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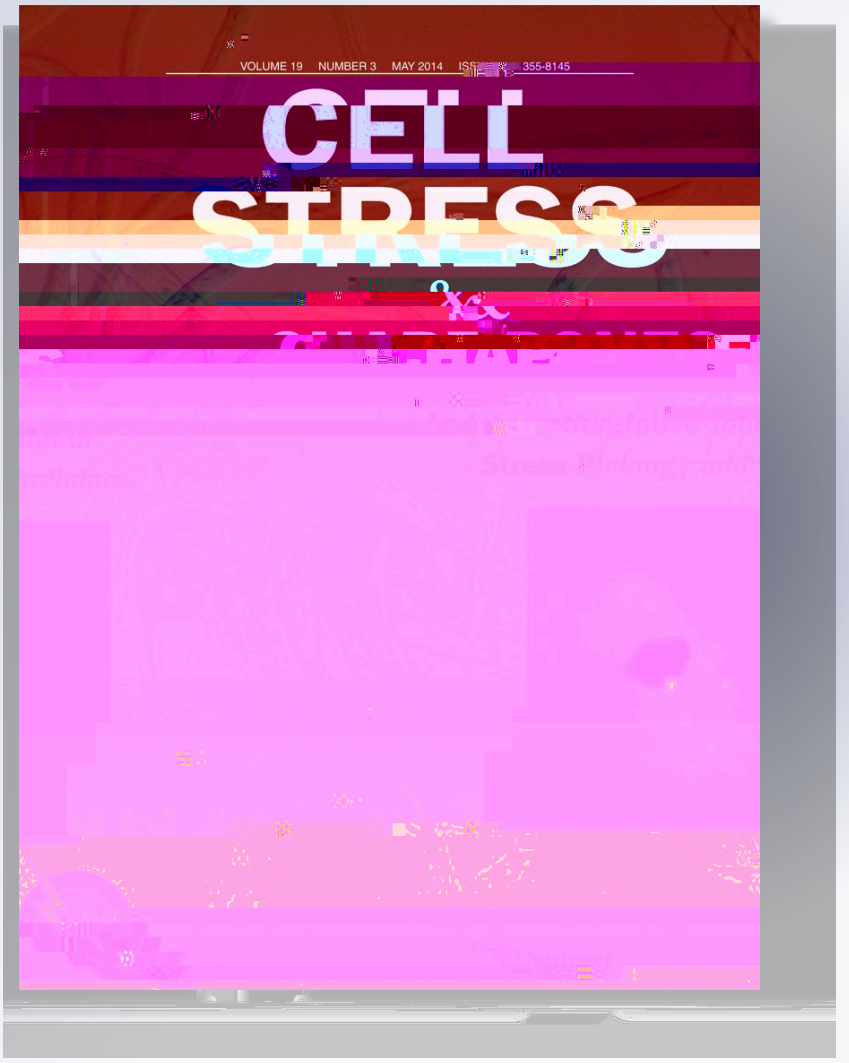

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ORIGINAL PAPER

Extracellular Hsp72 concentration relates to a minimum endogenous criteria during acute exercise-heat exposure

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catecholamines by supplementation (Whitham et al. 2006) or exercise response (Whitham et al. 2007), in addition to thermal change increase eHsp72. Acute exercise-heat stress presents both thermal and sympathetic challenge and as such, changes in concentration might be used to describe the magnitude of stress presented to an individual or system exercising in different environments.

eHsp72 has been detected in peripheral circulation of healthy individuals (Pockley et al. 1998) and is known to increase in response to single bouts of exercise (Walsh et al. 2001; Febbraio et al. 2002; Fehrenbach et al. 2005). Thermal, oxidative, metabolic and chemical stresses are well reported stimuli for increased concentrations of intracellular (iHsp72), and eHsp72 (Welch 1992; Morimoto et al. 1994). Exercise in hot and humid environments increases physiological strain on the body in comparison with temperate conditions (Galloway and Maughan 1997). Combined with exercise (exercise-heat stress), environmental manipulation to induce hyperthermia (Fehrenbach et al. 2001; Oishi et al. 2002; Moran et al. 2006; Whitham et al. 2007; Sandström et al. 2008; Iguchi et al. 2012) have been reported as stimuli for further increasing eHsp72 compared to exercise alone. Indeed a strong relationship exists between plasma eHsp72 and core temperature

