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ORIGINAL ARTICLE

Hypoxia-mediated prior induction of monocyte-expressed HSP72 and HSP32 provides protection to the disturbances to redox balance associated with human sub-maximal aerobic exercise

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Abstract HSP72 is rapidly expressed in response to a variety of stressors in vitro and in vivo (including hypoxia). This project sought a hypoxic stimulus to elicit increases in HSP72 and HSP32 in attempts to confer protection to the sub-maximal aerobic exercise-induced disturbances to redox balance. Eight healthy recreationally active male subjects were exposed to five consecutive days of once-daily hypoxia (2,980 m, 75 min). Seven days prior to the hypoxic acclimation period, subjects performed 60 min of cycling on a cycle ergometer (exercise bout 1—EXB1), and this exercise bout was repeated 1 day post-cessation of the hypoxic period (exercise bout 2—EXB2). Blood samples were taken immediately pre- and post-exercise and 1, 4 and 8 h post-exercise for HSP72 and immediately pre, post and 1 h post-exercise for HSP32, TBARS and glutathione [reduced (GSH), oxidised (GSSG) and total (TGSH)], with additional blood samples obtained immediately pre-day 1 and post-day 5 of the hypoxic acclimation period for the same indices. Monocyte-expressed HSP32 and HSP72 were analysed by flow cytometry, with measures of oxidative stress accessed by commercially available kits. There were significant increases in HSP72 ($P < 0.001$), HSP32 ($P = 0.03$), GSSG ($t = 9.5$, $P < 0.001$) and TBARS ($t = 5.6$, $P = 0.001$) in response to the 5-day hypoxic intervention, whereas no significant changes were observed for GSH ($P = 0.22$) and TGSH ($P = 0.25$). Exercise-induced significant increases in HSP72 ($P < 0.001$) and HSP32 ($P = 0.003$) post-exercise in EXB1; this response was absent for HSP72 ($P \geq 0.79$) and HSP32 ($P \geq 0.99$) post-EXB2. The hypoxia-mediated increased bio-available HSP32 and HSP72 and favourable alterations in glutathione redox, prior to exercise commencing in EXB2 compared to EXB1, may acquiesce the disturbances to redox balance encountered during the second physiologically identical exercise bout.

Keywords Stress protein · Oxidative stress · Glutathione · TBARS · Exercise · Heat shock proteins (HSP) · Oxidative stress · Hypoxia · Redox balance · Human

Abbreviations

EXB1	Exercise bout 1
EXB2	Exercise bout 2
GSH	Reduced glutathione
GSSG	Oxidised glutathione
HSP	Heat shock protein
HSP32	Heme oxygenase
iHSP	Intracellular heat shock protein
LT	Lactate threshold
Maximal oxygen consumption	$\dot{V}O_{2\,max}$
mHSP72	Monocyte heat shock protein

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PHE_R	Prolonged hypoxic exposure at rest
PO	Power output
RONS	Reactive oxygen and/or nitrogen species
TGSH	Total glutathione
WBGT	Wet bulb globe temperature
W	Watts

Introduction

In vivo hypoxia reduces the partial pressure of oxygen within the arterial blood, thus disturbing whole body homoeostasis (Taylor and Pouyssegur 2007). Hypoxia and exercise-mediated stress can alter in vivo redox balance (Bailey et al. 2000), which can lead to increased oxidative stress within the skeletal muscle (Powers et al. 2010a) and the blood (Nikolaidis and Jamurtas 2009). Hypoxia-mediated disturbances to redox balance within the blood have been postulated as a stimulus for increases in inducible heat shock protein 70 (HSP72) in vivo (Taylor et al. 2010b, 2011a).

Increases in intracellular HSPs (iHSP), including HSP72 (Morton et al. 2009; Yamada et al. 2008) and heme oxygenase-1 (iHSP32) (Fehrenbach et al. 2003), play central roles resisting the in vivo exercise-induced disturbances to whole body homoeostasis (thermal, Magalhães et al. 2010; mechanical, Vissing et al. 2009; substrate depletion Febbraio et al. 2004; oxidative stress, Morton et al. 2009, etc.). These increases in iHSP concentration during and post-exercise stress function to maintain cellular integrity and functioning (Fehrenbach et al. 2003; Magalhães et al. 2010; Selkirk et al. 2009).

Such stress-induced increases in HSPs have been utilised within various successful preconditioning strategies in vitro (Madden et al. 2008a) and in vivo (Yogaratnam et al. 2010). In vitro, thermal (Lepore et al. 2000) and pharmacological (Kume et al. 2000) stimuli have been used to increase basal iHSP72 levels, which, in light of further cellular stress, demonstrate enhanced cellular survival/resistance, i.e. conferred cellular tolerance (Lepore et al. 2000; Madden et al. 2008a). In vivo, clinical preconditioning has seen hyperbaric oxygen delivery (Yogaratnam et al. 2010), and pharmaceutical (Pignatelli et al. 2011) and ischaemic preconditioning (Pasupathy and Homer-Vanniasinkam 2005) utilised to augment resistance to ischaemic reperfusion injury and oxidative stress during and post-surgery (e.g. coronary artery bypass graft surgery)—some of which has been attributable to HSP72 (Yogaratnam et al. 2007). Many of these approaches have statistically and clinically relevant experimental outcomes such as reduced pain, improved clinical result and reduced time spent within high dependency wards (Pasupathy and Homer-Vanniasinkam 2005; Yogaratnam et al. 2010). Similarly, in vivo in rodents (Shima et al. 2008), a thermal preconditioning stimulus conferred tolerance to exercise-induced challenges to redox balance. However, to the authors' knowledge, there is no in vivo human empirical data investigating the efficacy of similar preconditioning strategies (i.e. those that increase basal iHSP72 expression, or other stress proteins) in providing protection to the redox balance disturbances associated with prolonged aerobic exercise performance (Fisher-Wellman and Bloomer 2009).

The foremost endogenous non-enzymatic antioxidant in the body is glutathione. During exercise and exposure to hypoxia, reduced glutathione (GSH) protects against stress-induced reactive oxygen and nitrogen species (RONS) formation, actively detoxifying components of the various RONS cascades such as hydroxyl radicals, hydrogen peroxide and lipid peroxides (Nikolaidis and Jamurtas 2009; Powers and Jackson 2008). This cyclic process constantly occurs during homoeostasis and is accelerated in response to disturbances in redox balance, such as that which occurs with exercise (Fisher-Wellman and Bloomer 2009) and during in vivo (Taylor et al. 2010b, 2011a) and in vitro (Kiang and Tsen 2006) hypoxia.

This increased production and concentration of RONS during and post-exercise are negatively related to cellular function and homoeostasis, particularly during recovery from exercise when high levels can inhibit the onset of recovery (Ascensao et al. 2008, 2011). However, when maximum physiological exercise adaptation is sought within a tissue (muscle or blood), it is acknowledged that oxidative stress is likely central to mediate such remodelling of the tissue in question, and thus provides the stimulus for enhanced cellular tolerance to develop (Nikolaidis and Jamurtas 2009; Powers et al. 2010a). Therefore, the presence and function of exercise-mediated pro-radical production and thus disturbances in redox balance within the tissues (muscle and blood originated) evidently have both positive (Powers et al. 2010a; Nikolaidis and Jamurtas 2009) and negative (Ascensao et al. 2008; Powers et al. 2007) cellular effects, dependent on type, duration and location of origin. Thus, a preconditioning strategy, which could reduce such exercise-induced disturbances to redox balance, may be advantageous in an applied exercise setting, whereby recovery and return to optimal performance (i.e. repeated match play) is sought (Ascensao et al. 2008, 2011) rather than maximal physiological adaptation (Nikolaidis and Jamurtas 2009; Powers et al. 2010a). Additionally, many surgical procedures disturb redox balance (increase oxidative stress) (Arsalani-Zadeh et al. 2011; Tsuchiya et al. 2008); so, a preconditioning strategy which could reduce oxidative stress and thus improve recovery time from surgery would be advantageous (Pasupathy and Homer-Vanniasinkam 2005; Pignatelli et al. 2011).

