Heliyon

Received:
6 June 2016
Revised:
21 November 2016
Accepted:
5 December 2016

Heliyon 2 (2016) e00211

# Long-term exposure to space's microgravity alters the time structure of heart rate variability of astronauts

**Kuniaki Otsuka** [a,b,*], **Germaine Cornelissen** [b], **Satoshi Furukawa** [c], **Yutaka Kubo** [d], **Mitsutoshi Hayashi** [d], **Koichi Shibata** [d], **Koh Mizuno** [c,e], **Tatsuya Aiba** [c,f], **Hiroshi Ohshima** [c], **Chiaki Mukai** [c]

[a] *Executive Medical Center, Totsuka Royal Clinic, Tokyo Women's Medical University, Tokyo, Japan*

[b] *Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA*

[c] *Space Biomedical Research Group, Japan Aerospace Exploration Agency, Tokyo, Japan*

[d] *Department of Medicine, Tokyo Women's Medical University, Medical Center East, Tokyo, Japan*

[e] *Faculty of Child and Family Studies, Tohoku Fukushi University, Miyagi, Japan*

[f] *Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan*

* Corresponding author at: Kuniaki Otsuka, Executive Medical Center, Totsuka Royal Clinic, Tokyo Women's Medical University, Related Medical Facility, Sinjuku City, Tokyo, Japan.

E-mail address: frtotk99@ba2.so-net.ne.jp (K. Otsuka).

## Summary

**Background:** Spaceflight alters human cardiovascular dynamics. The less negative slope of the fractal scaling of heart rate variability (HRV) of astronauts exposed long-term to microgravity reflects cardiovascular deconditioning. We here focus on specific frequency regions of HRV.

**Methods:** Ten healthy astronauts (8 men, 49.1 ± 4.2 years) provided five 24-hour electrocardiographic (ECG) records: before launch, 20.8 ± 2.9 (ISS01), 72.5 ± 3.9 (ISS02) and 152.8 ± 16.1 (ISS03) days after launch, and after return to Earth. HRV endpoints, determined from normal-to-normal (NN) intervals in 180-min intervals progressively displaced by 5 min, were compared in space versus Earth. They were fitted with a model including 4 major anticipated components with periods of 24 (circadian), 12 (circasemidian), 8 (circaoctohoran), and 1.5 (Basic Rest-Activity Cycle; BRAC) hours.

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Findings:** The 24-, 12-, and 8-hour components of HRV persisted during long-term spaceflight. The 90-min amplitude became about three times larger in space (ISS03) than on Earth, notably in a subgroup of 7 astronauts who presented with a different HRV profile before flight. The total spectral power (TF; $p < 0.05$) and that in the ultra-low frequency range (ULF, 0.0001–0.003 Hz; $p < 0.01$) increased from $154.9 \pm 105.0$ and $117.9 \pm 57.5$ $\text{msec}^2$ (before flight) to $532.7 \pm 301.3$ and $442.4 \pm 202.9$ $\text{msec}^2$ (ISS03), respectively. The power-law fractal scaling $\beta$ was altered in space, changing from $-1.087 \pm 0.130$ (before flight) to $-0.977 \pm 0.098$ (ISS01), $-0.910 \pm 0.130$ (ISS02), and $-0.924 \pm 0.095$ (ISS03) (invariably $p < 0.05$).

**Interpretation:** Most HRV changes observed in space relate to a frequency window centered around one cycle in about 90 min. Since the BRAC component is amplified in space for only specific HRV endpoints, it is likely to represent a physiologic response rather than an artifact from the International Space Station (ISS) orbit. If so, it may offer a way to help adaptation to microgravity during long-duration spaceflight.

Keywords: Health Sciences, Medicine, Cardiology

## 1. Introduction

In space, microgravity affects the central circulation in humans and induces a number of adaptive changes within the cardiovascular system. Previous investigations showed that the baroreflex sensitivity fluctuates along with altered blood volume distribution [1, 2, 3], which affects neural mechanisms involved in dynamic cardiovascular coordination. Several reports indicate that heart rate is maintained at preflight values [4, 5, 6] and that parasympathetic activity is reduced [4] in space. Cardiac output and stroke volume are reportedly increased in space as a result of an increase in preload to the heart induced by upper body fluid shift from the lower body segments with no major difference in sympathetic nerve activity [6]. However, high sympathetic nervous activity, measured invasively by microneurography in peroneal nerves, has been simultaneously detected in space in three astronauts [7] compared to the ground-based supine posture. Physiologic acclimation to space flight is a complex process involving multiple systems [8]. How the neural cardiovascular coordination adapts to the space environment is still poorly understood in humans.

When faced with a new environment, humans must first acclimate to it in order to survive. This includes the cardiovascular system. Adjustment to the new environment to improve quality of life follows, involving the autonomic, endocrine and immune systems, among others. But, as we reported previously [9], the "intrinsic" cardiovascular regulatory system, reflected by the fractal scaling of HRV [9, 10, 11], did not adapt to the new microgravity environment in space during long-duration (about 6-month) spaceflights. By contrast, after 6 months in space, the circadian rhythm of heart rate had adapted to the new microgravity environment in space [12], an important observation since disruption of circadian rhythms adversely affects human health [13, 14]. As humans plan for long-term space exploration, it is critical to ascertain that the regulatory system can function well in a microgravity environment.

The power-law fractal scaling of heart rate variability (HRV) relates to the autonomic [15], endocrine [15], immune, inflammatory [16, 17], mental, cognitive [18], and behavioral systems, which operate at multiple frequency ranges, from the 1 Hz cardiac cycle to circadian and even secular variations, as part of a broad time structure, the chronome [19]. Herein, we examine how the space environment affects HRV in specific frequency regions, broken down into 8 different frequency ranges. We focus on the basic rest-activity cycle (BRAC), well known since Kleitman [20], who showed regularly occurring alternations between non-REM and REM (Rapid Eye Movement) sleep. The BRAC is involved in the functioning of the central nervous system and manifests time-dependent changes in human performance, including oral activity cycles (e.g., eating, drinking, smoking).

## 2. Methods

### 2.1. Subjects

Ten healthy astronauts (8 men, 2 women) participated in this study. Their mean (± SD) age was 49.1 ± 4.2 years. Their mean stay in space was 171.8 ± 14.4 days. On the average, astronauts had already experienced spaceflight 0.9 ± 0.7 times and had passed class III physical examinations from the National Aeronautics and Space Administration (NASA). This study obtained consent from all subjects and gained approval from the ethics committee jointly established by the Johnson Space Center and Japan Aerospace Exploration Agency (JAXA). A detailed explanation of the study protocol was given to the subjects before they gave written, informed consent, according to the Declaration of Helsinki Principles.

### 2.2. Experimental protocols

Ambulatory around-the-clock 24-hour electrocardiographic (ECG) records were obtained by using a two-channel Holter recorder (FM-180; Fukuda Denshi). Measurements were made five times: once before flight (Control), three times during flight (International Space Station (ISS) 01, ISS02, and ISS03), and once after return to Earth (After flight). The before-flight measurement session (Control) was conducted on days 234.4 ± 138.4 (63 to 469) before launch in all but one astronaut who had technical problems with his before-flight record. In his case, a replacement control record was obtained 3.5 years after return to Earth. The three measurement sessions during flight were taken on days 20.8 ± 2.9 (18 to 28, ISS01), 72.5 ± 3.9 (67 to 78, ISS02) and 152.8 ± 16.1 (139 to 188, ISS03) after

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

launch, the latter corresponding to 19.1 ± 4.1 days (11 to 27) before return (ISS03). The last measurement session was performed on days 77.2 ± 14.4 (37 to 127 days) after return to Earth (After flight).

## 2.3. Analysis of heart rate variability and measurement of 1/f fluctuations in HR dynamics

The measurement procedures and data collection were conducted as previously reported [9, 12]. Briefly, for HRV measurements, QRS waveforms were read from continuous electrocardiographic (ECG) records. The RR intervals between normal QRS waveforms were extracted as the normal-to-normal (NN) intervals. The measured NN intervals were A/D converted (125-Hz) with 8-ms time resolution. After the authors confirmed that all artifacts were actually removed and that the data excluded supraventricular or ventricular arrhythmia, frequency-domain measures [15] were obtained with the MemCalc/CHIRAM (Suwa Trust GMS, Tokyo, Japan) software [21].

Time series of NN intervals covering 5-min intervals were processed consecutively, and the spectral power in different frequency regions was computed, namely in the "high frequency (HF)" (0.15–0.40 Hz; spectral power centered around 3.6 sec), "low frequency (LF)" (0.04–0.15 Hz; spectral power centered around 10.5 sec), and "very low frequency (VLF)" (0.003–0.04 Hz; 25 sec to 5 min) regions of the Maximum Entropy Method (MEM) spectrum. VLF power was further broken down into "VLF band-1" (0.005–0.02 Hz; 50 sec to 3.3 min), "VLF band-2" (0.02–0.03 Hz; 33 to 50 sec) and "VLF band-3" (0.03–0.15 Hz; 6.7 to 33 sec).

Time series of NN intervals were also processed consecutively in 180-min intervals, progressively displaced by 5 min, to estimate the "ultra-low frequency" (ULF) component (0.0001–0.003 Hz; periods of 2.8 hours to 5 min), further broken down into: "ULF band-1" (0.0001–0.0003 Hz; 166.7 to 55.5 min), "ULF band-2" (0.0003–0.001 Hz; 55.5 to 16.6 min), and "ULF band-3" (0.001–0.005 Hz; 16.6 to 3.3 min). Thus, 8 different frequency regions were examined: "HF", "LF", "VLF01", "VLF02", "VLF03", "ULF01", "ULF02", and "ULF03". Results representing each HRV component were averaged over the entire 24-hour.

To evaluate the $1/f^{\beta}$-type scaling in HRV, the $\log_{10}$[power] (ordinate) was plotted against $\log_{10}$[frequency] (abscissa) and a regression line fitted to estimate the slope $\beta$, as reported earlier [9]. Focus was placed on the frequency range of 0.0001–0.01 Hz (periods of 2.8 hours to 1.6 minutes), as previously reported [9].

## 2.4. Fit of 4-component cosine model

A multiple-component model consisting of cosine curves with anticipated periods of 24, 12, 8 and 1.5 hours was fitted to various HRV endpoints by cosinor [22] to

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

assess their time structure and to determine how the latter may have been modified in space. The model includes the usually prominent circadian rhythm (24-hour period) and its first two harmonic terms with periods of 12 (circasemidian) and 8 (circaoctohoran) hours, as well as the BRAC (with a period of about 90-min). Using a (least squares) regression approach, the cosinor does not require the data to be equidistant, and can thus handle missing values in cases when artifacts prevented the computation of HRV endpoints in some of the 5-min or 180-min intervals. Analyses considered primarily the Midline Estimating Statistic Of Rhythm (MESOR, a rhythm-adjusted mean) and the amplitude of each of the 4 components, as a measure of the extent of predictable change within each cycle. The 4-component model was fitted to 24-hour records of NN intervals, total power (TF), and power in the ULF (separately also in the ULF01, ULF02, and ULF03), VLF, LF, and HF regions of the MEM spectrum.

## 2.5. Inter-individual differences in HRV response to microgravity

Consistent differences in various HRV endpoints were noted in the way astronauts responded to microgravity. Examination of the inter-individual differences prompted the classification of the 10 astronauts into 2 clearly distinct groups. Hence, the influence of the space environment was also assessed separately in each group.

## 2.6. Statistical analyses

Since we previously showed that the fractal scaling of HRV did remain altered in space as compared to Earth during long-term (~ 6-month) spaceflights, this study specifically examines the behavior of HRV in 8 different frequency regions of the spectrum (ULF01, ULF02, ULF03, VLF01, VLF02, VLF03, LF, and HF), which can be considered to provide independent information. Adjustment for multiple testing thus uses a P-value of 0.05/8 to indicate statistical significance, using Bonferroni's inequality to adjust for multiple testing. The same correction is applied to other HRV endpoints shown for the sake of completeness, noting the high degree of correlation existing among different indices. We test whether HRV endpoints differ between space and Earth while showing no change among the 3 records obtained in space.

In order to do so, estimates of HRV endpoints averaged over 24 hours were expressed as mean ± SD (standard deviation). To minimize inter-individual differences in HR and HRV among the 10 astronauts that may obscure an effect of the space environment, 24-hour mean values of each variable were expressed as a percentage of mean, computed across the 5 sessions (before flight, ISS01, ISS02, ISS03, and after return to Earth) contributed by each astronaut. In this way, astronauts serve as their own longitudinal control. The two-sided paired-t and one-way analysis of variance (ANOVA) for repeated measures were applied on these relative values for the space vs. Earth difference and for comparing the 3 records in space, respectively.

