Impact of skin temperature and hydration on plasma volume responses during exercise

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Kenefick RW, Sollanek KJ, Charkoudian N, Sawka MN. Impact of skin temperature and hydration on plasma volume responses during exercise. J Appl Physiol 117: 413–420, 2014. First published July 3, 2014; doi:10.1152/japplphysiol.00415.2014.—Heat stress and hydration may both alter plasma volume (PV) responses during acute exercise; potential interactions have not been fully studied. The purpose of this study was to determine the effect of graded elevations in skin temperature (Tsk) on PV changes during steady-state exercise. Thirty-two men (22 ± 4 yr) were divided into four cohorts (n = 8 each) and completed EU and HYPO trials in one environment [ambient temperature (Ta) 10, 20, 30, and 40°C]. Thirty minutes of cycle ergometry (50% VO2peak) was performed. Core (Tc) and mean skin (Tsk) temperatures were measured; changes in PV, total circulating protein (TCP), and mean arterial pressure (MAP) were calculated; and skin blood flow (SkBF) was estimated. Hypohydration decreased (P < 0.05) PV by 200 ml (−5.7%) but did not alter TCP. Plasma loss was not different between EU and HYPO during exercise at any Ta. Plasma losses were greater (P < 0.05) with elevated Ta, with an average −130, −174, −294, and −445 ml losses during the 10, 20, 30, and 40°C trials, respectively. Significant (P < 0.05) correlations (r = 0.50 to 0.84) were found between ΔTCP and ΔPV during exercise when Tsk was cool/warm (<33°C; Tsk 10, 20, and 30°C), but not at 40°C (high Tsk). We conclude that 1) graded skin warming proportionally accentuated plasma loss; 2) plasma loss was associated with plasma protein efflux at lower Tsk and SkBF; 3) at high Tsk, additional plasma loss likely results from increased net filtration at the capillaries; and 4) HYPO did not alter vascular fluid loss during exercise in any environment.

PLASMA VOLUME (PV) IS IMPORTANT for supporting cardiovascular and thermoregulatory systems during exercise and particularly with heat stress (27). Body water deficits (hypohydration) will decrease the extracellular volume (8, 10) and its subcompartment, PV, in a proportionate manner (5, 27). During aerobic exercise, PV will further change (increase or decrease) depending upon the exercise type and intensity (20), posture (13), elevated ambient temperature (Ta) (11), and possibly, change in hydration status (28, 39).

Starling forces responsible for PV changes during exercise are oncotic and hydrostatic pressures (25). Oncotic pressure is directly related to the total circulating protein mass (33), which can be altered by either protein translocation between vascular and interstitial spaces, or by increased protein synthesis/breakdown. Hydrostatic forces include changes in mean arterial pressure (MAP) and tissue precapillary to postcapillary resistances (25). Dilation of cutaneous resistance vessels has been postulated to reduce the pre- to postcapillary resistance, which facilitates net filtration (25); thus elevated skin blood flow (SkBF) may facilitate plasma protein loss (11). Edwards and colleagues (11) reported in two subjects that plasma loss was greater during exercise with warm-hot (38°C) vs. cool (28°C) skin; however, it is unknown whether the plasma efflux or protein efflux, or both during exercise is proportionate to the magnitude of the SkBF response. Theoretically, loss of plasma in proportion to increases in SkBF could occur either due to plasma protein efflux, or to reductions in pre- to postcapillary resistances, or both (21, 25).

The effect of hypohydration (HYPO) on PV changes during exercise-heat stress is unclear. Sawka and colleagues (28, 30) reported that HYPO (3% to 7% body mass loss; BML) resulted in either plasma loss (28) or a smaller increase in PV (30) compared with euhydration (EU) during treadmill exercise in a hot (49°C) environment. Likewise, Harrison and colleagues (14) found a greater plasma loss in HYPO conditions (% BML) during cycle exercise in a hot (45°C) environment. Zappe and colleagues (39) reported that HYPO (2% BML) did not alter plasma loss, although PV loss increased during semisupine cycle exercise in a warm (30°C) environment. Differences in results among studies may have been due to exercise type, magnitude of HYPO, severity of heat stress, or some combination. If plasma loss is augmented during exercise with heat stress or HYPO, this could contribute to cardiovascular drift, and to the impairment in exercise performance in these conditions (4).