Therefore, a preconditioning strategy that elevates basal values of HSP32, HSP72 (Kalmar and Greensmith 2009) and GSH (Fisher-Wellman and Bloomer 2009) may augment the body's antioxidant capacity and widen the defence network, and thus could be beneficial in reducing the negative redox balance-associated effects on recovery from exercise and surgery.

It is hypothesised that the hypoxic acclimation period will increase basal iHSP72 and iHSP32 expression in addition to favourable alterations in blood redox balance status, which will blunt aerobic exercise-induced disturbances to redox balance and the HSP response within the blood. The aims of the present study were to investigate the effect of: (1) once-daily hypoxia for five consecutive days on basal HSP32 expression; (2) prior induction of HSP32 and HSP72 on the exercise-induced stress protein response; (3) prior induction of HSP32 and HSP72 on exercise-induced disturbances in redox balance.

Methods

Ethical approval

The protocol was approved by the University of Hull Sport, Health and Exercise Science Departmental Human Ethics Committee and all subjects signed informed consent following the principles outlined in the Declaration of Helsinki.

Experimental overview

Simplistically, an exercise bout was performed before and after 5 days of once-daily normobaric hypoxia (75 min, 2,980 m). The blood-borne variables to be assessed in response to both exercise bouts and the hypoxic acclimation period were iHSP72, iHSP32, glutathione and TBARS. Such an approach was used to assess the influence of the hypoxic acclimation period on exercise-induced disturbances to redox balance and basal HSP expression. Typical exercise physiology measures were assessed throughout both exercise bouts. Additionally, heart rate and oxyhemoglobin saturation were monitored during the daily hypoxic exposures.

Subjects and general experimental controls

Eight healthy recreationally active male subjects [mean ± SD 20.8 ± 3.2 years, 1.77 ± 15.7 cm, 72.1 ± 11.0 kg, physical activity 6.8 ± 1.8 h/week and power output (PO) at lactate threshold (LT) 184 ± 37 Watts (W)] volunteered to participate in the study. The confounding variables of smoking (Anbarasi et al. 2006), caffeine (Lu et al. 2008), glutamine (Singleton et al. 2004), generic supplementation (Hillman et al. 2011), thermal exposures (Selkirk et al. 2009), hypoxic exposures (Taylor et al. 2010b), hyperbaric exposures (Taylor et al. 2011b) and alcohol (Taylor et al. 2010a) were all controlled in line with previous work in the field (Taylor et al. 2011a). Compliance for all the aforementioned potential confounding variables, if not excluded during subject recruitment, was monitored via a questionnaire administered before, during and post the extended 13-day study period and was 100 % in all subjects.

Subjects remained within the temperature-controlled laboratory during the hypoxic exposures and exercise bout 1 (EXB1) and exercise bout 2 (EXB2; mean ± SD: WBGT 21.2 ± 0.2 °C, humidity 47 ± 4 %). Subjects did not sleep within the laboratory, but within a nearby university-owned accommodation block for the night prior to and during all days of the study. Subjects gained 9.8 ± 2.2 h of sleep prior to study commencement and repeated this sleep cycle on all study nights (10.7 ± 1.5 h). Methods, procedures and restrictions replicated those used previously to gain serial data for *mHSP72* (Taylor et al. 2010a, b, 2011a, b) and other physiological markers of homoeostasis (Madden et al. 2008b; Vince et al. 2009) to control the influence of confounding factors aside from the hypoxic exposures and the two exercise bouts on the blood-borne markers to be assessed. Due to experimental limitations (equipment and subject availability), a double-blind crossover design with a control normoxic intervention was unable to be employed.

Preliminary measurements

All subjects underwent initial LT testing on an SRM cycle ergometer (Schoberer Rad Mebtechnik, Konigskamp, Germany) using an incremental protocol starting at 125 W, increasing 20 W every 4 min until exhaustion. Capillary blood samples were collected every 2 and 4 min into lithium heparinised microvettes (Microvette CB300, Sarstedt, Nümbrecht, Germany) and analysed using a blood lactate analyser (YSI 2300 STAT, YSI Inc, Yellow Springs, OH). LT was calculated using the Dmax method (Cheng et al. 1992). All tests were conducted at sea level within a temperature-controlled laboratory. Upon completion of the LT test, subjects completed 30 min of cycling (familiarisation) at 90 % of their LT, on the cycle ergometer to be used for both exercise bouts. Subjects were also familiarised to the physiological data collection battery to be used (RPE, finger prick blood sampling, etc.) and the "medical type" of environment both exercise bouts would take place within.

Hypoxic protocol and blood sampling

Hypoxia was generated and delivered via a hypoxicator (HYP123 Hypoxicator, Hypoxico, New York, USA) which

utilises oxygen filtration to generate the necessary hypoxic load. The hypoxic exposure consisted of 75 min at a simulated altitude of 2,980 m, which equates to an oxygen concentration of 14.5 % at a barometric pressure of 775 mmHg, with the intervention commencing and ceasing at 0930 and 1045 hours, respectively. Heart rate and oxygen saturation were recorded every 5 min during the hypoxic exposure via a finger pulse oximeter (Nonin 9550 Onyx II Finger Pulse Oximeter, Nonin Medical Inc, Plymouth, USA).

Subjects were thoroughly habituated to the hypoxic protocol; such a protocol has been used previously (Taylor et al. 2010b, 2011a). The exposure was administered daily for five consecutive days at rest. In light of previous work (Taylor et al. 2011a) utilising a 10-day acclimation period, the current project employed only 5 days of exposures, due to the previous project (Taylor et al. 2011a) demonstrating an initial rapid phase of *mHSP72* accumulation in response to hypoxia within the first 5 days of exposures. The physiological response (mean oxyhaemoglobin saturation and heart rate) to an identical hypoxic exposure has been detailed elsewhere (Taylor et al. 2010b, 2011a), with the results within the present study in line with these previous observations.

Venous blood samples were taken immediately prior to the first hypoxic exposure (hypoxic day 1) and 30 min post-final hypoxic exposure (hypoxic day 5). The day-to-day changes in hypoxia-induced *mHSP72* expression and other haematological markers during repeated daily PHE_R have been demonstrated elsewhere in detail (Taylor et al. 2011a). All blood samples were drawn from the antecubital vein into potassium EDTA Vacuette tubes (HSPs and TBARS; Vacuette®, Greiner BIO-one, UK) or sodium citrate Vacuette tubes (glutathione; Vacuette®, Greiner BIO-one, UK) in both the exercise and hypoxic experimental periods.

Exercise protocol and blood sampling

Seven days prior to the hypoxic acclimation period, subjects performed 60 min of cycling on an SRM cycle ergometer at 90 % of their power output at LT (EXB1). This exercise bout was repeated 1 day post-cessation of the hypoxic period (EXB2). LT-determined exercise intensities are recognised as a more rigorously controlled exercise stress, than for example percentages of maximal oxygen consumption ($\dot{V}O_{2\,max}$), with this approach used previously in other HSP-related exercise studies (Morton et al. 2006, 2008). Exercise was conducted at sea level and within a temperature-controlled laboratory. Exercise commenced at 0930 hours on each exercise day; this consistent timing of blood sample collection was important to account for diurnal and circadian variations in basal *mHSP72* (Sandstrom et al. 2009; Taylor et al. 2010a), especially as basal values can dictate the magnitude of stressor-mediated changes in HSP72, both in vivo (Gjovaag and Dahl 2006) and in vitro (Vince et al. 2010). Physiological measures of exercise performance (heart rate, ratings of perceived exertion, blood lactate) in EXB1 and EXB2 were recorded throughout to ensure any changes in the biochemical measures of stress protein expression (*mHSP72* and *mHSP32*), and alterations in redox balance (plasma TBARS and whole blood glutathione) were not associated with variations in performance intensity between bouts. Venous blood samples were taken immediately pre-, post- and 1, 4 and 8 h post-exercise for *mHSP72* and immediately pre-, post- and 1 h post-exercise for *mHSP32*, plasma TBARS and glutathione. The sampling was conducted in this time-dependant manner due to previous literature demonstrating HSP32, plasma TBARS and glutathione returning to values approximate to control between 30 min and 1 h post-exercise (Fisher-Wellman and Bloomer 2009).

