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ORIGINAL ARTICLE

Changes in heart rate variability during the induction and decay of heat acclimation

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Abstract
Purpose We evaluated the changes in core temperature, heart rate, and heart rate variability (HRV) during the induction and decay of heat acclimation.
Methods Ten males (23 ± 3 years; 79.5 ± 3.5 kg; 15.2 ± 4.5 percent body fat; 51.13 ± 4.61 $mLO^2 \bullet kg^{-1} \bullet min^{-1}$ peak oxygen uptake) underwent a 14-day heat acclimation protocol comprising of 90-min cycling at ~50 % peak oxygen uptake at 40 °C and ~20 % relative humidity. Core temperature, heart rate, and 102 HRV measures were recorded during a heat tolerance test conducted at baseline (day 0) and at the end of the induction (day 14) and decay (day 28) phases.
Results Heat acclimation resulted in significantly reduced core temperature [rectal ($\chi^2 = 1298.14$, $p < 0.001$); esophageal ($\chi^2 = 1069.88$, $p < 0.001$)] and heart rate ($\chi^2 = 1230.17$, $p < 0.001$). Following the decay phase, 26, 40, and 60 % of the heat acclimation-induced reductions in rectal temperature, esophageal temperature, and heart rate, respectively, were lost. Heat acclimation was accompanied by profound and broad changes in HRV: at the end of the induction phase, 75 of the 102 variability measures computed were significantly different ($p < 0.001$), compared to only 47 of the 102 at the end of the decay phase.
Conclusions Heat acclimation is accompanied by reduced core temperature, significant bradycardia, and marked alterations in HRV, which we interpret as being related to vagal dominance. The observed changes in core temperature persist for at least 2 weeks of non-exposure to heat, while the changes in heart rate and HRV decay faster and are only partly evident after 2 weeks of non-exposure to heat.

Keywords Heart rate variability · HRV · Hot exposure · Acclimatization · Core temperature · Exercise

Abbreviations

ANS	Autonomic nervous system
HRV	Heart rate variability
NN	Normal-to-normal R–R intervals
R–R interval	Time between two consecutive R waves in the electrocardiogram
T_{es}	Esophageal temperature
T_{re}	Rectal temperature
$\dot{V}O_{2peak}$	Peak oxygen uptake

Introduction

Adaptation to higher ambient temperatures is achieved mainly through the process of repeated heat exposure (Pandolf 1998; Horowitz 2007; Armstrong and Maresh

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1991) which generates changes at all organismal levels to improve heat tolerance, including integrative physiological alterations, gene expression reprogramming and post-transcriptional mechanisms (Horowitz 2007). From an integrative physiology perspective, heat acclimation is characterized by increased sweating as well as attenuated heart rate, core temperature, skin temperature, and perceived exertion (Horowitz 2007; Armstrong and Maresh 1991; Flouris 2011).

The faster activation of heat loss mechanisms (i.e., vasodilation and sweating) and the delayed onset for the development of thermal injury in heat-acclimated individuals have been attributed—at least in part—to adaptations in autonomic nervous system (ANS) function (Horowitz 2007; Carrillo et al. 2013). Indeed, heat acclimation has been shown to attenuate heart rate during heat exposure in both humans (Eichna et al. 1950; Shvartz et al. 1975; Taylor et al. 1943; Yamazaki and Hamasaki 2003) and animals (Horowitz and Meiri 1993). This heat acclimation-induced bradycardia has been attributed to increased parasympathetic activity, as recorded through heart rate variability (HRV) measurements (Horowitz and Meiri 1993; Epstein et al. 2010). Nevertheless, the evidence on the effects of heat acclimation on ANS function appears to be inconclusive as some authors have reported an increase in sympathetic tone following heat acclimation (Frank et al. 2001), or alterations between sympathetic and parasympathetic dominance at different phases of heat acclimation (Horowitz and Meiri 1993).

While published data are not always consistent, it is generally agreed that a 10-day protocol consisting of 100-min exercise bouts in either hot-dry or hot-humid conditions results in near-complete phenotypic heat acclimation (Pandolf 1998; Lind and Bass 1963; Garrett et al. 2011)—yet improvements in sudomotor function may require up to 14 days of acclimation (Wendt et al. 2007). Ambient temperatures exceeding 35 °C are commonly used to provide a significant external heat load that raises skin temperature enough for complete heat acclimation to be attained (Regan et al. 1996; Shvartz et al. 1973). Moreover, it is proposed that continuous exercise be performed at an intensity equal to or exceeding 50 % of peak oxygen uptake ($\dot{V}O_{2peak}$) to attain complete acclimation (Wendt et al. 2007). Nevertheless, heat acclimation remains a transient state and its effects gradually disappear (Pandolf 1998; Garrett et al. 2011). This is particularly important for the autonomic regulation of heart rate because the heat acclimation-induced bradycardia is lost faster compared to other thermoregulatory adaptations when heat exposures cease (Pandolf 1998). Indeed, it has been reported that up to 85 % of the adaptation in heart rate is lost within 2 weeks of non-exposure to heat, in comparison to only 35 % of the adaptation in rectal temperature (Pandolf 1998). Nevertheless, the small number of studies investigating the rate of decay of heat acclimation is severely outdated and are limited by small sample sizes [i.e., 1–4 participants (Robinson et al. 1973; Bean and Eichna 1943; Eichna et al. 1945; Stein et al. 1949; Epstein et al. 2010)], incomplete heat acclimation [i.e., 2 days of heat acclimation or groups of unequal acclimation (Henschel et al. 1943; Lind and Bass 1963)], and use of measurements with low reliability [i.e., oral temperature (Wyndham and Jacobs 1957)].

