Heterogeneity of responses to orthostatic stress in homozygous twins

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ORTHOSTATIC TOLERANCE REFLECTS the effectiveness of integrated reflex cardiovascular control when central blood volume is reduced. These reflex adjustments, including tachycardia and elevated sympathetic vasomotor tone, maintain systemic blood pressure (BP) and, consequently, cerebral perfusion. In acute studies, there is considerable interindividual difference in orthostatic tolerance (29). However, we have reported that, within a given individual, orthostatic capacity varies by <10% on repeated tests separated by several weeks (29). These data suggest a characteristic response for a given individual that may be genetically influenced. There is evidence of plasticity in orthostatic tolerance in healthy individuals (12, 31), suggesting that these reflex responses are not entirely “programmed,” but are subject to considerable environmental influences, including level of deconditioning, hydration status, and disease (21, 26, 27). The relative contributions of genetics and other factors remain unclear and may interfere with assessments of how various interventions affect autonomic reflex cardiovascular function.

The genetic basis of BP regulation received preliminary attention by comparing homozygous twins in baseline or unstressed physiological conditions. Specifically, resting levels of arterial BP (ABP) (18, 40), plasma norepinephrine concentration (41), muscle sympathetic nerve activity (39), and central systolic BP (SBP) augmentation (35) all demonstrate a degree of heritability. The heredity estimates for resting ABP have been reported to range between 35 and 67% (38) and 11 and 67% (20), depending on the specific ABP component (systolic, diastolic, or mean), as well as the possible effects of age and gender. Monozygotic twins have higher heritability estimates compared with dizygotic twins (17, 20, 22, 35). Nevertheless, heredity estimates only account for, at most, one-half of resting ABP variability. Moreover, these studies failed to investigate the genetic contributions to ABP regulation when cardiovascular function is challenged across its range of regulatory capacity. As for heart rate (HR), the genetic basis of baseline levels is debatable (5, 10, 13, 14, 17).

In contrast to resting baseline measures, the heritability of BP regulation appears to vary under stressors that elicit pressor responses. In fact, Van den Bree and colleagues (37) suggest that a set of “work” vs. “resting” genes dominate ABP regulation during dynamic exercise. A similar phenomenon has been suggested with mental arithmetic (9, 10, 14) and the cold pressor test (5, 9, 14). In contrast, other studies have failed to find a genetic component for ABP reactivity (5, 9, 10), where environmental (i.e., individual habits and experiences, along with the reactions to these experiences) factors appear to be the main determinant of ABP regulation during handgrip exercise (14). As well, there is controversy regarding the degree to which genetics influences ABP during a pre-defined orthostatic stress (2, 22, 25). For example, Bielen and colleagues (2) found that the genetic trait attributed to BP regulation was greater in the standing vs. the supine position in monozygotic and dizygotic male twins. In contrast, Hunt et al. (22) observed a reduction in the SBP heritability estimate on going from a sitting to a standing position in both monozygous and dizygous twins.

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Therefore, the purpose of this investigation was to determine the cardiovascular and cerebrovascular responses in identical twins across the range of stress between supine rest and presyncope. We tested the hypothesis that the responses and tolerance for orthostatic stress would demonstrate a strong relationship between identical twins, implying a genetic component.

METHODS

Subjects. Sixteen healthy monozygotic twin pairs (8 male and 8 female pairs) from across the United States volunteered to participate in this study conducted at the University of California San Diego Medical Center. The average (mean ± SD) age, height, and weight were 27 ± 7 yr, 169 ± 11 cm, and 63 ± 12 kg, respectively. Zygosity was determined by self-report in all subjects. It has been reported that physical appearance similarity criteria yields a diagnoses in agreement with blood genotyping ~95% of the time (11, 30). To confirm, zygosity was further determined in seven twin pairs using DNA polymorphism analysis for short tandem repeat markers, D3 S1358, vWA, D16 S359, D2 S1338, D8 S1179, D21 S11, D18 S51, D19 S433, Thol, and PGA. The subject’s DNA was obtained using a cheek swab kit. All seven twin pairs were deemed identical. Subjects were not hypertensive, did not suffer from diabetes or cardiovascular disease, and were not on any medication known to alter cardiovascular function. To minimize sex hormone fluctuations, all female twin pairs and were not on any medication known to alter cardiovascular function. To minimize sex hormone fluctuations, all female twin pairs were placed on the same birth control medication of their choice 1 mo before data collection. All participants provided written, informed consent to participate. This research was approved by the University of California San Diego and NASA-Johnson Space Center, Houston, Texas, review boards.