Prior to reporting to the laboratory, subjects were instructed to drink 500 ml of water 2 h prior to all (including preliminary testing) exercise bouts and hypoxic exposures, in accordance with the ACSM position stand (Sawka et al. 2007). Hydration status prior to physical activity was assessed by plasma osmolarity utilising the freeze point depression method (Advanced Instruments Model 3320, Advanced Instruments Inc, Norwood, Massachusetts, USA). If subjects' plasma osmolarity was >279 mOsm/l, it was deemed that subjects were/or were approaching a hypohydrated state. This experimental control was not violated for any subjects for any experimental procedure or intervention.

Measurement of oxidative stress

Whole blood glutathione

It is important to note that sodium citrate-treated blood was collected for analysis of glutathione. Immediately after collection, a 2-ml aliquot of fresh blood was mixed with 8 ml of freshly prepared 5 % meta-phosphoric acid (Sigma-Aldrich Company Ltd., Dorset, England). This mixture was transferred to 1.5-ml eppendorf tubes, stored on ice for 15 min, and then centrifuged at 13,000×*g* and 4 °C for 15 min. The supernatant was collected and stored at −80 °C for later analysis of total glutathione (TGSH) and GSSG. This immediate preparation of glutathione-specific blood samples is important to ensure that glutathione reductase activity is limited to an absolute minimum and thus glutathione ratios are a true reflection of the

exercise and hypoxic intervention. The risk of artifactual alterations in redox balance as a consequence of inappropriate tissue handling is something which the present study has actively sought to neutralise (therefore, removing it as a potential confounding factor) in line with recent guidelines (Powers et al. 2010b).

TGSH and GSSG were analysed using a commercially available kit (Total Glutathione Detection Kit, Assay Designs, Ann Arbor, MI). Briefly, for determination of TGSH, previously prepared blood (described above) was diluted to 1:40 in a 1× assay buffer solution and transferred to a 96-well plate. A standard curve was produced from serially diluting 50 µl 1× assay buffer and 50 µl GSSG. A reaction mixture containing 20 µl of glutathione reductase was added to all wells on the plate, which was subsequently read at 405 nm every minute for 10 min in a microplate reader. All standards and samples were analysed in triplicate. TGSH laboratory (2.54 %; 2.41 %) and project-specific (1.87 %; 1.91 %) intra- and inter-assay coefficient of variation, respectively, were acceptable.

For determination of GSSG 1 µl of 2 M 4-vinylpyridine (Sigma-Aldrich Company Ltd., Dorset, England) solution was added to 50 µl of previously prepared blood samples (described previously). At the same time, 3 µl of 2 M 4-vinylpyridine solution was added to 150 µl GSSG for standard curve determination. Samples were incubated at room temperature for 1 h, after which 5 µl of 4-vinylpyridine-treated blood samples was added to 45 µl of assay buffer in a 96-well plate. To create a standard curve, 50 µl of the standard curve solution was added to 50 µl of assay buffer and serially diluted. All standards and samples were analysed in triplicate. GSSG laboratory (1.77 %; 1.76 %) and project-specific (1.76 %; 1.74 %) intra- and inter-assay coefficient of variation, respectively, were acceptable.

Plasma TBARS

An EDTA Vacuette tube (Vacuette®, Greiner BIO One, UK) filled with venous blood was mixed and then centrifuged at 1,500×*g* for 10 min, with the resulting EDTA plasma removed and stored at −80 °C. This plasma was, at a later date, analysed for lipid peroxidation using a commercially available kit following manufacturer's instructions (ZeptoMetrix, USA) as used previously elsewhere (Peart et al. 2011; Taylor et al. 2010a, b, 2011b; Hillman et al. 2011). Previously frozen plasma samples, obtained via centrifugation on their first thaw, were brought to room temperature. An aliquot (100 µl) was added to 100 µl of sodium dodecyl sulphate (SDS) solution and 500 µl of thiobarbituric acid solution. Samples were incubated for 1 h at 95 °C, after which time they were cooled to room temperature and centrifuged at 3,000×*g* for 15 min. Samples were added to a 96-well plate and read at 532 nm in a microplate reader (Biotek Synergy HT-R, Biotek Instruments, Vermont, USA). Results are expressed in malondialdehyde (MDA) equivalents. All standards and samples were analysed in triplicate. TBARS laboratory (1.96 %; 1.99 %) and project-specific (2.02 %; 1.98 %) intra- and inter-assay coefficient of variation, respectively, were acceptable.

Monocyte HSP72 and HSP32 assay

The choice, justification and measurement of *mHSP72* have previously been discussed in detail (Sandstrom et al. 2009; Taylor et al. 2010a). The assay has been extensively used within in vivo interventional experimental designs exploring exercise and environmental stress (hypoxia, hyperbaria) HSP responses (Hillman et al. 2011; Peart et al. 2011; Sandstrom et al. 2009; Taylor et al. 2010a, b, 2011a, b; Vince et al. 2010, 2011). An IgG1 (HSP72) or IgG2 (HSP32) isotype and concentration-matched FITC-conjugated negative control was used to asses non-specific binding. Briefly, cells obtained after red cell lysis were fixed and permeabilised (AbD Serotec, UK) and a negative control (FITC, AbD Serotec, UK) or anti-HSP72 antibody (SPA-810, Assay Designs, USA) or anti HSP32 antibody (OSA-111, Assay Designs, USA) was added to a final concentration of 100 µg/ml. This was used to label 1×10^6 cells according to the manufacturer's instructions and then incubated for 30 min in the dark. Samples were then analysed on a BDFACSCalibur (BD Biosciences) by flow cytometry with monocytes gated by forward/side scatter properties and further discriminated by CD14 expression. Mean fluorescence intensity (MFI) was then calculated using CELLQuest software (BD Biosciences) with a total of 50,000 cells counted.

Statistical analyses

All statistical analyses were completed using IBM SPSS Statistics 18 (SPSS Inc., Chicago, IL). Statistical assumptions were checked using conventional graphic methods and were deemed plausible unless stated otherwise. Central tendency and dispersion are reported as the mean (SD). The effect of exercise bout and time on *mHSP72*, *mHSP32*, GSSG, GSH, TGSH, plasma TBARS, lactate and heart rate were investigated using linear mixed models. The effect of the hypoxic intervention on *mHSP72*, *mHSP72*, GSSG, GSH, TGSH and plasma TBARS was investigated using paired *t* tests. The *mHSP72* and *mHSP32* expression were expressed as a percentage of the first measurement, which was in accordance with previous literature (Hillman et al. 2011; Morton et al. 2006; Peart et al. 2011; Taylor et al. 2010a, b, 2011a, b). The GSSG, GSH and TGSH data were

also expressed as a percentage of the first measurement in accordance with previous literature (Hillman et al. 2011). Relationships between the changes in *mHSP32* and *mHSP72* and markers of oxidative stress (GSH, GSSG and plasma TBARS) were investigated using Pearson's correlations. Two-tailed statistical significance was accepted as $P < 0.05$.

Results

There were significant mean increases of 67.2 % in *mHSP72* (95 % CI 52.9–81.4 %; $t = 11.1$, $P < 0.001$), 25.4 % in *mHSP32* (95 % CI 3.2–47.6 %; $t = 2.7$, $P = 0.03$), 7.1 % in GSSG (95 % CI 5.1–9.1 %; $t = 9.5$, $P < 0.001$) and 2.6 malondialdehyde equivalents in plasma TBARS (95 % CI 1.5–3.6 malondialdehyde equivalents; $t = 5.6$, $P = 0.001$) in response to the 5-day hypoxic intervention, whereas no significant changes were observed for GSH ($t = 1.3$, $P = 0.22$) and TGSH ($t = 1.2$, $P = 0.25$) (see insets of Figs. 1, 2, 3, 4, 5, 6).

There were significant exercise bout × time interaction effects for *mHSP72* ($F = 10.4$, $P < 0.001$) and *mHSP32* ($F = 3.9$, $P = 0.034$) (Figs. 1, 2). For the first exercise bout, a pronounced 105.5 % increase in *mHSP72* was observed from pre- to immediately post-exercise (95 % CI 54.2–156.9 %; $P < 0.001$), thereafter decreasing to about 150 % of the pre-exercise value and remaining relatively stable for the remainder of the post-exercise period.

