Hormonal and plasma volume changes after presyncope

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ABSTRACT

Background Aim of this study was to test the hypothesis that after presyncope, some blood hormone pools increase while others decrease.

Materials and methods In twelve healthy male adults, we determined plasma volume changes with plasma mass densitometry and hormone levels. The following were compared: supine rest, presyncope and 20-min post-presyncopal supine rest. We determined plasma renin activity (PRA), aldosterone, adrenocorticotropic hormone (ACTH), adrenomedulain and vasopressin (AVP) from venous blood samples.

Results Using passive 4-min 70° head-up tilt followed by 4-min sequences of additional lower body negative pressure of increasing intensity (15 mmHg steps), presyncope occurred after 11±6±8 min, at which time plasma volume was reduced by 15±5±7±4%, aldosterone increased by 37%, ACTH by 75%, PRA by 187% and AVP about 16-fold in average (all \( P < 0.01 \)); no significant changes in adrenomedullin were seen. Twenty-min post-presyncope, ACTH increased above presyncopal levels (+36%, \( P < 0.05 \)), aldosterone by 35% \( ( P = 0.07 ) \). PRA \( ( -47\% , P < 0.01 ) \) and AVP \( ( -84\% , P < 0.05 ) \) decreased below presyncopal but were still above supine control \( ( P < 0.01 ) \); similarly, plasma density fell by 2.17 ± 0.97 g L\(^{-1}\) below presyncopal \( ( P < 0.01 ) \), but above supine control \( ( P < 0.05 ) \), indicating rapid recovery (83% of initial plasma volume).

Conclusions We conclude that during the 20-min supine post-syncopal period, plasma volume, PRA and AVP return closer to baseline but aldosterone and ACTH continue increasing. The magnitude of observed concentration changes cannot be explained by haemoconcentration/haemodilution, rather it appears that the observed changes are indicative of hormone-specific endocrine activation patterns in the recovery phase.

Keywords ACTH, Aldosterone, Hormones, LBNP, Passive head-up tilt, PRA.


Introduction

Orthostasis moves blood to venous vessels below the heart, diminishing central blood volume and cardiac preload. Neuronal firing from both carotid and cardiopulmonary baroreceptors – with its inhibitory baroreflex effect on vascular resistance and heart rate – decreases because both receptor areas are located above the respective (arterial and venous) hydrostatic indifference levels [1]. This triggers corresponding sympathetic and humoral responses.

Maximal hormonal stimulation can be expected to occur when brain perfusion declines to a critical level and presyncope occurs. In healthy persons, this is hard to accomplish with head-up tilt (HUT) alone. Lower body ‘negative’ pressure (LBNP) simulates orthostasis by reducing cardiac preload as well [2]; in combination with HUT, it can be used to induce presyncope [3–5] in a reproducible manner [6].

Endocrine changes because of either HUT or LBNP have been repeatedly investigated [7–10] but barely any investigation dealt with endocrine changes that occur after presyncopal stress. This is important in physiological terms, i.e. cardiovascular readaptation to supine posture after orthostasis, and because recovery from stress is gaining grounds in its possible implication in the development of diseases. The aim of the present study was to determine plasma aldosterone, renin...
activity (PRA), adrenocorticotropic hormone (ACTH), adrenomedullin (ADM) and arginine vasopressin (AVP) at fully developed stage, and 20 min after, presyncopeal central hypovolemia along with haemodynamic and plasma volume changes.

**Methodology**

Twelve Caucasian men (24·8 ± 1·6 years, 76·5 ± 7·0 kg, 179 ± 4 cm) were familiarized with the test protocol and gave written informed consent. Inclusion: Age 20–30 years, physically fit, man, without medical condition. Exclusion: Consumption of coffee or other stimulants, unusual exercise in the week prior to the study. The protocol was approved by the Graz Medical University Ethics Board and performed in accordance with the 1989 WMA Declaration of Helsinki. Reporting of the study conforms to STROBE statement along with references to STROBE and the broader EQUATOR guidelines (Simera et al. January 2010 issue of EJCI).

