Am J Physiol Heart Circ Physiol 284: H1995–H2006, 2003.
First published January 30, 2003; 10.1152/ajpheart.00409.2002.

Microgravity alters respiratory sinus arrhythmia and short-term heart rate variability in humans

P-F. Migeotte,[1] G. Kim Prisk,[2] and M. Paiva[1]

[1]*Biomedical Physics Laboratory, Université Libre de Bruxelles, 1070 Brussels, Belgium; and*
[2]*Department of Medecine, University of California, San Diego, La Jolla, California 92093-0931*

Submitted 14 May 2002; accepted in final form 27 January 2003

Migeotte, P-F., G. Kim Prisk, and M. Paiva. Microgravity alters respiratory sinus arrhythmia and short-term heart rate variability in humans. *Am J Physiol Heart Circ Physiol* 284: H1995–H2006, 2003. First published January 30, 2003; 10.1152/ajpheart.00409.2002.—We studied heart rate (HR), heart rate variability (HRV), and respiratory sinus arrhythmia (RSA) in four male subjects before, during, and after 16 days of spaceflight. The electrocardiogram and respiration were recorded during two periods of 4 min controlled breathing at 7.5 and 15 breaths/min in standing and supine postures on the ground and in microgravity. Low (LF)- and high (HF)-frequency components of the short-term HRV (≤3 min) were computed through Fourier spectral analysis of the R-R intervals. Early in microgravity, HR was decreased compared with both standing and supine positions and had returned to the supine value by the end of the flight. In microgravity, overall variability, the LF-to-HF ratio, and RSA amplitude and phase were similar to preflight supine values. Immediately postflight, HR increased by ~15% and remained elevated 15 days after landing. LF/HF was increased, suggesting an increased sympathetic control of HR standing. The overall variability and RSA amplitude in supine decreased postflight, suggesting that vagal tone decreased, which coupled with the decrease in RSA phase shift suggests that this was the result of an adaptation of autonomic control of HR to microgravity. In addition, these alterations persisted for at least 15 days after return to normal gravity (1G).

heart rate; heart rate variability; respiratory sinus arrhythmia; controlled breathing; microgravity; spaceflight; gravity; autonomic control

ONE OF THE MOST IMPORTANT consequences on the cardiovascular system of exposure to weightlessness is the well-documented orthostatic intolerance experienced by many astronauts after spaceflight (6). This orthostatic hypotension is thought to be the result of the cardiovascular adaptation to microgravity that occurs in response to the cephalad shift of blood and body fluids. This fluid shift is the result of the removal of all hydrostatic pressure gradients and results in an increase of the stroke volume (SV) in microgravity (32). Heart rate (HR) and arterial pressure decrease (18) and cardiac output is elevated (39). The in-flight adaptation results in a reduction of plasma volume (23) and a tendency to return toward standing preflight control HR, SV, and cardiac output (39). Fritsch-Yelle et al. (18) have suggested that their observations of a decreased HR and arterial pressure are compatible with a cardiovascular system operating in microgravity with reduced sympathetic activity and vascular resistance.

On return to 1G conditions, Buckey et al. (6) reported a postural decrease of SV in all astronauts and found that those astronauts subject to orthostatic intolerance presented inadequate vasoconstrictor responsiveness. Previous studies have documented an impaired vagally mediated carotid-cardiac baroreflex after spaceflight (16) and an increased sensitivity of aortic and cardiopulmonary baroreflexes (12) after head-down bed rest. It has been suggested that plasma volume reduction resulting from microgravity exposure may be one underlying mechanism causing an alteration of the stimulus for the baroreceptors, therefore suggesting a chronic resetting. This suggests that reduction of plasma volume might be an important factor contributing to orthostatic intolerance after spaceflight. However, it seems unlikely to be the primary cause, and hypoadrenergic responsiveness, possibly centrally mediated, is likely a contributor (19). Thus it seems that the origin of orthostatic intolerance after spaceflight is multifactorial.

The demonstration by Akselrod et al. (1, 2) that power spectral analysis of R-R intervals (RRI) can be used to noninvasively investigate the autonomic control of the cardiovascular system leads to a multitude of publications on heart rate variability (HRV). It has been shown that respiration and postural changes have major influences on HRV and its components (9, 20, 22, 35). In particular, respiratory sinus arrhythmia (RSA), the high-frequency component of HRV, has been recognized as an index of the vagal-cardiac nerve traffic (2). Therefore, by analyzing its amplitude, we can infer changes in vagal tone or changes in the sensitivity of the sinus node to these vagal modulations, and its phase gives information about the duration of the integration of this cardiorespiratory reflex by the central nervous system. In addition, the spectral analysis of the RRI has been commonly applied in the last two

Address for reprint requests and other correspondence: P-F. Migeotte, Biomedical Physics Laboratory, CP 613/3, Université Libre de Bruxelles, 808, Route de Lennik, B-1070 Brussels, Belgium (E-mail: Pierre-Francois.Migeotte@ulb.ac.be).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

decades for the noninvasive assessment of the sympathovagal balance of the autonomic nervous system (29), although this remains somewhat controversial (14).

We hypothesized that in microgravity the parasympathetic component of cardiac autonomic control would be increased compared with 1G standing, and that, after exposure to microgravity, standing and supine autonomic control of HR would show a decreased vagal and increased sympathetic control of HR. In microgravity, these changes should result in increased amplitude and a phase shift of the RSA as well as in a decreased low frequency-to-high frequency ratio (LF/HF) compared with 1G standing. On return, this should lead to a decreased amplitude and phase shift of the RSA and increased LF/HF.

Therefore, we studied HR, HRV, and components of HRV during two controlled breathing protocols performed at two different breathing frequencies. Four astronauts repeated the same protocols in microgravity during the 16-day Neurolab STS-90 flight and on numerous occasions before and after the spaceflight in active standing and supine postures. Spectral analysis of RRI was used to assess the sympathovagal balance of the autonomic nervous system, and a time domain method, the polar representation of the RSA, was applied to estimate the amplitude and phase of the RSA (25).

The principal new results of this study are that *1*) the autonomic control of HR in microgravity was similar to that observed in the supine posture, and the most marked postflight changes in autonomic control were seen in the supine posture; *2*) RSA is decreased after exposure to microgravity, only in the supine posture, and this decrease does not depend on the breathing frequency; *3*) the phase of the RSA for the slowest breathing frequency is altered as well, primarily in the supine posture; and *4*) the subject's tachycardic responses do not require an orthostatic stress. In addition, our results suggest that, 15 days after return from 16 days of exposure to microgravity, subjects had still not returned to their preflight state.

METHODS

Experimental system. The equipment used is fully described elsewhere (33). Briefly, the subject breathed through a mouth piece connected to a valve unit equipped with a flowmeter in the wall of a bag in a box (linearized Fleisch no. 2 pneumotachograph). A valve placed in the inspired path allowed the subject to inspire and expire only during predefined periods of time that were under computer control. Hence a precise control of the breathing frequency was ensured. The subjects were equipped with two respiratory inductive plethysmograph (RIP) belts, placed respectively on the rib cage (RC) and the abdomen (AB) for the recording of the thoracic and abdominal respiratory movements. After calibration by an isovolume maneuver, the sum of these signals, each proportional to cross-section variation, is used as a measure of the lung volume variation (40). An electrocardiogram (ECG) was recorded with a traditional three-lead configuration. Data were digitized with a 12-bit analog-to-digital converter and recorded in digital format during the experiment. Sampling frequency was 400 Hz for the ECG and 100 Hz for the other signals.

Subjects and data collection. Subjects were four male crew members of the 16-day Neurolab STS-90 flight, average age 41 yr (range: 38–44 yr), average weight 82.4 kg (range: 75–88.5 kg), and average height 187 cm (range: 185–188 cm). Preflight data collection consisted of four sessions that were performed in the 3 mo before launch. In-flight experiments were performed on *days 4*, *6*, *11*, and *15* after launch. Postflight data were recorded 1, 2, 4, 5, and 15 days after landing. Testing was performed during the normal working period for a subject (~8:00 AM to 6:00 PM local time), at least 1 h after eating, and 2 h after exercise. Because of the logistical constraints associated with spaceflight, we were unable to fix testing to a particular time in the circadian phase. The protocols were approved by UCSD and NASA IRBs and subjects provided written informed consent.

