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Effect of Head-Down Bed Rest on the Neuroendocrine Response to Orthostatic Stress in Physically Fit Men

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Summary

The role of neuroendocrine responsiveness in the development of orthostatic intolerance after bed rest was studied in physically fit subjects. Head-down bed-rest (HDBR, –6 degrees, 4 days) was performed in 15 men after 6 weeks of aerobic training. The standing test was performed before, after training and on day 4 of the HDBR. Orthostatic intolerance was observed in one subject before and after training. The blood pressure response after training was enhanced (mean BP increments 18±2 vs. 13±2 mm Hg, $p<0.05$, means ± S.E.M.), although noradrenaline response was diminished (1.38±0.18 vs. 2.76±0.25 mol.l^{-1}, $p<0.01$). Orthostatic intolerance after HDBR was observed in 10 subjects, the BP response was blunted, and noradrenaline as well as plasma renin activity (PRA) responses were augmented (NA 3.10±0.33 mol.l^{-1}, $p<0.001$; PRA 2.98±1.12 vs. 0.85±0.15 ng.ml^{-1}, $p<0.05$). Plasma noradrenaline, adrenaline and aldosterone responses in orthostatic intolerant subjects were similar to the tolerant group. We conclude that six weeks of training attenuated the sympathetic response to standing and had no effect on the orthostatic tolerance. In orthostatic intolerance the BP response induced by subsequent HDBR was absent despite an enhanced sympathetic response.

Key words

Standing • Noradrenaline • Adrenaline • Plasma renin activity • Aldosterone

Introduction

Orthostatic intolerance and decreased exercise capacity develop during prolonged physical inactivity or space flight. Several mechanisms may contribute to orthostatic intolerance during bed-rest or weightlessness, including hypovolemia (Blomqvist 1983, Buckey *et al.* 1996), impaired baroreflex function (Hughson *et al.* 1994), increased venous compliance and compromised peripheral arterial vasoconstriction (Buckey *et al.* 1992), cardiac deconditioning (Levine *et al.* 1997), changes in proprioceptive and vestibular sensation (Convertino *et al.* 1997), and disturbances in the secretion and peripheral effects of catecholamines (Robertson *et al.* 1994, Shoemaker *et al.* 1999).

Reduced levels of plasma and urine catecholamines (Goldstein *et al.* 1995, Sigaudo *et al.* 1998) as well as a diminished response of plasma noradrenaline during cold pressure test (Convertino *et al.* 1998) might imply a direct inhibitory effect of bed-rest on sympathetic activity, resulting in decreased exercise capacity and orthostatic intolerance. On the other hand,

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the sympathoadrenal activity during space flight and immediately after landing may be enhanced (Robertson *et al.* 1999). After landing, an increased sympathoadrenal response was found in astronauts completing the orthostatic challenge, while the noradrenaline response was impaired in subjects exhibiting intolerance during orthostatic stress (Fritsch-Yelle *et al.* 1996). On the other hand, Convertino and Sather (2000) have observed an unchanged sympathoadrenal, but a reduced PRA response to the tilt test in subjects with low orthostatic tolerance. The blood samples in the aforementioned studies were taken only at the end of orthostatic challenge. Duration of the upright posture is one of the principal determinants of catecholamine levels with an increase within two minutes and the peak between five and twenty minutes (Saar and Gordon 1979). The question is arising whether orthostatic intolerance is due to reduced neuroendocrine responses or whether diminished hormonal response is the result of shorter duration of standing.

Candidates for space flight are generally selected from a population with excellent physical fitness. In endurance-trained subjects both poor (Williamson *et al.* 1992) and good (Cybulski *et al.* 1999) orthostatic tolerance was reported. Endurance training is known to significantly influence neuroendocrine responsiveness to several stimuli. Athletes have lower neuroendocrine responses to the same absolute workload than sedentary subjects (Hartley *et al.* 1972, Kjaer 1989), but they are prone to enhanced sympathoadrenal responses to exhaustive loading (Zouhal *et al.* 1998). In fact, a more pronounced plasma noradrenaline and plasma renin activity (PRA) responses to physical exercise after bed-rest were found in endurance-trained athletes compared to untrained or strength-trained subjects (Smorawinski *et al.* 2001).

