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EFFECTS OF MAXIMAL INTERMITTENT EXERCISE IN NORMOXIC AND HYPOXIC ENVIRONMENTS ON THE RELEASE OF CARDIAC BIOMARKERS AND THE POTENTIAL MECHANISM

LI FEIFEI

Ph. D. Thesis

HONG KONG BAPTIST UNIVERSITY

2014

Effects of Maximal Intermittent Exercise in Normoxic and Hypoxic Environments on the Release of Cardiac Biomarkers and the Potential Mechanism

LI Feifei

A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Professor FU Hoo Kin, Frank

Hong Kong Baptist University

May 2014

Declaration

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis, dissertation submitted to this or other institution for a degree, diploma or other qualification.

Signature: ______________________

Date: May 2014

Abstract

The purposes of this study were 1) to investigate the release of cardiac biomarkers resulting from acute bouts of maximal intermittent exercise in a laboratory-based setting and set up an exercise-induced cardiac biomarker release (EICBR) model; 2) to compare the changes in cardiac biomarkers in normoxic and hypoxic environments and determine the effects of hypoxia; 3) to investigate the changes in oxidative stress biomarkers resulting from acute bouts of maximal intermittent exercise in normoxic and hypoxic environments at multiple time points; and 4) to observe the relationship between oxidative stress and EICBR and explore the hypothesis that lipid peroxidation triggers the release of cardiac biomarkers from the cytosolic pool.

The maximal oxygen consumption (VO_{2max}) and the corresponding velocity of VO_{2max} (vVO_{2max}) of ten well-trained male marathon runners (age 22.1±2.6 y, body mass 64.0±4.9 kg and height 177.3±3.9 cm) was determined under normoxic (FIO_2=21.0%, VO_{2max}_N=64.72±5.63 ml·kg^{-1}·min^{-1} and vVO_{2max}_N=18.2±1.0 km·h^{-1}) and hypoxic (FIO_2=14.4%, VO_{2max}_H=62.16±6.74 ml·kg^{-1}·min^{-1} and vVO_{2max}_H=16.7±0.7 km·h^{-1}) conditions in two experimental trials. One set of conditions was tested in each trial. The order in which each participant faced each trial was selected at random and the trials were separated by 72 h.

The ten participants also completed three maximal intermittent exercise protocols, under normoxic (trial N, FIO_2=21.0%), absolutely hypoxic (trial AH, FIO_2=14.4%) and relatively hypoxic (trial RH, FIO_2=14.4%) conditions. The order in

which the participants faced the three conditions was once again selected at random and the protocols were separated by at least 7 d. Each bout of maximal intermittent exercise in trials N and AH consisted of a hard run of 16.4±0.9 $km \cdot h^{-1}$ (90% vVO_{2max}_N) for 2 min, followed by an easy run of 9.1±0.5 $km \cdot h^{-1}$ (50% vVO_{2max}_N) for 2 min with a 2% slope. In trial RH, each bout of exercise consisted of a hard run of 15.0±0.6 $km \cdot h^{-1}$ (90% vVO_{2max}_H) for 2 min, followed by an easy run of 8.4±0.3 $km \cdot h^{-1}$ (50% vVO_{2max}_H) for 2 min with a 2% slope. Each of the three trials consisted of 23 bouts of maximal intermittent exercise, performed over 92 min.

Measurements of the serum of the antecubital venous blood were performed pre- and post- (0 h, 2 h, 4 h and 24 h) exercise. The measurements were taken at five time points for each of the three conditions. The cardiac damage biomarkers of high sensitivity cardiac troponin T (hs-cTnT) and cardiac troponin I (cTnI) and the oxidative stress biomarkers of malondialdehyde (MDA), lipid hydroperoxide (LH), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and total antioxidant capacity (TAOC) were analysed. Heart rate (HR) and arterial oxygen saturation (SaO_2) were recorded before and during exercise.

Due to the skewed distribution of the data ($P<0.05$), a non-parametric Friedman's test was used to compare the differences in the levels of hs-cTnT and cTnI between pre- and post-exercise and at each time point for the three conditions. MDA, LH, SOD, CAT, GSH, TAOC and HR were normally distributed ($P>0.05$) and were analysed using one-way repeated ANOVA tests. Pearson's product moment correlation coefficients were used to determine the degree of association between the

peak levels of hs-cTnT and cTnI, and MDA, LH, SOD, CAT, GSH and TAOC.

In trial N, the level of hs-cTnT was elevated 0 h post-exercise (9.628±3.797 $pg{\cdot}ml^{-1}$ was significantly different from the pre-exercise level of 5.118±1.857 $pg{\cdot}ml^{-1}$, P=0.005), reached its peak level 2 h post-exercise (24.290±18.628 $pg{\cdot}ml^{-1}$ was significantly different from the pre-exercise level, P=0.005) and returned to the baseline level at 24 h post-exercise (5.978±1.849 $pg{\cdot}ml^{-1}$). The peak levels of hs-cTnT (N, AH 37.001±31.995 $pg{\cdot}ml^{-1}$, RH 28.614±23.628 $pg{\cdot}ml^{-1}$) and cTnI (N 0.0375±0.0437 $ng{\cdot}ml^{-1}$, AH 0.0475±0.0533 $ng{\cdot}ml^{-1}$, RH 0.0345±0.0375 $ng{\cdot}ml^{-1}$) did not significantly differ under the three conditions.

In trial AH, the peak levels of hs-cTnT (2 h, 4 h) and cTnI (2 h, 4 h) were highly related to the MDA_0h and the TAOC_24h. In trial RH, the peak levels of hs-cTnT (2 h, 4 h) and cTnI (2 h, 4 h) were highly related to the TAOC_4h.

It was concluded that maximal intermittent exercise can be used to trigger EICBR. The stimulus of hypoxia did not induce more cardiac damage in this exercise model. Maximal intermittent exercise potentially triggers EICBR through oxidative stress, especially lipid peroxidation.

Keywords: *cardiac biomarkers, hs-cTnT, cTnI, oxidative stress, hypoxia*

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List of Abbreviation

AMI	Acute myocardial infarction
a-vO2	Arterial-venous oxygen
BNP	B-type natriuretic peptide
CAT	Catalase
cTn	Cardiac troponin
cTnC	Cardiac troponin C
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
EICBR	Exercise-induced cardiac biomarkers release
EICF	Exercise-induced cardiac fatigue
GSH	Glutathione
HR	Heart rate
hs-cTnT	High sensitivity cardiac troponin T
LH	Lipid hydroperoxides
MDA	Malondialdehyde
NT-Pro-BNP	NT-Pro B-type natriuretic peptide
Q_{max}	Maximal cardiac output
ROS	Reactive oxygen species
SaO_2	Arterial oxygen saturation
SD	Standard deviation

SOD	Superoxide dismutase
sTnI	Skeletal troponin I
sTnT	Skeletal troponin T
TAOC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
TEAC	Tolox equivalent antioxidant capacity
TnI	Troponin I
TnT	Troponin T
URL	Upper reference limit
VO_2	Oxygen consumption
VO_{2max}	Maximal oxygen consumption
vVO_{2max}	Velocity of maximal oxygen consumption

Introduction

Background of the study

In 2000, 2007 and 2012, the Joint European Society of Cardiology, American College of Cardiology, American Heart Association and World Heart Federation recommended the use of the cardiac biomarkers cardiac troponin T (cTnT) and cTnI as the gold standard for the diagnosis of acute myocardial infarction (AMI) and cardiac necrosis in the setting of acute cardiac ischemia because of their high specificity and sensitivity (Alpert, Thygesen, Antman & Bassand, 2000; Thygesen, Alpert & White, 2007; Thygesen, Alpert, Jaffe, Simoons, Chaitman & White, 2012). The cardiac biomarkers are released in patients 2 h to 4 h after the onset of AMI and persist for up to 10 d to 14 d (Morrow, 2006), or even longer than 21 d (Wu & Ford, 1999). Cardiac biomarker release is related to AMI and cardiac necrosis because the myocardial sarcomere consists of seven actin monomers, double-stranded tropomyosin and a cardiac troponin (cTn) complex (Collinson, Boa & Gaze, 2001). The cTn complex is composed of three subunits. The cTnT subunit is a binding protein that attaches the cTn complex to tropomyosin. The cTnI subunit modulates the interaction between actin and myosin by acting as an inhibitor of actomyosin adenosine triphosphatase activity. The cardiac troponin C (cTnC) subunit is a calcium binding subunit. Most of the cTnT and cTnI subunits are bound to tropomyosin on the thin filament of the myofibril and have a structural function. A small pool of both subunits remains unbound in the cytosol, comprising approximately 5.0% (Adams,

Schechtman, Landt, Ladenson & Jaffe, 1994) or 6.0% to 8.0% (Kroff, Katus & Giannitsis, 2006) of the total cTnT population and 3.7% (Katus, Remppis, Scheffold, Diederich & Kuebler, 1991) or 2.0% to 8.0% (Kroff et al., 2006) of the total cTnI population. Different forms of troponin T (TnT) and I (TnI) are found in cardiac (cTnT, cTnI) and skeletal (sTnT, sTnI) muscle, encoded by separate genes and sharing some amino acid sequence homology (Shave, Baggish, George, Wood, Scharhag & Whyte et al., 2010). The state of the art second- and third- generation immunoassay techniques can differentiate between cTnT and sTnT without cross-reactivity (Shave, Dawson, Whyte, George, Ball & Collinson et al., 2000). A fourth-generation cTnT and hs-cTnT assay is now widely used in AMI diagnosis and research.

EICBR has been widely reported in elite and recreational athletes after long-term exhaustion competition events and a continuous exercise modality, which is of great concern to athletes, coaches and scientists. Intermittent exercise and altitude training programs are popular exercise modalities, but the data for these programs are more limited.

Intermittent training involves repeated short to long bouts of high intensity exercise, equal to or higher than the maximal intensity reached during competition, interspersed with recovery periods, light exercise or rest, during which oxygen consumption (VO_2) fluctuates (Billat, 2001). Intermittent exercise training was first described in 1959 by Reindell and Roskamm in Germany and is now popular with both runners and researchers. It can include aerobic (short and long aerobic intermittent training) and anaerobic (fixed intensity and all-out run) sessions. Aerobic

intermittent training is defined as a program that elicits a higher ratio of aerobic metabolism to anaerobic metabolism (Billat, 2001). Runners use this technique to train at a velocity (exercise intensity) close to their own competition velocity, interspersed with either a positive or negative recovery period to avoid an increase in blood lactate.

Altitude training programs, both in natural and simulated hypoxic environments, are used by elite athletes, and especially long-distance runners, to enhance their performance at sea level. A normobaric hypoxic environment provides simulated altitude with a reduction in the concentration of oxygen (replacing oxygen with nitrogen), but without any change in barometric pressure. A nitrogen apartment is a normobaric hypoxic apartment that simulates an altitude environment equivalent to approximately 8000 m (Willber, 2004). Athletes can train and sleep in a nitrogen apartment rather than having to travel to a high altitude location far away. Acute exposure to a hypoxic environment can impair an athlete's VO_{2max}, although there are large inter-individual variations in this impairment at a given level of hypoxia, from 2000 m to 3000 m (Billat, Lepretre, Heubert, Koralsztein & Gazeau, 2003; Friedmann, Frese, Menold, Kauper, Jost & Batrsch, 2005; Mollard, Woorons, Letourenl, Lamberto, Favret & Pichon et al., 2007). These inter-individual variations in the VO_{2max} change after acute exposure to hypoxia divide individuals into altitude training responders and non-responders. Altitude training responders are athletes who exhibit a large reduction in their VO_{2max} during moderate hypoxia and suffer from a significant reduction in both their maximal cardiac output (Q_{max}) and maximal

arterial-venous oxygen (a-vO_2) difference. Altitude training non-responders are able to tolerate hypoxia, as attested by a significantly smaller drop in their VO_{2max} during moderate hypoxia, display a blunted Q_{max} and are conversely able to maintain a maximal a-vO_2 difference. The a-vO_2 difference is the difference between the oxygen concentration in the arterial blood moving from the heart to the working muscles and the concentration of oxygen in the venous blood circulating from the working muscles back to the heart. Thus, the a-vO_2 difference provides an indirect measure of the amount of oxygen extracted by the working skeletal muscles (Wilber, 2004). Q_{max} represents the function of the cardiac muscle. The ability to maintain the maximal a-vO_2 difference suggests that altitude training non-responders may keep their skeletal muscle work in both normoxic and hypoxic environments.

The potential mechanisms underlying EICBR are ischemia-induced bleb formation, the force of cardiac contraction and myocardial stunning, among others, which can be explained by oxidative stress triggering lipid peroxidation. Oxidative stress is the result of an increased exposure to free radicals and their products. Free radicals are molecules or molecule fragments with one or more unpaired electrons that can be produced by the mitochondrial respiratory chain during oxygen metabolism. Free radicals are very unstable and reactive and have a short lifetime. Reactive oxygen species (ROS) are formed when free radicals react with oxygen. ROS contain both free radicals and reactive forms of oxygen species. Increased levels of ROS may be harmful to cells (Sen, 2001), as they can trigger lipid (cell membrane) peroxidation, which can result in myocyte cell membrane dysfunction and damage (Ji,

2001). The cell requires complex protection in the form of the antioxidant system to limit these harmful effects (Finaud, Lac & Filaire, 2006). Oxidative stress is a condition in which an imbalance exists between ROS production and the antioxidant defence system. A direct assessment of the quantity of ROS is extremely difficult due to their high reactivity and relatively short half-lives (Fisher-Wellman & Bloomer, 2009). The majority of ROS research related to exercise has used indirect methods that measure the more stable molecular products and oxidation target products formed via ROS reactions, including the lipid peroxidation end products MDA, LH and thiobarbituric acid reactive substances (TBARS). Oxidative stress can also be measured by observing alterations in the antioxidant system, including changes in certain antioxidant enzymes, such as SOD and CAT, and changes in reduced GSH, TAOC and the trolox equivalent antioxidant capacity (TEAC) (Nie, Close, George, Tong & Shi, 2010a; Tian, Nie, Tong, Baker, Thomas & Shi, 2010).

Statement of the research problem

EICBR has been widely reported after long-term endurance events, such as continuous (marathon, triathlon and cycling) and intermittent (basketball, rugby and football) exercise events, which is of great concern and is still under debate. In a laboratory-based setting, in which exercise duration and intensity can be carefully manipulated and measured, the EICBR model has been successfully established under continuous exercise modalities (21 km and 42 km run). EICBR is related to several factors, such as the exercise modality, intensity and duration, the subject's age,

gender, body mass and training status, and the environment. The exercise duration and intensity are the most important factors for eliciting a cTn release. The exercise intensity appears to cause a more pronounced increase in cTn levels than the exercise duration (Shave, George, Atkinson, Hart, Middleton & Whyte et al., 2007b; Fu, Nie & Tong, 2009; Serrano-Ostariz, Terreros-Blanco, Legaz-Arrese, George, Shave & Bocos-Terraz et al., 2009b). The majority of existing studies have reported descriptive data on subjects in a field setting during continuous prolonged endurance events. Few laboratory-based studies have observed EICBR resulting from intermittent exercise, although intermittent exercise is thought to be effective in causing cardiac biomarker release in the field (George, Dawson, Shave, Whyte, Jones & Hare et al., 2004; Nie, Tong, Shi, Lin, Zhao & Tian, 2007). HR and VO_2 are generally higher during intermittent exercise than during paired patterns (the same average power output) of continuous exercise (Edwards, Ekelund, Harris, Hesser, Hultman & Melcher et al., 1973). During intermittent exercise, fluctuations in VO_2 and a higher VO_{2max} are affected by central (stroke volume and HR) and peripheral (a-vO_2 difference) factors. The constant oxygen requirement during continuous training sessions is mainly associated with greater oxygen extraction (Daussin, Zoll, Dufour, Ponsot, Lonsdorfer-Wolf & Doutreleau et al., 2008). Intermittent exercise can therefore stimulate both the cardiac and skeletal muscle systems, whereas continuous exercise primarily affects only the skeletal muscle system.

Both intermittent and hypoxic training sessions are very popular in long-term endurance events, but the data on EICBR are limited. Acute hypoxic exposure may

result in an imbalance between ROS production and the antioxidant defence system and may therefore lead to oxidative stress compared with normoxia (Bailey, Davies & Young, 2001; Joanny, Steinberg, Robach, Richalet, Gortan & Gardette et al., 2001; Moller, Loft, Lundby & Olsen, 2001; Askew, 2002; Magalhaes, Ascensao, Viscor, Soares, Oliveira & Marques et al., 2004; Pialous, Mourier, Rock, Mazur, Schmitt & Richalet et al., 2009). Depending on the mechanism behind EICBR, exercise under hypoxic conditions may trigger more cardiac damage than under normoxic conditions. Shave et al. (2004) investigated the changes in cardiac biomarkers after cycling in a hypoxic environment and found that cTnT release was only detected under hypoxic conditions. However, there were no oxidative stress data to explain these findings.

