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Muscle redox signalling pathways in exercise. Role of antioxidants

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ABSTRACT

Recent research highlights the importance of redox signalling pathway activation by contraction-induced reactive oxygen species (ROS) and nitric oxide (NO) in normal exercise-related cellular and molecular adaptations in skeletal muscle. In this review, we discuss some potentially important redox signalling pathways in skeletal muscle that are involved in acute and chronic responses to contraction and exercise. Specifically, we discuss redox signalling implicated in skeletal muscle contraction force, mitochondrial biogenesis and antioxidant enzyme induction, glucose uptake and muscle hypertrophy. Furthermore, we review evidence investigating the impact of major exogenous antioxidants on these acute and chronic responses to exercise. Redox signalling pathways involved in adaptive responses in skeletal muscle to exercise are not clearly elucidated at present, and further research is required to better define important signalling pathways involved. Evidence of beneficial or detrimental effects of specific antioxidant compounds on exercise adaptations in muscle is similarly limited, particularly in human subjects. Future research is required to not only investigate effects of specific antioxidant compounds on skeletal muscle exercise adaptations, but also to better establish mechanisms of action of specific antioxidants *in vivo*. Although we feel it remains somewhat premature to make clear recommendations in relation to application of specific antioxidant compounds in different exercise settings, a bulk of evidence suggests that N-acetylcysteine (NAC) is ergogenic through its effects on maintenance of muscle force production during sustained fatiguing events. Nevertheless, a current lack of evidence from studies using performance tests representative of athletic competition and a potential for adverse effects with high doses ($>$ 70 mg/kg body mass) warrants caution in its use for performance enhancement. In addition, evidence implicates high dose vitamin C (1 g/day) and E ($\geq$ 260 IU/day) supplementation in impairments to some skeletal muscle cellular adaptations to chronic exercise training. Thus, determining the utility of antioxidant supplementation in athletes likely requires a consideration of training and competition periodization cycles of athletes in addition to type, dose and duration of antioxidant supplementation.

1. Introduction

It is well established that muscular contraction and intense exercise generate an increased production of reactive oxygen species (ROS) and nitric oxide (NO) and promote oxidative stress in skeletal muscle [1–8]. While the principal cellular and tissue sites of ROS production during exercise have been a matter of debate [9], recent evidence appears to indicate that exercise-induced ROS is primarily of non-mitochondrial origin, particularly from nicotinamide adenine dinucleotide phosphate (NADPH)

Abbreviations: Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; COX, cytochrome oxidase; DTT, dithiothreitol; ERK1/2, extracellular signal-regulated kinases 1/2; Gpx, glutathione peroxidase; Grx, glutaredoxins; GSH, glutathione; GSSG, glutathione disulphide; H_2O_2, hydrogen peroxide; IGF-1, insulin-like growth factor 1; JNK, c-Jun N-terminal kinase; L-NAME, N^G-nitro-L-arginine methyl ester; mTOR, mammalian target of rapamycin; NAC, N-acetyl cysteine; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, nuclear factor kappa B; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; NRF, nuclear respiratory factor; $O_2^{\bullet -}$, superoxide; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; p38 MAPK, p38 Mitogen-activated protein kinase; $p70^{S6K}$, ribosomal protein S6 kinase; PGC-1α, peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-alpha; PI3K, Phosphoinositide 3-kinase; PKA, protein kinase A; pO_2, partial pressure of oxygen; Prx, peroxiredoxins; ROS, reactive oxygen species; RyR1, ryanodine receptor/Ca^{2+} release channel; SERCA1, sarco(endo)plasmic reticulum Ca^{2+}-dependent ATPase, −2; SH, thiol; SIRT-1, sirtuin 1; SIRT-3, sirtuin 3; SOD, superoxide dismutase; Srx, sulfiredoxins; SSG, glutathionylation; TFAM, mitochondrial transcription factor A; TnI_f, fast twitch skeletal muscle-specific troponin I isoform; TRIM, 1-(2-trifluoromethyl-phenyl)-imidazole; TRPV1, transient receptor potential cation channel, subfamily V, member 1; Trx, Thioredoxins; VO_2, volume of oxygen consumed

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oxidases [10–12]. Historically, an increased level of ROS has been regarded as deleterious to cells [13,14]. Exposure to increased levels of ROS has been implicated in damage and modifications to cellular lipids, DNA and proteins [15]. ROS has also been implicated in chronic conditions such as cardiovascular disease [16–18] and type 2 diabetes [19]. Such negative effects of ROS might be related to an excessive level and/or duration of ROS exposure and to the cellular origin of ROS produced [11]. On the other hand, evidence has emerged over the past two decades showing that ROS and NO produced physiologically by cells are important signalling molecules, acting through mechanisms such as post-translational redox modifications of cysteine thiols on proteins [20,21]. Such signalling can regulate diverse biological functions such as the maintenance of tissue homoeostasis, regulation of transcriptional activity, cell proliferation and differentiation, and cell migration [15,22–26]. Recent research has also highlighted the potential importance of ROS and NO-mediated signalling in normal exercise-related molecular and cellular responses [14]. In particular, redox-signalling pathways have been implicated in several acute and chronic responses of skeletal muscle to exercise, including skeletal muscle glucose uptake and muscle insulin sensitivity [27,28]; modulation of endogenous antioxidant enzyme levels [6,29,30]; mitochondrial biogenesis [31–33]; muscle contraction force [34–36] and muscle hypertrophy [37,38].