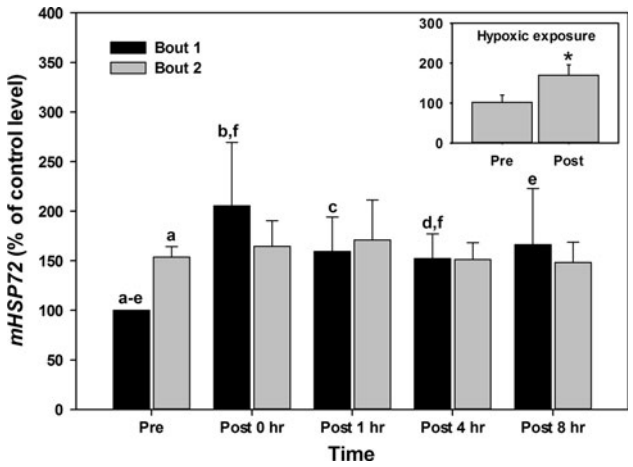

Fig. 1 Mean (SD) *mHSP72* expression immediately pre and post, and 1, 4 and 8 h post-exercise. Bout 1 and bout 2 refer to two bouts of 60 min of exercise at 90 % of lactate threshold, separated by 5-day hypoxic intervention period. The *inset graph* shows the mean (SD) *mHSP72* expression before and after the 5-day hypoxic intervention period. The *mHSP72* is expressed as a percentage of the first measurement. *Like letters* represent significant differences between mean values. Post significantly higher than pre (*$P < 0.05$)

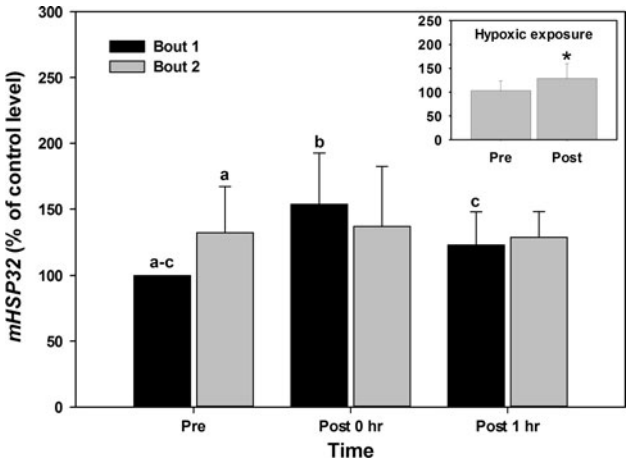

Fig. 2 Mean (SD) *mHSP32* expression immediately pre and post, and 1, 4, and 8 h post-exercise. Bout 1 and bout 2 refer to two bouts of 60 min of exercise at 90 % of lactate threshold, separated by the 5-day hypoxic intervention period. The *inset graph* shows the mean (SD) *mHSP32* expression before and after the 5-day hypoxic intervention period. The *mHSP32* is expressed as a percentage of the first measurement. *Like letters* represent significant differences between mean values. Post significantly higher than pre (*$P < 0.05$)

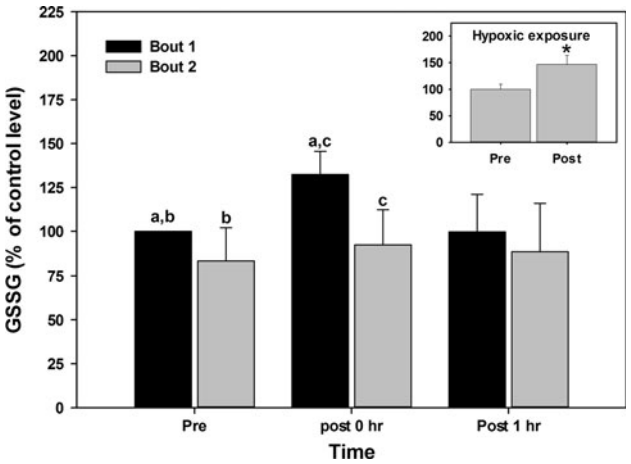

Fig. 3 Mean (SD) whole blood GSSG expression immediately pre, post and 1 h post-exercise. Bout 1 and bout 2 refer to two bouts of 60 min of exercise at 90 % of lactate threshold, separated by the 5-day hypoxic intervention. The *inset graph* shows the mean (SD) GSSG before and after the 5-day hypoxic intervention period. *Like letters* represent significant differences between mean values. Post significantly higher than pre (*$P < 0.05$)

A similar response also was observed for *mHSP32* ($P = 0.003$). In contrast, no significant changes were observed in response to the second exercise bout for *mHSP72* ($P \geq 0.79$) or *mHSP32* ($P \geq 0.99$). When comparing across exercise bouts, only the pre-exercise values were significantly different for *mHSP72* ($P < 0.001$) and *mHSP32* ($P = 0.024$), with higher values in EXB2 compared to EXB1.

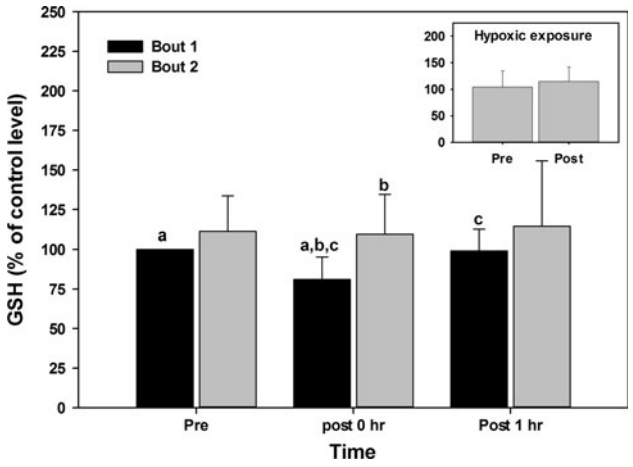

Fig. 4 Mean (SD) whole blood GSH expression immediately pre, post and 1 h post-exercise. Bout 1 and bout 2 refer to two bouts of 60 min of exercise at 90 % of lactate threshold, separated by the 5-day hypoxic intervention period. The *inset graph* shows the mean (SD) GSSG before and after the 5-day hypoxic intervention period. *Like letters* represent significant differences between mean values

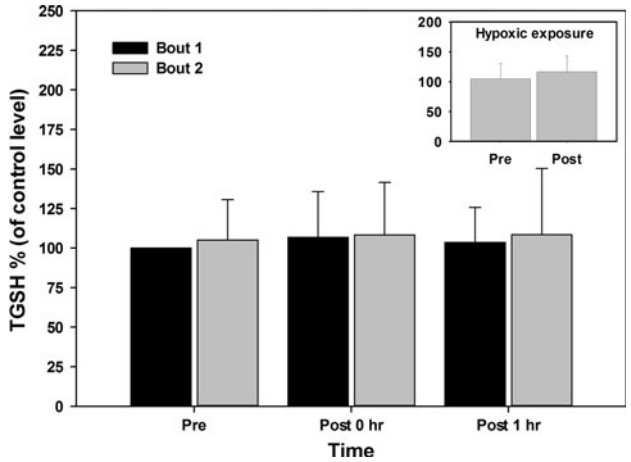

Fig. 5 Mean (SD) whole blood TGSH expression immediately pre, post and 1 h post-exercise. Bout 1 and bout 2 refer to two bouts of 60 min of exercise at 90 % of lactate threshold, separated by the 5-day hypoxic intervention. The *inset graph* shows the mean (SD) TGSH before and after the 5-day hypoxic intervention period

Significant main effects for exercise bout ($F = 23.4$, $P = 0.001$) and time ($F = 12.9$, $P = 0.001$), and a significant exercise bout × time interaction ($F = 21.5$, $P < 0.001$) were observed for GSSG (Fig. 3). The GSSG was 16.5 % lower pre-exercise (95 % CI 3.5–29.5 %; $P = 0.018$) and 39.9 % lower immediately post-exercise (95 % CI 27.2–52.6 %; $P < 0.001$) for the second exercise bout compared to the first. Furthermore, a significant 32.5 % increase in GSSG was observed from pre-exercise to immediately post-exercise for the first exercise bout (95 % CI 19.0–45.9 %; $P < 0.001$), whereas no significant change was observed for the second ($P = 0.26$).