Experiments were carried out in a dimmed quiet room (23–24 °C, 50–55% humidity) between 9 and 11 a.m. Each test started with a 30-min supine rest period to acquire cardiovascular data in steady-state conditions [11]. Tests were carried out using a tilt table-LBNP device equipped with an adjustable footrest; LBNP sealing was positioned at the iliac crest [12].

Monitoring of blood pressure and heart rate (3-lead ECG) was commenced during the supine control phase using both intermittent brachial cuff and continuous finger measurements (Task Force® Monitor; CNSystems, Graz, Austria). Subjects were instructed to avoid leg movements and to breathe normally. They were secured and had access to an emergency shut-down (automatic return to supine and pressure neutralization) at all times. The execution of the pre-programmed test protocol and synchronous data recording was performed using LabView® (National Instruments, Austin, TX, USA).

**HUT + graded LBNP protocol**

At minute zero, the tilt table was brought from 0° (supine) to 70° head-up position for 4 min, after which pressure in the LBNP chamber was reduced by 15 mmHg every 4 min until presyncopeal signs or symptoms occurred (Fig. 1). At this point, the table returned to 0° and LBNP was stopped at once. Data recording was continued for 20 more minutes. In all our test subjects, presyncope was reached. Presyncope was defined as the occurrence of at least one of the following conditions: (i) Blood pressure drop below systolic 80 mmHg or by ≥20 mmHg within 1 min, diastolic by ≥15 mmHg/min, heart rate by ≥15 bpm (nine subjects); (ii) Light-headedness, dizziness, visual disturbances, nausea, stomach awareness, clammy skin, excessive sweating or skin pallor (three subjects). Subjects were free to stop the test anytime at their request; however, all the experiments were continued until presyncope occurred.

**Blood sampling**

From an uncongested vein, maintained at the heart level during supine and upright positions to avoid erroneous readings because of hydrostatic effects [1], blood was sampled using a 17G-1 × 40 mm Teflon® catheter immediately before orthostatic stress was commenced, at presyncope, and a third one 20 min later (supine) into 10-ml syringes and transferred into aprotinin-/EDTA-treated tubes that were immediately put on ice and then spun for 15 min at 1500 G and 4 °C (Ivan Sorvall, New York, NY, USA, RC2-B). An aliquot was used for plasma mass densitometry, and remaining plasma was stored at –80 °C for later hormone analysis.

**Measurements and calculations**

Plasma volume shifts were estimated from plasma mass density (g L⁻¹, 37·00 ± 0·02 °C) measurements because of superior accuracy and precision of high-resolution oscillation-time determinations using the mass-spring principle: Mass density of the fluid moved into or from the circulating blood is assumed 1008 g L⁻¹, and its volume (FV) – expressed as relative change of plasma volume (% PV) – is computed (c = presyncope/haemoconcentrated, d = supine/haemodiluted) from plasma density (PD) changes according to [11]. For the upright phase where haemoconcentration occurs, calculation was carried out from Eq. 1:

\[
F_{\text{Vout}} = 100 \times \frac{PD_d - PD_c}{PD_c - 1008} \quad (\%PVd) \tag{1}
\]

Where PVd is plasma volume in the supine control condition. For (supine) haemodilution after presyncope, Eqn. 2 applies [13]:

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**Figure 1**  Test design and time protocol. 1: Control blood sampling after ≥30 min supine. The orthostatic stimulation phase started with passive 70° head-up for 4 min, then 15 mmHg LBNP was commenced in this position and increased by 15 mmHg every 4 min until presyncopeal signs or symptoms occurred (flash symbol). At this point, LBNP was stopped, the table tilted back and sample 2 drawn at once. Supine position was maintained for 20 min, at the end of which sample 3 was taken.
\[
FV_{\text{in}} = 100 \times \frac{PD_c - PD_d}{PD_d - 1008} (\%PV_c) \tag{2}
\]

\[
FV = (FV_{\text{out}}/100)(100 - FV_{\text{in}}) (\%PV_d) \tag{3}
\]

FV values are given as % PVd throughout to be quantitatively comparable.

