Mechanisms for greater insulin-stimulated glucose uptake in normal and insulin-resistant skeletal muscle after acute exercise

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Insulin and exercise can independently increase glucose uptake by skeletal muscle. The increased uptake coincides with greater Akt substrate of 160 kDa (AS160) phosphorylation without improved proximal insulin signaling at steps from insulin receptor binding to Akt activity. Causality remains to be established between greater AS160 phosphorylation and improved ISGU. The end effector for normal individuals is increased GLUT4 translocation, but this remains untested for insulin-resistant individuals postexercise. Following exercise, insulin-resistant individuals can attain ISGU values similar to nonexercising healthy controls, but after a comparable exercise protocol performed by both groups, ISGU for the insulin-resistant group has been consistently reported to be below postexercise values for the healthy group. Further research is required to fully understand the mechanisms underlying the improved postexercise ISGU in individuals with normal or subnormal insulin sensitivity and to explain the disparity between these groups after similar exercise.

Importance of Insulin-Stimulated Glucose Uptake by Skeletal Muscle

Understanding the mechanisms regulating insulin-stimulated glucose uptake by skeletal muscle is important because 1) muscle accounts for most insulin-mediated glucose disposal (21) and 2) muscle insulin resistance is a key defect in the progression to type 2 diabetes mellitus (20, 43, 97). Even in nondiabetic individuals, insulin resistance increases the risk for atherogenesis, cardiovascular disease, hypertension, cognitive dysfunction, and some cancers (27, 45, 69).

Insulin and exercise can independently increase glucose uptake secondary to redistribution of GLUT4 glucose transporters from the cell interior to cell surface membranes (CSM) (19, 25, 93). Elevated insulin-independent glucose uptake during exercise is mostly reversed by 2–3 h postexercise, whereas enhanced muscle and whole body insulin sensitivity, detectable at 1–4 h postexercise, can persist for up to 24–48 h (1, 12, 13, 79, 88, 92, 124).

Exercise capacity is related to muscle glycogen availability, and glycogen stores are diminished by vigorous exercise (59). Increased insulin-stimulated glucose uptake and enhanced glycogen synthase activity restore glycogen to preexercise levels. With sufficient postexercise dietary carbohydrate, muscle glycogen can be “supercompensated” above typical values, providing a performance advantage (13, 59). Because greater muscle glycogen stores can improve endurance capacity, it seems reasonable to speculate that from an evolutionary perspective, the processes leading to glycogen supercompensation might have conferred survival advantages (58).

In vivo insulin-stimulated glucose uptake depends on many factors, including capillary blood flow, endocrine and neural inputs, glucose transport by the GLUT4 glucose transporter, and glucose metabolism. This review extends earlier reviews that have focused on acute exercise effects on insulin-stimulated GLUT4 translocation and glucose uptake (33, 49, 52, 75, 128, 130) by including more recently published information, proposing a new model for elucidating acute exercise effects.
and comparing exercise effects on individuals with normal vs. subnormal insulin sensitivity.

**Enhanced Insulin-Stimulated Glucose Uptake by Skeletal Muscle Postexercise**

Richter et al. (92) discovered that insulin-stimulated glucose uptake of muscle, measured by perfused hindlimb model using arterial-venous glucose differences and muscle accumulation of radiolabeled glucose analogs, is substantially elevated after acute exercise (Fig. 1). Acute exercise can also elevate in vivo insulin-stimulated glucose disposal assessed by the euglycemic hyperinsulinemic clamp in humans (4, 79, 103, 125), rats (81), dogs (87), and sheep (77).

To determine human muscle glucose uptake, Pehmöller et al. (86), Richter et al. (94), Treebak et al. (118), and Wojtaszewski and colleagues (126, 127) measured quadriceps muscle blood flow and arterial-venous glucose concentration difference (Fig. 1). Relying on one-legged knee extension exercise, they demonstrated greater insulin-stimulated glucose uptake vs. nonexercised contralateral muscle at ~3–10 h postexercise. Perséghin et al. (88), using a hyperglycemic hyperinsulinemic clamp with labeled glucose infusion and nuclear magnetic resonance, found greater insulin-stimulated muscle glucose uptake in humans at 48 h postexercise vs. unexercised values.