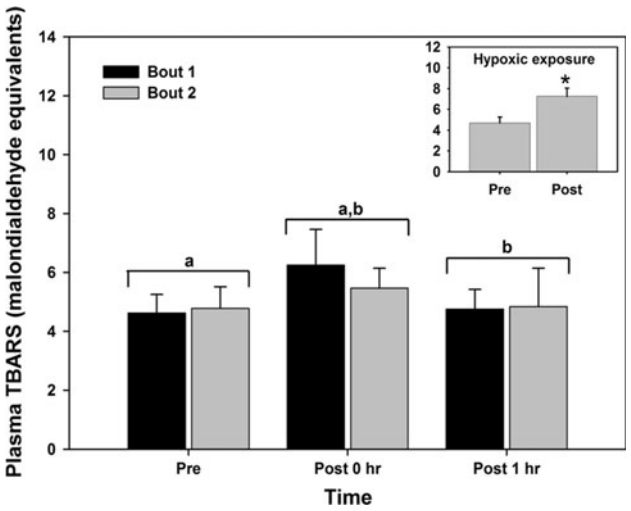

Fig. 6 Mean (SD) plasma TBARS expression immediately pre, post and 1 h post-exercise. BOUT 1 and bout 2 refer to two bouts of 60 min exercise at 90 % of lactate threshold, separated by the 5-day hypoxic intervention period. The *inset graph* shows the mean (SD) plasma TBARS before and after the 5-day hypoxic intervention period. *Like letters* represent significant differences across time (main effect). Post significantly higher than pre (*$P < 0.05$)

There was a significant main effect for time ($F = 7.0$, $P = 0.005$) and a significant exercise bout × time interaction ($F = 4.9$, $P = 0.02$) for GSH (Fig. 4), but the main effects for exercise bout failed marginally to reach statistical significance ($F = 4.0$, $P = 0.064$). The GSH was significantly higher for the second exercise bout compared to the first, only immediately post-exercise. This was because GSH decreased from pre-exercise to post-exercise and then significantly increased from immediately post-exercise to 1 h post-exercise for the first exercise bout, but no significant changes were observed for the second.

No statistically significant main effects of exercise bout ($F = 0.02$, $P = 0.89$) and time ($F = 0.1$, $P = 0.90$) or exercise bout × time interaction ($F = 0.01$, $P = 0.99$) were observed for TGSH (Fig. 5).

There was a significant main effect for time for plasma TBARS ($F = 10.3$, $P < 0.001$), where plasma TBARS increased significantly from pre- to post-exercise ($P = 0.001$) and then decreased from immediately post-exercise to 1 h post-exercise ($P = 0.002$) (Fig. 6). However, the main effect for exercise bout ($F = 0.5$, $P = 0.50$) and the exercise bout × time interaction ($F = 1.2$, $P = 0.32$) were not statistically significant.

No significant relationships were observed between the change in HSP32 and the changes in GSH ($r = 0.06$, $P = 0.88$), GSSG ($r = 0.03$, $P = 0.94$) or plasma TBARS ($r = 0.46$, $P = 0.25$). Similarly, no significant relationships were observed between the change in HSP72 and the changes in GSH ($r = 0.09$, $P = 0.83$), GSSG ($r = 0.28$, $P = 0.50$) or plasma TBARS ($r = -0.37$, $P = 0.36$).

There were significant differences across time for blood lactate during 60 min exercise periods ($F = 79.1$, $P < 0.001$), rising from 1.1 mM at rest to 2.6 mM after 30 min (mean difference = 1.5 mM; 95 % CI 1.2–1.8 mM; $P < 0.001$), and remaining stable for the last 30 min. No significant main effect for exercise bout ($F = 0.01$, $P = 0.91$) and no significant exercise bout × time interaction ($F = 0.003$, $P = 1.0$) were observed. No main effects for exercise bout ($F = 0.02$, $P = 0.88$) and time ($F = 0.2$, $P = 0.90$), and no exercise bout × time interaction ($F = 0.06$, $P = 0.98$), were observed for heart rate. Due to the small range in RPE data (2–3 units), it was not appropriate to report formal statistics on the data. However, it was clear by visually scrutinising the data that there were negligible differences between exercise bouts.

The change in oxyhaemoglobin saturation and heart rate during the 75 min of hypoxic exposure, for each of the 5 days of the hypoxic exposure period, has been presented elsewhere (Taylor et al. 2010b, 2011a), with the physiological responses within the present study in line with these prior observations. Oxyhaemoglobin saturation dropped from 99 % before each hypoxic exposure to around 90–91 % 15 min into hypoxic exposure and remained depressed for the remainder of the hypoxic exposure, before returning to baseline 10 min after exposure. Heart rate increased from around 70 beats/min 10 min before hypoxic exposure to around 81 beats/min during the first 5 min of exposure. Apart from a small initial decrease, heart rate remained relatively constant for the remainder of the hypoxic exposure, almost returning to baseline within 10 min after exposure.

Discussion

The principal finding of the present study was that a 5-day hypoxic acclimation period was sufficient to reduce the disturbance to redox balance of 60 min of prolonged aerobic exercise at 90 % of LT. This reduction was evident by the significant increase (32.5 %; 95 % CI 19.0–45.9 %; $P < 0.001$) in GSSG post-EXB1 being absent post-EXB2 ($P = 0.26$). Such a reduction in disturbance to redox balance post-exercise is likely attributable to the prior induction (increased content of pre-EXB2 compared to pre-EXB1) and thus bio-availability of the potently antioxidant stress protein *mHSP32* ($P = 0.024$) and the highly stress-inducible *mHSP72* ($P < 0.001$), in addition to favourable alterations in glutathione ratios.

It is likely that the increased co-bioavailability of *mHSP72* (Kalmar and Greensmith 2009), *mHSP32* (Rothfuss et al. 2001; Gozzelino et al. 2010) and GSH (Nikolaidis and Jamurtas 2009) may augment the antioxidant capacity of the blood. This augmentation may allow exercise-mediated increases in oxidant production (skeletal muscle, Powers et al. 2010a and blood originated, Nikolaidis and Jamurtas 2009), and thus disturbances to redox balance to be more readily dealt with post-hypoxic acclimation period. Despite these observations, establishment of cause and effect mechanistic evidence cannot be claimed, with such evidence requiring further, likely in vitro, experimentation.

HSP72

Within the present study, EXB1 demonstrated an increase of approximately 100 % in *mHSP72* immediately post-exercise, with values remaining elevated by approximately 50 % 1, 4, and 8 h post-exercise (Fig. 1), in line with previous increases seen in iHSP72 post-exercise (Fehrenbach et al. 2000). Seven days post-EXB1, before the commencement of the hypoxic acclimation period, basal *mHSP72* values returned to concentrations approximate to control (pre-EXB1 values). This return to baseline is pertinent within the study design, as basal values are known to be indicative of the magnitude of stress-mediated HSP72 response both in vivo (Gjovaag and Dahl 2006; McClung et al. 2008) and in vitro (Vince et al. 2010). The hypoxia-mediated increases (approximately, 60 %) in basal *mHSP72* remain elevated (approximately, 50 % higher compared to control and pre-EXB1) before the commencement of EXB2 (inset of Fig. 1). The expression kinetics in EXB2, compared to EXB1 did not demonstrate a significant increase in *mHSP72* immediately or 1, 4, or 8 h post-exercise. This difference is likely attributable to the prior induction and thus bio-availability of *mHSP72* conferring protection to the subsequent nonlethal stressor, as shown in vitro (Lepore et al. 2000; Madden et al. 2008a). However, in vivo, this is the first demonstration of prior induction of HSP72 via a non-thermal non-mechanical stressor likely conveying protection to one of the biochemical rigours (challenges to redox balance) associated with aerobic exercise.

