Improved Insulin Sensitivity After Exercise: Focus on Insulin Signaling

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After a single bout of exercise, the ability of insulin to stimulate glucose uptake is markedly improved locally in the previously active muscles. This makes exercise a potent stimulus counteracting insulin resistance characterizing type 2 diabetes (T2D). It is believed that at least part of the mechanism relates to an improved ability of insulin to stimulate translocation of glucose transporters (GLUT4) to the muscle membrane after exercise. How this is accomplished is still unclear; however, an obvious possibility is that exercise interacts with the insulin signaling pathway to GLUT4 translocation allowing for a more potent insulin response. Parallel to unraveling of the insulin signaling cascade, this has been investigated within the past 25 years. Reviewing existing studies clearly indicates that improved insulin action can occur independent of interactions with proximal insulin signaling. In contrast, more recent observations indicate that interactions exist at the distal signaling level of AS160 and atypical protein kinase C (aPKC). Although the functional interpretation is lacking, these novel observations may present a breakthrough in understanding the beneficial interplay between exercise and insulin action.

The prevalence of type 2 diabetes (T2D) is currently reaching epidemic proportions, not only in western countries, but throughout the world (1). In Europe and the United States, ~5–8% of the adult population is estimated to suffer from this disease, and in genetically prone subpopulations, the prevalence may be as high as ~50% (2). One of the first clinical traits of T2D is impaired ability of insulin to adequately regulate glucose homeostasis due to insulin resistance in multiple tissues. Skeletal muscle constitutes ~40% of human body mass and has been reported to account for ~50–75% of insulin-stimulated glucose uptake. From a quantitative perspective, skeletal muscle is therefore considered the most important tissue in regard to insulin-stimulated glucose disposal, and correspondingly maintenance of glucose homeostasis. Based on epidemiological evidence, T2D is strongly associated with adiposity and lack of physical activity. Conversely, reduced adiposity induced by diet interventions combined with physical training programs is considered a cornerstone in prevention and treatment of T2D (3). Clearly, increased energy turnover in response to exercise is a component in initiating and maintaining weight loss. However, independent of changes in adiposity, exercise has repeatedly been shown to improve regulation of glucose homeostasis in both healthy and T2D subjects (4,5). Considering the prevalence of T2D, much research interest is focused on elucidating the underlying molecular mechanism explaining this beneficial interaction between exercise and insulin action in skeletal muscle. Not only will this help designing the best exercise strategies in regard to prevent or treat T2D, but furthermore, this knowledge is valuable in regard to developing pharmaceutical and eventually genetic approaches to treat the disease. This article reviews the ongoing progress in defining the interaction between acute exercise and insulin-stimulated glucose uptake—with particular focus on the insulin signaling pathway.

IMPROVED INSULIN ACTION AFTER ACUTE EXERCISE

Improved insulin action after acute exercise was first demonstrated in 1982 in perfused isolated rat hindquarters. In that study, we observed that after treadmill exercise, the ability of insulin to stimulate glucose uptake as well as glycogen synthesis was markedly increased (6). Furthermore, the effect of prior exercise was mainly observed in glycogen-depleted muscle suggesting that this phenomenon is locally related to muscle actually contracting during exercise (7). Subsequently, it was demonstrated that following exercise, a residual effect on glucose uptake was still measured 1 h after termination of exercise even in the absence of insulin, whereas 2.5 h after exercise, increased glucose uptake was only observed in the presence of insulin (8,9). Within the past 25 years, characterizing the beneficial interaction between acute exercise and subsequent insulin action has been an area of much focus; although progress has been made recently, the underlying mechanisms are still poorly understood.

