

**Presyncopal cardiac contractility and autonomic activity in young
healthy males**

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Running head: Decreased cardiac contractility in presyncope

Summary

We investigated non-invasively cardiac contractility and autonomic nervous activity during presyncopal orthostatic stress induced in healthy humans. A graded orthostatic stress (GOS) paradigm, consisting of head-up tilt (HUT) combined with lower body negative pressure (LBNP) of increasing magnitude, was used to reach a presyncopal end-point in 15 healthy adults. Continuous beat-to-beat hemodynamic and autonomic parameters were recorded. From supine control (C1) to presyncope (PS), total peripheral resistance index (TPRI) decreased from 2300 ± 500 to 1910 ± 320 $\text{dyne} \cdot \text{s} \cdot \text{m}^2 / \text{cm}^5$ ($p=0.004$), index of contractility (IC) from 59 ± 14 to 27 ± 6 $1000/\text{s}$ ($p<0.0001$), left ventricular working index (LVWI) from 5.2 ± 1.3 vs. 3.6 ± 0.6 $\text{mmHg} \cdot \text{L} / (\text{min} \cdot \text{m}^2)$ ($p=0.0001$) and acceleration index (ACI) from 65 ± 18 vs. 54 ± 15 $100/\text{s}^2$ ($p=0.04$). Low frequency variation of diastolic blood pressure ($\text{LF}_{\text{nu}}\text{dBP}$) increased from 51 ± 14 to 67 ± 11 % ($p=0.0006$) and of systolic blood pressure ($\text{LF}_{\text{nu}}\text{sBP}$) from 50 ± 6 vs. 67 ± 8 % ($p<0.0001$). High frequency variation of RR-interval ($\text{HF}_{\text{ms}}\text{RRI}$) decreased from 385 ± 320 to 38 ± 43 ms^2 ($p=0.001$). From late GOS (G3) to PS, TPRI decreased from 2540 ± 640 to 1910 ± 320 $\text{dyne} \cdot \text{s} \cdot \text{m}^2 / \text{cm}^5$ ($p=0.003$), IC from 35 ± 6 to 27 ± 6 $1000/\text{s}$ ($p=0.003$), LVWI from 4.6 ± 0.9 to 3.6 ± 0.6 $\text{mmHg} \cdot \text{L} / (\text{min} / \text{m}^2)$ ($p=0.003$), $\text{LF}_{\text{nu}}\text{sBP}$ from 71 ± 8 to 67 ± 8 % ($p=0.03$), $\text{LF}_{\text{mmHg}^2}\text{dBP}$ from 6.6 ± 4.0 to 4.8 ± 2.9 mmHg^2 ($p=0.0001$), $\text{LF}_{\text{mmHg}^2}\text{sBP}$ from 9.7 ± 7.8 to 7.4 ± 4.8 mmHg^2 ($p=0.01$). $\text{HF}_{\text{nu}}\text{RRI}$ increased from 19 ± 8 to 28 ± 13 % ($p=0.008$). Myocardial contractility indices and parameters of sympathetic activity were reduced in the presyncopal state, while parasympathetic activity was increased. This suggests a decrease in cardiac contractility during orthostatically induced presyncope in healthy subjects.

Key words: Hemodynamics, Total peripheral resistance, blood pressure, impedance cardiography, sympathetic withdrawal, LBNP, HUT

Introduction

Presyncope is a state immediately preceding a syncopal event, defined as a sudden, brief, transient loss of consciousness, with loss of postural tone (Kapoor 2000). ‘Physiological’ syncope within the frame of an orthostatic stress paradigm develops as a result of critically diminished cardiac preload due to low venous return. Once brain perfusion is reduced below a critical level, a “neurocardiogenic” reflex (“vasovagal attack”) is triggered that causes vasodilation, reduces heart rate, cardiac output, and consequently blood pressure, and loss of consciousness is imminent. Although the trigger for the rapid and sudden switch in autonomic responses still remains elusive and poorly understood, it has frequently been emphasized that the autonomic nervous system plays a key role as a final common pathway leading to syncope (Furlan et al. 1998, Mangin et al. 2001, Morillo et al. 1994).

It is unclear how exactly sympathetic activity changes in a presyncopal situation: power spectral analysis of RR variability indicates increased, unchanged, or reduced sympathetic activity immediately preceding a vaso-vagal event, and microneurography has revealed rather unpredictable patterns (Mosqueda-Garcia et al. 1997). Pagani has concluded that sympathetic activation is associated with an increase of the LF component of both muscle nerve sympathetic activity (MSNA) and systolic arterial pressure (SAP), and suggested the use of changes in the LF of SAP variability as a marker of changes in sympathetic efferent activity (Pagani et al. 1997). They also found that the HF component of MSNA variability in normalized units was closely related with the HF component (in normalized units) of RRI (Pagani et al. 1997).

Vaso-vagal syncope might be triggered by a stimulation of cardiac receptors, and inadequate stimulation of mechanoreceptors may be caused by excessive myocardial contractility (Oberg and Thoren 1972). While some found powerful presyncopal myocardial activity (Shalev et al. 1991), others observed no difference (Bellard et al. 2003) or even decreased myocardial

contractility (Liu et al. 2000). Peak endocardial acceleration (PEA) measurements provided indication for decreased myocardial contractility during fainting (Mangin et al. 2001) as did impedance cardiography (Mitro et al. 2006). However, these studies were carried out in patients.

