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Exercise-induced oxidative stress: A tool for "hormesis" and "adaptive response"

Katsuhiro Koyama

Graduate School of Education, University of Yamanashi, 4-4-37 Takeda, Kofu, Yamanashi 400-8510, Japan

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Abstract Physical exercise-mediated production of reactive oxygen species has been shown to cause oxidative stress, particularly in contracting skeletal muscles. Growing evidence indicates that exercise-induced oxidative stress plays an indispensable role in upregulating signaling pathways required to promote not only skeletal muscle, but also whole body adaptation to physical exercise. It is becoming increasingly clear that exercise-related beneficial adaptations are strongly regulated by exercise-induced oxidative stress, consistent with hormesis theory. According to the hormesis hypothesis, exercise-induced mild to moderate oxidative stress through reactive oxygen species generation stimulates favorable exercise-related physiological adaptations. Additionally, repeated exposure to oxidative stress induced by physical training can trigger various hormesis-based adaptations (i.e., hormetic adaptive responses), including activation of antioxidative defense mechanisms. This brief review provides an overview of several conceptual frameworks related to exercise-mediated hormetic adaptive responses rather than a detailed critique of individual reports.

Keywords : oxidative stress, hormesis, adaptive response, reactive oxygen species (ROS)

Introduction

Free radicals possess one or more unpaired electrons in their outer orbits and are therefore highly reactive. Both free radicals and nonradical reactive oxygen derivatives (e.g., hydrogen peroxide) are collectively referred to as reactive oxygen species (ROS). The primary ROS generated at multiple locations in cells both during and after exercise is the superoxide anion radical ($O2•^-$), which is negatively charged and relatively membrane impermeable. Dismutation of superoxide, catalyzed by superoxide dismutase (SOD), spontaneously produces hydrogen peroxide (H_2O_2), which has a relative long half-life, thus enabling it to diffuse within the cell and across cell membranes. Further, although hydrogen peroxide is generally considered a relatively weak oxidizing agent, its cytotoxicity occurs through the formation of hydroxyl radicals (•OH) by metal-catalyzed reactions, such as the Fenton reaction and the Haber-Weiss reaction requiring iron and copper, respectively. The hydroxyl radical has strong oxidizing potential and can rapidly damage a number of target molecules, including lipids, proteins, and nucleic acids. It is now well established that physical exercise promotes the generation of all of these ROS, resulting in a harmful effect on many cellular components.

Most cells, including muscle fibers, contain various antioxidant defense mechanisms that can be broadly classified as enzymatic or nonenzymatic antioxidants; these reduce the risk of oxidative damage, especially during periods of ROS induction by stimuli such as exercise, smoking, and radiation exposure. The term "antioxidant" refers to any substance that retards, prevents, or reverses oxidative denaturation of a substrate[1]. Numerous antioxidant strategies are involved in protection against ROS-mediated toxicity. For instance, enzymatic antioxidants convert ROS to less reactive molecules (e.g., catalase converts hydrogen peroxide into H_2O and $•O_2^-$) and prevent the transformation of these less reactive products into more damaging molecules. A nonenzymatic antioxidant strategy is to minimize the availability of pro-oxidants such as iron and copper ions via metal-binding proteins. Moreover, numerous low-molecular-weight nonenzymatic antioxidants are capable of scavenging ROS; examples include endogenously synthesized molecules such as glutathione, uric acid, and bilirubin, as well as exogenous dietary agents such as vitamins C and E[2].

ROS were originally recognized as deleterious oxidants because of their strong association with oxidative damage to biological molecules. We must therefore consider whether the effects of ROS generated through physical exercise are adequately balanced by the total capacity of the antioxidant defense system. It is clear that a change in the oxidant–antioxidant balance leading to a shift toward a severely oxidizing environment (i.e., overproduction of ROS) results in oxidative damage, which has various negative effects on physiological function. However, a slight

Correspondence: koyama@yamanashi.ac.jp

imbalance caused by mild to moderate ROS production appears to trigger the activation of several signaling mechanisms necessary for a positive adaptive response[3,4]. Therefore, it is now widely accepted that ROS are not inherently harmful, but rather play a crucial role in controlling cellular function as a "double-edged sword". In living systems, the balance of ROS generation and removal predominantly modifies the redox status, which has a narrow physiological range; this phenomenon is called "redox homeostasis"[5,6]. The main focus of this review is to discuss growing evidence that transient exercise-induced alterations in the redox status under normal physiological conditions are essential for various positive adaptive responses such as hormesis-associated upregulation of the antioxidant defense system.

