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REVIEW

Redox biology of exercise: an integrative and comparative consideration of some overlooked issues

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Summary

The central aim of this review is to address the highly multidisciplinary topic of redox biology as related to exercise using an integrative and comparative approach rather than focusing on blood, skeletal muscle or humans. An attempt is also made to re-define 'oxidative stress' as well as to introduce the term 'alterations in redox homeostasis' to describe changes in redox homeostasis indicating oxidative stress, reductive stress or both. The literature analysis shows that the effects of non-muscle-damaging exercise and muscle-damaging exercise on redox homeostasis are completely different. Non-muscle-damaging exercise induces alterations in redox homeostasis that last a few hours post exercise, whereas muscle-damaging exercise causes alterations in redox homeostasis that may persist for and/or appear several days post exercise. Both exhaustive maximal exercise lasting only 30 s and isometric exercise lasting 1–3 min (the latter activating in addition a small muscle mass) induce systemic oxidative stress. With the necessary modifications, exercise is capable of inducing redox homeostasis alterations in all fluids, cells, tissues and organs studied so far, irrespective of strains and species. More importantly, 'exercise-induced oxidative stress' is not an 'oddity' associated with a particular type of exercise, tissue or species. Rather, oxidative stress constitutes a ubiquitous fundamental biological response to the alteration of redox homeostasis imposed by exercise. The hormesis concept could provide an interpretative framework to reconcile differences that emerge among studies in the field of exercise redox biology. Integrative and comparative approaches can help determine the interactions of key redox responses at multiple levels of biological organization.

Key words: antioxidant, biomarker, eccentric, free radical, training.

Introduction

During the last decade, the field of redox biology has witnessed many remarkable developments. Reactive species have been found to serve a multitude of diverse purposes, from controlling the signaling of intracellular pathways (Forman et al., 2010) to mediating enzyme activation (Stubbe and van Der Donk, 1998) and participating in antibiotic synthesis (Lesniak et al., 2005). The significance of reactive species has been further underlined by the emerging links between cellular redox events and the etiology of many human diseases (Valko et al., 2007). In addition, shifting from the initial perception that reactive species and oxidative stress were largely considered harmful entities [i.e. produced *via* uncontrolled processes (Southorn and Powis, 1988)] to the currently supported view that they also serve useful purposes [i.e. produced *via* controlled processes (Okegbe et al., 2011)], a lot of development has been attained. As a result of this progress in basic redox biology, the subfield of exercise redox biology has also markedly advanced. From the largely descriptive nature of the first attempts in this field using crude techniques to today's studies addressing the effects of reactive species using state-of-the-art analytical techniques, a large volume of information has been accumulated. It is worth mentioning that in a perspective article, the field of exercise redox biology has been recognized as one of the key themes that will drive the exercise science in the future (Baldwin and Haddad, 2010).

There are excellent reviews focusing on particular issues of exercise redox biology, including antioxidant supplementation (Gross et al., 2011; Powers et al., 2011a), skeletal muscle function (Reid, 2001; Powers and Jackson, 2008; Reid, 2008), intracellular signaling (Ji, 2008; Powers et al., 2011b) and muscle wasting (Pellegrino et al., 2011; Reid and Moylan, 2011). In addition, other reviews have been devoted to the analysis of the effects of acute and chronic exercise on the levels of oxidant biomarkers in blood and other tissues (Finaud et al., 2006; Bloomer, 2008; Nikolaidis et al., 2011). Alternatively, the central aim of this review is to address the highly multidisciplinary topics of redox biology as related to exercise using an integrative and comparative approach rather than focusing on blood, skeletal muscle or humans. More importantly, this review does not mean to be comprehensive; rather, it aims to highlight neglected, yet thought-provoking studies and novel findings, as well as to establish research hypotheses that hopefully will provide directions for future research in the field. An attempt is also made to re-define or refine some nebulous core concepts in the redox biology field.

The 'good' (antioxidants), the 'bad' (reactive species) and the 'ugly' (oxidative stress)[1]

The distinction drawn in the section heading is schematically used to parody the popular manichaeistic view that antioxidants are considered 'useful' entities, reactive species are considered 'harmful' entities and oxidative stress is considered a 'negative' state. Certainly, the reality is much more complicated and antioxidants, reactive species and oxidative stress can serve both useful and detrimental roles, which are dependent on the biological context (within an organism), which in turn are greatly dependent on the environmental context (outside an organism).

Antioxidants

Admittedly, to define the term 'antioxidant' is a difficult task. In this paper, antioxidant is defined as any mechanism, structure and/or substance that delays, prevents or removes oxidative modifications to a target molecule (Halliwell and Gutteridge, 2007; Pamplona and Costantini, 2011). Antioxidants can be complex molecules such as the superoxide dismutases and peroxiredoxins, or simpler ones such as uric acid and glutathione (Gutteridge and Halliwell, 2010). They can be broadly classified according to their function into: (1) free radical scavengers (e.g. ascorbic acid), (2) non-free radical scavengers (e.g. catalase) and (3) agents that inhibit generation of reactive species (e.g. metal chelators). The characterization of a molecule as an antioxidant is not constructive unless it is associated with the oxidant that has to be neutralized and the assay used is described (Azzi et al., 2004; Gutteridge and Halliwell, 2010). Moreover, a well-characterized antioxidant action of a molecule *in vitro* does not necessarily translate to cells, organs or animals without appropriate verification in these systems (Azzi et al., 2004). For example, even though the antioxidant action of vitamin E *in vitro* is undisputed, its role *in vivo* has for too long been a matter of controversial debate (Brigelius-Flohe and Galli, 2010). In addition, there is a tendency to include molecules with heterogenous properties such as vitamin C, vitamin E, β-carotene or selenium under the general heading of 'antioxidants'. This approach might lead to erroneous interpretations because these molecules have different mechanisms of action and the antioxidant activity is only one of their functions, if any (Fortes and Virgili, 2008). For more information on this topic, the reader is referred to the following reviews: Azzi et al. and Gutteridge and Halliwell (Azzi et al., 2004; Gutteridge and Halliwell, 2010).

