Cardiovascular and thermoregulatory dysregulation over 24 h following acute heat stress in rats

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Quinn CM, Audet GN, Charkoudian N, Leon LR. Cardiovascular and thermoregulatory dysregulation over 24 h following acute heat stress in rats. Am J Physiol Heart Circ Physiol 309: H557–H564, 2015. First published June 12, 2015; doi:10.1152/ajpheart.00918.2014.—The influences of severe heat stroke (HS) on cardiovascular function during recovery are incompletely understood. We hypothesized that HS would elicit a heart rate (HR) increase persisting through 24 h of recovery due to hemodynamic, thermoregulatory, and inflammatory events, necessitating tachycardia to support mean arterial pressure (MAP). Core temperature (Tc), HR, and MAP were measured via radiotelemetry in conscious male Fischer 344 rats (n = 22; 282.4 ± 3.5 g) during exposure to 37°C ambient temperature until a maximum Tc of 42.0°C, and during recovery at 20°C ambient temperature through 24 h. Rats were divided into Mild, Moderate, and Severe groups based on pathophysiology. HS rats exhibited hysteresis relative to Tc with HR higher for a given Tc during recovery compared with heating (P < 0.0001). “Reverse” hysteresis occurred in MAP with pressure during cooling lower than heating per degree Tc (P < 0.0001). Mild HS rats showed tachycardia [P < 0.01 vs. control (Con)] through 8 h of recovery, elevated MAP (P < 0.05 vs. Con) for the initial 5 h of recovery, with sustained hyperthermia (P < 0.05 vs. Con) through 24 h. Moderate HS rats showed significant tachycardia (P < 0.01 vs. Con), normal MAP (P > 0.05 vs. Con), and rebound hyperthermia from 4 to 24 h post-HS (P < 0.05 vs. Con). Severe HS rats showed tachycardia (P < 0.05 vs. Con), hypotension (P < 0.01 vs. Con), and hyperthermia for 24 h (P < 0.05 vs. Con). Severe HS rats showed 14- and 12-fold increase in heart and liver inducible nitric oxide synthase expression, respectively. Hypotension and hyperthermia in Severe HS rats was consistent with inducible nitric oxide synthase-mediated systemic vasodilation. These findings provide mechanistic insight into hemodynamic and thermoregulatory impairments during 24 h of HS recovery.

thermoregulation; heart rate; heat stroke; autonomic nervous system; blood pressure; iNOS

HEAT STROKE (HS) IS A DEBILITATING ILLNESS characterized by myriad thermoregulatory, cardiovascular, and systemic inflammatory abnormalities. Sustained exposure to uncompensable heat stress results in HS, which manifests as profound autonomic and thermoregulatory dysfunction, often complicated by coagulopathies and precipitating catastrophic multiorgan failure.

In response to whole body hyperthermia in humans and rats, heart rate (HR) increases linearly with increasing core temperature (Tc) (21, 30). This contributes to an increased cardiac output to support the demand for increased peripheral blood flow, which facilitates heat dissipation. A portion of the increased cutaneous perfusion can be attained through redirection of blood flow from other vascular beds, primarily compensatory splanchnic vasoconstriction (31, 32). These reciprocal alterations in blood flow allows for maintenance of mean arterial pressure (MAP) (i.e., MAP = cardiac output × TPR, where TPR is total peripheral resistance). In the absence of hemodynamic compensation, the decrease in vascular resistance within the peripheral circulation can lead to central hypovolemia (due to decreased venous return) and a fall in MAP.

Although the cardiovascular responses to heat stress are well recognized (7, 8, 19, 21, 25, 30, 34), the hemodynamic profile during recovery is less well understood, particularly following severe HS. If the hemodynamic adjustments observed in hyperthermia were solely caused by the influence of temperature on the sinoatrial (SA) node (9, 32), the values of HR and MAP per degree Tc during the immediate recovery period would mirror the linear response observed during heating. Similarly, the hemodynamic pattern during the first 24 h of recovery would fluctuate predictably with changes in Tc. However, data from rats and primates indicate that ~60% of hyperthermia-related tachycardia is autonomic in origin, and only 40% is the direct result of temperature on the SA node (16). Given the strong autonomic influence on hemodynamic responses to heat stress, it is likely that changes in HR during periods of changing Tc (e.g., during heat exposure and cooling) include a central role for autonomic mechanisms.

