Exercise Effects on Muscle Insulin Signaling and Action
Invited Review: Effect of acute exercise on insulin signaling and action in humans

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Wojtaszewski, Jørgen F. P., Jakob N. Nielsen, and Erik A. Richter. Invited Review: Effect of acute exercise on insulin signaling and action in humans. J Appl Physiol 93: 384–392, 2002; 10.1152/japplphysiol.00043.2002.—After a single bout of exercise, insulin action is increased in the muscles that were active during exercise. The increased insulin action has been shown to involve glucose transport, glycogen synthesis, and glycogen synthase (GS) activation as well as amino acid transport. A major mechanism involved in increased insulin stimulation of glucose uptake after exercise seems to be the exercise-associated decrease in muscle glycogen content. Muscle glycogen content also plays a pivotal role for the activity of GS and for the ability of insulin to increase GS activity. Insulin signaling in human skeletal muscle is activated by physiological insulin concentrations, but the increase in insulin action after exercise does not seem to be related to increased insulin signaling [insulin receptor tyrosine kinase activity, insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation (RS1), IRS-1-associated phosphatidylinositol 3-kinase activity, Akt phosphorylation (Ser473), glycogen synthase kinase 3 (GSK3) phosphorylation (Ser21), and GSK3a activity], as measured in muscle lysates. Furthermore, insulin signaling is also largely unaffected by exercise itself. This, however, does not preclude that exercise influences insulin signaling through changes in the spatial arrangement of the signaling compounds or by affecting unidentified signaling intermediates. Finally, 5′-AMP-activated protein kinase has recently entered the stage as a promising player in explaining at least a part of the mechanism by which exercise enhances insulin action.

glycogen synthase; muscle glucose transport; insulin sensitivity; glycogen
To discuss the effect of exercise on insulin action satisfactorily, it is necessary to briefly discuss the isolated effect of insulin and exercise on the two most studied physiological end points of insulin action: glucose transport and GS activity.

EFFECTS OF INSULIN ON MUSCLE GLUCOSE TRANSPORT AND GS ACTIVITY

Insulin facilitates muscle glycogen synthesis through its action on both glucose transport and on GS activity. Insulin stimulates translocation of glucose transporter proteins (GLUT-4) to the plasma membrane, thereby enhancing the glucose transport capacity (66). This has been shown in vitro in incubated human muscle strips, with the photolabeling technique; and in vivo after meal- and clamp-induced hyperinsulinemia with subcellular fractionation techniques (25, 42, 70). The cellular signals utilized to stimulate the translocation of the GLUT-4-containing vesicles and the vesicular docking apparatus likely involve activation of phosphatidylinositol 3-kinase (PI3K), Akt, and protein kinase C at a proximal and possibly phospholipase D at a further distal step of the insulin-signaling pathway (19, 39, 66). The synthesis of glycogen from endogenous glucose is under tight control by GS. GS activity is regulated both allosterically by glucose 6-phosphate and covalently by multisite phosphorylation. Insulin enhances the activity of GS by decreasing phosphorylation of the enzyme (12). The classical PI3K cascade also seems to be involved in the regulation of GS, possibly through deactivation of glycogen synthase kinase 3 (GSK3) and activation of protein phosphatase-1 (PP1) (8, 41, 84). In human muscle, physiological hyperinsulinemia leads to time-dependent activation of GSK3, which precedes the activation time course of both GS and glucose uptake (79, 80). Although these findings do not prove a causal link between the signal and the end point, they do support and extend the massive amount of data from studies performed in cell cultures and rodents, which suggest such links (reviewed in Refs. 20 and 66).

EFFECTS OF EXERCISE/CONTRACTION ON MUSCLE GLUCOSE TRANSPORT AND GS ACTIVITY

The factors thought to be involved in exercise-induced glucose transport have recently been reviewed (58). However, the regulation of glycogen synthesis and GS activity during exercise has received less attention; some detail of this matter is given here. During exercise, human skeletal muscle relies strongly on intramuscular glycogen stores as a source of energy for contractile activity. To facilitate net glycogen breakdown, one could expect that glycogen synthesis was shut down. However, experiments with NMR spectroscopy have shown that the rate of glycogen synthesis after glycogen depletion is actually higher during low-intensity exercise than at rest (54, 55) (reviewed in Ref. 65). This is probably because GS activity is also increased during dynamic exercise, which lowers the muscle glycogen content (4, 87).

