Role of individual predisposition in orthostatic intolerance before and after simulated microgravity

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Grenon, S. M., S. Hurwitz, N. Sheynberg, X. Xiao, C. D. Ramsdell, C. L. Mai, C. Kim, R. J. Cohen, and G. H. Williams. Role of individual predisposition in orthostatic intolerance before and after simulated microgravity. J Appl Physiol 96: 1714–1722, 2004; 10.1152/japplphysiol.01274.2003.—Orthostatic intolerance (OI) is a major problem after spaceflight. Its etiology remains uncertain, but reports have pointed toward an individual susceptibility to OI. We hypothesized that individual predisposition plays an important role in post-bed rest OI. Twenty-four healthy male subjects were equilibrated on a constant diet, after which they underwent tilt-stand test (pre-TST). They then completed 14–16 days of head-down-tilt bed rest, and 14 of the subjects underwent repeat tilt-stand test (post-TST). During various phases, the following were performed: 24-h urine collections and hormonal measurements, plethysmography, and cardiovascular system identification (a noninvasive method to assess autonomic function and separately quantify parasympathetic and sympathetic responsiveness). Development of presyncopal or syncopal defined OI. During pre-TST, 11 subjects were intolerant and 13 were tolerant. At baseline, intolerant subjects had lower serum aldosterone (P < 0.01), higher excretion of potassium (P = 0.01), lower leg venous compliance (P = 0.03), higher supine parasympathetic responsiveness (P = 0.02), and lower standing sympathetic responsiveness (P = 0.048). Of the 14 subjects who completed post-TST, 9 were intolerant and 5 were tolerant. Intolerant subjects had lower baseline serum cortisol (P = 0.03) and a higher sodium level (P = 0.02) compared with tolerant subjects. Thus several physiological characteristics were associated with increased susceptibility to OI. We propose a new model for OI, whereby individuals with greater leg venous compliance recruit compensatory mechanisms (activation of the renin-angiotensin-aldosterone system and sympathetic nervous system, and withdrawal of the parasympathetic nervous system) in the face of daily postural challenges, which places them at an advantage to face orthostatic stress. With head-down-tilt bed rest, the cardiovascular system deconditioning; renin-angiotensin-aldosterone system; leg venous compliance; autonomic function; head-down-tilt bed rest role of microgravity would play in orthostatic intolerance. Results of this study have implications for the development of an integrated predictive model of OI and for the design and interpretation of clinical studies of OI. ORTHOSTATIC INTOLERANCE (OI) is a debilitating problem after spaceflight, affecting 20–83% of astronauts and cosmonauts depending on the duration of exposure to the weightless environment (36). Several systems have been implicated in the pathophysiology of post-spaceflight OI, including the autonomic system through altered baroreflex reactivity (8, 17, 18) and adrenergic responsiveness (19, 22), leg venous compliance (60), cardiac pump function (35, 45, 46), volume-regulating systems (32), and, more recently, vascular function and reactivity through nitric oxide synthase-dependent mechanisms (62). In addition to the deconditioning effects of spaceflight on the cardiovascular system and other regulatory systems, reports have emerged on the contribution of interindividual variability in the tolerance to orthostatic stress both in simulated microgravity (25, 34, 43) and actual microgravity environments (19) and in clinical medicine (58), emphasizing the importance of the individual physiological “profile” in determining the response to orthostatic stress before and after spaceflight. Unfortunately, the contributions of the different physiological alterations induced by microgravity and of the individual’s predisposition to OI remain uncertain, which together may underlie the multifactorial pathophysiology of post-spaceflight OI.

In the history of aerospace medicine research, stand or tilt tests of 10- to 15-min duration have been the standard methods of evaluating OI, stemming from the protocols used in astronauts. As stated by Convertino and Sather (7), definition of the contributions of different physiological systems to orthostatic tolerance in healthy human subjects has been limited partly by failure to use tests designed specifically to induce presyncopal end points in all subjects. In clinical medicine, Streeter and Anderson (59) have reported the absence of published guidelines as to how many times or how long after standing blood pressure should be measured to detect orthostatic hypotension. In fact, they have described a subset of patients among whom orthostatic hypotension becomes evident and progressively severe after standing for >10 min, frequently escaping diagnosis. Only recently in cardiology were clinical practice guidelines published on tilt-table testing to evaluate patients with syncope (1).

The present study had two goals: 1) to evaluate the use of a long-duration orthostatic protocol in assessing OI in healthy subjects and to determine the reproducibility of these results after a period of simulated microgravity and 2) to study the role of individual predisposition in OI through an integrated assessment of the renal, hormonal, and autonomic variables, both at baseline and after simulated microgravity, to identify possible contributing factors. Factors were chosen based on their possible involvement in the pathogenesis of OI, supporting evidence in clinical medicine (57–59), prior spaceflight data (5, 19), and previous ground-based studies (25, 34, 43): physical

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characteristics, hormonal and renal factors, autonomic factors, hemodynamic factors, and factors related to the peripheral vascular system (venous compliance and vascular reactivity).

