Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans

ARTICLE *in* FOOD AND CHEMICAL TOXICOLOGY: AN INTERNATIONAL JOURNAL PUBLISHED FOR THE BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION · JUNE 2013

Impact Factor: 2.9 · DOI: 10.1016/j.fct.2013.05.046 · Source: PubMed

Food and Chemical Toxicology 61 (2013) 171–177

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans

G.C. Bogdanis [a,*], P. Stavrinou [a], I.G. Fatouros [b], A. Philippou [c], A. Chatzinikolaou [b], D. Draganidis [b], G. Ermidis [b], M. Maridaki [a]

[a] Department of Physical Education and Sport Science, University of Athens, Greece
[b] Department of Physical Education and Sport Science, Democritus University of Thrace, Komotini, Greece
[c] Department of Experimental Physiology, Medical School, University of Athens, Greece

ARTICLE INFO

Article history:
Available online 6 June 2013

Keywords:
Sprint cycling
Antioxidant capacity
Catalase
Glutathione
Protein carbonyls

ABSTRACT

This study investigated the changes in oxidative stress biomarkers and antioxidant status indices caused by a 3-week high-intensity interval training (HIT) regimen. Eight physically active males performed three HIT sessions/week over 3 weeks. Each session included four to six 30-s bouts of high-intensity cycling separated by 4 min of recovery. Before training, acute exercise elevated protein carbonyls (PC), thiobarbituric acid reactive substances (TBARS), glutathione peroxidase (GPX) activity, total antioxidant capacity (TAC) and creatine kinase (CK), which peaked 24 h post-exercise (252 ± 30%, 135 ± 17%, 10 ± 2%, 85 ± 14% and 36 ± 13%, above baseline, respectively; $p < 0.01$), while catalase activity (CAT) peaked 30 min post-exercise (56 ± 18% above baseline; $p < 0.01$). Training attenuated the exercise-induced increase in oxidative stress markers (PC by 13.3 ± 3.7%; TBARS by 7.2 ± 2.7%, $p < 0.01$) and CK activity, despite the fact that total work done was 10.9 ± 3.6% greater in the post- compared with the pre-training exercise test. Training also induced a marked elevation of antioxidant status indices (TAC by 38.4 ± 7.2%; CAT by 26.2 ± 10.1%; GPX by 3.0 ± 0.6%, $p < 0.01$). Short-term HIT attenuates oxidative stress and up-regulates antioxidant activity after only nine training sessions totaling 22 min of high intensity exercise, further supporting its positive effect not only on physical conditioning but also on health promotion.

1. Introduction

Low-volume, high-intensity interval training (HIT) has recently gained popularity as an effective method of improving anaerobic as well as aerobic fitness (Burgomaster et al., 2005, 2006) in only a few sessions. This type of training has not only been used in healthy trained individuals, but also in patient populations with metabolic disorders such as obesity and type II diabetes (Gibala et al., 2012). The effectiveness of this type of training stems from a combination of high anaerobic demand, mainly in the first bouts and an increasingly high aerobic contribution as the high intensity bouts are repeated (Bogdanis et al., 1996; Parolin et al., 1999). As a result, adaptations include rapid increases in muscle oxidative capacity, as reflected by key mitochondrial enzymes, glucose transporters, and muscle membrane lactate transporters (Burgomaster et al., 2007). The key factor for the success of this exercise model is the combination of high intensity, corresponding to >200% of the power output eliciting maximal oxygen uptake and resulting in large changes in ATP:ADP/AMP ratio and the activation of 5′-adenosine monophosphate-activated protein kinase (AMPK) and peroxisome-proliferator activated receptor γ coactivator (PGC)-1α (Gibala et al., 2009, 2012).

The large increase in metabolism during this type of exercise may cause increased production of reactive oxygen and reactive nitrogen species (RONS) that may not be counteracted by antioxidant defense systems, causing oxidative stress (Powers et al., 2011a,b; Powers and Jackson, 2008). The sources of RONS production during or after HIT may be both the high oxygen consumption, as well as the high anaerobic metabolism inducing RONS production from xanthine and NADPH oxidase, ischemic reperfusion conditions, altered calcium homeostasis and induced muscle damage (Bloomer and Goldfarb, 2004; Hellsten, 1994; Nikolaidis et al., 2007, 2008; Powers et al., 2011a,b). Although HIT is becoming a popular training modality for athletes as well as for the general population, there is very little and conflicting information regarding oxidative stress after an acute session (Bloomer et al., 2006;

Abbreviations: ANOVA, analysis of variance; CAT, catalase; CK, creatine kinase; GPX, glutathione peroxidase; HIT, high intensity training; PC, protein carbonyls; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances.

* Corresponding author. Address: Department of Physical Education and Sports Sciences, 41 Ethnikis Antistasis Str, Daphne, 17237, Athens, Greece. Tel.: +30 210 7276115.

E-mail addresses: gbogdanis@phed.uoa.gr, gregbogdanis@gmail.com (G.C. Bogdanis).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.05.046

Deminice et al., 2010; Farney et al., 2012) or short-term training (Fisher et al., 2011; Hellsten et al., 1996). Bloomer et al. (2006) found that there was no increase in protein carbonyls (PC) and malondialdehyde (MDA), a lipid peroxidation marker, after six 10 s sprints with 3 min recovery in anaerobically trained men. However, Shing et al. (2007) found significant increases in MDA when trained cyclists performed nine bouts lasting 30 s each at 150% of power output eliciting maximal oxygen consumption (VO_{2max}). The only study that examined oxidative stress and antioxidant enzyme activity [glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) activities] using the most popular protocol of HIT (i.e. repeated 30 s sprints) has followed individuals for only three sessions over one week period (Fisher et al., 2011). Antioxidant enzyme activity, measured in lymphocytes, was increased immediately after each exercise session, but returned to resting levels after 3–24 h post exercise, showing no significant training effect over the three sessions. However, oxidative stress measured indirectly in plasma as thiobarbituric acid reactive substances (TBARS) was attenuated after the third exercise session (Fisher et al., 2011). Therefore, the purpose of the present study was to investigate the acute (one session of four 30 s high intensity bouts) and the long-term changes in oxidative stress biomarkers and antioxidant status indices caused by a 3-week HIT regimen.

