IOP Publishing Physiol. Meas. 40 (2019) 104001 (9pp) https://doi.org/10.1088/1361-6579/ab4c18

Physiological Measurement

IPEM Institute of Physics and Engineering in Medicine

PAPER

Assessment of ventricular repolarization instability in terms of T-wave alternans induced by head-down bed-rest immobilization

Alba Martín-Yebra[1,2], Violeta Monasterio[3], Federica Landreani[4], Pablo Laguna[2,5], Juan Pablo Martínez[2,5] and Enrico G Caiani[4,6]

[1] Department of Biomedical Engineering, Lund University, Lund, Sweden
[2] BSICoS Group, Instituto de Investigación en Ingeniería de Aragón (I3A), IIS Aragón, Universidad de Zaragoza, Zaragoza, Spain
[3] Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain
[4] Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Milano, Italy
[5] Centro de Investigación Biomédica en Red—Bioingeniería, Biomateriales y Nanomedicina, (CIBER-BBN), Spain
[6] Author to whom any correspondence should be addressed.

E-mail: enrico.caiani@polimi.it

RECEIVED
25 June 2019

REVISED
19 September 2019

ACCEPTED FOR PUBLICATION
8 October 2019

PUBLISHED
30 October 2019

Keywords: microgravity, head-down bed-rest (HDBR), ventricular repolarization (VR), electrocardiogram (ECG), T-wave alternans (TWA), cardiac arrhythmias

Abstract

Objective: To assess the effects of different durations of simulated microgravity exposure on ventricular repolarization (VR) in terms of T-wave alternans (TWA) as well as to test whether an increase in VR heterogeneity could be detected once normal gravity was restored. *Approach*: A total of 63 healthy volunteers were recruited in several head-down bed-rest (HDBR) experiments in the context of the European Space Agency bed-rest strategy. TWA is evaluated during the night period using ambulatory ECG recordings, before, during and after long- (60 d), mid- (21 d) and short- (5 d) duration HDBR by the long-term averaging technique. *Main results*: 5–21 d of exposure to simulated microgravity by means of the HDBR model do not lead to a significant increase of cardiac electrical instability in healthy myocardial substrates up to the point of eliciting TWA on the surface ECG. However, TWA indices increased after long-term HDBR exposure, once normal gravity was re-established, indicative of incipient electrical instability on VR at the conclusion of 60 d of HDBR. *Significance*: The results of this work underline the importance of focusing future research on immediate effects after long-term microgravity exposure, both simulated by HDBR or from space mission scenarios, once partial gravity conditions are re-established. A deeper insight in the understanding of human body reactions in these scenarios results crucial in the design of future long-duration spaceflight missions, to mitigate any potential risk that can limit astronaut's performance.

1. Introduction

The control of autonomic and cardiovascular (CV) systems, even in the absence of disease, depends partly on a set of environmental conditions that, if modified, could produce unwanted changes. One of these conditions is gravity. It is well known that prolonged exposure to weightlessness (0 Gz) leads to CV deconditioning, inducing significant changes in autonomic control of the CV system (Convertino and Hoffler 1992), which can also have potential impact on cardiac electrical activity. Consequently, cardiac rhythm disturbances may occur if cardiac repolarization is adversely influenced. In this context, special attention is focused nowadays on the study of body reaction (including CV response) to gravity restoration after a long period of 0 Gz exposure.

Indeed, alterations on ventricular repolarization (VR) induced by both spaceflight and microgravity simulated on Earth (usually by means of the head-down bed-rest (HDBR) model (Pavy-Le Traon *et al* 2007)) have been investigated during the last two decades (Caiani *et al* 2016). In particular, long-duration spaceflight has been found to prolong the QTc interval in some crewmembers (D'Aunno *et al* 2003). Moreover, a reversible increase of spatial and temporal VR heterogeneity was observed after short (5 d) and long (90 d) HDBR experiments on healthy subjects (Sakowski *et al* 2011, Caiani *et al* 2013). Both studies evaluated VR from the electrocardiogram

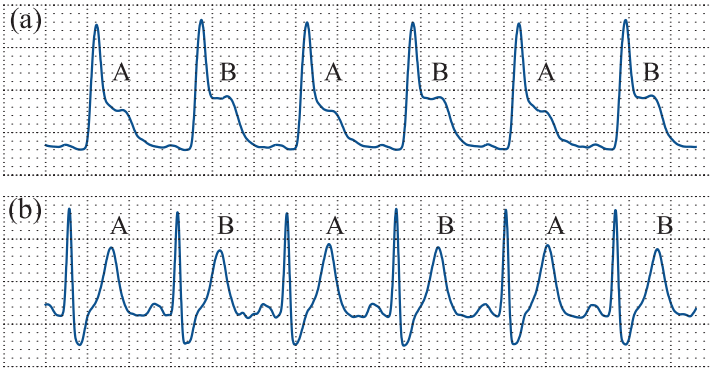

Figure 1. Two examples of real ECG signals with evident T-wave alternans (TWA) (a) and microvolt-level TWA (b) recorded during two percutaneous coronary interventions.

(ECG) signal by means of the QRS-T angle and the spatial ventricular gradient (SVG). All these results support the hypothesis that a weightlessness condition increases arrhythmic risk.

