Echocardiographic changes following Active Heat Acclimation

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Key Words

heat adaptation; heat acclimation; plasma volume; stroke volume; diastolic function; echocardiography; preload

Abstract

Heat adaption through acclimatisation or acclimation improves cardiovascular stability by maintaining cardiac output due to compensatory increases in stroke volume. The main aim of this study was to assess whether 2D transthoracic echocardiography (TTE) could be used to confirm differences in resting echocardiographic parameters, before and after active heat acclimation (HA). Thirteen male endurance trained cyclists underwent a resting blinded TTE before and after randomisation to either 5 consecutive daily exertional heat exposures of controlled hyperthermia at 32°C with 70% relative humidity (RH) (HOT) or 5-days of exercise in temperate (21°C with 36% RH) environmental conditions (TEMP). Measures of HA included heart rate, gastrointestinal temperature, skin temperature, sweat loss, total non-urinary fluid loss (TNUFL), plasma volume and participant's ratings of perceived exertion (RPE). Following HA, the HOT group demonstrated increased sweat loss (p=0.01) and TNUFL (p=0.01) in comparison to the TEMP group with a significantly decreased RPE (p=0.01). On TTE, post exposure, there was a significant comparative increase in the HOT group in left ventricular end diastolic volume (p=0.029), SV (p=0.009), left atrial volume (p=0.005), inferior vena cava diameter (p=0.041), and a significant difference in mean peak diastolic mitral annular velocity (e') (p=0.044). Cardiovascular adaptations to HA appear to be

predominantly mediated by improvements in increased preload and ventricular compliance.

TTE is a useful tool to demonstrate and quantify cardiac HA.

Introduction

Thermoregulatory induced cardiovascular insufficiency impairs the ability to exercise in the heat[1,2]. Heat adaption through acclimatisation or acclimation (HA) improves cardiovascular stability by maintaining cardiac output, despite lowering resting heart rate (HR)[3], due to compensatory increases in stroke volume (SV)[1,4]. The increase in SV is predominantly thought to be due to an increase in plasma volume (PV)[5,6] however the effect size detected by meta-analysis is small[1]. Improvements in cardiovascular efficiency following HA have also been attributed to falls in the core and skin temperature accommodating a lower HR and consequent increased SV[7]. Other mechanisms include a reduction in sympathetic stimulation[8] which has been shown to reduce HR and induce plasma volume expansion in animal models[9]. Fundamental changes in the cardiac morphology have also been identified, following HA, with increased ventricular compliance[9,10] secondary to a redistribution of cardiac myosin isoenzymes and alterations in sarcoplasmic proteins in rat models[11,12]. This allows the ventricle to accept an increased preload and then to deliver an increased SV without an increase in end-diastolic pressure. This increase in compliance improves cardiac efficiency and pressure generation[10,13] so advantageously increasing cardiac output without a significant increase in HR. This suite of cardiovascular changes has not been conclusively demonstrated in human subjects.

This study was performed to specifically assess whether resting 2D trans-thoracic echocardiography (TTE) could be used to identify differences in measurable echocardiographic cardiovascular parameters following active HA in comparison to exercise in temperate conditions. A secondary aim was to investigate whether TTE could provide further insight into cardiovascular adaptations to heat and the underlying mechanisms in human subjects.

Methods

Participants

The study protocol was approved by Leeds Beckett University Research and Ethics Committee. Thirteen healthy male participants volunteered and gave written consent. All participants were amateur endurance trained cyclists, or triathletes, classified as performance level 3 by training or $\dot{V}O_{2peak}$[14]. Each participant was previously unacclimated to the heat and the acclimation sessions occurred during the winter-spring to reduce seasonal acclimatisation effects[15]. Additionally, participants were asked to avoid prolonged thermal exposures[16] (i.e. baths, saunas and steam rooms), during the trial period. Participants were instructed to maintain their normal training routines in the interval between initial measures and the intervention. Participants were requested not to undergo any training during the intervention period. All participants were asked to refrain from alcohol consumption at least 24 hours prior to any testing and to abstain from caffeine/stimulant containing drinks 6 hours prior to TTE.

