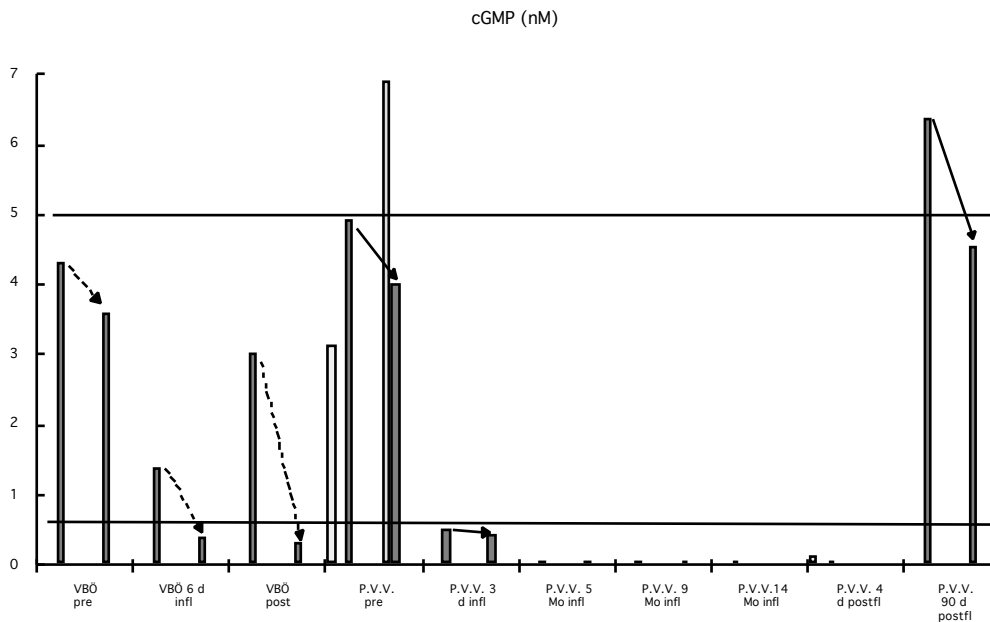


Figure 4. Basic levels and LBNP-induced effects (arrows) of pcGMP in one cosmonaut (VBÖ) before flight (pre), inflight, and after landing (post) during one short-term (9-day) and one long-term (438-day) flight (PVV), respectively. pcGMP is all but absent during and shortly after the long-term flight; however, LBNP always decreases pcGMP by 20%.

Although the physiological role of intracellular pcGMP is well known as a second messenger of ANP's biologic action, its extracellular significance is unclear. However, cGMP excretion has been used as a marker for changes in the ANP system during HDT bedrest studies (72). Acute stress produces different responses in pANP and pcGMP; dissociation of pcGMP and pANP has been observed in conjunction with postural changes and LBNP. Earlier findings of a parallel decrease of both pANP and pcGMP with 45 and 90 min LBNP may apply only to the low (-15 mmHg) suction level used (45).

These case-study endocrine stress-response data are the first from a flight exceeding one year. There was pronounced and consistent depression of plasma ANP and cGMP during flight while plasma catecholamines during LBNP were elevated mid-flight and after landing. There were no deteriorated LBNP-induced hormonal alterations during flight, indicating responsive regulatory status. Thus, long-term flight may not pose serious changes within steady-state variables and stimulus-response dynamics of fluid-electrolyte-endocrine control systems.

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