Measurements. HR was collected using a standard three-lead electrocardiogram. Noninvasive SBP, diastolic BP (DBP), and mean ABPs (MAP) were continuously collected (servo off) from a finger on the right hand, positioned at heart level using a Finapres (model 2300, Ohmeda). Finapress measures have been shown to accurately represent and track beat-by-beat BP during rest and orthostatic stress (23, 24). Finger BP estimates were adjusted to manual brachial artery sphygmomanometry measures during supine rest. Care was taken to ensure that the Finapres BP values did not drift throughout the testing procedure by comparing these values to manually obtained values throughout the course of the test. Transcranial pulsed-wave Doppler ultrasound (2 MHz probe; Transpect TCD, Medasonics) was used to measure middle cerebral artery mean blood flow velocity (MFV) on a beat-by-beat basis, which has been shown to be a valid means of measuring MFV (28, 33). Similarly, pulsed-wave Doppler ultrasound (2-MHz probe; Multiflow DWL, Elektronische Systeme Doppler) was used to obtain stroke volume velocity using the suprasternal notch approach. B-mode echo imaging of the aorta was performed (Sonos 5500, HP), allowing measurement of aortic root diameter in systole. By combining stroke volume velocity and diameter with changes in cardiac cycle, cardiac output (Q) was calculated. An index of cerebrovascular resistance (CVRi) was also calculated as the quotient of MAP adjusted for eye level (MAP_eve) and middle cerebral artery MFV. Total peripheral resistance (TPR) was calculated as MAP/Q. End-tidal carbon dioxide (ETCO2) was measured from a nasal cannula (CO2 Monitor, Ohmeda 5200) and adjusted for BTPS.

Experimental protocol. Subjects arrived several days before the initial testing session for dietary stabilization and familiarization with the surroundings and all procedures. For comparison purposes, members of each twin pair were randomly assigned as twin 1 and twin 2. On the test day, subjects had a catheter placed in their right antecubital vein, and they ate a defined meal at least 2 h before testing. The order in which a twin was tested was randomly determined by using the alphabetic order of the twin pairs names. A given twin pair was tested in either the morning or afternoon.

Each twin was placed supine inside a lower body negative pressure (LBNP) chamber positioned on a tilt table. The various data collection instruments were then placed on the subject. After at least 30 min of supine rest, 5 min of baseline data were collected, followed by 5 min of 60° head-up tilt (HUT). After 5 min of HUT, LBNP at 10 mmHg was applied for 3 min, followed every 3 min by an increase in LBNP of 10 mmHg until the onset of presyncope, at which time LBNP was stopped and the subject was returned to the supine position for a 5-min recovery period. At each stage of the protocol, subjects were asked to report their symptoms (i.e., nausea, sweatiness, tunnel vision, and limb numbness or pain) on a scale from 1 to 10. Presyncope, or termination of the test, was defined as follows: 1) a sudden drop in BP (SBP > 25 mmHg and DBP > 15 mmHg); 2) a sudden drop in HR (>15 beats/min); 3) sudden or extreme subject nausea, clammy skin, sweating, or pallor of the skin; 4) upon subject’s request; 5) SBP < 70 mmHg; 6) significant cardiac arrhythmias; or 7) loss of ECG signal.

Data analysis. Data were recorded in real time on digital format tape (Teac RD-111T Data Recorder) and then transferred to a computer at 100 Hz for analysis, except for the ECG signal, which was sampled at 1,000 Hz. R-wave-R-wave interval was taken as the time between successive R-waves, while HR was computed as the inverse. The average response for each variable was taken from the last 60 s of beat-by-beat data in each protocol stage. However, at presyncope, a 10-s average was used to provide a clearer estimate of rapidly changing events. Orthostatic tolerance time was measured from the onset of HUT until the cessation of HUT + LBNP, when the subject was returned to the supine position for recovery.

