Heart rate variability during cardiovascular reflex testing: the importance of underlying heart rate

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Abstract

Objectives: Heart rate variability (HRV) is often measured during clinical and experimental cardiovascular reflex tests (CRT), as a reflection of cardiac autonomic modulation, despite limited characterization of the rapid responses that occur. Therefore, we evaluated the responsiveness of HRV indices in 20 healthy young adults (age, 27 ± 6 y; mass, 76.9 ± 16.8 kg; height, 1.79 ± 0.12 m) during four separate established CRT.

Methods: These included the [I] orthostatic challenge, [II] isometric handgrip, [III] cold pressor and [IV] cold diving reflex tests. Electrocardiogram was recorded throughout, with HRV derived from RR intervals at rest and from each CRT. On a separate day, a subgroup of participants (n=9) completed the same protocol for a second time.

Results: The maximal slope of heart rate change (dTdt) was significantly different between all CRT, with the orthostatic challenge producing the fastest increase (2.56 ± 0.48) and the cold pressor the fastest reduction (−1.93 ± 0.68) in heart rate. Overall HRV, reflected by Poincaré plot ratio (SD1:SD2), was significantly reduced during all CRT ([I], −0.41 ± 0.12; [II], −0.19 ± 0.05; [III], −0.36 ± 0.12; [IV], −0.44 ± 0.11; p<0.05) relative to baseline and this was reproducible in time-series. However, when HRV indices were correlated to mean-RR an exponential growth-like relationship was evident (R² ranging from: 0.52–0.62).

Conclusions: These unique outcomes demonstrate that short-term alterations in HRV are evident during CRT, while indicating the importance of adjusting for, or at least reporting, underlying heart rate when interpreting such measures.

Keywords: autonomic nervous system; electrocardiogram; frequency domain; poincaré plot; sympatho-vagal balance; time domain.

Introduction

Cardiovascular reflex tests (CRT) offer a well-established, noninvasive method to assess autonomic nervous system function in healthy [1] and clinical populations [2]. A series of CRT first described by Ewing et al. [2] consisted of five separate exercises and included deep breathing, isometric muscle contractions and orthostatic challenge. When these CRT were performed in sequence, it was reported to produce predictable changes in heart rate and blood pressure useful for determination of subclinical cardiac autonomic nerve damage. The original set of tests is still widely used throughout the literature with modification, omission and addition of other exercises common when assessing cardiac autonomic nervous system function [3]. However, many researchers are now measuring and reporting heart rate variability (HRV), determined via continuous electrocardiogram recording, as a cardiac specific indicator of autonomic nervous system modulation [4]. In fact, it is a widely held view that variability in physiological measures such as heart rate can provide data which reflects parasympathetic (vagal) and sympathetic nervous system modulation. However, despite the great awareness of HRV and its association with autonomic control of heart rate, the analysis and relation of HRV to cardiac autonomic modulation is still controversial [5, 6].

Control of heart rate is modulated by the intrinsic spontaneous depolarization of pacemaker cells in the sinoatrial node [7], as well as moment-by-moment extrinsic changes in vagal (parasympathetic) and sympathetic autonomic tone, with sympathetic modulation occurring much slower than vagal [8]. Measurement of HRV is traditionally categorized within time, frequency and nonlinear mathematical domains, although recently other parameters have also been discussed [9]. While heart rate and some HRV responses to individual CRT have been characterized [10–12], to the authors knowledge, no studies...
have reported HRV indices in response to a variety of established CRT including the orthostatic challenge, isometric handgrip at maximal voluntary contraction (MVC), cold pressor test and cold diving reflex [13–15] collected within the same individuals and in time-series.

As such, this study aimed to characterize commonly reported time domain indices of HRV such as the standard deviation between RR intervals (SDNN) and the root mean square of successive differences (RMSSD) along with the well-established nonlinear Poincaré plot ratio (SD1:SD2). Time and nonlinear domain HRV indices were chosen for investigation as they are the preferred estimate of short-term HRV rather than frequency domain which is thought to reflect long-term (>5 min) components [4]. We hypothesized that each CRT would elicit predictable changes in heart rate, blood pressure, HRV and, secondly, these changes would be reproducible in time-series.

Materials and methods

Participants

Twenty young and healthy adults (12 males, 8 females) participated in this study (age 27 ± 6 y, mass 76.9 ± 16.8 kg and height 1.79 ± 0.12 m). All participants completed a medical screening questionnaire and provided written, informed consent to the procedures approved by the Human Research Ethics Committee (University of Wollongong) in accordance with the regulations of the National Health and Medical Research Council (Australia).

