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RESEARCH ARTICLE

Exercise as a model to study redox homeostasis in blood: the effect of protocol and sampling point

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Abstract
Twenty males ran either on a level treadmill (nonmuscle-damaging condition) or on a downhill treadmill (muscle-damaging condition). Blood and urine samples were collected before and after exercise (immediately after, 1h, 4h, 24h, 48h, and 96h). The following assays were performed: F_2-isoprostanes in urine, protein carbonyls in plasma, glutathione, superoxide dismutase, glutathione peroxidase, and catalase in erythrocytes. The main finding was that monophasic redox responses were detected after nonmuscle-damaging exercise compared to the biphasic responses detected after muscle-damaging exercise. Based on these findings, muscle-damaging exercise may be a more appropriate experimental model to induce physiological oxidative stress.
Keywords: Eccentric exercise, muscle damage, reactive oxygen species, isoprostanes, oxidative stress, biomarkers

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Introduction

The development of a valid and robust physiological model to induce oxidative stress would be very useful in redox biology. This is mainly because disturbing redox homeostasis has proved in many cases crucial to delineate the effects of several purported oxidant stressors. Indeed, many studies have shown that the effects of aging, obesity, cigarette smoking, or nutrition on redox homeostasis are manifested or further magnified after having the participants subjected to an exercise stimulus (Bejma & Ji, 1999; Bloomer et al., 2007a; Mastaloudis et al., 2004; Vincent et al., 2006). For example, although resting levels of lipid peroxides in human plasma were not different between normal-weight and overweight individuals at rest, overweight participants had significantly higher lipid peroxide values after acute exercise than their normal-weight counterparts (Vincent et al., 2006). Following exercise, greater reactive species production has been reported in skeletal muscle of old rats compared to young rats (Bejma & Ji, 1999). An exacerbation in exercise-induced oxidative stress has been reported in cigarette smokers compared to nonsmokers (Bloomer et al., 2007a). Although plasma F_2-isoprostane levels were similar between the placebo and the combined supplemented vitamin C and E groups at baseline, F_2-isoprostanes increased during exercise only in the placebo group (Mastaloudis et al., 2004). Based on these observations, it becomes clear that the resting levels of many redox biomarkers can give much less information compared to the ones modified by an acute exercise session. In other words, it may be easier to find an existing effect of a redox agent (e.g. antioxidant supplementation) on body fluids and tissue redox status after exercise than at rest, simply because the stimulus of exercise may extend the magnitude and the duration of change in redox homeostasis. This renders exercise as a convenient experimental model to study the dynamics of redox homeostasis.

Despite the fact that acute exercise is frequently used as a stimulus to produce physiologically a state of oxidative

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stress (Kerksick et al., 2008, 2010; Nikolaidis et al., 2008, 2011), rarely has the chosen exercise model been rigorously tested to see whether it suitably serves this purpose. For example, several studies have failed to induce oxidative stress after an acute bout of exercise (Dixon et al., 2006; Jimenez et al., 2000; Sacheck et al., 2000). In addition, most of the available biomarkers have not been validated for the exercise setting. For example, the time course and the magnitude of these biomarkers appear to be different after nonmuscle-damaging and muscle-damaging exercise protocols (Michailidis et al., 2007; Nikolaidis et al., 2008). Therefore, the aim of the present study was to thoroughly investigate the time-course changes of several commonly utilized oxidative stress biomarkers by performing serial measurements during a 96-h period after an acute bout of either a nonmuscle-damaging or a muscle-damaging exercise protocol. For this purpose, a battery of oxidative stress biomarkers was determined in different biological matrices (i.e. plasma, erythrocytes, and urine) so that the effects of exercise on redox homeostasis are more comprehensively described (Nikolaidis & Jamurtas, 2009). We believe that this is an important preliminary step in ascertaining the possible biological function of oxidative stress during and after acute exercise. In addition, this knowledge may provide useful information for designing sounder human experiments using exercise as a model of oxidative stress.

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Methods

Participants

In total, 20 male subjects (age, 19–30 years; body fat, 9%–19%; VO_{2max}, 41–49 $mL \cdot kg^{-1} \cdot min^{-1}$), recruited by an advertisement in the department, participated in this study. All subjects were in good health as assessed by medical history and physical examination. The subjects did not spend more than one time per week on sport activities. Subjects did not receive medication known to influence the variables measured. The subjects were matched with respect to age, body fat, and VO_{2max} and divided into two equal-sized groups (nonmuscle-damaging and muscle-damaging group). Subjects were stable in terms of their anthropometric characteristics for at least the last 2 years and had not experienced any eccentric exercise training or other activities with a large eccentric component for at least 6 months before the study. They were instructed to abstain from strenuous exercise for 3 days before and during data collection and were not taking antiinflammatory drugs. Subjects read and signed an informed-consent form approved by the local university ethics committee.