IMPROVED INSULIN ACTION IS MAINLY AN INTRACELLULAR PHENOMENON

Consistent with the early observations that improved insulin sensitivity is mainly observed in glycogen-depleted muscle, it is believed that this effect is due to a local contraction-induced mechanism. This interpretation is supported by the

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subsequent finding that improved insulin sensitivity in rodents is still observed after *in situ* contraction of one leg by electrical stimulation of the sciatic nerve followed by measurement of insulin sensitivity in both legs by hindquarter perfusion (7). Similarly, we have more recently repeated these findings in humans in response to one-legged exercise followed by measurement of insulin-stimulated glucose uptake in both legs (10–13). Because improved insulin sensitivity still exists when measured *in vitro* after *in vivo* exercise, intact systemic delivery does not appear to be a necessary component (14,15). In fact, intact systemic delivery is not necessary even during exercise because improved insulin sensitivity can be observed after *in vitro* contractions in serum (16). Interestingly, in a matching *in vitro* study, improved insulin sensitivity was not observed when muscle was incubated in Krebs–Henseleit bicarbonate buffer. This shows that the presence of a serum factor (likely a protein) is pivotal for contractions to improve insulin sensitivity (17). From the above mentioned, it seems evident that improved insulin action after exercise at least to a large extent relates to a local intracellular phenomenon, despite the requirement of systemically circulating protein. Consistent with this interpretation, based on exofacial photolabeling of GLUT4 in rodents, improved insulin-stimulated glucose uptake 2.5 h after exercise seems to be accounted for by changes in membrane content of GLUT4 (18,19).

INTRACELLULAR ADAPTATIONS LEADING TO IMPROVED GLUT4 TRANSLOCATION

When elucidating on intracellular mechanisms leading to improved insulin-stimulated GLUT4 translocation, two main sites of regulation may be involved. Either exercise interacts with the insulin signaling pathway stimulating this process or alternatively, exercise interacts with the GLUT4 translocation machinery allowing for the same insulin signal to recruit more GLUT4 to the membrane. In **Figure 1**, insulin signaling to glucose uptake in skeletal muscle is illustrated. In the following sections, interactions between exercise and insulin signaling will be evaluated focusing on the time point 3–4 h after exercise, when the acute effect of exercise on glucose uptake is reversed but before major alterations in protein composition of muscle should be expected.

INSULIN SIGNALING 3–4 HOURS AFTER EXERCISE

Proximal insulin signaling involves binding of insulin to the insulin receptor (IR). This leads to increased IR tyrosine phosphorylation, IR tyrosine kinase activity, IR substrate-1 (IRS-1) tyrosine phosphorylation, and IRS-1-associated phosphatidylinositol-3 kinase activity (see **Figure 1**). This signaling sequence does not appear to be acutely regulated by exercise or contractions either *in vitro* or *in vivo* (20,21). Despite this, prior exercise may alter the response to subsequent insulin stimulation. In a series of studies evaluating insulin action 3–4 h after termination of one-legged knee-extensor exercise, we have demonstrated that prior exercise does not improve insulin clearance, IR tyrosine kinase activity, IRS-1 tyrosine phosphorylation, IRS-1-associated phosphatidylinositol-3 kinase activity, Akt Ser^{473} phosphorylation, Akt thr^{308} phosphorylation, glycogen synthase kinase-3 (GSK-3)α Ser^{21} phosphorylation, or GSK-3 activity despite markedly increased glucose uptake in response to physiological insulin stimulation (11–13,22). In those studies, it was further demonstrated that in contrast to a maximal insulin stimulus in rodent muscle (23–25), physiological insulin