Based on the hypothesis of clear indication of decreasing presyncopal myocardial contractility together with a shift away from sympathetic towards parasympathetic activity, the aim of this study was to investigate non-invasively indices of cardiac contractility, autonomic nervous activity, and hemodynamics during and after presyncopal orthostatic stress in healthy humans.

Materials and Methods

Subjects: To avoid effects of confounding variables such as height, gender or athletic training on orthostatic tolerance (Goswami et al. 2008) we selected fifteen healthy males (31 ± 2 yr, 75 ± 3 kg, 182 ± 2 cm, BMI 23 ± 1 kg/m²) with no history of syncope, pathological condition (neurological, cardiovascular, endocrine) and not on any medication participated. Test subjects abstained from alcohol, smoking and caffeine as well as from vigorous exercise for 48 hours prior to examination, and were advised not to change their fluid and salt intake as governed by their usual dietary habits. A light breakfast with sufficient fluid intake was allowed pre-test. All studies occurred between 09.00 and 12.00 hrs in an air-conditioned, semi-dark room (Temp: $22 \pm 1^\circ\text{C}$ and humidity: 55%). The Graz Medical University's Ethics Committee approved the study, and written informed consent was obtained from each subject.

Experimental protocol: We used the Adaptive and Spaceflight Physiology Institute's multi-stimulation test device (www.meduni-graz.at/iap/AHST.htm) that allows for < 10 sec postural changes and LBNP build-up (Figure 1, below). The sealing was maintained at the iliac crest, as sealing position has been shown to affect hemodynamic responses (Goswami et al 2008). Test subjects were secured and had access to an emergency shutdown (automatic return to

supine and pressure neutralization) at all times. The experiment commenced with 5 min 70° passive head-up tilt (HUT). CNSystems, Graz, Austria). It was followed by graded LBNP, which started with 20mmHg suction, and was increased by 10 mmHg each in 3-min intervals, until presyncope occurred. The criteria of presyncope were a) Blood pressure drop below systolic 80 mmHg or by ≥ 25 mmHg/min, diastolic by ≥ 15 mmHg/min, and /or heart rate decrease by ≥ 15 bpm b) Lightheadedness, dizziness, visual disturbances, nausea, stomach awareness, clammy skin, excessive sweating, or skin pallor (Hinghofer-Szalkay et al 2006).

Hemodynamics: Blood pressure was monitored using the Penaz principle (Wesseling 1996). Total peripheral resistance index (TPRI) was calculated as mean arterial blood pressure / cardiac index and mean arterial blood pressure as calculated from diastolic and systolic pressures, respectively: $MAP = dBP + 1/3 (sBP - dBP)$. Cardiac index (CI), which relates heart performance to the size of the individual, was calculated as cardiac output/body surface area. Stroke index was calculated as stroke volume/body surface area. Impedance cardiography, in which the changes in thoracic impedance are converted to reflect changes in thoracic fluid/volume over time, was performed based on the original Kubicek approach but using an improved estimate of thoracic volume (Task Force Monitor, TFM®).

TFM® ECG / impedance electrodes were positioned together with upper arm and finger blood pressure cuffs (Fortin et al. 2006). Electrode strips were placed at the neck and thoracic regions, the latter specifically at the midclavicular line at the xiphoid process level. The method has been described in detail elsewhere (Fortin et al. 2006). Recorded and calculated data were stored real-time beat-to-beat throughout the entire experiment.

Heart rate and blood pressure variability: Low (LF: 0.05 - 0.17 Hz) and high frequency (HF: 0.17 - 0.40 Hz) power components of RR-intervals (RR), diastolic blood pressure (dBP) and systolic blood pressure (sBP) were evaluated and given in absolute values (ms^2) and normalized units (nu) applying an autoregressive method, as normalization minimizes disruptive effects of changes in total power (Camm et al. 1996). The referring frequency

bands indicate autonomic modulation of the sinoatrial node and of vasomotion (Camm et al. 1996). In the present study we used changes in the LF range of arterial blood pressure to assess information about sympathetic activity, as it is generally considered to be associated with sympathetic nerve activity (Malliani et al. 1991, Pagani et al. 1997). We used changes in the HF range of heart rate variability (HF_RRI) to assess parasympathetic activity because HF_RRI is primarily mediated by parasympathetic nerve modulation, whereas LF_RRI is probably affected by both parasympathetic and sympathetic modulations (Pagani et al. 1997, Stauss 2003).

Myocardial contractility parameters: Myocardial contractility parameters were derived through impedance cardiography measurement. The index of contractility (IC) reflects the aortic peak flow and it is the maximum impedance changes ($\Delta Z/\Delta t_{\max}$) normalized to the ground impedance Z_0 . The acceleration index (ACI) refers to the maximum aortic blood acceleration as a function of time and is defined as $\Delta^2 Z/\Delta t_{\max}^2$. The left ventricular work index (LVWI) was calculated as (mean blood pressure – pulmonary capillary occlusion pressure (fixed to 7 mmHg)) * cardiac index * const.

Data acquisition and analysis: Data from distinct protocol time points were used to obtain the defined parameter patterns, particularly from the period preceding and immediately after presyncope.

