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Additive Effects of Heating and Exercise on Baroreflex Control of Heart Rate in Healthy Males

Tiago Peçanha¹, Cláudia L. M. Forjaz¹, David. A. Low²

Affiliation:
¹Exercise Hemodynamic Laboratory, School of Physical Education and Sport, University of Sao Paulo, Brazil.
²Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, UK

Author’s contributions: TP, CLMF and DAL participated in the study conception and design, were responsible for the interpretation of data and contributed to the writing of the manuscript. TP and DAL were responsible for data collection and analyses. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Running head: Effects of Heating and Exercise on Baroreflex Function

Corresponding author: Tiago Peçanha. Exercise Hemodynamic Laboratory, School of Physical Education and Sport, University of São Paulo, Av. Professor Mello Moraes, 65 - Cidade Universitária, São Paulo, SP, Brazil. Zipcode: 05508-030. E-mail address: pecanhatiago@gmail.com
ABSTRACT

This study assessed the additive effects of passive heating and exercise on cardiac baroreflex sensitivity (cBRS) and heart rate variability (HRV). Twelve healthy young men (25±1 yrs, 23.8±0.5 kg/m²) randomly underwent two experimental sessions: heat stress (HS; whole-body heat stress using a tube-lined suit to increase core temperature by ~1 °C) and normothermia (NT). Each session was composed of a: pre-intervention rest (REST1); HS or NT interventions; post-intervention rest (REST2); and 14 min of cycling exercise [7 min at 40%HR_{reserve} (EX1) and 7 min at 60%HR_{reserve} (EX2)]. Heart rate and finger blood pressure were continuously recorded. cBRS was assessed using the sequence (cBRS_{SEQ}) and transfer function (cBRS_{TF}) methods. HRV was assessed using the indices SDNN (standard deviation of RR intervals) and RMSSD (root mean square of successive RR intervals). cBRS and HRV were not different between sessions during EX1 and EX2 (i.e. matched heart rate conditions: EX1=116±3 vs. 114±3, EX2=143±4 vs. 142±3 bpm; but different workloads: EX1=50±9 vs. 114±8, EX2=106±10 vs. 165±8 Watts; for HS and NT, respectively; P<0.01). However, when comparing EX1 of NT with EX2 of HS (i.e. matched workload conditions, but with different heart rates), cBRS and HRV were significantly reduced in HS (cBRS_{SEQ} = 1.6±0.3 vs. 0.6±0.1 ms/mmHg, P<0.01; SDNN = 2.3±0.1 vs. 1.3±0.2 ms, P<0.01). In conclusion, in conditions matched by HR, the addition of heat stress to exercise does not affect cBRS and HRV. Alternatively, in workload-matched conditions, the addition of heat to exercise results in reduced cBRS and HRV compared to exercise in normothermia.

Keywords: heat stress, baroreflex sensitivity, heart rate variability, core temperature, blood pressure
New & Noteworthy: The present study assessed cardiac baroreflex sensitivity during the combination of heat and exercise stresses. This is the first study to show that prior whole-body passive heating reduces cardiac baroreflex sensitivity and autonomic modulation of heart rate during exercise. These findings contribute to the better understanding of the role of thermoregulation on cardiovascular regulation during exercise.
INTRODUCTION

During exercise, the arterial baroreflex is reset in an intensity-dependent manner to operate at the prevailing blood pressure evoked by the exercise (17, 37). This exercise-induced baroreflex resetting is commonly accompanied by a reduction in cardiac baroreflex sensitivity (cBRS) around the operation point, as has been demonstrated by a variety of dynamic baroreflex analysis techniques (20, 31, 38). Two main mechanisms have been proposed for these baroreflex responses during exercise; central command, i.e. a feedforward mechanism that originates in the brain related to the perceived effort of the task (17, 32, 37), and the exercise pressor reflex, i.e., a feedback mechanism situated in skeletal muscle, which responds to mechanical (i.e. pressure, movement, etc), and chemical (i.e. pH, H+, ATP, acid lactic, diprotonated phosphate) stimuli (17, 20, 37). Research suggests that during dynamic exercise, the increased effort sensation alongside chemical and mechanical stimuli in the active muscle fibers trigger the reduction in cBRS and in parasympathetic nerve activity as well as the increase in sympathetic nerve activity to the heart, culminating in parallel increases in heart rate (HR) and blood pressure (17). While this model embraces most of the exercise-related stimuli, it does not consider the potential effect of increases in core temperature ($T_c$) on such autonomic responses (8).

