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Abnormalities of Angiotensin Regulation in Postural Tachycardia Syndrome

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Abstract

Background—Postural tachycardia syndrome (POTS) is a disorder characterized by excessive orthostatic tachycardia and significant functional disability. Previously, we reported that POTS patients have low blood volume and inappropriately low renin activity (PRA) and aldosterone. In this study, we sought to more fully characterize the renin-angiotensin-aldosterone system (RAAS), to gain a better understanding of the pathophysiology of POTS.

Objective—We prospectively assessed the plasma levels of Angiotensin (Ang) peptides and their relationship to other RAAS components in patients with POTS compared with healthy controls.

Methods—While on a sodium controlled diet, heart rate (HR), PRA, Ang I, Ang II, Ang (1–7) and aldosterone were measured in POTS patients (n=38) and healthy controls (n=13).

Results—POTS patients had larger orthostatic increases in HR than controls (52±3 [mean±SEM] bpm vs. 27±6 bpm; $P=0.001$). Plasma Ang II was significantly higher in POTS patients (43±3 pg/ml vs. 28±3 pg/ml; $P=0.006$), while plasma Ang I and Ang-(1–7) were similar between groups. Despite the two-fold increase of Ang II, POTS patients trended to lower PRA levels than controls (0.9±0.1 ng/mL/h vs. 1.6±0.5 ng/mL/h, $P=0.268$) and lower aldosterone levels (4.6±0.8 pg/ml vs. 10.0±3.0 pg/ml; $P=0.111$). Estimated angiotensin-converting enzyme-2 (ACE2) activity was significantly lower in POTS than controls (0.25±0.02 vs. 0.33±0.03; $P=0.038$).

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Conflicts of Interest - None

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Conclusions—Some patients with POTS have inappropriately high plasma angiotensin II levels, with low estimated ACE2 activity. We propose that these abnormalities in angiotensin regulation may play a key role in the pathophysiology of POTS in some patients.

Keywords

tachycardia; autonomic nervous system; angiotensin II; aldosterone; ACE2

Background

Postural tachycardia syndrome (POTS) is a chronic disorder characterized by an excessive increase in heart rate on standing, in the absence of orthostatic hypotension. It is estimated that 500,000 patients are affected in the United States alone ¹. This disorder disproportionately affects women of childbearing age ^{2–3}. Patients often suffer from palpitations, lightheadedness, and mental clouding ⁴, and POTS is associated with significant functional disability and diminished quality of life ⁵.

Multiple pathophysiological mechanisms may contribute to the orthostatic tachycardia intolerance in POTS. These include increased sympathetic tone (reflected by elevated plasma norepinephrine levels) ^{2–6}, partial autonomic neuropathy ⁷ and low blood volume ⁸. The renin-angiotensin-aldosterone system (RAAS) plays a vital role in blood volume regulation. Renin catalyzes the production of angiotensin (Ang) I, which is converted to Ang II by angiotensin-converting enzyme (ACE). Ang II then stimulates the production of aldosterone, which promotes renal sodium reabsorption. In response to a blood volume deficit, one would expect up-regulation of the RAAS in an effort to stimulate reabsorption of sodium and water and correct the blood volume. We previously reported that patients with POTS have inappropriately low levels of plasma renin activity (PRA) and aldosterone in response to the low blood volume (all assessed in the supine position) ^{9–10}. Ang levels were not assessed in that prior study. More recently, Stewart et al.¹¹ reported that a subgroup of POTS patients has increased plasma levels of Ang II. Using a skin model¹², Stewart et al. proposed decreased activity of angiotensin-converting enzyme 2 (ACE2) in POTS patients associated with skin blood flow abnormalities that could be rescued with Ang-(1–7). To date, similar abnormalities have not been demonstrated in the systemic circulation. Given the abnormal blood volume regulation that has already been documented in POTS, we sought to characterize angiotensin regulation in POTS to gain a better understanding of the pathophysiology, and identify novel targets for treatment.