2. Material and methods

2.1. Participants

Eight healthy young male volunteers (age: 24.3 ± 1.4 y, body mass: 77.9 ± 2.9 kg, height: 179 ± 1 cm, body fat: 9.7 ± 1.3%) participated in this study. Participants, although non-athletes, were physically active. Prior to data collection, a medical questionnaire was completed by the participants to exclude individuals taking medications or having recent musculoskeletal injuries. Written informed consent was obtained from each participant, after a thorough explanation of the testing and training protocol, the possible risks involved and the right to terminate participation at will. The study was approved by the local Institutional Review Board and all procedures were in accordance with the Helsinki declaration of 1975, as revised in 1996.

2.2. Experimental design and procedures

Initially, participants were familiarized with cycle ergometer sprinting by completing two separate sessions, including short (6–10 s) and longer (30 s) sprints. During the second familiarization session, anthropometric measurements were also performed (body mass, height) and body fat was estimated from skinfold thickness measurements (Jackson and Pollock, 1978). In the third preliminary visit, a baseline maximal 30 s all-out sprint was performed on a modified friction-loaded cycle ergometer (Monark, model 864), against a resistance equivalent to 0.075 kg/kg body mass. The angular velocity of the flywheel was detected using a photocell and the signal was sampled at 200 Hz using a Biopac data acquisition system (Biopac Systems Inc.). The highest power output generated over 1 s was defined as peak power output (PPO), while mean power output (MPO) over the 30 s sprint was also recorded. A standardized warm-up preceded each testing and training session, consisting of 5 min cycling at a pedal rate of 60 rpm, 30 s at 100 rpm and 30 s at 120 rpm, against a resistance of 1 kg. A restraining harness, passed around the waist, was used to stabilize the participant on the saddle and limit the exercise to the lower limbs. The two side straps of the belt were fixed to a metal rail, bolted on the floor behind the bicycle frame. Toe clips were used and the feet were also strapped to the pedal using strong tape. The saddle height and harness setting for each subject were noted and used for all trials.

2.2.1. Exercise testing and training

Participants performed nine HIT sessions spread over three weeks with 48–72 h between sessions. The first training session was performed three days after the baseline 30 s sprint test. Each session included four to six 30 s bouts of high intensity cycling. Venous blood samples were obtained at rest as well as 0.5, 24 and 48 h following the first and last training session, that included four 30 s sprints, interspersed with 4 min of recovery (Fig. 1).

Each 30 s bout of cycling was performed at a constant pace of 100 rpm separated by 4 min of recovery. Participants were instructed to reach a pedal rate of 100 rpm against the ergometer's inertial resistance and then the individually adjusted load was applied. In all training sessions, except the last (post-training), the resistive load was adjusted, so that the MPO generated in the baseline 30 s sprint by each participant was replicated at this constant pace. The number of sprints performed in each training sessions increased from four to six over the first six sessions, according to the principle of progressive training overload and then decreased to four in the last three training sessions with the aim to avoid overtraining (Fig. 1). An increase in the number of sprints from 4 to 7 over the first five training sessions is typically used in most short term high-intensity training studies (e.g. Burgomaster et al., 2005, 2006). In the final session (post-test), participants completed four 30 s bouts, as in the first session, but the load was re-adjusted according to their post-training MPO recorded during a post-training 30 s sprint, performed two days after the eighth session (Fig. 1).

In all training and testing sessions, participants were verbally encouraged to maintain the predetermined pedaling rate throughout each 30 s bout. A metronome was used to assist them in maintaining the steady cycling rate. During the 4-min recovery period between sprints, participants remained seated on the ergometer and either rested or cycled at a low cadence (~50 rpm) against a light resistance (50 W) to reduce venous pooling in the lower extremities and the feelings of light-headedness or nausea. Participants were instructed to perform active recovery for the middle 3 min of the 4 min recovery period. However, during the rest intervals between the last two or three sprints in the 5 and 6 sprints sessions, most participants were unwilling to perform active recovery and preferred to rest. We have allowed this passive recovery between the last sprints in these sessions to avoid undue pressure to the participants and to encourage them to complete the session. MPO during each 30 s bout was recorded as previously described.

2.3. Diet analysis

In order to standardize diet and antioxidant status, 2-day diet recalls were completed before each trial. Investigators monitored dietary consumption before the first testing session and participants replicated the same dietary plan for the two days before the last training session. A trained qualified dietician instructed participants how to complete diet recall questionnaires and quantify food serving sizes. Diet recalls were analyzed using the Science Fit Diet 200A (Science Technologies, Athens, Greece).

2.4. Blood sampling and analysis

Each participant reported to the laboratory between 8:00 and 9:00 a.m. after an overnight fast on two occasions for blood sampling and exercise testing (Fig. 1). Participants abstained from alcohol and caffeine consumption for at least 24 h, and did not perform any exercise for the last 72 h before testing. Venous blood samples (~10 mL) were drawn after 20 min of rest at a seated position and the same procedure was repeated after the 4 × 30 s high intensity cycling test (Fig. 1). Blood was collected via venipuncture from an arm vein with a disposable 20-gauge needle using a Vacutainer tube holder (Becton–Dickinson, Franklin Lakes, NJ, USA). Blood was collected in tubes with SST-Gel/clot activator for serum separation (allowed to clot at room temperature and then centrifuged at 1500 g and 4 °C for 15 min) for subsequent measurement of creatine kinase activity (CK), protein carbonyls (PC), thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC) and catalase (CAT). Whole blood (1 mL, stored at at −20 °C) was utilized for the measurement of glutathione peroxidase activity (GPX). Plasma CK activity was determined spectrophotometrically using a test kit at a stable temperature of 37 °C (Hitachi 917 analyzer, Roche Diagnostics GmbH, Mannheim, Germany).

Measurement of PC was performed as previously described (Margonis et al., 2007). Briefly, 20% TCA (50 μL) was added to serum samples (50 μL) and the resulting solution was incubated (in ice bath, 15 min) and centrifuged (15,000 g, 5 min, at 4 °C). The supernatant produced was discarded, and 2,4-dinitrophenylhydrazine in HCl (2.5 N) was added in the pellet (500 μL of 10 mM for the sample, or 500 μL of 2.5 N HCl for the blank). Samples were then incubated for 1 h in the dark at room temperature, vortexed intermittently (every 15 min) and centrifuged (15,000 g, 5 min, 4 °C). The supernatant was discarded again, TCA was added (1 mL, 10%) in the pellet, vortexed, and centrifuged (15,000 g, 5 min, 4 °C). The resulting supernatant was discarded, 1 mL of ethanol–ethyl acetate (1:1 v/v) was added and samples were vortexed and centrifuged (15,000 g, 5 min, 4 °C). This step was repeated two more times. The final supernatant was discarded, and urea (1 mL of 5 M solution at pH 2.3) was added, and samples were vortexed and incubated (37 °C, 15 min). Samples were finally centrifuged (15,000 g, 3 min, 4 °C) and their absorbance was read at 375 nm. A control sample was used that was mixed only with HCl while the rest of the samples were mixed with DNPH (freshly prepared in 2.5 N HCl). The carbonyl content (in nmol mg^{-1} of protein) of the resulting supernatant was calculated by measuring its absorbance at 375 nm against the absorbance reading of the control supernatant, using an extinction coefficient 22 mM^{-1} cm^{-1}.

