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Organelles in focus

Mitochondria: Mitochondrial participation in ischemia–reperfusion injury in skeletal muscle

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ABSTRACT

Irrespective of the organ involved, restoration of blood flow to ischemic tissue is vital, although reperfusion *per se* is deleterious. In the setting of vascular surgery, even subtle skeletal muscle ischemia contributes to remote organ injuries and perioperative and long-term morbidities. Reperfusion-induced injury is thought to participate in up to 40% of muscle damage.

Recently, the pathophysiology of lower limb ischemia–reperfusion (IR) has been largely improved, acknowledging a key role for mitochondrial dysfunction mainly characterized by impaired mitochondrial oxidative capacity and premature mitochondrial permeability transition pore opening. Increased oxidative stress triggered by an imbalance between reactive oxygen species (ROS) production and clearance, and facilitated by enhanced inflammation, appears to be both followed and instigated by mitochondrial dysfunction.

Mitochondria are both actors and target of IR and therapeutic strategies modulating degree of ROS production could enhance protective signals and allow for mitochondrial protection through a mitohormesis mechanism.

1. Organelle facts

- Mitochondria produce ATP, the main energy source of cells and generate ROS, acting either as causes of cellular injuries or as second messengers allowing mitohormesis.
- Mitohormesis is a phenomenon triggered by moderate oxidative stress activating mitochondrial biogenesis and therefore improving cellular and mitochondrial antioxidant capacities.
- Ischemia–reperfusion increases oxidative stress, inflammation and triggers oxidative damage in tissues.
- Lower limb ischemia–reperfusion initiates muscle mitochondrial dysfunctions including reduced oxidative capacity and mitochondrial pore transition opening.
- Skeletal muscle injuries aggravate the prognosis of patients suffering from peripheral arterial disease.
- Ischemic conditioning generally protects skeletal muscle, reducing ROS production, inflammation and mitochondrial dysfunctions.

2. Introduction

Life requires energy, and this energy is stored in adenosine triphosphate (ATP) molecules that are produced in the mitochondria by oxidative phosphorylation. The roles of mitochondria extend far beyond energy production, as they are important generators of reactive oxygen species (ROS), which can either act as second messengers or as a source of cellular damage, depending on the amount produced.

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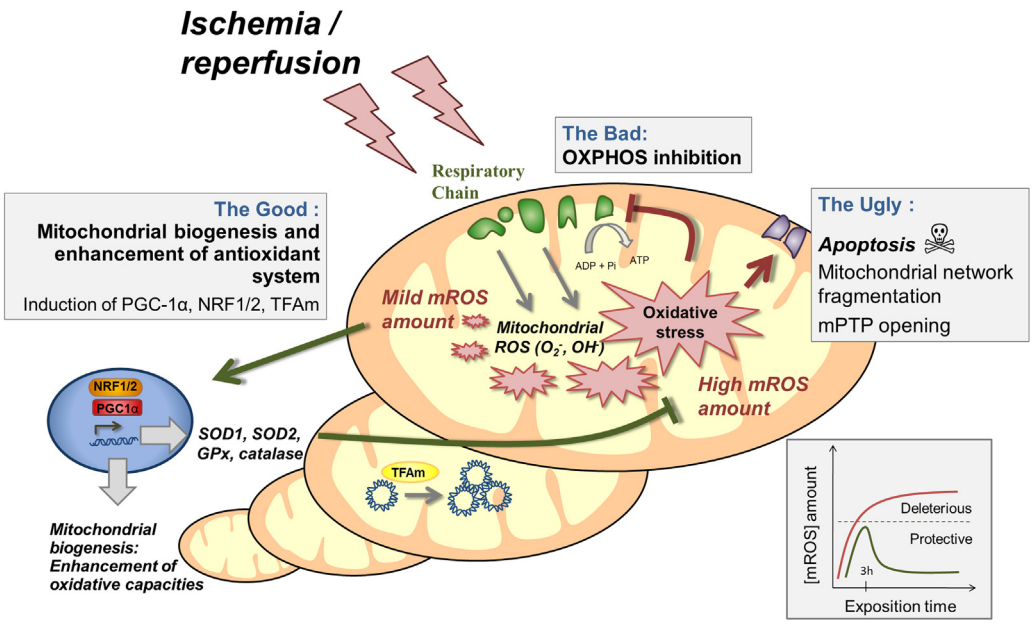

Fig. 1. Mitochondria and reactive oxygen species interactions: the good, the bad and the ugly. ADP, adenosine diphosphate; ATP, adenosine triphosphate; GPx, glutathione peroxidase; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; NRF: nuclear respiratory factor; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; Pi, inorganic phosphate; SOD, superoxide dismutase; TFAm, transcription factor A, mitochondrial.

