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

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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 days), mid- (21 days) and short- (5 days) duration HDBR by the long-term averaging technique.

Main results: 5 to 21 days 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 days of HDBR.

Significance: 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.

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

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 days) and long (90 days) HDBR experiments on healthy subjects (Caiani et al., 2013; Sakowski et al., 2011). Both studies evaluated VR from the electrocardiogram (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 days 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 days 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 (Maeda et al., 2009; Monasterio et al., 2012; Verrier et al., 2005; Verrier and Nearing, 2003).

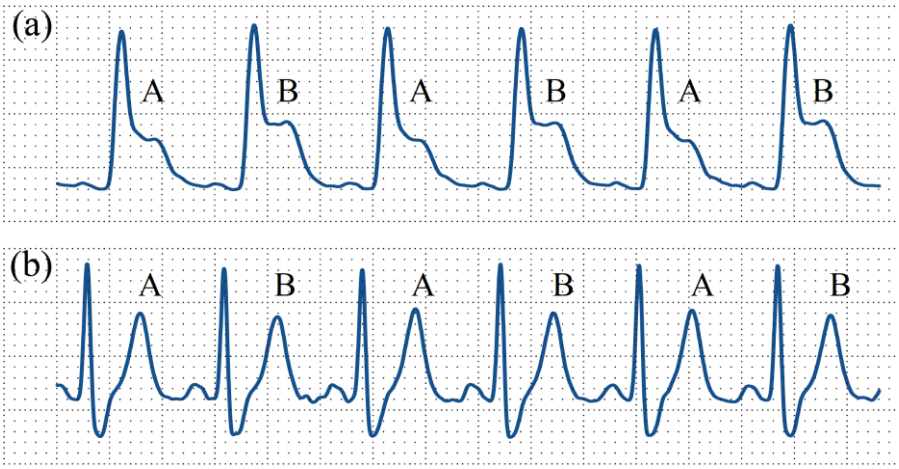

Figure 1: Two examples of real ECG signals with evident TWA (a) and microvolt-level TWA (b) recorded during two percutaneous coronary interventions.

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., 1Gz) 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 days), mid- (21 days) and short- (5 days) duration HDBR experiments.

2. Methods

2.1. The head-down bed rest strategy

Since on-orbit research is limited, the head-down (-6 degrees) 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-degree negative tilt for periods ranging from 5 days (short-term) to two months (long-term).

In particular, ECG data were acquired from two short-duration (5 days, denoted as SHORT from here on), two mid-duration (21 days, denoted as MID) and two long-duration (60 days, 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 days 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. During the HDBR period, all subjects adhered to a strict HDBR 24h a day for 5, 21 or 60 days 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 days (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.