Prior acute exercise leads to greater insulin-stimulated uptake of radiolabeled glucose analogs by isolated muscles from rat or mice (1, 12–14, 31, 37, 38, 44, 46, 47, 101, 102, 124). Isolated muscle experiments demonstrate that exercise effects (~3 to 48 h postexercise) do not require altered blood flow or other systemic factors. Comparable exercise-induced increases using 3-O-methylglucose (phosphorylated by hexokinase) vs. 2-deoxyglucose (phosphorylated by hexokinase) indicate that exercise regulates glucose transport per se. Although these experiments demonstrate that prior exercise can directly regulate subsequent insulin-stimulated glucose transport in isolated muscles, it remains possible that prior exercise can also influence other processes that enhance in vivo glucose uptake, including elevated muscle blood flow and hexokinase activity (36, 93).

**Enhanced Insulin-stimulated Glucose Uptake After Ex Vivo Contractions in Serum**

The complexity of in vivo exercise makes it challenging to identify the essential events for increased glucose uptake. To focus on contraction independent of systemic factors, isolated rat muscles were electrically stimulated to contract and allowed to recover for 3 h before insulin-stimulated glucose uptake was measured (12). Surprisingly, postcontraction insulin-stimulated glucose uptake did not exceed resting controls, suggesting that a humoral factor is required for the in vivo exercise effect. The lack of increased insulin-stimulated glucose uptake after prior contraction by isolated muscles in the absence of serum has been confirmed in several subsequent studies (26, 37, 39). Gao et al. (39) performed a similar experiment, except that contractions were performed in serum. Contractions in serum caused greater insulin-stimulated glucose uptake that apparently required a serum protein that remains unidentified.

It is important to note that in the experiments using muscles stimulated to contract ex vivo, the presence of serum during contraction was essential for the postcontraction increase in insulin-stimulated glucose uptake, but serum’s presence was not required during the postcontraction period or during the glucose uptake measurement.

Funai et al. (37) measured insulin-stimulated glucose uptake in muscles from rats that performed in vivo exercise, followed by their isolated muscles being stimulated to contract in serum. Exercise plus ex vivo contraction had an additive effect on subsequent insulin-mediated glucose uptake, and they interpreted these results to suggest that each stimulus may rely on distinct mechanisms to increase insulin sensitivity. An alternative interpretation is that the intense ex vivo contraction protocol would be predicted to recruit all muscle fibers, but that might not be true after the in vivo exercise protocol. If these assumptions are true, they may help explain the additive effect of exercise followed by contractions on subsequent insulin-stimulated glucose uptake. This scenario seems logical, but some additional observations from the same study (37) should be considered when interpreting the results. Although increasing exercise duration from 1 to 2 h would also be expected to cause greater muscle fiber recruitment, postexercise insulin-stimulated glucose uptake was not different after 1 vs. 2 h of exercise. In addition, insulin-stimulated glucose uptake was not greater for muscles after ex vivo contraction in serum compared with prior in vivo exercise, as might be predicted if greater recruitment by ex vivo contraction accounted for the additive glucose uptake with combined in vivo exercise and ex vivo contractions. The available evidence does not provide a conclusive explanation for the additive effects of prior in vivo exercise.
exercise and ex vivo contraction in serum on subsequent insulin-stimulated glucose uptake.

**Insulin Signaling and GLUT4 Vesicle Trafficking**

Recent reviews summarize the processes accounting for insulin-stimulated glucose transport (6, 32, 65, 70, 96, 108) (Fig. 2). In brief, insulin binds to its receptor, activating receptor kinase activity, and autophosphorylation of specific tyrosine residues, leading to greater tyrosine phosphorylation of insulin receptor substrate (IRS) proteins. IRS-1 is the predominant IRS isoform in skeletal muscle, with IRS-2 expressed at lower abundance. Tyrosine-phosphorylated IRS engages phosphatidylinositol 3-kinase (PI3K), resulting in the Ser/Thr kinase Akt2 being phosphorylated on Thr308 by phosphoinositide kinase-1 and Ser474 by mechanistic target of rapamycin 2. Of the three Akt isoforms (Akt1, Akt2, and Akt3), Akt2 is most important for insulin-stimulated glucose transport. Akt2 catalyzes Ser/Thr phosphorylation of many Akt substrates, including the early endosomes, the endosomal recycling compartment, and the trans-Golgi network, before returning back to the GSV. One GLUT4 molecule can recycle multiple times between the cell interior and CSM (32, 70).

Akt2-dependent events other than AS160 phosphorylation are required for insulin-stimulated glucose transport (42). The Ral-GAP complex (RGC) is a heterodimer composed of a regulatory subunit known as RGC1 (also called Ral-GAPβ) and a catalytic subunit known as RGC2 (70, 71). Ral-GAPα1 (also called GRNL1) is the RGC2 highly expressed in skeletal muscle (17, 70). Akt2-dependent phosphorylation of the catalytic subunit reduces Ral-GAPα1 activity against the small GTPase RalA, favoring GTP-bound RalA and targeting of GLUT4 to CSM (16, 17, 70, 112).

