Plasma hyperosmolality improves tolerance to combined heat stress and central hypovolemia in humans

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Gagnon D, Romero SA, Ngo H, Poh PY, Crandall CG. Plasma hyperosmolality improves tolerance to combined heat stress and central hypovolemia in humans. Am J Physiol Regul Integr Comp Physiol 312: R273–R280, 2017. First published December 21, 2016; doi:10.1152/ajpregu.00382.2016.—Heat stress profoundly impairs tolerance to central hypovolemia in humans via a number of mechanisms including heat-induced hypovolemia. However, heat stress also elevates plasma osmolality; the effects of which on tolerance to central hypovolemia remain unknown. This study examined the effect of plasma hyperosmolality on tolerance to central hypovolemia in heat-stressed humans. With the use of a counterbalanced and crossover design, 12 subjects (1 female) received intravenous infusion of either 0.9% iso-osmotic (ISO) or 3.0% hyperosmotic (HYPER) saline. Subjects were subsequently heated until core temperature increased ~1.4°C, after which all subjects underwent progressive lower-body negative pressure (LBNP) to presyncope. Plasma hyperosmolality improved LBNP tolerance (ISO: 288 ± 193 vs. HYPER: 382 ± 145 mmHg × min, P = 0.04). However, no differences in mean arterial pressure (P = 0.10), heart rate (P = 0.09), or muscle sympathetic nerve activity (P = 0.60; n = 6) were observed between conditions. When individual data were assessed, LBNP tolerance improved ≥25% in eight subjects but remained unchanged in the remaining four subjects. In subjects who exhibited improved LBNP tolerance, plasma hyperosmolality resulted in elevated mean arterial pressure (ISO: 62 ± 10 vs. HYPER: 72 ± 9 mmHg, P < 0.01) and a greater increase in heart rate (ISO: +12 ± 24 vs. HYPER: +23 ± 17 beats/min, P = 0.05) before presyncope. No differences in these variables were observed between conditions in subjects that did not improve LBNP tolerance (all P ≥ 0.55). These results suggest that plasma hyperosmolality improves tolerance to central hypovolemia during heat stress in most, but not all, individuals. 

blood pressure; heart rate; lower-body negative pressure; muscle sympathetic nerve activity

This raises the question as to whether hyperosmolality has an effect upon tolerance to central hypovolemia during heat stress. In humans, rapid infusion of hyperosmotic/oncotic solutions (~7% NaCl) is beneficial for the primary resuscitation from severe hypovolemia and hypovolemic shock (20). Moderate salt loading with sodium chloride tablets also improves orthostatic tolerance in individuals with recurrent syncope (4, 13). These beneficial effects are primarily attributed to osmotically mediated fluid shifts into the vascular space. However, a number of observations suggest that hyperosmolality may improve neural and cardiovascular responses to hypovolemia, independently of fluid shifts. In animal models, hyperosmolality causes sympathoexcitation through osmosensitive neurons located in the forebrain circumventricular organs (38, 39). Four weeks of dietary salt loading results in greater vasoconstriction of isolated rat mesenteric resistance arteries to given doses of norepinephrine (33). Finally, hyperosmolality improves cardiac function during resuscitation following hemorrhage in sheep, by stimulating cardiac sympathetic nerve activity (15). In humans, improved orthostatic tolerance with oral salt loading was accompanied by greater forearm vasoconstriction in individuals with recurrent syncope (4). Plasma hyperosmolality also increases resting muscle sympathetic nerve activity (MSNA) and improves the baroreflex control of MSNA (3, 14, 41). These findings are particularly relevant to the present investigation, as the extent to which MSNA increases is an important determinant of tolerance to central hypovolemia in normothermic (5, 32) and hyperthermic (7) conditions.

Overall, current evidence suggests that hyperosmolality stimulates physiological responses that could improve tolerance to central hypovolemia. Hyperosmolality accompanying heat stress may therefore attenuate the detrimental effect of heat-induced hypovolemia during further reductions in central blood volume. The purpose of this study was to examine the effect of plasma hyperosmolality on tolerance to central hypovolemia during heat stress. It was hypothesized that plasma hyperosmolality would improve tolerance to central hypovolemia of heat-stressed humans relative to an iso-osmotic state.