Methods

Subjects

Thirty-eight patients referred to the Vanderbilt University Autonomic Dysfunction Center with POTS between September 2005 and September 2009 and 13 healthy control subjects were included in this study. Patients with POTS met the conventional criteria ^{9–13}. Briefly, patients developed symptoms of orthostatic intolerance accompanied by a heart rate rise ≥ 30 bpm that occurred within the first 10 minutes of standing or head-up tilt, without any evidence of orthostatic hypotension (a fall in blood pressure of $\geq 20/10$ mmHg). Patients had at least a 6-month history of symptoms, in the absence of another chronic debilitating disorder or prolonged bed rest, and were at least 18 years of age. Healthy control subjects were similar in age to the POTS patients. None of the control subjects had symptoms of orthostatic intolerance. Due to the strong female predominance in POTS, only female control subjects were recruited. POTS patients and control subjects were free of medications that could impact cardiovascular tone for at least 5 half-lives and did not take fludrocortisone for at least 5 days before testing. Patients were allowed to remain on

selective serotonin reuptake inhibitors and oral contraceptives (that did not contain drospirone) at constant doses. The medication categories that subjects were taking (both prior to admission and during the study) are categorized in Supplemental Table 1. The Vanderbilt University Investigational Review Board approved this study, and written informed consent was obtained from each subject before the study began. The protocols reported here were parts of a study entitled —The Pathophysiology of Orthostatic Intolerance (ClinicalTrials.gov NCT00608725).

Protocol

Study investigations were performed on the Elliot V. Newman Clinical Research Center at Vanderbilt University. For at least 3 days before testing, study subjects consumed a standardized methylxanthine-free diet that provided 150 mEq/day of sodium and 70 mEq/day of potassium. On one day, each subject underwent a Stand Test with supine and upright vital signs and plasma catecholamines. This test is routinely used to characterize our patients with POTS. On a separate morning, while in a fasting state, each subject had her blood sampled for PRA, serum aldosterone and plasma angiotensin species while in a supine body position.

Stand Test with Supine and Upright Vitals and Catecholamines

The Stand Test was performed to assess the hemodynamic and biochemical responses to increased central hypovolemia (accentuated by the gravitational stress). Heart rate, blood pressure, and plasma norepinephrine and epinephrine were measured after overnight rest with subjects in the supine position and again after subjects had been standing for up to 30 minutes (as tolerated). For catecholamine measurements, blood was collected in plastic syringes and immediately transferred to chilled vacuum tubes containing sodium heparin. The plasma was separated by refrigerated centrifugation at -4°C , reduced glutathione (6%) was added, and samples were stored at -80°C until the assay. Concentrations of norepinephrine and epinephrine were quantified by high-performance liquid chromatography with electrochemical detection following adsorption of plasma catechols onto acid-washed alumina¹⁴.

Assessment of Menstrual Cycle Phase

All subjects were pre-menopausal. In order to account for variability related to the phases of the menstrual cycle, estradiol and progesterone levels were sampled simultaneously with the angiotensin species. Subjects were defined as being in the follicular phase if progesterone was <1.5 ng/ml and in the luteal phase if progesterone was >2.5 ng/ml. Estradiol and progesterone levels were measured by solid phase, competitive chemiluminescent enzyme immunoassays in the Vanderbilt Clinical Diagnostics Laboratory. The estradiol assay has a working range of 20 – 2000 pg/mL with intra- and inter-assay precision of approximately 5%. The progesterone assay has a working range of 0.2 – 40 ng/mL with intra- and inter-assay precision of approximately 10%. Both assays were performed on the Immulite 2000 instrument (Siemens Healthcare Diagnostics Inc., Los Angeles, CA).