Preliminary testing

Body composition was assessed by calibrated air displacement plethysmography (BOD POD, Life measurement systems, USA), and measurements of height (Seca, 220, Germany) and body mass (Seca, 770, Germany) were recorded. $\dot{V}O_{2peak}$($L.min^{-1}$) was determined from an incremental test to volitional exhaustion on a cycle ergometer (Wattbike, Trainer, UK) in temperate laboratory conditions (19.3 ± 1.6℃, 47± 6% relative humidity). Participants adjusted bike dimensions at baseline, and these then remained fixed for all experimental trials. Participants started cycling at ~150 watts, and increased the workload at a rate of 20 $W.min^{-1}$ until exhaustion or the individual's cadence dropped below 70 rpm[17]. Respiratory gases were measured continuously using breath-by-breath in-line gas analysis (Cortex, Metalyser, 3B, Germany), calibrated following the manufacturer's instructions. HR was

recorded continuously during all exercise tests (Polar, V800, Finland). $\dot{V}O_{2peak}$ was considered the highest $\dot{V}O_2$ in any 30s period in the last two minutes[18].

Echocardiography

Prior to intervention participants underwent a resting TTE (CX50, Phillips, USA) performed by a blinded British Society of Echocardiography (BSE) accredited practitioner. Echocardiography measures were performed in the resting partial left decubitus position with the couch head at 30° elevation (flat for subcostal views) satisfying the requirements of the BSE minimum data set[19]. Measures were averaged over 3 heart beats. TTE was performed at a similar time of day to avoid potential diurnal variation. Left ventricular stroke volume (SV; in cm^3) was calculated in two ways: 1) from the product of the velocity-time integral (cm) of the pulsed-wave Doppler in the left ventricular outflow tract (LVOT) and the LVOT cross sectional area (πr^2; in cm^2), determined by a TTE measurement of the LVOT in the parasternal long-axis view; 2) From the subtraction of the left ventricular end systolic volume (LVESV) from the left ventricular end diastolic volume (LVEDV) calculated using Simpson biplane method[19] in both two chamber and four chamber views, and then averaged. Atrial volumes were calculated using the area-length method[20]. The septal and lateral mitral annular peak velocities were averaged to give an overall e'.

Acclimation protocol

Participants were randomised to either an isothermic HA (HOT) (n=7) or temperate exercise intervention (TEMP) (n=6). The isothermic HA arm consisted of five consecutive daily exertional heat exposures performed in an environmental chamber at 32.1 ± 0.1℃ with 70.3 ± 1.1% relative humidity, utilising the controlled hyperthermia method which is potentially more effective in eliciting greater and more rapid HA than constant work or self-paced approaches[5,21]. This approach involves rapidly increasing core temperature (1-1.5℃) through

an active exercise phase, followed by a reduced exercise intensity to attain and maintain a core temperature (≥38.5°C). This was achieved by cycling at a relative exercise intensity of 2.0-2.7 W · kg^{-1} [22] to reach the target temperature in ~30-minutes and maintain it for 60 minutes with the work titrated to maintain this core temp. The TEMP group cycled for 90 minutes, every day for 5 days in temperate environmental conditions (21.5±1.2°C with 36.2 ± 5.6% relative humidity) at 2.0-2.7 W · kg^{-1}.

Two hours before arrival at each experimental exposure, participants consumed 500mL of water to ensure euhydration prior to each exposure. On arrival, participants provided a urine sample which was assessed for urine colour, osmolality (Osmocheck, Vitech, Scientific Ltd, Japan) and specific gravity (Hand refractometer, Atago, Tokyo, Japan). Participants were deemed euhydrated when urine osmolality was <700 mOsmol · kg^{-1}, specific gravity <1.020 and urine colour <3 on an 8-point scale[23].

Prior to commencing exercise, participants' nude body mass (NBM) was assessed and haemoglobin (Hb) concentration and haematocrit (Hct) measured, in triplicate, to calculate the plasma volume (PV) using the Dill and Costill method[24]. This was followed by 5-minutes rest in HOT or TEMP conditions, after which resting HR, gastrointestinal temperature (T_{gi}), skin temperature (T_{sk}), thermal sensation score (TSS)[25] and thermal comfort[26] (TC) were recorded and the physiological strain index (PSI) was calculated[27]. T_{gi} was measured by using a calibrated[28] ingestible core temperature pill (BodyCap, e-Celsius, France), set to record at 10s intervals, ingested >6 hours before the trial. Four skin temperature loggers (iButton TM, Integrated Products, USA) were attached to the right side of the body and mean skin temperature calculated from the equation by Ramanathan, 1964[29]. HR was monitored continuously whilst TSS, RPE, TC, Tgi, T_{sk} were recorded at 5-minute intervals.

