Amino Acids (2014) 46:1491–1499
DOI 10.1007/s00726-014-1721-3

ORIGINAL ARTICLE

Plasma and lymphocyte Hsp72 responses to exercise in athletes with prior exertional heat illness

Patricia A. Ruell · David Simar · Julien D. Périard · Stuart Best · Corinne Caillaud · Martin W. Thompson

Received: 1 August 2013 / Accepted: 3 March 2014 / Published online: 17 March 2014

Abstract We investigated the effect of exercise in the heat on both intracellular and extracellular Hsp72 in athletes with a prior history of exertional heat illness (EHI). Two groups of runners, one consisting of athletes who had a previous history of EHI, and a control group (CON) of similar age (29.7 ± 1.2 and 29.1 ± 2 years CON vs. EHI) and fitness [maximal oxygen consumption ($\dot{V}O_2max$) 65.7 ± 2 and 64.5 ± 3 ml kg^{-1} min^{-1} CON vs. EHI] were recruited. Seven subjects in each group ran on a treadmill for 1 h at 72 % $\dot{V}O_2max$ in warm conditions (30 °C, 40 % RH) reaching rectal temperatures of ~39.3 (CON) and ~39.2 °C (EHI). Blood was collected every 10 min during exercise and plasma was analysed for extracellular Hsp72. Intracellular Hsp72 levels were measured in both monocytes and lymphocytes before and immediately after the 60-min run, and then after 1 h recovery at an ambient temperature of 24 °C. Plasma Hsp72 increased from 1.18 ± 0.14 and 0.86 ± 0.08 ng/ml (CON vs. EHI) at rest to 4.56 ± 0.63 and 4.04 ± 0.45 ng/ml (CON vs. EHI, respectively) at the end of exercise ($p < 0.001$), with no difference between groups. Lymphocyte Hsp72 was lower in the EHI group at 60 min of exercise ($p < 0.05$), while monocyte Hsp72 was not different between groups. The results of the present study suggest that the plasma Hsp72 response to exercise in athletes with a prior history of EHI remained similar to that of the CON group, while the lymphocyte Hsp72 response was reduced.

Keywords Heat stroke · Plasma Hsp72 · Exercise · Peripheral blood mononuclear cells

P. A. Ruell (✉) · J. D. Périard · S. Best · C. Caillaud · M. W. Thompson
Exercise, Health and Performance Faculty Research Group, Faculty of Health Sciences, The University of Sydney, Lidcombe, P.O. Box 170, Sydney, NSW 1825, Australia
e-mail: patricia.ruell@sydney.edu.au

D. Simar
Inflammation and Infection Research, School of Medical Sciences, Faculty of Medicine, The University of NSW, Sydney, Australia

Introduction

Heat stroke is a potentially fatal illness which can present in two forms, classic heat stroke which affects the elderly or very young, and exertional heat stroke which is found mainly in young fit individuals following intense prolonged exercise (Moreau and Deeter 2005; Sawka et al. 2011). Diagnosis is based on an elevated body temperature (>40 °C) and the presence of neurological symptoms (Bouchama and Knochel 2002). Even with treatment, the illness is often fatal, or the patient is left with neurological sequelae (Leon and Helwig 2010). Postmortem analysis has shown that one of the most striking features of exertional heat stroke is that many organs are affected with haemorrhages (Malamud et al. 1946). Other findings include liver damage, renal dysfunction and disseminated intravascular coagulation that is a common, and in some cases fatal, complication of heat stroke (Sutton 1984; Leon and Helwig 2010).

The heat shock proteins (Hsps) range in size from 15 to 110 kDa and function by refolding other proteins and moving them between cellular compartments (Horowitz and Robinson 2007). The most studied member of this group, heat shock protein 72 (Hsp72), is strongly induced by heat, and can be protective against a subsequent stress that may be the same or of a different form (Kregel 2002).

In cellular models, injection of Hsp70 antibody renders the cell extremely sensitive to heat stress (Riabowol et al. 1988). Yang et al. (1998) found that upregulation of Hsp72 by both heat and chemical stress in a rodent model protected against a subsequent heat stress that normally induced heat stroke. Such studies point to the possible role of Hsp72 in the prevention of heat stroke in humans.

There is a natural variability in the basal synthesis and inducibility of Hsp72 in a human population (Lyashko et al. 1994; Boshoff et al. 2000) and it is possible that impaired Hsp72 expression and/or inducibility may lead to a greater susceptibility to exertional heat stroke. This is supported by observations in the elderly, where diminished induction of liver Hsp72 in response to heat stress is thought to be associated with increased mortality in conditions of high ambient temperature (Zhang et al. 2006).

While intracellular Hsps have a well-established role in protecting the cell against subsequent stress, the function of plasma Hsp72 is less well documented. It is thought that Hsp72 acts as a chemokine in the plasma, initiating an inflammatory response (Dybdahl et al. 2002). Recently, it has been suggested that lymphocytes are a major source of Hsp72 in the plasma during intense exercise (Heck et al. 2011). Hence, lower levels of leucocyte Hsp72 may also be reflected by reduced levels of plasma Hsp72. Analysis of plasma Hsp72 is more straightforward than leucocyte Hsp72, and this could form a useful screening test to identify athletes at risk of developing exertional heat illness (EHI).

