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Insulin signalling: effects of prior exercise

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Abstract
After the discovery and clinical use of insulin for treatment of diabetes it became clear that some of the biological effect of insulin was dependent on the circumstances under which it was given. Relevant for this review is the notion that physical activity, in addition to its own direct metabolic effects also markedly affects the ability of insulin to stimulate a range of metabolic processes. More specifically, during and for a prolonged period after, exercise elicits effects on processes such as insulin-induced muscle glucose uptake and glucose metabolism which influence systemic glucose homeostasis. These phenomena are probably responsible for the improvement in glucose homeostasis and metabolic control that typically occurs with exercise in people with insulin resistance and probably contributes to the reduced risk for development of type 2 diabetes in individuals who engage in regular exercise. Here we focus on the influence of a single bout of exercise on the action of insulin on processes such as glucose uptake and glucose storage in skeletal muscle.
Keywords AMPK, glucose uptake, glycogen, insulin sensitivity, PI3K skeletal muscle.

Thirty years ago, it was reported that habitual physically active subjects responded better to a certain dose of insulin than their sedentary counterparts (Bjorntorp *et al.* 1970). Further investigations on this phenomenon were performed by several groups, and led to the premise that the effect of exercise training seemed to affect insulin action in both healthy subjects as well as subjects with insulin resistance diseases (Bjorntorp *et al.* 1970, Ruderman *et al.* 1979, Saltin *et al.* 1979, Mondon *et al.* 1980). Ruderman *et al.* (1979) suggested that training had a transient effect and just a few days of physical inactivity led to a marked reduction in insulin action. In human beings the gold standard hyperinsulinaemic euglycaemic clamp technique has shown improved insulin sensitivity in relation to glucose clearance at the whole body level after a single bout of cycle or stair-climbing exercise (Bogardus *et al.* 1983, Devlin *et al.* 1987, Mikines *et al.* 1988, Perseghin *et al.* 1996) and suggests that the action of insulin may be improved as long as 2 days after a single exercise bout (Mikines *et al.* 1988, Perseghin *et al.* 1996). Some exceptions to this concept do however exist. For example, immediately after exercise insulin action on glucose clearance *in vivo* is impaired possibly because of elevated concentrations of catecholamines, free fatty acids and intracellular glucose metabolites (Kjær *et al.* 1986, 1990, Devlin *et al.* 1989). Likewise, eccentric exercise or physical activities with a dominant component of eccentric contractions elicit a prolonged decrease in insulin action, which may be caused by muscle damage and altered protein expression (e.g. GLUT4) and function [e.g. phosphatidyl inositol 3 kinase (PI3K)] (Asp *et al.* 1995, 1996, Tuominen *et al.* 1996, Del Aguila *et al.* 2000).

Increased insulin sensitivity enables muscle glycogen supercompensation

The recovery process from exercise is critical for skeletal muscle. When circumstances allow it, muscle cells will not only recover to a pre-exercise state, but will often overcompensate making it possible to better tolerate subsequent exercise bouts. For example, a range of genes can turn on during the recovery process aiming at

re-synthesis of proteins degraded during exercise, and are also likely to produce proteins to a level higher than before the exercise bout (Williams & Neufer 1996). Similarly, fuel storage in the form of muscle glycogen seems to recover to a level higher than before exercise, a process referred to as muscle glycogen super-compensation (Bergström & Hultman 1966, Richter 1996). Both of these responses probably enable the muscle to perform better during the next exercise bout. One of many acknowledged effects of insulin is to stimulate non-oxidative glucose metabolism in skeletal muscle, which, in most cases, primarily means glycogen synthesis. This action of insulin is improved after exercise and is believed to enable muscle glycogen storage to be supercompensated. The effect of insulin on the super-compensation of muscle glycogen is made possible by the up-regulation of the two primary regulatory factors involved in glycogen synthesis: (1) glucose transport over the sarcolemma membrane and (2) the activity of the enzyme glycogen synthase. (Richter *et al.* 1982, Mikines *et al.* 1988, Perseghin *et al.* 1996, Wojtaszewski *et al.* 2000a).