Experimental data exist in support of HSP72-mediated tolerance to oxidative and ischaemic stress (Shima et al. 2008). It has been established that functional impairment, whether that be in regulation or efficacy in HSP synthesis, is present in various diseases and pathological conditions, such as those affiliated with chronic oxidative stress pathology, i.e. in neurodegeneration and cardiovascular disease (Kalmar and Greensmith 2009). Mouse models with transgenic upregulation of HSP72 have demonstrated resistance to the biochemical rigours of oxidative/ischaemic stress compared to control animals (Marber et al. 1995; Plumier et al. 1995). Furthermore, within exercising rodents, animals demonstrating enhanced HSP72 expression also display augmented RONS scavenging capacity

and reduced muscle injury post-downhill running compared to those animals with low HSP72 expression (Shima et al. 2008). The authors (Shima et al. 2008) postulated that elevations of HSP72 may protect the antioxidant defence system in skeletal muscle by enhancing the adaptive HSP72 mRNA response. Mechanistically, in vitro, evidence exists that elevations in oxidative stress are a trigger for increases in HSP72 concentration (Ahn and Thiele 2003; Kukreja et al. 1994) with similar findings recently shown in vivo to acute (Taylor et al. 2010b) and repeated daily hypoxic exposures (Taylor et al. 2011a). Therefore, it is likely that the repeated disturbance to redox balance from the daily hypoxic exposure (Taylor et al. 2011a) may be acting as a stimulus for elevated HSP72 expression and as a potential stimulus for hormesis (conveyed cellular protection to exercise-mediated disturbances to redox balance) to occur in preparation for EXB2 (Radak et al. 2008a, b). However, caution is required when making such postulations based on plasma TBARS and whole blood glutathione data, without supporting data specifically reporting measures of protein oxidation (e.g. protein carbonyls). Such limitations of plasma TBARS are subsequently discussed in the glutathione and plasma TBARS section of the present discussion.

HSP32

Similarly to HSP72, the inducible isoform of HSP32 has been shown to increase in concentration under in vitro experimental conditions of ischaemia, hypoxia and inflammation within cell and tissue lines (Gozzelino et al. 2010; Rothfuss et al. 2001). A rodent exercise model demonstrated 700 and 400 % increases in HSP32 mRNA 1 and 3 h post-exercise, respectively (Essig et al. 1997). However, these conditions lack specificity to those experienced during in vivo exercise or sojourn to altitude within humans.

Within humans, post-half marathon completion, significant elevations in HSP32 have been shown in leukocytes, monocytes and granulocytes, as measured by flow cytometry (Niess et al. 1999). Findings within the current study demonstrate comparable findings to previous work (Fehrenbach et al. 2003; Niess et al. 1999), with significant increases in *mHSP32* seen immediately post (approximately 50 %) and 1 h post (approximately 30 %) EXB1. Interestingly, this increase in EXB1 (pre to post and 1 h post) is absent from EXB2. This absence of an increase (EXB1 compared to EXB2) post-exercise is likely attributable to the hypoxic intervention significantly increasing (approximately 30 %) basal *mHSP32* content (see inset of Fig. 2), with these elevations sustained (significantly increased pre-EXB2 compared to pre-EXB1) at comparable levels prior to commencement of EXB2 compared to pre-EXB1 (Fig. 2). This increase in bio-available *mHSP32* may likely ameliorate the disturbance in redox balance experienced during the exercise bout via the potent antioxidant capacity of HSP32 (Gozzelino et al. 2010; Rothfuss et al. 2001).

Glutathione and plasma TBARS

Measures of oxidative stress through indirect quantification of lipid peroxidation via plasma TBARS have been extensively used within the literature (Fisher-Wellman and Bloomer 2009). Most research has demonstrated elevations in plasma TBARS post-maximal (Bloomer and Goldfarb 2004) and sub-maximal exercise (Fisher-Wellman and Bloomer 2009), which return to baseline values within 1 h post-exercise (Bloomer and Goldfarb 2004; Fisher-Wellman and Bloomer 2009). However, the utilisation of plasma TBARS to measure exercise-induced changes in oxidative stress is not without limitations. The literature has reported concerns about the specificity of the TBARS assay and these considerations have been discussed in recent reviews (Fisher-Wellman and Bloomer 2009; Powers et al. 2010b). The present study demonstrated a significant increase in plasma TBARS post-EXB1 and EXB2, which returned to values approximate to baseline 1 h post-exercise. These results are in line with previous research for sub-maximal exercise (Fisher-Wellman and Bloomer 2009). However, no significant differences were evident in plasma TBARS data between the two exercise bouts within the present study. This may be attributable to the lack of specificity of the plasma TBARS assay in assessment of the exercise-mediated changes in oxidative stress (Powers et al. 2010b). The author has attempted to alleviate the problems associated with plasma TBARS analysis, via utilisation of measures of whole blood glutathione redox balance, which have been shown to be a more sensitive and specific measure of disturbances to redox balance in response to exercise, in comparison to plasma TBARS (Fisher-Wellman and Bloomer 2009; Powers et al. 2010b).

Within EXB1 post-exercise, there was a significant increase in GSSG, a decrease in GSH and no change in TGSH, with these exercise-induced permutations in GSSG returning to baseline 1 h post-exercise in both EXB1 and EXB2. These findings are in line with previous literature, whereby a multitude (Fisher-Wellman and Bloomer 2009) of non-eccentric aerobic exercise protocols elicit decreases in GSH, increases in GSSG and no change in TGSH. Interestingly in EXB2, despite comparable physiological performance data, the significant increase in GSSG is absent compared to EXB1 (Fig. 3). This may likely be attributable to the hypoxia-mediated changes in basal GSSG, whereby pre-exercise values are significantly lower within EXB2 compared to EXB1 (see inset Fig. 3). This

reduction in GSSG is seen in tandem with an elevation in basal GSH values pre-exercise in EXB1 compared to EXB2 (Fig. 4), although not statistically significant. Such alterations in GSH:GSSG are indicative of a more favourable basal antioxidant capacity (Ilhan et al. 2004). Therefore, the increase in bio-available GSH (failed marginally to reach significance) may present augmented resistance to exercise-induced disturbances to redox balance during EXB2. Previously, it has been shown in animals (Leeuwenburgh and Ji 1995) and humans (Sen et al. 1994a, b) the importance of GSH in protecting against resting and exercise-induced oxidative stress, with reduced availability of GSH correlated to the decreased ability of an organism to survive an oxidative cellular insult. The hypoxia-mediated changes in glutathione ratios, i.e. decreased GSSG and increased GSH, may provide a more favourable blood glutathione environment for the acquiescence of exercise-induced oxidative stress and thus disturbances to redox balance (Ilhan et al. 2004).

However, as discussed elsewhere (Fisher-Wellman and Bloomer 2009; Powers et al. 2010b), the TBARS assay has several fundamental issues regarding specificity and, although recognised as an inexpensive and simple biomarker of oxidative stress, TBARS, as discussed, does have some technical problems and these must be considered when interpreting the literature and any experimental findings (Powers et al. 2010b). The technical limitations of TBARS were sought to be minimised via the inclusion of whole blood glutathione measures, which is in line with recent recommendations to assess more than one biomeasure of oxidative stress when examining exercise-induced changes in redox balance (Powers et al. 2010b).

Experimental limitations

Future work should employ measures of protein oxidation (e.g. protein carbonyls) to more securely investigate the cause and effect mechanistic relationship between hypoxia-mediated disruptions in redox balance and *mHSP72*/*mHSP32* expression in both the blood and muscle in vitro and in vivo. Additionally, the study design could be improved via the incorporation of a double-blind crossover design with a control normoxic intervention. Such improvements would securely attempt to establish clear cause and effect mechanistic evidence for the novel findings within the present small novel human study.