Reactive species

'Reactive species' is a collective term that encompasses chemically reactive molecules, which can be either radicals or non-radicals. Reactive species is a highly heterogeneous classification and includes molecules that are characterized by very different chemical and biological properties. For example, the half-life of reactive species (which mainly depends on the rate constant of the reactive species and the concentration of the target molecule) can range from a few nanoseconds for the most reactive species (e.g. hydroxyl radical; $HO^{\bullet}$) to seconds and hours for rather stable reactive species (e.g. hydrogen peroxide; H_2O_2). Accordingly, the diffusion distances (i.e. the distance over which the reactive species concentration drops to a tenth) of reactive species can range from approximately $20\,\mu m^2\,s^{-1}$ for $HO^{\bullet}$ to $1500\,\mu m^2\,s^{-1}$ for H_2O_2 in aqueous solutions (Winterbourn, 2008). Probably because of the short half-life and the short diffusion distance of $HO^{\bullet}$, living organisms have not developed specific enzymatic systems for its detoxification (Lushchak, 2011). In contrast, H_2O_2 can be neutralized as it is converted to water and oxygen by the enzyme catalase. For more information on this topic, the reader is referred to specialized reviews (Buettner, 1993; Winterbourn, 2008).

Oxidative stress and alterations in redox homeostasis

Defining the term 'oxidative stress' has proved an even harder task. Stress is defined as displacement from homeostasis causing injury to a biological system (Kassahn et al., 2009). By analogy, Sies (Sies, 1985) defined oxidative stress as 'a disturbance in the prooxidant/antioxidant balance in favor of the oxidants, leading to potential damage'. This definition placed emphasis on macromolecular damage. Later, Jones (Jones, 2006) redefined oxidative stress as 'a disturbance in the prooxidant/antioxidant balance in favor of the oxidants, leading to a disruption of redox signaling and control'. This definition placed emphasis on redox regulation of cell signaling. More recently, the two authors have merged the two definitions in a joint article as 'a disturbance in the prooxidant/antioxidant balance in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage' (Sies and Jones, 2007). Thus, the current definition of oxidative stress accounts for two different mechanistic outcomes – macromolecular damage and disruption of redox circuits – which lead to aberrant cell signaling and dysfunctional redox control. Macromolecular damage is considered in terms of oxidative mechanisms linked to reactive species.

It is apparent that the terms 'disturbance', 'disruption' and 'damage' that are contained in the current definition of oxidative stress imply interruption and/or loss of normal function, which is not in tune with the presently accepted view that oxidative modifications and alterations in redox signalling are linked to both 'beneficial' and 'harmful' biological effects (Valko et al., 2007; Bashan et al., 2009). For these reasons, in the present review we will use the term oxidative stress only as 'an increase in the level of reactive species and/or oxidant biomarkers' (Table 1). Adopting this definition, oxidative stress is verified by increased formation of reactive species and/or increased levels of biochemical products (e.g. F_2-isoprostanes) formed by reacting with oxidants, ignoring the repercussions of this alteration. In practice, in our opinion, this definition is automatically used by most of the researchers in the field. But even this oxidative stress definition cannot adequately describe more complex situations. For example, concluding that an organism has reached a state of oxidative stress is not always straightforward. For example, Tweedie et al. (Tweedie et al., 2011) reported lower oxidative DNA modifications despite greater H_2O_2 production in skeletal muscles from rats selectively bred for high running capacity compared with low-capacity runners. In addition, changes in redox-active molecules (e.g. the redox-sensitive transcription factor NFκB or ferrous iron) or antioxidants (e.g. uric acid or glutathione reductase) cannot be readily attributed to increased reactive species production, despite having clear effects on the redox processes of organisms. Therefore, the term 'alterations in redox homeostasis' will be used throughout this manuscript to indicate 'a shift in the level of reactive species, oxidant biomarkers, antioxidants and/or redox active molecules'. In our opinion, unless we have misinterpreted or overlooked something, this definition is suitable to characterize changes in

[1]The title alludes to the Sergio Leone's spaghetti Western film *The Good, the Bad and the Ugly* (1966). In the film there are three main characters representing the 'good', the 'bad' and the 'ugly'. None of these characters, however, can be clearly identified as totally 'good', 'bad' or 'ugly'. In fact, the film's title reveals the director's criticism about the canonized heroes of classical westerns. Thus, the adjectives in the title are merely relative. We believe that this analogy is also valid for the terms 'antioxidants, 'reactive species', and 'oxidative stress', as the biological roles of each are blurred.

Table 1. Definitions and criteria for the terms ‘alterations in redox homeostasis’ and ‘oxidative stress’ used in the present review

Definition	Criteria
Alterations in redox homeostasis A shift in the level of reactive species, oxidant biomarkers, antioxidants and/or redox-active molecules	Increased or decreased level of reactive species (e.g. H_2O_2, $O_2^{\bullet-}$) and/or Increased or decreased level of oxidant biomarkers (e.g. F_2-isoprostanes, protein carbonyls) and/or Increased or decreased level of antioxidant molecules (e.g. catalase, glutathione) and/or Increased or decreased level of redox active molecules (e.g. ferrous iron, NFκB)
Oxidative stress An increase in the level of reactive species and/or oxidant biomarkers	Increased level of reactive species (e.g. H_2O_2, $O_2^{\bullet-}$) and/or Increased level of oxidant biomarkers (e.g. F_2-isoprostanes, protein carbonyls)

redox-related molecules where they cannot be directly attributed to alterations in reactive species production. The same term is particularly useful in cases where, in the same study, changes in some molecules indicate oxidative stress and changes in other molecules indicate reduced oxidative stress (i.e. reductive stress) or no stress. We believe that this ongoing search for an improved term to describe oxidative stress mainly stems from the lack of a specific quantitative component in the concept of oxidative stress and the fact that no common strategy exists for its measurement; as a result, other attempts will certainly follow. For more information and a critical discussion of the oxidative stress concept, the reader is referred to the following reviews: Azzi, and Sies and Jones (Azzi, 2007; Sies and Jones, 2007).