Experimental studies have shown that stimulation of the thermoregulatory center in the hypothalamus produces neural responses in the cardiovascular control medullary area, promoting changes in sympathetic nerve and baroreflex activities (16, 40, 42). In humans, passive heat stress has been shown to increase HR (10), cardiac sympathetic modulation (3), and skin and muscle sympathetic activities (28), and to decrease cardiac parasympathetic modulation (12). Investigations of cBRS responses to passive heat stress in humans have produced equivocal findings, e.g., decreases (7, 12-13, 26, 52), no change (7, 9, 49-51) or increases (24) in cBRS. These conflicting findings may be explained by the varied baroreflex assessment protocols (10), however, most
studies assessing integrated baroreflex responses using dynamic techniques to estimate spontaneous cBRS (i.e. sequential method and transfer function analysis) have found decreases in cBRS during passive heating (12, 26, 52).

Although the effects of hyperthermia alone on baroreflex function have previously been investigated (8, 10), there is scarcity of data investigating cBRS responses to combined heat stress and exercise. Due to the evidence displaying decreases in cBRS in response to separate effects of passive heating and exercise, it is tempting to hypothesize that the execution of exercise after passive heating will produce further reductions in cBRS relative to exercise without prior heat stress. However, there is no data so far to support such a hypothesis. Therefore, the aim of this study was to assess the effects of prior passive heating on cardiovascular autonomic and cBRS responses to exercise in healthy young subjects. To address such a question, we performed HR-matched comparisons of cBRS responses to exercise under heat-stress and normothermic conditions. However, since such a comparison is only possible through a reduction of the absolute workload during exercise under heat-stress, we also performed absolute exercise workload-matched comparisons between heat stress and normothermic conditions.

MATERIALS AND METHODS

Subjects

Twelve healthy young men (25±1 yrs; 77±2 kg; 1.80±0.01 m; 23.8±1.9 kg/m²) were recruited. Participants were recreationally active, had no history of cardiovascular disease or smoking and were not taking any form of medication. Prior to participation, subjects received a detailed explanation about the experimental procedures and provided their written informed consent. The study was conducted in accordance with
the Declaration of Helsinki and was approved by the local Institutional research ethics committee.

**Exercise Test**

Prior to the experimental sessions, subjects attended the laboratory to perform a maximal exercise test on a magnetically braked cycle ergometer (Corival 400, Lode, Groningen, The Netherlands) using an incremental step protocol. Initially, subjects remained seated for 3 min on the ergometer, after which they performed 5 min of warm-up at 50% of the expected maximal workload (i.e. 146 ± 8 Watts). Then, the workload was increased by 30 watts every 2 min until maximal effort was obtained. All subjects attained maximal workload within 8 – 12 min [rating of perceived exertion (RPE) = 19 – 20]. During the test, ventilatory variables were continuously measured using a metabolic cart (CPX Ultima, Medical Graphics Corporation, Minnesota, United States) and HR was recorded with a HR monitor positioned at the subject’s chest (Polar RS800cx, Kempele, Finland). Maximal oxygen consumption (VO\textsubscript{2max}) and HR (HR\textsubscript{max}) were determined by the maximal values attained at the end of exercise test (average of 30 s data).

**Experimental protocol**

All subjects performed two visits to the laboratory at the same time of day, conducted in a randomized balanced order and separated by 3 – 7 days. Temperature and humidity of the room were kept constant across the tests (temperature ≈ 22-23 °C; humidity ≈ 35%). Subjects were instructed to avoid alcohol and exercise for 24 h, caffeine ingestion for 12 h, and food intake for 2 h prior to the sessions.