In the present study, we tested the hypothesis that the increased HR observed during HS in rats would extend into the recovery period, due to persistent hemodynamic, thermoregulatory, and inflammatory events, necessitating tachycardia for maintenance of MAP. Furthermore, we sought to identify potential underlying mechanisms of hemodynamic instability and their effect on the pathology of HS.

NEW & NOTEWORTHY

The present study provides mechanistic insight into hemodynamic and thermoregulatory impairments during 24 h of heat stroke recovery. These findings are qualitatively similar to clinical manifestations observed in heat stroke patients and provide further insight into multiple stressors placed on integrative cardiovascular regulation during the 24 h following severe heat stress.

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METHODS

Animals

Thirty adult male Fischer 344 rats (Charles River Laboratories, Wilmington, MA) weighing 282.4 ± 3.5 g were used. Rats were housed individually in Nalgene polycarbonate cages (Ancare, Bellmore, NY) fitted with HEPA-filter cage tops and ALPHA-dri/Cob blend bedding (PharmaSrv, Framingham, MA). Environmental enrichment consisted of a rat igloo (Nalgene Nunc, Rochester, NY), a maplewood product containing a food treat to encourage foraging (W0002, BioServ, Frenchtown, NJ), and stainless steel rings for the support of postural adjustments. Rats were housed under standard laboratory conditions (20 ± 2°C, 12:12-h light-dark cycle; lights on at 0600) in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. Rodent laboratory chow (Harlan Teklad, LM-485, Madison, WI) and water were provided ad libitum, except during heat exposure. The Institutional Animal Care and Use Committee approved all experimental procedures, which were performed in accordance with the American Physiological Society’s guiding principles for research involving animals and adhered to the Guide for the Care and Use of Laboratory Animals.

Radiotelemetry Measurements

Ten days before arrival, rats were implanted intraperitoneally with TL11M2-C50-PXT Physiotel Multiplus Radiotelemetry Transmitters (Data Sciences International, St. Paul, MN) by Charles River Laboratory. Briefly, an abdominal midline incision was made, exposing the abdominal aorta. Aortic blood flow was temporarily restricted, and a small incision made into the vessel through which a catheter was fed for continuous measurement of MAP. The abdominal aortic catheter was secured by a cellulose patch using medical glue, and aortic blood flow was then restored. The radiotelemetry device was sutured to the abdominal wall, followed by closure of the peritoneal muscle and skin layers. Each transmitter device allowed for the simultaneous and continuous monitoring of Tc (±0.25°C), HR (beats/min), and MAP (±3 mmHg) in conscious, free-ranging animals. Tc, HR, and MAP were acquired continuously and averaged at 1-min intervals throughout experimentation.

Transmitters weighed ~11 g or ~4% of presurgical body weight (BW). Rats were housed for 7 days upon arrival before the start of experimentation to acclimate to the vivarium. Stable circadian Tc, HR, and MAP rhythms were required in all rats before experimentation, indicating full surgical recovery and acclimatization to our vivarium, as previously described (24). Each radiotelemetry device was calibrated before and following experimentation to ensure accurate measurements.

Heating Protocol

Of the 30 rats used for the study, 22 were randomized to the heating group and 8 to the nonheated control (Con) group. The heat stress protocol has been described elsewhere (29). Briefly, conscious, unrestrained rats were exposed to an ambient temperature (Ta) of 37.0 ± 0.2°C in a floor standing incubator, in the absence of food and water, until a maximum temperature (Te,max) of 42.0°C was attained. Following removal from heat exposure at Te,max, food and water were provided ad libitum during undisturbed recovery at Ta of 20 ± 2°C. Con animals were exposed to the same experimental conditions at Ta of 20 ± 2°C with the timing of all procedures matched to that of a heated rat.

RNA Extraction, Reverse Transcription, and Real-time PCR

Gene expression levels of rat inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), prostacyclin (PGI2), and 18s rRNA (Applied Biosystems, Foster City, CA) were determined for heart and liver samples collected 24 h post-HS. RNA was isolated from ~30 mg frozen heart and liver tissue using the Agilent Total RNA Isolation Mini Kit (Agilent Technologies, Santa Clara, CA) and QuickGene RNA Tissue Kit II (AutoGen, Holliston, MA), respectively. The 260/230 and 260/280 nm ratios were ≥1.8 for all RNA samples.

