

Improvement of obesity-linked skeletal muscle insulin resistance by strength and endurance training

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Abstract

Obesity-linked insulin resistance is mainly due to fatty acid overload in non-adipose tissues, particularly skeletal muscle and liver, where it results in high production of reactive oxygen species and mitochondrial dysfunction. Accumulating evidence indicates that resistance and endurance training alone and in combination can counteract the harmful effects of obesity increasing insulin sensitivity, thus preventing diabetes. This review focuses the mechanisms underlying the exercise role in opposing skeletal muscle insulin resistance-linked metabolic dysfunction. It is apparent that exercise acts through two mechanisms: (1) it stimulates glucose transport by activating an insulin-independent pathway and (2) it protects against mitochondrial dysfunction-induced insulin resistance by increasing muscle antioxidant defenses and mitochondrial biogenesis. However, antioxidant supplementation combined with endurance training increases glucose transport in insulin-resistant skeletal muscle in an additive fashion only when antioxidants that are able to increase the expression of antioxidant enzymes and/or the activity of components of the insulin signaling pathway are used.

Key Words

- ▶ insulin resistance
- ▶ obesity
- ▶ diabetes
- ▶ exercise
- ▶ mitochondria
- ▶ oxidative stress
- ▶ ROS production

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Introduction

Obesity is one of the most important public health problems in the world, reaching epidemic proportions in several industrialized countries (Ogden *et al.* 2014) and rising in many developing countries (Popkin 1994). Indeed, consequence of the obesity is the increased risk for various illnesses, such as diabetes mellitus, gallbladder disease, osteoarthritis, coronary artery disease and some forms of cancer (Vona-Davis *et al.* 2007).

In the last century, the disease that is increased the most in obese people, compared with lean ones, is type 2 diabetes mellitus (T2DM), a condition resulting from the metabolic changes associated with excess fat.

A pivotal role in T2DM development is played by insulin resistance (IR) that is the reduction of the

response of peripheral target tissues to a physiological concentration of insulin. Skeletal muscle plays a central role in whole body IR (Zierath *et al.* 2000), so that skeletal muscle IR is a predictor of the T2DM development and maintenance of adequate muscle glucose disposal may help to prevent diabetes.

The prevalent theory on impaired insulin signaling in obesity links IR to the increase of circulating FFA and excessive deposition of lipids in non-adipose tissues, including liver and skeletal muscle (Sethi & Vidal-Puig 2007). However, two different mechanisms centered on mitochondria function have been proposed to explain the onset of IR in skeletal muscle following lipid storage. Indeed, either a decrease in mitochondrial fatty

acid oxidation due to mitochondrial dysfunction or enhancement in mitochondrial oxidant production in response to excess fuel has been thought to contribute to IR development in skeletal muscle. Actually, the observation that increased production of radicals and other reactive oxygen species (ROS) is an early event in the development of IR (Houstis *et al.* 2006) suggests that mitochondrial dysfunction is a complication of the hyperlipidemia-induced ROS production, which might promote mitochondrial alterations, lipid accumulation and inhibition of insulin action.

Recently, due to the observation that IR and related disorders are growing dramatically all over the world, the efforts to identify and develop effective approaches for their treatment have been intensified. In addition to dietary regimes aimed at weight loss, two major non-pharmacological approaches to improve insulin sensitivity have included antioxidant supplementation and exercise training.

In recent years, antioxidants have been used extensively to overcome the effects of excess of ROS in several pathologies. However, antioxidant supplementation, used in an attempt to protect against IR and related complications, has supplied contrasting results (Abdali *et al.* 2015).

The health-promoting effects of the physical activity have been known for a longer time. Already in ancient China the need to promote and prescribe exercise for health-related benefits was recognized (Viña *et al.* 2012). Currently, physical inactivity is considered as a risk factor for cardiovascular disease and a widening variety of other chronic diseases, including diabetes, cancer (colon and breast), obesity, hypertension, bone and joint diseases (osteoporosis and osteoarthritis), and depression (Warburton *et al.* 2006). Conversely, regular physical activity is considered to produce healthy effects, including increases in tissue metabolism, due to mitochondrial proliferation (Venditti *et al.* 2014a,b, 2016), insulin sensitivity and cardiorespiratory fitness, so that it is able to prevent diabetes (Colberg 2007) and coronary heart disease (Wannamethee *et al.* 1998).

The beneficial effects of exercise are evident, not only in healthy persons but also in patients, because, suitably graded, exercise is useful as an adjunctive therapy in the treatment of patients with several chronic diseases (Warburton *et al.* 2006).

The mechanisms underlying obesity-induced IR development have been recently reviewed (Di Meo *et al.* 2017), so that this review, after briefly examining the link

among obesity, IR and ROS, focuses the attention on the potential role of exercise training in opposing metabolic dysfunction in patients with IR by describing possible cellular and molecular mechanisms.

Obesity, diabetes and insulin resistance

Obesity is a medical condition mainly due to a chronic imbalance between energy intake and energy expenditure, which causes or exacerbates several chronic diseases, including T2DM (Kopelman 2000). T2DM is characterized by too high plasma glucose levels and often it is the result of IR, i.e. tissue failure to respond to insulin, which is often accompanied by a variety of metabolic and cardiovascular abnormalities (DeFronzo *et al.* 1991).

It is also well established that skeletal muscle plays an important role in whole body IR (Zierath *et al.* 2000) so that many researchers focused on this tissue.

The mechanisms of IR remain largely unknown, but impaired glucose uptake characterizing skeletal muscle IR seems to result from defects in insulin receptor signaling (Goodyear *et al.* 1995, Bjornholm *et al.* 1997).

Insulin signaling in normal and insulin-resistant muscle

Normally, insulin regulates glucose transport into the muscle by activating a protein signaling cascade (Zierath *et al.* 2000). After binding with insulin, the insulin receptor is autophosphorylated on tyrosine residues allowing the binding and consequent phosphorylation of the insulin receptor substrates 1 and 2 (IRS-1, IRS-2). IRS binding to the phosphatidylinositol 3-kinase (PI3K) results in activation of a PI3K-dependent pathway comprising phosphoinositide-dependent kinase (PDK), atypical protein kinase C (aPKC) and protein kinase B (PKB/Akt). Among the Akt substrates, a protein of 160 kDa (AS160) was found to be an important molecule in the activation of glucose transport in muscle. AS160 phosphorylation by Akt removes inhibition of the glucose transporter (GLUT4) translocation from inner vesicles to plasma membrane where it promotes glucose uptake whose full stimulation also requires an atypical protein kinase C (PKC ζ) (Bandyopadhyay *et al.* 1997) (Fig. 1).

In obesity (Goodyear *et al.* 1995) and T2DM (Bjornholm *et al.* 1997) there is a reduction in IRS-1 and IRS-2 tyrosine phosphorylation, which has been related to their increased serine/threonine phosphorylation