**Hormones**

Aldosterone was measured using a competition assay (Immuno-tech RIA kit IM1664), plasma renin activity (PRA) with an angiotensin I-coated tube \(^{125}\)I RIA (DiaSorin), ACTH by means of an immunoradiometric assay (DiaSorin ACTH IRMA kit REF 27130), adrenomedullin with a Peninsula Laboratories RIA and vasopressin using RIA kits (Stillwater, MN, USA). Hormone levels were above detection limit in all samples.

**Data analysis**

Values are given as mean ± SD. A nonparametric paired Wilcoxon test was used to compare the related conditions (i) supine rest and presyncopal, (ii) supine rest and 20-min post-stress and (iii) presyncopal and 20-min post-stress.

**Results**

Test subjects reached presyncope after 11 ± 2.8 min progressive orthostatic loading (lowest, 7.5; highest, 16.3 min; tolerance times appeared evenly distributed within that range). Diastolic (supine, 82 ± 7) and mean arterial pressures (supine, 98 ± 8 mmHg) were reduced (on average by 6 mmHg each) at presyncope. Heart rate increased from 59 ± 7 (supine) to 114 ± 30 bpm (presyncopal) and returned to control levels 20-min post-stress, as did the pressure values. Hormonal concentration values and significant effects are given in Table 1.

### Presyncope

Aldosterone, PRA, vasopressin and ACTH were elevated above supine values at presyncope; plasma volume decreased 15.5 ± 7.4% (Eq. 1; all \(P < 0.01\)). ADM changes were not significant.

### Post-presyncopal (supine) phase

Twenty minutes following presyncope, plasma volume was mostly regained (12.8 ± 5.9% of initial supine control plasma volume) as calculated according to Eqns 2 and 3; plasma density values were still slightly above supine control (\(P < 0.05\)). ACTH (+15 pg mL\(^{-1}\), \(P < 0.05\)) and aldosterone (+37 pg mL\(^{-1}\), but \(P = 0.07\)) further increased above presyncopal values. In contrast, AVP (−108 pg mL\(^{-1}\), \(P < 0.05\)) and PRA values (−0.63 ng mL\(^{-1}\) per h, \(P < 0.01\)) fell below presyncopal, but still above supine control (Table 1).

### Discussion

The main new finding of this study is that during the 20-min supine post-syncopal period, plasma volume, PRA and AVP return closer to baseline but aldosterone and ACTH continue increasing. The varying hormonal responses do not appear to be explained by haemoconcentration: plasma volume gained 12.8%, averaging 2.7% below, of the initial supine value. **Correction added on 12 April 2011, after first online publication:** the sentence “plasma volume gained 12.8%, averaging 2.7% above, of the initial supine value” was changed to “plasma volume gained 12.8%, averaging 2.7% below, of the

| Table 1 Hormone plasma concentration and plasma mass density values, mean ± SD. Presyncopal values determined from sample immediately taken after test subject was brought back to supine position |
|-----------------|-----------------|-----------------|
| **Hormone**     | **Supine control** | **Presyncopal** | **20-min post-presyncope** |
| Aldosterone (pg mL\(^{-1}\)) | 78 ± 52 | 107 ± 63* | 144 ± 85* |
| PRA (ng ATI mL\(^{-1}\) per h) | 0.47 ± 0.34 | 1.35 ± 1.03* | 0.72 ± 0.56* ** |
| ACTH (pg mL\(^{-1}\)) | 24 ± 8 | 42 ± 24* | 57 ± 31* ** |
| ADM (pg mL\(^{-1}\)) | 59 ± 34 | 86 ± 52 | 62 ± 39 |
| AVP (pg mL\(^{-1}\)) | 0.74 ± 0.22 | 12.83 ± 11.60* | 2.04 ± 1.16* ** |
| PD (g L\(^{-1}\)) | 1018±50 ± 0.88 | 1020±79 ± 1.14* | 1018±62 ± 0.97* ** |

Twenty min post = 20 min after subject was brought back to supine rest after presyncope. PRA, plasma renin activity; ACTH, adrenocorticotropic hormone; ADM, adrenomedullin; AVP, vasopressin; PD, plasma density (g L\(^{-1}\), 37 °C). *Significant change compared with supine control; **Compared with presyncopal.
increased adrenomedullin has been reported after 2 min of 70 subjects’ average orthostatic tolerance times were < 12 min; post-stress. From the circulation to a higher degree than aldosterone 20-min times after stress release, PRA may already have been cleared lower in tolerant persons after 45-min HUT [16]. Twenty min-higher in persons who tolerated LBNP-50 (18 min) but orthostatic tolerance is not clear; however, PRA was found (releasing factor?) from the brainstem.