RNA was reverse-transcribed into cDNA (37°C for 2 h) using the High Capacity Reverse Transcription Kit (Applied Biosystems). TaqMan Expression Assays containing 0.9 μM of each primer and 0.25 μM of a TaqMan MGB probe, comprising a FAM reporter dye at the 5’-end and a nonfluorescent quencher at the 3’-end, were combined with 50 ng cDNA, Fast Advanced Master Mix, and water in a final reaction volume of 10 μl. The gene expression assays used in this study were as follows: Hs99999901_s1 (18S rRNA), Rn01410330_m1 (IL-6), Rn00561646_m1 (iNOS), Rn01438328_m1 (COX-2), and Rn00694461_m1 (PGI2 synthase). RT-PCR reactions were performed in duplicate under the following conditions: hold at 95°C for 20 s, 40 cycles of 95°C for 1 s, and 60°C for 20 s (StepOne Plus Real-Time PCR System; Applied Biosystems) with >90% efficiency for all genes. Details of gene expression analysis are described elsewhere (17).

Data Analysis and Statistics

Heating calculations. Time to Te,max represents the total heat exposure time. Thermal strain (°C-min) during heating (ascending) and cooling (descending) were calculated as intervals × 0.5 (°C above Ta = 39.5°C at the start of the interval) and (°C above Ta = 39.5°C at the end of the interval), respectively. Hemodynamic responses during immediate recovery. In terms of acute HS recovery, one goal was to evaluate potential baroreflex mechanisms contributing to the hysteresis. Therefore, for this portion of the analysis, we examined both systolic blood pressure (SBP) and MAP. Due to the neurophysiological mechanisms contributing to baroreflex control of HR, SBP is more strongly correlated with baroreflex control of HR than is MAP (13, 33). Although both variables gave similar results, the hysteresis analysis focuses on SBP due to this relationship. HR and SBP were plotted as functions of Te during heating and cooling (ascending and descending phases, respectively) to evaluate whether hemodynamic responses to Te were directionally different between phases; that is, whether the responses exhibited hysteresis.

Dehydration. BW was measured on a top-loading balance with an accuracy of ±0.1 g. Percent dehydration was calculated as [(preheat BW – postheat BW)/preheat BW] × 100, which is an estimate that did not account for BW loss from feces or urine. Preheat BW was obtained immediately before heat exposure; postheat BW was obtained immediately upon removal from the heating chamber at Te,max (i.e., onset of recovery). Water bottles were weighed immediately at the onset of recovery and 24 h post-heat stress to calculate water intake and to serve as an estimation of hydration over the 24-h period.

Statistics. Values are means ± SE, except PCR results, which are presented as mean fold-change without SE. Normalized cycle threshold values within the same tissue (heart or liver) were compared with Con using one-way ANOVA, then unpaired t-tests were used to determine differences between each severity group and controls. Changes in gene expression that were more than twofold and had a P ≤ 0.05 were considered significant. One-way ANOVA or paired t-tests were conducted to compare the heating and cooling phases for HR and MAP values at specific Te values. For multiple comparisons, Holm-Sidak post hoc test was used to identify significant differences.

RESULTS

Heat Characteristics

Based on Te profiles observed during acute recovery, results were stratified into Mild, Moderate, and Severe HS, as described previously (29). Briefly, Mild animals display a mono-
phasic thermoregulatory response maintaining ~1°C hyperthermia throughout recovery. Moderate animals display a rebound hyperthermia of ~1°C above Con after a brief return to baseline. Severe animals display a triphasic response with a brief return to baseline, followed by a rebound hyperthermia that transitioned into sustained hypothermia of ~1°C below Con. Following stratification of HS severity in the present study, cardiovascular and inflammatory responses were compared among groups. Table 1 outlines the thermal characteristics of heat exposure per HS severity. No between-group differences existed in any TcMax or thermal strain during heat exposure (P > 0.05; Table 1). Con rats maintained the normal daytime Tc of ~37°C throughout experimentation.

Heat stress resulted in BW loss of 7.9 ± 0.6, 7.8 ± 0.6, and 8.7 ± 0.7% for Mild, Moderate and Severe, respectively (Table 1). No between-group differences existed in BW loss during heat exposure; all heated groups had significantly greater BW loss than Con (1.5 ± 0.1%; P < 0.01). At 24 h, Severe animals had not regained BW (7.2 ± 0.7%); however, Mild and Moderate animals recovered to 3.1 ± 0.8 and 4.3 ± 0.7% below starting BW, respectively (P < 0.05 vs. Severe; Table 1).

