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Influence of postexercise cooling techniques on heart rate variability in men

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The reduction of core body temperature (T_C) is vitally important in the treatment of hyperthermia; however, little is known regarding the impact of cooling treatments on the autonomic control of heart rate (HR). The aim of the present study was to examine the influence of three field-based hyperthermia treatments on the neural control of HR via heart rate variability (HRV). Following exercise-induced hyperthermia (T_C ~40.0°C) in a warm environment (34.2 ± 0.5°C), nine healthy, active men were treated during recovery, in a randomized order, with intravenous cold saline infusion (IV) or ice packs (ICE) or fan cooling with intermittent water spray (FAN) for 40 min. During each treatment, HR dynamics via power spectral (VLF, LF, HF), Poincare plot (SD1, SD2), approximate entropy (ApEn) and short- (α_1) and long-term (α_2) fractal scaling analyses were determined every 10 min. At recovery onset, HR and T_C were similar between treatments and were significantly reduced over the 40 min recovery period. During recovery, HR and α_2 were significantly reduced from initial levels but were significantly greater for IV compared with ICE and FAN. In contrast, VLF, LF, HF, SD1, SD2 and ApEn increased during recovery, with all being significantly lower for IV compared with ICE and/or FAN. The present results demonstrated that IV, compared with ICE and FAN, resulted in significantly greater HR, reduced spectral and geometrical HRV, lower HR complexity and reduced long-term HR control, indicative of reduced vagal and/or increased sympathetic modulation. Specific treatments for exercise-induced hyperthermia may result in an altered sympathovagal balance that requires further examination.

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In tropical environments, the risks of heatstroke and hyperthermia are great, with treatment primarily based upon rapid cooling to reduce core body temperature (T_C) and tissue damage (Hadad *et al.* 2004*b*; Casa *et al.* 2007). Although of paramount importance, the best single cooling treatment for hyperthermia is currently unknown, with a variety of cooling techniques being used for heatstroke (Armstrong *et al.* 1996; Hadad *et al.* 2004*b*; Casa *et al.* 2007). Hadad and colleagues (2004*b*) commented that both evaporative and cold water immersion were effective in treating heatstroke, with water and air fanning recommended for treatment in the field. Recently, Casa and colleagues (2007) reviewed the benefits and misconceptions of cold water immersion for exertional heatstroke and concluded that based upon the current evidence, the best exertional heatstroke treatment was cold water and, if possible, cold water immersion, since it provided the fastest T_C cooling rate (Casa *et al.* 2007). Although ideal, cold water immersion facilities may be limited in the field and other treatment techniques, such as air fanning (Wyndham *et al.* 1959; Kielblock *et al.* 1986; Mitchell *et al.* 2001), intravenous cold saline (Wyndham *et al.* 1959; Frank *et al.* 1997) and ice packs (Kielblock *et al.* 1986; Armstrong *et al.* 1995) may be more applicable in the field treatment of hyperthermia. Recently, we examined the impact of three field-based cooling treatments (i.e. intravenous saline infusion, ice packs or water spray and fan cooling), noting that all

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significantly reduced T_C and were appropriate for first aid treatment in field settings (unpublished observations). Despite these techniques being readily available and used in the field, little is known of the impact of these treatments on the neural regulation of heart rate as determined via heart rate variability (HRV).