Evaluation of Renin Activity, Aldosterone and Angiotensin Species

PRA was assayed by conversion of angiotensinogen to Ang I by a radioimmunoassay technique (antibodies from IgG Corporation) and reported in nanograms of Ang I per milliliter per hour. Blood for aldosterone was collected in chilled vacuum tubes without preservative, and the serum was extracted and sent to the laboratory on ice. Serum aldosterone was measured by radioimmunoassay (DPC Coat-a-Count, Diagnostic Products Corp).

Blood for determination of Ang peptides (10 ml) was poured into pre-chilled tubes that contained 0.5 ml of an inhibitor solution composed of 25 mM NH₄-EDTA, 0.44 mM *o*-phenanthroline (Sigma, St. Louis MO), 0.12 mM pepstatin A (Sigma, St. Louis MO) and sodium p-hydroxymercuribenzoate (Sigma, St. Louis MO). This cocktail prevents the *in vitro* metabolism of Ang I during manipulation of the sample. Blood samples were centrifuged at 3000 rpm for 20 min at 4°C, and aliquots of plasma were stored at -80°C until assayed.

Angiotensin samples were analyzed at the Wake Forest Hypertension Core Laboratory. Plasma was extracted using Sep-Pak columns, as previously described^{15, 16}. The sample was eluted, reconstituted and split for the three radioimmunoassays. Recoveries of radiolabeled Ang added to the sample and followed through the extraction were 92% (n = 23). Samples were corrected for recoveries. Ang I was measured using a commercially available kit (Peninsula, Belmont, CA, USA). Ang II was measured using a kit produced by ALPCO Diagnostics (Windham, NH, USA) and Ang-(1-7) was measured using the antibody described previously^{17, 18}. The minimum detectable levels of the assays were 2.5 pg/tube for Ang-(1-7), 0.8 pg/tube for Ang II and 1.25 pg/tube for Ang I. Values at or below the minimum detectable level of the assay were arbitrarily assigned half that value for statistical analysis. The interassay coefficients of variation were 18% for Ang I, 12% for Ang II, and 8% for Ang-(1-7). The antibody used in the Ang II kit shows cross-reactivity with Ang III-(2-8) and Ang IV-(3-8), but no cross-reactivity with Ang I. Therefore the values reported for Ang II do not distinguish between Ang II, Ang III and Ang IV.

ACE 2 Enzyme Activity and Adrenal Responsiveness

Enzyme activity was estimated from the ratio of the product to substrate. ACE2 activity was estimated as the ratio of Ang-(1-7) to Ang II, reported without units.

Angiotensin II binds to the adrenal AT-1 receptor to signal the synthesis and release of aldosterone. We estimated adrenal responsiveness by calculating the ratio of aldosterone (output) to Ang II (receptor ligand), and was reported without units.

Sample-size determination

Stewart et.al.¹¹ observed Ang II values with a standard deviation of 13 pg/ml. This study was designed to have 90% power at the 5% level to detect a true difference in Ang II response between cases and controls of 13 pg/ml¹⁹.

Statistical considerations

Data including baseline characteristics (demographics, clinical and biochemical data) are expressed as mean ± SEM (unless otherwise noted). Groups were compared with the Student's *t* test. The Mann-Whitney *U* test was also used to confirm the results obtained from the Student's *t* test, and the significance of the reported parameters was not different between the two tests. Categorical data (e.g. menstrual cycle phase) were analyzed using a Fisher's Exact test. Statistical analyses were carried out using the statistical software SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL). All of the tests were 2-sided, and *P*<0.05 was considered statistically significant.

Results

Baseline characteristics

We studied 38 patients with POTS (36 females and 2 males) and 13 age-matched (all females) control subjects. Baseline characteristics were similar between the two groups and

are summarized in (Table 1). The majority of subjects in both groups were studied in the follicular phase of their menstrual cycle.