To our knowledge, no study has examined the integrated assessment of possible contributors to OI both before and after microgravity simulation while maintaining a constant diet. In fact, in one study that manipulated several of the above-mentioned factors, subjects with intolerance to orthostatic stress before simulated microgravity were eliminated from analysis (43). Hence, we hypothesized that I) a long-duration orthostatic stressor at baseline may be useful in predicting postsimulated microgravity OI and 2) two levels of factors influence postspaceflight OI: individual predisposition and the deconditioning effect of microgravity itself. Understanding the underlying etiological susceptibility to OI would allow us to improve individualization and targeting of countermeasures and treatment of both civilian patients and astronauts.

METHODS

Subjects

Twenty-four healthy, male subjects (age = 34 ± 2 yr; height = 177 ± 1 cm; weight = 78.4 ± 2.1 kg) were recruited for study. Screening procedures included a history and physical examination, 12-lead electrocardiogram, complete blood count with differential, chemistry profile, thyroid function tests, urinalysis, toxicology screen, and psychological evaluation. Subjects were nonsmokers and on no medication before enrollment in the study. The exclusion criteria included a history or evidence of hypertension, coronary artery disease, diabetes, renal insufficiency, thyroid disease, hepatitis, anemia, psychiatric disorder, and alcohol or drug abuse. Additional exclusion criteria included known sleep disorders, shift work, and transmeridian travel within the 6 mo before study. None of the subjects had a history of syncope.

Protocol

Subjects were admitted to the General Clinical Research Center (GCRC) at Brigham and Women’s Hospital and maintained on an isocaloric diet containing 200 meq of sodium, 100 meq of potassium, 1,000 mg of calcium, and 2,500 ml of fluid throughout the study period. The subjects were equilibrated on the diet in the GCRC for up to 5 days prebed rest (pre-HDTB), then entered a 14-day (subjects I–3) or 16-day (subjects 4–24) period of 4° head-down-tilt bed rest (HDTB). Bed rest was initiated at 1500 on the last day of pre-HDTB after the tilt-stand test (pre-TST) and ended on day 14 or 16 at 1000 with the post-HDTB tilt-stand test (post-TST). Sleep-wake cycles remained constant throughout the study, with 8 h of sleep each day between 2200 and 0600. Of these 24 subjects, 6 received a countermeasure during post-TST (subjects 4, 6, 7, 9, 12, and 14) and 4 were placed on a sleep-deprivation protocol (subjects 18–21). These were excluded from the post-TST analysis. Room temperature was maintained between 21 and 22°C. Subjects were strictly confined to bed for the entire HDTB period. They were allowed to lie on their side, back, or front. Voiding and defecation occurred in the supine position. Meals were eaten while subjects were lying on their sides, propped up on one elbow. No medications, smoking, alcohol, or caffeine were allowed during the study. The study protocol was approved in advance by the Institutional Review Board of the Brigham and Women’s Hospital. Each subject provided written, informed consent before participating in the study.

Tilt-Stand Tests

Pre-TST was performed at 1000 before initiation of bed rest and at the end of bed rest (post-TST) using a motorized tilt table (American Echo, model 9607). Subjects were tilted to the upright position with 10-min stops at 30, 60, and 90°, during which time hemodynamic, hormonal, and autonomic measurements were taken (see Measurements). After reaching the upright position (90°), subjects remained standing by themselves for an additional 120 min. Nontolerance to tilt-stand tests was defined as clinical signs of OI (diaphoresis, nausea, lightheadedness, or dizziness) accompanied by a decrease in systolic blood pressure (SBP) of >20 mmHg below baseline or an increase in heart rate (HR) of >20 beats/min above baseline. If signs of nontolerance were noted, subjects were returned to the supine position.

Measurements

Tilt-stand tests. Initiation of tilt-stand tests marked time 0. Blood samples were drawn for plasma renin activity (PRA), aldosterone (Aldo), cortisol (Cort), and catecholamines in the supine position (–10 min, –1 min, baseline), at 30° tilt (+5 min), 60° tilt (+15 min), 90° tilt (+20 min), and in the standing position (+40 min, +80 min).

Cardiovascular system identification. Before tilt-stand test and at 30, 60, and 90° tilt, data were recorded for cardiovascular system identification (CSI) analysis. Subjects were instrumented for continuous noninvasive monitoring of arterial blood pressure (ABP; Portapres, TNO, or Finapres, Ohmeda), instantaneous lung volume (ILV; respiration system, Ambulatory Monitoring Systems), and HR (surface electrocardiogram). During data collection, subjects were instructed to breathe in response to auditory tones spaced at random intervals ranging from 1 to 15 s, with a mean of 5 s. Subjects controlled their own tidal volume to maintain normal ventilation. This random breathing protocol excites a broad range of frequencies, thereby facilitating system identification (2). Previous work indicates that random interval breathing does not have a measurable effect on autonomic function (2). ABP, ILV, and HR data collected in supine and upright postures were saved for later CSI analysis (39, 40).