GS is influenced by both stimulatory and inhibitory factors during exercise, and the consequent effect of exercise on GS activity is a result of the relative strength of the various stimuli. Several human studies have shown that muscle GS activity is higher in a glycogen-depleted state compared with a glycogen-loaded state (7, 46, 79, 86, 89), and it has been suggested that exercise-induced GS activation is dependent on, and merely a result of, glycogen breakdown (48). The mechanism behind this dependency is unknown but at least in rodents seems to involve both covalent modifications of GS, as the apparent molecular mass of GS increases (83), and changes in the subcellular localization of GS (48). Whether these two changes are linked is unknown, but cellular redistribution of GS induced by glycogen depletion (48) could for instance make GS more susceptible to dephosphorylation. Conversely, the covalent modifications of GS, seen during conditions with high muscle glycogen content, are only partly reversible by phosphatase treatment (83), indicating the involvement of phosphorylation-dependent regulatory mechanisms in addition to other unknown factors. An important role of phosphatases in exercise-induced activation of GS is suggested by recent work in transgenic mice showing that the PP1 (GS phosphatase) targeting subunit Gm (RGl) is required for exercise to activate GS (1), although the mechanism by which exercise affects Gm is not clarified. In support of the notion that glycogen breakdown is a prerequisite for activation of GS, dynamic exercise that leads to GS activation in healthy subjects actually decreased GS activity in patients with McLeod’s disease, who are unable to break down muscle glycogen due to inborn glycogen phosphorylase deficiency (49a).

It is also noteworthy that isometric exercise decreases GS activity (31, 34). This has been ascribed to decreased PP1 activity (31, 34), but increased activity of protein kinase A has also been suggested to inhibit GS, at least during dynamic exercise (86). Isometric exercise is likely to be associated with some degree of hypoxia, and both hypoxia and exercise activate the 5’-AMP-activated protein kinase (AMPK) (reviewed in Refs. 28 and 77). Interestingly, AMPK has been shown to phosphorylate GS in vitro (9), and pharmacological AMPK activation decreases GS activity in rodent muscle (83). Thus activation of AMPK during exercise might work as an endogenously activated GS kinase. Another kinase known to phosphorylate and deactivate GS is GSK3. GSK3α has been suggested to take part in the regulation of GS activity during exercise in rats (43). However, in humans, GSK3α activity is unchanged by exercise and unaffected by marked differences in muscle glycogen content (Ref. 85 and J. P. Wojtaszewski, C. MacDonald, J. N. Nielsen, Y. Hellsten, D. G. Hardie, B. E. Kemp, B. Kiens, and E. A. Richter, unpublished observations). Therefore, GSK3α does not seem to be involved in GS regulation during moderate exercise in humans.
INSULIN ACTION AND INSULIN SIGNALING DURING EXERCISE

Ordinarily, the insulin concentrations in plasma decrease during exercise (22); relatively little attention has been given to the possible effects of the low circulating insulin concentrations during postabsorptive exercise. Nevertheless, in severely insulin-deficient animals, exercise-induced glucose uptake is decreased compared with when basal insulin concentrations are present (73), even when exaggerated hormonal and substrate responses to the very low insulin concentrations are avoided (75). In vitro, it has been convincingly demonstrated that muscle glucose uptake during contractions occurs by an insulin-independent mechanism (47, 52, 62, 74) and that insulin and contraction have additive effects on glucose transport (47, 53). Taken together with the in vivo findings, these in vitro findings suggest that, although insulin is not necessary for muscle contraction to increase muscle glucose transport, at least in vivo the full effect of exercise on muscle glucose uptake is only found when basal plasma insulin concentrations are present. In addition, in vivo, the effect of exercise and euglycemic hyperinsulinemia is synergistic (13), which could be interpreted as an increase in insulin sensitivity during exercise. However, whether insulin sensitivity in its strict sense (decreased half-maximally stimulating insulin concentration) is increased is not known because a full dose-response curve of insulin action on glucose uptake at rest and during exercise has not been performed in humans.