We recently used direct calorimetry to quantify whole-body heat loss enhancement during heat acclimation, as well as the decay in these improvements after the end of heat acclimation [Poirier et al. (2014)]. In light of the above unresolved questions, our objective in this paper was to evaluate the changes in HRV observed during the induction (14 days) and decay (14 days) of heat acclimation. Based on previous data demonstrating an acclimation-induced bradycardia (Horowitz and Meiri 1993; Epstein et al. 2010), we hypothesized that the induction of heat acclimation would be accompanied by increased parasympathetic activity, as recorded through HRV measurements. Moreover, as 85 % of the adaptation in heart rate is lost within 2 weeks of non-exposure (Pandolf 1998), we hypothesized that the ANS modulation would return to baseline by the end of the decay phase.

Materials and methods

Participants

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants who volunteered to take part in this study. Ten male participants were recruited within the University of Ottawa community and volunteered to take part in the study. All participants were assumed to be non-heat acclimatized as the testing took place from late September to mid-April. Furthermore, all participants were not endurance trained to avoid the potential for partial acclimation from endurance training (Gisolfi 1973). Mean ± standard deviation characteristics of the participants were as follows: age, 23 ± 3 years; height, 180 ± 5 cm; body mass, 79.52 ± 3.5 kg; Siri body fat, 15.18 ± 4.5 %; body surface area, 1.99 ± 0.1 m^2; and $\dot{V}O_{2peak}$, 51.13 ± 4.6 mLO^2 kg^{-1} min^{-1}. All participants were healthy, non-smoking, and did not have any cardiovascular, metabolic and respiratory diseases. The current data were collected as part of a larger study, the main paper of which is published elsewhere [Poirier et al. (in press)].

Experimental design

Each participant completed one preliminary session and 14 heat acclimation sessions that included three heat tolerance tests. During the preliminary session, participants received an orientation to the instrumentation and experimental protocols, completed the American Heart Association/American College of Sports Medicine Pre-Participation Screening (Balady et al. 1998) and the Canadian Society for Exercise Physiology Physical Activity Readiness (CSEP 2002) questionnaires to ensure their safety for participation, and performed body composition and fitness assessments. Body height was measured using a stadiometer (Detecto, model 2391, Webb City, MO, USA), while body mass was determined using a digital high-performance weighing platform (model CBU150X, Mettler Toledo Inc., Missisauga, ON, CAN). Subsequently, body surface area was calculated using these measurements (DuBois and DuBois 1916). Body density was measured using hydrostatic weighing and percentage of body fat was estimated via the Siri equation (Siri 1956). The $\dot{V}O_{2peak}$ was measured using indirect calorimetry (MOXUS system, Applied Electrochemistry, Pittsburgh, PA, USA) during a progressive incremental exercise protocol performed on an upright-seated constant-load cycle ergometer (Corival, Love B.V., Gronignen, Netherlands). The starting external workload was 80 W and participants were asked to maintain a cadence of 80 rpm. Thereafter, the workload was increased by 20 W every minute until the participant could no longer maintain a minimum cadence of 60 rpm (Canadian Society for Exercise Physiology 1986). The $\dot{V}O_{2peak}$ was defined as the highest oxygen uptake during the test, taken as the mean of the two highest consecutive 15-sec recordings. A second $\dot{V}O_{2peak}$ was performed after the induction phase to assess improvements in cardiorespiratory fitness following the 2 weeks of heat acclimation.

Following the preliminary session, all participants underwent 14 sessions of heat acclimation. Specifically, on days 1–13 inclusively, each participant performed 90 min of exercise at ~50% $\dot{V}O_{2peak}$ on an upright cycle ergometer located inside an environmental chamber set at an ambient temperature of 40 °C and ~20 % relative humidity. Thereafter, participants were asked to continue their normal daily life for 14 days while avoiding vigorous exercise and exposures to high ambient temperatures (i.e., >25 °C).