Thus, the aim of this study was to determine the intra- and extracellular Hsp72 responses during exercise in the heat in athletes with a prior history of EHI compared with a control group who had never suffered from heat illness. We hypothesised firstly that plasma Hsp72 concentration would be reduced during and following an exercise challenge in the heat in athletes with a history of EHI compared with control athletes, and secondly that a similar difference between these groups of athletes would be found in lymphocytes and monocytes.

Methods

Subjects

Fourteen healthy young men (<40 years) participated in the study which was approved by the University of Sydney Human Ethics Committee. All subjects were medically screened and written informed consent was obtained after an explanation of the experimental protocol and possible risks were given.

The EHI group consisted of seven subjects who had previously experienced an episode of heat illness. Criteria for inclusion in the EHI group included treatment during or after a running event by medical personnel as well as a high rectal temperature (>41.0 °C), and/or rhabdomyolysis, and/or signs of neurological dysfunction such as loss of consciousness or confusion and/or combative behaviour. The control group (CON) consisted of seven subjects of similar age and aerobic fitness level as the subjects with heat illness. The main criterion for inclusion in the CON group was that they were athletes in training who had not experienced an episode of heat illness. Subjects were excluded from the study if they were taking medication or if they were suffering from cardiovascular or metabolic disease. Anthropometric and aerobic fitness levels of all the subjects in the study are presented in Table 1.

Table 1 Anthropometric and aerobic fitness values

	Control	EHI
Age (years)	29.7 ± 1.2	29.1 ± 2.2
Weight (kg)	73.8 ± 3.4	77.8 ± 2.6
Height (m)	1.79 ± 0.03	1.81 ± 0.02
Vo_2 max (ml/kg/min)	65.7 ± 2.0	64.5 ± 2.7

Values are mean ± SE

EHI exertional heat illness group, $\dot{V}O_2$max maximal oxygen consumption

Exercise tests

On the first visit to the laboratory, all subjects undertook a standard incremental gradient exercise test to volitional exhaustion on a treadmill in a temperate environment [20 °C, 40 % relative humidity (RH)] to determine their maximal oxygen uptake ($\dot{V}O_2$max). The incremental test consisted firstly of running for 4 min at four submaximal velocities (10, 12, 14 and 16 km h^{-1}), followed by a rest period, and secondly an increase in incline (2 % at 2 min intervals) until volitional fatigue while running at 10 km h^{-1}. The running velocity corresponding to 72 % $\dot{V}O_2$max was calculated from the $\dot{V}O_2$: running velocity relationship. Expired respiratory gas was collected using Douglas bags during the last minute of each work load while ventilation volume was measured with a Harvard dry gas metre (Harvard Ltd., Edenbridge, UK) and the volume was corrected to standardised temperature, pressure and dry gas (STPD). Pm1111E oxygen and IrI507 carbon dioxide analysers (Servomex, Sugar Land, USA) enabled the fractions of oxygen and carbon dioxide, respectively, to be determined.

The subjects returned for the exercise-heat test approximately 1 week after the $\dot{V}O_2$max test. Subjects were asked to refrain from vigorous physical activity and consumption of caffeine and alcohol in the preceding 24 h. On

presentation to the laboratory, subjects were weighed, a cannula was inserted into an antecubital vein and a resting blood sample was collected. The exercise-heat test was performed at a running velocity that elicited 72 % of the subject's $\dot{V}O_2$max in a climate chamber set to 40 % RH and ambient temperature of 30 °C. A fan was turned on after 10 min of exercise and set to an air velocity of 10 km h^{-1}. A blood sample (5–10 ml) was taken at 10-min intervals during exercise and immediately after the end of the exercise-heat test. The test was terminated after 1 h of running, at exhaustion, if the subject's core temperature increased beyond 39.9 °C, or whichever occurred first. A final blood sample was taken 1 h after the cessation of exercise.

During the exercise-heat test, heart rate was monitored continuously using a T-31 Polar transmitter–receiver (Kempele, Finland) and recorded every 10 min. Every 15 min, subjects were asked to rate their level of perceived exertion using the Borg category scale (Borg 1982) and thermal comfort was evaluated using the Bedford Thermal Comfort Scale (Bedford 1936).

Rectal temperature (T_{rec}) was measured and recorded on a Digisense 400 series portable data logger (Cole Parmer, IL, USA) as an index of core temperature using a YSI 400 series thermistor probe (Mallinckrodt Medical, MO, USA) inserted to a depth of 10 cm past the anal sphincter. Skin temperature was measured at four sites using iButtonTM temperature sensors/data loggers (Maxim Integrated Products, Sunnyvale, USA). An area-weighted mean skin temperature was calculated from the four sites (Ramanthan 1964). Rectal temperature was measured every 5 min while skin temperature was acquired every minute. Sweat rate was estimated by weighing the subject and their clothes before and after exercise, with corrections made for the water ingested during exercise and sweat contained within running attire. Expired gases were collected during the heat test at 10, 20 and 55 min and analysed as described previously. Subjects were permitted to drink water ad libitum during the exercise period.