NBM was measured post exposure and whole body sweat rate and total non-urinary fluid loss were calculated. Fluid intake was consumed *ad libitum* by participants and weighed to the nearest (g) to calculate whole body sweat rate ((preNBM – postNBM) + (fluid intake - urine output)/time). Following completion of the five-day acclimation/control the TTE, and PV was re-assessed within 24 hours.

Statistical Analysis

Measures were assessed for normality using the Shapiro–Wilk test prior to data analysis. Physical characteristics of the two groups were compared using an unpaired t-test. Physiological measures were compared between the HOT and TEMP groups on Day 1 and Day 5 using a two-way ANOVA. Nominal categorical variables were measured using a Kruskal-Wallis ANOVA on the Δ change (Day 5- Day 1) between HOT and TEMP.

The difference in TTE measures (post exposure-pre exposure) was calculated and compared between HOT and TEMP groups. Cohen's d effect size (ES) was calculated, together with 95% confidence intervals, using a threshold scale where an ES of 0-0.2 was considered trivial; 0.2-0.6, a *small* effect; 0.6-1.2, a *moderate* effect; 1.2-2.0, a *large* effect; and >2, a *very large* effect[30]. A 2-way ANOVA of TTE parameters was performed using HOT and TEMP and pre and post exposure. ES and CI were also calculated for the Δ change in PV between HOT and TEMP.

The α level was set to 0.05. All statistical analyses were performed using GraphPad Prism 8.0, GraphPad Software, San Diego, California.

Results

All participants completed the exposures in their entirety. The mean age, body surface area (Mosteller) and $\dot{V}O_{2peak}$ was 28.5 (±9.9), 1.93±0.1 m^2 and 59.7±8.1ml·kg·min^{-1} respectively.

There were no significant differences in age, height, body mass, body surface area, body fat percentage, or $\dot{V}O_{2peak}$ between HOT and TEMP groups.

There was a (9±12%) increase in PV in the HOT group with a -0.2±6% increase in the TEMP with a *moderate,* but insignificant, trend towards a Δ increase in PV ES 1.02 (CI -0.19 to 2.24) (p=0.102) as assessed by the Dill and Costill method[24]. Following HA, the HOT group demonstrated increased sweat loss, from day 1 to day 5 (0.36±0.28 L/min), in comparison to the TEMP group (-0.07±0.19L/min) (p=0.01) with Δ increases in total non-urinary fluid loss (TNUFL) (HOT; 548ml±430ml, TEMP; -110ml±291ml, p=0.01). There was no significant change, from day 1 to day 5, in resting heart rate (HOT; -5±4.6bpm, TEMP; 1±7.4bpm) or peak heart rate (HOT; -4.9±6.2bpm, TEMP; -2±9.4bpm) between groups. There was a significantly decreased RPE (p=0.01) in the HOT group (-2±1)in comparison to the TEMP group (0±1) but no significant difference in the T_{gi}, T_{sk}, TSS, TC or PSI. With regard to hydration status, there was no significant difference in urine osmolality, urine colour, urine specific gravity or body mass in comparing day 1 and day 5 exposure values between HOT and TEMP groups.

TTE data are detailed in Table 1. Pre-exposure, indexed left ventricular volumes (LVEDV HOT 80±7ml/m^2, TEMP 80±11ml/m^2) and atrial volumes (LA HOT 30±6ml/m^2, TEMP 31±11ml/m^2) were elevated in both groups in comparison to normal reference values[19]. There were no significant differences between the HOT and TEMP groups in any of the baseline TTE variables. The change in significant echocardiographic variables (post exposure -pre exposure), comparing the HOT and TEMP group, for LA Volume, SV, e', LVEDV and IVC diameter can be visualised in Figure 1 .

