

ROS and myokines promote muscle adaptation to exercise

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Physical exercise induces a network of alterations in the transcriptome and proteome of the skeletal muscle, resulting in modifications of the muscle physiology. Intriguingly, exercise also transiently induces the production of both reactive oxygen species (ROS) and some inflammatory cytokines in skeletal muscle. In fact, it seems that exercise-induced ROS are able to stimulate cytokine production from skeletal muscle. Despite the initial view that ROS were potentially cell damaging, it now seems possible that these substances have important roles in the regulation of cell signaling. Muscle-derived cytokines, so-called 'myokines', are distinguished from inflammation and instead possess important anti-inflammatory and metabolic properties. In this opinion piece, we suggest that both ROS and myokines are important players in muscle adaptation to exercise.

Paradoxical roles of ROS and inflammatory cytokines in exercise and disease

Chronic states such as type 2 diabetes, aging, and neurodegenerative and cardiovascular disease all have aspects of metabolic dysfunction. Previously, the strongest focus has been on the role of nutrition and obesity in these states; however, more recent evidence has indicated that regular exercise has a major role in the prevention of these chronic diseases and premature mortality, independent of obesity [1]. In line with this notion was the recent demonstration that physical inactivity without weight gain for only two weeks induced a cluster of metabolic and physiological changes, including a decrease in muscle mass and insulin sensitivity and an increase in plasma triglycerides [2], indicating a rapid adaptation to a decreased energy level. Conversely, muscle metabolism transiently adapts to the higher energy demand during acute exercise by increasing mitochondrial activity. Repeated bouts of exercise will result in a gradual acclimation to a higher fitness level, represented by, for example, increased mitochondrial respiration, glucose utilization and fatty acid oxidation. At the molecular level, these adaptations include mobilization of mitochondria [3] and coordinated upregulation of extracellular matrix genes [4].

In addition to metabolic changes, exercise increases reactive oxygen species (ROS) and induces endogenous antioxidant defense mechanisms [5]. Given that ROS are thought to have a prominent role in the pathogenesis of diseases in which exercise seems to be preventative, their

induction by exercise is conventionally regarded as a consequence of damaging contractile activity and degenerating muscle fibers, thus potentially delaying muscle recovery. However, in contrast to this initial view, it is becoming apparent that these substances also play an important part in the regulation of gene transcription and protein synthesis [6,7]. This new theory challenges the current dogma that ROS always have a harmful effect on cells and introduces an important role for ROS in cell signaling, for example, during muscle adaptation to exercise. ROS can induce redox-sensitive transcription factors, resulting in the activation of inflammatory cytokines. This could potentially explain why ROS and inflammation are related in their etiology in chronic diseases.

A similar paradox is described for the actions of inflammatory cytokines as they relate to exercise. On one hand, infiltration of immune cells into white adipose tissue and its ensuing inflammation is correlated with the development of insulin resistance and type 2 diabetes [8]. In addition, activated immune cells and inflammation have an important role in cardiovascular diseases [9]. Moreover, Parkinson's and Alzheimer's diseases are linked to local inflammation in the brain, and systemic inflammation seems to exacerbate the progression of these diseases [10]. On the other hand, some exercise-induced cytokines, produced and secreted by skeletal muscle, seem to exert anti-inflammatory effects and alter cellular metabolism [11]. Thus, the anti-inflammatory effects of regular exercise will protect against chronic systemic low-grade inflammation. Crucial to this theory is the finding that skeletal muscle is an endocrine organ that produces and releases cytokines, termed 'myokines' [12–15] (Box 1).

In this opinion piece, we stress the role of ROS and myokines in muscle adaptation to exercise. In support of this view, we describe recent findings on peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1 α), a well-established exercise-related factor, and suggest a feedback relationship between PGC1 α and ROS. We further emphasize that ROS are likely to regulate the transcription of myokines and suggest a context- and concentration-dependent outcome of ROS and pro-inflammatory cytokines and a role for both of these as signaling molecules in skeletal muscle adaptation to exercise.

Exercise-induced reactive oxygen species can regulate cell signaling in muscle

'ROS' is a collective name for molecules that contain oxygen, which makes them chemically instable and reactive.