Insulin also stimulates GTP loading and activation of Rac1, a Rho-family GTase, by a PI3K-dependent and Akt-independent mechanism (18, 110, 111). Rac1 modulates nonsarcomeric actin-related proteins 2/3 that modify actin polymerization and cofillin that influences actin depolymerization (18). Activated Rac1 also promotes the autophosphorylation and activation of p21-activated kinase 1, which in turn phosphorylates multiple proteins that regulate actin dynamics (18, 57). Re-modeling actin and other microtubule-associated proteins influences insulin signaling and GLUT4 vesicle trafficking by mechanisms that are not fully understood (51).

Insulin signaling regulates the multistep exocytosis of GLUT4 storage vesicles (GSV) via 1) release from internal retention, 2) translocation using cytoskeletal-mediated processes, 3) tethering and docking to CSM, and 4) fusion with CSM (96). GLUT4 in CSM is internalized by cholesterol-dependent endocytosis or clathrin-mediated endocytosis, with the latter reportedly more important in muscle (32, 70). Insulin substantially elevates GLUT4 exocytosis, but there is some controversy if insulin also slows GLUT4 endocytosis (55, 62). Internalized GLUT4 can cycle through several locations, including the early endosomes, the endosomal recycling compartment, and the trans-Golgi network, before returning back to the GSV. One GLUT4 molecule can recycle multiple times between the cell interior and CSM (32, 70).

**Model for Greater Insulin-stimulated Glucose Uptake Postexercise**

The following model aims to encourage new ideas about the processes underlying increased insulin sensitivity postexercise. Triggers are initiating events that activate subsequent memory elements, which store information that is relayed to the mediator, which translates the memory into action by controlling an element, which store information that is relayed to the mediator. This model is based on a preexisting model for ischemic preconditioning of the heart whereby a few minutes of reduced cardiac blood flow produces 72 h of protection against damage to the heart upon subsequent ischemic challenge (131). A similar model is used because these disparate processes share similar temporal fea-

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**Fig. 2.** Insulin-signaling pathway for insulin-stimulated GLUT4 translocation and glucose transport in skeletal muscle. IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 2-phosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PAK1, p21-activated kinase 1; PKC, protein-kinase C; RalA, RalGTPase-activating protein-α1; Ral-GAP, Ral-GTPase-activating protein; GLUT4, glucose transporter 4; GSV, GLUT4 storage vesicle.
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was not essential for increased insulin sensitivity. Similar muscle glycogen concentration, indicating that glycogenolysis consequently increased insulin sensitivity. AICAR did not alter imidazoleacarboxamide riboside (AICAR; a compound that glycogen’s inhibitory effect on AMPK activity (106). Binding site (76, 89), but there are conflicting reports regarding activity. Interestingly, the improve insulin action, suggesting that prior AMPK activation AMPK, but some protocols that stimulated AMPK did not increased insulin-stimulated glucose uptake also stimulated that each of the multiple in situ contractile protocols that insulin sensitivity (1, 46, 67, 101–103). Kim et al. (63) found (AMPK) is frequently characterized by subsequently improved insulin sensitivity that is independent of exercise. Insulin-stimulated glucose transport in muscle (66, 82). These stimulations. Electrically stimulated contraction by isolated muscle in contractions, but these models do not perfectly replicate in vivo glycogen levels in rats can be approximately two- to fourfold greater for rats compared with mice (13, 22, 46, 74, 116, 126).

The relationship between glycogen and insulin sensitivity can be uncoupled, at least after artificially induced contractions. Electrically stimulated contraction by isolated muscle in a serum-free buffer reduces glycogen (3) without enhancing insulin-stimulated glucose transport (12). Kim et al. (63), using 20 different in situ contraction protocols, found that glycogen was lowered by each protocol that produced greater insulin-stimulated glucose uptake, but glycogen was also similarly reduced by several protocols that did not increase insulin-stimulated glucose transport. The decline in glycogen and increase in insulin-stimulated glucose transport were not significantly correlated. Reduced glycogen is apparently insufficient for improved insulin sensitivity after ex vivo or in situ contraction, but these models do not perfectly replicate in vivo exercise.

Another approach to probe glycogen’s relationship with insulin sensitivity is to inject unexercised rats with epinephrine to activate glycogenolysis. This protocol elevates subsequent insulin-stimulated glucose transport in muscle (66, 82). These experiments demonstrated a relationship between glycogen and insulin sensitivity that is independent of exercise.

