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Cardiovascular Autonomic Adaptation of the Mars500 Crew during Parabolic Flight

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Received: date / Accepted: date

Abstract Purpose: During spaceflight, astronauts are submitted to a combination of stressors, including weightlessness, confinement, radiation, sleep disturbances, etc. The aim of this study was a first attempt to disentangle the combined effects of weightlessness and confinement on the cardiovascular regulation system.

Methods: Autonomic cardiovascular control was determined with continuous ECG and noninvasive finger blood pressure measurements in six members of the Mars500 crew and six healthy control subjects in supine and standing positions during parabolic flight. Heart rate (HRV) and blood pressure variability (BPV) were determined in time and frequency domain.

Results: Before parabolic flight, HRV analyses showed higher vagal responses for the control group when the difference between supine and standing posture was considered. In accordance with this observation, the Mars500 crew expressed a higher sympathetic outflow via BPV in supine position, while vagal predominance was expected. In-flight, HRV analyses showed higher vagal indices for the control group during microgravity in standing position.

Conclusions: In the Mars500 crew, a reduced vagal and an increased sympathetic modulation were found compared to the control group. These findings suggest that even after six months completion of the confinement study, some influences on the ANS still remain.

Keywords parabolic flight · confinement · Mars500 · heart rate variability · blood pressure variability

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1 Introduction

Space has always intrigued mankind. Several space missions have been conducted in the last decades and now there is a desire to travel to Mars. However, such space missions are not without risks for the astronauts; the lack of gravity has proven to alter several systems in the human body, such as the renal, cardiopulmonary and musculoskeletal systems (Aubert et al., 2005). Moreover, upon return to earth, astronauts might suffer from orthostatic intolerance (Sides et al., 2005), which might be linked to an impaired cardiovascular autonomic control after prolonged spaceflight (Aubert et al., 2005; Baevsky et al., 2007; Cooke et al., 2000; Goldberger et al., 1994). However, there is another aspect in long-term spaceflights which is often not taken into account, i.e. that the astronauts are faced with several emotional and physical challenges. This long-term confinement might lead to alterations in the immune system (Chouker et al., 2002) and even elicit cardiac diseases (Malliani, 2000). Studies on the autonomic control of the heart during prolonged spaceflights thus not only study the effect of long-term exposure to microgravity, but also the response of the cardiovascular system to confinement.

In preparation for a future spaceflight to Mars, six subjects were selected to simulate a mission to Mars. This so-called Mars500 crew conducted numerous experiments and were confined for 520 days. Only the lack of gravity was not simulated (ESA, 2013). In this study, we aim to submit the same crew to microgravity to see how their autonomic nervous system (ANS) is altered under gravity transitions. This is a first step towards disentangling confinement effects from gravity effects. Up till now, space missions include simultaneously both effects. Now, the unique opportunity to submit a crew separately to microgravity and confinement is offered.

There are several ways to conduct research in microgravity, but ground-based studies are the most cost-efficient. They include head-out-of-water immersion, head-down bedrest and parabolic flights, and have each their advantages and disadvantages (Aubert et al., 2004). Parabolic flights offer 20 s-periods of microgravity, preceded and followed by periods of hypergravity (1.6-1.8 g) and are chosen to submit the Mars500 crew to weightlessness. Several studies already investigated the response of the ANS to changing gravity conditions; a decreased heart rate and a higher vagal modulation of the ANS during microgravity, and a reduction in vagal modulation during hypergravity were reported in (Beckers et al., 2003; Linnarsson et al., 1996; Verheyden et al., 2005). The hemodynamic response to changing gravity is described in (Liu et al., 2011; Mukai et al., 1991; Pump et al., 1999), with among others a decrease in mean arterial blood pressure during short-term weightlessness.

The goal of this study is to compare the response to gravity transitions of a crew that was in confinement for 520 days with a matched control group. Cardiac ANS modulation is evaluated by means of heart rate (HRV) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and blood pressure (Laitinen et al., 1999) variability (BPV) techniques. The parabolic flights took place six months after the Mars500 study. As results from the 105 days-pilot confinement study (Mars105) and Mars500 study report that 13 days after the confinement, there are no autonomic adaptations left (Vigo et al., 2012; Vigo et al., 2013; Wan et al., 2011), we hypothesize that there will be no different response of the Mars500 crew compared to the control group.

2 Data

2.1 Mars500 Crew

Six healthy, non-smoking, male subjects were selected to participate in a confinement study of 520 days during which a Mars mission was simulated (June 2010 - November 2011). The same selection criteria as for astronauts were followed. This Mars500 crew (mean $\pm$ SD; age: 34 ± 5 yr; height: 176 ± 4 cm; weight: 81 ± 5 kg; BMI: 26 ± 2 kg/m^2) participated for this study in the 56th ESA parabolic flight campaign (PFC) in May 2012. With the exception of one subject, all of them took part in parabolic flights for the first time.

2.2 Control Group

In order to make a comparison between the response of the Mars500 crew and a group that did not participate in the confinement study, data of six subjects that participated in previous PFC were selected, such that they match in age and posture (mean $\pm$ SD; age: 33 ± 6 yr; height: 177 ± 7 cm; weight: 77 ± 7 kg; BMI: 24 ± 2 kg/m^2. The data were recorded during the 29th (November 2000) and 34th (April 2002) ESA parabolic flight campaign (Verheyden et al., 2005). Only data of subjects that participated for the first time in a parabolic flight were used to make a fair comparison. This group will further be referred to as the control group.

All subjects gave their written informed consent to participate in the study. Each member of the Mars500 crew underwent a special medico-physical examination at the aeromedical center of the DLR, Cologne. Each participant of the control group underwent a special flight medico-physical examination at the 'Medical center of the Belgian Air Force, Brussels' one month before the flight campaign in order to pass FAA III tests. The study was approved from an ethical point of view by the 'Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale', the Ethical Committee of the Faculty of Medicine, KU Leuven, Belgium and the ESA medical board. All subjects were free of any cardiopulmonary or other systemic diseases, none were taking any medications. The subjects were not allowed to take either general medications or medications for the control of motion sickness before and during the parabolic flight sessions in order to eliminate the effects of pharmacological agents on cardiovascular control.