Some overlooked effects of exercise

Effects of non-muscle- and muscle-damaging exercise on redox homeostasis

It should be noted that we use the term ‘non-muscle-damaging exercise’ in an improper way only to serve the purposes of our discussion, as all types of exercise induce a certain degree of muscle damage. Nevertheless, some types of exercise induce greater muscle damage than others. Exercise comprising a great deal of shortening muscle contractions (e.g. horizontal running or cycling) induces only limited muscle damage (Mathur et al., 2011). In contrast, exercise comprising a great deal of lengthening muscle contractions (e.g. downhill running or walking downstairs) induces extensive muscle damage (Nikolaidis et al., 2008). However, it must be stressed that even a concentric contraction-biased exercise can induce extensive muscle damage when the intensity is very high and/or the duration is very long [e.g. repeated sprints or ultramarathons (Knez et al., 2006; Howatson and Milak, 2009)]. In the present review, only the effects of acute exercise (i.e. exercise that has been performed only once) in reference to the resting condition are presented. For brevity, we use the term ‘exercise’ to refer to ‘acute exercise’ unless otherwise specified.

The effect of non-muscle-damaging exercise on reactive species production and the levels of oxidant biomarkers have been studied extensively (Fisher-Wellman and Bloomer, 2009). The most frequently studied biological specimen is blood, because of its ease in sampling and analysis of its constituents. It is now well established that acute non-muscle-damaging exercise results in oxidative stress in blood and skeletal muscle (Fisher-Wellman and Bloomer, 2009). Despite the hundreds of animal (including human) studies that have investigated the effects of non-muscle-damaging exercise on redox homeostasis, the vast majority of them have collected blood samples just immediately post exercise (Miyazaki et al., 2001; Bloomer et al., 2006), or at some other early post-exercise point (Alessio et al., 2000; Watson et al., 2005). Nevertheless, a number of studies have collected more than two blood samples at later time points after non-muscle-damaging exercise (Weiss et al., 2002; Schneider et al., 2003; Bloomer et al., 2005; Bloomer et al., 2007; Michailidis et al., 2007). Based on these studies, it is evident that non-muscle-damaging exercise induces alterations in redox homeostasis that lasts a few hours post exercise.

Although not so intensively investigated as non-muscle-damaging exercise, there are several studies that have looked into the effects of muscle-damaging exercise on redox homeostasis (Nikolaidis et al., 2008). Again, the biological tissue that has been studied the most is blood. In these studies, more specimens during the recovery period have been generally collected, thus the potential role of muscle damage to induce long-term oxidative stress can be appreciated. The most interesting finding reported by these studies is that alterations in redox biomarkers may persist for and/or appear several days after muscle-damaging exercise. This is in direct contrast to the rapid return of redox biomarkers to the resting values within a few hours after an acute non-muscle-damaging exercise. In fact, several studies have revealed that eccentric contractions increased the production of reactive species (Close et al., 2004) and induced oxidative stress in the blood, peaking in most cases at 2–3 days after exercise and returning towards baseline afterwards (Close et al., 2004; Nikolaidis et al., 2007b; Theodorou et al., 2010; Theodorou et al., 2011). Similarly, several studies showed redox homeostasis alterations some days after muscle-damaging exercise in skeletal muscle (Cabral de Oliveira et al., 2001; Silva et al., 2011). It would be interesting to investigate whether isolated skeletal muscle exercise (e.g. eccentric contractions of elbow flexors) induces oxidative stress in distant tissues (e.g. knee extensor muscles or organs such as liver and kidney). This will elucidate whether the appearance of oxidative stress after muscle-damaging exercise is a phenomenon produced in isolated tissues only (i.e. the eccentrically contracted muscles), or is also a product of systemic response mediated at least in part *via* blood.

The major conclusion of the comparison between non-muscle- and muscle-damaging exercise is that the term ‘exercise’ must be specifically defined with regard to exercise-induced changes in redox homeostasis, otherwise erroneous results may be obtained. The present analysis indicates that sampling time after non-muscle-damaging exercise and muscle-damaging exercise may lead to different conclusions regarding exercise-induced redox homeostasis responses. In fact, as Fig. 1 depicts, changes in protein oxidation can be transient (in non-muscle-damaging exercise) or prolonged (in muscle-damaging exercise). Similar kinetics has been reported for other redox biomarkers (Michailidis et al., 2007; Nikolaidis et al., 2007b). Regarding the non-muscle-damaging exercise, collecting blood samples up to 4 h post exercise should be enough to satisfactorily describe the changes in oxidative stress after non-muscle-damaging exercise of moderate duration (approximately 1 h). Collecting blood samples at 24 h after non-muscle-damaging exercise or at some other (more delayed) time

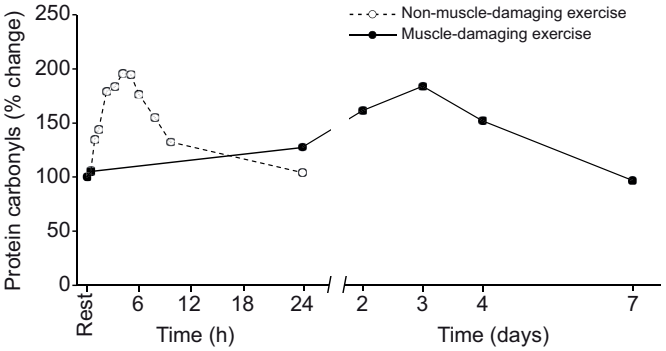

Fig. 1. The dichotomous effects of non-muscle-damaging exercise and muscle-damaging exercise on protein carbonyls in blood plasma. Protein oxidation was increased quantitatively similarly between the different types of exercise, though it exhibited a different time course. After non-muscle-damaging exercise, protein oxidation was increased at 30 min (35%), peaked at 4 h after exercise (96%) and declined thereafter, reaching resting levels by 24 h. After muscle-damaging exercise, protein oxidation was increased at day 1 (28%), peaked at day 3 after exercise (84%) and declined thereafter, reaching resting levels by day 7. Non-muscle-damaging exercise consisted of running for 45 min at 70–75% V_{O_2max} and then at 90% V_{O_2max} to exhaustion on a horizontal treadmill. Muscle-damaging exercise consisted of 75 maximal eccentric knee flexions on an isokinetic dynamometer. The 100% level corresponds to the protein carbonyl level at rest [calculations are based on data from Michailidis et al. and Nikolaidis et al. (Michailidis et al., 2007; Nikolaidis et al., 2007b) using the same assay].