In addition to the above sessions, participants underwent three heat tolerance tests in an environmental chamber during the induction (Day 0 and 14) and decay (Day 28) of heat acclimation. Each participant performed all of the calorimeter trials at the same time of day. On experimental testing days, participants were asked to arrive to the laboratory well hydrated and having avoided alcohol and caffeine for 12 h. Furthermore, they were asked to avoid any major thermal stimuli on their way to the laboratory. Clothing was standardized to running shorts and sandals for all experimental trials. While the nature of the experimental protocol made it difficult to maintain an elevated level of hydration, participants were asked to remain as hydrated as possible throughout the study (500 mL of water prior to bed and 500 mL upon waking up in the morning were the general guidelines to follow throughout the study).

The heat tolerance tests began with an instrumentation period at an ambient room temperature of ~24 °C. Once all the equipment and probes were in place and functioning, the participant entered the calorimeter which was regulated at an ambient temperature of 35.2 ± 0.1 °C and absolute humidity of 5.56 ± 2.39 g•kg^{-1} (~16 % relative humidity). The heat tolerance test protocol is illustrated in Fig. 1:A.

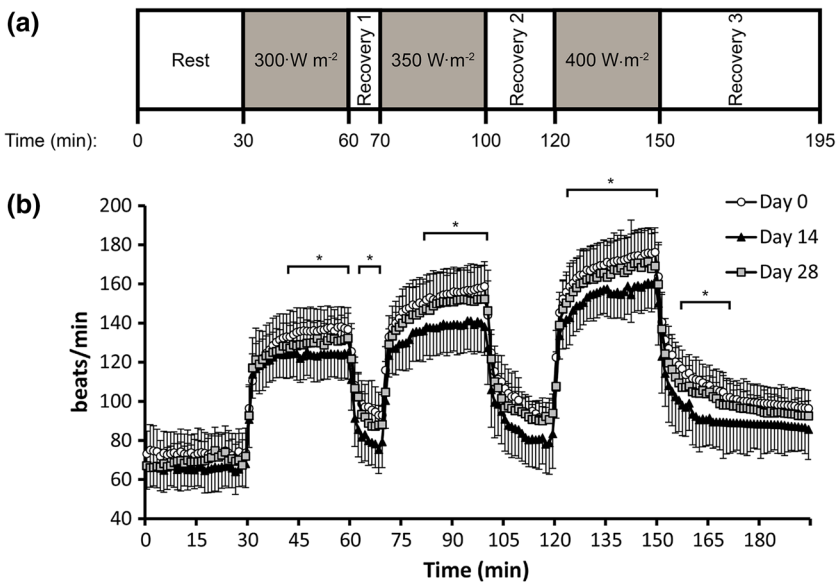

Fig. 1 The heat tolerance test protocol (**a**) and the heart rate (mean ± SD) of the participants during days 0, 14 and 28 (**b**). The *asterisk* indicates statistically significant difference between day 0 and day 14 ($p < 0.01$)

In brief, after a 30-min rest period in an upright-seated position, intermittent exercise was performed at fixed rates of metabolic rate heat production equal to 300, 350 and 400 W•m^{-2}, each level being 30 min in duration. The 1st and 2nd bout of exercise were separated by a 10-min recovery, while a 20-min recovery period was allocated between the second and third bouts. Following the last exercise bout, a 45-min resting period was completed to finish the trial (Fig. 1a). The rates of heat production in the heat tolerance tests were chosen to ensure that an uncompensable or near uncompensable heat stress condition was achieved in the first exercise bout, progressing to a fully uncompensable condition in the final exercise bout.

Physiological measurements

Core temperature

Esophageal (T_{es}) and rectal (T_{re}) temperatures were measured using pediatric thermocouple probes (Mon-a-therm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St. Louis, USA). The rectal probe was inserted ~15 cm beyond the anal sphincter and the esophageal probe was inserted through the nostril, during which time the participant was asked to swallow sips of water. Once fully inserted, the tip of the probe rested 40 cm past the entrance of the nostril, an estimation of heart height as position, neck length and head depth can vary between people. Both T_{es} and T_{re} were measured continuously during the heat tolerance tests.

Heart rate variability

R–R interval data were extracted from 175-Hz electrocardiogram waveforms throughout all heat tolerance tests using a 5-lead Holter DigiTrak XT (Philips, Andover, USA) and those deemed to be normal-to-normal (NN) were retained for further analysis. Using the NN interval time series of each subject (total of three NN interval series), HRV analyses were performed employing the Continuous Individualized Variability Analysis—CIMVA^{TM} software (http://ohridal.org/cimva/CIMVA-Core-Description.pdf) to extract a total of 102 measures of variability computed from the statistical, geometric, informational, energetic, and invariant domains. The list of all 102 variability measures computed is provided in an Online Data Supplement. We used a 5-min window analysis with 30-s time step to effectively assess the transients between the different phases of the protocol.