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Box 1. Myokines are not an inflammatory response

The first myokine to be identified was interleukin (IL)-6. At first, it was hypothesized that exercise-induced elevation of IL-6 was an inflammatory response caused by muscle damage. However, the increase in IL-6 was observed after both concentric (e.g. biking or leg-kicking) and eccentric (e.g. strength training or running) contractions and, thus, could not solely be due to muscle damage [46]. In addition, by comparing plasma from the femoral artery with plasma from the femoral vein, it became clear that the increased levels of plasma IL-6 during exercise originated from skeletal muscle [47]. Since this finding, several pieces of evidence have emerged that indicate a metabolic role, independent of its inflammatory effects, for IL-6, as well as for other potential myokines [15]. Although the cytokine responses to exercise and sepsis have many similarities, they seem to occur as distinct events. For example, during sepsis, there is a marked and rapid increase in circulating tumor necrosis factor (TNF)- α , which is followed by an increase in IL-6. By contrast, during exercise, the marked increase in IL-6 is not preceded by elevated TNF- α [15]. Another example is that the IL-6 signaling in macrophages is dependent on activation of the NF κ B signaling pathway [15], whereas intramuscular IL-6 expression is regulated by a network of signaling cascades that are likely to involve crosstalk between the Ca²⁺-activated nuclear factor of activated T cells pathway, muscle glycogen content and the p38 mitogen-activated protein kinases, among other pathways. The biological consequence is that the cytokine response in macrophages promotes inflammation, whereas the cytokine response elicited by non-damaging physical exercise has anti-inflammatory and metabolic properties.

The most reactive ROS are free radicals, which have one or more unpaired electrons, produced as by-products of oxidative metabolism. As their name implies, ROS are reactive and can harm the cell by causing an oxidizing cascade, resulting in DNA damage and protein and lipid oxidation, and ultimately inducing apoptosis or senescence. Indeed, ROS have an established role in muscle catabolism by inducing the proteasome pathway for protein degradation [16]. However, in healthy individuals, ROS are buffered by endogenous antioxidant defense mechanisms: for example, superoxide dismutase and nitric oxide synthase (NOS). The onset of exercise results in the production of ROS that, in turn, can initiate cell signaling and induce alterations in gene expression by directly modifying target proteins or by changing the intracellular redox state [7]. For example, ROS has been suggested to play a part in the signaling pathways of growth factors [17]. Thus, although the chronically elevated levels of ROS during degenerative disease might be harmful, we claim that the acute production of ROS during physical activity might be beneficial. In contrast to the currently held view of the role of ROS in suppressing muscle recovery (see Box 2), we believe that increased levels of ROS during exercise are not only harmless but also contribute to some of the adaptations occurring after physical exercise.

Do ROS induce mitochondria and antioxidant defense mechanisms?

The exercising muscle requires increased mitochondrial respiration, which subsequently results in elevated ROS production and induced antioxidant defense mechanisms (Figure 1). PGC1 α is a transcriptional co-activator of genes involved in mitochondrial respiration and biogenesis [18,19], and recent studies have speculated on its role in