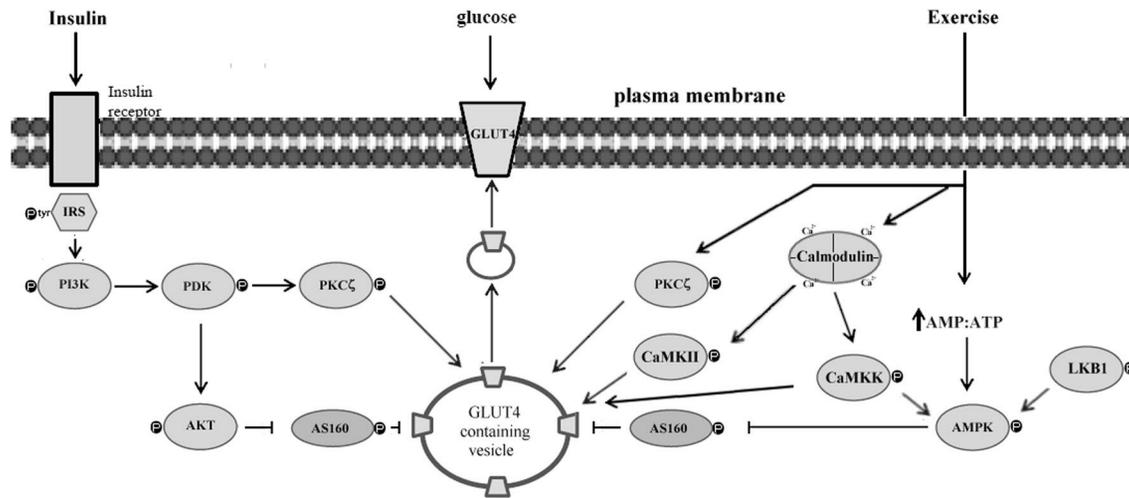


Figure 1

Schematic representation of the signaling pathways mediating insulin- and exercise-induced skeletal muscle glucose transport. In normal conditions, insulin binding to its receptor results in the phosphorylation of the insulin receptor substrate (IRS) on tyrosine residues allowing the activation of the phosphatidylinositol 3-kinase (PI3K), which leads to phosphorylation of phosphoinositide-dependent kinase (PDK). PDK, in turn, activates protein kinase B (Akt) and atypical protein kinase C (PKC ζ). Akt inhibits a 160kDa protein (AS160), thus promoting the translocation of the glucose transporter type 4 (GLUT4) to the plasma membrane, in which PKC ζ is also involved. PI3K also increases NADPH oxidase (NOX) activity, leading to increased production of O $_2^{\cdot-}$ that is converted to H $_2$ O $_2$ by superoxide dismutase (SOD). H $_2$ O $_2$ enhances glucose uptake by inhibiting protein tyrosine phosphatases (PTPs) and promoting tyrosine phosphorylation of IRS. Muscle contraction activates an insulin-independent mechanism that stimulates glucose transport. The two pathways converge in their distal parts in which AS160 and aPKC are involved. A pivotal role is played by AMP-activated protein kinase (AMPK), and Ca $^{2+}$ - and calmodulin-dependent protein kinases. AMPK is activated by an increase in the AMP:ATP ratio, a serine threonine kinase (LKB1) and calcium/calmodulin-dependent protein kinase kinase (CaMKK). The Ca $^{2+}$ /calmodulin-dependent kinase II (CaMKII) also seems to be implicated in glucose transport.

(Paz *et al.* 1997) by kinases, such as inhibitor kappa B kinase (IKK) (Gao *et al.* 2002), c-Jun amino-terminal kinases (JNK) (Aguirre *et al.* 2000) and mammalian target of rapamycin (mTOR) (Li *et al.* 1999). As a result PI3K levels are reduced with subsequent decrease in Akt (Kim *et al.* 1999) and atypical PKC (Kim *et al.* 2003) activity and glucose uptake, likely due to reduced GLUT4 activity/translocation (Shulman 2000).

Mechanisms of obesity-induced insulin resistance

One of the most harmful effects of obesity is the lipid deposition in non-adipose tissues that occurs when the capacity of adipose tissue to store lipids is overwhelmed (Sethi & Vidal-Puig 2007) and may lead to lipotoxicity and IR development (Shulman 2000).

The IR dependence on tissue lipid overload and the finding of mitochondrial dysfunction in obese and insulin-resistant patients (Kelley *et al.* 1999, Simoneau *et al.* 1999) suggested that a decrease in total mitochondrial oxidative capacity could contribute to IR reducing skeletal muscle capacity to manage increased FFA influx. In fact, mitochondrial dysfunction would

decrease lipid utilization thereby contributing to fatty acid overload and muscle IR development. In particular, when faced with a chronic dietary overload with saturated fatty acids, muscle cells produce many lipid metabolites, including diacylglycerol (DAG), ceramide (CER) and derived gangliosides, which are considered maladaptive signals arising from disordered lipid metabolism (Savage *et al.* 2007, Watt & Hoy 2012). The accumulation of DAG and CER is tightly associated with the IR development since such molecules impair insulin signaling activating aPKC isoforms that inhibit IRS1 and Akt, respectively (Powell *et al.* 2003, Li 2004). CER also achieves Akt inhibition through activation of protein phosphatase 2A (PP2A) (Stratford *et al.* 2004), while GM3, a ceramide-derived ganglioside, inhibits the insulin receptor (Lipina & Hundal 2015).

Reactive oxygen species production

Despite the strong association between IR and impaired oxidative capacity in skeletal muscle, the idea that in obesity IR is due to a mitochondrial dysfunction is not supported by the observation that high ROS production, but not low oxidative capacity, is a requirement for the

early phases of IR development in obesity (Fisher-Wellman *et al.* 2014).

ROS, which are generated in processes occurring in mitochondria and other cellular sites (Venditti *et al.* 2015, Di Meo *et al.* 2016), include superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$). Of these, $\bullet OH$ is the most reactive and initiates chain reactions leading to cell structural and functional alterations (Valko *et al.* 2007).

When ROS generation exceeds cellular antioxidant defenses, oxidative stress develops (Sies 1997), a detrimental process that has been related to many pathological conditions (Valko *et al.* 2007, Reed 2011, He & Zuo 2015), and is also involved in the IR etiology (Henriksen *et al.* 2011).

It is now clear that ROS can be either harmful or beneficial to living systems because they may cause organ dysfunction if occurring in a massive extent, but they are essential for several physiological processes when moderately produced. Their role also depends on the type of reactive species produced and localization of their source and their targets (Finkel & Holbrook 2000). Thus, H_2O_2 can exert stimulatory or inhibitory effects on insulin signaling, depending on its concentration and the site of production relative to various components of insulin signaling pathway(s) (Rindler *et al.* 2013).

Dual role of NADPH oxidase-derived H_2O_2

Since the 1970s it was shown that exogenously added H_2O_2 could mimic the insulin signaling activity (Czech *et al.* 1974a) and oxidation of key fat cell sulfhydryls in response to insulin-stimulated glucose transport (Czech *et al.* 1974b). Subsequently, it was found that H_2O_2 produced by a plasma membrane NADPH oxidase facilitated normal signal transduction by insulin (May & de Haen 1979). NADPH activity is increased by PI3K activation resulting from its binding to IRS-1 and leads to increased H_2O_2 concentration near the receptor (Espinosa *et al.* 2009).

Actually, NADPH oxidase-derived H_2O_2 can also reduce insulin sensitivity as demonstrated by the observation that cultured cardiomyocyte exposure to high glucose, which activates NOX2, impairs insulin signaling that is rescued by treatment with a NOX2 activation inhibitor (Balteau *et al.* 2011).

A NOX-mediated contribution to skeletal muscle IR seems to be provided by the overactivity of the renin-angiotensin system (RAS). Indeed, angiotensin II (Ang II)

impairs glucose transport system in skeletal muscle (Ogihara *et al.* 2002). Such an effect is due to Ang II ability to inhibit the insulin-PI3K signaling pathway stimulating serine phosphorylation of IRS-1 (Wei *et al.* 2006). The Ang II effects seem to depend on capacity of Ang II to generate ROS in various cell types including skeletal muscle (Shiuchi *et al.* 2004, Blendea *et al.* 2005). Moreover, the observations that obesity is associated with overactivation of both systemic and adipose RAS in humans and animals (Kalupahana & Moustaid-Moussa 2011) and blockade of Ang II type 1 receptor (AT1R) improves IR and glucose intolerance in obese rodents (Chu *et al.* 2006) suggest that obesity plays a role in RAS stimulation and IR onset.

Mitochondria-derived H_2O_2

Houstis and coworkers (2006) first showed a link between mitochondrial-derived ROS and IR and subsequent studies using MnSOD mimetics (Chen *et al.* 2008) and MnSOD overexpression (Hoehn *et al.* 2009, Boden *et al.* 2012) suggested a role for $O_2^{\bullet-}$ in the impairment of insulin sensitivity. However, the observation that targeted overexpression of the catalase gene to mitochondria (MCAT) protected from lipid-induced muscle IR (Lee *et al.* 2010), whereas SOD2 overexpression did not alleviate muscle IR alone and did not increase the effect of H_2O_2 scavenging (Lark *et al.* 2015) demonstrated H_2O_2 involvement in IR.

The obesity-induced increase in mitochondrial ROS production could be due to the inverse relationship between electron flow rate and electron leak along the respiratory chain. *In vitro*, in presence of ADP (State 3), respiration rate is high and ROS generation is low, whereas, in the absence of ADP (State 4), respiration rate is low and ROS production is high (Chance & Williams 1956). *In vivo*, near state 4 conditions and high ROS production likely occur during periods of nutrient overload combined with minimal ATP demand, that is, high caloric intake combined with a sedentary lifestyle.

Increase in mitochondrial ROS generation might also depend on the enhanced circulating levels of triglycerides and free fatty acids that induce a metabolic shift in skeletal muscle toward increased reliance on fat relative to glucose for energy production (Muoio & Neufer 2012). Indeed, mitochondrial H_2O_2 production is greater when fatty acids relative to the glycolytic metabolite pyruvate are oxidized (Anderson *et al.* 2007, Seifert *et al.* 2010).