Estimates of the MESOR and of the relative amplitude of each of the 4 anticipated components (with periods of 24, 12, 8, and 1.5 hours, expressed as a percentage of MESOR) of the selected HRV endpoints were considered as imputations for a comparison of HRV endpoints obtained during ISS03 versus before-flight. The statistical significance of change between the two sessions was determined using the 2-tailed paired t test. Inter-group differences were determined using the two-tailed Student t-test. P-values less than 0.05, adjusted for multiple testing according to Bonferroni's inequality, were considered to indicate statistical significance. The Stat Flex (Ver. 6) software (Artec Co., Ltd., Osaka, Japan) was used.

## 3. Results

### 3.1. Change in time structure of heart rate variability during long-duration spaceflight

Average HRV endpoints during each of the 5 sessions are shown in Table 1A. Results from a comparison of their relative values between space and Earth and across the 3 sessions on the ISS are summarized in Table 1B. On average, among the 10 astronauts, no differences were found in HR (or NN) or in SDNN, the standard deviation of NN intervals. As reported earlier, the fractal scaling of HRV (slope $\beta$) was statistically significantly less steep in space than on Earth, while no changes were observed across the 3 records obtained in space, Tables 1A and 1B. This result may be accounted for by the large space-Earth difference observed in the ULF frequency region of the spectrum, which is statistically significant for ULF02 and ULF03, as well as for ULF01 once it is normalized by the total spectral power (TF). These HRV endpoints did not differ among the 3 sessions recorded on the ISS, Tables 1A and 1B. Of all the HRV endpoints considered herein, apart from $\beta$ and the spectral power in the 3 ULF bands, only SDmean5 and SDmean30 show a lasting difference in space as compared to Earth, Tables 1A and 1B.

Differences in $\beta$ and the spectral power in the 3 ULF bands may stem from changes occurring around a frequency of one cycle in about 90 min. Indeed, $\beta$ is computed over a frequency range centered around one cycle in about 90 min (1.7–166 min). Its absolute value decreased from $1.087 \pm 0.130$ (control, before flight) to $0.924 \pm 0.095$ (ISS03) ($p < 0.01$). Correspondingly, ULF01/TF, also centered around 90 min, increased from $0.207 \pm 0.053$ to $0.310 \pm 0.090$, whereas ULF02/TF and ULF03/TF decreased from $0.189 \pm 0.037$ to $0.136 \pm 0.030$ and from $0.219 \pm 0.035$ to $0.151 \pm 0.034$, respectively.

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

## Table 1A. Change in characteristics of heart rate variability associated with 6-month mission in space: Numerical results.*

| Variable | | Units | Target period (range) | n | Control (Before flight) | | ISS01 | | ISS02 | | ISS03 | | After flight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Time- domain measures | HR | (beats/min) | 24 hours | 10 | 69.9 | 10.9 | 66.7 | 8.5 | 66.9 | 7.0 | 66.6 | 7.4 | 69.2 | 8.9 |
| | NN-interval | (msec) | 24 hours | 10 | 878.2 | 146.7 | 914.1 | 126.4 | 906.4 | 97.6 | 911.9 | 104.3 | 880.5 | 120.9 |
| | SDNN | (msec) | 24 hours | 10 | 132.5 | 45.2 | 148.4 | 29.5 | 140.1 | 52.6 | 151.0 | 43.2 | 144.7 | 43.5 |
| | SDANN (5 min) | (msec) | 24 hours | 10 | 115.8 | 43.6 | 129.0 | 27.0 | 121.4 | 46.0 | 130.0 | 39.3 | 125.1 | 43.2 |
| | SDANN (30 min) | (msec) | 24 hours | 10 | 109.3 | 44.2 | 125.2 | 27.1 | 117.9 | 44.7 | 129.1 | 38.5 | 116.8 | 44.6 |
| | TINN | (msec) | 24 hours | 10 | 571.5 | 178.9 | 638.0 | 144.9 | 523.5 | 186.7 | 552.7 | 128.7 | 612.3 | 146.9 |
| | HRVI | (–) | 24 hours | 10 | 35.7 | 11.2 | 39.9 | 9.1 | 32.7 | 11.7 | 34.5 | 8.0 | 38.3 | 9.2 |
| | Triangular Index (TI) | (–) | 24 hours | 10 | 34.2 | 10.4 | 38.3 | 9.2 | 30.8 | 10.8 | 31.8 | 7.2 | 36.8 | 9.0 |
| | Lorenz Plot Length | (msec) | 24 hours | 10 | 627.9 | 228.3 | 690.7 | 160.7 | 659.0 | 284.5 | 745.0 | 252.2 | 707.1 | 234.9 |
| | Lorenz Plot Width | (msec) | 24 hours | 10 | 54.9 | 16.5 | 50.9 | 15.6 | 51.5 | 13.7 | 61.7 | 15.9 | 58.9 | 15.8 |
| | Length/Width ratio | (–) | 24 hours | 10 | 11.5 | 2.5 | 14.5 | 4.2 | 13.0 | 4.9 | 12.5 | 4.2 | 12.5 | 4.8 |
| | SDNN index (30 min) | (msec) | 30 min | 10 | 72.3 | 19.1 | 66.6 | 16.7 | 63.9 | 15.1 | 68.0 | 17.6 | 76.6 | 17.4 |
| | SDNN index (5 min) | (msec) | 5 min | 10 | 56.5 | 14.8 | 53.1 | 13.2 | 50.9 | 11.3 | 55.1 | 13.4 | 58.8 | 13.4 |
| | CVNN | (%) | 5 min | 10 | 16.3 | 5.1 | 17.5 | 4.9 | 16.0 | 4.6 | 17.3 | 3.6 | 17.7 | 5.9 |
| | r-MSSD | (msec) | 5 min | 10 | 23.9 | 5.9 | 23.1 | 5.9 | 22.6 | 5.2 | 26.4 | 5.8 | 24.7 | 6.6 |
| | NN50 | (number) | 5 min | 10 | 4048.2 | 2841.3 | 3603.4 | 2514.5 | 3142.4 | 2605.1 | 4442.1 | 2610.0 | 4226.3 | 2616.1 |
| | pNN50 | (%) | 5 min | 10 | 4.360 | 3.536 | 4.050 | 3.143 | 3.430 | 2.819 | 5.820 | 3.594 | 5.090 | 4.260 |
| Frequency- domain measures | \|β\| | (log(msec$^2$)/log(Hz)) | 90 min (1.7–166 min) | 10 | 1.087 | 0.130 | 0.977 | 0.098 | 0.910 | 0.130 | 0.924 | 0.095 | 1.135 | 0.147 |
| | TF-component | msec$^2$ | 90 min (2 sec–166 min) | 10 | 6417.1 | 3238.0 | 5932.1 | 2453.4 | 5297.5 | 2806.2 | 6530.9 | 3562.3 | 6897.6 | 2823.3 |
| | ULF-component | msec$^2$ | 90 min (5–166 min) | 10 | 3479.8 | 1636.4 | 3255.5 | 1295.1 | 2857.4 | 1982.5 | 3624.3 | 2362.4 | 3815.4 | 1605.2 |
| | ULF01 | msec$^2$ | 90 min (55–166 min) | 10 | 1361.2 | 775.7 | 1788.0 | 747.4 | 1450.9 | 1146.7 | 2080.8 | 1399.7 | 1389.3 | 640.7 |
| | ULF02 | msec$^2$ | 36 min (17–55 min) | 10 | 1190.3 | 561.6 | 885.2 | 433.2 | 849.6 | 561.5 | 878.2 | 520.4 | 1378.1 | 548.7 |
| | ULF03 | msec$^2$ | 10 min (3–17 min) | 10 | 1360.3 | 596.7 | 920.2 | 522.1 | 868.0 | 488.2 | 1034.7 | 764.3 | 1533.5 | 860.7 |

*(Continued)*

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 1A.** (*Continued*)

| Variable | | Units | Target period (range) | n | Control (Before flight) | | ISS01 | | ISS02 | | ISS03 | | After flight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| | VLF-component | msec$^2$ | 5 min (25 sec–5 min) | 10 | 2113.7 | 1361.6 | 1928.7 | 1034.7 | 1741.5 | 827.4 | 2105.8 | 1211.2 | 2210.5 | 1127.5 |
| | VLF01 | msec$^2$ | 2 min (50 sec-3.3 min) | 10 | 1177.2 | 834.2 | 1114.4 | 605.5 | 1002.5 | 464.8 | 1245.3 | 758.5 | 1209.6 | 666.5 |
| | VLF02 | msec$^2$ | 42 sec (33–50 sec) | 10 | 291.9 | 185.9 | 275.2 | 151.7 | 250.3 | 132.3 | 287.6 | 147.5 | 297.8 | 134.0 |
| | VLF03 | msec$^2$ | 20 sec (6.7–33 sec) | 10 | 911.3 | 425.2 | 836.9 | 416.2 | 773.5 | 367.4 | 864.6 | 389.8 | 960.4 | 391.9 |
| | LF-component | msec$^2$ | 15 sec (6–25 sec) | 10 | 698.8 | 316.1 | 635.8 | 329.3 | 595.8 | 296.1 | 661.1 | 306.0 | 742.9 | 310.6 |
| | HF-component | msec$^2$ | 4.3 sec (2.5–6 sec) | 10 | 116.5 | 55.1 | 104.6 | 60.2 | 94.9 | 45.9 | 127.6 | 63.7 | 120.1 | 52.2 |
| | LF/HF ratio | (–) | | 10 | 6.428 | 2.711 | 6.398 | 1.760 | 6.305 | 0.744 | 5.606 | 1.761 | 6.506 | 2.129 |
| | ULF/TF | (–) | 90 min (5–166 min) | 10 | 0.549 | 0.079 | 0.556 | 0.071 | 0.511 | 0.123 | 0.542 | 0.091 | 0.557 | 0.092 |
| | ULF01/TF | (–) | 90 min (55–166 min) | 10 | 0.207 | 0.053 | 0.314 | 0.078 | 0.251 | 0.095 | 0.310 | 0.090 | 0.202 | 0.070 |
| | ULF02/TF | (–) | 36 min (17–55 min) | 10 | 0.189 | 0.037 | 0.145 | 0.025 | 0.154 | 0.051 | 0.136 | 0.030 | 0.204 | 0.045 |
| | ULF03/TF | (–) | 10 min (3–17 min) | 10 | 0.219 | 0.035 | 0.151 | 0.034 | 0.164 | 0.024 | 0.151 | 0.034 | 0.219 | 0.047 |
| | VLF-/TF | (–) | 5 min (25 sec–5 min) | 10 | 0.316 | 0.057 | 0.319 | 0.064 | 0.347 | 0.088 | 0.323 | 0.055 | 0.312 | 0.065 |
| | VLF01/TF | (–) | 2 min (50 sec-3.3 min) | 10 | 0.173 | 0.041 | 0.186 | 0.043 | 0.200 | 0.050 | 0.189 | 0.039 | 0.169 | 0.042 |
| | VLF02/TF | (–) | 42 sec (33–50 sec) | 10 | 0.044 | 0.010 | 0.045 | 0.013 | 0.051 | 0.021 | 0.045 | 0.011 | 0.043 | 0.013 |
| | VLF03/TF | (–) | 20 sec (6.7–33 sec) | 10 | 0.147 | 0.045 | 0.139 | 0.038 | 0.159 | 0.055 | 0.143 | 0.055 | 0.143 | 0.047 |
| | LF-/TF | (–) | 15 sec (6–25 sec) | 10 | 0.114 | 0.039 | 0.106 | 0.034 | 0.122 | 0.044 | 0.110 | 0.047 | 0.111 | 0.040 |
| | HF-/TF | (–) | 4.3 sec (2.5–6 sec) | 10 | 0.019 | 0.009 | 0.018 | 0.007 | 0.020 | 0.008 | 0.023 | 0.014 | 0.018 | 0.008 |

---

[*] For definition of HRV endpoints, see [15].