Discussion

The significant positive findings of this study are that a relatively short 5-day isothermic HA protocol, compared to equivalent temperate exercise, increases LA volume, SV, LVEDV, e' and IVC diameter. The LA and LV volumes were elevated in both groups at baseline, consistent with trained amateur athletes at performance Level 3. In this context, it is striking that the left atrial volumes and SV rose further following active HA in the HOT group; an effect that was not seen in the TEMP group who underwent matched exercise exposure in temperate conditions. The significant differences in thermal comfort, sweat loss and TNUFL following the active HA in the HOT group suggest at least partial acclimation.

The significant increase in LA volume and IVC diameter would suggest an increase in preload secondary to increased PV[6]. PV expansion is also likely to be at least partially responsible for the elevated SV[1,6,31]. However, despite a *moderate* effect size, the trend towards increased PV in the HOT group was insignificant when assessed by the Dill and Costill method[24]. This apparent contradiction might be explained by the small sample size as well as inaccurate methodology where the haemoconcentration effect is estimated by calculations based on haemoglobin concentration and haematocrit before and after exposure using the Dill and Costill method. Whilst inexpensive and rapidly repeatable there are multiple potential errors associated with this method[32–35]. Alternatively, trained individuals, such as in this study, may also have a lower PV adaptation response as measured by this methodology due to higher levels of background adaption secondary to the physical training[36] so requiring a longer acclimation protocol, and more participants, to exhibit the full phenotype.

PV expansion alone may not account for all the observed TTE data. The rise in the speed of early LV relaxation (e') during diastole reflects increased ventricular compliance or reduced LV stiffness. Increased compliance permits the heart to convert an elevated venous return, and consequently increased LV preload, into a higher end-diastolic volume, without elevation

of LV end-diastolic pressure (LVEDP). It is known that e', the average of septal and lateral peak mitral annular velocity during early LV relaxation, increases following long-term physical training[37] and increases further during exercise[38]. However e' transiently decreases immediately following high-volume exercise[39]. HA appeared to protect against this effect when comparing e' between HOT and TEMP groups. In rats, HA redistributes cardiac myosin isoenzymes from the fast V_1 isoform with high ATPase activity to the predominance of the slow V_3 form with low ATPase activity resulting in increased contractile efficiency[9] and increased LV compliance. This is not thought to be due to volume increases but a true change in heat-acclimated myocardial elastic properties[9]. The combined heat exposure in the HOT group (450 minutes) was significantly less than the stimulus in rodent studies[10,11] where intrinsic myocardial effects were demonstrated and arguably insufficient to alter myocardial elastic properties. However a high level of physical fitness is thought to improve the physiological response to exercise in the heat[40], perhaps by 'priming', leading to a more rapid acclimation[36] although possibly a lower magnitude of response[36] following full HA.

Furthermore e' is a relatively, but not absolutely, load (preload and afterload) independent measure of LV relaxation[41]**.** There was a non-significant (p=0.099) *moderate* increase in E (rapid early diastolic filling) in the HOT group post-exposure which was mediated, in part, by a reduction in E (post-exposure) in the TEMP group. Albeit non-significant, the increase in E-wave velocity following HOT exposure and the relative fall in E-wave following TEMP exposure appear to reflect the LV filling effects of increased and decreased PV respectively. These parallel changes in E and e' within the HOT group explain the minimal change in their ratio: E/e'. E/e' is commonly recognised as a surrogate of LV filling pressure and a predictor of LA pressure[42]. This would indicate that following isothermic HA, the increased preload, potentially mediated by an increased PV, was balanced by an increase in LV compliance so as to permit increased LV filling without an increase in LA pressure[43]. This balanced response is

mirrored by the behaviour of the left ventricle during exercise. In the healthy heart, the increase in velocity of early mitral inflow (E) during exercise, is balanced by the energy-requiring increase in the velocity of early ventricular relaxation (e'). The overall effect is that the ratio E/e' is maintained, or even falls slightly, during exertion. This reflects a maintenance of (or slight reduction in) LA pressure, in stark contrast to the rise in E/e' seen in the failing ventricle.