Several studies have examined HRV during exposure to hot and cold environments (Lindqvist *et al.* 1991; Lossius *et al.* 1994; Brenner *et al.* 1997; Thayer *et al.* 1997; Kinugasa & Hirayanagi, 1999; Frank *et al.* 2001; Liu *et al.* 2008). For example, Liu *et al.* (2008) demonstrated that HRV (as measured by the low to high frequency ratio) and sympathetic activity were significantly greater when subjects experienced thermal discomfort at low and high air temperatures (21 and 29–30°C) compared with thermal comfort (24–28°C) despite similar thermal sensation. Despite these many studies examining the impact of environmental temperature on HRV, very few, if any, have examined HRV during hyperthermia and its subsequent cooling treatment. Previously, HRV and, in particular, the very low frequency component of HRV (0.0039–0.04 Hz) was reported to be greater during core and skin surface cooling, suggesting a direct modulation of cardiac function by thermal stimuli (Fleisher *et al.* 1996). In contrast, mild core cooling via cold saline infusion resulted in an increased adrenergic response exemplified by an increase in noradrenaline-mediated peripheral vasoconstriction and increased arterial blood pressure (Frank *et al.* 1997). Recently, cold water (14°C) immersion following supramaximal exercise resulted in greater HRV and parasympathetic reactivation compared with passive rest in a hot environment (∼35°C; Buchheit *et al.* 2009*b*). These prior studies (Fleisher *et al.* 1996; Frank *et al.* 1997; Buchheit *et al.* 2009*b*) were conducted in normothermic individuals, with results difficult to extrapolate to hyperthermic individuals. To our knowledge, no studies have examined HRV during the cooling treatment of hyperthermia. Given the relationships between thermoregulation and the neural regulation of the cardiovascular system (Kitney, 1974, 1975; Kitney & Rompelman, 1977; MacKenzie *et al.* 1992), and between HRV, myocardial electrical stability and morbidity (Huikuri *et al.* 1992; Billman, 2006), alterations in T_C via cooling treatment may significantly impact the neural control of HR (i.e. HRV) and subsequent recovery from hyperthermia. Therefore, the aim of the present study was to examine HRV during field-based hyperthermia treatments (Sinclair *et al.* 2009).

Methods

Participants and ethical approval

Eleven healthy, male participants, with a mean ± S.D. age of 23.5 ± 2.3 years, height 181.8 ± 8.3 cm, mass 79.8 ± 7.5 kg, body fat percentage 13.9 ± 3.7% and maximal oxygen uptake 54.8 ± 2.5 ml kg min^{-1}, volunteered for this study as part of a larger study (Sinclair *et al.* 2009). The study was conducted within the standards set by the latest version of the Declaration of Helsinki and approved by the James Cook University Human Ethics and the Australian Defence Human Research Ethics committees. After being briefed, all participants completed a pre-screening questionnaire and provided informed written consent.

Procedure

As previously described (Sinclair *et al.* 2009), participants completed three sessions in a climate control chamber (34.2 ± 0.5°C, 62.3 ± 3.1% relative humidity and circulating airspeed ≤ 0.5 m s^{-1}), with each session separated by at least 72 h. Briefly, each session comprised a repeated intermittent exercise protocol consisting of walk–run (2 min at 6 km h^{-1} and 4 min at 10 km h^{-1}) on a motorized treadmill until T_C was elevated to near 40°C, followed by 40 min of recovery with a cooling treatment. Core temperature was measured every minute via a telemetric system comprising of an ingestible temperature pill (2 cm × 1.2 cm diameter covered in a silicone rubber; HQ Inc., Palmetto, FL, USA) that was ingested at least 5 h before the session to ensure the pill had passed the stomach, and a data logging unit (BCTM, Fitsense, Southborough, MA, USA) situated in close proximity to the participant. This telemetric system has been confirmed as a valid index of T_C with excellent applicability to ambulatory monitoring (Byrne & Lim, 2007). The cooling treatments were undertaken in a randomized order and consisted of: fridge-cold saline infusion (2 l of 0.9% sodium chloride solution, 'IV'), ice packs administered to the armpits, groins and back of the neck ('ICE') and industrial fan cooling with intermittent water spray ('FAN'). Upon T_C reaching 40°C, participants stopped exercise, removed all clothing except bike shorts and undertook a supine position for recovery. The transition from exercise cessation to treatment commencement was 5 min. Recovery HR was monitored from an ECG signal (lead II configuration) obtained using a bioamp and PowerLab 4sp system (Chart v5.1) at a sampling rate of 1000 Hz (ADInstruments Inc., Castle Hill, NSW, Australia). The ECG recordings were analysed offline for frequency-domain, geometrical and non-linear measures of HRV every 10 min using customized software (Heart Signal Co., Kempele, Finland) as previously described (Huikuri *et al.* 1992). All R–R intervals were automatically detected and visually confirmed, with R–R intervals associated with artifact removed. No ectopic beats were exhibited, with only normal-to-normal R–R intervals (≥98% of recording) included for analysis.

Frequency-domain HR dynamics

During recovery, frequency-domain measures of HRV were determined via power spectral analysis using an autoregressive model order of 20 (Huikuri *et al.* 1992). Power spectral density within the very low frequency (VLF; 0.003–0.04 Hz), low frequency (LF; 0.04–0.15 Hz) and high frequency bandwidths (HF; 0.15–0.4 Hz; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) was determined. All frequency-domain components were expressed in absolute units (ms^2), with the LF/HF ratio calculated as a measure of sympathovagal balance (Pagani *et al.* 1986).

