Insulin signalling, exercise and cellular integrity

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Abstract

Although the effects of exercise on insulin sensitivity are generally positive, eccentric exercise presents a paradox because it induces a transient state of insulin resistance that persists for up to 48 h after the exercise bout. Excessive eccentric contractions, such as prolonged downhill running, or marathon running, causes muscle damage and disruption of the integrity of the cell. Down-regulation of insulin receptor tyrosine phosphorylation and subsequent steps in the insulin signalling pathway, including insulin receptor substrate-1 (IRS-1)-associated phosphoinositide 3-kinase (PI3K), Akt kinase serine phosphorylation and activity and glucose transporter (GLUT-4) protein content, are evident in skeletal muscle after eccentric exercise. Furthermore, increased tumour necrosis factor α (TNF-α) expression from monocytes is associated with the decrease in PI3K activity after this type of exercise. Recent studies have shown that TNF-α can increase IRS-1 serine/threonine phosphorylation, which impairs IRS-1 docking to the insulin receptor, and this inhibits insulin signalling. Thus a unifying hypothesis to explain insulin resistance after eccentric exercise may include inflammation arising from the disruption of muscle-cell integrity, leading to an acute-phase response that includes TNF-α, with the latter inhibiting insulin signalling and subsequent metabolic events. In contrast, exercise training increases insulin signalling and GLUT-4 expression, decreases TNF-α expression in skeletal muscle, and is associated with enhanced insulin sensitivity. These observations highlight the complexity of the cellular and molecular adaptations to exercise. Understanding these adaptations is essential in order to establish a sound theoretical basis for recommending exercise as a therapeutic intervention for insulin resistance and type 2 diabetes.

Introduction

Resting and postprandial metabolism in mammalian skeletal muscle cells requires effective insulin-mediated glucose regulation. By definition, these cells are considered ‘insulin-sensitive’ if the hormone produces a normal physiological response and maintains glucose homeostasis [1]. In contrast, ‘insulin resistance’ is characterized by a less-than-normal physiological response to normal hormone levels. Exercise and a balanced energy diet promote insulin sensitivity, while inactivity and excess caloric intake are associated with insulin resistance. However, recent studies have shown that, when exercise features a major eccentric component, which requires the muscles to lengthen as tension develops, the outcome is transient insulin resistance. This insulin resistance remains evident for up to 2 days after the exercise bout. Eccentric exercise also causes extensive muscle damage [2–4], muscle soreness [5], oedema [6] and elevation of plasma myocellular proteins [3]. This review will outline the beneficial effects of exercise on insulin-mediated glucose metabolism, and highlight the effects of eccentric exercise on insulin signalling and cellular integrity in skeletal muscle.

Cellular insulin signalling

Insulin signal transduction and the effects of exercise on insulin signalling have been the subject of several recent reviews [7–12]. Briefly, the pathway is activated once insulin binds to its receptor on the plasma membrane. The receptor is a transmembrane heterotetrameric glycoprotein with two extracellular α-subunits that serve as insulin-binding sites and two intracellular β-subunits that contain tyrosine residues [13–15]. The receptor is capable of autophosphorylation through these tyrosine residues leading to phosphorylation of insulin receptor substrate-1 (IRS-1) and its related family of substrate proteins [16,17]. IRS-1 then acts as a docking site for a number of proteins with Src homology 2 (SH2) domains, including the dual-function lipid kinaseserine protein kinase, phosphoinositide 3-kinase (PI3K), growth-factor-receptor-bound protein 2 (Grb2), and the SH2-domain-containing protein tyrosine phosphatase (SHP2) [18–20]. Signalling through PI3K initiates several biological effects, including glucose metabolism, glycogen, lipid and protein synthesis, gene expression and cell growth/differentiation. Once PI3K is activated, it produces lipid second messengers that stimulate downstream proteins, which include the serine/threonine kinase known as Akt kinase (also known as protein kinase B),

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Abbreviations used: Grb2, growth-factor-receptor-bound protein 2; GSK-3, glycogen synthase kinase 3; IL, interleukin; IRS-1, insulin receptor substrate-1; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; SH2, Src homology 2; SHP2, SH2-domain-containing protein tyrosine phosphatase-2; TNF-α, tumour necrosis factor α.