During exercise, the perfusion of the muscle is dramatically increased compared with at rest; in this way, the delivery of insulin (plasma flow \times \text{plasma insulin concentration}) is markedly enhanced during exercise even when plasma insulin concentrations are reduced by up to 50% during exercise. It has been suggested that this increase in delivery of insulin during exercise is the reason for the apparent marked effect of basal insulin concentrations during exercise compared with when insulin is virtually absent (73, 75). Still, if this were the case, one might expect insulin signaling to be upregulated during exercise compared with at rest. In rodents, this is apparently not the case, at least for the most proximal parts of the insulin-signaling cascade. Thus treadmill running did not increase insulin receptor and insulin receptor substrate 1/2 (IRS-1/2) tyrosine phosphorylation, total and IRS-1/2-associated PI3K activity, Akt activity, and GSK3 serine phosphorylation in rodent muscle (30, 82, 90). This is in agreement with studies of the intact rat, the perfused hindlimb rat model, and the incubated rat muscle preparation in which electrically stimulated muscle showed no activation or deactivation of proximal insulin signaling elements (24, 67, 76, 81, 88). Only few human studies have been done, and the results are less consistent. Thus moderate exercise has been found to induce a modest phosphorylation of Akt, whereas in another study tyrosine phosphorylation of the insulin receptor was decreased and IRS-1 tyrosine phosphorylation and IRS-1-associated PI3K activity were unchanged (38, 70).

INSULIN ACTION AND SIGNALING IN THE POSTEXERCISE PERIOD

Low muscle glycogen availability limits the ability to perform exercise (5). Resynthesis of muscle glycogen after exercise is therefore important. However, if no carbohydrate is ingested after exercise, glycogen stores are only rebuilt slowly and incompletely. This is because the process of glucose uptake rapidly normalizes after exercise mainly due to a decline in glucose delivery (muscle perfusion) and a decline in glucose transport capacity of the cell surface membrane (reviewed in Ref. 57). On the other hand, mechanisms exist to secure a high rate of glycogen storage once carbohydrate becomes available, and these mechanisms are so efficient that glycogen levels can be rebuilt to levels higher than the normal resting level (supercompensation). The phenomenon primarily involves an enhancement of the metabolic action of insulin in the previously exercised muscles (previously reviewed in Refs. 29 and 78). It should be noted that exercise does not always increase insulin action. For example, immediately after exercise, insulin action in vivo is impaired possibly due to elevated concentrations of catecholamines and free fatty acids (17, 36, 37). 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 altered protein expression and function (2, 3, 14, 71).

Studies in humans during hyperinsulinemic euglycemic clamp conditions revealed improved insulin sensitivity of glucose clearance at the whole body level in the period after a single bout of cycle or stair-climbing exercise (7, 18, 45, 51). These changes have been observed as long as 2 days after the exercise bout (45, 51). Skeletal muscle is normally the major tissue targeted by insulin, and changes in this tissue are likely the predominant causes of the effect seen at the whole body level. Improved insulin action is primarily seen in the previously active rather than the inactive muscles. For example, in postabsorptive healthy subjects, glucose uptake across the rested and exercised leg was similar when measured 3 h after one-legged exercise. However, insulin’s ability to stimulate glucose uptake at this point was twofold higher in the exercised leg compared with the rested leg (61). In that study, insulin action was elevated both at submaximal and maximal effective insulin concentrations; however, a clear shift of the dose-response curve to the left was still evident, indicating an increased insulin sensitivity of muscle glucose uptake (Fig. 1) (61).

What is the mechanism behind the enhanced metabolic effect of insulin (Fig. 2)? At early time points (3–4 h) after exercise, during which insulin sensitivity is increased, protein expression of GLUT-4 and insulin signaling intermediaries and total activity of GS are
Fig. 1. Exercise improves 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 (glucose uptake is given as % of maximal increase). Data are extracted from Richter et al. (61).

not elevated in human muscle (79). At later time points, enhanced protein expression of GLUT-4 is much more likely to have occurred and, although not yet reported in humans, apparently may be an important mechanism in increasing insulin action in rodents (56). Still, in isolated muscle, in which protein synthesis is inhibited by incubation with cycloheximide, muscle contraction leads to a normal increase in insulin sensitivity (21). This indicates that protein synthesis-independent mechanisms may also improve insulin sensitivity. The classical PI3K signaling cascade proposed to activate some metabolic events in skeletal muscle, including glucose transport and GS activity, has been studied in humans during hyperinsulinemic muscle, including glucose transport and GS activity. Similarly, when the metabolic action of insulin is decreased for example by prior caffeine ingestion (69) or in relatives to patients with Type 2 diabetes (68), signals induced by physiological concentrations of insulin were also unchanged. Thus, in a range of conditions with changed metabolic action of insulin, it has not been possible to point out signaling alterations in total muscle lysates.