Experimental design and overview

The study involved two phases and each participant was their own control. Phase one required all participants (n=20) complete a baseline (quiet supine rest) heart rate assessment followed by four CRT (orthostatic challenge, isometric handgrip [100% MVC], cold pressor test, cold diving reflex). The CRT were presented in the same order and were separated by 10 min of quiet rest (blind folded and either supine or seated upright). Phase two comprised of a random selection of participants (n=9, age 25 ± 4 y, mass 72.4 ± 13.6 kg, height 1.76 ± 0.12 m) who return to the laboratory following 8 weeks. For the returning female participants (n=4), they were asked to return in the same phase of the menstrual cycle. These participants completed a second baseline (quiet supine rest) heart rate assessment and repeated the four CRT in the same order. An overview of the experimental design is provided in Figure 1.

Experimental protocols

Quiet supine rest: Participants lay supine on a plinth, arms by their side, legs straight and head was supported by a pillow, for a duration of 20 min. A blindfold was placed over eyes and a metronome was used to pace breathing frequency (15 breaths per minute) while sound was kept to a minimum.

Orthostatic challenge: On verbal command, participants were required to stand rapidly, from the supine body position, and place their back on the wall with arms loosely hanging to the side of their torso for a duration of 3 min. Heels of their feet were placed 30 cm from the wall, while maintaining straight knees. This body position eliminates any lower limb muscle contractions that may contribute to enhanced venous return, thus augmenting the reflex response. The participants continued to wear the blindfold over eyes and pace their breathing frequency (15 breaths per minute).

Isometric handgrip [100% MVC]: Participants were returned to the supine position. A handgrip dynamometer (SM-K series, Total Patient Care, Sydney, Australia), previously adjusted for hand size, was placed in their dominant hand. Participants then adopted a 90° flexion of the elbow so that the dynamometer was pointing toward the ceiling. On command, participants were required to sustain a maximal isometric handgrip (MVC) for a duration of 60 s. As the participants continued to wear the blindfold, feedback was provided verbally regarding the time (15, 30, 45 s) and also the isometric force. Peak isometric force (kg) was recorded.

Cold pressor test: Participants, still wearing the blindfold, were transferred to a laboratory wheelchair and then relocated to the cold-water bath, while maintaining a seated position. This procedure eliminated any excessive skeletal muscle contractions of the limbs or torso. The nondominant arm (not involved in the handgrip assessment) was placed in a horizontal position next to the pre-prepared cold-water bath (custom built 90 cm length, 30 cm width, 25 cm height). The water bath was set at 5 °C and the water was continually circulated using a constant flow pump with in-built thermistor (Uni-stat, Thermoline, Aus). Participants were directed to immerse their upper limb (hand to mid-brachium) into the water for a duration of 5 min.

Cold diving reflex: Participants were repositioned, using the wheelchair, to be directly facing the cold-water bath as described above. The height of the bath was adjusted so that each subject could lean easily forward and their face be at the level of the water. The water bath was set at 5 °C and the water remained circulating using the constant flow pump. Participants were directed to inspire to total lung capacity, close their glottis, and then immerse their face into the water. The water covered frontal, maxillary and mandibular regions of the face. Participants sustained this breath hold position until volition. As a safety requirement, the participants maintain a gentle tapping of their right index finger (once every 5 s) to indicate consciousness and a pulse oximeter (Nellcor PM100 N Covidien, Aus) was worn on the left index finger to monitor arterial oxygen saturation (%). Duration of breath hold time (s) was recorded.

Electrocardiograph and blood pressure

Participants were fitted with a 3-lead electrocardiograph device (eMotion Faros 180, Mega Electronics Ltd, Finland) using disposable electrode patches (Ambu BlueSensor VLC Electrodes, Ballerup Denmark). The electrocardiograph device was set to continuously record lead II (1000 Hz). A blood pressure cuff was placed around the brachium and connected to a portable device (Omron, Australia). Blood pressure was recorded during the orthostatic challenge (between 15 and 45 s), the cold pressor test (between 2 and 3 min) and the cold diving reflex (whilst face was submerged; 15–45 s) but was not recorded during the handgrip test, due to the nature of the test.
Heart rate variability analysis