CSI evaluates interactions between physiological signals (HR, ABP, and ILV) on a second-to-second basis to enable dynamic assessment of physiological mechanisms. CSI generates a closed-loop model of cardiovascular regulation specific for the individual subject at the time the signals were collected. The model characterizes the dynamic coupling between physiological signals in terms of impulse response functions. These couplings include the HR baroreflex (the autonomically mediated baroreflex coupling between fluctuations in ABP and fluctuations in HR), respiration-induced HR variability (ILV → HR: the autonomically mediated coupling between respiration and HR), the mechanical effects of respiration on ABP due to the alterations in venous return and the filling of intrathoracic vessels and heart chambers associated with the changes in intrathoracic pressure (ILV → ABP), and circulatory mechanics (the relationship between cardiac contraction and the generation of the ABP waveform) (39, 40). The impulse response functions are obtained by solving a set of causal autoregressive moving average equations, which relate the noninvasively measured signals. The model orders of these equations are determined by using a parameter reduction algorithm in conjunction with Rissanen’s minimum-description length criterion (47).

Because the HR baroreflex and ILV → HR couplings are regulated by the autonomic system, the features of these impulse responses reflect autonomic responsiveness. CSI may also be used to quantify the parasympathetic responsiveness and the sympathetic responsiveness separately on the basis of analysis of the ILV → HR impulse response function. This approach has been validated with animal and human data in Xiao et al. (64). Sympathetic and parasympathetic responsiveness are unitless.

Plethysmography. During pre-HDTB and at the end of HDTB on the days before the orthostatic testing, venous occlusion plethysmography was performed to measure calf compliance. A strain gauge (EC5R plethysmograph, Hokanson, Bellevue, WA) was placed around the calf at its maximal circumference. External pressure was applied on the thigh at 30, 60, and 90°, during which time hemodynamic, hormonal, and autonomic measurements were taken (see Measurements).
through an occlusion cuff, which was attached to an electronically controlled air pump. Pressure levels of 30, 40, and 50 mmHg were delivered consecutively after having reached a steady state at the previous level. Venous compliance corresponds to the ratio of the change in calf volume over the change in external pressure. Tau (time constant of the exponential function) was obtained by measuring the time constant for filling of veins on application of external pressure.

Vascular reactivity: angiotensin II and norepinephrine infusions. Blood pressure, HR, and Aldo responses to angiotensin II infusion were examined before (pre-HDTB) and at the end of bed rest on the days before orthostatic testing. Subjects remained supine from 2200 the previous day throughout the infusion protocol. Angiotensin II amide (Ciba, Summit, NJ) infusion testing was performed in two consecutive stages: a 30-min control period starting at 0800 followed by an infusion of 3 ng · kg⁻¹ · min⁻¹ for 30 min. Blood pressure and HR were recorded every 2 min by indirect sphygmomanometer. Blood samples for Aldo were collected from a peripheral venous catheter 5 min before the end of each period.

Blood pressure and HR responses to norepinephrine infusion were then examined after a 30-min washout period of angiotensin II with the subjects remaining in the supine position. Norepinephrine infusion testing was performed in two consecutive stages: a 30-min control period and an infusion of 0.03 mcg · kg⁻¹ · min⁻¹ for 15 min. Blood pressure and HR were recorded every 2 min by indirect sphygmomanometer. Blood samples for Aldo were collected from a peripheral venous catheter 5 min before the end of each period.

Blood pressure and HR responses to norepinephrine infusion were then examined after a 30-min washout period of angiotensin II with the subjects remaining in the supine position. Norepinephrine infusion testing was performed in two consecutive stages: a 30-min control period and an infusion of 0.03 mcg · kg⁻¹ · min⁻¹ for 15 min. Blood pressure and HR were recorded every 2 min by indirect sphygmomanometer. Blood samples for Aldo were collected from a peripheral venous catheter 5 min before the end of each period.

Hemodynamic, renal, and cardioendocrine measurements. Blood pressure and HR were recorded by indirect sphygmomanometer at 0600, 1400, and 2200 on all study days. Body weight was determined every morning at 0600 after a morning void (subjects remained supine during HDTB). The 24-h urine samples were collected by voluntary micturition for measurements of daily urine volume, sodium, potassium, Aldo, Cort, chloride, and creatinine excretion. Blood samples were collected at 0600 from a peripheral venous catheter after the previous level. Venous compliance corresponds to the ratio of the change in calf volume over the change in external pressure. Tau (time constant for filling of veins on application of external pressure) was obtained by measuring the time constant for filling of veins on application of external pressure.