2.3 Instrumentation

Data of the Mars500 subjects were collected using a Nexfin monitor (BMEYE, Amsterdam, The Netherlands). Pregelled Ag/AgCl electrodes (Red Dot, 3M, Saint Paul, MN, USA) were pasted on the thorax for 6-lead electrocardiogram (ECG) recordings. Continuous blood pressure (BP) measurements were obtained with an inflatable finger cuff with infrared plethysmography using Finapres technology (Finapres Medical Systems, Amsterdam, The Netherlands). ECG and BP recordings were sampled at 1000 Hz and 200 Hz respectively. Accceleration data were also recorded and matched with the acceleration signal by ESA.

The recordings of the control subjects contain ECG measurements with a lead II derivation. The analog output of the amplified ECG was connected to an A/D converter (DATAQ Instruments Inc., Akron, OH, USA) and sampled at 1000 Hz. In addition, blood pressure recordings were obtained with a photo-plethysmograph (Portapres, TNO, Amsterdam, The Netherlands).

2.4 Experimental Protocol

The subjects participated in a parabolic flight during which microgravity was obtained. The flights were organized by ESA and Novespace in Bordeaux, France. An Airbus A300 followed a parabolic trajectory, yielding a gravity profile as shown in Fig. 1. Each flight consisted of 31 parabolas. Between two parabolas, there was a break of almost 2 minutes. After every five parabolas, a longer break of 4 to 8 minutes was held. The data of the first nine parabolas were not used, such that the subjects could become accustomed to the microgravity experience. Next, the subjects were respectively standing and in supine position, each during 8 parabolas and were instructed not to talk during the parabolas. To prevent free floating during microgravity, the subjects were attached with belts around the feet in standing position and around the chest in supine position.

Prior (pre-flight) and immediately after the flight (post-flight), reference measurements were recorded in two positions (standing and supine), each during 10 minutes.

During the previous campaigns (29th and 34th PFC), only ECG and systolic blood pressure measurements in standing and supine position were recorded pre- and in-flight. Thus, no comparison of diastolic blood pressure measurements, as well as of the post-flight recordings could be implemented.

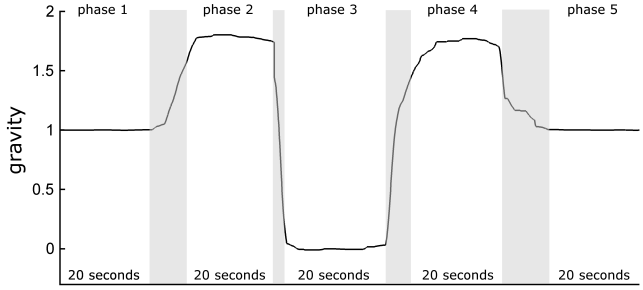


Fig. 1: Gravity profile of one parabola. The gray areas indicate the transition periods.

The data acquisition of the Mars500 crew was in collaboration with research on the acute stress response (id:9335) (Erasmus Experiment Archive (EEA), 2013) and on the coherence between brain cortical function and neuro-cognitive performance (id:9336) (Erasmus Experiment Archive (EEA), 2013) in the Mars500 crew.

2.5 Data Segmentation

Each parabola is divided in five phases, as shown in Fig. 1: 1. pre-normogravity (1 g), 2. pre-hypergravity (1.7-1.8 g), 3. microgravity (0 g), 4. post-hypergravity (1.6-1.8 g), and 5. post-normogravity (1 g). The segmentation was performed in a similar manner as described in (Beckers et al., 2003), i.e. >1.6 g for pre-hypergravity, <0.1 g for microgravity and >1.5 g for post-hypergravity. Each phase has an average duration of 20 s.

3 Methods

The data recorded with the Nexfin monitor are converted with FrameInspector (v1.32, BMEYE), such that further processing of the data could be performed in MATLAB R2012a (MathWorks, Natick, MA, USA).

3.1 Heart Rate Variability

Lead II of the ECG was used to determine the variability of the heart rate. For that purpose, the QRS complexes are detected using the Pan-Tompkins algorithm (Pan and Tompkins, 1985). All detections are corrected according to (Widjaja et al., 2010) and visually verified. The intervals between adjacent QRS complexes are the so-called normal-to-normal (NN) interval series and form a tachogram.

The quantification of HRV is performed using measures that are described in the Task Force on HRV (Task

Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Because we have segments of 20 seconds during the parabolic flights, we only use the following short-time HRV measures: meanNN [ms], SDNN [ms], RMSSD [ms], pNN50 [%], SDSD [ms], LF_{NN} [ms^2], HF_{NN} [ms^2], $LFnorm_{NN}$ [nu], $HFnorm_{NN}$ [nu] and LF/HF_{NN} [-]. Parasympathetic modulation is measured by RMSSD, pNN50, SDSD, HF_{NN} and $HFnorm_{NN}$, while the sympathovagal balance can be derived from $LFnorm_{NN}$ and LF/HF_{NN}.

During the pre- and post-flight recordings, the power spectrum is computed via Welch's method after resampling of the tachogram at 2 Hz using cubic spline interpolation. In the case of the in-flight recordings, the segments only have a duration of 20 seconds. Therefore, an appropriate technique to compute the power spectrum is needed. The procedure as described in (Verheyden et al., 2005) is followed and includes linear trend removal, tapering with a Hamming window, assessment of stationarity and zero padding.

3.2 Blood Pressure Variability

From the continuous blood pressure recordings, a systogram and diastogram are constructed. The systogram consists of consecutive systolic blood pressure values, while the diastogram comprises consecutive diastolic values. During the blood pressure recordings, there are periods to recalibrate the system. In those periods, cubic spline interpolation was performed using the time information derived from the heart rate.

Measures that quantify BPV are described in (Laitinen et al., 1999; Parati et al., 2013; Parati et al., 1995) and include meanBP [mmHg], SDBP [mmHg], LF_{BP} [mmHg^2], HF_{BP} [mmHg^2] and LF/HF_{BP} [-]. The same procedure as in the frequency domain HRV analysis is applied to compute the power spectrum of the systogram and diastogram. Note that in the remainder of the study, the abbreviation BP will be used when concerning both the systogram and diastogram. When it only concerns the systogram or diastogram, this will be abbreviated by SBP and DBP respectively.

