Review article

Skeletal muscle reactive oxygen species: A target of good cop/bad cop for exercise and disease

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Metabolic stresses associated with disease, ageing, and exercise increase the levels of reactive oxygen species (ROS) in skeletal muscle. These ROS have been linked mechanistically to adaptations in skeletal muscle that can be favourable (i.e. in response to exercise) or detrimental (i.e. in response to disease). The magnitude, duration (acute versus chronic), and cellular origin of the ROS are important underlying factors in determining the metabolic perturbations associated with the ROS produced in skeletal muscle. In particular, insulin resistance has been linked to excess ROS production in skeletal muscle mitochondria. A chronic excess of mitochondrial ROS can impair normal insulin signalling pathways and glucose disposal in skeletal muscle. In contrast, ROS produced in skeletal muscle in response to exercise has been linked to beneficial metabolic adaptations including mitochondrial biogenesis and muscle hypertrophy. Moreover, unlike insulin resistance, exercise-induced ROS appears to be primarily of non-mitochondrial origin. The present review summarizes the diverse ROS-targeted metabolic outcomes associated with insulin resistance versus exercise in skeletal muscle, thus, presenting two contrasting perspectives of pathologically harmful versus physiologically beneficial ROS. Here, we discuss the key sites of ROS production during exercise and the effect of ROS in skeletal muscle of people with type 2 diabetes.

Keywords: Reactive oxygen species, Mitochondria, Skeletal muscle, Exercise

Introduction

Skeletal muscle is a primary site of adaptation to metabolic insults that can occur as a result of disease or physical stressors such as exercise.[1,2] Individuals with type 2 diabetes (T2D) have an impaired capacity of skeletal muscle to transport glucose and store it as glycogen in response to hyperinsulinaemia.[3] On the other hand, skeletal muscle becomes more efficient at glucose disposal in response to a programme of endurance exercise training;[4] and mitochondrial content and function also become enhanced.[5] As polarized as the skeletal muscle adaptations to insulin resistance/T2D and exercise training are, one likely mediating factor in both biological cases in point is an excess of reactive oxygen species (ROS).

ROS are produced at multiple cellular sites including in the mitochondria during oxidative phosphorylation,[6] in the endoplasmic reticulum in response to protein folding stresses,[7] in membrane-bound NADPH oxidases,[8] through xanthine oxidase (XO) activation,[9] and via phospholipase A_2 (PLA_2)-dependent processes.[10]

In basal conditions, ROS produced in the mitochondria constitute a major source of cellular ROS.[6] During oxidative phosphorylation, reducing equivalents (NADH, $FADH_2$) formed during energy metabolism provide electrons to be transferred along a series of electron carriers within the mitochondrial respiratory chain. The transfer of electrons generates increased membrane potential, increased oxygen consumption, and ultimately the conversion of ADP to ATP.[11] However, the process is not perfect and a small percentage (~0.15%) of electrons leak from the respiratory chain complexes (I and III), resulting in the production of ROS including superoxide ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2).[12]

Interestingly, an excess of ROS has been implicated as both physiologically beneficial and pathologically harmful in the body. ROS have been shown to act as important intracellular signalling molecules for

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insulin signalling transduction in healthy tissues.[13,14] In addition, exercise is known to increase the levels of ROS in skeletal muscle and other tissues.[15] ROS produced during exercise are also thought to play a key role in skeletal muscle adaptations associated with exercise training.[16]

In contrast to this physiologically beneficial ROS, a chronic over-production of ROS systemically and in skeletal muscle promotes oxidative stress and might contribute to the pathogenesis of T2D,[2,17] ageing,[18,19] cancer cachexia,[20] and sarcopenia.[21] Excess ROS produced during metabolic and cellular processes can oxidatively damage macromolecules including DNA, lipids, and proteins, modify cellular redox status, and alter cellular functions.[22] Furthermore, by acting as signalling molecules,[23] excess ROS might act as important causative secondary messengers in the impaired insulin signalling pathways associated with insulin resistance in T2D.[11]

The seemingly paradoxical role of excess ROS in physiological versus pathological states in skeletal muscle could be a function of magnitude, duration, and/or cellular origin of ROS produced.[9] In the present review, we focus on two divergent but key areas that appear to shed some light on this apparent paradox: insulin resistance-related ROS in T2D and contraction-induced ROS.