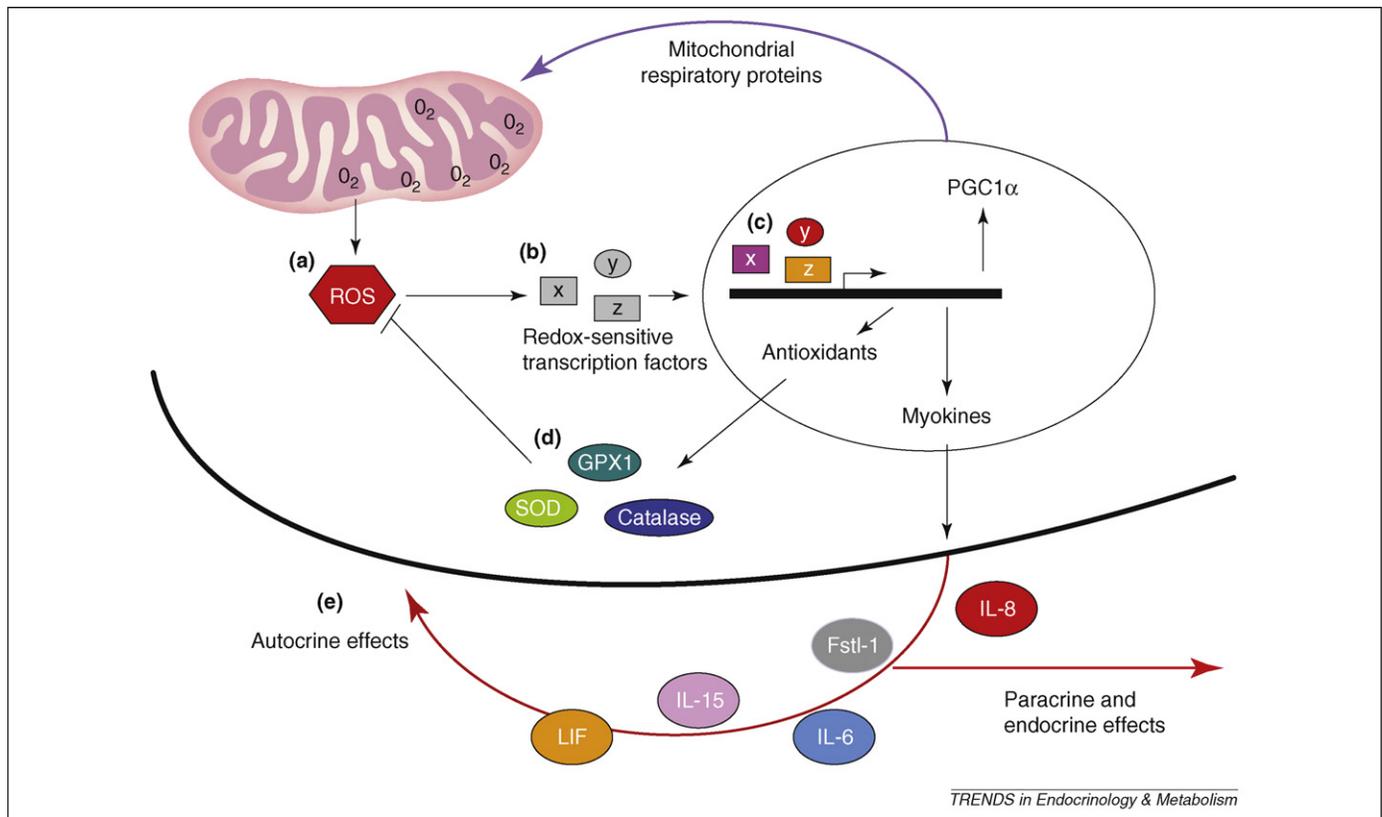
Box 2. Current viewpoints of ROS in exercise

The idea that ROS can promote muscle adaptation is controversial. In sharp contrast to our viewpoint, others regard ROS as having an inhibitory effect on muscle recovery and suggest antioxidant treatment for muscle fatigue [48]. Yet others argue for low levels of ROS to be required in muscle metabolism, whereas higher levels are suggested to promote contractile dysfunction resulting in muscle weakness and fatigue [49]. These assumptions are supported by the following observations:

- ROS have been suggested to act as an endogenous mediator of muscle fatigue during exercise by inhibiting calcium sensitivity [48];
- ROS can cause DNA damage, lipid peroxidation and protein carbonyls [50];
- ROS and inflammatory cytokines can induce proteasome expression through activation of the transcription factor nuclear factor kappa B, which can result in muscle wasting during chronic disease [50].

regulating antioxidant defense genes. PGC1 α -null mice express lower levels of the antioxidant enzymes glutathione peroxidase-1 and superoxide dismutase-2 (SOD2) and are sensitized to ROS-dependent neurodegeneration [20]. Conversely, increasing PGC1 α levels affords protection from oxidative-stressor-mediated death to neural cells in culture [20]. Furthermore, overexpressing PGC1 α in murine skeletal muscle resulted in increased expression of the ROS-scavenging enzymes SOD2, thioredoxin reductase-2 and catalase [21]. Thus, decreased expression of PGC1 α , which leads to decreased mitochondrial respiration (and, thus, a decreased production of ROS), also results in decreased expression of antioxidant defense genes, whereas an increase in PGC1 α demonstrates a converse relationship. These observations imply a potential feedback loop in which PGC1 α regulates antioxidant mechanisms via regulation of mitochondrial respiration and ROS. In turn, ROS might regulate antioxidant defense genes through activation of redox-sensitive transcription factors, supporting our view that ROS are important signaling molecules in adaptation to exercise (Figure 1).