In each test, the following protocol points were identified: Supine control (C1), early tilt (G1), late tilt (G2), late Graded orthostatic stress [GOS] (G3), presyncope (PS), early recovery (PG1) and late control (PG2). All data are means from observation periods: C1, sixty seconds before GOS; G1, the immediate 60 s after commencing HUT; G2, the last sixty seconds of HUT; G3, the last minute of LBNP 20 (this was the only LBNP step finished by all test subjects); PS, the last minute of GOS preceding presyncope; PG1, the first sixty seconds after

GOS; and PG2, the last sixty seconds before termination of the experiment, which was five minutes after return to supine.

INSERT FIGURE 1

Statistical analysis: Variables were tested for normality using the D'Agostino & Pearson omnibus normality test and expressed as mean value \pm SD. Repeated measures ANOVA with Dunnett post-hoc or the Friedman test with Dunn post hoc testing were used to test for changes in all tested variables and parameters with orthostatic loading (C1 compared to all other time periods). All reported p values are two-sided. For all tests, significance was set at $p \leq 0.05$. All analyses were performed using GraphPad Prism 5 software.

Results

Mean orthostatic tolerance time was 13 ± 1 min. Six test subjects showed a pure vasodepressor reaction prior to presyncope, five a mixed (cardioinhibitory and vasodepressor) reaction and four showed symptoms like lightheadedness, sweating, nausea, and visual disturbances.

Hemodynamic changes during and after GOS: Compared to supine control (C1) SI, CI, TPRI and blood pressure values were reduced and HR and TI increased in presyncopal state (PS) (Tab 1). After GOS, HR, SI, and CI quickly returned to normal (not different from C1), while mean arterial blood pressure and TPRI was below C1 levels at PG1, and TI remained elevated at PG1 and PG2 (Table 1). In comparing late GOS (G3) with PS, diminished TPRI was observed (Table 1).

INSERT TABLE 1

Changes in myocardial contractility parameters during and after GOS: Figure 2 shows the myocardial contractility indices IC, ACI, LVWI at defined protocol times. IC decreased stepwise during GOS (59 ± 14 to 27 ± 6 1000/s; $p < 0.0001$). ACI was significantly decreased (65 ± 18 to 54 ± 15 100/s²; $p = 0.04$) only at PS, and increased above C1 at PG1 (65 ± 18 to 80 ± 17 100/s²; $p = 0.002$). Similarly, LVWI was reduced at PS (5.2 ± 1.3 to 3.6 ± 0.6 mmHg*L/min/m²; $p < 0.0001$) (Fig 1). In late GOS (G3), as compared with PS, IC (35 ± 6 to 27 ± 6 1000/s; $p = 0.003$) and LVWI (4.6 ± 0.9 to 3.6 ± 0.6 mmHg*L/(min*m²); $p = 0.003$) were decreased, whereas ACI was unchanged (Figure 2).

INSERT FIGURE 2

Autonomic changes during and after GOS: LF_{nu}_dBP and LF_{nu}sBP increased in a stepwise fashion with a peak at G3 (51 ± 14 to 70 ± 15 %; $p < 0.0001$ and 50 ± 6 to 71 ± 8 %; $p < 0.0001$, respectively) and returned to supine rest values after GOS (Figure 3). Even at presyncope (PS), both LF_{nu}dBP (51 ± 14 to 67 ± 11 %; $p = 0.0006$) and LF_{nu}sBP (50 ± 6 to 67 ± 8 %; $p < 0.0001$) increased compared to supine control. Compared late GOS (G3) with presyncope (PS) LF_{nu}sBP fell from 71 ± 8 to 67 ± 8 % ($p = 0.03$). There was a presyncopal decrease during G3, in particular LF_{mmHg}²dBP fell from 6.6 ± 4.0 to 4.8 ± 2.9 mmHg² ($p = 0.0001$) and LF_{mmHg}²sBP from 9.7 ± 7.8 to 7.4 ± 4.8 mmHg² ($p = 0.01$) (Figure 3).

HF_{nu}RRI decreased significantly at G2 (35 ± 15 to 17 ± 7 %; $p = 0.0002$) and G3 (35 ± 15 to 18 ± 8 %; $p = 0.003$) compared to supine control (C1) and increased from G3 (19 ± 8 to 28 ± 13 %; $p = 0.01$) to presyncopal point (PS) (Figure 4). During GOS HF_{ms}²RRI decreased in a stepwise fashion (385 ± 320 to 38 ± 43 ms²; $p = 0.0009$) and returned quickly, after cessation of GOS, to C1 levels (Fig 3). In comparing late GOS (G3) with presyncope (PS), diminished values of HF_{ms}²RRI were observed (103 ± 77 to 39 ± 43 ms²; $p = 0.003$) (Fig 4).

INSERT FIGURE 3 AND 4

Discussion

We present evidence of reduced myocardial contractility, decreased total peripheral resistance and diminished sympathetic activity in healthy men undergoing orthostatically induced presyncope.