**Table 1B.** Comparison of relative HRV endpoints in Space and on Earth.[*]

| | *Means (10 astronauts)* | | | | | | | Space vs. Earth | | ISS01-03 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **F** | **P** |
| | Primary endpoints | | | | | | | | | | |
| ULF01 | 83.36 | 121.19 | 81.97 | 124.52 | 88.95 | 86.16 | 109.23 | 1.933 | NS | 3.106 | NS |
| ULF02 | 115.85 | 85.24 | 76.54 | 85.12 | 137.25 | 126.55 | 82.30 | 6.265 | 0.001 | 0.431 | NS |
| ULF03 | 123.56 | 80.66 | 75.23 | 84.86 | 135.70 | 129.63 | 80.25 | 7.344 | < 0.001 | 0.924 | NS |
| VLF01 | 97.16 | 99.97 | 91.02 | 107.01 | 104.83 | 101.00 | 99.34 | 0.250 | NS | 2.135 | NS |
| VLF02 | 100.69 | 97.34 | 90.57 | 103.60 | 107.80 | 104.24 | 97.17 | 1.354 | NS | 2.141 | NS |
| VLF03 | 104.67 | 94.54 | 90.20 | 99.38 | 111.21 | 107.94 | 94.71 | 2.345 | NS | 1.327 | NS |
| LF | 105.48 | 93.15 | 90.01 | 98.99 | 112.37 | 108.92 | 94.05 | 2.160 | NS | 1.153 | NS |
| HF | 103.99 | 90.11 | 85.09 | 112.54 | 108.27 | 106.13 | 95.91 | 1.121 | NS | 4.582 | NS |
| | Secondary endpoints | | | | | | | | | | |
| TF | 102.32 | 97.44 | 83.44 | 103.19 | 113.61 | 107.96 | 94.69 | 2.482 | NS | 3.778 | NS |
| ULF | 102.95 | 99.96 | 78.42 | 103.52 | 115.15 | 109.05 | 93.97 | 1.910 | NS | 2.621 | NS |
| VLF | 100.96 | 96.99 | 88.87 | 103.20 | 109.98 | 105.47 | 96.36 | 1.630 | NS | 2.321 | NS |
| ULF/TF | 101.16 | 103.07 | 93.37 | 99.96 | 102.44 | 101.80 | 98.80 | 0.906 | NS | 1.214 | NS |
| ULF01/TF | 81.13 | 124.29 | 96.43 | 119.89 | 78.25 | 79.69 | 113.54 | 4.376 | 0.014 | 2.416 | NS |
| ULF02/TF | 114.49 | 88.38 | 91.39 | 82.87 | 122.87 | 118.68 | 87.55 | 6.199 | 0.001 | 0.562 | NS |
| ULF03/TF | 121.54 | 83.79 | 91.27 | 83.26 | 120.15 | 120.84 | 86.11 | 6.945 | 0.001 | 1.100 | NS |
| VLF/TF | 97.60 | 99.31 | 107.00 | 100.06 | 96.03 | 96.81 | 102.12 | 1.145 | NS | 0.555 | NS |
| VLF01/TF | 93.69 | 101.89 | 109.48 | 103.35 | 91.59 | 92.64 | 104.91 | 2.137 | NS | 0.397 | NS |
| VLF02/TF | 96.91 | 100.07 | 108.81 | 100.47 | 93.74 | 95.32 | 103.12 | 1.530 | NS | 0.621 | NS |
| VLF03/TF | 101.92 | 96.84 | 107.65 | 97.09 | 96.50 | 99.21 | 100.53 | 0.175 | NS | 1.169 | NS |
| LF/TF | 102.99 | 95.35 | 107.26 | 96.97 | 97.42 | 100.21 | 99.86 | 0.038 | NS | 1.189 | NS |
| HF/TF | 101.09 | 92.76 | 102.21 | 109.37 | 94.58 | 97.83 | 101.45 | 0.436 | NS | 1.312 | NS |
| LF/HF | 100.55 | 102.16 | 103.28 | 89.54 | 104.47 | 102.51 | 98.33 | 0.484 | NS | 1.974 | NS |
| HR | 102.68 | 98.28 | 98.82 | 98.22 | 102.00 | 102.34 | 98.44 | 1.793 | NS | 0.043 | NS |
| NN | 97.40 | 101.77 | 101.19 | 101.64 | 98.00 | 97.70 | 101.53 | 1.788 | NS | 0.035 | NS |
| CVRR | 94.98 | 103.46 | 94.89 | 102.43 | 104.23 | 99.61 | 100.26 | 0.119 | NS | 0.613 | NS |
| SDNN | 91.44 | 106.14 | 96.17 | 105.38 | 100.86 | 96.15 | 102.57 | 1.139 | NS | 1.053 | NS |
| r-MSSD | 98.74 | 95.33 | 93.78 | 110.11 | 102.04 | 100.39 | 99.74 | 0.161 | NS | 4.545 | NS |
| NN | 105.87 | 100.09 | 97.18 | 96.38 | 100.48 | 103.17 | 97.88 | 1.544 | NS | 0.698 | NS |
| NN50 | 104.53 | 92.78 | 79.83 | 113.64 | 110.59 | 107.56 | 94.28 | 1.093 | NS | 1.980 | NS |
| NN50+ | 96.13 | 92.98 | 78.64 | 126.63 | 105.63 | 100.88 | 99.42 | 0.113 | NS | 2.541 | NS |
| NN50- | 95.24 | 82.15 | 75.63 | 137.51 | 109.47 | 102.36 | 98.43 | 0.257 | NS | 4.117 | NS |
| pNN50 | 90.37 | 87.90 | 77.29 | 135.50 | 108.94 | 99.65 | 100.23 | 0.035 | NS | 3.388 | NS |
| pNN50+ | 87.99 | 93.91 | 79.83 | 130.65 | 107.61 | 97.80 | 101.46 | 0.233 | NS | 2.578 | NS |
| pNN50– | 90.21 | 86.15 | 73.10 | 140.81 | 109.74 | 99.97 | 100.02 | 0.002 | NS | 4.296 | NS |

(*Continued*)

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 1B**. (*Continued*)

| | *Means (10 astronauts)* | | | | | | | Space vs. Earth | | ISS01-03 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **F** | **P** |
| SDANN5 | 92.10 | 107.08 | 95.96 | 104.85 | 100.01 | 96.05 | 102.63 | 1.046 | NS | 1.187 | NS |
| SDANN30 | 90.01 | 107.93 | 96.77 | 108.65 | 96.63 | 93.32 | 104.45 | 1.537 | NS | 1.339 | NS |
| SDmean5 | 102.51 | 96.81 | 93.19 | 100.27 | 107.22 | 104.87 | 96.76 | 4.004 | 0.025 | 3.693 | NS |
| SDmean30 | 103.63 | 95.80 | 92.12 | 97.68 | 110.77 | 107.20 | 95.20 | 6.551 | 0.001 | 1.954 | NS |
| N | 94.98 | 99.95 | 106.92 | 107.43 | 90.72 | 92.85 | 104.77 | 2.359 | NS | 1.181 | NS |
| X | 98.29 | 100.10 | 100.54 | 98.89 | 102.18 | 100.24 | 99.84 | 0.184 | NS | 0.237 | NS |
| M | 96.69 | 105.11 | 98.51 | 101.23 | 98.46 | 97.58 | 101.61 | 2.598 | NS | 3.447 | NS |
| TINN | 97.65 | 111.12 | 88.84 | 95.94 | 106.45 | 102.05 | 98.63 | 0.898 | NS | 6.227 | 0.048 |
| HRVI | 97.64 | 111.14 | 88.83 | 95.95 | 106.44 | 102.04 | 98.64 | 0.891 | NS | 6.241 | 0.047 |
| TI | 98.60 | 112.06 | 88.28 | 93.14 | 107.92 | 103.26 | 97.83 | 1.374 | NS | 7.098 | 0.027 |
| Length | 90.65 | 103.84 | 94.15 | 108.23 | 103.14 | 96.89 | 102.07 | 0.823 | NS | 1.231 | NS |
| Width | 97.90 | 91.20 | 92.98 | 111.42 | 106.50 | 102.20 | 98.53 | 0.766 | NS | 4.756 | NS |
| Len/Wid | 91.94 | 113.80 | 101.01 | 96.52 | 96.73 | 94.34 | 103.78 | 1.606 | NS | 1.371 | NS |
| Trend (β) | 108.03 | 97.23 | 90.33 | 91.80 | 112.61 | 110.32 | 93.12 | 4.298 | 0.016 | 1.958 | NS |

P-values adjusted for multiple testing, using Bonferroni's inequality, considering that 8 different tests were conducted (in 8 independent frequency regions).

Secondary endpoints also used the same correcting factor, considering the large correlation among different endpoints, shown here for sake of completeness only (rather than for testing per se).

For definition of HRV endpoints, see [15].

[*] 24-hour mean HRV endpoints expressed as a percentage of 5-session average for each astronaut, then averaged during each session across the 10 astronauts.

## 3.2. Individual HRV response to microgravity associated with change in parasympathetic nerve activity

Individual 24-hour records of NN intervals (and hence instantaneous HR values) showed striking differences among the 10 astronauts. In 7 of them (Group 1), the 24-hour standard deviation (SD) of NN intervals was much lower (74.7–105.4 msec) than in the other 3 (Group 2) (171.7–196.0 msec) (Student $t = 10.462$, $p < 0.001$). The two groups also differed in their average NN intervals (820. 8 ± 44.6 vs. 1023.2 ± 54.2, Student $t = 2.610$, $p = 0.031$). The inter-group difference in SD (NN) persisted during ISS01 ($t = 3.451$, $p = 0.009$), ISS02 ($t = 4.615$, $p = 0.002$), and ISS03 ($t = 3.430$, $p = 0.009$), as well as after return to Earth ($t = 3.287$, $p = 0.011$), when a difference in average NN intervals was also observed ($t = 2.610$, $p = 0.031$). Moreover, astronauts in Group 1 tended to respond to the space environment by increasing their average NN interval (decreasing their HR). The inter-group difference in response was statistically significant during ISS02

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

(t = 2.814, p = 0.023) and ISS03 (t = 3.515, p = 0.008), when the average NN intervals of all 7 astronauts of Group 1 was increased (on average by 85.4 ± 59.0 msec, t = 3.825, p = 0.009) and that of all 3 astronauts of Group 2 was decreased (on average by 41.9 ± 23.6 msec, t = 3.072, p = 0.092). Table 2 lists individual results during each of the 5 recordings, illustrating strong inter-individual differences in the HRV response to the space environment.

### 3.3. Power-law scaling β and ULF component of astronauts whose heart rate decreased in space

As seen for all 10 astronauts, the absolute value of β was also statistically significantly decreased in space (ISS03: 0.944 ± 0.097) as compared to preflight (1.144 ± 0.102) for the 7 astronauts of Group 1. Their ULF02 and ULF03 power was statistically significantly decreased from 915.0 ± 320.4 $msec^2$ to 673.6 ± 275.3 $msec^2$ and from 1017.4 ± 268.1 $msec^2$ to 647.6 ± 192.5 $msec^2$, respectively. In Group 2, there were no statistically significant differences in any of the HRV endpoints.

### 3.4. Change in chronome components (notably the basic rest-activity cycle) of heart rate variability during long-duration exposure to microgravity in space

Changes during the 6-month spaceflight in the relative amplitudes of the 24-, 12-, 8-, and 1.5-hour components, expressed as a percentage of the MESOR, are shown in Table 3 for NN intervals, β, TF, and the different frequency ranges of the spectrum. On the average, the 90-min amplitude of TF, ULF and ULF01 increased 2- to 3-fold in space in astronauts of Group 1, whereas it decreased in those of Group 2, Table 3. During ISS03 as compared to preflight, the BRAC amplitude of TF increased from 154.9 ± 105.0 to 532.7 ± 301.3 $msec^2$, or from 3.2 to 11.3% of MESOR (n = 7), that of ULF increased from 117.9 ± 57.5 to 442.4 ± 202.9 $msec^2$, or from 4.1 to 15.8% of MESOR (n = 7) and that of ULF01 increased from 124.3 ± 82.8 to 427.6 ± 214.8 $msec^2$, or from 8.9 to 31.2% of MESOR (n = 7). In astronauts of Group 2, the 90-min amplitude of ULF01 decreased from 801.6 ± 155.6 before flight to 452.0 ± 239.9 during ISS02, or from 30.8 to less than 20% of the MESOR in space (n = 3), Table 3.

Two examples of the fitted model to the TF data are shown in Fig. 1, comparing the record during ISS03 (right) with the preflight record (left). In one case (Fig. 1A), the 90-min amplitude increased from 59.5 to 684.5 $msec^2$, with practically no change in the circadian amplitude. In another case (Fig. 1B), the 90-min amplitude also increased from 71.4 to 754.5 $msec^2$, but it was accompanied by an increase in the 24-hour amplitude from 529.8 to 3196.4 $msec^2$.