Design

All measurements were performed between 08:00 and 11:00 h after overnight fasting, except for the measurements at 4 h post exercise. Subjects visited the laboratory twice. In the first visit, VO_{2max} was determined to ensure that the subjects of the two groups ran at similar exercise intensities when the actual running protocols were performed. Subjects visited the laboratory for a second time 7–14 days after VO_{2max} determination and ran either on a level treadmill (nonmuscle-damaging group) or on a downhill treadmill at –15% gradient (muscle-damaging group). Exercise included 45 min of running at an intensity corresponding to 70%–75% of their VO_{2max}. Participants had access to water ad libitum during exercise. Subjects stayed within the laboratory area and remained seated or lying during the first 4 h of postexercise period. Subjects left the lab after the 4-h blood sampling. To control for the effect of prior diet on the outcome measures of the study, subjects followed individualized isoenergetic dietary plans (35% fat, 50% carbohydrate, 15% protein) for 2 days before the trial and during the days of blood sampling. They were asked to record their food intake and the time of food consumption during those days. They were also asked to abstain from alcohol during the 2 days and the days of blood samplings as well as to abstain from caffeine during the day of the trial. Finally, the participants were instructed to abstain from intensive and/or structured physical activities for 2 days before the trial and during the days of blood sampling. Blood and urine samples were collected before and post exercise (immediately post, 1 h, 4 h, 24 h, 48 h, and 96 h post).

Muscle damage assessment

Each participant assessed delayed-onset muscle soreness (DOMS) during squat movement (90° knee flexion) and perceived soreness was rated on a scale ranging from 1 (normal) to 10 (very sore). Creatine kinase (CK) was assayed spectrophotometrically using a kit from Spinreact (Sant Esteve, Spain).

Blood and urine collection

Before each exercise protocol and within 2 min from the completion of exercise, a blood sample was drawn from a forearm vein. A portion of blood was collected into a tube containing EDTA and was placed immediately on ice for the determination of hematocrit and hemoglobin. The remaining blood was collected in EDTA tubes, centrifuged immediately at 1,370 g for 10 min at 4°C and the plasma was collected. The packed erythrocytes were lysed with 1:1 (v/v) distilled water, inverted vigorously and centrifuged at 4,000 g for 15 min at 4°C. Urine spot samples were collected in clean, dry containers either from the first morning void (for the time points rest, 24 h, 48 h, and 96 h) or within 10 min from the indicated postexercise time points. Urine samples were briefly centrifuged to remove sediment (1,000 g for 5 min at 4°C) and were stored in the presence of BHT 0.005%. Plasma, erythrocyte, and urine samples were stored at –80°C and thawed only once before analysis.

Assays

A competitive immunoassay was used for the quantitation of F_2-isoprostanes in urine (Cayman Chemical, Charlotte, USA). Urine was purified using the solid-phase

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extraction cartridges. The purification and the subsequent ELISA assay were performed following the manufacturer's recommendations. Plasma protein carbonyls, erythrocyte glutathione (GSH), and erythrocyte catalase were determined spectrophotometrically as described previously (Theodorou et al., 2010). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured in erythrocytes using commercially available kits (Cayman Chemical, Charlotte, USA). Briefly, SOD activity was measured utilizing a tetrazolium salt that produces a water-soluble formazan dye on reduction with superoxide anion. The rate of the reduction with O_2 is linearly related to the xanthine oxidase activity and is inhibited by SOD. The SOD activity can be quantified by measuring the decrease in absorbance at 440 nm. GPx is indirectly measured by a coupled reaction with glutathione reductase. Oxidized glutathione, produced on reduction of hydroperoxide by GPx, is recycled to its reduced state by glutathione reductase and NADPH. The conversion of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. For standardizing the dilution of urine, creatinine levels were measured using a kit (Fisher Diagnostics, Middletown, USA). Postexercise plasma volume changes were computed based on hematocrit and hemoglobin using the following formula: $PV_{post} / PV_{pre} = Hb_{pre} / Hb_{post} \times (100 - Hct_{post}) / (100 - Hct_{pre})$. Hematocrit was measured by microcentrifugation and hemoglobin was measured using a kit from Spinreact (Sant Esteve, Spain).