Moreover, accumulation of various lipid metabolites that are able to interact with the mitochondrial inner

membrane disrupts electron transport and stimulates mitochondrial ROS production (Schonfeld & Wojtczak 2008, Seifert *et al.* 2010).

The ROS effect on IR has been attributed to alterations in various intracellular signaling pathways. Among the proposed effectors there are various kinases such as PKCs, IKK β , JNK, p38 mitogen-activated protein kinase (MAPK), which catalyze the phosphorylation of serine residues in IRS-1, thus reducing the phosphorylation of its tyrosine residues by insulin and inhibiting its activity (Paz *et al.* 1997).

Several reports suggest that high mitochondrial H₂O₂ release could contribute to IR via dissociation of hexokinase II (HKII) from mitochondria. Glucose phosphorylation is impaired in high-fat diet-fed rats (Furler *et al.* 1997, Halseth *et al.* 2000) and T2DM patients (Bonadonna *et al.* 1996). HkII expression is increased by insulin (Vogt *et al.* 2000) and decreased in patients with T2DM (Kruszynska *et al.* 1998, Pendergrass *et al.* 1998). HKII association with the mitochondrial outer membrane is promoted by insulin (Chen-Zion *et al.* 1992, Vogt *et al.* 1998) via Akt phosphorylation of HKII (Pastorino *et al.* 2005), whereas exogenous H₂O₂ dissociates HKII from mitochondria in cultured cardiomyocytes (Wu *et al.* 2011).

The increased production of ROS can also contribute to IR development causing mitochondrial dysfunction. Indeed, when ROS are produced at high rate, a substantial part of them escapes the mitochondrial antioxidant systems oxidatively damaging DNA, lipids and proteins, and leading to impairment of important mitochondrial functions. Oxidative damage to mitochondria is due to \cdot OH radicals, which can also damage other cellular structures. Indeed, although it is unlikely that such radicals are released by mitochondria, other ROS, such as H₂O₂, are able to cross mitochondrial membranes and reach such structures where, in the presence of Fe²⁺ ligands, it can generate \cdot OH radical.

In conclusion, mitochondrial dysfunction found in IR is a complication of the hyperlipidemia-induced ROS production in skeletal muscle, even though, when the mitochondrial oxidative capacity decreases, the lipid metabolism also decreases leading to fat accumulation thus contributing to the IR development.

Exercise and insulin resistance

It is known that regular exercise elicit adaptive responses that improves the metabolism of glucose

and lipids in skeletal muscles during the resting state. Moreover, it is well established that health preservation and prevention of age-related disorders requires the adoption of appropriate lifestyles including a habitual exercise regimen (Ciolac 2013). Indeed, regular aerobic physical activity (training) induces cardiorespiratory and musculoskeletal adaptive responses (Warburton *et al.* 2006), which increase resistance to conditions leading to increased ROS production including prolonged or strenuous exercise (Ebbeling & Clarkson 1989). Moreover, it maintains insulin sensitivity and cardiorespiratory fitness, preventing T2DM (Colberg 2007, Colberg & Grieco 2009) and coronary heart diseases (Thompson *et al.* 2003). Regular physical activity can also be used in the management of patients affected by T2DM (O'Gorman & Krook 2008) and chronic heart failure (Edelmann *et al.* 2014). This suggests that the improvement, produced by exercise, in insulin action on skeletal muscle glucose metabolism in insulin-resistant individuals could decrease conversion rates to overt diabetes, as well as reduce cardiovascular mortality.

The beneficial effects of training could appear to conflict with reports indicating that physical unaccustomed or strenuous exercise, particularly that characterized by remarkable component of eccentric contraction, causes damage, including structural and functional alterations in skeletal muscle and other tissues (Gollnick & King 1969, King & Gollnick 1970, McCutcheon *et al.* 1992, Clarkson 1997).

The opposite effects of acute exercise and training are in great part due to the ability of ROS to play a dual role in animal organisms. Indeed, the high levels of ROS produced during a single session of acute exercise leads to cellular damage and dysfunction, whereas the low levels of ROS produced during the single sessions of a training program can induce adaptive responses beneficial for the organism (Di Meo *et al.* 2016). During physical activity, several ROS sources are activated contributing to the oxidative damage and/or to the adaptive processes (Di Meo *et al.* 2016). However, the existence of a substantial interplay between various ROS sources suggests that the activation of one can lead to activation of the others inducing a positive feedback loop (Di Meo *et al.* 2016). This phenomenon, strengthening cellular oxidative damage, makes it more difficult to identify the primary generator of ROS activated by exercise unless fluorescent protein-based probe is used to obtain a reliable organelle specific measurement.

Contraction-induced glucose transport

In skeletal muscle, glucose transport can be activated by at least two separate pathways, one stimulated by insulin, insulin-mimicking agents and insulin-like growth factors, and one activated by muscle exercise and hypoxia (Zierath *et al.* 2000). Although it has been long known that glucose transport into muscle can also be stimulated by an insulin-independent mechanism activated by contractions (Goldstein *et al.* 1953), only relatively recently it has been shown that the increase in muscle glucose transport elicited in response to contractions is primarily due to translocation of GLUT4 from cellular storage sites (Goodyear *et al.* 1991). Much less is currently understood regarding the intracellular signaling mechanisms responsible for this contraction-dependent pathway. However, it is generally believed that the molecular signaling mechanism leading to GLUT4 translocation during muscle contraction is distinct from that of insulin (Lund *et al.* 1995). The two pathways appear at least partially converge in their distal parts, and the possibility has been raised that AS160 operates as a common, downstream point of convergence mediating the effects of both insulin and contraction on GLUT4 translocation (Kramer *et al.* 2006). Contraction-stimulated glucose uptake also seems to be mediated by aPKC because increased activity of the protein has been demonstrated in skeletal muscle of endurance-trained humans (Nielsen *et al.* 2003).

There is evidence indicating roles of AMP-activated protein kinase (AMPK), and several Ca^{2+} -dependent mechanisms, including calcineurin and Ca^{2+} - and calmodulin-dependent protein kinase in contraction-induced glucose transport (Wright *et al.* 2004) (Fig. 1).

AMPK is activated by a complex mechanism involving an increase in the AMP:ATP ratio and allosteric modification and phosphorylation by one or more upstream kinases, including the serine threonine kinase named liver kinase B1 (LKB1) (Sanders *et al.* 2007) and the calcium/calmodulin-dependent protein kinase kinase β (CaMKK β) activated in response to Ca^{2+} signaling (Jorgensen & Rose 2008). Protein kinases, such as Ca^{2+} /calmodulin-dependent kinase II (CaMKII), have also been implicated as critical molecules underlying contraction-stimulated glucose transport in skeletal muscle (Witczak *et al.* 2007), even though the mechanism of the glucose transport stimulation is still a matter of debate.

The observation that glucose transport can be stimulated by contractions through an insulin-independent mechanism suggests that muscle contraction

can be one alternative mean to activate glucose transport in insulin-resistant conditions which are not consequence of generalized resistance in the mechanism(s) involved in GLUT4 translocation. In fact, it has been demonstrated that short-term exercises of moderate-to-heavy intensity stimulate glucose transport in skeletal muscle from diabetic rats (Wallberg-Henriksson & Holloszy 1984, 1985) and insulin-resistant patients (Wahren *et al.* 1975).

The improvement in glucose uptake induced by a single bout of exercise also continues for several hours after exercise and often persists until the next day. The increase in post-exercise glucose uptake is mainly mediated by insulin-dependent glucose uptake (Hamada *et al.* 2006).

It is worth nothing that strenuous or exhaustive exercise results in muscle fatigue and frequently causes post-exercise muscle damage that does not induce metabolic improvement but rather impairs it. For example, muscle damage suppresses insulin sensitivity compared to the resting state and it has been proposed that the increase in ROS produced by exercise not only generates muscle damage but also impairs insulin-stimulated glucose uptake (Kirwan *et al.* 1991). This idea is consistent with the suggested close relationship between oxidative stress and IR (Fisher-Wellman & Neuffer 2012).

However, in order to establish the obesity-linked alterations that result in IR, it is necessary to examine what alterations are prevented or attenuated by the chronic exercise which is the only able to elicit adaptive responses in skeletal muscle resulting in IR improvement.

