BED REST IMMOBILIZATION WITH VARIOUS ORAL SODIUM SUPPLY: PLASMA HORMONES AND BODY FLUIDS

HELMUT G. HINGHOFER-SZALKAY[1,2], ZOLTÁN LÁSZLÓ[3], DANIELA JEZOVA[4], ANDREAS RÖSSLER[2], BERND HADITSCH[1], KARL PILZ[1], HERFRIED PASSATH[1], HERMANN SCHARFETTER[5]

[1]Institute for Adaptive and Spaceflight Physiology, Graz, Austria (http://www.asm.at/iap); [2]Department of Physiology, Medical Faculty, University of Graz, Austria; [3]Csepeli Weiss Manfréd Hospital, Budapest, Hungary; [4]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [5]Institute for Electro- and Biomedical Engineering, Technical University Graz, Austria Email: helmut.hinghofer@uni-graz.at

Objective. To answer the question if plasma hormone concentrations (plasma renin activity – PRA, vasopressin – pAVP, and aldosterone concentration) due to antiorthostatic immobilization (8 days -6° head-down tilt bed rest – HDBR) are altered by oral salt load, we provided constant sodium supply during 4 days ambulatory conditions followed by 8 days HDBR in 10 normotensive men.

Methods. A ‚low' (LS: 143±10 mM) and ‚high' (HS: 434±17 mM Na^+/d excreted) sodium treatment were provided in randomized order, separated ≥1 mo. Before and at the end of HDBR, hemodynamic variables and thoracic impedance were determined, and blood was taken for aldosterone and PRA, venous hematocrit, and plasma mass density. Extracellular fluid volume and pAVP were determined every second day. Whole body electrical impedance spectroscopy was employed to assess changes in extracellular volume, hormone determinations were done with radioimmunoassay, mass density measurements with the mechanical oscillator technique.

Results. Extracellular volume decreased with HDBR (LS: -4.0%, p=0.002; HS: -5.8%, p=0.018) without significant difference between salt treatments. Resting hormone levels were not altered by HDBR, but pAVP was lower (5.5±0.1 pg/ml) in HS than in LS (7.2±0.3 pg/ml) as was plasma aldosterone (HS: 69±7 pg/ml, LS: 180±24 pg/ml). On the other hand, HDBR reduced extracellular volume by ?5% irrespective of dietary sodium supply.

Conclusions. Our data support the hypothesis that hormonal activities are more affected by oral salt load than by simulated short-term space flight, and suggest that the reduction of extracellular fluid volume due to head down bed rest is not influenced by moderate changes of dietary sodium supply.

Key words: Renal function – Extracellular volume – Bed rest – Immobilization – Salt intake – Vasopressin – Plasma renin activity – Aldosterone – Bioelectrical impedance spectroscopy – Sodium excretion – Cardiovascular – Extracellular fluid volume

Bed rest, particularly in head down (-3 to -10°) position (antiorthostatic bed rest, HDBR) minimizes Gz-effects on the circulatory systems (NORSK 1992; FORTNEY et al. 1996) and produces cardiovascular, deconditioning' that is in certain aspects similar to the effects of spaceflight microgravity (BUCKEY et al. 1996). It reduces extracellular volume (ECV) (GREENLEAF 2001) as early as after 2 days (FORTNEY et al. 1991) together with a ?10% plasma volume loss (MAURAN et al. 1999). It has been suggested that after an initial fluid loss, adaptation to HDBR leads to a new steady state within ≥24 hours and elicits antinatriuretic endocrine signals (BESTLE et al. 2001), resulting in decreased plasma and ECV (CONVERTI-

no et al. 1990; Fortney et al. 1991; Johansen et al. 1997). According to previous investigations, a loss of total body water of up to 5 % (Blanc et al. 1998) is mirrored by a commensurate body mass decline (Blanc et al. 1998 Mauran et al. 1999 Bestle et al. 2001). But it has also been suggested that total body water stays unchanged despite diminished ECV during bed rest, which could be explained by a fluid shift from extra- to intracellular compartments (Fortney et al. 1991, 1996; Millet et al. 2001; Ksinantova et al 2002). Although this dispute has not yet been resolved, HDBR-induced ECV loss is a well established effect.