Statistical Analysis

The raw data were examined for outliers and validity. Means and standard errors were used to describe the data at baseline and at the end of HDTB. The main analytic tools were the unpaired t-test to compare the groups and the paired t-test for within-group comparisons, since normality was not rejected. Because the sample sizes were relatively small, the comparisons were repeated with rank methods without contradictions. In addition, the rate of change during orthostatic challenge was reported as the linear regression coefficient or slope using data through 80 min. The rates of change were summarized by using the median and the interquartile range, and the groups were compared by Wilcoxon’s rank sum test. There was a large number of hypothesis tests. To offer the opportunity to judge the strength of the relationships, we reported the P values from planned hypothesis-driven tests without adjusting for multiple-hypothesis testing. The conventional criterion of 5% alpha for limiting the probability of a type I error may be too liberal; therefore, a smaller criterion can be used if strict dichotomous hypothesis testing is desired. Furthermore, small sample sizes resulted in a relatively lower power; therefore, it is important to review the magnitudes of the effects in conjunction with the P values.

RESULTS

Tables 1–7 present the results of the tilt-stand tests (Table 1); pre- and post-HDTB hormonal and renal measurements (Table 2); plethysmography (Table 3); cardiovascular system identification (Table 4); hemodynamic measurements (Table 5); urinary and serum catecholamines (Table 6); and rate of change of hormones, catecholamines, and hemodynamic measurements during orthostatic challenge (Table 7).

Orthostatic Tolerance

Table 1 displays the response of subjects to orthostatic stress. During the pre-TST, 13 of 24 subjects completed the tilt-stand test (pre-Tol), whereas 11 became presyncopal (pre-Int). Although presyncopal symptoms were monitored for >2 h for each subject, presyncope always occurred during either tilt or the early standing period. The average time of presyncope pre-HDTB was 23.5 ± 2.8 min. Of the 14 subjects who underwent post-TST after HDTB, 9 were intolerant (post-Int) and 5 were tolerant (post-Tol). Of these 14 subjects, 2 shifted from a tolerant status before HDTB to an intolerant status after HDTB; the others who remained tolerant or intolerant,
respective, during both studies. This increase in tilt-stand-test pressure of 50 mmHg by tolerance status
pre-HDTB
Factors Associated With Tilt-Stand-Test Tolerance
were further examined to evaluate differences between them.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-HDTB (n = 24)</th>
<th>End-HDTB (n = 14)</th>
<th>Pre-HDTB Measurements Predicting Post-Tilt-Stand Test Tolerance (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Int (n = 11)</td>
<td>Tol (n = 13)</td>
<td>P value</td>
</tr>
<tr>
<td>Cardioendocrine panel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA, ng/ml·min⁻¹·mmHg</td>
<td>1.0±0.2</td>
<td>1.4±0.2</td>
<td>0.17</td>
</tr>
<tr>
<td>Aldo, ng/ml</td>
<td>8.0±0.6</td>
<td>13.3±1.6</td>
<td>0.009</td>
</tr>
<tr>
<td>Urine Aldo, µg/dl</td>
<td>11.9±2.5</td>
<td>12.6±1.4</td>
<td>0.73</td>
</tr>
<tr>
<td>Cort, µg/dl</td>
<td>18.6±1.4</td>
<td>19.8±1.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Urine Cort, µg/total volume</td>
<td>57.2±4.6</td>
<td>48.7±5.6</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum electrolytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na, meq/l</td>
<td>140±1</td>
<td>140±1</td>
<td>0.89</td>
</tr>
<tr>
<td>K, meq/l</td>
<td>4.3±0.1</td>
<td>4.5±0.1</td>
<td>0.43</td>
</tr>
<tr>
<td>Cl, meq/l</td>
<td>106±1</td>
<td>104±1</td>
<td>0.39</td>
</tr>
<tr>
<td>Creat, mg/dl</td>
<td>0.82±0.04</td>
<td>0.88±0.03</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine electrolytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na, meq/total volume</td>
<td>181±13</td>
<td>167±13</td>
<td>0.46</td>
</tr>
<tr>
<td>K, meq/total volume</td>
<td>86.9±3.7</td>
<td>70.5±4.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Cl, meq/total volume</td>
<td>217±8</td>
<td>209±9</td>
<td>0.53</td>
</tr>
<tr>
<td>Creat, mg/total volume</td>
<td>1693±91</td>
<td>1594±89</td>
<td>0.45</td>
</tr>
<tr>
<td>Urine volume, ml</td>
<td>2451±151</td>
<td>2294±167</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Values are means ± SE. Int, intolerant; Tol, tolerant; PRA, plasma renin activity; Aldo, aldosterone; Cort, cortisol; Creat, creatinine.

respectively, during both studies. This increase in tilt-stand-test tolerance with HDTB did not reach statistical significance. The average time to presyncope within the group of 14 who participated in both tilt-stand tests was 21.6 ± 3.0 min pre-HDTB and 21.7 ± 2.3 min post-HDTB. The characteristics of intolerant and tolerant subgroups during the pre- and post-TST were further examined to evaluate differences between them.