Measurement of TBARS was performed as described previously (Margonis et al., 2007). Briefly, 100 μL of serum was mixed with TCA (500 μL, 35%) and Tris–HCl (500 μL, 200 mM at pH 7.4) and the final solution was incubated (10 min at room temperature). Samples were then mixed with Na_2SO_4 (1 mL, 2 M) and thiobarbituric acid solution (55 mM) and the final solution was incubated (95 °C, 45 min). Thereafter, samples were cooled on ice (5 min) and vortexed after adding TCA (1 mL, 70%). The samples were then centrifuged (15,000 g, 3 min), and the absorbance of the resulting supernatant was read at 530 nm. A blank was run with each

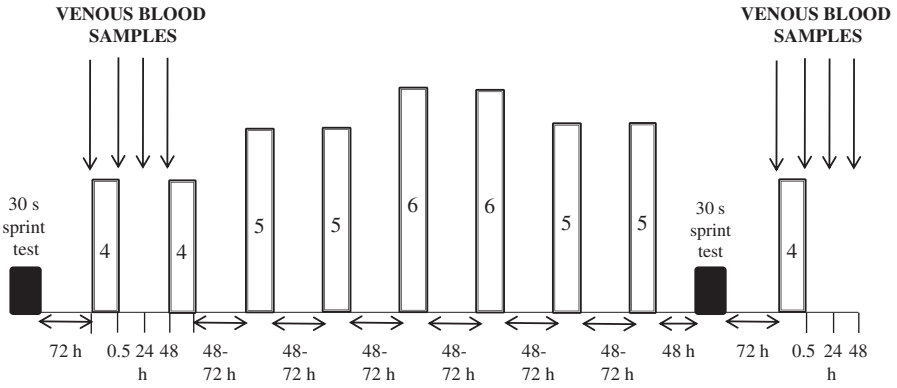

Fig. 1. Schematic representation of the study protocol. Training sessions are depicted as open bars and the numbers inside bars indicate the number of 30 s sprints performed during each session. Venous blood samples were taken during the first and last training sessions at the following time points: at rest and 0.5, 24 and 48 h after the fourth 30 s sprint. Dark bars indicate performance of a 30 s maximal sprint test, where the power output used in training and testing sessions was determined.

sample so that a baseline shift in absorbance was estimated. A standard curve was then created by using a malondialdehyde standard at concentrations of 0, 1.25, 2.5, 5, and 10 μM.

Measurement of TAC was performed as described previously (Margonis et al., 2007). In brief, serum (20 μL) was added to sodium potassium phosphate (480 μL, 10 mM, pH 7.4) and 2,2-diphenyl-1-picrylhydrazyl (DPPH·; 500 μL, 0.1 mM) free radical and the final solution was incubated in the dark (30 min at room temperature). Then, samples were centrifuged at 20,000 g for 3 min and the absorbance of the supernatant was read at 520 nm. TAC was estimated as mM of DPPH reduced to 1,1-diphenyl-2-picrylhydrazine (DPPH:H) by the antioxidants contained in the samples. Catalase activity was determined as described previously (Margonis et al., 2007). Sodium–potassium phosphate (2975 μl, 67 mM, pH 7.4) was added in serum samples (20 μL) and the mixture was incubated (37 °C, 10 min). Samples were then mixed with H_2O_2 (5 μL, 30%) and the change in absorbance was immediately read at 240 nm for 1.5 min. GPX activity was measured spectophotometricaly, at 37 °C as previously described (Margonis et al., 2007) with a commercial kit (Ransel RS 505, Randox, Crumlin, UK).

2.5. Statistical analyses

All statistical analyses were performed using the STATISTICA v.8.0 software (StatSoft Inc., Tulsa, OK, USA). Data are presented as means and standard error of the mean. A two-way repeated measures ANOVA (training x time) was used to examine the effects of training and sampling time for PC, TBARS, GPX, TAC and CK. Tukey's post–hoc tests were performed when a significant main effect or interaction was obtained ($p < 0.05$) to locate differences between means. Effect size for main effects and interaction was estimated by calculating partial eta squared (η^2) values using the STATISTICA v.8.0 software. Effect sizes were classified as small (0.2), medium (0.5) and large (0.8). Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Power output

Participants performed a total of 44 high intensity 30 s cycling bouts, adding up to 22 min of pure exercise time over the 3-week training period. The pure exercise time in each session was between 2 and 3 min, while training session duration including the 4 min recovery intervals between bouts was between 14 and 23 min, adding up to 162 min over the 3-week training period.

Mean power output was increased in all four 30 s bouts post-training compared with pre-training (main effect training: $p = 0.015$; $\eta^2 = 0.59$, "medium"). This is translated into a 10.9 ± 3.6% increase in total work done during the four 30 s bouts from 60.8 ± 2.5 to 67.1 ± 2.4 kJ.

3.2. Creatine kinase activity (CK)

A training by sampling time interaction ($p = 0.004$, $\eta^2 = 0.45$, "medium"), with no main effects, was found for serum CK activity. Post-hoc comparisons showed that CK activity was elevated by 36 ± 13% ($p < 0.01$) 24 h after the pre-training test and returned to resting values at 48 h (Fig. 2). In contrast, after the post training 4 × 30 s test, the exercise induced increase in CK activity was abolished and thus CK activity at 24 h was significantly lower compared with the corresponding pre-training value (Fig. 2).

3.3. Indices of oxidative stress

The 2-way ANOVA revealed significant main effects for training (pre vs. post; $p = 0.016$, $\eta^2 = 0.53$, "medium") and sampling time ($p < 0.001$, $\eta^2 = 0.88$, "large") protein carbonils (PC). Also there was a training by sampling time interaction ($p = 0.025$, $\eta^2 = 0.32$, "medium"). Before training, PC peaked at 24 h (252 ± 30% increase above baseline) and remained elevated (31 ± 16% above baseline) for 48 h (Fig. 3). Training resulted in an overall 13.3 ± 3.7% attenuation of the post-exercise PC elevation, and a 15.5 ± 5.2% decrease of the peak value ($p < 0.01$). PC concentration returned to baseline after 48 h only in the post-training test (Fig. 3).