There is some evidence (12, 23, 28, 30) that hot Ta and HYPO may accentuate plasma loss during exercise; however, no investigations have examined the effect of altering both variables concurrently and in a systematic manner. Therefore, the purpose of this study was to determine the effect of graded skin temperature (Tsk) elevations (and thus SkBF) in EU and HYPO conditions on PV changes during steady-state exercise. In addition, we estimated hydrostatic and oncotic pressures to provide insight into physiological mechanisms possibly responsible for these expected plasma losses during exercise. We hypothesized that during exercise the plasma losses would be greater at higher Tsk (skin blood flows) and that HYPO would further accentuate this plasma loss. In addition, we hypothesized that total circulating protein losses would be solely responsible for any plasma loss.

METHODS

Subjects. Thirty-two men (age 22 ± 4.2 yr, height 180 ± 0.1 cm, weight 85.4 ± 10.8 kg, % body fat 14.5 ± 3.8%) volunteered to participate in this study.

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The 32 volunteers were later divided into four cohorts \((n = 8\) each) matched for \(\dot{V}_{O_2}\text{peak}\), body mass, and body surface area. Before participation, each volunteer attended briefings informing them of the purpose of the experiment and possible risks, and provided written informed consent. Appropriate institutional review boards approved the study. Investigators adhered to policies for protection of human subjects as prescribed in Army Regulation 70–25 and U.S. Army Medical Research and Materiel Command Regulation 70–25. The research was conducted in adherence with the provisions of 45 Code of Federal Regulations Part 46.

Preliminary procedures and familiarization. Volunteers were unacclimatized to heat at the time of familiarization and experimental testing. In addition, all testing occurred during fall, winter, and early spring months. Two weeks of preliminary testing and training preceded the experimental trials. Volunteers wore T-shirts and shorts during preliminary testing, familiarization sessions, and all experimental testing. On the initial day of preliminary testing, body mass was determined for each volunteer using an electronic scale (WSI-600; Mettler Toledo, Toledo, OH), followed by measures of body composition and \(\dot{V}_{O_2}\text{peak}\). Body density was estimated using a skinfold caliper (Lange; Beta Technology, Cambridge, MD) with procedures and equations as described by Jackson and Pollock (15). Percent fold caliper (Lange; Beta Technology, Cambridge, MD) with procedures and equations as described by Jackson and Pollock (15). Percent body fat was calculated using the equations provided by Siri (37).

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For 5 consecutive days, volunteers consumed 2 liters of sports drink after 6:00 P.M. The following morning, volunteers provided a first morning void for urine specific gravity (USG) analysis and had a blood sample (<5 ml) taken to measure plasma osmolality (\(P_{\text{osm}}\)). In addition, nude body mass was measured before breakfast and after voiding. These measures of USG, \(P_{\text{osm}}\), and body mass were used to provide feedback to the volunteers regarding their hydration state to help ensure that they would be well hydrated on the day of experimental testing. On the day of testing, volunteers were considered euhydrated with a combination of any two of the following: USG <1.020, \(P_{\text{osm}} <290 \text{ mmol/kg}, or nude body mass within 1% of the 5-day average (26).

Experimental Testing

Each subject cohort performed experimental testing in a randomized, counterbalanced design in one of four environments: 10°, 20°, 30°, and 40°C (\(T_a\)) while euhydrated and hypohydrated (~4% body mass).

HYP0 and heat exposure. On the morning of each experimental testing trial, USG, \(P_{\text{osm}}\), and body mass were measured for comparison against the preceding week’s 5-day average. Volunteers consumed a standardized breakfast of 540 kcal (16 g fat, 94 g carbohydrate, 8 g protein) and 250 ml of water. They were then instrumented for measurements of HR and rectal temperature (\(T_r\)) before entering the environmental chamber set at 50°C, ~20% RH, 1.6 m/s air speed. \(T_r\) was obtained from a telemetric temperature sensor (VitalSense Jonah Ingestible Capsule; Minimitter, Bend, OR), representative of true \(T_{sk}\) inserted 8–10 cm (length of gloved index finger) beyond the anal sphincter. This approach used simultaneously against a conventional rectal probe yields excellent agreement, with differences ≤0.05°C, and has been used in prior investigations (16, 17).