Muscle adaptive responses to training

Exercise training is able to induce major adaptations in skeletal muscle, which are dependent on the nature of the adaptive stimulus.

Heavy resistance exercise, also referred to as strength training, typically consists of a small number of contractions (often fewer than 10–20) with development of a relatively high force. It results in hypertrophy of the muscle cells with an increase in strength, without major changes in biochemical makeup. The gain in muscle cross-sectional area found in resistance-trained subjects is mainly due to an increase in the number of myofibrils, whereby the fast fiber types (type IIA and type IIX) are mostly responsible for the net increase in muscle size (Gonyea & Sale 1982).

Endurance exercise training is characterized by a large number of contractions (often many thousand) performed with development of a relatively low force. It increases

the capacity of muscle for aerobic metabolism promoting its adaptation toward a more oxidative phenotype without muscle hypertrophy or an increase in strength. Specifically, endurance exercise training leads to fiber-type transformation, mitochondrial biogenesis, angiogenesis, and other adaptive changes in skeletal muscles (Yan *et al.* 2011). These adaptations make it possible for previously untrained individuals to markedly increase their ability to exercise for prolonged periods at exercise intensities that could be maintained for only a few minutes in the untrained state.

Both types of training can improve insulin action and glucoregulation, although it is not clear if these methods of training achieve the same outcome by identical mechanisms.

Resistance exercise

Skeletal muscle is a plastic tissue that rapidly adapts to its mechanical environment (Goldberg 1968). Increased load across a muscle, such as from strength exercise, results in a compensatory increase in muscle size. The size increase comes largely from the growth of existing cells rather than an increase in cell number (Goodman *et al.* 2011), even though eccentric strength training seems to be able to cause muscle fiber neof ormation (Antonio & Goneya 1993).

Tissue size is determined by the balance between the rates of protein synthesis and degradation within the cells (Rennie *et al.* 2004) so that in muscle a net positive or net negative myofibrillar protein balance results in hypertrophy or atrophy, respectively. In healthy adults, rates of myofibrillar protein synthesis fluctuate between periods of net positive balance after protein feeding, and net negative balance during fasting and the change in muscle mass over time is very small (Phillips *et al.* 2011). While both rates of myofibrillar protein synthesis and breakdown fluctuate during anabolic and catabolic conditions, in the non-diseased state the regulation of skeletal muscle mass is primarily dictated by the regulation of muscle protein synthesis (Greenhaff *et al.* 2008, Phillips 2009).

Adaptive response to resistance exercise is not limited to the increase in muscle mass and strength. Indeed, recent prospective study, examining the role of weight training in the primary prevention of T2DM, suggests that the training is associated with a significantly lower risk of T2DM independent of aerobic exercise (Grøntved *et al.* 2012).

Moreover, in the past decades several studies demonstrated that resistance training is able to lower the percentage of glycosylated hemoglobin, increase glucose disposal, and improve metabolic and lipidic profiles decreasing cardiovascular disease risk in patients with T2DM (Zanuso *et al.* 2010). However, the mechanisms underlying such changes were not clear. Because body sensitivity to insulin appeared to be directly proportional to muscle mass the effects of resistance training on insulin sensitivity were initially attributed to the increase in muscle mass (Miller *et al.* 1984). Subsequently, however, it was reported that resistance training can increase insulin sensitivity by qualitative changes independent of a gain in muscle mass (Ishii *et al.* 1998).

The increase in lean mass also results in an increased resting metabolic rate, triggering an upward spiral of metabolic health (Speakman & Selman 2003). Moreover, because skeletal muscle mass declines at a rate of 3–8% each decade after 30 years of age (Lexell *et al.* 1988), leading to increased risk of IR and T2DM, muscle mass gain elicited by resistance training remains an important objective in the elderly.

Although classic strength training protocols impact predominantly on muscle and cross-sectional area of its fibers, it has been shown that regular resistance training is effective in reducing abdominal fat among individuals with T2DM (Kwon *et al.* 2010), thus contributing to the decrease of one of the major risk factors for IR.

Mechanisms responsible for increase in muscle mass

One of the main pathways responsible for muscle hypertrophy via increased protein synthesis is the insulin-like growth factor 1 (IGF-1)/PI3K/Akt pathway. IGF-1 is a factor promoting the growth which is involved in growth and regeneration of the skeletal muscle. IGF-1 binding to its receptor triggers intracellular signaling pathways ultimately leading to tyrosine residue phosphorylation on Akt activating this kinase (Schiaffino & Mammucari 2011). Akt in turn acts on proteins including the forkhead box O (FoxO) transcription factor family, which is involved in several cellular processes including protein metabolism (Tzivion *et al.* 2011). Akt phosphorylates and represses FoxO thus leading to protein degradation inhibition. Furthermore, Akt stimulates protein synthesis through mammalian target of rapamycin (mTOR), which is constituted by two multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), characterized by different sensitivities to rapamycin

(Toschi *et al.* 2009). Activation of mTORC1 leads to phosphorylation of ribosomal protein S6 as well as other factors involved in translation initiation and elongation which results in increased protein synthesis (Schiaffino & Mammucari 2011).

Although this pathway is essential for muscle growth during development and regeneration, its role in adult muscle response to mechanical load is less clear.

Mechanisms responsible for increase in glucose clearance

The examination of the available literature shows that resistance training induces several changes in skeletal muscle, which are relevant to increase glucose clearance. An important adaptation elicited by resistance training in normal (Yaspelkis *et al.* 2002) and high-fat fed (Krisan *et al.* 2004) rodent skeletal muscle is an increase in total skeletal muscle GLUT4 protein concentration. Such an increase is significant because GLUT4 concentration is directly related to the rate of insulin-stimulated skeletal muscle glucose transport. However, this change alone not fully accounts for increased rates of insulin-stimulated glucose transport in the skeletal muscle from resistance-trained animals. In fact, resistance training increases insulin-stimulated carbohydrate metabolism in normal skeletal muscle and reverses high-fat diet-induced skeletal muscle IR by altering the activity of components of the insulin signaling cascade, such as PI3K, PKC ζ and Akt (Krisan *et al.* 2004).

Another effect of resistance training, which is important for its benefits on glucose metabolism, is the stimulation of the glycogen synthesis. It is well known that skeletal muscle is the largest reservoir of glycogen in the human body and glycogen synthase (GS) is the enzyme responsible for catalyzing the (1 \rightarrow 4) linkage in the formation of glycogen. Glucose storage in the form of glycogen is regulated by the protein kinase glycogen synthase kinase-3 β (GSK-3 β), another direct target of PI3K/Akt. Increased expression and activity of skeletal muscle GSK-3 β have been implicated in the pathogenesis of IR (Nikoulina *et al.* 2000). Conversely, resistance training results in stimulation by Akt of glycogen synthesis via inhibition of GSK-3 β (Case *et al.* 2011). Akt can inhibit GSK3 β by phosphorylation at a serine residue (Ser9 in GSK-3 β) and GSK3 β deactivation leads to dephosphorylation and activation of GS, thus contributing to the stimulation of glycogen synthesis. The increase in Akt-mediated GS activity plays a major role in non-oxidative glucose

disposal and is therefore an important adaptation toward glycemic control in response to resistance training.

It has been observed that, during a single bout of resistance exercise, AMPK activity is increased (Dreyer *et al.* 2006). This activation suppresses muscle protein synthesis by inhibiting mTOR signaling pathway through reduced phosphorylation of downstream components of the mTOR pathway. However, 1–2 h after exercise, inhibition is removed and protein synthesis can occur in the muscle in association with mTOR activation (Dreyer *et al.* 2006). The transient activation of AMPK also leads to phosphorylation of target proteins involved in a number of metabolic pathways, which result in an increase of ATP-generating processes such as fatty acid oxidation and glucose uptake, the rate-limiting step in the hexose utilization, via increased GLUT4 translocation (Mu *et al.* 2001). However, the absence of active AMPK only partially suppresses the contraction ability to activate glucose transport, suggesting the existence of AMPK-independent signaling pathways for a full biological response (Mu *et al.* 2001).

Hyperinsulinemia decreases β -oxidation in insulin-resistant subjects thus reducing fatty acid utilization (Xu *et al.* 1995). The insulin-sensitizing effect of resistance training releases the brake on β -oxidation and contributes to improved metabolic flexibility and a more balanced utilization of fatty acids as substrates. Increased insulin sensitivity might therefore contribute to increased lipid clearance from the blood. Another important adaptation responsible for the insulin-sensitizing effect of the training independent of an increase in muscle mass might be the enhanced insulin receptor protein expression (Holten *et al.* 2004). These adaptations might be responsible for restoring metabolic flexibility in T2DM in response to resistance training.