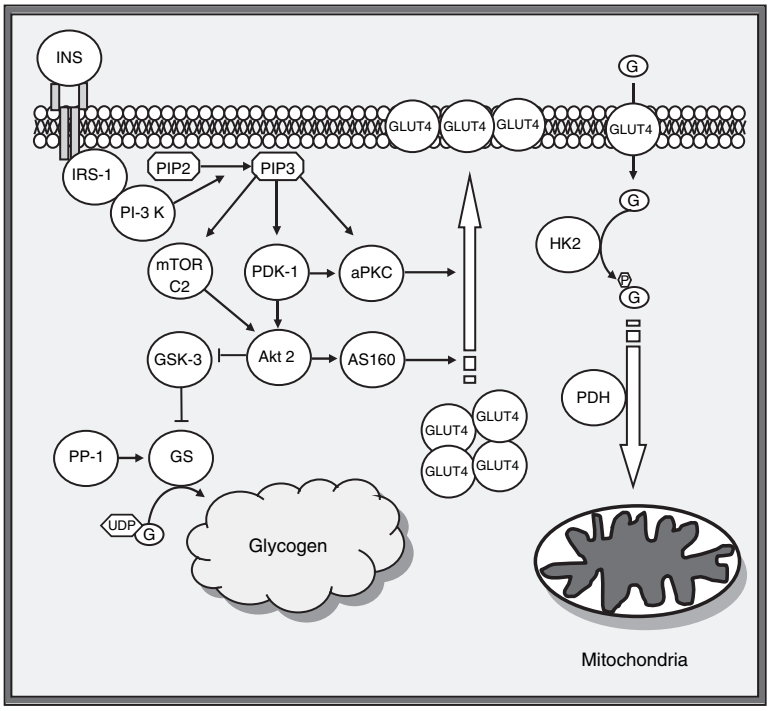

Figure 1 Insulin signaling to GLUT4 translocation and stimulation of glucose uptake.

stimulation in humans leads to a sustained activation of insulin signaling. Thus, based on the available data from human studies, 3–4 h after exercise, improved insulin-stimulated glucose uptake is not associated with improved proximal insulin signaling from the association of insulin to the receptor to activation of phosphatidylinositol-3 kinase. Furthermore, based on phosphorylation markers, neither Akt, nor PDK-1, nor mTORC2, the two upstream kinases responsible for phosphorylation of Akt seem to be more potently regulated by insulin after exercise (11,13). This would also be consistent with normal Ser^{21} phosphorylation and deactivation of GSK-3 as observed. It should be emphasized that these observations are based on *in vitro* analyses on whole muscle preparations. Thus, it cannot be ruled out if interactions localized in specific cellular compartments are not detected. However, the consistency of the findings as well as the repeated use of site-specific phosphorylations as markers of *in vivo* upstream regulation support this interpretation. In this context, it has been speculated whether exercise results in alterations in subcellular localization of insulin signaling molecules allowing for more potent distal actions (26). Consistent with this proposal, IRS proteins have been detected in plasma membrane, nucleus, and a cytosol fraction. However, when evaluating protein content in these fractions of IR, IRS-1, Akt, and GSK-3, no changes in localization were observed in response to 1 h of cycling (~70% VO_2 peak) (26). Collectively, if prior exercise results in positive interaction with insulin signaling to GLUT4 translocation in human skeletal muscle, this most likely occurs at a signaling step not yet investigated.

THE SEARCH FOR NEW CANDIDATES

Within recent years, several novel insulin signaling molecules have been characterized as downstream of phosphatidylinositol-3 kinase. In particular, emerging evidence suggests that atypical protein kinase C (aPKC) and "Akt substrate of 160 kDa" (AS160) play key roles for normal insulin-stimulated glucose uptake as reviewed (27,28). As described, acute exercise has not been observed to regulate proximal insulin signaling adding support to the concept of two conceptually different signaling cascades stimulating GLUT4 translocation. This view is further supported by the observation of distinct pools of GLUT4 with different potential for translocation in response to insulin and exercise allowing for an additive effect of these stimuli on GLUT4 translocation and glucose uptake. However, as understanding of intracellular signaling to GLUT4 translocation is evolving, it has become clear that signaling is not a linearly propagating process, and furthermore, that several branches of signaling probably have to be coordinated for normal GLUT4 translocation to occur. This allows for the speculation that some branches of insulin and exercise signaling may converge at distal levels.