For all environmental conditions, during the morning of both the EU and HYPO trial days, volunteers walked on a treadmill at 3 mph at 3.5% grade for 30 min, followed by 30 min of seated rest. This work/rest cycle continued for the duration of the 3-h dehydration protocol. The purpose of light walking exercise was to increase core temperature and initiate sweating, so that a ~4% BML could be achieved before experimental testing, a model that allows examination of how HYPO affects performance in the absence of other confounding factors (29). Throughout the heat exposure, if \(T_r\) reached 39.5°C, walking was discontinued and volunteers sat in the chamber for the remaining duration of the exercise cycle. Sweat loss volume was determined from changes in body mass measured every 30 min. In the EU trials, sweat volume and BML was considered equivalent so that volunteers drank 1 ml of 0.05% NaCl in water solution to replace every 1 g of mass that was lost. After heat exposure, the volunteers were allowed a 90-min break, during which they showered and rested to return to normothermia. Following the break, nude body mass was again measured, and this value was compared with the preheat exposure value. If body mass was not within 0.5% of preheat exposure values in the EU trial, additional fluid was provided. In the HYPO condition, \((\Delta BM/\text{pre-BM}) \times 100 = \%\text{HYPO}, where BM is body mass.

Submaximal exercise testing. Experimental testing consisted of 30 min of submaximal cycle ergometer exercise. Before experimental testing, volunteers were fitted with an HR monitor and \(T_{sk}\) thermistors (YSI, Yellow Springs, OH) on the left chest, arm, calf, and thigh. Volunteers then entered the environmental chamber set at one of four \(T_a\) and mmHg vapor pressures: 10°C, 4.6 mmHg, 50% RH, 20°C, 8.8 mmHg, 50% RH; 30°C, 9.5 mmHg, 30% RH; and 40°C, 11.0 mmHg, 20% RH. These conditions were chosen so that the skin-to-air vapor pressure gradient for each \(T_a\) tested would allow for sweat evaporation from the skin such that there would be no accumulation or dripping that could alter \(T_{sk}\) (1). Volunteers sat for approximately 30 min to equilibrate to the testing conditions and control for the hemodilution associated with change of \(T_{sk}\) from cool to warm (25), after which a blood draw was taken immediately before and after exercise.

Cycle ergometry was performed using the hyperbolic mode. Because exercise intensity affects blood pressure (BP) responses (7, 39), the Lode Linear Factor was individualized to elicit a 50% \(\dot{V}_{O_2}\text{peak}\) workload for each volunteer established during familiarization, to hold BP relatively constant. Although exercise intensity was independent of pedal cadence, 60 ± 5 rpm was encouraged to standardize mechanical efficiency (19, 34).

Physiological measures. During the 30-min cycle ergometer exercise, \(T_{sk}\) was monitored continuously using a data acquisition system, and mean weighted \(T_{sk}\) was calculated according to the method described by Ramanathan (22). Measures of HR and \(T_{r}\) were recorded preexercise and every 5 min. BP (Cycle automated cuff; SunTech Medical, Morrisville, NC) measures were made preexercise and at ~25 min of exercise. MAP was calculated as the following: (systolic BP – diastolic BP) * 0.33 + diastolic BP. An estimate of whole body SkBF was made using the \(T_{sk}\) and \(T_{sk}\) measurements at ~30 min using the equation provided by Rowell (24): \(Q_{sk} = \frac{1}{C} \times h(T_{sk} - T_{sk})\), where \(C\) is the specific heat of blood (~0.87 kcal°C–1 liter–1), \(h\) is heat production (\(\dot{V}_{O_2}\), in liter/min), and \(Q_{sk}\) is skin blood flow.
Blood Samples

Four blood sample collections were used for analysis. Before exposure to heat (dehydration protocol), a catheter was placed in an antecubital vein for blood sampling. The first 10 ml blood sample (before heat exposure) was taken after 20 min of seated rest in the temperate antechamber to allow for equilibration. Ninety minutes following the dehydration protocol, a second 10-ml blood sample was taken after 20 min of seated rest in a temperature environment. For the experimental trials, after entering the environmental chamber set at one of the four designated ambient temperatures, a 10-ml blood draw was taken after sitting for 30 min (preexercise) and immediately after cycle exercise while still seated. Both pre- and postexercise blood draws were taken with controlled posture (sitting) and arm position.