Upon arrival to the laboratory, subjects had their nude weight measured and collected their urine for urine osmolality assessment (U\textsubscript{osm}; Osmocheck pocket pal OSMO, Vitech Scientific Ltd, Horsham, United Kingdom). Subjects were admitted to the protocol if their U\textsubscript{osm} ranged from 200 to 600 mOsmol/kg\textsubscript{H\textsubscript{2}O}. Experimental measurements started
with a 10-min baseline supine rest assessment (REST1). Thereafter, subjects were exposed to the heat stress (HS) or normothermia (NT) interventions in the supine position. For HS, subjects were dressed in a water-perfused tube-lined suit (Med-Eng, Ottawa, Canada) covering the entire body, except for the head, face, hands, feet and the right forearm. This system controls skin temperature by changing the temperature of the water perfusing the suit. Subjects were exposed to HS by perfusing 48°C water through the suit until $T_c$ had increased $\approx 1°C$ or up to 60 min. The 1°C target increase in $T_c$ was chosen in order to promote a moderate heat stress in the participants that could be tolerated during the ensuing exercise. Once the target $T_c$ was reached, the temperature of the water perfusing the suit was reduced to $\sim 42°C$ to limit any further increase in $T_c$. For NT, subjects remained under laboratory temperature without using the suit for a similar timeframe. Subjects wore the suit during the whole protocol of the HS session (e.g., during exercise). For NT, since the use of the suit proved to promote undesirable heat storage in pilot tests, we decided to perform this entire session without using the suit in order to maximize the difference in thermal stress between sessions. After HS/NT interventions, subjects rested in the supine position for a second 10-min rest assessment (REST2). Subjects were then transferred to the cycle ergometer (Corival 400, Lode, Groningen, The Netherlands) to perform 14 min of exercise. The first 7 min of exercise were performed at 40% of the subject's HR reserve (EX1), whereas the last 7 min were performed at 60% of HR reserve (EX2). These exercise intensities were chosen in order to elicit light and moderate physiological overloads, respectively, and to allow reliable assessment of cardiac autonomic modulation (39). The target HR for each exercise bout ($HR_{ex}$) was calculated prior to the experimental sessions using the following equation: $HR_{ex} = [(HR_{max} - HR_{rest}) \times \%] + HR_{rest}$ (22). The $HR_{rest}$ and $HR_{max}$, respectively, referred to the HR recorded prior to the exercise test and at maximal effort. Workload was set based on the relationship of HR and workload obtained in the maximal exercise test and was adjusted during the first 3 min of each exercise bout to maintain the target HR. After
exercise, subjects had their nude weight reassessed and were instructed to rehydrate accordingly (Figure 1).

Measurements

During the experimental sessions, $T_c$ was measured in intervals of 10 s using a telemetric temperature pill (CorTemp® Wireless Ingestible Temperature Sensor, HQInc., Palmetto, Estados Unidos) swallowed by the subjects at least 2 hours prior to the experiments. This system has been shown to provide a valid $T_c$ measurement at rest and during exercise (5). Mean skin temperature ($T_{sk}$) was measured through the weighted average of six thermocouples (Surface temperature probe, Ellab, Norwich, United Kingdom) (44) and recorded continuously online (E-Val Pro, Ellab, Norwich, United Kingdom). HR was obtained using a 3 lead electrocardiogram (Powerlab, AD Instruments, Oxford, United Kingdom) and arterial blood pressure was measured on a beat-by-beat basis on the middle finger of the right hand using photoplethysmography (Finometer, Finapress Medical System, Amsterdam, The Netherland). Intermittent brachial blood pressure was also monitored by an automated sphygmomanometer (GE Pro300V2; Dinamap, Tampa, United States) positioned on the left arm. Skin blood flow (SKBF) was measured via laser-Doppler flowmetry using an integrated flow probe (Periflux System 5001, Perimed, Jarfalla, Sweden) attached to the right forearm, and cutaneous vascular conductance (CVC) was calculated from the ratio of SKBF and brachial mean arterial pressure (MAP). HR, beat-to-beat blood pressure, $T_c$ and SKBF were recorded continuously online (Powerlab, AD Instruments, Oxford, United Kingdom; 1 KHz sampling rate). Thermal discomfort was measured using a 9-point thermal discomfort scale (0 = unbearably cold, 9 = unbearably hot). All of the aforementioned parameters were recorded for REST1, REST2, EX1 and EX2 and data
analyses were performed in the last five minutes of each period (i.e. steady state condition). Additionally, subject’s RPE was recorded for EX1 and EX2 using Borg’s 6-20 scale (2).

**Cardiovascular autonomic analysis**

HR and blood pressure signals were exported into Heart Scope software (v 1.3.0.1, AMPS-LLC, New York, NY, USA) for the generation of RR intervals (RRi) and beat-by-beat systolic blood pressure (SBP) time series. The time series were visually inspected and occasional misdetections were manually corrected. Ectopic beats were identified and replaced by interpolated RRi values (less than 2% of the signal). Spontaneous cBRS was calculated using the sequence (cBRS$_{seq}$) and transfer function (cBRS$_{TF}$) techniques. For cBRS$_{seq}$ analysis, the software identified sequences of three or more consecutive beats in which SBP and RRi changed in the same direction (at least 1 mmHg for SBP and 4 ms for RRi). In each sequence, the slope of the linear regression line between SBP and RRi was determined (only sequences with $r^2 > 0.8$ were used) and the mean of the slopes was determined as the mean cBRS$_{seq}$ (33). For cBRS$_{TF}$, the transfer function between RRi and beat-by-beat SBP variabilities was obtained by a bivariate spectral analysis. The greatest magnitude of this function at the low frequency band was accepted as the cBRS$_{TF}$ (34). For estimations of parasympathetic modulations to the heart, time-domain HR variability was analyzed through the calculations of the standard deviation of RRi intervals (SDNN) and the square root of the mean of the sum of the squares of differences between adjacent normal RR intervals (RMSSD) (43).