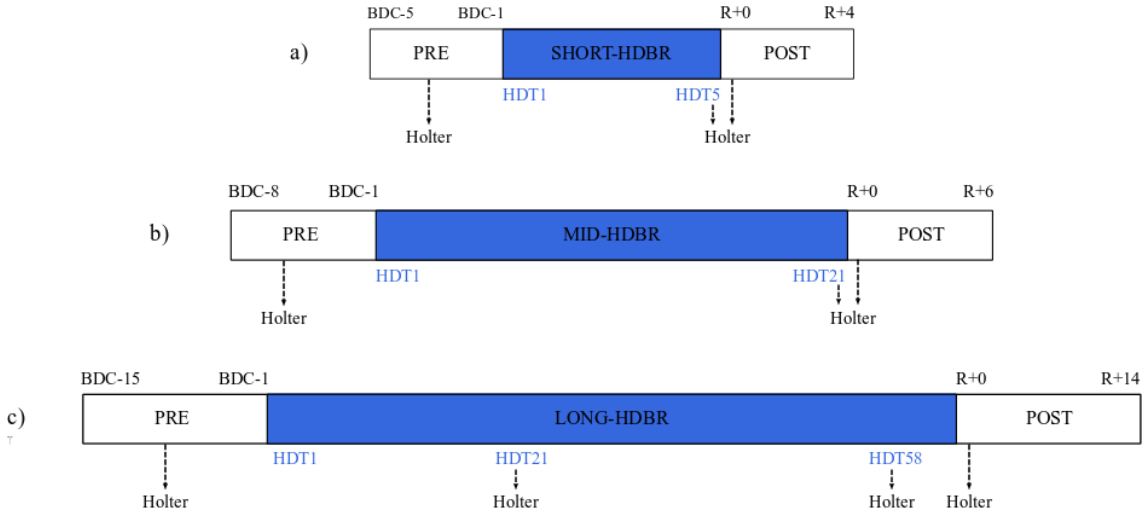

Figure 2: Head-down bed-rest protocols with timing of the Holter ECG recordings acquisitions considered in this manuscript, for (a) SHORT, (b) MID and (c) LONG duration campaigns.

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.

Short-duration HDBR campaigns

One SHORT campaign (3 repetitions) was performed at MEDES (ESA acronym: BR-AG1), in which 12 subjects (age range 21-41 years) were enrolled. An additional SHORT campaign (3 repetitions) was organized at DLR (ESA acronym: SAG), including 10 subjects (age range 25-44 years).

Mid-duration HDBR campaigns

One MID campaign (3 repetitions) was performed at MEDES (ESA acronym: MNX), in which 12 subjects (age range 20-44 years) were enrolled. An additional MID campaign (2 repetitions) was organized at DLR (ESA acronym: MEP), including 10 subjects (age range 23-42 years).

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). 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.

Table 1: Anthropometric data of subjects in control group participating in SHORT (5 days), MID (21 days) and LONG (60 days) duration head-down bed rest ESA campaigns. Data are presented as median (25^{th};75^{th} percentiles).

	SHORT (n=22)	**MID** (n=20)	**LONG** (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)

2.3. ECG acquisition

In this work, we have analyzed 24h 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 days 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 3 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, and (ii) at least 80% of the beats needs to fulfill two additional conditions: a) the difference between the i^{th} and the $(i-1)^{th}$ RR intervals is less than 150 ms; b) the difference between the baseline voltage measured at the i^{th} 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 k^{th} ECG segment, $\boldsymbol{y}_k$, was estimated following the same multilead approach as described in (Monasterio et al., 2012). First, the 8 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 $\boldsymbol{y}_k^a = [y_k^a(1), \cdots, y_k^a(N)]^T$.

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

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

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 ($IAAn$). 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 $p \leq \alpha_c$, with $\alpha_c = 0.05/M$ being M the total number of multiple comparisons.

3. Results

The distributions of average HR, IAA and $IAAn$ computed during the night period are shown in Figure 3.