Resistance training and mitochondria

For a long time a predominant view has been that endurance and strength training are distinct exercise modalities, whose effects on skeletal muscle are related to increase in mitochondrial density (Davies *et al.* 1981) and increase in myofibrillar units (Gonyea & Sale 1982), respectively. More recent researches, however, suggest that mitochondrial biogenesis is stimulated by both training modalities (Pesta *et al.* 2011). Indeed, 10-week resistance training was found to enhance mitochondrial respiration to the same extent as aerobic training in skeletal muscle of lean, previously sedentary adults. The enhanced

oxidative capacity was mainly due to qualitative mitochondrial changes, whereas tissue mitochondrial density contributed to a smaller extent (Pesta *et al.* 2011). Subsequent study, investigating effects of different types of exercise on mitochondrial content and substrate oxidation in T2DM patients (Sparks *et al.* 2013), found that 9-month resistance or endurance training increased mitochondrial content in the skeletal muscle, and this change was associated with clinical improvements. Furthermore, combined training improved all measures of lipid and carbohydrate oxidation as well as mitochondrial content and enzyme activity, indicating that long-term combined training is an effective lifestyle therapy for T2DM (Sparks *et al.* 2013).

These reports are of great interest and support the view that mitochondrial derangement can be one of the factors responsible of IR and T2DM, even though they do not address the problem of the ROS production and its possible involvement in mitochondrial dysfunction.

At present, whether resistance training reduces the mitochondrial ROS production remains unknown. A recent report (Flack *et al.* 2016) suggests that resistance training does not reduce ROS production in older adults. This observation appears inconsistent with previous observations suggesting that lipid (Vincent *et al.* 2002) and DNA (Parise *et al.* 2005a) oxidative damage was reduced, whereas antioxidant enzyme activity was increased (Parise *et al.* 2005b) following resistance training in older adults. Moreover, it has been reported that resistance training combined with antioxidant supplementation significantly increases free fat mass without concomitant improvement in insulin sensitivity in older adults (Bobeuf *et al.* 2010).

Endurance exercise

Evidence that the increase produced by endurance training in skeletal muscle metabolic capacity was due to increases in both tissue mitochondrial protein content and mitochondrial respiratory capacity was initially obtained in rats subjected to a program of treadmill running (Holloszy 1967). Subsequent studies confirmed the finding of exercise-induced mitochondrial biogenesis (Baldwin *et al.* 1972), but showed that swim training induces an increase in the mitochondrial population which is characterized by a lightly reduced aerobic capacity (Venditti *et al.* 1999, 2014b).

Despite increase in mitochondria, aerobic exercise training reduces lipid and protein oxidative damage in skeletal muscle (Venditti *et al.* 2014b). Furthermore, it

renders tissue less susceptible to the oxidative damage in conditions leading to increased ROS production. For example, it was found that training prevented lipid peroxidation increase induced by moderate intensity exercise in rat muscle (Alessio & Goldfarb 1988). However, other studies suggested that training did not affect the extent of lipid peroxidation due to exhaustive swimming but decreased the rate of the peroxidative reactions, thus allowing trained animals to sustain the activity for a longer period before the fatigue became limiting (Venditti & Di Meo 1996, 1997). The increase induced by exercise in tissue resistance to oxidative challenges seems to be associated with increased cellular antioxidant defenses. Several studies examined the effect of endurance training on the activities of antioxidant enzymes. Much of these studies cannot be directly compared to each other because of the differences in experimental design, animal model and analytical procedures. However, in the whole they show that training results in an increase in activity of skeletal muscle antioxidant enzymes, such as SOD, GPX, CAT and glutathione reductase (GR) (Powers *et al.* 1999), even though some studies failed to find enhanced antioxidant activity after training. In fact, the determination of single enzyme activity and single scavenger concentration provides only a limited assessment of the tissue ability to prevent generation of or scavenge ROS. However, measurement of total antioxidant capacity of skeletal muscle indicates that such a capacity is increased by endurance training (Venditti & Di Meo 1996, 1997), supporting the view that the resistance of skeletal muscle against free radical-induced lipid peroxidation is at least in part due to increased antioxidant defenses.

Surprisingly, there are few studies concerning the effect of training on antioxidant enzyme expression. Such studies showed increases in Cu/ZnSOD and MnSOD protein level only in some muscles but not in others (Gore *et al.* 1998, Hollander *et al.* 1999).

It is likely that training exerts its protective effects also decreasing H₂O₂ production, even though scarce information is available on training impact on cellular ROS sources. The rate of mitochondrial H₂O₂ release was decreased in skeletal muscle (Venditti *et al.* 1999, 2014b) from rats trained to swim. Conversely, no effect on H₂O₂ release by intermyofibrillar and subsarcolemmal mitochondria was found in skeletal muscle mitochondria following voluntary wheel training (Servais *et al.* 2003).

Measurements of H₂O₂ release rate in the presence of respiratory inhibitors suggested that training reduces the concentration of the autoxidizable electron carriers

located at Complexes I and III in the muscle mitochondria (Venditti *et al.* 2014b).

However, other swim training-induced adaptations can contribute to the reduction of the H₂O₂ release rate found in the muscle mitochondria. These could include the increased activity of the H₂O₂-metabolizing enzyme GPX, which is coupled with the increase in GR activity, in the muscle mitochondria (Venditti *et al.* 2014b).

The decrease in H₂O₂ release might also be due to increased uncoupling of the inner mitochondrial membrane linked to increased levels of members of the mitochondrial transporters family, uncoupling protein 3 (UCP3) and uncoupling protein 2 (UCP2), which are primarily expressed in skeletal muscle. However, this idea is not supported by the observation that endurance training decreases Ucp2 and Ucp3 expression in fast-twitch fibers of skeletal muscle in both rodents (Boss *et al.* 1998) and humans (Schrauwen *et al.* 1999) and UCP3 protein content in all types of fibers in humans (Russell *et al.* 2003).

Studies dealing with exercise training effects on NADPH oxidase activity are limited but some data suggest that exercise training is able to modulate NADPH oxidase activity in obese but not in lean subjects. Indeed, it has been reported that endurance training reduced microvascular endothelial NOX content in muscle biopsies from vastus lateralis of obese (Cocks *et al.* 2016) but not of lean men (Cocks *et al.* 2013).

Endurance training in T2DM: prevention and management

The benefits of physical activity are evident in healthy people but there is also strong evidence of the effectiveness of regular physical activity in the primary and secondary prevention of several chronic diseases such as diabetes (Pedersen & Saltin 2006, Warburton 2006).

Several studies support the idea that aerobic exercise, as well as resistance exercise, is associated with a decreased risk of type 2 diabetes. In early prospective study, each increase of 500kcal in energy expenditure per week was associated with a decreased incidence of type 2 diabetes of 6% (Helmrich *et al.* 1991). The observation that such a benefit was particularly evident among people with a high body mass index was subsequently supported by several other studies. For example, randomized trials found that lifestyle interventions including physical activity, combined with diet-induced weight loss, reduced the risk of type 2 diabetes by 42% (Pan *et al.* 1997) and

58% (Tuomilehto *et al.* 2001), respectively, in high-risk subjects. On the other hand, interventions of exercise alone appeared to be just as effective in terms of prevention of T2DM as programs of diet alone or diet and exercise combined (Pan *et al.* 1997, Tuomilehto *et al.* 2001).

Physical activity has also been recommended by physicians in managing patients with T2DM because improvements in insulin sensitivity through regular exercise could overcome defects in insulin signal transduction noted in muscle from T2DM patients. Thus, endurance exercise training was shown to improve insulin sensitivity in patients with T2DM (Dela *et al.* 1995, Lazarevic *et al.* 2006) and to enhance both insulin-stimulated and non-insulin-mediated glucose uptake in skeletal muscle (Dela *et al.* 1992, 1994).

These results agree the observations of the existence of a strong association between exercise and reduced rates of death from any cause and from diabetes in particular. Indeed, a prospective cohort study showed that physically inactive men with established T2DM had a 1.7-fold increased risk of premature death compared with physically active men with T2DM (Wei *et al.* 2000). Another cohort study showed that walking at least 2 h per week was associated with a reduction in the incidence of premature death of 39–54% from any cause and of 34–53% from cardiovascular disease among patients with diabetes (Gregg *et al.* 2003). Moreover, walking that led to moderate increases in heart and breathing rates was associated with significant reductions in all-cause mortality and cardiovascular-related mortality (Gregg *et al.* 2003).