Several clinical studies have investigated myocardial contractility and changes in autonomic variables. Mizumaki et al. used echocardiography to assess the contractility status and power spectral analysis for HRV measurements (Mizumaki et al. 1995). Mangin et al. employed peak endocardial acceleration (PEA) as an index of myocardial contractility and measured HRV through power spectral analysis (Mangin et al. 2003). However, some did not incorporate autonomic variables in their myocardial contractility studies but used impedance cardiography to assess myocardial contractility (Mitro et al. 2006). In the present investigation, we focussed on changes in hemodynamic and myocardial contractility combined with autonomic activity using graded orthostatic stress (GOS) to provoke presyncope in unmedicated healthy male test subjects. We have previously used the combined head up tilt and graded lower body suction to study hemodynamic and neurohormonal responses to extreme orthostatic stress (Grasser et al, unpublished observations), effect of Chinese herbs on cardiovascular responses (Gao et al 2008) and more recently, to demonstrate post reactive hyperemia in the human liver (Hinghofer-Szalkay et al. 2008).

Hemodynamic changes: With increasing orthostatic stress there was a steady increase in thoracic impedance from supine control to presyncope, suggesting increasing central

hypovolemia (Cai et al. 2000, Pomerantz et al. 1970). Neurally mediated syncope is usually triggered by peripheral blood pooling, low venous return, and a “partially emptied” (Zaqqa et al. 2000) heart; increased parasympathetic activity finally reduces heart rate, rendering cardiac output insufficient to support proper brain perfusion (Lurie et al. 1996). Brown et al. (Brown et al. 2000) observed total peripheral resistance to fall from a level elevated 40% above supine values two minutes before, to 20% one minute before presyncope, indicating build-up of peripheral loss of vascular tone. We found total peripheral resistance index already decreased below supine control values one minute before presyncopal signs occurred.

Differences are conceivably protocol dependent: Brown et al. (Brown et al. 2000) used 20 mmHg steps every 10 minutes; we increased LBNP intensity by 3-minute steps. We used impedance cardiography to calculate total peripheral resistance index from the baseline; Brown et al. used brachial artery ultrasonography to indirectly assess cardiac output. We found a marked drop in resistance index from the last minute of tolerated LBNP to presyncope, indicating sympathetic withdrawal shortly before presyncope. It is possible that the elevated circulating Norepinephrine (NE) and Epinephrine (E) contributed to a beta-adrenergic receptor (β AR) downregulation in humans (Bristow et al. 1982). When receptors in intact cells or tissues are exposed to agonists, there is often a rapid decline in responsiveness. This process is called receptor desensitization (Pippig et al. 1993). The time frames over which these processes occur range from seconds (phosphorylation) to minutes (endocytosis) and hours (down-regulation) and in terms of β_2 -AR the extent of receptor desensitization reaches to attenuation of agonist potency and maximal responsiveness (Fergusson 2001, Pippig et al. 1993). The same might hold for α_1 -AR subtypes, which are the prime mediator of smooth muscle contraction (Piascik et al. 2001). Experiments with knockout mice suggest that α_{1A} -AR is the dominant AR for vascular smooth muscle contraction (Piascik et al. 2001). Rudner and colleagues hypothesized that α_{1A} -AR mediated contraction may account for generalized splanchnic vasoconstriction during stress in human

(Rudner et al 1999). It is conceivable that elevated NE and E levels (both were observed elevated at presyncope compared to baseline, data not shown) led to a desensitization of adrenergic receptors, which would explain our observation of decreased myocardial contractility indices and peripheral resistance preceding syncope.

Another possible explanation could be metabolically driven vasodilation, which was found after strenuous exercise (Clifford et al. 2004). Convertino has proposed that under orthostatic stress, beta adrenergic-induced tachycardia is a primary mechanism for the maintenance of arterial blood pressure (Convertino et al. 2000). However, our observed total peripheral index drop decreased the mean arterial pressure in the last 60 seconds before presyncope, in agreement to previous findings (El-Bedawi et al. 1994, Julu et al. 2003, Leloir et al. 2003) so arterial pressure was no longer stabilized in this situation.

After the combined head up tilt and LBNP, the heart rate, stroke index and cardiac index returned to supine control within one minute, whereas blood pressure was still reduced. This could be attributed to the still below supine control levels of peripheral resistance at this point. Thoracic impedance stayed elevated above baseline for 15 minutes (PG2), probably due to fluid loss during GOS (Evans et al. 2004). However, cardiac output was not different from supine control.

Myocardial contractility indicators changes: Sudden reductions in venous return as caused by orthostatic stress primarily involve chronotropic instead of inotropic compensation (Guazzi et al. 1995). Indeed, all contractility indices decreased with presyncope in our study; index of contractility decreased progressively with increasing orthostatic load. Similarly, Acceleration index and left ventricular working index were also decreased, only significantly, though, at presyncope. Overall, the observations on cardiac contractility with (pre) syncope are controversial: Shalev et al. (Shalev et al. 1991) found powerful myocardial activity using echocardiography, others observed no difference in myocardial contractility (Bellard et al. 2003) or even decreased contractility (Liu et al. 2000) also using echocardiography. Using

impedance cardiography, Mitro et al. (Mitro et al. 2006) also provided evidence against increased contractility. Mangin et al. (Mangin et al. 2001), using peak endocardial acceleration as index of myocardial contractility, found that cardiac inotropy increased gradually during the entire testing in a head up tilt positive subgroup but they also observed a decrease in myocardial contractility during fainting.