In our previous work (Martín-Yebra *et al* 2015), we studied HDBR-induced changes on T wave alternans (TWA) activity, defined as a consistent alternation in the amplitude, duration and/or morphology of the ST-T complex in a beat-to-beat basis (figure 1), before and after 5 and 21 d of HDBR. TWA has been postulated as an ECG marker of cardiac electrical instability and ventricular vulnerability, associated to the occurrence of ventricular arrhythmias (Verrier *et al* 2011). In that study (Martín-Yebra *et al* 2015), short-term TWA analysis was performed under two stress conditions, orthostatic tolerance and bicycle exercise stress tests, performed both before the start of the HDBR and immediately after its conclusion. Results suggested that neither 5 nor 21 d of HDBR were long enough periods to induce a significant increase in VR heterogeneity in terms of TWA under stress conditions, in contrast to previous observations (Grenon *et al* 2005).

In clinical practice, TWA analysis was originally performed under controlled heart rate conditions, typically by exercise-induced stress. However, this approach led to an elevated number of indeterminate TWA tests due to the non-stationarity conditions, as ECG signals commonly appear corrupted by excessive noise and by the presence of ectopic beats. As an alternative, and in order to reduce the number of indeterminate tests, the long-term analysis of TWA in 24 h ambulatory ECG recordings has become a matter of increasing interest with promising results (Verrier and Nearing 2003, Verrier *et al* 2005, Maeda *et al* 2009, Monasterio *et al* 2012).

Based on these observations, we hypothesized that an increase in VR heterogeneity could be related to the length of microgravity exposure, and reveal itself only once normal gravity conditions (i.e. 1 Gz) are restored.

Accordingly, the aim of this work is to assess the effects of different durations of simulated microgravity exposure on ventricular repolarization in terms of TWA by the long-term averaging technique in ambulatory ECG recordings (Monasterio *et al* 2012), as well as to test whether an increase in VR heterogeneity could be detected once HDBR was concluded and normal gravity restored. In particular, TWA will be evaluated during the night period before, during and after long- (60 d), mid- (21 d) and short- (5 d) duration HDBR experiments.

2. Methods

2.1. The head-down bed rest strategy

Since on-orbit research is limited, the head-down (−6°) bed rest (HDBR) model has been proved to be a reliable ground-based simulation analogue for most physiological effects of spaceflight (Pavy-Le Traon *et al* 2007). This manoeuvre represents a model of chronic circulatory unloading, simulating sustained exposure to microgravity, and offers a unique opportunity for studying the effects of prolonged space-flight on the CV system, as well as for testing potential countermeasures (CM) to prevent CV deconditioning. Since 2001, the European Space Agency (ESA) has conducted such HDBR studies, where subjects are placed on a bed with a 6° negative tilt for periods ranging from 5 d (short-term) to 2 months (long-term).

In particular, ECG data were acquired from two short-duration (5 d, denoted as SHORT from here on), two mid-duration (21 d, denoted as MID) and two long-duration (60 d, denoted as LONG) HDBR campaigns, using the same equipment and experimental protocols in all of them. These campaigns were hosted by two specialized centers: the ':envihub' at the German Aerospace Center (Deutsches Zentrum für Luft-und Raumfahrt e.V, DLR) in Cologne (Germany) and the Institut de Médecine et de Physiologie Spatiales (MEDES) in Toulouse (France).

The protocols, depicted in figure 2, included several days of acclimation to the bed rest facility, referred as PRE period (5, 8 and 15 d respectively for SHORT, MID and LONG). Those days are denoted as baseline data collection, BDC-X to BDC-1, where *X* stands for the *X*th day before the beginning of the uninterrupted HDBR period.

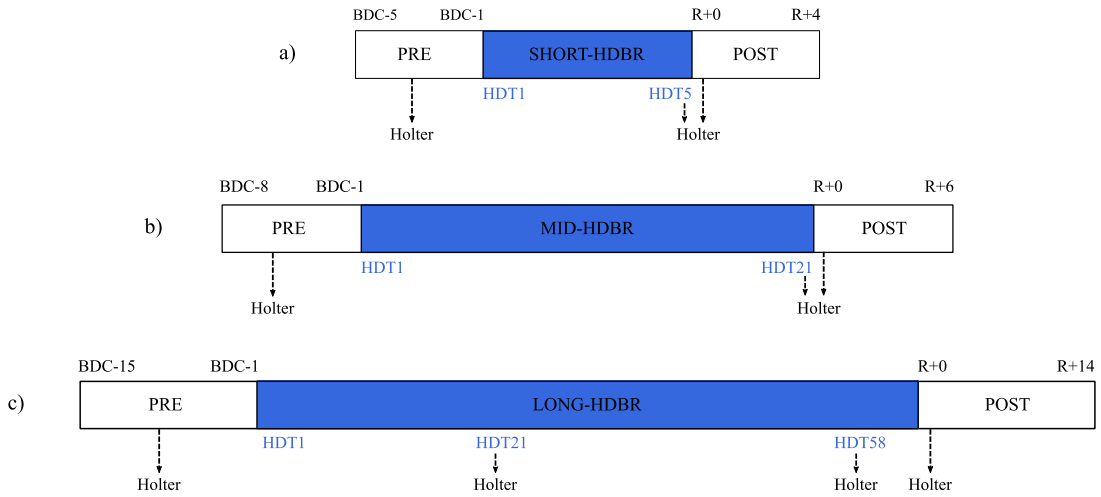

Figure 2. Head-down bed-rest (HDBR) protocols with timing of the Holter ECG recordings acquisitions considered in this manuscript, for (a) SHORT, (b) MID and (c) LONG duration campaigns.

