Application of the IAP cardiovascular fitness test protocol for AustroMars candidate screening

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Abstract

Rationale: The purpose of our research was to test for cardiovascular stability within 16 Austromars candidates, and to determine hemodynamic variables and hormones in a presyncopal state as evoked by a specific test protocol.

Procedures and methods: We used a graded orthostatic stress (GOS) paradigm consisting of head-up tilt (HUT) combined with lower body negative pressure (LBNP) up to a presyncopal end-point on 15 males and one healthy female. Hemodynamic parameters were monitored and venous blood samples taken.

Results: From supine control to presyncope, mean standing time was 12.3 ± 1.2 min, heart rate (HR) increased by 68 ± 12% ($p < 0.0001$) and thoracic impedance (TI) rose by 12 ± 1% ($p < 0.0001$), whereas following parameters decreased: stroke volume index (SI) 44 ± 4% ($p < 0.0001$), systolic pressure (SBP) 26 ± 3% ($p < 0.0001$), diastolic pressure (DBP) 16 ± 5% ($p = 0.004$), mean arterial blood pressure (MAP) 19 ± 3% ($p < 0.0001$), pulse pressure 41 ± 8% ($p = 0.0003$) and total peripheral resistance index (TPRI) 11 ± 5% ($p = 0.03$). Heart rate and blood pressure variabilities decreased together with pulse pressure. Plasma volume decreased by 11 ± 2% ($p = 0.0004$). Plasma norepinephrine (NE) increased by 86 ± 16% ($p = 0.0001$), epinephrine (E) by 460 ± 266% ($p = 0.06$), cortisol by 10 ± 6% ($p = 0.02$), plasma renin activity by 147 ± 26% ($p = 0.002$) and aldosterone by 24 ± 21% ($p = 0.2$). Conclusion: Our combined HUT- graded LBNP paradigm is useful to study CV regulation and hormonal responses under severe stress conditions.

Key terms: Graded orthostatic stress, presyncope, hormones, hemodynamics

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Introduction:

During simulated spaceflight missions, it is necessary to create, and to deal with, situations that produce high levels of cardiovascular stress.
Extreme cardiovascular stress can be produced by increasing orthostatic challenge via hypergravity (human centrifuge), hypovolemia, or a combination of passive head-up tilt and lower body suction. We use the latter approach to drive cardiovascular regulation to a presyncopal point.
Presyncope is a state immediately preceding a syncopal event, defined as a sudden, brief, transient loss of consciousness, with loss of postural tone [1, 2]. Typical syncope within the frame of an orthostatic stress paradigm, i.e. in physiological terms, develops because cardiac preload critically decreases due to low venous blood return. This ultimately reduces brain perfusion below a critical level, and triggers a "neurocardiogenic" reflex response ("vasovagal attack") that reduces heart rate, cardiac output, and consequently blood pressure. Cerebral underperfusion finally leads to loss of consciousness [3].
Orthostatic stress (OS) – as caused by head-up tilt (HUT) or lower body negative pressure (LBNP) – induces cardiovascular and neuroendocrine changes in an intensity and duration dependent order. Post-spaceflight orthostatic intolerance has been explained with hemodynamic mechanisms (hypovolemia, reduced venous capacitance, cardiac atrophy, decreased TPR response), reflex mechanisms and morphological changes in vestibular otolith organs [4, 5]. The initial effects of upright posture are mostly related to hydrostatic changes: Carotid baroreceptor pressure and cardiac preload both decrease because the arterial hydrostatic indifference point between supine and upright is located at, while the venous hydrostatic indifference point is located below heart level [6, 7] rendering diastolic filling reduced upon assumption of an upright position.
A fall in cardiac filling pressure reduces the firing rate from cardiopulmonary receptors, alters medullar feedback patterns, and triggers neurohumoral responses, like sympathetic activation and hormonal release mechanisms [8-10]. Hormones like catecholamines, renin activity, aldosterone and cortisol respond in specific ways to gravitational stress.
Additional LBNP [11, 12] diminishes cardiac preload even further – e.g. LBNP of 40 mmHg *per se* reduces cardiac output by approximately 25%, similar to passive HUT [13] -, so the stimulus combination eventually leads to cerebral underperfusion and consequently a presyncopal situation.
Changes in circulatory norepinephrine concentration correlate well with sympathetic traffic to resistance blood vessels [14] and are often used as an indirect measure of sympathetic activity. Muscular sympathetic nervous activity (MSNA) mirrors sympathetic traffic to peripheral blood vessels [15, 16] but the measurements require a rather invasive procedure. Forearm venous norepinephrine plasma concentration and MSNA are closely correlated in healthy persons [17].
This report provides data on hemodynamic and hormonal changes observed during the screening process of 16 Austromars candidates. The data prove that the chosen protocol provided extreme CV stress.