**Statistical Analysis**

Normal distribution was checked using the Shapiro-Wilk test and was rejected for SDNN and RMSSD. Thus, these variables were log-transformed (ln) and normality was achieved. Paired T-Tests were employed to compare descriptive data between HS and
NT sessions, and to compare cardiovascular autonomic variables between EX1 of NT and EX2 of HS (i.e. matched-workload condition). A two-way ANOVA (session vs. time) was employed for comparing responses between HS and NT sessions across the different time points. When a main effect or an interaction was significant, post hoc comparisons were performed using the Newman-Keuls test. For all analyses, a p ≤ 0.05 was considered statistically significant. All analyses were performed online using the software STATISTICA (v 8.0, StatSoft, Tulsa, United States). Data are presented as mean ± SE.

**RESULTS**

The subject's VO$_{2\text{max}}$, HR$_{\text{max}}$ and maximal workload were 47.3 ± 2.3 ml.kg$^{-1}$.min$^{-1}$, 185 ± 2 bpm and 294 ± 13 Watts, respectively. Subject’s initial hydration status was similar between the sessions as demonstrated by the similar values of U$_{\text{osm}}$ (P = 0.72) and initial body mass (P = 0.87) in HS and NT sessions (Table 1). However, the HS session promoted greater body mass loss compared to NT (P<0.01). There were no differences in EX1 and EX2 HRs (either for absolute HR or % of HR reserve) and RPE between HS and NT sessions (P = 0.16-0.99). On the other hand, exercise workload was significantly lower in HS for both EX1 and EX2 (P < 0.01).

[insert Table 1 here]

The responses of T$_{c}$ and T$_{sk}$ are presented in Figure 2. In HS, T$_{c}$ (Fig 2a) significantly increased from REST1 to REST2 (+0.8 ± 0.0 °C; P < 0.01) and this increase persisted for EX1 and EX2 (P < 0.01). On the other hand, in NT, T$_{c}$ did not change from REST1 to EX1 (+0.0 ± 0.0 °C; P = 0.65 – 0.86) but slightly increased at the end of EX2 compared with REST2 (+0.3 ± 0.0 °C; P < 0.01). As a consequence, T$_{c}$ was
significantly higher in HS compared with NT from REST2 to EX2 ($P = 0.04$ for session vs. time). For $T_{sk}$ (Fig 2b), in the HS session, $T_{sk}$ significantly increased from REST1 to REST2 (+3.6 ± 0.2 °C; $P < 0.01$), and then slightly decreased in EX1 and EX2 (-1.0 ± 0.2 °C; $P < 0.01$), but still remained above resting levels ($P < 0.01$). In the NT session, $T_{sk}$ did not change from REST1 to REST2 (+0.1 ± 0.0 °C; $P = 0.70$), but then decreased at EX1 (-0.6 ± 0.2 °C; $P = 0.02$) and returned to resting levels at EX2 ($P = 0.39 – 0.43$). Consequently, $T_{sk}$ was significantly higher in HS than NT from REST2 to EX2 ($P < 0.01$ for session x time). Thermal discomfort was significantly greater in HS compared with NT session from REST2 until EX2 (6.6 ± 0.2 vs. 5.6 ± 0.1 for all moments pooled, $P < 0.01$).

[insert Figure 2 here]

Hemodynamic Responses

Figure 3 presents the hemodynamic responses to the HS and NT protocols. HR (Fig3a) increased from REST1 to REST2 in HS ($P < 0.01$), and then increased to the target HRs for EX1 and EX2 (i.e. 40% and 60% $HR_{reserve}$; $P < 0.01$). In NT, HR did not change from REST1 to REST2 ($P = 0.91$), and likewise increased to the target HRs for EX1 and EX2 ($P < 0.01$). Consequently, HR was significantly greater at REST2 in HS than NT, but there were no difference between the sessions for the other time points ($P < 0.01$ for session x time). In both sessions, MAP (Fig3b) did not change from REST1 to REST2 ($P = 0.52 – 0.97$) and then increased at EX1 and EX2 ($P < 0.01$). However, the increase in MAP during exercise was lower in HS, and for this reason, MAP was significantly lower at EX1 and EX2 in HS than NT ($P < 0.01$ for session x time). SKBF and CVC (Fig3c and 3d) increased from REST1 to REST2 ($P < 0.01$) in HS and remained increased at EX1 and EX2 ($P < 0.01$). In NT, SKBF and CVC did not change from REST1 to EX1 ($P = 0.90 – 0.91$) and then slightly increased at EX2 ($P = 0.03$).
Therefore, SKBF and CVC were significantly higher in HS vs. NT from REST2 to EX2
\((P < 0.01\) for session x time).\)