Geometrical and non-linear HR dynamics

The short/minor (SD1) and long/major (SD2) axes of the Poincare plot and the ratio of these axes (SD1/SD2) were determined as geometrical measures of HR dynamics reflecting short-term instantaneous R–R modulation, long-term R–R modulation and the balance between the two measures, respectively (Tulppo *et al.* 1996). Non-linear HR dynamics were also examined, since previously these measures were reported to be superior to conventional power spectral components for documentation of HR behaviour (Tulppo *et al.* 2001*a*), while the thermoregulatory system was reported to influence HR via non-linear means (Kitney, 1974; Kitney & Rompelman, 1977). The non-linear measures examined were short- (α_1) and long-term (α_2) fractal scaling exponents (Peng *et al.* 1995) and approximate entropy (ApEn; Pincus, 1991). Fractal scaling examination was conducted via detrended fluctuation analyses that calculated the slope of the relationship between the (log) integrated and detrended fluctuation and (log) window size (Peng *et al.* 1995). The α_1 and α_2 values were computed based upon window sizes of $\leq$11 beats and $\geq$12 beats, respectively (Peng *et al.* 1995). Heart rate regularity and complexity were determined via ApEn, with input variables (m, length of compared runs at each time point of the time series = 2; r, tolerance for judging the similarity of runs = 20%) based upon a previous study (Pincus, 1991). Greater ApEn indicated more irregularity and greater complexity in the HR signal (Pincus, 1991).

Heart rate dynamics at the same HR

Since previous studies have reported different HR dynamics at the same HR (Tulppo *et al.* 1999; Leicht *et al.* 2008), and to provide further information concerning HR behaviour during cooling treatment, HR dynamics were examined during a 10 min epoch of similar HR for each treatment. The 10 min epochs selected for this comparison were based upon identification of a similar average HR per treatment for each participant ($\sim$100 beats min^{-1}; e.g. 30–40 min for IV *versus* 10–20 min for ICE *versus* 10–20 min for FAN for participant 2).

Statistical analysis

All analyses were calculated using the Statistical Package for the Social Sciences software (SPSS version 16, SPSS Inc., Chicago, IL, USA), with data examined for outliers and distribution. Normality of the data was confirmed via the Kolmogorov–Smirnov statistic with a Lilliefors significance correction. Data not normally distributed were transformed using a natural logarithm prior to further statistical analysis. All variables were analysed using a one-way (treatment) or two-way (time × treatment) repeated measures analysis of variance (ANOVA) and Tukey's honestly significant difference *post hoc* tests. Statistical significance was accepted at $P < 0.05$, and all data are presented as means ± S.D.

Results

Owing to consistent outlier values (i.e. values 2–3 times the group average) for some HRV variables, results for two participants were excluded, with the remaining results for nine participants presented. The intermittent exercise-induced heating phase lasted on average 58.5 ± 11.8 min, was similar between sessions ($P > 0.05$) and resulted in a similar T_C (39.9 ± 0.1 *versus* 39.9 ± 0.2 *versus* 39.9 ± 0.2°C, $P > 0.05$) and HR (180.9 ± 18.1 *versus* 184.4 ± 14.3 *versus* 190.3 ± 11.7 beats min^{-1}, $P > 0.05$) for IV, ICE and FAN treatments, respectively. During recovery, T_C and HR were significantly reduced over time (time main effect, $P < 0.001$; Table 1) while significantly greater HR (treatment effect, $P < 0.001$) was exhibited for IV compared with ICE and FAN during each 10 min epoch of recovery (time × treatment effect; Table 1).

Frequency-domain HR dynamics

At treatment commencement, all frequency-domain measures of HRV were low, with VLF, LF and HF increasing over time (time main effect, $P < 0.001$; Table 1). There was a significant main treatment effect ($P < 0.001$) for VLF, LF and HF, with greater values for FAN compared with ICE, which was greater than IV (Table 1). These treatment differences in HRV, particularly VLF and LF, were most apparent during the latter stages of recovery, although VLF during FAN was greater than IV for each 10 min epoch (time × treatment effect; Table 1). The LF/HF ratio was not significantly changed during recovery and was similar between treatments, although LF/HF for FAN was non-significantly lower compared with IV and ICE treatments (Table 1).