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 2.** Individual HRV responses of astronauts.*

| **Subjects** | | **Variables** | **units** | **Control (Before flight)** | | **ISS01** | | **ISS02** | | **ISS03** | | **After flight** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| Group 1 | Case 1 | Heart Rate | (b/min) | 74.8 | 10.6 | 81.5 | 16.1 | 75.5 | 7.3 | 71.6 | 12.2 | 73.5 | 16.9 |
| | | r-MSSD | (msec) | 23.1 | 8.2 | 22.6 | 5.7 | 23.3 | 5.3 | 27.0 | 7.1 | 28.9 | 9.3 |
| | | pNN50 | (%) | 3.9 | 5.9 | 3.2 | 2.8 | 3.4 | 2.5 | 5.4 | 3.8 | 8.0 | 6.2 |
| | | HF-component | $msec^2$ | 127.5 | 112.9 | 92.1 | 54.3 | 100.4 | 49.7 | 127.5 | 61.1 | 193.1 | 103.4 |
| | | LF/HF ratio | (–) | 6.0 | 3.4 | 6.6 | 3.0 | 7.1 | 2.7 | 7.3 | 3.7 | 4.9 | 2.5 |
| | Case 2 | Heart Rate | (b/min) | 78.8 | 13.5 | 67.0 | 9.2 | 77.7 | 9.8 | 74.8 | 7.1 | 78.1 | 10.0 |
| | | r-MSSD | (msec) | 23.3 | 7.6 | 29.5 | 6.7 | 23.6 | 6.3 | 26.1 | 6.6 | 23.8 | 7.4 |
| | | pNN50 | (%) | 4.5 | 4.8 | 8.4 | 5.2 | 4.4 | 3.8 | 6.0 | 5.9 | 4.8 | 4.7 |
| | | HF-component | $msec^2$ | 169.1 | 114.7 | 235.0 | 106.9 | 162.5 | 101.9 | 239.4 | 170.0 | 165.1 | 123.1 |
| | | LF/HF ratio | (–) | 6.0 | 4.1 | 6.0 | 3.0 | 6.5 | 3.5 | 5.4 | 3.1 | 6.3 | 4.4 |
| | Case 3 | Heart Rate | (b/min) | 89.9 | 11.9 | 72.2 | 14.4 | 70.9 | 8.0 | 78.5 | 12.5 | 82.3 | 7.5 |
| | | r-MSSD | (msec) | 22.9 | 3.9 | 20.3 | 6.6 | 18.2 | 4.5 | 32.9 | 13.7 | 19.2 | 4.7 |
| | | pNN50 | (%) | 3.0 | 2.5 | 2.4 | 3.7 | 1.4 | 2.2 | 13.1 | 13.8 | 1.5 | 2.0 |
| | | HF-component | $msec^2$ | 105.3 | 48.0 | 86.0 | 49.3 | 71.3 | 34.3 | 180.1 | 153.0 | 98.0 | 64.0 |
| | | LF/HF ratio | (–) | 5.8 | 2.8 | 5.8 | 3.6 | 6.2 | 3.8 | 3.3 | 2.6 | 6.2 | 3.2 |
| | Case 4 | Heart Rate | (b/min) | 77.3 | 10.0 | 70.3 | 15.0 | 64.9 | 6.2 | 66.4 | 14.6 | 66.9 | 6.1 |
| | | r-MSSD | (msec) | 16.8 | 4.9 | 17.8 | 3.9 | 19.7 | 3.7 | 20.2 | 4.4 | 23.0 | 6.2 |
| | | pNN50 | (%) | 1.3 | 1.9 | 1.2 | 1.4 | 1.5 | 1.6 | 1.9 | 1.8 | 3.8 | 3.8 |
| | | HF-component | $msec^2$ | 55.7 | 33.2 | 56.3 | 27.7 | 74.0 | 30.1 | 69.2 | 29.3 | 102.7 | 66.9 |
| | | LF/HF ratio | (–) | 13.4 | 7.2 | 10.7 | 6.1 | 7.4 | 3.2 | 9.2 | 5.5 | 8.1 | 4.6 |
| | Case 5 | Heart Rate | (b/min) | 61.6 | 5.1 | 59.9 | 6.7 | 56.5 | 9.0 | 57.2 | 6.7 | 62.1 | 10.6 |
| | | r-MSSD | (msec) | 15.9 | 2.9 | 11.9 | 2.2 | 15.1 | 3.7 | 15.2 | 3.6 | 13.1 | 3.8 |
| | | pNN50 | (%) | 0.5 | 0.6 | 0.2 | 0.4 | 0.7 | 1.2 | 0.6 | 1.5 | 0.3 | 0.7 |
| | | HF-component | $msec^2$ | 37.9 | 15.7 | 22.0 | 10.3 | 31.5 | 15.3 | 31.8 | 17.1 | 26.2 | 19.2 |
| | | LF/HF ratio | (–) | 4.6 | 2.7 | 7.5 | 4.6 | 7.5 | 4.6 | 6.6 | 4.7 | 7.4 | 4.8 |
| | Case 6 | Heart Rate | (b/min) | 69.0 | 7.4 | 62.4 | 6.7 | 67.7 | 12.9 | 63.4 | 12.4 | 71.7 | 12.0 |
| | | r-MSSD | (msec) | 19.0 | 4.4 | 22.3 | 7.1 | 20.4 | 5.7 | 31.1 | 8.5 | 23.4 | 5.6 |
| | | pNN50 | (%) | 1.8 | 1.8 | 3.5 | 4.1 | 2.3 | 2.6 | 10.2 | 6.8 | 4.0 | 3.6 |
| | | HF | $msec^2$ | 69.1 | 38.6 | 90.6 | 54.7 | 84.7 | 62.6 | 144.5 | 88.9 | 110.2 | 55.0 |
| | | LF/HF ratio | (–) | 10.8 | 6.4 | 8.7 | 5.4 | 8.9 | 5.2 | 5.7 | 3.3 | 12.2 | 7.3 |
| | Case 7 | Heart Rate | (b/min) | 64.6 | 5.4 | 66.2 | 10.4 | 64.5 | 5.9 | 62.9 | 5.6 | 66.6 | 6.4 |
| | | r-MSSD | (msec) | 21.3 | 3.8 | 25.2 | 7.1 | 18.1 | 5.5 | 22.6 | 4.6 | 18.8 | 3.5 |
| | | pNN50 | (%) | 2.2 | 1.8 | 5.7 | 5.4 | 1.5 | 2.1 | 3.2 | 3.1 | 1.4 | 1.3 |
| | | HF-component | $msec^2$ | 79.1 | 32.9 | 85.6 | 42.3 | 50.4 | 25.2 | 78.2 | 30.6 | 62.6 | 25.5 |
| | | LF/HF ratio | (–) | 5.9 | 3.8 | 4.7 | 3.0 | 6.1 | 3.7 | 4.8 | 2.7 | 7.5 | 4.7 |
| Group 2 | Case 8 | Heart Rate | (b/min) | 65.8 | 13.5 | 65.5 | 11.2 | 69.4 | 15.8 | 67.7 | 14.4 | 64.5 | 7.2 |
| | | r-MSSD | (msec) | 28.8 | 9.7 | 21.0 | 4.8 | 28.1 | 4.7 | 25.2 | 6.3 | 36.8 | 6.0 |

(*Continued*)

Table 2. (*Continued*)

| Subjects | Variables | units | Control (Before flight) | | ISS01 | | ISS02 | | ISS03 | | After flight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| | pNN50 | (%) | 8.4 | 9.1 | 2.4 | 2.4 | 6.5 | 3.7 | 4.8 | 4.8 | 15.3 | 6.2 |
| | HF-component | msec$^2$ | 160.9 | 129.4 | 73.4 | 39.9 | 91.7 | 39.5 | 90.7 | 48.2 | 163.1 | 65.9 |
| | LF/HF ratio | (–) | 8.6 | 6.9 | 10.3 | 7.3 | 6.7 | 3.3 | 8.4 | 4.9 | 5.9 | 2.7 |
| Case 9 | Heart Rate | (b/min) | 67.8 | 16.5 | 71.8 | 19.0 | 65.2 | 10.5 | 66.4 | 13.0 | 76.6 | 23.0 |
| | r-MSSD | (msec) | 33.6 | 12.5 | 27.6 | 8.4 | 32.9 | 8.0 | 32.7 | 10.6 | 27.7 | 10.3 |
| | pNN50 | (%) | 12.2 | 11.3 | 7.0 | 6.4 | 11.1 | 8.0 | 10.9 | 9.7 | 7.3 | 8.8 |
| | HF-component | msec$^2$ | 200.7 | 137.5 | 147.7 | 80.0 | 179.8 | 92.5 | 201.3 | 135.5 | 123.1 | 90.0 |
| | LF/HF ratio | (–) | 7.8 | 4.2 | 7.5 | 3.7 | 7.2 | 3.1 | 6.3 | 2.7 | 8.9 | 4.5 |
| Case 10 | Heart Rate | (b/min) | 51.6 | 8.5 | 49.8 | 7.0 | 57.1 | 17.9 | 54.4 | 13.5 | 53.1 | 7.4 |
| | r-MSSD | (msec) | 35.3 | 8.8 | 32.9 | 6.5 | 26.5 | 6.3 | 31.6 | 7.7 | 31.7 | 6.6 |
| | pNN50 | (%) | 13.2 | 8.8 | 10.9 | 6.4 | 5.0 | 4.3 | 9.7 | 6.7 | 9.7 | 6.2 |
| | HF-component | msec$^2$ | 161.7 | 78.7 | 157.7 | 55.6 | 102.4 | 42.6 | 119.9 | 45.2 | 139.8 | 60.2 |
| | LF/HF ratio | (–) | 6.7 | 4.7 | 5.7 | 3.6 | 6.0 | 3.6 | 6.3 | 3.6 | 6.9 | 4.7 |

r-MSSD: square root of mean squared differences of successive NN intervals; pNN50: fraction of consecutive NN intervals that differ by more than 50 ms; HF-component: spectral power centered around 3.6 sec; LF/HF ratio: ratio of low-frequency (LF, centered around 10.5 sec) and high-frequency (HF) spectral power; all indices obtained from 5-min segments, averaged over the entire 24-hour span.

[*] Astronauts were grouped in terms of their NN records (see text). Each record contains 254 to 286 values, except for case 8 after return to Earth (N = 70 or 71).

### 3.5. Implications of heart rate response to space environment for adaptation to microgravity

To better understand the meaning of a difference in HRV response to the space environment, we compared the characteristics of the 4-component model fitted to some HRV endpoints before flight and during ISS03 between Groups 1 and 2. Before flight, the MESOR of TF, ULF and VLF spectral power was statistically significantly lower, on average, in astronauts of Group 1 as compared to those of Group 2, Table 4. These differences became smaller during ISS03, to the point of no longer reaching statistical significance, except for TF and VLF spectral power, Table 4. In other words, the two groups differed less in space (ISS03) than before flight.

Before flight, the BRAC amplitude was found to be much smaller in Group 1 as compared to Group 2, the difference being statistically significant for all considered HRV endpoints, except for LF, Table 4 (left). During ISS03, the 90-min amplitude increased in Group 1 and mostly decreased in Group 2 (except for LF), so that differences between the two groups were no longer statistically significant after spending several months in space, Table 4 (right). Similar results

**Table 3.** Change in relative amplitude of 24-, 12-, 8-, and 1.5-hour components of some HRV endpoints during 6-month mission in space.[*]

| | Group 1 (N = 7) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Space vs. Earth | | ISS03 vs. Before | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| NN | | | | | | | | | | | |
| 24h-A | 8.07 | 12.52 | 9.80 | 12.51 | 9.85 | 8.96 | 11.61 | 1.940 | NS | 2.332 | NS |
| 12h-A | 5.14 | 5.95 | 6.26 | 6.70 | 6.70 | 5.92 | 6.30 | 0.399 | NS | 1.749 | NS |
| 8h-A | 3.74 | 4.42 | 3.03 | 4.31 | 3.66 | 3.70 | 3.92 | 0.219 | NS | 0.831 | NS |
| 1.5h-A | 0.94 | 1.16 | 0.99 | 1.61 | 1.42 | 1.18 | 1.25 | 0.294 | NS | 1.977 | NS |
| β | | | | | | | | | | | |
| 24h-A | 18.35 | 20.67 | 20.86 | 20.48 | 19.60 | 18.98 | 20.67 | 0.275 | NS | 0.250 | NS |
| 12h-A | 13.31 | 19.97 | 20.67 | 19.06 | 12.45 | 12.88 | 19.90 | 1.195 | NS | 0.843 | NS |
| 8h-A | 13.67 | 13.35 | 12.31 | 12.07 | 12.25 | 12.96 | 12.58 | 0.380 | NS | 0.581 | NS |
| 1.5h-A | 2.07 | 1.68 | 1.76 | 1.99 | 1.33 | 1.70 | 1.81 | 0.326 | NS | 0.222 | NS |
| TF | | | | | | | | | | | |
| 24h-A | 24.59 | 56.39 | 53.79 | 62.60 | 33.99 | 29.29 | 57.59 | 3.978 | **0.044** | 2.326 | NS |
| 12h-A | 23.94 | 43.43 | 44.11 | 49.68 | 28.03 | 25.98 | 45.74 | 2.531 | NS | 1.916 | NS |
| 8h-A | 18.99 | 38.89 | 39.86 | 41.00 | 19.76 | 19.38 | 39.92 | 5.149 | **0.013** | 2.877 | 0.169 |
| 1.5h-A | 3.17 | 8.14 | 7.76 | 11.29 | 4.93 | 4.05 | 9.06 | 3.367 | ***0.091*** | 3.240 | 0.106 |
| ULF | | | | | | | | | | | |
| 24h-A | 37.33 | 97.94 | 72.79 | 104.14 | 49.16 | 43.25 | 91.62 | 3.686 | ***0.062*** | 2.382 | NS |
| 12h-A | 33.64 | 84.49 | 66.30 | 88.82 | 37.76 | 35.70 | 79.87 | 3.417 | ***0.085*** | 2.644 | NS |
| 8h-A | 30.91 | 63.26 | 54.71 | 60.19 | 32.81 | 31.86 | 59.39 | 3.484 | ***0.078*** | 2.278 | NS |
| 1.5h-A | 4.06 | 11.14 | 8.47 | 15.80 | 5.71 | 4.88 | 11.80 | 7.485 | **0.002** | 4.923 | **0.016** |