Whole blood was transferred to tubes containing lithium heparin, and samples of whole blood were taken for analysis of hemoglobin (Hb) and hematocrit (Hct) (iSTAT 1 Analyzer; Abbott Point of Care, East Windsor, NJ). Although use of automated analyzers for measurement of Hct has been called into question, this method is acceptable for the range of $P_{osm}$ observed in the current study (38). Whole blood was then centrifuged for 10 min at 4°C, and the plasma was aliquoted for $P_{osm}$ measurement in triplicate by freezing point depression (210 Micro-Osmometer; Fiske, Norwood, MA). Initial PV (absolute) was calculated using the equation provided by Dill and Costill (9) from Hct and Hb values obtained in temperate conditions either preceding or after the dehydration sessions. Similarly, percent change in PV (% $\Delta PV$) from EU to HYPO was calculated for the range of Posm observed in the current study (38).

Statistical Analysis

Sample size and power analyses for statistical significance were driven by estimates of practical significance. Briefly, associations between $\Delta$PV (y-axis) and primary outcomes (x-axis) were considered meaningful when $r \geq 0.5$ (6). Based on the relationship between Cohen’s $r$ and $d$ (6, 18), an equivalent effect size of 1.2 was used to calculate (6) the need for ~12 to 18 subjects per group, assuming $\alpha = 0.05$ and $\beta = 0.20$ when comparing four independent groups. EU and HYPO were collapsed ($n = 16$) for this comparison. Because our interest was in differences between EU and HYPO conditions at discrete time points (0 and 30 min) within each $T_a$, these comparisons were made via paired t-test. All analyses were performed using GraphPad, v 4.0. All data are presented as means ± SD except where indicated.

RESULTS

Volunteers in each of the four cohorts completed all aspects of testing.

Descriptive characteristics of each of the four subject cohorts are present in Table 1 and were not statistically different. HYPO and heat exposure. All volunteers were euhydrated prior to heat exposure: USG was <1.020. $P_{osm}$ was <290 mmol/kg, and body mass was <1% of the 5-day average. In the HYPO trials, there was a 4.1 ± 0.03%, 4.2 ± 0.01%, 4.0 ± 0.01%, and 4.1 ± 0.01% BML in 10°, 20°, 30°, and 40°C conditions, respectively. Prior to the experimental exercise trials, HYPO resulted in a significant ($P < 0.05$) PV reduction of ~6.5% ($PV = 3.3 ± 0.3$ liter HYPO vs. 3.5 ± 0.3 liter EU) and a significant increase ($P < 0.05$) in $P_{osm}$ (~300 mmol/kg) compared with EU trials (~284 mmol/kg).

Physiological responses. During the 30-min steady-state submaximal cycle ergometry bouts, the percent change in BML (preexercise to postexercise) was <1% for all EU and HYPO trials. Oxygen uptake (~2.0 liter/min) was not affected by environmental condition.

Table 2 displays thermal and cardiovascular variables preexercise (0 min) and at 30 min of cycle ergometer exercise. $T_a$ values were not different ($P > 0.05$) between EU vs. HYPO at rest or exercise in any environment. Overall, $T_a$ increased ~4.0°C with each 10°C increase in $T_a$. $T_{ce}$ values were greater ($P < 0.05$) preexercise and at 30 min of exercise in the HYPO vs. EU trials within each $T_a$. $T_{ce}$ values were not different either before or after exercise between the environmental conditions. The $T_{ce}$-$T_{sk}$ gradient was not different ($P > 0.05$) between EU and HYPO preexercise or at 30 min within each $T_a$, except at 40°C, when at 30 min the HYPO value was greater ($P < 0.05$) compared with EU (2.6 ± 0.4 vs. 2.0 ± 0.4°C, respectively). Estimated SkBF was not different between EU and HYPO trials in the 10, 20, and 30°C environments but increased in a stepwise manner as $T_a$ increased. In both EU and HYPO conditions, estimated SkBF at 40°C was three to six times greater than the other conditions tested; however, at 40°C, HYPO values were significantly lower ($P < 0.05$) than those in the EU trials (Table 2). Preexercise (0 min) HR values and those at 30 min of steady-state cycling were greater ($P < 0.05$) under HYPO conditions vs. EU conditions within each $T_a$ and were significantly elevated ($P < 0.05$) with higher $T_a$. Table 3 provides absolute PV, TCP, and MAP before and after exercise. In addition, PV loss and percent change in PV and absolute change ($\Delta$) in TCP and MAP from preexercise (0 min) to 30 min of exercise for both EU and HYPO conditions