Peripheral arterial disease (PAD) is a very common manifestation of atherosclerosis related to lower limb arterial stenosis or occlusions. The resulting ischemia leads to exercise or resting pain and, ultimately, to tissue necrosis resulting in leg amputation.

Insufficient oxygen supply was long presumed to be the main and sole cause for the manifestations of PAD; however, reperfusion-related impairment in skeletal muscle mitochondria associated with oxidative stress now appear as key mechanisms.

Such recent advances in mitochondrial participation to IR injury in skeletal muscle support new therapeutic approaches targeting mitochondria. Reducing the amount of ROS perceived by the cells might allow for a shift from a vicious (increased ROS, increased mitochondrial dysfunction, further ROS increase and oxidative damage) to a virtuous cycle (ROS signaling, mitochondrial protection and antioxidative system stimulation) (Fig. 1).

2.1. Organelle function and cell physiology

The physiological functions of mitochondria include ATP production, ROS generation and detoxification, apoptosis involvement, regulation of cytoplasmic and mitochondrial matrix calcium, metabolite synthesis and catabolism. An abnormality in any of these processes can be termed as mitochondrial dysfunction and can impair cell physiology which, when considering skeletal muscle cells, includes contractility and participation in glycemic control.

2.1.1. Oxidative phosphorylation

This process allows the production by the mitochondrial respiratory chain complexes of cellular free energy in the form of ATP and is one of the most prominent functions of mitochondria. Maximal oxidative capacity varies widely depending on the prominence of muscle fiber types (Meyer et al., 2014) and exercise capacity appears linked to skeletal muscle mitochondrial oxidative capacities and coupling. Interestingly, type I muscle fibers (slow-twitch oxidative fibers with high mitochondrial content) participate in euglycemia maintenance, as opposed to the more glycolytic type II fibers (Dela and Helge, 2013).

2.1.2. Mitochondrial permeability transition pore (mPTP)

An increase in calcium concentration in the mitochondrial matrix triggers this high-conductance inner membrane channel opening. While transient openings may serve the purpose of providing a fast Ca^{2+} release mechanism, persistent mPTP opening is followed by a deregulated release of matrix Ca^{2+}, termination of oxidative phosphorylation, matrix swelling with inner membrane unfolding and eventually outer membrane rupture with release of apoptotic proteins and cell death. Pore opening can also cause production of reactive oxygen species, as shown by the occurrence of "superoxide flashes" triggered by transient openings of the mPTP in cardiomyocytes (Wang et al., 2008). The molecular nature of mPTP remains under debate. The long-standing notion that mPTP formation occurs at contact sites of the inner and outer membranes through voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator (ANT) is unlikely since VDAC- and ANT-null mitochondria still display a cyclosporin A permeability transition. Interestingly, reconstituted dimers of F_0F_1 ATP synthase form a channel with properties identical to those of the mPTP, leading to the hypothesis that complex V dimers may actually form the pore (Bernardi, 2013).

2.1.3. Mitochondrial dynamics

Mitochondria are highly dynamic organelles that undergo fission (division) and fusion (joining). Mitochondrial fission and fusion play critical roles in maintaining functional mitochondria in stress conditions. Fusion helps mitigate stress by mixing the contents of partially damaged mitochondria. Fission enables the removal of damaged mitochondria and is necessary to create new mitochondria, but can also facilitate apoptosis during high levels of cellular stress (Youle and van der Bliek, 2012). These processes are regulated by GTPases including optic atrophy protein and mitofusin 1 and 2 for fusion, and dynamin-related protein 1 (Drp1) and the Drp1 targeting molecule fission 1 (Fis1) for fission.