Biochemical analysis

Samples for flow cytometry, plasma Hsp72 and for haematology measures were collected into EDTA tubes at rest, after 60 min of exercise and 1 h post exercise (1 h post) with additional samples for plasma Hsp72 collected every 10 min during the exercise period. The sample for plasma Hsp72 was immediately placed on ice, while samples for haematology and flow cytometry were kept at room temperature. Haematology analysis was performed using a Sysmex KX-21N instrument (Sysmex, Kobe, Japan) and lymphocyte and monocyte counts were corrected for changes in plasma volume as described previously (Dill and Costill 1974). Plasma Hsp72 was analysed using a commercial kit (catalogue number DYC1663, R and D Systems, MN, USA). Samples were diluted 1/5 in PBS containing 1 mM EDTA and 0.5 % Triton X-100 and assayed according to the manufacturer's instructions and previous research (Molvarec et al. 2009).

The expression of Hsp72 in monocytes and lymphocytes was measured by flow cytometry on a FACSCalibur (BD Biosciences, USA) as previously described (Simar et al. 2007). Briefly, 100 µl of EDTA-treated blood was mixed with 5 µl of anti-CD14 phycoerythrin (PE) conjugated or the corresponding isotype control and incubated in the dark for 20 min at room temperature. The erythrocytes were then lysed by incubating the cells in a solution consisting of 0.1 mM NaEDTA, 166 mM NH_4Cl and 10 mM $KHCO_3$, and then washing with phosphate-buffered saline (PBS, pH 7.4) supplemented with 1 % bovine serum albumin (BSA). Leucocytes were fixed using 100 µl of the reagent A from an Intrastain Fixation and Permeabilization Kit for flow cytometry (Dako, Glostrup, Denmark). For the permeabilisation step, 100 µl reagent B was mixed with the cells and 1 µg of either anti-Hsp72 FITC conjugated (Assay Designs, MI, USA) or the FITC-labelled mouse isotype control (BD Biosciences Pharmingen, CA, USA) were added and the cells incubated for 20 min on ice. After washing with PBS, the cells were fixed with 1 % paraformaldehyde (PFA) and stored at 4 °C in the dark until analysis. Monocytes and lymphocytes were differentiated according to size and granularity and using CD14 positive staining with a minimum of 10,000 cells included in the analysis. Data were analysed using Cytobank software (Cytobank Inc, CA, USA). The expression level of Hsp72 within each cell population was measured using the median fluorescence intensity (MFI) and background fluorescence was accounted for using the isotype control.

In vitro heat shock experiment

A resting blood sample from each subject was heated for 2 h at 42 °C and the cells were analysed by flow cytometry for monocyte and lymphocyte Hsp72. A similar heat shock protocol has been used previously (Fehrenbach et al. 2001).

Statistics

Statistical analysis was carried out using SPSS 19.0. Non-paired Student's *t* test was used to compare anthropometric parameters including weight, height, age, $\dot{V}O_2$max, maximum heart rate, skin temperature and sweat rate. A comparison was made between the two groups for monocyte and lymphocyte Hsp72 in the heat-shocked blood samples

using Student's t test. For other measures, a two-way ANOVA was conducted with repeated measures on time. To correct for violations of the assumption of sphericity with the repeated factor, the Huynh–Feldt correction was applied to the F ratio. Post hoc comparisons (Bonferroni) were used to further analyse the time effect where it was significant. When a group difference was found, each time point was assessed using a non-paired Student's t test. Plasma Hsp72 during exercise was tested for a linear trend with respect to time. A significance level of $p < 0.05$ was selected and results are reported as mean ± SE.

Results

Subjects

All runners in the EHI group had serious symptoms at the time of collapse (either signs of CNS dysfunction, and/or rhabdomyolysis). T_{rec} at collapse was 41.5 ± 0.4 °C (range 39.7–42.9 °C, $n = 6$). Rectal temperature was obtained either from the medical record ($n = 2$), or by self report. Time since collapse ranged from 6 weeks to 10 years.

There were no significant differences between the groups with respect to age, weight, height and aerobic fitness (Table 1).

Physiological responses to heat stress

Two subjects in the EHI group and one in the CON group reached a T_{rec} of 39.9 °C resulting in an early termination of the exercise-heat test at 50–55 min. Sweat rate at the end of exercise did not differ between groups (1.7 ± 0.1 vs. 1.4 ± 0.2 l/h CON vs. EHI). Heart rate increased over time during the exercise test ($p < 0.001$) with no difference between the two groups.

T_{rec} is shown in Fig. 1 with a similar response for the two groups. The T_{rec} after 60 min of exercise was 39.32 ± 0.18 and 39.19 ± 0.41 °C ($p > 0.05$, CON vs. EHI). Time since collapse did not affect T_{rec} since when the EHI group was further divided into those with collapse <1 year and >1 year, there was no difference when these subgroups were compared with the CON group ($p > 0.05$). This suggests that runners had recovered from any abnormal thermoregulatory responses that may have precipitated their collapse previously. Skin temperature was 33.43 ± 0.42 vs. 33.28 ± 0.35 °C after 60 min of exercise with no difference between groups ($p > 0.05$) (CON vs. EHI, respectively). There was a significant time effect for the rating of perceived exertion ($p < 0.001$) increasing from 12.4 ± 0.4 and 11.5 ± 0.5 (15 min) to 15.3 ± 0.6 and 14.7 ± 0.8 (CON vs. EHI) after 60 min of exercise with no difference between groups. There was a time effect for thermal comfort ($p < 0.005$) but no significant difference between groups.