METHODS

Experimental overview. The data presented in this study were collected as part of an experimental protocol designed to examine a separate question (the effect of plasma hyperosmolality on skin sympathetic nerve activity during heat stress), the results of which have been published previously (16). As part of the protocol, skin

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sympathetic nerve activity was examined during passive heating until a core temperature increase of ~0.6°C. For the current study, heating was continued until an increase in core temperature of ~1.4°C, at which heating was stopped. For hyperosmotic (HYPER, 3.0% NaCl) saline (Baxter, Deerfield, IL) was subsequently initiated and continued for 90 min. Infusion rates for the ISO (0.2 ml·kg body wt⁻¹·min⁻¹) and HYPER (0.125 ml·kg body wt⁻¹·min⁻¹) solutions were chosen based on previous studies showing these rates minimize differences in plasma volume expansion between conditions (2, 24, 30, 34–36).

During the infusion period, skin sympathetic nerve activity was initially adjusted to achieve the primary aim of the previously published study (16). At the end of the infusion period, a blood sample was drawn after which baseline data were collected for 10 min. The temperature of the water circulating through the tube-lined suit was then increased to ~50°C, and whole body heating continued until an increase in core temperature ~1.2°C was achieved. At this point, the temperature of the water perfusing the suit was reduced to 44–46°C to minimize further increases in core temperature (~0.2°C, for a total increase of ~1.4°C) while the microelectrode was adjusted to obtain an MSNA signal. An appropriate MSNA signal during both conditions could only be obtained in six subjects, as we limited the search to ~10 min. A venous blood sample was then drawn and incremental LBNP to presyncope was performed. The protocol began at 20 mmHg LBNP, with pressure in the chamber subsequently decreasing by 10 mmHg every 3 min until presyncope. Criteria for determining presyncope included continued self-reporting by the subject of feeling faint, sustained nausea, rapid and progressive decrease in blood pressure resulting in a sustained systolic blood pressure <80 mmHg, and/or relative bradycardia accompanied by a narrowing of pulse pressure. Immediately after the release of LBNP, a final venous blood sample was drawn.

Data analysis. Data were collected with data acquisition hardware (MP150, Biopac, Santa Barbara, CA) at a sampling frequency of 50–200 Hz. To account for differences in LBNP tolerance time between conditions (see RESULTS), the data were analyzed as a function of relative LBNP time. To do so, total LBNP time in seconds was divided into increments of 20%. For all variables (except MSNA), data from the last 15 s of each increment were averaged and used for analyses, the exception being that the last 5 s were averaged at presyncope (i.e., 100% of LBNP time). For MSNA data, a 20-s sample was used for analyses at each LBNP increment and at presyncope. This time frame was chosen to best reflect MSNA at presyncope, while keeping a consistent time frame across all time points. Nonetheless, it should be noted that shorter time frames for MSNA analyses increases measurement variability (25). LBNP tolerance was quantified using the cumulative stress index (CSI), calculated by summing the product of LBNP level and absolute time at each level (23).

Statistical analysis. Core and mean skin temperatures, cutaneous vascular conductance, heart rate, mean arterial blood pressure, MSNA, plasma volume, and plasma osmolality were analyzed using a two-way repeated measures analysis of variance with the factors of condition (levels: ISO, HYPER) and relative LBNP time [levels: 0% (pre-LBNP), 20%, 40%, 60%, 80%, 100% (presyncope)]. If a significant condition × LBNP time interaction was observed, a Holm–Sidak post hoc analysis was performed. Heating time and LBNP tolerance were analyzed using paired samples t-tests. Comparisons between “responders” and “nonresponders” (see RESULTS) for physical characteristics, LBNP tolerance, plasma volume, and osmolality were per-

**Experimental protocol.** All subjects volunteered for two experimental trials, performed as a counterbalanced and crossover design. Both trials were separated by a minimum of 48 h to avoid physiological adaptation (21). After instrumentation, subjects assumed a supine position on a patient bed and mean skin temperature was clamped at ~33–34°C by adjusting the temperature of the water circulating through the tube-lined suit. An intravenous catheter was inserted into a superficial vein of the arm and a preinfusion blood sample was drawn a minimum of 30 min after the subject assumed the supine posture. An intravenous infusion of either ISO (0.9% NaCl) or hyperosmotic (HYPER, 3.0% NaCl) saline (Baxter, Deerfield, IL) was subsequently initiated and continued for 90 min. Infusion rates for the ISO (0.2 ml·kg body wt⁻¹·min⁻¹) and HYPER (0.125 ml·kg body wt⁻¹·min⁻¹) solutions were chosen based on previous studies showing these rates minimize differences in plasma volume expansion between conditions (2, 24, 30, 34–36).