Linking mitochondrial ROS to skeletal muscle insulin resistance

Skeletal muscle is quantitatively the most important tissue in peripheral insulin resistance *in vivo*.[24] In fact, in T2D, impairments in skeletal muscle glucose disposal account for ~80% of the defective insulin mediated whole-body glucose disposal.[25]

Possible causative factors in the pathophysiology of skeletal muscle insulin resistance include ectopic lipid- or lipid intermediate (notably diacylglycerol and ceramide)-induced impairments in insulin signalling pathways via protein kinase C activation[26,27] and mitochondrial dysfunction.[28] Different experimental models have also linked excess ROS production with the development of skeletal muscle insulin resistance. These models include (a) excess ROS production by the mitochondria in experimental murine models of excess nutrient intake and T2D;[2,23,28–30] (b) excess ROS production by NADPH oxidases due to the over-activity of the renin–angiotensin system and increased angiotensin II levels;[31] and more recently (c) excess ROS production by XO in experimental diabetes models.[32] The present review will focus only on excess ROS production according to model (a) above given the abundance of research that has focused on mitochondrial ROS as being implicated in skeletal muscle insulin resistance.

The relation between increased mitochondrial ROS and skeletal muscle insulin resistance has been well established *in vitro* and *in vivo*.[17,23,28,29] In humans, it was demonstrated that both acute and chronic high-fat dietary intakes can increase mitochondrial H_2O_2 production and oxidative stress in the skeletal muscle of healthy, insulin-sensitive individuals.[23] Moreover, obese, insulin-resistant humans have increased the levels of mitochondrial skeletal muscle H_2O_2 emission compared with lean insulin-sensitive individuals.[23,33] Hoehn *et al.*[29] demonstrated an increased mitochondrial $\cdot O_2^-$ production in palmitate-treated L6-myotubes *in vitro* and in high-fat-fed experimental mice *in vivo*. Furthermore, approaches aimed at the selective quenching of mitochondrial $\cdot O_2^-$ including the genetic overexpression of manganese superoxide dismutase (MnSOD) or supplementation with the mitochondrial $\cdot O_2^-$ targeted antioxidant Mn(III)-tetrakis (4-benzoic acid) porphyrin were shown to improve or prevent skeletal muscle insulin resistance in the high-fat-fed mice.[29] In addition, Anderson *et al.*[23] employed the use of both a small antioxidant peptide (SS31) targeted to the mitochondrial inner membrane and the genetic overexpression of mitochondrial-targeted human catalase (MCAT) in experimental rats and mice respectively, fed high-fat diets. All the treatments resulted in a reduction of mitochondrial H_2O_2 emission by >50%. Moreover, chronic intake of SS31 or genetic overexpression of MCAT prevented the onset of high-fat-diet-induced insulin resistance in skeletal muscle in the experimental animals.[23] Together, these and other[17] studies implicate excess mitochondrial ROS as both a causative factor in skeletal muscle insulin resistance and a key target for antioxidant-based prevention and treatment strategies.

In contrast to these findings, two recent studies found no benefit of mitochondrial-targeted antioxidant treatments in C57BL/6 mice[28,30] for improving skeletal muscle insulin resistance, despite marked reductions in mitochondrial ROS and improvements in skeletal muscle oxidative stress levels. These findings thus raise the possibility that targeting elevated mitochondrial ROS levels associated with skeletal muscle insulin resistance might not in fact be a fruitful treatment for improving insulin sensitivity in disease states (such as T2D) characterized by skeletal muscle insulin resistance. The failure of large-scale antioxidant supplementation trials for T2D prevention[34–36] appears to support the futility of this approach for diabetes prevention. However, it should be noted that many of these large studies were limited by study design and subject selection factors.[37] Moreover, most studies lacked insight into the mode of action of specific antioxidants and their biological targets,[37,38] thus making the interpretation of the findings difficult.

Pathways through which ROS might impair insulin signalling have not been well defined, but ROS have been shown to activate stress-sensitive molecules including mitogen-activated protein kinase (MAPK)[39] and c-Jun N-terminal kinase (JNK).[40] JNK can phosphorylate the insulin receptor substrate (IRS) subunit of the insulin signalling cascade at serine residues, thus attenuating key metabolic pathways of the insulin signalling cascade (Fig. 1).[41] Moreover, antioxidant supplementation in rats was shown to inhibit high-fat-diet-induced activation of JNK and IRS-1 serine phosphorylation in skeletal muscle, thus preventing a reduction in insulin sensitivity.[42] Mechanisms through which ROS activate JNK in tissues are unclear.[43] However, it has been shown that ROS can activate JNK via oxidation and inactivation of specific JNK-inactivating phosphatases.[43] Furthermore, it has been shown that tumour necrosis factor-α-induced mitochondrial ROS can activate JNK through activation of the apoptosis signal-regulating kinase -1 in human hepatoma cells.[44]

Do people with T2D have increased levels of ROS in skeletal muscle?