Geometrical and non-linear HR dynamics

Similar to the frequency-domain HRV measures, the geometrical measures of HR dynamics (SD1, SD2 and SD1/SD2) were low at the start of treatment and increased

Table 1. Core body temperature, heart rate and frequency-domain heart rate variability measures at 0–10 min (A), 10–20 min (B), 20–30 min (C) and 30–40 min (D) of IV, ICE and FAN treatments

	A	B	C	D	Treatment effect ($P < 0.05$)	Time effect ($P < 0.05$)
T_C (°C)						
IV	39.6 ± 0.2	38.7 ± 0.2[a]	38.1 ± 0.2[ab]	37.8 ± 0.1[abc]		
ICE	39.8 ± 0.3	39.0 ± 0.5[a]*	38.3 ± 0.6[ab]	37.8 ± 0.4[abc]		
FAN	39.6 ± 0.3	38.4 ± 0.4[a]*†	37.9 ± 0.3[ab]†	37.7 ± 0.3[ab]	—	A > B > C > D
HR (beats min^{-1})						
IV	120.6 ± 7.3	109.6 ± 6.5[a]	103.8 ± 6.5[ab]	99.1 ± 5.3[ab]		
ICE	113.9 ± 7.8*	100.3 ± 11.2[a]*	90.5 ± 11.1[ab]*	84.5 ± 10.4[abc]*		
FAN	107.8 ± 6.3*†	91.9 ± 5.7[a]*†	87.0 ± 5.4[ab]*	83.3 ± 6.5[ab]*	IV > ICE, FAN	A > B > C > D
ln VLF (ms^2)						
IV	5.31 ± 0.52	5.33 ± 0.68	5.13 ± 0.46	5.46 ± 0.81		
ICE	5.44 ± 0.43	6.22 ± 0.77*	6.40 ± 0.86[a]*	6.22 ± 0.64[a]		
FAN	6.25 ± 0.58*	6.45 ± 0.50*	6.79 ± 0.64*	7.08 ± 0.51*	IV < ICE < FAN	A < B, C, D
ln LF (ms^2)						
IV	3.18 ± 0.55	3.92 ± 1.04	4.23 ± 0.77[a]	4.69 ± 0.83[a]		
ICE	3.58 ± 0.65	5.33 ± 1.24[a]*	6.01 ± 1.14[a]*	6.75 ± 0.97[ab]*		
FAN	4.61 ± 0.65	6.36 ± 0.76[a]*†	6.97 ± 0.66[a]*†	7.25 ± 0.66[ab]*	IV < ICE < FAN	A < B < C < D
ln HF (ms^2)						
IV	1.26 ± 0.95	2.48 ± 1.89	3.10 ± 1.45	3.17 ± 1.15		
ICE	1.65 ± 0.72	3.58 ± 1.91	4.48 ± 1.40	5.23 ± 1.17		
FAN	3.76 ± 0.89	5.19 ± 1.12	5.88 ± 0.94	6.13 ± 0.75	IV < ICE < FAN	A < B, C A, B < D
LF/HF						
IV	8.47 ± 5.85	6.26 ± 5.47	4.84 ± 3.61	5.77 ± 2.74		
ICE	7.57 ± 3.27	8.53 ± 8.64	5.73 ± 3.91	5.34 ± 2.92		
FAN	3.24 ± 2.39	4.56 ± 3.66	3.93 ± 2.51	3.89 ± 2.32	—	—

Values are means ± S.D. [a]$P < 0.05$ *versus* A; [b]$P < 0.05$ *versus* B; [c]$P < 0.05$ *versus* C; *$P < 0.05$ *versus* IV; †$P < 0.05$ *versus* ICE. Abbreviations: T_C, core body temperature; HR, heart rate; VLF, very low frequency; LF, low frequency; and HF, high frequency.

significantly over time (time main effect $P < 0.05$; Table 2). There was a significant treatment effect ($P < 0.01$), with SD1 and SD2 being significantly greater during FAN compared with ICE, which was greater than IV (Table 2). This treatment effect did not, however, result in a significant difference between treatments for SD1/SD2 (Table 2).