After a full description of experimental procedures the protocol was approved by the institutional ethics committee and all subjects completed medical questionnaires and provided signed informed consent following the principles outlined by the Declaration of Helsinki of 1975, as revised in 2008.

Preliminary testing

Prior to undertaking the experimental trials of the study, volunteers attended the laboratories whereby their anthropometric data was collected for height (centimeter) using a fixed stadiometer (Detecto Physicians Scales; Cranlea & Co., Birmingham, UK), and body density using calipers (Harpenden, Burgess Hill, UK) and a four-site skin fold calculation (Durnin and Womersley 1974). Following determination of body density, % body fat was calculated according to the method described by Siri (1956). Nude body mass (NBM) was recorded to 0.01 kg from digital scales (ADAM GFK 150, USA).

$\dot{V}O_{2peak}$ was determined as a means for estimating pre testing aerobic capacity and exercise intensity for the subsequent testing protocols. Volunteers performed an incremental $\dot{V}O_{2peak}$ test on a cycle ergometer (Monark e724, Vansbro, Sweden) at a starting intensity of 80 W in temperate laboratory conditions (20 °C, 40 % relative humidity (RH)). Resistance was applied to the flywheel to elicit an increase of 24 W min^{-1} whilst the volunteer was informed to maintain a constant cadence of 80 rpm. The $\dot{V}O_{2peak}$ was considered as the highest $\dot{V}O_2$ obtained in any 10-s period and in line with the end-point criteria guidelines of the British Association of Sport and Exercise Sciences (Winter et al. 2007). Expired metabolic gas was measured using online gas analysis (Metamax 3X, Cortex, Germany). All preliminary testing was performed on the same ergometer (Monark, e724, Vansbro, Sweden). Heart rate (HR) was recorded during all exercise tests by telemetry (Polar Electro Oyo, Temple, Finland). Power outputs corresponding to 50 % $\dot{V}O_{2peak}$ were calculated from the $\dot{V}O_2$ /power output relationship. Saddle position was adjusted by the volunteer to their preferred cycling position and remained unchanged for all trials. During all trials, volunteers wore shorts, socks, and shoes.

Experimental protocol

Blood sampling and analysis

Venous blood samples were taken immediately pre- and post- and 24 h post-test TEMP, HOT and VHOT exercise. A 10-ml whole blood sample was drawn from the antecubital fossa. Each sample was divided equally into 5-ml tubes (Starstedt, Germany) containing EDTA as anticoagulant. Whole blood samples were centrifuged (Eppendorf 5804 R Centrifuge) at 4, 500 rpm for a period of 15 min to separate plasma. Plasma was pipetted (Eppendorf Research/Research Pro) into 1.5 ml microtubes (Eppendorf) and stored at −86 °C (Sanyo Ultra Low, VIP Series) until analysis which utilised a commercially available HSP70 high sensitivity enzyme immunometric assay kit (Enzo Life Sciences, Michigan, USA). Quantitative determination of the inducible Hsp72 was performed according to manufacturer's guidelines. Incubation of the 96 well kit, including the required quality control standards was performed on an orbital shaker (Heidolph Titramax 1000) at 600 rpm, and read by a plate reader using absorption at 450 nm (El_x 800 Universal Microplate reader, Bio-Tek Instruments). Plasma Hsp72 concentrations were corrected for changes in venous plasma volume (Dill and Costill 1974) with haemoglobin collected in duplicate using a microcuvette and analysed using a B-Haemoglobin Photometer (Hemocue Limited, Ängelholm, Sweden) and haematocrit collected in triplicate (~50 μl) with glass capillary tubes and analysed following centrifugation at 12 14,000 rpm for 3 min (Haemotospin 1300 Centrifuge, Hawksley & Sons Ltd, West Sussex, UK).