AS160 AS A CONVERGING SIGNALING MOLECULE

Within the past years, several potential target molecules downstream of Akt have been identified, including Synip and PIKfyve (29–31). In particular, an important role of AS160 in regulation of insulin-stimulated glucose uptake has become evident as reviewed (27). Presently, the model of regulation of AS160 suggests that AS160 in the basal nonphosphorylated state acts as a brake inhibiting GLUT4 translocation. When phosphorylated by Akt in response to insulin stimulation, a Rab GTPase–activating protein domain on AS160 is inactivated, allowing for activation of one or more Rab molecules, likely including Rab8A, 10, and 14. This model is intriguing because Rab molecules have previously been shown to play crucial roles in regard to vesicle docking and fusion (32–34); thus, AS160 may be the first molecule identified to be directly involved in transferring the insulin signal to GLUT4 membrane-trafficking events. In response to insulin stimulation, AS160 is phosphorylated on five of six phospho-Akt substrate motifs (RXRXXS*/T*) (Ser^{318}, Ser^{570}, Ser^{588}, Thr^{642}, and Thr^{571}) (29). Using site-specific antibodies, results from our laboratory have confirmed that in human skeletal muscle, all five sites are phosphorylated in response to physiological insulin stimulation (35). Interestingly, it appears that phosphorylation of AS160 is also involved in the signal leading to contraction-stimulated glucose uptake in rodent muscle, at least in part mediated by AMPK (36,37). However, extrapolation to humans should be done with caution particularly because AS160 phosphorylation is observed in response to low (but not high) exercise intensities in humans and not as an early response to exercise initiation (38,39). These observations do not support that AS160 phosphorylation is necessary for exercise-induced glucose uptake in muscle.

Disregarding the role of AS160 in exercise-stimulated glucose uptake, it is interesting to note that moderate exercise induces a time-dependent effect on AS160 phosphorylation in muscle. This delayed effect of exercise on AS160 phosphorylation could be of importance in regard to subsequent insulin-stimulated glucose uptake. In fact, 4 h after a 2-h swim in rat epitrochlearis muscle, glucose uptake in response to a physiological insulin dose is significantly increased coinciding with increased basal as well as insulin-stimulated AS160 phosphorylation (40). In that study, a greater Thr^{308} phosphorylation of Akt was observed. As described, Akt does not appear to be activated to a greater extent in human skeletal muscle in response to insulin stimulation 4 h after acute exercise (11,13). However, data from our laboratory show that in human muscle, prior exercise leads to increased basal as well as insulin-stimulated site-specific phosphorylation on Ser^{318}, Ser^{570}, Ser^{588}, and Thr^{571} (35). Considering the role of AS160 for insulin-stimulated glucose uptake, these findings may well be important in regard to improving insulin sensitivity at this time point. It should be emphasized that the effects of exercise on AS160 phosphorylation are of a minor magnitude (10–20%), whereas insulin-stimulated glucose uptake is increased by ~70% in the previously exercised muscle. Thus, either AS160 phosphorylation is not linearly related to glucose uptake or spatial localization of a subfraction of AS160 proteins results in altered functional impact. Alternatively, the observed additive effect of prior exercise and AS160 phosphorylation is not causally linked to improved insulin-stimulated glucose uptake.

APKC AS A CONVERGING SIGNALING MOLECULE

In response to insulin stimulation, activation of aPKC is thought to involve both allosteric binding of PIP3 in the vicinity of the membrane as well as phosphorylation of Thr^{410} by PDK-1 collectively leading to autophosphorylation on Thr^{560} (41–43). These observations are primarily based on studies in rat adipocytes, and thus, *in vivo* activation of aPKC in skeletal muscle and human skeletal muscle in particular is not well documented. The precise role of aPKC in regulating GLUT4 translocation is still at an evolving state; however, several lines of evidence suggest that parallel to Akt, activation of aPKC is critical in both the process of translocation and docking/fusion of GLUT4 to the plasma membrane (44).