Comparable to the geometrical and frequency-domain HR measures, ApEn was low initially but increased during recovery (time main effect $P < 0.05$; Table 2), with greater ApEn exhibited for FAN compared with IV (time × treatment effect $P < 0.05$; Table 2). In contrast, α_1 and α_2 were high at treatment commencement, with only α_2 significantly decreasing over time (time main effect, $P < 0.001$; Table 2). There were no significant treatment differences for α_1, while α_2 during IV was significantly greater than ICE (treatment effect, $P < 0.01$) and in particular FAN during recovery (time × treatment effect, $P < 0.05$; Table 2).

Heart rate dynamics at the same HR

At a HR of approximately 98 beats min^{-1} for each treatment, all measures where similar between treatments except for a significantly (treatment main effect, $P < 0.05$) greater VLF, LF, HF, SD1 and SD2 for FAN compared with IV (Table 3).

Discussion

The present results demonstrated that during cooling treatment of exercise-induced hyperthermia, altered cardiovascular dynamics occurred, with IV resulting in a greater HR and lower HRV compared with ICE and FAN treatments. Of particular importance was that IV resulted in significantly lower VLF, LF, HF, SD1, SD2 and HR complexity (ApEn) compared with FAN and/or ICE, indicative of reduced vagal and/or increased sympathetic modulation during recovery. Although each cooling treatment resulted in a similar T_C reduction and treatment of exercise-induced hyperthermia over the 40 min, the autonomic control of cardiac function was significantly different between cooling treatments, which may have implications for cardiac function and recovery from hyperthermia.

Frequency-domain and geometrical HR dynamics

Following exercise in thermoneutral conditions, sympathetic activity and parasympathetic activity remain

Table 2. Geometrical and non-linear heart rate variability measures at 0–10 min (A), 10–20 min (B), 20–30 min (C) and 30–40 min (D) of IV, ICE and FAN treatments

	A	B	C	D	Treatment effect ($P < 0.05$)	Time effect ($P < 0.05$)
SD1 (ms)						
IV	2.66 ± 0.74	5.97 ± 5.83	8.24 ± 7.78	6.46 ± 4.97		
ICE	3.01 ± 0.98	8.17 ± 4.63	11.80 ± 6.03	17.12 ± 7.67		
FAN	8.87 ± 4.97	15.21 ± 7.21	20.76 ± 11.21	24.53 ± 10.85	IV < ICE < FAN	A < B, C A, B < D
SD2 (ms)						
IV	31.42 ± 8.74	30.11 ± 10.24	26.86 ± 8.21	29.93 ± 10.72		
ICE	41.62 ± 9.91	50.67 ± 16.94	55.02 ± 25.36	61.02 ± 21.37		
FAN	69.19 ± 12.50	56.78 ± 15.49	72.39 ± 21.22	81.72 ± 20.12	IV < ICE < FAN	A < D
SD1/SD2						
IV	0.09 ± 0.03	0.20 ± 0.20	0.28 ± 0.21	0.21 ± 0.11		
ICE	0.07 ± 0.02	0.15 ± 0.06	0.21 ± 0.05	0.27 ± 0.06		
FAN	0.13 ± 0.07	0.26 ± 0.11	0.28 ± 0.08	0.29 ± 0.07	—	A < B, C, D
ApEn						
IV	0.49 ± 0.15	0.54 ± 0.21	0.67 ± 0.28	0.79 ± 0.21		
ICE	0.41 ± 0.12	0.63 ± 0.20	0.88 ± 0.21[a]	0.95 ± 0.15[a]		
FAN	0.37 ± 0.15	0.81 ± 0.15[a]	0.89 ± 0.10[a]	0.92 ± 0.14[a]	IV < FAN	A < B < C, D
α_1						
IV	1.50 ± 0.14	1.58 ± 0.20	1.33 ± 0.46	1.48 ± 0.26		
ICE	1.55 ± 0.14	1.58 ± 0.12	1.48 ± 0.18	1.46 ± 0.21		
FAN	1.24 ± 0.38	1.37 ± 0.27	1.42 ± 0.19	1.35 ± 0.22	—	—
α_2						
IV	1.21 ± 0.15	1.10 ± 0.32	1.02 ± 0.22	1.02 ± 0.13		
ICE	1.15 ± 0.12	0.94 ± 0.20	0.88 ± 0.13[a]	0.69 ± 0.09[abc*]		
FAN	0.97 ± 0.20*	0.84 ± 0.17*	0.79 ± 0.14*	0.82 ± 0.14	IV > ICE, FAN	A > B, C, D

Values are means ± S.D. [a]$P < 0.05$ *versus* A; [b]$P < 0.05$ *versus* B; [c]$P < 0.05$ *versus* C; and *$P < 0.05$ *versus* IV. Abbreviations: SD1, short axis of Poincare plot; SD2, long axis of Poincare plot; ApEn, approximate entropy; α_1, short-term fractal scaling exponent; and α_2, long-term fractal scaling exponent.