Factors Associated With Tilt-Stand-Test Tolerance pre-HDTB

The pre-INT group had a lower serum Aldo (P = 0.009) and a higher baseline excretion of potassium (P = 0.01). PRA was not significantly different during pre-HDTB (P = 0.17) (Table 2). The pre-Int group also had a lower venous compliance (P = 0.034) compared with the pre-Tol group (Table 3). When CSI was used to evaluate autonomic function, pre-Int was shown to have higher parasympathetic responsiveness in the supine position compared with pre-Tol at baseline (P = 0.02) and lower sympathetic responsiveness in the upright position during pre-TST (P = 0.048) (Table 4). There was a trend toward a lower baseline HR in the pre-Int group compared with the pre-Tol group (Table 5), but there was no significant difference in baseline blood pressure. There was no significant difference in urinary or serum catecholamines at baseline between the two groups (Table 6).

There were no differences at baseline in age (pre-Int: 34 ± 3 yr; pre-Tol: 35 ± 4 yr; P = 0.94), height (pre-Int: 178 ± 2 cm; pre-Tol: 177 ± 2 cm; P = 0.89), weight (pre-Int: 78 ± 3 kg; pre-Tol: 79 ± 3 kg; P = 0.82), or body mass index (pre-Int: 25 ± 1 kg/m²; pre-Tol: 25 ± 1 kg/m²; P = 0.65) based on tolerance grouping.

There was no difference in the HR, SBP, or diastolic blood pressure in response to norepinephrine infusion, or in HR, SBP, diastolic blood pressure, or Aldo response to angiotensin II infusion between pre-Int and pre-Tol groups before HDTB.

During the pre-TST, pre-Int had a significantly higher rate of rise of norepinephrine compared with pre-Tol (P < 0.001) (Table 7) and demonstrated a higher rate of increase of HR (P = 0.04). They also demonstrated a trend toward slower increase in PRA relative to pre-Tol (P = 0.09) and toward a

Table 3. Leg venous compliance pre-HDTB and end-HDTB using plethysmography at an external pressure of 50 mmHg by tolerance status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-HDTB (n = 24)</th>
<th>End-HDTB (n = 14)</th>
<th>Pre-HDTB Measurements Predicting Post-Tilt-Stand Test Tolerance (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Int (n = 11)</td>
<td>Tol (n = 13)</td>
<td>P value</td>
</tr>
<tr>
<td>Tau, s Complacency, %/mmHg</td>
<td>22.92±8.19</td>
<td>61.47±9.40</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>0.004±0.0002</td>
<td>0.006±0.001</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Values are means ± SE. Tau, time constant of the exponential function.
faster decrease in SBP \((P = 0.09)\) and diastolic blood pressure \((P = 0.08)\) (Table 7).

**Factors Associated with Tilt-Stand-Test Tolerance Post-HDTB**

During the post-TST, a lower baseline Cort \((P = 0.03)\) and a higher serum sodium \((P = 0.02)\) among post-Int were the only significant differences between post-Int and post-Tol (Table 2).

During post-TST, post-Int demonstrated a faster decrease in SBP \((P = 0.008; \text{Table 7})\).

**Pre-HDTB Factors Associated With Post-HDTB Tolerance Status**

Lower Aldo \((P = 0.035)\) and higher sodium \((P = 0.05)\) at baseline during pre-HDTB were associated with post-Int (Table 2). A lower supine parasympathetic responsiveness \((P = 0.03)\) and lower standing sympathetic responsiveness \((P = 0.06)\) during pre-HDTB testing were also associated with post-Int to orthostatic stress (Table 4).

**DISCUSSION**

This study provides an integrated assessment of individual physical and physiological factors that could contribute to OI and evaluates the use of a long-duration orthostatic tilt-stand-test protocol to assess orthostatic tolerance before and after a period of simulated microgravity. We observed that several baseline physiological characteristics were associated with poorer tolerance to orthostatic stress. We also observed that the use of a long-duration orthostatic stress protocol may be useful for predicting OI after exposure to simulated microgravity.

**Proposed Model: Role of Individual Predisposition to OI**

We have observed that subjects who were tilt-intolerant pre-HDTB, compared with subjects who were tilt-tolerant pre-HDTB, had a lower leg venous compliance, a suppressed renin-angiotensin-Aldo axis (RAAS), a higher excretion of potassium, a higher parasympathetic responsiveness in the supine position, and a lower sympathetic responsiveness in the standing position. These differences were attenuated when such measures obtained post-HDTB in subjects who were tilt intolerant post-HDTB were compared with those of subjects who were tilt tolerant post-HDTB. These observations have been incorporated into the model shown in Fig. 1. According to this model, individuals who have a greater leg venous compliance (as a result of daily postural challenges to cardiovascular homeostasis) recruit a variety of compensatory mechanisms, including activation of the RAAS and the sympathetic nervous system and downregulation of the parasympathetic nervous system. As a result, these individuals are better able to tolerate an orthostatic challenge than individuals with a lower baseline venous compliance, who do not similarly recruit these compensatory mechanisms. However, during a period of HDTB, individuals with a greater leg venous compliance are no longer exposed to daily orthostatic challenges and thus no longer recruit these compensatory mechanisms. In addition, other changes known to occur with microgravity and simulated microgravity take effect, such as changes in hormonal, renal, and autonomic function (24). Thus post-HDTB measures of leg venous compliance, sympathetic responsiveness, and parasympathetic responsiveness are no longer significantly different between individuals who are tilt intolerant compared with individuals who are tilt tolerant post-HDTB.