Two peak values can be detected in the serum of AMI patients, which may represent different sources and release mechanisms related to the mechanism resulting in EICBR. In athletes, cardiac biomarkers can be detected 1 h after the onset of exercise (Middleton, George, Whyte, Gaze, Collinson & Shave, 2008). The highest levels of cardiac biomarkers are measured after 5 h (Fu et al., 2009) and the levels then decrease significantly over 24 h to return to baseline values (Herrmann, Scharhag, Miclea, Urhausen, Herrmann & Kindermann, 2003). In patients, cTnT and cTnI measurements are used to diagnose cardiac necrosis, which is due to the irreversible injury of the cardiomyocytes and the release of structurally bound proteins into the serum during acute cardiac ischemia. However, in athletes, the cytosolic pool of cTnT and cTnI may be of some importance in distinguishing between reversible and irreversible cardiomyocyte damage (Wu & Ford, 1999; Koller,

2003; Hickman, Potter, Aroney, Koerbin, Southcott & Wu et al., 2010). The exercise-induced release of cTnT and cTnI in obviously healthy athletes may be due to an exercise-induced reversible increase in the membrane permeability of the cardiomyocytes, resulting in a transient release of unbound cTn from the cytosolic pool into the serum (Wu & Ford., 1999; Neumayr, Pfister, Mitterbauer & Hoetnagl, 2005; Scharhag, Herrmann, Urhausen, Haschke, Herrmann & Kindermann, 2005; Scharhag, Urhausen & Schneider, 2006). The underlying mechanisms responsible for this transient cardiomyocyte cell membrane dysfunction may be related to elevated ROS production, driven by the oxidative stress that occurs during prolonged exercise (Nie et al., 2010a; Sahlin, Shabalina, Mattson, Bakkman, Fernstrom & Rozhdestvenskaya et al., 2010). There is as yet little evidence to explain the mechanisms by which cardiac biomarkers are released after prolonged exercise. Researchers are trying to show that this phenomenon is indeed part of a physiological response or reversible process that does not result in clinically significant cardiac damage, as exercise-induced oxidative stress is known to frequently occur after prolonged exercise. Nie et al. (2010a) determined the temporal association between myocardium oxidative stress and serum cTnT levels following prolonged exercise in an animal model. As studying the effect of oxidative stress on cardiac damage biomarker release in humans after performing prolonged exercise is clearly limited by the ability to assess specific changes in cardiac tissue and the interactive effect of skeletal muscle, only one human study has been conducted thus. Whyte et al. (2005) suggested that oxidative stress may be unrelated to the observed cardiac damage

following prolonged exercise, as skeletal muscle may be the main source of the oxidative stress biomarkers released into the serum during exercise. The observed biomarkers may therefore not reflect the myocardium and cardiomyocyte environment. However, the evidence in athletes is still limited.

The differences between EICBR in altitude training responders and non-responders are thus still unknown.

Purpose of the study

The purposes of this study were 1) to investigate the release of the cardiac biomarkers hs-cTnT and cTnI resulting from acute bouts of maximal intermittent exercise in a laboratory-based setting and set up an EICBR model; 2) to compare the changes in cardiac biomarkers in normoxic and hypoxic environments and determine the effects of hypoxia; 3) to compare the changes in oxidative stress biomarkers at various time points and under different environment conditions; 4) to observe the relationship between the oxidative stress biomarkers MDA, LH, SOD, CAT, GSH, TAOC and EICBR and to explore the hypothesis that lipid peroxidation triggers the release of cardiac biomarkers from the cytosolic pool.

Significance of the study

This study was designed to investigate the changes in cardiac biomarkers resulting from intermittent exercise under normoxic and hypoxic environments at multiple time points until 24 h post-exercise. Both intermittent training and hypoxic

training are very popular training methods used by elite athletes, especially long-distance runners, to enhance their exercise performance. However, there is limited data on the EICBR resulting from these training programs. The results of this study will enhance our understanding of this issue and provide recommendations regarding recovery periods after intermittent training and hypoxic training for marathon runners and coaches.

The hypothesis that exercise-induced oxidative stress triggers the release of cardiac biomarkers has not been much studied in humans because of limitations in our ability to assess specific changes in cardiac tissue *in vivo*. The results of this study will determine the mechanism for EICBR and provide suggestions for strategies to protect the heart from fatigue and minor damage before and after endurance training.

The concept of altitude training responders and non-responders is used to explain inter-individual variations after acute exposure to a hypoxic environment and in the improvement of performance after altitude training. This study will provide more information on the difference in cardiac damage experienced by these two groups.

Literature Review

The positive consequences and benefits of physical activity in the prevention, management and treatment of cardiovascular disease have been well documented. Participation in sports events, especially prolonged exercise such as long-distance running, is on the rise all over the world. Both recreational and competitive athletes, especially elite professionals, are constantly striving to optimise their fitness and performance.

The physiological foundations and determinants of prolonged exercise performance, especially long-distance running, are VO_{2max}, the relative VO_2 sustained during long-distance running (the maximal steady state), the skeletal muscle fibre type and running economy (Fallowfield & Wilkinson, 1999; Tian, 2003). The VO_{2max} of most elite male long-distance runners is greater than 70 $ml \cdot kg^{-1} \cdot min^{-1}$ (Noakes, 1991) and of elite female runners is greater than 65 $ml \cdot kg^{-1} \cdot min^{-1}$ (Costill, 1986). A runner with a VO_{2max} of 50 $ml \cdot kg^{-1} \cdot min^{-1}$ will not be able to complete a marathon within 2.5 h (which requires an average speed of 16.88 $km \cdot h^{-1}$), as this will demand a rate of oxygen uptake of 55 $ml \cdot kg^{-1} \cdot min^{-1}$ (Fallowfield & Wilkinson, 1999). It is therefore obvious that the higher a runner's VO_{2max}, the higher their peak running velocity and the better their endurance performance potential.

VO_{2max} is the best objective measurement of performance and is a widely used index for the integrity of cardiovascular function (Snell & Mitchell, 1984). According to the Fick principle, VO_{2max} is the product of cardiac output and the a-vO_2 difference

across the body (Wilber, 2004; Fukuda, Taketeru, Matsumoto, Komatsu, Nakajima & Nagai et al., 2010). Thus, there are both central (oxygen delivery) and peripheral (oxygen extraction) factors that determine systemic oxygen transport. However, the single most important peripheral factor that limits the ability of skeletal muscle to use oxygen in normal, healthy individuals without any disease (Lewis & Haller, 1989) is Q_{max} (Saltin, 1985). Thus, the measurement and training of the maximal exercise capacity, or VO_{2max}, may be viewed as a surrogate for the measurement and training of maximal cardiac function (Thompson, 2001). The stimulation and enhancement of cardiac function will therefore play an important role in improving prolonged exercise performance in both recreational and competitive athletes, especially elite professionals.

VO_{2max} is only one of the key physiology factors determining the average speed sustained during long-distance running. The performance achieved during a competition also depends on the skeletal muscle fibre type, running economy and anaerobic capacity. However, a small improvement in VO_{2max}, such as 1% to 2%, can significantly improve race performance. It is therefore not surprising that increasing VO_{2max}, especially through cardiac function, is frequently the principal target of prolonged exercise training programs.

Intermittent exercise and continuous exercise

Continuous and intermittent training programs are both established exercise modalities widely used in the preparation of athletes for prolonged exercise

competition events. Continuous training is characterised by a constant sub-maximal intensity and VO_2, whereas intermittent training involves repeated short to long bouts of high intensity exercise, equal or superior to the maximal competition intensity, interspersed with recovery periods, light exercise or rest, during which VO_2 fluctuates (Billat, 2001). For similar energy expenditures, training durations and frequencies, the two training modalities result in different endurance training adaptations and there is considerable debate as to which training modality, continuous or intermittent, will have a greater effect on VO_{2max}. Daussin et al. (2007) demonstrated that intermittent training sessions improved both the central and peripheral factors controlling VO_{2max}, whereas the constant oxygen requirement during continuous training sessions was mainly associated with greater oxygen extraction. Daussin et al. (2008) used a cross-over research design and found that VO_{2max} increased after both continuous (9%, $P<0.050$) and intermittent (15%, $P<0.010$) exercise training over 8 w, with no significant difference between the two modalities. Only the intermittent training program was associated with improvements in Q_{max}, which increased from 18.1±1.1 $L \cdot min^{-1}$ to 20.1±1.2 $L \cdot min^{-1}$ ($P<0.010$), as measured using a test to exhaustion. Q_{max} improved with intermittent exercise training through an increase in both HR (from 164±3 $beats \cdot min^{-1}$ to 168±4 $beats \cdot min^{-1}$, $P<0.050$) and stroke volume (from 110±7 ml to 119±8 ml, $P<0.050$). No significant changes were observed in these parameters after continuous training, as the HR changed from 165±4 $beats \cdot min^{-1}$ to 165±4 $beats \cdot min^{-1}$ and the stroke volume changed from 113±8 ml to 112±6 ml. An increase in Q_{max} is correlated with an increase in VO_{2max}. Intermittent training, especially low

volume, high intensity training, induces rapid improvements in the muscle's oxidative capacity (affecting the peripheral factors of VO_{2max}) and VO_{2max} itself (Harmer, McKenna, Sutton, Snow, Ruell & Booth et al., 2000; Burgomaster, Hughes, Heigenhauser, Bradwell & Gibala, 2005).

Participation in long-distance running events at moderate to high altitude and the use of both continuous and intermittent exercise sessions to optimise performance by elite long-distance runners is increasing. As mentioned, training to improve the maximal endurance performance, or VO_{2max}, may be viewed as training to improve Q_{max} and cardiac function. The work placed on the heart when cardiac function is enhanced will be considerable. Contrary to the positive consequences of acute and chronic exercise for the heart and circulatory system, there has been speculation in recent years that prolonged exercise, whether through acute exposure (marathon events) or chronic exercise (continuous and intermittent training), may actually have some negative consequences for cardiac function and health (George, Shave, Warburton, Scharhag & Whyte, 2008).

EICBR

Exercise-induced cardiac fatigue (EICF) is represented by a reduction in left ventricular function subsequent to prolonged exercise, such as long-distance running. EICF has been recently reported in both healthy recreational and competitive athletes, with limited success in elucidating its mechanisms (Dawson, George, Shave, Whyte & Ball, 2003). EICBR has been implicated in previous studies as a potential

mechanism for EICF. The evidence pertaining to this premise has been the identification of cardiac biomarkers, such as cTnT, cTnI, B-type natriuretic peptide (BNP), NT-Pro B-type natriuretic peptide (NT-Pro-BNP), creatine kinase and creatine kinase myocardial band mass, released post-exercise (Shave, George & Gaze, 2007a).

Following prolonged and progressive ischemia, cardiomyocytes undergo necrosis, damage and cardiac remodelling. Damaged myocardium then develops an abnormality in its cardiac function, which may lead to a failure in the heart's ability to pump blood at a rate that is commensurate with the requirements of the metabolism. A number of cardiac biomarkers can be detected in the serum that reflect the different stages of cardiac damage, including markers of inflammation (such as C-reactive protein), cardiac ischemia (such as ischemia modified albumin), cardiac cell necrosis (such as the cytosolic enzyme creatine kinase and the creatine kinase myocardial band mass), small protein myoglobin, the structural proteins cTnT and cTnI and markers of cardiac dysfunction (BNP and NT-Pro-BNP) (Shave et al., 2007a).

Other than in patients suffering from coronary syndromes, a range of minor EICBR caused by acute long-term endurance events has been reported in recreational and competitive athletes, including elite athletes. Most existing studies are primarily descriptive and use a broad range of assessment techniques, focusing on subjects in field-based environments and in prolonged endurance events, both in continuous (marathon, triathlon and cycling) and intermittent exercise events (George et al., 2004; Nie et al., 2007). Many confounding factors that may influence physiological stress should be considered, such as the exercise modality, intensity and duration, the

subject's age, gender, body mass and training status, and the environment (Dawson et al., 2003). Few of the studies related to this issue have used a laboratory-based setting and most have used continuous exercise to induce physiological stress, for example, 45 min and 90 min run at 80% and 100% of the ventilatory threshold (Fu et al., 2009); 45 min, 90 min and 180 min run at 85% and 95% of the anaerobic threshold (Serrano-Ostariz et al., 2009b) and a 50 mile cycle trial at the lactate threshold (Shave et al., 2004). As a result, the findings on the relationship between cardiac biomarkers and depressed cardiac function have been mixed (Scharhag, George, Shave, Urhausen & Kindermann, 2008).

Shave et al. (2007b) conducted a meta-analysis exploring the incidence of cTnT release after prolonged exercise, measured using a third-generation immunoassay, and demonstrated that approximately 47% (95% CI=39-56%) of the participants studied in endurance events finished the prescribed exercise with a serum cTnT greater than 0.01 $\mu g \cdot l^{-1}$ (the detection limit for the third-generation immunoassay used). The detection of post-exercise cTnT by a third-generation immunoassay after cycling events was approximately half that of running events (27% vs. 52%, P=0.042), and 53% for triathlon events. Lower rates of post-exercise cTnT release during cycling events may be explained by lower exercise intensities, as the exercise intensity appears to cause a more pronounced increase in cTnT levels than the exercise duration (Fu et al., 2009). The meta-analysis further showed that the detection of post-exercise cTnT decreased slightly as the event duration increased (P=0.022) and the mean body mass decreased (P=0.003). There was, however,

significant colinearity between the moderator variables of exercise duration and mean body mass (Kendall's tau=-0.346, P=0.0207). The post-exercise release of cTnT was therefore associated with body mass and with less well-trained individuals or with those competing at a higher intensity, but was not affected by age (P=0.309). It was only slightly higher (the mean slope of the meta-regression line=-0.007) for studies that included more male participants (P=0.028) (Shave et al., 2007b).

A similar meta-analysis of cTnI levels across different studies cannot be conducted because the numerous analysis platforms and different standards used preclude a direct comparison of the data. However, the post-exercise cTnI level may be similar to or higher than that of cTnT. Scharhag et al. (2005) examined cTnT and cTnI in one hundred and five endurance athletes after running a marathon, a 100 km ultra-marathon and a long-distance mountain bike race. They found that the level of cTnI increased by 74% and that 47% of the athletes exceeded the detection limit of cTnT (the coefficient of correlation between cTnT and cTnI: r=0.79, P<0.001).

BNP and NT-Pro-BNP are present in resting athletes' serum and increase after prolonged exercise. They are affected by the duration of the endurance exercise (Scharhag et al., 2008; Serrano-Ostariz, Legaz-Arrese, Terreros-Blanco, Lopez-Ramon, Cremades-Arroyos & Alvarez-Izquierdo et al., 2009a) and the athlete's age (Scharhag et al., 2005). BNP and NT-pro-BNP are hormones secreted predominantly by the myocytes in the ventricular wall in response to increased ventricular stretch and stress (Hall, 2004). Measurements of these two hormones are used in the diagnosis of suspected acute or chronic heart failure (Hobbs, Davis, Roalfe, Hare,

Davies & Kenkre, 2002; Hobbs, Davis, Roalfe, Hare, Davies & Heart, 2004; Zaphiriou, Robb, Murray-Thomas, Mendez, Fox & McDonagh et al., 2005). NT-pro-BNP is more widely used as a marker, as it has a longer half-life (120 min) than active BNP (20 min).

The relationship between BNP and NT-Pro-BNP and cTn has been the subject of some interest (Fu, Nie, George, Tong, Lin & Shi, 2010). Significant increases in BNP or NT-Pro-BNP and cTnT in heart failure patients (Sahinarslan, Guz, Okyay, Torer, Bali & Sindel et al., 2007) may suggest myocardial injury and decreased cardiac function (Kociol, Pang, Gheorghiade, Fonarow, Connor & Felker, 2010). Ohba et al. (2001) reported a relationship between exercise-induced increases in BNP and cTnT in healthy athletes, but this relationship could not be substantiated in larger studies (Scharhag et al., 2005; Fu et al., 2010).