The electrocardiograph signal was downloaded from the device and imported into HRV-Scanner Software (Biosign, Ottenhofen Germany) enabling wave visualization and beat-by-beat detection of RR interval time (ms). The electrocardiograph signals were then exported into Kubios (Department of Physics of the University of Kuopio, Finland) and passed through an automatic filter which removed artefacts due to ectopic beats, missed beats etc., and replaced them using cubic spline interpolation leaving only physiologically normal interval time series before HRV analysis was completed. RR interval time (mean-RR), time series (minimum heart rate, peak heart rate, mean heart rate, SDNN, RMSSD and the maximal slope of heart rate change [dTdt]) and the non-linear Poincaré plot ratio (SD1:SD2) were extracted from the beat-by-beat data. These extracted indices were derived from time periods as follows. Supine rest: final 5 min. Orthostatic challenge: initiation of standing to peak heart rate. Isometric handgrip: 60 s of MVC gripping. Cold pressor test: commencement of cold-water exposure to the peak heart rate response (approximately 3 min). Cold diving reflex: peak heart rate when the face entered the water to the completion of the heart rate response (approximately 3 min). Cold diving reflexes.

Experimental standardization

A familiarization session ensured participants were accustomed to the equipment and the protocols. Specifically, participants were required to visit the laboratory prior to the commencement of the study, when they were explained each CRT, demonstrated the required body position and performed an isometric handgrip assessment to establish 100% MVC. On the day of the testing for either phase one or phase two, participants were asked to refrain from strenuous exercise on the day of the testing, and not to consume alcohol for 12 h, and caffeine for 4 h prior to an experiment. All experiments were performed in a temperature-controlled thermal chamber (24 °C, 35% relative humidity) and participants were placed in this chamber 30 min prior to the commencement of testing. Aural temperature was continuously recorded (Squirrel SQ2040, Grant Instruments, UK) to monitor body core temperature (°C) and this was an important and unique aspect to the study of HRV.

Data analysis

An a priori power analysis was completed and indicated that a minimum of 16 subjects were required to detect differences in HRV with at least 90% statistical power. For comparison between CRT, data were analyzed using repeated measures one-way analysis of variance (ANOVA). For comparisons between conditions, the P value was adjusted with the Bonferroni procedure as a multiple comparison. For reproducibility within CRT, data were analyzed using a two-way repeated measure ANOVA with the factors of time (2 levels: day 1 & day 2) and condition (5 levels: baseline, orthostatic challenge, isometric handgrip [MVC], cold pressor test, cold diving reflex). When a significant interaction or main effect was detected, post-hoc comparisons were carried out using paired t-tests. For correlations between HRV indices and mean-RR, data were fit with a second order nonlinear polynomial (quadratic) curve. Data were inspected for normality prior to analysis using quantile comparison plots. Alpha (significance level) was set at 0.05 for all comparisons, with data being reported as means [SD]. All analyses were performed using GraphPad Prism version 8 (GraphPad Software, La Jolla, California, USA).

Results

All participants successfully completed each test. In addition, aural temperature was maintained for each participant in a thermoneutral state (36.3–36.6 °C), during the time course of the assessments, thus reducing the influence of vasomotor function on absolute heart rate at rest or during the reflexes.
Heart rate and blood pressure during cardiovascular reflex tests

Peak heart rate was increased in all CRT, whereas minimum heart rate was increased during the isometric handgrip and cold pressor test but reduced in the cold diving test, relative to baseline (Table 1; p<0.05). Diastolic, systolic and mean arterial blood pressure were all raised on average during each test, with the cold pressor producing the largest and orthostatic producing the smallest changes to mean arterial pressure, relative to baseline (Table 1; p<0.05). The orthostatic challenge produced the greatest positive dTdt; however the cold pressor test produced an approximately equal negative dTdt (Figure 2; p<0.05).

Change in heart rate variability during cardiovascular reflex tests

For ease of reading all the following comparisons of HRV measures during CRT are the mean difference relative to baseline. The cold diving reflex produced significant increases in SDNN, whereas SDNN remained stable during all other CRT (Table 2; p<0.05). The isometric handgrip test significantly reduced RMSSD, whereas it was significantly increased during the cold diving test and remained stable in both the other CRT (Table 2; p<0.05). The SD1:SD2 ratio was significantly lower in all CRT with very little difference between the conditions (Table 2; p<0.05). Finally, within the subgroup of participants (n=9) that returned and repeated the CRT on a second day, there were no significant differences in any of the HRV indices calculated relative to the first day (Table 2; all p>0.05).