During the HDBR period, all subjects adhered to a strict HDBR 24 h a day for 5, 21 or 60 d for SHORT, MID and LONG campaigns, respectively. Those days are denoted as HDTX (head-down tilt). Subjects underwent a strict monitoring and controlled diet in order to prevent body weight changes. Sleeping hours were scheduled from 11:00 pm to 6:30 am at DLR and from 11:00 pm to 7:00 am at MEDES, and napping was not allowed during the day. After concluding the HDBR period, subjects had to remain in the facility for additional 5, 7 or 15 d (referred as POST period), respectively for SHORT, MID and LONG, denoted as R+X, where R+0 is the day when the immobilization period ended by an orthostatic tolerance (OT) test performed in the morning. During the PRE and POST periods, lying on bed during the day was not allowed. Figure 2 illustrates the protocols for SHORT, MID and LONG campaigns including the specific time points of ECG acquisition considered in this manuscript.

For SHORT and MID campaigns, the protocol was designed as a crossover study: every subject repeated the HDBR two or three times, one time with no intervention (CONTROL) and in the other(s) with specific CM(s) applied during HDBR, with a washout period (1.5 months for SHORT and 4 months for MID) between the end of one repetition to the onset of the next one. The order of inclusion in any intervention group was randomly assigned to each subject. Due to the long washout period (>2 years) required in the case of long-duration recruitments, a multi-group design (one control and one intervention group) was adopted for LONG campaigns.

2.2. Study population

An only-male population was recruited, after multiple screening and psychological tests. Subjects had no history of CV disease and were not taking medications of any kind. The choice of including only males was driven by ESA standardization plan. For each HDBR campaign, all volunteers provided written informed consent to participate in the study, approved by the respective Ethical Committee for Human Research at each of the hosting institutions.

2.2.1. Short-duration HDBR campaigns

One SHORT campaign (three repetitions) was performed at MEDES (ESA acronym: BR-AG1), in which 12 subjects (age range 21–41 years) were enrolled. An additional SHORT campaign (three repetitions) was organized at DLR (ESA acronym: SAG), including ten subjects (age range 25–44 years).

2.2.2. Mid-duration HDBR campaigns

One MID campaign (three repetitions) was performed at MEDES (ESA acronym: MNX), in which 12 subjects (age range 20–44 years) were enrolled. An additional MID campaign (two repetitions) was organized at DLR (ESA acronym: MEP), including ten subjects (age range 23–42 years).

2.2.3. Long-duration HDBR campaigns

One LONG campaign was performed at DLR (ESA acronym: RSL), including 24 subjects (12 in control, 12 in the CM group, age range 20–45 years). An additional LONG campaign was organized at MEDES (ESA acronym: Cocktail) in which 20 subjects (10 in control, 10 in the CM group, age range 20–45 years) were enrolled.

As different CMs were adopted in the different campaigns, only data from the CONTROL group were analysed in the present study. For the SHORT campaigns, all the 22 subjects in the CONTROL group completed the experiments, while for the MID, 20 out of 22 subjects in CONTROL did (1 withdrawal in each location).

Table 1. Anthropometric data of subjects in control group participating in SHORT (5 d), MID (21 d) and LONG (60 d) duration head-down bed-rest European Space Agency campaigns. Data are presented as median (25th;75th percentiles).

	Short	Mid	Long
	($n = 22$)	($n = 20$)	($n = 21$)
Age (years)	31.6 (25.4;35.8)	32.0 (28.25;40.0)	28.0 (27.3;36.0)
Weight (kg)	76.2 (73.6;80.4)	68.2 (63.9;76.8)	76.6 (68.7;83.4)
Height (m)	1.79 (1.75;1.82)	1.77 (1.74;1.83)	1.78 (1.72;1.81)

In LONG, one subject dropped out on BCD-4 for medical reasons not related to the study in RLS campaign. Therefore, the final study population is composed of 22 subjects in SHORT, 20 subjects in MID and 21 subjects in LONG. Anthropometric data of the final population included in this study for each HDBR campaign duration is presented in table 1.

2.3. ECG acquisition

In this work, we have analyzed 24 h Holter ECG recordings (H12+, Mortara Instrument Inc., Milwaukee, WI, 12 leads, 1000 Hz sampling frequency) acquired at PRE, at the end of the HDBR period and at POST (R+0). In LONG, additional Holter ECG acquisitions recordings were also available at 21 and 57 d from the beginning of HDBR. The timing of the ECG Holter acquisitions taken into consideration in this study at each campaign is indicated in figure 2. In order to avoid potential effects on cardiac response derived from the multiple concomitant experiments scheduled during the day, which also differed among the different periods of the HDBR (PRE, HDT and POST), and among the study campaigns, only the portion of ECG acquisitions during the night period (23:00–06:00) were selected for this analysis.

2.4. Prepocessing

Preprocessing of ECG recordings included QRS detection using a wavelet-based ECG delineator (Martínez *et al* 2004) and baseline wander removal in each lead using a cubic spline interpolation technique. Then, ECG was low-pass filtered (cut-off frequency: 15 Hz) to attenuate out-of-band noise and artefacts, and downsampled to 125 Hz, reducing the computational cost without compromising the TWA analysis. Finally, the ventricular repolarization phase (ST-T complex) was segmented at each beat, by defining a fixed interval of 350 ms starting at the end of the QRS complex.

2.5. TWA analysis

Automatic TWA analysis was performed in three steps: (1) selection of signal segments suitable for further analysis; (2) estimation of the TWA amplitude for each segment; (3) computation of the nightly index of average alternans activity that characterizes each Holter (Monasterio *et al* 2012). Each step is explained next.

