Original article

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

Tiago Peçanha[1], Cláudia L. M. Forjaz[1], David. A. Low[2]

Affiliation:

[1]Exercise Hemodynamic Laboratory, School of Physical Education and Sport, University of Sao Paulo, Brazil.

[2]Research 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/m^2) 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/m^2) 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 Netherland) 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_{2max}) and HR (HR_{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_{osm}; Osmocheck pocket pal OSMO, Vitech Scientific Ltd, Horsham, United Kingdom). Subjects were admitted to the protocol if their U_{osm} ranged from 200 to 600 mOsmol/kgH_2O. 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 ≈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 ~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).

[insert Figure 1 here]

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 $p \leq 0.05$ 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_{2max}, HR_{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_{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 ºC; P < 0.01). As a consequence, T_c was

241 significantly higher in HS compared with NT from REST2 to EX2 (P = 0.04 for session
242 vs. time). For T_{sk} (Fig 2b), in the HS session, T_{sk} significantly increased from REST1 to
243 REST2 (+ 3.6 ± 0.2 °C; P < 0.01), and then slightly decreased in EX1 and EX2 (-1.0 ±
244 0.2 °C; P < 0.01), but still remained above resting levels (P < 0.01). In the NT session,
245 T_{sk} did not change from REST1 to REST2 (+0.1 ± 0.0 °C; P = 0.70), but then
246 decreased at EX1 (-0.6 ± 0.2 °C; P = 0.02) and returned to resting levels at EX2 (P =
247 0.39 – 0.43). Consequently, T_{sk} was significantly higher in HS than NT from REST2 to
248 EX2 (P < 0.01 for session x time). Thermal discomfort was significantly greater in HS
249 compared with NT session from REST2 until EX2 (6.6 ± 0.2 vs. 5.6 ± 0.1 for all
250 moments pooled, P < 0.01).

251 [insert Figure 2 here]

252

253 **Hemodynamic Responses**

254 Figure 3 presents the hemodynamic responses to the HS and NT protocols. HR (Fig3a)
255 increased from REST1 to REST2 in HS (P < 0.01), and then increased to the target
256 HRs for EX1 and EX2 (i.e. 40% and 60% $HR_{reserve}$; P < 0.01). In NT, HR did not change
257 from REST1 to REST2 (P = 0.91), and likewise increased to the target HRs for EX1
258 and EX2 (P < 0.01). Consequently, HR was significantly greater at REST2 in HS than
259 NT, but there were no difference between the sessions for the other time points (P <
260 0.01 for session x time). In both sessions, MAP (Fig3b) did not change from REST1 to
261 REST2 (P = 0.52 – 0.97) and then increased at EX1 and EX2 (P < 0.01). However, the
262 increase in MAP during exercise was lower in HS, and for this reason, MAP was
263 significantly lower at EX1 and EX2 in HS than NT (P < 0.01 for session x time). SKBF
264 and CVC (Fig3c and 3d) increased from REST1 to REST2 (P < 0.01) in HS and
265 remained increased at EX1 and EX2 (P < 0.01). In NT, SKBF and CVC did not change
266 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 ($P < 0.01$; 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 ± 8 vs. 106 ± 10 watts, respectively, P = 0.38), but with different HRs (114 ± 3 vs. 143 ± 4 bpm, respectively, $P < 0.01$). In this comparison, T_c (38.0 ± 0.3 vs.

294 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
295 CVC (2.14 ± 1.25 vs. 0.55 ± 0,29 a.u./mmHg) were all significantly higher ($P < 0.05$),
296 and MAP was significantly lower (92 ± 6 vs. 115 ± 18 mmHg; $P < 0.01$) in EX2 of HS in
297 comparison with EX1 of NT. Regarding the comparisons of autonomic variables,
298 BRS_{seq}, BRS_{TF}, SDNN and RMSSD were lower in HS in comparison with NT ($P < 0.01$;
299 Figs 5a, 5b, 5c and 5d).

300

301 [insert Figure 5 here]

302

303 **DISCUSSION**

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

313 The separate effects of exercise and passive heating on cardiovascular autonomic
314 function have been widely explored (7, 9, 12-13, 20, 26, 37-38, 50-52). Studies using
315 dynamic techniques of spontaneous BRS analysis have consistently demonstrated a
316 reduction in cBRS in response to both exercise (20, 31, 38) and passive heating (12,
317 26, 52). In the present study, the reduction in cBRS from REST2 to exercise (EX1/EX2)
318 in NT confirms the isolated effect of exercise on cBRS, while the reduction in cBRS
319 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 $_{C}$BRS 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

401 preparations and observed that HR is a major determinant of HRV responses to
402 several stimuli. So, the greater HR can partially explain the reduced HRV observed in
403 the HS session when comparisons were matched by absolute workload. However, as
404 acknowledged by Monfredi et al. (29) and demonstrated by other studies (41, 45), a
405 reduced HRV does not only result from an increased HR, but still is an independent
406 predictor of cardiovascular risk, providing useful information on cardiac autonomic
407 modulation.