The kinetic profile of EICBR during and after exercise

Very few studies have investigated the changes in serum cardiac biomarkers during exercise. Middleton et al. (2008) found that inter-individual variations affect the kinetic profile of cTnT release. During marathon running on a motorised treadmill for between 60 min and 120 min, cTnT (measured using a third-generation immunoassay) increased in all of the participants. The elevation in cTnT began after 60 min (in five subjects) and 120 min (in four subjects) of exercise and returned to the baseline level in all of the subjects within 1 h post-exercise. All but one subject showed a further release of cTnT after the completion of the marathon within the 24 h

recovery period, with five of these subjects showing an elevated cTnT 24 h after exercise. Unlike in the usual pre- and post-exercise studies, the serum levels of cTnT in this study peaked at between 90 min to 150 min of exercise in seven subjects during marathon run, at 12 h after the completion of the marathon in one subject, and at 24 h post-exercise in one subject. The release of cTnT within the first 60 min of exercise indicated that exercise-induced cTnT release is not necessarily limited to prolonged endurance exercise.

Nevertheless, the clinical significance of EICBR is still under debate because different release kinetics has been reported. After 2 h to 4 h of AMI, the cytosolic unbound cTn in patients is released, followed by the structurally bound troponin of the thin filament. Release into the serum persists up to 10 d to 14 d, or even as long as 21 d. In athletes, exercise-induced increases in cardiac biomarkers typically decrease significantly within 24 h after exercise and usually reach baseline values within this period (Herrmann et al., 2003). The relationship between raised cardiac biomarkers and depressed cardiac function (BNP and NT-Pro-BNP) has been reported with little consequence (Scharhag et al., 2008).

Potential mechanisms underlying EICBR

Based on the bimodal release kinetics recorded in AMI patients, it is assumed that there are at least two pools of cTn, the cytosolic and structural pools (Katus et al., 1991; Adams et al., 1994). Analyses performed during the development of the troponin assays suggest that approximately 5.0% or 6.0% to 8.0% of the total cTnT

found in the heart is in the cytosolic pool, which is perhaps better termed the early releasable pool, and similarly approximately 3.7% or 2.0% to 8.0% of the total cTnI is in the cytosolic pool. Although little is known about the renewal and regeneration process of the thin filament, which is fundamental for understanding the origin of these two physically different pools, the two pools are thought to be important in the kinetics of EICBR because of the prompt increase in cTn in the clinical setting and the long persistence of cTn in the circulation after an acute incident. The early releasable pool of cTnT and cTnI is very similar to the amount of creatine kinase myocardial band mass found in the myocytes, which is thought to cause the initial increases. The subsequent release and elevation of cTn, which can persist for a longer time, are due to the degradation of the structurally bound pool. A persistent elevation of cTn probably reflects continuing degradation, rather than delayed clearance, because even in patients with renal dysfunction, clearance curves do not appear to be altered (Ellis, Dreisbach & Lertora, 2001).

The cytosolic pool may be of some importance in differentiating between reversible (ischemia) and irreversible (necrosis) cardiomyocyte injury. EICBR in obviously healthy athletes may be due to an exercise-induced reversible increase in the membrane permeability of the cardiomyocytes, resulting in a transient release in unbound cTn from the cytosolic pool into the serum (Wu & Ford, 1999; Neumayr et al., 2005; Scharhag et al., 2005; Scharhag et al., 2006). Any extrapolation from findings on cardiovascular patients with AMI to healthy athletes should be performed cautiously, as there is only limited direct evidence of myocardial ischemia resulting

from prolonged exercise (Scharhag et al., 2008). The underlying mechanisms responsible for exercise-induced cTn release, both ischemic development and transient changes in membrane permeability, could be related to elevated ROS production caused by the oxidative stress that occurs commonly with prolonged exercise (Nie et al., 2010a; Sahlin et al., 2010).

Nie et al. (2010a) mentioned that the underlying mechanisms responsible for EICBR are unknown. Previous authors have speculated that cTnT and cTnI release during prolonged exercise is mediated through myocardial stunning (Starnes & Bowles, 1995), the ischemic development of blebs (Hickman et al., 2010; Lippi & Banfi, 2010) and transient changes in membrane permeability (Neumayr, Pfister, Mitterbauer, Maurer, Gaenzer & Sturm 2002), among others. It is possible that all of these potential mechanisms are related to the elevated ROS production that occurs with prolonged exercise. The heart may be vulnerable to oxidative damage due to oxidative stress (Kakarla, Vadluri & Reddy, 2005) because it is highly aerobic (Chance, Sies & Boveries, 1979), has metabolic processes that produce ROS at rest and during exercise (Di Meo & Venditti, 2001) and has reduced antioxidant enzyme activity compared to other tissues (Kakarla et al., 2005). Increased levels of ROS can harm cells by triggering lipid cell membrane peroxidation, which may result in myocyte cell membrane dysfunction and damage (Sen, 2001).

There is as yet little evidence for this hypothesis. One human study conducted by Whyte et al. (2005) examined the relationship between cardiac damage and the markers of oxidative stress, TEAC, MDA and 4-hydroxynoenal, after the Flora

London Marathon in 2003. Thirty-two of the thirty-nine runners studied had cTnT (measured using a third-generation immunoassay) levels above the assay's detection limit (0.01 $\mu g \cdot l^{-1}$; detected levels ranged from 0.01 $\mu g \cdot l^{-1}$ to 0.73 $\mu g \cdot l^{-1}$) and twenty runners had cTnT levels above the AMI cut-off (0.05 $\mu g \cdot l^{-1}$). The TEAC (1.80±0.12 $mmol \cdot l^{-1}$ pre-exercise to 1.89±0.21 $mmol \cdot l^{-1}$ post-exercise) and MDA (2.90±1.58 $\mu mol \cdot l^{-1}$ pre-exercise to 3.59±1.47 $\mu mol \cdot l^{-1}$ post-exercise) levels were significantly increased post-marathon, but were unrelated to the changes in cTnT (TEAC, r=0.10, P>0.050; MDA, r=0.40, P>0.050; 4-hydroxynoenal, r=0.39, P>0.050). These results suggest that ROS production and formation may be unrelated to the minimal cardiac damage observed after prolonged exercise. However, TEAC, MDA and 4-hydroxynoenal are global biological measures and may not reflect the tissue and cellular environment, specifically that of the myocardium and cardiomyocytes. The time course used only revealed the situation immediately after exercise. Nie et al. (2007, 2010b) provided evidence that cTn concentration peaks at 5 h after the onset of exercise.

Analysing the effect of ROS production on EICBR in humans who perform prolonged exercise is clearly limited by the ability to assess tissue specific changes. Nie et al. (2010a) observed the temporal association between myocardium oxidative stress and serum cTnT levels following prolonged exercise in an animal model. Male Sprague-Dawley rats were accessed for serum cTnT at 0 h (0.053±0.023 $ng \cdot ml^{-1}$; ranging from 0.020 $ng \cdot ml^{-1}$ to 0.100 $ng \cdot ml^{-1}$, P<0.050) and 2 h (0.329±0.678 $ng \cdot ml^{-1}$; ranging from 0.016 $ng \cdot ml^{-1}$ to 2.110 $ng \cdot ml^{-1}$, P<0.050) after a 3 h acute bout of

swimming with 5% of the body weight attached to the tail. In the heart tissue, the myocardial MDA level (baseline 1.3±0.3 nmol·mg·pro^{-1}) was elevated at 0 h (1.7±0.2 nmol·mg·pro^{-1}, P<0.050) and 2 h (1.6±0.3 nmol·mg·pro^{-1}, P<0.050) post-exercise. The authors concluded that temporal changes in the serum cTnT matched the increase in the myocardial tissue concentration of MDA. The temporal association between the levels of cTnT and MDA suggests a role for the exercise-induced increase in ROS in the mediation of the cTnT release from cardiomyocytes that is commonly recorded after prolonged exercise. The study was, however, limited in terms of the number of markers of ROS damage and antioxidant activity that were measured and in that the duration, intensity and volume of cardiac activity could not be carefully manipulated.

Howatson et al. (2011) challenged the results of Nie et al. (2010a). Antioxidant supplementation has been shown to attenuate oxidative stress, inflammation and muscle damage indices following strenuous exercise. Therefore, if there is a temporal relationship between cTn release and lipid peroxidation in cell membranes (measured through myocardial MDA), antioxidant supplementation should reduce cTn release. Howatson et al. (2011) administered an antioxidant or a placebo supplement each day from 5 d prior to 48 h following a marathon. The antioxidant supplement raised the TAOC at 24 h post-exercise and reduced the markers of inflammation post-exercise (interleukin 6) and 24 h after exercise (C-reactive protein). However, no difference in the cTnI release was observed between the groups. Howatson et al. (2011) suggested that the mechanisms responsible for EICBR are not related to exercise-induced oxidative stress and inflammation damaging the cardiomyocyte membrane. The

integrity of the cardiomyocyte membrane may be compromised by mechanical stress associated with sustained elevations in the HR, the formation of membrane blebs or the stimulation of integrin. The mechanisms responsible for EICBR are still unknown and are currently a matter of debate.

Oxidative stress biomarkers and exercise

ROS are naturally produced in the human body and exert both positive (immune system) and negative (lipid, protein or DNA oxidation) effects (Finaud et al., 2006). In the immune system, neutrophils and macrophages are in charge of destroying foreign substances. They produce free radicals through the reduced nicotinamide-adenine dinucleotide phosphate-oxidase system, which is present in the leukocytes. The programmed formation of ROS during the immunity process plays an essential biological role in homeostasis control. During oxygen metabolism, ischemia reperfusion and haemoglobin and myoglobin oxidation, the formation of ROS is not programmed.

The biological effects of ROS can be positive or negative. As mentioned, ROS are involved in the immunity phenomenon, in particular by acting against antigens during phagocytosis (Jenkins, 1988; Cheeseman & Slater, 1993; George et al., 2008). ROS play an important role in cellular signals and in the biogenesis of cells, because they can serve as cell messengers and modify the oxidation-reduction status (Rimbach, Hohler & Fischer, 1999; Sen, 2001). ROS can also have possible harmful effects, because they can alter the size and shape of the compounds they interact with

(Jenkins, 1988; Cooper, Vollaard & Choueiri, 2002). ROS can induce apoptosis in healthy cells, provoke inflammation or altered cellular functions and have the ability to oxidise poly-unsaturated free fatty acids, which take part in the organisation of the cell membrane (Alessio, 1993; Cheeseman & Slater, 1993; Jenkin & Goldfarb, 1993).

The oxidation of poly-unsaturated free fatty acids initiates lipid peroxidation, a chain reaction that produces more ROS and substances such as conjugated dienes, MDA, TBARS and LH (Young & McEneny, 2001). Lipid peroxidation changes the fluidity of the cell membrane, reduces the membrane's capacity to maintain an equilibrated concentration gradient and increases membrane permeability and inflammation (Radak, Kaneko & Tahara, 1999). Consequently, it is possible to detect a loss of intracellular liquids, a decrease in calcium transport in the endoplasmic reticulum, alterations in mitochondrial functions, cell alterations and the loss of cryptozoic proteins and enzymes. Every type of cell can be damaged by ROS, including cardiac muscle. Along with the lipid peroxidation effects, which may result in cell membrane dysfunction and damage to cells, including myocytes (Ji, 2001), ROS also oxidise the blood and structural proteins (Szweda, Frigust & Szweda, 2002) and cause DNA strand breaks and damage (Renke, Popadiuk & Korzon, 2000; Levine, 2002).

An antioxidant can be defined as a substance that helps to reduce the severity of oxidative stress, either by forming a less active radical or by quenching the damaging ROS chain reactions on substrates. A range of antioxidants are active in the body, such as the antioxidant enzymes SOD, CAT and GSH and the non-enzymatic

antioxidants vitamin A, vitamin C, vitamin E, uric acid and ferritin. The efficiency of the antioxidant system depends on nutritional intake and on endogenous antioxidant enzyme production, which can be modified by exercise, training and ageing (Dekkers, Doornen & Kemper, 1996). Oxidative stress is a condition in which an imbalance exists between ROS production and the antioxidant defence system.

Since the initial finding of increased lipid peroxidation following acute aerobic exercise in 1978 (Dillard, Lotov, Savin, Dumelin & Tappel, 1978), the field of oxidative stress and exercise has expanded substantially. A direct assessment of ROS is extremely difficult due to their high reactivity and relatively short half-lives. The majority of ROS research related to exercise has used indirect methods, monitoring the levels of more stable molecular products and oxidation target products formed during ROS reactions, such as the lipid peroxidation end products MDA, TBARS and LH. Oxidative stress can be measured by observing the alterations in the antioxidant system, such as changes in the antioxidant enzymes SOD, CAT, GSH and TAOC.

Oxidative damage after acute bouts of exercise

The most common method used to determine exercise-induced oxidative stress damage after aerobic exercise is the assessment of lipid peroxidation using MDA, LH and TBARS levels (Fisher-Wellman & Bloomer, 2009). MDA is a three carbon chain aldehyde produced during the decomposition of LH. The majority of existing studies have noted no increase in the levels of MDA following maximal exercise (Niess, Hartmann, Grunert-Fuchs, Poch & Speit, 1996), whereas a few studies have reported a significant increase (Braun, Clarkson, Freedson & Kohl, 1991; Ashton, Young,

Peters, Jones, Jackson & Davies et al., 1999; Fatouros, Jamutas, Villiotou, Pouliopoulou, Fotinakis & Taxildaris et al., 2004). The studies reporting significant increases typically used maximal or near maximal exercise protocols, indicating the role of exercise intensity in MDA formation. Some studies noted an increase in LH levels (Ashton et al., 1999; Radak et al., 1999) or no change in LH (Quindry, Stone, King & Broeder, 2003; Gochman, Reznick, Avizohar, Ben-Amotz & Levy, 2007) post-exercise.

Antioxidant capacity after acute bouts of exercise

The body's antioxidant capacity may be temporarily decreased by strenuous physical work, as the components of the antioxidant defence system are used to quench the harmful radicals produced. SOD is the major defence against superoxide radicals and is the first defensive line against oxidative stress. In all cells at rest, the majority of ROS produced in the mitochondria are reduced by the mitochondrial SOD. In muscular cells, 65% to 85% of the SOD activity is performed in the cytosol (Power & Lennon, 2000a). CAT is present in every cell, particularly in the peroxisomes, which are cell structures that use oxygen to detoxify toxic substances and produce H_2O_2 (Antunes, Derick & Cadenas, 2002). The GSH peroxidase in the cell cytosol and mitochondria has the ability to transform H_2O_2 into water, transforming GSH into oxidised GSH in the process. The measurement of GSH can also reveal lipid peroxidation.

Vitamin E terminates the chain reaction of lipid peroxidation by reacting with the peroxyl radical. Vitamin E then becomes a radical itself and is subsequently

reduced by vitamin C, forming yet another radical, which is further reduced by GSH. Vitamin E is a fat-soluble vitamin made up of several isoforms known as tocopherols and has been called the most important chain-breaking antioxidant because of its abundance in cells and the mitochondrial membrane and its ability to act directly on ROS (Evans, 2000). Vitamin E interacts with numerous antioxidants, such as vitamin C and GSH, which have the capacity to regenerate vitamin E from its oxidised form (Coombes, Powers & Rowell, 2001). Vitamin E thus plays an important role in cell membranes because it stops lipid peroxidation.

During and immediately after exercise, it appears that the antioxidant capacity of the body may be reduced (Tozzi-Ciancarelli, Penco & Massimo, 2002; Di Massimo, Scarpelli & Tozzi-Ciancarelli, 2004; Steinberg, Delliaux & Jammes, 2006). The capacity then typically increases above basal conditions during the recovery period (Young & MeEneny, 2001; Watson, Callister, Taylor, Sibbeitt, MacDonald-Wicks & Garg, 2005; Steinberg et al., 2006). However, conflicting findings have been reported regarding the main enzyme SOD (Tozzi-Ciancarelli et al., 2002) and no change after exercise has also been reported for SOD and CAT (Laaksonen, Atalay, Niskanen, Uusitupa, Hanninen & Sen, 1999; Miyazaki, Oh-ishi, Ookawara, Kizaki, Toshinai & Ha et al., 2001). Typically, a decrease in GSH (Szczesniak, Karolkiewicz, Deskur, Rychlewski, Konys & Stankiweicz, 1998; Steinberg, Ba, Bregeon, Deiliaux & Jammes, 2007) has been reported following a variety of aerobic exercise protocols. The GSH status typically returns to the baseline within 15 min to 30 min of recovery (Steinberg et al., 2006; Steinberg et al., 2007).