Protocols and measurements. Two controlled breathing protocols, each of them lasting ~4 min, were part of a larger set of lung function studies lasting a total of ~20 min. They were separated by a period of ~3 min of normal uncontrolled breathing and were always performed in the same order. Data were first recorded in the standing posture and then in the supine posture, ~10 min after the subjects assumed that posture.

During the normal-paced breathing protocol (NPB) the subject inspired at a constant flow rate (~0.25 l/s) for 2 s. The flow was controlled by the subject watching a flowmeter. He inspired through a mouthpiece, until the closure of a valve, and then he relaxed and expired to functional residual capacity (FRC) during an equal period of 2 s without attempting to control expiratory flow. For NPB, the breathing cycle lasted 4 s, corresponding to 15 breaths/min (0.25 Hz), and leading to ~0.5 liters tidal volume.

The slow-paced breathing protocol (SPB) followed NPB. It consisted in the same controlled flow rate (0.25 l/s) of inspiration for a period of time of 4 s, and expiration also lasted 4 s. Therefore, the breathing period was 8 s, corresponding to 7.5 breaths/min (0.125 Hz), and to ~1-liter tidal volume.

Subjects were trained on the system to perform these maneuvers as naturally as possible, and special attention was paid to avoid breathing against the valve. During the maneuver, a constant posture was maintained as follows: standing on the ground and in microgravity with their hands at the level of the mouthpiece. In the supine posture, the subjects' arms were resting at their sides. Accurate pacing and depth of breathing were obtained through the visual feedback of the flow during the experiment. The measured breathing frequency for all recordings was 0.25 ± 0.002 (SD) Hz for NPB and 0.1249 ± 0.0008 Hz for SPB.

Before the paced breathing protocols, three standard isovolume maneuvers were performed at FRC with a closed glottis, which allowed the calibration of RIP on the AB and RC signals to give a signal proportional to the respired volume. During the isovolume maneuver, the ratio of volume motion coefficients (X/Y) is equal to the ratio of RC/AB signal amplitude (40). The respired volume was computed using the following equation: $\Delta Vol = X\,AB + Y\,RC$ where X and Y are the RIP volume motion coefficients.

Data analysis. All data analysis was carried out by Matlab 5.2 with Signal Processing Toolbox 4.1 (The Mathworks). An automated algorithm for the detection of inspiration and expiration was applied to the respiration signal. Two time series were constructed with occurrences of inspiration and expiration.

An automated algorithm for the detection of the QRS wave in the ECG was applied, and a time series of occurrences of R

waves was constructed (t_{rr}^{k} represents the occurrence of the kth R wave). The RRI time series were then computed as the time differences between two successive R waves ($RRI_k = t_{rr}^{k} - t_{rr}^{k-1}$). Premature ventricular contraction and/or ectopic beats were automatically detected [RRI outside the range (350–1,500 ms)], and the RRI was linearly interpolated with the surrounding values. All detected events and interpolated values were visually inspected. Recordings presenting more than one abnormal heartbeat per 30 s were removed from the analysis. For each test, time domain and frequency domain parameters (see below) were computed on a segment of 180 s of continuous RRI.

Frequency domain analysis. The power spectral analysis of RRI was performed according to the recommendations of the "Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology" (38). Briefly, a high-pass digital filtering (cut off = 0.033 Hz) and detrending procedure was applied to remove the continuous trend and very low oscillations in the nonequidistant RRI time series. Continuous RRI (180 s) was then interpolated (cubic spline), resampled at 2 Hz, and divided in five overlapping segments of 60 s. Each of them was in turn detrended, Hanning windowed, and fast Fourier transformed. We used the Welch algorithm for an averaging periodogram to estimate the power distribution.

The stationarity of time series was evaluated by computing the variance of the signal removed by the detrending procedures, i.e., difference between the total variance of the nondetrended time series and the total variance (total power density). Recordings with large removed variance were classified as nonstationary and removed from the analysis. The coefficient of variation (CV) was computed as the SD of the detrended RRI divided by mean RRI and was used to assess overall variability. The areas under the LF ($0.04\ Hz < f < 0.15\ Hz$) and HF ($0.15\ Hz < f < 0.4\ Hz$) spectral components were then computed. LF/HF was calculated only for the NPB protocol, in which respiratory frequency was in the HF range.

Time domain analysis. The polar representation of the RSA is a method for the analysis of the respiratory component of HRV without making assumptions regarding the breathing frequency (25). It was applied to the same segments of 180 s of stationary RRI for the NPB and SPB protocols after application of the data exclusion criteria (see above). This method is fully described elsewhere (25) and has previously been used to analyze uncontrolled breathing experiments performed during a 6-mo spaceflight.

Briefly, the method consists of a time alignment of the RRI with respect to the occurrence of the heartbeat in the breath cycle. For each heartbeat, its phase in the breath cycle (θ) is defined by normalizing the delay between its occurrence and the beginning of the breath with the total duration of the actual breath. Hence, RRI occurring in the inspiration will have a phase between 0 and 50%, and those occurring in expiration, a phase between 50 and 100%. Pooling together heartbeats from different breaths and plotting the RRI against its phase in breath (Fig. 1A), we obtain a graph on which RSA, if present, appears as a consistent oscillation in the breath cycle. Hence, RSA can be modeled by the following cosine curve

$$RRI = R + \rho_c \times \cos[(\theta - \theta_c) \times 2\ \pi/100] \qquad (1)$$

where R is the mean RRI and ρ_c and θ_c are, respectively, the amplitude and phase of the cosine. The use of a cosine curve at the respiratory frequency in the model corresponds to the analysis of the respiratory peak in the traditional Fourier spectral analysis. The superposition of data from different breaths implies that phases of 0 and 100% of the breath cycle represent the same physiological situation, the FRC of the subject. Taking advantage of this situation, we use a polar coordinate representation where the radial coordinate ρ is the RRI and the angular coordinate θ is the phase in breath cycle. In this representation (Fig. 1*B*) the RSA is modeled by a circle with radius R and center c with coordinates ρ_c,θ_c, in which a large RSA appears as a large offset of the center of the fitted circle in relation to the origin. Hence ρ_c is a measure of the RSA amplitude and θ_c a measure of the RSA phase in the breath cycle.

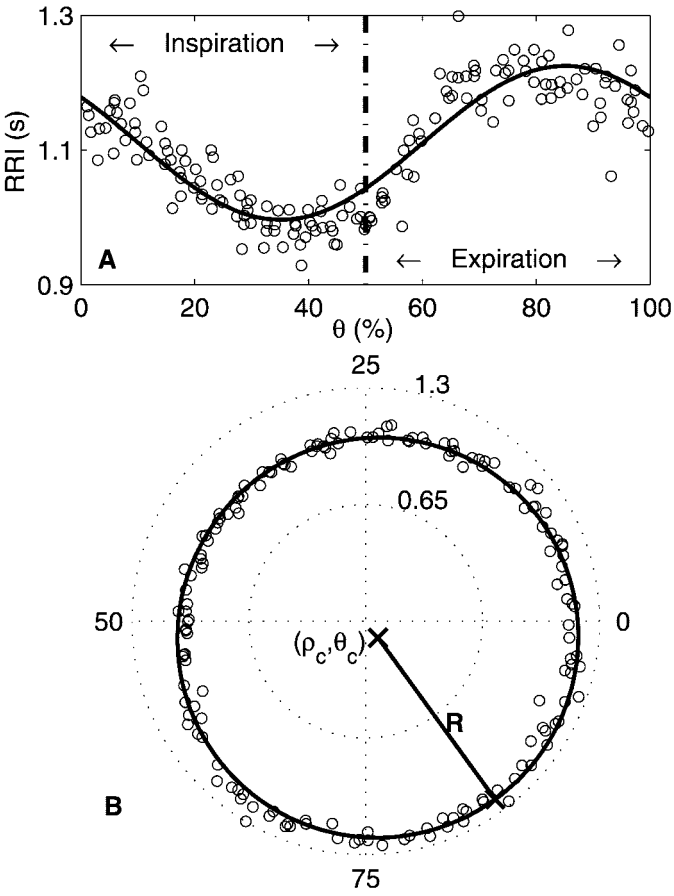

Fig. 1. Respiratory sinus arrhythmia for *subject 3*; slow breathing protocol in standing position on preflight. R-R interval (RRI) vs. its phase in breath cycle (θ; % of breath). *A*: Cartesian representation with a fitted cosine curve. *B*: polar representation with RRI as ρ coordinate and θ its phase in breath and the fitted circle. Duration of the respiratory cycle is normalized to 100, 0 and 50% corresponding to the onset of inspiration and expiration. R is the mean RRI. The offset of the center of the fitted circle gives the amplitude (ρ_c) and phase (θ_c) of the RSA. Fitted parameters (±95% confidence interval): $R = 1.11 \pm 0.003$ s, $\rho_c = 0.114 \pm 0.005$ s, $\theta_c = -14.9 \pm 0.6$%. The dotted line at R = 1.3 provides a visual reference in which there is no respiratory sinus arrhythmia (RSA).