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and the protein kinase C atypical subgroup, PKCζ and PKCα [21]. Akt plays an important role in skeletal muscle glycogen synthesis. Glycogen synthase is the key regulatory enzyme in the glycogenic process and is regulated by its inhibitory enzyme glycogen synthase kinase 3 (GSK-3). Phosphorylation of Akt leads to inactivation of GSK-3, thus facilitating glycogen synthase activation and glycogen synthesis.

Signalling through Grb2 and SHP2 activates the GTP-binding protein, Ras, which in turn stimulates the mitogen-activated protein kinase (MAPK) pathway leading to cell growth and differentiation [22–24]. Both the PI3K and MAPK arms of the insulin-signalling pathway have been implicated in the metabolic response to the cellular stress induced by eccentric exercise [25–27].

**Acute exercise and insulin-mediated PI3K signalling**

A single bout of exercise can have a very beneficial effect on glucose metabolism and increases insulin sensitivity in sedentary subjects [28–32]. Enhanced glucose metabolism is evident for up to 48 h, but the response appears to have an insulin-independent and an insulin-dependent phase. During exercise, circulating insulin is suppressed as part of an integrated physiological response that facilitates increased substrate availability to the contracting muscle [33]. Suppression extends into the immediate post-recovery period and may contribute to the insulin-independent regulation of glucose, which lasts for 3–6 h after the exercise bout [30]. During this time, skeletal muscle glucose regulation remains independent of insulin and the exercise/contraction pathway that mediates glucose uptake during exercise appears to prevail. Investigations focusing on insulin signalling after exercise suggest that neither a single bout of exercise nor muscle contraction induce an increase in the levels of insulin-receptor autophosphorylation, tyrosine phosphorylation of the insulin receptor, IRS-1 phosphorylation or PI3K activation [34–37]. Indeed, insulin-stimulated insulin receptor tyrosine kinase and PI3K activity in human skeletal muscle are reported to be lower after exercise [37]. Studies from our own laboratory have also shown a decrease in IRS-1-associated PI3K activity after an acute bout of intense cycling exercise, and the restoration of activity was still incomplete 30 min after the exercise bout [38]. In contrast, an increase in insulin-stimulated PI3K has been reported in rat skeletal muscle after acute exercise [39]. However, the antibody used in this study precipitated both IRS-1 and IRS-2-associated PI3K, making it difficult to determine whether either or both substrate–enzyme complex was affected. A recent study by Christ-Roberts et al. [40] attempted to examine the acute effects of exercise on insulin signalling by measuring PI3K activity in human skeletal muscle before and during simultaneous exercise and insulin infusion. Since insulin is normally suppressed during exercise, the conditions of this experiment do not represent a true physiological state. Nevertheless, PI3K activity and Akt serine phosphorylation were increased to a greater extent when exercise was performed during insulin infusion compared with a non-exercise control trial. This suggests that the suppression of insulin during exercise may be partially responsible for the decrease in insulin signalling.