Stand Test with Supine and Upright Vitals and Catecholamines

POTS patients had a greater increment in heart rate than control subjects on standing (52 ± 3 bpm vs. 27 ± 6 bpm; $P=0.001$), as would be expected given the diagnostic criteria for POTS. Supine heart rate was higher in POTS patients compared to control subjects (70 ± 2 bpm vs. 62 ± 3 bpm; $P=0.022$), while the standing heart rate was markedly higher in POTS than control subjects (122 ± 4 bpm vs. 89 ± 5 bpm; $P<0.001$). The supine systolic blood pressure was similar between POTS and control subjects (107 ± 2 mmHg vs. 104 ± 4 mmHg, $P=0.439$), and was not significantly different on standing (107 ± 4 mmHg vs. 102 ± 4 mmHg; $P=0.450$). There was a non-significant trend toward a higher supine diastolic blood pressure in patients with POTS in comparison to control subjects (67 ± 1 mmHg vs. 62 ± 2 mmHg, $P=0.081$). This difference diminished with standing, and upright values were not significantly different (72 ± 2 mmHg vs. 70 ± 3 mmHg; $P=0.633$).

Plasma norepinephrine was significantly higher in POTS patients than control subjects both when supine (261 ± 44 pg/ml vs. 134 ± 10 pg/ml; $P=0.009$) and while standing upright (794 ± 87 pg/ml vs. 399 ± 36 pg/ml; $P<0.001$). Plasma epinephrine values were not different between the two groups in either body position (Table 1).

PRA and Aldosterone

There was a non-significant trend toward lower PRA (0.9 ± 0.1 ng/mL/h vs. 1.6 ± 0.5 ng/mL/h, $P=0.268$) and serum aldosterone (4.6 ± 0.8 pg/ml vs. 10.2 ± 3.1 pg/ml; $P=0.111$) in POTS compared to control subjects.

Angiotensin Species in Plasma

Ang II levels were significantly higher in POTS patients when compared to control subjects (43 ± 3 pg/ml vs. 28 ± 3 pg/ml; $P=0.006$; Figure 1B). In contrast, Ang I levels were not different between POTS and control subjects (78 ± 10 pg/ml vs. 57 ± 6 pg/ml; $P=0.311$; Figure 1A), nor were Ang-(1–7) levels (8 ± 1 pg/ml vs. 8 ± 1 pg/ml; $P=0.837$; Figure 1C).

ACE2 Activity and Adrenal Responsiveness

As seen in Figure 1D, POTS patients had lower estimated ACE2 activity (Ang-(1–7)/Ang II ratio) compared with control subjects (0.25 ± 0.02 vs. 0.33 ± 0.03 ; $P=0.038$). Adrenal responsiveness was assessed as a ratio of aldosterone:Ang II. This was significantly lower in POTS patients than control subjects (0.14 ± 0.03 vs. 0.36 ± 0.08 ; $P=0.004$).

Discussion

In an adult cohort of patients with POTS, we have reproduced our previously reported findings⁹ that PRA and aldosterone levels are inappropriately low in POTS, and have now documented abnormal regulation of Ang II. The main new findings of this study are that: 1) circulating Ang II levels are increased in an unselected cohort of POTS patients; 2) estimated systemic ACE2 activity is reduced in POTS, as estimated from the circulating Ang-(1–7) and Ang II plasma levels; and 3) there is decreased estimated adrenal responsiveness to Ang II.

High Ang II in POTS

PRA and serum aldosterone concentration were both reduced in POTS patients in agreement with our prior findings⁹. In the traditional model of the renin-angiotensin-aldosterone