Studies that have directly measured ROS production in human T2D skeletal muscle have been scant, limited methodologically by *ex vivo* measurement techniques, and have produced uncertain findings.[45–47] Abdul-Ghani *et al.*[45] reported no difference in overall mitochondrial ROS production in skeletal muscle of people with T2D versus healthy controls, although the individuals with T2D had an increased mitochondrial H_2O_2 generation per unit of ATP production. Given that ATP production is driven by cellular energy demand *in vivo*, these findings suggest an increased *in vivo* ROS production in the skeletal muscle of people with T2D.[45] Another study[46] reported a tendency for increased mitochondrial

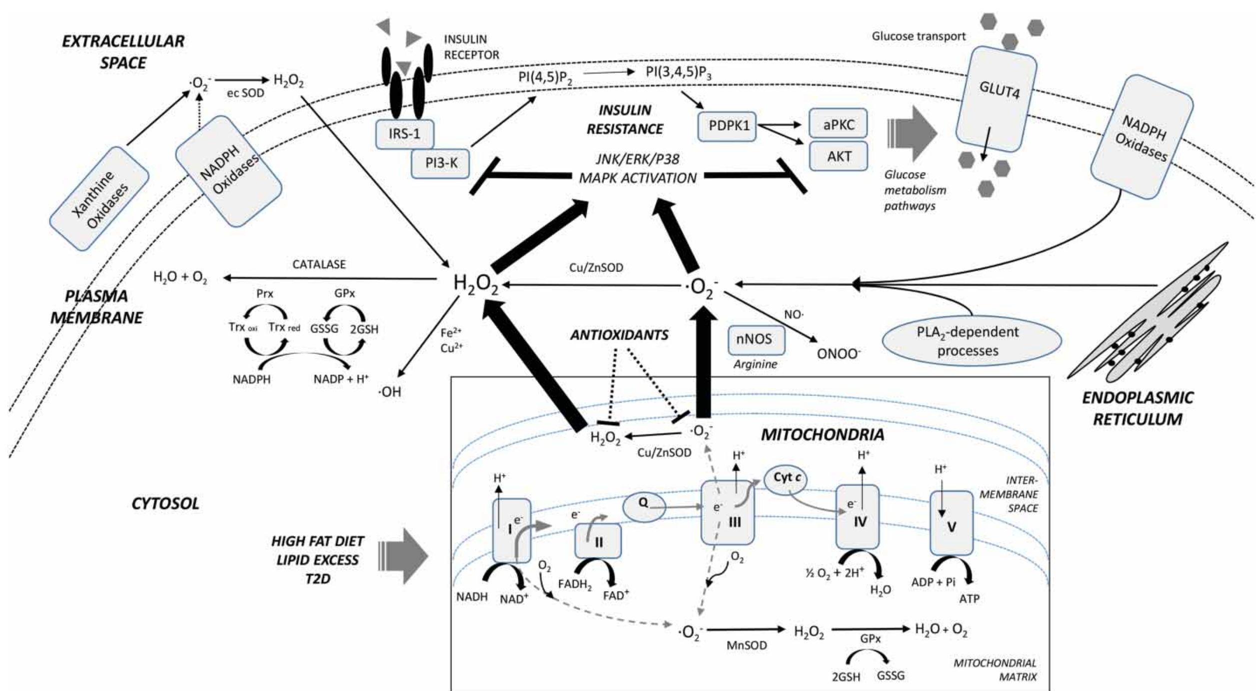

Figure 1 Cellular origins and effects of ROS in skeletal muscle during insulin resistance. Excess ROS produced in the mitochondria in response to a high-fat diet, excess lipid levels, or T2D promotes activation of major stress pathways that can impair normal insulin signalling, skeletal muscle glucose transport, and intracellular glucose metabolism. A network of endogenous antioxidants acts to scavenge these ROS, but the levels are relatively deficient when burdened with a chronic excess of ROS. Exogenous antioxidant therapy targeting mitochondrial ROS might attenuate this insulin resistance in skeletal muscle (refer to the text for a detailed discussion). Thick arrows indicate primary cellular ROS sources. Broken lines indicate some uncertainty. NADH, reduced nicotinamide adenine dinucleotide; NAD^+, oxidized nicotinamide adenine dinucleotide; $FADH_2$, reduced flavin adenine dinucleotide; FAD^+, oxidized flavin adenine dinucleotide; I–V, complexes of electron transport/oxidative phosphorylation; Cyt c, cytochrome C; GSH, reduced glutathione; GSSG, glutathione disulphide; GPx, glutathione peroxidase; Prx, peroxiredoxin reductase; Trx oxi, oxidized thioredoxin; Trx red, reduced thioredoxin; MnSOD, manganese superoxide dismutase; Cu/ZnSOD, copper/zinc superoxide dismutase; ec SOD, extracellular superoxide dismutase; nNOS, neuronal nitric oxide synthase; PLA_2, phospholipase A_2; $\cdot O_2^-$, superoxide anion; H_2O_2, hydrogen peroxide; NO·, nitric oxide; $ONOO^-$, peroxynitrite; ·OH, hydroxyl radical; IRS-1, insulin receptor substrate 1; PI3-K, phosphoinositide 3-kinase; $PI(4,5)P_2$, phosphatidylinositol (3,4)-bisphosphate; $PI(3,4,5)P_3$, phosphatidylinositol (3,4,5)-triphosphate; PDPK1, 3-phophoinositide-dependent protein kinase-1; aPKC, atypical protein kinase C; AKT, serine/threonine protein kinase B; GLUT4, glucose transporter type 4; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; p38 MAPK, p38 mitogen-activated protein kinase.