There are several possible reasons for this negative outcome. The first is that there is not necessarily a linear relationship between insulin signaling and insulin action. The second possibility might be that the assays used to measure signaling simply are not sensitive enough to pick up possibly small but significant changes in signaling. Third, signaling today is measured in muscle lysates. It might be that the subcellular localization of the signaling intermediaries is important for action, and this is completely missed by homogenizing the muscle before analysis. Finally, an obvious possibility is that the important signaling related to exercise-induced increased insulin sensitivity is downstream of the Akt/GSK3 step, which is as far down as the signaling cascade presently is revealed.

Recent human (11) and rodent (72) data suggest that insulin in rested muscle may increase microvascular blood volume (capillary recruitment) even in the absence of changes in total blood flow. Thus it is likely that improved delivery and distribution of both insulin and glucose may enhance insulin action on muscle glucose uptake in vivo. Especially after exercise, improved insulin-mediated capillary perfusion might be of importance for increased insulin sensitivity and could potentially explain the faster activation of glucose transport and of GS in the exercised compared with the rested leg (79, 80). If so, one might also expect a faster activation of the insulin signaling cascade, which, however, was not a general finding (79, 80). Therefore, the effect of previous exercise on capillary perfusion during insulin stimulation awaits direct experimental evidence. Evidence for a blood flow-independent effect of exercise on insulin action is the observation that insulin action on glucose uptake and transport is also increased after contraction in perfused rat muscles (59) in the absence of increased muscle perfusion (62) as well as in the isolated and incubated muscle preparation (23). Thus cellular changes in the exercised muscles seem to prime the glucose transport system and GS for activation when subsequently stimulated by insulin. In incubated muscle isolated from swim-exercised rats, enhanced insulin-stimulated recruitment of glucose transporter proteins (GLUT-4) to the plasma membrane was evident; this seems to be the major mechanism responsible for the enhanced insulin-stimulated glucose transport after exercise (27).

Nonoxidative glucose metabolism is the predominant pathway by which exercised muscles handle the increased amount of glucose taken up (7, 45, 51). This is due to at least two different mechanisms increasing GS activity. The first is increased insulin-induced activation of GS after exercise, which has been observed in both rodents and humans (59, 79). The second is
increased GS activity because of glycogen depletion, as discussed above in EFFECTS OF EXERCISE/CONTRACTION ON MUSCLE GLUCOSE TRANSPORT AND GS ACTIVITY. The interesting thing about GS activation and exercise is that GS activity is tightly coupled to glycogen concentration (48) and therefore is increased after exercise for as long as it takes to replenish muscle glycogen. In this respect, the response is much different from the response of glucose transport, which always decreases rapidly and markedly (but not necessarily completely) after exercise, even in the absence of glycogen repletion (reviewed Ref. 57).

It has long been known that exercise-induced changes in insulin sensitivity of muscle glucose transport determined in vitro are linked to carbohydrate availability in the period after exercise. Thus, in rodent muscle, carbohydrate deprivation after exercise extends the period of improved insulin sensitivity (10, 26). In humans, intake of 100 g of glucose 3 h after cycle exercise eliminated the increase in whole body insulin action 12 h later (7). In this context, it is noteworthy that in both human and rodent muscle insulin’s ability to activate the glucose transport process is enhanced when glycogen storage is decreased. For example, in rodents, glycogen levels have been manipulated by exercise and diet in the days up to the experiment. In both perfused and incubated muscles, enhanced stimulatory effects of insulin on glucose transport/GLUT-4 recruitment were found when glycogen levels were decreased below normal, whereas decreased insulin action was observed when glycogen was supercompensated (16, 32, 33). When glycogen content is decreased in human muscle by dynamic knee extensions and insulin action subsequently is evaluated by the hyperinsulinemic euglycemic clamp technique, the increase in glucose uptake is correlated to the amount of glycogen used during the exercise bout (58). Furthermore, muscle glycogen content also affects the insulin-induced GS activity and rate of glycogen synthesis. Thus an inverse relationship be-
tween GS activation and muscle glycogen content exists during euglycemic hyperinsulinemia when studied 15 h after glycogen-depleting exercise or a similar period of rest (7). In addition, glycogen-dependent insulin-induced GS activation has been observed in humans after exercise when the endogenous insulin concentration was elevated in response to a meal (85). However, from these studies, it is difficult to distinguish between the role of glycogen and the role of enhanced insulin sensitivity induced by the prior exercise itself. Nevertheless, decreasing muscle glycogen concentrations by incubation with epinephrine also increases subsequent insulin sensitivity (50), supporting a role of glycogen of its own in regulation of insulin sensitivity. Patients with muscle glycogen phosphorylase deficiency (McArdle’s disease) have muscle glycogen levels in the basal state that are approximately twofold higher than those in healthy matched control subjects. Thus these patients offer the possibility to elucidate the role of high-glycogen concentrations without the confounding influence of prior exercise and diet manipulation. In the McArdle patients, the basal activity of GS was low; however, more importantly, the increase in whole body glucose clearance and GS activity in response to stimulation by a physiological insulin concentration was impaired (49). When the difference in muscle glycogen level between McArdle patients and control subjects was correlated to the difference in insulin-induced increase in GS activity between McArdle patients and control subjects, the high glycogen levels in these patients could to a great extent explain the impaired increase in insulin-induced GS activity ($r^2 = 0.91, P = 0.002$). In concordance, it has also been reported in healthy humans that insulin-stimulated glycogen synthesis measured by $^{31}$P-NMR spectroscopy was reduced in a glycogen-loaded condition (40). The reduction was not due to increased turnover of glycogen, pointing to an impaired action of GS. Thus a range of experimental evidence suggests that “simply” lowering muscle glycogen will enhance insulin’s activation of glucose transport, GS activity, and glycogen synthesis, whereas increasing glycogen concentrations will result in opposite changes.