point after exercise does not seem to offer any additional information regarding redox homeostasis alterations. The practice of many relevant studies to collect one blood sample immediately after exercise can lead to inaccurate deductions, because only a part of the entire picture is depicted. Concerning the practical applications relevant to muscle-damaging exercise, the present analysis indicates that measuring the levels of blood redox parameters only immediately and/or for some hours after muscle-damaging exercise produces results of limited value, because the levels of these parameters may remain altered even for 4 days after this type of exercise. Therefore, future relevant studies should perform multiple blood samplings late into recovery (at least up to 4 days post exercise) to describe the effects of muscle-damaging exercise in more complete dimensions. Additionally, researchers designing experiments in the field of redox biology should take care to have all participants abstain from muscle-damaging exercise for more than 4 days before enrollment in the study, to ensure the 'resting' condition of the blood redox homeostasis. Finally, taking into account that muscle-damaging exercise induces long-lasting and extensive changes in redox homeostasis, muscle-damaging exercise might be a more appropriate model with which to study the effects of experimental interventions (e.g. administration of redox agents or exposure to environmental oxidant stimuli such as smoking) on redox biology of blood and other tissues.

Effects of very short duration exercise on redox homeostasis

As we have shown above, there is consensus in the relevant literature that exercise (either muscle-damaging or not) is capable of inducing alterations in redox homeostasis. In addition, many studies have shown that the magnitude of redox homeostasis alterations increases as the intensity and duration of exercise increase (Quindry et al., 2003; Wang et al., 2006; Bloomer et al., 2007; Lamprecht et al., 2008). The latter finding may have led to the well-held idea that only exercise of sufficient intensity and duration induces alterations in redox homeostasis. Although this seems to hold true for the intensity component of exercise, it may not be exactly the case regarding the role of duration in the appearance of exercise-induced alterations in redox homeostasis. In fact, many studies have reported that an acute exhaustive maximal exercise task lasting only 30 s (i.e. the Wingate test) is capable of inducing systemic changes in redox homeostasis (Groussard et al., 2003a; Groussard et al., 2003b; Baker et al., 2004; Cuevas et al., 2005; Cooke et al., 2008; Bloomer and Smith, 2009; Arent et al., 2010). For example, Groussard et al. (Groussard et al., 2003b), employing electron paramagnetic resonance (EPR) spectroscopy, reported a twofold increase in lipid-derived radicals in plasma after 30 s of cycling. Moreover, other studies have reported increases in F_2-isoprostanes (the reference biomarkers of lipid peroxidation) in plasma and decreases in reduced glutathione (GSH; a valid biomarker of the redox state) in erythrocytes after the same 30 s cycling test (Groussard et al., 2003a; Groussard et al., 2003b; Cuevas et al., 2005; Cooke et al., 2008; Arent et al., 2010) (Fig. 2). Similarly, several studies have reported increases in reactive species production determined by EPR in whole blood (Peters et al., 2006) and changes in the redox couple of reduced glutathione and oxidized glutathione (glutathione disulfide; GSSG) toward a more oxidized redox potential after isometric handgrip exercise (at 50% of maximal voluntary contraction to exhaustion) lasting from 84 to 170 s (Matuszczak et al., 2005; Steinberg et al., 2006). Based on this evidence, it seems that even very short isometric exercise activating a relatively small muscle mass is able to increase reactive species production and induce oxidative stress systemically.

Looking beyond blood, muscle and humans

Exercise alters redox homeostasis across body fluids, organs and tissues

In almost every exercise biology subfield, the vast majority of studies have focused on blood (for practical sampling reasons) and skeletal muscle (as the tissue most closely related to fatigue and performance). Nevertheless, the influence of exercise on redox homeostasis of other tissues is of equal importance considering that exercise affects virtually every tissue of the body in multiple ways (Booth and Laye, 2009). For example, acute prolonged exercise decreases the glycogen content of liver with easily comprehensible repercussions to fatigue and performance (Baldwin et al., 1975). Considering that reactive species have been found to regulate glycogen metabolism in the liver (Gao et al., 2010), regulation of glycogen depletion and/or replenishment through alterations in redox homeostasis seems likely. Based on studies that have measured redox homeostasis in tissues other than blood and skeletal muscle, it is certain that acute exercise alters redox homeostasis in practically every fluid, blood cell, tissue and organ. In fact, several studies have found alterations in redox homeostasis after acute exercise in exhaled breath (Mercken et al., 2005), urine (McAnulty et al., 2010), lymphocytes (Boudreau et al., 2005), neutrophils (Sureda et al., 2005), diaphragm (Itoh et al., 2004), heart (Nie et al., 2010), liver (Liu et al., 2000), lung (Prigol et al., 2009), spleen (Kruger et al., 2009), thymus (Quadrilatero and Hoffman-Goetz, 2005), kidney (Leeuwenburgh and Ji, 1995) and brain (Lappalainen et al., 2010). This is not to imply that all tissues respond both qualitatively and quantitatively in a similar way to the same exercise stimulus. In fact, the expression of many redox proteins has proved to be highly cell, tissue, organ and species specific (Aon-Bertolino et al., 2011; Dammeyer and Arner, 2011; Godoy et al., 2011). Moreover, fluid-, tissue- and organ-specific redox homeostasis changes to an oxidant stimulus have been described in