3.3 Statistical Analysis

The non-parametric Friedman's test with repeated measures is used for the paired statistical analyses to study postural and gravitational effects in the Mars500 crew. Statistical significance is considered when $p < 0.05$. Due to the small number of subjects in the Mars500 crew and the control group, it is difficult to conduct reliable unpaired statistical analysis (Ioannidis, 2005; Sterne and Smith, 2001). Therefore, the differences between the Mars500 crew and the control group are described and interpreted based on tendencies in the parameters.

4 Results

Fig. 2 displays the signals that are recorded with the Nexfin monitor during one parabola in standing position. ECG and blood pressure recordings are shown, as well as their deduced tachogram, systogram and diastogram.

Fig. 3 depicts the values of HRV and BPV measures during the pre-flight recordings for the Mars500 crew and the control group, both in standing and supine position. Figs. 4 and 5 display boxplots of in-flight measures of respectively HRV and BPV.

4.1 Postural and Gravitational Effects in Mars500 crew

Prior to the analysis of postural and gravitational effects in the Mars500 crew, the effect of the parabolic flight is assessed by comparison of the pre- and post-flight recordings. For every position and for every HRV and BPV measure, no significant difference is found between pre- and post-flight. Therefore, postural effects during normo-gravity are analyzed using the pre- and post-flight data as repeated measures.

Effect of posture

All HRV measures, except for SDNN and LF_{NN}, show significant differences between standing and supine posture during the pre- and post-flight recordings. Increased values are observed in the supine position for meanNN, RMSSD, pNN50, SDSD, HF_{NN} and $HFnorm_{NN}$, while $LFnorm_{NN}$ and LF/HF_{NN} are suppressed. The effect of posture on the blood pressure is found in LF_{BP}, where a significant decrease is observed in supine position in both the systogram and diastogram. In the diastolic BPV, we also find reductions in meanDBP and SDDBP in supine position. The influence of posture in meanNN, HF_{NN}, meanSBP and LF_{SBP} during the preflight recordings are shown in Fig. 3. Significant differences are indicated by an asterisk (*). The other HRV and BPV measures exhibit a similar behaviour, but are not shown here.

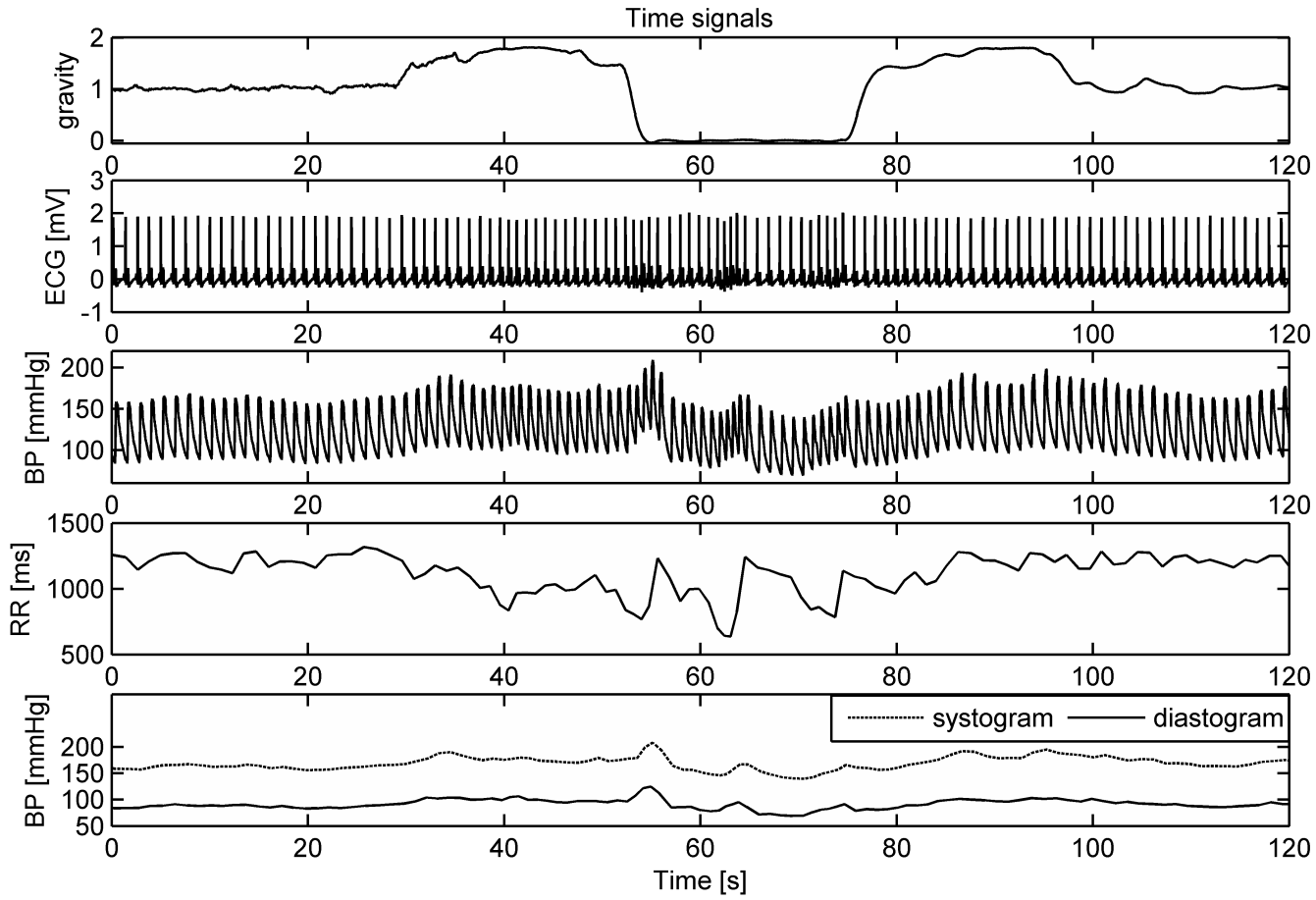


Fig. 2: Time signals acquired with the Nexfin monitor during one parabola in standing position. From top to bottom: gravity profile; lead II ECG signal; blood pressure signal; tachogram; systogram and diastogram.