Statistical methods. Data were grouped according to protocols and posture and recording sessions as follows: preflight (all 4 baseline data collections), in-flight (early = *days 5* and *6*, late = *days 11* and *15*), and postflight (early = *return days 1* and *2*, mid = *return days 4* and *5*, late = *return day 15*). To account for intersubject variability, all parameters (except for the RSA phase) were normalized by each subject's own average upright control value. Preflight, no differences were observed between NPB and SPB HR, and these data were grouped. Hence, the 100% preflight value corresponds to

Table 1. *Heart rate, heart rate variability, sympathovagal balance, and RSA*

			Inflight		Postflight					
	Preflight		Early	Late	Early		Mid		Late	
Parameter	Standing	Supine	μG	μG	Standing	Supine	Standing	Supine	Standing	Supine
HR, beats/min										
NPB	73.3±3.2	57.1±2.4‡‡‡	53.4±3.8***	58.1±3.7***	86.5±5.7**	65.6±3.5‡††	75.5±2.5*	62.0±2.8‡†	79.5±2.3	63.0±4.1‡
SPB	72.0±3.8	56.6±2.6‡‡	54.4±3.0***	58.6±4.1***	82.2±5.1**	66.7±3.8‡†††	79.1±3.6*	64.2±3.0‡††	76.3±4.9	62.4±3.3‡
CV RRI, %										
NPB	5.41±0.75	5.36±0.55	4.25±0.44	3.87±0.26	4.31±0.74	3.19±0.40**‡	7.31±2.0	4.31±0.65‡	6.39±1.6*	5.26±0.8‡
SPB	7.96±0.76	8.30±0.80	6.38±0.60	7.68±1.1	7.05±0.62	5.59±1.2**‡	8.82±0.94	6.33±1.2*‡	9.09±1.9	7.83±1.9‡
LF/HF										
NPB	4.83±0.70	1.67±0.40‡‡	1.77±0.76**	1.68±0.77**	9.99±1.6**	2.12±0.44‡	10.1±2.3*	1.89±0.64‡	9.14±3.9	0.99±0.56‡
RSA amplitude, ms										
NPB	11.0±2.6	33.7±6.9‡‡‡	28.3±6.6*	28.2±7.3*	5.43±1.2	17.4±4.4‡	13.7±4.3	21.5±4.1‡	9.4±3.4	30.2±6.7†
SPB	56.9±8.8	90.3±14‡‡	72.9±11	77.4±20	47.7±8.2	46.2±15††	51.6±7.1	56.1±15†	49.3±8.8	82.7±23
RSA phase, % of breath cycle										
NPB	−3.32±3.6	−5.03±3.8	−0.04±4.7	−2.02±3.8	−2.52±5.4	−9.57±4.6	0.6±3.9	−6.86±3.5	−6.10±5.4	−7.20±5.1
SPB	−25.1±2.3	−16.7±2.6‡‡	−18.8±0.8	−16.5±1.7*	−31.6±3.0	−25.3±3.3†	−28.9±4.0	−25.6±3.0†	−31.3±9.1	−22.3±5.2
No. of observations accepted/total										
NPB	16/16	15/16	7/8	8/8	7/8	8/8	6/8	7/8	4/4	4/4
SPB	15/16	14/16	8/8	7/8	6/8	8/8	7/8	8/8	3/4	4/4

Values are means ± SE; $n = 4$ subjects. HR, heart rate; CV RRI, coefficient of variation of R-R intervals; LF/HF, ratio of low frequency to high frequency; RSA, respiratory sinus arrhythmia; NPB, normal paced breathing protocol; SPB, slow paced breathing protocol; in-flight early = launch (L) + 4 and 6 days; in-flight late = L + 11 and 15 days; postflight early = return (R) + 1 and 2 days; postflight mid = R + 4 and 5 days; postflight late = R + 15 days. *Significant compared with preflight standing: *$P < 0.05$, **$P < 0.01$, ***$P < 0.01$. Significant compared with preflight supine: †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$. Significant paired comparison supine vs. corresponding standing values: ‡$P < 0.05$, ‡‡$P < 0.01$, ‡‡‡$P < 0.001$.

mean standing HR (NPB and SPB grouped), mean CV for NPB, mean RSA amplitude standing, and mean LF/HF NPB standing. All results are presented as averages ± SE. All statistical comparisons were performed on the raw and normalized data with the statistical software SPSS 6.1 (SPSS, Chicago, IL). Because there were only minor differences, with a smaller intersubject variability for normalized data, only statistics computed on normalized data are reported in Figs. 1–6 and Table 1. The nonparametric Mann and Whitney-Wilcoxon rank sum test was computed to test the time course of each parameter. When there was no significant time course, data on the figures were pooled for comparisons with preflight. Therefore, except for HR, the time course on Figs. 1–6 is presented in a unified way with in-flight (early and late pooled) and postflight mid- and late data pooled. Paired comparisons between standing and supine values were made with the Wilcoxon matched-pairs signed-rank test. In addition, standing minus supine differences were computed for each test and each subject on every parameter. On return (early + mid), changes from control were studied using the unpaired Mann and Whitney-Wilcoxon rank sum test, and standing minus supine differences significantly different from zero were tested using the signed-rank sum test. $P \leq 0.05$ was considered as significant, and, given the small sample size, the value of P is indicated.

Exclusion of recordings from the analysis was based on *1*) the poor quality of breathing data that did not allow accurate detection of inspiration and expirations (7 recordings), *2*) the presence of more than six premature ventricular contractions in the 3-min analyzed data (2 postflight recordings on *return days 1* and *4*) and *3*) the analysis of stationarity of the time series that showed that three NPB recordings and two SPB recordings presented removed variance >60 and 40%, respectively, of their total variance. The 60 and 40% limits were chosen as a compromise between ensuring stationarity and not rejecting too many recordings. The few recordings that are lost because of failure to meet the quality criteria imply that comparisons and statistical inference between different conditions are made on a different number of observations. The divergence from the total number of recordings is reported in Table 1. This divergence is small and unlikely to significantly affect the overall statistical interpretation.

RESULTS

Table 1 presents a summary of the mean values ± SE of each parameter and the number of accepted recordings in each protocol, posture condition, and all experimental sessions.

HR. Because there was no significant difference between NPB and SPB, these were grouped for statistical testing. Hence, Fig. 2 presents the time course of average HR normalized with respect to preflight standing of grouped NPB and SPB. The 100% normalized value corresponds to an average HR of 73 ± 3 beats/min, and supine HR was significantly lower (57 ± 2 beats/min). Early in-flight, HR was decreased to 54 ± 3 beats/min [a 26% decrease compared with preflight standing ($P < 0.001$) and 5% compared with preflight supine ($P < 0.01$)]. HR late in-flight increased to 58.3 ± 3 beats/min [a 5% increase ($P < 0.001$) compared with early in-flight] and was no longer different from preflight supine. Early postflight, standing HR increased to 85 ± 4 beats/min (15% increase compared with preflight) and supine HR increased to 66 ± 3 beats/min (14% compared with preflight). A trend to return to preflight values was seen for both postures, although HR remained significantly higher during late postflight for supine data compared with preflight. There were no

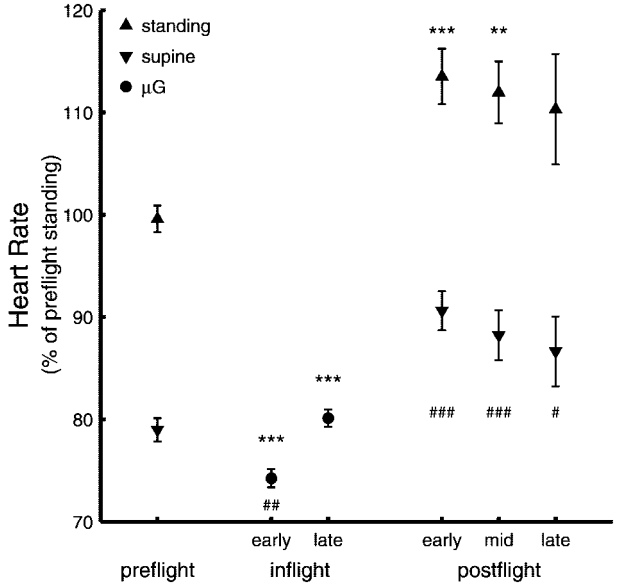

Fig. 2. Heart rate as a function of data collection period. Data are normalized to each subject's preflight standing control value. Normal and slow-paced breathing values are pooled. Values are means ± SE. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, significant compared with standing preflight. #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$, significant compared with supine preflight. In addition to the differences shown, the trend between early and late in-flight data is significant at $P < 0.001$.

postflight changes in the difference between standing and supine values.