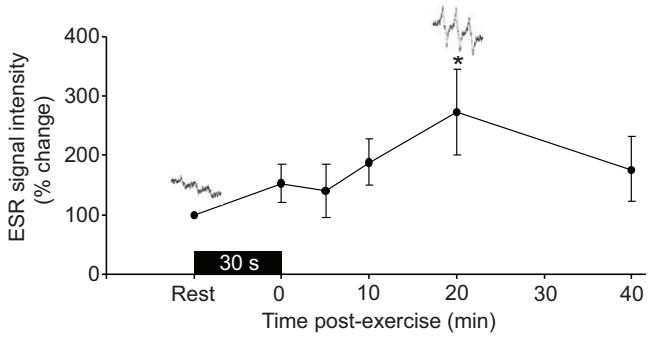

Fig. 2. Time-course changes in reactive species production detected by electron paramagnetic resonance (EPR) after cycling at maximum speed for 30 s in the blood serum of humans (means ± s.e.m.). The intensity of the EPR signal was the greatest at 20 min post-exercise. The 100% level corresponds to the EPR signal intensity level at rest. Typical spectra are shown for a single individual pre-exercise and at 20 min post-exercise [modified from Groussard et al. (Groussard et al., 2003b) with kind permission from Springer Science and Business Media]. *Statistically significant compared with pre-exercise (P<0.05).

the literature (Liu et al., 2000; Dyson et al., 2011). Nevertheless, it is evident that metabolic perturbations after virtually every type of exercise lead to alterations in redox homeostasis.

One of the intriguing questions in the field of exercise redox biology is the origin of oxidative stress in blood plasma that appears after exercise (Nikolaidis and Jamurtas, 2009). A muscle-centric point of view is frequently adopted to explain reactive species generation and oxidative modifications in plasma, thus obscuring the possibility that sources of reactive species and oxidative modifications other than skeletal muscle may be also at work during exercise. In our opinion, however, this frequently adopted muscle-centric perspective has not been put into rigorous test. The muscle-centric approach has been easily adopted to explain alterations in redox homeostasis measured in plasma primarily due to the fact that in many other popular physiological measurements the changes seen in plasma are indeed affected to a great extent by skeletal muscle. For example, the very large increases in blood lactate appearing after short-duration high-intensity exercise (van Hall, 2010) and in creatine kinase after muscle-damaging exercise (Nikolaidis et al., 2007b) are cases where skeletal muscle is indeed the main contributor to what is measured in the blood. The fact that exercise induces alterations in redox homeostasis in all tissues studied so far indirectly supports the idea that exercise-induced oxidative modifications detected in the plasma do not necessarily stem from oxidants produced inside skeletal muscle.

Another noteworthy observation stemming from the ubiquity of exercise-induced alterations in tissue redox homeostasis is that these changes occur despite the large differences in the rate of reactive species production, redox potential, antioxidant environment and levels of oxidant biomarkers among fluids and tissues at rest (Fig. 3). For example, the basal rate of $O_2^{\bullet-}$ production in the liver is more than fourfold higher than that found in the brain of the sperm whale (Cantu-Medellin et al., 2011). Similarly, the basal rate of $NO^{\bullet}$ production in the cerebral cortex of the rat is more than ninefold higher than that in the kidney (Dambrova et al., 2003). The redox potential (a measure of the tendency of a chemical species to acquire electrons using the major redox couple GSH/GSSG) also differs across tissues: the redox potential of the liver is approximately 28 mV more negative (i.e. more oxidized) than that of the brain in the rat (–246 mV *vs* –218 mV, respectively) (Giustarini et al., 2011). Even more negative redox potential values appear in erythrocytes (–193 mV) and plasma (–140 mV) in humans (Kemp et al., 2008). In addition, total ascorbate concentration is more than three orders of magnitude higher in the brain compared with in erythrocytes (Dyson et al., 2011), whereas liver contains approximately 4.5-fold higher GSH concentration compared with the brain in the rat (Giustarini et al., 2011). Similarly, the activity of extracellular superoxide dismutase (SOD) is approximately 20-fold higher in the lung compared with skeletal muscle in the mouse (Ookawara et al., 1998), whereas the activity of extracellular SOD is approximately sixfold higher in the heart compared with skeletal muscle in the sperm whale (Cantu-Medellin et al., 2011). The levels of oxidant biomarkers are also different across tissues at rest. For instance, the level of protein carbonyls (an index of protein oxidation) is more than twofold higher in the spleen compared with the liver in the rat (Arguelles et al., 2004). Similarly, the level of F_2-isoprostanes is more than 12-fold higher in the liver compared with skeletal muscle in the rat (Morrow et al., 1992). Finally, molecules constituting the chemical

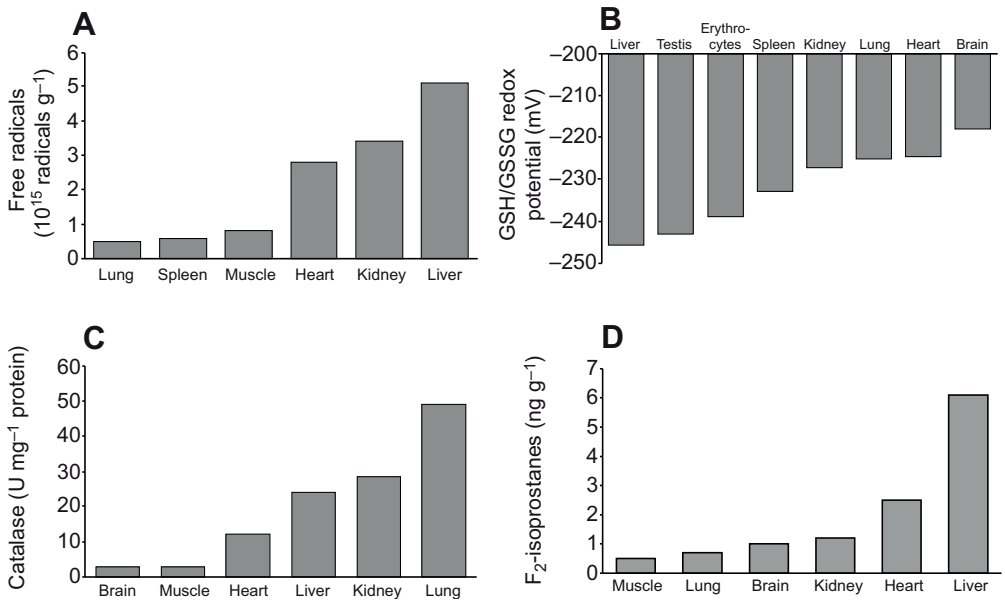

Fig. 3. Tissue redox composition at rest. (A) Concentration of free radicals detected by electron paramagnetic resonance (EPR) in rat tissues (Mallard and Kent, 1966; Wyard, 1968). (B) Redox potential of the glutathione (GSH)/glutathione disulfide (GSSG) couple in rat tissues. Redox potential was calculated using the Nernst equation, E_{hc}=–240–(59.1/2)×(log[GSH]2/[GSSG]) (Schafer and Buettner, 2003). GSH and GSSG values are derived from Giustarini et al. (Giustarini et al., 2011). Note that changes in the concentration of GSH and/or GSSG result in only a small change in redox potential because of the logarithmic form of equation. (C) Catalase activity (a major antioxidant enzyme) in sperm whale tissues (Cantu-Medellin et al., 2011). (D) Concentration of F_2-isoprostanes (a reference biomarker of lipid peroxidation) in rat tissues (Morrow et al., 1992).