Mechanisms of adaptive responses to endurance training

Mitochondrial biogenesis

Although it was long known that endurance exercise induces an increase in muscle mitochondria number (Holloszy 1967), the mechanisms underlying such an increase were elucidated only at the beginning of the twenty-first century. This was due to a lack of information regarding how mitochondrial biogenesis, which requires the orchestrated expression of the genes encoded in the mitochondrial genome and the nuclear genes encoding mitochondrial proteins, is regulated.

Advancement in the understanding of the mitochondrial biogenesis regulation was reached with the discovery of the transcription factors, nuclear respiratory

factors 1 (NRF-1) (Evans & Scarpulla 1990) and 2 (NRF-2) (Virbasius *et al.* 1993). Further advancement was reached with the discovery of an inducible coactivator, the peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α), which activates the transcription factors NRF-1 and NRF-2 (Puigserver *et al.* 1998). These in turn promote the transcription of oxidative phosphorylation genes, and other specific mitochondrial genes, including those involved in the transcription and replication of mtDNA, such as the factor of transcription A (TFAM) (Wu *et al.* 1999).

PGC-1 protein expression increases quickly in muscle cells stimulated to contract (Irrcher *et al.* 2003). Moreover, Pgc-1 gene expression increases in rat skeletal muscle after a single bout of exercise (Baar *et al.* 2002) and in human skeletal muscle after endurance training (Wang *et al.* 2011). Resistance training, performed after endurance exercise, amplifies the adaptive signaling response of mitochondrial biogenesis compared with single-mode endurance exercise, suggesting that concurrent training may be beneficial for the adaptation of muscle oxidative capacity (Wang *et al.* 2011). Increase in PGC-1 α level was also found in rat skeletal muscle after 10 weeks of training to swimming, and such an increase was associated with increases in NRF-1 and NRF-2 levels (Venditti *et al.* 2014b).

It is worth noting that stimuli, activated during exercise, can contribute to eliciting the PGC-1 gene response. First, acute exercise results in rapid activation of p38 MAPK (Boppart *et al.* 2000) which activates PGC-1 α by phosphorylation (Puigserver *et al.* 2001) and mediates the increase in its expression (Knutti *et al.* 2001). Then, the activated PGC-1 α moves into the nucleus and coactivates the transcription factors that regulate expression of mitochondrial proteins.

Other exercise-activated stimuli inducing Pgc-1 gene response include: (i) increase in cytosolic calcium concentration, which activates various signaling pathways regulated by the calcineurin phosphatase and the calmodulin-modulated kinase, (ii) the decrease in levels of high-energy phosphates, leading to the activation of AMPK, (iii) stimulation of the adrenergic system, leading to cyclic AMP synthesis, and activation of protein kinase A and other kinases (Kang & Ji 2012).

Furthermore, PGC-1 α function is not only regulated by phosphorylation, but also by other covalent modifications among which acetylation, methylation and ubiquitination (Fernandez-Marcos & Auwerx 2011).

It seems that Pgc-1 expression is also upregulated by ROS. Indeed, the observation that the increase in Pgc-1 α mRNA, induced by electrical stimulation in cell

culture of rat skeletal muscle, is prevented by antioxidant incubation (Silveira *et al.* 2006) suggests ROS involvement in exercise-induced stimulation of Pgc-1 α expression. Thus, the observation that the H₂O₂-induced increase in the mRNA content of Sod, Cat and Gpx in Pgc-1 α KO fibroblasts is lower than that in wild-type fibroblasts (St-Pierre *et al.* 2006) indicates that the upregulation of antioxidant enzymes can be mediated by PGC-1 α .

Moreover, notwithstanding disagreeing results exist in literature (Higashida *et al.* 2011), several experimental studies reported that antioxidant supplementation attenuated the Pgc-1 gene expression (Ristow *et al.* 2009, Paulsen *et al.* 2014) and PGC-1 protein content (Gomez-Cabrera *et al.* 2008, Venditti *et al.* 2014a,b, 2016). It was also reported that vitamin E supplementation prevented the increase in activator and coactivator levels induced by physical training in rat liver, muscle and heart (Venditti *et al.* 2014a,b, 2016). These results suggest that the ROS produced during each session of exercise are able to modify mitochondrial population acting as signals regulating molecular events crucial for adaptive responses to training of rat tissues.

Although ROS ability to act as signaling molecules for the tissue adaptation induced by training seems to contrast with the oxidative damage and dysfunction elicited by acute exercise, an explanation can be provided by differences in extent and temporal pattern of ROS generation. Thus, a moderate, intermittent ROS production during short time periods in a program of graduate aerobic training can activate signaling pathways leading to cellular adaptation and protection against future stresses. Conversely, moderate levels of ROS production over long time periods (e.g. hours) or high levels produced during brief strenuous exercise can lead to structural and functional tissue damage inactivating important cellular molecules.

Insulin resistance improvement

The training-induced increase in PGC-1 expression seems to be also involved in signaling pathways, which might result in improvement of skeletal muscle insulin sensitivity. Indeed, besides regulating the mitochondrial biogenesis, PGC-1 is able to regulate expression of endogenous antioxidants, such as Cu/ZnSod, MnSod and Gpx, in skeletal muscle (Olesen *et al.* 2010, Kang & Ji 2012). It is likely that this coordination of the proliferation of ROS-producing organelles with the increase in the antioxidant levels helps to maintain redox homeostasis.

In addition, PGC-1 α promotes mSirt3 gene expression, which is mediated by an endoplasmic reticulum-binding element mapped to the SIRT3 promoter region (Kong *et al.* 2010). In turn, SIRT3 binds to, deacetylates and activates mitochondrial enzymes, including MnSOD, through a posttranslational mechanism (Shi *et al.* 2005).

Because PGC-1 α is able to regulate the mRNA expression of Ucp2 and Ucp3 in muscle cell culture (St-Pierre *et al.* 2003), it was suggested that PGC-1 α could increase the uncoupling capacity and concomitantly reduce mitochondrial ROS production (Kang & Ji 2012). However, it is doubtful that an increase in uncoupling protein expression is elicited by endurance training since an increase in UCP3 protein has been observed in rat muscle only after acute exercise (Zhou *et al.* 2000) and short-term training (10 days) (Jones *et al.* 2003).

The molecular mechanism for enhanced glucose uptake with long-term exercise training can be in part related to increased expression and activity of key proteins involved in the regulation of glucose uptake and metabolism in skeletal muscle. Molecular candidates for improved glucose homeostasis in connection with exercise include GLUT4, HK and GS.

GLUT4 has been identified as a key player in the exercise regulation of glucose transport, because the protein, recruited from intracellular sites by exercise, moves to the cell surface, where it mediates glucose transport into the muscle cells (Holloszy 2003). A direct connection between increased GLUT4 protein expression and increased basal and insulin-stimulated glucose transport and metabolism was found in GLUT4-transgenic mouse models (Hansen *et al.* 1995). Furthermore, it was shown that GLUT4 overexpression in skeletal muscle could prevent impaired whole-body glucose homeostasis associated with various states of IR. For example, modest overexpression (twofold) of GLUT4 in heart, skeletal muscle and adipose tissue in transgenic mice prevented the impairment of glycemic control and the associated hyperglycemia caused by high-fat feeding (Ikemoto *et al.* 1995).

The effect of high-fat diet on GLUT4 expression in skeletal muscle appears to be variable because either decrease or lack of change of GLUT4 expression has been reported (Zorzano *et al.* 2005). However, in IR conditions, the ability of insulin to stimulate GLUT4 translocation decreases, resulting in a reduced GLUT4 content at the plasma membrane (Zierath & Wallberg-Henriksson 2002).

Conversely, besides increasing mitochondrial biogenesis, exercise enhances expression of the GLUT4 glucose transporter (Rodnick *et al.* 1990). The increase

in GLUT4 occurs in parallel with, and is mediated by, the same signals and some of the same transcription factors as the increase in mitochondrial biogenesis. Thus, PGC-1 stimulates Glut4 expression (Lehman *et al.* 2000) by activating NRF-1, which in turn increases myocyte enhancer factor 2A (MEF-2A) expression (Baar *et al.* 2003), and by coactivating MEF-2A, which further increases GLUT4 transcription.