ECG signals were processed in segments of 128 consecutive beats, with 50% overlap between adjacent segments. In order to exclude possible transient segments present in the signal from the analysis, a stability criterion based on heart rate (HR) and baseline wander was defined, as in as in Monasterio *et al* (2012). In particular, a suitable ECG segment must accomplish these conditions: (i) the difference between the maximum and minimum instantaneous HR in the segment less than 20 beats min^{-1}, and (ii) at least 80% of the beats needs to fulfill two additional conditions: (a) the difference between the ith and the $(i-1)$ th RR intervals is less than 150 ms; (b) the difference between the baseline voltage measured at the ith PQ segment and the one measured in the $(i-1)$ th beat before baseline wander removal is lower than 300 μV.

The TWA waveform associated to the kth ECG segment, $\boldsymbol{y}_k$, was estimated following the same multilead approach as described in Monasterio *et al* (2012). First, the eight independent standard leads (V1 to V6, I and II) were linearly combined using periodic component analysis, which maximizes the 2-beat periodicity of the ECG signal (the TWA periodicity) in the combined lead (Monasterio *et al* 2010). Then, the Laplacian likelihood ratio method (LLRM) (Martínez and Olmos 2005) was applied in the combined lead to estimate the TWA waveform $\boldsymbol{y}_k$ for each segment, denoted as

$$\boldsymbol{y}_k = [y_k(1), \cdots, y_k(N)]^T,$$

with N the total number of samples within the ST-T complex.

Before averaging the estimated TWA waveforms, a phase-alignment method (17) was applied so that all of them presented a positive polarity. As they may be computed from non-consecutive segments (that is, some

unstable segments are discarded), they could present opposite polarity depending on the initial phase of the TWA sequence inside each segment, which might lead to cancellation when averaging.

This phase aligment step was performed according to the cross-correlation of each TWA waveform with the 'dominant waveform', obtained by a classical principal component analysis (we refer the reader to Martín-Yebra *et al* (2018) for more details). In brief, we maintained or changed the TWA waveform polarity depending on whether its correlation with the dominant waveform was positive or negative, respectively. The *k*th phase-aligned TWA waveform is denoted as $\boldsymbol{y}_k^a = \left[y_k^a(1), \cdots, y_k^a(N)\right]^T$.

Finally, the index of average alternans (IAA) was defined as the mean absolute value of the average of all phase-aligned waveforms:

$$\mathrm{IAA} = \frac{1}{N}\sum_{n=1}^{N}\left|\frac{1}{K}\sum_{k=1}^{K} y_k^a(n)\right|.$$

Similarly to our previous study (Martín-Yebra *et al* 2015), where we evaluated the short-term TWA analysis under stress conditions, the IAA normalized by the average T-wave amplitude of the recording was also computed (IAA*n*). This average T-wave amplitude was computed as the mean value of the ST-T complex in the first principal component obtained by PCA analysis in the available standard leads.

2.6. Statistical analysis

Data are presented as median (25th and 75th percentiles), unless otherwise specified. To evaluate differences in TWA activity among the different stages of the HDBR (PRE, HDT and POST), the non-parametric Friedman test and Wilcoxon signed rank paired test with Bonferroni correction were applied for repeated measurements. In addition, to evaluate the effect of different HDBR durations (SHORT, MID and LONG) in TWA activity, the non-parametric Kruskal–Wallis test and Mann–Whitney test were applied. For all tests, the null hypothesis was rejected when $p \leqslant \alpha_c$, with $\alpha_c = 0.05/M$ being M the total number of multiple comparisons.

3. Results

The distributions of average HR, IAA and IAA*n* computed during the night period are shown in figure 3.

3.1. Short-duration HDBR

A significant increase in average HR at POST (70 (53.7;67.1) beats min^{-1}) was found both compared to PRE (59.5 (50.5;66.5) beats min^{-1}, $p = 0.009$, $\alpha_c = 0.0166$) and to HDT5 (54.9 (51.0;63.2), $p = 0.001$, $\alpha_c = 0.0166$). No significant differences induced by HDT were found in IAA, neither in absolute nor in normalized amplitudes (figure 3, top panels).

3.2. Mid-duration HDBR

The same effect was observed after 21 d of HDBR (figure 3, middle panels). Average HR increased at POST (66.2 (58.2;73.5) beats min^{-1}) compared to PRE (57.3 (52.6;62.9) beats min^{-1}, $p = 0.002$, $\alpha_c = 0.0166$) and compared to HDT21 (54.6 (46.1;59.6) beats min^{-1}, $p < 0.001$, $\alpha_c = 0.0166$) but no significant differences induced by HDT in terms of TWA were found.

3.3. Long-duration HDBR

A significant increase in HR was found at POST (73.6 (69.5;79.6) beats min^{-1}) compared to PRE (57.2 (52.3;61.0) beats min^{-1}, $p < 0.001$, $\alpha_c = 0.0083$), HDT21 (54.7 (53.3;58.6) beats min^{-1}, $p < 0.001$) and HDT57 (58.6 (54.7;63.6) beats min^{-1}, $p < 0.001$, $\alpha_c = 0.0083$). A similar tendency was observed with TWA indices: IAA showed an increasing trend at POST (0.462 (0.334; 0.542) μV) compared to PRE (0.398(0.280;0.490 μV, NS)) and to the end of the HDBR (HDT57: 0.248 (0.190;0.347) μV, $p = 0.0325$, $\alpha_c = 0.0083$), although differences did not reach the significance level (figure 3, bottom panels). These increases became significant when TWA was normalized by the T-wave amplitude (IAA*n*: 0.15(0.085;0.23)%) at POST versus 0.09(0.05;0.13)% at PRE ($p = 0.0046$), 0.09(0.04;0.13)% at HDT21 ($p = 0.0046$), and 0.08 (0.04;0.11)% at HDT57 ($p = 0.0033$, in all cases $\alpha_c = 0.0083$). Linear correlation with HR was $r^2 = 0.189$ and $r^2 = 0.255$, for IAA and IAA*n*, respectively.