Overall variability. Preflight CV for SPB was ~150% of the NPB value, and there was no significant difference between standing and supine values for either protocol. Figure 3 presents the time course of average CV normalized with respect to preflight NPB standing data. In-flight CV was decreased significantly (−21%) for NPB ($P < 0.05$) and only decreased slightly (−16%) for SPB (not significant). Early postflight, supine CV for NPB and SPB decreased significantly to 63% ($P < 0.01$) and 102% ($P = 0.02$), respectively. Although not significant, a similar trend was seen for standing NPB ($P = 0.09$). In addition, during early and mid plus late postflight, there was a standing to supine difference that was different from preflight. This difference is best seen for standing minus supine differences (Fig. 3*B*) where an increase of this difference on return (early + mid) compared with preflight is seen for SPB and less significantly for NPB.

Sympathovagal balance. Figure 4*A* presents the time course of the normalized LF/HF for the NPB protocol only. Indeed, for SPB the respiratory component was in the LF band, and the LF/HF ratio would not be interpretable in the same way. During preflight, supine LF/HF was significantly lower (38 ± 8%) than the standing value ($P < 0.01$). In-flight LF/HF did not change with time in microgravity, was similar to preflight supine, and remained significantly lower than preflight standing. On return, standing LF/HF increased to ~2 and ~3.6 times its preflight value during early and mid-postflight, respectively, and remained elevated on late postflight. A large intersubject variability of the postflight response was present. All supine postflight LF/HF were similar to preflight values. The differences between standing and supine LF/HF increased on return (Fig. 4*B*) to ~2.8 times its preflight value.

Amplitude of the RSA. For the clarity of the presentation, NPB and SPB data were normalized with respect to their respective preflight standing values and are presented in Fig. 5, *A* and *B*. This hides the large

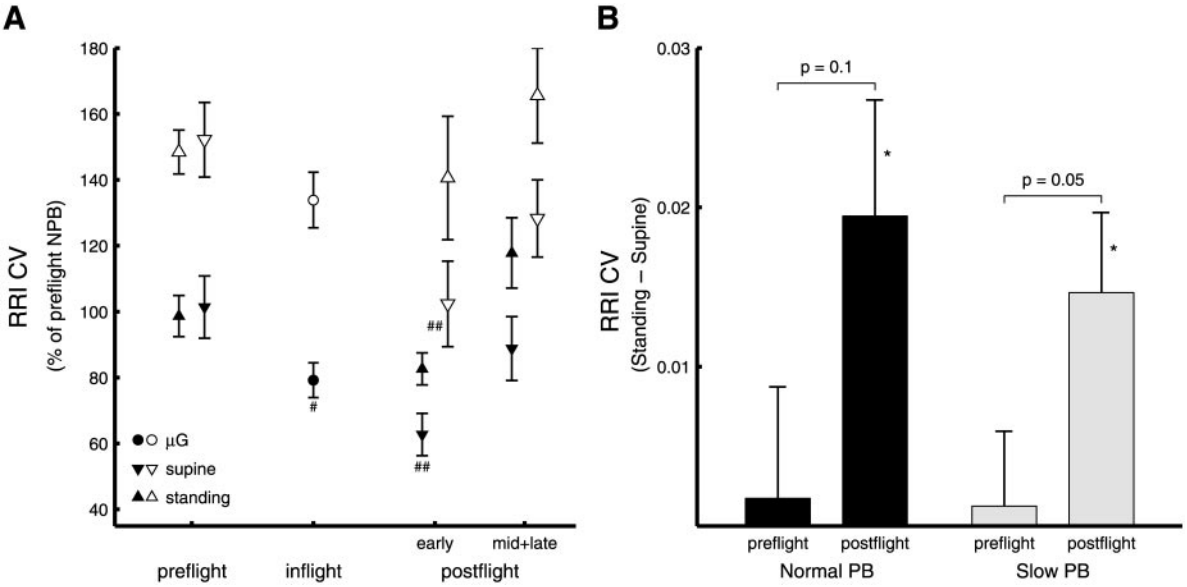

Fig. 3. *A*: RRI coefficient of variation (CV) as a function of data collection period. Data are normalized to each subject's preflight standing value for normal-paced breathing (PB) protocol. Filled symbols, NPB. Open symbols, slow-paced breathing (SPB). #$P < 0.05$ and ##$P < 0.01$, significant compared with supine preflight. *B*: CV standing − supine difference ± SE. *$P < 0.05$, significantly different from 0.

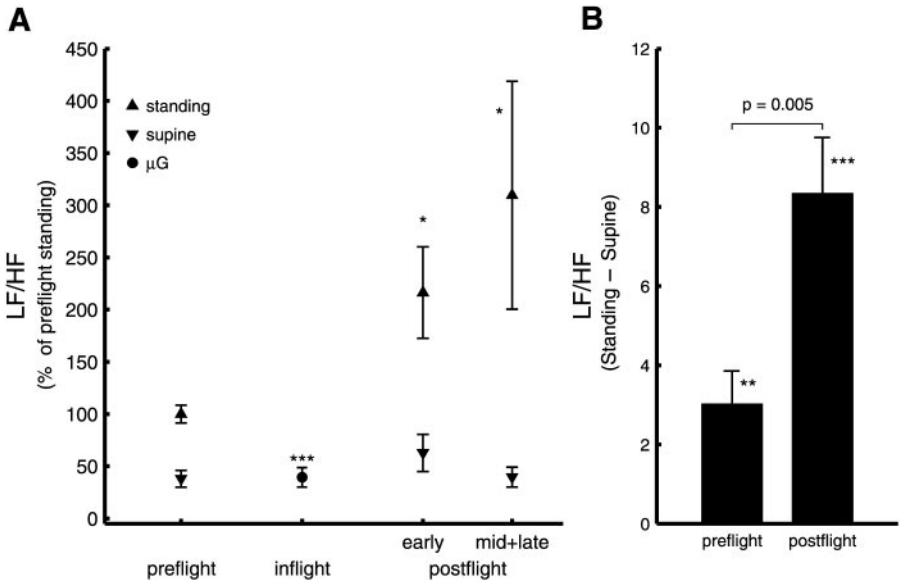

Fig. 4. *A*: low frequency-to-high frequency ratio (LF/HF) for NPB as a function of data collection period. Data are normalized to each subject's preflight standing control value. $*P < 0.05$ and $***P < 0.001$, significant compared with standing preflight. *B*: LF/HF standing − supine difference. $**P < 0.01$ and $***P < 0.001$, significantly different from 0.

difference in the absolute values in this parameter (see Table 1). Thus preflight for NPB (Fig. 5*A*), the 100% standing normalized value corresponded to an average amplitude of RSA of 11 ± 3 ms, and RSA amplitude supine was approximately three times its standing value ($P < 0.001$). Preflight SPB RSA amplitude was also larger supine (~150%) than standing ($P < 0.01$). For both postures, RSA amplitude was larger for SPB compared with NPB ($P < 0.001$; significance not shown in Table 1). In-flight for both protocols, RSA amplitude was comparable to preflight supine values. No significant difference between early and late in-flight data was seen, and these data were pooled. In-flight RSA amplitude was increased compared with preflight standing for NPB ($P < 0.001$) and for SPB ($P < 0.05$). Early postflight supine values were decreased to 183 ± 27% for NPB compared with preflight 289 ± 19%, and to 83 ± 17% for SPB compared with preflight 152 ± 12% (both $P < 0.01$). A progressive return to the preflight value was seen. Postflight for both protocols, standing values were similar to preflight. With the decrease of the supine value, this leads to a decrease of the standing to supine difference (Fig. 5*C*), which vanishes for SPB ($P = 0.02$) and decreases less significantly for NPB ($P = 0.1$).