environment (which are potential oxidizable substrates) also affect redox homeostasis. For example, considering that polyunsaturated fatty acids are susceptible to peroxidation, unlike saturated and monounsaturated fatty acids, membranes containing less polyunsaturated fatty acids are more resistant to peroxidative modifications by reactive species produced in their vicinity (Nikolaidis and Mougios, 2004; Hulbert, 2005; Pamplona, 2008). Indeed, the degree of polyunsaturation of phospholipid fatty acids has been reported to be 68% in the heart and just 48% in plasma, rendering the phospholipids contained in the latter less susceptible (at least relying solely on this aspect) to peroxidation (Nikolaidis et al., 2006a). Based on the aforementioned data, it is evident that exercise-induced changes in redox homeostasis are a well-conserved phenomenon among fluids and tissues irrespective of substantial differences in redox composition.

Acute exercise alters redox homeostasis across strains and species

The fact that exercise-induced oxidative stress is a fundamental biological response (in a sense, similar to the increased lactate production or increased mitochondrial respiration that follows exercise) is further verified by the fact that exercise-induced oxidative stress is a well-conserved response across strains and species. In fact, acute exercise has been reported to induce redox homeostasis alterations in body fluids, tissues and organs in humans, laboratory rodents of several strains as well as non-laboratory animals (both domestic and wild animals). Regarding humans, most of the available studies suggest that acute exercise indiscriminately induces oxidative stress in both sexes (Pepe et al., 2009), juveniles and adults (Nikolaidis et al., 2006b; Nikolaidis et al., 2007a), and in young and old (Bailey et al., 2010) as well as healthy and diseased (Puente-Maestu et al., 2011) individuals. Regarding laboratory animals, several studies have reported exercise-induced alterations in redox homeostasis in Wistar rats (Veskoukis et al., 2008), Sprague-Dawley rats (Gul et al., 2006; Nie et al., 2010) and Fischer 344 rats (Bejma et al., 2000). Similarly, alterations in redox homeostasis have been reported in mdx mice [an animal model for Duchenne muscular dystrophy, which lacks dystrophin (Radley-Crabb et al., 2011)], C57/BL6J mice (Yokota et al., 2009), BALB/c mice (De la Fuente et al., 1995), Swiss mice (Prigol et al., 2009), Hsd:ICR mice, which had been selected for high wheel-running activity (Vaanholt et al., 2008), and guinea pigs (De la Fuente et al., 1995). Finally, exercise has been reported to induce alterations in redox homeostasis in fish (Aniagu et al., 2006), birds (Costantini et al., 2008; Costantini and Lipp, 2010; Larcombe et al., 2010), dogs (Wyse et al., 2005) and horses (Kinnunen et al., 2009). Based on the above-mentioned analysis, it is evident that exercise-induced changes in redox homeostasis are a ubiquitous fundamental response of most (if not all) animal species, irrespective of definite differences in redox composition across species and between non-human animal models and humans. Alternatively, though, this common link among the species (i.e. alterations in redox homeostasis) may partly indicate that the current tools applied to assess redox homeostasis are too crude to delineate subtle differences among strains and species.

Multiple sources and tissues generate reactive species during exercise

All tissues and cells produce various reactive species under normal and stressed conditions. The primary reactive species produced are superoxide anion ($O_2^{\bullet-}$) and nitric oxide ($^{\bullet}NO$). Subsequently, the rest of the reactive species are mostly produced from $O_2^{\bullet-}$ and $^{\bullet}NO$ (Turrens, 2003; Tennyson and Lippard, 2011). $O_2^{\bullet-}$ – among others – is generated by the mitochondrial electron transport chain, auto-oxidation reactions of many molecules such as cysteine and $FADH_2$, oxidation of haemoglobin and myoglobin as well as by various enzymes such as xanthine oxidoreductase, NADPH oxidase and cytochrome P450 peroxidase (Halliwell and Gutteridge, 2007). It is important to emphasize that the degree of $O_2^{\bullet-}$ generation in mitochondria (considered a major source) may be indirectly regulated by uncoupling proteins. Uncoupling proteins reduce the number of protons flowing through the ATP synthase (Buttemer et al., 2010). Consequently, the leak of protons through uncoupling proteins dissociates substrate oxidation from phosphorylation of ADP to ATP, thus decreasing the production of $O_2^{\bullet-}$ (Buttemer et al., 2010). Most $^{\bullet}NO$ is synthesized from L-arginine by $^{\bullet}NO$ synthases (Bredt, 1999). However, $^{\bullet}NO$ can be regenerated from nitrite (NO_2^-) by acidic disproportionation as well as by enzymatic reduction *via* xanthine oxidoreductase, mitochondrial enzymes or deoxygenated hemoglobin and myoglobin (Jensen, 2009). In addition, endothelial $^{\bullet}NO$ synthase was found to be capable of reducing NO_2^- to $^{\bullet}NO$ under anoxia (Jensen, 2009).