Methods and procedures

Subjects

Fifteen healthy males and one healthy female participated (Age: mean ± S.E.M., 31 ± 4 years; BMI: mean ± S.E.M., 22,8 ± 2,2 kg/m^2; height: mean ± S.E.M., 182 ± 5 cm; weight: mean ± S.E.M., 75 ± 9 kg; body surface: mean ± S.E.M., 1,96 ± 0,12 m^2). They were examined specifically for their circulation status and the possibility of posturally related syncopes (Investigator: Medical School Innsbruck, Department of Physiology, Chair: Prof. Hörtnagel). They were informed about the nature and purpose of the study and gave their written consent and were restrained from alcohol, smoking and caffeine for 48 hours prior to the examination. Moreover, we advised our test subjects do not have exercise in past 72 hours prior to the test. Since there is an influence of salt intake on baroreceptor

sensitivity [18] and because the person's volume status influences hormonal basal levels as well as responses to OS, test subjects were advised not to change their fluid and salt intake as governed by their usual dietary habits. Our test subjects were advised only to have a light breakfast and sufficient water ingestion [19]. All studies occured in the morning hours (0900 -1200 hrs.) in an air-conditioned room (22°C ± 1°C). This study was approved by the ethical committee of the Medical School Graz and written informed consent was obtained from each subject.
To avoid confusion with (pre)syncope other than strictly orthostatic in physiological terms, we only accepted test subjects without any pathological event in their past.

Experimental Protocol

This was a randomised and open study. All experiments were carried out between 0900 - 1200 hrs. During supine rest a 17-gauge 1,4×40-mm 3-way-stopcock Teflon catheter (TriCath In®, Codan Steridex) was inserted in the right cubital vein. The TFM® ECG and impedance electrodes were positioned together with a blood pressure-cuff and a finger-cuff. After a 30 min supine rest five minutes passive HUT at 70° was followed by additional 20mmHg that was increased by additional 10mmHg every three minutes until presyncope occurred. Both postural changes and LBNP pressure build up occurred in less then 10 sec.
Two antecubital venous blood samples were taken one minute before HUT and one minute after presyncope (Fig.1).

Presyncope was defined by the following signs and symptoms: Lightheadedness, sweating, nausea, weakness and hyperventilation, a sensation of physical alienation or visual disturbances preceding actual syncope further a sudden drop in arterial blood pressure and heart rate [2]. As soon as one or more of these criteria occured the subject was brought back to supine and the second blood sample collected at + 1min.
Orthostatic tolerance was defined as time (in minutes) from commencing HUT until presyncopal signs or symptoms become evident.

Treatment of blood samples

Samples were collected in ice chilled EDTA test tubes, blood cells separated from plasma immediately and stored at -80°C until analysis.