Table 3. Frequency-domain, geometrical and non-linear heart rate variability measures at the same heart rate following IV, ICE and FAN treatments

	IV	ICE	FAN
HR (beats min^{-1})	99.9 ± 3.8	97.1 ± 5.2	98.6 ± 7.7
ln VLF (ms^2)	5.47 ± 0.83	5.94 ± 0.77	6.54 ± 0.53*
ln LF (ms^2)	4.54 ± 0.65	5.24 ± 1.07	5.72 ± 1.30*
ln HF (ms^2)	3.07 ± 1.11	3.60 ± 1.45	4.78 ± 1.37*
LF/HF	5.44 ± 2.48	6.54 ± 3.74	3.30 ± 2.27
SD1 (ms)	6.06 ± 4.98	7.49 ± 3.52	13.07 ± 5.64*
SD2 (ms)	29.85 ± 10.57	45.79 ± 18.40	67.92 ± 17.12*†
SD1/SD2	0.20 ± 0.11	0.16 ± 0.05	0.20 ± 0.09
ApEn	0.75 ± 0.23	0.72 ± 0.17	0.57 ± 0.27
α_1	1.48 ± 0.26	1.54 ± 0.14	1.31 ± 0.30
α_2	1.03 ± 0.13	0.87 ± 0.16	0.89 ± 0.25

Values are means ± S.D. *$P < 0.05$ *versus* IV: †$P < 0.05$ *versus* ICE. Abbreviations: HR, heart rate; VLF, very low frequency; LF, low frequency; HF, high frequency; SD1, short axis of Poincare plot; SD2, long axis of Poincare plot; ApEn, approximate entropy; α_1, short-term fractal scaling exponent; and α_2, long-term fractal scaling exponent.

high and low, respectively, with both responding in a reciprocal fashion (i.e. decreased sympathetic and increased parasympathetic activity) with recovery (Brenner *et al.* 1997, 1998). With the addition of a hot environment during exercise, greater cardiac sympathetic activity has been observed during and following exercise, with a gradual decrease in sympathetic activity and reciprocal increase in parasympathetic modulation during recovery (Brenner *et al.* 1997, 1998). In the present study, recovery was initially dominated by low HRV that increased over time with the three cooling techniques effective in reducing T_C at rates similar to

or greater than that previously reported for air fanning (Wyndham *et al.* 1959; Mitchell *et al.* 2001), saline infusion (Frank *et al.* 1997) and ice packs (Kielblock *et al.* 1986). However, unique to the present study was that the autonomic control of HR was significantly different between treatments, with greater VLF, LF, HF, SD1 and SD2, indicating greater vagal modulation and/or reduced sympathetic modulation, for ICE and FAN compared with IV over the 40 min period.