**Heat Exposure and Immediate Recovery**

**Hemodynamic adjustments during immediate recovery.** Figure 1 is a representative example of HR and SBP responses during and immediately following heat exposure. During the ascending phase (acute heating), increasing Tc corresponded with an increase in HR (Fig. 1, solid circles) until TcMax. In contrast, SBP displayed a biphasic response with an initial increase in pressure, followed by a decrease at TcMax (Fig. 1, solid inverted triangles). HR responses exhibited hysteresis relative to Tc such that HR was higher for a given Tc during the descending phase (cooling; Fig. 1, open circles) compared with the ascending phase (acute heating). A “reverse” hysteresis occurred in the SBP profile during the descending phase such that recovery pressure was lower than that achieved during passive heating per degree Tc (Fig. 1, open triangles).

Figure 2 illustrates the group mean differences in HR (△HR) and SBP (△SBP) between the ascending and descending phases at arbitrarily chosen Tc values of 39.5 and 41.0°C. For both 39.5 and 41.0°C, we observed significant △HR and △SBP between the ascending and descending phases (P < 0.0001), independent of HS severity. Since there were no differences among groups, data from all three groups were collapsed for further analysis. The △HR between ascending and descending phases at 39.5 and 41.0°C were 121 ± 14 and 115 ± 8 beats/min, respectively. These △HR responses were not different from each other (P = 0.754). In contrast, the △SBP between ascending and descending at 39.5 and 41.0°C were −14 ± 3 and −37 ± 2 mmHg, respectively, representing a significant return in SBP toward baseline values during descent at 39.5°C (P < 0.0001).

**24-h Recovery**

**Hemodynamic adjustments throughout 24 h of heat stress recovery.** Figure 3 depicts the hemodynamic profile throughout 24 h of recovery per HS severity. Mild HS rats displayed tachycardia (P < 0.01 vs. Con; Fig. 3, top) that persisted through 8 h of recovery before normalizing to Con values, and moderate hypertension (P < 0.05 vs. Con; Fig. 3, bottom). Severe rats exhibited sustained hypertension (P < 0.05 vs. Con; Fig. 3, bottom) that lasted throughout 24 h of recovery.

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**Table 1. Physiological response to uncompensable heat stress as a function of severity of response to heat stroke**

<table>
<thead>
<tr>
<th>Heat stress</th>
<th>Nonheated Control</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcMax, °C</td>
<td>37.17 ± 0.1</td>
<td>42.00 ± 0.02*</td>
<td>42.01 ± 0.02*</td>
<td>42.00 ± 0.02*</td>
</tr>
<tr>
<td>Time to TcMax, min</td>
<td>N/A</td>
<td>241 ± 26</td>
<td>216 ± 18</td>
<td>235 ± 17</td>
</tr>
<tr>
<td>Total thermal strain, °C-min</td>
<td>N/A</td>
<td>194.1 ± 24.9</td>
<td>183.6 ± 10.8</td>
<td>173.3 ± 10.2</td>
</tr>
<tr>
<td>Ascending thermal strain, °C-min</td>
<td>N/A</td>
<td>181.5 ± 33.2</td>
<td>154.4 ± 16.4</td>
<td>162.9 ± 10.1</td>
</tr>
<tr>
<td>Descending thermal strain, °C-min</td>
<td>N/A</td>
<td>24.5 ± 5.2</td>
<td>28.0 ± 3.2</td>
<td>22.0 ± 1.9</td>
</tr>
<tr>
<td>In-heat BW loss, %</td>
<td>1.5 ± 0.1</td>
<td>4.0 ± 0.6</td>
<td>4.0 ± 0.6</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>24-h BW loss, %</td>
<td>−0.0 ± 0.2</td>
<td>3.1 ± 0.8</td>
<td>4.3 ± 0.7</td>
<td>7.2 ± 0.7††</td>
</tr>
<tr>
<td>Maximum SBP, mmHg</td>
<td>421 ± 7</td>
<td>222 ± 2*</td>
<td>226 ± 1*</td>
<td>227 ± 1*</td>
</tr>
<tr>
<td>SBP @ TcMax, mmHg</td>
<td>384 ± 38*</td>
<td>210 ± 37††</td>
<td>200 ± 38††</td>
<td>190 ± 38††</td>
</tr>
<tr>
<td>Maximum MAP, mmHg</td>
<td>136 ± 4</td>
<td>182 ± 2*</td>
<td>186 ± 1*</td>
<td>188 ± 1*</td>
</tr>
<tr>
<td>MAP @ TcMax, mmHg</td>
<td>111 ± 3</td>
<td>175 ± 3*</td>
<td>174 ± 3*</td>
<td>171 ± 2*</td>
</tr>
<tr>
<td>Maximum HR, beats/min</td>
<td>391 ± 14</td>
<td>577 ± 6*</td>
<td>609 ± 8††</td>
<td>618 ± 8††</td>
</tr>
</tbody>
</table>