Antioxidants play an important role in regulating tissue levels of ROS through free radical scavenging and adaptive electrophilic-like mechanisms (interested readers are referred to ref. [13] for a comprehensive review). Acute and chronic exercise tends to up-regulate endogenous antioxidant enzyme abundances and activities in skeletal muscle [6,29,30], therefore enabling an improved capacity to decrease adverse effects of increased ROS production. Moreover, the common supplementation of antioxidants by elite and recreational athletes [39,40] may also enhance the capacity of skeletal muscle to neutralize ROS produced during exercise. Benefits might relate to an improvement in cellular redox state and decreased oxidative modifications to DNA, lipids and proteins. Some evidence shows an ameliorating effect of antioxidant supplementation on muscle damage associated with delayed onset muscle soreness [41], although other evidence does not support a protective effect of supplementary antioxidants [42–44]. ROS has also been implicated in premature muscular fatigue during sustained submaximal muscle contraction and exercise [45–47]. Therefore, the use of supplementary antioxidants might help to delay muscular fatigue and improve exercise performance.

Despite the aforementioned potential benefits of antioxidant supplementation in exercising humans, recent research has implicated the use of antioxidants in impairments rather than improvements in some acute and chronic responses of skeletal muscle to exercise [31,33,35,48,49]. These impairments in adaptive changes within skeletal muscle are presumably a result of an attenuation of normal redox-signalling pathways in muscle by antioxidants [14]. In particular, antioxidant supplementation has been found in some studies to impair some adaptive responses to endurance exercise training [33,48,49] and resistance exercise training [35,38]. Nonetheless, study findings overall remain equivocal in human participants in relation to effects of antioxidants on skeletal muscle adaptations and performance outcomes following exercise training [50–52].

The present review aims to firstly present a discussion of some important redox-signalling-related pathways implicated in acute and chronic responses of skeletal muscle to muscle contraction and exercise; and secondly, to discuss the impact of antioxidants on these redox-signalling-related pathways. Where possible, we have focussed on evidence arising from studies using healthy human participants, given the potential applicability of such findings to human athletic endeavours. However, considering existing ethical and methodological limitations in human-based studies, a vast amount of important mechanistic information can only currently be gathered using *in vitro* models, *ex vivo* models, *in-situ* models and *in vivo* animal models. Additionally, a wealth of information exists in studies concerned with elderly or infirm populations. Thus, we wish for readers to bear in mind the inherent limitations of translating findings from discrete populations, or from non-*in vivo*, non-human studies directly to human athletes.

2. Exercise-related redox signalling pathways in skeletal muscle

ROS including superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), NO, and reactive NO derivatives including peroxynitrite ($ONOO^-$) have been implicated in redox signalling in cells either directly or indirectly [53]. Key sites of ROS production during exercise include NADPH oxidase enzymes (which are associated with the sarcoplasmic reticulum [SR], transverse tubules and plasma membrane), phospholipase A_2 and xanthine oxidase [12,46]. Skeletal muscle mitochondria are also important biological generators of ROS, however *in vitro* and *ex vivo* evidence suggests that they are not likely to be key contributors to the increased muscle ROS production during exercise given higher mitochondrial ROS production at rest compared with exercise [9,54,55]. In terms of NO, neuronal nitric oxide synthase (nNOS) is the likely key generator of NO in skeletal muscle during contraction [46,56]. NADPH oxidase production of ROS and nNOS production of NO appears to be especially important for redox signalling in muscle during exercise [57–61].

Transient and reversible post translational chemical modifications of reactive cysteine thiol residues on cell proteins, such as through processes including S-nitrosylation, S-glutathionylation, sulfenylation and disulphide formation, likely constitute important redox modifications through which cells respond to altered levels of ROS and NO [21,36,62]. S-nitrosylation of proteins involves the coupling of a NO group to a reactive cysteine thiol to form an S-nitrosylated protein [63]. Protein S-nitrosylation can produce diverse cellular effects, including altered regulation of enzyme activities, altered receptor and transporter activities, altered gene transcription and translation, and protein–protein interactions [63]. S-nitrosylation has been observed in numerous proteins associated with skeletal muscle contraction and exercise, including the skeletal muscle ryanodine receptor/Ca^{2+} release channel (RyR1), myosin, cAMP response binding protein (CREB), calpain-2, caspase-3, sarco(endo)plasmic reticulum Ca^{2+}-dependent ATPase (SERCA1a), histone deacetylase-2 (HDAC2), plasma membrane Ca^{2+}-ATPase type-1 (PMCA1), and specific insulin-signalling proteins [64–73]. S-glutathionylation involves the formation of mixed disulphides between GSH and cysteine thiol groups of proteins [74]. S-glutathionylation of a protein can result in its activation or deactivation, which may be important in the regulation of cell signalling mediators [21,74]. S-glutathionylation is known to interface with protein phosphorylation via modulation of cellular kinases and phosphatases, including protein kinase A (PKA), creatine kinase, mitogen-activated protein kinase kinase kinase 1 (MEKK1), phosphatase and tensin homologue deleted from chromosome 10 (PTEN), adenosine monophosphate-activated protein kinase (AMPK), and protein tyrosine phosphatase 1B (PTP1B) [75–81]. Post-translational redox modifications such as S-nitrosylation and S-glutathionylation may therefore play important molecular signalling roles in skeletal muscle given their intricate associations with key proteins linked to muscle contraction and exercise adaptations (Fig. 1).

In addition to laying the foundation for cellular redox signalling