Exercise that stimulates AMP-activated protein kinase (AMPK) is frequently characterized by subsequently improved insulin sensitivity (1, 46, 67, 101–103). Kim et al. (63) found that each of the multiple in situ contractile protocols that increased insulin-stimulated glucose uptake also stimulated AMPK, but some protocols that stimulated AMPK did not improve insulin action, suggesting that prior AMPK activation may be necessary but not sufficient for elevated insulin sensitivity. Interestingly, the β-subunit of AMPK has a glycogen-binding site (76, 89), but there are conflicting reports regarding glycogen’s inhibitory effect on AMPK activity (106).

Fisher et al. (31) incubated rat muscle with 5-amino-4-imidazoleacarboxamide riboside (AICAR; a compound that can lead to AMPK stimulation) with serum and found subsequently increased insulin sensitivity. AICAR did not alter muscle glycogen concentration, indicating that glycogenolysis was not essential for increased insulin sensitivity. Similar results were reported for isolated mouse extensor digitorum longus (EDL) muscles incubated with AICAR plus serum (64). To test whether AMPK activation is essential for improved insulin sensitivity, experiments were performed using EDL muscles from mice genetically deficient in AMPK. Prior AICAR treatment did not produce enhanced insulin-stimulated glucose uptake in AMPK-deficient mice. Although intriguing, AICAR experiments do not directly test exercise-related mechanisms.

Immediately after acute exercise, AS160 phosphorylation was increased on Ser588 and Thr642 in rat epitrachelaris muscle in the absence of added insulin (14, 38, 101) and on Ser341, Ser588, Thr642, and Ser704 in human skeletal muscle without insulin infusion (119). Therefore, it is possible that increased insulin-independent AS160 phosphorylation may be a trigger for increased insulin sensitivity when muscle is subsequently stimulated by insulin.

Geiger et al. (41) evaluated the effects of exposing isolated muscle to a supraphysiological insulin dose and serum, removing the insulin and serum for 3 h, and finally restimulating the muscle with a physiological insulin dose. Remarkably, muscles were more sensitive to the physiological insulin dose after exposure to supraphysiological insulin. They hypothesized that elevated insulin sensitivity was secondary to GLUT4 recruitment to CSM by various stimuli (including exercise, AICAR, and insulin), followed by GLUT4 internalization to an unspecified intracellular location, where GLUT4 is more susceptible to exocytosis upon exposure to a physiological insulin concentration. These provocative results suggest that various stimuli causing GLUT4 exocytosis may be considered a trigger. Apparently no subsequent studies designed to directly test this idea have been published. However, research on humans has provided information about the effects of sequential in vivo exposure of muscle to elevated circulating insulin concentration on glucose metabolism. Studying humans undergoing two sequential euglycemic hyperinsulinemic clamps that were 15 h apart, Lucidi et al. (73) found no enhancement of glucose disposal during the second clamp compared with the first clamp. Jovanovic et al. (60) studied the second-meal phenomenon, which refers to the well-documented observation that postmeal glycemia is lower after the second of two similar meals that are spaced several hours apart. They found that muscle glycogen accumulation during the 5-h period after lunch was approximately twofold greater when healthy humans had consumed breakfast 4 h earlier compared with a lunch trial without prior breakfast. The greater glycogen accumulation after the breakfast-plus-lunch trial vs. the lunch-only trial was characterized by similar insulinemia, lower glycemia, and lower nonesterified fatty acids. The results of these studies using nonexercised rats or humans are interesting (41, 60, 73), but it is unclear whether these experiments are truly relevant to the postexercise increase in insulin sensitivity.

Potential Memory Element(s)

Although the proposed model suggests that the trigger may be a transient event that reverses shortly after cessation of exercise, another possibility is that the event is relatively persistent with the dual functions of both trigger and memory element. In this context, a sustained low glycogen concentration can be considered as a potential memory element. If
sustained low glycogen is acting as a memory element, then restoring glycogen concentration would be predicted to reverse the increased insulin sensitivity. Consistent with that interpretation, earlier research demonstrated that feeding rats a high-carbohydrate diet postexercise speeds glycogen resynthesis concomitant with reversal of increased insulin-stimulated glucose uptake (13, 38). Also consistent with the possibility that low glycogen may serve as a memory element, incubation of isolated rat muscles with glucose and insulin postexercise accelerated reversal of increased insulin-stimulated glucose uptake along with glycogen supercompensation (44). Carbohydrate feeding of humans postexercise also produced glycogen repletion and eliminated the increase in insulin sensitivity (4). The magnitude of muscle glycogen decrement at 3–4 h after one-legged exercise was moderately correlated ($r^2 = 0.53$) with the insulin-stimulated glucose uptake in thigh muscles of men during a euglycemic hyperinsulinemic clamp (91). Although multiple results support the idea that sustained low glycogen may be a memory element, the evidence is coincidental and does not establish a causal relationship.