The molecular mechanisms utilized by glycogen to influence the action of insulin are not known, but two observations suggest that changes in insulin signaling levels could be involved. In rodent muscle with lowered glycogen content, Akt is activated by insulin to a greater extent than in muscle with normal glycogen levels (15, 33). Likewise, rodent and human muscles with low glycogen content have higher basal AMPK activity (unpublished observations and Refs. 33, 60, 83). Because prior 5-aminoimidazole-4-carboxamide riboside (AICAR, a pharmacological AMPK activator) treatment leads to enhanced insulin sensitivity of glucose transport in incubated rodent muscle (21), it may be that glycogen-dependent insulin action is mediated by AMPK. Whether glycogen-induced alterations in signaling levels of Akt and AMPK are linked is unknown.

Other stimuli of glucose transport such as hypoxia, which like exercise enhances glycogenolysis, also lead to subsequent enhancement of insulin sensitivity, at least in rodent muscle (21). Nevertheless, some evidence suggests that glycogen breakdown is not necessary for enhancement of insulin action in muscle. For example, as mentioned above, AICAR treatment of incubated muscle results in enhanced insulin sensitivity of glucose transport without any measurable changes in glycogen content (21). In addition, after exercise, the increase in insulin sensitivity seems to persist beyond the point of glycogen normalization in both human (6) and rodent muscle (59). Therefore, evidence suggests that glycogen breakdown is a major but not the only contributor to enhancement of insulin sensitivity.

PERSPECTIVES

The effect of prior exercise to enhance insulin action has been well documented for 20 years and has been known as a clinical fact by diabetologists for much longer. Nevertheless, despite intense research efforts, the observation still stands fairly unexplained in terms of the molecular mechanisms involved. It is clear that the lowered glycogen content of the muscle after exercise plays a pivotal role in determining the insulin response of glucose transport and glycogen synthesis, but how this is brought about in molecular terms remains elusive. Rather disappointingly, the prevailing finding has been that postexercise-increased insulin action in muscle is not related to increased insulin signaling, as studied today in muscle lysates. Perhaps we need to look more at the spatial arrangements of the signaling molecules in the muscle cell and study whether this might be changed by exercise in a way that makes signaling more effective. Changes in insulin signaling that are important for insulin sensitivity could also lie beyond the Akt/GSK3 steps. Alternatively, because exercise, by depletion of glycogen stores, creates a state of fuel deficit in the muscle, incoming glucose is diverted to glycogenesis and incoming fatty acids primarily to oxidation (35). In this way, the conditions are quite opposite to those of lipid oversupply, recently suggested to cause insulin resistance (44, 63, 64), and therefore might in fact result in increased insulin sensitivity. Finally, activation of AMPK with AICAR also causes increased insulin sensitivity without glycogen depletion (21), and this observation suggests that targets downstream of AMPK may be involved in determining insulin sensitivity, which is another venue to explore.

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