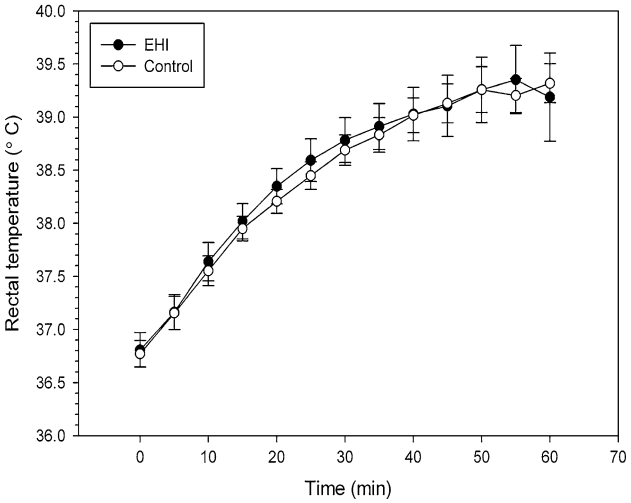

Fig. 1 Rectal temperature (T_{rec}) during prolonged exercise in the heat. *EHI* exertional heat illness group. A significant time effect was observed in both groups ($p < 0.001$)

Haematology

Lymphocyte and monocyte counts increased with time (Table 2; $p < 0.001$). Lymphocyte numbers were the same for both groups while there was a group effect for monocyte number ($p < 0.05$). There was no significant interaction between group and time. Post hoc analysis found a higher monocyte count in EHI compared with the CON group at 60 min and 1 h post exercise ($p < 0.05$).

Hsp72 expression

There was a significant time effect for plasma Hsp72 ($p < 0.001$) with no difference between groups (Fig. 2). Further post hoc analysis of the time effect showed that plasma Hsp72 was higher at 10 min of exercise compared to pre ($p < 0.005$) and all other time points were higher than pre ($p < 0.001$). The increase during exercise could be described by a linear trend line ($p < 0.001$).

Lymphocyte Hsp72 was significantly lower in EHI compared with the CON group (Fig. 3a, group effect, $p < 0.05$) with no time effect and no group by time interaction. The groups were significantly different at 60 min of exercise ($p < 0.05$) with a trend to significance at pre- and post-1 h ($p = 0.060$ and 0.065, respectively). Hsp72 expression in monocytes was not significantly different between groups ($p > 0.05$) and showed a significant increase with time (Fig. 3b; $p < 0.005$), with post hoc analysis demonstrating that post 1 h was greater than rest and 60 min (Fig. 3b; $p < 0.005$).

Table 2 Haematological parameters

	Group	0 min	60 min	1 h post	Group effect	Time effect
Monocyte count ($\times 10^9$/l)	Control	0.4 ± 0.1	0.5 ± 0.1**	0.4 ± 0.1**	$p < 0.05$*	$p < 0.005$
	EHI	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1		
Lymphocyte count ($\times 10^9$/l)	Control	1.5 ± 0.2	2.8 ± 0.3	1.1 ± 0.1	NS	$p < 0.001$
	EHI	1.5 ± 0.1	3.2 ± 0.3	1.2 ± 0.1		

Values are mean ± SE

Hsp72 heat shock protein 72, *EHI* exertional heat illness group, *NS* not significant

*Groups were significantly different $p < 0.05$; **post hoc testing showed significant differences between Control and EHI groups at these time points ($p < 0.05$)

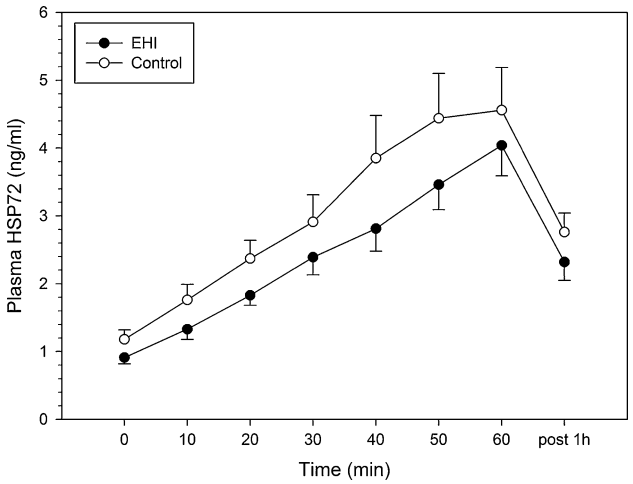

Fig. 2 Plasma Hsp72 during prolonged exercise in the heat. There was a significant time effect ($p < 0.001$) with no difference between groups

In vitro heat shock experiment

Blood collected at rest was heated in a water bath for 2 h and the leucocytes analysed for Hsp72 expression. There was no significant change in leucocyte number following in vitro heat shock ($p > 0.05$, $n = 13$). There was a significant induction of Hsp72 in both lymphocytes and monocytes with heating (Fig. 4; $p < 0.001$). In the heat-shocked samples, there was no difference between groups for monocyte Hsp72 while there was a trend for lymphocyte Hsp72 to be lower in the EHI group ($p = 0.086$).