ROS production in individuals with T2D when compared with control participants matched for age, body mass index (BMI), and physical fitness (and mitochondrial content). In contrast to these studies, Minet and Gaster[47] found reduced absolute mitochondrial H_2O_2 production in primary myotubes from individuals with T2D and no difference in the ratio of mitochondrial H_2O_2 production per unit of ATP production when compared with the control participants. However, as discussed by the authors, different findings in the latter *in vitro* study compared with the previous studies could relate to the absence of *in vivo* metabolic conditions relevant to ROS production in T2D.[47] Overall, these studies might also be limited by the accuracy of assays used to assess ROS production from isolated mitochondria. In particular, commonly used redox-sensitive probes such as Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) could be cross-reactive with lipid hydroperoxides and/or other redox-related metabolites[11,48] and are prone to artefactual ROS formation.[49] Thus, a degree of caution is required in the interpretation of these findings.[11]

In addition to the direct measurements of ROS production, indirect oxidative stress measurements reflective of excess ROS including protein carbonyls and 8-hydroxy-2′-deoxyguanosine were shown to be increased in skeletal muscle of people with T2D when compared with non-diabetics.[32,50,51] In contrast to these findings, Brinkmann *et al.*[52] reported reduced levels of F2-isoprostanes in the skeletal muscle of people with T2D when compared with age and BMI-matched controls.

Oxidative stress is defined broadly as a suprathreshold imbalance between the production and the scavenging of ROS by the body's antioxidant defence network, favouring ROS production.[23] Thus, a limitation of using these oxidative stress markers as indicators of ROS production is that reduced antioxidant levels could promote oxidative stress without a concomitant increase in ROS production. Studies investigating the levels of antioxidant enzymes in the skeletal muscle of people with T2D have produced mixed findings when compared with the levels of insulin-sensitive controls. The protein levels of MnSOD were found to be reduced in the skeletal muscle of individuals with T2D in some studies[32,53] but not others.[52] On the other hand, Brinkmann *et al.*[52] reported increased levels of peroxiredoxins (PRDX2 and PRDX6) but unaltered levels of glutathione peroxidase (GPx) in the skeletal muscle of people with T2D by using immunohistochemical methods. Collectively, these findings suggest that, compared with the healthy controls, the skeletal muscle antioxidant status in people with T2D is unclear and further studies are required that closely match age, body mass, and physical activity levels.

A lack of comprehensive data on the oxidative stress milieu in the skeletal muscle of people with T2D coupled with the absence of currently available accurate *in vivo* measurement techniques for the probing of ROS[11] makes it difficult to establish a clear conclusion on alterations in the levels of ROS in the skeletal muscle of people with T2D. Moreover, considering potentially complicating factors between studies such as metabolic heterogeneity and the possibility of confounding factors such as the presence of diabetic complications, comparisons between current and future investigations will require some caution.