Statistical analysis

Initial testing using the Friedman test was performed to compare days 0, 14 and 28 using T_{re}, T_{es}, heart rate, as well as the entire distribution computed for each of the 102 variability time series. The robust false discovery rate method (Pounds and Cheng 2006) was used to adjust for multiple comparisons, suggesting that the appropriate threshold to reject the null hypothesis of equal medians was $p < 0.01$. Two-tailed Wilcoxon signed-rank tests were used for post hoc analysis between days (i.e., day 0 vs. day 14, day 0 vs. day 28, and day 14 vs. day 28). Thereafter, Friedman tests were used to compare T_{re}, T_{es}, heart rate, as well as of the 102 variability time series between days 0, 14 and 28 during specific periods of the heat tolerance tests; that is, rest, exercise at 300 W•m^{-2}, recovery 1, exercise at 350 W•m^{-2}, recovery 2, exercise at 400 W•m^{-2}, and recovery 3 (e.g., comparison of T_{re} during the rest period between days 0, 14 and 28). Two-tailed Wilcoxon signed-rank tests were used for post hoc comparisons between days within a specific period. The aforementioned robust false discovery rate method was used to adjust for multiple comparisons and a rate of 0.001 false positives was applied.

Results

The minute-by-minute heart rate of the participants during days 0, 14 and 28 is illustrated in Fig. 1b, while the mean T_{re}, T_{es}, and heart rate during days 0, 14 and 28 is illustrated in Fig. 2. Friedman tests demonstrated a statistically significant effect of "day" on T_{re} ($\chi^2 = 1298.14$, $p < 0.001$), T_{es} ($\chi^2 = 1069.88$, $p < 0.001$), and heart rate ($\chi^2 = 1230.17$, $p < 0.001$). Post hoc Wilcoxon signed-rank tests demonstrated significant differences between days 0 and 14 in T_{re}, T_{es}, and heart rate, as well as significant differences between days 0 and 28 in T_{re} and T_{es} ($p < 0.001$; Fig. 2). Period-specific Friedman tests used to compare T_{re}, T_{es}, and heart rate between days 0, 14 and 28 demonstrated statistically significant differences in all variables at all periods of the heat tolerance test ($p < 0.001$; Fig. 2). Two-tailed Wilcoxon signed-rank tests used for post hoc comparisons between days within a specific period revealed significant differences between days 0 and 14 in T_{re}, T_{es}, and heart rate, as well as significant differences between days 0 and 28 in T_{re} and T_{es} across most periods of the thermal tolerance test ($p < 0.001$; Fig. 2).

The data analysis included all 102 indices of HRV extracted (a list is provided in an Online Data Supplement). Although analyses were conducted on all HRV variables, given the inability to graphically illustrate the results for all the indices extracted, selected indices covering most HRV domains are presented in Fig. 3 (mean NN: statistical domain; standard deviation of NN: statistical domain; root mean square of successive differences: statistical domain; coefficient of variation: statistical domain; detrended fluctuation analysis α1 and α2: invariant domain) and Fig. 4

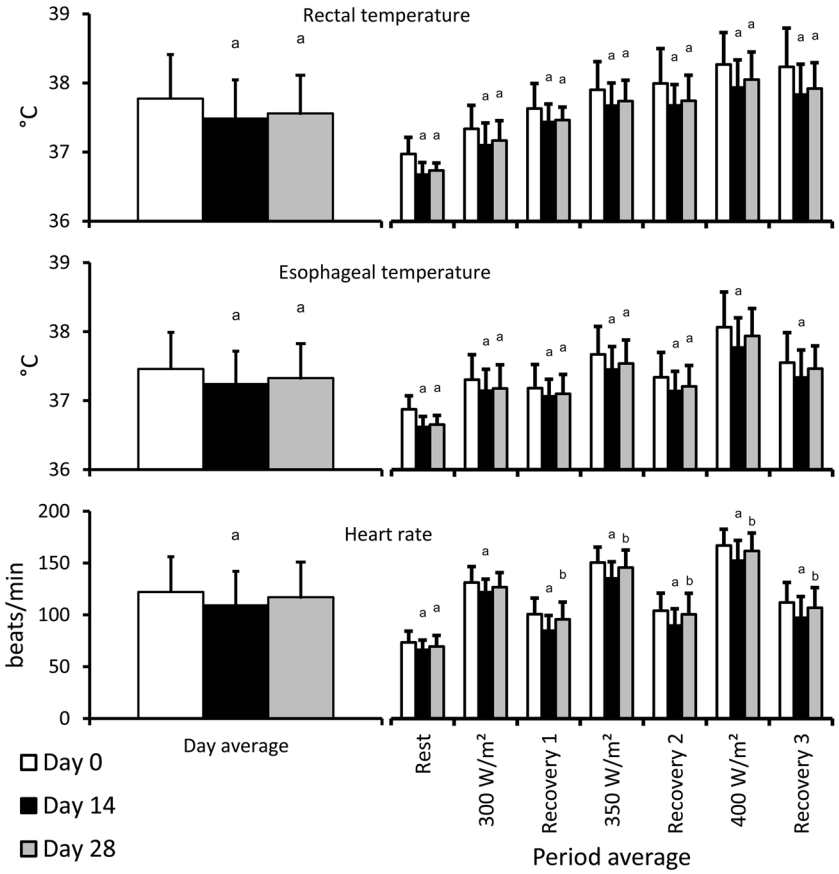

Fig. 2 Rectal temperature, esophageal temperature, and heart rate during days 0, 14 and 28 illustrated as whole-day (*left* side graphs) and period-specific (*right* side graphs) values (mean ± SD). *a* Statistically significant difference from day 0 ($p < 0.001$). *b* Statistically significant difference between days 14 and 28 ($p < 0.001$)