2.1.4. Reactive oxygen species (ROS)

Under resting conditions, over 90% of cellular ROS is produced in the mitochondria. The major sites for ROS generation are electron transport chain complexes I and III. Interestingly, ROS are a

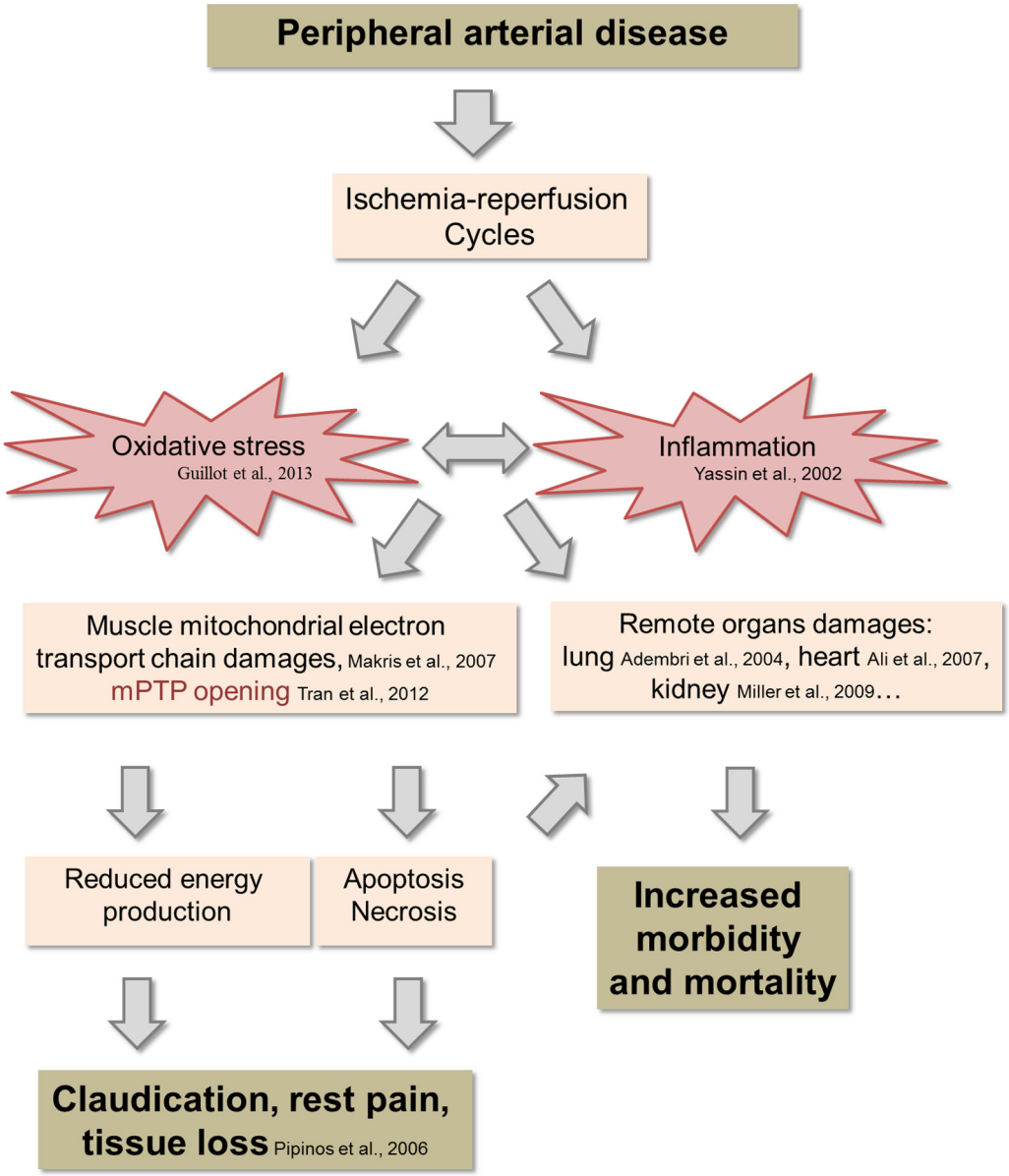

Fig. 2. Implication of mitochondria in the pathophysiology of peripheral arterial disease. Modified from Pipinos et al. (2006).

double-edged sword. They are beneficial *via* cell signaling involved in the antioxidant defense network, but can be harmful by inducing oxidative stress (Zorov et al., 2006). Superoxide anion ($O_2^{\bullet -}$) appears to be a highly important ROS, its toxicity being related to the generation of further reactive species able to attack intracellular biomolecules and resulting in protein carboxylation, lipid peroxidation and DNA damage.