To understand why these contradictory results have been obtained, it is necessary to understand the current limitations of oxidative stress and acute exercise research. The multiple body systems, including the antioxidant defence system, function in a complex, interconnected fashion. Concrete conclusions regarding precisely how and why ROS are produced during exercise therefore require further research. Any current proposed complete explanation of these processes may underestimate the complexity of the human body and the associated redox systems. It is clear that acute exercise has the potential to result in increased ROS production, which may or may not result in acute oxidative stress. As stated earlier, for oxidative stress to occur, the ROS produced during exercise must exceed the antioxidant defence system present, thereby resulting in oxidative damage to specific biomolecules (Fisher-Wellman & Bloomer, 2009). Different exercise protocols may induce different levels of ROS production, as oxidative damage has been shown to be dependent on both exercise intensity and duration (Fisher-Wellman & Bloomer, 2009). Other factors appear to affect the degree of antioxidant defence present, including age (Sacheck, Cannon, Hamada, Vannier, Blumberg & Roubenoff, 2006), training status (Elosua, Molina, Fito, Arquer, Sanchez-Quesada & Covas et al., 2003) and dietary and supplement intake (Watson et al., 2005). If oxidative stress does occur, detection depends to a large degree on the tissue sampled and the specificity of the biomarker chosen (Dalle-Donne, Rossi, Colombo, Giustarini & Milzani, 2006).

Oxidative stress in the myocardium and exercise

Cardiac muscle tissue has a high oxidative metabolic rate and relatively low

activity of the main antioxidant enzymes, which may enhance its susceptibility to oxidative injury after acute exercise (Ascensao, Magalhaes, Sores, Oliveira & Duarte, 2003). The post-neonatal heart normally has a low cell growth rate and slow protein turnover, suggesting that the myocardium may have a limited ability to adapt to acute oxidative stress (Ji, 2000). As the myocardium consists of predominantly aerobic tissue, most of its antioxidant enzymes have a higher absolute activity than those in skeletal muscle (Ascensao et al., 2003). Given the high rates of oxygen consumption and ROS production, the ability of cardiac muscle to neutralise ROS appears to be limited (Ascensao et al., 2003). GSH is crucial to tissue antioxidant activity in general, and the concentration of GSH in the myocardium is well below that found in other tissues, such as skeletal muscle, liver and kidneys (Powers, Ji & Leeuwenburgh, 1999; Powers & Sen, 2000b).

There is direct evidence that acute exercise can induce increased ROS generation in the myocardium, as confirmed by Ohkuwa et al. (1997), who found an increase in the cardiac levels of free radicals. The increase in the tissue metabolic rate obviously represents a major assault on cardiac muscle. Alterations in the antioxidant system and myocardial injury markers have been reported following acute exercise. Ohkuwa et al. (1997) and Liu et al. (2000) observed an increase in the cardiac concentration of GSH in young rats subjected to a short period of intense exercise. Venditti and Di Meo (1996) found an increase in the cardiac markers for lipid peroxidation in rats after a period of exhausting swimming. This exercise-induced increase in the myocardial lipid peroxidation products, MDA and LH, supports the

hypothesis that exercise increases oxidative stress and results in structural damage to the cardiomyocytes.

The role of exogenous vitamin E, a non-enzymatic antioxidant, in protecting cardiac tissue during and after intense exercise has also been studied. Benderitter et al. (1996) analysed the relationship between the variations induced by exercise in the cardiac concentration of vitamin E and the markers for lipid peroxidation of TBARS in rats. The relationship reflected the contribution of vitamin E in combating exercise-induced cardiac lipid peroxidation. It therefore seems clear that cardiac muscle tissue, when stimulated by acute exercise, presents increases in the markers of cell damage due to oxidative stress, notably lipid peroxidation. The non-enzymatic antioxidant GSH and vitamin E system appears to play a crucial role in protecting the heart in the harmful situation arising from acute exercise. Howatson et al. (2011) did not obtain positive results because they did not include vitamin E for ingestion as the exogenous factor.

Normoxia, absolute hypoxia and relative hypoxia

Acute exposure to a hypoxic environment (both at natural altitude and in a nitrogen apartment) can impair VO_{2max} with a progressively increasing altitude, although with large inter-individual variations (Billat et al., 2003; Friedmann et al., 2005). Discussions continue regarding how each step in the oxygen transport pathway contributes to this loss in exercise capacity and the disruptions to homeostasis. The current working model uses the passive diffusive steps in the lungs and muscles to

explain most of the decrease in oxygen availability (Wagner, 2010). This mechanism, especially the diffusion limitation in the lung, is exaggerated in athletes with relative high pulmonary blood flows (Johnson, 1967; Torre-Bueno, Wagner, Saltzman, Gale & Moon, 1985; Levine & Stray-Gundersen, 1999) and who may develop exercise-induced hypoxaemia even at sea level (Dempsey & Wagner, 1999). VO_{2max} is reduced progressively and linearly by 0.6% per 100 m altitude in direct proportion to the reduction in SaO_2 (Wehrlin & Hallen, 2006). With an increasing ascent to a higher altitude, there is a progressive decline in the amount of oxygen in the ambient or inspired air (PIO_2). At low altitudes, the resting SaO_2 is generally well maintained and thus results in only a marginal disruption in homeostasis. As one ascends to more moderate altitudes (up to 3000 m), a slight, but significant, decrease in the resting SaO_2 (from 95% to 92%) is observed and the ambient PIO_2 can decrease from 159 mmHg at sea level to 110 mmHg. At even higher altitudes (5000 m), the ambient PIO_2 can decrease further to 85 mmHg and the resting SaO_2 approaches 80% and lower. This decrease in the inspired oxygen pressure and the resulting drop in the SaO_2 with high altitude exposure represent a significant disruption in homeostasis (Mazzeo, 2008). As the SaO_2 decreases immediately following acute exposure to hypoxia, the resting cardiac output increases to maintain normal oxygen consumption. Naeije et al. (1982) found a typical response in twenty-four subjects acutely breathing a fraction of inspired oxygen of 12% (4100 m). The subjects' cardiac output increased by 22%, which was entirely explained by an 18% increase in HR, whereas the stroke volume did not change. It is remarkable that the increase in cardiac output exactly matched

the decrease in SaO_2, ensuring that the product of the two, oxygen delivery to the tissue, remained unchanged. However, it must be noted that when the same absolute workload is performed in hypoxia and in normoxia, a greater relative exercise intensity is elicited in hypoxia, accompanied by a significantly greater disruption in homeostasis and a lower VO_{2max}. A greater physiological and metabolic adjustment is required when performing similar exercise tasks in hypoxia than in normoxia. For example, a series of studies (Mazzeo, Bender & Brooks, 1991; Wolfel, Groves & Brooks, 1991; Mazzeo, Brooks & Butterfield, 1995; Wolfel, Selland & Cymerman, 1998) conducted in the Pikes Peak, Colorado, USA, found that cycling at a power output of 100 W at sea level elicited a VO_2 of 1.5 $L \cdot min^{-1}$, which represented a relative work intensity of 50% of the VO_{2max} in normoxia. When cycling at an identical workload of 100 W at the summit of Pikes Peak (4300 m), a VO_2 similar to that observed at sea level (1.5 $L \cdot min^{-1}$) was found. However, due to the reduction in the VO_{2max} in hypoxia (a 25% decrease compared to sea level), this represented a significantly greater relative exercise intensity of 65% of the VO_{2max} in hypoxia.

Consequently, an individual's VO_{2max} declines progressively with increasing altitude. A particular absolute workload performed in normoxia will result in a greater relative exercise intensity when performed in hypoxia. When exercising in hypoxia, the body must respond and adapt to two independent stresses, hypoxia and exercise. The simultaneous presence of the two stressors will have an additive effect that will influence the maximal exercise capacity and the overall exercise performance.

Cardiac biomarkers and oxidative stress biomarkers in a hypoxic environment

Acute hypoxic exposure may result in an imbalance between ROS production and the antioxidant defence system, leading to oxidative stress (Bailey, Davies & Young, 2001; Joanny et al., 2001; Moller et al., 2001; Askew, 2002; Magalhaes et al., 2004; Pialoux et al., 2009). Even 3 h of resting at 3000 m hypoxia or 10 min of 4800 m hypoxic exercise at an intensity of 30% VO_{2max} can induce oxidative stress, significantly increase the levels of MDA and oxidation protein products and decrease the alpha-tocopherol in highly trained athletes. Changes in the MDA and SaO_2 levels are significantly correlated, suggesting that ROS production mechanisms are different during hypoxia and exercise responses (Pialoux et al., 2009).

Davison et al. (2006) used electron spin resonance spectroscopy to directly detect ROS and found that a single bout of 2 h of exercise at 55% VO_{2max} intensity and at 2000 m under hypoxic conditions resulted in no observable increases in the free radical concentration from the normoxic baseline. The authors found that other hypoxia studies had largely used intermittent exhaustive protocols, at or near an intensity of 100% VO_{2max}, to generate oxidative stress (Bailey et al., 2000; Bailey et al., 2001), suggesting that both the exercise modality and SaO_2 (degree of hypoxia) play an important role in the generation of oxidative stress. During low intensity and duration protocols, the antioxidant defence system appears sufficient to meet the ROS production. As the intensity and duration of the exercise increases, the defence system is no longer adequate, potentially resulting in oxidative damage to the surrounding tissues (Fisher-Wellman & Bloomer, 2009). Other factors, such as dietary intake, will

affect the degree of antioxidant defence present.

If oxidative stress occurs, detection depends on the tissue or serum sample, the timing of a given sample and the sensitivity and specificity of the biomarkers chosen (Dalle-Donne et al., 2006). Significant or null findings in previous studies may be related to the lack of specificity of the chosen biomarker (such as TBARS (Oh-ishi, Heinecke, Ookawara, Miyazaki, Haga & Radak et al., 2002)) or an improper sampling protocol (Fisher-Wellman & Bloomer, 2009).

The mechanism underlying endurance exercise-induced oxidative stress is thought to be elevated VO_2 and mitochondrial oxygen flux, but is still under debate. Studies conducted in a hypoxic environment have found that exercise-induced increases in lipid peroxidation are consistently associated with a decrease in SaO_2 but not in VO_2. A systemic increase in VO_2 is therefore not the exclusive mediator of exercise-induced oxidative stress under hypoxic conditions. Exercise and hypoxia may play different roles in the increase of ROS (Alessio, Hagerman, Fulkerson, Ambrose, Rice & Wiley, 2000; Bailey et al., 2000). An acute bout of exercise performed in hypoxia may selectively increase oxidative stress compared to normoxia, although this is still under debate.

If cardiac biomarker release is related to oxidative stress, does exercise in hypoxia trigger more cardiac damage than normoxia? Shave et al. (2004) investigated the changes in cardiac biomarkers after cycling in a hypoxic environment and found that cTnT release (measured using a third-generation immunoassay) was only detected in hypoxia. However, there were no oxidative stress data to explain the

findings. Eight trained male triathletes completed two 50 mile cycle trials at the same absolute intensity, equivalent to the lactate threshold (previously determined in normoxia), randomly assigned from normoxia and hypoxia (FIO_2=15%, simulating an altitude of 2500 m). Blood was collected pre-exercise and at 0 h and 24 h post-exercise. The completion times for the normoxic (126±7 min) and hypoxic (125±6 min) trials were not significantly different. HR was not recorded during the continuous exercise. The cTnT level (measured using a third-generation immunoassay) was only increased in the youngest, least trained subject (0.016 $\mu g \cdot L^{-1}$) 0 h after the hypoxic trial. The results demonstrated that 2 h of continuous exercise by trained subjects in a hypoxic environment did not have a greater effect on EICBR.

Altitude training responders and non-responders

As mentioned, altitude training programs in both natural and simulated normobaric hypoxic environments are widely used by elite athletes, especially long-distance runners to enhance sea-level performance. Acute exposure to a hypoxic environment may impair VO_{2max}, although there is large inter-individual variation. Altitude training responders are subjects who exhibit a large reduction in VO_{2max} and suffer from reductions in both Q_{max} and maximal a-vO_2 difference. Altitude training non-responders are subjects who can tolerate hypoxia, exhibit a small drop in VO_{2max}, mostly suffer from reductions in Q_{max}, but are able to maintain their maximal a-vO_2 difference. The ability to maintain the maximal a-vO_2 difference suggests that altitude training non-responders may keep their skeletal muscle work in hypoxia.

Participation in prolonged exercise may increase the risk of cardiovascular emergency, such as AMI. Over 1,500,000 cases of myocardial infarction occur annually in the USA (American Heart Association, 1992). Over 25,000 sudden death cases may occur soon after exercise (Chen, Serfass, Macker-Bojack, Kelly, Titus & Apple, 2000). Unlike skeletal muscle, in which fatigue and damage are natural physiological adaptations to exercise, the heart does not have the luxury of stopping for rest and massage therapy after running a marathon (George et al., 2008). The work placed on the heart when cardiac function is enhanced is considerable. The usual cause of an elevation in cTn in cardiovascular patients is myocyte necrosis. The appearance of cTn in apparently healthy athletes, including adolescents (Tian, Nie, Tong, Cao, Gao & Man et al., 2006; Nie et al., 2007; Fu et al., 2009; Fu et al., 2010), participating in prolonged exercise has therefore raised concerns about the cardiovascular health consequences of such exercise (La Gerche & Prior, 2007). It has been hypothesised that cTn release is most likely related to cytosolic pool leakage from the cardiac myocyte membranes due to membrane dysfunction induced by oxidative stress, rather than myocyte necrosis. However, Harper (2010) believed that, in the absence of a long-term follow-up study, this hypothesis should be viewed with caution. Both animal (Chen et al., 2000) and human case studies (McKechnie, Leary, Noakes, Kallmeyer, MacSearraigh & Olivier, 1979; Harper, 2010) have directly or indirectly shown evidence of cardiac necrosis after prolonged exercise. Due to the potential importance of this research topic and the limited current findings, care must be taken when discussing the controversy that the release of cTn cardiac damage

biomarkers is an indicator of risk of a cardiovascular event or is a cardiac adaptation to prolonged exercise.

Statement of hypotheses

Research hypotheses

Acute bouts of maximal intermittent exercise can increase the serum levels of hs-cTnT and cTnI, both in normoxic and hypoxic trials, with the highest peak levels observed in an absolutely hypoxic trial. The levels of the oxidative stress biomarkers MDA, LH, SOD, CAT, GSH and TAOC will change post-exercise and the highest peak levels will be observed in an absolutely hypoxic trial. Correlations will be observed between hs-cTnT, cTnI and MDA, LH, SOD, CAT, GSH and TAOC in the global circulation.

Statistical hypotheses

1. There will be no significant differences between the pre- and post-exercise levels of hs-cTnT and cTnI from acute bouts of maximal intermittent exercises in normoxic, absolutely hypoxic and relatively hypoxic trials.

2. There will be no significant differences in the hs-cTnT and cTnI serum levels at each time point between the normoxic, absolutely hypoxic and relatively hypoxic trials.

3. There will be no significant differences between the pre- and post-exercise levels of MDA, LH, SOD, CAT, GSH and TAOC from acute bouts of maximal intermittent exercise in normoxic, absolutely hypoxic and relatively hypoxic trials.

4. There will be no significant differences in the levels of MDA, LH, SOD, CAT, GSH and TAOC at each time point between the normoxic, absolutely hypoxic and relatively hypoxic trials.

5. There will be no relationship between the peak levels of hs-cTnT and cTnI and MDA, LH, SOD, CAT, GHS and TAOC at any time point.

6. There will be no significant differences in the levels of hs-cTnT and cTnI in altitude training responders and non-responders.

Methods

Pilot study

The purposes of the pilot study were to test the protocol for determining VO_{2max} and corresponding vVO_{2max} and to test the maximal intermittent exercise protocol.

Three well-trained male marathon runners (Table 1) took part in the pilot study in a normoxic environment.

Table 1. Participants' physical characteristics and training information (N=3)

Participants	A	B	C
Age (y)	26	23	22
Body mass (kg)	58.6	58.0	64.0
Body height (cm)	173.0	173.0	176.0
Training years (y)	8	6	8
Training volume ($km \cdot w^{-1}$)	70	50	60
Personal best in a marathon race (min)	170	179	160

The method of the pilot study is as follows.

1) Determination of the VO_{2max} and corresponding vVO_{2max}. A treadmill was used to determine the VO_{2max} and corresponding vVO_{2max}. An initial speed of 10 $km \cdot h^{-1}$ with a 0 slope was used. The participants ran for 3 min at each stage and the speed was increased by 1 $km \cdot h^{-1}$ after each stage (every 3 min), without any pause between stages. When the respiratory exchange ratio reached 1.00, the stages were shorten to 2 min and the speed increments continued at 1 $km \cdot h^{-1}$ until exhaustion and

vVO_{2max} was the minimum speed at which the participant was running when VO_{2max} occurred, provided that this speed was sustained for at least 1 min (Billat, Flechet, Petit, Muriaux & Koralsztein, 1999).