Summary: HSP32, HSP72 and glutathione

The combination of increased bio-available *mHSP32* and *mHSP72* prior to exercise commencing in EXB2 compared to EXB1 may acquiesce the disturbance to redox balance during the second, physiologically identical exercise bout. Furthermore, the favourable alterations in whole blood glutathione redox balance, before commencement of EXB2 compared to EXB1 (i.e. a reduction in GSSG and increase in GSH) may, in tandem with elevated basal stress protein levels (*mHSP32* and *mHSP72*), or independently, potentially augment the body's ability to deal with exercise-induced disturbances to redox balance. This highlights a current topic of interest within the literature, whereby maximising oxidative stress is viewed as a positive physiological cascade to procure maximal physiological adaptation (Nikolaidis and Jamurtas 2009; Powers et al. 2010a). However, using a practical example, within a competitive season, i.e. a soccer season, limitation of the disturbance to redox balance from a match may be desirable, as this exercise-induced disturbance in redox balance is known to limit the time taken for full recovery (Ascensao et al. 2007, 2008, 2011), and thus reduction in such disturbances may ensure that players return to optimal performance with the lowest decrement in repeat performance (i.e. repeated match performance). Additionally, reductions in disturbances to redox balance are desirable in various surgical procedures (Arsalani-Zadeh et al. 2011) and thus the results presented here may be of use in such fields, as postulated elsewhere (Pignatelli et al. 2011). This dichotomy in the physiological in vivo effects of oxidative stress is a point of interest within the current literature and has been addressed in recent reviews (Nikolaidis and Jamurtas 2009; Powers et al. 2007, 2010a).

To the authors' knowledge, this is the first human in vivo study to demonstrate prior hypoxia-mediated elevation/induction of basal *mHSP32* and *mHSP72* and favourable adaptations in whole blood glutathione redox potentially conferring tolerance to sub-maximal aerobic exercise-induced disturbances to redox balance.

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References

Ahn SG, Thiele DJ (2003) Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. Genes Dev 17(4):516–528

Anbarasi K, Kathirvel G, Vani G, Jayaraman G, Devi CSS (2006) Cigarette smoking induces heat shock protein 70 kDa expression and apoptosis in rat brain: modulation by bacoside A. Neuroscience 138(4):1127–1135

Arsalani-Zadeh R, Ullah S, Khan S, MacFie J (2011) Oxidative stress in laparoscopic versus open abdominal surgery: a systematic review. J Surg Res 169(1):E59–E68. doi:10.1016/j.jss.2011.01.038

Ascensao A, Ferreira R, Magalhaes J (2007) Exercise-induced cardioprotection—biochemical, morphological and functional

evidence in whole tissue and isolated mitochondria. Int J Cardiol 117(1):16–30. doi:10.1016/j.ijcard.2006.04.076

Ascensao A, Rebelo A, Oliveira E, Marques F, Pereira L, Magalhaes J (2008) Biochemical impact of a soccer match—analysis of oxidative stress and muscle damage markers throughout recovery. Clin Biochem 41(10–11):841–851. doi:10.1016/j.clinbiochem.2008.04.008

Ascensao A, Leite M, Rebelo AN, Magalhaes S, Magalhaes J (2011) Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. J Sports Sci 29(3):217–225. doi:10.1080/02640414.2010.526132

Bailey DM, Davies B, Young IS (2000) Evidence for reactive oxidant generation during acute physical exercise and normobaric hypoxia in man. Journal of Physiology-London 528P:45P-45P

Bloomer RJ, Goldfarb AH (2004) Anaerobic exercise and oxidative stress: A review. Can J Appl Physiol Rev Can Physiol Appl 29(3):245–263

Cheng B, Kuipers H, Snyder AC, Keizer HA, Jeukendrup A, Hesselink M (1992) A new approach for the determination of ventilatory and lactate thresholds. Int J Sports Med 13(7):518–522

Essig DA, Borger DR, Jackson DA (1997) Induction of heme oxygenase-1 (HSP32) mRNA in skeletal muscle following contractions. Am J Physiol Cell Physiol 272(1):C59–C67

Febbraio MA, Mesa JL, Chung J, Steensberg A, Keller C, Nielsen HB, Krustrup P, Ott P, Secher NH, Pedersen BK (2004) Glucose ingestion attenuates the exercise-induced increase in circulating heat shock protein 72 and heat shock protein 60 in humans. Cell Stress Chaperones 9(4):390–396

Fehrenbach E, Passek F, Niess AM, Pohla H, Weinstock C, Dickhuth HH, Northoff H (2000) HSP expression in human leukocytes is modulated by endurance exercise. Med Sci Sports Exerc 32(3):592–600

Fehrenbach E, Niess AM, Passek F, Sorichter S, Schwirtz A, Berg A, Dickhuth HH, Northoff H (2003) Influence of different types of exercise on the expression of haem oxygenase-1 in leukocytes. J Sports Sci 21(5):383–389. doi:10.1080/0264041031000071164

Fisher-Wellman K, Bloomer R (2009) Acute exercise and oxidative stress: a 30 year history. Dynamic Medicine 8(1):1

Gjovaag TF, Dahl HA (2006) Effect of training and detraining on the expression of heat shock proteins in m. triceps brachii of untrained males and females. Eur J Appl Physiol 98(3):310–322

Gozzelino R, Jeney V, Soares MP (2010) Mechanisms of cell protection by heme oxygenase-1. Annu Rev Pharmacol Toxicol 50:323–354. doi:10.1146/annurev.pharmtox.010909.105600

Hillman AR, Vince RV, Taylor L, McNaughton L, Mitchell N, Siegler J (2011) Exercise-induced dehydration with and without environmental heat stress results in increased oxidative stress. Appl Physiol Nutr Metab 36(5):698–706. doi:10.1139/h11-080

Ilhan N, Kamanli A, Ozmerdivenli R (2004) Variable effects of exercise intensity on reduced glutathione, thiobarbituric acid reactive substance levels, and glucose concentration. Arch Med Res 35(4):294–300

Kalmar B, Greensmith L (2009) Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 61(4):310–318. doi:10.1016/j.addr.2009.02.003

Kiang JG, Tsen KT (2006) Biology of hypoxia. Chin J Physiol 49(5):223–233

Kukreja RC, Qian YZ, Kontos MC, Yang G, Loesser KE, Yoshida K, Hess ML (1994) Time-course of increase of heat-shock protein-70 messenger-RNA during ischemia–reperfusion and preconditioning in rat-heart. FASEB J 8(5):A857

Kume M, Yamamoto Y, Yamagami K, Ishikawa Y, Uchinami H, Yamaoka Y (2000) Pharmacological hepatic preconditioning: involvement of 70-kDa heat shock proteins (HSP72 and HSP73) in ischaemic tolerance after intravenous administration of doxorubicin. Br J Surg 87(9):1168–1175. doi:10.1046/j.1365-2168.2000.01509.x

Leeuwenburgh C, Ji LL (1995) Glutathione depletion in rested and exercised mice—biochemical consequence and adaptation. Arch Biochem Biophys 316(2):941–949

Lepore DA, Hurley JV, Stewart AG, Morrison WA, Anderson RL (2000) Prior heat stress improves survival of ischemic–reperfused skeletal muscle in vivo. Muscle Nerve 23(12):1847–1855

Lu PZ, Lai CY, Chan WH (2008) Caffeine induces cell death via activation of apoptotic signal and inactivation of survival signal in human osteoblasts. Int J Mol Sci 9(5):698–718. doi:10.3390/ijms9050698

Madden LA, Sandstrom E, Lovell RJ, McNaughton L (2008a) Inducible heat shock protein 70 and its role in preconditioning and exercise. Amino Acids 33(4):511–516

Madden LA, Vince RV, Sandstrom ME, Taylor L, McNaughton L, Laden G (2008b) Microparticle-associated vascular adhesion molecule-I and tissue factor follow a circadian rhythm in healthy human subjects. Thromb Haemost 99(5):909–915

Magalhães FC, Amorim F, Passos R, Fonseca M, Oliveira K, Lima M, Guimarães J, Ferreira-Júnior J, Martini A, Lima N, Soares D, Oliveira E, Rodrigues L (2010) Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. Cell Stress Chaperones 15(6):885–895. doi:10.1007/s12192-010-0197-7

Marber MS, Mestril R, Chi SH, Sayen MR, Yellon DM, Dillmann WH (1995) Overexpression of the rat inducible 70-kd heat-stress protein in a transgenic mouse increases the resistance of the heart to ischemic-injury. J Clin Investig 95(4):1446–1456

McClung JP, Hasday JD, He JR, Montain SJ, Cheuvront SN, Sawka MN, Singh IS (2008) Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. Am J Physiol Regul Integr Comp Physiol 294(1):R185–R191

