Update on Thiol Status and Supplements in Physical Exercise

Chandan K. Sen

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Abstract/Résumé

Strenuous physical exercise represents a condition that is often associated with increased production of reactive oxygen species in various tissues. One of the most reliable indices of exercise-induced oxidant production is tissue glutathione oxidation. In humans, exercise-induced blood glutathione oxidation is rapid and subject to control by antioxidant supplementation. The objective of this brief review is to provide an update of our current understanding of cellular thiols and thiol antioxidants. Cellular thiols are critically important in maintaining the cellular antioxidant defense network. In addition, thiols play a key role in regulating redox-sensitive signal transduction process. Lipoic acid is a highly promising thiol antioxidant supplement. Recent studies have clarified that while higher levels of oxidants may indeed inflict oxidative damage, oxidants are not necessarily deleterious. Under certain conditions oxidants may function as cellular messengers that regulate a multitude of signal transduction pathways. In light of this, the significance of oxidants in various aspects of biology needs to be revisited.

L'effort physique intense est souvent associé à une augmentation de la production d'espèces réactives de l'oxygène dans divers tissus. L'oxydation du glutathion tissulaire est un des indicateurs les plus fiables de la production d'oxydants induite par l'effort. L'oxydation du glutathion sanguin induite par l'effort est rapide chez les humains ; un contrôle par un supplément d'antioxydants s'avère donc possible. Le but de cette brève synthèse est de

The author is with the Laboratory of Molecular Medicine, Department of Surgery, 512 Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, OH 43210.

mettre à jour les connaissances sur les thiols intracellulaires et les antioxydants de thiol. En plus de jouer un rôle majeur dans la protection du réseau cellulaire contre l'oxydation, les thiols cellulaires sont d'importants acteurs dans la régulation du signal de transduction sensible aux réactions d'oxydoréduction. L'acide lipoïque est, sous forme de supplément, un antioxydant de thiol très prometteur. D'après des études récentes, même si des concentrations élevées d'oxydants peuvent causer des dommages par oxydation des cellules, les oxydants ne sont pas toujours nuisibles. Dans certaines conditions, les oxydants peuvent constituer des messagers cellulaires qui régularisent de nombreuses voies de transduction du signal. Par conséquent, il serait pertinent de revoir le rôle des oxydants dans les différents champs de la biologie.

Introduction

During the last three decades, the study of reactive oxygen species with respect to human health and disease has gained remarkable momentum. Early studies in this field primarily focussed on oxidative stress—a condition wherein oxidants overwhelm the antioxidant defenses leading to tissue damage. Strenuous physical exercise represents a condition that is often associated with increased production of reactive oxygen species in various tissues (Sen, et al., 1994b). Since this observation was reported in the early 1980s, there has been a steadily growing interest to develop an understanding of the significance of oxidants in exercise biology. Issues such as mechanisms involved in exercise-induced oxidant production, oxidative stress, and strategies to minimize exercise-induced oxidative stress using antioxidant supplements have been addressed in numerous studies that have been reviewed (Sen, 1995a,1995b, in press; Sen, et al., 1994b, 2000a). Many of the earlier studies related to exercise-induced oxidative stress are riddled with poor quality of interpretation of results primarily because of limitations in the analytical approaches that were used to quantify oxidant generation or oxidative stress. The last 5–10 years have witnessed a significant improvement in the methods used to study oxidant biology (Sen and Packer, in press; Sen et al., 2000a). This development has been instrumental in shedding new light on oxidant biology. As we uncover novel fundamental essentials in oxidant biology, it is important to revisit older findings in light of current knowledge. One of the most reliable indices of exercise-induced oxidant production is tissue glutathione oxidation (Sen, 1995a, 1997; Sen et al., 1994c). In humans, exercise-induced blood glutathione oxidation is rapid and subject to control by antioxidant supplementation (Sen et al., 1994c). The objective of this brief review is to provide an update of our current understanding of cellular thiols and thiol antioxidants (Sen, 1997, 1998, 2000; Sen and Packer, 1996, 2000) and their possible relation to physical exercise.