Statistical analysis

Biochemical data and DOMS were analyzed by a two-way repeated-measures (2 groups × 7 times) ANOVA. If a significant interaction was obtained, pairwise comparisons were performed through simple main-effects analysis. Physical characteristics, fitness level, and diet between groups were examined by unpaired Student's *t*-test. Plasma volume changes post exercise was examined by one-way repeated measures ANOVA. The statistical power required to detect a 20% postexercise change in each biomarker was calculated using G*Power 3 (Faul et al., 2007). The pooled standard deviation of the resting and exercise values was used for these calculations, while the α level was set at 0.05. Data are presented as means ± SEM.

Results

The characteristics of the nonmuscle-damaging group were as follows: age, 25 ± 5 years; height, 176 ± 4 cm; weight, 77 ± 5 kg; body fat, 13% ± 4%; maximal oxygen consumption (VO_{2max}) 47 ± 6 mL·kg^{-1}·min^{-1}. The characteristics of the muscle-damaging group were as follows: age, 27 ± 6 years; height, 174 ± 3 cm; weight, 74 ± 5 kg; body fat, 14% ± 4%; VO_{2max}, 46 ± 5 mL·kg^{-1}·min^{-1}. There were no significant differences in any of the physical characteristics and fitness levels between the two groups. Energy, macronutrient, and antioxidant intake did not differ between the two groups (Table 1). Regarding the nonmuscle-damaging group, during its first 45 min, the participants ran at an intensity corresponding to 72.7% ± 1.5% VO_{2max}, whereas the participants of the muscle-damaging group ran at 72.1% ± 2.4% VO_{2max}. During the testing of the nonmuscle-damaging group, the heart rate was 162.2 ± 6.1, the rating of exertion on Borg's scale (6–20) was 12.5 ± 2.0, and the respiratory exchange ratio was 0.94 ± 0.06. The respective values for the muscle-damaging group were 159.9 ± 5.5, 12.3 ± 2.1, and 0.93 ± 0.07. There were no significant differences in exercise intensity between the groups. Plasma volume did not change during the 96-h postexercise period (data not shown). The statistical power to detect a 20% postexercise change was 77% for F_2-isoprostanes, 92% for protein carbonyls, 99% for GSH, 89% for SOD, 90% for GPx, and 84% for catalase. Judging from the values of the statistical power (>80% in most of the cases), the present study has sufficient power to detect a significant effect if it existed.

Table 1. Analysis of daily energy intake of individuals 2 days before and during the day of the trials (mean ± SEM).

	Nonmuscle-damaging	Muscle-damaging
Energy (kcal)	2800 ± 91	2729 ± 109
Carbohydrate (% energy)	52.3 ± 1.9	52.1 ± 1.5
Fat (% energy)	32.1 ± 1.6	34.5 ± 1.1
Protein (% energy)	15.6 ± 1.1	13.4 ± 1.2
Vitamin C (mg)	133 ± 15	114 ± 9
Vitamin E (mg, α-TE[a])	7.0 ± 0.7	7.0 ± 0.3
Selenium (μg)	39.9 ± 2.1	37.9 ± 1.8

[a]α-TE, alpha-tocopherol equivalents.

Muscle damage

With regard to DOMS and CK, the main effects of group and time as well as the group by time interaction were significant ($p < 0.001$ for both main effects and the interaction in both DOMS and CK; Figures 1A and 1B). In the nonmuscle-damaging group, DOMS significantly increased 24 h and 48 h post exercise (30% and 140%). In the muscle-damaging group, DOMS significantly increased immediately after exercise (110%) returned toward baseline levels at 1 h and 4 h and increased again 24 h and 48 h post exercise (260% and 580%). In the nonmuscle-damaging group, CK activity significantly increased 24 h and 48 h post exercise (143% and 341%). In the muscle-damaging group, CK activity significantly increased 24–96 h post exercise (from 186% to 1029%).