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Statistical analysis. Core and mean skin temperatures, cutaneous vascular conductance, heart rate, mean arterial blood pressure, MSNA, plasma volume, and plasma osmolality were analyzed using a two-way repeated measures analysis of variance with the factors of condition (levels: ISO, HYPER) and relative LBNP time [levels: 0% (pre-LBNP), 20%, 40%, 60%, 80%, 100% (presyncope)]. If a significant condition × LBNP time interaction was observed, a Holm–Sidak post hoc analysis was performed. Heating time and LBNP tolerance were analyzed using paired samples t-tests. Comparisons between “responders” and “nonresponders” (see RESULTS) for physical characteristics, LBNP tolerance, plasma volume, and osmolality were per-
formed using independent samples t-tests. For all analyses, the level of significance was set at an α of P ≤ 0.05. Statistical analyses were performed using commercially available statistical software (Prism 6, Graphpad Software, La Jolla, CA). All variables are reported as means ± SD.

RESULTS

All subjects combined. The heating period lasted 64 ± 12 and 66 ± 11 min for the ISO and HYPER conditions, respectively (P = 0.61). During the ISO condition, whole body heating increased mean skin temperature from 33.5 to 38.5°C and core temperature by 1.39 ± 0.18°C (from 36.35 to 37.75°C). During the HYPER condition, mean skin temperature increased from 33.5 to 38.3°C and core temperature by 1.39 ± 0.17°C (from 36.42 to 37.81°C). There were no differences between conditions in mean skin and core temperatures throughout subsequent LBNP to presyncope (both P > 0.30). Intravenous infusion of hyperosmotic saline increased plasma osmolality and resulted in greater plasma volume expansion compared with the infusion of ISO saline (both P < 0.01, Fig. 1). Plasma osmolality and changes in plasma volume remained greater during HYPER, relative to ISO, just before the onset of LBNP (both P < 0.01). At the end of LBNP, plasma osmolality remained greater during HYPER (P < 0.01), whereas there were no differences between conditions for change in plasma volume (P = 0.36). Overall, LBNP tolerance improved during HYPER, expressed as both time (ISO: 553 ± 238 vs. HYPER: 670 ± 164 s, P = 0.03) and CSI (ISO: 288 ± 193 vs. HYPER: 382 ± 145 mmHg × min, P = 0.04). Despite the improvement in tolerance, there were no differences in mean arterial pressure (P = 0.10, Fig. 2), heart rate (P = 0.09, Fig. 2), cutaneous vascular conductance (P = 0.55, Fig. 2), and MSNA (P = 0.60, Table 1) between conditions during the LBNP period. When assessing the individual data (Fig. 3), the CSI of eight subjects improved by 25% or more during HYPER (range: +26 to +180%), whereas it did not change or decreased in four subjects (range: −28% to +5%). We therefore pursued the statistical analyses by separating subjects into “responders” and “nonresponders.”

Responders. In eight subjects, LBNP tolerance increased by 25% or more with plasma hyperosmolality (ISO: 177 ± 63 vs. HYPER: 340 ± 117 mmHg × min, P < 0.01). In these subjects, the absolute level of LBNP was greater during HYPER, relative to ISO, at 80% of LBNP time (43 ± 5 vs. 31 ± 6 mmHg, P < 0.01) and at presyncope (48 ± 9 vs. 39 ± 6 mmHg, P < 0.01). Changes in blood pressure and heart rate differed between conditions during LBNP (both P = 0.05, Fig. 4). During the HYPER condition, mean arterial pressure was greater between 20% and 100% of LBNP time. For heart rate, no individual time points were statistically different according to the post hoc analysis. However, the increase in heart rate from 0 to 80% of LBNP time was greater during HYPER relative to ISO (+23 ± 17 vs. +12 ± 24 beats/min, P = 0.05). In contrast, reductions in cutaneous vascular conductance during LBNP did not differ between conditions (P = 0.41, Fig. 5). Of the six subjects from whom appropriate MSNA signals were obtained, three were classified as responders. During LBNP, MSNA appeared greater during HYPER in these subjects, although no statistical analyses were performed on these data (Table 1).