**Table 4. Cardiovascular system identification pre-HDTB and end-HDTB by tolerance status**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Int ((n = 24))</th>
<th>Tol ((n = 13))</th>
<th>(P) value</th>
<th>Int ((n = 14))</th>
<th>Tol ((n = 5))</th>
<th>(P) value</th>
<th>Int ((n = 14))</th>
<th>Tol ((n = 5))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasympathetic</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>responsiveness</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>0.030±0.004</td>
<td>0.018±0.002</td>
<td>0.02</td>
<td>0.019±0.004</td>
<td>0.012±0.002</td>
<td>0.31</td>
<td>0.027±0.004</td>
<td>0.012±0.002</td>
<td>0.03</td>
</tr>
<tr>
<td>Standing</td>
<td>0.015±0.004</td>
<td>0.014±0.004</td>
<td>0.78</td>
<td>0.003±0.002</td>
<td>0.004±0.002</td>
<td>0.65</td>
<td>0.016±0.008</td>
<td>0.006±0.002</td>
<td>0.23</td>
</tr>
<tr>
<td>Sympathetic</td>
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<tr>
<td>Supine</td>
<td>0.017±0.008</td>
<td>0.031±0.017</td>
<td>0.49</td>
<td>0.006±0.015</td>
<td>0.018±0.025</td>
<td>0.40</td>
<td>0.021±0.021</td>
<td>0.037±0.019</td>
<td>0.63</td>
</tr>
<tr>
<td>Standing</td>
<td>−0.007±0.011</td>
<td>0.038±0.015</td>
<td>0.048</td>
<td>0.036±0.007</td>
<td>0.027±0.015</td>
<td>0.58</td>
<td>0.039±0.013</td>
<td>0.039±0.013</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE.
The greater excretion of potassium at baseline among tilt-intolerant individuals compared with tilt-tolerant individuals is intriguing. Because all subjects were on a constant potassium intake, this fact suggests that the tilt-intolerant subjects had a decreased total body potassium at the end of the control period. Previous studies have reported a decreased vascular response to pressors, specifically angiotensin II in subjects with a decreased vs. increased total body potassium (26). However, serum potassium levels were similar in the two groups. An alternate possibility is that the tilt-intolerant actually had an increased total body potassium, and the increased potassium excretion was secondary to coming into balance on a lower potassium intake than usual for them. Potassium channels, sensitive to concentrations of potassium, have been recognized as important regulators of arterial tone (41). Thus higher potassium levels may predispose vessels for dilation and lead to inadequate increases in peripheral resistance (41).

Prior Studies on Association Between the Renin-Angiotensin-Aldo System and OI

Jacob et al. (28, 29) reported that, in patients with well-documented OI, PRA was low and did not rise as much as expected during assumption of upright posture. In studies assessing the role of the RAAS in OI, low PRA was correlated with lower levels of Aldo and hypovolemia (29, 48). Similarly, in patients with chronic fatigue syndrome and delayed orthostatic hypotension, low Aldo (57) and low PRA (12) have been observed. In healthy subjects, Shvartz et al. (51) found that “fainters” had a smaller increase in PRA during tilt-table test than “nonfainters.” However, Harrison et al. (25) described evidence for an upregulated RAAS with a higher PRA in response to tilt in intolerant compared with tolerant individuals. It should be noted that, in the latter study, the subjects were previously dehydrated, and sodium and potassium homeostasis was not attained. It is interesting to note that the sympathetic nervous system is a known regulator of RAAS function (10, 23) and that patients with autonomic failure usually present exceptionally low renin levels, even in the face of low blood pressure on standing (3). Hence, the autonomic nervous system may further contribute to a downregulated RAAS seen in OI.

Prior Studies on Association Between the Autonomic Nervous System and OI

Using CSI to assess sympathetic and parasympathetic function, we observed that a higher parasympathetic responsiveness at rest and a lower sympathetic responsiveness in the upright posture were associated with tilt-stand-test intolerance. This observation is supported by data from Fritsch-Yelle et al. (19) and Waters et al. (63), who reported that astronauts unable to complete the orthostatic challenge test after spaceflight had a lower index of sympathetic function. However, Cooke and Convertino (9) found that subjects susceptible to fainting show an increased adrenergic response compared with nonfainters, a response also seen in acute central hypovolemia (13). These apparently divergent results can be reconciled as follows. Individuals susceptible to fainting have decreased sympathetic (and increased parasympathetic) responsiveness. However, the very large hypotensive stimulus associated with tilt, which occurs in fainters, elicits a large adrenergic response (as manifested in this study by the higher rate of rise of serum norepinephrine). Nonfainters have a much smaller hypotensive stimulus and thus evoke a smaller adrenergic response to tilt even though they have greater sympathetic responsiveness (reserve) than fainters.