Half-life, suggesting there was still increased ACTH output that tolerance limit was reached in our test subjects at the presyncopal event had prolonged after-effects in terms of hypothalamic stress responses.

Adrenomedullin levels were expected to rise during orthostatic stress because of earlier observations [7,20] but our presyncopal adrenomedullin was not significantly elevated. Our subjects’ average orthostatic tolerance times were < 12 min; increased adrenomedullin has been reported after 2 min of 70° passive HUT, a condition of simple central hypovolemia that evokes moderate sympathoadrenal activation [20]. On the other hand, two other studies did not observe any ADM increase during 20 or 30 min of 70 HUT° [21,22]. Thus, two studies found an effect of central hypovolemia on adrenomedullin whereas three others, including the present one, did not. The kit we used in this study measures total adrenomedullin. There are two molecular forms of adrenomedullin present in human plasma, active ‘mature’ and inactive glycine extended [22]. These authors found both of these forms to be unaffected by HUT.

Plasma vasopressin concentration (AVP) has repeatedly been reported to increase with orthostatic stress, particularly at presyncope [14,17,23]. The rise in AVP seems to be greater in less tolerant people undergoing LBNP [15] or head-up tilt [10]. We wanted to use the presyncopal rise of AVP as a reference and found a 16-fold increase compared with supine rest, suggesting this phase. A post-orthostatic aldosterone increase is in line with earlier observations [7,19]. Aldosterone after presyncope was clearly elevated (P < 0.01) compared with supine control, indicating an increased circulating aldosterone pool.

ACTH and aldosterone relationship
ACTH and aldosterone profiles seem to be similar during and after intense orthostatic stress. Causal connections between ACTH and aldosterone release cannot be concluded just on the basis of time courses, however. The fact that aldosterone half-life is longer (approximately 30 min) than that of ACTH (5–10 min) or PRA (10–15 min) [14,24,25] does neither explain further aldosterone increase nor a seemingly continuing ACTH output after presyncopal orthostatic stress. Perhaps, the presyncopal event had prolonged after-effects in terms of hypothalamic stress responses.

We previously observed a similar close correlation between aldosterone and ACTH during- and between-repeated HUT and LBNP sessions [26]. Because a relationship between the rate of blood flow through the adrenal gland and the rate of glucocorticoid secretion has been proposed since the early 1950s [27], elevated ACTH levels might be mainly responsible for the observed aldosterone levels. Our findings of increases in ACTH and aldosterone during repetitive orthostatic stresses [26] and during recovery (such as in this study) seem to suggest that as well. However, while correlations can indicate a predictive relationship, they do not mean a causal relationship.

The zona glomerulosa is the primary site of renin formation in humans [28], and this adrenocortical renin production is able to regulate aldosterone synthesis directly from the zona glomerulosa [29]. This adrenal RAAS not only generates Ang II but may also respond to physiological demands from the circulatory system. This adrenal RAAS supports aldosterone synthase expression in this way, representing a chronic action instead of the acute effects known from the systemic RAAS [30].

Finally, the chemical nature of the hormonal cascade components could be responsible for these differences. For example, renin, Ang II and ACTH are peptide products; however, aldosterone is steroidal in structure. This situation implies that synthesis of aldosterone within the zona glomerulosa is perhaps determined by different enzymatic activities. It can be speculated that an initial activation of enzymatic cascade for aldosterone synthesis might last longer than comparable half-lives of other peptide hormones.