Several studies have reported that AMPK activation can reverse within 30–60 min of postexercise/contraction (54, 80, 90, 123), suggesting AMPK is an unlikely memory element, consistent with several studies that reported increased insulin-stimulated glucose uptake at ~3–4 h postexercise without persistent AMPK activation (1, 101, 129). A small increase in the phosphorylation of the AMPK substrate acetyl-CoA carboxylase-$\beta$ was detected in human muscle ~4 h after acute exercise prior to insulin infusion, but this increase was eliminated at the end of a 100-min euglycemic hyperinsulinemic clamp (34). However, AMPK activation during exercise could trigger a more persistent phosphorylation of another protein substrate that may act as a memory element.

A portion of the increase in insulin-independent AS160 phosphorylation that is evident immediately postexercise can be evident for many hours after exercise is completed. Studies using a phospho-Akt substrate antibody that appears to bind phosphorylated Thr642 have also detected a sustained increase in insulin sensitivity (86, 118) and glucose uptake have been studied in humans after one-legged exercise (86, 118). Phosphorylation of AS160 on Thr642 or Ser588 (14, 38). However, high-intensity insulin-mediated glucose uptake coincided with elevated p-AS160 Thr642 in rat muscle studied 3 or 27 h postexercise (38). Insulin-stimulated AS160 phosphorylation (p-AS160) and glucose uptake have been studied in humans after one-legged exercise (86, 118). Phosphorylation of AS160 on Ser318, Ser341, Ser588 Thr642, Ser704, and Ser751 of insulin stimulated in exercised muscle exceeded resting values (86). Muscle from the exercised leg exceeded resting levels for several hours postexercise, insulin-stimulated glucose uptake is enhanced without increasing proximal insulin signaling, including insulin receptor binding, IRS-1 tyrosine phosphorylation, IRS-1/PI3K activity, and Akt phosphorylation (72). Greater AS160 phosphorylation postexercise could trigger a more persistent phosphorylation of another protein substrate that may act as a memory element.

Another potential memory element was suggested by the hypothesis that GLUT4 recruited to CSM is subsequently internalized to an intracellular location, conferring greater susceptibility to recruitment by insulin (41). GLUT4 residing in the putative high-susceptibility site is a potential memory element. It will be challenging to experimentally determine whether the specific GLUT4 transporters initially recruited to CSM are rerecruited by the subsequent insulin stimulus.

In theory, the synthesis of new protein(s) may serve as a memory element, but isolating muscles immediately postexercise and incubating them with the protein synthesis inhibitor cycloheximide did not attenuate subsequent insulin-stimulated glucose uptake (31). Consistent with this result, greater insulin-stimulated glucose uptake several hours postexercise can occur with unaltered GLUT4 abundance (11, 14).

Potential Mediators

To identify the mediators of increased insulin sensitivity after exercise, it is necessary to study muscles under insulin-stimulated conditions. For these experiments, relying exclusively on supraphysiological insulin concentrations can be misleading. For example, many insulin signaling end points (including insulin receptor or IRS-1 tyrosine phosphorylation, phosphotyrosine-associated PI3K activity, and Akt phosphorylation) can continue to increase sharply at supraphysiological insulin doses that exceed the insulin concentration that is sufficient for maximal or nearly maximal insulin-stimulated glucose uptake (61, 68). Accordingly, caution is appropriate when interpreting insulin signaling at supraphysiological insulin doses, and the following discussion emphasizes research using physiological insulin concentrations.