Defense systems can reduce ROS-induced damage. Briefly, $O_2^{\bullet -}$ in the matrix is converted to H_2O_2 by matrix MnSOD (SOD2), while $O_2^{\bullet -}$ released in the intermembrane space is partly dismuted by intermembrane space CuZnSOD (SOD_1). Any residual $O_2^{\bullet -}$ that diffuses into the cytosol is converted by cytosolic CuZnSOD. If mitochondrial $O_2^{\bullet -}$ reaches the extracellular space, it is detoxified by extracellular CuZnSOD (SOD_3). Glutathione-based systems constitute the major redox buffer in the cytosol and H_2O_2 can also be reduced to water by catalase or glutathione peroxidase.

3. Organelle pathology

There are a number of muscular pathologies associated with mitochondrial dysfunction due to either a primitive defect in mitochondrial protein or to secondary injury in the setting of general disease such as diabetes, cardiac or pulmonary failure, cancer and/or inflammatory diseases (Moylan and Reid, 2007). With particular focus oriented toward cardiovascular diseases, data indeed demonstrate an impairment of skeletal muscle mitochondria with decreased mitochondrial oxidative capacities in patients suffering from peripheral arterial disease (PAD) (Brass and Hiatt, 2000; Brass et al., 2001; Pipinos et al., 2000, 2006; Makris et al., 2007). This dependence on oxygen and ATP is critical in skeletal muscle. In PAD muscle, suboptimal energy production from defective mitochondria participates in pathogenesis in addition to reduced oxygen supply (Pipinos et al., 2006) while damage to the mitochondria enhances the production of ROS. Furthermore, MnSOD (the initial

line of ROS defense in mitochondria) has been found to be deficient in PAD muscles (Pipinos et al., 2006; Makris et al., 2007). These findings reflect an impaired mitochondrial antioxidant defense system which is unable to respond to abnormally elevated ROS production, leading to significant oxidative damage to muscle proteins and lipids. Of note, IR-induced injury differs according to the skeletal muscle phenotype and thus, type II fibers probably sustain more damage than type I muscle fibers.

Although not fully elucidated in skeletal muscle, the origin of mitochondrial dysfunction related to ischemia–reperfusion has been studied in the myocardium and can occur at several levels. A decrease in electron transport chain (ECT) protein may result from increased autophagy and/or from decreases in PGC1-α protein content (Lee et al., 2012). Posttranslational alterations of ETC in the ischemic myocardium are also involved, including increased protein tyrosine nitration of complex I and complex II, decreased protein S-glutathionylation of complex II, inactivation of Fe–S protein of complex III and increased hyperphosphorylation of complex IV. Finally, during ischemia and/or reperfusion phases, a loss of mitochondrial cardiolipin (a mitochondrial membrane phospholipid) has also been proposed to explain the decline in complex I (Paradies et al., 2004), III and complex IV activities.

Accordingly, ischemia–reperfusion of the lower limb, largely studied in experimental models using either aortic cross-clamping or leg tourniquet, has confirmed the reduction in mitochondrial oxidative capacity associated with increased ROS production (Brandão et al., 2003; Charles et al., 2011). Although endogenous up-regulation of mitochondrial biogenesis and enhancement of the antioxidant defense may delay muscle injury, IR also increases the Bax/Bcl2 ratio and reduces the capacity for mitochondrial calcium retention thus favoring early mPTP opening and apoptosis (Mansour et al., 2012a; Tran et al., 2012). Interestingly, reduced CyP-D expression in gastrocnemius muscle likely prevented cyclosporin A from blocking mPTP opening during lower-limb IR (Pottecher et al., 2013).

Taken together, both apoptosis and necrosis can lead to tissue loss and ultimately to leg amputation. In addition, nerve, skin and subcutaneous tissue damages also occur, leading to the characteristic “trophic legs” of patients with advanced PAD having thin and brittle muscles, and thin hairless skin with impaired sensorimotor function. Interestingly, surgical by-pass or endovascular therapies do not always relieve patient symptoms. Leg pain has consequently been related to metabolic alterations rather than to reduced blood flow. Furthermore, even subtle skeletal muscle alterations participate in inflammation and remote organ injuries aggravating patient morbidity and mortality rates through multi-organ failure (Yassin et al., 2002; Adembri et al., 2004; Fowkes et al., 2006; Ali et al., 2007; Miller et al., 2009) (Fig. 2).

Importantly, a better understanding of PAD pathophysiology and particularly in view of the fact that defective mitochondria and oxidative stress are central to this myopathy allows for new therapeutic approaches. Increased ROS production occurs before mitochondrial dysfunction (Guillot et al., 2013) suggesting that strategies aiming to reduce ROS production might be successful.