[insert Figure 3 here]

Cardiovascular Autonomic Responses

Cardiovascular autonomic responses to HS and NT sessions are depicted in Figures 4 and 5. In HS, \(^{BRS_{seq}}\) and \(^{BRS_{TF}}\) (Fig4a and 4b) significantly decreased from REST1 to REST2 \((P \leq 0.01)\), further decreased from REST2 to EX1 \((P < 0.01)\) and remained similar in EX2 \((P = 0.85 – 0.90)\). In NT, \(^{BRS_{seq}}\) and \(^{BRS_{TF}}\) did not change from REST1 to REST2 \((P = 0.13 – 0.39)\), decreased at EX1 \((P < 0.01)\) and remained similar in EX2 \((P = 0.90 – 0.92)\). As a result, \(^{BRS_{seq}}\) and \(^{BRS_{TF}}\) were significantly lower in HS than NT at REST2, but there were no differences between sessions during exercise \((P = 0.01 – 0.02\) for time vs. session). For SDNN and RMSSD (Fig4c and 4d), in HS these variables progressively decreased \((REST1 > REST2 > EX1 > EX2; P < 0.01)\), while in NT, SDNN and RMSSD did not change from REST1 to REST2 \((P = 0.90 – 0.92)\) and then progressively decreased at EX1 and EX2 \((P < 0.01)\). Therefore, SDNN and RMSSD were significantly lower in HS than NT at REST2, but there were no differences between sessions during exercise \((P < 0.01\) for time x session).

[insert Figure 4 here]

The matching of HR between the sessions was possible by the manipulation of exercise workload, which was significantly lower in HS in comparison with NT for both EX1 and EX2 \((P < 0.01; \text{Table 1})\). So, cardiovascular autonomic responses to EX1 of NT and EX2 of HS were compared to allow a comparison of similar workloads between conditions \((114 \pm 8 \text{ vs. } 106 \pm 10 \text{ watts, respectively, } P = 0.38)\), but with different HRs \((114 \pm 3 \text{ vs. } 143 \pm 4 \text{ bpm, respectively, } P < 0.01)\). In this comparison, \(T_c\) \((38.0 \pm 0.3 \text{ vs.} \)
37.0 ± 0.4 °C), \( T_{sk} \) (36.5 ± 0.6 vs. 32.7 ± 1.2 °C), SKBF (163 ± 91 vs. 51 ± 26 a.u.) and CVC (2.14 ± 1.25 vs. 0.55 ± 0.29 a.u./mmHg) were all significantly higher \((P < 0.05)\), and MAP was significantly lower (92 ± 6 vs. 115 ± 18 mmHg; \( P < 0.01 \)) in EX2 of HS in comparison with EX1 of NT. Regarding the comparisons of autonomic variables, \( BRS_{seq} \), \( BRS_{TF} \), SDNN and RMSSD were lower in HS in comparison with NT \((P < 0.01; \text{Figs 5a, 5b, 5c and 5d}).\)

DISCUSSION

The aim of this study was to assess the additive effects of exercise and passive heating on baroreflex function in healthy young subjects. Subjects underwent passive heat stress or normothermia and then performed short-term exercise. HS produced expected thermoregulatory changes (1, 14, 19), such as elevations in \( T_{c} \), \( T_{sk} \), HR, SKBF and CVC. In addition, HS attenuated the increases in MAP to exercise. Despite these thermal and hemodynamic differences, cBRS and HRV during exercise were not different between sessions when comparisons were matched by HR. However, when similar absolute workloads were compared (i.e. EX1 of NT x EX2 of HS), both BRS and HRV were reduced in HS compared with NT.

The separate effects of exercise and passive heating on cardiovascular autonomic function have been widely explored (7, 9, 12-13, 20, 26, 37-38, 50-52). Studies using dynamic techniques of spontaneous BRS analysis have consistently demonstrated a reduction in cBRS in response to both exercise (20, 31, 38) and passive heating (12, 26, 52). In the present study, the reduction in cBRS from REST2 to exercise (EX1/EX2) in NT confirms the isolated effect of exercise on cBRS, while the reduction in cBRS from REST1 to REST2 in HS demonstrates the isolated effect of heating on BRS.
Despite these findings outlining the independent effects of both exercise and passive heating on cBRS responses, there is a scarcity of information on the additive effects of heating and exercise on cBRS. In one of the few studies investigating such a question, Norton et al. (30) observed a progressive baroreflex resetting and an increase in $T_c$ during prolonged exercise, and this response was independent of the reduction in central venous pressure, raising the possibility that the $T_c$ increase accompanying the exercise could have been responsible for the progressive baroreflex resetting. However, it is not possible to rule out the potential concurrent influence of increased central command to this progressive baroreflex resetting, since RPE also increased during exercise (30). In the present study, when comparisons were matched by HR, RPE was similar between sessions, suggesting similar central command activation (47). In this comparison, cBRS was similar between the sessions, contradicting the hypothesis of an additive effect of exercise and heating on the BRS response.