In contrast to the volume of information on insulin-stimulated aPKC activation, only a few studies have looked into exercise regulation of aPKC. In human skeletal muscle, *in vitro* aPKC activity is increased in response to acute exercise (45–48), although not in an exercise intensity–dependent manner when the activity of aPKC is evaluated *in vitro* (48). Whether this truly reflects *in vivo* regulation is not established, but this suggests that activation of aPKC in response to exercise may only be part of the signal leading to glucose uptake.

Considering the important role of aPKC for insulin-stimulated glucose uptake and the observation that aPKC is activated in response to exercise makes aPKC another potential candidate linking exercise and insulin signaling. We recently evaluated this 4 h after exercise, using the one-legged knee-extension protocol (11). Interestingly, in that study, we observed that immunoprecipitated aPKC from previously exercised muscle was more potently activated by the allosteric activator PIP3 when compared to rested muscle (**Figure 2**). Changes in PIP3 responsiveness under these conditions should be considered as a consequence of altered functional properties of the enzyme. Furthermore, because increased PIP3 responsiveness is observed after immunoprecipitation including several washing steps, the interaction seems robust and thus may be of a covalent nature. Interestingly, PIP3 stimulation leads to increased Thr^{560} autophosphorylation of purified aPKC when investigated *in vitro* (49). An obvious target of future investigations in this regard is the impact of prior exercise on Thr^{560} phosphorylation during insulin stimulation *in vivo*. However, functional antibodies targeting this site on human skeletal muscle aPKC are not available. Currently, no absolute measure of PIP3 concentrations in skeletal muscle exists, but if improved responsiveness of aPKC to PIP3 is supposed to play a physiologically relevant role, it would be expected to also reflect on aPKC activity in response to insulin stimulation. Curiously, we do not observe this. However, based on a ~40% greater ($P = 0.17$) delta increase in aPKC activity in exercised muscle in response to insulin stimulation, it could be hypothesized that sensitivity of the *in vitro* assay does not allow for detection of improved PIP3 responsiveness within a lower physiological range of PIP3 concentrations.

SUMMARY AND PERSPECTIVES

The effect of acute exercise to improve insulin-stimulated glucose uptake has been known for >25 years; however, despite considerable scientific interest, the underlying molecular mechanisms are still poorly understood. The present consensus is that improved insulin action should primarily be ascribed to increased translocation of GLUT4 to the membrane. This does not rule out that other coinciding adaptations may be involved. Thus, improved capacity for delivery and intracellular metabolism of glucose may be necessary coadaptations in order to optimally benefit from improved insulin-stimulated glucose membrane permeability after exercise. In this context, a well-described effect of exercise is to reduce glycogen content in muscle. As previously reviewed (50), this not only leads to a marked increase in glycogen synthase activity *per se*, but also the effect of insulin to further increase glycogen synthase activity is improved under these conditions (6,13). In order to understand how insulin-stimulated GLUT4 translocation might be improved after exercise, an obvious strategy has been to evaluate whether interactions exist between prior exercise and the signaling pathway utilized by insulin to stimulate GLUT4 translocation. Within the past 10 years, we have examined this possibility