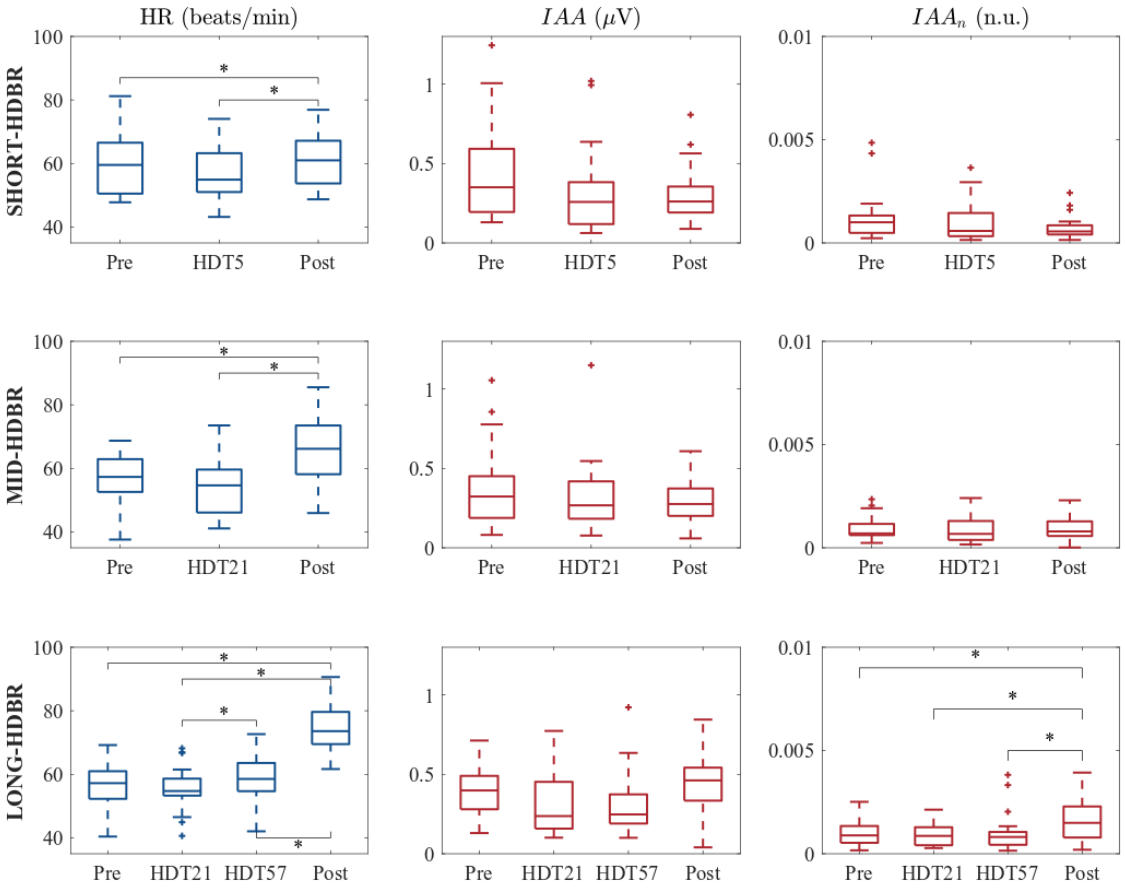

Figure 3: Distribution of average HR, IAA and $IAAn$ (normalized by T-wave amplitude) computed before (PRE), the last day of HDBR, and after (POST), in SHORT (top panels), MID (middle panels) and LONG (bottom panels) of HDBR. *: $p<\alpha_c$.

Short-duration HDBR

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

Mid-duration HDBR

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

Long-duration HDBR

A significant increase in HR was found at POST (73.6 (69.5;79.6) beats/min) compared to PRE (57.2 (52.3;61.0) beats/min, p<0.001, α_c = 0.0083), HDT21 (54.7 (53.3;58.6) beats/min, p<0.001) and HDT57 (58.6 (54.7;63.6) beats/min, p<0.001, α_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, α_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 ($IAAn$: 0.15(0.085;0.23)% at POST vs 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 α_c = 0.0083). Linear correlation with HR was r^2 =0.189 and r^2 =0.255, for IAA and $IAAn$, respectively.