Oxidative stress and exercise

Oxidative stress. The term "oxidative stress" has been defined as the "imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage"[7]. More recently, it was redefined as "a disturbance in the oxidants and antioxidants balance in favor of the oxidants, leading to disruption of redox signaling and control and/or molecular damage"[8]. However, this definition implies that oxidative stress is inextricably linked to negative responses such as the disruption of cellular signaling pathways, macromolecule damage, and disturbance of homeostasis. This is inconsistent with the commonly accepted view that oxidative modifications and alterations in redox signaling are linked to both "beneficial" and "harmful" biological effects[6,9,10]. Because the description of oxidative stress is subject to revision as our knowledge of redox biology advances, it is essential that researchers clearly define this term within the framework of their research[11]. Thus, the present review has used the term "oxidative stress" to simply mean "an increase in the level of ROS and/or oxidant biomarkers". Practically, because the direct measurement of short-lived ROS is a difficult task, an increased concentration of macromolecule oxidation products is used as an indirect biomarker in most studies. According to the definition adopted in this review, it is considered that there is a threshold that distinguishes "physiological oxidative stress" leading to positive adaptive responses from "pathological oxidative stress" causing oxidative damage, physiological dysfunction, and injury.

Exercise-induced oxidative stress. To put it simply, any circumstance in which oxygen consumption is increased (such as during exercise) may result in oxidative stress. It has been assumed that increased ROS generation in contracting skeletal muscles is directly related to elevated oxygen consumption associated with enhanced mitochondrial respiration (i.e., oxidative phosphorylation). In particular, most aerobic exercises, generally characterized by long-term physical exertion, consume more oxygen to supply their greater energy requirements; this can lead to a substantial increase in ROS formation by as much as 50- to 100-fold[12]. Thus, electron loss resulting from the consumption of oxygen is greater during aerobic exercise than at rest[13]. On this basis, it was previously thought that mitochondrial electron transport chain reactions are the most important source of ROS during aerobic exercise. However, recent findings suggest that this is not the case[14,15]. Regardless of whether mitochondrial ROS generation is the primary source, elevated oxidative stress is acknowledged to be a primary outcome of aerobic exercise[16]. Furthermore, it is important to emphasize that the complex antioxidant defense systems that protect against ROS under resting conditions are often insufficient to counteract oxidative stress during or after exercise.

Anaerobic exercise is commonly described as short-term, high-intensity physical activity, and sometimes as exhausting exercise. Similar to aerobic exercise, all forms of anaerobic exercise have the potential to increase ROS formation and thus induce oxidative stress. Since oxygen consumption is increased during and after anaerobic exercise to a lesser extent than in aerobic exercise, activated mitochondrial respiration is not thought to be the major endogenous cause of ROS production. Instead, anaerobic exercise-induced increases in ROS are suggested to largely derive from activated radical-generating enzymes (xanthine oxidase and NADPH oxidase), prostanoid metabolism, phagocytic respiratory burst, disruption of iron-containing proteins, and a loss of calcium homeostasis[17]. For instance, xanthine oxidase, a major contributory enzyme to ROS formation during exercise, is produced by the ischemia-triggered proteolysis of xanthine dehydrogenase that occurs during strenuous exercise; its use of molecular oxygen as the electron acceptor results in the production of $O_2^{\bullet-}$ and H_2O_2[18-20].

Sources and tissue distribution of exercise-generated ROS. One of the intriguing questions in the field of exercise physiology is the site of ROS production in exercise-induced oxidative stress. Although it is generally accepted that ROS generation predominantly occurs in contracting skeletal muscles, the primary organ/tissue of endogenous ROS production has not been clearly identified. The lack of in vivo studies is due to the inherent difficulties in investigating exercise, a process that involves many interrelated physiological systems affected by the increased energy requirement of contracting skeletal muscles. Actually, it is feasible that as well as skeletal muscle, tissues such the heart, lungs, liver, and white blood cells may also contribute to systemic ROS generation during and after exercise[2].