Hemodynamic measurements

We used the Task Force Monitor (TFM®; CnSystems, Austria) to monitor beat to beat heart rate (HR) with ECG and stroke index (SI) with impedance cardiography. Blood pressure was also monitored beat-to-beat by the "vascular unloading technique" [20] which applies

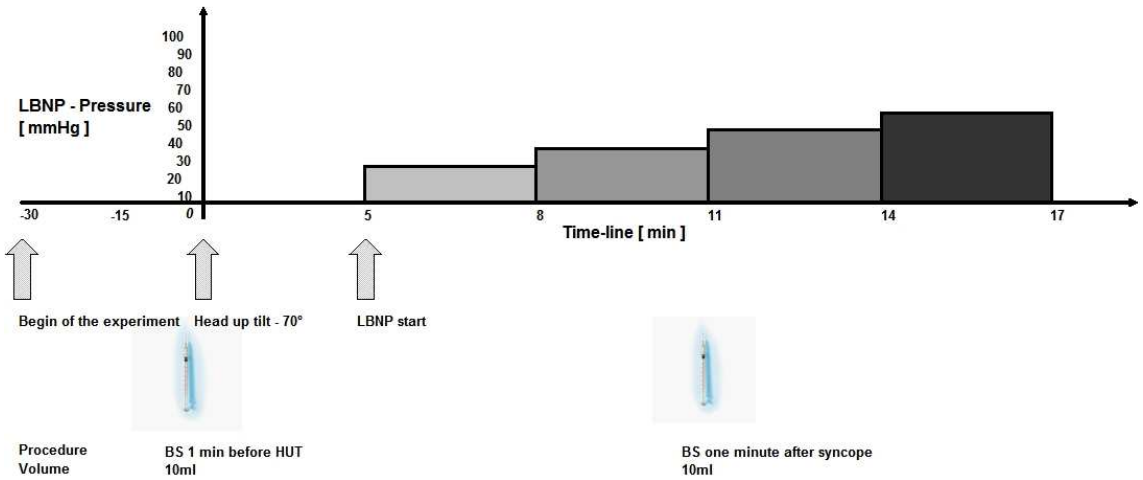

Fig. 1: *GOS protocol and blood sampling procedure.*

a continuous self-calibration against conventional oscillometric pressure determination on contralateral arm. Total peripheral resistance index (TPRI) was calculated as mean arterial blood pressure/cardiac index [21]. We used thoracic fluid content (TFC) to estimate changes in thoracic impedance (TI). Mean arterial blood (MAP) was calculated through
MAP = ((SPB-DBP) / 3) + DBP.

LBNP and HUT: Graded orthostatic stress (GOS)

We use a specially designed LBNP seal (Institute for Adaptive and Spaceflight Physiology, Graz, Austria) that allows for precise positioning at the crista ilica superior level, without relocation during LBNP. The abdominal region remained outside of the negative pressure area at all times. The entire GOS protocol was performed computer controlled, employing the IAP`S automated human multistimulation test device (www.meduni-graz.iap Fig. 2)

A MatLab based program both executes the test protocol and allows for synchronous recording of the data from the cardiovascular monitoring system (TFM®). Test subjects were secured and had access to an emergency shutdown (automatic return to supine and pressure neutralisation) at all times.