408 There are some limitations in this study that should be highlighted. Firstly, the results
409 are limited to healthy young men, and future studies are necessary to assess the
410 additive effects of passive heating and exercise on cardiac autonomic function in other
411 populations (e.g., women, elderly, individuals with cardiovascular diseases, etc).
412 Secondly, in order to equalize HR between sessions we had to manipulate the exercise
413 workloads, which were significantly lower in HS. In an attempt to overcome such a
414 limitation, we also performed comparisons between matched workload conditions, but
415 with these comparisons HR and RPE were significantly increased in HS. The difficulty
416 of matching HR, RPE and workload in a single comparison between different thermal
417 conditions is present in most thermoregulation and exercise studies (6). An alternative
418 could be to assess the additive effects of heating and exercise during the post-exercise
419 phase instead of during the exercise period. Using this approach, the concerns about
420 workload and RPE would be absent, with only HR remaining to be matched. Other
421 limitations involve the methods employed for BRS assessment during exercise.
422 Although spontaneous methods have advantages over other methods, including their
423 simplicity, noninvasive nature, low operational cost, good reproducibility and capacity to
424 assess the integrated baroreflex responses to exercise (21, 25), these methods do not
425 allow the assessment of baroreflex responses along its full stimulus-response curve,
426 including the analysis of baroreflex resetting; only providing information on baroreflex
427 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.

GRANTS

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).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. **Binder K, Gagnon D, Lynn AG, Kondo N, and Kenny GP**. Heat stress attenuates the increase in arterial blood pressure during isometric handgrip exercise. *Eur J Appl Physiol* 113: 183-190, 2013.
2. **Borg G**. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 2: 92-98, 1970.
3. **Brenner IK, Thomas S, and Shephard RJ**. Spectral analysis of heart rate variability during heat exposure and repeated exercise. *European Journal of Applied Physiology & Occupational Physiology* 76: 145-156, 1997.
4. **Bull RK, Davies CT, Lind AR, and White MJ**. The human pressor response during and following voluntary and evoked isometric contraction with occluded local blood supply. *J Physiol (Lond)* 411: 63-70, 1989.
5. **Byrne C, and Lim CL**. The ingestible telemetric body core temperature sensor: a review of validity and exercise applications. *BJSM online* 41: 126-133, 2007.
6. **Cheuvront SN, Kenefick RW, Montain SJ, and Sawka MN**. Mechanisms of aerobic performance impairment with heat stress and dehydration. *J Appl Physiol* 109: 1989-1995, 2010.
7. **Crandall CG**. Carotid baroreflex responsiveness in heat-stressed humans. *Am J Physiol Heart Circ Physiol* 279: H1955-1962, 2000.
8. **Crandall CG**. Heat stress and baroreflex regulation of blood pressure. *Med Sci Sports Exerc* 40: 2063-2070, 2008.
9. **Crandall CG, Cui J, and Wilson TE**. Effects of heat stress on baroreflex function in humans. *Acta Physiol Scand* 177: 321-328, 2003.
10. **Crandall CG, and Wilson TE**. Human cardiovascular responses to passive heat stress. *Compr Physiol* 5: 17-43, 2015.
11. **Crandall CG, Wilson TE, Marving J, Vogelsang TW, Kjaer A, Hesse B, and Secher NH**. Effects of passive heating on central blood volume and ventricular dimensions in humans. *J Physiol (Lond)* 586: 293-301, 2008.
12. **Crandall CG, Zhang R, and Levine BD**. Effects of whole body heating on dynamic baroreflex regulation of heart rate in humans. *Am J Physiol Heart Circ Physiol* 279: H2486-2492, 2000.
13. **Cui J, Shibasaki M, Davis SL, Low DA, Keller DM, and Crandall CG**. Whole body heat stress attenuates baroreflex control of muscle sympathetic nerve activity during postexercise muscle ischemia. *J Appl Physiol* 106: 1125-1131, 2009.
14. **Cui J, Shibasaki M, Low DA, Keller DM, Davis SL, and Crandall CG**. Heat stress attenuates the increase in arterial blood pressure during the cold pressor test. *J Appl Physiol* 109: 1354-1359, 2010.
15. **Cui J, Wilson TE, and Crandall CG**. Muscle sympathetic nerve activity during lower body negative pressure is accentuated in heat-stressed humans. *J Appl Physiol* 96: 2103-2108, 2004.
16. **Evans MH**. Potentiation of a cardioinhibitory reflex by hypothalamic stimulation in the rabbit. *Brain Res* 154: 331-343, 1978.
17. **Fisher JP, Young CN, and Fadel PJ**. Autonomic adjustments to exercise in humans. *Compr Physiol* 5: 475-512, 2015.
18. **Gallagher KM, Fadel PJ, Smith SA, Stromstad M, Ide K, Secher NH, and Raven PB**. The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in humans. *Exp Physiol* 91: 79-87, 2006.
19. **Gonzalez-Alonso J**. Human thermoregulation and the cardiovascular system. *Exp Physiol* 97: 340-346, 2012.
20. **Hartwich D, Dear WE, Waterfall JL, and Fisher JP**. Effect of muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity during exercise in humans. *J Physiol (Lond)* 589: 6157-6171, 2011.