(*Continued*)

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 3**. (*Continued*)

| | **Group 1 (N = 7)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **Space vs. Earth** | | **ISS03 vs. Before** | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| ULF01 | | | | | | | | | | | |
| 24h-A | 42.91 | 158.90 | 95.59 | 166.67 | 43.62 | 43.26 | 140.39 | 4.465 | **0.026** | 2.601 | NS |
| 12h-A | 41.33 | 144.99 | 92.88 | 145.30 | 43.58 | 42.46 | 127.72 | 3.302 | ***0.098*** | 2.188 | NS |
| 8h-A | 39.90 | 110.80 | 74.42 | 105.45 | 40.78 | 40.34 | 96.89 | 3.014 | 0.141 | 2.145 | NS |
| 1.5h-A | 8.87 | 22.71 | 16.30 | 31.32 | 11.55 | 10.21 | 23.44 | 4.706 | **0.020** | 4.052 | **0.040** |
| ULF02 | | | | | | | | | | | |
| 24h-A | 44.92 | 61.12 | 70.78 | 51.16 | 71.49 | 58.20 | 61.02 | 0.549 | NS | 0.742 | NS |
| 12h-A | 44.73 | 55.49 | 58.22 | 48.84 | 49.83 | 47.28 | 54.18 | 0.778 | NS | 0.426 | NS |
| 8h-A | 34.01 | 32.05 | 47.92 | 35.97 | 51.07 | 42.54 | 38.65 | 0.655 | NS | 0.183 | NS |
| 1.5h-A | 4.44 | 4.71 | 4.79 | 6.13 | 4.76 | 4.60 | 5.21 | 0.917 | NS | 1.647 | NS |
| ULF03 | | | | | | | | | | | |
| 24h-A | 62.70 | 36.69 | 40.41 | 49.54 | 67.57 | 65.13 | 42.22 | 2.574 | NS | 0.772 | NS |
| 12h-A | 41.72 | 32.60 | 26.97 | 28.96 | 34.97 | 38.35 | 29.51 | 1.480 | NS | 1.653 | NS |
| 8h-A | 29.02 | 19.52 | 26.48 | 33.48 | 26.66 | 27.84 | 26.49 | 0.178 | NS | 0.529 | NS |
| 1.5h-A | 3.01 | 2.01 | 2.38 | 3.01 | 2.50 | 2.75 | 2.47 | 0.621 | NS | 0.008 | NS |
| ULF/TF | | | | | | | | | | | |
| 24h-A | 17.86 | 28.05 | 20.27 | 18.86 | 14.31 | 16.09 | 22.39 | 1.938 | NS | 0.228 | NS |
| 12h-A | 16.58 | 25.73 | 20.24 | 22.24 | 15.18 | 15.88 | 22.74 | 1.659 | NS | 1.168 | NS |
| 8h-A | 16.19 | 15.74 | 15.62 | 13.76 | 11.14 | 13.67 | 15.04 | 0.514 | NS | 0.557 | NS |
| 1.5h-A | 3.97 | 4.83 | 4.87 | 5.28 | 3.75 | 3.86 | 4.99 | 1.753 | NS | 0.869 | NS |
| ULF01/TF | | | | | | | | | | | |
| 24h-A | 45.30 | 68.04 | 31.24 | 71.25 | 88.99 | 67.14 | 56.84 | 0.507 | NS | 1.408 | NS |

(*Continued*)

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 3.** (*Continued*)

| | **Group 1 (N = 7)** | | | | | | | **Space vs. Earth** | | **ISS03 vs. Before** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| 12h-A | 32.19 | 67.20 | 41.99 | 59.94 | 27.71 | 29.95 | 56.38 | 2.437 | NS | 1.721 | NS |
| 8h-A | 34.96 | 41.44 | 30.34 | 47.92 | 29.27 | 32.11 | 39.90 | 0.950 | NS | 1.299 | NS |
| 1.5h-A | 10.25 | 10.52 | 11.04 | 14.97 | 7.09 | 8.67 | 12.18 | 2.771 | NS | 1.772 | NS |
| ULF02/TF | | | | | | | | | | | |
| 24h-A | 35.43 | 29.49 | 33.98 | 18.14 | 30.42 | 32.93 | 27.20 | 1.266 | NS | 3.291 | 0.100 |
| 12h-A | 33.34 | 30.88 | 26.52 | 28.13 | 27.31 | 30.32 | 28.51 | 0.319 | NS | 0.646 | NS |
| 8h-A | 15.88 | 15.95 | 18.20 | 27.22 | 33.30 | 24.59 | 20.45 | 0.963 | NS | 1.769 | NS |
| 1.5h-A | 6.22 | 6.01 | 5.94 | 6.98 | 7.83 | 7.02 | 6.31 | 0.658 | NS | 0.424 | NS |
| ULF03/TF | | | | | | | | | | | |
| 24h-A | 53.53 | 24.10 | 26.26 | 41.43 | 39.95 | 46.74 | 30.60 | 3.376 | **0.090** | 1.266 | NS |
| 12h-A | 26.80 | 22.56 | 13.09 | 16.51 | 18.06 | 22.43 | 17.39 | 1.215 | NS | 1.599 | NS |
| 8h-A | 16.01 | 13.82 | 14.19 | 17.23 | 23.01 | 19.51 | 15.08 | 1.052 | NS | 0.363 | NS |
| 1.5h-A | 5.33 | 4.07 | 5.23 | 6.62 | 4.51 | 4.92 | 5.31 | 0.491 | NS | 1.837 | NS |
| VLF | | | | | | | | | | | |
| 24h-A | 29.89 | 41.13 | 38.29 | 42.63 | 34.64 | 32.27 | 40.68 | 0.859 | NS | 1.013 | NS |
| 12h-A | 20.71 | 24.11 | 20.84 | 29.59 | 21.58 | 21.15 | 24.85 | 0.964 | NS | 1.102 | NS |
| 8h-A | 17.50 | 26.51 | 25.08 | 33.56 | 10.67 | 14.08 | 28.38 | 2.857 | NS | 1.621 | NS |
| 1.5h-A | 9.68 | 17.06 | 14.18 | 17.14 | 11.36 | 10.52 | 16.13 | 1.717 | NS | 1.436 | NS |
| LF | | | | | | | | | | | |
| 24h-A | 18.12 | 15.07 | 18.21 | 24.30 | 27.81 | 22.97 | 19.19 | 1.267 | NS | 0.760 | NS |
| 12h-A | 14.30 | 18.91 | 13.14 | 17.69 | 24.28 | 19.29 | 16.58 | 0.729 | NS | 0.639 | NS |
| 8h-A | 19.79 | 14.11 | 11.82 | 17.08 | 22.48 | 21.14 | 14.34 | 1.485 | NS | 0.524 | NS |
| 1.5h-A | 7.24 | 7.96 | 5.75 | 8.92 | 5.88 | 6.56 | 7.54 | 0.802 | NS | 0.952 | NS |

(*Continued*)

**Table 3**. (*Continued*)

| | Group 1 (N = 7) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Space vs. Earth | | ISS03 vs. Before | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| HF | | | | | | | | | | | |
| 24h-A | 26.30 | 27.31 | 26.59 | 55.76 | 36.39 | 31.35 | 36.56 | 0.482 | NS | 1.266 | NS |
| 12h-A | 18.17 | 17.89 | 20.99 | 38.32 | 30.69 | 24.43 | 25.73 | 0.152 | NS | 1.794 | NS |
| 8h-A | 16.34 | 17.95 | 16.92 | 25.72 | 18.22 | 17.28 | 20.20 | 0.635 | NS | 1.502 | NS |
| 1.5h-A | 6.79 | 6.77 | 6.85 | 10.87 | 12.54 | 9.67 | 8.16 | 0.647 | NS | 1.358 | NS |
| LF/HF | | | | | | | | | | | |
| 24h-A | 24.67 | 24.76 | 14.48 | 26.18 | 23.65 | 24.16 | 21.81 | 0.564 | NS | 0.289 | NS |
| 12h-A | 11.93 | 15.23 | 10.84 | 15.63 | 12.33 | 12.13 | 13.90 | 0.596 | NS | 1.087 | NS |
| 8h-A | 10.44 | 13.20 | 13.98 | 10.21 | 8.82 | 9.63 | 12.46 | 1.959 | NS | 0.054 | NS |
| 1.5h-A | 9.06 | 11.16 | 5.31 | 11.39 | 11.76 | 10.41 | 9.29 | 1.775 | NS | 0.875 | NS |

| | Group 2 (N = 3) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Space vs. Earth | | ISS03 vs. Before | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| NN | | | | | | | | | | | |
| 24h-A | 19.82 | 15.86 | 17.48 | 18.32 | 17.99 | 18.91 | 17.22 | 0.621 | NS | 0.499 | NS |
| 12h-A | 5.80 | 8.78 | 9.45 | 8.40 | 8.80 | 7.30 | 8.88 | 1.113 | NS | 0.777 | NS |
| 8h-A | 3.61 | 4.28 | 4.35 | 3.83 | 4.14 | 3.88 | 4.15 | 0.271 | NS | 0.129 | NS |
| 1.5h-A | 1.06 | 1.62 | 1.90 | 2.40 | 1.90 | 1.48 | 1.97 | 1.907 | NS | 2.546 | NS |
| β | | | | | | | | | | | |
| 24h-A | 37.55 | 24.94 | 30.95 | 35.59 | 31.28 | 34.42 | 30.49 | 0.406 | NS | 0.210 | NS |

(*Continued*)

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 3**. (*Continued*)

| | **Group 2 (N = 3)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **Space vs. Earth** | | **ISS03 vs. Before** | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| 12h-A | 23.78 | 29.58 | 30.72 | 27.21 | 17.56 | 20.67 | 29.17 | 1.394 | NS | 0.486 | NS |
| 8h-A | 19.68 | 17.39 | 15.66 | 16.70 | 18.84 | 19.26 | 16.58 | 0.455 | NS | 1.043 | NS |
| 1.5h-A | 2.24 | 2.11 | 1.86 | 1.67 | 1.75 | 1.99 | 1.88 | 0.317 | NS | 0.557 | NS |
| TF | | | | | | | | | | | |
| 24h-A | 60.22 | 31.50 | 51.61 | 39.07 | 35.60 | 47.91 | 40.73 | 0.760 | NS | 1.368 | NS |
| 12h-A | 48.94 | 17.76 | 45.86 | 50.37 | 33.90 | 41.42 | 38.00 | 0.338 | NS | 0.043 | NS |
| 8h-A | 47.92 | 26.41 | 35.01 | 33.84 | 18.12 | 33.02 | 31.75 | 0.916 | NS | 7.198 | **0.002** |
| 1.5h-A | 9.80 | 3.28 | 5.68 | 7.49 | 4.73 | 7.27 | 5.48 | 2.802 | 0.186 | 0.732 | NS |
| ULF | | | | | | | | | | | |
| 24h-A | 87.09 | 53.23 | 73.05 | 58.51 | 45.77 | 66.43 | 61.60 | 0.283 | NS | 0.861 | NS |
| 12h-A | 75.45 | 44.29 | 65.30 | 71.00 | 38.67 | 57.06 | 60.20 | 0.238 | NS | 0.128 | NS |
| 8h-A | 76.57 | 46.30 | 55.71 | 63.55 | 35.12 | 55.85 | 55.19 | 0.085 | NS | 2.082 | NS |
| 1.5h-A | 13.05 | 8.83 | 8.23 | 5.60 | 6.02 | 9.54 | 7.55 | 1.275 | NS | 3.629 | ***0.066*** |
| ULF01 | | | | | | | | | | | |
| 24h-A | 108.64 | 71.78 | 88.03 | 96.08 | 32.58 | 70.61 | 85.30 | 0.967 | NS | 0.375 | NS |
| 12h-A | 120.47 | 64.16 | 92.57 | 121.20 | 57.76 | 89.12 | 92.64 | 0.269 | NS | 0.050 | NS |
| 8h-A | 114.42 | 72.49 | 91.25 | 107.47 | 59.20 | 86.81 | 90.40 | 0.182 | NS | 0.339 | NS |
| 1.5h-A | 30.75 | 17.55 | 16.54 | 12.27 | 12.29 | 21.52 | 15.45 | 2.639 | NS | 6.623 | **0.003** |
| ULF02 | | | | | | | | | | | |
| 24h-A | 115.88 | 41.77 | 77.19 | 41.78 | 79.06 | 97.47 | 53.58 | 3.810 | ***0.053*** | 4.063 | **0.040** |
| 12h-A | 103.51 | 39.62 | 54.65 | 41.31 | 47.27 | 75.39 | 45.19 | 4.227 | **0.033** | 3.225 | 0.108 |
| 8h-A | 64.38 | 33.04 | 41.18 | 37.45 | 36.76 | 50.57 | 37.22 | 2.777 | NS | 2.236 | NS |