The importance of ROS in normal skeletal muscle insulin signalling

It was a goal of the above discussion to review the detrimental effects of excess ROS production on skeletal muscle insulin sensitivity in T2D. However, it is apparent that ROS are not inherently detrimental to insulin action. In fact, some evidence tends to the contrary, at least in healthy tissues.[13,14]

In contrast to potentially harmful excess mitochondrial ROS production, the generation of ROS from non-mitochondrial sources has been shown to be important in relation to healthy insulin signalling in insulin-sensitive tissues.[8,54] In particular, H_2O_2 production by plasma membrane-bound NADPH oxidases has been shown to increase in response to insulin stimulation both in adipocytes and skeletal muscle.[8,54] A recent study also implicated XO as a key ROS generator in response to insulin stimulation in skeletal muscle.[9] Increased insulin-stimulated H_2O_2 in skeletal muscle might occur via a phosphoinositide 3-kinase (PI3-K)- and/or protein kinase C-induced calcium release mechanism of action.[54] Moreover, H_2O_2 has been shown to oxidatively modify and inactivate key protein tyrosine phosphatases (PTEN and PTP1B) in adipocytes and skeletal muscle, thus resulting in enhanced insulin signalling.[8,14] Although this physiological H_2O_2 appears to be primarily of NADPH oxidase[8,54] or XO[9] origin, it was demonstrated that the levels of H_2O_2, PI3-K/Akt pathway activation, and insulin sensitivity were increased in the skeletal muscle of high-fat-fed transgenic mice lacking the antioxidant enzyme GPx.[14] Moreover, the treatment of $GPx^{-/-}$ mice with the antioxidant *N*-acetyl cysteine promoted increased insulin resistance in the skeletal muscle.[14] GPx is ubiquitously expressed both in the mitochondria and the cytosol, thus implying the possible involvement of mitochondrial ROS in the maintenance of normal insulin sensitivity also.

The apparent paradoxical role of ROS in the improvement versus impairment of insulin sensitivity in skeletal muscle could be related to the magnitude and/or duration (acute versus chronic) of excess

ROS produced[9,14] as well as the cellular origin of ROS (i.e. mitochondrial ROS versus XO-derived ROS).[9] To further our discussion on physiologically beneficial ROS, we now proceed with a detailed review of exercise and skeletal muscle ROS production. A discussion of the major cellular sites of ROS production during muscular contraction is followed by a review of several key potential ROS-mediated adaptive responses to exercise.

Sites of skeletal muscle ROS production during contraction

Since oxygen consumption increases during exercise, it is often assumed that the mitochondria are the major source of ROS production during contraction. However, the mitochondria make a large contribution to ROS production at rest, but not during contraction in mouse myotubes.[55] Also, mitochondrial oxygen consumption can increase up to 100-fold during contraction, whereas ROS production only increases 2- to 4-fold.[55–57] Indeed, recent work suggests that the majority of ROS production during contraction is from non-mitochondrial sources and includes NADPH oxidase, nitric oxide synthase (NOS), calcium-dependent 14 kDa isoform of PLA_2 and XO.[55–58] Contracting rat and mouse muscle cells have been shown by several authors by using the ROS-sensitive fluorescent probe dichloro-dihydro-fluorescein diacetate (DCFH-DA) to increase intracellular ROS levels, with the fluorescence being detected throughout the muscle fibre and not localized to the mitochondria.[55–57] Thus, the increase in ROS during contraction is probably not from the mitochondria and involves other potential sites of $\cdot O_2^-/H_2O_2$ production (Fig. 2).

Potential site(s) of ROS production during contraction

NADPH oxidase

Of the potential sources of ROS production during contraction, it appears that NADPH oxidase (NOX) plays a key role, since blocking its activity in rat primary muscle cells with the NOX inhibitor,