Following 60 seconds of stress cessation, the index of contractility and left ventricular working index were not different from baseline levels; Acceleration index, however, was increased 60 seconds following the termination of graded orthostatic stress. A recent study, which also used impedance cardiography, observed a trend towards increased myocardial contractility one minute after syncope (Mitro et al. 2006). The authors speculated that the increased inotropy might represent a compensatory mechanism originating from a sympathetic reflex response to increased peripheral blood pooling.

Heart rate and blood pressure variability: Vasovagal syncope is associated with a sudden onset of hypotension, often in combination with bradycardia or transient asystole, probably due to sympathetic withdrawal. Shortly before presyncope, we observed low frequency power components of diastolic and systolic blood pressure increased, whereas absolute values showed no change, compared to baseline. As total power variance is reduced, the absolute power of low frequency appears unchanged compared to rest (Camm et al. 1996). Low frequency power components of diastolic and systolic blood pressure both decreased between late graded stress and presyncope, indicating sudden sympathetic withdrawal which is in agreement with previous findings and matches the decrease in vascular resistance index, acceleration index and left ventricular working index. The high frequency power component of RR-interval ($HF_{ms}RRI$) was below baseline levels at presyncope, while $HF_{nu}RRI$ remained unchanged. Using power spectral analysis of heart rate variability, Mizumaki et al. (Mizumaki et al. 1995) also found decreased presyncopal high frequency power component of RR-interval in their control group of healthy men. Similarly, Mangin et al. (Mangin et al. 2001)

found a marked presyncopal drop in the high frequency power component of RR-interval in subjects prone to syncope. However, they used isoproterenol infusion to provoke syncope in their test subjects and this could influence the autonomic system. We observed $HF_{nu}RRI$ decreased at end of tilt phase and at late graded orthostatic stress compared to control, which suggests low parasympathetic activity. From late graded stress to presyncope, however, $HF_{nu}RRI$ increased, indicating vagal activation shortly before presyncopal signs or symptoms occurred. This is in agreement with previous observations (Furlan et al. 1998, Kamiya et al. 2005). Piccirillo found diminished $HF_{nu}RRI$ in the early stage of HUT but no change from their first recording to their second, denoted as the last 256 beats before syncope (Piccirillo et al. 2004). This discrepancy to our observation could be attributed to the breathing patterns, as our subjects were allowed to breathe freely. Higher breathing frequency in the presyncopal state elevates the $HF_{nu}RRI$ (Piccirillo et al. 2004).

Limitations: We studied healthy males with no history of syncope. We cannot generalize our findings to patients with vasovagal syncope or orthostatic intolerance. To simulate real life scenario characterized by spontaneous ventilatory activity, we did not control breathing, as this is known to influence heart rate and blood pressure variability (Piccirillo et al. 2004).

Conclusion: Myocardial contractility indices and parameters of sympathetic activity were reduced in the presyncopal state, while indication of parasympathic activity was increased. This suggests a decrease in cardiac contractility during orthostatically induced presyncope in healthy young subjects. Additional investigations are warranted to confirm these findings across a larger age range, in both men and women and in non-healthy subjects.

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Conflict of interest: None

References

- BELLARD E, FORTRAT JO, SCHANG D, DUPUIS JM, VICTOR J, LEFTHERIOTIS G: Changes in the transthoracic impedance signal predict the outcome of a 70 degrees head-up tilt test. *Clin Sci* **104**: 119-126, 2003.
- BRISTOW MR, GINSBURG R, MINOBE W, CUBICCIOTTI RS, SAGEMAN WS, LURIE K, BILLINGHAM ME, HARRISON DC, STINSON EB: Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med* **307**: 205-211, 1982.
- BROWN CM, HAINSWORTH R: Forearm vascular responses during orthostatic stress in control subjects and patients with posturally related syncope. *Clin Auton Res* **10**: 57-61, 2000.
- CAI Y, HOLM S, JENSTRUP M, STROMSTAD M, EIGTVED A, WARBERG J, HOJGAARD L, FRIBERG L, AND SECHER NH: Electrical admittance for filling of the heart during lower body negative pressure in humans. *J Appl Physiol* **89**: 1569-1576, 2000.
- CAMM AJ, MALIK M, BIGGER JT, BREITHARDT G, CERUTTI S, COHEN RJ, COUMEL P, FALLEN EL, KENNEDY HL, KLEIGER RE, LOMBARDI F, MALLIANI A, MOSS AJ, ROTTMAN JN, SCHMIDT G, SCHWARTZ PJ, SINGER DH: Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* **17**: 354-381, 1996.
- CLIFFORD PS, HELLSTEN Y: Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol* **97**: 393-403, 2004.
- CONVERTINO VA, SATHER TM: Effects of cholinergic and beta-adrenergic blockade on orthostatic tolerance in healthy subjects. *Clin Auton Res* **10**: 327-336, 2000.
- EL-BEDAWI KM, HAINSWORTH R: Combined head-up tilt and lower body suction: A test of orthostatic tolerance. *Clin Auton Res* **4**: 41-47, 1994.

EVANS JM, STENGER MB, MOORE FB, HINGHOFFER-SZALKAY H, ROSSLER A, PATWARDHAN AR, BROWN DR, ZIEGLER MG, KNAPP CF: Centrifuge training increases presyncopal orthostatic tolerance in ambulatory men. *Aviat Space Environ Med* **75**: 850-858, 2004.