Values are means ± SE. TcMax, maximum core temperature; BW, body weight; SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate. *P < 0.05 vs. Control; †Mild, and ‡Moderate.
elevated MAP ($P < 0.05$ vs. Con; Fig. 3, middle) for the initial 5 h of recovery, and sustained hyperthermia ($P < 0.05$ vs. Con; Fig. 3, bottom) persisting throughout the 24-h recovery period. Moderate HS animals displayed significant tachycardia ($P < 0.01$ vs. Con; Fig. 3, top) throughout 24 h of recovery, normal MAP ($P > 0.05$ vs. Con; Fig. 3, middle), with a rebound hyperthermia beginning 4 h post-HS ($P < 0.05$ vs. Con; Fig. 3, bottom) that was not different from Mild and was maintained throughout 24 h of recovery. Severe HS was characterized by sustained tachycardia ($P < 0.05$ vs. Con; Fig. 3, top), profound hypotension within 3 h post-HS ($P > 0.01$ vs. Con; Fig. 3, middle) and maintained through 24 h of recovery, accompanied by hyperthermia within 5 h post-HS and sustained for 24 h of recovery ($P < 0.05$ vs. Con; Fig. 3, bottom).

Systemic inflammatory gene expression. Figure 4 illustrates inflammatory gene expression 24 h post-HS per heat stress severity. Cardiac and liver expression of iNOS, IL-6, COX-2, and PGI$_2$ demonstrated a dose-response relationship such that increasing HS severity correlated with a stepwise increase in gene expression. Cardiac iNOS expression was elevated 14-fold in Severe animals and was not significantly changed in Moderate or Mild HS (significance was defined as ≥2-fold change; Fig. 4, dotted line), whereas liver iNOS expression was elevated in Moderate and Severe groups (3- and 13-fold, respectively) with a substantially greater elevation in Severe animals. Cardiac IL-6 and COX-2 expression were elevated in Moderate HS animals seven- and eightfold, respectively; however, liver expression was not significant in Moderate animals. Cardiac IL-6 and COX-2 expression in Severe animals was elevated 51- and 24-fold, respectively, with liver expression elevated 4- and 3-fold. Moderate and Severe HS correlated with increased cardiac expression of PGI$_2$ (4- and 7-fold, respectively) that was not noted in the liver.

Fig. 2. HR (top) and SBP (bottom) values at 39.5 and 41.0°C T$_c$ during heating and cooling (ascending and descending phases, respectively). Note that HR was significantly higher for a given T$_c$ during recovery, and SBP was significantly lower, suggesting baroreflex mechanisms contribute to the HR hysteresis (see text for discussion). Values are means ± SE. *$P < 0.001$, ascending vs. descending.

Fig. 3. Sixty-minute averages for HR (top), MAP (middle), and $T_c$ (bottom) during 24-h recovery from heat stroke (HS) in each severity group and in nonheated control rats. Values are means ± SE. *$P < 0.05$ vs. *Con, ¶Mild, §Moderate, and ¶¶Severe.
The primary findings from this study are as follows: 1) a significant HR-Tc hysteresis exists in response to severe heat stress such that, during immediate recovery, HR is higher for any given Tc compared with during heat exposure; 2) arterial pressure displays a “reverse” hysteresis relative to Tc such that recovery MAP is lower per given Tc; 3) Severe HS responses were associated with major impairments in baroreflex and thermoregulatory compensation throughout 24 h of recovery; and 4) our data are consistent with the existence of a systemic inflammatory response, which was dependent on the severity of HS responsiveness. These findings provide translational insight into clinically relevant cardiovascular pathophysiology during and after HS and also provide novel mechanistic insight into cardiovascular responses to severe hyperthermia.