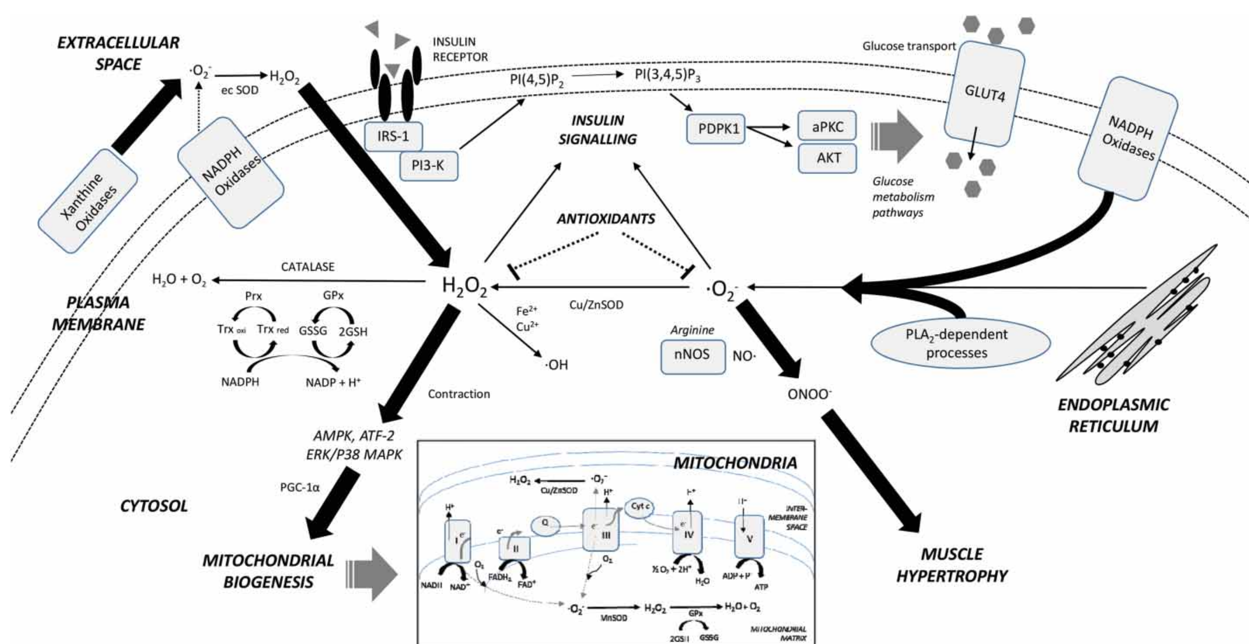

Figure 2 Cellular origins and effects of ROS in skeletal muscle during exercise contraction. Excess ROS produced in non-mitochondrial sources in response to exercise can promote adaptations including mitochondrial biogenesis through stress kinase activation and muscle hypertrophy via peroxynitrite-related signalling pathways. ROS (primarily from non-mitochondrial sources) also promotes insulin signalling transduction. A network of endogenous antioxidants acts to scavenge these ROS, but an excess intake of exogenous antioxidants might impair exercise adaptations (refer to the text for a detailed discussion). Thick arrows indicate primary cellular ROS sources. Broken lines indicate some uncertainty. NADH, reduced nicotinamide adenine dinucleotide; NAD^+, oxidized nicotinamide adenine dinucleotide; $FADH_2$, reduced flavin adenine dinucleotide; FAD^+, oxidized flavin adenine dinucleotide; I–V, complexes of electron transport/oxidative phosphorylation; Cyt c, cytochrome C; GSH, reduced glutathione; GSSG, glutathione disulphide; GPx, glutathione peroxidase; Prx, peroxiredoxin reductase; Trx oxi, oxidized thioredoxin; Trx red, reduced thioredoxin; MnSOD, manganese superoxide dismutase; Cu/ZnSOD, copper/zinc superoxide dismutase; ec SOD, extracellular superoxide dismutase; nNOS, neuronal nitric oxide synthase; PLA_2, phospholipase A_2; $\cdot O_2^-$, superoxide anion; H_2O_2, hydrogen peroxide; NO·, nitric oxide; $ONOO^-$, peroxynitrite; ·OH, hydroxyl radical; IRS-1, insulin receptor substrate 1; PI3-K, phosphoinositide 3-kinase; $PI(4,5)P_2$, phosphatidylinositol (3,4)-bisphosphate; $PI(3,4,5)P_3$, phosphatidylinositol (3,4,5)-triphosphate; PDPK1, 3-phophoinositide-dependent protein kinase-1; aPKC, atypical protein kinase C; AKT, serine/threonine protein kinase B; GLUT4, glucose transporter type 4; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; p38 MAPK, p38 mitogen-activated protein kinase; AMPK, AMP-activated protein kinase; ATF-2, activating transcription factor-2; PGC-1α, peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α.