An illustration of TWA measurement in one subject from LONG campaign is shown in figure 4. The ECG segment with highest alternans value was selected both from PRE and POST recordings from which median ST-T complexes of even (blue dotted line) and odd beats (grey line) are plotted, with a detailed view around the T peak. While no difference between median beats at PRE is visible, a few microvolt-level difference is visible at POST. To note also here the decrease in the T-wave amplitude from PRE to POST.

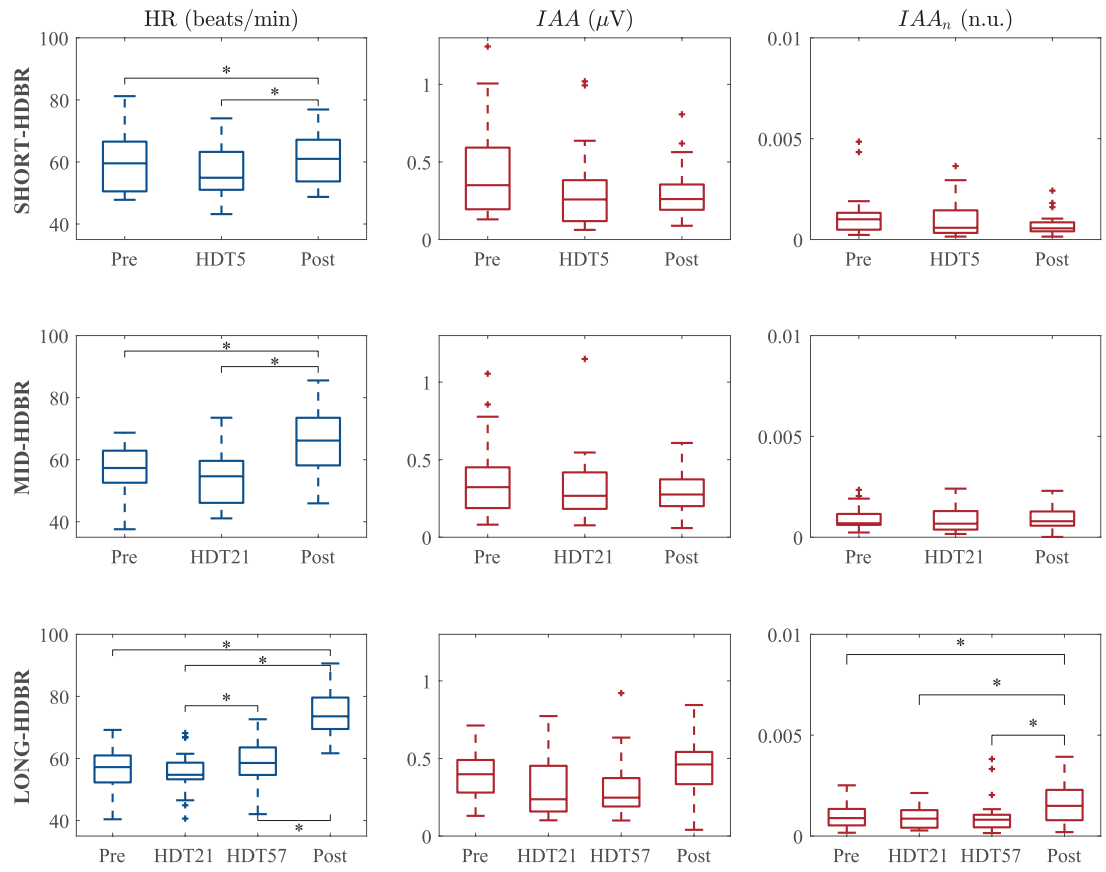

Figure 3. Distribution of average HR, IAA and IAA*n* (normalized by T-wave amplitude) computed before (PRE), the last day of head-down bed-rest (HDBR), and after (POST), in SHORT (top panels), MID (middle panels) and LONG (bottom panels) of HDBR. $*$: $p < \alpha_c$.