Exercise-induced hormesis and adaptive response

Hormesis and adaptive response. "Hormesis" was originally defined in the field of toxicology as a phenomenon

in which small amounts of a harmful substance (e.g., ionizing radiation, heavy metals, and toxins) have positive stimulatory effects on living organisms[21]. Hormesis is therefore characterized by a dose-response relationship in which low-dose stimulation induces various positive physiological responses, but high-dose stimulation has harmful consequences. Thereafter, the concept of hormesis has been extended to "adaptive response", in which living organisms chronically exposed to low-dose hazardous substances become resistant to subsequent high-dose exposure to the same stimulant[22]. In other words, repeated low-dose stimulations can trigger various hormesis-based adaptations (hormetic adaptive responses), including the activation of antioxidative capacity, DNA repair function, and immune function.

Hormetic adaptive responses to exercise. According to the principle of hormesis, mild to moderate oxidative stress (i.e., increased ROS generation) induced by exercise is a necessary stimulus for most exercise-related physiological responses. Although the antioxidant defense system may be temporarily inhibited by ROS production through acute exercise of low to moderate intensity, it may be adversely enhanced during the recovery phase as a result of the initial oxidant stimulation. Furthermore, repeated exposure to increased ROS through chronic physical exercise (training) induces adaptive responses that upregulate the antioxidant defense system[3,23], leading to a shift in the redox state toward a more reducing environment. These changes provide adaptive resistance to oxidative stress during subsequent training sessions, as well as during exposure to other severe oxidative conditions unrelated to exercise[3,24,25]. Taken together, it is thought that exercise-induced mild or moderate oxidative stress may operate as a trigger for hormetic adaptive responses. Thus, in order to obtain health-promoting adaptation (e.g., enhanced antioxidant defense capacity), the physiological stimulus (in this case, exercise-induced oxidative stress) is essential, but levels must never exceed the upper threshold for oxidative damage (Fig. 1).

Many in vitro studies have examined the effects of ROS on hormetic adaptive responses, mainly in myocyte cell cultures. H_2O_2 exposure has been shown to augment the expression of key antioxidant enzymes in cultured myotubes[26]. In addition, a recent study revealed that myotube exposure to exogenous H_2O_2 increased peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α: one of the most important transcriptional coactivators) promoter activity and mRNA expression[27]. Remarkably, these effects were inhibited when the antioxidant *N*-acetylcysteine was added to culture medium. Moreover, ROS are also thought to serve as critical signaling events involved in regulating other physiological functions of skeletal muscles, such as contraction and glucose uptake[28,29]. A low concentration of H_2O_2 increases both Ca^{2+} release from the sarcoplasmic reticulum and muscle force, whereas a large increase in H_2O_2 concentration results in a sharp decrease in force output[30]. Indeed, one previous study showed that H_2O_2 facilitates glucose uptake by skeletal muscles in a dose-dependent manner up to an optimal concentration; thereafter, it becomes inhibitory[31]. Collectively, numerous in vitro studies strongly support the notion that ROS can stimulate various cytoprotective functions through hormesis-based adaptation.

Similarly, in vivo studies support the hypothesis that exercise-induced oxidative stress leads to adaptive responses in a hormetic manner, ultimately mediating the favorable effects of exercise on physical performance, health promotion, and disease prevention[24,25]. Recently, two important studies were conducted by Gomez-Cabrera

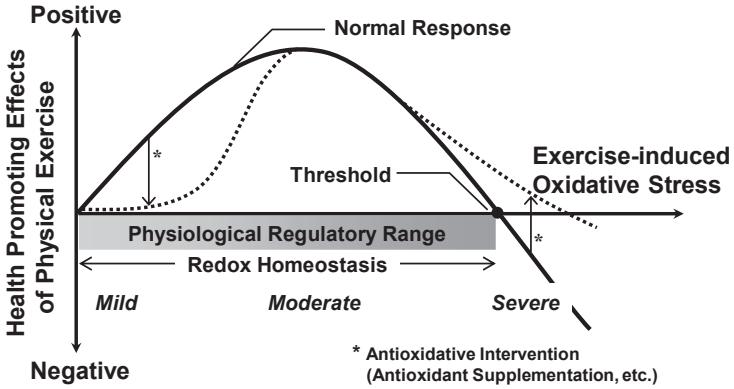

Fig. 1 Schematic diagram of the relationship between exercise-induced oxidative stress and health-promoting effects.
The solid line describes the health-promoting effects of physical exercise as a function of the degree of exercise-induced oxidative stress. A mild to moderate level of oxidative stress (ROS production) leads to positive effects, whereas severe oxidative stress exceeding the threshold results in negative effects that cause oxidative damage. The dotted line represents the influence of antioxidative intervention on health-promoting effects. When exercise-induced oxidative stress is mild, some antioxidative intervention (e.g., antioxidant supplementation) attenuates exercise-induced hormetic adaptive responses. In contrast, when exercise is likely to be exhaustive or severe, antioxidative intervention may be recommended to suppress the negative effects of exercise (i.e., oxidative damage).