2) Maximal intermittent exercise protocol. The maximal intermittent exercise consisted of a hard run at 100% vVO_{2max} for 3 min, followed by an easy run at 50% vVO_{2max} for 3 min, forming a 6 min bout. The protocol continued for 10 bouts of 60 min in total (Middleton et al., 2008). The HR was recorded pre-exercise and 30 min after the onset of exercise. Five millilitres of antecubital venous blood was collected pre-exercise and 4 h and 24 h post-exercise. The results are presented in Table 2.

Table 2. The results of the pilot study

Participants	A	B	C
Time spent in the VO_{2max} test (min)	22	21	21
Terminal speed of the VO_{2max} test ($km \cdot h^{-1}$)	22	21	21
Bouts completed in the maximal intermittent exercise (n)	8	9	7
cTnI_4h ($ng \cdot ml^{-1}$)	<0.010*	0.040	<0.010*

* The detection limit of cTnI was 0.010 $ng \cdot ml^{-1}$.

Both protocols were altered based on the results of the pilot study. The initial speed of the VO_{2max} test was increased to 12 $km \cdot h^{-1}$ with a 2% slope to decrease the duration and terminal speed of the test. A bout in the maximal intermittent exercise was redefined as a hard run at 90% vVO_{2max} for 2 min, followed by an easy run at 50% vVO_{2max} for 2 min, and the protocol was increased to 23 bouts without any pause between bouts.

Participants

Twelve well-trained marathon runners were recruited from the local Physical Education Department of the Beijing Sport University and a running club in Beijing. Ten of the runners completed all of the preliminary testing, experimental trials and protocols in the normoxic and hypoxic environments for this study (Table 3). These ten participants were free of any disease, non-smokers and did not take any antioxidant supplements within the month before the study. The participants gave written consent and were fully informed about the potential cardiovascular risks and the purpose and procedures of the study. The study was approved by the Ethical Committee on the Use of Human and Animal Subjects in Research at Hong Kong Baptist University. The participants' physical characteristics and training information are shown in Table 3.

Table 3. Participants' physical characteristics and training information

N	10
Age (y)	22.1±2.6
Gender	Male
Body mass (kg)	64.0±4.9
Body height (cm)	177.3±3.9
VO_{2max}_N in normoxia ($ml \cdot kg^{-1} \cdot min^{-1}$)	64.72±5.63
vVO_{2max}_N in normoxia ($km \cdot h^{-1}$)	18.2±1.0
VO_{2max}_H in hypoxia ($ml \cdot kg^{-1} \cdot min^{-1}$)	62.16±6.74
vVO_{2max}_H in hypoxia ($km \cdot h^{-1}$)	16.7±0.7
Training years (y)	5.6±2.3
Training volume ($km \cdot w^{-1}$)	51.1±3.3
Personal best in a marathon race (min)	169.7±9.5

Values are means ± standard deviation (SD).

Preliminary testing and familiarisation

An initial medical examination, including any history of cardiac symptoms, resting blood pressure, electrocardiography and standard echocardiography, was performed by a team of medical doctors and technicians at the Beijing Sport University to exclude any acute or chronic potential cardiac diseases. All of the participants were given enough time to familiarise themselves with the treadmill, facemask and other equipment used in the study before the experimental trials commenced.

Experimental trials – determination of the VO_{2max} and corresponding vVO_{2max}

A treadmill was used to determine the VO_{2max} and corresponding vVO_{2max}. The participants performed a general 5 min to 10 min warm up at a self-set speed. The test then used an initial speed of 12 $km \cdot h^{-1}$ with a 2% slope. The participants performed stages of 3 min and the speed increased by 1 $km \cdot h^{-1}$ after every stage (every 3 min), without pauses between stages. When the respiratory exchange ratio reached 1.00, the stages were shorted to 2 min and continued to increase by 1 $km \cdot h^{-1}$ after every stage. The test stopped either when the increase in VO_2 was less than 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ while the respiratory exchange ratio was >=1.15 with an increase in speed, or when exhaustion was reached. VO_{2max} was the highest 30 s average value of the recorded VO_2.

The corresponding vVO_{2max} was the minimal speed at which the participant was running when VO_{2max} was reached, as long as this speed was sustained for at least

1 min. Ventilatory, metabolic and HR data were recorded during the test.

This test was conducted in both normoxic (FIO_2=21.0%) and hypoxic (FIO_2=14.4%, simulating an altitude of 3000 m) environments in the nitrogen apartment of the Research Centre in the Beijing Sport University. The order of the two environments was selected at random and the two tests were separated by at least 72 h (Levine & Stray-Gundersen, 2009).

Protocols – maximal intermittent exercise

Each participant completed three trials of maximal intermittent exercise, separated by at least 7 d. The trials were conducted in three conditions, normoxia (trial N, FIO_2=21.0%), absolute hypoxia (trial AH, FIO_2=14.4%) and relative hypoxia (trial RH, FIO_2=14.4%), and the order in which each participant faced the conditions was selected at random. The VO_{2max} tests were conducted in both the normoxic (VO_{2max}_N and vVO_{2max}_N) and hypoxic environments (VO_{2max}_H and vVO_{2max}_H). Trials N and AH used the VO_{2max}_N and vVO_{2max}_N and trial RH used the VO_{2max}_H and vVO_{2max}_H to calculate the exercise intensity. As mentioned in the literature review, VO_{2max} decreases in a hypoxic environment. The exercise intensity was therefore the same in trials N and RH and higher in trial AH. The differences between trial N and trial RH therefore reflect the real hypoxic effects at the same exercise intensity.

The maximal intermittent exercise tests were conducted in the afternoon. The participants were instructed to refrain from exercise and alcohol for 48 h before each

test and to maintain their usual dietary pattern. A standardised breakfast and lunch were provided to all participants on a test day. Prior to the start of each trial, the participants completed a 1000 m warm-up at their own pace.

In trials N and AH, the bout of maximal intermittent exercise was a hard run (16.4±0.9 $km \cdot h^{-1}$) at 90% vVO_{2max}_N for 2 min, followed by an easy run (9.1±0.5 $km \cdot h^{-1}$) at 50% vVO_{2max}_N for 2 min with a 2% slope. The test covered 23 bouts of 92 min in total, without pauses between bouts.

In trial RH, the bout of maximal intermittent exercise was a hard run (15.0±0.6 $km \cdot h^{-1}$) at 90% vVO_{2max}_H for 2 min, followed by an easy run (8.4±0.3 $km \cdot h^{-1}$) at 50% vVO_{2max} _H for 2 min with a 2% slope. The test covered 23 bouts of 92 min in total, without pauses between bouts.

Table 4. Exercise intensities in trials N, AH and RH

	Intermittent exercise intensity ($km \cdot h^{-1}$)	FIO_2 of the nitrogen apartment	Simulated Altitude
N	2 min 90% vVO_{2max}_N : 2 min 50% vVO_{2max}_N 2 min 16.4±0.9 : 2 min 9.1±0.5	21.0%	0 m
AH	2 min 90% vVO_{2max}_N : 2 min 50% vVO_{2max}_N 2 min 16.4±0.9 : 2 min 9.1±0.5	14.4%	3000 m
RH	2 min 90% vVO_{2max}_H : 2 min 50% vVO_{2max}_H 2 min 15.0±0.6 : 2 min 8.4±0.3	14.4%	3000 m

Measurements

Venous blood samples (5 ml) were taken from the antecubital vein using

venous punctures before the maximal intermittent exercise (pre-exercise), immediately after the maximal intermittent exercise (0 h) and 2 h, 4 h and 24 h post-exercise in all of the trials (Nie et al., 2007; Middleton et al., 2008; Fu et al., 2010; Nie et al., 2010; Tian et al., 2010). Each blood sample was allowed to clot at room temperature (20°C), and then centrifuged at 3000 g for 15 min. The separated serum was drawn and stored at -80°C for further analysis.

The cardiac damage biomarkers hs-cTnT and cTnI in the serum were analysed at every time point. A new highly sensitive enzyme immunoassay based on electrochemiluminescence technology was used in the Elecsys 2010 automated batch analyser (Roche Diagnostics, Basel, Switzerland). The detection limit for the assay was 3 pg·ml^{-1}. The 99th percentile cut-off concentration was 14 pg·ml^{-1} and the level at 10% coefficient of variation was 13 pg·ml^{-1}. The upper reference limit (URL) was set at 14 pg·ml^{-1} (Giannitsis, Kurz, Hallermanyer, Jarausch, Jaffe & Katus, 2010). The recommended diagnostic threshold for AMI using cTnT, according to the World Health Organisation guidelines, is 100 pg·ml^{-1}. Serum cTnI was measured using the AccuTnI assay method in an Access analyser (Beckman Coulter, Miami, FL, USA). The detection limit for cTnI was 0.01 ng·ml^{-1}. The reported URL for cTnI ranges from 0.04 to 0.80 ng·ml^{-1}, depending on the laboratory test used. Here, the URL for cTnI was set at 0.04 ng·ml^{-1} (Apple, Quist, Doyle, Otto & Murakami, 2003; Scharhag et al., 2008; Serrano-Ostariz et al., 2009a; Serrano-Ostariz et al., 2009b).

The oxidative stress biomarkers MDA, LH, SOD, CAT, GSH and TAOC (Nie et al., 2010a; Tian et al., 2010) in the serum at each time point were determined using

a commercial assay kit from the Nanjing Jiancheng Institute, China, in a spectrophotometer (DU7400 Beckman Co. USA). Lipid peroxidation was evaluated using the TBARS method and was expressed as the MDA concentration. A spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid with MDA at 535 nm was used to determine the MDA level, which was expressed in $nmol \cdot ml^{-1}$. The level of GSH was determined colourimetrically at 412 nm with the spectrophotometer, following the reaction of GSH with 5,5-dithiobes (2-ni-trobenzoic acid), and was expressed in $mg \cdot L^{-1}$. TAOC was measured using the method of Benzie and Strain (1996). Ferric ions in the reaction mixture were reduced with antioxidant reducing agents to form the complex ferrous-tripyridyltriazine, the concentration of which was estimated using a colourmetric assay and used to reflect the concentration of TAOC, expressed in $U \cdot ml^{-1}$. The SOD activity was measured with a xanthine-xanthine oxidase system, which is a superoxide anion generator that follows the augmentation and reduction of the corresponding coloured compounds. SOD activity was expressed in $U \cdot ml^{-1}$ and determined by a decrease in H_2O_2 absorption at 240 nm.

HR (S810, Polar, Finland) and SaO_2 (Nonin Medical Inc., Plymouth, MN, USA) were recorded before and during the maximal intermittent exercise.

Statistical analysis

A statistical analysis was performed using the software programme SPSS 19.0. All of the results were expressed as means ± SD. A Kolmogorov-Smirnov test was

used to evaluate the normality of the data. As the data followed a skewed distribution (P<0.050), a non-parametric Friedman's test was used to compare hs-cTnT and cTnI pre- and post- (0 h, 2 h, 4 h and 24 h) exercise and a Wilcoxon signed rank test was used for pair comparisons where appropriate. The Bonferonni adjusted P value was 0.050/4. A non-parametric Friedman's test was used to compare the hs-cTnT and cTnI levels at each time point across the three conditions (N, AH and RH) and a Wilcoxon signed rank test was used for pair comparisons where appropriate. The Bonferonni adjusted P value was 0.050/3. The serum cTnI was reported as <0.010 $ng{\cdot}ml^{-1}$ and was therefore represented as 0.005 $ng{\cdot}ml^{-1}$. The positive rates in the three conditions, which are the percentage of participants with hs-cTnT and cTnI values above the URL of 14 $pg{\cdot}ml^{-1}$ and 0.04 $ng{\cdot}ml^{-1}$, were compared using Fisher's exact test. The P value was set at 0.050.

As the data were normally distributed (P>0.050), a one-way repeated ANOVA test was used to compare the MDA, LH, SOD, CAT, GSH and TAOC levels pre- and post- (0 h, 2 h, 4 h and 24 h) exercise. A post hoc least significant difference test was used where appropriate. The Bonferonni adjusted P value was 0.050/4. A one-way repeated ANOVA test was then used to compare the MDA, LH, SOD, CAT, GSH, TAOC, HR and SaO_2 levels at each time point across the three conditions (N, AH and RH). A post hoc Bonferroni test was used where appropriate. The P value was set at 0.050.

Pearson's product moment correlation coefficients were used to determine the degree of association between the peak levels of hs-cTnT and cTnI, and MDA, LH,

SOD, CAT, GSH and TAOC. The P value was set at 0.050.

As the data were normally distributed (P>0.050), an independent sample t-test was used to compare the differences between VO_{2max} and the percentage change in VO_{2max} in the altitude training responders and non-responders. A non-parametric Mann-Whitney U test was used to compare the differences in hs-cTnT and cTnI between the responders and non-responders. The P value was set at 0.050.

Two participants had extreme outlier results. Appendix II shows an alternative analysis after the deletion of these two participants.

Appendix III shows a one-way repeated ANOVA test comparing the levels of hs-cTnT and cTnI pre- and post- (0 h, 2 h, 4 h and 24 h) exercise across the three conditions (N, AH and RH).

Appendix IV shows four 2×3 two-way repeated ANOVA tests using time (pre- and post-exercise) and condition (N, AH and RH) to compare the levels of MDA, LH, SOD, CAT, GSH and TAOC.

Appendix V shows a 2×3×5 factorial ANOVA test using group (responders and non-responders), condition (N, AH and RH) and time (pre- and post- (0 h, 2 h, 4 h and 24 h) exercise) to compare the levels of MDA, LH, SOD, CAT, GSH and TAOC in the altitude training responders and non-responders.

Results

Ten participants completed the 92 min maximal intermittent exercise for trials N, AH and RH. No participants reported cardiac symptoms, either during or after the exercise. In trial N, the SaO_2 was 98.10±0.88% before exercise and 95.96±1.23% during exercise. In trial AH, the SaO_2 was 93.10±2.85% before exercise and 86.23±2.71% during exercise. In trial RH, the SaO_2 was 93.50±1.51% before exercise and 86.61±3.91% during exercise. During exercise, the SaO_2 in trial N was significantly higher (*P*=0.000) than that in trial RH.

As shown in Table 5, the HR during exercise in trial AH was significantly higher than in trial N and trial RH, but there were no significant differences between the HR in trials N and RH.

Table 5. HR during maximal intermittent exercise in trials N, AH and RH

		HR (beats·min^{-1})	*P*
Hard run			
	Trial N	170.7±1.8	0.040 (N vs. AH)
	Trial AH	176.1±1.6	
	Trial RH	170.4±1.6	0.012 (RH vs. AH)
Easy run			
	Trial N	138.1±4.3	0.004 (N vs. AH)
	Trial AH	147.3±3.2	
	Trial RH	138.0±3.3	0.000 (RH vs. AH)

Changes in cardiac biomarkers

Changes in hs-cTnT

The changes in hs-cTnT pre- and post-exercise and the differences between the three conditions are shown in Table 6. Compared with the baseline level (5.118±1.857 pg·ml^{-1}), hs-cTnT increased 0 h post-exercise in both trials N and AH. In trial RH, hs-cTnT began to increase 2 h post-exercise. The level peaked 2 h post-exercise, remained at this high level until 4 h post-exercise and returned to the baseline level 24 h post-exercise. When the three trials were compared, hs-cTnT_AH_0h was significantly higher than hs-cTnT_RH_0h (P=0.011).

In trial N, seven participants (70%) had hs-cTnT levels above the URL. Trial AH recorded 90% and trial RH 50% such participants. There was no significant difference in the numbers of hs-cTnT-positive rates between the three conditions. The positive rate in trial AH was not significantly higher than that in trial RH (P=0.051).

Table 6. Changes in hs-cTnT (pg·ml^{-1})

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	5.118±1.857	9.628±3.797* (P=0.005)	24.290±18.628* (P=0.005)	22.563±16.493* (P=0.005)	5.978±1.849
AH	5.118±1.857	11.486±4.861*† (P=0.008)	37.001±31.995* (P=0.005)	34.593±27.632* (P=0.005)	7.803±3.886
RH	5.118±1.857	8.221±3.513†	28.614±23.561* (P=0.005)	26.132±18.453* (P=0.005)	6.222±2.463

* Significantly different from pre-exercise, P<0.050/4;

† Significantly different from another condition, P<0.050/3.