21. **Iellamo F, Legramante JM, Raimondi G, Castrucci F, Massaro M, and Peruzzi G**. Evaluation of reproducibility of spontaneous baroreflex sensitivity at rest and during laboratory tests. *J Hypertens* 14: 1099-1104, 1996.
22. **Karvonen J, and Vuorimaa T**. Heart rate and exercise intensity during sports activities. Practical application. *Sports Med* 5: 303-311, 1988.
23. **Kinugasa H, and Hirayanagi K**. Effects of skin surface cooling and heating on autonomic nervous activity and baroreflex sensitivity in humans. *Exp Physiol* 84: 369-377, 1999.
24. **Krnjajic D, Allen DR, Butts CL, and Keller DM**. Carotid baroreflex control of heart rate is enhanced, while control of mean arterial pressure is preserved during whole body heat stress in young healthy men. *Am J Physiol-Reg I* 311: R735-R741, 2016.
25. **La Rovere MT, Pinna GD, and Raczak G**. Baroreflex sensitivity: Measurement and clinical implications. *Ann Noninvas Electro* 13: 191-207, 2008.
26. **Lee K, Jackson DN, Cordero DL, Nishiyasu T, Peters JK, and Mack GW**. Change in spontaneous baroreflex control of pulse interval during heat stress in humans. *J Appl Physiol* 95: 1789-1798, 2003.
27. **Leshnower BG, Potts JT, Garry MG, and Mitchell JH**. Reflex cardiovascular responses evoked by selective activation of skeletal muscle ergoreceptors. *J Appl Physiol* 90: 308-316, 2001.
28. **Low DA, Keller DM, Wingo JE, Brothers RM, and Crandall CG**. Sympathetic nerve activity and whole body heat stress in humans. *J Appl Physiol* 111: 1329-1334, 2011.
29. **Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, and Boyett MR**. Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. *Hypertension* 64: 1334-1343, 2014.
30. **Norton KH, Gallagher KM, Smith SA, Querry RG, Welch-O'Connor RM, and Raven PB**. Carotid baroreflex function during prolonged exercise. *J Appl Physiol* 87: 339-347, 1999.
31. **Ogoh S, Fisher JP, Dawson EA, White MJ, Secher NH, and Raven PB**. Autonomic nervous system influence on arterial baroreflex control of heart rate during exercise in humans. *J Physiol (Lond)* 566: 599-611, 2005.
32. **Ogoh S, Wasmund WL, Keller DM, A OY, Gallagher KM, Mitchell JH, and Raven PB**. Role of central command in carotid baroreflex resetting in humans during static exercise. *J Physiol (Lond)* 543: 349-364, 2002.
33. **Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Groppelli A, Pedotti A, Zanchetti A, and Mancia G**. Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension* 12: 214-222, 1988.
34. **Parati G, Di Rienzo M, and Mancia G**. How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life. *J Hypertens* 18: 7-19, 2000.
35. **Petrofsky JS, Phillips CA, Sawka MN, Hanpeter D, Lind AR, and Stafford D**. Muscle fiber recruitment and blood pressure response to isometric exercise. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology* 50: 32-37, 1981.
36. **Pichon AP, de Bisschop C, Roulaud M, Denjean A, and Papelier Y**. Spectral analysis of heart rate variability during exercise in trained subjects. *Med Sci Sports Exerc* 36: 1702-1708, 2004.
37. **Raven PB, Fadel PJ, and Ogoh S**. Arterial baroreflex resetting during exercise: a current perspective. *Exp Physiol* 91: 37-49, 2006.
38. **Sala-Mercado JA, Ichinose M, Hammond RL, Ichinose T, Pallante M, Stephenson LW, O'Leary DS, and Iellamo F**. Muscle metaboreflex attenuates spontaneous heart rate baroreflex sensitivity during dynamic exercise. *Am J Physiol Heart Circ Physiol* 292: H2867-2873, 2007.

570 39. **Sandercock GR, and Brodie DA**. The use of heart rate variability measures to
571 assess autonomic control during exercise. *Scand J Med Sci Sports* 16: 302-313, 2006.
572 40. **Simon OR, Basuray BN, West WL, and Copeland R**. Interaction between the
573 baroreflex and anterior hypothalamic stimulation. Demonstration of a noradrenergic
574 involvement. *Neuropharmacology* 24: 665-675, 1985.
575 41. **Stauss HM**. Heart rate variability: just a surrogate for mean heart rate?
576 *Hypertension* 64: 1184-1186, 2014.
577 42. **Takeda K, and Bunag RD**. Sympathetic hyperactivity during hypothalamic
578 stimulation in spontaneously hypertensive rats. *J Clin Invest* 62: 642-648, 1978.
579 43. **Task-Force**. Heart rate variability. Standards of measurement, physiological
580 interpretation, and clinical use. Task Force of the European Society of Cardiology and
581 the North American Society of Pacing and Electrophysiology. *Eur Heart J* 17: 354-381,
582 1996.
583 44. **Taylor WF, Johnson JM, Kosiba WA, and Kwan CM**. Cutaneous vascular
584 responses to isometric handgrip exercise. *J Appl Physiol* 66: 1586-1592, 1989.
585 45. **Tsuji H, Venditti FJ, Jr., Manders ES, Evans JC, Larson MG, Feldman CL,**
586 **and Levy D**. Reduced heart rate variability and mortality risk in an elderly cohort. The
587 Framingham Heart Study. *Circulation* 90: 878-883, 1994.
588 46. **White DW, and Raven PB**. Autonomic neural control of heart rate during
589 dynamic exercise: revisited. *J Physiol (Lond)* 592: 2491-2500, 2014.
590 47. **Williamson JW, McColl R, Mathews D, Mitchell JH, Raven PB, and Morgan**
591 **WP**. Brain activation by central command during actual and imagined handgrip under
592 hypnosis. *J Appl Physiol* 92: 1317-1324, 2002.
593 48. **Winchester PK, Williamson JW, and Mitchell JH**. Cardiovascular responses
594 to static exercise in patients with Brown-Sequard syndrome. *J Physiol (Lond)* 527 Pt 1:
595 193-202, 2000.
596 49. **Yamazaki F, Matsumura F, Nagata J, Ando A, and Imura T**. Spontaneous
597 arterial baroreflex control of the heart rate during head-down tilt in heat-stressed
598 humans. *Eur J Appl Physiol* 85: 208-213, 2001.
599 50. **Yamazaki F, Sagawa S, Torii R, Endo Y, and Shiraki K**. Effects of acute
600 hyperthermia on the carotid baroreflex control of heart rate in humans. *Int J*
601 *Biometeorol* 40: 200-205, 1997.
602 51. **Yamazaki F, and Sone R**. Modulation of arterial baroreflex control of heart rate
603 by skin cooling and heating in humans. *J Appl Physiol* 88: 393-400, 2000.
604 52. **Yamazaki F, and Sone R**. Thermal stress modulates arterial pressure
605 variability and arterial baroreflex response of heart rate during head-up tilt in humans.
606 *Eur J Appl Physiol* 84: 350-357, 2001.
607
608