system, renin converts angiotensinogen to Ang I, the precursor to Ang II, and Ang II stimulates aldosterone production via the angiotensin receptor – Type 1 (Figure 2 TOP). This pathway is normally stimulated by a decrease in blood volume. Levels of PRA and aldosterone are therefore paradoxically low in patients with POTS, given their low blood volume⁹. It is interesting that despite their low PRA and aldosterone, POTS patients had significantly elevated levels of plasma Ang II (Figure 2 BOTTOM). These high Ang II levels are similar to those reported by Stewart et al. in a subset of their adolescent POTS patients¹¹. The discordance of Ang II vis-à-vis PRA and aldosterone suggests that there may be a primary defect in the regulation of Ang II – either overproduction of Ang II or decreased degradation of Ang II. Given that Ang-(1–7) levels were not increased in POTS patients in proportion to the increases in Ang II, it is more likely that the problem is diminished Ang II degradation (due to decreased ACE2 activity) rather than Ang II overproduction (Figure 2 BOTTOM). These findings are in keeping with the hypothesis that relative Ang peptide levels are determined by the balance between ACE and ACE2 activity¹⁶.

Low ACE2 Activity in POTS

ACE2 is a recently identified carboxypeptidase that catalyzes the production of Ang-(1–7) from Ang II²⁰. ACE2 is the primary catabolic pathway for Ang II, and mice with disrupted ACE2 genes have increased plasma Ang II levels²¹. In this study, ACE2 activity was indirectly assessed as the ratio of Ang-(1–7) to Ang II (enzyme product to substrate). The fact that the plasma levels of Ang-(1–7) did not rise in parallel with Ang II suggests that ACE2 activity is diminished in POTS patients. Using a skin blood flow model, Stewart et al. reported that while healthy control subjects had greater skin blood flow than POTS patients at baseline, their skin blood flow decreased to the same level as POTS patients with administration of an ACE2 inhibitor¹². The POTS patients did not experience a change in skin blood flow in response to ACE2 inhibition. The investigators concluded that POTS patients had blunted ACE2 activity. Our data are consistent with the findings of Stewart et al., and extend them from the skin to the systemic circulation. The cause of the decreased ACE2 activity in POTS is not clear. It could reflect down-regulation of ACE2 by high Ang II²² or negative feedback resultant from the low blood volume. The function and regulation of ACE2 under conditions of reduced blood volume, as in POTS, requires further investigation.

Pathophysiological Role of Ang II in POTS

Ang II is a potent vasoconstrictor and important regulator of plasma volume; it also plays an important role in supporting blood pressure during various physiological stresses including standing. The mechanism by which elevated plasma Ang II might contribute to the pathophysiology of POTS is unclear, but several underlying processes could be operative. Ang II is known to regulate its receptors^{23, 24}. The prolonged presence of high plasma Ang II has been shown to induce a relative resistance to Ang II due to increased occupancy of the receptors or receptor downregulation in the vasculature, with resultant impairment of vasoconstrictive capacity on orthostatic challenge²⁵. Downregulation of receptors in the adrenal cortex might partially explain the paradoxically high levels of Ang II and low levels of aldosterone^{24, 26}. A defect in signal transduction pathways downstream of the receptors could contribute to the lack of tissue stimulation by the high Ang II. The pressor reactivity to Ang II may be reduced with blood volume depletion, and may be enhanced with conditions of volume and sodium excess²⁷. This might explain in part the amelioration of symptoms with volume replacement and high sodium intake in POTS²⁸.

Alternatively, increased Ang II can create a state of generalized vasoconstriction with consequently reduced additional vasoconstrictive capacity on upright posture (fewer

receptors available for recruitment), which manifests as orthostatic intolerance. While arterial resistance would be expected to increase significantly with upright posture, Stewart et al. reported little change in peripheral arterial resistance on upright tilt in POTS patients¹⁵. This sustained vasoconstriction, and increased vascular resistance, may contribute to reduced blood volume in POTS. Reduced perfusion of capillary beds during vasoconstriction can lead to a decrease of the vascular surface area, and hence decreases plasma volume²⁹. A decrease in perfused vascular beds could also increase the hydrostatic pressure in the remaining vascular beds, which could then lead to a decreased vascular refilling and lower blood volume.