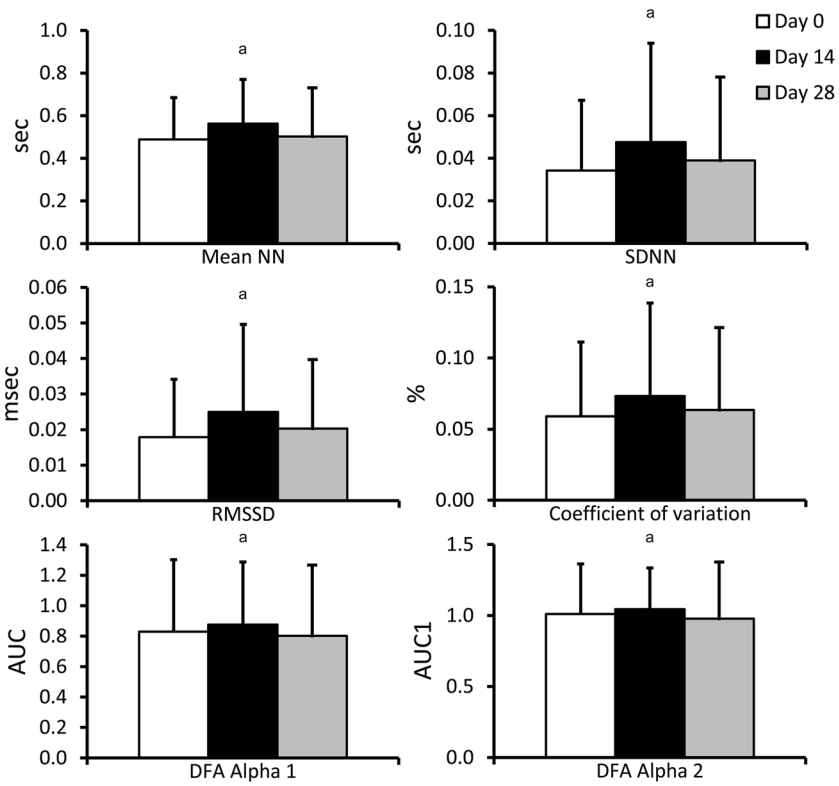

Fig. 3 Mean normal-to-normal intervals (NN), standard deviation of NN (SDNN), root mean square of successive differences (RMSSD), coefficient of variation, as well as detrended fluctuation analysis α1 (DFA Alpha 1) and α2 (DFA Alpha 2) during days 0, 14 and 28 (mean ± SD). *a* Statistically significant difference compared to day 0 ($p < 0.001$). *b* Statistically significant difference between days 14 and 28 ($p < 0.001$)