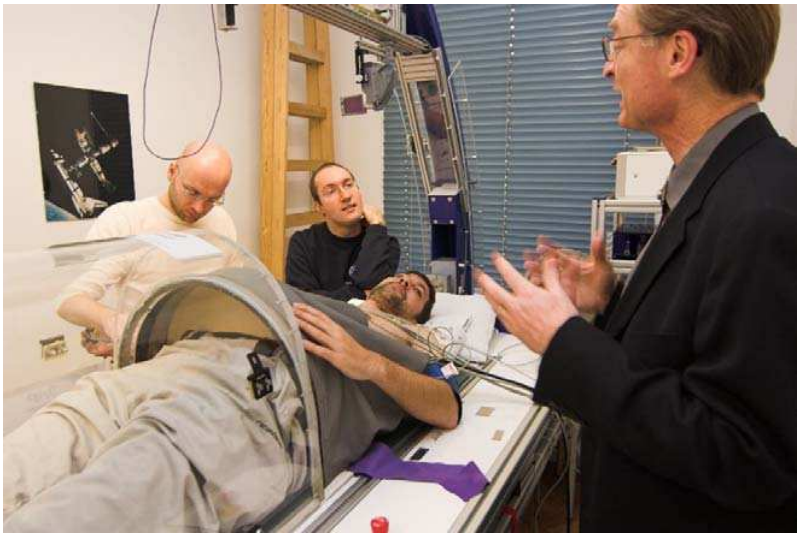

Fig. 2: *AMSD – Automated human multi-stimulation test device.*

Hematocrit and plasma density

Micro-hematocrit was determined in triplicate (10 min at 11.000 rpm) without corrections for trapped plasma or F_C – ratio. Blood plasma mass density (PD; $g \times L^{-1}$ at 37.0°C) was measured based on high-resolution oscillation-time determinations using the mass-spring principle [22]. Plasma samples (0.2ml) were prepared from heparinized blood samples and measured ≤ 60min after spinning. Plasma volume changes (Δ PV) were calculated from the density changes according to

$$FV = 100 \times \frac{PD_1 - PD_2}{PD_2 - 1008} PVd \qquad (\%\ PVd)$$

where PD_1 and PD_2 refer to the hemodiluted and hemoconcentrated state (supine vs. presyncopal), respectively.

Hormone measurements

Measurement of plasma renin activity (PRA) (RENCTK, DiaSorin S.p.A., Italy) is based on competition between labeled angiotensin-1 and native (sample) angiotensin-1 for a limited number of the RIA antibody binding sites. PRA is denoted as ng angiotensin-1 generated per ml plasma per hour incubation ($ngANG1*ml^{-1}*hr^{-1}$). Plasma aldosterone was determined with a modified RIA (ALDOCTK-2, DiaSorin S.p.A., Italy). Serum cortisol was measured by RIA (GammaCoat™, Cortisol, ^{125}I RIA Kit, and DiaSorin S.p.A., Italy).
Catecholamines were determined in one run by isocratic HPLC with electrochemical detection at an oxidation potential of 400-500 mV (Chromsystems CLC 100, solid phase extraction: Chromsystems, Munich, Germany). The mobile phase was pumped through the system (1ml/min) by an Agilent 1100 pump (Hewlett Packard GmbH, Germany). Evaluation was performed using

Waters Empower™ Software (Waters, Milford, MA).

Statistical analysis

Unless otherwise stated, values are given as means ± S.E.M. We used the GraphPad Prism 4 for hypothesis testing, for which we generally used normalized values, i.e., deviations from pre-stimulus control (after 30 minutes supine). Comparisons were made using Student's t-test or Two-way ANOVA with Bonferroni posttest. Single column test was performed with Wilcoxon signed rank-test. Statistical significance was assumed when $p < 0.05$

Results

Mean standing time: Mean standing time was (from begin HUT to presyncope) 12.3 ± 1.2 min.
Hemodynamics: Heart rate increased 68 ± 12% ($p < 0.0001$) whereas stroke volume index fell by 44 ± 4% ($p < 0.0001$), thoracic impedance increased by 12 ± 1% ($p < 0.0001$). Total peripheral resistance index decreased by 11 ± 5% ($p = 0.03$). Systolic blood pressure decreased by 26 ± 3% ($p < 0.0001$), diastolic by 16 ± 5% ($p = 0.0001$), mean by 19 ± 3% ($p < 0.0001$) and pulse pressure by 41 ± 8% ($p = 0.0003$). (Fig. 3)