Accuracy of the sample data was ensured by plotting a graph for linearity between known sample concentrations and optical density. A linear trendline and equation was used to translate raw plate reader result into Hsp72 units (nanograms per milliliter). The intra/inter-assay variability was 10.5/17.36 %, respectively. The assay sensitivity is described by the manufacturer as 0.09 ng ml^{-1} and the detection range of the assays were 0.20 12.5 ng ml^{-1} for Hsp72.

Statistical analysis

All statistical calculations were performed using PASW software version 18.0 (SPSS, Chicago, IL, USA). All outcome variables were assessed for normality of distribution and sphericity prior to further analysis and deemed plausible in all instances unless otherwise stated. A two-way (time×trial) repeated-measures analysis of variance (ANOVA) was performed to test significance between and within trials. One-way ANOVA with repeated measures was used to compare physiological, perceptual and thermal data between exogenous environments, Bonferroni pairwise comparisons compared between separate exogenous temperature conditions.

Stepwise multiple regression analysis was performed for the six dependent variables which yielded the strongest relationship to the increase in eHsp72 concentration (rate of change in T_{rec} (degrees Celsius per hour), peak T_{rec} (degrees Celsius), mean T_{rec} for the final 60 min (degrees Celsius), duration $T_{rec} \geq 39.0$ °C (minutes), change in T_{rec} (degrees Celsius), duration $T_{rec} \geq 38.5$ °C (minutes)). Nine volunteers' data were used for the model as no eHsp72 was detected for one volunteer. Data was reported as mean±SD, with two

The change in T_{rec} was significantly different between conditions ($f = 33.621$, $p < 0.001$), but post hoc analysis only observed significantly greater differences between VHOT, and TEMP and HOT ($p < 0.001$). This was also true of the rate of T_{rec} increase ($f = 37.475$, $p < 0.001$), where VHOT elicited a significantly greater rate compared to TEMP and HOT ($p < 0.001$).

Area under the curve for T_{rec} of 38.5 °C ($f = 4.045$, $p = 0.035$) and 39.0 °C ($f = 7.163$, $p = 0.005$) (degrees Celsius per minute) were significantly different between conditions overall, VHOT was significantly greater compared with TEMP and HOT ($p = 0.003$ and $p = 0.013$), but no difference was observed between TEMP and HOT.

Duration spent with rectal temperatures of ≥38.5 °C ($f = 18.475$, $p < 0.001$) and ≥39.0 °C ($f = 9.631$, $p = 0.001$) (minutes) displayed significant main effect difference but was not different between TEMP and HOT, however VHOT was significantly longer than TEMP and HOT ($p = 0.014$ and $p = 0.06$).

Main effect for end T_{mu} was observed as significant ($f = 36.381$, $p < 0.001$). Significant difference was also found