F_2-isoprostanes

Concerning F_2-isoprostanes, the main effect of time as well as the group by time interaction was significant ($p < 0.001$ for both; Figure 2A). The main effect of group was not significant ($p = 0.292$). In the nonmuscle-damaging group, F_2-isoprostane concentration significantly increased immediately after exercise (21%) and returned

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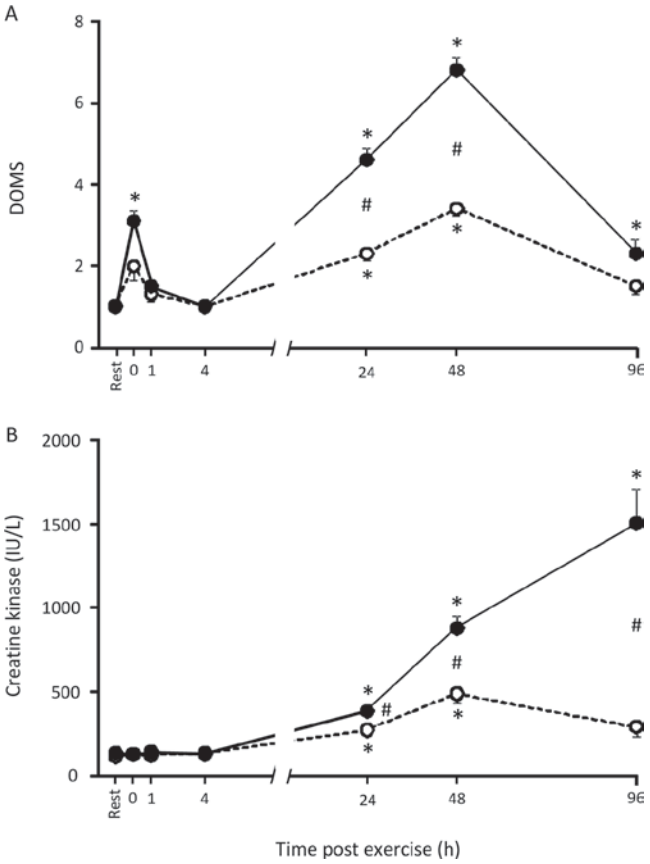

Figure 1. Delayed-onset muscle soreness (DOMS; A) and creatine kinase activity (B) after nonmuscle-damaging exercise (open circles) and muscle-damaging exercise (closed circles; mean ± SEM). *Significantly different from the preexercise value in the same exercise protocol ($p < 0.05$). #Significant difference between the two exercise protocols at the same time point ($p < 0.05$).

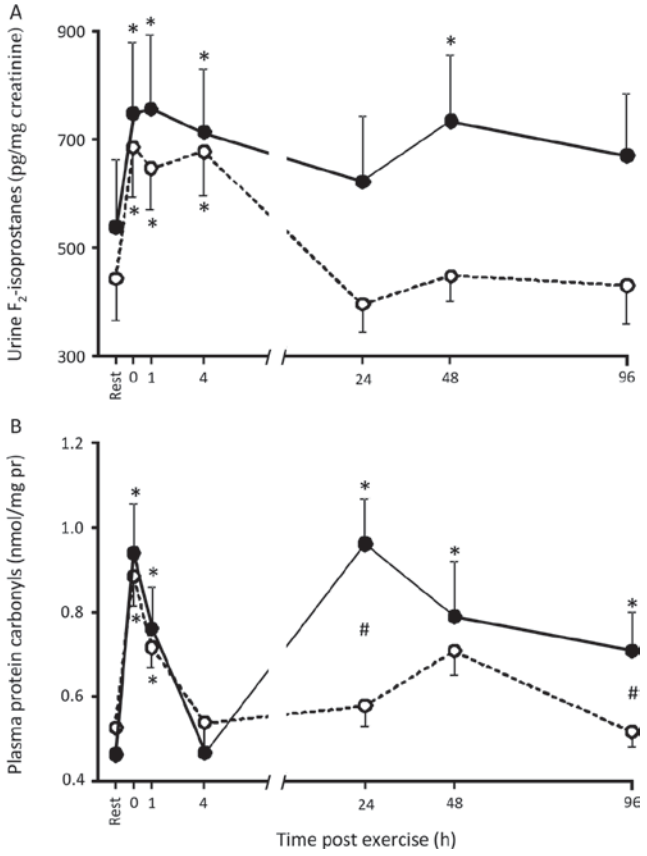

Figure 2. F_2-isoprostane (A) and protein carbonyl (B) concentration after nonmuscle-damaging exercise (open circles) and muscle-damaging exercise (closed circles; mean ± SEM). *Significantly different from the preexercise value in the same exercise protocol ($p < 0.05$). #Significant difference between the two exercise protocols at the same time point ($p < 0.05$).

toward baseline levels thereafter. In the muscle-damaging group, F_2-isoprostane concentration significantly increased immediately after exercise (23%), returned toward baseline values at 1 h and 4 h, and increased again 24–96 h post exercise (from 26% to 39%).