Discussion

The aim of this study was to determine the Hsp72 responses in lymphocytes, monocytes and plasma during exercise in the heat in athletes with a prior history of EHI compared with a control group who had never suffered from heat illness. A unique feature of the present study was that the EHI athletes in this study were recruited on the basis of their medical history, and were not heat intolerant as determined from their rectal temperature rise during the exercise session (Fig. 1). Our study found that lymphocyte Hsp72 was lower in the EHI compared with the CON group, while no difference was found for monocyte and plasma Hsp72.

Plasma Hsp72 increased approximately fourfold with exercise, with no observed group difference. Similar increases in plasma Hsp72 have been noted previously at this exercise intensity and duration (Whitham et al. 2006). It has been suggested that Hsp72 is secreted into plasma via exosomes which are small vesicles that cross the plasma membrane (de Maio 2011). Various cells and organs have been identified as possible sources of plasma Hsp72 including lymphocytes (Heck et al. 2011), liver (Febbraio et al. 2002) and brain (Lancaster et al. 2004). Plasma Hsp72 functions as a signalling molecule, potentially acting as a danger signal in the cell and activating a pro-inflammatory response (Asea et al. 2002). An interesting observation was the rapid increase in plasma Hsp72 compared with rest after only 10 min of exercise ($p < 0.005$) (Fig. 2). Over the course of the exercise period, the plasma Hsp72 increased in a linear function. Most studies have only collected data on plasma Hsp72 before and after exercise, so serial data points every 10 min during exercise add to the limited information on the kinetics of plasma Hsp72. Further studies are needed to determine the source and role of Hsp72 in plasma.

In the present study, we found that athletes with a history of prior EHI had lower lymphocyte Hsp72 levels compared with the CON group. The finding of a lower lymphocyte Hsp72 level in EHI runners is consistent with previous research linking higher Hsp72 levels with protection against thermal stress (Yang et al. 1998). A previous study that categorised participants as heat intolerant in response to a 2 h walk in the heat found lower lymphocyte Hsp72 1 h post exercise (Moran et al. 2006), which is consistent with the present study. However, the athletes in the present study were no longer heat intolerant, suggesting

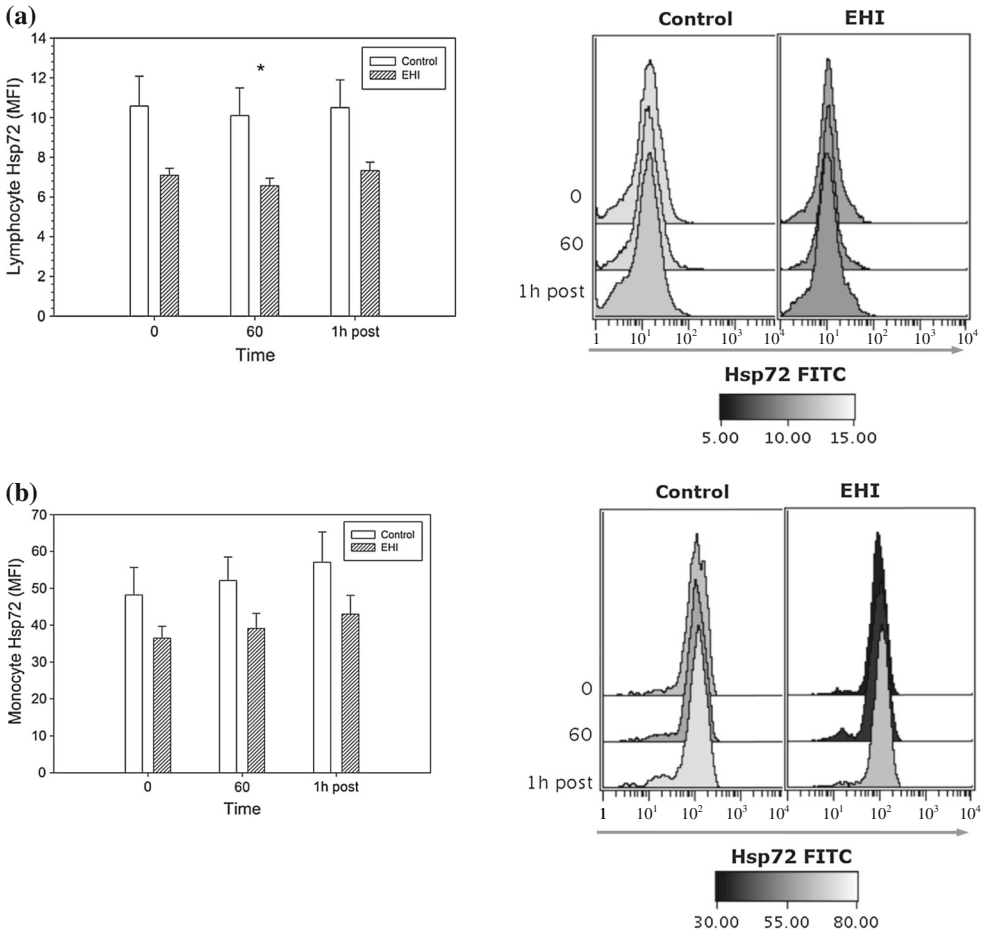

Fig. 3 Lymphocyte and monocyte heat shock protein 72 (Hsp72) expression as measured using median fluorescence intensity (MFI) before and after prolonged exercise in the heat in Control and exertional heat illness (EHI) groups. A typical example of flow cytometric analysis of blood from a Control and EHI athlete is shown to the right of each graph (arbitrary units). *FITC* fluorescein isothiocyanate, 0, resting sample; 60, immediately post exercise samples; 1 h post, 1 h post-exercise sample. **a** *Significant group effect (Control vs. EHI) ($p < 0.05$). Post hoc analysis demonstrated a difference between Control and EHI groups at 60 min of exercise. **b** Monocyte Hsp72 showed no difference between groups (Control vs. EHI)