609

610 **FIGURE CAPTIONS**

611 **Figure 1 –** Experimental protocol. Subjects performed two randomized visits in separate days
612 (heat stress – HS and normothermia – NT). REST1, resting prior to the HS or NT interventions.
613 REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise performed at
614 40% of the subject's heart rate reserve. EX2, final 7 min of the exercise performed at 60% of the
615 subject's heart rate reserve

616 **Figure 2 –** Core (T_c) and mean skin (T_{sk}) temperature measured (mean ± SE) in the heat stress
617 (HS; n = 12) and normothermia (NT; n = 12) sessions. REST1, resting prior to the HS or NT
618 interventions. REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise
619 performed at 40% of the subject's heart rate reserve. EX2, final 7 min of the exercise performed
620 at 60% of the subject's heart rate reserve. A two-way ANOVA (session vs. time) was employed
621 for comparing responses between HS and NT sessions across the different time points. * $p \leq$
622 0.05 vs. REST1. # $p \leq 0.05$ vs. REST2. † $p \leq 0.05$ vs. NT.

623 **Figure 3 –** Hemodynamic responses (mean ± SE) in the heat stress (HS; n = 12) and
624 normothermia (NT; n = 12) sessions. HR, heart rate. MAP, mean arterial pressure. SKBF, skin
625 blood flow. CVC, cutaneous vascular conductance. REST1, resting prior to the HS or NT
626 interventions. REST2, resting after the HS or NT interventions. EX1, first 7 min of the exercise
627 performed at 40% of the subject's heart rate reserve. EX2, final 7 min of the exercise performed
628 at 60% of the subject's heart rate reserve. A two-way ANOVA (session vs. time) was employed
629 for comparing responses between HS and NT sessions across the different time points. * $p \leq$
630 0.05 vs. REST1. # $p \leq 0.05$ vs. REST2. ‡ $p \leq 0.05$ vs. EX1. † $p \leq 0.05$ vs. NT.

631 **Figure 4 –** Cardiovascular autonomic measures (mean ± SE) in the heat stress (HS; n = 12)
632 and normothermia (NT; n = 12) sessions. BRS_{seq}, cardiac baroreflex sensitivity assessed
633 through the sequence method. BR_{STF}, cardiac baroreflex sensitivity assessed through the
634 transfer function method. SDNN, standard deviation of the RR intervals. RMSSD, square root of
635 the mean of the sum of the squares of differences between adjacent normal RR intervals.
636 REST1, resting prior to the HS or NT interventions. REST2, resting after the HS or NT
637 interventions. EX1, first 7 min of the exercise performed at 40% of the subject's heart rate
638 reserve. EX2, final 7 min of the exercise performed at 60% of the subject's heart rate reserve. A

639 two-way ANOVA (session vs. time) was employed for comparing responses between HS and
640 NT sessions across the different time points. * $p \leq 0.05$ vs. REST1. # $p \leq 0.05$ vs. REST2. ‡ $p \leq$
641 0.05 vs. EX1. † $p \leq 0.05$ vs. NT.

642 **Figure 5 –** Comparison of the cardiovascular autonomic variables (mean ± SE) measured at
643 EX1 of the normothermic session (NT EX1; n = 12) and EX2 of the heat stress session (HS
644 EX2; n = 12) (i.e. matched-workload conditions with different heart rates). BRS_{seq}, cardiac
645 baroreflex sensitivity assessed through the sequence method. BRS_{TF}, cardiac baroreflex
646 sensitivity assessed through the transfer function method. SDNN, standard deviation of the RR
647 intervals. RMSSD, square root of the mean of the sum of the squares of differences between
648 adjacent normal RR intervals. Paired T-Tests were employed to compare data between NT EX1
649 and HS EX2. $ $p \leq 0.05$ vs. NT EX1.