It is known that HA leads to relative bradycardia[7]. It is not possible, in the current study, to distinguish whether heat-induced bradycardia, resulting in prolonged diastole has enhanced filling, so as to augment SV, or whether a combination of enhanced LV preload and contractility have augmented SV sufficiently to permit the attainment of the body's cardiac output at a lower resting heart rate. Regardless of the relative contribution of these two mechanisms, it is interesting to observe the trend ($p=0.056$) toward a lower peak HR in the HOT group in comparison to the TEMP condition. Heat-mediated decrease in sympathetic outflow[5] is another potential mechanism for the augmentation of SV, via enhanced cardiac compliance.

Whilst changes of increased LA volume, LVEDV, SV and increased e' seen in the HOT group may all be seen following exercise training[37,44] we believe there are a number of reasons that a HA effect is a much more compelling explanation. First, the TEMP group underwent a very similar training stimulus over the 5-day intervention and they did not exhibit the same augmented chamber volumes or improved diastolic function. Second, all participants were performance Level 3 athletes and exhibited a degree of cardiac adaptation to exercise, which did not differ betwen groups. Finally, the speed of adaptation is more in keeping with HA than training adaptation, which can take up to a year[44].

Overall, these data demonstrate several concurrent cardiovascular adaptations occurring in response to HA. These findings are supported by the only other study, to our knowledge, to assess echocardiographic responses to heat exposure. These include a significantly increased LA volume, LVEDV and LV lateral e' following passive heat exposure in 12 male participants without a control group or markers of HA[45]. A further study assessed the effects of 14 – 21 days of passive HA with mild exercise routines in 8 patients prior to bypass surgery in comparison to ambient non-exercising controls. Here there was less diastolic dysfunction- by generated diastolic pressure curves in comparison to LV area- following surgery in the HA group in comparison to controls.

There are several limitations to this study which mean that whilst these data is hypothesis forming drawing firm conclusions on the observed changes on echocardiographic parameters is not possible. Our sample size was small (and curtailed by COVID-19 pandemic) and so likely lacked statistical power to show statistically significant changes in PV as well as HR (both peak and resting). Due to the limited prior studies assessing echocardiography in heat acclimation no formal power calculations were performed prior, however similar changes were observed in twelve participants without a control group with less robust passive heat stimulus[45]. Furthermore echocardiography has significant interobserver and intraobserver variability particularly in the measurement of LV volumes[46]. The measurement of SV by doppler assessment of the LVOT can be compromised by technical factors such as angular acuity of aortic blood flow, and/or off-axis aortic annular dimensions but has been shown to be reproducible. Studies have shown reasonable reproducibility with respect to diastolic function however[47].

A longer acclimation protocol may have more effectively demonstrated the underlying mechanisms. We chose a deliberately short but stressful humid isothermic HA exposure in

trained individuals which we considered might induce more rapid HA, particularly when incorporated with physical exercise[48–50], in comparison to passive heat exposure[1,51–53]. Furthermore the cardiac adaptations to HA are thought to manifest in the first 4-7 days[5]. Whilst we instructed participants to continue normal training patterns before initial measures, and not to exercise during the interventions, we did not record their exercise activity outside of the study nor ensure that there was no significant changes between groups which potentially could have introduced bias- particularly if the TEMP group continued to exercise. This may explain the trend in reductions in echocardiographic parameters in the TEMP group (Figure 1, Table 1).

Conclusion

This study is the first to show differences in TTE parameters following active HA and the first to control for a training effect with a temperate exercise arm. It also gives further insight into the cardiovascular adaptations to HA which are characterised by an increase in both pre-load and ventricular compliance as demonstrated by elevated LV chamber volumes, increased SV and an increase in e', with no concomitant change in E/e'. TTE is a useful tool to demonstrate and quantify cardiac adaptation. This has several implications to utilise TTE as a research tool in elite athletes[1,5] firefighters, miners, the military or aid workers[54] particularly in the development of tailored rapid HA regimes. Echocardiography in heat acclimation merits further study.

Conflict of Interest

The authors declare no conflict of interest.