The present study was aimed to test the hypothesis that the orthostatic intolerance after bed-rest is related to attenuation of the neuroendocrine responsiveness. To diminish the effect of previous training status on the response variability, the volunteers before being subjected to bed-rest underwent training of the same type, duration and individual intensity. To avoid the effect of the duration of standing on the magnitude of neuroendocrine response, a blood sample was collected not only at the end of standing but another sample was withdrawn after three minutes of standing.

Methods

Fifteen jet aircraft pilots of an Army Air Base volunteered for the investigation as approved by the Ethical Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences. The subjects underwent detailed medical examination once a year and they performed sports activities according to their individual choice twice a week. Two subjects were occasional smokers, but no subject took any medication. The subjects had similar dietary habits and equal professional requirements.

They were of average age 34±1 years, height 177±1 cm and weight 81±3 kg (means ± S.E.M). The aerobic capacity was determined using an incremental exercise test on motor driven treadmill (Laufergotest, Jaeger, Germany). The initial velocity of the treadmill was 6 km per hour and increased by one km per hour every minute until the subject was unable to maintain the speed. The subjects were verbally encouraged to exercise until exhaustion. Ventilatory gas exchange parameters were measured every 30 s using an automated system (Ergo-Oxyscreen, Jaeger, Germany), which was calibrated according to the manufacturer's instructions. The heart rate (HR) was measured using an ECG monitor (Servomed, Hellige, Germany).

Training consisted of one hour of cross-country running at least 4 days of a week at the intensity of 70-80% of maximal oxygen uptake controlled by HR. This period lasted 6 weeks. One or two days after the last bout of the training the subjects were admitted to hospital and underwent HDBR. They remained in the –6 degrees tilt position, with one pillow, with a minimum of physical activity.

Each subject underwent orthostatic test before onset of the training (control), after finishing the training, and on day 4 of HDBR. The investigation started between 07:30 and 08:00 h after an overnight fast. A catheter (TriCath, Codan, Espergaerde, Denmark) was inserted into a forearm vein at least 30 min before control blood sampling. After 15 min in the sitting position in the control and training examinations, or in –6 degrees head-down tilt for the HDBR examination, the subjects were placed for 15 min in the supine position with the legs placed on a pad and the knees in 90° flexion. Then they were asked to stand for 10 min without any movement in silence without eye contact with the investigator. After 10 min of standing, the subjects were asked to lie in the supine position for 10 min. Blood samples were taken immediately before standing, after 3 min, in the last minute of standing, and after 10 min of rest. Orthostatic intolerance (OI) was defined as dizziness with swaying and/or loss of verbal contact. In cases of OI, the blood sample was taken immediately and the subject was placed in the supine position. Blood pressure (BP) and HR were

measured on the brachial artery with an automated sphygmomanometer (Dinamap, Johnson & Johnson, USA) before standing, every 2 min during standing and after 10 min of the subsequent rest.

Blood was collected into cooled polyethylene tubes. The samples were centrifuged at 4 °C and plasma was stored at –70 °C until analyzed. All samples were measured in duplicates. Concentration of noradrenaline (NA) and adrenaline (A) in the plasma was determined by the radioenzymatic method (Peuler and Johnson 1977). Plasma renin and aldosterone were measured using commercial radioimmunoassay kits (Immunotech, France).

Statistical analysis was performed with the Sigma Stat 2.0 program (Jandel Scientific, USA). A two-way ANOVA for repeated measurements with consecutive post-hoc tests was used for determining 1) differences from baseline, and 2) differences between the training and control as well as between the training and bed-rest conditions. The Students paired t-test or Wilcoxon signed rank test, if appropriate, were used to evaluate the differences of single variables. Two-sided values of $p<0.05$ were considered to be significant.