There is much less agreement on hemodynamic and hormonal effects of bed rest. Heart rate and blood pressure were reported both unchanged and increased, vasopressin unchanged or reduced, norepinephrine reduced but microneurographic sympathetic burst rate elevated (Arbeille et al. 1992; Gharib et al. 1992; Schmedtje et al. 1996; Kamiya et al. 2000; Bestle et al. 2001; Millet et al. 2001a,b). in most of these studies, oral sodium supply was not controlled, and different sodium load might explain at least some of these contradictory observations. Changing sodium balance as influenced by dietary sodium supply alters body fluid volume, hormonal, and possibly hemodynamic regulations. This determines cardiovascular function as characterized by altered cardiac preload pressures and cardiovascular responses (Roos et al. 1985; Hall et al. 1986; Epstein 1992; Luetkemeier 1995; Miller 1995). Rising salt intake has been found to reduce plasma renin activity, aldosterone and catecholamines (Mark et al. 1978; Roos et al. 1995; Bech et al. 1998; Heer et al. 2001) while to enhance atrial natriuretic peptide concentration (Buckley et al. 1994; Bech et al. 1998). Plasma volume and central venous pressure also depend on sodium intake (Miller 1995; Andersen et al. 1998), and resting heart rate has been shown to be higher with very low as compared to normal salt consumption (Schorr et al. 1997).

Increased saline ingestion also has been said to raise extracellular volume (Manning and Guyton 1982; Roos et al. 1995; Thompson et al. 1990; Miller 1995); however, a recent study demonstrated that changing sodium supply did not have an effect on total body water (Heer et al. 2001). It has been hypothesized that extra sodium might be stored in the interstitial space during low-salt conditions, suggesting the existence of a reservoir able to store sodium in an osmotically inactive form (Titze et al. 2002) and fluid retention in spite of sodium depletion, with the result of stabilizing ECV. This issue does not seem to be resolved yet.

Therefore, the hypothesis tested in this study was that along with corresponding hormonal responses, salt intake would influence the reduction of extracellular volume due to head down tilt bed rest. Specifically, we expected larger ECV loss with 'low' as compared to 'high' salt during HDBR, together with increased levels in aldosterone concentration and plasma renin activity. Further, we wanted to clarify if resting (morning) levels of vasopressin are influenced by salt treatment during 8 days head down bed rest.

Subjects and methods

The study was approved by the University of Graz Ethical Board. 10 healthy male lean normotensive test persons - 27±6 y, 72±11 kg, 178±5 cm - were informed about nature and purpose of the study, refrained from alcohol, smoking, and medication, and gave their written consent. They all finished the study according to its design.

Sodium status. Dietary sodium supply was set during the entire 4+8 day time frame either to 'low' (LS: ?120 mM Na^+/d ingested) or 'high' (HS: ?420 mM Na^+/d ingested) by a professional dietitian. The menus differed in sodium content but were equal in terms of energy supply, with protein/fat/carbohydrate contributing approximately 15/35/50 %. Five men received HS first, the five others started with LS; the treatments were set more than 1 month apart. Water or juice was allowed ad libitum during the entire experiment duration. Sodium excretion was measured to verify salt intake (Roberts et al. 1991).

Study protocol. The protocol aimed to answer two questions: First, how do vasopressin concentrations and extracellular volumes develop over time under the conditions of this study? Every second day, blood was taken for AVP determination at 0900 hrs in supine position, and body impedance measurements performed. Second, how do – after a 4-day salt adaptation period – other hormonal, blood volume sensitive, and hemodynamic variables change due to

8 days HDBR with continued salt treatment? On day 5 (immediately pre-HDBR) and 13 (end-HDBR), according blood sampling and measurements were performed between 0900 and 1200 hrs.

The entire experiment lasted 12 days (day 1 through day 13 – Fig. 1). An ambulatory phase with moderate physical activity allowed for adaptation to controlled salt intake - adaptation to clamped salt intake needs about 4 days (day 1-5) to assume full effect (Roos et al. 1985; Armstrong et al. 1987; Buckley et al. 1994). This was followed by an 8-day HDBR phase (day 5-13): Each bed was put in a reclined –6° position. A pillow provided neck support. Eating, drinking and all personal hygiene was done while strictly avoiding upright posture. There was 24-hour camera surveillance and continuous supervision by a professional nurse staff.