Insulin sensitivity is dependent on glucose availability

Studies in rodents and humans have revealed that the reversal of insulin sensitivity to stimulate muscle glucose transport is linked to glucose metabolism. For example, depriving rats of carbohydrate in the post-exercise period results in a prolonged increase in insulin sensitivity compared with the carbohydrate-fed state (Cartee *et al.* 1989). In fact, replacement of glucose by the non-metabolized 2-deoxy-glucose analogue in the incubation media significantly lengthened the period of increased insulin sensitivity (Gulve *et al.* 1990). In contrast, muscle incubation in conditions beneficial to glucose uptake results in a faster normalization of muscle insulin sensitivity (Gulve *et al.* 1990). Likewise, in human settings, Bogardus *et al.* (1983) observed that whole body insulin sensitivity 15 h after a single bout of exercise was normalized when human subjects ingested 100 g of carbohydrate 3 h after exercise whereas when the subjects fasted in the post-exercise period enhanced insulin sensitivity was maintained.

Muscle glycogen regulates exercise-induced insulin sensitivity

Muscle glycogen itself might be an important regulator of enhanced insulin action on glucose metabolism following exercise. This is because both muscle glucose uptake and glycogen synthase activity in response to insulin are dependent on the level of glycogen in the muscle. For example in human experiments, the ability of physiological levels of insulin to stimulate muscle glucose uptake after exercise is positively correlated with the amount of glycogen used during the prior exercise bout (Wojtaszewski *et al.* 1997, 2000a, Richter *et al.* 2001a) (Fig. 1). Likewise, an inverse relationship between glycogen synthase activation and muscle glycogen content exists during euglycaemic hyperinsulinaemia when studied 15 h after glycogen-depleting exercise or a similar period of rest (Bogardus *et al.* 1983). In addition food intake and accompanying hyper-insulinaemia 3 h after exercise activate glycogen synthase inversely related to the muscle glycogen content (Wojtaszewski *et al.* 2001). In line with these observations, we and others have recently found that the ability of a variety of stimuli [insulin, contractions and 5-aminoimidazole-4-carboxamide-1-b-4-ribofuranoside (AICAR)] to regulate glycogen synthase activity and glucose transport is increased in glycogen-depleted vs. glycogen-loaded fast-twitch rat muscle (Jensen *et al.* 1997, Derave *et al.* 1999, Kawanaka *et al.* 2000, Nielsen *et al.* 2001, Wojtaszewski *et al.* 2002a). The cellular mechanism for this is not fully understood. However, the amount of GLUT4 translocated to the surface membrane in response to insulin and contraction varies inversely with the muscle glycogen content (Derave *et al.* 1999, 2000). In addition, glycogen-dependent changes in glycogen synthase activity may

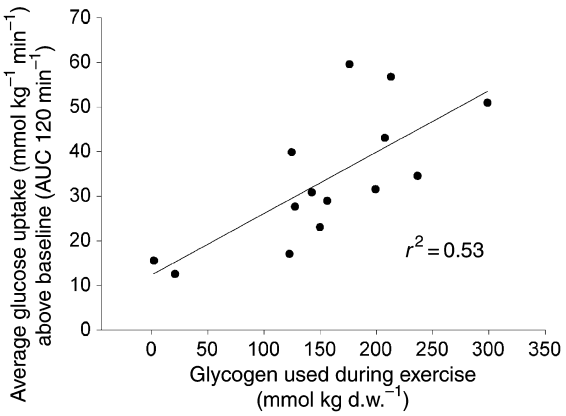

Figure 1 Correlation between the degree of glycogen depletion and the insulin-stimulated glucose uptake in thigh muscles of healthy men. Subjects performed a 60-min one-legged knee-extensor exercise, and glycogen depletion was measured as the difference in glycogen content between the rested and exercised leg 3–4 h following exercise. The insulin-stimulated glucose uptake was measured as the area under curve (AUC) after baseline subtraction for glucose uptake (A − V difference × flow) in the exercised leg during a 120-min hyperinsulinaemic (~100 μU mL^{-1}) euglycaemic clamp starting 3–4 h post-exercise. $n = 14$ and $r^2 = 0.53$. The figure is reproduced from Richter *et al.* (2001a) with permission. Data are combined from Wojtaszewski *et al.* (1997, 2000a).

relate to changes in both sub-cellular localization as well as covalent modifications of glycogen synthase (Nielsen *et al.* 2001). The above-mentioned observations indicate that the glycogen content after exercise or the amount of glycogen used during exercise is linked to the enhanced metabolic action of insulin in the post-exercise time period. However, glycogen levels alone cannot entirely explain the changes in insulin sensitivity following exercise. This has been demonstrated clearly by the fact that rodents continue to show a significantly elevated insulin sensitivity beyond the point of full glycogen re-synthesis (Cartee *et al.* 1989). Recently, this was supported by the finding that muscles pre-treated with the AMPK activating agent AICAR subsequently exhibit enhanced insulin sensitivity for activation of glucose transport. These changes occurred despite similar glycogen content in the AICAR-treated and -untreated muscles (Fisher *et al.* 2002). Whether the mechanism elicited by AICAR to regulate insulin sensitivity is similar to one of the mechanisms activated in response to exercise remains to be proved.