The exact time course that describes the transition between exercise-related insulin-independent and insulin-dependent glucose regulation remains to be established. However, based on studies published to date, it appears that the period beginning 3–6 h after exercise best describes the insulin-dependent phase. Early studies by Bogardus et al. [41] and Devlin and colleagues [29] established that a single bout of exercise resulted in increased insulin sensitivity. In human muscle, Cusi et al. [42] have shown that insulin-stimulated tyrosine phosphorylation of both the insulin receptor and IRS-1 is also increased for at least 24 h after a single bout of exercise. Surprisingly, and in contrast with the findings of Devlin et al. [29], insulin-mediated glucose metabolism was not improved in this study, and likewise, IRS-1-associated PI3K activity was also unchanged by exercise [42]. However, while both studies examined insulin-resistant type 2 diabetic subjects, the exercise intensity and the duration between the exercise bout and measurement of insulin sensitivity differed. When exercise intensity is moderate, it may take more than one bout of exercise to impact insulin sensitivity in people with marked insulin resistance. Accordingly, it may be preferable to use several bouts of exercise to examine the acute effects of exercise on insulin sensitivity and insulin signalling in these subjects [43,44].

**Exercise training and insulin-mediated PI3K signalling**

Exercise training is typically associated with a 20–30% increase in insulin-stimulated glucose metabolism [45–50]. Several cellular, molecular and physiological adaptations contribute to this enhanced metabolic state including increased glucose and insulin delivery to the muscle, greater blood flow and capillarization, increased enzyme activity within the muscle, increased GLUT-4 translocation and increased insulin signalling [51,52]. However, the effects of exercise training on insulin signalling are not consistent. One of the first studies on this topic was performed on skeletal muscle from rats trained by treadmill running, and found that exercise increased IR, IRS-1 and PI3K mRNA [53]. In contrast, exercise training does not appear to increase gene expression of IR or IRS-1 and may even attenuate expression of IRS-2 and the p85α regulatory subunit of PI3K in humans [54]. Cross-sectional studies comparing trained and sedentary subjects have found that exercise training is associated with a decrease in IR, IRS-1 and IRS-2, with no effect on Akt and an increase in GLUT-4 [55]. In contrast with gene and protein expression data, both cross-sectional and exercise-training studies in humans show that insulin-stimulated IRS-1-associated PI3K activity in the vastus lateralis muscle is significantly greater among exercise-trained subjects. This increase in signalling activity is associated with greater insulin sensitivity in the exercise-trained subjects [46]. These data are congruent with the work of Houmard et al. [45], which showed an increase in insulin-stimulated...
PI3K activity in previously sedentary subjects after short-term (7 days) exercise training. Although there are only a limited number of studies to date, there is good evidence that exercise training can modulate insulin sensitivity in human skeletal muscle by enhancing the dynamic processes of protein phosphorylation and activation in the insulin/PI3K signalling cascade. Additional investigations are necessary to determine whether these improvements occur in conjunction with changes in gene or protein expression.

**Eccentric exercise disrupts cellular integrity and induces insulin resistance in skeletal muscle**

The physiological stress associated with unfamiliar or very intense exercise causes skeletal muscle damage and transient insulin resistance [25,56,57]. We have previously shown a decrease in glucose disposal 48 h after eccentric exercise [56]. When compared with non-exercise control or concentric exercise trials, eccentric exercise resulted in a 37% decrease in insulin-mediated glucose disposal. However, glucose uptake was not different between control or concentric exercise. The effect of eccentric exercise on pancreatic β-cell function has also been examined. Using the hyperglycaemic clamp procedure (180 min, 10 mM), we have shown that eccentric exercise is linked to increased pancreatic β-cell secretion in young individuals [4]. It has been suggested that this effect could be an attempt to compensate for the insulin-resistant state observed following muscle damage. Other investigators using similar exercise protocols have reproduced the phenomenon of transient insulin resistance following eccentric exercise [57,58].

We have provided further evidence for the decrease in insulin action following eccentric exercise at the cellular level. A hyperinsulinaemic–euglycaemic clamp was performed on young, healthy sedentary males before and 24 h after eccentric exercise [25]. Muscle biopsies were taken to determine the effect of the eccentric exercise bout on insulin action in skeletal muscle. Insulin-stimulated IRS-1 tyrosine phosphorylation, IRS-1-associated PI3K, Akt serine phosphorylation and Akt activity were reduced following *in vivo* stimulation of the muscles. In addition, insulin-stimulated glucose disposal was also markedly impaired, which confirmed the development of insulin resistance in the muscle cell after eccentric exercise. However, the molecular mechanism(s) that triggers the insulin resistance that follows eccentric exercise is only beginning to be understood.