Changes in cTnI

The changes in cTnI pre- and post-exercise and the differences in the three conditions are shown in Table 7. In trial AH, cTnI increased 2 h post-exercise from the baseline level (equal to or lower than 0.010 ng·ml^{-1}), peaked 4 h post-exercise and returned to the baseline level 24 h post-exercise. There were no significant changes in trial N and RH and no significant differences between the three conditions.

In trial N, five participants (50%) had a cTnI level above the URL. Trial AH recorded 90% and trial RH 60% such participants. There was no significant difference between the cTnI-positive rates in the three conditions. The positive rate in trial AH was not significantly higher than that in trial N (P=0.051).

Table 7. Changes in cTnI (ng·ml^{-1})

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	0.0050	0.0065±0.0047	0.0275±0.0324	0.0375±0.0437	0.0050
AH	0.0050	0.0095±0.0072	0.0475±0.0533* (P=0.007)	0.0695±0.0786* (P=0.007)	0.0050
RH	0.0050	0.0065±0.0047	0.0345±0.0375	0.0540±0.0573	0.0050

* Significantly different from pre-exercise, P<0.050/4.

Changes in oxidative stress biomarkers

The changes in the oxidative stress biomarkers (MDA, LH, SOD, CAT, GSH and TAOC) and the differences between the three conditions are shown in Table 8-13.

Table 8. Changes in MDA ($nmol \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	4.794±0.533	4.715±0.413	4.721±0.613	4.614±0.411	4.870±1.160
AH	4.794±0.533	4.775±0.438	4.941±0.572	4.539±0.616	4.607±0.598
RH	4.794±0.533	4.797±0.757	5.253±0.883	4.705±0.563	5.028±0.510

Table 9. Changes in LH ($mIU \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	17.841±1.327	19.564±1.992	18.339±0.929	18.742±2.098	18.374±1.680
AH	17.841±1.327	19.040±1.860	18.522±1.909	18.605±2.006	19.838±2.951
RH	17.841±1.327	19.037±3.041	18.622±1.838	19.529±2.461	19.835±1.533

Table 10. Changes in SOD ($U \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	82.084±7.891	83.604±12.070	81.055±6.883	88.094±8.734	79.839±9.508
AH	82.084±7.891	85.113±12.291	78.770±9.447	87.821±8.831	85.549±10.443
RH	82.084±7.891	86.235±11.821	85.674±7.934	91.499±7.023* (P=0.003)	84.765±11.964

* Significantly different from pre-exercise, P<0.050/4.

Table 11. Changes in CAT ($U \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	57.977±5.580	55.982±3.912	56.002±2.607	60.287±5.793	58.133±3.516
AH	57.977±5.580	58.305±3.651	57.732±3.978	57.596±4.471	57.968±5.205
RH	57.977±5.580	53.837±5.026	58.547±4.839	60.644±3.668	58.379±3.458

Table 12. Changes in GSH (mg·L^{-1})

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	6.822±0.734	7.836±0.691* (P=0.010)	7.675±0.731	7.280±0.750	7.174±0.668
AH	6.822±0.734	7.474±0.378	7.548±0.580	7.326±0.483	7.433±0.470
RH	6.822±0.734	7.628±0.376	7.891±0.742	7.635±0.720	7.844±0.633

* Significantly different from pre-exercise, P<0.050/4.

Table 13. Changes in TAOC (U·ml^{-1})

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	6.049±1.086	6.613±0.851	5.488±0.836	7.106±0.830	6.554±0.741
AH	6.049±1.086	6.222±0.822	5.173±0.701	6.056±0.786	6.721±0.974
RH	6.049±1.086	6.735±1.035	5.742±0.851	6.587±0.943	6.590±0.519

Relationships between the cardiac biomarkers and oxidative stress biomarkers

Tables 14 and 15 show the correlations between the peak levels of the cardiac biomarkers (hs-cTnT_2h, hs-cTnT_4h, cTnI_2h and cTnI_4h) and oxidative stress biomarkers (MDA, LH, SOD, CAT, GSH and TAOC).

Table 14. Correlations in trial AH

	cTnI_2h	cTnI_4h	MDA_0h	TAOC_24h
hs-cTnT_2h	0.970(0.000)		0.652(0.057)	0.794(0.011)
hs-cTnT_4h		0.975(0.000)	0.714(0.031)	0.714(0.031)
cTnI_2h			0.699(0.036)	0.795(0.010)
cTnI_4h			0.725(0.027)	0.769(0.015)

The results are shown in r (P).

Table 15. Correlations in trial RH

	cTnI_2h	cTnI_4h	TAOC_4h
hs-cTnT_2h	0.952(0.000)		0.650(0.042)
hs-cTnT_4h		0.933(0.000)	0.705(0.023)
cTnI_2h			0.765(0.011)
cTnI_4h			0.665(0.036)

The results are shown in *r* (*P*).

Differences between the altitude training responders and non-responders

Based on the percentage change in VO_{2max} (ΔVO_{2max}), the participants were divided into altitude training responders, those with a large reduction in VO_{2max} in a hypoxic environment (ranging from -14.6% to -6.2%, median -11.1%), and non-responders, those with a small reduction or no change in VO_{2max} in a hypoxic environment (ranging from -5.6% to 14.1%, median -0.7%). Table 16 shows that there was a significant difference between the ΔVO_{2max} (P=0.019) in the responders and non-responders, but no significant difference in the hs-cTnT and cTnI levels (Tables 17 and 18).

Table 16. Differences in VO_{2max} between the responders and non-responders

	VO_{2max}_N ($ml \cdot kg^{-1} \cdot min^{-1}$)	VO_{2max}_H ($ml \cdot kg^{-1} \cdot min^{-1}$)	ΔVO_{2max} (%)
Responders(N=5)			
Means ± SD	65.14±4.57	58.22±4.49	-10.6±3.0*
Range	60.1 - 70.7	51.3 - 61.5	-14.6 - -6.2
Non-responders(N=5)			
Means ± SD	64.30±7.06	66.10±6.59	3.1±8.4*
Range	56.4 - 74.6	51.3 - 61.5	-5.6 - 14.1

*Significant difference between the responders and non-responders, $P<0.050$;

ΔVO_{2max}= (VO_{2max}_H - VO_{2max}_N)/VO_{2max}_N.

Table 17. Differences in hs-cTnT ($pg \cdot ml^{-1}$) levels between the responders and non-responders

		Pre-exercise	Post-exercise			
			0 h	2 h	4 h	24 h
N	R	5.030±2.080	8.340±4.361	16.384±9.874	16.352±8.892	5.612±2.462
	NR	5.206±1.847	10.916±3.046	32.196±22.956	28.774±20.893	6.344±1.137
AH	R	5.030±2.080	10.770±6.285	39.930±46.662	34.778±40.225	7.206±4.810
	NR	5.206±1.847	12.202±3.518	34.072±10.223	34.408±9.993	8.400±3.153
RH	R	5.030±2.080	8.446±3.668	25.164±24.471	23.892±19.240	5.838±2.989
	NR	5.206±1.847	7.996±3.766	32.064±24.908	28.372±19.582	6.606±2.085

R = responders; NR = non-responders.

Table 18. Differences in cTnI ($ng \cdot ml^{-1}$) levels between the responders and non-responders

		Pre-exercise	Post-exercise			
			0 h	2 h	4 h	24 h
N	R	0.005	0.005	0.011±0.008	0.020±0.018	0.005
	NR	0.005	0.012±0.004	0.046±0.038	0.060±0.052	0.005
AH	R	0.005	0.008±0.007	0.053±0.077	0.079±0.113	0.005
	NR	0.005	0.022±0.016	0.042±0.019	0.060±0.029	0.005
RH	R	0.005	0.008±0.007	0.023±0.028	0.049±0.053	0.005
	NR	0.005	0.012±0.004	0.048±0.043	0.066±0.060	0.005

R = responders; NR = non-responders.

Discussions

This is the first study to investigate the release of the cardiac biomarkers hs-cTnT and cTnI during acute bouts of maximal intermittent exercise in a laboratory-based setting, and to succeed in setting up an EICBR model. This study also investigates the effect of maximal intermittent exercise in a hypoxic environment on cardiac biomarkers. The relationship between cardiac damage and oxidative stress is revealed for the first time in a human study, providing evidence that oxidative stress, especially lipid peroxidation, is responsible for EICBR.

During the maximal intermittent exercise, the HR recorded in trial AH was significantly higher than that in trial N and RH, and no significant differences were recorded in the HR in trials N and RH. The exercise output intensity or human physiological response was therefore similar in trials N and RH. The SaO_2 recorded during exercise in trial RH was lower than that in trial N. Trials N and RH had the same exercise output intensity at different levels of hypoxia. Any differences between the two trials should reveal the effects of a hypoxic environment on EICBR without the interaction of exercise intensity.

The EICBR model

The results of this study indicate that maximal intermittent exercise over 92 min, at an intensity of 90% vVO_{2max} hard run interspersed with 50% vVO_{2max} easy run, both in normoxic and hypoxic environments, can induce the release of cardiac

biomarkers.

The hs-cTnT biomarker

The novel, specific and sensitive marker hs-cTnT was developed in 2010 (Giannitsis et al., 2010). The first- (1987) and second- (1992) generation cTnT assays use bovine TnT as a standard and concern has been raised over the potential cross-reactivity with sTnT in the presence of skeletal muscle damage (Wu, Valdes & Apple, 1994). The third- (2000) generation, highly specific cTnT assay uses recombinant human cTnT as the calibration material and has been clinically validated to have cardiac specificity in the presence of exercise-induced skeletal muscle damage (Shave et al., 2000).

The fourth- (2004) generation cTnT assay uses monoclonal antibodies that recognise epitopes five residues apart in the central part of the molecule: M7, which recognises an epitope at residues 125-131, and M11.7, which recognises an epitope at residues 136-147. This antibody selection corresponds to cardiospecific sequences that have no sequence homology to foetal TnT. A recent analysis reported, however, that a protein found in the skeletal muscle of patients with primary skeletal muscle disease is captured by all of these antibodies, again raising the possibility that the specificity of cTnT for cardiac muscle may be absolute (Giannitsis et al., 2010).

The hs-cTnT (2010) assay uses the fragment-antigen binding portions of two cTnT-specific mouse antibodies directed against epitopes in the central region of human cTnT. The capture antibody (M7) is biotinylated and directed against an epitope at amino acid residues 125-131 and is identical to that in the fourth-generation

assay. The detection antibody is directed against an epitope at amino acid residues 136-147. The original antibody (M11.7) has been reengineered, replacing the constant C1 region of the fragment-antigen binding region with a human IgG C1 region to produce a mouse-human chimeric detection antibody. The assay sensitivity has been increased by increasing the sample volume from 15 μL to 50 μL, increasing the ruthenium concentration of the detection antibody and lowering the background signal by buffer optimisation (Giannitsis et al., 2010).

This study uses this more specific and sensitive biomarker. As mentioned, the level seen in the 99th percentile of healthy subjects is defined as the URL and concentrations above the URL are considered elevated. The positive rate of hs-cTnT (those exceeding the URL of 14 pg·ml^{-1}) in this study was 70% (seven out of ten participants) after 92 min of maximal intermittent exercise consisting of hard run at 16.4±0.9 km·h^{-1} with an HR of 170.7±1.8 beats·min^{-1} and easy run at 9.1±0.5 km·h^{-1} with an HR of 138.1±3.3 beats·min^{-1}. The hs-cTnT level increased 0 h post-exercise and peaked 2 h after the maximal intermittent exercise, approximately 3.5 h after the onset of exercise. The hs-cTnT remained at the peak high level until 4 h post-exercise and returned to the baseline level within 24 h after the maximal intermittent exercise.

Using limited adult data obtained with the hs-cTnT immunoassay, Tian et al. (2012) observed that 85% of adults studied (eleven out of thirteen subjects) had hs-cTnT levels above the URL (14 pg·ml^{-1}) after a 90 min continuous run at an exercise intensity of 95% of the ventilatory threshold. The running velocity was 12.1±0.9 km·h^{-1} and the HR during exercise was 161±13 beats·min^{-1}. The peak level of hs-

cTnT occurred at 3 h post-exercise and returned to the baseline level 24 h after continuous exercise. The hs-cTnT peaked at 19.1 pg·ml^{-1} (ranging from 9.7 pg·ml^{-1} to 305.6 pg·ml^{-1}) after continuous exercise, compared with 24.290±18.628 pg·ml^{-1} (ranging from 6.170 pg·ml^{-1} to 61.970 pg·ml^{-1}) after intermittent exercise.

It is difficult to compare these two studies, as there are large individual variations in the cTnT released (Shave et al., 2007b; Scharhag et al., 2008). It appears that, compared with similar continuous exercise, intermittent exercise can trigger a higher peak level of cTnT and a faster rate of release into the serum, which may be related to a higher exercise intensity (Shave et al., 2007b; Fu et al., 2009; Serrano-Ostariz et al., 2009b). This issue must be investigated in further studies.

It is also difficult to compare this study with those previously conducted, as the immunoassay technology has improved dramatically. The meta-analysis conducted by Shave et al. (2007b) demonstrated that approximately 47% of participants in long-term run, 52% of participants in cycling and 53% of participants in triathlons finished their exercise with a serum cTnT level (measured using a third-generation assay) greater than the detection limit. Mingels et al. (2009) demonstrated that after running a marathon, 86% of runners had hs-cTnT levels greater than the URL, whereas only 45% of the runners showed an increased cTnT level when measured using a fourth-generation cTnT assay. Further, the hs-cTnT assay is the only assay shown to have a performance capability sufficient to detect the cTnT concentration in healthy individuals before exercise. This newly developed assay therefore has greater sensitivity for calculating the rate of cTnT-positive cases.

Intermittent exercise can also trigger EICBR. This result enhances some contradictory field-based studies that are still under debate. George et al. (2004) found that, after competing in an inter-university game (80 min to 90 min), nine rugby and ten soccer players had no change in their cTnT levels (measured using a third-generation assay). Nie et al. (2007) found that, after a 66 min training game, four out of ten male adolescent basketball players showed an increased serum cTnT level above the myocardial injury cut-off of 0.01 ng·ml^{-1}. As EICBR is related to a subject's age and training experience, the peak levels of cardiac biomarkers and their positive rates are lower in adults than in adolescents (Tian et al., 2012). When research is conducted in adults, the exercise intensity should therefore have an important effect on the EICBR.

The cTnI biomarker

The positive rate of exercise-induced increases in cTnI in this study was 50% (five out of ten participants) after 92 min of maximal intermittent exercise in a normoxic environment. The cTnI level increased 2 h post-exercise and peaked (0.0275±0.0324 ng·ml^{-1}) 4 h post-exercise, approximately 5.5 h after the onset of exercise. The serum cTnI returned to the baseline level and was below the detection limit within 24 h. Further, cTnI_2h was strongly related to hs-cTnT_2h (r=0.970, P<0.000) and cTnI_4h was strongly related to hs-cTnT_4h (r=0.975, P<0.000).

The positive rate of exercise-induced increases in cTnI cannot be calculated using a meta-analysis, because existing studies use different immunoassays with different standards, resulting in incomparable cTnI values. However, it has been

demonstrated that the post-exercise cTnI positive rate may be similar to that of cTnT, as confirmed by this study, or may be higher than that of cTnT (measured using a third-generation assay). Scharhag et al. (2005) examined cTnT and cTnI levels in one hundred and five endurance athletes after a marathon run, a 100 km ultra-marathon or a long-distance mountain bike race and found that the URL of cTnI (0.04 $ng \cdot ml^{-1}$; AccuTnI, Beckman Coulter) was exceeded by 74% of the athletes, whereas only 47% of the athletes exceeded the URL of cTnT (measured using a third-generation assay, the coefficient of correlation between cTnT and cTnI was r=0.79, P<0.001). Carranza-Garcia et al. (2010) found that, after an indoor soccer match, which is a kind of field-based intermittent exercise, cTnI increased to 0.033±0.051 $\mu g \cdot L^{-1}$ in both males and females, but that there was no change in cTnT (measured using a third-generation assay). Nie et al. (2007) found that, after a basketball training program, 30% of subjects had a serum cTnI concentration greater than 0.06 $ng \cdot ml^{-1}$, which is the standard for the diagnosis of a myocardial injury.

Effect of maximal intermittent exercise in a hypoxic environment on cardiac biomarkers

The results of this study suggest that the additional stimulus of hypoxia does not induce more EICBR. The hs-cTnT-positive rates were 70% in trial N, 90% in trial AH and 50% in trial RH and there was no significant difference between the three conditions. The cTnI-positive rates were 50% in trial N, 90% in trial AH and 60% in trial RH and there was no significant difference between the three conditions.