When looking at the in-flight recordings, we observe that the differences in posture are more pronounced in the hypergravity phases. Then, significant differences between standing and supine are found for all HRV measures, except for SDNN and LF_{NN}, with always higher values in the supine than standing position, except for $LFnorm_{NN}$ and LF/HF_{NN}. Fig. 4 displays those differences for meanNN, RMSSD, pNN50 and HF_{NN} (indicated by *). During microgravity, we note significantly higher values in supine position for meanNN, RMSSD, pNN50, SDSD, HF_{NN} and $HFnorm_{NN}$, and lower values for $LFnorm_{NN}$ and LF/HF_{NN}. BPV displays a similar behaviour; the difference between standing and supine is significant for all BPV measures in hypergravity, except for LF/HF_{BP}, with always higher values in standing than supine position. During microgravity, all BPV measures except for LF_{SBP} and HF_{BP} show significantly higher values during standing than supine. These results are shown for the systogram for meanSBP, SDSBP, LF_{SBP} and HF_{SBP} in Fig. 5.

Effect of gravity transitions

The effect of gravity transitions during a parabolic flight is clearly seen in the standing position when we consider the HRV measures. Hypergravity (phases 2 and 4) reduces HRV compared to normo- and microgravity. This is significant for meanNN, SDNN, RMSSD, pNN50, SDSD, LF_{NN} and HF_{NN}. On the other hand, microgravity increases HRV compared to preceding and following hypergravity phases, but is only significantly higher than normogravity for SDNN. Also meanNN is significantly different during microgravity, but is higher than during hypergravity, and lower than during normogravity. These results are shown in Fig. 4. In the supine position, apart from meanNN that is significantly lower during the microgravity phase,

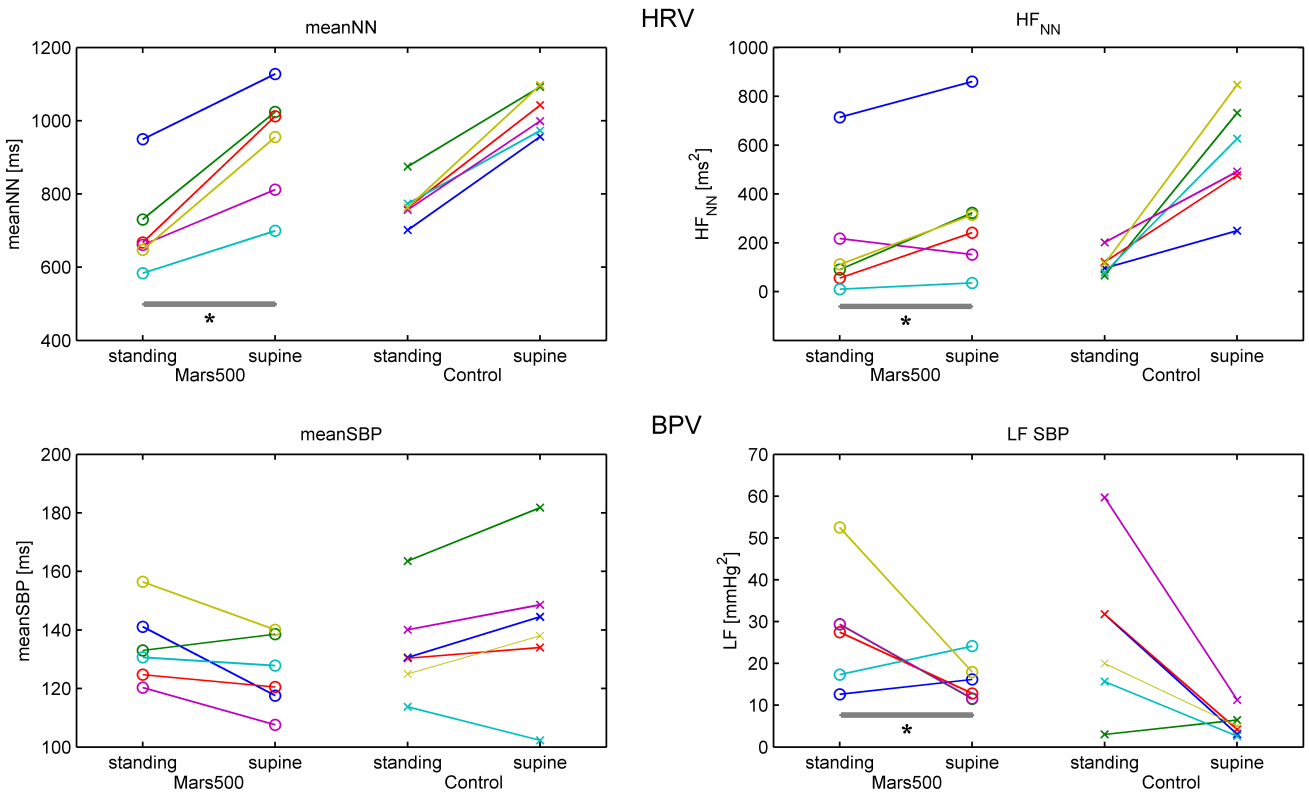


Fig. 3: Plots of pre-flight HRV and BPV measures of Mars500 crew and the control group in standing and supine position. (*: postural difference in the Mars500 crew)

no effects of gravity transitions are found in the HRV measures.

Gravity effects in BPV are only observed in the microgravity phase in standing position, where SDSBP and SDDBP are significantly higher, as given in Fig. 5. Only the diastolic meanBP is significantly lower in microgravity compared to the other phases. In the supine position, no differences in BPV are found.

4.2 Mars500 Crew versus Control Group

The comparison between the Mars500 crew and the control group is carried out on the pre- and in-flight data in both standing and supine position.

Pre-flight

No differences are found in supine and standing position during the pre-flight recordings when HRV measures are visually compared. We do find some dissimilarities between both groups when the difference between standing and supine is observed. We find that the postural differences tend to be smaller for pNN50, HF_{NN}, $LFnorm_{NN}$ and $HFnorm_{NN}$ for the Mars500 crew, as can be seen for HF_{NN} in Fig. 3.