Although cardiovascular responses to whole body hyperthermia are reasonably well understood in humans (8, 10, 11, 14, 30, 32, 38) and rats (20–22, 25, 26, 28), the integrative mechanisms controlling HR and MAP responses during recovery are not completely clear. With regard to the observed hysteresis in the immediate recovery period, several studies have demonstrated the preservation of baroreflex control of HR during heat stress (8, 11, 38, 40, 41). Interestingly, although vascular sympathetic nerve activity is increased during heat stress (12), heat-induced attenuation of postsynaptic vasoconstrictor responsiveness results in progressive and profound reductions in MAP (14) in rats (20, 26, 28) and humans (11, 14, 39, 42). Kregel et al. (20, 26, 27) demonstrated reduced vascular reactivity during exposure to high local temperatures in the rat mesenteric arteries, both in vivo and in isolated vessel preparations. The significance of this finding lies in the potential for a similar reduction in global vasoconstrictor responsiveness, contributing to a reduction in TPR and, subsequently, MAP. It is plausible that this vasodilation led to the relative hypotension observed during the late stages of heat stress, and the early recovery phase elicited an error signal, resulting in baroreflex-mediated tachycardia. When HR was plotted as a function of Tc, this resulted in a HR-Tc hysteresis until the error signal was corrected and MAP returned to baseline values.

Notably, after normalization of the HR-Tc and MAP-Tc relationships in the immediate recovery phase, the hemodynamic response of Severe HS animals again fell into a dysregulated pattern of tachycardia and hypotension that was sustained throughout 24 h. The increase in iNOS expression may be representative of a systemic inflammatory response, which could have contributed to the loss of hemodynamic compensation via widespread peripheral vasodilation. The associated decrease in TPR would then result in a sustained decrease in MAP that was unable to be corrected through an increase in HR, as we observed in our Severe group of rats.

An alternative (or concurrent) possibility is that the sustained hypotension seen in the Severe group was due to greater sustained hypovolemia in this group. This could have been another consequence of the reduction in vasoconstrictor responsiveness at the level of the kidney. Other alterations in renal mechanisms (not specifically studied here) could have contributed to a hypovolemia-dependent hypotension in this group as well. Thus both renal and vascular impairment could have contributed to the sustained hypotension that was apparently too substantial to be corrected by the sustained tachycardia in the Severe group (Fig. 3).

The hemodynamic and thermoregulatory responses noted in the present study are consistent with, and extend, the findings of Gorman and Proppke (16). If we make the assumption that the heated animals are attempting to regulate MAP at a similar set point, it would follow that Moderate animals have a higher HR than Mild or Severe, as follows. In Moderate animals, TPR and MAP responses were similar to those in Mild animals. In this scenario, it is likely that increased HR would be sufficient to maintain MAP in the Moderate group. However, in Severe animals, the increase in HR was insufficient, and the systemic vasodilation led to both hypotension and hypothermia (increased peripheral heat loss). This suggests that Moderate HS animals retain the ability to compensate for the HS-related reduction in TPR and maintain MAP, whereas Severe animals cannot.
Interestingly, compared with the Mild group, the Moderate rats showed higher HR throughout 24-h recovery, despite a similar $T_c$ profile. This disconnect between the $T_c$ and HR responses based on HS severity suggests that the elevation in HR in Mild animals was driven primarily by the increase in temperature, and that of Moderate animals was the combined result of hyperthermia (thermoregulatory tachycardia) and autonomic baroreflex adjustments necessary for the preservation of MAP in the face of decreased TPR. In this context, we note that we did not directly assess the influence of temperature on the SA node; thus the tachycardic response in Mild HS could be attributed to factors other than hyperthermia.

Importantly, Leon et al. (23) demonstrated an inverse relationship between IL-6 concentrations and $T_c$ in a mouse model of HS. The results of that study showed a substantial increase in IL-6 during post-HS hyperthermia in mice (~115 min post-HS), and a moderate elevation at 24 h when the animals had resolved into a fever response. Bouchama et al. (3) showed that, after HS, there was an increase in plasma IL-6 in heated baboons, and that those animals having the highest IL-6 levels also had the lowest overall MAP, highest HR, and most severe alterations in organ biomarkers during recovery. This response was blunted under more moderate HS conditions. Similarly, the present study shows a substantial increase in IL-6 expression in the hypothermic Severe HS animals and a less significant response in the hyperthermic Moderate HS rats at 24 h. Furthermore, Wele and colleagues (36, 37) have also shown that IL-6 is increased in heated mouse skeletal muscle via mechanisms that include heat shock factor-1-mediated upregulation of IL-6 promoter activity. To confirm the links between upregulation of iNOS, IL-6, COX-2, and PGH$_2$, systemic inflammation, and vasodilation, future studies should analyze the protein expression and circulating levels of these key mediators.