Phase of the RSA. During preflight, the supine and standing RSA phase (Fig. 6*A*) for NPB was similar at about −5% of the breath cycle. This means that max-

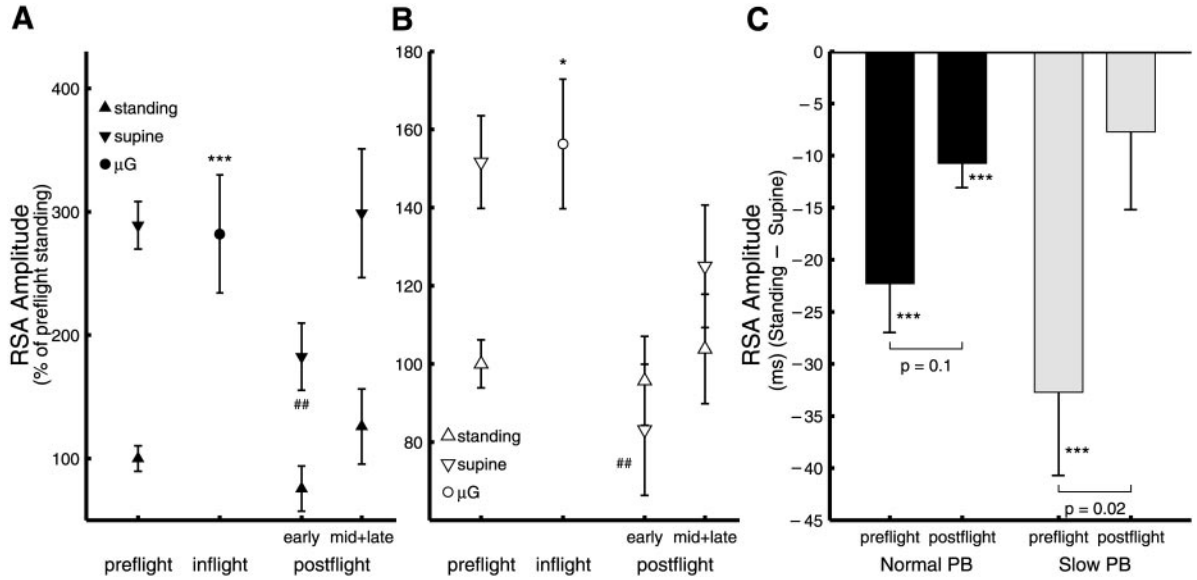

Fig. 5. *A* and *B*: RSA amplitude as a function of data collection period for NPB protocol (*A*) and SPB protocol (*B*). Data are normalized to each subject's preflight standing control value. $*P < 0.05$ and $***P < 0.001$. Significant compared with standing preflight. $##P < 0.01$. Significant compared with supine preflight. *C*: RSA amplitude standing − supine difference. $***P < 0.01$, significantly different from 0.

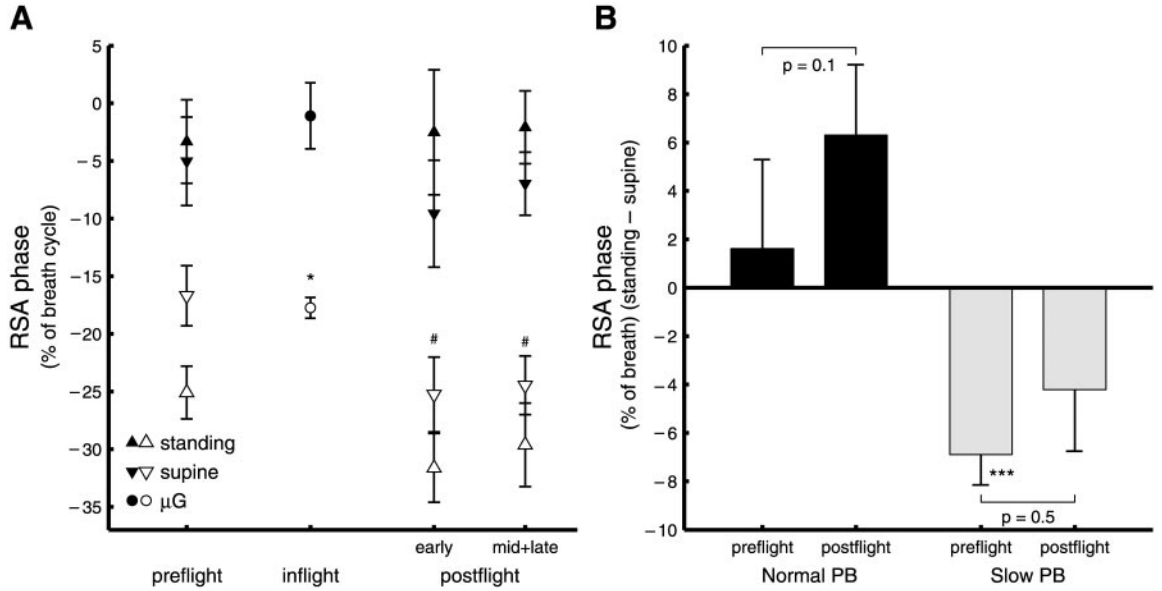

Fig. 6. *A*: RSA phase in the breath cycle as a function of data collection period. Values are means ± SE of the measured phase for each data collection period (values are not normalized). *$P < 0.05$, significant compared with standing preflight, #$P < 0.05$, significant compared with supine preflight. *B*: RSA phase standing − supine difference. ***$P < 0.001$, significantly different from 0.

imum HR (minimum RRI) slightly precedes the onset of expiration (RSA phase = 0% means that HR follows the fluctuations of instantaneous lung volume and that RRI is minimum at the end of inspiration and corresponds to an in-phase relationship). RSA phase for SPB standing was about −25%, which means that HR reaches its maximum in the middle of inspiration (this corresponds to a 90° out of phase relationship). In addition, maximum HR was reached later in inspiration in the supine posture, which was different from standing ($P = 0.002$). For NPB, there were no changes in the time course for RSA phase. SPB phase in flight was similar to preflight supine and significantly different from preflight standing ($P < 0.05$). Postflight, there was no difference between periods. For SPB, the RSA supine was more out of phase than during preflight ($P < 0.05$). This means that HR reaches its maximum earlier in the inspiration. For SPB, the standing to supine difference, which was highly significant preflight (Fig. 6*B*), did not reach significance postflight, and the comparison between pre- and postflight was also not significant.

DISCUSSION

HR. Preflight, the increased HR standing compared with supine is likely the result of sympathetic activation, which is the normal orthostatic response to prevent blood from pooling in the legs and arterial blood pressure from falling (10). The fact that average HR is the same in the two different breathing protocols (Table 1) is evidence that the protocols themselves did not induce a different tonic level of autonomic control of HR. Our result showing a decreased HR in microgravity (Fig. 2) is in agreement with results from previous studies (18, 39), and the change we observed is of similar magnitude.

Previous findings of increased SV and cardiac output (32, 39) as well as a decrease in mean arterial pressure (36) are consistent with a decreased HR in microgravity compared with standing and supine values. This change in HR can be interpreted as a fast response of the autonomic nervous system trying to maintain a normal cardiac output and normal blood perfusion to the brain in microgravity while facing contradictory inputs such as increased SV and decreased central venous pressure (5). This decrease in HR can be attributed to a decreased sympathetic activity and increased vagal tone. The fact that our result of a decreased HR seen during the controlled breathing experiment is in agreement with the result of Fritsch-Yelle et al. (18) obtained during normal activity in space shows that this HR response to microgravity exposure is not affected by our choice of breathing protocol.

The significant trend toward control values in HR seen in microgravity is in agreement with Verbanck et al. (39) and may be explained by the plasma volume reduction that occurs in-flight (23). This is also consistent with adaptation of the autonomic control of HR, which likely occurs in response to the new environment of weightlessness.