Table 1. Subject characteristics for each cohort

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23 ± 5</td>
<td>23 ± 3</td>
<td>24 ± 6</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180.0 ± 0.1</td>
<td>179.0 ± 0.1</td>
<td>179.0 ± 0.1</td>
<td>182.0 ± 0.1</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>87.3 ± 13.7</td>
<td>79.8 ± 4.9</td>
<td>85.4 ± 14.5</td>
<td>89.1 ± 6.7</td>
</tr>
<tr>
<td>VO$_{peak}$, ml·kg$^{-1}$·min$^{-1}$</td>
<td>43.6 ± 4.1</td>
<td>45.3 ± 4.6</td>
<td>46.3 ± 5.2</td>
<td>43.7 ± 7.0</td>
</tr>
<tr>
<td>Peak power, W</td>
<td>313 ± 35</td>
<td>300 ± 20</td>
<td>318 ± 24</td>
<td>314 ± 28</td>
</tr>
<tr>
<td>Fat, %</td>
<td>14.6 ± 3.6</td>
<td>14.5 ± 2.8</td>
<td>14.0 ± 4.0</td>
<td>15.0 ± 4.0</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>2.1 ± 0.2</td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD ($n = 8$). $T_a$, ambient (air) temperature.
Table 2. Skin temperature, core temperature, core-to-skin gradient, whole body sweat rate, heart rate, and estimated whole body skin blood flow responses before (0 min) and 30 min after submaximal cycle ergometry exercise testing

<table>
<thead>
<tr>
<th>T&lt;sub&gt;r&lt;/sub&gt;, °C</th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>30 min</td>
<td>0 min</td>
<td>30 min</td>
<td>0 min</td>
</tr>
<tr>
<td>T&lt;sub&gt;r&lt;/sub&gt;, °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>24.8 ± 0.8</td>
<td>25.0 ± 0.8</td>
<td>28.7 ± 0.6</td>
<td>29.0 ± 0.7</td>
</tr>
<tr>
<td>HYPO</td>
<td>25.0 ± 1.0</td>
<td>24.7 ± 0.6</td>
<td>29.3 ± 0.8</td>
<td>29.3 ± 0.8</td>
</tr>
<tr>
<td>T&lt;sub&gt;r&lt;/sub&gt;, °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>37.2 ± 0.2</td>
<td>37.4 ± 0.5</td>
<td>37.1 ± 0.3</td>
<td>37.7 ± 0.2</td>
</tr>
<tr>
<td>HYPO</td>
<td>37.7 ± 0.1*</td>
<td>38.2 ± 0.3*</td>
<td>37.7 ± 0.2*</td>
<td>38.2 ± 0.2*</td>
</tr>
<tr>
<td>ΔT&lt;sub&gt;r&lt;/sub&gt;-T&lt;sub&gt;sk&lt;/sub&gt;, °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>12.4 ± 0.8</td>
<td>13.0 ± 0.5</td>
<td>8.4 ± 0.5</td>
<td>8.7 ± 0.7</td>
</tr>
<tr>
<td>HYPO</td>
<td>12.7 ± 1.0</td>
<td>13.5 ± 0.8</td>
<td>8.4 ± 0.9</td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td>Whole body SR, liter/hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>0.3 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>HYPO</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1*</td>
<td>0.7 ± 0.2*</td>
<td>1.1 ± 0.2*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>78 ± 15</td>
<td>127 ± 12</td>
<td>66 ± 11</td>
<td>132 ± 7</td>
</tr>
<tr>
<td>HYPO</td>
<td>87 ± 15*</td>
<td>141 ± 20*</td>
<td>86 ± 14*</td>
<td>152 ± 11*</td>
</tr>
<tr>
<td>Estimated SkBF, liter/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>HYPO</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>2.1 ± 0.4</td>
<td>5.6 ± 0.9*</td>
</tr>
</tbody>
</table>