Correlation between heart rate variability and mean-RR during cardiovascular reflex tests

Despite the difference in conditions that HRV indices were collected, all the data approximately fall along a common exponential growth-like curve (Figure 3). As such, a second order polynomial (quadratic) curve was fit to the data to determine the goodness of fit (R²). RMSSD showed the

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**Table 1**: Heart rate and blood pressure responses during cardiovascular reflex testing.

<table>
<thead>
<tr>
<th>Variable, unit</th>
<th>Baseline</th>
<th>Orthostatic</th>
<th>Handgrip [MVC]</th>
<th>Cold pressor</th>
<th>Cold diving reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood pressure, mm Hg</strong></td>
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</tr>
</tbody>
</table>

Presented data are means [SD]. Twenty young adults (n=20) completed an assessment of heart rate during baseline (quiet supine rest) and followed by four cardiovascular reflex tests (orthostatic challenge, isometric handgrip [100% MVC], cold pressor test and cold diving reflex) relative to baseline (supine quiet rest). Each individual response is plotted (grey circles) along with the group mean and standard deviation (solid black line). One-way repeated measures ANOVA with Bonferroni’s post hoc analysis was completed; a, b, c, d Labelled columns without a common letter differ, p<0.05. Map, Mean arterial pressure; MVC, Maximal voluntary contraction.
Table 2: Heart rate variability indices collected during cardiovascular reflex testing on separate days.

<table>
<thead>
<tr>
<th>Variable, unit</th>
<th>Baseline</th>
<th>Orthostatic</th>
<th>Orthostatic vs. Baseline</th>
<th>Handgrip [MVC]</th>
<th>Handgrip vs. Baseline</th>
<th>Cold pressor</th>
<th>Cold pressor vs. Baseline</th>
<th>Cold diving reflex</th>
<th>Cold diving vs. Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean [SD]</td>
<td>Mean [SD]</td>
<td>Mean diff. [95% CI]</td>
<td>Mean [SD]</td>
<td>Mean diff. [95% CI]</td>
<td>Mean [SD]</td>
<td>Mean diff. [95% CI]</td>
<td>Mean [SD]</td>
<td>Mean diff. [95% CI]</td>
</tr>
<tr>
<td>Mean diff. [95% CI]</td>
<td>-4 [-72, 65]</td>
<td>-7 [-75, 62]</td>
<td>-</td>
<td>13 [-55, 82]</td>
<td>-</td>
<td>20 [-48, 89]</td>
<td>-</td>
<td>12 [-80, 57]</td>
<td>-</td>
</tr>
<tr>
<td>Mean diff. [95% CI]</td>
<td>-3 [-39, 33]</td>
<td>-3 [-38, 34]</td>
<td>-</td>
<td>1 [-35, 37]</td>
<td>-</td>
<td>0 [36, 36]</td>
<td>-</td>
<td>32 [-4, 68]</td>
<td>-</td>
</tr>
<tr>
<td>SD1:SD2</td>
<td>Day 1</td>
<td>0.63 [0.40]</td>
<td>0.22 [0.13]</td>
<td>-0.41 [-0.60, -0.23]^a</td>
<td>0.19 [0.10]</td>
<td>-0.44 [-0.62, -0.26]^a</td>
<td>0.27 [0.11]</td>
<td>-0.36 [-0.54, -0.19]^a</td>
<td>0.19 [0.09]</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>0.81 [0.43]</td>
<td>0.24 [0.13]</td>
<td>-0.57 [-0.89, -0.24]^a</td>
<td>0.21 [0.12]</td>
<td>-0.60 [-0.93, -0.28]^a</td>
<td>0.22 [0.06]</td>
<td>-0.59 [-0.92, -0.27]^a</td>
<td>0.30 [0.09]</td>
</tr>
<tr>
<td>Mean diff. [95% CI]</td>
<td>0.18 [-0.04, 0.40]</td>
<td>0.02 [-0.19, 0.24]</td>
<td>-0.02 [-0.20, 0.24]</td>
<td>-0.05 [-0.26, 0.18]</td>
<td>-0.11 [-0.10, 0.34]</td>
<td>-</td>
<td>0.11 [-0.10, 0.34]</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Assessment of heart rate variability on two separate days (day 1, n=20; day 2, n=9) during baseline (quiet supine rest) and four cardiovascular reflex tests (orthostatic challenge, isometric handgrip [100% MVC], cold pressor test and cold diving reflex). For comparisons between conditions on Day 1 a repeated measures one-way ANOVA with Bonferroni’s post hoc analysis was completed; ^a Labelled columns differ from baseline, p<0.05. For reproducibility testing within CRT, data were analyzed using a two-way repeated measure ANOVA with the factors of time (2 levels: day 1 & day 2) and condition (5 levels: baseline, orthostatic challenge, isometric handgrip [MVC], cold pressor test, cold diving reflex); no significant differences were observed. SDNN, standard deviation of normal to normal intervals; RMSSD, root mean square of successive differences; SD1:SD2, Poincaré plot ratio.
strongest correlation ($R^2$: 0.62), followed by SD1 ($R^2$: 0.56), SDNN ($R^2$: 0.54) and finally SD2 ($R^2$: 0.53).