There were significant main effects for training (pre vs. post; $p = 0.034$, $\eta^2 = 0.45$, "medium") and sampling time ($p < 0.001$, $\eta^2 = 0.90$, "large") for thiobarbituric acid reactive substances (TBARS). The training by sampling time interaction was not statistically significant ($p = 0.13$, $\eta^2 = 0.21$, "small"). TBARS peaked at 24 h after exercise (135 ± 17% increase above baseline) and remained elevated (54 ± 13% above baseline) for 48 h (Fig. 3). TBARS concentration was reduced post-training by an overall value of 7.2 ± 2.7%, ($p < 0.01$) and a 9.0 ± 2.8% decrease of the peak value.

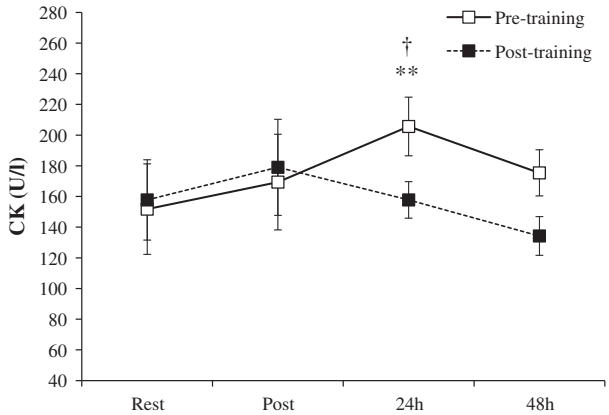

Fig. 2. Creatine kinase (CK) activity at rest and 30 min (Post), 24 h and 48 h after a 4 × 30 s high intensity cycling test performed before (pre-) and after (post-) training ($n = 8$). $^{**}p < 0.01$ from the corresponding post-training value. †$p < 0.01$ from the corresponding resting value.

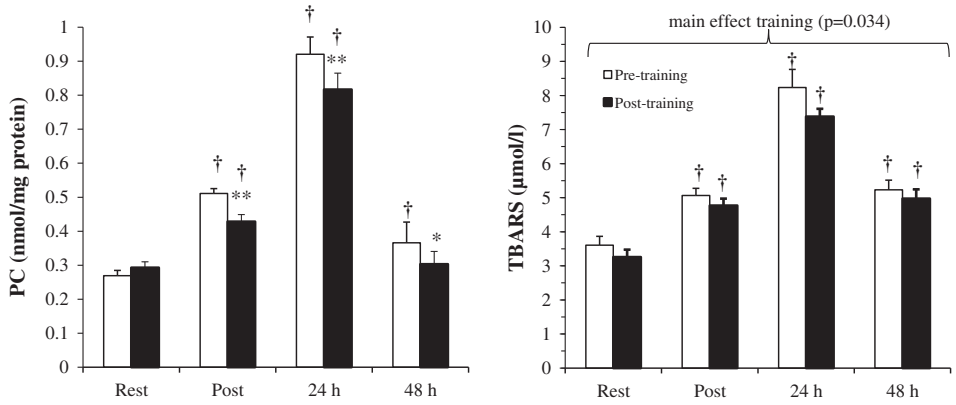

Fig. 3. Oxidative stress markers at rest and 30 min (Post), 24 h and 48 h after a 4 × 30 s high intensity cycling test performed before (pre-) and after (post-) training ($n = 8$). Left panel: Protein carbonyl (PC) concentration; Right panel: Thiobarbituric acid reactive substances (TBARS) concentration. * and **$p < 0.05$ and $p < 0.01$ from the corresponding pre-training value. †$p < 0.01$ from the corresponding resting value.

3.4. Antioxidant status

Glutathione peroxidase (GPX) activity analysis showed significant main effects for training (pre vs. post; $p < 0.001$, $\eta^2 = 0.80$, "large") and sampling time ($p < 0.001$, $\eta^2 = 0.77$, "large"), but not a training by sampling time interaction ($p = 0.096$, $\eta^2 = 0.22$, "small"). GPX activity was not increased 30 min post-exercise compared to rest, but peaked at 24 h (10.2 ± 1.7% above baseline) and remained elevated (4.8 ± 1.3%% above baseline) for 48 h (Fig. 4A). Training resulted in an overall 3.0 ± 0.6% ($p < 0.01$) increase of the post-exercise GPX elevation, and a 4.9 ± 1.0% increase of the peak value (Fig. 4A)

The 2-way ANOVA for Catalase (CAT) activity showed significant main effects for training (pre vs. post; $p = 0.033$, $\eta^2 = 0.45$, "medium") and sampling time ($p < 0.001$, $\eta^2 = 0.62$, "medium"), but not a training by sampling time interaction ($p = 0.42$, $\eta^2 = 0.11$, "small"). CAT activity peaked 30 min post-exercise (55.7 ± 17.7% above baseline), remained 21.4 ± 8.3% above baseline at 24 h and returned to resting values at 48 h post exercise (Fig. 4B). As a result of training, the overall post-exercise CAT responses were enhanced by 26.2 ± 10.1% ($p < 0.01$) compared to pre-training (Fig. 4B).

Significant main effects for training (pre vs. post; $p < 0.001$, $\eta^2 = 0.88$, "large") and sampling time ($p < 0.001$, $\eta^2 = 0.63$, "medium"), as well as a training by sampling time interaction ($p < 0.001$, $\eta^2 = 0.47$, "medium") were found for total antioxidant capacity (TAC). Before training, TAC peaked at 24 h (85 ± 14% increase above baseline) and remained elevated (62 ± 17% above baseline) for 48 h (Fig. 4C). Training resulted in an increase in TAC at all sampling points by an average of 26.2 ± 10.1%, including a large elevation of the resting TAC by 84 ± 9% compared with the pre-training values (Fig. 4C).

4. Discussion

The present study was designed to investigate both the acute (single session) and the longer-term (training) responses to a popular and highly effective HIT program. Currently, there is limited information regarding oxidative stress responses following one session of this type of training. One main finding was that a single session of HIT, comprising four 30 s bouts, with a pure exercise time of only 2 min and a total duration of 16 min (including the three 4-min recovery periods), induces a large (1.5–2.5-fold) increase of oxidative stress markers of lipid peroxidation (TBARS) and protein carbonylation (PC), as well as a concomitant elevation of antioxidant defence mechanism, that peak 24 h post-exercise. The other main finding of the present investigation was that a 3-week HIT resulted in a reduction of oxidative stress markers and a marked elevation of antioxidant status indices, despite the fact that total work done was greater in the post- compared with the pre-training exercise test. These results are in accordance with and add to the findings of Fisher et al. (2011), who has followed individuals for only three training sessions over one week period and did not measure oxidative stress 24 h post exercise, which is the time point that we observed the peak concentration of TBARS and PC.