The kinetics profile of hs-cTnT release after maximal intermittent exercise was almost the same under the three conditions. The peak levels at 2 h post-exercise were 24.290±18.628 pg·ml^{-1} in trial N, 37.001±31.995 pg·ml^{-1} in trial AH and 28.614±23.561 pg·ml^{-1} in trial RH and there was no significant difference between the three conditions. The three conditions showed similar changing trends in cTnI and the peak levels at 4 h post-exercise were 0.0375±0.0437 ng·ml^{-1} in trial N, 0.0695±0.0786 ng·ml^{-1} in trial AH and 0.0540±0.0573 ng·ml^{-1} in trial RH. There was again no significant difference between the three conditions.

The HR_AH during the hard run was significantly higher than the HR_N (*P*=0.040) and HR_RH (*P*=0.012). However, the hs-cTnT and cTnI levels, which are strongly related to exercise HR and intensity (Serrano-Ostariz et al., 2009a; Legaz-Arrese, George, Carranza-Garcia, Munguia-Izquierdo, Moros-Garcia & Serrano-Ostariz, 2011), were not significantly different between the three conditions. Shave et al. (2004) studied eight trained male triathletes who completed two 50 mile cycle trials, randomly selected to be under normoxic or hypoxic conditions (FIO_2=15.0%, simulating an altitude of 2500 m), at the same absolute intensity (input stimulus) equivalent to their lactate threshold, which was previously determined in a normoxic environment. Blood was collected pre-exercise and 0 h and 24 h post-exercise. The completion times for the normoxic trial (126±7 min) and hypoxic trial (125±6 min) were not significantly different. The HR was not recorded during the continuous exercise. The cTnT levels (measured using a third-generation assay) increased (0.016 μg·L^{-1}) immediately after the hypoxic trial in only the youngest, least well-trained

subject. Shave et al. (2004) therefore demonstrated that 2 h of continuous exercise in a hypoxic environment did not have a significant effect on EICBR in trained subjects, which was also confirmed by this study.

It appears that exercise intensity plays a more important role in EICBR than a hypoxic stimulus. The 24 h kinetic profile of hs-cTnT release was approximately the same in trials N and AH, but not in trial RH. The hs-cTnT level did not increase 0 h post-exercise in trial RH and the hs-cTnT_AH0h (11.486±4.861 $pg \cdot ml^{-1}$) was significantly higher than the hs-cTnT_RH0h (8.221±3.513 $pg \cdot ml^{-1}$, $P=0.033$). The running speed in trial RH was lower than in trial AH. This result agrees with Fu et al. (2009), who showed that cTnT levels (measured using a third-generation assay) were related to exercise speed (input stimulus) in a laboratory-based setting, and with Serrano-Ostariz et al. (2009a), who showed that cardiac biomarker release was related to exercise HR in a field-based setting.

Effect of maximal intermittent exercise on oxidative stress

Previous studies that also use multiple time point measurements have defined 0 h, 2 h and 4 h post-exercise as the early recovery period and 24 h post-exercise as the late recovery period (Tian et al., 2010).

The findings of this study suggest that the antioxidant defence capacity increases during the early recovery period after maximal intermittent exercise in marathon runners. The significant increases in the serum GHS (from 6.822±0.734 $mg \cdot L^{-1}$ to 7.836±0.691 $mg \cdot L^{-1}$, $P=0.010$) 0 h post-exercise support this hypothesis.

If the antioxidant defence capacity increases in a hypoxic environment, SOD levels may change during the early recovery period following maximal intermittent exercise in a hypoxic environment to defend against the resulting oxidative stress. Trial RH was performed under relatively the same exercise intensity as trial N, but at a lower SaO_2. During the early recovery period, SOD increased 4 h post-exercise in trial RH (from 82.084±7.891 $U \cdot ml^{-1}$ to 91.499±7.023 $U \cdot ml^{-1}$, P=0.003).

GSH is considered to be the main endogenous antioxidant (Fisher-Wellman & Bloomer, 2009). GSH peroxidase uses GSH as a substrate to transform H_2O_2 into water and oxidised GSH. GSH can directly detoxify ROS (of which H_2O_2 is one), thereby enhancing the functional antioxidant capacity of the non-enzymatic antioxidant system (Finaud et al., 2006). Although GSH peroxidase and CAT have the same action upon H_2O_2, GSH peroxidase is more efficient at higher ROS concentrations, whereas CAT has an important action at lower H_2O_2 concentrations (Jenkins & Goldfarb, 1993; Antunes et al., 2002). Typically, a decrease in GSH (Szczeniak et al., 1998; Nikolaidis, Jamurtas, Paschalis, Kostaropoulos, Kladi-Skandali & Balamitis et al., 2006; Steinberg et al., 2006; Michailidis, Jamurtas, Nikolaidis, Fatouros, Koutedakis & Papassotiriou et al., 2007; Steinberg et al., 2007), an increase in oxidised GSH (Laaksonen, Atalay, Niskanen, Uustipa, Hanninen & Sen, 1996; Laaksonen et al., 1999; Nikolaidis et al., 2006; Michailidis et al., 2007) and no change in the total GSH concentration (Sen, Rankinen, Vaisanen & Rauramaa et al., 1994; Laaksonen et al., 1996; Laaksonen et al., 1999; Goldfard, Patrick, Bryer & You, 2005) have been reported following a variety of aerobic exercise protocols.

Studies that have reported null findings (Marin, Hanninen, Muller & Klinger, 1990; Laires, Madeira, Sergio, Colaco, Vaz & Felisberto et al., 1993; Nikolaidis et al., 2006) for GSH status may have been affected by the timing of their sampling, as oxidised GSH is rapidly reduced *in vivo* by GSH reductase, by the training status of their subjects (Kretzschmar, Muller, Hubscher, Marin & Klinger, 1991) and by the intensity of the exercise performed by their subjects (Marin et al., 1990; Laires et al., 1993).

Regular aerobic exercise helps guard against cell ageing and the appearance of some cancers. Trained marathon runners have a greater antioxidant capacity, as confirmed by Nieman et al. (2002), who showed that oxidative stress does not increase in highly trained triathletes despite the occurrence of inflammation. In this study, GSH increased rather than decreased after maximal intermittent exercise. It is reasonable to postulate that increased GSH augments the capacity of the antioxidant defence system and thus improves resistance to oxidative stress damage during the early recovery period.

Tian et al. (2010) investigated oxidative stress in twelve male adolescent marathon runners during the early and late recovery periods following a 21 km continuous run. The increase in the 4 h post-exercise GSH level (P<0.050) was considered part of a self-protecting mechanism against EICBR. Changes in the GSH levels in the cardiac tissue were also observed. The antioxidant response to a 3 h bout of swimming was a significant decrease in GSH 2 h and 24 h post-exercise in rats. The TAOC did not alter across the trial in this study. Leeuwenburgh et al. (1996) also reported a small decrease in the antioxidant capacity of the myocardium after a 210 m

swim in rats. The TAOC tends to increase after endurance exercise (Liu, Bergholm & Makinattila, 1999, Mastaloudis, Leonard & Traber, 2001) and appears to be mobilised from its reserves to preserve the body against the harmful effects of ROS.

SOD is the major defence against superoxide radicals and is the first line of defence against oxidative stress. SOD represents a group of enzymes that catalyse the conversion of oxygen with an extra electron in its outer orbit (forming a ROS) and the formation of H_2O_2. In all cells, at rest, the majority of the ROS produced by the mitochondria are reduced by mitochondrial SOD. The remainder diffuse into the cytosol (Das, Lewis-Milock & White, 1997). Although some studies have shown an increase in SOD activity after hypoxic training (Higuchi, Cartier, Chen & Holloazy, 1985; Pushpalatha, Nishanth & Sathyavelu, 2007), other studies claim that SOD activity does not change after training (Ji, Stratman & Lardy, 1992; Gul et al., 2006). Existing studies focusing on SOD and lipid peroxidation levels in the heart have shown contradictory findings. Burneiko et al. (2004) identified a decrease in heart SOD enzyme activity after swimming, whereas Gul et al. (2006) found no change in the heart tissue SOD activity. In this study, the SOD increase in trial RH represents an increase in oxidative stress in a hypoxic environment.

CAT is present in all cells and is found particularly in the peroxisomes, which are cell organelles that use oxygen to detoxify toxic substances and produce H_2O_2 (Antunes et al., 2002). CAT converts H_2O_2 into water and oxygen. Finaud et al. (2006) found that CAT was primarily located in the peroxisomes to reduce organic hydroperoxides such as hydrogen peroxide, which are capable of activating lipid

peroxidation, resulting in increased serum MDA, TBARS or LH. In this study, the CAT levels did not significantly change and there were no changes in the levels of MDA and LH, which are markers of lipid peroxidation. Tian et al. (2010) found that, 2 h after a 21 km run, the CAT activity of adolescent runners increased 50% from the pre-exercise level. The increase in CAT activity may be partly responsible for the absence of oxidative stress, as there was no evident increase in TBARS during this period. The only available data for CAT activity in runners 6 h after exercise showed that the activity remained at pre-exercise levels following a half marathon run (Marzatico, Pansarasa, Bcrtorclli, Somenzini & Dclla-Vallc, 1997).

LH is a marker of the initial reaction in lipid peroxidation and is a specific marker for cellular membrane damage (Ashton et al., 1999; Rimbach et al., 1999). Lipid peroxidation leads to the breakdown of lipids and to the formation of a wide array of primary oxidation products, such as LH, and secondary oxidation products, such as MDA. Some studies have demonstrated an initial increase in LH concentration after intense exercise (5 min stages of treadmill run at 40%, 70% and 100% VO_{2max} with 15 min rest between stages) (Fogarty, Hughes, Burke, Brown, Trinick & Duly et al., 2011). Miller et al. (2013) observed that LH increased 257% post-exercise and demonstrated that, due to the rate of appearance and degradation of this labile redox compound, LH was relatively unstable in the hours after exercise. Dalle-Donne et al. (2003) also confirmed that LH may be degraded within minutes of its formation.

Relationship between EICBR and oxidative stress

The release of hs-cTnT was strongly related to cTnI (r=0.933~0.975, P<0.000) in this study. Scharhag et al. (2005) detected the same relationship and found that the coefficient correlation between cTnT (measured using a third-generation assay) and cTnI was 0.79 (P<0.001). It is assumed that oxidative stress triggers lipid peroxidation-induced reversible membrane leakage and that the cTn in the cytosolic pool, rather than the structural pool, is released into the serum, resulting in EICBR. Scharhag et al. (2008) suggested that this reversible membrane leakage can also be indirectly explained by the cTnT and cTnI release kinetics and the relationship between them. They found that the smaller molecule cTnI has an earlier, more prolonged release and a higher post-exercise incidence rate than cTnT.

However, in this study, the relationship between hs-cTnT and cTnI was even closer and the kinetics of cTnI release did not reveal the earlier, more prolonged release or the higher post-exercise incidence rate. Both hs-cTnT and cTnI increased 0 h post-exercise and the level of hs-cTnT peaked 2 h post-exercise, which is earlier than the 4 h post-exercise peak in cTnI levels. The 24 h post-exercise levels of cTnI returned to the baseline levels and could not be detected in the participants' serum, whereas hs-cTnT did not return to its detection limits within 24 h post-exercise. Scherr et al. (2011) and Tian et al. (2012) observed changes in the level of hs-cTnT up to 72 h post-exercise. This change is probably due to the improved sensitivity of the updated cTnT immunoassay detection method. The hypothesis that oxidative stress is the mechanism for cTn release cannot be simply explained by the descriptive data

collected.

For the first time, the relationship between MDA, which is a product of lipid peroxidation, and cTn is revealed in a human study. In trial AH, the peak levels of hs-cTnT (r=0.714, P=0.031) and cTnI (r=0.725, P=0.027) 4 h after maximal intermittent exercise were related to the serum MDA levels 0 h post-exercise. Serum cTnI 2 h (r=0.699, P=0.036) post-exercise was also related to the levels of MDA 0 h post-exercise.

Nie et al. (2010a) observed a temporal association between elevations in serum cTnT (measured using a third-generation immunoassay) and changes in the MDA levels in myocardial tissue. Serum cTnT and myocardial tissue MDA followed the same release kinetics after 3 h of strenuous swimming in male Sprague-Dawley rats. The released cTnT was present in the circulation of all of the animals at 0 h and 2 h post-exercise. Middleton et al. (2008) observed cTnT elevations in all of their subjects both during and after a treadmill marathon using multiple time point measurements. Previous human studies have reported that between 0 and 78% of participants have cTnT positive rates after bouts of prolonged exercise (Shave et al., 2007b). This discrepancy may be related to the limited number of blood samples taken. The release of cTnT release after exercise was common in all of the subjects. A partial observation of cTnT release may miss the true relationship between oxidative stress and cardiac damage. As oxidative stress has been demonstrated to be a normal physiology response to exercise, cTnT release was only partially observed.

Whyte et al. (2005) conducted a human study and found no relationship

between MDA and cTnT (measured using a third-generation assay) and that the cTnT positive rate was lower than 50% following a marathon. A failure to observe cTnT release in some subjects may be the cause of the negative results. In this study, there was no relationship between hs-cTnT and MDA in trials N and RH. Whyte et al. (2005) demonstrated that oxidative stress data collected in the serum of human subjects are global biological measures and may not reflect the tissue and cellular environment and, specifically, the environment of the cardiomyocytes. Although Nie et al. (2010a) collected MDA in the myocardium, it appears impossible to collect cardiac tissue *in vivo* in a human study. To my knowledge, no existing study has tested the relationship between the serum and tissue concentrations of oxidative stress markers, although some studies have provided indirect evidence. Altan et al. (2009) showed that only the heart tissue levels of lipid peroxidation biomarkers were significantly higher in a hypoxic exercise group than in a normoxic exercise group. This finding supported the view that an increase in oxygen consumption leads to an increase in the production of ROS in heart tissue, paving the way for lipid oxidation (Liu et al., 2000). Other data sets have included lung tissue and skeletal muscle.

The heart is one of the organs with a high mass specific oxygen consumption and it is therefore natural that the heart is confronted with more pro-oxidant production and hence oxidative stress. The literature also records some negative results in heart tissue. Metin et al. (2002) did not find significant changes in the lipid peroxidation levels of the heart in female rats. As the rats were female, oestrogen may have played a protective role against the antioxidant effect (Ide, Tsutasui, Ohashi,

Hayashidani, Suematsu & Tsuchihashi et al., 2002). Nakanishi et al. (1995) found that lipid peroxidation production was high in the heart and lung tissue of rats exposed to a 5500 m altitude, but did not observe a significant change in the muscle tissue. In a hypoxic environment, lipid oxidation will be enhanced in cardiac tissue compared with skeletal muscle, which may make a greater contribution to the serum concentration of MDA in a hypoxic environment. This may explain why the relationship between cTn and serum MDA was revealed in trial AH, but not in trial N.

Another important finding of this study is a relationship between the release of cTn and the level of TAOC during the late recovery period in a hypoxic environment. In trial AH, the serum hs-cTnT (r=0.794, P=0.011) and cTnI (r=0.795, P=0.010) 2 h after maximal intermittent exercise were related to the serum TAOC level 24 h post-exercise. The serum hs-cTnT (r=0.714, P=0.031) and cTnI (r=0.769, P=0.015) 4 h after exercise were also related to the level of TAOC 24 h post-exercise. In trial RH, the serum hs-cTnT (r=0.650, P=0.042) and cTnI (r=0.765, P=0.011) 2 h after maximal intermittent exercise were related to the serum TAOC level 4 h post-exercise. The serum hs-cTnT (r=0.705, P=0.023) and cTnI (r=0.665, P=0.036) 4 h post-exercise were also related to the TAOC level 24 h post-exercise. The overall value for TAOC corresponds to the sum of all of the antioxidants. However, interpreting the relationship between cTn release and antioxidant capacity is difficult, because such increases may be a result of nutritional effects or of an adaptation towards oxidative stress.

Difference between EICBR in the altitude training responders and non-responders

There were no significant differences in the levels of hs-cTnT and cTnI between the altitude training responders and non-responders.

Although a number of investigators have coupled cTn release with a non-invasive assessment of cardiac function after exercise, the results from these efforts have been inconsistent. Neilan et al. (2006) measured cTnT (measured using a third-generation assay) and performed echocardiography 20 min after exercise in non-elite participants completing the Boston Marathon in an average time of approximately 4 h. The serum cTnT levels were increased in 63% of the participants. The increases in cTnT were associated with a reduction in right ventricular contractility, as measured by a strain echocardiograph. However, although the concomitant changes in cardiac function and cTn release were shown after prolonged exercise, a correlation analysis did not prove cause and effect between Q_{max} and cTn release.