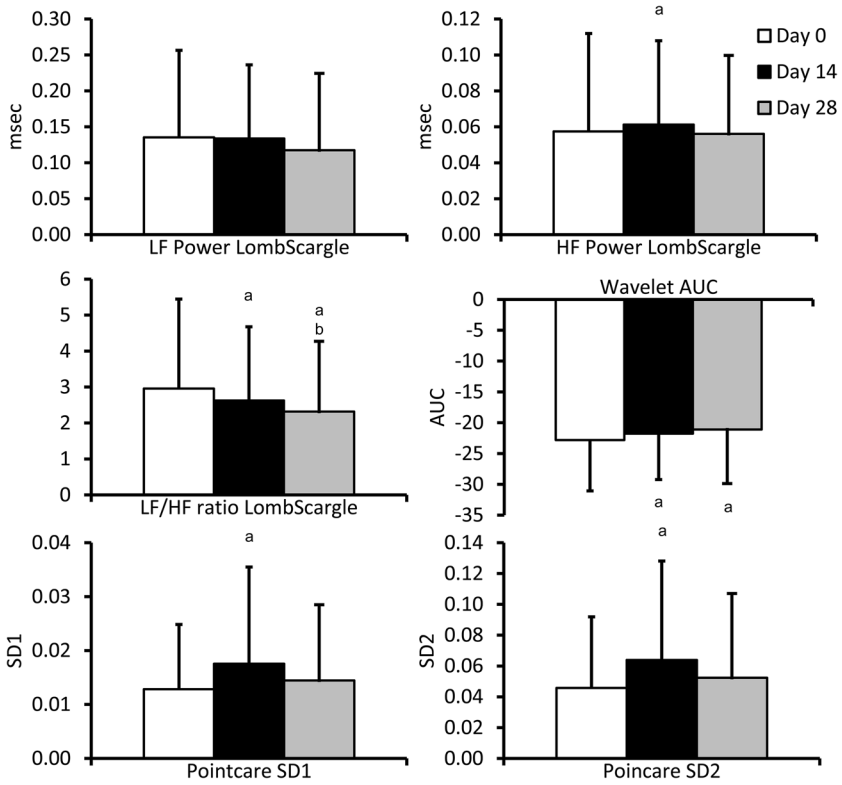

Fig. 4 Low (LF Power LombScargle), high (HF Power LombScargle), and low/high (LF/HF Power LombScargle) frequency ratio derived from the Lomb-Scargle periodogram, wavelet area under the curve (Wavelet AUC), as well as Poincaré plot SD1 and SD2 during days 0, 14 and 28 (mean ± SD). *a* Statistically significant difference compared to day 0 ($p < 0.001$). *b* Statistically significant difference between days 14 and 28 ($p < 0.001$)

(low, high, and low/high frequency ratio derived from the Lomb-Scargle periodogram: energetic domain; wavelet area under the curve: energetic domain; Poincaré plot SD1 and SD2: geometric domain). Friedman tests demonstrated a statistically significant effect of "day" on 90 out of the 102 variability measures computed ($p < 0.01$), including all the measures illustrated in Figs. 3 and 4 with the exceptions of low frequency and detrended fluctuation analysis α2 ($p > 0.01$). Post hoc Wilcoxon signed-rank tests demonstrated significant differences between all days in 50 out of the 102 variability measures computed ($p < 0.001$). Significant differences between days 0 and 14 were detected in 75 out of the 102 variability measures computed ($p < 0.001$). Significant differences between days 0 and 28 were detected in 47 out of the 102 variability measures computed ($p < 0.001$). Significant differences between days 14 and 28 were detected in 68 out of the 102 variability measures computed ($p < 0.001$).

The analysis of HRV variables was extended via period-specific comparisons. Given the inability to graphically illustrate the period-specific results for all the indices extracted, selected indices covering different HRV domains are presented in Fig. 5 (mean NN: statistical domain; root mean square of successive differences: statistical domain; Poincaré plot SD1: geometric domain). Period-specific Friedman tests used to compare HRV at rest between days 0, 14 and 28 demonstrated a significant effect of "day" on 69 out of the 102 variability measures computed ($p < 0.001$). During exercise at 300 W•m^{-2} and recovery 1, there was a significant effect of "day" on 76/102 and 55/102 variability measures, respectively ($p < 0.001$). During exercise at 350 W•m^{-2}, recovery 2, exercise at 400 W•m^{-2}, and recovery 3, there was a significant effect of "day" on 72/102, 68/102, 70/102, and 71/102, respectively ($p < 0.001$). It should be noted that the variables illustrated in Fig. 5 demonstrated significant effects of "day" on all periods ($p < 0.001$). In 74 out of the 102 variability indices extracted (including those illustrated in Fig. 5), post hoc Wilcoxon signed-rank tests demonstrated significant differences between days 0 and 14 for most periods ($p < 0.001$). Similar comparisons between days 0 and 28 demonstrated significant differences in 45 out of the 102 variability indices computed ($p < 0.001$).

Discussion

The main finding of the present study is that heat acclimation generates thermoregulatory alterations that are accompanied by profound adaptations in HRV, considered to be indicative of altered ANS function. Specifically, we observed a significant reduction in T_{re} and T_{es} at the end of the induction phase, which outlasted the decay phase. In addition, heat acclimation was accompanied by bradycardia—attributed to altered ANS, specifically vagal dominance—which was present at the end of the decay

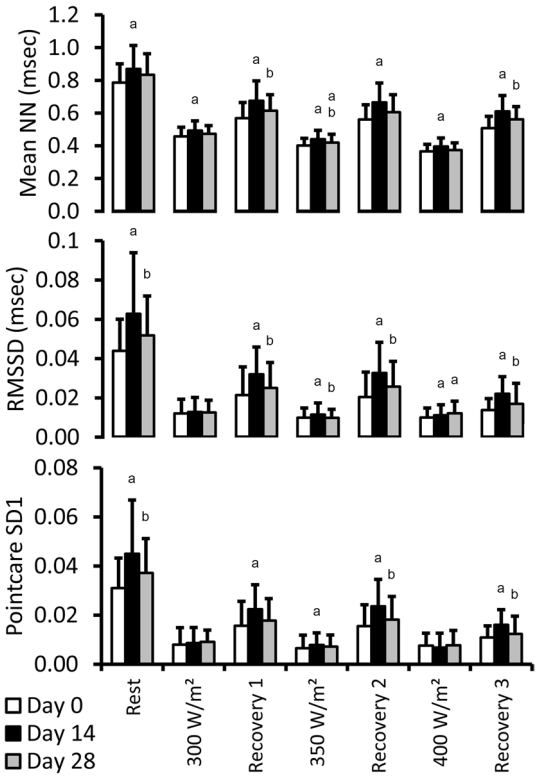

Fig. 5 Period-specific mean normal-to-normal intervals (NN), root mean square of successive differences (RMSSD), and Poincaré plot SD1 during days 0, 14 and 28 (mean ± SD). *a* Statistically significant difference compared to day 0 ($p < 0.001$). *b* Statistically significant difference between days 14 and 28 ($p < 0.001$)