Acute exercise-induced elevation of oxidative stress may be explained by several mechanisms. The rise of oxidative stress markers immediately post-exercise may be attributed either to the pro-oxidant activity of protons that are accumulated in intra- and extra-muscular compartments during high intensity exercise (Siesjo et al., 1985; Bogdanis et al., 1996) or to catecholamine autooxidation, since catecholamines are markedly elevated during this type of exercise (Zouhal et al., 1998). Although less likely, aerobic metabolism during recovery between sprints may indirectly contribute to ROS production by down-regulating cytochrome oxidase, thereby increasing coenzyme Q utilization as an electron acceptor that forms semiquinones which are well-known pro-oxidants as they reduce oxygen to superoxide radicals (Loschen et al., 1974; Hellsten et al., 1996).

However, one of the major factors of elevated oxidative stress immediately post-exercise is the up-regulation of the xanthine oxidase system due to an instant and stringent deoxygenation in active muscles during sprinting as the one occurring during cuff-induced ischaemia that is followed by a reoxygenation due to reperfusion (Nioka et al., 1998). Anaerobic exercise elevates purine catabolism which is associated with a vast increase of circulating hypoxanthine and uric acid (Starling et al., 1996). In fact, hypoxanthine concentration in plasma peaks approximately 20 min following anaerobic exercise (Hellsten-Westing et al., 1989) which is in agreement with the increased oxidative stress markers shortly after exercise. A rise in lipid peroxidation, as evidenced by the increased TBARS, results in a loss of plasma memberane's fluidity which ultimately increases its permeability that drives the efflux of cytosolic proteins such as CK (Sjodin et al., 1990). This explains the CK rise following the pre-training exercise test and absence of CK rise in the circulation following the post-training exercise test probably due to a lower elevation of lipid peroxidation. An up-regulation of lipid peroxidation and protein oxidation has been previously demonstrated, 6–48 h following sprint-type exercise (Marzatico et al., 1997; Radák et al., 1998).

Another reason for the delayed rise of oxidative stress markers observed in this study may be the ROS generated by mechanism of

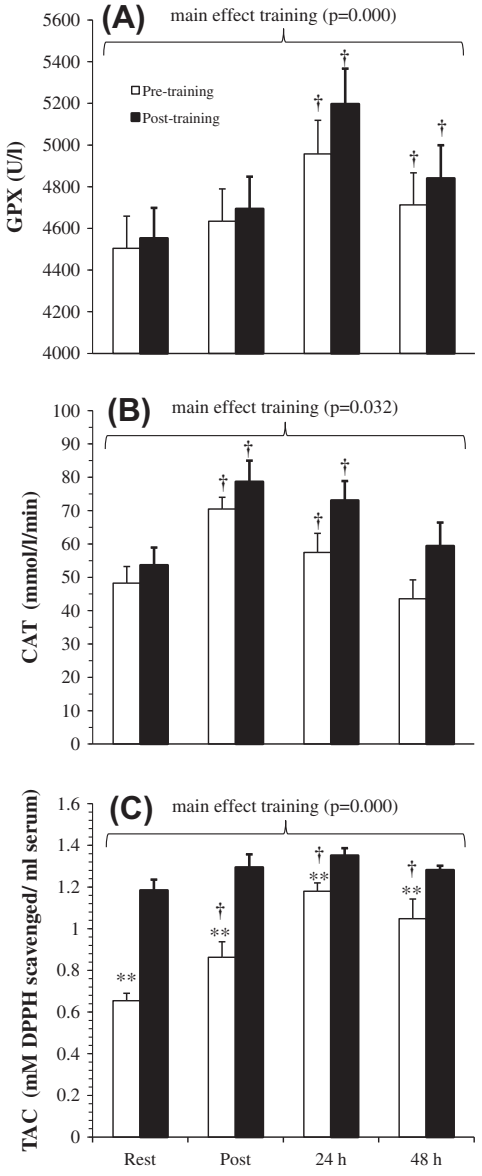

Fig. 4. Antioxidant status indices at rest and 30 min (Post), 24 h and 48 h after a 4 × 30 s high intensity cycling test performed before (pre-) and after (post-) training ($n = 8$). Top panel (A): Glutathione peroxidase (GPX) activity; Middle panel (B): Catalase (CAT) activity; Bottom panel (C): Total antioxidant capacity (TAC). $^{**}p < 0.01$ from the corresponding post-training value. †$p < 0.01$ from the corresponding resting value.

NADPH oxidase located on the membrane of leukocytes recruited during the initial phase of the inflammatory response associated with exercise-induced muscle damage, as evidenced by the rise in CK, at least pre-training (Jaeschke, 1995; Nikolaidis et al., 2008). The rise of PC and TBARS was less pronounced and faster than has been usually observed in other exercise models (i.e. football, plyometrics etc.) with a stronger eccentric component (Chatzinikolaou et al., 2010; Fatouros et al., 2010; Ispirlidis et al., 2008). Protein oxidation responses may be related to elevated oxidation of albumin and other serum proteins (Miyamoto et al., 2003).

The marked increase of uric acid observed previously (Baker et al., 2004; Chatzinikolaou et al., 2010; Starling et al., 1996), in some cases for 24–48 h, after this type of exercise, explains the TAC elevation during recovery since uric acid has been estimated to account for one third of TAC concentration (Whitehead et al., 1992). Erythrocyte antioxidant system has been shown to be altered following supramaximal-type exercise which explains the elevated GPX activity probably due to a profound decline of reduced glutathione (GSH) in erythrocytes (acts as a cofactor of GPX and is consumed to scavenge ROS or re-synthesize a-tocopherol and ascorbic acid) during post-exercise recovery (Groussard et al., 2003). As shown in Figs. 3 and 4, peak CAT activity was observed immediately after exercise and before the peak of the oxidative stress markers PC and TBARS. This is commonly observed after a single bout of both aerobic and anaerobic exercise (Inal et al., 2001; Michailidis et al., 2007) and may be attributed to a rapid mobilization of tissue antioxidant stores in the circulation, possibly due to a leakage of CAT from muscle fibers or erythrocytes into the circulation, since CAT is an intracellular enzyme (Michailidis et al., 2007).