## Discussion

The homeostatic responses, involving the adjustment of the cardiovascular system, to acute CRT are of significant interest across many domains of clinical and physiological science. In this study, our focus was to better characterize the rapid changes that occur in commonly reported short-term HRV indices in response to CRT and determine their reproducibility in time-series. Moreover, given the most recent debate concerning the validity of HRV to depict changes in the cardiac autonomic responsiveness, this study uniquely considered the recommendations to report relative changes HRV whilst addressing the influence of absolute heart rate. In line with our working hypothesis, heart rate acutely responded during all four CRT. However, alterations in short-term HRV indices were predominately only evident with CRT that provoked strong withdrawal or activation of cardiac vagal modulation such as the cold diving reflex. Further, while we demonstrate that these alterations are reproducible in time-series, we also note a strong relationship observed between short-term HRV indices and the underlying heart rate (mean-RR) across CRT. These unique outcomes advance understanding by contextualizing short-term HRV alterations in response to CRT provocation in young healthy adults.

This study used four well established CRT, controlling specially for thermal and body movement artefacts. The cold diving reflex produced the greatest changes to short-term measures of HRV. During this CRT, overall or ‘continuous’ HRV was increased, as reflected by SDNN, with the increased variability attributable to rapid or ‘instantaneous’ cardiac vagal slowing of heart rate, as reflected by RMSSD [4]. Previously, cold compresses applied to the face have been demonstrated to reduce the short-term fractal scaling exponent (DFA$\alpha_1$) in parallel with reduced low frequency to high frequency (LF/HF) ratio in healthy adults [11]. In that study, the authors concluded that the changes observed were associated with sympathetic activation in the presence of enhanced vagal outflow. We extend these findings by demonstrating even further augmentation of vagal outflow, reflected by increased RMSSD, when including breath hold as part of the CRT.

In contrast to the cold diving reflex, the current study demonstrated that overall HRV, as reflected by SDNN, remained equivalent in all other CRT. Earlier studies have demonstrated frequency domain HRV following the orthostatic challenge [10] and DFA$\alpha_1$ during cold pressor test [11, 12] to be altered, with the authors of each study suggesting the changes reflected increased cardiac sympathetic modulation. Despite seeing no change in overall HRV, our data showed that RMSSD was lower, relative to baseline, in all three CRT that elicit an increased heart rate, but only reaching significance in the isometric handgrip test. This highlights the known withdrawal of vagal influence and shift in favor of sympathetic cardiac modulation during these three tests but indicates a limited ability to quantify changes in cardiac sympathetic influence using HRV [16]. This is possibly explained by the use of recording durations which were not equivalent. However, while our relative change comparisons used a 5 min baseline recording, in line with the Task Force guidelines [4] for standardization, the nature of each CRT dictates the duration of ECG available for the analysis (i.e., approx. 45 s was the longest common time point for all participants during their breath hold). Notwithstanding, as argued by Malik et al. [6], HRV as a tool to quantify ‘responsiveness’ has some clinical merit, if change in the parameter of...
interest is assessed rather than absolute values. The current observations suggest that the stimulus should be carefully selected, where the maximal isometric handgrip offers some degree of change in HRV differentiation, when compared to other known sympathetic stimuli, and offers reproducibility. However, there is still need for larger confirmatory studies, including a broader array of HRV indices and studies which complete comparisons of absolute HRV using recordings of equivalent duration.