Statistics. Paired t-tests were used to assess weight, height, and orthostatic tolerance times between randomly assigned twin groups. The effect of tilt phase on the measured variables across all subjects was assessed using a repeated-measures one-way analysis of variance (SAS version 9.1). Relationships between the responses from each twin pair across the consecutive levels of the tilt test were assessed in two manners. First, a general view of how the twin pairs were related was assessed using least squares linear regression analysis of paired data for each variable and level of the orthostatic tilt test, with emphasis on the slope of the relationship and how this was similar to, or deviated from, the line of unity. Subsequently, Bland-Altman analysis (1, 3) was performed to assess the difference scores between the twin pairs (twin 1-twin 2) for each variable at each level of the tilt test. In particular, we were interested in the bias and the degree of error that might be involved, if it was assumed that the two twins responded identically. The bias was determined as the mean of the difference between the twin pairs for each tilt test level. Error was defined as the standard deviation of the difference in scores. Subsequently, an error coefficient was calculated as the error divided by the mean value at a particular level of the tilt test. Unfortunately, technical difficulties arose with the LBNP protocol where destructive interference waves were introduced into the stroke volume velocity Doppler ultrasound signal. Therefore, Q and TPR were determined only during supine rest and 60° HUT in 10 twin pairs. Cerebral blood flow and CVRi were obtained in 15 of the 16 twin pairs at various time points, whereas all other variables were obtained in all twin pairs during each study phase. The level of statistical probability was set at P < 0.05, and all data are reported as means ± SE, unless otherwise stated.
This pattern is replicated for all variables. relative to the mean overall level of a particular variable. error coefficient, when the degree of variability is assessed orthostatic stress to presyncope is particularly evident in the test progressed. This increase in variability with progressive responses. The error estimate tended to increase as the tilt indicating little or no systematic difference in the twin pair estimates were relatively consistent and small over time, bias and error estimates for each stress level. The bias corresponded to unity. Table 3 outlines the Bland-Altman estimates were moderately related in homozygous twin pairs while supine, but became progressively less similar as level of orthostatic stress increased. Nonetheless, the time to presyncope was very similar in the twin pairs. Thus, while orthostatic capacity per se appears to be quite similar, the

<table>
<thead>
<tr>
<th>Variable</th>
<th>MFV, cm/s</th>
<th>CVRi, mmHg/cm⁻¹s</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>MAPeye, mmHg</th>
<th>ETCO₂, mmHg</th>
<th>Q, l/min</th>
<th>TPR, mmHg⁻¹min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine</td>
<td>61.4±1.7</td>
<td>1.42±0.04</td>
<td>68.9±2.1</td>
<td>85.0±1.5</td>
<td>85.0±1.5</td>
<td>37.2±0.5</td>
<td>6.49±0.36</td>
<td>14.1±0.8</td>
</tr>
<tr>
<td>HUT 58.5±1.8</td>
<td>65.9±1.5</td>
<td>1.10±0.04</td>
<td>84.2±3.1</td>
<td>85.3±1.8</td>
<td>63.6±1.8</td>
<td>35.5±0.5</td>
<td>3.97±0.21</td>
<td>22.4±1.5</td>
</tr>
<tr>
<td>−10 mmHg</td>
<td>56.3±1.5</td>
<td>1.11±0.04</td>
<td>91.9±3.7</td>
<td>83.7±2.0</td>
<td>62.0±1.9</td>
<td>33.5±0.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>−20 mmHg</td>
<td>54.8±1.5</td>
<td>1.10±0.04</td>
<td>100.9±3.5</td>
<td>82.1±2.2</td>
<td>60.4±2.1</td>
<td>32.4±0.7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Presyncope</td>
<td>46.3±1.4</td>
<td>0.92±0.07</td>
<td>121.3±5.63</td>
<td>64.2±2.7</td>
<td>42.5±2.7</td>
<td>27.8±0.9</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are means ± SE. HUT, head-up tilt; MFV, middle cerebral artery mean blood flow velocity; CVRi, cerebrovascular resistance index; HR, heart rate; MAP, mean arterial blood pressure; MAPeye, mean arterial blood pressure at eye level; ETCO₂, end-tidal carbon dioxide; Q, cardiac output; TPR, total peripheral resistance; N/A, not available. *P < 0.05 vs. supine; †P < 0.05 vs. 20 mmHg; ‡P < 0.05 vs. all conditions.

RESULTS

Mean data analysis. There were no significant differences in height, weight, or age between the two twin groups (P > 0.2). Compared with supine, the normotensive components of the tilt test (i.e., before presyncope) were characterized by increased HR and TPR, unchanged MAP, and decreases in ETCO₂, middle cerebral artery MFV, CVRi, and Q (Table 1: all P < 0.05). At presyncope, middle cerebral artery MFV, MAP, MAPeye, and ETCO₂ were reduced below levels measured at all prior time points, whereas CVRi was lower at presyncope compared with baseline only. In contrast, HR at presyncope was greater than supine baseline and HUT+LBNP periods (P < 0.05; Table 1). Average orthostatic tilt tolerance time was 954 ± 65 and 893 ± 70 s for twin 1 and twin 2 groups, respectively (P = 0.08). Figure 1 demonstrates both the linear regression and Bland-Altman plots for individual tilt tolerance times between each twin pair. The slope and coefficient of the regression relationship were both strong. Moreover, the difference scores varied within ±2 SD of the mean, suggesting similarity between twins (4).