During the first 10 min of recovery, T_C and HR were reduced from exercise levels for all treatments, with the lowest HR exhibited for FAN and a concomitant greater VLF compared with IV. A significant relationship between VLF and thermoregulation has been reported previously, with greater VLF exhibited in cold (12.8°C) compared with hot (35°C) environments (Fleisher *et al.* 1996; Thayer *et al.* 1997). Fleisher *et al.* (1996) reported that core, rather than skin, cooling was necessary for an increased VLF. In the present study, T_C was reduced to a similar extent for FAN and IV despite significantly different HR and VLF. Therefore, the greater VLF for FAN may be a result of non-thermoregulatory mechanisms, since this HRV component also reflects activities of the parasympathetic and renin–angiotensin–aldosterone systems (Taylor *et al.* 1998). Possible changes in hydration and/or plasma volume (Burklow *et al.* 1999; Frank *et al.* 2001; Carter *et al.* 2005) may have impacted on both the parasympathetic and renin–angiotensin–aldosterone systems, resulting in a greater VLF for FAN compared with IV. Greater parasympathetic activity and baroreflex gain have been observed in the hypohydrated compared with the euhydrated state (Charkoudian *et al.* 2003; Carter *et al.* 2005), and greater sympathetic activity exhibited with increases in plasma volume due to saline infusion (Burklow *et al.* 1999) and heat acclimation (Frank *et al.* 2001). During recovery in the present study, hydration status was not examined; however, given that the exercise responses were similar prior to each treatment (Sinclair *et al.* 2009) and that 1 l of saline was infused during IV in the first 10 min, it may be expected that overall hydration status and plasma volume were greater for IV. Subsequently, greater hydration/plasma volume for IV may have inhibited renin–angiotensin–aldosterone and/or parasympathetic modulations, resulting in an enhanced sympathetic activity and greater HR for IV compared with FAN. Furthermore, the significant and dramatic increase in plasma volume (1 l in 10 min) during IV may have activated cardiopulmonary reflexes (e.g. Bainbridge reflex), resulting in greater sympathetic activity and reflex tachycardia (Billman *et al.* 1981) compared with the FAN and ICE states. Significantly lower VLF, lower LF and HF and greater LF/HF for IV compared with FAN provide support for the lower parasympathetic and greater sympathetic modulation during IV compared with FAN. Infusion of the second litre of saline (10–20 min) would then maintain the volume-dependent regulatory mechanisms (i.e. lower parasympathetic and greater sympathetic modulation), resulting in lower HRV for IV compared with FAN throughout the remainder of the 40 min recovery. Although we have proposed a volume-dependent mechanism for the reduced HRV during IV compared with FAN, others have reported greater HRV and parasympathetic modulations following increased plasma volume at rest (Spinelli *et al.* 1999) and following maximal exercise (Buchheit *et al.* 2009*a*). Enhanced baroreflex inhibition of sympathetic efferent activity due to small plasma volume changes was suggested for the greater parasympathetic activity (Spinelli *et al.* 1999; Buchheit *et al.* 2009*a*). It may be that small plasma volume changes stimulate a baroreflex-mediated parasympathetic activation, whereas large and/or dramatic plasma volume changes result in an opposing effect (i.e. parasympathetic inhibition and/or sympathetic activation). Further studies may clarify this paradox.

Despite the aforementioned proposed volume–autonomic interaction, HR was progressively reduced for IV during recovery and may reflect a direct temperature influence on the sino-atrial node (Nishikawa & Namiki, 1988; Oyston *et al.* 1989). Since T_C and HR were reduced during recovery for all treatments, a direct influence of T_C on sino-atrial node function for all treatments cannot be excluded (Nishikawa & Namiki, 1988; Oyston *et al.* 1989) and may occur simultaneously with the previously mentioned volume–autonomic regulation of HR for all treatments. Furthermore, peripheral mechanisms may also contribute to HR dynamics during treatment of hyperthermia. During the initial 10 min of recovery, HR for ICE was significantly lower, with similar levels of HRV to that of IV. Given the proposed greater and similar level of dehydration for ICE and FAN, respectively, compared with IV, we would have expected a similar increase in VLF and HRV for ICE and FAN in line with the volume–autonomic interaction suggested above. However, this was not the case initially in recovery for ICE and may indicate competition between the volume–autonomic interaction and enhanced sympathetic activity via peripheral cold stimulation of thermoreceptors (Yu & Lumbers, 2000; Tulppo *et al.* 2005). Subsequent melting of the ice packs during recovery would then reduce this peripheral influence and result in a greater exhibition of the volume–autonomic interaction, greater HRV and greater parasympathetic modulations, similar to that exhibited during FAN.

Non-linear HR dynamics

During the 1970s, Kitney and colleagues (Hyndman *et al.* 1971; Kitney, 1975; Kitney & Rompelman, 1977) reported that thermoregulation influenced HR in a non-linear manner. Since then, non-linear dynamics and the quality of the HR signal have been examined, with particular