Morton JP, MacLaren DP, Cable NT, Bongers T, Griffiths RD, Campbell IT, Evans L, Kayani A, McArdle A, Drust B (2006) Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. J Appl Physiol 101(1):176–182

Morton JP, Maclaren DPM, Cable NT, Campbell IT, Evans L, Kayani AC, Mcardle A, Drust B (2008) Trained men display increased basal heat shock protein content of skeletal muscle. Med Sci Sports Exerc 40(7):1255–1262. doi:10.1249/Mss.0b013e31816a7171

Morton JP, Kayani A, Mcardle A, Drust B (2009) The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med 39(8):643–662

Niess AM, Passek F, Lorenz I, Schneider EM, Dickhuth HH, Northoff H, Fehrenbach E (1999) Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes—acute and adaptational responses to endurance exercise. Free Radic Biol Med 26(1–2):184–192

Nikolaidis MG, Jamurtas AZ (2009) Blood as a reactive species generator and redox status regulator during exercise. Arch Biochem Biophys 490(2):77–84. doi:10.1016/j.abb.2009.08.015

Pasupathy S, Homer-Vanniasinkam S (2005) Surgical implications of ischemic preconditioning. Arch Surg 140(4):405–409. doi:10.1001/archsurg.140.4.405

Peart DJ, McNaughton LR, Midgley AW, Taylor L, Towlson C, Madden LA, Vince RV (2011) Pre-exercise alkalosis attenuates the heat shock protein 72 response to a single-bout of anaerobic exercise. J Sci Med Sport 14(5):435–440. doi:10.1016/j.jsams.2011.03.006

Pignatelli P, Tellan G, Marandola M, Carnevale R, Loffredo L, Schillizzi M, Proietti M, Violi F, Chirletti P, Delogu G (2011)

Effect of L-carnitine on oxidative stress and platelet activation after major surgery. Acta Anaesthesiol Scand 55(8):1022–1028. doi:10.1111/j.1399-6576.2011.02487.x

Plumier JCL, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN (1995) Transgenic mice expressing the human heat-shock protein-70 have improved postischemic myocardial recovery. J Clin Investig 95(4):1854–1860

Powers SK, Jackson MJ (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 88(4):1243–1276. doi:10.1152/physrev.00031.2007

Powers SK, Kavazis AN, McClung JM (2007) Oxidative stress and disuse muscle atrophy. J Appl Physiol 102(6):2389–2397. doi:10.1152/japplphysiol.01202.2006

Powers SK, Duarte J, Kavazis AN, Talbert EE (2010a) Reactive oxygen species are signalling molecules for skeletal muscle adaptation. Exp Physiol 95(1):1–9. doi:10.1113/expphysiol.2009.050526

Powers SK, Smuder AJ, Kavazis AN, Hudson MB (2010b) Experimental guidelines for studies designed to investigate the impact of antioxidant supplementation on exercise performance. Int J Sport Nutr Exerc Metab 20(1):2–14

Radak Z, Chung HY, Goto S (2008a) Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med 44(2):153–159. doi:10.1016/j.freeradbiomed.2007.01.029

Radak Z, Chung HY, Koltai E, Taylor AW, Goto S (2008b) Exercise, oxidative stress and hormesis. Ageing Res Rev 7(1):34–42. doi:10.1016/j.arr.2007.04.004

Rothfuss A, Radermacher P, Speit G (2001) Involvement of heme oxygenase-1 (HO-1) in the adaptive protection of human lymphocytes after hyperbaric oxygen (HBO) treatment. Carcinogenesis 22(12):1979–1985

Sandstrom ME, Madden LA, Taylor L, Siegler JC, Lovell RJ, Midgley AW, Mcnaughton L (2009) Variation in basal heat shock protein 70 is correlated to core temperature in human subjects. Amino Acids 37(2):279

Sawka MN, Burke L, Eichner R, Maughan R, Montain SJ, Stachenfeld N (2007) Exercise and fluid replacement. Med Sci Sports Exerc 39(2):377–390

Selkirk GA, McLellan TM, Wright HE, Rhind SG (2009) Expression of intracellular cytokines, HSP72, and apoptosis in monocyte subsets during exertional heat stress in trained and untrained individuals. Am J Physio Regul Integr Comp Physiol 296(3):R575–R586. doi:10.1152/ajpregu.90683.2008

Sen CK, Atalay M, Hanninen O (1994a) Exercise-induced oxidative stress—glutathione supplementation and deficiency. J Appl Physiol 77(5):2177–2187

Sen CK, Rankinen T, Vaisanen S, Rauramaa R (1994b) Oxidative stress after human exercise—effect of N-acetylcysteine supplementation. J Appl Physiol 76(6):2570–2577

Shima Y, Kitaoka K, Yoshiki Y, Maruhashi Y, Tsuyama T, Tomita K (2008) Effect of heat shock preconditioning on ROS scavenging activity in rat skeletal muscle after downhill running. J Physiol Sci 58(5):341–348. doi:10.2170/physiolsci.RP004808

Singleton KD, Ziegler TR, Luo M, Fernandez-Estivariz C, Wischmeyer PE (2004) Intravenous glutamine increases serum HSP 72 levels in both humans and rodents following systemic inflammation. In: 27th Annual conference on shock, Halifax, Canada, 05–08 June 2004, p 250

Taylor CT, Pouyssegur J (2007) Oxygen, hypoxia, and stress. Stress Responses Biol Med 1113:87–94

Taylor L, Midgley A, Chrismas B, Madden L, Vince RV, McNaughton L (2010a) Daily quadratic trend in basal monocyte expressed HSP72 in healthy human subjects. Amino Acids 38(5):1483–1488

Taylor L, Midgley A, Chrismas B, Madden L, Vince RV, Mcnaughton L (2010b) The effect of acute hypoxia on heat shock protein 72 expression and oxidative stress in vivo. Eur J Appl Physiol 109(5):849–855

Taylor L, Midgley A, Chrismas B, Hillman A, Vince RV, Madden L, Mcnaughton L (2011a) Daily hypoxia increases basal monocyte HSP72 expression in healthy human subjects. Amino Acids 40(2):393–401

Taylor L, Midgley A, Sandstroem ME, Chrismas B, McNaughton L (2011b) The effect of the hyperbaric environment on heat shock protein 72 expression in vivo. Res Sports Med (in press)

Tsuchiya M, Sato EF, Inoue M, Asada A (2008) Open abdominal surgery increases intraoperative oxidative stress: can it be prevented? Anesth Analg 107(6):1946–1952. doi:10.1213/ane.0b013e318187c96b

Vince RV, McNaughton L, Taylor L, Midgley AW, Laden G, Madden LA (2009) Release of VCAM-1 associated endothelial microparticles following simulated SCUBA dives. Eur J Appl Physiol 105:507–513

Vince RV, Oliver K, Midgley A, McNaughton L, Madden L (2010) In vitro heat shock of human monocytes results in a proportional increase of inducible Hsp70 expression according to the basal content. Amino Acids 38(5):1423–1428

Vince RV, Midgley AW, Laden G, Madden LA (2011) The effect of hyperbaric oxygen preconditioning on heat shock protein 72 expression following in vitro stress in human monocytes. Cell Stress Chaperones 16(3):339–343. doi:10.1007/s12192-010-0246-2

Vissing K, Bayer ML, Overgaard K, Schjerling P, Raastad T (2009) Heat shock protein translocation and expression response is attenuated in response to repeated eccentric exercise. Acta Physiol 196(3):283–293. doi:10.1111/j.1748-1716.2008.01940.x

Yamada P, Amorim F, Moseley P, Schneider S (2008) Heat shock protein 72 response to exercise in humans. Sports Med 38(9):715–733

Yogaratnam JZ, Laden G, Guvendik L, Cowen M, Cale A, Griffin S (2007) Can hyperbaric oxygen be used as adjunctive heart failure therapy through the induction of endogenous heat shock proteins? Adv Therapy 24(1):106–118

Yogaratnam JZ, Laden G, Guvendik L, Cowen M, Cale A, Griffin S (2010) Hyperbaric oxygen preconditioning improves myocardial function, reduces length of intensive care stay, and limits complications post coronary artery bypass graft surgery. Cardiovascu Revasc Med 11(1):8–19. doi:10.1016/j.carrev.2009.03.004