Nonresponders. In four subjects, LBNP tolerance either decreased or improved by no more than 5% with plasma hyperosmolality (ISO: 510 ± 171 vs. HYPER: 466 ± 176 mmHg × min, P = 0.40). In these subjects, the absolute level of LBNP at a given percentage of LBNP time did not differ between conditions (P = 0.32). Blood pressure and heart rate during LBNP were similar between conditions (both P ≥ 0.55, Fig. 4). Reductions in cutaneous vascular conductance also did not differ between conditions (P = 0.51, Fig. 5). In the three nonresponders from whom appropriate MSNA signals were obtained, MSNA did not appear to be affected by plasma hyperosmolality, although no statistical analyses were performed on these data (Table 1).

Responders vs. nonresponders. Of the eight responders, four underwent the ISO trial first and the other four underwent the HYPER trial first. Of the four nonresponders, one underwent the ISO trial first and the other four underwent the HYPER trial first. There were no differences (all P ≥ 0.09) in physical characteristics between responders (7 males/1 female, age: 34 ± 9 y, height: 173 ± 10 cm, weight: 73 ± 15 kg) and nonresponders (4 males, age: 27 ± 5 y, height: 176 ± 7 cm, weight: 78 ± 16 kg). However, responders displayed lower LBNP tolerance during the ISO condition (177 ± 63 mmHg × min) relative to nonresponders (510 ± 171 mmHg × min, P < 0.01). During the HYPER condition, plasma osmolality and relative changes in plasma volume were similar between responders and nonresponders before LBNP (osmolality: 305 ± 3 vs. 305 ± 2 mosmol/kg; plasma volume: +4.4 ± 2.5 vs. +6.7 ± 3.6%, both P > 0.21) and immediately following LBNP (osmolality: 307 ± 5 vs. 306 ± 3 mosmol/kg; plasma volume: −7.3 ± 5.9 vs. −10.8 ± 3.4%, both P > 0.31).

Fig. 1. Relative changes in plasma volume from baseline (left) and plasma osmolality (right) after 90 min of intravenous infusion (injection) of either iso-osmotic (ISO) or hyperosmotic (HYPER) saline and a subsequent passive heating period followed by lower-body negative pressure (LBNP) to presyncope. Values are means ± SD for 12 subjects. Data were analyzed with a two-way repeated measures ANOVA. Pre-LBNP: values immediately before the LBNP protocol. End-LBNP: values immediately after the LBNP protocol. *P ≤ 0.05 between conditions.
HYPEROSMOLALITY AND LBNP TOLERANCE

In the current study, we examined the effect of plasma hyperosmolality on LBNP tolerance in heat-stressed humans. Infusion of hyperosmotic saline improved overall LBNP tolerance, relative to the infusion of ISO saline that minimized differences in plasma volume expansion between conditions. However, an improvement in LBNP tolerance with plasma hyperosmolality was not observed in all subjects. Some subjects demonstrated large improvements in LBNP tolerance (≥25%, responders), whereas little to no improvement was observed in others (≤5%, nonresponders). In the group of responders, plasma hyperosmolality was accompanied by a greater blood pressure and a greater increase in heart rate up to the point of presyncope. These results suggest that plasma hyperosmolality improves tolerance to central hypovolemia during heat stress in most, but not all individuals.

Heat stress profoundly impairs LBNP tolerance in humans (28). A primary factor contributing to this effect is central hypovolemia, as heat stress reduces central blood volume even in the absence of additional reductions in central blood volume (6). Restoration of blood volume, either through rapid bolus or continuous ISO saline infusion, improves LBNP tolerance in heat-stressed humans (18, 22). However, heat stress also increases plasma osmolality, which could have beneficial effects for tolerance during a central hypovolemic challenge. In animal models, hyperosmolality can be sympathoexcitatory through osmosensitive neurons located in the forebrain circumventricular organs (38, 39). In sheep, hyperosmotic saline infusion results in greater cardiac sympathetic nerve activity and a greater heart rate response during resuscitation from hemorrhage (15). In normothermic humans, oral salt loading results in greater forearm vasoconstriction during orthostatic stress and it improves orthostatic tolerance (4, 13). Infusion of hyperosmotic saline also increases resting MSNA and improves the baroreflex control of MSNA (3, 14, 41). The current study extends these observations by demonstrating that physiological responses mediated by hyperosmolality translate into improved LBNP tolerance during heat stress in humans. However, it should be noted that this observation was not consistent in all subjects.