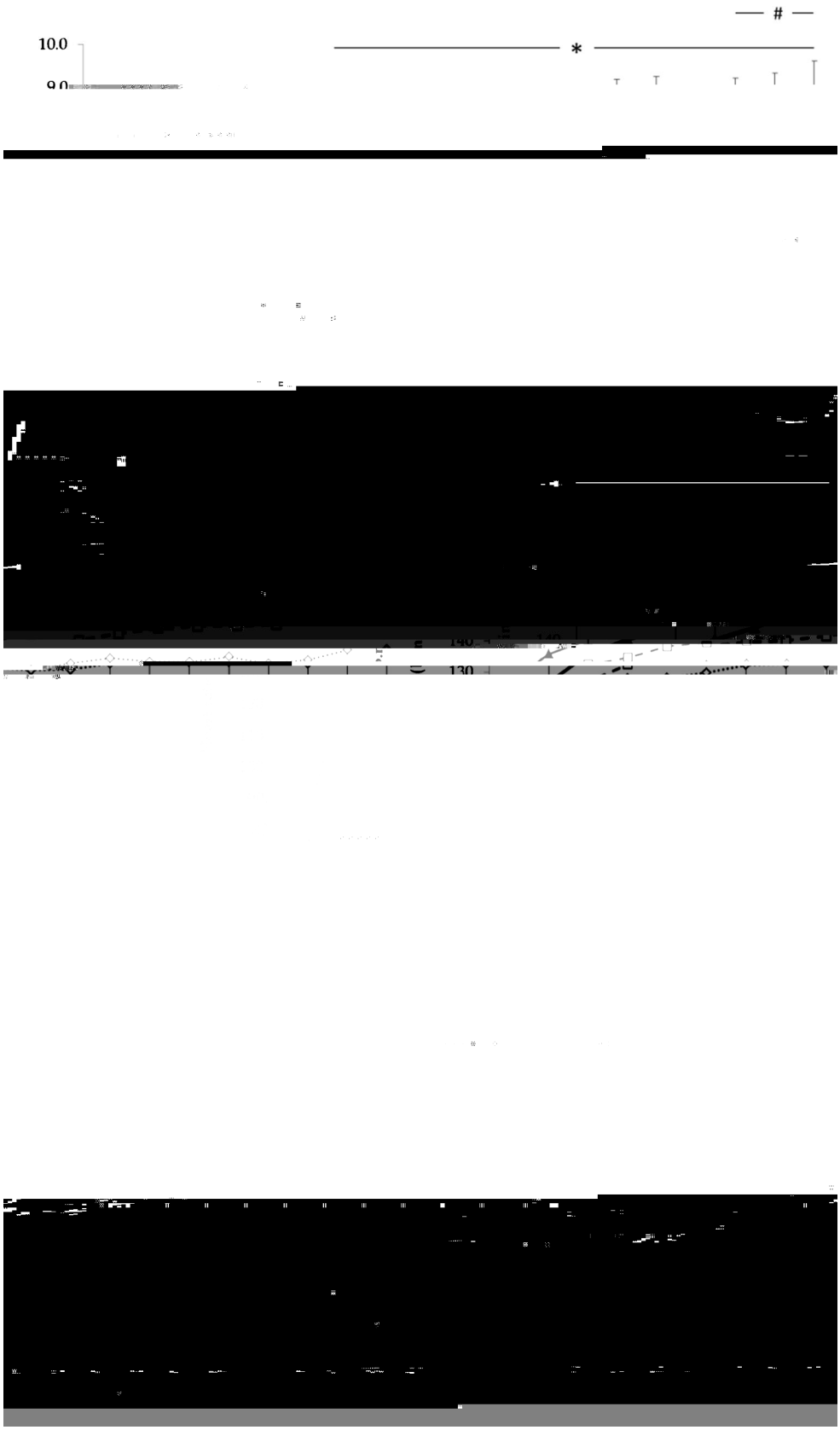

Fig. 1

to the present study (McClung et al. 2008; Magalhães et al. 2010; Périard et al. 2012). Established endogenous physiological and thermoregulatory parameters, particularly those less commonly reported in literature determining eHsp72 changes (rate of T_{rec} increase, area under the curve (AUC) for T_{rec} of 38.5 and 39.0 °C, duration $T_{rec} \geq 38.5$ and ≥39.0 °C), taken during each condition were analysed to determine whether they could be used to describe more effectively endogenous conditions leading to increased eHsp72 concentration.