(*Continued*)

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 3.** (*Continued*)

| | Group 2 (N = 3) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Space vs. Earth | | ISS03 vs. Before | |
| HRV endpoint | Before | ISS01 | ISS02 | ISS03 | After | Earth | Space | paired t | P | paired t | P |
| 1.5h-A | 10.37 | 5.16 | 7.12 | 3.82 | 5.24 | 7.81 | 5.37 | 1.047 | NS | 2.217 | NS |
| ULF03 | | | | | | | | | | | |
| 24h-A | 65.84 | 32.72 | 48.60 | 36.56 | 44.94 | 55.39 | 39.29 | 0.675 | NS | 0.810 | NS |
| 12h-A | 79.34 | 15.71 | 44.70 | 43.96 | 26.36 | 52.85 | 34.79 | 1.003 | NS | 0.886 | NS |
| 8h-A | 55.65 | 21.07 | 21.60 | 35.66 | 26.79 | 41.22 | 26.11 | 1.023 | NS | 0.677 | NS |
| 1.5h-A | 2.73 | 3.12 | 1.96 | 2.71 | 2.02 | 2.37 | 2.60 | 0.483 | NS | 0.008 | NS |
| ULF/TF | | | | | | | | | | | |
| 24h-A | 24.97 | 22.74 | 29.37 | 31.76 | 29.00 | 26.99 | 27.96 | 0.107 | NS | 0.958 | NS |
| 12h-A | 23.48 | 26.92 | 26.49 | 26.60 | 15.56 | 19.52 | 26.67 | 3.320 | ***0.096*** | 0.328 | NS |
| 8h-A | 23.18 | 20.18 | 17.73 | 17.54 | 19.29 | 21.23 | 18.48 | 0.726 | NS | 1.441 | NS |
| 1.5h-A | 3.93 | 4.16 | 8.16 | 5.99 | 6.37 | 5.15 | 6.11 | 0.615 | NS | 0.676 | NS |
| ULF01/TF | | | | | | | | | | | |
| 24h-A | 42.32 | 44.30 | 48.37 | 50.48 | 31.22 | 36.77 | 47.72 | 0.776 | NS | 1.437 | NS |
| 12h-A | 47.50 | 46.01 | 42.67 | 58.27 | 39.76 | 43.63 | 48.98 | 0.904 | NS | 1.354 | NS |
| 8h-A | 50.49 | 55.09 | 50.52 | 49.11 | 49.18 | 49.83 | 51.57 | 0.267 | NS | 0.354 | NS |
| 1.5h-A | 9.85 | 11.57 | 18.14 | 11.70 | 9.94 | 9.90 | 13.81 | 1.159 | NS | 0.450 | NS |
| ULF02/TF | | | | | | | | | | | |
| 24h-A | 30.10 | 29.26 | 41.42 | 27.29 | 54.72 | 42.41 | 32.66 | 1.034 | NS | 0.825 | NS |
| 12h-A | 56.17 | 26.10 | 26.97 | 25.47 | 35.02 | 45.60 | 26.18 | 2.146 | NS | 2.269 | NS |
| 8h-A | 22.72 | 13.42 | 10.26 | 17.50 | 42.38 | 32.55 | 13.73 | 13.648 | <**0.001** | 1.159 | NS |
| 1.5h-A | 8.32 | 4.50 | 5.87 | 7.41 | 5.46 | 6.89 | 5.93 | 0.276 | NS | 0.167 | NS |

(*Continued*)

**Table 3.** (*Continued*)

| | **Group 2 (N = 3)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **Space vs. Earth** | | **ISS03 vs. Before** | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| ULF03/TF | | | | | | | | | | | |
| 24h-A | 28.57 | 13.69 | 19.92 | 8.49 | 36.54 | 32.55 | 14.03 | 2.902 | NS | 2.763 | NS |
| 12h-A | 24.10 | 13.50 | 16.68 | 19.87 | 24.19 | 24.14 | 16.69 | 1.471 | NS | 0.489 | NS |
| 8h-A | 16.60 | 21.78 | 19.32 | 18.71 | 16.52 | 16.56 | 19.94 | 1.459 | NS | 0.378 | NS |
| 1.5h-A | 8.47 | 3.22 | 5.00 | 8.68 | 5.01 | 6.74 | 5.63 | 1.081 | NS | 0.192 | NS |
| VLF | | | | | | | | | | | |
| 24h-A | 67.35 | 36.26 | 43.68 | 58.98 | 44.16 | 55.76 | 46.31 | 0.654 | NS | 0.982 | NS |
| 12h-A | 43.61 | 25.58 | 22.78 | 46.55 | 33.76 | 38.68 | 31.64 | 0.948 | NS | 0.219 | NS |
| 8h-A | 22.40 | 23.38 | 11.35 | 30.15 | 20.26 | 21.33 | 21.63 | 0.052 | NS | 1.717 | NS |
| 1.5h-A | 15.94 | 8.09 | 13.54 | 13.61 | 14.55 | 15.25 | 11.75 | 3.172 | 0.116 | 2.117 | NS |
| LF | | | | | | | | | | | |
| 24h-A | 31.02 | 20.76 | 29.00 | 38.00 | 29.65 | 30.34 | 29.25 | 1.026 | NS | 1.241 | NS |
| 12h-A | 23.15 | 14.79 | 15.99 | 23.03 | 23.85 | 23.50 | 17.94 | 2.142 | NS | 0.018 | NS |
| 8h-A | 24.38 | 11.74 | 11.28 | 15.81 | 18.18 | 21.28 | 12.95 | 2.199 | NS | 2.031 | NS |
| 1.5h-A | 9.27 | 6.77 | 12.33 | 10.12 | 6.35 | 7.81 | 9.74 | 1.401 | NS | 0.185 | NS |
| HF | | | | | | | | | | | |
| 24h-A | 62.47 | 24.21 | 30.45 | 42.66 | 35.59 | 49.03 | 32.44 | 1.170 | NS | 1.314 | NS |
| 12h-A | 38.30 | 20.22 | 16.52 | 21.89 | 34.08 | 36.19 | 19.55 | 3.440 | ***0.083*** | 1.211 | NS |
| 8h-A | 22.78 | 16.29 | 13.50 | 11.87 | 14.90 | 18.84 | 13.89 | 1.065 | NS | 1.861 | NS |
| 1.5h-A | 13.45 | 5.53 | 3.74 | 8.55 | 4.95 | 9.20 | 5.94 | 2.947 | 0.154 | 3.345 | ***0.093*** |

(*Continued*)

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

**Table 3**. (*Continued*)

| | Group 2 (N = 3) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Space vs. Earth | | ISS03 vs. Before | |
| **HRV endpoint** | **Before** | **ISS01** | **ISS02** | **ISS03** | **After** | **Earth** | **Space** | **paired t** | **P** | **paired t** | **P** |
| LF/HF | | | | | | | | | | | |
| 24h-A | 32.88 | 14.76 | 11.37 | 7.33 | 15.48 | 24.18 | 11.15 | 4.222 | **0.033** | 9.487 | <**0.001** |
| 12h-A | 15.90 | 24.65 | 7.34 | 8.31 | 10.68 | 13.29 | 13.43 | 0.079 | NS | 1.407 | NS |
| 8h-A | 11.69 | 9.97 | 12.10 | 6.25 | 19.31 | 15.50 | 9.44 | 1.242 | NS | 1.233 | NS |
| 1.5h-A | 10.80 | 10.41 | 12.23 | 6.91 | 9.03 | 9.91 | 9.85 | 0.018 | NS | 0.535 | NS |

[*] Amplitudes expressed as a percentage of MESOR, P-values adjusted for multiple testing, considering 6 different frequency regions (ULF01, ULF02, ULF03, VLF, LF, and HF). Based on results from Tables 1A, 1B and 2, significant results were anticipated to be found in the ULF rather than in other spectral regions. NN: normal-to-normal intervals; β: slope of fractal scaling; TF: total spectral power; ULF, VLF, LF and HF: spectral power in ultra-low, very low, low, and high frequency regions of the spectrum. Non-sinusoidal waveform may occasionally be associated with overfit (A > 100%).

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

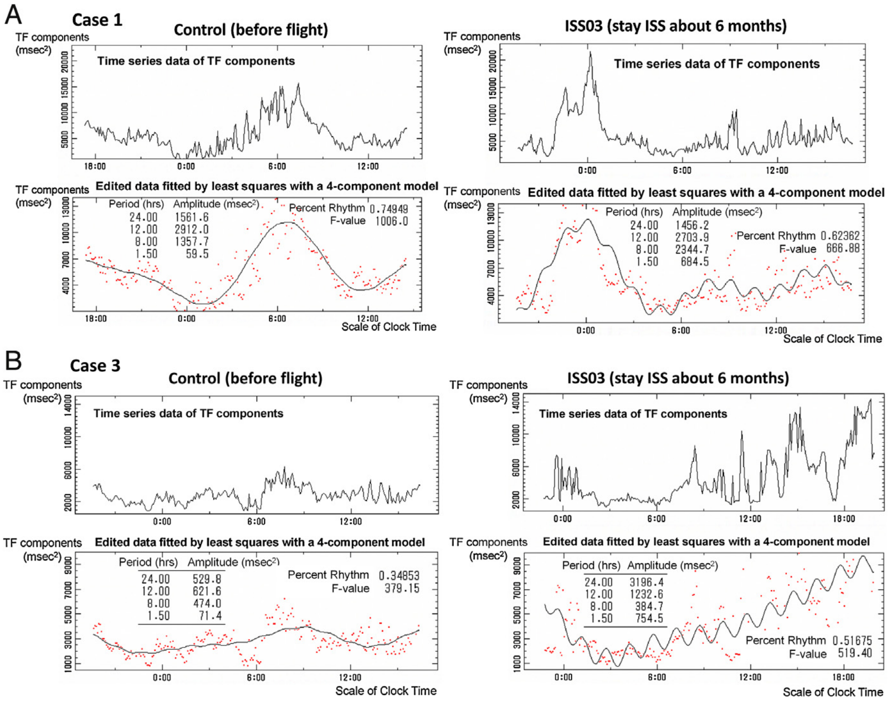

**Fig. 1.** Illustrative examples of the 4-component model fitted to the TF spectral power of two astronauts during a 6-month spaceflight. As compared to preflight (left), the 90-min component is amplified during session ISS03 in space (right). Whereas the circadian amplitude is mostly unchanged in one case (Fig. 1A), it is also amplified in another case (Fig. 1B). A fixed model is used, considering only anticipated periodicities. As such, the model is not optimal for any given record, even if on a group basis it conveys the behavior of components that are the most commonly detected in such records. Because it is a fixed model, the residual variance may exhibit lack of fit. Nevertheless, the amplitude of the about 90-min component is increased during ISS03 in astronauts of Group 1, when it resembles that of astronauts of Group 2.

were observed for the 24-hour amplitude, and to a lesser extent for the 12-hour and 8-hour amplitudes of these HRV endpoints. These results suggest that the HRV of astronauts in Group 2, but not in Group 1, may have been sufficiently large to be exposed to the space environment.