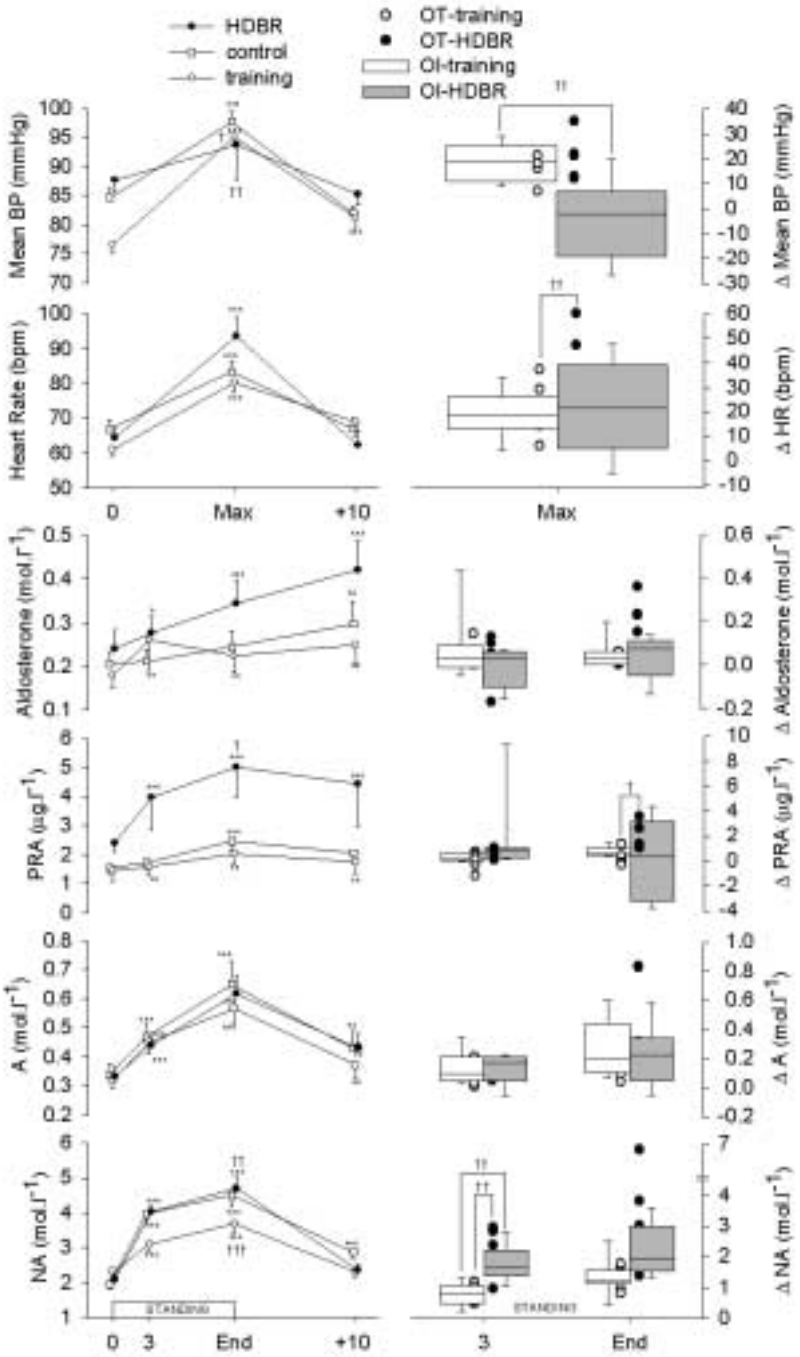

Fig. 1. *Left panel: Blood pressure, heart rate – baseline, maximal value, post-standing, plasma aldosterone, PRA, adrenaline and noradrenaline – all samples, in control period, after the training and subsequent bed rest. Data are expressed as means ± S.E.M. Statistical significance: p< 0.05, 0.01 and 0.001, *vs. baseline, †training vs. control, bed rest vs. training. Responses of the variables versus baseline in orthostatic intolerant subjects (OI, displayed as medians and 5th, 25th, 75th and 95th percentiles) compared to orthostatic tolerant subjects (OT, individual values) after training and subsequent bed rest.*