Because AVP is a potent stimulator of aldosterone production in human glomerulosa cells and is also formed in the adrenal medulla [31], AVP levels may be co-responsible for elevated aldosterone concentrations. However, in our experiments, during recovery AVP levels had returned close to baseline levels.
Changes in circulating blood volume
Hormones in the blood plasma have a low transcapillary escape rate because of their nature as peptides, or they are partly protein bound, such as steroids (aldosterone approximately 60%; [32]). Consequently, plasma hormone concentration changes may be caused by capillary filtration, as it occurs with haemoconcentration of orthostasis [11]. We, therefore, wanted to assess the degree of fluid loss from the intravascular compartment under HUT + LBNP.

Haemoconcentration because of capillary filtration with orthostasis increases plasma protein concentration and mass density, the measurement of which allows for the computation of plasma volume changes. Plasma mass density rose by an average 2.29 ± 1.14 g L⁻¹ from supine control to presyncope, indicating an average 15.5 ± 7.4% plasma volume loss, similar to previous observations with passive head-up tilt [9,14,33]. Twenty minutes after the orthostatic challenge had ceased, plasma density decreased by 2.17 ± 0.97 g L⁻¹ below presyncopal, indicating that most (83%) of the fluid lost during upright plus LBNP was regained at that point in time. Similar effects have been found in earlier head-up tilt studies as well [11,33]. None has yet investigated plasma volume changes during and after combined HUT+LBNP up to syncopal effect, however. The fast return of plasma density – an indicator of plasma protein concentration [13] – to supine control values is still puzzling. The reason for this quick volume gain is unclear; exchange between microcirculation and macrocirculation [34] that have different red cell concentrations would influence haematocrit but not protein concentration. Lymphatic return of fluid back to the circulation would be expected to take much longer than 20 min.

We, therefore, speculate that capillary return of fluid that was filtered out during orthostasis is the most probable explanation for the fast fall in plasma density after presyncope, indicating capillary inward filtration in the post-stress supine phase as the main mechanism for the observed haemodilution.

Limitations
Plasma hormone concentrations change in hormone-specific manners when physiological conditions are altered, depending on individual hormone kinetics (incretion rate, fractional clearance, total mass, distribution volume). Relatively rapid changes mainly reflect an altered release rate to the circulation. Mere concentration measurements do not allow discerning between effects because of release and clearance changes, however. Plasma volume changes were determined in this study because they too influence hormone concentration values. Increasing concentration vis-à-vis rising plasma volume, as seen with ACTH and aldosterone in the recovery phase, can only mean an even larger increase in the respective circulating hormone pool. We did not measure angiotensin that acts as direct stimulus for extrinsic aldosterone secretion, and its possible role for aldosterone release remains speculative.

In summary, the present results show that circulating hormone pools after presyncope have different time courses between hormones. Twenty minutes after central hypovolemia was released, with plasma volume nearly restored, aldosterone and ACTH levels further increased – indicating an even larger fractional increase in the respective circulating pool – while PRA and AVP partly returned to supine control levels. The magnitude of observed concentration changes cannot be explained by haemoconcentration/haemodilution, rather it appears that the observed changes are indicative of hormone-specific endocrine activation patterns in the recovery phase after presyncopal central hypovolemia.

Perspectives
This study investigated changes in hormone levels and plasma volume occurring from supine control to the point preceding imminent syncpe, and in the early supine recovery phase thereafter. We found a clear dissociation in the post-presyncopal changes between hormones. To explain these unexpected results, it would be important to gather data on angiotensin and cortisol changes as well, which will be carried out in a follow-up study. Further, as hormone levels conceivably continue to increase during longer durations of orthostatic load before presyncope, different presyncopal levels and possibly different time courses after the orthostatic stimulus would be seen. This assumption remains to be tested. Further, additional studies are needed to investigate the full time course of hormone pools in the post-presyncopal recovery period. Such data might also help to create a simplified model of fluid and hormonal pool kinetics.

Acknowledgements
We thank our participants for their time and patience.

Financial disclosure
This study was done as part of a European Space Agency sponsored project and was supported by the Austrian Research Promotion Agency (FFG project 817086 ‘Orthocap’).

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Received 9 December 2010; accepted 22 February 2011

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