Exercise-Induced Oxidant Production

Studies originating from several independent laboratories provide firm support to the contention that strenuous exercise is associated with increased generation of reactive oxygen species in tissues (Sen et al., 1994b, 2000a). Intense training may deplete tissue antioxidant defenses (Subudhi et al., 2001). However, little is known about the various sources of exercise-induced reactive oxygen species formation. Although recent studies have attempted to clarify this issue, we are yet to have a

firm consensus. While Bejma and Ji (1999) allude to the mitochondria and NADPH oxidase as potential sources of exercise-induced oxidant formation, Vina et al. (2000) claim that xanthine oxidase is the primary contributor and that mitochondrial sources are negligible. Vina et al. reported that exercise caused an increase in blood xanthine oxidase activity in rats. In addition, inhibiting xanthine oxidase with allopurinol prevented exercise-induced oxidation of glutathione in both rats and humans. Furthermore, inhibiting xanthine oxidase prevented tissue damage as manifested by increases in the plasma activity of cytosolic enzymes (lactate dehydrogenase, aspartate aminotransferase, and creatine kinase) following exhaustive exercise. Based on these findings it was concluded that xanthine oxidase is responsible for the free radical production and tissue damage during exhaustive exercise and that mitochondria play a minor role as a source of free radicals during exhaustive physical exercise (Vina et al., 2000). Consistently, it has been shown by the same group that xanthine oxidase is an important source for free radical generation during exercise in chronic obstructive pulmonary disease (Heunks et al., 1999).

Moller et al. (2001) demonstrated that the risk for exercise-induced oxidative stress is higher under conditions of altitude hypoxia. This group investigated the effect of a single bout of exhaustive exercise on the generation of DNA strand breaks and oxidative DNA damage under normal conditions and at high-altitude hypoxia (4559 meters for 3 days). Twelve healthy subjects performed a maximal bicycle exercise test. Urinary excretion of 8-hydroxy-2'-deoxyguanosine increased during the first day in altitude hypoxia, and there were more endonuclease III-sensitive sites in urinary DNA on day 3 at high altitude. The subjects had more DNA strand breaks in altitude hypoxia than at sea level. The level of DNA strand breaks further increased immediately after exercise in altitude hypoxia. Exercise-induced generation of DNA strand breaks was not seen at sea level. Hypoxia appeared to diminish the capacity to withstand oxidative stress produced by exhaustive exercise (Moller et al., 2001).

A recent study by McArdle et al. (2001) provides substantial support to the contention that muscular contraction is associated with reactive oxygen species production. Fifteen minutes of aerobic contractile activity induced a rapid release of superoxide anions from mouse skeletal muscle *in vivo*. Studies with contracting cultured skeletal muscle myotubes confirmed that this was due to release from myocytes rather than other cell types present within muscle tissue *in vivo*. This increased oxidant production caused a rapid, transient decrease in muscle protein thiol content (Mcardle et al., 2001).

Venditti et al. (1999) demonstrated that hepatic antioxidant defense systems are able to withstand oxidative challenge due to low-intensity exercise of moderate duration. In contrast, the free radical production associated with long-lasting exercise causes oxidative injury in hepatic cellular components and in particular induces protein degradation in the heavy mitochondrial fraction characterized by higher susceptibility to oxidative stress (Venditti et al., 1999).

Is exercise-induced oxidative stress sensitive to gender difference? Tiidus et al. (1999) addressed this issue in rats. Female rats had higher levels of vitamin E in liver and heart tissues than males, males had significantly more vitamin C in the plantaris muscle than females, and female rats also had less liver glutathione than males. Despite these differences in tissue antioxidant status at rest, acute exercise

resulted in significant and equal tissue oxidative stress in both genders as indicated by tissue glutathione status (Tiidus et al., 1999). Aging increases the susceptibility of the heart to exercise-induced oxidative stress (Bejma et al., 2000).