for all four environmental conditions are presented. During exercise, there were greater (P < 0.05) PV losses and thus a larger %Δ in PV with increased T<sub>r</sub> under either EU or HYPO. Hydration state did not alter (P > 0.05) plasma losses or the %Δ in PV during exercise between the EU and HYPO conditions; thus data were collapsed across groups for further analysis. Average PV losses (EU and HYPO combined) were −130 ± 112 (−1.8%), −174 ± 163 (−4.7%), −294 ± 183 (−6.6%), and −445 ± 135 (−8.3%) ml in the 10, 20, 30, and 40°C environments, respectively. There were no differences (P > 0.05) in TCP or ΔTCP between the EU and HYPO conditions for each environment tested. The TCP loss (P > 0.05) was −9, −1, −17, and −19 g in the 10, 20, 30, and 40°C environments, respectively. There were no differences in MAP (P > 0.05) between EU and HYPO conditions in the 10, 20, or 30°C environments; however, there was a smaller (P < 0.05) for all four environmental conditions are presented. During exercise, there were greater (P < 0.05) PV losses and thus a larger %Δ in PV with increased T<sub>r</sub> under either EU or HYPO. Hydration state did not alter (P > 0.05) plasma losses or the %Δ in PV during exercise between the EU and HYPO conditions; thus data were collapsed across groups for further analysis. Average PV losses (EU and HYPO combined) were −130 ± 112 (−1.8%), −174 ± 163 (−4.7%), −294 ± 183 (−6.6%), and −445 ± 135 (−8.3%) ml in the 10, 20, 30, and 40°C environments, respectively. There were no differences (P > 0.05) in TCP or ΔTCP between the EU and HYPO conditions for each environment tested. The TCP loss (P > 0.05) was −9, −1, −17, and −19 g in the 10, 20, 30, and 40°C environments, respectively. There were no differences in MAP (P > 0.05) between EU and HYPO conditions in the 10, 20, or 30°C environments; however, there was a smaller (P < 0.05)


**Table 4. Correlation matrix of collapsed EU and HYPO values for \( \Delta \text{MAP}, \Delta \text{TCP}, \text{and } \Delta \text{SkBF to } \Delta \text{PV} \) within each \( T_a \) tested and overall**

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta \text{MAP vs. } \Delta \text{PV} )</td>
<td>0.12</td>
<td>-0.01</td>
<td>0.12</td>
<td>0.19</td>
<td>0.40</td>
</tr>
<tr>
<td>( \Delta \text{TCP vs. } \Delta \text{PV} )</td>
<td>0.50*</td>
<td>0.72*</td>
<td>0.84*</td>
<td>0.10</td>
<td>0.50*</td>
</tr>
<tr>
<td>( \Delta \text{SkBF vs. } \Delta \text{PV} )</td>
<td>0.03</td>
<td>-0.07</td>
<td>-0.32</td>
<td>-0.22</td>
<td>-0.41*</td>
</tr>
<tr>
<td>( \Delta \text{SkBF vs. } \Delta \text{TCP} )</td>
<td>0.36</td>
<td>0.27</td>
<td>0.20</td>
<td>0.60*</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Significant relationship (\( P \leq 0.05 \)).

\( \Delta \text{MAP} \) during exercise under HYPO vs. EU conditions at 40°C (Table 3).