During the hyperosmotic condition, a group of responders had relatively large improvements in LBNP tolerance (+106%), whereas little to no change was observed in a group of nonresponders (~8%). The most obvious difference between these groups is the markedly lower LBNP tolerance of responders, relative to nonresponders, during the iso-osmotic condition (~threefold). In fact, the four nonresponders had the four highest LBNP tolerances during the iso-osmotic condition. Thus hyperosmolality may only improve LBNP tolerance during heat stress in otherwise low-tolerant individuals. Alternatively, we cannot rule out the possibility of interindividual sensitivity to plasma hyperosmolality, analogous to the effects of salt consumption on blood pressure where individuals are often defined as “salt-sensitive” or “salt-insensitive” (40). For example, plasma hyperosmolality resulted in greater blood pressure and greater increases in heart rate up to the point of presyncope in responders. These effects were not observed in nonresponders, despite similar plasma osmolality before and immediately after the LBNP protocol. It is therefore possible that plasma hyperosmolality only improves tolerance to central hypovolemia in individuals who are physiologically sensitive to its effects.

The precise physiological mechanism(s) by which plasma hyperosmolality improves LBNP tolerance remains to be determined. As discussed, greater blood pressure and a greater increase in heart rate were observed up to the point of presyncope (Fig. 4). Preliminary observations suggest these responses could be driven by greater sympathetic activation, evidenced by elevated MSNA (Table 1). Such results are consistent with...
the observation that the increase in MSNA is an important determinant of LBNP tolerance in normothermic (5, 32) and heat stress (7) conditions and that hyperosmolality improves the baroreflex control of MSNA at rest under normothermic conditions (41). However, future studies are needed to test this hypothesis. In contrast, plasma hyperosmolality did not affect the reduction in cutaneous vascular conductance during LBNP (Fig. 4). The neural control of the cutaneous circulation is achieved by vasconstrictor and active vasodilator systems (12, 27). Reductions in cutaneous vasodilation during combined heat stress and LBNP have been ascribed to both increased vasconstrictor activity (31) and withdrawal of active vasodilation (19). Therefore, plasma hyperosmolality does not appear to result in greater cutaneous vasconstrictor activity and/or greater withdrawal of active cutaneous vasodilation during combined heat stress and LBNP.

**Perspectives and Significance**

The infusion of hyperosmotic/oncotic solutions is favorable for the early resuscitation from severe hypovolemia (20) and oral salt loading improves orthostatic tolerance (4, 13). However, the general premise for using hyperosmotic solutions/oral salt loading is to create an osmotic gradient that draws fluid from the intracellular/interstitial compartments into the vascular space. It is therefore challenging to differentiate the effect of plasma hyperosmolality from that of hypervolemia on orthostatic tolerance. In the current study, we employed different infusion rates for ISO and HYPER saline to minimize differences in plasma volume expansion relative to when the same solution is used for both solutions (34, 35). Thus the current study extends previous findings by demonstrating that plasma hyperosmolality has a favorable effect upon LBNP tolerance during heat stress conditions, even when changes in intravascular volume are minimized. Given that hypovolemia and hyperosmolality often occur concurrently during heat stress (1, 17), these results suggest that the physiological effects of hyperosmolality may counter the detrimental effects of hypovolemia on LBNP tolerance in heat stressed humans.

**Considerations.** It is recognized that the expansion of plasma volume was not perfectly matched between trials before the LBNP protocol. Previously, we found that heat stress-induced reductions in LBNP tolerance were mitigated by ~36% before the LBNP protocol. Previously, we found that heat stress-induced reductions in LBNP tolerance were mitigated by ~36%

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Values are means ± SD and presented as a percentage of total lower-body negative pressure (LBNP) time for three subgroups: 1) all subjects from whom appropriate muscle sympathetic nerve activity (MSNA) signals were obtained (n = 6; 2) subjects in whom LBNP tolerance improved during hyperosmotic (HYPER) (responders, n = 3), and; 3) subjects in whom LBNP tolerance did not improve during HYPER (nonresponders, n = 3). Pre-LBNP: values immediately before the LBNP protocol. ISO, iso-osmotic. 