Exercise and Thiols

The low molecular weight thiol glutathione (GSH) is present in all animal cells often in quite high (mM) concentrations. GSH is known to have multifaceted physiological functions including antioxidant defense, drug detoxification, regulation of signal transduction, storage and transport of cysteine, regulation of cell proliferation, deoxyribonucleotide synthesis, regulation of immune response, and regulation of leukotriene and prostaglandin metabolism (Sen, 1995a, 1995b, 1997, 1998, 1999, 2000, 2001a, 2001b, in press; Sen et al., 1992, 1994b, 2000a; Sen and Hanninen, 1994; Sen and Packer, 1996, 2000). A competition for glutathione precursors between the immune system and the skeletal muscle is thought to be responsible for skeletal muscle fatigue (Bounous and Molson, 1999). A key mechanism that accounts for much of the metabolic and cell regulatory properties of glutathione is thiol-disulfide exchange equilibria. Physical exercise has been shown to affect this equilibrium by causing thiol oxidation (Sen and Packer, 2000).

Physical exercise induced tissue glutathione oxidation has been observed in numerous studies. A recent work by Laaksonen et al. (1999) further underscores the critical role of glutathione homeostasis in modulating exercise-induced oxidative stress and, conversely, the effect of oxidative stress at rest on exercise-induced changes in glutathione redox status (Laaksonen et al., 1999). Recently it has been shown that oxidation of protein thiols and glutathione may be involved in the secondary damage following pliometric contractions (Mcardle et al., 1999).

Cysteine is the rate limiting amino acid for intracellular GSH synthesis (Sen, 1997). Whey protein concentrate has been shown to represent an effective and safe cysteine donor for GSH replenishment during GSH depletion in immune deficiency states (Bounous, 2000). The effect of whey protein concentrate on exercise-induced perturbation of tissue thiol status remains to be tested. Pro-glutathione antioxidant supplements such as lipoic acid and N-acetylcysteine have shown beneficial effects (Khanna et al., 1998, 1999a; Sen, 1997; Sen and Packer, 2000). N-acetylcysteine has been shown to improve human limb muscle performance during fatiguing exercise (Reid et al., 1994). In addition, N-acetylcysteine supplementation spared exercise-induced blood glutathione oxidation in humans (Sen et al., 1994c). Lipoic acid is a potent pro-glutathione agent and its mechanism of action provides it with a significant advantage over N-acetylcysteine (Sen, 1997, 2000; Sen and Packer, 2000; Sen et al., 1997c, 1999). Because lipoic acid is reduced by enzymes in the human cell, its potent reduced form can be continuously regenerated in the cell. Thus, lipoic acid is able to increase cellular GSH levels at low micromolar concentrations while mM concentration of N-acetylcysteine is used in most studies (Sen et al., 1997c). In addition to its antioxidant properties, lipoic acid has potent insulin-mimetic properties. Lipoic acid has been shown to stimulate glucose uptake in muscle cells. This property of lipoic acid is retained even under conditions of insulin resistance (Khanna et al., 1999b). Several studies also demonstrate outstanding efficacy of lipoic acid to prevent or correct age related disorders such as lowering of tissue vitamin C status and mitochondrial

dysfunction (Ames, 1998; Hagen et al., 1999, 2000; Lykkesfeldt et al., 1998; Shi et al., 1999; Suh et al., 2001). Taken together, lipoic acid has an outstanding potential in exercise nutrition and this area remains to be explored.