Several factors may explain why cBRS was not different during exercise between the thermal conditions when matched by HR. The interaction between passive heating- and exercise-induced changes in cBRS may not simply be additive but rather complimentary or even redundant (32, 48). So, in the absence of one mechanism, other mechanisms could work in concert to provide the required response (48). Based on that hypothesis, in the NT session of the present study non-thermoregulatory mechanisms could have predominated in lieu of the absent thermoregulation-driven autonomic responses, resulting in equivalent cBRS responses to exercise in comparison to the HS session. Studies testing the interaction of various mechanisms in the baroreflex responses to exercise, for example central command and the exercise pressor reflex, have given support to this “redundancy hypothesis” (4, 18, 27, 48). A ‘basement’ effect in the responses should not be disregarded as well. BRS values during exercise without heating approached near-to-zero values. So, even with a potential additive effect of heating on such responses, this response could be virtually
restrained by the minimum achievable values. Finally, it is important to highlight that in
order to match HR between the sessions, the workload had to be reduced in HS
session. This reduced workload in HS might have elicited reduced muscle fiber
recruitment, ultimately leading to a reduced exercise pressor reflex (35). Since the
exercise pressor reflex also influences BRS responses to exercise (17, 20, 37), this
reduced workload in HS might have prevented differences in BRS responses between
sessions.

In order to balance the influence of different workloads and the potential influence of
exercise pressor reflex on the study’s main outcomes, we also compared the
differences in cBRS between EX1 of NT and EX2 of HS (i.e. similar absolute workloads
but higher HR during EX2 of HS). It could be argued that differences in time of
assessment between EX1 of NT and EX2 of HT (i.e., first 7 min vs. last 7 min of the 14
min of exercise) might have differently affected cBRS and HRV between sessions.
However, after initial adjustments (i.e., first 3 min of exercise) duration does not seem
to affect autonomic measurements during short-term exercise (36), and for this reason
the observed differences between sessions most likely result from different thermal
stresses. In this sense, absolute workload-matched comparisons revealed a reduced
cBRS in HS compared with NT, suggesting an additive effect of heating during exercise
when workload-matched conditions are used.

The mechanisms whereby heat stress might affect cBRS responses during exercise in
workload-matched conditions are not well known. Experimental studies have shown
that stimulation of thermoregulatory-related areas in the brain promote neural
responses in cardiovascular control medullary areas (16, 40, 42). Although this has not
been investigated, this relationship might also be present during exercise under heat
stress conditions and, for this reason, the hyperthermia might be partly responsible for
the changes in cBRS via central interactions. Other mechanisms might include the
parasympathetic and sympathetic responses to exercise and heating. It is well
demonstrated that exercise and heat stress independently reduce parasympathetic and increase sympathetic nerve activity to the heart and vasculature (3, 28, 46). So, it is possible that during both exercise and heat stress, these responses will be accentuated, reducing the capacity for additional parasympathetic withdrawal and sympathetic activation to sequences of blood pressure decay. The parasympathetic withdrawal occurring during exercise might be specifically related to BRS responses, since Ogoh et al. (31) demonstrated that parasympathetic blockade significantly reduced cBRS at rest and prevented further reductions during exercise. The greater reduction of the time-domain HRV indices SDNN and RMSSD in HS compared with NT (using matched workload comparisons) supports a reduction in parasympathetic modulation to the heart at rest and during exercise under heat stress, and are in line with previous studies using passive heat stress (12, 23). Additionally, it is also not possible to rule out the chance that increased central command activation may be behind the reduced BRS and HRV in HS compared with NT in workload-matched conditions. In such a comparison, RPE was significantly greater in HS than NT (13±1 vs. 11±1, \( P < 0.01 \)), which suggests greater central command activation in the former. Future studies are needed to directly assess the effects of heating on central command activation in response to exercise. Finally, a decrease in central venous pressure secondary to the cardiovascular drift promoted by HS (i.e., increases in skin blood flow and conductance) might also partially explain the decrease in cBRS and HRV in this session via cardiopulmonary baroreflex deactivation (11, 15).