650

651

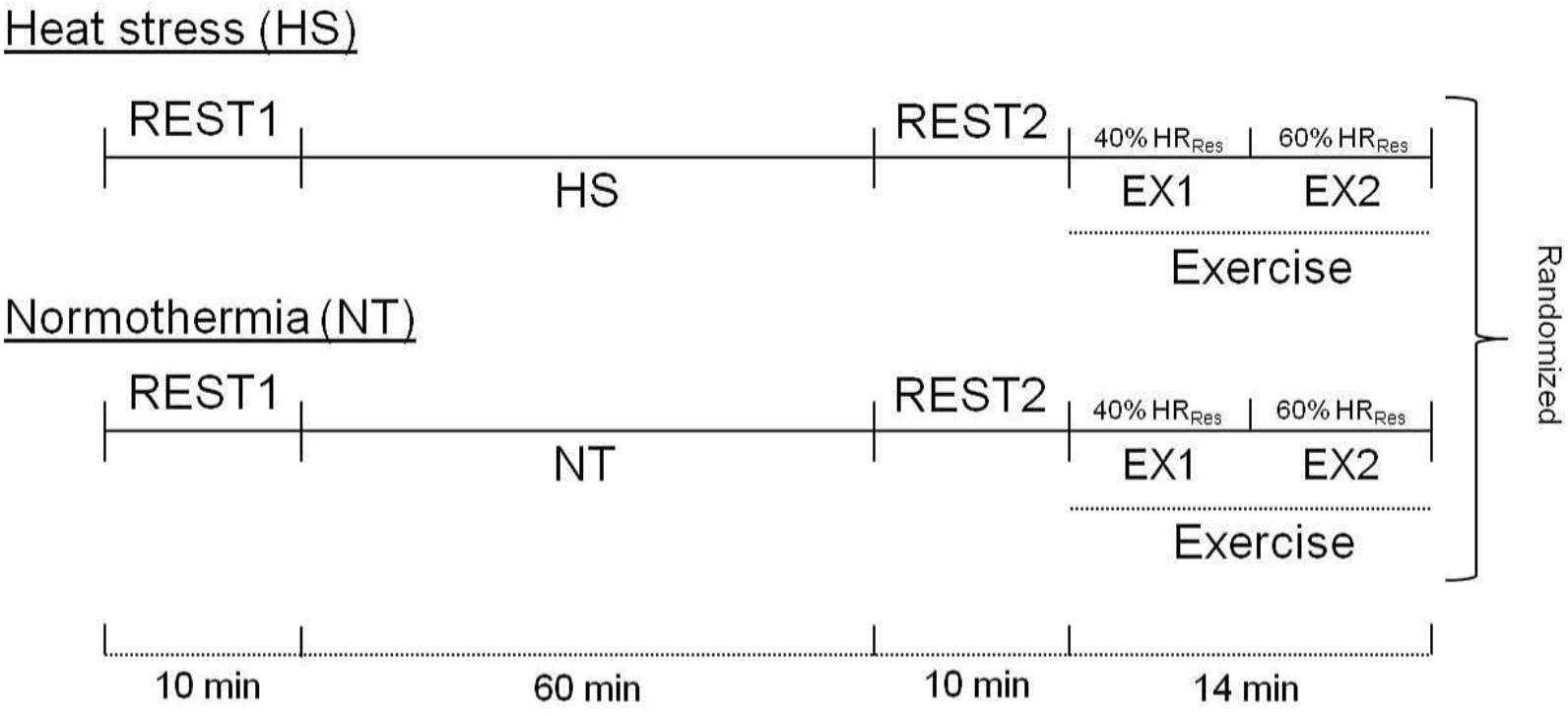
652 **TABLES**

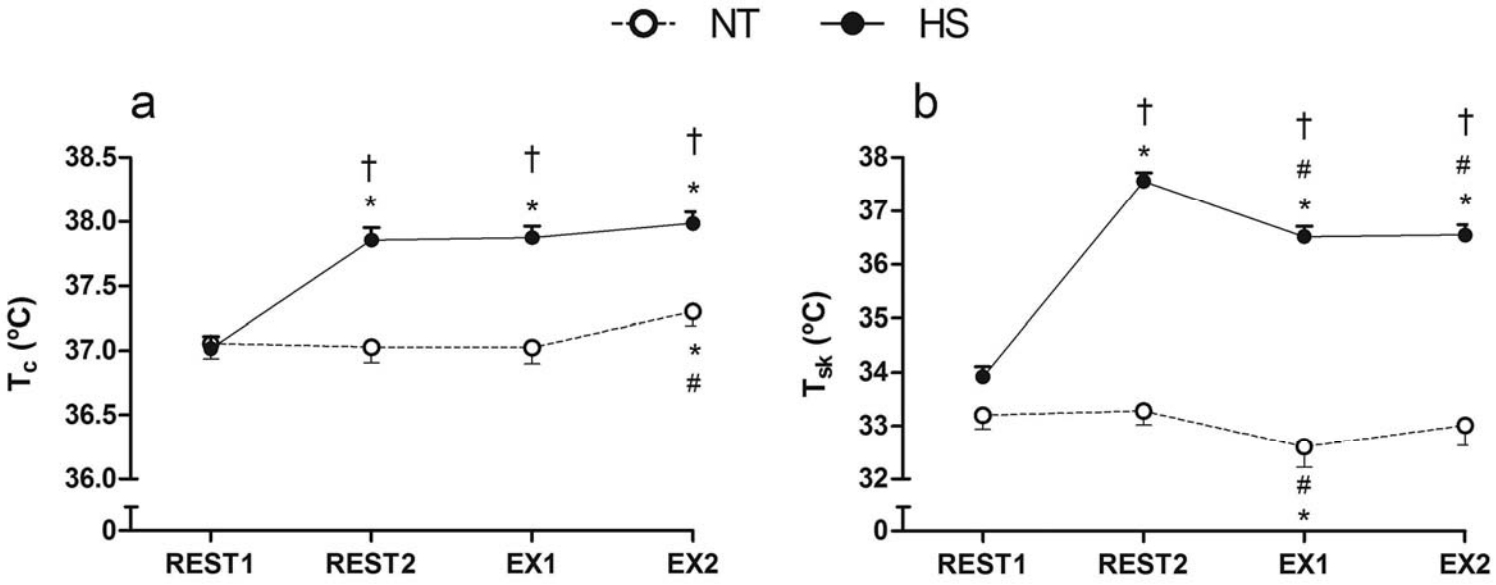
653 Table 1 – Subject's hydration status and exercise parameters (mean ± SE) during the
654 heat stress (HS) and normothermia (NT) sessions. P values are for HS vs. NT.