George et al. (2008) observed no correlation between a post-race troponin elevation and the echocardiographic parameters for left ventricular function in a small cohort of athletes completing the Comrades Marathon. Very little evidence for impaired cardiac function and cTn release was found, resulting in no difference in cTn release in the altitude training responders and non-responders. This further supported the view of Nie et al. (2010b) that exercise-induced cTn release is a natural or normal adaptation to the biochemical and mechanical stress of exercise and is preparation for the next bout of prolonged exercise.

Summary

Major findings

1. Maximal intermittent exercise over 92 min, consisting of a hard run at 90% vVO_{2max} alternating with a positive recovery easy run of 50% vVO_{2max}, can induce hs-cTnT and cTnI release.

2. The additional stimulus of a hypoxic environment does not induce more cardiac damage after maximal intermittent exercise. It appears that exercise intensity plays a more important role in EICBR than a hypoxic stimulus.

3. The relationship between MDA, which is a production of lipid peroxidation, and cTn release is revealed in an absolutely hypoxic experiment trial. The highest level of cTn release is related to TAOC, which is a marker of the antioxidant capacity during the late recovery period after exercise.

4. There are no significant differences in EICBR between altitude training responders and non-responders.

Limitations and suggestions

1. The study includes a small number of participants and a limited number of markers for ROS damage and antioxidant activity. Some of the most recently reported oxidative stress biomarkers, such as F_2-isoprostanes, are not included.

2. It is difficult to demonstrate that cTn release is related to antioxidant capacity as a result of nutritional effects or because of an endogenous antioxidant. It is therefore difficult to give suggestions on how to prevent this transient cardiac damage.

3. Other potential mechanisms of cTn release, such as ischemia-induced bleb formation, the force of cardiac contraction, induced cell wounds and myocardial stunning, are not studied.

4. Although the study carefully manipulated the exercise intensity and altitude level, the levels of hs-cTnT and cTnI release were not significantly different in the normoxic and hypoxic trials.

5. HR and oxidative stress levels are higher in a hypoxic environment. It appears that there is a threshold for cTn release. Further studies should test the effects of higher altitude levels on cTn changes.

Conclusions

Maximal intermittent exercise can be used as an exercise model to trigger EICBR. The hypoxic environment stimulus does not induce more cardiac damage after maximal intermittent exercise. Exercise intensity appears to play a more important role in EICBR than a hypoxic stimulus. The mechanism for EICBR may be related to oxidative stress, especially lipid peroxidation, and the antioxidant system can be used to reduce EICBR.

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Appendix I

实验同意书

研究题目及参与者

The changes in cardiac biomarkers resulting from intermittent exercise at sea level and simulated high altitude environments（常氧与低氧环境下，间歇运动对心肌损伤标志物的影响）。参与者为签署实验同意书之男性中长跑运动员

研究程序

研究程序开始之前，研究人员会向实验参与者解释实验目的及程序，实验带来的益处及危害。实验前，研究人员会量度实验参加者之安静血压、超声心动，心电。正式实验由 5 次急性实验组成，2 次最大摄氧量的测试 ，分别在常氧和低氧（3000 模拟海拔）进行。间歇运动 3 次，1 次在常氧环境下进行，2 次在低氧环境下进行。90%最大跑速跑 2 分钟，50%最大跑速休息 2 分钟，4 分钟为一组，总共 15 组，大概 1 小时左右。实验结束后，实验参加者需要维持低强度整理运动，直至血压及心率恢复实验前水平。如果实验参加者无法持续完成至少 3 组运动，试验实时终止，但实验参加者应接受研究人员完成低低强度整理运动，直至血压及心率恢复实验前水平，才结束运动

风险评估

存在之风险/不适 常见有大强度运动所引起之风险/不适均有可能出现于本研究过程中。如呼吸强度过大，肌肉疲劳、酸痛。这些不适均可以得到缓和。呼吸强度过大可于运动测试结束即刻得到缓和。肌肉疲劳、酸痛可能会持续几个小时。

风险/不适之缓和方法 测试前适当的热身运动以及测试后的缓和运动有助缓解存在之风险/不适。研究人员亦会于测试过程中密切观察实验对象的生理反应，同时，实验对象请保持与研究人员的良好沟通，并遵守研究人员的指示。

紧急医疗操施

万一于测试过程中发生由测试引起的身体损伤及其他医疗状况，会根据实验室的紧急医疗程序进行处理，并及时汇报。实验对象会获得免费的医疗保障，但不会获得赔偿。此保障将于实验对象离开实验室时失效。

研究效益

实验对象可进一步了解自身的身体状况，挑战极限。

隐私保障及参与条款

实验对象的私隐，会得到保障。所有数据将只应用于本研究，并在研究结束时销毁。参与此研究纯属自愿。实验对象可以在任何时间退出此研究而不需受到任何限制。

联络数据

如有任何有关此研究项目之查询，请联络项目执行人李飞霏，电话：132 6318 3657。

Appendix II

Removing the two participants with extreme outlier results (N=8)

Additional significant differences were not detected after the removal of the two extreme scores. No differences were seen between the three conditions during the hard run trial (HR) after the removal of the two extreme scores, as shown in Table II_1.

Table II_1. HR during maximal intermittent exercise in trials N, AH and RH (N=8)

		HR (beats·min^{-1})	*P*
Hard run			
	Trial N	170.9±6.4	
	Trial AH	176.7±5.0	
	Trial RH	171.1±5.0	
Easy run			
	Trial N	138.8±13.7	0.013 (N vs. AH)
	Trial AH	148.6±10.0	
	Trial RH	139.5±9.8	0.001 (RH vs. AH)

Table II_2. Changes in hs-cTnT (pg·ml^{-1}, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	5.196±1.862	10.379±3.689* (*P*=0.004)	26.644±19.820	24.458±17.518	6.206±1.696
AH	5.196±1.862	11.591±5.158	29.811±10.670* (*P*=0.012)	29.170±11.280* (*P*=0.012)	13.980±7.536
RH	5.196±1.862	8.321±3.973	26.284±21.172* (*P*=0.012)	25.424±18.178* (*P*=0.012)	6.134±2.026

* Significantly different from pre-exercise, *P*<0.050/4.

Table II_3. Changes in cTnI ($ng{\cdot}ml^{-1}$, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	0.005	0.0069±0.0053	0.0313±0.0345	0.0425±0.0476	0.005
AH	0.005	0.0086±0.0069	0.0350±0.0177* (*P*=0.011)	0.0513±0.0253* (*P*=0.011)	0.005
RH	0.005	0.0069±0.0053	0.0338±0.0387	0.0494±0.0529	0.005

* Significantly different from pre-exercise, $P<0.050/4$.

Table II_4. Changes in MDA ($nmol{\cdot}ml^{-1}$, N=8).

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	4.743±0.555	4.686±0.438	4.781±0.637	4.610±0.444	5.091±1.055
AH	4.743±0.555	4.664±0.329	4.919±0.614	4.577±0.655	4.486±0.530
RH	4.743±0.555	4.855±0.788	5.318±0.920	4.781±0.550	5.007±0.541

Table II_5. Changes in LH ($mIU{\cdot}ml^{-1}$, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	18.274±1.594	19.235±1.962	18.356±1.016	18.518±2.205	18.135±1.705
AH	18.274±1.594	19.220±1.932	18.625±2.037	18.643±2.164	19.966±3.163
RH	18.274±1.594	18.630±2.977	18.248±1.557	19.704±2.570	20.180±1.210* (*P*=0.006)

* Significantly different from pre-exercise, $P<0.050/4$.

Table II_6. Changes in SOD ($U \cdot ml^{-1}$, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	81.421±8.452	81.345±11.060	81.710±7.160	87.317±9.130	81.704±8.545
AH	81.421±8.452	83.103±11.770	79.149±10.139	86.259±8.261	83.676±9.721
RH	81.421±8.452	84.886±11.874	86.535±8.019	91.000±7.334* (*P*=0.006)	83.611±12.242

* Significantly different from pre-exercise, $P<0.050/4$.

Table II_7. Changes in CAT ($U \cdot ml^{-1}$, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	57.616±6.330	55.923±4.221	56.423±2.505	59.580±5.873	57.536±3.331
AH	57.616±6.330	58.828±3.606	58.159±4.094	58.228±4.427	57.527±5.458
RH	57.616±6.330	54.667±4.667	59.397±4.398	59.986±3.306	58.746±3.505

Table II_8. Changes in GSH ($mg \cdot L^{-1}$, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	6.904±0.752	7.959±0.645	7.815±0.663	7.351±0.788	7.277±0.649
AH	6.904±0.752	7.514±0.390	7.643±0.555	7.332±0.522	7.438±0.507
RH	6.904±0.752	7.624±0.402	7.777±0.705	7.635±0.770	7.850±0.675

Table II_9. Changes in TAOC ($U \cdot ml^{-1}$, N=8)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	6.000±1.106	6.634±0.961	5.564±0.873	7.030±0.866	6.405±0.660
AH	6.000±1.106	6.140±0.854	5.268±0.700	5.955±0.790	6.437±0.594
RH	6.000±1.106	6.725±1.106	5.765±0.907	6.420±0.853	6.611±0.551

Table II_10. Correlations in trial N (N=8)

	cTnI_2h	cTnI_4h	MDA_24h
hs-cTnT_2h	0.957(0.000)		0.914(0.001)
hs-cTnT_4h		0.964(0.000)	0.845(0.008)
cTnI_2h			0.904(0.002)
cTnI_4h			0.848(0.008)

The results are shown in *r* (*P*).

Table II_10. Correlations in trial AH (N=8)

	cTnI _2h	cTnI _4h	MDA _4h	SOD _2h	CAT _4h	GSH _24h	TAOC _4h
hs-cTnT_2h							
hs-cTnT_4h		0.876 (0.01)			0.782 (0.038)		
cTnI_2h				-0.858 (0.006)		-0.720 (0.044)	0.812 (0.027)
cTnI_4h			0.864 (0.012)	-0.777 (0.023)			

The results are shown in *r* (*P*).

Table II_11. Correlations in trial RH (N=8)

	cTnI_2h	cTnI_4h	MDA_2h	CAT_24h
hs-cTnT_2h	0.984(0.000)		0.723(0.043)	0.717(0.045)
hs-cTnT_4h		0.918(0.001)	0.759(0.029)	
cTnI_2h				0.770(0.026)
cTnI_4h				0.743(0.035)

The results are showed in *r* (*P*).

Appendix III

Changes in hs-cTnT and cTnI analysed by one-way repeated ANOVA tests

A one-way repeated ANOVA test was used to compare hs-cTnT and cTnI pre- and post- (0 h, 2 h, 4 h and 24 h) exercise. A post hoc least significant difference test was used where appropriate. The Bonferonni adjusted P value was 0.050/4. A one-way repeated ANOVA test was used to compare hs-cTnT and cTnI at each time point under the three conditions (N, AH and RH). A post hoc Bonferroni test was used where appropriate. The P value was set to 0.050. The results are shown in Table III_1 and Table III_2.

Table III_1. Changes in hs-cTnT (pg·ml^{-1})

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	5.118±1.857	9.628±3.797* (P=0.002)	24.290±18.628* (P=0.011)	22.563±16.493* (P=0.010)	5.978±1.849
AH	5.118±1.857	11.486±4.861*† (P=0.001)	37.001±31.995* (P=0.011)	34.593±27.632* (P=0.007)	7.803±3.886
RH	5.118±1.857	8.221±3.513†	28.614±23.561* (P=0.012)	26.132±18.453* (P=0.006)	6.222±2.463

* Significantly different from pre-exercise, P<0.050/4;

† Significantly different from another condition, P<0.050/3.

Table III_2. Changes in cTnI (ng·ml^{-1})

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	0.005	0.0065±0.0047	0.0275±0.0324	0.0375±0.0437	0.005
AH	0.005	0.0095±0.0072	0.0475±0.0533	0.0695±0.0786	0.005
RH	0.005	0.0065±0.0047	0.0345±0.0375	0.0540±0.0573	0.005

Appendix IV

Four 2×3 two-way repeated ANOVA tests, using time (pre- and post-exercise) and condition (N, AH and RH)), were used to compare the MDA, LH, SOD, CAT GSH and TAOC levels. The results are shown in the following tables.

Table IV_1. Changes in MDA ($nmol \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	4.809±5.001	4.837±0.533	4.721±0.613	4.589±0.360	4.917±1.094
AH	4.809±5.001	4.817±0.429	4.980±0.638	4.539±0.616	4.661±0.582
RH	4.809±5.001	4.797±0.757	5.265±0.948	4.693±0.601	5.028±0.510
Time		0.953	0.308	0.117	0.701
Condition		0.990	0.428	0.761	0.501
Interaction		0.990	0.428	0.761	0.501

Table IV_2. Changes in LH ($mIU \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	18.044±1.384	19.462±1.886	18.263±0.886	18.531±2.057	18.125±1.708
AH	18.044±1.384	18.914±1.780	17.912±1.297	18.605±2.006	19.888±2.958* (*P*=0.030)
RH	18.044±1.384	19.037±3.041	18.385±1.813	19.796±2.488	19.988±1.591* (*P*=0.023)
Time		0.224	0.969	0.096	0.028
Condition		0.651	0.771	0.678	0.311
Interaction		0.651	0.771	0.678	0.311

* Significantly different from pre-exercise, $P<0.050$.

Table IV_3. Changes in SOD ($U \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	82.084±7.891	85.373±12.476	81.055±6.883	89.056±7.190	79.839±9.508
AH	82.084±7.891	84.141±11.861	77.217±8.307	87.821±8.831	84.226±10.544
RH	82.084±7.891	86.235±11.821	84.615±7.772	93.057±5.604* (P=0.003)	84.765±11.964
Time		0.181	0.653	0.038	0.809
Condition		0.581	0.368	0.076	0.703
Interaction		0.581	0.368	0.076	0.703

* Significantly different from pre-exercise, P<0.050.

Table IV_4. Changes in CAT ($U \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	57.977±5.580	55.450±3.992	56.002±2.607	60.633±5.308	57.893±3.366
AH	57.977±5.580	57.982±3.550	58.062±4.209	57.596±4.471	57.423±5.580
RH	57.977±5.580	53.837±5.026	58.906±5.045	60.408±3.848	58.379±3.458
Time		0.315	0.898	0.763	0.965
Condition		0.194	0.144	0.472	0.846
Interaction		0.194	0.114	0.472	0.846

Table IV_5. Changes in GSH ($mg \cdot L^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	6.943±0.778	7.911±0.684* (P=0.006)	7.675±0.731* (P=0.030)	7.199±0.646	7.183±0.625
AH	6.943±0.778	7.527±0.387	7.569±0.609* (P=0.028)	7.326±0.483	7.535±0.535
RH	6.943±0.778	7.628±0.376	7.844±0.779* (P=0.043)	7.570±0.740	7.844±0.633
Time		0.021	0.018	0.373	0.134
Condition		0.064	0.674	0.229	0.078
Interaction		0.064	0.674	0.229	0.078

* Significantly different from pre-exercise, P<0.050.

Table IV_6. Changes in TAOC ($U \cdot ml^{-1}$)

	Pre-exercise	Post-exercise			
		0 h	2 h	4 h	24 h
N	6.049±1.086	6.496±0.870	5.488±0.836	6.986±0.960	6.450±0.750
AH	6.049±1.086	6.312±0.818	5.244±0.699	6.056±0.786	6.734±0.912
RH	6.049±1.086	6.735±1.035	5.676±0.884	6.466±0.929	6.590±0.519
Time		0.202	0.142	0.243	0.169
Condition		0.641	0.138	0.077	0.609
Interaction		0.641	0.138	0.077	0.609

Appendix V

Differences between the oxidative stress biomarkers in the altitude training responders and non-responders

A 2×3×5 factorial ANOVA test using group (responders and non-responders), condition (N, AH and RH) and time (pre-exercise and 0 h, 2 h, 4 h and 24 h post-exercise) was used to compare the MDA, LH, SOD, CAT, GSH and TAOC levels in the altitude training responders and non-responders. The results are shown in Table V_1.

Table V_1. *P* value of a factorial 2×3×5 ANOVA test.

	MDA	LH	SOD	CAT	GSH	TAOC
Time*Group	0.572	0.650	0.998	0.017*	0.140	0.504
Condition*Group	0.164	0.333	0.147	0.420	0.484	0.809
Time*Condition	NA	NA	NA	NA	NA	NA
Time*Condition*Group	NA	NA	NA	NA	NA	NA

* $P<0.050$.

Curriculum Vitae

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1. Received the degree of Master of Sports Science from the Beijing Sport University, Beijing, June 2008.
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