Muscle tissue damage initiates a series of immune reactions known as the acute-phase response [59]. The acute-phase response is critical to promote clearance of damaged muscle tissue, as well as to induce muscle repair and growth [60]. The acute-phase response includes the activation of the complement system, neutrophils and monocytes. The magnitude of the acute-phase response is dependent on the extent of muscle-cell disruption [61]. Within the tissue, monocytes undergo morphological and functional differentiation, inducing macrophage formation. Macrophage infiltration into the muscle peaks 24–48 h after damage [62]. The most important role of macrophages is in cytokine production [63]; tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β being chief among them [64].

Recent investigations have implicated the cytokine TNF-α as a modulator of insulin-mediated glucose metabolism. *In vitro* studies show that TNF-α can induce insulin resistance and down-regulate insulin receptor signalling in a variety of cell types, including adipocytes, hepatocytes and skeletal muscle [65–67]. Furthermore, increased TNF-α is associated with insulin resistance in obesity, pregnancy,
sepsis, exercise-induced muscle damage and aging [25, 68–71]. Receptors on the plasma membrane bind TNF-α and activate a pathway that includes sphingomyelinase and ceramide [72, 73]. It has been proposed that TNF-α promotes Ser207 phosphorylation of IRS-1, thus impairing its association with the insulin receptor, which in turn would inhibit subsequent downstream signalling [74].

Examination of the effect of elevated TNF-α on insulin signalling in C2C12 muscle cells revealed that insulin stimulation of IRS-1 and IRS-2-associated PI3K activation was impaired by 54% and 55% respectively [67]. Furthermore, treatment with TNF-α decreased insulin-stimulated IRS-1 tyrosine phosphorylation by 40% and also resulted in a 27% decrease in 2-deoxyglucose uptake. Having demonstrated the inhibitory effects of TNF-α on glucose uptake and insulin signalling in cultured muscle cells, we examined whether a similar mechanism could account for insulin resistance in human muscle after exercise-induced muscle damage [67]. Venous blood samples were obtained before and 24 h after eccentric exercise to evaluate ex vivo endotoxin-induced mononuclear cell secretion of the cytokines TNF-α, IL-6 and IL-1β. TNF-α increased more than 2-fold within 24 h of the eccentric exercise bout, but no change was seen in IL-6 or IL-1β production. TNF-α production was positively correlated with decreased insulin action on PI3K. In summary, there is strong evidence that eccentric exercise results in muscle-tissue damage, leading to muscle-cell disruption, impaired glucose homeostasis, and an acute-phase response that is associated with mononuclear cell release of TNF-α (Scheme 1). This cytokine, and not IL-6 or IL-1β, may modulate insulin signalling and provide insight into the mechanism responsible for the transient insulin resistance in skeletal muscle following exercise-induced muscle damage.

Conclusions

Exercise training leads to enhanced insulin sensitivity, which appears to be mediated by increased post-receptor insulin signalling, specifically at the distal steps of the insulin/PI3K signalling cascade. To date, studies in human skeletal muscle do not support the paradigm that exercise training increases expression of the proteins that comprise the pathway, but exercise training does enhance insulin-mediated phosphorylation and protein activation. We have also noted that, although exercise generally exerts a positive effect on insulin sensitivity, it may paradoxically promote insulin resistance if it causes disruption of muscle-cell integrity. The cellular mechanism associated with exercise-induced insulin resistance appears to be related to the acute-phase response and increased TNF-α secretion. These effects are relatively transient (<72 h) and can be avoided by minimizing the eccentric, or lengthening contractions, during the exercise bout.

References


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