Considerable evidence indicates that several hours postexercise, insulin-stimulated glucose uptake is enhanced without increasing proximal insulin signaling, including insulin receptor binding, IRS-1 tyrosine phosphorylation, IRS-1/PI3K activity, and Akt activity (Fig. 3) (5, 14, 31, 38, 47, 86, 126). In contrast, multiple studies have reported that several hours postexercise, insulin-stimulated AS160 phosphorylation and glucose uptake are enhanced (1, 10, 14, 37, 38, 86, 101, 118). Insulin-stimulated muscles from rats studied 3–4 h postexercise had elevated phosphorylated p-AS160 (p-AS160) Thr642 (14, 37, 38, 101) and p-AS160 Ser588 (14, 101). Increased insulin-mediated glucose uptake coincided with elevated p-AS160 Thr642 in rat muscle studied 3 or 27 h postexercise (38). Insulin-stimulated AS160 phosphorylation (p-AS160) and glucose uptake have been studied in humans after one-legged exercise (86, 118). Phosphorylation of AS160 on Ser318, Ser341, Ser588 Thr642, Ser704, and Ser751 of insulin stimulated in exercised muscle exceeded resting values (86). Muscle from the exercised leg exceeded resting levels for insulin-stimulated p-AS160 on Ser318, Ser341, and Ser751, with a trend for elevated p-AS160 Ser588 and unaltered p-AS160 Ser660 or p-AS160 Thr642 (118). However, high-intensity interval cycle ergometry led to greater glucose disposal during a euglycemic hyperinsulinemic clamp without elevated muscle p-AS160 Thr642 at 3 h postexercise (72). Greater AS160 phosphorylation has often, but not always, been found after exercise that improved insulin sensitivity. Causality remains to be established between greater AS160 phosphorylation and elevated insulin-stimulated glucose uptake.

Protein phosphorylation status represents the balance between actions of kinases and phosphatases. Prior exercise does not appear to enhance subsequent (~3–4 h postexercise) Akt...
activity or the activation of other known AS160 kinases, including AMPK, SGK1, or RSK1, in insulin-stimulated rat muscle (101). The role of Ser/Thr protein phosphatase(s) is uncertain because the specific enzymes regulating AS160 dephosphorylation are unidentified. No research has tested whether AS160 becomes a better substrate for kinases or worse substrate for phosphatases postexercise.

Prior exercise does not uniformly enhance insulin-stimulated phosphorylation of every Akt substrate. No increase in TBC1D1 phosphorylation was detected with the phospho-Akt substrate antibody in insulin-stimulated rat muscle at 3 or 27 h postexercise (38). Human muscle pTBC1D1 Thr596 was unaltered for exercised vs. nonexercised insulin-stimulated samples at ~5–7 h postexercise (86). Exercise also did not elevate insulin-mediated phosphorylation of Ser21/9-glycogen synthase kinase 3 (GSK3) or TSC2 Ser239 in rats at ~4 h postexercise (1, 15) or GSK3 Ser21 in humans at ~4–6 h postexercise (126). However, exercise led to greater insulin-stimulated proline-rich Akt substrate of 40 kDa Thr246 phosphorylation (15). The functional consequences of this effect are unknown. Exercise can also influence Akt-independent insulin signaling. Frøsig et al. (35) reported that acute one-legged exercise by men did not alter subsequent (4 h postexercise) insulin-stimulated atypical PKC (aPKC) activity without exogenous phosphatidylinositol-3,4,5-trisphosphate (PIP3), but aPKC activity measured with exogenous PIP3 was greater for the exercised vs. unexercised leg. Conflicting results implicate aPKC as being positively related, negatively related, or unrelated to insulin-stimulated glucose uptake (28, 29, 109, 120). Frøsig et al. (35) also reported greater IRS-2/Pi3K for the exercised vs. unexercised leg, but IRS-1 appears to be more important than IRS-2 for insulin-stimulated muscle glucose uptake (7, 53, 115).

Several insulin-signaling proteins downstream of PI3K that regulate insulin-stimulated glucose transport have not been studied postexercise. Among these proteins is Rac1, an insulin-regulated, PI3K-dependent insulin-signaling protein that is not directly controlled by Akt (18, 110, 111, 121).

Studies performed in the absence of exercise demonstrate that insulin’s ability to increase GLUT4 in CSM relies primarily on enhanced GLUT4 exocytosis (30, 62, 107). However, the effects of prior exercise on specific insulin-regulated GLUT4 trafficking steps have not been reported. It is possible that prior exercise influences GSV release, interactions of the GSV with cytoskeletal-regulated processes, tethering or docking of GSV to CSM, and/or fusion of GSV with CSM. It also remains possible that prior exercise attenuates GLUT4 endocytosis in insulin-stimulated muscles.

**End Effector**

Although the specific insulin-stimulated GLUT4 trafficking steps that are influenced by prior exercise remain to be identified, it has been demonstrated in rat skeletal muscle that the increased insulin-stimulated glucose transport ~4 h postexercise is accompanied by a proportional increase in GLUT4 in CSM (47). These results implicate greater GLUT4 translocation as a major end effector for elevated insulin-stimulated glucose transport postexercise.