It should be noted that exercise-induced improvement in insulin signaling is not exclusively restricted to increased GLUT4 protein expression. Indeed, it seems that, although exercise increases GLUT4 protein in diabetic patients (Dela *et al.* 1995), the main mechanism is the increase in post-receptor insulin signaling, especially at the distal step of the insulin PI3K cascade (which results in GLUT4 translocation and glucose uptake) (Zierath 2002).

Increase in GLUT4 transcription and expression of Glut4 mRNA has been shown to persist for 3–24 h after single bout of exercise (Kraniou *et al.* 2006, Richter & Hargreaves 2013). In this way, regular exercise translates into a steady-state increase of GLUT4 protein expression, and subsequent improvement in glucose control over time (Richter & Hargreaves 2013).

It is worth noting that even resistance training increases the rate of insulin-stimulated glucose transport and GLUT-4 protein concentration, but, differently from aerobic exercise, such changes are not associated to changes in oxidative capacity in rodent (Krisan *et al.* 2004) and human (Holten *et al.* 2004) skeletal muscles. This difference between endurance- and resistance-trained skeletal muscle was difficult to explain considering the effect of resistance training on levels of PGC-1, which regulates the expression of genes involved in oxidative phosphorylation (Bonen 2009). A study using stimulations at different frequencies, simulating strength and endurance training in isolated rat extensor digitorum longus and soleus muscles, showed selective activation of the Akt-mTOR signaling cascade with the strength and preferential activation of AMPK-PGC-1 α signaling with the endurance protocol (Atherton *et al.* 2005). However, subsequently, it was reported that resistance exercise, as well as endurance training, enhanced Pgc-1 α expression due to transcriptional regulation (Deldicque *et al.* 2008). More recently, a form of Pgc-1 α (Pgc-1 α 4) has been identified, which is preferentially induced in mouse and human muscle during resistance exercise (Ruas *et al.* 2012). PGC-1 α 4 does not regulate most of the known PGC-1 α targets such as the mitochondrial genes of oxidative phosphorylation, but rather regulates the insulin-like

growth factor-1 and myostatin pathways (Ruas *et al.* 2012), both of which are known regulators of muscle size and strength (Florini 1987, McPherron *et al.* 1997).

Glucose phosphorylation appears to be another site of regulation and a potential barrier to glucose uptake and utilization. It is well known that glucose, which is entered into the muscle cell, undergoes phosphorylation to glucose 6-phosphate (G-6-P) by HKII. Glucose is then metabolized through the glycolytic and oxidative pathways leading to energy generation during exercise or is converted to glycogen in the post-exercise period. Although glucose transport is generally believed to be rate limiting for insulin-mediated glucose metabolism in muscle (Fink *et al.* 1992), the rate-limiting step could shift beyond transport under hyperglycemic or hyperinsulinemic conditions (Yki-Jarvinen *et al.* 1987).

A possible role for HK in training-linked improvement of glucose homeostasis was supported by the observation that transgenic overexpression of HKII led to increased basal and insulin-stimulated glucose uptake (Chang *et al.* 1996). Theoretically, impairment in insulin-stimulated muscle glucose uptake shown in animals fed a high-fat diet can be due to deficits in one or more of the steps required for glucose uptake: (i) delivery of glucose to the muscle membrane, (ii) transport across the muscle membrane, (iii) intracellular phosphorylation by HK, and (iv) glycogen synthesis.

It was demonstrated that the impairment in muscle glucose uptake induced by consumption of a high-fat diet was due not only to impairment in glucose transport but also to defects in glucose delivery and phosphorylation (Halseth *et al.* 2000). Subsequent work showed that transgenic HKII overexpression was able to improve exercise-stimulated but not insulin-stimulated glucose uptake in mice fed a high-fat diet (Fueger *et al.* 2004). A difference between stimulation by exercise vs insulin is that exercise results in a massive hyperemia that lowers the barrier to glucose delivery. Therefore, the above results confirm that high-fat feeding increases the resistance to muscle glucose uptake even at sites upstream of glucose phosphorylation (i.e., glucose delivery and membrane transport). However, they also indicate that when the barriers to glucose transport and delivery are minimized during exercise as a result of hyperemia and contraction-stimulated GLUT4 translocation, impairment in glucose phosphorylation, which is improved by HKII overexpression, can be shown.

Some reports show that even glycogen storage is involved in training-induced improvement in glucose

disposal. Traditionally, GS has been considered to catalyze the key step of glycogen synthesis and exert most of the control over this metabolic pathway. Insulin signaling stimulates the non-oxidative glucose metabolism involving GS activation, the rate-limiting enzyme in the storage of glucose in glycogen particles (Roach 2002). It has been reported that, in response to 3 weeks of one-legged endurance exercise training, insulin-stimulated glucose uptake markedly increased in trained compared with untrained muscle (Frøsig *et al.* 2007). This increase coincided with an increase in protein expression of GLUT4, and HK II, as well as increased GS total activity in skeletal muscle. These adaptations are likely able to improve the intracellular conditions for uptake and metabolism of glucose (Frøsig *et al.* 2007). Increased expression and activity/phosphorylation of Akt and AS160 were also evident after training. However, the sequence leading from Akt activation to GS activation in skeletal muscle was not affected by endurance training, suggesting that Akt1 is not a major kinase regulating GS in human skeletal muscle in response to insulin stimulation (Frøsig *et al.* 2007).

The increase in GS activation was also observed in response to strength training regimen (Holten *et al.* 2004), but, in contrast to endurance training, strength training also led to detectable significant change in GS protein expression. Furthermore, it was shown that endurance training increased GS activity and GLUT4 expression and improved glucose disposal in diabetic patients (Christ-Roberts *et al.* 2004). In the whole, the results suggest that exercise training increases insulin-stimulated glucose disposal primarily by increasing GLUT4 protein expression without enhancing insulin-stimulated PI3K signaling, and that once the glucose enters the myocyte, increased GS activity preferentially shunts it into glycogen synthesis.

Interestingly, evidence is available implicating an inhibitory role in insulin signaling for mTOR pathway via an increased serine phosphorylation of IRS-1 (Tzatsos & Konstantin 2006). Such a process can have many consequences, including dissociation of IRS proteins from the insulin receptor, blockage of certain Tyr phosphorylation sites of IRS, and induction of IRS protein degradation (Pederson *et al.* 2001). Furthermore, phosphorylation of IRS-1 at Ser 636–639 was found to be involved in cases involving obesity-linked IR and T2DM (Khamzina *et al.* 2005). Both mTOR and the ribosomal S6 kinase 1 (S6K1), a downstream effector of mTOR, appeared to be involved in the Ser 636–639 phosphorylation of IRS-1 (Khamzina *et al.* 2005). It was also found that

a chronic increase in physical activity inhibited fed-state mTOR/S6K1 signaling and reduced IRS-1 serine phosphorylation in rat skeletal muscle (Glynn *et al.* 2008). Reduced mTOR/S6K1 signaling during chronic increases in physical activity may play an important regulatory role in the serine phosphorylation of IRS-1, which should be examined as a potential mechanism for IR attenuation associated with increased IRS-1 serine phosphorylation.

PGC-1 and training protection against insulin resistance

Endurance training induces increases in muscle levels of PGC-1 α which mediates a series of changes which potentially could protect the tissue against IR. Therefore, it is opportune to examine data dealing with the effects of possible changes of PGC-1 α levels in untrained and trained insulin-resistant animals.

Reductions in expression of Pgc-1 α and genes encoding for oxidative phosphorylation were found in skeletal muscle of T2DM patients and it was proposed that such reductions explained the alterations in mitochondrial metabolism in T2DM (Mootha *et al.* 2003, Patti *et al.* 2003). Interestingly, diabetes is also associated with reduced expression of mitofusin 2 (Mfn2) (Bach *et al.* 2005), which belongs to the families of dynamin-like proteins required for mitochondrial outer membrane fusion (Palmer *et al.* 2011). The observation that Mfn2 can be induced by PGC-1 through interaction with the transcription factor ERR α (Liesa *et al.* 2008), and that inhibition of fusion results in decreased mitochondrial size and function (Palmer *et al.* 2011) suggests the existence of another mechanism linked to PGC-1 deficiency which can contribute together with dysregulation of respiratory chain to the development of IR and T2DM.

In the light of the above considerations it is surprising that overexpression of Pgc-1 α in skeletal muscle results in an increase in IR in response to a high-fat diet, an effect which has been attributed to an increased fatty acid influx in myocytes which exceeds their capacity of consumption (Choi *et al.* 2008, Wong *et al.* 2015).