interest associated between short-term fractal properties of HR and adverse clinical events (Mäkikallio *et al.* 1997, 1999). To our knowledge, HR fractal properties have not been examined during hyperthermia or its treatment, with the present study being the first to document both short- and long-term HR fractal properties following heat-induced stress. In the present study, α_1 was near 1.5 at treatment commencement, reflecting a strong correlation of short-term HR dynamics and enhanced sympathetic modulation (Tulppo *et al.* 2001*a*, 2005) that was slowly reduced towards a fractal signal (∼1.0) and greater vagal modulation (Tulppo *et al.* 2001*b*) during recovery. Notably, this α_1 reduction was very slow over the 40 min recovery period, indicative of a continual sympathovagal imbalance that was also reflected by LF/HF values >1. The α_1 reduction was similar for each treatment, although it was non-significantly lower for the FAN compared with IV and ICE. Despite the lower values for FAN, no significant differences were noted for α_1 between treatments or over time despite simultaneous changes for spectral HRV components. This result was surprising given the advantages of non-linear HR measures over conventional power spectral HRV measures (Mäkikallio *et al.* 1996, 1997; Tulppo *et al.* 2001*a*), particularly at times of high sympathetic stimulation (Hautala *et al.* 2003). The lack of α_1 change with concurrent spectral HRV changes may reflect the level of HR disturbance following exercise-induced hyperthermia and a limitation of α_1 to demonstrate HR behaviour following heat-induced stress or the small sample size used in the present study.

In contrast to the results for α_1, long-term fractal scaling behaviour (α_2) was initially high and decreased over time, with greater values for IV compared with ICE and FAN. These results, along with greater ApEn for FAN, provide further support for greater vagal modulation, randomness and complexity in HR behaviour during FAN treatment compared with IV. Of particular interest was that HR complexity (ApEn) was substantially low for all treatments, indicating a significant and continual disturbance that increased over time towards normal resting ApEn values (Tulppo *et al.* 2001*a*). However, ApEn values were substantially lower for IV compared with FAN, and may indicate a potential concern during hyperthermia treatment, particularly in those with autonomic imbalance or myocardial electrical instability. Lower ApEn values have been observed in patients with cardiovascular disease (Mäkikallio *et al.* 1996, 1998) and prior to the development of cardiac arrhythmias (Vikman *et al.* 1999, 2001). As previously stated, the main goal of hyperthermia treatments is to reduce T_C in the shortest possible time and reduce the level of tissue damage (Hadad *et al.* 2004*b*). However, such treatments may also impact on the HR behaviour. The present results of unique HR dynamics following different cooling treatments, possibly via non-autonomic (temperature), autonomic activation and peripheral mechanisms, indicates a potential element that requires monitoring during treatment.

Heart rate dynamics at the same HR

Previously, similar (Kinugasa & Hirayanagi, 1999; Leicht *et al.* 2003) and varying levels of HR control (Tulppo *et al.* 1999; Leicht *et al.* 2008), as reflected by HRV, have been reported at the same HR. In the present study, HRV was significantly greater during FAN compared with IV, which provides further evidence of greater vagal modulation during FAN treatment. Furthermore, these results supplement the results of prior studies (Tulppo *et al.* 1999; Leicht *et al.* 2008) showing that autonomic regulation, as measured by HRV, can be completely different at the same HR and that HRV *per se* provides important regulatory information, since a pure HR does not reflect these potential differences in autonomic regulation.

Study limitations

Firstly, the present results were limited to a small sample size and to the treatment of exercise-induced hyperthermia in young healthy individuals. To our knowledge, HRV during treatment of heat stroke and/or greater T_C has not been examined; however, given that the cooling rates exhibited in the present study were similar to (Kielblock *et al.* 1986) or less than that previously reported during treatment of heat stroke (Hadad *et al.* 2004*a*,*b*), it may postulated that similar or greater HRV responses may also occur during heat stroke treatment that remain to be clarified. Secondly, the cooling treatments used in the present study were limited to simple field-based treatments, with other treatments, such as cold water immersion and/or treatment combinations (e.g. ICE + FAN) yet to be examined. Possibly, other cooling treatments or combinations may alter HR control to a larger extent and impact on cardiac function. Thirdly, respiration was not controlled during the hyperthermia treatments, with HR dynamics possibly influenced by differences in respiration (Brown *et al.* 1993; Penttilä *et al.* 2003). Since similar HRV was reported during metronome and spontaneous breathing rates up to 21 breaths min^{-1} (Patwardhan *et al.* 1995) and similar exercise was conducted prior to each treatment, the present results were likely to reflect the influences of the cooling treatments rather than possible respiration differences.

Conclusion

In conclusion, the present study demonstrated that HR dynamics vary with cooling techniques when treating exercise-induced hyperthermia. Although preliminary, these results may indicate an altered autonomic control of HR during certain cooling treatments that may

influence cardiac electrical stability in susceptible persons. Further study is necessary to examine the physiological mechanisms for the altered HR behaviour during cooling treatment of exercise-induced hyperthermia.

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