In addition to its peripheral effects, both locally formed and circulating Ang II can act centrally to increase the sympathetic outflow via binding to AT-1 receptors in the circumventricular organs of the brain. Elevated plasma norepinephrine on standing, an indirect biochemical marker of increased sympathetic nervous system activity³⁰, was present in our POTS cohort. Local brain Ang II may also be elevated in POTS patients as a result of decreased metabolism by ACE2^{11, 12}. Over-expression of brain ACE2 (which would lead to decreased Ang II) has been recently reported to attenuate the development of neurogenic hypertension³¹ in mice. Conversely, reduced ACE2 activity may contribute to the high sympathetic tone in POTS. Ang II facilitates peripheral noradrenergic neurotransmission by both augmenting norepinephrine release and putatively inhibiting norepinephrine reuptake in the nerve terminals^{32, 33}. Whether the later effect of Ang II on the adrenergic nerves contributes to the high norepinephrine in POTS is unknown.

Stigmata of High Angiotensin II in POTS

Patients with POTS trended toward a higher diastolic blood pressure in the supine position (Table 1) than the healthy control subjects, consistent with our prior reports of elevated diastolic blood pressure in patients with POTS². These data are consistent with the aforementioned hypothesis of increased baseline vasoconstriction in POTS. This vasoconstriction could be due to a direct vascular effect of the Ang II, or due to increased sympathetic nervous system activity (which could be stimulated by CNS effects of Ang II).

Limitations

One limitation of this study was that we used estimated ACE2 activity (ratio of Ang-(1-7)/Ang II) rather than measuring the soluble ACE2, a recently reported technique³⁴. Most of the subjects in this report were studied prior to the publications of reports of soluble ACE2 assay. The soluble ACE2 level in the plasma is influenced by shedding of the ACE2 expressed on the plasma membrane, which is thought to be a mechanism to regulate ACE2 activity³⁵. It is not yet known if circulating levels of soluble ACE2 and the Ang-(1-7)/Ang II ratio are comparable indicators of ACE2 activity, nor which is the superior technique.

Another limitation is that the RAAS hormone assessments were all performed with subjects in a supine body position, and not while standing. Our prior studies that found abnormalities of plasma volume, PRA and aldosterone while supine, and there were no difference in plasma volume shifts with upright posture⁹. This study was designed to better probe the RAAS system by assessing the angiotensin system in a similar context. It would also be interesting, however, to understand the behavior of the angiotensin system with upright posture. Although the time courses of adaptation to upright posture by angiotensin species are not known, this should be assessed in future studies.

Future Directions

Further studies probing the role of Ang II in blood volume regulation in POTS are needed to better understand the pathophysiological implications of our findings. The first prong would

be to probe the Ang II and ACE2 relationship. While we estimated ACE2 activity, it may be optimal to measure ACE2 activity more directly. It is also important to investigate whether inhibition of ACE2 creates a POTS phenotype. Second, POTS patients appear to have an inadequate aldosterone response for the Ang II level. Future investigations could determine whether the blunted aldosterone production in POTS relates to problems with the AT1 receptor and downstream signaling, or whether the problems in POTS may relate to the synthesis of aldosterone itself.

Conclusion

In summary, we report that patients with POTS have increased plasma levels of Ang II, despite inappropriately low renin and aldosterone on the background of low blood volume. Our results suggest that some patients with POTS have reduced ACE2 activity and reduced adrenal responsiveness. These findings support the hypothesis that abnormal angiotensin regulation contributes to the pathophysiology of POTS in some patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary of Abbreviations (alphabetical)

ACE	Angiotensin converting enzyme
ACE2	Angiotensin converting enzyme 2
Ang	Angiotensin
Ang I	Angiotensin I (aka Angiotensin 1–10)
Ang II	Angiotensin II (aka Angiotensin 1–8)
Ang III	Angiotensin III (aka Angiotensin 2–8)
Ang IV	Angiotensin IV (aka Angiotensin 3–8)
Ang-(1–7)	Angiotensin 1–7
AT-1 receptor	Angiotensin II Type I receptor
POTS	Postural Tachycardia Syndrome
PRA	Plasma renin activity
RAAS	Renin-Angiotensin-Aldosterone System
SEM	standard error of the mean

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Figure 1.