Overall, with the exception of \( \Delta \text{MAP} \) at 40°C, there were no overall EU vs. HYPO differences in \( \Delta \text{MAP}, \Delta \text{TCP}, \text{and } \Delta \text{PV} \) (0 to 30 min exercise). Thus EU and HYPO conditions were collapsed to examine the relationships between \( \Delta \text{MAP}, \Delta \text{TCP}, \text{and } \Delta \text{PV} \). Table 4 presents a correlation matrix of \( \Delta \text{MAP}, \Delta \text{TCP}, \text{and } \Delta \text{SkBF to } \Delta \text{PV} \) values within each \( T_a \) tested and for all \( T_a \) combined. Overall \( \Delta \text{TCP} \) was significantly related (\( P \leq 0.05 \)) to overall \( \Delta \text{PV} \) (Table 4; Fig. 1); however, \( R \) values within each \( T_a \) tested were significant at 10, 20, and 30°C. The relationship between \( \Delta \text{TCP} \) and \( \Delta \text{PV} \) at 40°C was not significant (\( P > 0.05 \); Table 4; Fig. 2). Overall \( \Delta \text{SkBF} \) was significantly related (\( P \leq 0.05 \)) to overall \( \Delta \text{PV} \) (Table 4).

**DISCUSSION**

This was the first study to systematically evaluate the independent and combined effects of skin warming and HYPO on plasma losses during acute steady-state exercise. Additionally, we sought to determine the influence of transcapillary Starling forces on PV losses during steady-state exercise. We employed compensable heat stress conditions so that core temperature would be similar in each environment but \( T_a \) would be elevated with environmental temperature. This effectively narrowed the core-to-skin temperature gradient, resulting in progressively higher SkBF responses with each increment in \( T_a \) (24). In addition, we controlled for posture and \( T_a \) effects while carefully controlling for hydration status. Our novel finding is that during exercise 1) graded skin warming proportionally accentuates plasma loss; 2) plasma loss is associated with plasma protein efflux; 3) at high skin temperatures, additional plasma loss is likely mediated by large decreases in cutaneous pre- to postcapillary resistances due to pronounced cutaneous vasodilation; and 4) HYPO per se did not alter vascular fluid loss during steady-state exercise.

Following our standardized HYPO protocol, BML, increase in USG (>1.020), and plasma parameters were consistent with the subjects being hypohydrated (3). We found that at rest, HYPO reduced PV and TCP. The HYPO-mediated PV reduction of 5.7% with a 4% BML is consistent with previously reported values (3, 31). The HYPO increase in \( P_{\text{osm}} \) of 16 mosmol/kg is slightly greater than previously reported (3, 31).

Our study is the first to clearly demonstrate that during exercise, greater plasma loss occurs when the \( T_{sk} \) is elevated, and that this plasma loss is accentuated in a graded manner with \( T_{sk} \) and SkBF requirements. At higher ambient temperatures, higher SkBF likely increased the cutaneous capillary surface area for filtration and caused an additional lowering of pre- to postcapillary resistances (25), which both act to increase net capillary filtration and thus plasma loss. In an earlier study with a limited number of subjects (\( n = 2 \)), Edwards et al. (11) reported ~9% hemoconcentration during prolonged cycling (90 min at 50% VO\text{2peak}) at 38°C (\( T_{sk} \sim 37°C \)) compared with ~6% hemoconcentration in an environment at 28°C (\( T_{sk} \sim 34°C \)). They attributed the greater hemoconcentration in 38°C to a higher \( T_{sk} \) and a resulting greater net filtration and plasma protein loss between the vasculature and the interstitium through cutaneous capillaries. In addition, Senay (35) postulated that the cutaneous vasodilation resulting from a rise in \( T_{sk} \) due to heat exposure will be accompanied by an increase in capillary filtration coefficient, thus possibly increasing the amount of plasma fluid filtering into the interstitial space.

We have expanded these previous limited findings in a much larger subject population over a range of environmental temperatures and by defining the skin temperatures (estimated skin blood flows) that augment plasma efflux during prolonged steady-state exercise. The difference in plasma loss between environments was likely mostly oncotically mediated, because the protein loss can account for the plasma loss on the basis of Scatchard relationships (32). In addition, significant relationships between change in TCP and change in PV were found in the experiments at 10, 20, and 30°C environmental temperatures. Furthermore, the change in MAP during exercise was similar in each environment (or tended to be smaller in the hotter environments), so increased hydrostatic forces were not likely contributing to any differences in plasma loss among environments. However, the change in SkBF and change in PV relationship was overall significant although not within any particular environment (Table 4). The stronger relationship in the larger group analysis may have been related to a larger total range of each variable (PV and SkBF), thus allowing the relationship to emerge when it was not possible to observe it in the individual groups.