On-site measurements. Hemodynamic measurements were performed on a semi-continuous basis with an automated cuff oscillometry (Dynamap) and monitoring of ECG, heart sounds, and thoracic impedance performed using a commercially available setup (ICG-M401, AKG, Budapest, Hungary) on days 5 (per-HDBR) and 13 (post-HDBR). Whole body multi-frequency impedance measurements were performed on days 1, 3, 5, 7, 9, 11, and 13 between 1000-1200 hrs after lying supine ≥10 mins for extracellular fluid assessment (Deurenberg and Schouten 1994; Lichtenbelt et al. 1994; Deurenberg et al. 1995; Scharfetter et al. 1997). For this purpose, electrodes were attached at the right hand and foot, and measurements started within 3 min. A commercially available body impedance spectrometry system (XITRON 4000B, Xitron Technologies, San Diego) was used. For each measurement point, 6 datasets were generated, with each dataset obtained by averaging 5 spectra sampled at 50 logarithmically spaced frequencies between 5 and 500 kHz. ECV was estimated from impedance data which have been extrapolated to the theoretical frequencies zero and infinity by use of Cole-Cole modeling (Scharfetter et al. 1997).

Blood / urine sampling and processing. Urine samples were collected on a void-by-void basis throughout the day, volumes documented, and samples stored at –20 °C for later determination of daily sodium excretion from 24-h urine aliquots, using flame photometry.

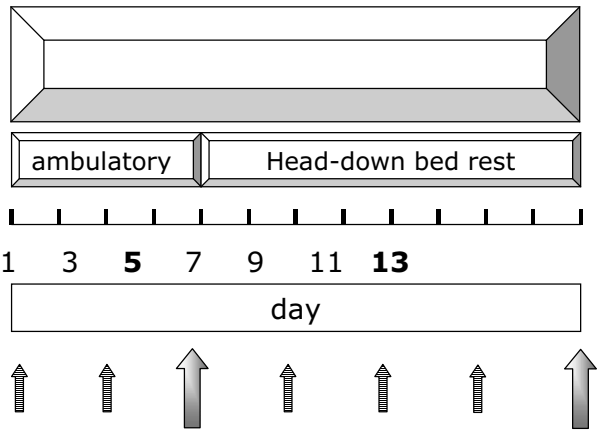

Fig.1 Test protocol outline. Every second day (1, 3, 5,…) blood was taken at 0900 hrs for vasopressin measurement, and body electrical impedance determinations performed in supine position which was commenced at least 30 mins prior to sampling / measurements. On days 5 and 13 between 0900 and 1200 hrs (i.e., pre- and post-bed rest), blood was taken for additional hormone, plasma density, and hematocrit measurements, thoracic impedance was determined, and ECG and heart sounds were taken together with forearm arterial blood pressure for hemodynamic monitoring.

Antecubital venous blood was sampled generally after 30 min supine rest at 0900 hrs in order to circumvent the influence of circadian rhythms, using a 17G-1.4x40 mm Teflon catheter. Heparinized blood was used for hematocrit and for spinning to platelet-poor plasma for plasma density measurement. Another part was collected in EDTA test tubes, spun immediately, and stored at -80°C until hormone analyses were performed.

Measurements on blood and plasma. Hematocrit was measured in triplicate using microcentrifugation; no adjustments for trapped plasma or Fc-ratio were done. Blood plasma mass density (g/l, 37 °C, 0.2 ml samples) was measured based on high-resolution oscillation-time determinations using the mass-spring principle because changes reflect, i.a., blood volume changes due to capillary filtration phenomena (Hinghofer-Szalkay 1986).

Hormone measurements were performed in duplicate using competitive or sandwich immunoassays for plasma renin activity (PRA), aldosterone, and arginine vasopressin (AVP). Total plasma volume required for single determinations for aldosterone and PRA was 0.8 ml (combined) and an additional 1.0

ml for vasopressin. The procedures were identical as employed by us earlier (László et al. 2001).

Biometry. All data distributions are indicated as mean ± SEM. We applied two-way ANOVA hypothesis testing with salt treatment (LS / HS) and orthostatic condition (pre- vs. post-HDBR) as between group factors and plasma hormone concentration, hemodynamic data, thoracic impedance, plasma density, and hematocrit as dependent variables. Differences between mean values were evaluated by a post-hoc multiple range test (Newman-Keuls). One-tailed, paired t-tests were applied for testing ECV effects from body electrical impedance. An alpha error cut-off level of 0.05 was used for hypothesis testing, and p values are indicated only in case of significant effects.