In the systolic BPV measures, no differences are found between both groups in the standing position. In the supine position, a trend towards higher values for the Mars500 crew is found for LF_{SBP}, as shown in the bottom right panel in Fig. 3.

In-flight

The comparison in-flight between the Mars500 and the control subjects is performed per HRV and BPV measure and per position.

In the HRV measures when standing, we observe differences during microgravity for meanNN, RMSSD, SDSD and LF/HF_{NN}, with always lower vagal indices and higher indices for sympathovagal balance for the Mars500 crew, as can be seen in Fig. 4 for meanNN and RMSSD. During hypergravity, we notice differences between both groups for pNN50 and HF_{NN}, as shown in

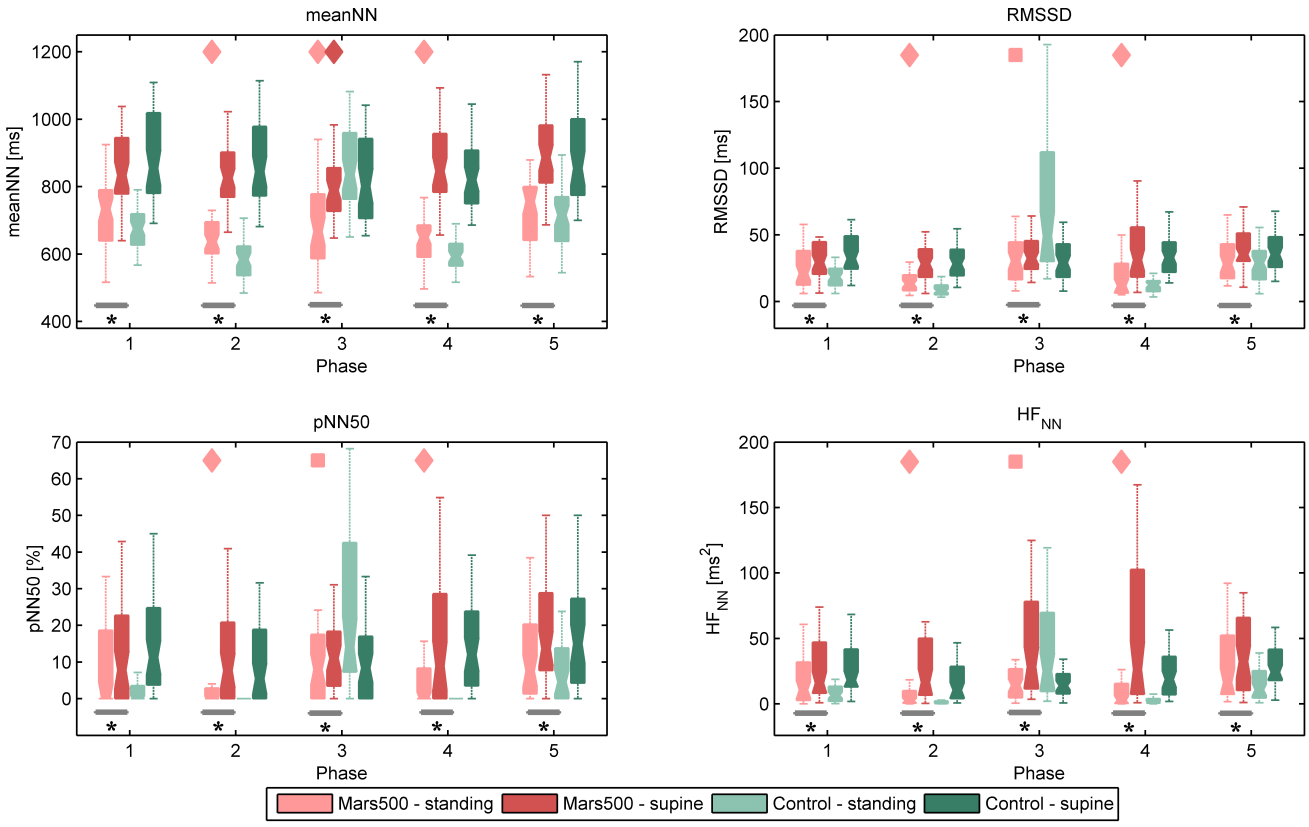


Fig. 4: Boxplots of in-flight HRV measures of Mars500 crew and the control group in standing and supine position. (*: postural difference in the Mars500 crew; ♦: gravitational difference with respect to the other phases in the Mars500 crew; ■: gravitational difference only with respect to the hypergravity phases in the Mars500 crew)

the bottom panels in Fig. 4. Here, higher vagal indices are observed for the Mars500 crew. In the supine position, only a difference in HF_{NN} is found during microgravity.

In the systolic BPV measures (see Fig. 5), we find a higher meanSBP and a lower SDSBP in the Mars500 subjects during microgravity in standing position. In supine position, we observe higher values in the Mars500 crew during the hyper- and microgravity phases for SDSBP and HF_{SBP}. During microgravity, also a higher LF_{SBP} is found in the Mars500 subjects.

5 Discussion

The aim of this study was to investigate if and how the autonomic adaptation during parabolic flight of a crew that was in confinement for 520 days is altered with respect to a control group. In order to characterize cardiovascular functioning, we continuously recorded ECG and blood pressure during parabolic flight in standing and supine position in the Mars500 crew. From the ECG and blood pressure measurements, we computed the tachogram, systogram and diastogram, from which several standardized HRV and BPV measures were derived. These indices were then used to evaluate the effect of posture and gravity on the ANS of the Mars500 crew. Next, differences in autonomic functioning between the Mars500 crew and the control group were evaluated on pre- and in-flight HRV and BPV measures.

The main findings of this study are firstly the observation of postural and gravitational effects in the Mars500 crew. Secondly, we found that the pre-flight recordings of the Mars500 crew and the control group are highly similar. Lastly and most importantly, we observed a long-term confinement effect on cardiovascular ANS control to transitions into weightlessness, even though the parabolic flights took place six months after the confinement study.