Notwithstanding the changes observed in HRV during the cold diving and maximal isometric handgrip test in the current study, our data also demonstrated a strong nonlinear relationship between HRV and the underlying heart rate. While Malik et al. [6] argues that HRV, regardless of underlying heart rate, is a valid measure of cardiac autonomic responsiveness to provocation, our observation alongside other studies [17, 18], support the argument offered by Boyett et al. [5] that HRV is primarily a nonlinear surrogate of heart rate itself. Further, variability in the beat rate remains evident in the human transplanted heart [19], isolated rabbit hearts [20] and isolated cardiomyocytes derived from human stem cells in culture [21]. As such, HRV is likely influenced by both autonomic modulation and intrinsic mechanisms, including the calcium clock [7], within the sino-atrial node of the heart. However, we suggest that the maximal slope of heart rate change (dTdt) may provide a more straightforward and appropriate method to quantify cardiac autonomic modulation in response to CRT.

In this study, we were able to compare the rate of rise of heart rate between three distinct CRT, each with unique sensor-integration-effector loop. For example, the distinctive and rapid increase in heart rate due to the challenge to mean arterial blood pressure offers a unique opportunity for longitudinal cardiovascular reflex assessments. The slope by which heart rate increases, in response to a prescribed stimulus, has been proposed as marker of physiological mal-adaptation with respect to the autonomic nervous system. For example, a reduced maximum rate of increased heart rate during submaximal exercise bout has been reported to be associated with over-reaching, particularly in females, in the context of endurance training [22]. Furthermore, in middle age males, a blunted heart rate response to a submaximal aerobic exercise bout has been associated with an increased cardiovascular and all-cause mortality [23]. HRV across any of the domains, is limited by the sensitivity to detect and quantify ‘sympatho-vagal balance’ [24] and this is supported by the current study, in the context of rapid and reproducible cardiovascular reflexes. Therefore, given the maximal slope of the change in heart rate is a reproducible positive chronotropic effect, the rate of change during these assessments could prove to be a valuable non-invasive surrogate to the ‘sympatho-vagal effect’ [16]. Such potential for a rapid and non-invasive marker of training mal-adaptation is of particular interest.

For example, the very demanding physical and occupational training of Defence and Emergency Services populations could possibly be monitored in individuals, where perturbations to such chronotropic responses may be factored into training prescription. Obviously, such hypotheses must now be validated in such contexts, including over-reaching and severe perturbations to sleep deprivation and fatigue.

Although we included female participants in our study, examining sex as a factor which influences HRV responses to CRT was beyond the scope of the current study. Yet importantly, we did ask that our female participants (n=4), who returned for visit two, were in the same phase of their menstrual cycle. We recognize that males are over represented in previous studies completed and that there may be divergent effects of CRT on cardiac autonomic modulation in men and women, especially given the potential effects of menstrual cycle phase and oral contraceptive use on cardiovascular function [25]. In addition, HRV for females differs to males, particularly in the first decades of life, during ambulatory activity [26]. However, we would raise that in line with HRV being a non-linear surrogate of heart rate, many studies have not controlled for the latter. As females, on average, are smaller in stature, they are also more likely to have higher resting heart rates, thus raising if the reported differences may be partly heart rate derived. Likewise, and in addition, participant fitness levels should be considered in future research. It is well-established that cardiac vagal modulation is strong in aerobically trained and healthy subjects [27], which likely contributes to improved cardiovascular regulation in fit relative to unfit participants. As such, alterations of vagal modulation attributable to CRT in participants of our study may have been amplified in comparison to less aerobically-trained populations. Yet, notably, for this study we controlled both thermal and body movement artefacts, as they were more likely to contribute to differing perturbations in short-term HRV.

Conclusions

Inappropriate sympahto-vagal balance may contribute to increased risk of adverse cardiac events [28], making noninvasive characterization of cardiac autonomic
modulation, via HRV, an important endeavor. In summary, we demonstrate that CRT-elicted alterations in short-term HRV strongly reflect vagal mediated slowing of heart rate but provide limited ability to quantify changes in cardiac sympathetic modulation. Further, the strong nonlinear relationship between HRV and heart rate highlights the importance of adjusting, or at least reporting, underlying heart rate when interpreting such measures. Finally, while the maximal slope of heart rate change is commonly reported alongside aerobic exercise, we suggest that investigation of dTdt during CRT may provide a more controlled assessment of pathophysiological and/or maladaptive alterations in cardiac autonomic modulation.

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Author contributions: GP conceived and designed the study. GP, PMcL and MM contributed to the acquisition, analysis, assembly and interpretation of the data. MM completed the statistical analysis. GP and MM drafted the manuscript. All authors reviewed and approved the submission of the final manuscript

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The research related to human use has complied with all the relevant national regulations, institutional policies, and in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors’ Institutional Review Board (University of Wollongong) or equivalent committee. (HE/17-100).

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