Protein carbonyls

With regard to protein carbonyls, the main effect of time as well as the group by time interaction were significant ($p < 0.001$ and $p = 0.002$, respectively; Figure 2B). The main effect of group was not significant ($p = 0.422$). In the nonmuscle-damaging group, protein carbonyl concentration significantly increased immediately after exercise (68%) and at 1 h (36%) and returned toward baseline levels thereafter. In the muscle-damaging group, protein carbonyl concentration significantly increased immediately after exercise (104%) and at 1 h (65%), decreased toward baseline values at 4 h, and increased again 24–96 h post exercise (from 54% to 108%).

GSH

Referring to GSH, the main effect of time as well as the group by time interaction were significant ($p < 0.001$ and $p = 0.032$, respectively; Figure 3). The main effect of group was not significant ($p = 0.306$). In the nonmuscle-damaging group, GSH concentration significantly decreased immediately after exercise (18%), at 1 h (21%) and at 4 h (14%) and did not differ from the baseline values

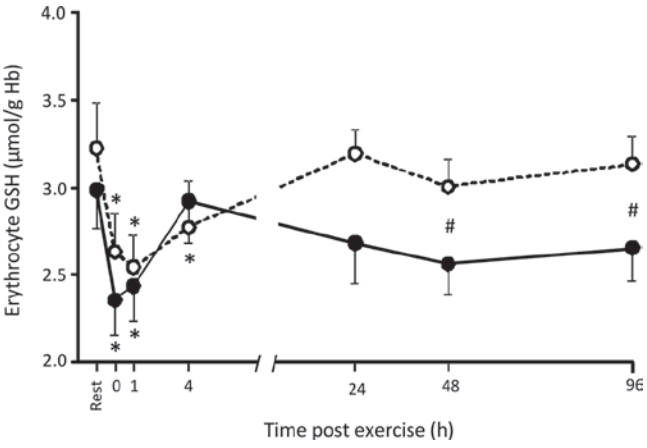

Figure 3. GSH concentration after nonmuscle-damaging exercise (open circles) and muscle-damaging exercise (closed circles; mean ± SEM). *Significantly different from the preexercise value in the same exercise protocol ($p < 0.05$). #Significant difference between the two exercise protocols at the same time point ($p < 0.05$).

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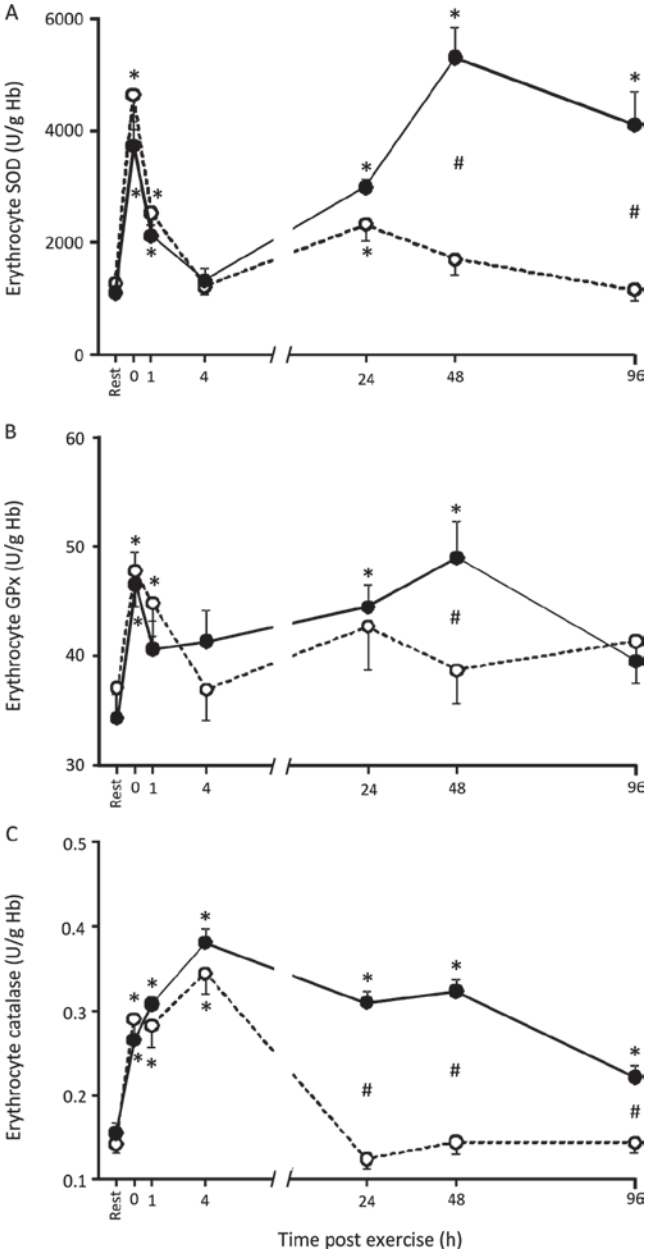