Prior Studies on Associations Between Venous Leg Compliance and OI

The role of lower extremity venous capacitance in OI is still debated. We found that lower leg compliance was associated with poorer tolerance to orthostatic stress pre-HDTB but not post-HDTB. Our findings are supported by a report from Ludwig and Convertino (34) in which higher compliance was associated with higher tolerance during lower body negative pressure. However, other reports, such as the one by Fu et al. (20), do not support this association. In the latter study, the authors report that a larger leg compliance pre-bed rest was associated with post-bed rest OI (20). However, the results of the pre-bed rest tilt-test tolerance were not reported. Pavy-Le Traon et al. (43) reported that change in leg compliance over bed rest and an actual higher leg compliance measurement after bed rest were associated with orthostatic tolerance. However, the effect of leg compliance on pre-bed rest tilt was not reported. Although we did not observe a significant relationship between leg venous compliance and OI after bed rest, we have previously reported an increase in leg venous compliance with bed rest (24). In fact, our model does not exclude the contribution of an increased venous compliance after bed rest.

Reports in clinical medicine have also brought important insight to OI and venous compliance. Farquhar et al. (14) and Freeman et al. (16) have reported, against expectations, that calf venous compliance was lower in patients with idiopathic OI compared with a group of age-matched controls. Stewart

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-HDTB (n = 24)</th>
<th>P value</th>
<th>End-HDTB (n = 14)</th>
<th>P value</th>
<th>Pre-HDTB Measurements Predicting Post-Tilt-Stand Test Tolerance (n = 14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi serum, pg/ml</td>
<td>27.0±7.6</td>
<td>0.24</td>
<td>60.5±21.3</td>
<td>0.23</td>
<td>57.1±25.0</td>
<td>0.46</td>
</tr>
<tr>
<td>Epi urine, µg/total volume</td>
<td>12.5±1.8</td>
<td>0.66</td>
<td>15.2±5.2</td>
<td>0.77</td>
<td>11.0±1.9</td>
<td>0.41</td>
</tr>
<tr>
<td>NE serum, pg/ml</td>
<td>220.9±64.7</td>
<td>0.92</td>
<td>136.6±25.0</td>
<td>0.73</td>
<td>235.0±77.8</td>
<td>0.29</td>
</tr>
<tr>
<td>NE urine, µg/total volume</td>
<td>57.4±9.4</td>
<td>0.41</td>
<td>81.6±17.4</td>
<td>0.67</td>
<td>53.8±12.8</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values are means ± SE. Epi, epinephrine; NE, norepinephrine.
Table 7. Rate of change in hormones, catecholamines, and hemodynamic measurements during pre-TST and Post-TST by tolerance status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-TST (n = 24)</th>
<th>Post-TST (n = 14)</th>
<th>P value</th>
<th>Pre-HDTB Measurements Predicting Post Tilt-Stand Test Tolerance (n = 14)</th>
<th>Post-HDTB Measurements Predicting Post Tilt-Stand Test Tolerance (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA, ng/ml</td>
<td>0.10 (0.09, 0.12)</td>
<td>0.08 (0.06, 0.11)</td>
<td>0.001</td>
<td>0.10 (0.09, 0.11)</td>
<td>0.08 (0.06, 0.12)</td>
</tr>
<tr>
<td>Aldo, ng/ml</td>
<td>0.13 (0.12, 0.14)</td>
<td>0.08 (0.06, 0.10)</td>
<td>0.001</td>
<td>0.12 (0.11, 0.13)</td>
<td>0.07 (0.06, 0.10)</td>
</tr>
<tr>
<td>Cort, g/dl</td>
<td>14.14 (10.5, 23.1)</td>
<td>2.79 (2.31, 4.17)</td>
<td>0.001</td>
<td>12.27 (2.99, 24.55)</td>
<td>9.12 (5.17, 9.24)</td>
</tr>
<tr>
<td>NE, pg/min -1</td>
<td>1.97 (0.54, 0.51)</td>
<td>0.52 (0.44, 0.47)</td>
<td>0.001</td>
<td>1.04 (0.31, 2.40)</td>
<td>0.52 (0.31, 0.47)</td>
</tr>
<tr>
<td>Epi, pg/ml</td>
<td>1.17 (0.24, 1.50)</td>
<td>0.20 (0.01, 0.27)</td>
<td>0.001</td>
<td>1.94 (1.43, 2.60)</td>
<td>0.35 (0.18, 0.50)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>11.91 (12.36, 13.47)</td>
<td>8.29 (7.90, 13.09)</td>
<td>0.001</td>
<td>12.27 (10.5, 23.1)</td>
<td>16.24 (15.43, 34.2)</td>
</tr>
<tr>
<td>SBP, mmHg/min</td>
<td>7.04 (7.23, 7.04)</td>
<td>6.64 (6.44, 6.84)</td>
<td>0.001</td>
<td>9.12 (5.17, 9.24)</td>
<td>7.04 (7.23, 7.04)</td>
</tr>
<tr>
<td>MAP, mmHg/min</td>
<td>0.30, 0.08)</td>
<td>0.06 (0.05, 0.07)</td>
<td>0.001</td>
<td>0.52 (0.44, 0.47)</td>
<td>0.06 (0.05, 0.07)</td>
</tr>
</tbody>
</table>