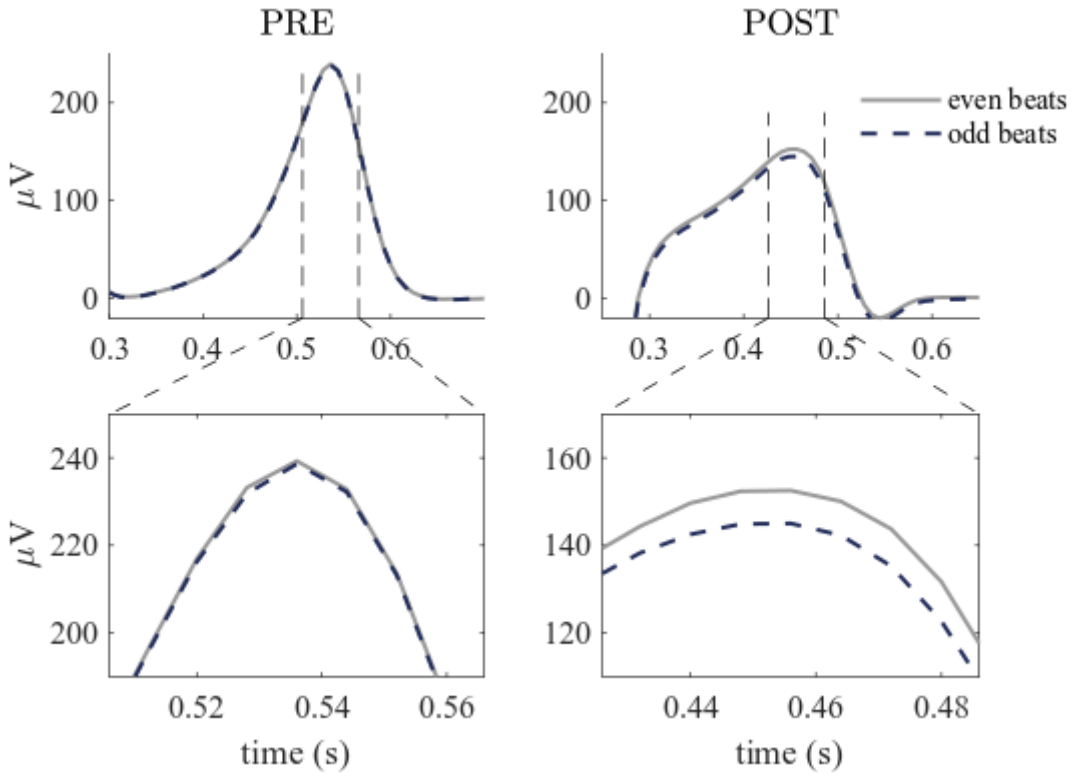

Figure 4: Illustration of TWA measurement on the πCA combined lead in one particular subject. The plot shows the median ST-T complex of even beats (blue dotted line) and odd beats (grey line) computed in a 128-beat ECG segment from one particular subject at PRE (left column) and at POST (right column). 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 $\boldsymbol{IAA}$ = 0.13 μV at PRE and and $\boldsymbol{IAA}$ = 0.59 μV at POST.

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.

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).

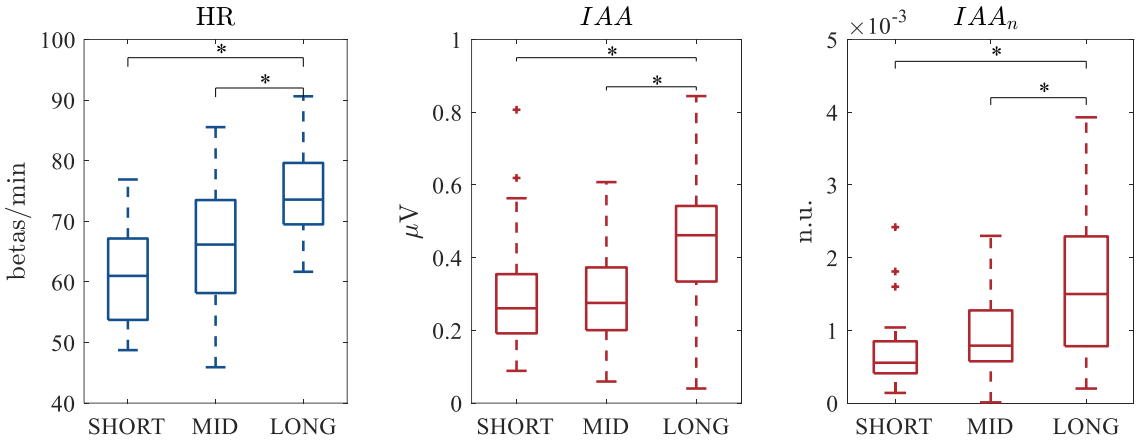

Figure 5: Distribution of average HR, IAA and $IAAn$ (normalized by T-wave amplitude) at POST for the different HDBR durations (SHORT, MID and LONG). *: p<α_c.

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 (Caiani et al., 2013, 2016; Sakowski et al., 2011). However, we did not find a clear significant increase in the index of average alternans (IAA) between PRE and POST after 5- and 21-day

HDBR. Nonetheless, an increasing tendency in IAA at POST with respect to both PRE and to the end of the 60-day 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 (Caiani et al., 2013; Sakowski et al., 2011), 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 $IAAn$ 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 $IAAn$ after 60-day 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 to 16 days 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, 4 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-days 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, results of this study indicate that 5 to 21 days 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 days 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.

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