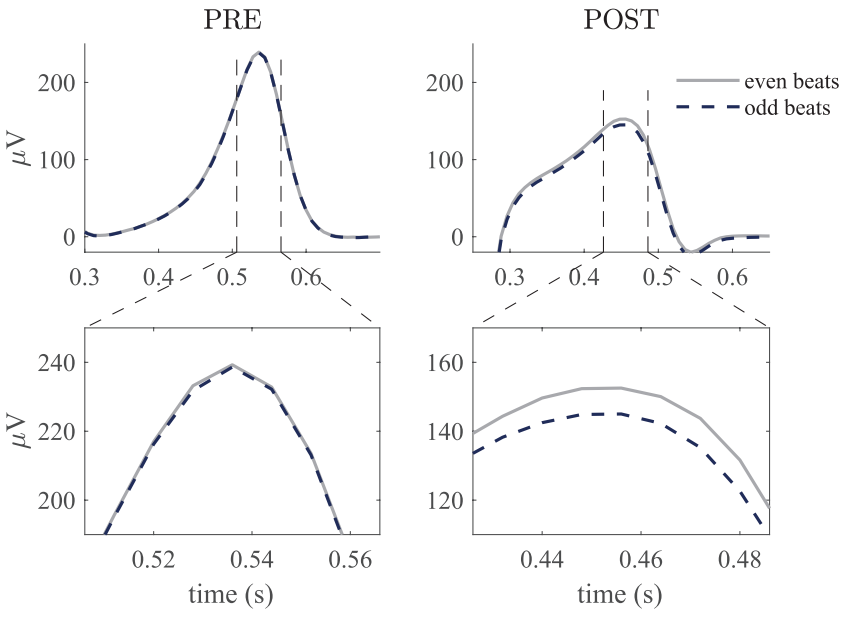

Figure 4. Illustration of T-wave alternans (TWA) measurement on the πCA combined lead in one particular subject. The plot shows the median ST-T complex of even beats (blue dotted line) and odd beats (grey line) computed in a 128-beat ECG segment from one particular subject at PRE (left column) and at POST (right column). ECG segments were selected according to the highest magnitude of the alternans waveform for each recording. A close-up of the ST-T segments, delimited by the vertical lines, is shown in bottom panels. In the example, average TWA amplitude of the segment was 0.45 μV at PRE and 5.22 μV at POST. In the same patient, the TWA indices after averaging the whole recording were **IAA** = 0.13 μV at PRE and and **IAA** = 0.59 μV at POST.

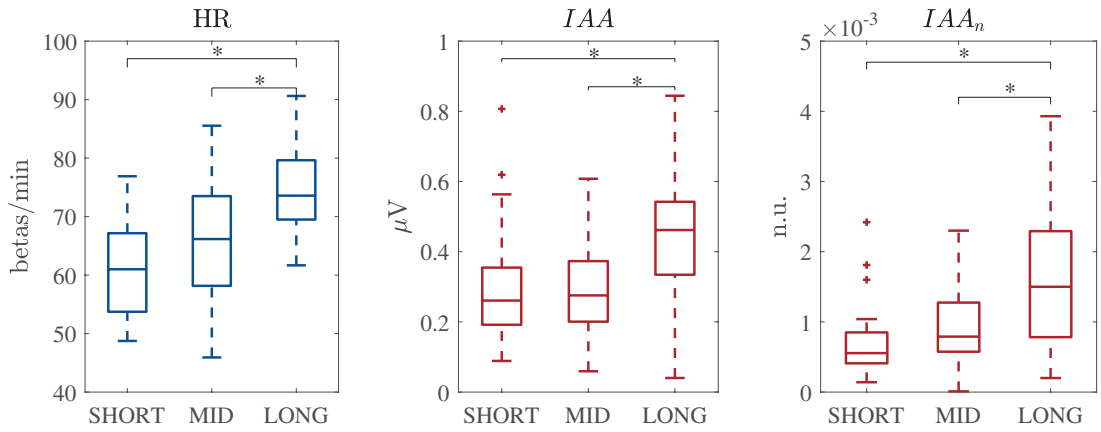

Figure 5. Distribution of average HR, IAA and IAA*n* (normalized by T-wave amplitude) at POST for the different HDBR durations (SHORT, MID and LONG). $*: p < \alpha_c$.

3.4. Effect of HDBR duration

We also compared the effect of HDBR duration on HR and TWA activity at three different stages of BR (PRE, last day of HDBR and POST). While no significant differences among the three groups (SHORT, MID and LONG) were found at PRE or at the end of the HDBR (results not shown), we found that subjects undergoing LONG-HDBR had significantly higher HR and IAA the first day of the recovery period (i.e. at POST) in comparison to subjects in both SHORT and MID campaigns (figure 5).

4. Discussion

In this study, we focused on the analysis of ventricular repolarization alterations induced by HDBR in terms of TWA activity. TWA was assessed at rest, by long-term averaging of ambulatory ECG recordings during the night hours. The analysis was restricted to the night period in order to avoid any potential confounding effects on cardiac activity elicited by other experiments, scheduled mainly during the day period.

Sedentary HDBR led to an increase in average nocturnal heart rhythm at POST in all SHORT, MID and LONG campaigns. TWA in Holter ECGs has been found to increase with HR in the range between 70 and 110 in heart failure patients (Monasterio *et al* 2012). In the present study, HR was generally lower and subjects were healthy volunteers. Despite that, we would have expected some HDBR-induced changes, as it has been reported that HDBR reversibly increases ECG repolarization heterogeneity and, consequently, potentially ventricular arrhythmic risk (Sakowski *et al* 2011, Caiani *et al* 2013, 2016). However, we did not find a clear significant increase in the index of average alternans (IAA) between PRE and POST after 5- and 21 d HDBR. Nonetheless, an increasing tendency in IAA at POST with respect to both PRE and to the end of the 60 d immobilization period (i.e. HDT57) was observed in LONG campaings, although it did not reach statistical significance after Bonferroni correction. It should be noted that Bonferroni correction could be overconservative in practical situations where repeated tests are not truly independent (as it happens in our case, where the comparison between PRE and HDT57 is not completely independent from the comparison between PRE and HDT21, for example).

Interestingly, those changes became significant when IAA was normalized by changes on T-wave amplitude (IAA). T-wave morphology has been previously reported to be altered by HDBR (Sakowski *et al* 2011, Caiani *et al* 2013), as a result of the fluid loss and hypovolemia, which in turn resulted in a diminished plasma volume and shrinking of heart cavities (Caiani *et al* 2014). Normalization allowed then to discount the effect of T-wave amplitude changes in TWA amplitude estimation.