that the impaired Hsp72 response was not a temporary response to the EHI episode, but an innate characteristic. A refinement of the present study was that lymphocytes and monocytes were differentiated by flow cytometry while the results in the aforementioned study (Moran et al. 2006) were based on a mixed lymphocyte/monocyte preparation. Such a difference in lymphocyte Hsp72 may prove useful in screening athletes at risk of developing EHI especially when simpler methods become available for performing the analysis. It should be noted that the present study is limited by the small number of subjects, and the fact that only young males were included. Further studies are needed with more subjects including females and older individuals to confirm the study findings.

Many studies on heat stress in athletes have examined the leucocytes partly because of their accessibility, but also because these are circulating cells that are exposed to the same temperatures and stressors as the various organs of the body (Sonna et al. 2002; Lovell et al. 2008). Leucocytes are often used in human studies because of their ease of isolation and it is tempting to infer that lymphocyte Hsp72 levels could be a reflection of levels in other tissues, although this is yet to be confirmed. In patients who underwent cardiac surgery, no correlation between heart muscle and lymphocyte Hsp72 levels was reported, suggesting that lymphocyte Hsp72 levels could not predict myocardial levels (Demidov et al. 1999). While our study has identified lower levels of Hsp72 in lymphocytes from the EHI group, this cannot be generalised to other organs. Hsp72 levels were approximately four times higher in monocytes than in lymphocytes and this is consistent with other studies (Oehler et al. 2001; Bautmans et al. 2005; Simar et al. 2007). During the inflammatory process, higher Hsp72 levels may be important in protecting monocytes against reactive oxygen species (Oehler et al. 2001). Surprisingly, we did not find a difference between EHI and CON subjects in monocyte Hsp72. Monocytes have a shorter life cycle in the circulation compared with lymphocytes, and they are more responsive to stress with increases in monocyte Hsp72 occurring at lower temperatures than lymphocyte Hsp72 (Oehler et al. 2001). It is possible that monocyte Hsp72 is more variable than lymphocyte Hsp72 with a rapid response to local stressful events.

We did not find any difference between the two groups in terms of Hsp72 inducibility in monocytes following a