Change in insulin sensitivity is primarily a local phenomenon

Improvement of insulin sensitivity takes place primarily in the exercised, rather than the rested, muscles. This is suggested from experiments using both rodent and human models where muscles in only one limb have performed work prior to evaluation of insulin action in both limbs. This enables a distinction between humoral influence to the muscle vs. locally induced changes. In both humans and rodents the prior exercised leg takes up glucose to a far greater extent and with enhanced insulin sensitivity compared with the rested leg (Richter *et al.* 1984, 1989) (Fig. 2). These models prove the involvement of locally induced factors, but do not exclude the possibility that changes also take place in rested muscle (to a lesser extent) or other tissues. These changes might be small in magnitude, but, by mass and over time, may become important at the whole body level. Moreover, the one limb-exercise models do not exclude that a humoral factor is needed to achieve full effect of prior exercise on insulin action. In fact, recent work suggests that in order to obtain increases in insulin sensitivity of glucose transport after *in vitro* contraction or after *in vitro* treatment with AICAR, rodent muscles need to be incubated in the presence of serum during the period of stimulation (Cartee & Holloszy 1990, Gao *et al.* 1994, Fisher *et al.* 2002). The nature of this serum factor is not reported yet, but apparently exists in serum from non-exercised animals, and is either not needed in the perfused rat hind limb model or is present in the serum-free medium used for perfusion (Richter *et al.* 1984).

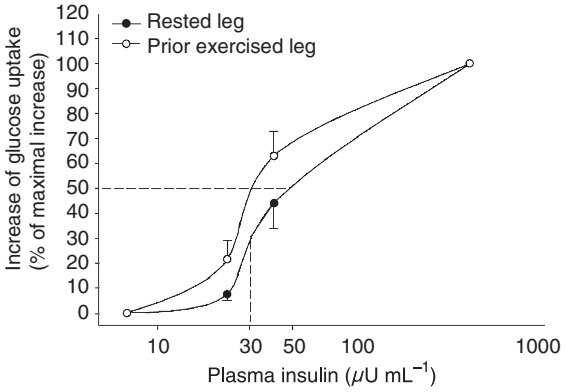

Figure 2 Exercise-improved insulin sensitivity of glucose uptake in human muscle. Prior one-legged exercise enhances sensitivity of glucose uptake to insulin in the exercised compared with the rested leg, in healthy human subjects as indicated by the decreased insulin concentration eliciting a half maximal glucose uptake response, when glucose uptake is given as percentage of maximal increase. The figure is reproduced from Wojtaszewski *et al.* (2002c) with permission. Data are extracted from Richter *et al.* (1989) and represent mean ± SE for $n = 6$.

Protein synthesis is not needed for exercise-induced insulin sensitivity

Mechanisms seem to exist whereby insulin action is enhanced independent of protein synthesis. In favour of this is the observation that at early time points (3–4 h) after exercise, during which insulin sensitivity is increased, protein expression of GLUT4, of key insulin signalling intermediaries and total activity of glycogen synthase (reflection of glycogen synthase protein content) are not elevated in human muscle (Wojtaszewski *et al.* 2000a). In line with this observation, in isolated muscle, in which protein synthesis is inhibited by incubation with cycloheximide, muscle contraction still leads to a normal increase in insulin sensitivity (Fisher *et al.* 2002). Still, at later time points enhanced protein expression of key proteins, e.g. GLUT4, is much more likely to have occurred and, although not yet reported in humans, apparently is an important mechanism in increasing insulin action in rodents after a single bout of exercise (Ren *et al.* 1994, Hansen *et al.* 1998a).

Different signals regulate glucose transport and glycogen synthase activity by insulin and exercise

Evidence suggests that the enhanced insulin-stimulated glucose transport post-exercise is because of an enhanced recruitment of the GLUT4 protein to the muscle plasma membrane in response to insulin (Hansen *et al.* 1998a, Thorell *et al.* 1999). This facilitates