phase, yet to a smaller degree. It is important to note that the present HRV analysis went beyond the typical time and frequency domain measures (Dinas et al. 2011), assessing for the first time in the heat acclimation literature a comprehensive panel of 102 variability markers. This is crucial because the sensitivity to detect heat acclimation-induced ANS adaptations may vary across the extracted HRV indices and should be addressed by analyzing both well-established as well as recently developed markers (Bravi et al. 2011).

Our HRV analysis demonstrated that the heat acclimation-induced augmented thermal tolerance was accompanied by bradycardia and parasympathetic nervous system dominance. The attenuated heart rate following heat acclimation has been a common finding observed in both humans (Eichna et al. 1950; Shvartz et al. 1975; Taylor et al. 1943; Yamazaki and Hamasaki 2003) and animals (Horowitz and Meiri 1993). Previous research suggested that up to 85 % of the adaptation in heart rate is lost within 2 weeks of non-exposure to heat (Pandolf 1998). Our results partly support this notion demonstrating that 60 % of the heat acclimation-induced bradycardia was lost within 2 weeks. Nevertheless, while some authors proposed that this heat acclimation-induced heart rate reduction is caused by increased parasympathetic activity (Horowitz and Meiri 1993; Epstein et al. 2010), others reported an increase in sympathetic tone following heat acclimation (Frank et al. 2001), or alterations between sympathetic and parasympathetic dominance at different phases of heat acclimation (Horowitz and Meiri 1993). Our study clearly demonstrated that heat acclimation was accompanied by vagal dominance, which was observed as early as the rest period of the heat tolerance test conducted during day 14 and remained apparent until the end of the test for the majority of the HRV indices extracted. Indeed, after extracting a large number of variability indices covering all known HRV domains, we found an increased HRV, standard deviation of HRV, low/high frequency ratio, Poincaré plot SD1 and SD2, sample entropy, and root mean square of differences of successive NN intervals indicating a marked ANS adaptation following heat acclimation characterized by parasympathetic dominance. At the end of the decay phase, this vagal dominance was evident at a much smaller degree, since only 45/102 variability indices showed a significant difference from day 0 (compared to 74/102 at the end of the induction phase).

The current design cannot provide conclusive evidence regarding the mechanisms involved in the observed heat acclimation-induced bradycardia. Recent data suggested that the most important adaptations induced by heat acclimation are local, specifically in the sweat gland apparatus (increased cholinergic sensitivity of the eccrine sweat gland or increased glandular hypertrophy) and the skin microcirculation (augmented skin blood flow response to acetylcholine caused by up-regulation of the cyclooxygenase pathway) (Lorenzo and Minson 2010). Nevertheless, these local adaptations can have profound central effects. Indeed, the direct calorimetry data from the current trials [presented in (Poirier et al. 2013)] demonstrated an 11 % increase in the body's evaporative capacity following heat acclimation, leading to a 26 % reduction in the cumulative change in body heat content across the three exercise periods. This is in line with the present reduction in T_{re} and T_{es}, demonstrating that heat acclimation resulted in a significant reduction in thermal strain. It is possible, therefore, that the attenuated thermal strain following heat acclimation led to an adrenergic withdrawal due to a reduced competition between the skin microcirculation and the exercising muscles for the available cardiac output. In turn, this adrenergic withdrawal translated into the bradycardia and vagal dominance recorded by our HRV measurements which was reflected by the increased HRV, standard deviation of HRV, low/high frequency ratio, Poincaré plot SD1 and SD2, sample entropy, and root mean square of differences of successive NN intervals. This is clearly evident also in the period-specific data illustrated in Fig. 5 where indices

from the statistical (mean NN and root mean square of successive differences) and the geometric (Poincaré plot SD1) domain are attenuated during exercise, yet following heat acclimation—and, sometimes, after the decay phase—there are significantly higher values indicating vagal dominance. Collectively, our calorimetry (Poirier et al. 2013; Poirier et al. (in press)), thermometry, and HRV data suggest that the observed bradycardia and altered ANS function occurred as a functional response to enhanced evaporative heat loss and not as a physiological adaptation to heat acclimation per se.