Generation of $O_2^{\bullet-}$ and $^{\bullet}NO$ has generally been reported to increase during exercise in various tissues in animals, including humans. $O_2^{\bullet-}$ has been reported to be increased in the brain (Pedreanez et al., 2006), skeletal muscle (Brooks et al., 2008), peritoneal macrophages (De la Fuente et al., 1995), neutrophils (Garcia et al., 2011) and lymphocytes (Tanimura et al., 2008). Similarly, $^{\bullet}NO$ has been reported to be increased in skeletal muscle (Brooks et al., 2008), $CD34^-$ blood mononuclear cells (Jenkins et al., 2011), putative endothelial progenitor cells (Jenkins et al., 2009), plasma (Yang et al., 2007) and exhaled breath (Persson et al., 1993). Regarding the effects of exercise on uncoupling proteins, in a detailed study, Jiang et al. (Jiang et al., 2009) found dramatic increases in uncoupling protein 3 (the isoform expressed primarily in skeletal muscle) after exercise in rat muscle. More importantly, based mainly on the parallel increase in uncoupling protein 3 and the reactive species production, these authors suggested that the decreased efficiency of oxidative phosphorylation due to uncoupling protein 3 upregulation may serve an antioxidant function to protect muscle mitochondria from exercise-induced oxidative stress (Jiang et al., 2009). In addition to these primary reactive species, it should be highlighted that many other reactive species are produced in cells under physiological and stressed conditions. Similarly, many other mechanisms control their rate of production and decomposition. It is noteworthy that, despite the importance of $O_2^{\bullet-}$, $^{\bullet}NO$ and the other reactive species to cellular activities, the molecular mechanisms of their production, accumulation, function and degradation remain insufficiently understood, largely because of the limited specificity of many of the inhibitors, scavengers and detectors used in the studies (Winterbourn, 2008). For a detailed description of the potential sources of exercise-induced reactive species production, the reader is referred to specific reviews (Sjodin et al., 1990; Jackson et al., 2007; Sachdev and Davies, 2008; Lamb and Westerblad, 2011; Powers et al., 2011c).

Possible biological importance of exercise-induced alterations in redox homeostasis

A major research objective in redox biology is to delineate the biological consequences of alterations in redox homeostasis. Despite its importance, the biological outcomes of alterations in redox homeostasis are hard to define and are mostly based on

mere temporal associations. This is largely because of the difficulty in linking the inherently complex alterations in redox homeostasis with physiological processes. We believe that for an accurate description of the biological effects of reactive species, (at least) the following experimental variables must be explicitly determined: (1) the type of reactive species generated (e.g. $O_2^{\bullet-}$ or $HO^{\bullet}$); (2) the amount of reactive species generated; (3) the duration for which reactive species remain increased; (4) the type of cell in which reactive species are generated (e.g. erythrocyte or muscle cell); (5) the cellular compartment in which reactive species are generated (e.g. mitochondria or cytosol); (6) the type (e.g. enzymatic or not), concentration and location (e.g. lipophilic or hydrophilic) of antioxidants; (7) the type of molecule that has undergone oxidation (e.g. receptor or transfer protein); (8) the type of the functional group that has undergone oxidation (e.g. sulfhydryl group or hydroxyl group); and (9) whether the oxidation is reversible or irreversible (e.g. oxidation of cysteine residues, a reversible oxidation, or carbonylation, an irreversible oxidation) (Table 2). Remarkably, it appears that reactive species may induce physiological effects either in increased or decreased concentrations. To our knowledge, no study has yet described the effect of exercise or any other stimulus fulfilling all of the aforementioned requirements. Consequently, the role of reactive species generation during exercise remains, to some degree, elusive.

Although a considerable number of issues remain open, reactive species have been implicated in a vast array of diverse biological mechanisms and functions. Only in skeletal muscle is there increasing evidence to support the hypothesis that reactive species play an essential role in regulating hypertrophy (Powers et al., 2011b), atrophy (Pellegrino et al., 2011), force (Westerblad and Allen, 2011), fatigue (Reid, 2008), damage and repair (Nikolaidis et al., 2008), cytokine production (Scheele et al., 2009), fatty acid metabolism (Silveira et al., 2008), glucose uptake (Merry and McConell, 2011) as well as adaptations to chronic exercise (Jackson, 2009). Despite the fact that tissues other than muscle and blood have drawn much less attention in the field of exercise redox biology, accumulating reports indicate that the overall redox-related adaptations in the liver of exercise-trained animals might lead to a reduced DNA mutation rate and attenuate the age-induced inflammation (Radak et al., 2008a). In addition, evidence indicates that accumulation of oxidative damage in aged brain impairs its function, and exercise can attenuate the accumulation of oxidative damage, improving brain function (Radak et al., 2008a). Moreover, exercise-induced reactive species production could play a role in the induction of neurotrophins, which might be important for neurogenesis (Radak et al., 2008a). In addition, it should be taken into account that many tissues exhibit increased levels of oxidative damage during exercise [e.g. heart, spleen and lungs (Veskoukis et al., 2008; Kruger et al., 2009)] and, as a result, reactive species may contribute to the responses and adaptations seen in these tissues during exercise. For an authoritative treatise on the reconciliation of chemistry and biology of reactive species the reader is referred to specific reviews (Winterbourn, 2008; Dickinson and Chang, 2011; Floyd et al., 2011; Murphy et al., 2011).

Table 2. Essential experimental variables to be determined for more accurate appreciation of alterations in redox homeostasis

1. The type of reactive species generated
2. The amount of reactive species generated
3. The duration for which reactive species remain increased
4. The type of cell in which reactive species are generated
5. The cellular compartment in which reactive species are generated
6. The type, concentration and location of antioxidants
7. The type of molecule that has undergone oxidation
8. The type of the functional group that has undergone oxidation
9. Whether the oxidation is reversible or irreversible

Integrating the redox homeostasis responses to exercise within a hormetic framework

Despite the lack of a generally agreed definition, most researchers concur that hormesis is a dose–response phenomenon characterized by either a U-shaped or an inverted U-shaped dose response depending on the end-point measured (Calabrese and Baldwin, 2001). In hormesis, dose response is characterized by low dose stimulation and high dose inhibition, leading to the biphasic, hormetic dose–response curve (Calabrese and Baldwin, 2001). It is important to emphasize that in order to characterize a dose–response relationship as hormetic, both the stimulus (e.g. exercise) and the response (e.g. an oxidant biomarker) should be measured concurrently and repeatedly over an adequate time interval. Equally important is that the stimulus must be monotonic (i.e. either only increasing or decreasing) whereas the response must be non-monotonic (i.e. increase followed by decrease or the reverse). That said, a biphasic change (i.e. increase followed by decrease) of an oxidant biomarker during recovery from a single bout of exercise (e.g. Uchiyama et al., 2006) does not constitute a hormetic effect of exercise. Similarly, a biphasic change of an oxidant biomarker after chronic exercise of progressively increased and decreased training load does not constitute a hormetic effect of exercise (Margonis et al., 2007). For a detailed discussion on hormesis, the reader is referred to specialized reviews (Calabrese and Baldwin, 2002; Goto et al., 2007; Costantini et al., 2010; Kendig et al., 2010).