glycogen synthesis by increasing glucose availability. Glycogen synthesis is further stimulated by increased activation of glycogen synthase, most likely induced by dephosphorylation (Richter *et al.* 1982, Wojtaszewski *et al.* 2002c). Thus, the regulation of these processes is not altered in nature by prior exercise, but simply more potently activated by insulin. In theory, such increases in potency could be mediated by changes in the cellular signals activating these processes. Despite tremendous progress, it is still rather unclear how insulin signals to regulate glucose transport and also glycogen synthase activity. Exercise *per se* also stimulates glucose transport and glycogen synthase activity and evidence suggests that the signals used by exercise are not the same as those utilized by insulin. Furthermore, the signals turned on by exercise that might regulate these processes, and which might influence/potentiate the insulin-regulated signals, are unknown. For example, experiments using several models, including transgenic animals and kinase inhibitors *in vitro*, indicate that the insulin receptor, insulin receptor substrate (IRS)-1/2 and phosphatidylinositol 3 kinase (PI3K) are not necessary for exercise-induced muscle glucose transport and/or glycogen synthase activity (reviewed in (Richter *et al.* 2001a, Sakamoto & Goodyear 2002). Likewise, the glycogen-associated protein phosphase 1 ($PP1G_m$) seems to be involved in exercise-induced glycogen synthase activation, but apparently not that of insulin (Aschenbach *et al.* 2001, Suzuki *et al.* 2001). Finally, the fact that AMPK activity is regulated by exercise but not by insulin also indicates differences in regulation of glucose and glycogen metabolism by these two stimuli in skeletal muscle (Hayashi *et al.* 1998).

Improved insulin sensitivity despite similar activation of the classical PI3K cascade

In the past, insulin signal components stimulating glucose transport (IR, IRS1/2, PI3K) have been investigated in prior exercised muscle. Furthermore, downstream elements, like Akt and glycogen synthase kinase (GSK) 3, but not atypical PKCs, have also been investigated because of their apparent role in glycogen synthase activation by insulin (reviewed by Saltiel & Pessin 2002). Since the acute effect of exercise on muscle glucose transport is rather rapidly declining even without glycogen normalization (Ploug *et al.* 1987) (in contrast to glycogen synthase activation which stays high until glycogen is replenished) we and others examined muscle signalling in response to insulin 3–4 h after exercise. At this point a clear enhancement of insulin action on glucose uptake in human and rodent muscle is evident and an enhancement of glycogen synthesis/glycogen synthase activity is detectable. In human muscle we studied the classical PI3K signalling cascade during hyperinsulinaemic clamp conditions 3–4 h after one-legged knee extensor exercise (Thong *et al.* 2002, Wojtaszewski *et al.* 1997, 2000a). Using this model, only a small muscle group performs work, and as mentioned, systemic changes induced by exercise are minimal and not present at the time when the clamp is initiated. In whole muscle lysates, the activity or degree of phosphorylation of the insulin receptor, IRS1, IRS1 associated PI3K, Akt and GSK3, was determined. In neither step of the cascade was the level of steady-state activation/phosphorylation enhanced in the exercised muscle compared with the resting muscle. Thus, in muscle lysates, no changes were observed that could explain the enhanced glycogen synthase activation or the enhanced glucose uptake in the exercised muscle. Similar observations have been reported from studies of rodent muscle stimulated *in vivo* with supra-maximal or *in vitro* with submaximal effective insulin concentrations (Goodyear *et al.* 1995, Hansen *et al.* 1998a). Interestingly, in other circumstances with measurable changes in insulin sensitivity of glucose metabolism, it has so far not been possible to detect explanatory changes in insulin-induced signals. For example, when the metabolic action of insulin is decreased by prior caffeine ingestion (Thong *et al.* 2002) or in relatives to patients with type II diabetes (Storgaard *et al.* 2001), signals induced by physiological concentrations of insulin were also largely unchanged.

Insulin-induced signalling immediately after exercise

Athletes are encouraged to ingest carbohydrates immediately after exercise rather than hours later. One reason for this is to take advantage of the acute effects of exercise *per se* to stimulate both glucose transport and glycogen synthase activity, promoting a faster glycogen resynthesis. Superimposing the effects of exercise and insulin on glucose transport makes it experimentally difficult to define changes in insulin sensitivity at this point. However, under some experimental conditions a synergistic effect on glucose transport may indicate cross talk between the regulatory mechanisms utilized by these two stimuli (Berger *et al.* 1975, DeFronzo *et al.* 1981, Wasserman *et al.* 1991, Vergauwen *et al.* 1994). Several studies have looked at insulin signalling in skeletal muscle immediately after exercise, and as discussed below a very different signal response to insulin is evident at this point compared to several hours after exercise. If insulin is administered immediately after treadmill running, an enhanced insulin-activated signalling is observed in the rat muscle at the level of phosphotyrosine (PY)-associated PI3K activity and in the human muscle at the level of Akt-ser^{473} phosphorylation (Zhou & Dohm 1997, Thorell *et al.* 1999). This could be due to an enhanced flow and insulin delivery in the exercised,