PGC1 α is transiently induced by acute exercise [22,23]. This induction has been suggested to occur in response to altered energy demands and because of PGC1 α interacting with the metabolic enzyme AMP-activated protein kinase [19]. PGC1 α has also been proposed to be a key mediator of long-term adaptation to exercise [19]. Indeed, basal levels of PGC1 α gene expression increased approximately two-fold after a period of endurance training in humans [24]. In addition, endurance-trained transgenic mice overexpressing PGC1 α in muscle demonstrate increased endurance capacity and VO₂max [21]. This increased endurance could occur because of increased muscle mitochondrial respiration enabling these mice to train at a more intense level, which would support the notion of a direct relationship between PGC1 α gene expression and altered energy demands. In humans, this relationship was further assessed utilizing a five-week muscle inactivity model. Unilateral lower-limb unloading was applied by suspending one lower limb and having the subjects walk on crutches, a model inducing muscle atrophy and alterations in contractile protein expression [25]. Long-term muscle inactivity results in many changes in skeletal muscle, involving not only a lack of sustained mitochondrial bio-



TRENDS in Endocrinology & Metabolism

Figure 1. Proposed model of signaling activation by ROS in skeletal muscle in response to exercise. (a) Reactive oxygen species (ROS) are produced from mitochondrial respiration by a leakage of electrons from the electron-transport chain to oxygen. This process increases during exercise as a consequence of increased mitochondrial activity. (b) ROS have the potential to regulate cell signaling by affecting redox-sensitive transcription factors (here assigned x, y and z), inducing PGC1 α , antioxidant defense mechanisms and myokines. (c) PGC1 α activates genes involved in mitochondrial respiration, enabling increased mitochondrial activity, whereas (d) endogenous antioxidants as superoxide dismutase (SOD), glutathione peroxidase-1 (GPX1) and catalase buffer the increased amount of ROS. (e) Myokines act in an autocrine, paracrine or endocrine fashion to stimulate hypertrophy (IL-6, LIF and IL-15) or angiogenesis (IL-8 and Fstl-1).

genesis and activity but also a degeneration of the muscle and a dramatic decrease in energy demand [26]. Indeed, there was a coordinated downregulation of PGC1 α and its target genes in this model [27]. We suggest, therefore, that the central role of PGC1 α in muscle adaptation is to adjust mitochondrial respiration to altered energy demands and that this occurs in coordination with ROS and antioxidant defense mechanisms.

ROS induce myokines in response to exercise

Inflammation is necessary for wound healing and clearance of infections by leukocyte infiltration into damaged or infected tissue. This process is tightly regulated, and a loss of this homeostasis can lead to chronic inflammation and degenerative diseases, such as atherosclerosis and rheumatoid arthritis. In degenerative diseases, elevated levels of ROS are commonly accompanied by chronic inflammation, including elevated levels of pro-inflammatory cytokines. Furthermore, it has been suggested that ROS induce muscle-produced cytokines during exercise [15]. We have suggested recently that these cytokines and other peptides produced, expressed and released by muscle fibers that exert either paracrine or endocrine effects should be classified as 'myokines' [14] (Box 1). Thus, it seems that skeletal muscle has the capacity to express several myokines and that these take part in the exercise-induced muscle adaptation network (Figure 1).

Human intervention studies support the idea that ROS might stimulate the production of myokines in skeletal muscle in response to exercise [28,29]. Exercise-induced interleukin (IL)-6 was ablated after simultaneous treatment with antioxidants (vitamins A, C and E) in humans [28], a result that indicates a ROS-dependent induction of IL-6 in response to exercise. A similar conclusion was reached in another study, which showed that supplementation with vitamins C and E inhibited the release of IL-6 from contracting human skeletal muscle [29]. Furthermore, inactivation of NOS by N^G-nitro-L-arginine methyl ester (L-NAME) resulted in an attenuated exercise induction of IL-6, IL-8 and pyruvate dehydrogenase kinase gene expression, indicating that these were all NO-induced target genes in response to exercise [6]. Conversely, femoral artery infusion of the NO donor nitroglycerin significantly augmented expression of the same genes that were attenuated by L-NAME [6]. Together, these data indicate a role for ROS as an inducer of myokine production, as well as a mediator of cell signaling, in skeletal muscle.

The discovery that exercise provokes an increase in cytokine production established a potential link between contractile activity and immune function. However, further studies led to the discovery that not only could muscle-derived cytokines account for exercise-associated immune changes but also they play a part in mediating the metabolic changes associated with physical activity and

exercise training adaptation. Some examples are discussed below.