Hormetic effects of reactive species on muscle function and fatigue date back to at least the early 1990s. Reid et al. (Reid et al., 1993) first proposed an innovative theoretical model based on the positive effects of reactive species on the contractile force of skeletal muscle. Their model actually predicted a hormetic effect of reactive species on muscle function. That is, a modest increase in reactive species causes muscle force enhancement, whereas this positive effect is reversed at higher reactive species concentrations in a dose-dependent manner (Reid et al., 1993). Shortly afterwards, several studies verified this hormetic effect of reactive species on muscle force. Oba et al. (Oba et al., 1996) reported that incubation of intact single frog fibers in a solution containing H_2O_2 induced a biphasic effect on muscle force. Initially, muscle force increased, but as the exposure to H_2O_2 continued, muscle force declined (Oba et al., 1996). Likewise, Andrade et al. (Andrade et al., 1998) reported that single mouse fibers responded to H_2O_2 exposure in a time-dependent manner, showing biphasic changes. These single fiber findings were extended to intact skeletal muscle by Lawler et al. (Lawler et al., 2010). These researchers found that the diaphragm exhibited a similar biphasic contractile response to reactive species because lower levels of xanthine oxidase (a major $O_2^{\bullet-}$ producer) markedly enhanced diaphragm contractility, whereas high levels of xanthine oxidase did not (Lawler et al., 2010). Recently, Wei et al. (Wei et al., 2011) reported that the effects of contractile activity on $O_2^{\bullet-}$ production also occur in a biphasic manner. Specifically, $O_2^{\bullet-}$ production (detected using a novel mitochondrial-targeted biosensor) increased following brief tetanic stimulation (five tetani) but markedly decreased following prolonged tetanic stimulation (40 tetani) (Wei et al., 2011). Moving on to *in vivo* findings, Jonsdottir et al. (Jonsdottir et al., 1998)

reported that chronic exercise decreased the levels of nitrate (a stable metabolite of •NO) in blood plasma at 7 days but increased thereafter (up to 35 days).

The conceptual framework provided by these (admittedly fragmented) data can potentially reconcile differences that emerge among relevant studies. For example, the hormesis concept could provide an interpretative framework for one of the most intriguing questions in the field of exercise redox biology: the effect of antioxidant supplementation on exercise adaptations. Indeed, an old and active debate exists in the literature regarding the effect of antioxidant supplementation on the biology of animals (including humans). Despite the progress of analytic techniques and the refinement of study designs, striking disagreement exists among studies regarding the influence of antioxidant supplementation on physical performance and redox homeostasis. Indeed, several studies have indicated that antioxidant supplementation induces a positive effect (Jakeman and Maxwell, 1993; Shafat et al., 2004), a negative effect (Gomez-Cabrera et al., 2008; Ristow et al., 2009) or no effect (Yfanti et al., 2010; Kyparos et al., 2011; Theodorou et al., 2011) on muscle performance. Likewise, several studies have reported that antioxidant supplementation attenuates oxidative stress (Close et al., 2006; Kinnunen et al., 2009), others have reported that it induces a pro-oxidant effect (McAnulty et al., 2005; Versari et al., 2006) and others have reported that it does not affect redox homeostasis (Rytter et al., 2010; Theodorou et al., 2011). The hormetic concept predicts that the effects of antioxidant supplementation on muscle performance and redox homeostasis are dependent on the antioxidant dose. In fact, the limited evidence indicates that too much generation of reactive species may be harmful whereas modest generation may be beneficial (Radak et al., 2008b). Based on this evidence, it is plausible to assume that the divergence regarding the effects of antioxidant supplementation on exercise adaptations and redox homeostasis may partly be explained by the different degree of reactive species decomposition. Despite the promising first data and the appealing nature of the hormesis concept, the establishment of deviation from linearity in dose–response relationships for exercise-induced alterations in redox homeostasis requires studies specifically designed to locate and describe the possible hormetic effects of exercise.

Conclusions and the way ahead

In this review we attempted to designate the fundamental nature of oxidative stress using exercise as a model of an oxidative stressor. Despite obvious differences in details, we have shown that diverse types of exercise (from extreme muscle-damaging exercise to that lasting only few seconds) are all associated with alterations in redox homeostasis and oxidative stress. With the necessary modifications, exercise is capable of inducing redox homeostasis alterations in all fluids, cells, tissues and organs studied so far, irrespective of strain and species. The most important message coming from these observations is that ‘exercise-induced oxidative stress’ is not an ‘oddity’ associated with a particular type of exercise, tissue or species. Rather, we believe that ‘oxidative stress’ constitutes a ubiquitous fundamental biological response to the alteration of redox homeostasis imposed by the physical stress of exercise. In support to this, oxidative stress has been reported after every possible stress employed, such as increased temperature (Lushchak and Bagnyukova, 2006); decreased temperature (Qiu et al., 2011), acidic pH and alkaline pH (Wang et al., 2009); as well as hypoxia (Sharma et al., 2011) and hyperoxia (Krnic et al., 2011). There is no doubt that although diverse stimuli induce redox homeostasis alterations and/or oxidative stress, the effects of these alterations on biological adaptations are translated differently in many (or all) cases. The example of exercise highlights, among others, the interconnections and complexity of redox biology and may indicate the inability of the current tools to reveal subtle differences. We believe that integrative and comparative approaches could shed light on the interactions of key redox responses at multiple levels of biological organization.

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