A possible explanation for the striking difference in the \( \Delta \text{TCP}-\Delta \text{PV} \) relationship in the 40°C condition (Fig. 2) may lie in differences in blood flow distribution between this and all other environmental conditions. Progressive increases in environmental temperature result in large increases in SkBF via both local and reflex mechanisms (2). Thus during moderate exercise at high environmental temperatures, a larger relative...
proportion of the cardiac output is directed to the skin compared with during cooler conditions (2). Senay (35) suggested that the cutaneous vasodilation and rise in $T_{sk}$ with heat exposure is accompanied by an increase in capillary filtration coefficient, thus increasing the amount of fluid passing into the interstitial space. In the present study, using Rowell’s calculations (24), we estimated that whole body SkBF was approximately three times higher in the 40°C condition compared with the 30°C condition (Table 2). Although we did not conduct specific measurements of Starling forces, it may be that oncotic pressure was relatively less of a driving force for hemoconcentration in the skin vasculature compared with that of active skeletal muscle. In this scenario, a greater proportion of the total blood flow (cardiac output) would be experiencing skin-specific Starling forces in the 40°C condition compared with the other three conditions.

Our present data are inconsistent with prior observations (14, 28, 30) that when one is hypohydrated during exercise, a reduced plasma hemodilution (28, 30) or greater plasma loss (14, 28, 30) will occur compared with when one is euhydrated. Sawka and colleagues (28, 30) reported greater plasma loss or an increase in total circulating proteins and PV. In these conditions, therefore, a larger preexercise and early exercise restriction, the rate of hemoconcentration during cycling exercise in the heat was similar.

Reasons for differences among studies are unclear but may be influenced by environmental temperature, a subject’s heat acclimation status, duration of recovery from the dehydration sessions, or a combination of these. Our current results are most similar to those of Zappe and colleagues (39), with the exception that our initial HYPO state was larger (4%) than that of the previous study (1.8%). With regard to the other studies, it is possible that the markedly higher environmental temperatures (49 and 45°C vs. 30 and 40°C) of studies demonstrating greater plasma loss when hypohydrated (14, 28, 30) had an effect on PV via augmented sweating and SkBF responses.

Another possibility is that studies tending to demonstrate the additional plasma loss (or smaller gain) when hypohydrated usually employed heat-acclimated subjects (28, 30). Additionally, a longer period of recovery was employed (overnight); this additional time may have allowed for protein synthesis and an increase in total circulating protein. Both heat acclimation (36) and extended recovery from dehydration (25) can contribute to increased total circulating proteins and PV. In these conditions, therefore, a larger preexercise and early exercise TCP and PV may allow for a larger delta during the acute exercise bout.

Interpretation of our present data is subject to certain limitations. First, we did not directly measure SkBF. SkBF was estimated on the basis of core to skin temperature gradients as originally calculated by Rowell (24). Although these estimates are likely robust, there is interindividual variability in SkBF responses, which makes any individual absolute value difficult to predict or model. It will be important to follow up with specific SkBF measurements. A second caveat is that our
present conclusions regarding a causative influence of ΔTCP on ΔPV must be tempered by the fact that these conclusions are based on correlative data. Third, it is important to note that the conclusions about hydrostatic pressure influences were based on systemic (brachial artery) measurements of MAP, not pressures measured directly at the capillary level, which are likely to be slightly lower at any given brachial artery pressure.

In summary, we found that during acute, steady-state cycle exercise, 1) graded skin warming proportionally accentuates plasma loss; 2) plasma loss is associated with plasma protein efflux; 3) at high skin temperatures, additional plasma loss is likely mediated by large decreases in cutaneous pre- to post-capillary resistances due to profound cutaneous vasodilatation; and 4) HYPO per se did not alter vascular fluid loss during exercise in any environment. The additional plasma losses during steady-state exercise with increasing levels of heat stress likely contribute to cardiovascular drift (progressive increases in HR) and could potentially contribute to impairment in exercise performance as we have previously observed (16).

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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