An important result from our analysis is that subjects undergoing LONG HDBR presented significantly higher average HR, IAA and IAA*n* values on the first day of the recovery period (i.e. at POST) than subjects that were immobilized during shorter periods (SHORT and MID). Looking at the distribution of HR and IAA indices, the question of whether the increase in TWA could have been induced by the same pattern observable on average HR arises. However, the low linear correlation found between both parameters seems to indicate that TWA changes are likely related to a more complex and heart-rate unrelated mechanisms. The increased electrical instability at POST reflected by the increase in IAA and IAA*n* after 60 d exposure to simulated microgravity, once normal gravity conditions have been restored, reveals that ventricular repolarization mechanisms may also be altered during this period. Such finding could provide a deeper insight into the understanding of human body reaction after a long period of weightlessness condition once gravity is restored, which results crucial in the design of long-duration space missions.

Already in Kramer *et al* (2017), subjects from the RSL study (11 out of them were included in our LONG group) were reported to present similar behaviour on daily resting HR. In that study, differences in average HR between control and the CM group (intense jump training program, 12 subjects) were also observed. In particular, resting HR increased slowly from the middle of the HDBR, with a more pronounced rise at the beginning of the recovery period in the control group. In the CM group, the HR increase was not so evident, since only a slight increase was observed at the beginning of the recovery phase, while the HR was significantly lower than in control. Increased HR during or after HDBR has been associated with decreased stroke volume and maximal cardiac output, as well as with a decreased cardiac vagal tone together with increased sympathetic stimulation and beta-receptor sensitivity (Convertino and Hoffler 1992). Since all those factors, which are also known to play a role in the appearance of TWA phenomenon, can be affected by physical exercise, the inclusion of exercise-based countermeasures, as in case of RSL study, may have a positive impact on preventing or reducing cardiovascular deconditioning, as well as in reducing risk relevant to TWA once gravity level is re-established. Indeed, when we evaluated IAA in both control and JUMP groups (Martín-Yebra *et al* 2017), the increase at POST was no longer visible in the CM group, thus suggesting that a 5/6 jump exercise sessions per week during HDBR may be an effective CM.

In SHORT and MID campaigns, the absence of significant increase in IAA after HDBR measured from Holter ECG recordings was in agreement with the fact that no increase of TWA was found under stress conditions (i.e. orthostatic tolerance and aerobic power tests) in the same campaigns (Martín-Yebra *et al* 2015). This supports the hypothesis that the duration of exposure to microgravity is an important factor in influencing the underlying phenomena. To the best of our knowledge, only one previous work assessed TWA before and after 9–16 d of HDBR (Grenon *et al* 2005). In contrast to our findings, in Grenon *et al* (2005) Grenon and colleagues reported an increased number of subjects with positive TWA test after the immobilization period, concluding that simulated microgravity induces microvolt TWA. However, the different responses of the subjects to the HDBR did not support a clear evidence of an adverse effect of HDBR on cardiac electrical instability in terms of TWA in that study population. In particular, four out of 24 subjects (17%) already had a positive TWA test at PRE, while this number increased up to 10 (41.7%) at POST. Unexpectedly, two of the TWA positive subjects at PRE were labelled as TWA negative at POST.

These results underline the importance of focusing future research on immediate effects following the end of long-term microgravity exposure, both simulated by LONG HDBR and resulting from space mission scenarios, where Earth or partial gravity conditions are re-established. A deeper insight in the understanding of human body reactions in these scenarios could be crucial in the design of future long-duration space flight missions (to Moon or Mars), where landing on a partial gravity environment is expected. As depicted by our results, an increase in TWA indices was visible at R+0 after 60 d HDBR, thus possibly highlighting an increase risk in arrhythmia susceptibility that could pose a risk, or limit the astronaut's performance, in this particular scenario.

In any case, the complex nature of these kind of experiments limits the amount of data available in each HDBR campaign in terms of number of subjects. Hence we tried to cope with this by pulling together subjects of different campaigns with the same duration, considering only the control group, this retrospective analysis cannot take into account specific conditions relevant to each HDBR (different bed rest facility and setting, possible seasonal effects in the subject's behaviour, etc) that could have influenced the participating subjects in different ways. Moreover, the multiple experiments scheduled during the day period (from 10 to 16), required the subjects to actively perform a set of activities or being subjected to particular procedures, such as orthostatic tilt table test, exercise stress tests and sometimes also minimally invasive (i.e. muscle biopsies), with known potential impact on the CV system. Those experiments differed among the different periods of the HDBR (PRE, HDT and POST) and among the study campaigns. Therefore, in order to guarantee the most comparable scenario among the different periods of HDBR, i.e. PRE, HDT and POST, we decided to limit the TWA analysis to the night period, when the subjects were lying on bed in resting conditions, avoiding any potential interference due to the complex experimental set-up. Finally, we limited our analysis to TWA as defined in the literature (Verrier *et al* 2011), that is, as an ABABAB… alternating pattern. The analysis of other repetitive patters, which were considered out of the scope of this study, remains to be investigated.

5. Conclusion

In conclusion, the results of this study indicate that 5–21 d of exposure to simulated microgravity by means of the HDBR model do not lead to a significant increase of cardiac electrical instability in healthy myocardial substrates up to the point of eliciting TWA on the surface ECG. However, an increasing tendency was observed in TWA indices, after long-term HDBR exposure once normal gravity was re-established, which became significant after normalization by the average T-wave amplitude. Such finding can be indicative of incipient electrical instability on VR at the conclusion of 60 d of HDT. Further investigations, perhaps considering additional ECG-risk markers and a larger population, would be required in order to confirm this microgravity-induced effect on

cardiac repolarization, which could explain a potentially increased arrhythmia propensity after weightlessness condition.