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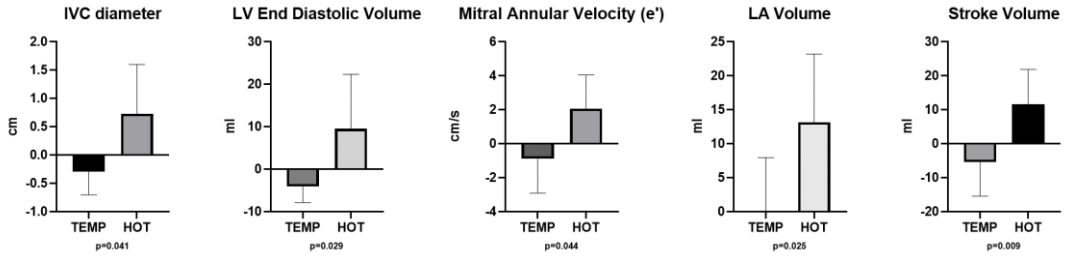

Figure 1: The change in significant ($p<0.05$) echocardiographic parameters from pre exposure to post exposure in the HOT (heat acclimation) and TEMP (temperate exercise) groups. LV; left ventricle, IVC; inferior vena cava.

		TEMP Mean (SD)		HOT Mean (SD)		Δ Cohen's D	P value
		Pre	Post	Pre	Post		
LV volumes							
EDV	(ml)	154 (13)	150 (15)	152 (21)	161 (16)	1.65 (0.39-2.91)	0.03*
ESV	(ml)	63 (4)	62 (6)	62 (12)	66 (12)	0.98 (-0.18-2.13)	0.17
SV (LVEDV-LVESV)	(ml)	91 (10)	88 (12)	90 (13)	96 (13)	1.29 (0.09-2.49)	0.05
SV (LVOT area x LVOT VTI)	(ml)	108 (16)	103 (9)	102 (9)	113 (8)	1.69 (0.42-2.96)	0.01*
Ejection fraction	(%)	59 (2)	58 (4)	60 (5)	59 (5)	0.17 (-0.92-1.27)	0.77
LV longitudinal function							
MAPSE (lateral)	(cm)	1.9 (0.4)	1.8 (0.2)	1.7 (0.3)	1.8 (0.3)	-0.06 (-1.19-1.07)	0.56
MAPSE (medial)	(cm)	1.4 (0.1)	1.5 (0.4)	1.5 (0.2)	1.4 (0.1)	0.11 (-1.02-1.24)	0.86
LV S'	(cm/s)	14 (2)	12 (1)	12 (2)	12 (2)	0.77 (-0.45-2.00)	0.97
RV longitudinal function							
TAPSE	(cm)	2.5 (0.3)	2.6 (0.3)	2.4 (0.2)	2.3 (0.3)	-0.75 (-1.88- 0.37)	0.17
RV S'	(cm)	16 (3)	13 (2)	15 (2.2)	13 (2.0)	0.41 (-1.71- 2.53)	0.50
Diastology							
E	(cm/s)	83 (20)	74 (8)	78 (13)	83 (6)	1.12 (-0.14-2.38)	0.10
A	(cm/s)	39 (12)	35 (7)	44 (14)	37 (7)	-0.29 (-1.48- 0.9)	0.11
E/A		2.3 (0.9)	2.2 (0.6)	1.8 (0.4)	2.3 (0.6)	1.08 (-0.18-2.33)	0.11
E'	(cm/s)	23 (4)	16 (4)	19 (2)	16 (4)	1.40 (0.10-2.71)	0.04*
A'	(cm/s)	10 (2)	8 (3)	10 (3)	9 (2)	0.65 (-0.57 -1.86)	0.31
Atrial Volumes							
LA volume	(ml)	57 (10)	53 (11)	58 (20)	67 (19)	1.91 (0.6-3.23)	0.005**
RA Volume	(ml)	60 (17)	60 (19)	68 (16)	76 (19)	0.57 (-0.54-1.68)	0.33
Inferior Vena Cava							
IVC diameter expiration	(cm)	2.0 (0.3)	1.7 (0.5)	1.8 (0.4)	2.5 (0.6)	1.60 (0.26-2.94)	0.03*
IVC diameter inspiration	(cm)	0.9 (0.2)	0.6 (0.4)	0.9 (0.3)	1.2 (0.3)	1.98 (0.6-3.36)	0.03*