The main finding of the present investigation was that a 3-week HIT resulted in a reduction of oxidative stress markers (by 7–13%) and a marked elevation of antioxidant status indices (by 3–38%), despite the fact that total work done was 10.9 ± 3.6% greater in the post- compared with the pre-training exercise test. Most importantly, these adaptations were achieved in only nine exercise sessions with 22 min of pure exercise time and a total of 162 min time commitment in the whole 3-week period. These adaptations of the pro-oxidant and anti-oxidant balance with short-term HIT may be considered as an additional beneficial response to this type of exercise, adding up to the effectiveness of this model for healthy and patient populations. Previous studies using endurance exercise have shown that a several fold greater exercise time is required to achieve similar adaptations in oxidative stress and antioxidant status (Fatouros et al., 2004). For example, in the study of Miyazaki et al. (2001) a 12-week training program, with running for 60 min per day at 80% of maximal heart rate, 5 days per week was used to attain similar responses to the present study. Training time in that study adds up to 3600 min compared with 162 min in the present study, stressing the efficiency of HIT. Therefore, the present study suggests that the a large part of the favorable adaptations of pro- and antioxidant mechanisms seen in well trained individuals (Conti et al., 2012; Farney et al., 2012) may be achieved relatively quickly by a time-efficient training method such as HIT. A potential limitation of the present study is the lack of a true control group. A non-exercise group would have strengthened even further this compelling data.

Currently, there is limited information on the effects of HIT on the development of oxidative stress in humans. A human study showed that only three consecutive anaerobic training sessions elicited a significant decrease of oxidative stress markers with a concomitant increase of antioxidant status suggesting that this type of training induces rapid adaptations (Shing et al., 2007). Another human study showed that seven weeks of training resulted in substantial rise of skeletal muscle antioxidant enzymes (Hellsten et al., 1996). These results were similar to those reported by Atalay et al. (1996) who submitted rats to sprint training for six weeks and demonstrated a marked up-regulation of the antioxidant defence system in heart muscle and fast and mixed fiber skeletal muscles known to be recruited during sprinting. Anaerobic-type exercise utilizes the antioxidant system in order to counteract increased ROS production by mechanisms mentioned before (Sen et al., 1994). Therefore, it appears that chronic exposure to augmented ROS production induces a chronic up-regulation of the antioxidant system, also seen in this study. In fact, sprint training of animals elicited a marked increase in total muscle GSH as well as in GPX (Atalay et al., 1996). Antioxidant enzyme up-regulation in muscle may be related to the level of oxygen consumption by the specific tissue (Jenkins et al., 1984) since a relationship between antioxidant enzyme activity and succinate dehydrogenase activity as well as with fiber type composition in muscle has been

reported (Laughlin et al., 1990). However, this cannot be verified in this study since muscle oxidative capacity was not determined, but data from similar investigations support this notion (Burgomaster et al., 2005, 2008; Gibala et al., 2012).

Oxidative stress is generally considered harmful to the cell, by damaging cell proteins, DNA and lipids. Indeed, reactive oxygen species (ROS) have been implicated in disuse muscle atrophy (Pellegrino et al., 2011). However, there is growing evidence that intracellular ROS production constitutes an important signal for skeletal muscle remodeling (Powers et al., 2011a,b). This is because ROS are involved in the regulation of cell signaling pathways that increase muscle oxidative capacity (Jackson, 2009) and promote antioxidant capacity, possibly through redox control of two ROS sensitive signaling molecules: (a) the nuclear factor κB (NF-κB) that is involved in the expression of genes involved in inflammation, stress responses and apoptosis, and (b) the key regulator of mitochondrial biogenesis, i.e. peroxisome proliferator activated receptor γ coactivator 1 α (PGC)-1α (Powers et al., 2011a,b). The fact that adaptations on both muscle oxidative capacity and antioxidant defense occur in such a short time frame compared with the traditional endurance training programs make this type of exercise attractive for both healthy and patient populations, such as COPD patients (Rodriguez et al., 2012)

An important feature of high intensity cycle ergometer exercise is that it causes little or no muscle damage. This may be a characteristic of this type of exercise that includes only concentric muscle contractions, while the absence of muscle damage is indicated by the small (pre-training) or no changes (post-training) in CK, which is an indirect muscle damage marker. In practical terms, this means that cycling may be used as a training modality that causes all the adaptations of HIT, but avoids muscle damage and its consequences. It is well known that eccentric exercise and muscle damage are typically related to greater oxidative stress. For example, following a soccer match that involves both high and low intensity aerobic exercise, as well as anaerobic activities with high intensity eccentric contractions (e.g. sprints, jumps, changes of direction), there is considerable muscle damage and oxidative stress for up to 72 h post exercise (Ascensao et al., 2008; Fatouros et al., 2010). The fact that cycling causes minimal or no muscle damage may be used as a criterion to choose cycling vs. treadmill running, for HIT of healthy and patient populations who wish to avoid the combined effects of inflammation, oxidative stress and muscle damage.

One of the possible negative consequences of HIT may be physical overtraining, which is characterized by declining performance and transient inflammation. Previous studies have shown that ATP and total adenine nucleotide content may decline by about 20% following repeated sprint training for 6–7 weeks (Burgomaster et al., 2008; Stathis et al., 1994). Although the lower total adenine nucleotide pool is no sign of overtraining, it reflects a high metabolic stress and it may indicate that the frequency of HIT sessions should be determined with caution. Overtraining is accompanied by sustained leucocytosis, increased oxidative stress markers such as PC, TBARS and isoprostanes and a decline in reduced glutathione and total antioxidant capacity (Margonis et al., 2007). However, in the present study oxidative stress was reduced and antioxidant status was improved, suggesting that this HIT scheme is not causing any signs of overtraining, possibly due to its low exercise volume. A lack of lymphocytopenia and immune suppression following HIT has also been reported by Fisher et al. (2011), suggesting that a HIT protocol similar to the one used in the present study, may be an ideal mode of training for improving health, with less immunosuppression compared with traditional endurance training.

In summary, the results of the present study showed one session of HIT causes a large increase in oxidative stress markers and also an increase in antioxidant status that last for 24–48 h after exercise. Furthermore, short-term HIT attenuates oxidative stress and up-regulates antioxidant activity after only nine training sessions totaling 22 min of high intensity exercise, further supporting its effectiveness for not only in improving muscle oxidative capacity and performance but also in attenuating exercise induced oxidative stress.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Ascensao, A., Rebelo, A., Oliveira, E., Marques, F., Pereira, L., Magalhaes, J., 2008. Biochemical impact of a soccer match – analysis of oxidative stress and muscle damage markers throughout recovery. Clin. Biochem. 41, 841–851.