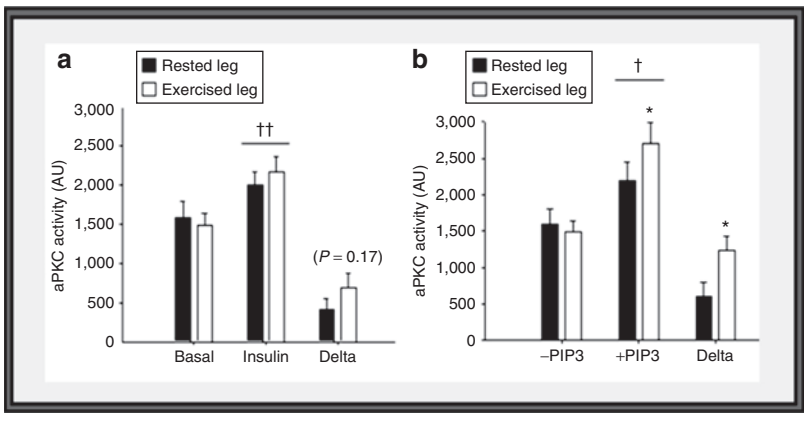

Figure 2 aPKC activity measured *in vitro*. (**a**) aPKC activity in basal and insulin-stimulated samples. (**b**) aPKC activity in basal samples, with or without addition of 10 µmol/l PIP3. Black bars are values in the rested leg. White bars are values in the previously exercised leg. $^{††}P < 0.005$ vs. basal values. $^{†}P < 0.05$ vs. basal values. $^{*}P < 0.05$ vs. response to PIP3 in the rested leg. Values are means ± s.e., $N = 12$. AU, arbitrary units. (Reproduced from ref. 11.)

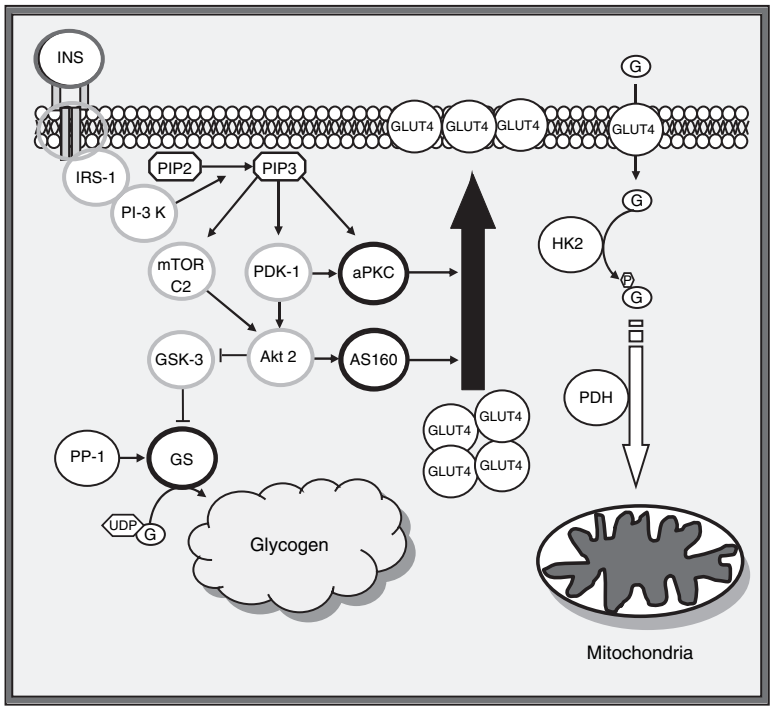

Figure 3 Insulin signaling to GLUT4 translocation and stimulation of glucose uptake after prior exercise. Gray circles: signaling not influenced by exercise. Bold black circles: Signaling more potently regulated after exercise. Normal black circles: Signaling components not evaluated.

locally in human muscle inspired by the parallel unraveling of the insulin signaling cascade. As illustrated in **Figure 3**, it seems evident that proximal insulin signaling is not influenced by prior exercise when evaluated 3–4 h after termination of exercise—a time point where insulin-stimulated glucose uptake is markedly improved. In contrast, interactions appear to exist in regard to regulation of AS160 and aPKC, proteins likely involved in both the GLUT4 translocation process as well as the process of docking/fusion of GLUT4 to the membrane. This may explain how more GLUT4 can be translocated to the muscle membrane in response to the same proximal insulin signal after exercise. Clearly, a causal relation needs to be established, and furthermore, the exercise-induced signal responsible for these interactions needs to be identified. However, considering the important role of both AS160 and aPKC for insulin-stimulated glucose uptake, these observations should inspire future research.

DISCLOSURE

The authors declared no conflict of interest.

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