**Translational Perspective**

Consistent with our findings, a substantial number of case studies report persistent sinus tachycardia and hypotension in HS patients upon clinical presentation (1, 2, 6, 7, 15, 35). Furthermore, if cardiovascular disease is present, increases in cardiac output via changes in HR, stroke volume, and contractility can be severely impaired, which significantly limits a patient’s ability to meet the demands placed on the cardiovascular system during exposure to hyperthermia. While we were limited in our ability to directly measure myocardial function, including changes in contractility, we previously demonstrated significant myocardial damage through histopathological examination in Severe animals at 24 h postheat (29). Importantly, our present findings extend our understanding of the significant burden on the cardiovascular system by demonstrating a persistent increased cardiovascular strain (i.e., tachycardia) through 24 h of recovery from uncompensable heat stress. In this context, several patient populations are at increased risk for heat illness. For example, hypertension in rats is associated with increased heat storage and more rapid increases in body temperature during exercise in a warm environment (4). Similarly, hypertensive humans show significant impairments in thermoregulatory vasodilation, putting them at greater risk for heat illness (18). These observations further emphasize the need for post-heat stress monitoring of patients with suspected HS, particularly those patients with underlying cardiovascular pathologies. Future studies should examine the time course of hemodynamic and thermoregulatory alterations beyond 24 h in an attempt to further characterize significant alterations in these parameters.

**Experimental Considerations**

We recognize that rats are not small humans, and, therefore, cardiovascular responses recorded in rats may have some comparative physiological limitations when extrapolating to human integrative mechanisms. While we are cognizant of these considerations, we propose that this rat model closely mimics clinical presentation, from molecular alterations to systemic organ dysfunction. Additional measurements that would provide further insight, but were not conducted here, include immunofluorescence assays for expression of iNOS in the heart and other tissues and assays to directly evaluate myocardial cell death (e.g., terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling) in the three groups. Additionally, it would be of value in future studies to evaluate whether physiological effects of iNOS upregulation could be blocked by competitive inhibition with L-arginine analogs such as N$^G$-nitro-L-arginine methyl ester.

With these limitations in mind, a substantial benefit of the experimental design in the present study is the ability to record data under conditions of extreme, uncompensable heat stress. Prospective studies of humans in conditions such as these are not possible due to the high levels of risk associated with this degree of heating. Thus our present model allowed us to increase our understanding of the acute physiological responses to such exposure in a model well accepted for mechanistic studies of cardiovascular responses and blood pressure control (5).

Two important follow-up questions remain. First, why did the rats exhibit such interindividual variability in hemodynamic responses to HS? Second, why did some rats end up in the Severe group, while others only sustained mild responses, when all heat exposures were identical? Our present data are consistent with previous work (5) regarding interindividual variability in systemic hemodynamics that is similar in humans and rats. While we do not provide evidence regarding the source of this variability, the present work is consistent with the previous report in emphasizing the interindividual variability present in the rat model, which may contribute to its strength as a model for human hemodynamics and thermoregulation.

In summary, we report significant changes in cardiovascular function immediately following and during the 24 h after severe heat stress in rats. These included hysteresis in the HR response as a function of $T_c$, such that HR was substantially higher for a given $T_c$ during acute recovery compared with the period of heat exposure. The existence of a concurrent “reverse” MAP-$T_c$ hysteresis suggests that the HR response was, at least in part, baroreflex mediated. Furthermore, the sustained disruption in blood pressure regulation through 24 h of recovery in the Severe HS group suggests autonomic dysfunction is not resolved following normalization of the hysteresis response. HS-mediated changes in vascular responsiveness, including widespread vasodilation, likely contribute to decreased TPR and sustained hypotension. Our findings are qualitatively
similar to clinical manifestations observed in HS patients and provide further insight into multiple stressors placed on integrative cardiovascular regulation during the 24 h after severe heat stress.

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DISCLAIMER

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REFERENCES