## 4. Discussion

Spaceflight dramatically alters cardiovascular dynamics, as illustrated by changes in HRV [12] and a less negative slope β of the fractal scaling [9] confirmed herein. Kleitman's about 90-min BRAC [20] was found to be amplified about 3-fold in space, notably among astronauts of Group 1, in keeping with a corresponding increase in ULF01/TF (0.0001–0.0003 Hz, i.e., 55–166 min) and corresponding decreases in ULF02/TF and ULF03/TF. Major changes observed in space all relate to the same frequency range centered around one cycle in about 90 min, including β.

**Table 4.** Characteristics of model of 4 anticipated components fitted to some HRV endpoints compared between astronauts whose HR did or did not decrease in space.*

| | | Control (Before flight) | | | | | | ISS03 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Group 1 (Decreased HR in Space) (n = 7) | | Group 2 (Increased HR in Space) (n = 3) | | Student t-test | | Group 1 (Decreased HR in Space) (n = 7) | | Group 2 (Increased HR in Space) (n = 3) | | Student t-test | |
| | | **mean** | **SD** | **mean** | **SD** | **t-value** | **p-value** | **mean** | **SD** | **mean** | **SD** | **t-value** | **p-value** |
| MESOR | NN-interval | 837.1 | 102.1 | 1021.9 | 143.6 | −2.35 | NS | 904.1 | 97.6 | 1002.0 | 134.5 | −1.31 | NS |
| | TF | 4674.1 | 1216.8 | 12924.0 | 2750.1 | −6.90 | **0.0005** | 5040.0 | 1631.2 | 10287.5 | 3043.6 | −3.66 | **0.0320** |
| | ULF | 2686.4 | 798.7 | 7229.4 | 1661.5 | −6.09 | **0.0015** | 2893.6 | 1138.2 | 5478.2 | 2099.0 | −2.60 | NS |
| | ULF01 | 971.8 | 443.0 | 2161.8 | 632.8 | −3.47 | **0.0425** | 1744.5 | 1054.8 | 2889.3 | 1064.6 | −1.57 | NS |
| | VLF | 1337.1 | 337.0 | 4536.4 | 1425.1 | −6.02 | **0.0015** | 1431.4 | 348.7 | 3687.2 | 894.7 | −6.06 | **0.0015** |
| | LF | 572.7 | 220.4 | 1067.8 | 268.3 | −3.08 | 0.0760 | 575.4 | 291.2 | 853.1 | 302.4 | −1.37 | NS |
| | HF | 92.4 | 45.4 | 174.6 | 23.4 | −2.91 | 0.0985 | 122.7 | 70.5 | 137.3 | 56.9 | −0.31 | NS |
| 24-hour Amplitude | TF | 1182.7 | 493.5 | 6430.0 | 2363.0 | −6.05 | **0.0015** | 3120.4 | 2248.0 | 4525.0 | 3652.8 | −0.76 | NS |
| | ULF | 1066.3 | 570.5 | 5055.7 | 1652.0 | −6.01 | **0.0015** | 2947.5 | 1944.6 | 3891.7 | 3382.3 | −0.57 | NS |
| | ULF01 | 566.9 | 444.9 | 2885.1 | 974.0 | −5.41 | **0.0030** | 2322.2 | 1631.2 | 2698.9 | 2172.9 | −0.31 | NS |
| | VLF | 407.2 | 220.1 | 2514.0 | 1794.6 | −3.33 | 0.0520 | 580.3 | 385.9 | 2149.4 | 1842.9 | −2.32 | NS |
| | LF | 116.7 | 84.9 | 297.9 | 209.0 | −2.05 | NS | 126.6 | 93.9 | 377.4 | 329.6 | −1.98 | NS |
| | HF | 30.8 | 34.3 | 88.8 | 41.3 | −2.32 | NS | 61.5 | 63.7 | 65.0 | 58.0 | −0.08 | NS |
| 12-hour Amplitude | TF | 1255.5 | 1006.9 | 6072.5 | 7806.4 | −1.75 | NS | 2558.4 | 1965.8 | 5357.6 | 1545.4 | −2.17 | NS |
| | ULF | 1006.0 | 784.8 | 5433.8 | 6761.2 | −1.86 | NS | 2601.2 | 1900.6 | 4454.7 | 2538.6 | −1.29 | NS |
| | ULF01 | 575.4 | 512.8 | 3333.7 | 2524.9 | −2.99 | 0.0870 | 2054.6 | 1686.9 | 3336.2 | 2088.9 | −1.03 | NS |
| | VLF | 287.0 | 219.6 | 1662.6 | 964.5 | −3.85 | **0.0246** | 385.1 | 184.9 | 1731.1 | 1259.6 | −3.00 | 0.0851 |
| | LF | 73.6 | 59.9 | 214.6 | 110.8 | −2.69 | NS | 86.3 | 56.3 | 231.2 | 209.1 | −1.82 | NS |
| | HF | 20.2 | 25.2 | 53.7 | 21.8 | −1.99 | NS | 42.3 | 33.2 | 34.0 | 32.3 | 0.37 | NS |

*(Continued)*

## **Table 4**. (*Continued*)

| | | Control (Before flight) | | | | | | ISS03 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Group 1 (Decreased HR in Space) (n = 7) | | Group 2 (Increased HR in Space) (n = 3) | | Student t-test | | Group 1 (Decreased HR in Space) (n = 7) | | Group 2 (Increased HR in Space) (n = 3) | | Student t-test | |
| | | **mean** | **SD** | **mean** | **SD** | **t-value** | **p-value** | **mean** | **SD** | **mean** | **SD** | **t-value** | **p-value** |
| 8-hour Amplitude | TF | 924.6 | 503.4 | 5448.3 | 4121.8 | −3.11 | 0.0720 | 2105.5 | 1417.6 | 3907.8 | 3526.6 | −1.22 | NS |
| | ULF | 898.5 | 423.2 | 4904.8 | 3394.5 | −3.34 | 0.0510 | 1834.5 | 1428.1 | 4036.8 | 2549.7 | −1.80 | NS |
| | ULF01 | 545.8 | 258.6 | 3025.5 | 1192.0 | −5.64 | **0.0025** | 1507.4 | 1222.9 | 2962.5 | 1889.2 | −1.49 | NS |
| | VLF | 251.7 | 164.4 | 847.5 | 445.7 | −3.27 | 0.0570 | 455.0 | 247.3 | 1133.0 | 633.0 | −2.57 | NS |
| | LF | 111.0 | 72.6 | 229.6 | 150.4 | −1.75 | NS | 87.4 | 29.8 | 150.0 | 93.6 | −1.70 | NS |
| | HF | 16.3 | 16.9 | 32.1 | 7.5 | −1.53 | NS | 28.9 | 23.8 | 18.4 | 17.8 | 0.67 | NS |
| 90-min Amplitude | TF | 154.9 | 105.0 | 1063.1 | 549.0 | −4.55 | **0.0095** | 532.7 | 301.3 | 872.3 | 738.1 | −1.09 | NS |
| | ULF | 117.9 | 57.5 | 790.4 | 292.0 | −6.32 | **0.0010** | 442.4 | 202.9 | 303.2 | 84.7 | 1.12 | NS |
| | ULF01 | 124.3 | 82.8 | 801.6 | 155.6 | −9.28 | **0.0001** | 427.6 | 214.8 | 303.0 | 74.2 | 0.95 | NS |
| | VLF | 126.0 | 77.2 | 629.5 | 554.7 | −2.56 | NS | 236.3 | 121.3 | 544.1 | 565.3 | −1.48 | NS |
| | LF | 38.6 | 31.7 | 73.2 | 33.1 | −1.57 | NS | 48.3 | 28.6 | 86.4 | 63.8 | −1.36 | NS |
| | HF | 6.0 | 3.7 | 19.4 | 9.1 | −3.39 | **0.0470** | 12.9 | 14.8 | 12.8 | 9.6 | 0.04 | NS |

NN: Normal-to-normal inter-beat interval; TF: Total spectral power; ULF: Ultra low frequency spectral power (0.0001–0.003 Hz).

ULF01: ULF band-1 (0.0001–0.0003 Hz); VLF: very low frequency spectral power (0.005–0.02 Hz).

LF: low frequency spectral power (0.04–0.15 Hz); HF: high frequency spectral power (0.15–0.40 Hz).

P-values adjusted for multiple testing, using Bonferroni inequality (considering 5 tests per endpoint: MESOR, amplitude of each of 4 anticipated components).

[*] MESOR: Midline Estimating Statistic Of Rhythm, a rhythm-adjusted mean.

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

Beyond the partly built-in circadian rhythms [23], there are many other oscillations of different frequencies, including the BRAC, observed in the sleep-wake (REM/NREM) cycle and also in heart rate variability. Some neuropeptides can have more prominent ultradian (with a frequency higher than one cycle per day; e.g., 8-hour periodicity) than circadian changes [24]. We previously showed that the circadian rhythm persisted in space in HR and $\beta$ [9, 12]. Herein, we confirm the presence in space of 24-, 12-, and 8-hour components in several HRV endpoints by the fit of a model including 4 anticipated components.

The question may be raised, however, whether different daily routines before and during flight (including higher or lower frequency of physical activities) as well as different sleep patterns in space may have contributed to the findings [25]. Whereas further work is needed to address this question, it should be noted that the space environment had a different effect on astronauts from the 2 groups. Amplitudes of all 4 anticipated components were markedly increased in astronauts of Group 1, whereas they were mostly decreased in astronauts of Group 2. It thus seems unlikely that the daily routine on the ISS fully accounts for the results observed in this study.

Unlike short-term (<24 h) analysis of HRV [25, 26, 27], transient changes of body movement related to the daily routine were not associated with measurements of long HRV signals, including the ULF and VLF components and the slope $\beta$. Aoyagi et al. [10, 11] reported that during both usual daily-routine and constant-routine protocols in healthy men, HRV at frequencies between 0.0033 Hz and $10^{-3.5}$ Hz (25 sec to 57 min periods) was behavior-independent, possibly reflecting intrinsic mechanisms of the regulatory system. Amaral LAN et al. [28] also reported that the complexity of heartbeat dynamics showed behavioral-independent features during a constant-routine protocol. As reported previously [25, 26, 27], however, body movement was lower and the HF component of HRV was higher during sleep than during wakefulness. The less negative slope $\beta$ in space versus Earth was also seen more prominently during the awake span [9]. Future studies are thus needed to examine how different daily routines before and during flight, including different sleep patterns in space, may contribute to our findings herein.

The presence of the BRAC in HRV endpoints observed herein is supported by different studies in a number of physiological systems. Based on 24-hour polygraphic tracings, Othmer E et al. [29] inferred that the so-called sleep-dream cycle of human sleep is a general activity pattern of the brain. Bailey D et al. [30] found regular oscillations with periods of 1–2 hours in their subjects' oxygen consumption. Orr WC et al. [31] noted that their subjects' heart rate showed the same about 90-min periodicity in performance of a complex vigilance task. Hiatt JF and Kripke DF [32] reported on 90- to 120-min ultradian rhythms in gastric motility. Lavie P and Kripke DF [33] discerned a cycle of 80–133 min in urine

http://dx.doi.org/10.1016/j.heliyon.2016.e00211

flow of awake subjects. The rhythm in urine flow was, however, clearly out of phase with those of electrolyte concentrations and osmolarity. Lavie P and Scherson A [34] observed rhythmic variations in subjects' ability to fall asleep throughout the day. Conversely, an expected variation in vigilance was reported by Okawa M et al., [35]; the ultradian rhythms in vigilance had periods of 90–120 min.

The BRAC may play an important and unique role in keeping the quality of life in space independently of or in conjunction with the circadian rhythm. It is involved in the functioning of the central nervous system which integrates many somatic, visceral, and neurobehavioral functions and manifests itself in the alternation of non-REM and REM sleep. Ultradians may be the basic signature of life [36].

Effects of space weather are enormous, which have acted as selective forces in humans on Earth and shaped human life as we know it today. Using 61 worldwide populations, Hancock AM et al. [37] elucidated the genetic basis for adaptation to the climate-mediated selection in a scan of the human genome. They identified genes that are key to the differentiation of brown adipocytes, and genes whose regulation makes a difference in response to ultraviolet radiation [37]. Among the circadian clock components, cryptochrome may have played a pivotal role in evolution because it coordinates light-induced effects and protects from hazards of ultraviolet radiation [38]. Brown adipocytes and their cryptochromes may not only be relevant to survival and adaptation, but they may also be targeted by natural selection [39]. Circadian clocks in brown adipocytes are relevant to mammalian adaptation and the cryptochromes in particular are of key importance because of their evolutionary roots of circadian clocks.