FERGUSON SSG: Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev* **53**: 1-24, 2001.

FORTIN J, HABENBACHER W, HELLER A, HACKER A, GRULLENBERGER R, INNERHOFER J, PASSATH H, WAGNER C, HAITCHI G, FLOTZINGER D: Non-invasive beat-to-beat cardiac output monitoring by an improved method of transthoracic bioimpedance measurement. *Comput Biol Med* **36**: 1185-1203, 2006.

FURLAN R, PIAZZA S, DELL'ORTO S, BARBIC F, BIANCHI A, MAINARDI L, CERUTTI S, PAGANI M, MALLIANI A: Cardiac autonomic patterns preceding occasional vasovagal reactions in healthy humans. *Circulation* **98**: 1756-1761, 1998.

GAO Y, GOSWAMI N, GRASSER E, ROESSLER A, STOEGER E, SCHWABERGER G, HINGHOFFER-SZALKAY H. Radix Astragali and orthostatic response: a double masked crossover study. *Aviat Space Environ Med* 2008; **79**:94-8.

GOSWAMI N, LOEPPKY J, HINGHOFFER-SZALKAY H. LBNP: Past protocols and technical considerations for experimental design. *Aviat Space Environ Med* **79**: 1-13, 2008.

GOSWAMI N, GRASSER E, ROESSLER A, SCHNEDITZ D, HINGHOFFER-SZALKAY H. The cardiovascular response to lower body negative pressure in humans depends on seal location *Physiol Res* 2008 Jul 18 [Epub ahead of print]

GUAZZI M, PEPI M, MALTAGLIATI A, CELESTE F, MURATORI M, TAMBORINI G: How the two sides of the heart adapt to graded impedance to venous return with head-up tilting. *J Am Coll Cardiol* **26**: 1732-1740, 1995.

HINGHOFFER-SZALKAY HG, ROESSLER A, EVANS JM, STENGER MB, MOORE FB, KNAPP CF: Circulatory galanin levels increase several fold with intense orthostatic challenge in healthy humans *J Appl Physiol* **100**: 844-849, 2006.

HINGHOFFER-SZALKAY H, GOSWAMI N, ROESSLER A, GRASSER E, SCHNEDITZ D. Reactive hyperemia in the human liver. *Am J Physiol: Gastrointest Liver Physiol* (doi: 10, 1152/ajpgi.0042.2008)

JULU POO, COOPER VL, HANSEN S, HAINSWORTH R: Cardiovascular regulation in the period preceding vasovagal syncope in conscious humans. *J Physiol* **549**: 299-311, 2003.

KAMIYA A, HAYANO J, KAWADA T, MICHIKAMI D, YAMAMOTO K, ARIUMI H, SHIMIZU S, UEMURA K, MIYAMOTO T, AIBA T, SUNAGAWA K, SUGIMACHI M: Low-frequency oscillation of sympathetic nerve activity decreases during development of tilt-induced syncope preceding sympathetic withdrawal and bradycardia. *Am J Physiol Heart Circ Physiol* **289**: 1758-1769, 2005.

KAPOOR WN: Primary care - Syncope. *N Engl J Med* **343**: 1856-1862, 2000.

LELORIER P, KLEIN G, KRAHN A, RAYMOND Y, SKANES AC, SHOEMAKER KJ: Combined head-up tilt and lower body negative pressure as an experimental model of orthostatic syncope. *J Cardiovasc Electrophysiol* **14**: 920-924, 2003.

LIU JE, HAHN RT, STEIN KM, MARKOWITZ SM, OKIN PM, DEVEREUX RB, AND LERMAN BB: Left ventricular geometry and function preceding neurally mediated syncope. *Circulation* **101**: 777-783, 2000.

LURIE KG, BENDITT D: Syncope and the autonomic nervous system. *J Cardiovasc Electrophysiol* **7**: 760-776, 1996.

MALLIANI A, PAGANI M, LOMBARDI F, CERUTTI S: Cardiovascular neural regulation explored in the frequency domain. *Circulation* **84**: 482-492, 1991.

MANGIN L, KOBEISSI A, LELOUCHE D, D'HEROUVILLE Y, MANSIER P, SWYNGHEDAUW B, MACQUIN-MAVIER I: Simultaneous analysis of heart rate

variability and myocardial contractility during head-up tilt in patients with vasovagal syncope.

J Cardiovasc Electrophysiol **12**: 639-644, 2001.

MITRO P, HIJOVA E: Myocardial contractility and cardiac filling measured by impedance cardiography in patients with nitroglycerine-induced vasovagal syncope. *Pacing Clin Electrophysiol* **29**: 1-8, 2006.

MIZUMAKI K, FUJIKI A, TANI M, SHIMONO M, HAYASHI H, INOUE H: Left-ventricular dimensions and autonomic balance during head-up tilt differ between patients with isoproterenol-dependent and isoproterenol-independent neurally mediated syncope. *J Am Coll Cardiol* **26**: 164-173, 1995.

MORILLO CA, KLEIN GJ, JONES DL, YEE R: Time and frequency-domain analyses of heart-rate-variability during orthostatic stress in patients with neurally-mediated syncope. *Am J Cardiol* **74**: 1258-1262, 1994.