**Improved Insulin-Stimulated Glucose Uptake Postexercise in Insulin-Resistant Individuals**

Improved insulin sensitivity in healthy individuals postexercise is striking, but a more urgent issue is to elucidate mechanisms for exercise effects on insulin resistance. One exercise session can increase insulin-stimulated glucose disposal in insulin-resistant humans (9, 23, 24, 88, 103) and rats (2, 14, 40, 84, 95, 114). Insulin-resistant humans and rats after acute exercise can reach glucose uptake values similar to healthy but unexercised controls (2, 14, 24, 40, 95). However, when both insulin-resistant and healthy groups perform similar exercise protocols, exercise consistently fails to equalize insulin-stimulated glucose uptake between the groups (Fig. 4) (14, 24, 86, 88, 114).

What are the potential triggers for enhanced postexercise insulin sensitivity in insulin-resistant individuals? Similarly low glycogen levels were reported immediately postexercise by insulin-resistant vs. normal rats (14, 114). Acute exercise activated AMPK in the soleus from normal but not insulin-resistant [high fat diet (HFD)] rats (114). In contrast, exercise elicited similar AMPK activation in predominantly fast-twitch epimyseal muscle of rats with normal vs. reduced (HFD)
insulin sensitivity (14). Apparently, no published studies have directly compared normal vs. insulin-resistant individuals for GLUT4 in CSM immediately postexercise, but insulin-independent glucose uptake by isolated muscles immediately postexercise likely reflects CSM GLUT4. Insulin-independent glucose uptake of soleus immediately postexercise was lower for normal vs. insulin-resistant rats (114), whereas values for epitrochlearis did not differ between normal and insulin-resistant rats (14). In both muscles, acute exercise induced greater insulin-stimulated glucose uptake vs. unexercised insulin-resistant rats, and insulin-stimulated glucose uptake of healthy controls exceeded insulin-resistant rats after comparable exercise.

What about potential memory element candidates in insulin-resistant individuals? Similar reductions in muscle glycogen were evident on the day postexercise in humans with normal vs. reduced insulin sensitivity (23, 24). Muscle glycogen was also similar 2 h postexercise in soleus from control vs. insulin-resistant HFD rats (114). Unlike normal rats, insulin-resistant rats had a sustained elevation of AMPK at 1, 2, 4, and 16 postexercise (85). Another potential memory element is CSM GLUT4 during exercise and then internalized postexercise to a location with high susceptibility for future insulin-mediated recruitment, but this remains untested in both normal and insulin-resistant individuals. Three studies have evaluated potential postexercise mediators in insulin-resistant individuals [induced by 2-wk HFD (14), 4-wk HFD (114), or 7-h intralipid infusion (86)] by studying muscles exposed to a physiological insulin dose. These studies found that although prior exercise caused greater insulin-stimulated glucose uptake by muscle, it did not enhance proximal signaling at insulin receptor tyrosine phosphorylation, IRS-1/Pi3K, Akt phosphorylation, or Akt activity in insulin-stimulated muscle (14, 86, 114). Prior exercise increased AS160 Ser588 phosphorylation but not Thr642 phosphorylation in muscle from insulin-resistant rats (14) and increased AS160 phosphorylation on both Ser588 and Thr642 in muscle from insulin-resistant humans (86). In each study, exercise had no effect on proximal signaling in controls with normal insulin sensitivity, and prior exercise increased insulin-stimulated phosphorylation of both AS160 Ser588 and Thr642. Studies using a supraphysiological insulin concentration reported that exercise by insulin-resistant rats (~3–4.5 mo of HFD) led to greater insulin receptor, IRS-1, and IRS-2 tyrosine phosphorylation with increased Akt phosphorylation (84, 85, 95). It would be valuable to perform a similar experiment and assess proximal insulin signaling and AS160 phosphorylation with a physiological insulin dose.

Regarding the end effector, apparently no studies have tested whether prior exercise leads to greater CSM GLUT4 in insulin-stimulated muscle of insulin-resistant individuals. However, increased insulin-stimulated glucose uptake postexercise occurred without elevated total GLUT4 abundance in insulin-resistant rats (14).

It is often assumed that the exercise benefits on insulin resistance are attributable to correcting the cellular defects responsible for insulin resistance. In this context, various lipid metabolites, including ceramide, diacylglycerol (DAG), and long-chain acyl-CoA species, are believed to induce insulin resistance (48) secondary to activating Ser/Thr kinases (e.g., JNK, mTORC1, IKK) that catalyze site-selective Ser phosphorylation of the insulin receptor and IRS-1, resulting in attenuated insulin signaling via IRS-1/Pi3K, Akt, and other key steps (6, 105, 122). Lipotoxicity is also linked to inflammation and/or reactive oxygen species-related pathways that can produce insulin resistance (6, 100, 104). It should be noted that insulin resistance has not uniformly been accompanied by evidence of lipotoxicity and inflammation. For example, Høeg et al. (50) found that insulin resistance induced by infusing intralipid for 7 h was not characterized by elevated muscle ceramides, DAG, or long-chain fatty acyl-CoA concentration, plasma levels of inflammatory cytokines, or insulin-stimulated increases in insulin signaling (IRS-1/Pi3K phosphorylation of Akt or AS160).