apocynin, or in isolated muscle fibres with NOX inhibitors such as diphenyleneiodonium chloride, apocynin, or a specific peptide inhibitor of NOX, gp91ds-tat, completely prevents the electrically stimulated increase in ROS.[56,59,60]

Nitric oxide synthase

The free radical, nitric oxide (NO), is produced in the skeletal muscle fibres during contraction[59] from the neuronal NOS isoform.[61] Furthermore, the highly reactive ROS peroxynitrite ($ONOO^-$) is produced from the reaction of NO with $\cdot O_2^-$.[62] Interestingly, the production of NO and ROS do not always occur concomitantly. NO production is only detected with fluorescent probes in muscle fibres at moderate-to-high contractile intensities, whereas ROS production can be detected throughout low-to-high contractile intensities.[59] This would imply that $ONOO^-$ production is also not likely to occur until contraction intensities reach at least moderate levels.

Xanthine oxidase

The production of ROS from XO is well matched with exercise intensity, since the precursors of hypoxanthine, such as inosine monophosphate, only accumulate following moderate-to-high intensity exercise.[63] However, the contribution of XO to skeletal muscle ROS production in humans during exercise is probably small due to XO being localized in the endothelial cells of the skeletal muscle rather than within the muscle fibre.[64]

Phospholipase A_2

Inhibitors of the calcium-dependent PLA_2 during *in vitro* contraction of rat diaphragm blocks ROS formation in muscle homogenates.[10] However, selective inhibitors of PLA_2 recently have been shown to be ineffective at preventing contraction-induced ROS formation in single muscle fibres, suggesting a limited role for PLA_2 in skeletal muscle ROS production during contraction.[59]

Exercise intensity and ROS production

Over 30 years ago, Davies *et al.*[15] published the first evidence that free radical levels were elevated in rat skeletal muscle following exhaustive exercise. However, it was only recently that Bailey *et al.*[65] provided the first direct evidence in humans that maximal exercise increases intramuscular free radical accumulation by using electron paramagnetic resonance spectroscopy. Exhaustive exercise is well documented to increase markers of oxidative stress in skeletal muscle, such as oxidized glutathione (GSSG) and lipid peroxidation.[66–68] However, the actual exercise intensity whereby ROS are increased in skeletal muscle is difficult to precisely quantify. Nevertheless, submaximal exercise has been shown to increase the markers of skeletal muscle oxidative stress. Treadmill running in rodents at ~70% of maximal oxygen uptake (VO_{2max}) significantly increases skeletal muscle GSSG.[69,70] In humans, cycling exercise at ~85% VO_{2max} significantly increases skeletal muscle GSSG.[71] By using interstitial dialysis techniques, Karamouzis *et al.*[72] have shown cycling at ~70% VO_{2max} is sufficient to significantly increase skeletal muscle F2-isoprostane levels. Thus, it would appear that moderate-to-high intensity endurance exercise is sufficient to induce skeletal muscle oxidative stress.

Regulation of contraction-induced mitochondrial biogenesis by ROS

Although pathologically high chronic levels of ROS are cytotoxic, it is also now clear that at low (physiological) levels they play an important role in cell signalling in normal healthy skeletal muscle.[73] Increasing mitochondrial content in cultured cells reduces oxygen consumption per unit of mitochondria,[19] thus reducing mitochondrial ROS production and oxidative stress.[74] Increasing mitochondrial content also protects mice against diet-induced obesity and insulin resistance and increases lifespan in a number of organisms.[18] Endurance training increases skeletal muscle mitochondrial biogenesis (the synthesis of new mitochondria)[75,76] and antioxidant enzymes.[70,77] It was Davies *et al.* in 1982,[15] who initially proposed that this could be a stimulus for the mitochondrial biogenesis observed following endurance training. However, it has been only in the past several years that evidence has emerged that the small, temporary, and perhaps localized ROS production during exercise is involved in regulating these beneficial effects involving the mitochondria,[73] which could potentially act to reduce mitochondrial ROS production.