et al.[32] and Ristow et al.[10]. In the former study, oral administration of vitamin C lowered training efficiency related to endurance capacity in both humans and rats. Furthermore, vitamin C inhibited exercise-induced PGC-1α upregulation and subsequent mitochondrial biogenesis, and inhibited exercise-induced expression of several antioxidant enzymes in rat skeletal muscles. In the latter study, similar findings in humans revealed that vitamin C and E supplementation prevents the induction of molecular regulators of insulin sensitivity (i.e., peroxisome proliferator-activated receptor γ and its coactivator, PGC-1α) and endogenous antioxidant defense enzymes (SOD and glutathione peroxidase) by physical exercise. Additionally, ROS generation during acute sprinting exercise has been confirmed to activate the PGC1-α pathway and stimulate mitochondrial biogenesis, because reduced ROS generation by blocking nonmitochondrial enzyme xanthine oxidase (one of the main sources of ROS in anaerobic exercise) with the specific inhibitor allopurinol attenuates these responses[33]. These results are extremely important as they suggest that the stimulus for exercise-induced mitochondrial biogenesis might not be muscle contractile activity per se, but rather nonmitochondrial ROS generation and the resultant alteration in intracellular redox status. In addition, a large number of experiments have revealed the importance of ROS induced by physical exercise in achieving optimal adaptive potential and physiological function[34-36]. Together, these important findings support the hypothesis that exercise-mediated hormetic adaptive responses are strongly regulated by exercise-induced ROS production (i.e., oxidative stress).

However, various favorable positive effects of exercise-induced oxidative stress are likely to depend on both the magnitude and duration of changes in ROS concentration[5] (Fig. 2). For example, a short-term, moderate increase in ROS production that can be controlled via the antioxidative defense system can activate signaling pathways leading to cellular adaptation and thus protect against later stress stimuli. In contrast, high levels of ROS production over long periods may result in chronic high levels of oxidative stress that eventually yields deleterious oxidative damage. These consequences of exercise are consistent with the concept of hormesis. It is possible that oxidative stress levels resulting from high-intensity or prolonged exercise will be sufficiently large to overwhelm the antioxidative defense capacity; under such circumstances, antioxidant supplementation may be recommended before and after exhausting or competitive exercise to mitigate exercise-induced oxidative damage (Fig. 1). In support of this, my research group[37] has already suggested that antioxidant administration to attenuate high-intensity exercise-induced hippocampal 4-hydroxy-2-nonenal (a marker of lipid oxidative damage) overproduction results in enhanced brain-derived neurotrophic factor expression in the rat hippocampus.

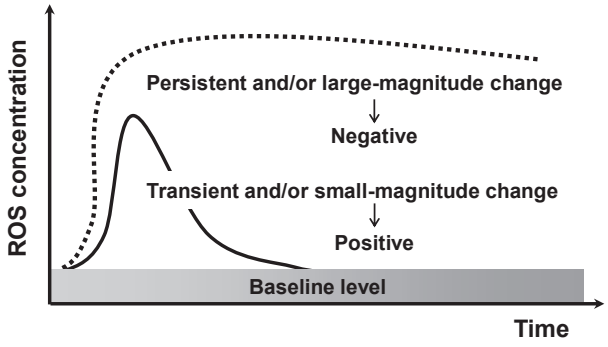

Fig. 2 Schematic diagram showing changes in both the temporal pattern and the magnitude of reactive oxygen species (ROS) production caused by physical exercise.
ROS normally occur in living tissues at relatively low steady-state levels. Transient and/or slight changes in ROS generation give rise to a temporary imbalance in the redox status and subsequent positive physiological adaptation. In contrast, persistent production of abnormally large amounts of ROS leads to dysregulated oxidative stress, which results in negative pathological events. (Modified from Dröge[5])