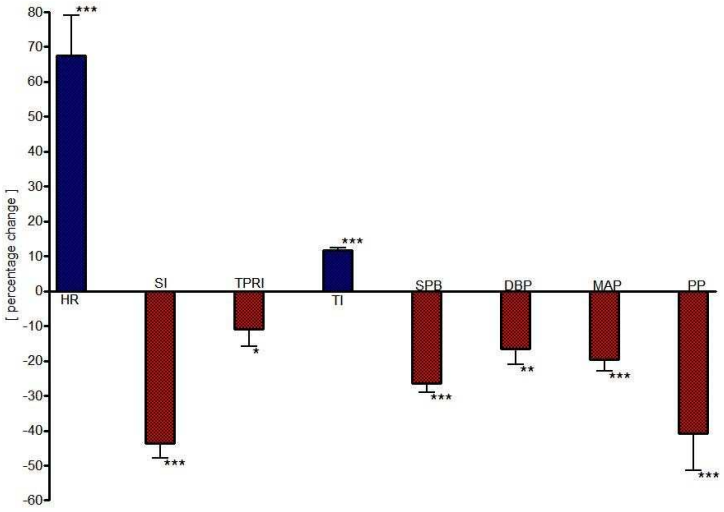

Fig.3: *GOS dependent changes in heart rate (HR), stroke index (SI), total peripheral resistance index (TPRI), thoracic impedance (TI) as well as systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and pulse pressure (PP). Bars show means ± SEM, N=16. ***, P < 0.0005; **, P < 0.005.*

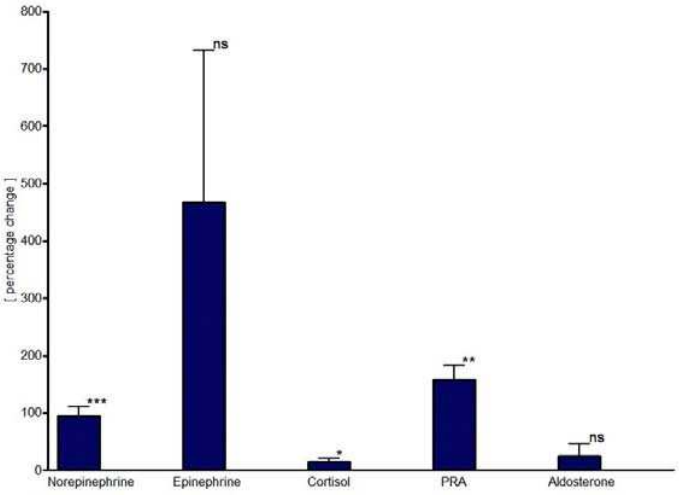

Fig. 4: *GOS dependent changes in norepinephrine (NE), epinephrine (E), cortisol, plasma renin activity (PRA) and aldosterone. Bars represent means ± SEM, N=13. ***, P < 0.0005; **, P < 0.005; *, P < 0.05.*

Hormones and plasma volume loss: Because of blood collect problems three of sixteen test subjects were excluded from further analysis. All syncope values were corrected due to the loss in plasma volume. We have seen a highly significant increase in norepinephrine (86 ± 16%, $p = 0.0001$) and a significant rise in cortisol (10 ± 6%, $p = 0.02$) whereas epinephrine (460 ± 266%, $p = 0.06$) showed no significant difference. Plasma renin activity showed a significant increase (147 ± 26%, $p = 0.002$) but aldosterone remained without a significant change (24 ± 21%, $p = 0.2$). (Fig. 4)

As a result of combined HUT and LBNP paradigm plasma volume decreased by 11 ± 2% ($p = 0.0003$) and hematocrit rose 7 ± 1% ($p < 0.0001$). (Fig. 5)

Discussion:

There exists a considerably body of evidence regarding hormonal and

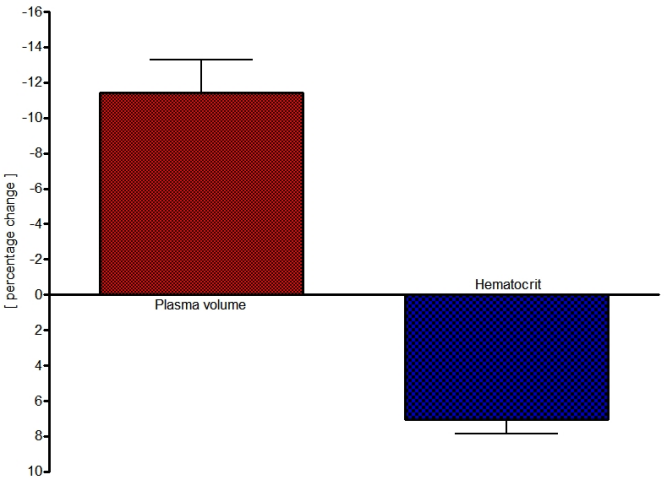

Fig. 5: *GOS dependent changes in plasma volume (PV) and hematocrit. Bars represent means ± SEM, N=13. ***, P < 0.0005;*