Accordingly, ischemic preconditioning (i.e. repeated short lasting ischemia–reperfusion cycles applied before sustained ischemia) has been shown to protect both ischemic muscles and remote organs in experimental animals and humans (Eberlin et al., 2008; Mansour et al., 2012a; Ali et al., 2007). Controlled reperfusion and ischemic post-conditioning (applied at the onset of reperfusion) was also shown to protect skeletal muscle from IR injuries (Beyersdorf and Schlensak, 2009; McAllister et al., 2008). This is clinically relevant since improved skeletal muscle mitochondrial function is associated with enhanced walking capacities, both in healthy subjects and in patients suffering from PAD.

Similarly, pharmacological approaches using a mimetic of the antioxidant system also decrease ROS production, protect muscle mitochondrial function and reduce infarct size (Tran et al., 2012).

During IR, mitochondria undergo fission that is dependent on Drp1 activation. Inhibition of Drp1 activation by Mdivi-1 (mitochondrial division inhibitor 1) was indeed shown to preserve mitochondrial morphology, lower mitochondrial reactive oxygen species, reduce cytosolic calcium (Sharp et al., 2014), prevent mPTP opening as well as reduce infarct size. Mdivi-1 was conversely protective if administered prior to or following myocardial ischemia, thereby opening promising therapeutic strategies in other organs submitted to IR (Sharp et al., 2014).

4. Future outlook

Controversial data have nevertheless been reported. Local and remote ischemic post-conditioning was found to decrease muscle mitochondrial function and trigger ROS production and inflammation (Mansour et al., 2012b). The temporal relationship between inflammation, oxidative stress and mitochondrial function hence deserves further investigation. There is also a need to investigate the main source of ROS arising in skeletal muscle during IR and, in particular, the specific extent of mitochondrial, xantine and NAPDH oxidase involvement. Further studies with regard to molecular pathways (SAFE, RISK, etc.) ultimately acting on the mPTP are also warranted in order to determine whether these endogenous protective pathways are impaired by IR.

Finally, defining the threshold level of ROS that will induce mitohormesis rather than oxidative stress is of key importance. This represents an obvious challenge knowing that such a threshold may vary according to the metabolic phenotype of the organ involved in IR and to comorbidity factors (hypertension, diabetes, old age, etc.) often present in patients suffering from PAD.

In summary, mitochondrial dysfunction resulting from- and enhancing ROS production is clearly a key mechanism involved in the deleterious effects of IR on skeletal muscles. While accessible to therapy, further knowledge is nonetheless needed to allow a broader translation of ischemic conditioning into clinical practice.

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References

Adembri C, Kastamoniti E, Bertolozzi I, Vanni S, Dorigo W, Coppo M, et al. Pulmonary injury follows systemic inflammatory reaction in infrarenal aortic surgery. Crit Care Med 2004;32:1170–7.

Ali ZA, Callaghan CJ, Lim E, Ali AA, Nouraei SA, Akthar AM, et al. Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: a randomized controlled trial. Circulation 2007;116:I98–105.

Bernardi P. The mitochondrial permeability transition pore: a mystery solved? Front Physiol 2013;4:95.

Beyersdorf F, Schlensak C. Controlled reperfusion after acute and persistent limb ischemia. Semin Vasc Surg 2009;22:52–7.

Brandão ML, Roselino JES, Piccinato CE, Cherri J. Mitochondrial alterations in skeletal muscle submitted to total ischemia. J Surg Res 2003;110:235–40.

Brass EP, Hiatt WR. Acquired skeletal muscle metabolic myopathy in atherosclerotic peripheral arterial disease. Vasc Med 2000;5:55–9.

Brass EP, Hiatt WR, Gardner AW, Hoppel CL. Decreased NADH dehydrogenase and ubiquinol-cytochrome *c* oxidoreductase in peripheral arterial disease. Am J Physiol Heart Circ Physiol 2001;280:H603–9.

Charles AL, Guilbert AS, Bouitbir J, Goette-Di Marco P, Enache I, et al. Effect of postconditioning on mitochondrial dysfunction in experimental aortic cross-clamping. Br J Surg 2011;98:511–6.

Dela F, Helge JW. Insulin resistance and mitochondrial function in skeletal muscle. Int J Biochem Cell Biol 2013;45:11–5.

Eberlin KR, McCormack MC, Nguyen JT, Tatlidede HS, Randolph MA, Austen WG Jr. Ischemic preconditioning of skeletal muscle mitigates remote injury and mortality. J Surg Res 2008;148:24–30.