Brown adipose tissue expressing BRAC may be an active pacemaker tissue, participating in the arrangement of ultradian [40] to infradian [41] oscillations. Circadian clocks may thus be built on properties generating metabolic oscillations in the ultradian range [38]. Brown adipose tissue may be a site of interaction between metabolic and circadian systems. A non-transcriptional pathway for the metabolic cycle engages the circadian clock, thereby enhancing clock performance [42]. As cryptochromes are key components of the core of the transcription-translation feedback loops on which circadian clocks are built, the question may thus be raised whether the amplification of the BRAC in space observed herein is a sign of early adaptation to microgravity.

## 5. Conclusion

Whether the increase in space of the BRAC amplitude is a sign that the intrinsic autonomic regulatory system may start to adapt requires further investigation, as β remains disturbed throughout the 6-month spaceflight. Whether some features of the HRV may indicate suitability for space travel also deserves further work as the

BRAC amplification in space was only observed in some but not all astronauts. Most HRV changes observed in space relate to a frequency window centered around one cycle in about 90 min, although astronauts follow regular 24-hour rest-activity and feeding schedules on the ISS. Since the BRAC component is amplified in space for only specific HRV endpoints, it is likely to represent a physiologic response rather than an artifact from the ISS orbit. If so, it may offer a way to help adaptation to microgravity during long-duration spaceflight.

# Declarations

## Author contribution statement

Kuniaki Otsuka: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Germaine Cornelissen, Yutaka Kubo, Mitsutoshi Hayashi, Koichi Shibata, Koh Mizuno: Analyzed and interpreted the data; Wrote the paper.

Satoshi Furukawa, Tatsuya Aiba, Hiroshi Ohshima, Chiaki Mukai: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

## Competing interest statement

The authors declare no conflict of interest.

## Additional information

No additional information is available for this paper.

## Funding statement

The JAXA Chronobiology Project was supported by the Japan Aerospace Exploration Agency (KO, YK, MH, NY, KS, TA, SF, HO, CM), Halberg Chronobiology Fund (GC).

## Acknowledgements

The authors thank Dr. I. Tayama and S. Ishida from the Space Biomedical Research Group, Japan Aerospace Exploration Agency (JAXA), for cooperation in our study. The authors also acknowledge the cooperation of the astronauts, the engineers, staff and managers of JAXA and NASA.

## References

[1] R.M. Baevsky, V.M. Baranov, I.I. Funtova, A. Diedrich, A.V. Pashenko, A. G. Chernikova, J. Drescher, J. Jordan, J. Tank, Autonomic cardiovascular and

respiratory control during prolonged spaceflights aboard the International Space Station, J. Appl. Physiol. 103 (2007) 156–161.

[2] B. Verheyden, J. Liu, F. Beckers, A.E. Aubert, Operational point of neural cardiovascular regulation in humans up to 6 months in space, J. Appl. Physiol. 108 (2010) 646–654.

[3] R.L. Hughson, J.K. Shoemaker, A.P. Blaber, P. Arbeille, D.K. Greaves, P.P. Pereira-Junior, D. Xu, Cardiovascular regulation during long-duration spaceflights to the International Space Station, J. Appl. Physiol. 112 (2012) 719–727.

[4] D. Xu, J.K. Shoemaker, A.P. Blaber, P. Arbeille, K. Fraser, R.L. Hughson, Reduced heart rate variability during sleep in long-duration spaceflight, Am. J. Physiol. Regul. Integr. Comp. Physiol. 305 (2013) R164–R170.

[5] A.L. Goldberger, M.W. Bungo, R.M. Baevsky, B.S. Bennett, D.R. Rigney, J. E. Mietus, G.A. Nikulina, J.B. Charles, Heart rate dynamics during long-term space flight: report on Mir cosmonauts, Am. Heart J. 128 (1994) 202–204.

[6] P. Norsk, A. Asmar, M. Damgaard, N.J. Christensen, Fluid shifts, vasodilatation and ambulatory blood pressure reduction during long duration spaceflight, J. Physiol. 593 (2015) 573–584.

[7] A.C. Ertl, A. Diedrich, I. Biaggioni, B.D. Levine, R.M. Robertson, J.F. Cox, J.H. Zuckerman, J.A. Pawelczyk, C.A. Ray, J.C. Buckey Jr., L.D. Lane, R. Shiavi, F.A. Gaffney, F. Costa, C. Holt, C.G. Blomqvist, D.L. Eckberg, F.J. Baisch, D. Robertson, Human muscle sympathetic nerve activity and plasma norepinephrine kinetics in space, J. Physiol. 538 (2002) 321–329.

[8] D. Williams, A. Kuipers, C. Mukai, R. Thirsk, Acclimation during space flight: effects on human physiology, CMAJ 180 (2009) 1317–1323.

[9] K. Otsuka, K. Cornelissen, Y. Kubo, M. Hayashi, N. Yamamoto, K. Shibata, T. Aiba, S. Furukawa, H. Ohshima, C. Mukai, Intrinsic cardiovascular autonomic regulatory system of astronauts exposed long-term to microgravity in space: observational study, npj Microgravity 1 (2015) 15018, doi:http://dx.doi.org/10.1038/npjmgrav.2015.18.

[10] N. Aoyagi, K. Ohashi, S. Tomono, Y. Yamamoto, Temporal contribution of body movement to very long-term heart rate variability in humans, Am. J. Physiol. Heart Circ. Physiol. 278 (2000) H1035–H1041.

[11] N. Aoyagi, K. Ohashi, Y. Yamamoto, Frequency characteristics of long-term heart rate variability during constant-routine protocol, Am. J. Physiol. Regul. Integr. Comp. Physiol. 285 (2003) R171–R176.

[12] N. Yamamoto, K. Otsuka, Y. Kubo, M. Hayashi, K. Mizuno, H. Ohshima, C. Mukai, Effects of long-term microgravity exposure in space on circadian rhythms of heart rate variability, Chronobiol. Int. 32 (2015) 327–340.

[13] M. Hastings, J.S. O'Neill, E.S. Maywood, Circadian clocks: regulators of endocrine and metabolic rhythms, J. Endocrinol. 195 (2007) 187–198.

[14] C. Scheiermann, Y. Kunisaki, P.S. Frenette, Circadian control of the immune system, Nat. Rev. Immunol. 13 (2013) 190–198.

[15] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation 93 (1996) 1043–1065.

[16] J.M. Huston, K.J. Tracy, The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy, J. Intern. Med. 269 (2010) 45–53.

[17] V. Papaioannou, I. Pneumatikos, N. Maglaveras, Association of heart rate variability and inflammatory response in patients with cardiovascular diseases: current strength and limitations, Front. Physiol. 4 (1) (2013) 174–213, doi:http://dx.doi.org/10.3389/fphys.2013.00174 Article 174.

[18] A.I. Hazzouri Zeki, M.N. Haan, Y. Deng, J. Neuhaus, K. Yaffe, Reduced heart rate variability is associated with worse cognitive performance in elderly Mexican Americans, Hypertension 63 (2014) 181–187.

[19] F. Halberg, G. Cornelissen, R.B. Sothern, G.S. Katinas, O. Schwartzkopff, K. Otsuka, Cycles tipping the scale between death and survival (= life), Prog. Theor. Phys. (Suppl. 173) (2008) 153–181.

[20] N. Kleitman, Biological rhythms and cycles, Physiol. Rev. 29 (1949) 1–30.

[21] A recent Advances in Time Series Analysis by Maximum Entropy Method, In: K. Saito, A. Koyama, K. Yoneyama, Y. Sawada, N. Ohtomo (Eds.), Hokkaido University Press, Sapporo, 1994.

[22] F. Halberg, Chronobiology: methodological problems, Acta. Med. Rom. 18 (1980) 399–440.

[23] F. Halberg, M.B. Visscher, Regular diurnal physiological variation in eosinophil levels in five stocks of mice, Proc. Soc. Exp. Biol. (N.Y.) 75 (1950) 846–847.

[24] M. Herold, G. Cornelissen, A. Loeckinger, D. Koeberle, P. Koenig, F. Halberg, About 8-hourly variation of circulating human endothelin-1 (ET-1) in clinical health, Peptides 19 (1998) 821–825.

[25] P.C. Ivanov, A. Bunde, L.A.N. Amaral, S. Havlin, J. Fritsch-Yelle, R.M. Baevsky, H.E. Stanley, A.L. Goldberger, Sleep-wake differences in scaling behavior of the human heartbeat: analysis of terrestrial and long-term space flight data, Europhys. Lett. 48 (1999) 594–600.

[26] R. Furlan, S. Guzzetti, W. Crivellaro, S. Dassi, M. Tinelli, G. Baselli, S. Cerutti, F. Lombardi, M. Pagani, A. Malliani, Continuous 24-h assessment of the neural regulation of systemic arterial pressure and RR variability in ambulant subjects, Circulation 81 (1990) 537–547.

[27] L. Bernardi, F. Valle, M. Coco, A. Calciati, P. Sleight, Physical activity influences heart rate variability and very-low-frequency components in Holter electrocardiograms, Cardiovasc. Res. 32 (1996) 234–237.

[28] L.A.N. Amaral, P.C. Ivanov, N. Aoyagi, I. Hidaka, S. Tomono, A.L. Goldberger, H.E. Stanley, Y. Yamamoto, Behavioral-independent features of complex heartbeat dynamics, Phys. Rev. Lett. 86 (2001) 6026–6029.

[29] E. Othmer, M.P. Hayden, R. Segelbaum, Encephalic cycles during sleep and wakefulness in humans: a 24-hour pattern, Science 164 (1969) 447–449.

[30] D. Bailey, D. Harry, R.E. Johnson, I. Kupprat, Oscillators in oxygen consumption of man at rest, J. Appl. Physiol. 34 (1973) 467–470.

[31] W.C. Orr, H.J. Hoffman, F.W. Hegge, The assessment of time-dependent changes in human performance, Chronobiologia 3 (1976) 293–309.

[32] J.F. Hiatt, D.F. Kripke, Ultradian rhythms in waking gastric activity, Psychosom. Med. 37 (1975) 320–325.

[33] P. Lavie, D.F. Kripke, Ultradian rhythms in urine flow in waking humans, Nature 269 (1977) 142–144.

[34] P. Lavie, A. Scherson, Ultrashort sleep-waking schedule: I. Evidence of ultradian rhythmicity in sleepability, Electroencephalogr. Clin. Neurophysiol. 52 (1981) 163–174.

[35] M. Okawa, M. Matousek, A.L. Nueth, I. Petersen, Changes of daytime vigilance in normal humans, Electroencephalogr. Clin. Neurophysiol. 52 (1981) S17.

[36] F.E. Yates, L.B. Yates, Ultradian rhytyhms as the dynamic signature of life, In: D. Lloyd, E.L. Rossi (Eds.), Ultradian Rhythms from Molecules to Mind, Springer, London, 2008, pp. 249–260.

[37] A.M. Hancock, D.B. Witonsky, G. Alkorta-Aranburu, C.M. Beall, A. Gebremedhin, R. Sukernik, G. Utermann, J.K. Pritchard, G. Coop, A. Di Rienzo, Adaptations to climate-mediated selective pressures in humans, PLoS

Genet. 7 (2011) e1001375, doi:http://dx.doi.org/10.1371/journal.pgen.1001375.

[38] M. Heijde, G. Zabulon, F. Corellou, T. Ishikawa, J. Brazard, A. Usman, F. Sanchez, P. Plaza, M. Martin, A. Falciatore, T. Todo, F.-V. Bouget, C. Bowler, Characterization of two members of the cryptochrome/photolyase family from *Ostreococcus tauri* provides insights into the origin and evolution of cryptochromes, Plant Cell Environ. 33 (2010) 1614–1626.

[39] T. Partonen, Hypothesis: cryptochromes and brown fat are essential for adaptation and affect mood and mood-related behaviors, Front. Neur. 3 (2012) 157, doi:http://dx.doi.org/10.3389/fneur.2012.00157.

[40] W. Blessing, M. Mohammed, Y. Ootsuka, Heating and eating brown adipose tissue thermogenesis precedes food ingestion as part of the ultradian basic rest-activity cycle in rats, Physiol. Behav. 105 (2012) 966–974.

[41] K.A. Zukotynski, F.H. Fahey, S. Laffin, R. Davis, S.T. Treves, F.D. Grant, L. A. Drubach, Seasonal variation in the effect of constant ambient temperature of 24 degree C in reducing FDG uptake by brown adipose tissue in children, Eur. J. Nucl. Med. Mol. Imaging 37 (2010) 1854–1860.

[42] G. van Ooijen, A.J. Millar, Non-transcriptional oscillators in circadian timekeeping, Trends Biochem. Sci. 37 (2012) 484–492.