Lipoic acid is taken up by cells and reduced to its potent dithiol form dihydrolipoate (DHLA) much of which is rapidly effluxed out from cells (Sen, 1997; Sen, et al., 1997b, 1997c). To improve retention in cells, the lipoic acid molecule has been modified to confer a positive charge at physiological pH. The result is the synthesis of N,N-dimethyl,N'-2-amidoethyl-lipoate. This protonated form of lipoic acid is commonly referred to as lipoic acid plus (LA-Plus; US patent 6,090,842; 2000), or lipoic acid with a positive charge (Sen et al., 1998). The uptake of LA-Plus by human T cells as well as murine neuronal cells is much higher compared to that of lipoic acid. Several-fold higher amounts of DHLA-Plus, the corresponding reduced form of LA-Plus, has been detected in LA-Plus treated cells compared to the amount of DHLA found in cells treated with lipoic acid. Thus, LA-Plus seems to be an improved form of lipoic acid (Sen et al., 1998; Tirosh et al., 1999). Extensive toxicity and *in vivo* studies are required to establish the overall significance of LA-plus as a therapeutic agent.

Oxidant Generation Does Not Necessarily Lead to Oxidative Stress

Generation of oxidants in a biological tissue is often incorrectly assumed to be necessarily leading to oxidative stress. It is important to recognize that during the course of their routine life every aerobic cell is expected to generate oxidants at certain levels. During the last 5 years or so, convincing evidence has accumulated to support the hypothesis that at levels much below that required to inflict oxidative damage, oxidants serve as messengers within the cell. In this way oxidants regulate a large variety of redox-sensitive signal transduction pathways (Sen, 1998, 2000, 2001a; Sen and Packer, 1996; Sen et al., 2000a). While some oxidant-driven pathways may indeed lead to pathological consequences, oxidants regulate normal physiological processes as well. In light of this information it is important to shun the false notion that oxidants necessarily cause stress. Oxidative stress is observed only under conditions when the generation oxidant is exaggerated to a point that overwhelms cellular antioxidant defenses and causes oxidative damage.

Redox based regulation of signal transduction and gene expression has emerged as a fundamental regulatory mechanism in cell biology. Electron flow through side chain functional CH_2-SH groups of conserved cysteinyl residues in proteins account for their redox-sensing properties (Sen, 1998). Thus thiols such as glutathione are critical regulators of redox-sensitive signal transduction pathways. For the most part, oxidation of glutathione is viewed as impairment of cellular antioxidant defenses. While that is true, it is important to recognize that changes in the glutathione level or redox-state within the cell may have other key effects. For example, it has been shown that the level of glutathione and its redox state in the muscle cell has a major influence of inducible NF-κB activity (Sen, et al., 1997a; Sen and Packer, 1996). Exposure to low amounts of oxidants triggers the expression of several defense proteins conferring protection against future challenges. Evolution of the concept that oxidants may have a messenger function

within the cell may be viewed as a sharp turn in our understanding of oxidant biology. This major development calls for a more critical analysis of the physiological and pathological roles of oxidants. While excess oxidants may indeed trigger oxidative tissue damage and limit performance, it is equally plausible that lower levels of oxidants confer protection. For example, we know that oxidants stimulate inducible vascular endothelial growth factor expression (Khanna et al., 2001). Is it then unrealistic to hypothesize that exercise-induced oxidants may drive angiogenesis and collateral formation in the microvasculature? These represent new horizons in the field that will provide us with critical insights.

Antioxidant supplements are likely to protect against the ravages of oxidant attack say during the course of strenuous exercise (Sen et al., 2000a). The question that follows is what antioxidant supplement regimen is expected to be most effective. Given that antioxidants act in a network (Sen, 1995a; Sen and Packer, 2000), it would be fair to speculate that taking a combination of the various classes of antioxidants would be prudent. While antioxidants are mostly safe even when consumed at moderately high doses, it should be noted that long term effects of mega doses have not been tested in humans.

Net antioxidant protection is represented by the sum of antioxidant defenses provided by endogenous and nutritional antioxidants. Endogenous antioxidant defense is heavily dependent on specific proteins encoded by specific genes (Sen and Hanninen, 1994). Given the genetic heterogeneity in the human population, endogenous antioxidant defense systems are expected to considerably vary from person to person. Other factors that may contribute to the individual variation of endogenous antioxidant defense profile include dietary habits and lifestyle (stress, smoking, alcohol consumption, UV exposure). Thus, efforts to assess individual antioxidant supplement needs of athletes should be emphasized.

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