On return to 1G, the tachycardia seen during both protocols and in standing as well as supine supports the hypothesis of a sympathetic activation. The fact that HR is similarly affected in supine and standing postures and presents the same pattern of progressive return to preflight values shows that the postflight tachycardia is not only the result of a postflight orthostatic stress but also the result of an autonomic adap-

tation. On return plus 15 days (late postflight), that supine HR was still significantly different from preflight shows that, after a 16-day spaceflight, 15 days of recovery are not sufficient to return to preflight control conditions.

Overall variability. During supine preflight, longer RRI (lower HR) was associated with a higher total variability (total power), which resulted in the absence of significant differences in CV (total power/mean RRI) between standing and supine conditions (Fig. 3*B*). Although there was no difference in the average HR between the NPB and SPB protocols, the observed differences in CV between these protocols is evidence that SPB allows the examination of different aspects of the autonomic control mechanisms, consistent with numerous studies on HRV associated with different breathing protocols (9, 20, 28, 35).

In microgravity, the significant (17%) decrease in CV for NPB supports the hypothesis of an in-flight adaptation of autonomic control of HR. Indeed, without changes of the LF/HF and HF component compared with supine, the decrease in CV must be attributed to increased RRI (decreased HR), which is the case on early in-flight (see Table 1), or to the smaller increase (compared with standing) of very low frequency (VLF), LF, and HF components, which is likely the case for late in-flight measurements in which HR is similar to supine preflight.

Early postflight, the decreased CV in the supine posture for both protocols is the result of a decrease in the RSA amplitude (HF), likely because of decreased vagal modulation of HR (see *Amplitude of RSA*). The markedly increased standing-supine differences compared with preflight (Fig. 3*B*) are also in agreement with a decreased vagal modulation when supine. That this difference does not exist preflight and that there are no corresponding differences in the HR change between standing and supine preflight versus postflight suggest that a different mix of autonomic control of HR is present postflight. Surprisingly, the most marked changes occurred in the supine measurements, confirming our hypothesis of a postflight alteration of autonomic control for the supine control of HR.

Sympathovagal balance. During NPB, HRV shows at least two components, one slow (LF) and one fast (HF). The VLF component, which was apparent in some recordings, will not be discussed here since these recordings were not long enough to accurately estimate this VLF component (38) and were removed by the detrending process. When the breathing frequency is in the HF range (as in the NPB), HF power is the result of the RSA and is assumed to reflect parasympathetically mediated oscillations, i.e., vagal modulation of HR (1, 2, 20, 35). LF power is assumed to be the result of modulations of the sympathetic and parasympathetic nervous activity (1, 31, 35).

When in a standing position, a situation of known sympathetic activation and increased HR, LF is dominant over HF oscillation (10, 31), which leads to a larger LF/HF than in supine posture (Fig. 4*A*). The LF/HF values we observed (Table 1) were similar to those reported by others (27).

In microgravity, LF/HF was similar to supine values, which were significantly lower than standing. This suggests that, in microgravity, HR is predominantly under vagal control. During microgravity, the absence of posture changes, which are causing variations in LF/HF, suggests that HR may predominately stay in one state of autonomic control and that fewer excursions to the sympathetic state will be made. This might be thought to lead to an adaptation process by which, for example, the decreased sympathetic stimulation during spaceflight could lead to an increased sensitivity of the noradrenergic receptors (34). Although no such adaptation was seen in our results on LF/HF, the increase in HR over the course of microgravity may be suggestive of such a process.

This hypothesis of an increased receptor sensitivity is in agreement with recent results from Ertl et al. (15) obtained by direct measurements of muscle sympathetic activity. Those studies showed that, in the same subjects, the sympathetic response to orthostatic stress (provoked by lower body suction) was increased on *day 12* or *13* during the same spaceflight. However, their measurement of an increased muscle sympathetic activity at rest in microgravity (compared with preflight supine) is in apparent contradiction with our measurements of LF/HF in microgravity, which were similar to supine values, and to a decreased HR in microgravity compared with supine. Both observations are evidence of decreased sympathetic outflow to the heart early in-flight with a return to values similar to supine late in-flight. Possible explanations of such apparent discrepancies are as follows: *1*) the measurements of the peroneal nerve muscle activity through microneurography and the plasma norepinephrine spillover and clearance reflect the overall sympathetic activity, whereas ours concern only the autonomic control of HR; supportive of this explanation are the measurements of resting HR (11, 15), which were similar to preflight supine and which are not supportive of an increased sympathetic outflow to the heart; *2*) their measurements, performed on *days 12* and *13*, reflect the influence of microgravity and the autonomic adaptation, which had already occurred by that time; and *3*) the increased sympathetic outflow may be accompanied by increased vagal modulation of HR, which is prevented from producing larger LF oscillations because RRI is already increased, in which case there would be no more reserve for additional (LF) oscillations in RRI.

On return to 1G, the large increased LF/HF standing suggests a sympathetic activation (increased LF), which is in agreement with observed postflight increases in plasma catecholamine concentrations (41) and supports the hypothesis of increased sensitivity of the noradrenergic receptors after exposure to microgravity (34). The similar, although not significant, increase in supine LF/HF early postflight (see Table 1) is in agreement with the results of Fritsch-Yelle et al. (17). They reported an increased LF/HF in supine pos-

ture for the landing day and 1–2 days after landing, which corresponds to our early postflight measurement, but they did not have comparable results during standing. These results are in agreement with an increased sympathetic and decreased vagal modulation of HR. As previously mentioned, this increased sympathetic modulation of HR can be attributed to an increased reactivity of the sympathetic nervous system because of its low level of stimulation during exposure to weightlessness and a possible resetting process, in agreement with results from head-down bed rest (4). In addition, a postflight increase in sympathetic nerve activity was also measured by Levine et al. (24) in the same subjects.

Amplitude of RSA. Among the three components present in the spectral analysis of HRV, the HF component, which corresponds to RSA, is the component in which the most widely accepted interpretation has been developed; it is a valid index of cardiac vagal modulation (21). Therefore, by analyzing its amplitude, we can infer changes in vagal tone or changes in the sensitivity of the sinus node to these vagal modulations or in the generation of these vagal modulations by the baroreflex. In addition, the phase, which was never analyzed before, gives information on the duration of the processing of this cardiorespiratory interaction by the entire loop, independently of its origin, which is still a matter of debate (21, 41).

During preflight, larger RSA amplitude for SPB than for NPB (see Table 1) is the result of the frequency dependency of the RSA (28, 35). This and the larger supine than standing values for both protocols (Fig. 5) are qualitatively in agreement with published models of RSA (9, 20, 35). The model of Hayano et al. (20) presents RSA as the result of phasic (excitatory/inhibitory) modulation of vagal control of HR, yielding maximum vagal excitation during expiration and inhibition during inspiration. Because the sinus node presents a low-pass filter property to vagal stimulation with a decay constant of ~1 s, the larger RSA at low breathing frequencies does not reflect a larger vagal tone per se but rather a larger expression of vagal modulation. In contrast, supine vagal control of HR is more pronounced than standing, and a larger RSA amplitude is a marker of larger vagal modulation of HR. This, together with the slower HR, is in agreement with a higher vagal tone supine than standing (10).

In microgravity, for both protocols, RSA amplitude was similar to that measured supine preflight and was increased compared with standing. This confirms our hypothesis that, in microgravity, autonomic control of HR is similar to that in the supine posture, suggesting that the cardiovascular control is more in a state of vagal activation, consistent with the in-flight decrease in HR. This interpretation is in agreement with previous results from spaceflight (17, 18) and head-down bed rest (7), all in agreement with an increased vagal activity at true or simulated microgravity.

In contrast to this result, Cooke et al. (8) found on a longer spaceflight a reduction of RSA compared with preflight supine, whereas our measurements, with more subjects and repetitions, do not show a significant decrease (see Table 1). It is unclear whether this difference is the result of an adaptation that occurred after being exposed to >15 days of microgravity in their study or is the result of methodological differences.

Early postflight, the decreased RSA amplitude in the supine posture (Fig. 5, *A* and *B*) supports our hypothesis of a decreased vagal tone after spaceflight, already evidenced by the increased HR and increased LF/HF standing. The decreased differences between standing and supine, which one observes for RSA amplitude (Fig. 5*C*), are mainly because of the decreases in the supine values, suggesting that no decreased vagal tone was present standing and contradicting our hypothesis of an overall reduced vagal tone. For SPB standing, a decreased vagal modulation could be counterbalanced by increased sympathetic modulation in the same LF band, therefore contributing to the absence of a standing decrease in RSA amplitude.