Increasing the ROS levels in skeletal muscle cells activates several redox-sensitive kinases such as AMP-activated protein kinase (AMPK), activating transcription factor-2 (ATF-2), and the members of the MAPK family including p38 MAPK, JNK, and extracellular signal-regulated kinase (ERK, also called p44/42 MAPK).[78,79] All these kinases are activated in skeletal muscle during exercise[70] and are at least partly involved in the regulation of mitochondrial biogenesis,[75,80] primarily via the transcriptional coactivator, peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which is a key regulator of mitochondrial biogenesis.[81,82] Increasing ROS levels in skeletal muscle cells activates AMPK, resulting in elevated PGC-1α,[78] with these ROS effects being blocked by co-treatment with antioxidants.[78] Silveira *et al.*[73] published the first clear evidence linking the regulation of contraction-induced mitochondrial biogenesis to ROS. Electrostimulation that induces

contraction of rat primary muscle cells increased ROS and also PGC-1α mRNA that could be prevented by treatment with antioxidants.[73]

The regulatory role for ROS in contraction-induced mitochondrial biogenesis has led to considerable controversy in the literature regarding the potential for antioxidant supplements to prevent these skeletal muscle adaptations to endurance training. There are some training studies that support such a role[16,83] and others that do not.[84–86] Furthermore, other studies have reported that antioxidants can inhibit the gene expression response of specific metabolic genes following training, but they also find no effect on functional muscle adaptations such as increased peak power or mitochondrial enzyme activity.[77] There are also several acute exercise studies showing that inhibition of XO-derived ROS with the XO inhibitor, allopurinol, can inhibit redox-sensitive kinases known to regulate mitochondrial biogenesis, such as the exercise-induced phosphorylation of p38 MAPK and ERK.[67,70,87] However, long-term treatment with allopurinol does not prevent the increases in skeletal muscle mitochondrial proteins or antioxidant enzymes following endurance training.[70] The lack of the effect of allopurinol on skeletal muscle adaptations following endurance training suggests considerable redundancy in the mitochondrial biogenesis pathways. These findings also highlight that exercise studies need to examine both acute (a single bout) and chronic (i.e. training) exercise to obtain a full understanding of the adaptive responses in skeletal muscle.

Regulation of contraction-induced antioxidant enzymes by ROS

The gene expression of MnSOD, extracellular SOD, and GPx are all increased following a single bout of exercise[70] with several weeks of endurance training known to increase skeletal muscle antioxidant enzymes, particularly MnSOD and GPx.[70,85,88] Furthermore, although still largely undefined, mitochondrial biogenesis appears to be involved in the regulation of antioxidant enzymes, since PGC-1α is required for the induction of Cu/ZnSOD, MnSOD, GPx1, and catalase in response to oxidative stress.[89]

Regulation of muscle hypertrophy by ROS

Recently, the highly reactive oxidant $ONOO^-$, which is formed by the reaction of O_2^- with NO, has been shown to regulate skeletal muscle hypertrophy induced by overload.[90] The signalling pathway appears to be via the $ONOO^-$ stimulated activation of the transient receptor potential cation channel, subfamily V, member 1 (Trpv1) to release intracellular Ca^{2+}, which then activates mammalian target of rapamycin (mTOR) to increase protein synthesis. A role for ROS in the regulation of protein synthesis is supported by Makanae *et al.*,[91] who found that a high daily oral dose of the antioxidant vitamin C not only attenuated skeletal muscle oxidative stress but also the hypertrophy normally observed following the mechanical overloading of the plantaris by hindlimb ablation. Understanding the molecular pathways regulating skeletal muscle protein synthesis are important for the treatment of muscle disorders, particularly the age-related loss of muscle mass, known as sarcopenia. Indeed, in elderly subjects, the normal increase in muscle protein synthesis is blunted in response to resistance exercise when compared with young subjects.[92] Studies are now required in humans to determine whether $ONOO^-$ is involved in the muscle hypertrophy response to resistance training, as this may provide novel targets for pharmacological interventions that augment the training response for the elderly.

Conclusion

While solid experimental data mechanistically links excess mitochondrial ROS production to the pathogenesis of insulin resistance and T2D, some recent findings in rodents interject some uncertainty into the once promising approach of targeting mitochondrial ROS as a means to improving insulin resistance. Moreover, it is not clear from the studies undertaken to date if in fact mitochondrial ROS production is increased in the skeletal muscle of people with T2D.

In contrast to insulin resistance/T2D, non-mitochondrial sites of ROS are primarily responsible for the ROS produced during exercise. These sites include NADPH oxidase, NOS, and perhaps to a lesser extent XO and are probably playing a role in the regulation of key adaptations to exercise, such as mitochondrial biogenesis, increased antioxidant defences, and perhaps muscle hypertrophy. However, further research is required to resolve the effect, if any, that antioxidant supplementation might have on preventing beneficial endurance training adaptations.

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