Myokines and exercise training

The first myokine to be identified was IL-6, a pleiotropic cytokine induced by muscle contractions [30] that stimulates both glucose and lipid metabolism [31,32]. IL-6 is induced in skeletal muscle via a Ca²⁺-dependent pathway [33] and acts through AMP-activated kinase [31,32,34]. It was suggested recently that IL-6 promotes hypertrophy by stimulating satellite-cell proliferation via STAT3 signaling [35]. Another member of the IL-6 superfamily, LIF, was induced *in vivo* in human skeletal muscle after strength training [36]. LIF has also been demonstrated to stimulate satellite-cell proliferation through the Janus kinase 2-signal transducer and activator of transcription 3 (JAK2-STAT3)-signaling pathway [37]. Another cytokine, IL-15, has been identified as an anabolic factor, is highly expressed in skeletal muscle [38] and is upregulated in human skeletal muscle after a bout of strength training [39]. These findings indicate that cytokines are potent metabolic signaling molecules that stimulate muscle hypertrophy in response to strength training. Although the metabolic effects of IL-6, LIF and IL-15 seem to occur more prominently during strength training, the actions of other cytokines might enhance the adaptations occurring during endurance training. Increased angiogenesis occurs as an important adaptation in skeletal muscle after a period of endurance training [40], and cytokines such as IL-8 and the newly identified Follistatin-like 1 (Fstl1) seem to have angiogenic properties. IL-8, previously classified as an angiogenic factor in cancer [41], is also upregulated in skeletal muscle in response to exercise [42]. The pro-angiogenic activity of IL-8 occurs after binding to one of its receptors, CXCR2 [41], which is also induced by exercise [43]. The recently identified Fstl1 might have similar effects on angiogenesis [44]. Fstl1 is induced by transgenic overexpression of Akt1, which coordinates blood vessel recruitment with normal tissue growth, a stimulatory effect that is dependent on nitric-oxide synthase activity. Further identification of myokines might lead to the discovery of their additional metabolic roles in response to exercise training.

ROS induce cell signaling and metabolic acting myokines

In this opinion piece, we emphasize the important roles of mitochondrial activation, ROS and antioxidant defense mechanisms in response to physical exercise. Given the many and diverse effects of physical exercise, we further suggest that muscle cells release several biologically active substances, including myokines, which participate in cell-to-cell and organ-to-organ crosstalk. The 'myokine concept' represents a paradigm shift, implying that the muscle has a central role in regulating its own metabolism. The intriguing point of this concept is that among the myokines, so-called pro-inflammatory cytokines are represented. However, it seems that muscle contractions elicit a cytokine response through a TNF- α - and NF κ B-independent mechanism and that both upstream and downstream signaling pathways for IL-6 differ markedly between immune-com-

petent cells and muscle cells. Consequently, the cytokine response to exercise seems to have important anti-inflammatory, metabolic and physiologic roles. Given that skeletal muscle comprises a major portion of the human body, our discovery that contracting skeletal muscle secretes proteins sets a novel paradigm: skeletal muscle is an endocrine organ producing and releasing myokines in response to contraction, thus influencing the metabolism in other tissues and organs. We believe that the myokines described to date represent only the tip of the iceberg and it is necessary to screen for additional factors produced and secreted by skeletal muscle that regulate hypertrophy, angiogenesis and insulin sensitivity and possibly, other mechanisms not yet discovered.

A somewhat provocative view is that both ROS and the induction of a cytokine response during exercise represent important mechanisms in training adaptation. In support of this, it seems that antioxidants attenuate some of the normal physiological responses to non-damaging exercise. In fact, antioxidant supplementation might even increase mortality [45], possibly reflecting a disturbed balance between endogenous antioxidant mechanisms and ROS caused by antioxidant supplementation. To summarize, we propose that ROS, well balanced by an endogenous antioxidant system, has an important role in muscle adaptation to exercise by regulating cell signaling. We further suggest that myokines represent an important link between contracting muscle and metabolic homeostasis, providing a molecular explanation to the well-established beneficial effects of physical exercise.

Acknowledgements

The Centre of Inflammation and Metabolism is supported by a grant from the Danish National Research Foundation (DG 02-512-555). The Copenhagen Muscle Research Centre is supported by grants from the University of Copenhagen, the Faculty of Science and the Faculty of Health Sciences at this university, and the Copenhagen Hospital Corporation. In addition, support was obtained from the Danish Medical Research Council, the Commission of the European Communities (contract no. LSHM-CT-2004-005272 EXGENESIS), and from grants from the National Health and Medical Research Council (NHMRC Project Grant Nos 251558, 342115 and 392206).

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