"End" describes the value in min 10 (OT) or immediately after OI occurrence. Statistical significance: p< 0.05, 0.01, and 0.001, †bed rest vs. training in each group. See legend in Fig 1.

Results

During standing before as well as after training, orthostatic intolerance occurred in one subject and after HDBR orthostatic intolerance developed in 10 out of 15 subjects. Training increased the aerobic capacity (VO_{2max} 0.060±0.002 vs. 0.055±0.003 $l.kg^{-1}.min^{-1}$, $p<0.01$). After training, resting BP and HR were reduced (mean BP, 77±1 vs. 85±2 mm Hg, $p< 0.01$; HR 61±2 vs. 67±3 bpm, $p<0.05$). After HDBR blood pressure was elevated (88±2 mm Hg, $p<0.001$), while resting HR was not changed (65±3 bpm). Training did not significantly influence baseline values of neuroendocrine parameters. After HDBR, plasma PRA was increased (2.5±0.3 vs. 1.4±0.4 $ng.ml^{-1}$, $p<0.05$).

Cardiovascular and neuroendocrine responses to standing in the whole group are displayed in the left panel of Figure 1. Mean BP increased similarly in both pre- and post-training examinations ($p<0.001$). HR rose during standing in all investigations ($p<0.001$). Training slightly decreased the HR response ($p<0.05$) and the highest increment of HR was found after HDBR ($p<0.01$). Neuroendocrine parameters rose during all periods of the examination. Training lowered the plasma NA response ($p<0.001$), but had no effect on responses of adrenaline, PRA and aldosterone. After HDBR the responses of plasma NA ($p<0.001$), aldosterone ($p<0.05$) and PRA ($p<0.05$) were enhanced. The plasma adrenaline response also tended to be augmented (N.S.).

The responses before and after HDBR of the group completing the standing (OT) after HDBR vs. the responses in OI subjects are displayed in the right panel of Figure 1. After HDBR a decrement of mean BP was observed in OI ($p<0.01$). The augmentation of HR and PRA responses after HDBR was significant only in OT (maximal HR value during standing, $p<0.01$; PRA at the end of standing $p<0.05$). NA response was augmented in both groups in the third minute of standing ($p<0.01$).

Discussion

The effect of head-down bed-rest on the neuroendocrine response during orthostatic stress was studied in healthy men after 6-week endurance training. The training was followed by an attenuation of the sympathoadrenal response to orthostatic stress. After bed-rest, a decrease of orthostatic tolerance was observed, although the plasma catecholamine response to standing up was increased in both the subjects who completed the test and in those who developed orthostatic intolerance.

Several studies have reported a significant effect of physical fitness on orthostatic tolerance and neuroendocrine responsiveness (Hartley *et al.* 1972, Kjaer 1989, Williamson *et al.* 1992, Zouhal *et al.* 1998, Cybulski *et al.* 1999, Smorawinski *et al.* 2001). The subjects were recruited from a population with higher physical fitness than in the average population (Macek *et al.* 1979). Six weeks of training increased their aerobic capacity by more than 10 %. Diminished baseline values of HR and BP as well as reduced HR response to orthostatic stress after training are in agreement with the cross-sectional data of Williamson *et al.* (1992) on untrained versus trained individuals. These authors reported serious impairment of orthostatic tolerance in their endurance-trained subjects after HDBR lasting 4 hours. On the other hand, after 10 weeks of the aerobic training program, the increases of aerobic capacity even larger than in our study were reported to have no negative effect on orthostatic tolerance and cardiovascular countermeasures (Lightfoot *et al.* 1989, Cybulski *et al.* 1999).