This is the first study in the heat acclimation literature to use an incremental heat tolerance test with fixed rates of metabolic rate heat production to assess improvements, if any, in the body's physiological capacity to dissipate heat. This is noteworthy, as the existing controversy regarding the effects of heat acclimation on ANS function may stem—at least in part—from the exercise protocols adopted in previous studies. To date, the effects of heat acclimation on ANS function have been investigated through absolute exercise intensity protocols that are based on a fixed rate of external work (Pandolf 1998; Epstein et al. 2010). However, inter-individual variation in mechanical efficiency, body composition, aerobic fitness, sex, age, chronic disease, hydration, and cardiovascular function can significantly alter the remaining constituents of the heat balance equation (i.e., the rate of metabolic energy expenditure as well as the rates of radiant, convective, conductive and evaporative heat exchange) (Kenny and Jay 2013). In turn, this results in significantly different levels of thermal strain that can severely affect the obtained results since it is difficult to determine if any observed effects on ANS function are truly associated with heat acclimation, or if they simply result from a greater thermal strain in certain subjects (Kenny and Jay 2013). In the current study, we used a heat tolerance test which included 30-min exercise bouts at fixed rates of metabolic rate heat production interspersed by recovery periods that provides a fixed thermal stimulus thereby allowing us to assess the 'true' physiological changes in the body's physiological capacity to improve its capacity to dissipate heat (Kenny and Jay 2013). This approach is more appropriate (from a heat balance perspective) and realistic (individuals working in the heat frequently use resting periods).

At the end of the heat acclimation induction and decay phases, both T_{re} and T_{es} were significantly reduced compared to day 0. Interestingly, this thermal adaptation was evident as early as the rest period of the heat tolerance test conducted on day 14 and remained apparent until the end of the intermittent exercise test. It is confirmed, therefore, that our heat acclimation protocol—comprising of 90 min of cycling at ~50 % $\dot{V}O_{2peak}$ at an ambient temperature of 40 °C and ~20 % relative humidity for 14 consecutive days—resulted in improved heat tolerance during rest, exercise, and recovery. The environmental parameters of the heat acclimation protocol were selected to create a strong driving force for evaporative heat loss and ensure an extensive thermal "training". Acclimation in a hot/humid condition does indeed stimulate improvements in sweating; however, the ambient relative humidity will limit the amount of sweat that can be evaporated for a given sweat production. In fact, there is evidence to suggest that non-evaporated sweat that sits on the skin surface can cause a suppression of sweat production (Candas et al. 1980), which could limit the development of the sweat glands in hot/humid conditions. On the other hand, previous research suggested that 35 % of the heat acclimation-induced adaptation in T_{re} is lost within 2 weeks of non-exposure to heat (Pandolf 1998). Our results partly support this notion demonstrating that 26 and 40 % (calculated as percent change from baseline), of the heat acclimation-induced reductions in T_{re} and T_{es}, respectively, were lost within 2 weeks.

The current 14-day heat acclimation protocol resulted in a 5 % increase in $\dot{V}O_{2peak}$ (Poirier et al. 2013). While the current design cannot independently assess the effects of heat acclimation and repeated physical training, it is well established that these two factors independently enhance whole-body heat loss capacity through distinct mechanisms. The classic work by Nadel and colleagues (Nadel et al. 1974) showed that physical exercise can augment sweating activity via increased sensitivity of the sweating response per unit change of central sweating. On the other hand, heat acclimation increases evaporated loss by lowering the core temperature at which onset for sweating occurs (Nadel et al. 1974). These distinct adaptations were confirmed in a recent study reporting that control subjects exercising at the same intensity in a cool environment show no significant thermoregulatory adaptations compared to individuals exercising in a hot-dry (40 °C, 30 % relative humidity) environment (Lorenzo and Minson 2010). Moreover, we recently showed that the evaporative requirement for heat balance alone describes ~90 % of all variability in whole-body sweat rate during steady-state exercise, whereas <2 % of variation is independently described by cardiorespiratory fitness (Gagnon et al. 2013). Therefore, we feel that the small increase in $\dot{V}O_{2peak}$ in this study as a result of physical training did not contribute significantly to the observed adaptations in HRV.

The practical application of the current results relates to the increasing popularity of heat acclimation protocols in sports (Buchheit et al. 2011; Racinais et al. 2013). As the heat acclimation-induced benefits are characterized by significant inter-individual variation (Racinais et al. 2012), ANS function—as evaluated through HRV measurements—obtained during a heat tolerance test may be used as an indicator of heat acclimation completeness. This is

because our current results suggest that the restoration of ANS function during the decay phase of heat acclimation precedes the restoration of heat loss mechanisms in heat-acclimated individuals.

It is concluded that heat acclimation is accompanied by reductions in T_{re} and T_{es}, significant bradycardia, and marked alterations in HRV, which we interpret as being related to vagal dominance. The observed changes in T_{re} and T_{es} persist for at least 2 weeks of non-exposure to heat, while the changes in heart rate and HRV decay faster and are only partly evident after 2 weeks of non-exposure to heat.

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Conflicts of interest Andrew J.E. Seely is the founder and Chief Science Officer, and Geoffrey Green is the Product Manager of Therapeutic Monitoring Systems (TMS). TMS aims to commercialize patented Continuous Individualized Multi-organ Variability Analysis (CIMVA) technology, with the objective of delivering variability-directed clinical decision support to improve quality and efficiency of care. All the other authors have no conflicts of interest to disclose.

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