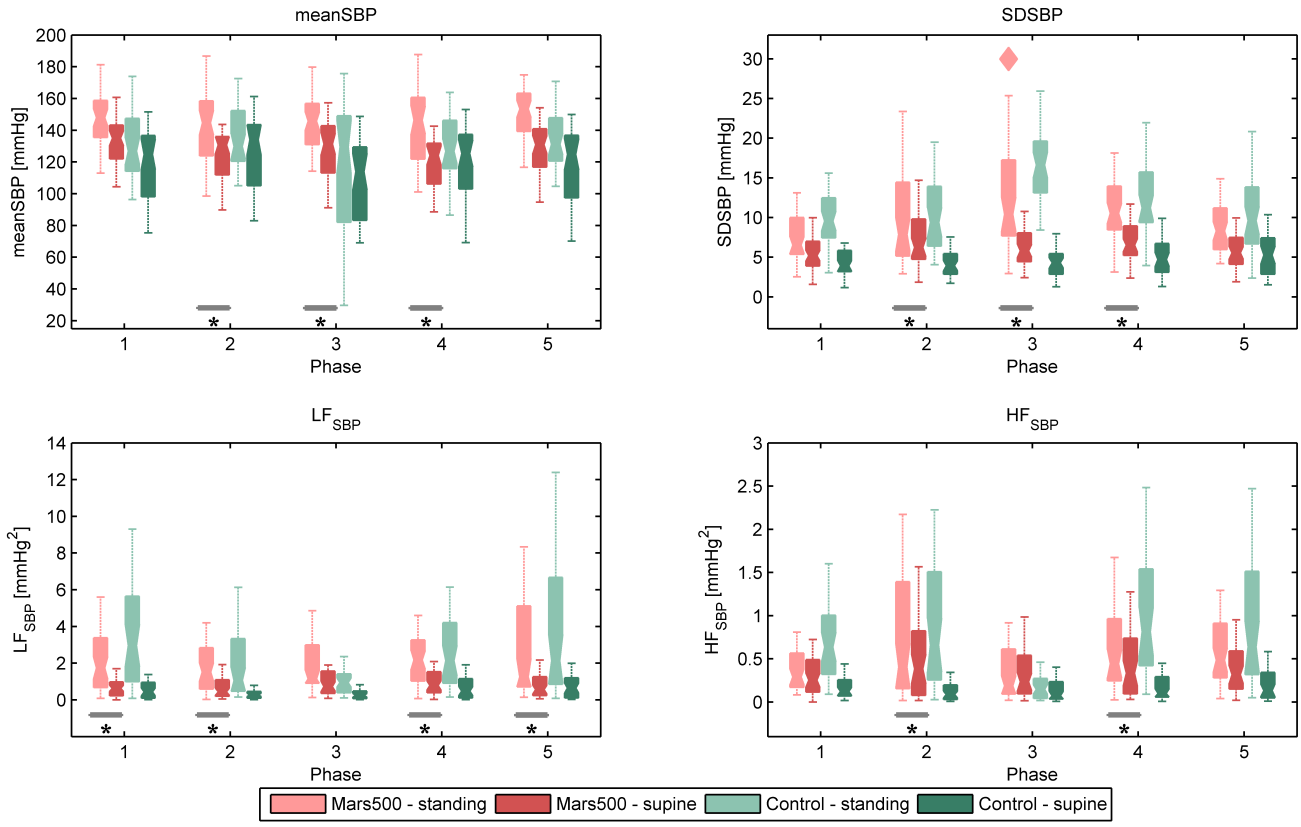


Fig. 5: Boxplots of in-flight BPV measures of Mars500 crew and the control group in standing and supine position. (*: postural difference in the Mars500 crew; ♦: gravitational difference with respect to the other phases in the Mars500 crew)

5.1 Postural Effects

The position of the body affects the autonomic nervous system; in supine position, there is a higher vagal outflow compared to the standing posture. Our comparisons between the positions using the pre- and post-flight recordings agree with this general autonomic response to posture. All vagal HRV indices increase in supine position, while indices for sympathovagal balance decrease. These observations in pre-flight data were also made in (Beckers et al., 2003; Verheyden et al., 2005).

The effects of posture on the blood pressure are limited. In the systogram, only a significant difference in LF_{SBP} was observed, where a reduction in sympathovagal balance was found in supine position. When analyzing the diastogram, differences were found in meanDBP, SDDBP and LF_{DBP}, with always higher values in standing than supine position. These results are in line with the publication by Netea *et al.* (Netea et al., 1998); they compared meanBP values in supine and sitting posture and found that meanSBP is not affected by posture. MeanDBP on the other hand appeared to be significantly higher seated than in supine. The increase in LF_{SBP} and LF_{DBP} from supine to standing is also reported in (Barnett et al., 1999).

When postural effects were studied during the different phases of a parabola, we found that both hypergravity phases magnify the differences between standing and supine. This was hypothesized as in the standing position, there is a blood shift towards the feet during hypergravity. This blood redistribution resulted in a decreased meanNN and vagal indices (RMSSD, pNN50, SDSD, HF_{NN}, $HFnorm_{NN}$) and increased values for $LFnorm_{NN}$ and LF/HF_{NN}. In addition, SDNN and LF_{NN}, which were not affected by postural differences in the pre- and post-flight recordings, exposed differences between standing and supine. The effect of posture during hypergravity on the time domain HRV measures was also reported by Beckers *et al.* (Beckers et al., 2003). Although they did not find any postural effects during hypergravity, they found "a tendency towards lower values for SDNN and RMSSD

in the standing position". The difference between standing and supine in the frequency domain HRV measures was observed in (Verheyden et al., 2005) and agrees with our findings.

The magnification of postural differences during hypergravity was also found in all BPV measures, with always higher values when standing. These differences are also a result of the redistribution of the blood.

During microgravity, we found higher values in supine position for all vagal HRV indices, and lower values for HRV indices for sympathovagal balance. The postural difference in meanNN, and the lack of a difference in SDNN is in contrast with what was reported by Beckers *et al.* and Verheyden *et al.* in (Beckers et al., 2003; Verheyden et al., 2005). They found no effect of posture in microgravity in meanNN and a significant difference in SDNN. The discrepancy in results possibly arises from the impaired ANS in the Mars500 crew, as will be discussed in Par. 5.3. Another explanation can be found in the fact that, apart from one Mars500 subject, it was the first time that the Mars500 crew experienced a parabolic flight. Beckers *et al.* showed that when subjects participate in two flights, there is a large inter-flight difference in among others meanNN (Beckers et al., 2003). Therefore, they only analyzed the data of the second flight. The detected differences in results can possibly be caused by this observation. In addition, note that the limited population size might also be responsible for differing findings.