Figure 4. SOD (A), GPx (B), and catalase (C) activity after nonmuscle-damaging exercise (open circles) and muscle-damaging exercise (closed circles; mean ± SEM). *Significantly different from the preexercise value in the same exercise protocol ($p < 0.05$). #Significant difference between the two exercise protocols at the same time point ($p < 0.05$).

thereafter. In the muscle-damaging group, GSH concentration significantly decreased immediately after exercise (21%) and at 1 h (19%) and did not differ from the baseline values thereafter.

Antioxidant enzymes

With respect to SOD, the main effects of group and time as well as the group by time interaction were significant ($p = 0.033$, $p < 0.001$ and $p < 0.001$, respectively; Figure 4A). In the nonmuscle-damaging group, SOD activity significantly increased immediately after exercise (270%) and at 1 h (101%) and returned toward baseline levels thereafter. In the muscle-damaging group, SOD activity significantly increased immediately after exercise (241%) and at 1 h (93%), decreased toward baseline values at 4 h and increased again 24–96 h post exercise (from 173% to 387%).

Concerning GPx, the main effects of time as well as the group by time interaction were significant ($p < 0.001$ for both; Figure 4B). The main effect of group was not significant ($p = 0.803$). In the nonmuscle-damaging group, GPx activity significantly increased immediately after exercise (270%) and at 1 h (101%) and returned toward baseline levels thereafter. In the muscle-damaging group, GPx activity remained significantly increased at all postexercise time points (from 173% to 387%).

Relating to catalase, the main effects of group and time as well as the group by time interaction were significant ($p = 0.001$, $p < 0.001$ and $p < 0.001$, respectively; Figure 4C). In the nonmuscle-damaging group, catalase activity significantly increased up to 4 h post exercise (from 99% to 142%) and returned toward baseline levels thereafter. In the muscle-damaging group, catalase activity remained significantly increased at all postexercise time points (from 43% to 146%).

Discussion

To our knowledge, this is the first attempt to thoroughly compare the short- and long-term redox biomarkers responses of two exercise models, namely level running versus downhill running, which cause entirely different degree of muscle damage in humans. Although hundreds of studies have determined the effects of acute exercise on blood redox homeostasis (Fisher-Wellman & Bloomer, 2009), most of them have employed a single exercise mode and collected only a few samples after exercise—at best, up to four samples (Finaud et al., 2006; Radak et al., 2000, 2003). This may explain, at least in part, the large discrepancy in the relevant literature. In the present study, a nonmuscle-damaging versus a muscle-damaging exercise protocol were compared side by side, samples from three biological matrices (i.e., plasma, erythrocytes, and urine) were collected for up to 96 h post exercise and several commonly used biomarkers of oxidative stress were measured. Our findings clearly indicate that exercise protocol greatly affects the choice of sampling time point. In fact, our data depict monophasic changes in redox biomarkers after nonmuscle-damaging exercise, whereas the changes in redox biomarkers were biphasic after muscle-damaging exercise. In general, our findings support that the greatest changes in redox biomarkers took place in the same time window; that is immediately after to 1 h post exercise (for both the nonmuscle-damaging and muscle-damaging exercise). In addition, a second change was noticed either 24 h or 48 h post exercise (muscle-damaging exercise). In contrast to the relative uniformity in the time course

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of biomarkers manifestation, the magnitude of change was completely different across the biomarkers regardless of the exercise protocol.