Acknowledgments

This work was supported by the Italian Space Agency (contracts 2018-7-U.0, 2013-033-R.0, I/047/10/0, recipient E G Caiani). Also, by CIBER in Bioengineering, Biomaterials & Nanomedicne (CIBER-BBN) through Instituto de Salud Carlos III and FEDER (Spain), project DPI2016-75458-R funded by MINECO and FEDER, and by Gobierno de Aragón (Reference Group BSICoS T39-17R) cofunded by FEDER 2014–2020 'Building Europe from Aragon'. The computation was performed by the ICTS NANBIOSIS, specifically by the High Performance Computing Unit of the CIBER-BBN at the University of Zaragoza.

ORCID iDs

Alba Martín-Yebra https://orcid.org/0000-0003-0226-4950
Violeta Monasterio https://orcid.org/0000-0001-5343-5671
Pablo Laguna https://orcid.org/0000-0003-3434-9254
Juan Pablo Martínez https://orcid.org/0000-0002-7503-3339
Enrico G Caiani https://orcid.org/0000-0002-1770-6486

References

Caiani E G, Martin-Yebra A, Landreani F, Bolea J, Laguna P and Vaïda P 2016 Weightlessness and cardiac rhythm disorders: current knowledge from space flight and bed-rest studies *Frontiers Astron. Space Sci.* **3** 27

Caiani E, Massabuau P, Weinert L, Vaïda P and Lang R 2014 Effects of 5 days of head-down bed rest, with and without short-arm centrifugation as countermeasure, on cardiac function in males (BR-AG1 study) *J. Appl. Physiol.* **117** 624–32

Caiani E, Pellegrini A, Bolea J, Sotaquira M, Almeida R and Vaïda P 2013 Impaired T-wave amplitude adaptation to heart-rate induced by cardiac deconditioning after 5-days of head-down bed-rest *Acta Astronaut.* **91** 166–72

Convertino V and Hoffler G 1992 Cardiovascular physiology. Effects of microgravity *J. Florida Med. Assoc.* **79** 517–24 (https://www.ncbi.nlm.nih.gov/pubmed/1402772)

D'Aunno D, Dougherty A, DeBlock H and Meck J 2003 Effect of short- and long-duration spaceflight on QTc intervals in healthy astronauts *Am. J. Cardiol.* **91** 494–7

Grenon S, Xiao X, Hurwitz S, Ramsdell C, Sheynberg N, Kim C, Williams G and Cohen R 2005 Simulated microgravity induces microvolt T wave alternans *Ann. Noninvasive Electrocardiol.* **10** 363–70

Kramer A, Kümmel J, Mulder E, Gollhofer A, Frings-Meuthen P and Gruber M 2017 High-intensity jump training is tolerated during 60 days of bed rest and is very effective in preserving leg power and lean body mass: an overview of the cologne RSL study *PLOS One* **12** e0169793

Maeda S *et al* 2009 Ambulatory ECG-based T-wave alternans and heart rate turbulence predict high risk of arrhythmic events in patients with old myocardial infarction *Circ. J.* **73** 2223–8

Martínez J and Olmos S 2005 Methodological principles of T wave alternans analysis: a unified framework *IEEE Trans. Biomed. Eng.* **52** 599–613

Martínez J, Almeida R, Olmos S, Rocha A and Laguna P 2004 A wavelet-based ECG delineator: evaluation on standard databases *IEEE Trans. Biomed. Eng.* **51** 570–81

Martín-Yebra A, Caiani E G, Monasterio V, Pellegrini A, Laguna P and Martínez J P 2015 Evaluation of T-wave alternans activity under stress conditions after 5 d and 21 d of sedentary head-down bed rest *Physiol. Meas.* **36** 2041

Martín-Yebra A, Monasterio V, Cygankiewicz I, Bayés-de-Luna A, Caiani E G, Laguna P and Martínez J P 2018 Post-ventricular premature contraction phase correction improves the predictive value of average T-wave alternans in ambulatory ECG recordings *IEEE Trans. Biomed. Eng.* **65** 635–44

Martín-Yebra A, Monasterio V, Laguna P, Martínez J P and Caiani E G 2017 Evaluation of changes in T-wave alternans induced by 60-days of immobilization by head-down bed-rest *Presented at the 2017 Computing in Cardiology (CinC)* pp 1–4

Monasterio V, Clifford G, Laguna P and Martínez J 2010 A multilead scheme based on periodic component analysis for T-wave alternans analysis in the ECG *Ann. Biomed. Eng.* **38** 2532–41

Monasterio V, Laguna P, Cygankiewicz I, Vázquez R, Bayés-Genís A, Bayés-de-Luna A and Martínez J 2012 Average T-wave alternans activity in ambulatory ECG records predicts sudden cardiac death in patients with chronic heart failure *Heart Rhythm* **9** 383–9

Pavy-Le Traon A, Heer M, Narici M, Rittweger J and Vernikos J 2007 From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006) *Eur. J. Appl. Physiol.* **101** 143–94

Sakowski C, Starc V, Smith S and Schlegel T 2011 Sedentary long-duration head-down bed rest and ECG repolarization heterogeneity *Aviat. Space Environ. Med.* **82** 416–23

Verrier R and Nearing B 2003 Ambulatory ECG monitoring of T-wave alternans for arrhythmia risk assessment *J. Electrocardiol.* **36** 193–7

Verrier R *et al* 2011 Microvolt T-wave alternans physiological basis methods of measurement and clinical utility—consensus guideline by International Society for Holter and Noninvasive Electrocardiology *J. Am. Coll. Cardiol.* **58** 1309–24

Verrier R, Nearing B and Kwaku K 2005 Noninvasive sudden death risk stratification by ambulatory ECG-based T-wave alternans analysis: evidence and methodological guidelines *Ann. Noninvasive Electrocardiol.* **10** 110–20