Insulin-resistant rats (~3–4.5 mo of HFD) that were exercised and compared with diet-matched sedentary controls had reduced markers of proinflammatory pathways, including phosphorylation of IKKβ, JNK, and IRS-1 Ser307 (84, 85, 95). In women made insulin resistant by overnight lipid infusion, whole body insulin sensitivity was improved the day after exercise concomitant with reduced DAG and ceramide concentrations, lower JNK phosphorylation, and attenuated inflammatory markers in muscle (99). Prior exercise also increased intramuscular triacylglyceride (TAG) concentration
and muscle abundance of enzymes controlling TAG synthesis (DAG acyltransferase 1, mitochondrial glycerol-3-phosphate acyltransferase, and sterol-CoA desaturase 1). These authors hypothesized that enhanced lipogenic capacity preferentially directed fatty acids to TAG synthesis and oxidation, leading to less DAG and ceramide accumulation and attenuated activation of proinflammatory pathways. Oakes et al. (83) reported that increased insulin-stimulated glucose uptake after exercise by insulin-resistant rats (3 wk HFD) was accompanied by reduced muscle total long-chain fatty acyl-CoA levels. Castorena et al. (14, 15) did not detect significant exercise effects on levels of ceramide, DAG, or triacylglyceride species, markers of activation of JNK, IKKα/B, or mTORC1, or proximal insulin signaling in muscles from insulin-resistant rats (2 wk of HFD). However, prior exercise lowered muscle 16:0 acyl-CoA concentration (15).

Exercise can reduce indices of lipotoxicity and inflammation, but at least with brief HFD, increased insulin sensitivity can occur after exercise even when many of these markers are unaltered (14, 15). Studies of insulin-resistant rats or humans that did not detect exercise-induced improvement in proximal insulin signaling (14, 86, 117) do not align with the idea that exercise-induced decrements in insulin sensitivity are always attributable to lowering lipotoxicity and inflammation, leading to improved proximal insulin signaling. Because each of these studies evaluated models of relatively short-term insulin resistance, it seems possible that exercise’s improvement in insulin sensitivity of insulin-resistant individuals occurs via multiple mechanisms that depend on the duration/nature of the insulin resistance and/or the characteristics of the exercise protocol.

It is reasonable to suggest that exercise benefits may be attributable to reversing the defects causing insulin resistance, but another plausible scenario is that exercise may indirectly compensate by enhancing other processes that are parallel or distal to the defect. Compelling evidence indicates that exercise can improve insulin sensitivity independent of directly reversing insulin resistance; i.e., exercise by highly insulin-sensitive humans, rats, and mice (13, 46, 124, 126, 127), which do not suffer from insulin resistance, produces robustly increased insulin-stimulated glucose uptake. As discussed above, insulin-stimulated glucose uptake is not equalized between insulin-resistant and non-insulin-resistant muscle after comparable exercise is performed (Fig. 4) (14). This residual disparity implies that exercise fails to completely eliminate the original defect causing insulin resistance. Although the apparent “ceiling effect” of exercise on insulin-resistant muscle is notable, it is nonetheless remarkable that acute exercise can “normalize” glucose uptake compared with sedentary controls.

Conclusion

This review is intended to spur new ways of seeing and thinking about current and future knowledge related to a major health benefit of exercise. Hopefully, new ideas, creative experiments, and/or alternative models will arise to elucidate the increased insulin-stimulated glucose uptake after acute exercise.

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AUTHOR CONTRIBUTIONS

G.D.C. interpreted results of experiments; G.D.C. prepared figures; G.D.C. drafted manuscript; G.D.C. edited and revised manuscript; G.D.C. approved final version of manuscript.

REFERENCES


Frosig C, Roepstorff C, Brandt N, Maarbjerg SJ, Birk JB, Wojtaszewski JF, Richter EA, Kiens B. Lipid-induced insulin resistance affects women less than men and is not accompanied by inflammation or impaired proximal insulin signaling. Diabetes 60: 64–73, 2011.


Kim J, Solis RS, Arias EB, Cartee GD. Postcontraction insulin sensitivity: relationship with contraction protocol, glycogen concentration,
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