MOSQUEDA-GARCIA R, FURLAN R, FERNANDEZ-VIOLANTE RD, TUSHAR SM, JARAI Z, ANANTHRAM V, ROBERTSON RM, ROBERTSON D: Sympathetic and baroreceptor reflex function in neurally mediated syncope evoked by tilt. *J Clin Invest* **99**: 2736-2744, 1997.

OBERG B, THOREN P: Increased activity in left ventricular receptors during hemorrhage or occlusion of caval veins in cat: A possible cause of vaso-vagal reaction. *Acta Physiol* **85**: 164-173, 1972.,

PAGANI M, MONTANO N, PORTA A, MALLIANI A, ABBOUD FM, BIRKETT C, SOMERS VK: Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation* **95**: 1441-1448, 1997.

PIASCIK MT, PEREZ DM: Alpha(1)-adrenergic receptors: New insights and directions. *J Pharmacol Exp Ther* **298**: 403-410, 2001.

- PICCIRILLO G, NASO C, MOISE A, LIONETTI M, NOCCO M, DI CARLO S, DE LAURENTIS T, MAGRI D, CACCIAFESTA M, MARIGLIANO V: Heart rate and blood pressure variability in subjects with vasovagal syncope. *Clin Sci* **107**: 55-61, 2004.
- PIPPIG S, ANDEXINGER S, DANIEL K, PUZICHA M, CARON MG, LEFKOWITZ RJ, LOHSE MJ: Over expression of beta-arrestin and beta-adrenergic receptor kinase augment desensitization of beta 2-adrenergic receptors. *J Biol Chem* **268**: 3201-3208, 1993.
- POMERANTZ M, DELGADO F, EISEMAN B: Clinical evaluation of transthoracic electrical impedance as a guide to intrathoracic fluid volumes. *Ann Surg* **171**: 686-694, 1970.
- RUDNER XL, BERKOWITZ DE, BOOTH JV, FUNK BL, COZART KL, D'AMICO EB, EL-MOALEM H, PAGE SO, RICHARDSON CD, WINTERS B, MARUCCI L, SCHWINN DA: Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. *Circulation* **100**: 2336-2343, 1999.
- SHALEV Y, GAL R, TCHOU PJ, ANDERSON AJ, AVITALL B, AKHTAR M, JAZAYERI MR: Echocardiographic demonstration of decreased left ventricular dimensions and vigorous myocardial contraction during syncope induced by head-up tilt. *J Am Coll Cardiol* **18**: 746-751, 1991.
- STAUSS HM: Heart rate variability. *Am J Physiol Regul Integr Comp Physiol* **285**: 927-931, 2003.
- WESSELING KH: Finger arterial pressure measurement with Finapres. *Z Kardiol* **85**: 38-44, 1996.
- ZAQQA M, MASSUMI A: Neurally mediated syncope. *Tex Heart Inst J* **27**: 268-272, 2000.

Figure legend

Figure 1: Experimental protocol and details of specific points at which the data were analyzed. C1: supine control; G1: early tilt; G2: late tilt; G3: late GOS; PS: presyncope; PG1: early recovery; PG2: late control.

Figure 2: Changes in myocardial contractility parameters during and after GOS. IC: index of contractility, ACI: acceleration index, LVWI: left ventricular working index. C1: supine control; G1: early tilt; G2: late tilt; G3: late GOS; PS: presyncope; PG1: early recovery; PG2: late control. Bars show means \pm SD. ***, $P < 0.0005$; **, $P < 0.005$; *, $P < 0.05$.

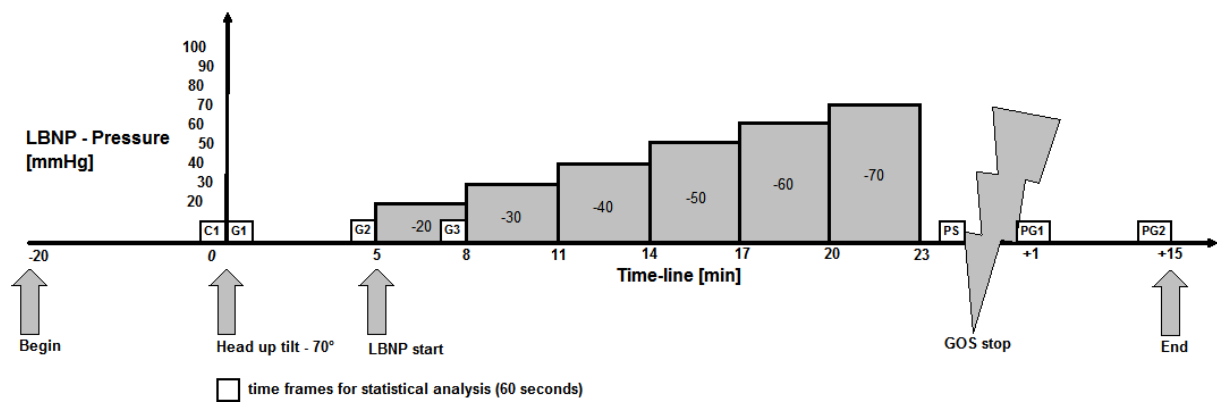
Figure 3: Autonomic changes during and after GOS. $LF_{nu}dBP$: low frequency of diastolic blood pressure, normalized values. $LF_{nu}sBP$: low frequency of systolic blood pressure, normalized values. $LF_{mmHg^2}dBP$: low frequency of diastolic blood pressure, absolute values. $LF_{mmHg^2}sBP$: low frequency of systolic blood pressure, absolute values. Bars show means \pm SD. ***, $P < 0.0005$; **, $P < 0.005$; *, $P < 0.05$.