**Table 1. Muscle sympathetic nerve activity during progressive lower-body negative pressure to presyncope performed under hyperthermic conditions after intravenous infusion of either iso-osmotic or hyperosmotic saline**

![Fig. 3. Individual (left) and mean (right) values of tolerance to central hypovolemia under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. Tolerance to central hypovolemia was quantified using the cumulative stress index (CSI). Values are means ± SD for 12 subjects. Data were analyzed with a paired samples t-test. *P ≤ 0.05 between conditions.](http://ajpregu.physiology.org/)

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when saline was infused to offset sweat losses (22). In that study, heat stress alone decreased plasma volume by ~6% from baseline, whereas saline infusion maintained plasma volume at baseline levels. In contrast, plasma volume in the current study was ~5% greater than baseline before the onset of LBNP during the hyperosmotic condition, whereas it remained at baseline values during the iso-osmotic condition. During the iso-osmotic condition, LBNP tolerance was 27% lower overall (46% in responders) relative to the hyperosmotic condition. An important distinction between our previous (22) and current studies is the fact that the differences in plasma volume were due to a relatively greater plasma volume in the current study versus a relatively lower plasma volume in our previous study (22). Although a reduction in plasma volume from baseline can compromise LBNP tolerance (22, 26), it is unclear if and/or to what extent the 5% plasma volume expansion observed in the current study affects LBNP tolerance during heat stress relative to a condition in which plasma volume is maintained.

Despite the potential modulating effect of differences in plasma volume expansion, we propose that hyperosmolality was the primary factor responsible for the observed differences in LBNP tolerance for the following reasons. A greater increase in heart rate was observed during LBNP in the group of responders. Similarly, preliminary observations suggest that plasma hyperosmolality also led to greater MSNA in the group of responders, although we recognize that the interpretation of these data is limited by the low number of subjects from which appropriate signals could be obtained. Nonetheless, these responses occurred despite subjects beginning the LBNP protocol with a greater relative plasma volume expansion. Such observations support a direct effect of hyperosmolality on heart rate and MSNA, as these responses would be expected to be

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**Fig. 4.** Mean arterial pressure (top) and heart rate (bottom) during progressive LBNP to pre-syncope performed under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. The data are plotted as a percentage of total LBNP time for 8 subjects in whom LBNP tolerance improved during HYPER (responders, left) and for 4 subjects in whom LBNP tolerance did not improve during HYPER (nonresponders, right). Values are means ± SD. Data were analyzed with a two-way repeated measures ANOVA. *P ≤ 0.05 between conditions at the indicated time point. **P ≤ 0.05 condition × time interaction.

**Fig. 5.** Changes in cutaneous vascular conductance (CVC) during progressive LBNP to pre-syncope performed under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. The data are changes from pre-LBNP values and are plotted as a percentage of total LBNP time for 8 subjects in whom LBNP tolerance improved during HYPER saline (responders, left) and for 4 subjects in whom LBNP tolerance did not improve during HYPER saline (nonresponders, right). Values are means ± SD. Data were analyzed with a two-way repeated measures ANOVA.
accompanied by relative hypovolemia as opposed to the observed hypervolemia. It should also be considered that relative changes in plasma volume were similar between conditions immediately following LBNP. This observation suggests that differences in plasma volume expansion evident before LBNP did not persist throughout the LBNP protocol. However, the greater LBNP levels reached during the hyperosmotic condition could contribute to this observation.

In conclusion, the current study evaluated the effect of plasma hyperosmolality on LBNP tolerance in heat-stressed humans. The main findings show that plasma hyperosmolality improves LBNP tolerance during heat stress in most, but not all, individuals. In individuals who improved LBNP tolerance, plasma hyperosmolality resulted in greater blood pressure and a greater increase in heart rate up to the point of presyncope. Preliminary observations suggest that these responses could be mediated by greater sympathetic activation, although the precise physiological mechanism(s) remain to be determined.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