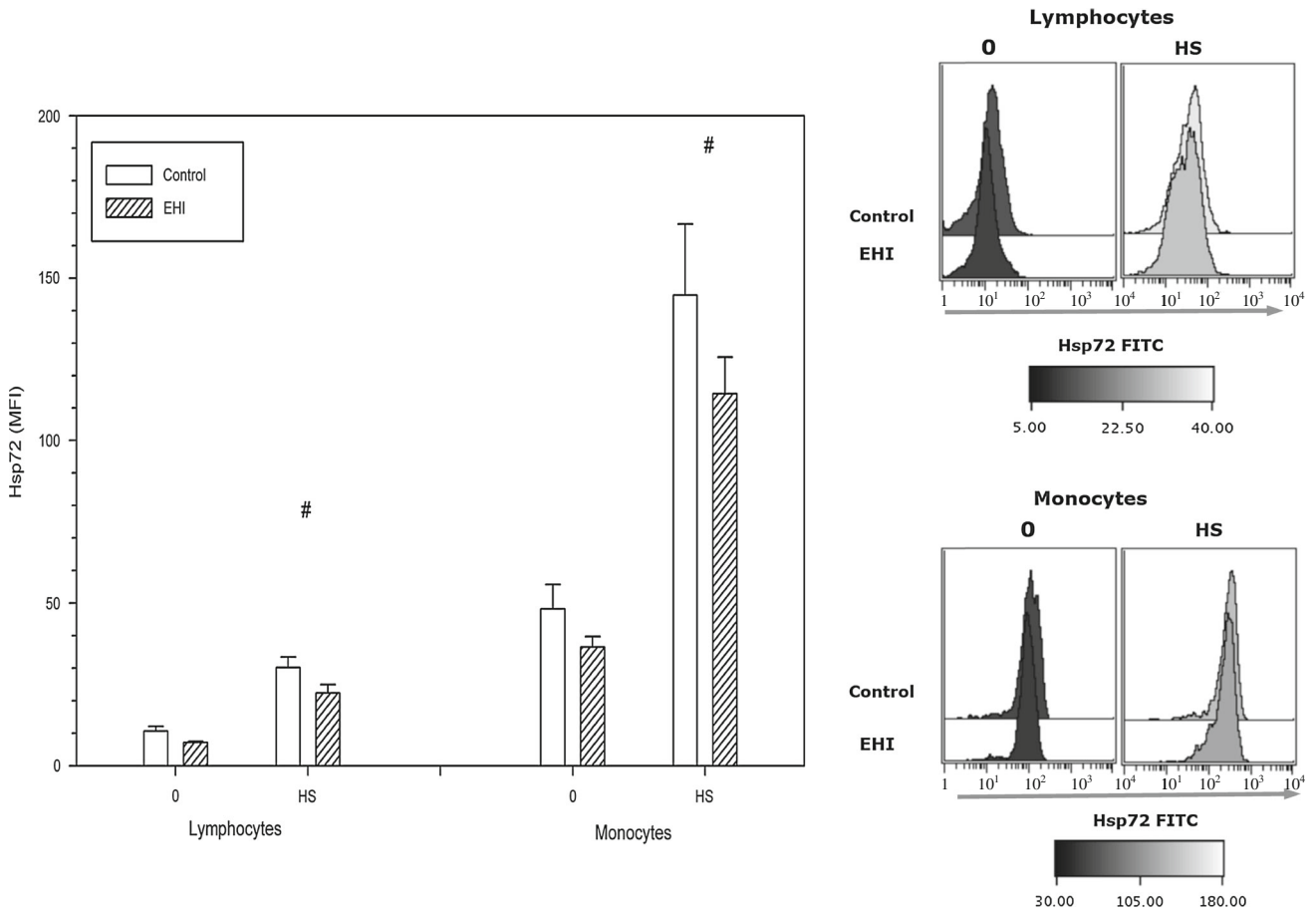

Fig. 4 Lymphocyte and monocyte heat shock protein 72 (Hsp72) expression as measured using median fluorescence intensity (MFI) before and after heat stress (HS) in Control and exertional heat illness (EHI) groups. A typical example of flow cytometric analysis of blood from a Control and EHI athlete is shown to the right of each graph (arbitrary units). 0, resting sample; HS, sample incubated for 2 h at 42 °C. #Significant temperature effect ($p < 0.001$). *FITC* fluorescein isothiocyanate

2 h in vitro heat shock, while lymphocyte Hsp72 levels tended to be lower in the EHI group ($p = 0.086$). In another study, induction of Hsp72 in lymphocytes from EHI patients did not differ from a control group shortly after the heat stress episode; however, 6 months later the recovered EHI patients tended to show higher levels of Hsp72 following heat shock (Xiao et al. 2003). The different trend in the present study may be due to regional/seasonal differences (the previous study was conducted in China) as it has been shown that the climatic conditions in which people live can have an impact on the cellular response to heat shock (Lyashko et al. 1994). The severity of the EHI suffered by the athletes prior to the present study was also greater with core temperatures usually exceeding 41 °C at the time of collapse, whereas in the Xiao et al. (2003) study mean oral temperature was 39.1 °C.

As leucocytes are a source of extracellular Hsp72, it was of interest to determine white blood cell number. A higher monocyte count was found in the EHI athletes with no difference in lymphocyte count between the two groups. It is known that trained athletes have higher numbers of total monocytes as well as the inflammatory subset CD14+CD16+ compared with untrained individuals (Selkirk et al. 2009). However, both groups of subjects in the present study were well matched in terms of $\dot{V}O_2max$ and age, and as such it is unlikely that training status can explain the difference in monocyte count. Inflammatory monocytes are responsible for transcription of cytokines (Selkirk et al. 2009) and are more sensitive to lipopolysaccharide. It should be noted that monocyte number for EHI runners was still in the normal range established for athletes previously (Horn et al. 2010). A higher monocyte count is potentially significant, given the ability of these cells to synthesise proinflammatory cytokines—thus further research is needed to measure monocyte number (both total and the inflammatory subset) in individuals with a prior history of EHI.

Conclusion

Our hypothesis that athletes with a prior history of EHI would demonstrate lower plasma Hsp72 concentration during exercise in the heat was not confirmed in this study. Our second hypothesis was partly confirmed with a significantly lower lymphocyte Hsp72 concentration in EHI runners, while monocyte Hsp72 was unchanged. The interesting possibility that a lower lymphocyte Hsp72 concentration during exercise might predispose these runners to develop EHI needs to be confirmed by further research.

Acknowledgments This study was supported by a grant from the NSW Sporting Injuries Committee. We are very grateful to the athletes for their time and effort in taking part in this study.

Conflict of interest The authors declare that they have no conflict of interest.

References

Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK (2002) Novel signal transduction pathway utilized by extracellular Hsp70. J Biol Chem 277:15028–15034

Bautmans I, Njemini R, Vasseur S, Chabert H, Moens L, Demanet C, Mets T (2005) Biochemical changes in response to intensive resistance exercise training in the elderly. Gerontology 51:253–265

Bedford T (1936) The warmth factor in comfort at work: a physiological study of heating and ventilation. In: McIntyre DA (ed) Indoor climate Industrial Health Research Board Report No 76, vol 76. HMSO, London

Borg G (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381

Boshoff T, Lombard F, Eiselen R, Bornman JJ, Bachelet M, Polla BS, Bornman L (2000) Differential basal synthesis of Hsp70/Hsc70 contributes to interindividual variation in Hsp70/Hsc70 inducibility. Cell Mol Life Sci 2000:1317–1325

Bouchama A, Knochel JP (2002) Medical progress: heat stroke. N Engl J Med 346:1978–1988

de Maio A (2011) Extracellular heat shock proteins, cellular export vesicles, and the stress observation system: a form of communication during injury, infection, and cell damage. Cell Stress Chaperones 16:235–249

Demidov ON, Tyrenko VV, Svistov AS, Komarova YY, Karpishenko AI, Margulis BA, Shevchenko YL (1999) Heat shock proteins in cardiosurgery patients. Eur J Cardiothorac Surg 16:444–449

Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37:247–248

Dybdahl B, Wahba A, Lien E, Trude H, Waage A, Qureshi N, Sellevold O, Espevik T, Sundan A (2002) Inflammatory response after open heart surgery: release of heat shock protein 70 and signalling through toll-like receptor 4. Circulation 105:685–690

Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH, Pedersen BK (2002) Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. J Physiol 544(3):957–962

Fehrenbach E, Niess AM, Veith R, Dickhuth H-H, Nothoff H (2001) Changes of Hsp72 expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature. J Leukoc Biol 69:747–754

Heck TG, Scholer CM, Bittencourt PIH (2011) Hsp70 expression: does it a novel fatigue signalling factor from immune system to brain? Cell Biochem Funct 29:215–226

Horn PL, Pyne DB, Hopkins WG, Barnes CJ (2010) Lower white blood cell counts in elite athletes training for highly aerobic sports. Eur J Appl Physiol 110:925–932

Horowitz M, Robinson SDM (2007) Heat shock proteins and the heat shock response during hyperthermia and its modulation by altered physiological conditions. Prog Brain Res 162:433–446

Kregel KC (2002) Invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92:2177–2186

Lancaster GI, Moller K, Nielsen B, Secher NH, Febbraio MA, Nybo L (2004) Exercise induces the release of heat shock protein 72 from the human brain in vivo. Cell Stress Chaperones 9:276–280

Leon LR, Helwig BG (2010) Heat stroke: role of the systemic inflammatory response. J Appl Physiol 109:1980–1988

Lovell R, Madden LA, McNaughton LR, Carroll S (2008) Effects of active and passive hyperthermia on heat shock protein 70 (Hsp70). Amino Acids 34:203–211

Lyashko VN, Vikulova VK, Chernicov VG, Ivanov VI, Ulmasov KA, Zatsepina OG, Evgen'ev MB (1994) Comparison of the heat shock response in ethnically and ecologically different human populations. Proc Natl Acad Sci USA 91:12492–12495

Malamud N, Haymaker W, Custer RP (1946) Heat stroke: a clinicopathologic study of 125 fatal cases. Military Surg 99:397–449

Molvarec A, Rigó J Jr, Lázár L, Balogh K, Makó V, Cervenak L, Mézes M, Prohászka Z (2009) Increased serum heat-shock protein 70 levels reflect systemic inflammation, oxidative stress and hepatocellular injury in preeclampsia. Cell Stress Chaperones 14:151–159

Moran DS, Eli-Berchoer L, Heled Y, Mendel L, Schocina M, Horowitz M (2006) Heat intolerance: does gene transcription contribute? J Appl Physiol 100:1370–1376

Moreau TP, Deeter M (2005) Heatstroke-predictable, preventable, treatable. JAAPA 18:30–35

Oehler R, Pusch E, Zellner M, Dungel P, Hergovics N, Homoncik M, Eliasen MM, Brabec M, Roth E (2001) Cell type-specific variations in the induction of Hsp70 in human leukocytes by feverlike whole body hyperthermia. Cell Stress Chaperones 6:306–315

Ramanthan NL (1964) A new weighting system for mean surface temperature of the human body. J Appl Physiol 19:531–533

Riabowol KT, Mizzen LA, Welch WJ (1988) Heat shock is lethal to fibroblasts microinjected with antibodies against Hsp70. Science 242:433–436

Sawka MN, Leon LR, Montain SJ, Sonna LA (2011) Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heatstress. Comp Physiol 1:1883–1928

Selkirk GA, MacLellan TM, Wright HE, Rhind SG (2009) Expression of intracellular cytokines, Hsp72, and apotosis in monocyte subsets during exertional heat stress in trained and untrained individuals. Am J Physiol 296:R575–R586

Simar D, Malatesta D, Badiou S, Dupuy AM, Caillaud C (2007) Physical activity modulates heat shock protein 72 expression and limits oxidative damage accumulation in a healthy elderly population aged 60–90 years. J Gerontol 62A:1413–1419

Sonna LA, Gaffin SL, Pratt RE, Cullivan ML, Angel KC, Lilly CM (2002) Effect of acute heat shock on gene expression by human peripheral blood mononuclear cells. J Appl Physiol 92:2208–2220

Sutton JR (1984) Heat illness. In: Strauss RH (ed) Sports medicine. W.B. Saunders, Philadelphia, pp 307–322

Whitham M, Walker GJ, Bishop NC (2006) Effect of caffeine supplementation on the extracellular heat shock protein 72 response to exercise. J Appl Physiol 101:1222–1227

Xiao C, Wu T, Ren A, Pan Q, Chen S, Wu F, Li X, Wang R, Hightower LE, Tanguay RM (2003) Basal and inducible levels of Hsp70 in patients with acute heat illness induced during training. Cell Stress Chaperones 8:86–92

Yang YL, Lu KT, Tsay HJ, Lin CH, Lin MT (1998) Heat shock protein expression protects against death following exposure to heatstroke in rats. Neurosci Lett 252:9–12

Zhang HJ, Doctrow SR, Oberley LW, Kregel KC (2006) Chronic antioxidant enzyme mimetic treatment differentially modulates hyperthermia-induced liver Hsp70 expression with aging. J Appl Physiol 100:1385–1391