Conversely, controversial results were obtained studying the effect of training on skeletal muscle IR in PGC-1 overexpressing mice. Indeed, it was reported that insulin action remained lower in the transgenic compared with the control group, even after voluntary exercise (Wong *et al.* 2015). It was also found that in insulin-resistant subjects a bout of exercise induced a delayed and reduced response in Pgc-1 α mRNA

and protein and transient phosphorylation of AMP-dependent protein kinase, and did not increase none of the genes downstream of PGC-1 α (De Filippis *et al.* 2008). In contrast, in another work, endurance training was found not only to rescue the IR, but in fact render the mice more insulin sensitive than similarly exercised wild-type controls (Summermatter *et al.* 2013). Thus, it is unclear whether muscle PGC-1 α overexpression results in enhancement of the insulin-sensitizing effects of exercise, and whether PGC-1 insufficiency ultimately contributes to muscle IR. Conversely, the aforementioned observations clearly indicate that the increases in PGC-1 levels obtained by pharmacological exercise mimetics are different from those obtained by habitual physical activity in which other factors contribute to salutary effects normally attribute to PGC-1. In fact, the activity and expression of PGC-1 α not only respond to a variety of positive and negative signaling pathways (Boppart *et al.* 2000, Knutti *et al.* 2001, Puigserver *et al.* 2001, Fernandez-Marcos & Auwerx 2011, Kang & Ji 2012), but it is also not exclusively required for the expression of respiratory chain and antioxidant proteins (Leick *et al.* 2010), although it is required for normal basal expression levels (Adhietty *et al.* 2009). Because of the complexity of the signaling pathways involved in the regulation of the mitochondrial biogenesis as well as antioxidant defense system, other studies are necessary to establish the mechanisms underlying the muscle response to programs of endurance training.

Because ROS seem to be involved in exercise-induced stimulation of PGC-1 α expression, the question arises whether antioxidant integration can block the adaptive responses to endurance training mediated by PGC-1 in insulin-resistant muscle. Previous works examining the impact of antioxidants on exercise-induced adaptations showed varying response to antioxidant supplementation on exercise-induced effects in healthy humans and rodents.

Thus, it was reported that combination of vitamin C and vitamin E blocked training-induced increases in insulin sensitivity, mitochondrial biogenesis, and MnSod, Cu/ZnSod and Gpx expression as well as increases in PGC-1 expression in healthy humans (Ristow *et al.* 2009). Similar results were found in rats trained to run, where vitamin C supplementation blunted exercise-induced increases in endurance capacity, in MnSod and Gpx expression, and mitochondrial biogenesis markers NRF-1 and Tfam (Gomez-Cabrera *et al.* 2008). Moreover, in rats trained to swim vitamin E supplementation attenuated the exercise-induced reduction in mitochondrial H₂O₂ release rate and prevented the increases in GPX and GR

activities, and mitochondrial biogenesis, as well as PGC-1, NRF-1 and NRF-2 protein content (Venditti *et al.* 2014b).

In contrast, other studies reported that vitamin E and vitamin C supplementation had no effect on exercise adaptations in both humans and rodents. Indeed, antioxidant supplementation did not alter exercise-induced increases in oxygen consumption, insulin sensitivity and markers of muscle adaptation, including GLUT4 and HKII in subjects trained to bicycle exercise (Yfanti *et al.* 2010, 2011), and insulin sensitivity, mitochondrial protein content, MnSOD, Cu/ZnSOD, and GLUT4 protein content in rats trained to swim (Higashida *et al.* 2011).

Although there is the possibility that the contradictory results are due to differences in the exercise protocols, antioxidant supplementation doses and methods for parameter measurement, the problem of the effects of antioxidants on adaptive responses to training and therefore role of ROS as initial stimuli for such adaptations remains controversial.

Controversy also exists on the effects of antioxidant supplementation on the response to training in obese, insulin-resistant and diabetic animals and humans.

It was previously shown that the antioxidant α -lipoic acid (LA) combined with endurance training increased glucose transport in insulin-resistant skeletal muscle in an additive fashion (Saengsirisuwan *et al.* 2001). The possible cellular mechanisms responsible for this interactive effect were investigated evaluating the effects of LA, endurance training, and the two interventions combined on insulin-stimulated glucose transport, protein expression and functionality of specific insulin signaling factors in soleus muscle of obese Zucker rats. The results indicated that the improvements of insulin action in insulin-resistant skeletal muscles after α -LA or endurance alone and in combination were associated with increases in expression of IRS-1 protein and IRS-1 associated with p85 regulatory subunit of PIK3 (Saengsirisuwan *et al.* 2004). However, endurance training also resulted in increases in GLUT4 protein and activities of total hexokinase, which were not modified following treatment combination.

Improvement of insulin action in the obese Zucker rats was obtained by additive interactions between exercise training and angiotensin-converting enzyme inhibitors (Steen *et al.* 1999), and exercise training and thiazolidinediones, a class of antidiabetic drugs that improve metabolic control in patients with T2DM increasing insulin sensitivity (Hevener *et al.* 2000).

In contrast, more recent data indicate that vitamin E and vitamin C supplementation in obese rodents does not modify exercise-induced improvements in insulin sensitivity but reduces mitochondrial biogenesis and mitochondrial protein expression (Picklo & Thyfault 2015).

It is unclear why the effects of vitamin E and vitamin C supplementation are so different from those of α -LA. However, many of effects of α -LA, which has long been touted only as an antioxidant, are not due to its antioxidant properties. Beneficial effects are achieved with low micromolar levels of LA, suggesting that some of its therapeutic potential extends beyond the strict definition of an antioxidant. Moreover, it has also been shown to improve glucose and ascorbate handling, to increase eNOS activity, to activate Phase II detoxification via the transcription of the nuclear factor erythroid 2-related factor 2 (Nrf2) (Shay *et al.* 2009).

Normally, Nrf2 is located in the cytoplasm and kept dormant by a cytoplasmic repressor named Kelch-like ECH-associated protein 1 (Keap1). A variety of activators, including oxidative free radicals, release and translocate Nrf2 into the nucleus where it regulates the expression of antioxidant enzymes such as NAD(P)H quinone dehydrogenase 1, glutathione s-transferase, GPX, and heme oxygenase 1 (Lee *et al.* 2011).

LA, acting as a pro-oxidant, may increase Nrf2-dependent transcriptional activity by forming lipoyl-cysteinyl mixed disulfides on Keap1 protein that sequesters Nrf2 (Dinkova-Kostova *et al.* 2002). The effect of LA on Nrf2 is interesting because a critical role has recently been described for the transcription factor against oxidative stress in health and during diabetes.

However, this is not the only mechanism by which LA is able to improve glucose uptake in skeletal muscle. Studies using insulin-sensitive adipocyte and muscle cell lines indicated that *in vitro* LA exposure increased phosphorylation and/or the activity of several components of the insulin signaling pathway, including the insulin receptor, IRS-1, type I PI3K, Akt and p38 AMPK (Yaworsky *et al.* 2000, Konrad *et al.* 2001). Subsequently, LA was found to both enhance the IRS1 protein expression in muscle of obese Zucker rats and association of IRS1 with the p85 regulatory subunit of PI3K (Saengsirisuwan *et al.* 2001) and activate AMPK (Lee *et al.* 2005).

The observation that α -LA enhances antioxidant enzyme expression and activates AMPK indicates that the acid is able to improve insulin sensitivity through mechanisms similar to those put in motion by the endurance training. Therefore, it is understandable that

the effects of LA and training are additive, differently from what occurs with other antioxidants and thus such effects are not at odd with the idea that ROS are able to function as an initial stimulus for the increased PGC-1 expression and adaptive responses to training.

Conclusions

Available evidence indicates that both resistance and endurance training are able to counteract the harmful effects of obesity, which predisposes to IR and T2DM. However, at present, there is no evidence that the beneficial effects of two types of training depend on similar mechanisms even though in the skeletal muscle both exhibit as a common effect the stimulation of mitochondrial biogenesis and the increase in respiratory capacity. The protection exerted by endurance training seems to be due to ROS produced in low amount during the single sessions of exercise, which can activate signaling pathways leading to both increased capacity to counteract oxidative stress and increased mitochondrial biogenesis. Conversely, disagreeing reports are available about the role played by ROS in the protection offered by resistance training against obesity-linked IR skeletal muscle so that further studies are necessary to clarify this topic.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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