hemodynamic changes due to HUT and LBNP in presyncopal state, but little is known about cardiovascular AND hormonal changes in a combined HUT and graded LBNP paradigm. Focussing on hemodynamic effects, El-Bedawi et al. [12] were the first to apply a combined HUT and LBNP protocol to induce (84% of their test subjects) presyncopal signs and symptoms. Lelorier et al. [23] demonstrated that combined HUT and graded LBNP protocol can reliably provoke orthostatic syncope in 100% of test subjects. Julu et al. [24] used a similar protocol and studied beat-to-beat cardiovascular regulation. We used a similar protocol and discovered a significant involvement of a neurohormone called galanin [11]. Our goal in this study was to investigate both cardiovascular and endocrine changes in a presyncopal state.

Neurally mediated syncope is presumably a result of a massive blood pooling in the lower body [25] that causes a sharp decline in venous return and a "partially emptied"[26] heart. Parasympathic traffic between heart and brainstem interferes with sympathetic activity, resulting in a drop in peripheral vascular resistance and bradycardia [27]. Blood pressure decreased in the last 60 seconds before presyncope in all 16 test subjects, which is in agreement to previous findings [12, 23, 24].

The sympathetic nervous system plays an important role in maintaining homeostatic conditions. Exposure to our combined HUT and graded LBNP paradigm inhibits arterial and cardiac baroreceptors which results in an overflow of released NE and E. The main part of NE in the circulation is derived from sympathetic nerves [28] and of E from the adrenal medulla [29]. During neurally mediated syncope the response of plasma catecholamines has been examined extensively and, in terms of NE, produced contradictory results: Decreased [30-32], unchanged [33] or elevated levels have been reported [34-36] . This might be due to confounding factors such as sampling time, potential changes in NE clearance and alterations in spillover to the general circulation [37]. It has even been suggested that the interpretation of plasma NE without consideration of associated hemodynamic factors is meaningless [38]. All our test subjects showed elevations in plasma NE and E levels.

Elevations of plasma epinephrine are in agreement with earlier reports on vasovagal syncope [30, 33-35]. It is conceivable that the rise in epinephrine enhances the cardiac contractility which results in promoting reflex bradycardia, vasodilation through peripheral stimulation of β_2- receptors and hypotension (Bezold-Jarisch reflex) [39].

The renin-angiotensin aldosterone system plays an important role in blood pressure regulation. We found an almost 3-fold PRA increase with presyncope. This is in accordance with Harrison et al. [19], who reported a significant rise in PRA in orthostatically intolerant subjects, which has also been repeatedly observed in normal persons with standing alone [40, 41]. Greenleaf et al. [42] compared a high-tolerance group (LBNP was uneventful) with a low-tolerance group (standing time was 11 ± 1 min) which experienced presyncope. Both groups showed an increase in PRA from baseline to test stop but PRA in the low tolerance group was significantly lower than that in the high tolerance group. They concluded that an attenuated activation of PRA facilitates syncope. A relation between hypovolemia and PRA in terms of orthostatic stability was suggested by Jacob et al. [43] who proposed abnormalities in the renin-angiotensin-aldosterone system in orthostatic intolerance.

Surprisingly, we did not find a significant rise in plasma aldosterone for tilt-induced hypovolemia. Dopamine is an aldosterone inhibitor [44] but we did not find dopamine significantly increased either. Angiotensin II was not measured in our study.
Cortisol plasma levels increased significantly in our study which is in agreement with Theodorakis et al. [45] and Wallbridge et al [36].

Conclusion:
The combined HUT- graded LBNP paradigm is a useful tool to provoke syncope in all test subjects, and to study features of cardiovascular and humoral regulation under severe stress conditions.

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