Postural BPV differences during microgravity were also observed, except for LF_{SBP} and HF_{BP}, although Liu *et al.* reported no differences in meanSBP and meanDBP during microgravity (Liu et al., 2011). In addition, it was hypothesized that there would be no differences in other BPV measures due to the same blood distribution in supine and standing posture during microgravity. These dissimilarities can be attributed to the same explanations as given above.

5.2 Gravitational Effects

The effects of gravity transitions on HRV and BPV in the Mars500 crew were also investigated. The largest effects were hypothesized to occur in the standing position as there is a considerable blood redistribution during the different phases of each parabola. During hypergravity, the blood is pulled towards the feet, which causes a reduction in HRV, and vagal tone (Verheyden et al., 2005). Opposed to what was expected and what was found by Liu *et al.* (Liu et al., 2011), hypergravity did not exhibit effects on BPV.

The onset of weightlessness is characterized by a sympathetic withdrawal and acute activation of the vagal nervous system. Later in the microgravity phase, there is an increasing dominance of the sympathetic nervous system (Liu et al., 2011). Vagal modulation was found to be significantly higher compared to hypergravity. However, significant differences with normogravity were only found for SDNN and meanNN, where only SDNN was larger in microgravity. In contrast to what was found in previous studies, meanNN was reduced compared to normogravity (Verheyden et al., 2005).

The effect of microgravity on BPV was only apparent by significantly higher values for SDSBP and SDDBP. Short-term BPV is suggested to be related to central sympathetic drive (Parati et al., 2013). An increase in sympathetic modulation might explain why during microgravity, meanNN was reduced compared to normogravity. Also a decrease in meanDBP was reported during microgravity, which is in agreement with (Fritsch-Yelle et al., 1996; Liu et al., 2011).

In the supine position, it was hypothesized that there would be no effect of gravity in HRV or BPV because the blood is constantly equally distributed over the body. Our results confirm this. Only during microgravity, when standing, a significantly lower meanNN was observed. A possible explanation of this effect might be found in the excitement or stress when experiencing microgravity for the first time. As mentioned before, Beckers *et al.* showed that meanNN is largely influenced by repetitive exposure to microgravity (Beckers et al., 2003).

Note that between the pre-flight and post-flight recordings, no differences in HRV and BPV were found. This shows that immediately after the flight there were no effects in the ANS from the parabolic flight.

5.3 Confinement Effects

In prolonged spaceflight, changes in cardiovascular autonomic control have been observed, such as a decrease in heart rate and mean arterial blood pressure during space missions up to six months (Verheyden et al., 2009); a decreased HRV during controlled respiration (6 breaths/min), which indicates reduced vagal reserve in space (Baevsky et al., 2007); and a reduction in vagal nerve traffic and vagal baroreflex gain at least up to two weeks after return to Earth (Cooke et al., 2000). It is, however, not certain which physiological effects are related to the prolonged

exposure to weightlessness and which effects are linked to the long-term confinement. This is a first attempt to disentangle microgravity and confinement effects, where we submitted the six subjects of the Mars500 crew that were confined for 520 days to gravity transitions, including microgravity.

The Mars500 study revealed that during the wake periods of the confinement, there was an augmented parasympathetic modulation, as meanNN, SDNN, RMSSD, HF_{NN} were significantly higher compared to pre-confinement (one day of Holter recording within two months before confinement). During the sleep periods, a diminished parasympathetic modulation was observed via a decreased $HFnorm_{NN}$. In addition, the sleep-wake differences of $LFnorm_{NN}$, $HFnorm_{NN}$ and LF/HF_{NN} diminished during confinement (Vigo et al., 2013). These effects were also found during prolonged spaceflight, which indicates that the long-term confinement of the astronauts is at least partially responsible for the reduced vagal response.

Although Vigo *et al.* did not report pre- and post-confinement differences (Vigo et al., 2013), we compared the Mars500 crew to a matched control group. The effect of the 520 days in confinement was at first studied on the pre-flight data by comparing them in standing and supine position. In HRV, no differences in both the standing and supine position were found. We did observe dissimilarities between the groups when the differences in HRV measures between standing and supine were considered; a trend towards higher differences in vagal HRV indices was observed for the control group. This result can be linked to the diminished sleep-wake differences that were found during confinement and might indicate that the control group has a better adaptation of the ANS than the Mars500 crew. A similar observation was made when comparing the BPV; a significant increase in LF_{SBP} was found in the Mars500 crew in the supine position. LF_{SBP} is linked to sympathetic outflow and should be low in supine position. This might also point to a less efficient ANS of the Mars500 subjects.

When assessing whether long-term confinement has an impact on the ANS response to changing gravity, we found significant differences between the Mars500 crew and the control group during hyper- and microgravity. In the hypergravity phases, the blood is shifted towards the feet when standing, causing reduced vagal modulation. A larger reduction in vagal indices was observed for the control group. In addition, several vagal indices and meanNN appeared to be higher for the control group. These observations indicate that the ANS might be better adapted in the control group.

In the BPV measures, we found a higher meanSBP and a lower SDSBP in the Mars500 subjects during microgravity in standing position compared to the control group. Possibly, this is a result of the confinement, as Wan *et al.* noted a tendency to higher BP values during post-confinement than during pre-confinement (Wan et al., 2011). They also reported decreases in BP during confinement and seeing that meanDBP also decreased during microgravity, we can conclude that both confinement and weightlessness are responsible for the decrease in BP during prolonged space missions (Verheyden et al., 2009). In supine position, we found a higher LF_{SBP} in the Mars500 crew during the microgravity phase. This is consistent with the pre-flight comparison to the control group, and suggests a higher sympathetic outflow for the Mars500 crew when vagal predominance was expected.