The results of the present study indicated that cBRS and HRV did not differ between HS and NT when conditions were matched by HR, but were lower in HS when conditions were matched by workload but with different HRs. Apart from the potential mechanisms discussed above to explain the effects of heating on cardiac autonomic responses, a potential HR-dependence of the assessed autonomic variables should not be neglected. In this sense, Monfredi et al. (29) tested a variety of cardiac
preparations and observed that HR is a major determinant of HRV responses to several stimuli. So, the greater HR can partially explain the reduced HRV observed in the HS session when comparisons were matched by absolute workload. However, as acknowledged by Monfredi et al. (29) and demonstrated by other studies (41, 45), a reduced HRV does not only result from an increased HR, but still is an independent predictor of cardiovascular risk, providing useful information on cardiac autonomic modulation.

There are some limitations in this study that should be highlighted. Firstly, the results are limited to healthy young men, and future studies are necessary to assess the additive effects of passive heating and exercise on cardiac autonomic function in other populations (e.g., women, elderly, individuals with cardiovascular diseases, etc). Secondly, in order to equalize HR between sessions we had to manipulate the exercise workloads, which were significantly lower in HS. In an attempt to overcome such a limitation, we also performed comparisons between matched workload conditions, but with these comparisons HR and RPE were significantly increased in HS. The difficulty of matching HR, RPE and workload in a single comparison between different thermal conditions is present in most thermoregulation and exercise studies (6). An alternative could be to assess the additive effects of heating and exercise during the post-exercise phase instead of during the exercise period. Using this approach, the concerns about workload and RPE would be absent, with only HR remaining to be matched. Other limitations involve the methods employed for BRS assessment during exercise. Although spontaneous methods have advantages over other methods, including their simplicity, noninvasive nature, low operational cost, good reproducibility and capacity to assess the integrated baroreflex responses to exercise (21, 25), these methods do not allow the assessment of baroreflex responses along its full stimulus-response curve, including the analysis of baroreflex resetting; only providing information on baroreflex sensitivity around the operating gain of HR and blood pressure. The decreased MAP in
conjunction with similar HR during exercise in the HS session compared with NT suggest a baroreflex resetting produced by HS. Studies involving more intricate techniques such as neck chamber or pharmacological approaches could help to clarify the effects of heating and exercise on full baroreflex stimulus-response curves. Finally, using the present design it is not possible to distinguish the isolated effects of body temperature and cardiovascular drift on the main outcomes. Future studies should employ strategies to maintain central venous pressure while testing the effects of heating on cBRS responses to exercise.

The present study assessed the additive effects of heating and exercise on autonomic responses to exercise. Distinct conclusions can be made depending on the factors used for comparison. In conditions matched by HR, the addition of heat to exercise does not promote further decreases in baroreflex sensitivity, probably because of redundancy among mechanisms, a 'basement effect' and/or a reduction in workload. On the other hand, in conditions matched by workload but with different HRs, the addition of heat to exercise culminates in reduced baroreflex sensitivity and reduced parasympathetic modulation to the heart. This latter result opens up the perspective of the prevailing thermal stress being an active mechanism modulating baroreflex responses to exercise. Future studies should try to investigate the additive effects of heating and exercise in conditions matched by workload, HR and RPE, analyzing the full baroreflex stimulus-response curves.

ACKNOWLEDGEMENTS

We thank Tyrone Miah and Ceri Atkinson for their help with data collection, Gemma Miller and Dean Morrey for the technical assistance, and all volunteers for their willingness to participate in the study.
The present study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2015/15466-0; 2015/13800-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

The authors declare no conflict of interest.


FIGURE CAPTIONS

**Figure 1** – Experimental protocol. Subjects performed two randomized visits in separate days (heat stress – HS and normothermia – NT). REST1, resting prior to the HS or NT interventions. REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise performed at 40% of the subject’s heart rate reserve. EX2, final 7 min of the exercise performed at 60% of the subject’s heart rate reserve.

**Figure 2** – Core ($T_c$) and mean skin ($T_{sk}$) temperature measured (mean ± SE) in the heat stress (HS; n = 12) and normothermia (NT; n = 12) sessions. REST1, resting prior to the HS or NT interventions. REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise performed at 40% of the subject’s heart rate reserve. EX2, final 7 min of the exercise performed at 60% of the subject’s heart rate reserve. A two-way ANOVA (session vs. time) was employed for comparing responses between HS and NT sessions across the different time points. * p ≤ 0.05 vs. REST1. # p ≤ 0.05 vs. REST2. † p ≤ 0.05 vs. NT.