Figure 4: Autonomic changes during and after GOS. $HF_{nu}RRI$: high frequency variation of RR-interval, normalized values. $HF_{ms^2}RRI$: high frequency variation of RR-interval, absolute values. C1: supine control; G1: early tilt; G2: late tilt; G3: late GOS; PS: presyncope; PG1: early recovery; PG2: late control. Bars show means \pm SD. ***, $P < 0.0005$; **, $P < 0.005$; *, $P < 0.05$.

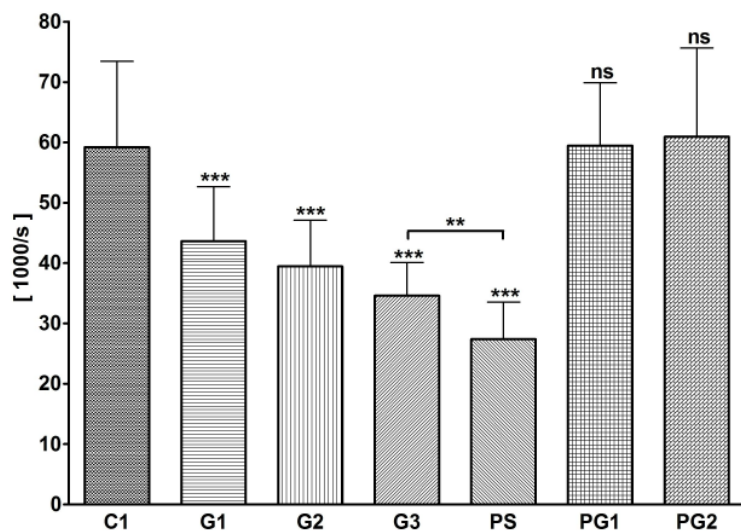
Table 1 Hemodynamic changes during and after GOS (graded orthostatic stress)

	HR [bpm]	SI [mL/m ²]	CI [L/(min* m ²)]	TI [Ω]	sBP [mmHg]	dBp [mmHg]	MAP [mmHg]	TPRI [dyne*s*m ² /cm ⁵]
C1	75±15	50±10	3.7±0.7	34.7±3.8	137±17	90±16	106±16	2306±496
G1	91±18**	37±5***	3.4±0.5	36.3±4.1***	136±21	95±22	109±20	2573±586
G2	93±14**	34±4***	3.2±0.4***	36.7±4.2***	135±17	95±12	109±12	2691±448*
G3	106±18***	31±4***	3.3±0.4*	37.8±4.4***	129±20	95±22	106±20	2543±642
PS	128±26***	26±4***	3.2±0.3**	38.9±4.6***	101±18***	75±14*	83±14***	1913±321*
PG1	87±20	46±8	3.8±0.7	36.2±4.2***	121±21	76±15	91±14*	1966±289*
PG2	65±10	53±10	3.4±0.7	35.4±4.2*	123±38	79±26	94±30	2375±554

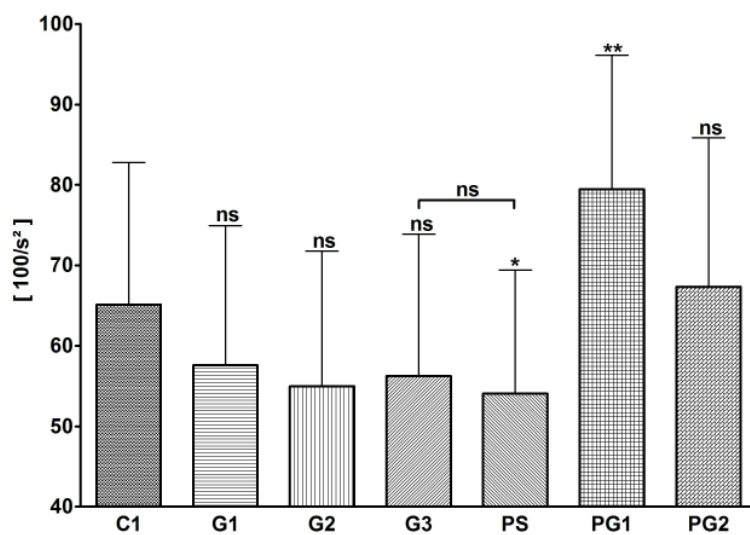
Table legend: Heart rate (HR), stroke index (SI), cardiac index (CI), thoracic impedance (TI), systolic blood pressure (sBP), diastolic blood pressure (dBp), mean arterial blood pressure (MAP), total peripheral resistance index (TPRI). Values are mean ± SD. C1: supine control; G1: early tilt; G2: late tilt; G3: late GOS; PS: presyncope; PG1: early recovery; PG2: late control.***, P < 0.0005; **, P < 0.005; *, P < 0.05.



IC



ACI



LVWI