A two-step approach was employed to assess extracellular volume from impedance data: First, the intra- and extracellular resistances were determined by fitting a Cole-Cole model to the measured data. From these resistances, volumes were then calculated with a fluid distribution model (Scharfetter et al. 1997). Data fitting was done by using an iterative method for nonlinear parameter identification from the optimization toolbox of Matlab™ 5.1. For ECV changes during ambulatory conditions, i.e. due to sodium treatment *per se* (day 1-5: 3 data points) and for differences between LS-HDBR and HS-HDBR (day 5-13: 5 data points per subject and treatment), non-linear fits were applied to assess ECV before and after HDBR.

Table 1
Venous hematocrit (volume %, means±SEM from micro-centrifugation; no corrections applied), plasma mass density (G/l, 27.0 °C. means±SEM), extracellular volume (liters, means±SEM) and thoracic electrical impedance (Ohms, means±SEM) with commencement (day 5) and finishing (day 13) o head down bed rest with low (LS) vs. high (HS) oral salt supply. ECV decreases with HDBR, no significant changes within other comparisons.

Venous hematocrit (Hct)

Sodium status	Day 5 (pre-HDBR)	Day 13 (post-HDBR)
LS	43.6 ± 0.9	44.6 ± 0.8
HS	42.6 ± 0.7	44.1 ± 0.6

Plasma mass density (PD)

Sodium status	Day 5 (pre-HDBR)	Day 13 (post-HDBR)
LS	1020.1 ± 0.2	1020.3 ± 0.2
HS	1019.5 ± 0.2	1019.3 ± 0.4

Extracellular volume (ECV)

Sodium status	Day 5 (pre-HDBR)	Day 13 (post-HDBR)	p
LS	19.8 ± 0.8	19.0 ± 0.7	0.00
HS	20.9 ± 0.8	19.6 ± 0.6	0.02

Thoracic impedance (Z_0)

Sodium status	Day 5 (pre-HDBR)	Day 13 (post-HDBR)
LS	27.3 ± 0.6	28.1 ± 0.8
HS	25.8 ± 0.7	27.7 ± 0.9

Results

Mean sodium excretion was 143±10 mM/d in the LS and 434±17 mM/d in the HS group from day 1-13, respectively. Urine output was 1.9 ± 0.6 liters/day in the LS, and 2.5 ± 0.3 liters in the HS group, respectively.

Extracellular volume changes. ECV decreased during HDBR by 0.79±0.32 l (-4.0%; p=0.002) in the LS, and by 1.21±0.31 l (-5.8 %; P=0.018) in the HS treatment, with no significant difference between the two. During 4 days pre-HDBR, there was a 3.3 % ECV decrease (P=0.02) in the LS but no change in the HS group. Data are given in Tab. l.

Hematocrit (Hct) and plasma mass density (PD). Measurements were done at the end of the AMB (day 5) and of the HDBR periods (day 13), respectively. The results are displayed in Table 1.

Effect of sodium supply: Hct and PD values were higher with LS as compared to HS, suggesting a state of lower plasma volume with salt depletion. However, statistical significance was only reached on day 5 for Hct, and on day 13 for PD.

Effect of antiorthostatic bed rest: Only the Hct increase with HS was statistically significant. HDBR did not produce consistant results in terms of blood volume change indicators.

Plasma hormone concentrations. Taking the individual means from measurements between day 1-13 (N=7), the pAVP level was higher in the LS than in the HS group (7.0 ± 0.5 vs. 5.1 ± 0.9 pg/ml, P=0.033 – Fig. 2).

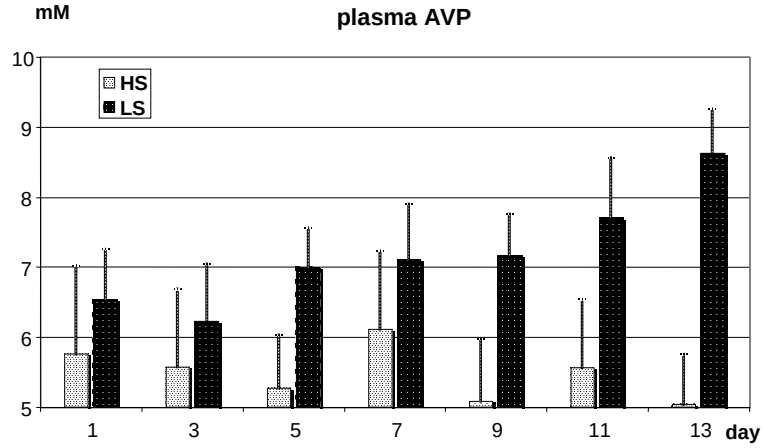

Fig. 2 Plasma vasopressin (means ± SEM) with time and low (LS) vs. high (HS) oral salt supply.