Fowkes FG, Anandan CL, Lee AJ, Smith FB, Tzoulaki I, Rumley A, et al. Reduced lung function in patients with abdominal aortic aneurysm is associated with activation of inflammation and hemostasis, not smoking or cardiovascular disease. J Vasc Surg 2006;43:474–80.

Guillot M, Charles AL, Chamaraux-Tran TN, Bouitbir J, Meyer A, Zoll J, et al. Oxidative stress preceedes skeletal muscle mitochondrial dysfunction during experimental aortic cross-clamping but is not associated with early lung, heart, brain, liver or kidney mitochondrial impairment. J Vasc Surg 2013, in press.

Lee HL, Chen CL, Yeh ST, Zweier JL, Chen YR. Biphasic modulation of the mitochondrial electron transport chain in myocardial ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2012;302:H1410–22.

Makris KI, Nella AA, Zhu Z, Swanson SA, Casale GP, Gutti TL, et al. Mitochondriopathy of peripheral arterial disease. Vascular 2007;15:336–43.

McAllister SE, Ashrafpour H, Cahoon N, Huang N, Moses MA, Neligan PC, et al. Postconditioning for salvage of ischemic skeletal muscle from reperfusion injury: efficacy and mechanism. Am J Physiol Regul Integr Comp Physiol 2008;295:681–9.

Mansour Z, Bouitbir J, Charles AL, Talha S, Kindo M, Pottecher J, et al. Remote and local ischemic preconditioning equivalently protects rat skeletal muscle mitochondrial function during experimental aortic cross-clamping. J Vasc Surg 2012a;55:497–505.

Mansour Z, Charles AL, Bouitbir J, Pottecher J, Kindo M, Mazzucotelli JP, et al. Remote and local ischemic postconditioning further impaired skeletal muscle mitochondrial function after ischemia–reperfusion. J Vasc Surg 2012;56:774–82.

Meyer A, Charles AL, Zoll J, Guillot M, Lejay A, Singh F, et al. Cryopreservation with dimethyl sulfoxide prevents accurate analysis of skinned skeletal muscle fibers mitochondrial respiration. Biochimie 2014., http://dx.doi.org/10.1016/j.biochi.2014.01.014, in press, PMID: 24472439.

Miller CC 3rd, Villa MA, Sutton J, Lau D, Keyhani K, Estrera AL, et al. Serum myoglobin and renal morbidity and mortality following thoracic and thoraco-abdominal aortic repair: does rhabdomyolysis play a role? Eur J Vasc Endovasc Surg 2009;37:388–94.

Moylan JS, Reid MB. Oxidative stress, chronic disease, and muscle wasting. Muscle Nerve 2007;35:411–29.

Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A, Ruggiero FM. Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart: involvement of reactive oxygen species and cardiolipin. Circ Res 2004;94:53–9.

Pipinos II, Judge AR, Zhu Z, Selsby JT, Swanson SA, Johanning JM, et al. Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. Free Radic Biol Med 2006;41:262–9.

Pipinos II, Shepard AD, Anagnostopoulos PV, Katsamouris A, Boska MD. Phosphorus 31 nuclear magnetic resonance spectroscopy suggests a mitochondrial defect in claudicating skeletal muscle. J Vasc Surg 2000;31:944–52.

Pottecher J, Guillot M, Belaidi E, Charles AL, Lejay A, Gharib A, et al. Cyclosporine A normalizes mitochondrial coupling, reactive oxygen species production, and inflammation and partially restores skeletal muscle maximal oxidative capacity in experimental aortic cross-clamping. J Vasc Surg 2013;57:1100–8.

Sharp WW, Fang YH, Han M, Zhang HJ, Hong Z, Banathy A, et al. Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia–reperfusion injury: therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. FASEB J 2014;28:316–26.

Tran TP, Tu H, Liu J, Muelleman RL, Li YL. Mitochondria-derived superoxide links to tourniquet-induced apoptosis in mouse skeletal muscle. PLoS ONE 2012;7:e43410.

Wang W, Fang H, Groom L, Cheng A, Zhang W, Liu J, et al. Superoxide flashes in single mitochondria. Cell 2008;134:279–90.

Yassin MM, Harkin DW, Barros D'Sa AA, Halliday MI, Rowlands BJ. Lower limb ischemia–reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. World J Surg 2002;26:115–21.

Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science 2012;337:1062–5.

Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. Biochim Biophys Acta 2006;1757:509–17.