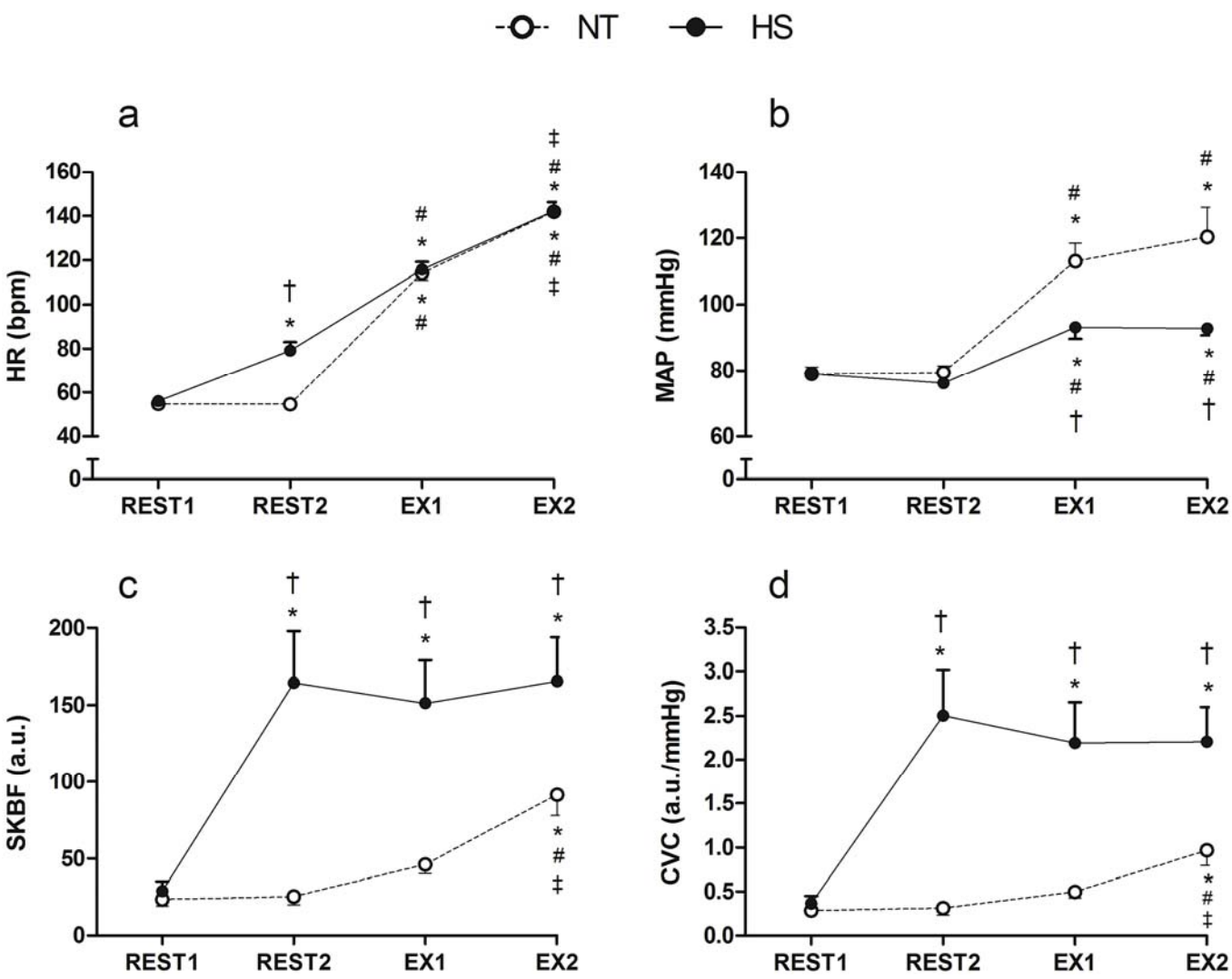
	HS	**NT**	***P***
Hydration Status			
U_{osm} (mOsmol/kgH_2O)	449 ± 60	470 ± 58	0.72
Initial body mass (kg)	77.0 ± 2.4	77.0 ± 2.5	0.87
Final body mass (kg)	76.1 ± 2.4 †	76.7 ± 2.5	< 0.01
Body mass loss (kg)	0.9 ± 0.1 †	0.3 ± 0.1	< 0.01
Exercise Parameters			
EX1			
HR (bpm)	116 ± 3	114 ± 3	0.73
HR (%$HR_{reserve}$)	40 ± 1	40 ± 1	0.49
Workload (Watts)	50 ± 9 †	114 ± 8	< 0.01
RPE (6-20)	11 ± 1	11 ± 1	0.63
EX2			
HR (bpm)	143 ± 4	142 ± 3	0.61
HR (%$HR_{reserve}$)	62 ± 1	62 ± 1	0.99
Workload (Watts)	106 ± 10 †	165 ± 8	< 0.01
RPE (6-20)	13 ± 1	13 ± 1	0.16