Four days of HDBR are sufficient to induce cardiovascular deconditioning (Sigaudo *et al.* 1996). After HDBR, orthostatic intolerance was found in two thirds of our subjects. In healthy individuals, the reduction of stroke volume is counteracted by reflex-mediated increases in heart rate and peripheral vascular resistance (Streeten 1990, Červenáková *et al.* 2001). Both cardiac and vascular responses are controlled by the sympathetic nervous system. Excretion rate of noradrenaline decreases within 4 hours of exposure to HDBR and remains low throughout 14 days of bed-rest (Goldstein *et al.* 1995). Reduced levels of urinary catecholamines are found even after HDBR lasting 42 days (Sigaudo *et al.* 1998). Urinary catecholamines are useful in monitoring long-lasting changes of sympathetic activity. To measure short-lasting changes during orthostatic load, we determined plasma levels of catecholamines, which can be used as an indicator of sympathetic activity (Yamanouchi *et al.* 1998). Attenuated NA together with exaggerated adrenaline responses were considered to be the principal triggering mechanisms for neurally mediated syncope (Kikushima *et al.* 1999). Indeed, in the control period of our study, the highest adrenaline increment was observed in the subject developing orthostatic intolerance. This was, however, not observed in the OI subjects after training and bed-rest.

An unchanged adrenaline response with impaired noradrenaline response to standing was

observed in astronauts developing pre-syncope on the first day after landing (Fritsch-Yelle *et al.* 1996). On the other hand, Robertson *et al.* (1999) concluded that enhanced rather than reduced sympathetic activation accompanies orthostatic intolerance following a space flight. An enhanced NA response to the orthostatic challenge after HDBR was also found in our study irrespective of the fact whether the subjects developed orthostatic intolerance or not. An impaired BP response in intolerant subjects, as compared to the tolerant subjects, and a comparable increment of plasma NA supports a decreased vasoconstrictor response to sympathetic stimulation in the development of syncopes after space flight or bed-rest (Convertino and Sather 2000). Sigaudo *et al.* (1998) have observed a greater sympathetic response and lower baroreflex sensitivity in subjects developing orthostatic intolerance after 42 days HDBR.

The values of PRA at baseline and after standing as well as the plasma aldosterone response after standing were all augmented after HDBR in our study. Since the renin-angiotensin-aldosterone system is characterized by a bidirectional relationship with the sympathetic system (Zimmerman 1981), its active end-products (angiotensin II and aldosterone) could be involved in the enhancement of noradrenaline release and *vice versa*. Sigaudo *et al.* (1998) reported increased baseline levels of active renin after 45 days of HDBR. The enhancement of the response to standing after bed-rest was most pronounced at the end of standing (PRA) and in the post-standing period (aldosterone). The lack of statistical significance of augmentation in OI subjects might be due to the shorter duration of standing before "final" blood sampling. This could be the crucial point in the studies reporting diminished neuroendocrine response to standing in OI subjects, with the blood samples taken only at the end of the load (Fritsch-Yelle *et al.* 1996). Blunted sympathetic nerve response at the end of tilt test was also found in orthostatic intolerance after 14 days HDBR (Shoemaker *et al.* 1999). To lower the effect of standing duration on the magnitude of the response we took blood samples after 3 min of standing and found unaltered responses in the subjects subsequently developing the symptoms of orthostatic intolerance.

Our results suggest that orthostatic intolerance after head-down bed-rest in physically well-prepared subjects is not caused by inhibition of neuroendocrine responses. Even the increased response of sympathoadrenal system after bed-rest, which yielded similar results in both orthostatic tolerant and intolerant subjects, did not prevent failure in orthostatic tolerance. The effect of bed-rest on orthostatic tolerance and neuroendocrine responsiveness has to be considered in the indication and interpretation of orthostatic testing in endocrine and cardiovascular diagnostics.

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