In contrast to what we hypothesized, the overall comparison between the Mars500 crew and the control group indicated that the Mars500 crew exhibits smaller differences in both sympathetic and parasympathetic responses. A healthy cardiovascular system is able to fastly and adequately respond to the needs of the human body, such as parasympathetic predominance in supine position. A logic conclusion of these results would be that the cardiovascular autonomic control mechanisms, although post-confinement did not differ significantly from pre-confinement, were still affected by long-term confinement. We, however, want to add that the Mars500 subjects were highly selected, as they had to pass the same tests as astronauts, whereas the control group consists of subjects that only have to pass FAA III tests. Possibly, the obtained results are related to the selection procedure. As the pre-confinement recordings of the Mars500 crew and the pre-flight recordings of the control group followed a completely different protocol, it was impossible to assess whether those two groups, prior to any confinement or parabolic flight study, exhibit any differences.

This research, however, shows that in future studies towards efficient countermeasures for the deconditioning of the cardiovascular system in prolonged spaceflights, not only the effects of microgravity on the cardiac autonomic control should be considered (Antonutto and Di Prampero, 2003; Hargens et al., 2013); also the prolonged confinement might have long-term implications for cardiovascular control mechanisms.

5.4 Limitations and Future Work

One limitation of this study includes the use of parabolic flights. They offer the possibility to obtain microgravity, but in order to produce these microgravity conditions, hypergravity phases precede and follow the microgravity phase. Although the response of the autonomic nervous system is fast, we can not guarantee that the changes we observed during microgravity were not caused by the preceding hypergravity phase. Due to the fastly changing gravity periods, there was also a methodological challenge to compute power spectra. However, Verheyden *et al.* showed that their computation is able to reliably compute spectra with ultrashort data sets of 20 s (Verheyden et al., 2005).

Experiencing microgravity for the first time also means that the subjects were probably excited in the beginning. This might have influenced the ANS response. In order to make a fair comparison, we chose to use also control subjects that flew parabolic flights for the first time. In addition, in order to not influence ANS activity, the subjects were not allowed to take medication against motion sickness. As a consequence, two subjects of the Mars500 crew were nauseated during the flight. However, we evaluated the data of the nauseated persons and found no differences with the other subjects.

A last limitation of this study is the population size which is very small. The Mars500 crew only consists of six members. This is inherent in this type of research. Statistical analysis should therefore be interpreted with care. In addition, no statistical analysis could be conducted when comparing the Mars500 crew to the control group, which lead to results based on visual tendencies. However, considering the uniqueness of the experiment, analyses based on these six subjects are believed to be highly valuable.

In future work, the influence of respiration on heart rate and blood pressure variability will be studied during changing gravity; it is well-known that respiration is an important modulator of the heart rate, and recent research demonstrated that inclusion of respiratory information can greatly complement traditional HRV analyses during stress monitoring (Widjaja et al., 2013). We hypothesize that application of this new approach will be benificial for parabolic flight data, in order to gain more knowledge about ANS functioning during microgravity. In addition, time-frequency methods will be applied to track changes in spectral power in time (Orini et al., 2012); the fastly changing gravity levels make time-frequency methods an interesting tool as they allow us to study the dynamic response of the autonomic nervous system.

6 Conclusion

This study aimed at assessing the autonomic adaptation of the Mars500 crew during microgravity. To the best of our knowledge, this is the first time that effects of long-term confinement (as in a voyage to Mars) and weightlessness on autonomic cardiovascular control mechanisms have been studied separately.

Although the parabolic flights took place six months after the Mars500 study, we found a reduced vagal and increased sympathetic outflow when vagal activation was expected, e.g. in supine position or during microgravity. These observations suggest that the autonomic adaptation of the Mars500 crew might still be impaired after six months and they may possibly lead to a reconsideration of the development of optimal and efficient countermeasures.

Acknowledgements

We thank the subjects who volunteered for this study. We acknowledge the support from the European Space Agency and Novespace and the collaboration of the crew of the Airbus A300 ZERO-G, and a special thanks to Mr. Vladimir Pletser (ESA). Research supported by

- Research Council KUL: GOA MaNet, PFV/10/002 (OPTEC), several PhD/postdoc & fellow grants;
- Flemish Government:
  - FWO: Postdoc grants, projects: G.0427.10N (Integrated EEG-fMRI), G.0108.11 (Compressed Sensing), G.0869.12N (Tumor imaging), G.0A5513N (Deep brain stimulation);
  - IWT: PhD grants, projects: TBM 070713-Accelero, TBM 080658-MRI, TBM 110697-NeoGuard; D. Widjaja and K. Van Hoorde are supported by an IWT PhD grant;
  - iMinds: SBO dotatie 2013, ICONs: NXT_Sleep, FallRisk;
  - Flanders Care: Demonstratieproject Tele-Rehab III (2012-2014);
- Belgian Federal Science Policy Office: IUAP P719 (DYSCO, 2012-2017); ESA AO-PGPF-01, PRODEX (CardioControl) C4000103224;
- EU: RECAP 209G within INTERREG IVB NWE programme, EU HIP Trial FP7-HEALTH/ 2007-2013 (no. 260777), EU MC ITN TRANSACT 2012 (no. 16679), ERC Advanced Grant: BIOTENSORS (no. 39804), ERASMUS EQR: Community service engineer (no. 539642-LLP-1-2013).

List of abbreviations

ANS	autonomic nervous system
BPV	blood pressure variability
$[-]_{DBP}$	concerns measures computed from the diastolic blood pressure series
HF	power in the high frequency range [0.15-0.40 Hz]
HFnorm	relative HF power to the total power (calculated as LF+HF)
HRV	heart rate variability
LF	power in the low frequency range [0.04-0.15 Hz]
LFnorm	relative LF power to the total power (calculated as LF+HF)
LF/HF	ratio between LF and HF power
$[-]_{NN}$	concerns measures computed from the NN interval series
PFC	parabolic flight campagin
pNN50	percentage of NN intervals that differs more than 50 ms from the preceding NN interval
RMSSD	square root of the mean squared differences of successive NN intervals
$[-]_{SBP}$	concerns measures computed from the systolic blood pressure series
SD	standard deviation
SDSD	standard deviation of successive differences

The scientific responsibility is assumed by its authors.

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