F_2-isoprostanes

Results from the present study indicate that systemic lipid peroxidation, as measured by F_2-isoprostanes (currently considered the reference method for redox status assessment; Halliwell & Lee, 2010) in urine, was elevated after both protocols of exercise. Nevertheless, the two protocols produced completely different kinetics of F_2-isoprostanes. In fact, nonmuscle-damaging exercise induced only short-term effects (i.e. 0 h and 4 h post exercise) on the concentration of F_2-isoprostanes in urine, whereas muscle-damaging exercise induced both short- and long-term effects (i.e., 0 h and 4 h as well as 24 h and 96 h post exercise, respectively) on the concentration of F_2-isoprostanes. Only two studies have measured the short-term effects of acute exercise on urine F_2-isoprostane levels employing protocols of extreme duration (from 12 h to 26 h of continuous exercise) and reported divergent results (McAnulty et al., 2007; Nieman et al., 2004). Nieman et al. (Nieman et al., 2004) determined the response of F_2-isoprostane levels after a triathlon race lasting approximately 12 h, collecting urine samples shortly after exercise. F_2-isoprostane concentration markedly increased by 89% at 5–10 min post race and by 107% at 1.5 h post race. On the contrary, McAnulty et al. (McAnulty et al., 2007) did not report any effect of a 160-km ultramarathon lasting approximately 26 h on F_2-isoprostane levels in urine collected 5–15 min after the race. The fact that in our study, increased levels of F_2-isoprostane were detected immediately after both exercise protocols indicates there was enough time for F_2-isoprostanes to accumulate in urine during the 45 min of the run. In addition, judging from the fact that F_2-isoprostane levels remained elevated 0 h to 4 h post exercise, it appears that the effect of acute exercise on F_2-isoprostane levels of urine is generally long lived. Considering the rapid elimination half life of F_2-isoprostanes in plasma (≈4 min in rabbits; (Basu, 1998) and ≈16 min in rats; Morrow et al., 1992), the persistent presence of high F_2-isoprostane levels in urine indicates increased production of these compounds after exercise.

There are some reservations regarding the validity and reliability of F_2-isoprostane ELISA kits (Nikolaidis et al., 2011). Nevertheless, a recent study (Klawitter et al., 2011) showed that the kit employed in the present study for F_2-isoprostanes resulted in less variability among the ELISAs and reduced the positive analytical bias between the ELISA and LC/LC-MS/MS results in urine samples. In addition, it is essential to consider that measuring F_2-isoprostanes even with the reference method (i.e. MS) has provided inconsistent results, largely due to the variable methods for extraction (solid-phase or affinity chromatography) and separation (HPLC or GC followed or not followed by TLC). This may result in measuring a mixture of different F_2-isoprostanes and/or other isoprostane isomers and related metabolites (Nikolaidis et al., 2011).

Protein carbonyls

Protein carbonyls is a generic biomarker of protein oxidation and are mainly generated by multiple free radicals, excited state species, and singlet oxygen (as a result of secondary reactions; Hawkins et al., 2009). Similarly to F_2-isoprostanes, both types of exercise protocols increased the levels of protein carbonyl in plasma, though exhibiting different kinetics. Indeed, nonmuscle-damaging exercise induced only short-term effects (i.e. 0 h and 1 h post exercise) on the concentration of protein carbonyls, whereas muscle-damaging exercise induced both short- and long-term effects (i.e. 0 h and 1 h as well as 24 h–96 h post exercise) on the concentration of protein carbonyls. We are aware of four studies that have investigated the effects of aerobic exercise on plasma protein carbonyls (Alessio et al., 2000; Bloomer et al., 2005, 2006, 2007b). These studies generally have reported increases in protein carbonyls similar to that found in our study immediately after exercise, whereas the increases in protein carbonyls mostly disappeared after 0.5 h to 6 h of recovery (Alessio et al., 2000; Bloomer et al., 2005, 2006, 2007b).

GSH

GSH is the most abundant low-molecular weight thiol (Halliwell & Gutteridge, 2007b). GSH can react *in vitro* with almost all reactive species and, taking into account that its concentration in erythrocytes is at almost millimolar concentrations (in the present study ≈0.45 mM in whole blood considering the concentration of hemoglobin 150 g/L), scavenging of reactive species is feasible *in vivo* (Halliwell & Gutteridge, 2007b). In addition, it can also act as a copper chelator and can diminish copper ability to generate hydroxyl radical from hydrogen peroxide (Halliwell & Gutteridge, 2007). Both exercise protocols significantly decreased the levels of GSH only in the short term, despite the fact the levels of GSH remained nonsignificantly depressed 24 h to 96 h after muscle-damaging exercise. These results denote that after exercise, hepatic GSH supply may not be sufficient to match the enhanced use resulting in reduction of erythrocyte GSH concentration. There might also be an increase in erythrocyte GSH clearance (e.g. increased consumption by muscle) after exercise. The majority of relevant studies suggest that GSH levels decrease during exercise, at least partly because of GSH use against free radicals and its consumption to regenerate ascorbic acid and alpha tocopherol (Finaud et al., 2006).