**Figure 3** – Hemodynamic responses (mean ± SE) in the heat stress (HS; n = 12) and normothermia (NT; n = 12) sessions. HR, heart rate. MAP, mean arterial pressure. SKBF, skin blood flow. CVC, cutaneous vascular conductance. REST1, resting prior to the HS or NT interventions. REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise performed at 40% of the subject’s heart rate reserve. EX2, final 7 min of the exercise performed at 60% of the subject’s heart rate reserve. A two-way ANOVA (session vs. time) was employed for comparing responses between HS and NT sessions across the different time points. * p ≤ 0.05 vs. REST1. # p ≤ 0.05 vs. REST2. ‡ p ≤ 0.05 vs. EX1. † p ≤ 0.05 vs. NT.

**Figure 4** – Cardiovascular autonomic measures (mean ± SE) in the heat stress (HS; n = 12) and normothermia (NT; n = 12) sessions. BRS$_{seq}$, cardiac baroreflex sensitivity assessed through the sequence method. BR$_{STF}$, cardiac baroreflex sensitivity assessed through the transfer function method. SDNN, standard deviation of the RR intervals. RMSSD, square root of the mean of the sum of the squares of differences between adjacent normal RR intervals. REST1, resting prior to the HS or NT interventions. REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise performed at 40% of the subject’s heart rate reserve. EX2, final 7 min of the exercise performed at 60% of the subject’s heart rate reserve. A
two-way ANOVA (session vs. time) was employed for comparing responses between HS and NT sessions across the different time points. * p ≤ 0.05 vs. REST1. # p ≤ 0.05 vs. REST2. ‡ p ≤ 0.05 vs. EX1. † p ≤ 0.05 vs. NT.

Figure 5 – Comparison of the cardiovascular autonomic variables (mean ± SE) measured at EX1 of the normothermic session (NT EX1; n = 12) and EX2 of the heat stress session (HS EX2; n = 12) (i.e. matched-workload conditions with different heart rates). BRS_{seq}, cardiac baroreflex sensitivity assessed through the sequence method. BRS_{TF}, cardiac baroreflex sensitivity assessed through the transfer function method. SDNN, standard deviation of the RR intervals. RMSSD, square root of the mean of the sum of the squares of differences between adjacent normal RR intervals. Paired T-Tests were employed to compare data between NT EX1 and HS EX2. $ p ≤ 0.05 vs. NT EX1.
Table 1 – Subject’s hydration status and exercise parameters (mean ± SE) during the heat stress (HS) and normothermia (NT) sessions. P values are for HS vs. NT.

<table>
<thead>
<tr>
<th></th>
<th>HS</th>
<th>NT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydration Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( U_{osm} ) (mOsm/kgH(_2)O)</td>
<td>449 ± 60</td>
<td>470 ± 58</td>
<td>0.72</td>
</tr>
<tr>
<td>Initial body mass (kg)</td>
<td>77.0 ± 2.4</td>
<td>77.0 ± 2.5</td>
<td>0.87</td>
</tr>
<tr>
<td>Final body mass (kg)</td>
<td>76.1 ± 2.4 †</td>
<td>76.7 ± 2.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Body mass loss (kg)</td>
<td>0.9 ± 0.1 †</td>
<td>0.3 ± 0.1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Exercise Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EX1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>116 ± 3</td>
<td>114 ± 3</td>
<td>0.73</td>
</tr>
<tr>
<td>HR (%HR(_{reserve}))</td>
<td>40 ± 1</td>
<td>40 ± 1</td>
<td>0.49</td>
</tr>
<tr>
<td>Workload (Watts)</td>
<td>50 ± 9 †</td>
<td>114 ± 8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RPE (6-20)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
<td>0.63</td>
</tr>
<tr>
<td>EX2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>143 ± 4</td>
<td>142 ± 3</td>
<td>0.61</td>
</tr>
<tr>
<td>HR (%HR(_{reserve}))</td>
<td>62 ± 1</td>
<td>62 ± 1</td>
<td>0.99</td>
</tr>
<tr>
<td>Workload (Watts)</td>
<td>106 ± 10 †</td>
<td>165 ± 8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RPE (6-20)</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>0.16</td>
</tr>
</tbody>
</table>

\( U_{osm} \), urine osmolality. EX1, first 7 min of the exercise performed at 40% of the subject’s heart rate reserve. EX2, final 7 min of the exercise performed at 60% of the subject’s heart rate reserve. HR, heart rate. RPE, rating of perceived exertion. † \( p \leq 0.05 \) vs. NT session.
Heat stress (HS)

REST1   HS   REST2  40% HR_{Res}  60% HR_{Res}  EX1  EX2  Exercise

Normothermia (NT)

REST1   NT   REST2  40% HR_{Res}  60% HR_{Res}  EX1  EX2  Exercise

10 min  |  60 min  |  10 min  |  14 min  |