compared with the rested, state. If so, insulin stimulation of the proximal signals would be expected to be elevated in prior exercised muscle as well. However, this was not observed at the level of the IR tyrosine phosphorylation or IRS1-associated PI3K activity in a study of treadmill-exercised mice, suggesting flow- and insulin delivery-independent mechanisms (Zhou & Dohm 1997, Wojtaszewski *et al.* 1999). Still, in this study, an elevated signalling at the level of PY-associated PI3K activity, Akt activity as well as GSK3 phosphorylation was evident in response to insulin in the prior exercised animals compared with rested animals. Apparently a significant part of this enhanced PI3K signalling was associated with IRS-2, although not to an extent by which it could account for the entire enhancement of PY-PI3K activity (Howlett *et al.* 2002), indicating that another, yet unknown, tyrosine phosphorylated protein may associate with PI3K in muscle. Thus, apparently neither IR nor IRS1 take part in the alterations necessary for enhanced metabolic action immediately after exercise. Nevertheless, as indicated above, the signal alterations measured are not long-term and thus cannot account for the prolonged increase in insulin sensitivity after exercise. Both human and rodent data support this view. Thus, insulin signalling through the classical PI3K cascade is apparently normal 3–4 h after exercise in humans (Wojtaszewski *et al.* 1997, 2000a, 2001), and in rodents insulin signalling is normalized 3 h after swimming exercise (rats) (Hansen *et al.* 1998b), and 30 min after treadmill exercise (mice) (Wojtaszewski *et al.* 1999, J.F.P. Wojtaszewski & L.J. Goodyear unpublished observations).

Does exercise mediate insulin sensitivity through activation of AMPK?

Based on recent observations that AMPK activity is activated during exercise and that AMPK is an important regulator of cell metabolism, an interesting link between a signal and metabolic regulation during/after exercise has developed (Winder & Hardie 1999, Richter *et al.* 2001a). The idea that AMPK may be a regulator of muscle insulin sensitivity has also developed and a hypothesis suggesting AMPK to be a mediator of exercise-induced insulin sensitivity has emerged. Fisher's *et al.* (2002) observation that *in vitro* treatment of isolated muscles with AICAR led to a subsequent increase in insulin sensitivity, similar to what is seen after *in vitro* contraction, best supports this view. Besides improved sensitivity for activation of glucose transport, AICAR treatment did not interfere with insulin signalling in the classical PI3K cascade, in line with the findings after exercise. However, more studies are needed to prove this link, because AICAR is not a specific AMPK agonist, and because some of the mechanisms utilized by AICAR are not similar to those turned on by exercise. For example, the regulation of the muscle glucose transport step is partially different (Mu *et al.* 2001). Two recent studies suggest that AICAR, perhaps through stimulation of AMPK activity, also acutely improves insulin sensitivity in two models of lipid-induced insulin resistance (Iglesias *et al.* 2002, Olsen & Hansen 2002). Interestingly, when rats performed exercise *in vivo*, AMPK was activated for some time in the period after cessation, not only in the prior exercised muscles (Rasmussen *et al.* 1998), which has also been demonstrated in human experiments (Fujii *et al.* 2000, Wojtaszewski *et al.* 2000b, 2002b, Richter *et al.* 2001b) but also in adipose tissue and liver (Park *et al.* 2002). As exercise *in vivo* is associated with an elevated adrenergic drive and because some findings suggest that AMPK activity can be regulated by adrenergic stimulation (Moule & Denton 1998, Minokoshi *et al.* 2002, Park *et al.* 2002), these events could be linked. Following adrenergic stimulation, insulin action in isolated muscle is enhanced probably due to the β-adrenergic-induced glycogen depletion (Nolte *et al.* 1994). Thus, although enhanced adrenergic drive during and immediately after exercise *in vivo* may act against improvement of insulin action (Kjær *et al.* 1986, 1990, Devlin *et al.* 1989) by time, this may convert to a stimulatory effect.

Conclusion

The exploration of the cellular mechanisms behind exercise-induced changes in insulin action is at its beginning and a range of possibilities exists explaining how insulin signalling is modulated after exercise in a time-dependent manner. Thus, future studies should address the relationship between level of signal and magnitude of action, sub-cellular localization/translocation of signalling molecules and the role of yet unexplored signals [e.g. aPKC (Farese 2002)]. The AMPK is a promising candidate mediating insulin sensitivity. Further studies are needed to firmly establish whether this signal is utilized during exercise to modulate insulin sensitivity in skeletal muscle.

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