There is currently considerable debate regarding the effects of oral antioxidant supplementation (e.g., vitamin C or E) on physical performance, health promotion, and disease prevention in humans. Most randomized controlled trials including different types of patients have concluded that antioxidant supplementation has a strong deleterious influence on morbidity and mortality associated with a variety of diseases[38-40]. Moreover, there is no strong evidence to support the use of antioxidant supplements as primary and secondary preventive measures, particularly in normal healthy people[41,42]. These findings indicate that active antioxidative intervention under mild to moderate oxidative stress conditions may abrogate the various health-promoting effects of physical exercise in humans (Fig. 1). Basically, exercise-induced oxidative stress itself is not necessarily harmful; instead, it is a fundamental ubiquitous biological response to exercise-induced changes in redox homeostasis and functions as a valuable mechanism driving hormetic adaptive responses.

Closing remarks

It is now thought that all modes of exercise, whether aerobic or anaerobic, have the potential to produce ROS and thus induce oxidative stress. Of course, exercise-induced oxidative stress is influenced by several other factors, including the mode of exercise (duration, intensity, and frequency), specific biomarkers chosen, time course of tissue sampling, age, training status, and dietary intake. Since the optimal level of ROS production (i.e., mild to moderate oxidative stress) may function as an indispensable mechanism in exercise-related hormetic adaptive responses, a strategy to maintain exercise-induced oxidative stress within the most suitable range must be established. In other words, it is necessary that exercise-induced oxi-

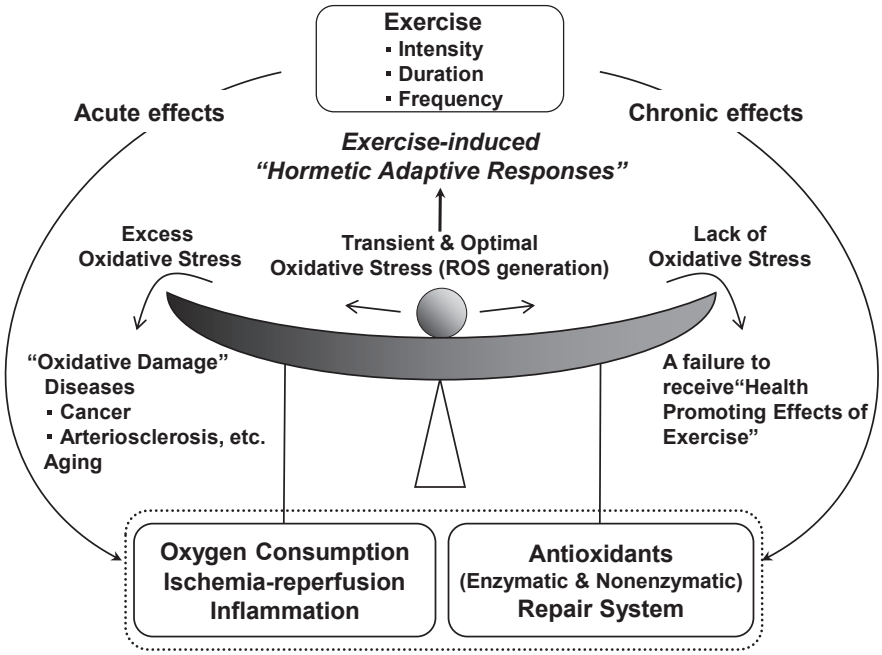

Fig. 3 Model of dynamic equilibrium in exercise-related redox homeostasis. ROS, reactive oxygen species.

dative stress is dynamically regulated within a physiological regulatory range to ensure redox homeostasis (Fig. 3). However, it is difficult to identify the threshold between beneficial physiological oxidative stress and pathological oxidative stress for each individual. To specifically obtain exercise-mediated benefits, new surrogate markers to predict the individual threshold of oxidative stress may also be required.

It is important to appreciate that exercise-induced oxidative stress-associated adaptations are systemic[43-46]. Since most investigations into the relationship between exercise-induced oxidative stress and hormetic adaptive responses have so far mainly focused on skeletal muscles, it is now imperative to evaluate systemic effects on the whole body, including internal organs (such as the heart, liver, kidney) and specifically the brain, in future research efforts[37,45,46]. Although the detailed role of oxidative stress in the mechanism of exercise-induced hormetic adaptations in humans remains to be completely elucidated, there is no doubt that exercise-induced oxidative stress, a tool for "hormesis" and "adaptive response", has tremendous potential to upregulate various biological functions.

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