The physiological and thermoregulatory responses to each exercise-heat stress condition were as expected for matched exercise in increasing thermal environments (Galloway and Maughan 1997; Maughan et al. 2012). Data observed three levels of strain between TEMP, HOT and VHOT conditions for peak HR, T_{rec}, PSI, and end T_{mu} [illegible]

has been shown to be exercise intensity and duration dependent in temperate conditions (Fehrenbach et al. 2005

Hepatosplanchnic, vascular and brain tissue, and peripheral blood mononuclear cells appear the principle sources of Hsp72 release into the systemic circulation (Febbraio et al. 2002; Lancaster et al. 2004; Lancaster and Febbraio 2005b; Johnson and Fleshner 2006). Concise reviews of the proposed active and passive mechanisms of eHsp72 release are presented by Lancaster and Febbraio (2005a), Fleshner and Johnson (2005) and Asea (2007). Briefly, it is proposed (Multhoff and Hightower 1996) that exosomes secreted following the fusion of multivesicular bodies with the plasma membrane, provide the secretory pathway for cells to activelyfollowing(9)-201.71.59acing(9)-201.71.d2-200-201.7mH620y e

changes in core temperature as a consequence of increased plasma catecholamines. Our data acknowledges the role of HR, and more specifically the elevated cardiac contribution to exercise in the VHOT condition in comparison to HOT and TEMP conditions as a further endogenous descriptor of eHsp72 release. It is therefore proposed that sympathetic activity, most rudimentarily measured from exercising HR, is an important component of the minimum endogenous criteria for increasing eHsp72 during exercise-heat stress, alongside the thermal criteria. Rather than increased temperature directly modulating elevated eHsp72 expression, it appears to be indirectly modulating it via increased HR, a reflection of increased adrenergic/catecholamine contribution to exercise-heat stress (Rowell et al. 1987).

It has been acknowledged by our data and others that core temperature (Ruell et al. 2006; Périard et al. 2012), rate of core temperature increase (Périard et al. 2012), and interestingly, aerobic capacity (Périard et al. 2012) are endogenous factors relating to Hsp72 increases. Périard et al. (2012) observed significant differences in HR despite no difference in eHsp72 increases between groups. In light of this, further work appears warranted to determine the role parasympathetic/sympathetic drive has in determining eHsp72 release during exercise-heat stress.

It is known that training status influences the basal and eHsp72 stress response to exercise-heat stress. In addition, prior HA, or progress towards the phenotype via endurance training may increase the immune response threshold for inducement of eHsp72 via exercise-heat stress. Njemini et al. (2004) also observed that inflammatory status, and its variable nature is also linked to eHsp72. Selkirk et al. (2008, 2009) acknowledged that the threshold for enhanced iHsp72 response, endotoxin leakage and inflammatory activation during exertional heat stress, in similar exogenous conditions to the present study, occurs at a lower temperature in untrained compared with trained subjects and support the endotoxin translocation hypothesis of exertional heat stroke, linking endotoxin tolerance and heat tolerance.

This individual and changing threshold along a continuum modulated by thermotolerance, inflammatory, and training status, suggests that prescription of exercise-heat stress exposure, administered controlling only simple parameters such as exogenous environment and work rate, may ultimately fail to stress sufficiently some individuals. The present data can therefore be used as a guide towards acute exercise-heat stress prescription. It is also important to consider that parameters appropriate for acute interventions shift with repeated exposures, as the HA phenotype and concurrent acquired cellular thermotolerance is enhanced (Sandström et al. 2008; Magalhães et al. 2010; Hom et al. 2012). Based upon these comments and the observation from the regression analysis that the rate of increase in T_{rec} (VHOT 1.56±0.53 °C h^{-1}) and the delta change in T_{rec} (VHOT 2.22±0.65 °C), it may be more appropriate to implement an isothermic (controlled hyperthermia) model of exercise-heat exposure (Garrett et al. 2009, 2011, 2012) where the rate of heat production can be accelerated (Amorim et al. 2008) and proposed minimum endogenous temperatures targeted (Amorim et al. 2011). This model requires greater exercise intensity during the early stages of the exposure, thus ensuring a more rapid increase 074776.98899

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