Atalay, M., Seene, T., Hanninen, O., Sen, C.K., 1996. Skeletal muscle and heart antioxidant defences in response to sprint training. Acta. Physiol. Scand. 158, 129–134.

Baker, J.S., Damian, Z., Bailey, M., Hullin, Z.D., Young, I., Davies, B., 2004. Metabolic implications of resistive force selection for oxidative stress and markers of muscle damage during 30 s of high-intensity exercise. Eur. J. Appl. Physiol. 92, 321–327.

Bloomer, R.J., Goldfarb, A.H., 2004. Anaerobic exercise and oxidative stress: a review. Can. J. Appl. Physiol. 29, 245–263.

Bloomer, R.J., Falvo, M.J., Fry, A.C., Schilling, B.K., Smith, W.A., Moore, C.A., 2006. Oxidative stress response in trained men following repeated squats or sprints. Med. Sci. Sports Exerc. 38, 1436–1442.

Bogdanis, G.C., Nevill, M.E., Boobis, L.H., Lakomy, H.K., 1996. Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. J. Appl. Physiol. 80, 876–884.

Burgomaster, K.A., Hughes, S.C., Heigenhauser, G.J., Bradwell, S.N., Gibala, M.J., 2005. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. J. Appl. Physiol. 98, 1985–1990.

Burgomaster, K.A., Heigenhauser, G.J., Gibala, M.J., 2006. Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. J. Appl. Physiol. 100, 2041–2047.

Burgomaster, K.A., Cermak, N.M., Phillips, S.M., Benton, C.R., Bonen, A., Gibala, M.J., 2007. Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. Am. J. Physiol. 292, 1970–1976.

Burgomaster, K.A., Howarth, K.R., Phillips, S.M., Rakobowchuk, M., Macdonald, M.J., McGee, S.L., Gibala, M.J., 2008. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J. Physiol. 586, 151–160.

Chatzinikolaou, A., Fatouros, I.G., Gourgoulis, V., Avloniti, A., Jamurtas, A.Z., Nikolaidis, M.G., Douroudos, I., Michailidis, Y., Beneka, A., Malliou, P., Tofas, T., Georgiadis, I., Mandalidis, D., Taxildaris, K., 2010. Time course of changes in performance and inflammatory responses after acute plyometric exercise. J. Strength Cond. Res. 24, 1389–1398.

Conti, V., Corbi, G., Russomanno, G., Simeon, V., Ferrara, N., Filippelli, W., Limongelli, F., Canonico, R., Grasso, C., Stiuso, P., Dicitore, A., Filippelli, A., 2012. Oxidative stress effects on endothelial cells treated with different athletes' sera. Med. Sci. Sports Exerc. 44, 39–49.

Deminice, R., Trindade, C.S., Degiovanni, G.C., Garlip, M.R., Portari, G.V., Teixeira, M., Jordao, A.A., 2010. Oxidative stress biomarkers response to high intensity interval training and relation to performance in competitive swimmers. J. Sports Med. Phys. Fitness. 50, 356–362.

Farney, T.M., McCarthy, C.G., Canale, R.E., Schilling, B.K., Whitehead, P.N., Bloomer, R.J., 2012. Absence of blood oxidative stress in trained men after strenuous exercise. Med. Sci. Sports Exerc. 44, 1855–1863.

Fatouros, I.G., Jamurtas, A.Z., Viliotou, V., Pouliopoulou, S., Fotinakis, P., Taxildaris, K., Deliconstantinos, G., 2004. Oxidative stress responses in older men during endurance training and detraining. Med. Sci. Sports Exerc. 36, 2065–2072.

Fatouros, I.G., Chatzinikolaou, A., Douroudos, I., Nikolaidis, M.G., Kyparos, A., Margonis, K., Michailidis, Y., Vantarakis, A., Taxildaris, K., Katrabasas, I., Mandalidis, D., Kouretas, D., Jamurtas, A.Z., 2010. Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. J. Strength Cond. Res. 24, 3278–3286.

Fisher, G., Schwartz, D.D., Quindry, J., Barberio, M.D., Foster, E.B., Jones, K.W., Pascoe, D.D., 2011. Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise. J. Appl. Physiol. 110, 730–737.

Gibala, M.J., McGee, S.L., Garnham, A.P., Howlett, K.F., Snow, R.J., Hargreaves, M., 2009. Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. J. Appl. Physiol. 106, 929–934.

Gibala, M.J., Little, J.P., Macdonald, M.J., Hawley, J.A., 2012. Physiological adaptations to low-volume, high-intensity interval training in health and disease. J. Physiol. 590, 1077–1084.

Groussard, C., Rannou-Bekono, Z.F., Machefer, Z.G., Chevanne, M., Vincent, Z.S., Sergent, Z.O., Cillard, Z.J., Gratas-Delamarche, A., 2003. Changes in blood lipid