Our result of a postflight decrease in RSA amplitude for supine NPB seems to be in contradiction with the study of Fritsh-Yelle et al. (17), which showed no postflight decrease in the HF component of HRV in supine posture. This discrepancy might be explained by methodological differences. Although they estimated the HF through the computation of an average power spectra for all subjects and integration of the resulting averaged HF frequency range, we estimated RSA amplitude for each subject before any averaging procedure, preserving the inter- and intrasubject variability. The results in the previous study (17) would likely have blurred individual differences, such as small variations in the individual breathing frequency, which was controlled in this study. Nevertheless, our result is consistent with their conclusion of a reduction in parasympathetic and increase in sympathetic influences on arterial pressure control after spaceflight.

Phase of RSA. The literature on phase of RSA is very sparse. Saul et al. (35) and, more recently, Cooke et al. (9) investigated a phase relationship between instantaneous lung volume and RRI variability through the estimation of transfer functions. However, the later studies were performed using broad-band-frequency breathing protocols, which are very difficult to compare with the single controlled frequency protocols used in this study. Despite these methodological issues, the postural differences and differences between NPB and SPB seen in the phase of RSA preflight (Fig. 6*A*) are consistent with previous results (3, 9). During preflight, the observed phase advance for SPB compared with NPB is in agreement with the low-pass filter model of RSA (20). With the same decay constant of ~1 s, the longer breathing period leads to larger RSA with a phase advance in the breath cycle. In addition, for SPB, that during standing RSA phase is more advanced than in the supine posture can be attributed to larger vagal tone in supine than in standing posture. Preflight for SPB, that standing RSA phase is more advanced than supine is also in agreement with previous results (20). Indeed the smaller amplitude of the

RSA observed while standing (Fig. 5*B*) means that the saturation level was reached more rapidly, which translates into an increased phase advance.

In microgravity, RSA phase for SPB is similar to preflight supine. This is consistent with our hypothesis that cardiovascular control mechanisms in microgravity have similarities with supine control.

Postflight, more out-of-phase supine values during NPB and SPB cause a significant standing to supine difference during NPB and a decrease of the standing to supine difference during SPB (Fig. 6*B*). This together with the significant difference seen in the supine RSA phase for SPB is further evidence that the vagal control of HR was altered by exposure to microgravity.

RSA is known to be the result of modulation of the cardiac vagal efferent activity by the central respiratory drive and gating of excitatory input to the vagal motor neurons by the lung inflation reflex (21). Therefore, the observed changes in the amplitude suggest a decreased cardiac vagal modulation possibly because of decreased vagal tone. The phase shift observed suggests a central adaptation of the timing of the gating or might be because of a change in delay in the afferent path of the lung inflation reflex. The presence of a phase shift also suggests that alterations of RSA are more likely to have their origin in the plasticity of the reflexes originating in the autonomic nervous system rather than in a mechanical change, such as plasma volume variations. Indeed, one expects mechanical changes to produce amplitude changes but not phase shifts in the reflex response.

In addition, if RSA bears an active physiological role (21), benefiting pulmonary gas exchange by matching perfusion to ventilation within each respiratory cycle, the observed phase shift on return might be at the origin of an adverse effect on the ventilation of the subjects. This in turn could lead to a competition between increased sympathetic activity (and decreased vagal tone) to increase the total peripheral resistance in response to orthostatic stress and increased RSA (increased vagal tone) to maintain this benefit. This competition could be a new factor contributing to the orthostatic intolerance. In addition, we might speculate that such a competition could contribute to the longer readaptation to 1G than the time it took to adapt to microgravity.

Surprisingly, the most important postflight changes were not seen on standing, but seen in the supine measurements, which is evidence that they did not result from an orthostatic stress, again pointing to a central adaptation. Therefore, we believe that our results support the notion that the cardiovascular changes observed after spaceflight are made of a complex interaction between a central autonomic disorder in which cardiorespiratory interactions might be an important contributing factor, differential adaptations of vessels in different anatomic regions (42), as well as plasma volume reduction and baroreflex alteration.

Limitations. This discussion about modifications of HRV and its components induced by microgravity as observed in this study is based on the hypothesis that RSA is exclusively mediated by vagal modulation of HR (1, 2). This is strongly supported by the fact that large doses of atropine (a parasympathetic muscarinic blocker) nearly abolish HR fluctuations in the HF (respiratory) band (26, 31). Whether the cause of these oscillations is to be found in the respiratory-induced pressure modulation of aortic and carotid baroreceptors (the peripheral hypothesis; see Ref. 30) or is the result of a central phenomenon (37) is not answered by these data. In both cases, the RSA is generated in, or mediated by, the central nervous system. In addition, we assumed that the LF/HF, under the condition that breathing frequency is >0.15 Hz (9), can be considered as a marker of the sympathovagal balance (29).

In conclusion, in this study of HR, spectral analysis of HRV, and RSA in four male subjects before, during, and after 16 days of microgravity, HR was decreased in microgravity and there was a significant trend for HR to increase during the flight. Overall variability was decreased in-flight, with no further adaptation. During microgravity, the spectral analysis showed that the sympathovagal balance, RSA amplitude, and phase were similar to supine values. These observations are consistent with an autonomic adaptation to weightlessness and evidence that in microgravity the control of HR is in a state of increased vagal activation and decreased sympathetic control compared with preflight standing. Postflight, the changes in amplitude and phase of the RSA are compatible with a reduced vagal activity after spaceflight and suggest a decreased vagal tone. This is consistent with the observed large increase of the sympathovagal balance during standing and with observed increased HR for standing and supine postures. In addition the postflight changes in RSA phase, in the protocol with the largest vagal influence (supine SPB), speak for an autonomic origin for the cardiovascular adaptation to microgravity. Surprisingly the most important postflight changes were not seen on standing, but in the supine measurements, which is evidence that they did not result from an orthostatic stress.

The persistent increase in HR for all measurements, absent differences in the RSA amplitude between standing and supine under the slow-breathing protocol, and the persistent differences in the CV between standing and supine postures suggest that after 15 days of reexposure to 1G the subjects are still not back to their preflight baseline condition.

We acknowledge the sustained and excellent support and cooperation of the crew of Neurolab and the many National Aeronautics and Space Administration (NASA) and Lockheed Martin Engineering Services personnel who supported the mission. We also thank F. Colin, Y. Verbandt, and P. Van de Borne for fruitful discussions and well-advised comments.

This work was supported by the Belgian Federal Office of Scientific, Technical, and Cultural Affairs under a PRODEX contract. G. K. Prisk was supported by NASA contracts NAS9-18764 and NAS9-19434 and by National Heart, Lung, and Blood Institute Grant U01-HL-53208-01.