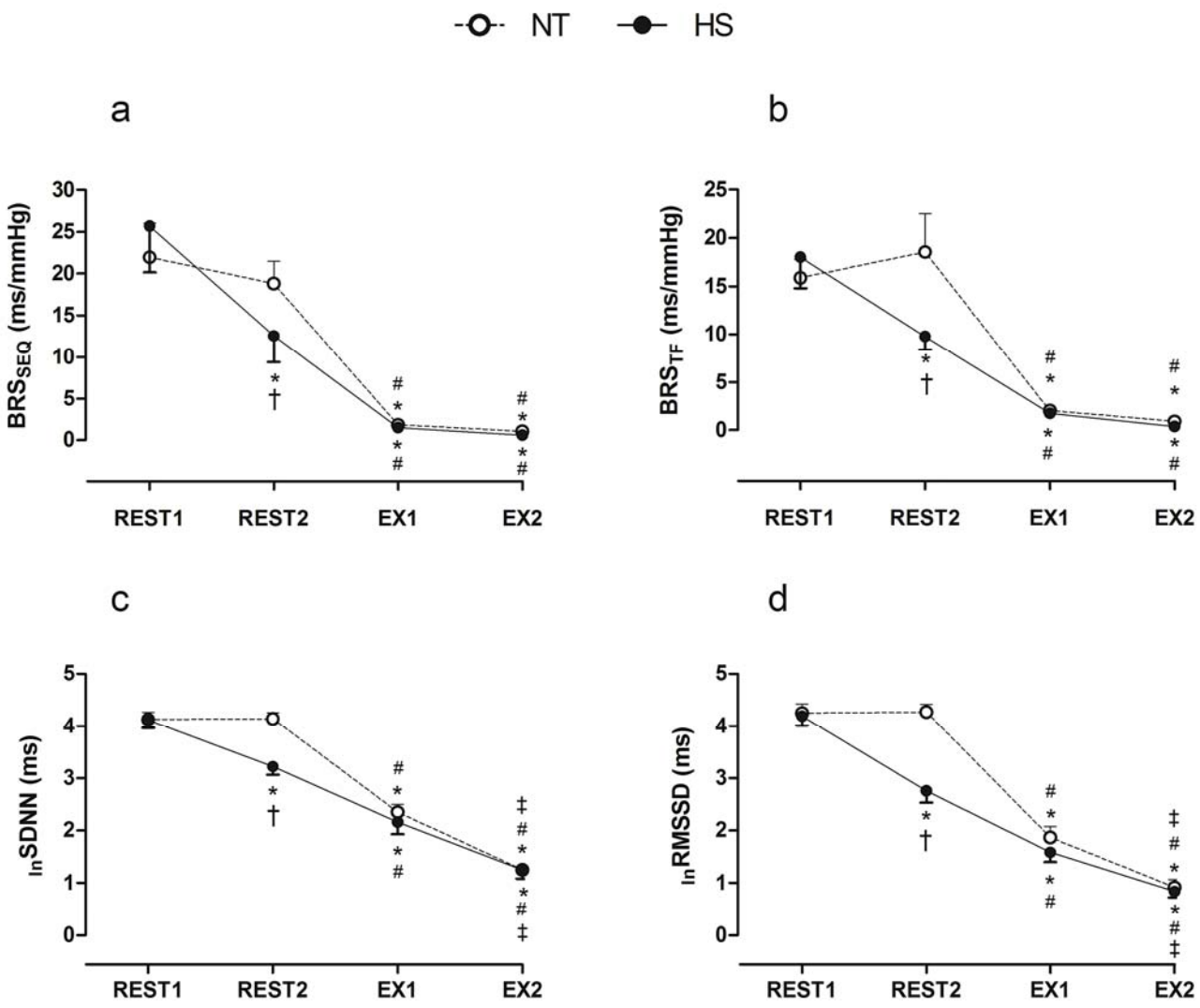
655 U_{osm}, urine osmolality. EX1, first 7 min of the exercise performed at 40% of the subject's heart
656 rate reserve. EX2, final 7 min of the exercise performed at 60% of the subject's heart rate
657 reserve. HR, heart rate. RPE, rating of perceived exertion. † $p \leq 0.05$ vs. NT session.