Admittedly, more complete information would have been obtained regarding glutathione redox status if both GSH and the oxidized form of glutathione (GSSG) had been measured. However, measuring GSSG has proved a challenging task. In fact, a GSH/GSSG ratio in the erythrocyte of about 10–20 is frequently reported in the

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literature (Vollaard et al., 2005) when the "true" ratio is supposed to be about 400 (Giustarini et al., 2011). In addition, all spectrophotometric assays do not have sufficient sensitivity to measure GSSG (Jones et al., 1998; Rossi et al., 2002). However, we have to underline that the maintenance of adequate levels of GSH is fundamental in that the GSH decrease may lead to an impaired antioxidant defense (Giustarini et al., 2011). In addition, many studies have shown decreased values of GSH after exercise (Gohil et al., 1988; Michailidis et al., 2007; Viguie et al., 1993). As a result, we believe that measurement of GSH in blood can be considered a valid redox biomarker.

Antioxidant enzymes

The three antioxidant enzymes followed a similar time course after exercise in qualitative terms, but not in quantitative terms. The enzymes increased monophasically after nonmuscle-damaging exercise, whereas increased biphasically after muscle-damaging exercise. SOD activity exhibited the highest increases after exercise (270% after nonmuscle-damaging exercise and 387% after muscle-damaging exercise), whereas GPx activity exhibited the lowest increases after exercise (29% after nonmuscle-damaging exercise and 42% after muscle-damaging exercise). Catalase activity exhibited moderate increases after exercise (142% after nonmuscle-damaging exercise and 146% after muscle-damaging exercise). SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, whereas catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen (Halliwell & Gutteridge, 2007). Considering that these two enzymes scavenge two reactive species (i.e. superoxide and hydrogen peroxide) with considerable half lives and ability to cross membranes (Nikolaidis & Jamurtas, 2009) render them potential oxidant scavengers not only for the erythrocyte but also for the circulation in general. Nevertheless, the increase in erythrocyte SOD, GPx, and catalase levels after exercise did not prove efficient at preventing the decrease in erythrocyte GSH or preventing the increase in plasma protein carbonyls and urine F_2-isoprostane levels.

Muscle-damaging exercise induced significant and large changes in redox biomarkers both shortly (immediately after to 4 h post exercise) and later into recovery (from 24 h to 96 h post exercise), whereas nonmuscle-damaging exercise induced oxidative stress only for some hours after exercise. The similarity in the short-term responses between the two protocols may have arisen because of the fact that both types of exercise contained a similar volume of aerobic component. However, the discrepancy in the long-term responses may have arisen due to differences in the degree of muscle damage. This has merit because it indicates that the redox responses may vary even after different muscle-damaging exercise protocols. For example, muscle-damaging exercise that is highly anaerobic in nature (i.e. isolated eccentric actions in a dynamometer) has been repeatedly shown to induce only delayed oxidative stress and not short-term oxidative stress (Nikolaidis et al., 2008; Theodorou et al., 2010). However, as the present study showed, muscle-damaging exercise including a high aerobic component was managed to induce both early and delayed perturbations in redox homeostasis.

Conclusions

The major conclusion of the present study is that the term *exercise* must be specifically defined with regards to exercise-induced changes in redox homeostasis, otherwise erroneous results may be obtained. Indeed, two almost identical running protocols with the same exercise intensity and duration but different running slope caused very diverse redox responses in plasma, erythrocytes, and urine. Nonmuscle-damaging exercise induced monophasic alterations in six of the most commonly used redox biomarkers lasting up to 4 h post exercise, whereas muscle-damaging exercise induced biphasic alterations lasting up to 96 h post exercise. The alterations in the levels of redox biomarkers occurred in a similar time window in the same exercise protocol, whereas the magnitude of alterations was different across biomarkers regardless of the exercise protocol. In addition, it was revealed that different biological matrices (i.e. plasma, erythrocytes, and urine) can be used interchangeably. The fact that F_2-isoprostane levels increased shortly after exercise indicate that urine can also be used for assessing the effects of acute exercise and not only the effects of the chronic exercise as is usually the case in the relevant literature. Taking into account that downhill running induced long-lasting and extensive changes in redox homeostasis, muscle-damaging exercise may be a more appropriate model to study the effects of experimental interventions (e.g. administration of redox agents or exposure to environmental oxidant stimuli) on free radical biology of blood and urine.

Declaration of interest

The authors report no conflict of interest.

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