REFERENCES

1. **Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, and Cohen RJ.** Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol Heart Circ Physiol* 249: H867–H875, 1985.
2. **Akselrod S, Gordon D, Ubel FA, Shannon DC, Barger AC, and Cohen RJ.** Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213: 220–222, 1981.
3. **Angelone A and Coulter NA.** Respiratory sinus arrhtythmia: a frequency dependent phenomenon. *J Appl Physiol* 19: 479–482, 1964.
4. **Barbe P, Galitzky J, Thalamas C, Langin D, Lafontan M, Senard JM, and Berlan M.** Increase in epinephrine-induced responsiveness during microgravity simulated by head-down bed rest in humans. *J Appl Physiol* 87: 1614–1620, 1999.
5. **Buckey JC Jr, Gaffney FA, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Yancy CW Jr, Meyer DM, and Blomqvist CG.** Central venous pressure in space. *J Appl Physiol* 81: 19–25, 1996.
6. **Buckey JC Jr, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Moore WE, Gaffney FA, and Blomqvist CG.** Orthostatic intolerance after spaceflight. *J Appl Physiol* 81: 7–18, 1996.
7. **Convertino VA, Doerr DF, Eckberg DL, Fritsch JM, and Vernikos-Danellis J.** Head-down bed rest impairs vagal baroreflex responses provokes orthostatic hypotension. *J Appl Physiol* 68: 1458–1464, 1990.
8. **Cooke WH, Ames JE, IV, Crossman AA, Cox JF, Kuusela TA, Tahvanainen KU, Moon LB, Drescher J, Baisch FJ, Mano T, Levine BD, Blomqvist CG, and Eckberg DL.** Nine months in space: effects on human autonomic cardiovascular regulation. *J Appl Physiol* 89: 1039–1045, 2000.
9. **Cooke WH, Cox JF, Diedrich AM, Taylor JA, Beightol LA, Ames IV JE, Hoag JB, Seidel H, and Eckberg DL.** Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am J Physiol Heart Circ Physiol* 274: H709–H718, 1998.
10. **Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KUO, and Eckberg DL.** Human responses to upright tilt: a window on central autonomic integration. *J Physiol* 517: 617–628, 1999.
11. **Cox JF, Tahvanainen KUO, Kuusela TA, Levine BD, Cooke WH, Mano T, Iwase S, Saito M, Sugiyama Y, Ertl AC, Biaggioni I, Diedrich AM, Robertson RM, Zuckerman JH, Lane LD, Ray CA, White RJ, Pawelczyk JA, Buckey JC, Baisch F, Blomqvist CG, Robertson D, and Eckberg DL.** Influence of microgravity on astronaut's sympathetic and vagal responses to Valsalva's manoeuvre. *J Physiol* 538: 309–320, 2002.
12. **Crandall CG, Engelke KA, Convertino VA, and Raven PB.** Aortic baroreflex control of heart rate after 15 days of simulated microgravity exposure. *J Appl Physiol* 77: 2134–2139, 1994.
14. **Eckberg DL.** Sympathovagal balance: a critical appraisal. *Circulation* 96: 3224–3232, 1997.
15. **Ertl AC, Diedrich A, Levine BD, Biaggioni I, Levine BD, Robertson RM, Cox JF, Zuckerman JH, Pawelczyk JA, Ray CA, Buckey JC Jr, Lane LD, shiavi R, Gaffney FA, Costa F, Holt C, Blomqvist CG, Eckberg DL, Baisch F, and Robertson D.** Human muscle sympathetic nerve activity and plasma noradrenaline kinetics in space. *J Physiol* 538: 321–329, 2002.
16. **Fritsch JM, Charles JB, Bennett BS, Jones MM, and Eckberg DL.** Short-duration spaceflight impairs human carotid baroreceptor-cardiac reflex responses. *J Appl Physiol* 73: 664–671, 1992.
17. **Fritsch-Yelle JM, Charles JB, Jones MM, Beightol LA, and Eckberg DL.** Spaceflight alters autonomic regulation of arterial pressure in humans. *J Appl Physiol* 77: 1776–1783, 1994.
18. **Fritsch-Yelle JM, Charles JB, Jones MM, and Wood ML.** Microgravity decreases heart rate and arterial pressure in humans. *J Appl Physiol* 80: 910–914, 1996.
19. **Fritsch-Yelle JM, Whitson PA, Bondar RL, and Brown TE.** Subnormal norepinephrine release relates to presyncope in astronauts after spaceflight. *J Appl Physiol* 81: 2134–2141, 1996.
20. **Hayano J, Mukai S, Sakakibara M, Okada A, Takata K, and Fujinami T.** Effects of respiratory interval on vagal modulation of heart rate. *Am J Physiol Heart Circ Physiol* 267: H33–H40, 1994.
21. **Hayano J, Yasuma F, Okada A, Mukai S, and Fujinami T.** Respiratory sinus arrhythmia a phenomenon improving pulmonary gas exchange and circulatory efficiency. *Circulation* 94: 842–847, 1996.
22. **Iwase S, Mano T, Cui J, Kitawaza H, Kamiya A, Miyazaki S, Sugiyama Y, Mukai C, and Nagaoka S.** Sympathetic outflow to muscle in humans during short periods of microgravity produced by parabolic flight. *Am J Physiol Regul Integr Comp Physiol* 277: R419–R426, 1999.
23. **Johnson PC, Driscoll TB, and LeBlanc AD.** Blood volume changes. In: *Biomedical Results from Skylab*, edited by Johnston RS and Dietlein LF. Washington, DC: NASA SP-377, 1977, p. 235–241.
24. **Levine BD, Pawelczyk JA, Ertl AC, Cox JF, Zuckerman JH, Diedrich A, Biaggioni I, Ray CA, Smith ML, Iwase S, Saito M, Sugiyama Y, Mano T, Zhang R, Iwasaki K, Lane LD, Buckey JC, Cooke WH, Baisch F, Robertson D, Eckberg DL, and Blomqvist CG.** Human muscle sympathetic neural and haemodynamic responses to tilt following spaceflight. *J Physiol* 538: 331–340, 2002.
25. **Migeotte PF and Verbandt Y.** A novel algorithm for the heart rate variability analysis of short-term recordings: polar representation of respiratory sinus arrhythmia. *Comput Biomed Res* 32: 56–66, 1999.
26. **Montano N, Cogliati C, Porta A, Pagani M, Malliani A, Narkiewicz K, Abboud FM, Birkett C, and Somers VK.** Central vagotonic effects of atropine modulate spectral oscillations of sympathetic nerve activity. *Circulation* 98: 1394–1399, 1998.
27. **Mukai S and Hayano J.** Heart rate and blood pressure variabilities during graded head-up tilt. *J Appl Physiol* 78: 212–216, 1995.
28. **Novak V, Novak P, de Champlain J, Robert Le Blanc A, Martin R, and Nadeau R.** Influence of respiration on heart rate and blood pressure fluctuations. *J Appl Physiol* 74: 617–626, 1993.
29. **Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell' Orto S, Piccaluga E, Turiel M, Baselli G, Cerutti S, and Malliani A.** Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 59: 178–193, 1986.
30. **Piepoli M, Sleight P, Leuzzi S, Valle F, Spadacini G, Passino C, Johnston J, and Bernardi L.** Origin of respiratory sinus arrhythmia in conscious humans an important role for arterial carotid baroreceptors. *Circulation* 95: 1813–1821, 1997.
31. **Pomeranz B, Macaulay RJB, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, and Benson H.** Asessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol Heart Circ Physiol* 248: H151–H153, 1985.
32. **Prisk GK, Guy H, Elliott AR, Deutschman RAI, and West JB.** Pulmonary diffusing capacity, capillary blood volume and cardiac output during sustained microgravity. *J Appl Physiol* 75: 15–26, 1993.
33. **Prisk GK, Guy HJB, Elliott AR, Paiva M, and West JB.** Ventilatory inhomogeneity determined from multiple-breath washouts during sustained microgravity on Spacelab SLS-1. *J Appl Physiol* 78: 597–607, 1995.
34. **Robertson D, Convertino VA, and Vernikos J.** The sympathetic nervous system and the physiologic consequences of spaceflight: a hypothesis. *Am J Med Sci* 308: 126–132, 1994.
35. **Saul JP, Berger RD, Albrecht P, Stein SP, MH Chen, and Cohen RJ.** Transfer function analysis of the circulation: unique insights into cardiovascular regulation. *Am J Physiol Heart Circ Physiol* 261: H1231–H1245, 1991.

36. **Shykoff BE, Farhi LE, Olszowka AJ, Pendergast DR, Rokitka MA, Eisenhardt CG, and Morin RA.** Cardiovascular response to submaximal exercise in sustained microgravity. *J Appl Physiol* 81: 26–32, 1996.
37. **Shykoff BE, Naqvi SS, Menon AS, and Slutsky AS.** Respiratory sinus arrhythmia in dogs effects of phasic afferents and chemostimulation. *J Cin Invest* 87: 1621–1627, 1991.
38. **Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology.** Heart rate variability standards mf Measurement, physiological interpretation, and clinical use. *Circulation* 93: 1043–1065, 1996.
39. **Verbanck S, Larsson H, Linnarsson D, Prisk GK, West JB, and Paiva M.** Pulmonary tissue volume, cardiac output, and diffusing capacity in sustained microgravity. *J Appl Physiol* 83: 810–816, 1997.
40. **Wantier M, Estenne M, Verbanck S, Prisk GK, and Paiva M.** Chest wall mechanics in sustained microgravity. *J Appl Physiol* 84: 2060–2065, 1998.
41. **Whitson PA, Charles JB, Williams WJ, and Cintron NM.** Changes in sympathoadrenal response to standing in humans after spaceflight. *J Appl Physiol* 79: 428–433, 1995.
42. **Zhang LF.** Vascular adaptation to microgravity: what have we learned? *J Appl Physiol* 91: 2415–2430, 2001.