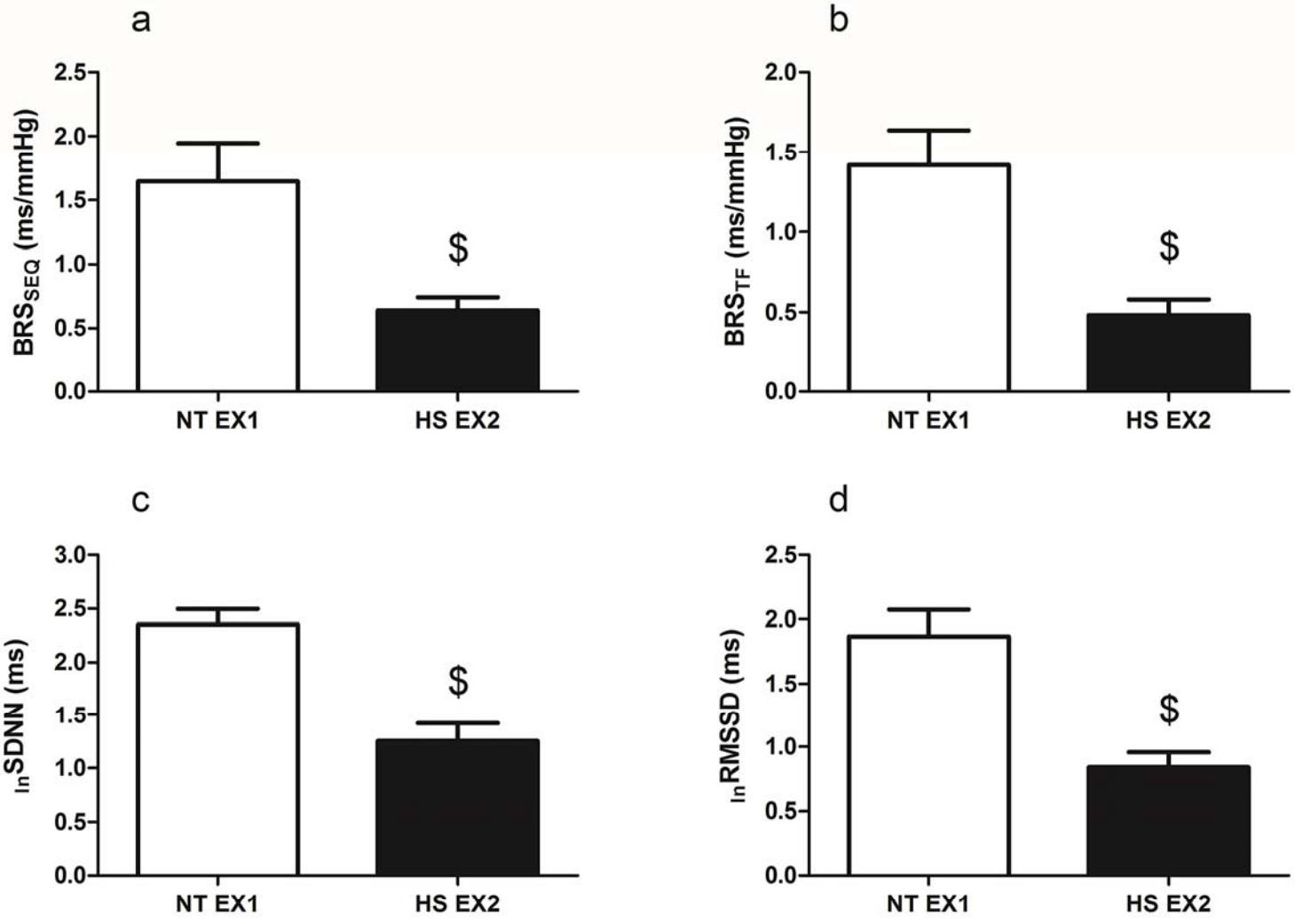
658


Heat stress (HS)
REST1
HS
REST2
40% HRRes
60% HRRes
EX1
EX2
Exercise
Normothermia (NT)
REST1
NT
REST2
40% HRRes
60% HRRes
EX1
EX2
Exercise
Randomized
10 min
60 min
10 min
14 min


NT
HS
a
b
T_c (ºC)
T_{sk} (ºC)
38.5
38.0
37.5
37.0
36.5
36.0
38
37
36
35
34
33
32
0
REST1
REST2
EX1
EX2
†
*
#


NT
HS
a
HR (bpm)
160
140
120
100
80
60
40
0
REST1
REST2
EX1
EX2
b
MAP (mmHg)
140
120
100
80
60
0
REST1
REST2
EX1
EX2
c
SKBF (a.u.)
200
150
100
50
0
REST1
REST2
EX1
EX2
d
CVC (a.u./mmHg)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
REST1
REST2
EX1
EX2


NT
HS
a
b
c
d
BRS$_{SEQ}$ (ms/mmHg)
BRS$_{TF}$ (ms/mmHg)
$_{ln}$SDNN (ms)
$_{ln}$RMSSD (ms)
REST1
REST2
EX1
EX2


a
BRS$_{SEQ}$ (ms/mmHg)
2.5
2.0
1.5
1.0
0.5
0.0
$
NT EX1
HS EX2
b
BRS$_{TF}$ (ms/mmHg)
2.0
1.5
1.0
0.5
0.0
$
NT EX1
HS EX2
c
$_{ln}$SDNN (ms)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$
NT EX1
HS EX2
d
$_{ln}$RMSSD (ms)
2.5
2.0
1.5
1.0
0.5
0.0
$
NT EX1
HS EX2