peroxidation markers and antioxidants after a single sprint anaerobic exercise. Eur. J. Appl. Physiol. 89, 14–20.
Hellsten, Y., 1994. The role of xanthine oxidase in exercise. In: Sen, C.K., Packer, L., Hanninen, O. (Eds.), Exercise and Oxygen Toxicity. Elsevier Science, Amsterdam, pp. 211–234.
Hellsten, Y., Apple, F.S., Sjödin, B., 1996. Effect of sprint cycle training on activities of antioxidant enzymes in human skeletal muscle. J. Appl. Physiol. 81, 1484–1487.
Hellsten-Westing, Y., Ekblom, B., Sjodin, B., 1989. The metabolic relation between hypoxanthine and uric acid in man following maximal short-distance running. Acta Physiol. Scand. 137, 341–345.
Inal, M., Akyuz, F., Turgut, A., Getsfrid, W.M., 2001. Effect of aerobic and anaerobic metabolism on free radical generation swimmers. Med. Sci. Sports Exerc. 33, 564–567.
Ispirlidis, I., Fatouros, I.G., Jamurtas, A.Z., Nikolaidis, M.G., Michailidis, Y., Douroudos, I., Margonis, K., Chatzinikolaou, A., Kalistratos, E., Katrabasas, I., Alexiou, V., Taxildaris, K., 2008. Time-course of changes in performance and inflammatory responses following a football game. Clin. J. Sports Med. 18, 428–431.
Jackson, M.J., 2009. Redox regulation of adaptive responses in skeletal muscle to contractile activity. Free Radic. Biol. Med. 47, 1267–1275.
Jackson, A.S., Pollock, M.L., 1978. Generalized equations for predicting body density of men. Br. J. Nutr. 40 (3), 497–504.
Jaeschke, H., 1995. Mechanisms of oxidant stress-induced acute tissue injury. Proc. Soc. Exp. Biol. Med. 209, 104–111.
Jenkins, R.R., Friedland, R., Howald, H., 1984. The relationship of oxygen uptake to superoxide dismutase and catalase activity in human skeletal muscle. Int. J. Sports Med. 5, 11–14.
Laughlin, M.H., Simpson, T., Sexton, W.L., Brown, O.R., Smith, J.K., Korthuis, R.J., 1990. Skeletal muscle oxidative capacity, antioxidant enzymes, and exercise training. J. Appl. Physiol. 68, 2337–2343.
Loschen, G., Azzi, A., Richter, C., Flohe, L., 1974. Superoxide radicals as precursors of mitochondrial hydrogen peroxide. FEBS Lett. 42, 68–72.
Margonis, K., Fatouros, I.G., Jamurtas, A.Z., Nikolaidis, M.G., Douroudos, I., Chatzinikolaou, A., Mitrakou, A., Mastorakos, G., Papassotiriou, I., Taxildaris, K., Kouretas, D., 2007. Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis. Free Radic. Biol. 43, 901–910.
Marzatico, F., Pansarasa, O., Bertorelli, L., Somenzini, L., Della Valle, G., 1997. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. J. Sports Med. Phys. Fitness 37, 235–239.
Michailidis, Y., Jamurtas, A.Z., Nikolaidis, M.G., Fatouros, I.G., Koutedakis, Y., Papassotiriou, I., Kouretas, D., 2007. Sampling Time is Crucial for Measurement of Aerobic Exercise–Induced Oxidative Stress. Med. Sci. Sports Exerc. 39, 1107–1113.
Miyamoto, Y., Koh, Y.H., Park, Y.S., Fujiwara, N., Sakiyama, H., Misonou, Y., Ookawara, T., Suzuki, K., Honke, K., Taniguchi, N., 2003. Oxidative stress caused by inactivation of glutathione peroxidase and adaptive responses. Biol. Chem. 384, 567–574.
Miyazaki, H., Oh-ishi, S., Ookawara, T., Kizaki, T., Toshinai, K., Ha, S., Haga, S., Ji, L.L., Ohno, H., 2001. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. Eur. J. Appl. Physiol. 84, 1–6.
Nikolaidis, M.J., Paschalis, V., Giakas, G., Fatouros, I.G., Koutedakis, Y., Kouretas, D., Jamurtas, A.Z., 2007. Decreased blood oxidative stress after repeated muscle-damaging exercise. Med. Sci. Sports Exerc. 39, 1080–1089.
Nikolaidis, M.G., Jamurtas, A.Z., Paschalis, V., Fatouros, I.G., Kouretas, D., 2008. The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations. Sports Med. 38, 579–606.
Nioka, S., Moser, D., Lech, G., Evengelisti, M., Chance, B., Kuno, S., 1998. Muscle deoxygenation in aerobic and anaerobic exercise. In: Plenum, Oxygen transport to tissue XX. New York, pp. 63–70.
Parolin, M.L., Chesley, A., Matsos, M.P., Spriet, L.L., Jones, N.L., Heigenhauser, G.J., 1999. Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. Am. J. Physiol. 277, 890–900.
Pellegrino, M.A., Desaphy, J.F., Brocca, L., Pierno, S., Camerino, D.C., Bottinelli, R., 2011. Redox homeostasis, oxidative stress and disuse muscle atrophy. J. Physiol. 589, 2147–2160.
Powers, S.K., Jackson, M.J., 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol. Rev. 88, 1243–1276.
Powers, S.K., Nelson, W.B., Hudson, M.B., 2011a. Exercise-induced oxidative stress in humans: cause and consequences. Free Radic. Biol. Med. 51, 942–950.
Powers, S.K., Talbert, E.E., Adhihetty, P.J., 2011b. Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle. J. Physiol. 589, 2129–2138.
Radák, Z., Nakamura, A., Nakamoto, H., Ohno, H., Goto, S., 1998. A period of anaerobic exercise increases the accumulation of reactive carbonyl derivatives in the lungs of rats.. Pflügers Arch – Eur. J. Physiol. 435, 439–441.
Rodriguez, D.A., Kalko, S., Puig-Vilanova, E., Perez-Olabarría, M., Falciani, F., Gea, J., Cascante, M., Barreiro, E., Roca, J., 2012. Muscle and blood redox status after exercise training in severe COPD patients. Free Radic. Biol. Med. 52, 88–94.
Sen, C.K., Atalay, M., Hanninen, O., 1994. Exercise induced oxidative stress: glutathione supplementation and deficiency. J. Appl. Physiol. 77, 2177–2187.
Shing, C.M., Peake, J.M., Ahern, S.M., Strobel, N.A., Wilson, G., Jenkins, D.G., Coombes, J.S., 2007. The effect of consecutive days of exercise on markers of oxidative stress. Appl. Physiol. Nutr. Metab. 32, 677–685.
Siesjo, B.K., Bendek, G., Koide, T., Westerberg, E., Wieloch, T., 1985. Influence of acidosis on lipid peroxidation in brain tissues in vitro. J. Cereb. Blood Flow Metab. 5, 253–258.
Sjodin, B., Westing, Y.H., Apple, F.S., 1990. Biochemical mechanisms for oxygen free radical formation during exercise. Sports Med. 10, 236–254.
Starling, R.D., Trappe, T.A., Short, K.R., Sheffield-Moore, M., Jozsi, A.C., Fink, W.J., Costill, D.L., 1996. Effect of inosine supplementation on aerobic and anaerobic cycling performance. Med. Sci. Sports Exerc. 28, 1193–1198.
Stathis, C.G., Febbraio, M.A., Carey, M.F., Snow, R.J., 1994. Influence of sprint training on human skeletal muscle purine nucleotide metabolism. J. Appl. Physiol. 76, 1802–1809.
Whitehead, T.G., Thorpe, H.G., Maxwell, S., 1992. Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. Anal. Chim. Acta 226, 265–277.
Zouhal, H., Rannou, F., Gratas-Delamarche, A., Monnier, M., Bentue-Ferrer, D., Delamarche, P., 1998. Adrenal medulla responsiveness to the sympathetic nervous activity in sprinters and untrained subjects during a supramaximal exercise. Int. J. Sport Med. 19, 172–176.