Hemodynamic variables: linear regression and Bland-Altman analysis. As an example, Figure 2 demonstrates the linear regression analysis and the Bland-Altman plots for MAPeye comparisons across twin pairs at each level of the tilt test. Visually, the linear regression analysis demonstrates that the slope of this relationship varies from one level of the test to another and often varies importantly from unity. Similarly, the Bland-Altman plots depict a varying degree of variability in the consecutive tilt-test periods. Table 2 presents the regression slope and coefficient values for each variable across the test levels and illustrates that the degree of similarity between twin pairs is highly variable in this protocol. For example, slope values of MAPeye comparing twin 1 to twin 2 ranged from 0.32 to 0.97 and rarely corresponded to unity. Table 3 outlines the Bland-Altman bias and error estimates for each stress level. The bias estimates were relatively consistent and small over time, indicating little or no systematic difference in the twin pair responses. The error estimate tended to increase as the tilt test progressed. This increase in variability with progressive orthostatic stress to presyncope is particularly evident in the error coefficient, when the degree of variability is assessed relative to the mean overall level of a particular variable. This pattern is replicated for all variables.

DISCUSSION

The main finding of the present study was that, in general, hemodynamic variables were moderately related in homozygous twin pairs while supine, but became progressively less similar as level of orthostatic stress increased. Nonetheless, the time to presyncope was very similar in the twin pairs. Thus, while orthostatic capacity per se appears to be quite similar, the
mechanism(s) by which that tolerance was achieved varied in the twin pairs.

The physiological response of the twin pairs to combined HUT/H11001 LBNP (Table 1) mirrors that previously reported in young, healthy individuals (15, 29, 32). LeLorier et al. (29) used a similar protocol of HUT/H11001 LBNP to induce presyncope. They reported HR to increase throughout the entire protocol, while middle cerebral artery MFV and ETCO2 decreased, with the largest change occurring at presyncope. SBP did not change until the onset of presyncope, where it significantly decreased (29). Their observations were similar to those of the present study using identical twins (Table 1). However, one major difference was the slightly longer average time to presyncope and larger tilt tolerance variability between subjects in their study. These observational differences could be the result of differences in testing protocol, such as longer data collection periods (5 min vs. 3 min) at each level of LBNP (29). Subjects in this study were introduced to higher levels of LBNP more quickly. Quicker transitions to higher orthostatic stress levels could have impacted orthostatic adaptation and tolerance. In addition, the fact that identical twins are genetically the same and were compared on the same day at relatively the same time, compared with repeating the tests in the same subjects 1 mo apart, could have played a major role in reducing tolerance time variability.

In addition to morphometric measures of body height, weight, and body mass index (2, 13, 16, 19, 22), heritability

![Fig. 2. Least square linear regression analysis (A) and Bland-Altman plots (B) for mean arterial blood pressure at eye level (mmHg) at each level of the orthostatic tolerance test. Symbols in A each represent a twin pair. HUT, head-up tilt; All Conditions, entire orthostatic tolerance test.]

**Table 2. Least square linear regression coefficient of determination and slope at each level of orthostatic stress**

<table>
<thead>
<tr>
<th>Variable</th>
<th>MFV, cm/s</th>
<th>CVRL, mmHg·cm⁻¹·s</th>
<th>HR, beats/min</th>
<th>MAPsys, mmHg</th>
<th>ETCO2, mmHg</th>
<th>Q, l/min</th>
<th>TPR, mmHg·l⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r^2 )</td>
<td>Slope</td>
<td>( r^2 )</td>
<td>Slope</td>
<td>( r^2 )</td>
<td>Slope</td>
<td>( r^2 )</td>
</tr>
<tr>
<td>Supine</td>
<td>0.30</td>
<td>0.35*</td>
<td>0.35</td>
<td>0.47*</td>
<td>0.64</td>
<td>1.05*</td>
<td>0.71</td>
</tr>
<tr>
<td>HUT</td>
<td>0.52</td>
<td>0.52*</td>
<td>0.34</td>
<td>0.41*</td>
<td>0.69</td>
<td>0.91*</td>
<td>0.46</td>
</tr>
<tr>
<td>−10 mmHg</td>
<td>0.64</td>
<td>0.80*</td>
<td>0.36</td>
<td>0.57*</td>
<td>0.68</td>
<td>0.65*</td>
<td>0.14</td>
</tr>
<tr>
<td>−20 mmHg</td>
<td>0.30</td>
<td>0.43</td>
<td>0.46</td>
<td>0.67*</td>
<td>0.58</td>
<td>0.50*</td>
<td>0.44</td>
</tr>
<tr>
<td>Presyncope</td>
<td>0.15</td>
<td>0.36</td>
<td>0.12</td>
<td>0.37</td>
<td>0.29</td>
<td>0.55*</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* \( P < 0.05. \)
appears to determine a large degree of variation in resting ABP (18, 38, 40), HR (14), sympathetic nerve activity (39), and plasma norepinephrine concentration (41). HR variability (6, 34), arterial compliance (35), and baroreflex function (36) have also been reported to be genetically influenced. However, heritability estimates are often <60%. Such estimates are consistent with the present findings, where coefficient of determination values ranged from 0.3 to 0.71 for MFV, CVRi, HR, and MAPeye during supine rest.