Aldosterone and PRA levels were lower (significantly in the case of aldosterone) in the HS as compared with LS treatments; in neither case did HDBR produce significant effects on basal aldosterone or PRA levels (Table 2).

Hemodynamic statistics are given in Table 3. Multivariate ANOVA did not show any significant changes in hemodynamic variables either with salt treatment nor bed rest.

Discussion

Bed rest removes much of the gravitational stimulation and is considered an analogue to weightlessness. Many effects of bed rest have been observed but give a heterogenous picture. Amongst others, it has been reported that heart rate, blood pressure, and plasma concentration of volume effective hormones remain unchanged after 2 days; that renin, aldosteron (Fortney et al. 1996) and angiotensin II levels are increased after one week (the duration as used in this study) while vasopressin is reduced (Bestle et al. 2001); that vasopressin remains unchanged while plasma ANP and norepinephrine are reduced along

Table 2
Venous plasma renin activity and aldosterone concentration (means±SEM) with commencement (day 5) and finishing (day 13) of head down bed rest with low (LS) vs. high (HS) oral salt supply

Plasma aldosterone concentration

Sodium status	Day 5 (pre-HDBR)	Day 13 (post-HDBR)
LS	200 ± 45	160 ± 17
HS	57 ± 7	81 ± 10
p	0.01	0.01

Plasma renin activity

Sodium status	Day 5 (pre-HDBR)	Day 13 (post-HDBR)
LS	2.1 ± 0.4	1.9 ± 0.2
HS	1.2 ± 0.3	2.1 ± 0.7

Table 3
Hemodynamic variables and thoracic electrical impedance (means±SEM) with commencement (day 5) of head down bed rest with low (LS) vs. high (HS) salt supply

	Heart rate (min^{-1})	Mean arterial pressure (mm Hg)	Pulse pressure (mm Hg)	Stroke volume index (ml/m^2)	Cardiac index ($l/min/m^2$)
LS, day 5	68.3 ± 0.2	91.5 ± 0.2	55.2 ± 0.1	27.3 ± 0.3	2.52 ± 0.01
LS, day 13	71.1 ± 0.4	89.7 ± 0.1	57.4 ± 0.4	36.2 ± 0.3	2.56 ± 0.01
HS, day 5	71.3 ± 0.3	96.8 ± 0.1	64.5 ± 0.2	45.5 ± 0.3	3.23 ± 0.02
HS, day 13	69.4 ± 0.4	91.2 ± 0.2	61.1 ± 0.2	43.3 ± 0.5	2.97 ± 0.02

with increased basal renin and aldosterone levels after 1-4 weeks (Arbeille et al. 1992; Gharib et al. 1992; Schmedtje et al. 1996; Millet et al. 2001); that blood pressure, heart rate, microneurographic sympathetic burst rate, and calf vascular resistance are increased after 14 days (Kamiya et al. 2000); and that systolic blood pressure and heart rate are unaffected after 17 days bed rest (Millet et al. 2001).

Different experimental conditions, one of them being variable sodium supply, may have contributed to those conflicting results. Resting heart rate was shown to be higher with very low (19 mM/d) as compared to normal sodium input ('high': 215 mM/d, Schorr et al. 1997). In our study, no consistent heart rate differences were observed when LS and HS conditions were compared. It is conceivable because we did not employ extreme salt regimes. This applies to the other hemodynamic variables as well. The multivariate analysis of our data indicate that irrespective of dietary sodium supply, 8 days head down tilt bed rest does not produce any hemodynamic changes.

Both bed rest immobilization and altered sodium availability impinge upon body fluid volume dynamics and renal / cardiovascular functioning; combined effects have not received much attention yet. Recent studies have shown that in the adapted ‚steady' state (i.e., after ≥24 hrs HDBR) the natriuretic response to saline infusion is unchanged (Drummer et al. 1992) or even augmented after approximately 1 week (Bestle et al. 2001), despite reduced extracellular and plasma volumes. Obviously, sodium and fluid volume regulation during HDBR are intertwined and need to be studied in more detail.

One of our working hypotheses was that different sodium supply might explain such contradictory observations from earlier investigations. Our data, however, do not indicate significant changes within plasma concentration of these hormones with HDBR, neither in the LS nor in the HS groups. Endocrine responses to bed rest do not seem to be significantly altered by sodium chloride intake, at least not in the range chosen in this study. This fits well with the lack of hemodynamic changes with bed rest.