Plasma levels of angiotensin (Ang) peptides (pg/ml) including Ang I (Panel A), Ang II (Panel B), Ang-(1–7) (Panel C) and angiotensin converting enzyme 2 (ACE2; Panel D) activity for patients with POTS and healthy control subjects. ACE2 activity was estimated as the Ang-(1–7):Ang II ratio. Note that estimated ACE2 activity is reduced in POTS, which may explain the elevated Ang II levels.

Figure 2.

Schematic diagram of the renin-angiotensin-aldosterone (RAAS) system profile in healthy individuals (TOP) and the proposed RAAS profile in patients with POTS (Bottom). Vertical arrows indicate up- or down-regulation of RAAS components. Patients with POTS have high levels of Ang II despite low levels of PRA. The high Ang II might be due to low ACE2 activity with decreased clearance. Despite the high Ang II levels, however, this aldosterone levels are low in the patients with POTS. AGT = angiotensinogen; PRA = plasma renin activity; ACE = angiotensin converting enzyme; ACE2 = angiotensin converting enzyme 2; Ang = angiotensin; AT₁R = angiotensin receptor type 1.

Table 1

Baseline demographics, phases of menstrual cycle, hemodynamic parameters and catecholamines of patients with POTS and control subjects

	POTS (n=38)	Control (n=13)	P
Demographics			
Female (n)	36	13	
Age (years)	32 ± 1	29 ± 2	0.174
Height (cm)	169 ± 1	168 ± 1	0.812
Weight (kg)	65 ± 2	63 ± 2	0.625
Body mass index (kg/m ²)	23 ± 0.7	22 ± 0.6	0.641
Progesterone (ng/ml)	3.5±0.8	4.0±2.0	0.807
Estradiol (ng/ml)	52.9±6.3	71.8±25.3	0.307
Phase of Menstrual Cycle			
Follicular Phase	66%	77%	0.727
Luteal Phase	34%	23%	
Supine			
Heart Rate (bpm)	70 ± 2	62 ± 3	0.022*
Systolic Blood Pressure (mmHg)	107 ± 2	104 ± 4	0.439
Diastolic Blood Pressure (mmHg)	67 ± 1	62 ± 2	0.081
Norepinephrine (pg/ml)	261 ± 4	134 ± 1	0.009*
Epinephrine (pg/ml)	18 ± 2	14 ± 2	0.332
Standing			
Heart Rate (bpm)	122 ± 4	89 ± 5	<0.001**
Systolic Blood Pressure (mmHg)	107 ± 4	102 ± 4	0.450
Diastolic Blood Pressure (mmHg)	72 ± 2	70 ± 3	0.633
Norepinephrine (pg/ml)	794 ± 87	399 ± 36	<0.001**
Epinephrine (pg/ml)	55 ± 8	57 ± 23	0.897
Change from Supine to Standing			
Heart Rate (bpm)	52 ± 3	27 ± 6	0.001**
Systolic Blood Pressure (mmHg)	0 ± 3	-3 ± 1	0.423
Diastolic Blood Pressure (mmHg)	5 ± 2	8 ± 2	0.535
Norepinephrine (pg/ml)	533 ± 87	269 ± 33	0.007*
Epinephrine (pg/ml)	37 ± 8	22 ± 7	0.353

POTS – Postural Tachycardia Syndrome; bpm= beats per minute. Continuous data are presented as the mean ± SEM, and were analyzed using Student's t-test. Categorical data are presented as percentages and were analyzed using the Fisher's Exact test. Phases of the menstrual cycle were defined as outlined in the text based on progesterone levels.

* P<0.05;

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P≤0.001.