Many factors, including spontaneous cognitive aspects and environmental influences, contribute to supine or resting hemodynamic variables. Therefore, a more thorough assessment of genetic influence on cardiovascular function should incorporate a degree or range of stress. In the present study, the stress was applied progressively to maximal tolerance to assess similarities or dissimilarities between twin pairs. With this approach, it was observed that the apparent similarities between the twin pairs regarding baseline or resting cardiovascular function deteriorates under progressive levels of orthostatic stress. This conclusion is supported by previous data from Hunt and colleagues (22), who reported rather low intraclass correlation values for ABP in monozygotic twins, ranging between 0.14 and 0.5 for sitting, standing, arithmetic, bicycle, and static handgrip exercise protocols. Of note, these authors observed that orthostasis reduced heritability estimates, derived from the intraclass correlation coefficients, for SBP in monozygotic twins when adjusted for age and environment (22).

The above discussion pertains to the majority of variables assessed in this study. However, it is noteworthy that the variability of HR and CVRi between twin pairs remained fairly consistent throughout the orthostatic test. This result supports an earlier finding that the intrapair similarity for HR increased on going to an upright posture (2). The mechanism for these divergent results is not clear and may include neural and end-organ controls.

The lack of similarity in twin responses during stress suggests that the neural, cardiac, and vascular mechanisms that are engaged to control BP during orthostasis are malleable and sensitive to environmental stimuli. Certainly, cardiovascular deconditioning, such as occurs in exposure to real or simulated microgravity, results in altered BP regulation and one or more of neural, cardiac, and/or vascular end points. These twins were not consistently engaged in identical lifestyles. Therefore, the heterogeneity in the identical twins might be explained by the propensity of integrated mechanisms for BP regulation to be sensitive to environmental influences. The rapid changes that can occur in orthostatic tolerance (7, 8) are further evidence of the important environmental influence on BP regulatory systems. The present data, therefore, indicate that such malleability of orthostatic tolerance in healthy individuals undergoing bed rest or space flight is less influenced by genetics than other predispositions to adaptation. However, the role of genetics in such adaptability requires further study.

**Limitations.** Both males and females were included in this study. However, sex-dependent differences were not the focus of our study, and, based on earlier results, it is not expected that heritability estimates for ABP reflect a strong gender basis (20). Age reportedly affects the heritability of some cardiovascular indexes (e.g., SBP) in some (38), but not all, studies (20). Nonetheless, this potential impact should not have factored importantly in the present analysis because of the relatively narrow age range of the volunteers. Twenty-four-hour variations in ABP and heritability estimates have been demonstrated (13, 16). However, this too should not have impacted on the within-twin pair variability, as both individuals were tested in either the morning or afternoon sessions. In support of this, there is no indication in the bias estimates of difference scores of a systematic or directional impact of twin 1 or twin 2. Finally, the level of fitness and/or physical activity patterns may affect orthostatic tolerance (42). Although reports of physical activity patterns were not assessed in the present study, measures of actual physical fitness (which will encompass all physical activity patterns) were not different between twin pairs.

In conclusion, the combined data indicate a higher than expected level of variance between identical twins with respect to both cardiovascular and cerebrovascular responses to a stress. The elevated variance with increasing stress may be due to an increase in the role of environmental factors (2, 37) as a genetic component nears a functional limit. Thus, although orthostatic tolerance times were similar within a twin pair, the mechanism involved in sustaining cardiovascular/cerebrovascular function was not.

**GRANTS**

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REFERENCES