Values are means (range), pre-TST, before tilt-stand test; post-TST, after tilt-stand test.

Fig. 1. Proposed model for the contribution of individual predisposition to orthostatic intolerance before and after simulated microgravity. RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; PNS, parasympathetic nervous system.

and Weldon (56) have reported a trend toward lower distensibility and capacitance in orthostatic tachycardia syndrome but no difference in compliance. In another study (55), the same authors demonstrated a lower leg venous compliance in OI patients but attributed this finding to a shift in compliance curves due to higher venous pressure in orthostatic patients. More recently, Stewart (54) reported the lack of contribution of the venous system of the limbs to OI. Hence, there is still controversy as to the contribution of venous compliance to OI.

OI: Using a Long-Duration Stressor

In this study, a long-duration tilt-stand test at baseline was used. It allowed us to identify in a population of healthy, male subjects those likely to experience OI after a period of simulated microgravity. In the past, most studies related to post-spaceflight OI have used a tilt test or stand test lasting 10–15 min (4–6, 11, 15, 19, 27, 30, 31, 33, 36–38, 50, 53, 61, 63), and few have used longer orthostatic stress periods (44, 49, 52). In some studies, when OI occurred during the pre-bed rest study, subjects were excluded from statistical analysis (43). The American College of Cardiology has recently suggested in an expert consensus document (1) that the tilt-table testing duration should be at least 30–45 min (drug-free tilt period) in the assessment of patients with syncpe. Hence, there may be a place for the use of longer periods of orthostatic stress in the prediction of OI after simulated microgravity. Such protocols could also facilitate the study of the contributions of different physiological parameters to OI. This may become important, especially for exploratory missions, which may involve the need to stand for longer periods of time on, for example, the lunar or martian surface.

Limitations

One of the major limitations of this study is sample size. Although we had a relatively high number of subjects for the baseline tilt-stand test, several subjects either received a countermeasure or followed a different bed-rest protocol and were subsequently excluded from analysis. Another variable that
may have influenced our results is the complexity of our experimental orthostatic challenge. We used a longer and more complex protocol, which may have influenced our ability to detect a significant increase in OI after HDBT. However, this design enabled us to more accurately determine an individual’s orthostatic threshold, thereby facilitating characterization of important physiological factors associated with OI. Yet another limitation of this study was that we did not measure blood volume, which would have been useful to correlate with hormonal measurements. Furthermore, an important confounder in this study is instrumentation, because it is well known that instrumentation, such as the placement and presence of intravenous catheters, can affect orthostatic tolerance. However, no presyncopal symptoms were experienced by any subjects during screening blood sampling. Last, the validity of hormonal measurements. Furthermore, an important confounder in this study is instrumentation, because it is well known that instrumentation, such as the placement and presence of intravenous catheters, can affect orthostatic tolerance. However, no presyncopal symptoms were experienced by any subjects during screening blood sampling. Last, the validity of the bed-rest model used for this study has been questioned over the last few decades. One confounding factor in the model is the presence of a transverse G force on the heart, which is known that instrumentation, such as the placement and presence of intravenous catheters, can affect orthostatic tolerance. However, no presyncopal symptoms were experienced by any subjects during screening blood sampling. Last, the validity of the bed-rest model used for this study has been questioned over the last few decades. One confounding factor in the model is the presence of a transverse G force on the heart, which is known that instrumentation, such as the placement and presence of intravenous catheters, can affect orthostatic tolerance. However, no presyncopal symptoms were experienced by any subjects during screening blood sampling. Last, the validity of the bed-rest model used for this study has been questioned over the last few decades. One confounding factor in the model is the presence of a transverse G force on the heart, which is

In summary, the present findings allow us to suggest a new model, whereby individual predisposition plays an important role in OI. In this model, a greater leg venous compliance acts to recruit compensatory mechanisms such as RAAS and the sympathetic nervous system to face postural challenges, and hence positively contribute to greater orthostatic tolerance. Second, we believe that there is a role for longer-duration tilt or stand test protocols in the study of OI to better define characteristics that predispose one to this condition. These findings may improve our understanding of OI affecting clinical patients and astronauts, define more accurately those at risk, and increase our ability to develop targeted countermeasures.

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