Our plasma vasopressin data suggest higher morning values in the LS vs. HS treatment. Also there was a tendency towards increasing AVP with time in the LS group. Since this effect was not seen in HS, this is conceivably not an effect of HDBR *per se*. We are not aware of investigations that would clarify if chronic salt intake, under conditions comparable to those used in this study, can influence plasma AVP levels during a time course of several days. Seckl et al (1986) have reported a fall of vasopressin with oral hypertonic saline in humans, these effects were transient in nature, however. It seems that additional investigations into possible long-term plasma vasopressin effects caused by various sodium supply are warranted to clarify this issue.

Since plasma volume has been reported to decrease with bed rest (Hyatt 1969; Fortney et al. 1991; Mauran et al. 1999), we expected changes in hematocrit (Hct) and plasma mass density with HDBR. This did not occur, with the exception of hematocrit in the HS group (3.5 % increase, P=0.004). It must be kept in mind that plasma mass density is primarily influenced by total protein concentration (Hinghofer-Szalkay 1986), which might be influenced by altered protein synthesis and/or removal kinetics over several days independently of capillary fluid shifts. Similarly, hematocrit is potentially influenced by other factors next to plasma volume over an extended time frame. Thus, plasma volume might have decreased without increasing plasma density and hematocrit.

Carefully applied impedance spectroscopy is sensitive to ECV changes in persons in supine position (Deurenberg and Schouten 1994; Scharfetter et al. 1997) and given appropriate model assumptions (Lichtenbelt et al. 1994; Deurenberg et al. 1995). Since we found an equal ECV decrease during 8 days HDBR both with high and low salt treatment, it needs to be discussed why salt treatment did not result in different ECV decrease with HDBR. Increased saline ingestion has been thought to raise extracellular volume, but a recent study conducted in more than 30 test subjects (Heer et al. 2001) demonstrated that changing sodium supply did not have an effect on total body water. Rather, fluid seemed to been shifted from the interstitial to the intravascular compartment with high salt load, increasing plasma volume. If extra sodium can be stored in the interstitial space during low-salt conditions without being osmotically active vis-ŕ-vis the circulatory system, as suggested in a recent investigation (Titze et al. 2002), an extra amount of fluid might be retained in spite of sodium depletion and ultimately stabilize extracellular fluid volume.

The results of our study add credibility to this hypothesis because there was no significant difference between the ECV effect of HDBR with high vs. low salt intake. Thus, contrary to the commonly held view that extracellular fluid volume rises with salt intake, our data seem to corfirm a lack of such an effect. The ECV constriction due to HDBR was unaffected by oral sodium supply in the range used. Significant ECV changes from whole body impedance spectrometry with larger differences in sodium balance cannot be excluded; indeed, we found a significant (albeit small) ECV decrease during the 4 pre-HDBR days of adaptation to LS. No effect was seen in the HS group, obviously because HS more closely matched the test person's everyday salt intake.

Plasma volume and central venous pressure also depend on oral sodium intake: A 250 mM/d sodium diet resulted in higher central venous pressure than 20-mM/d dietary sodium (Miller 1995). Another study chose 75 vs. 300 mM/d sodium, whithout significant effects on central venous pressure (Andersen et al. 1998). From this it seems that rather drastic alterations in salt supply are required to induce significant preload changes. Indeed, our data do not indicate an effect on thoracic hydration because thoracic electrical impedance (Z_0) was not significantly different in HS as compared to LS treatment. Neither did HDBR produce significant changes of thoracic impedance.

In conclusion, we show that after simulated weightlessness (8 days head-down bed rest), aldosterone levels are increased above pre-bed rest levels only when test subjects were given 'high' doses of dietary sodium. According to our whole body impedance data, this cannot be explained by differences in extracellular volume as expected, since the ECV decrease due to bed rest was not different in the HS vs. LS situation. However, an ECV decrease during 4 days adaptation to LS was found. Further, we could demonstrate higher vasopressin levels with LS vs. HS but without influence of HDBR. This lends support to the hypothesis that hormonal activities are more affected by oral salt load than by simulated short-term space flight.

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Corresponding author: Helmut G. Hinghofer-Szalkay
Institut für Physiologie, Uni Graz
Harrachgasse 21/5, A-8010 Graz, Austria
Phone: +43 (316) 383638
Fax: +43 (316) 381270
Email: helmut.hinghofer@uni-graz.at