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SYMPOSIUM REVIEW

Regulation of glucose and glycogen metabolism during and after exercise

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Abstract Utilization of carbohydrate in the form of intramuscular glycogen stores and glucose delivered from plasma becomes an increasingly important energy substrate to the working muscle with increasing exercise intensity. This review gives an update on the molecular signals by which glucose transport is increased in the contracting muscle followed by a discussion of glycogen mobilization and synthesis by the action of glycogen phosphorylase and glycogen synthase, respectively. Finally, this review deals with the signalling relaying the well-described increased sensitivity of glucose transport to insulin in the post-exercise period which can result in an overshoot of intramuscular glycogen resynthesis post exercise (glycogen supercompensation).

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Introduction

Carbohydrate in the form of glucose and intramuscular glycogen becomes an increasingly important energy substrate with rising exercise intensity (Holloszy & Kohrt, 1996). Carbohydrate oxidation accounts for 10–15% of total energy production during low intensity aerobic exercise (~30% $\dot{V}_{O_2max}$), increasing progressively to roughly 70–80% of total energy during exercise of about 85% $\dot{V}_{O_2max}$ to about 100% of energy consumption at exercise intensities of 100% of $\dot{V}_{O_2max}$ and above (Romijn *et al.* 1993; Holloszy & Kohrt, 1996). There are two sources of glucose molecules available to the working muscle; plasma glucose and muscle glycogen. While very little net glycogen breakdown is observed at low-intensity exercise, glycogen-breakdown becomes the predominant glucose source at higher intensities (Hargreaves & Richter, 1988). In terms of athletic performance, low muscle glycogen depots seem detrimental to both high and moderate intensity exercise performance (Hargreaves & Richter, 1988). This has resulted in the widespread practice of high-carbohydrate diet regimens to increase pre-exercise glycogen levels (carbohydrate loading) (Hargreaves & Richter, 1988). In this review we discuss the current thinking on the molecular signals that acutely control glucose uptake and glycogen use by the working muscle. Then we discuss the mechanisms by which skeletal muscle may accomplish an increase in glycogen stores above pre-exercise levels, focusing on the mechanisms enhancing insulin-stimulated glucose uptake post-exercise.

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Glucose metabolism during exercise-regulation of glucose transport

Glucose delivery to the working muscle is increased by a marked increase in capillary perfusion during exercise as originally described by August Krogh in frog muscle and recently confirmed by real time contrast enhanced ultrasound in humans (Sjoberg *et al.* 2011). Another way to increase delivery is to increase plasma glucose concentrations by ingestion of carbohydrate rich meals or drinks. The magnitude of increase depends on the type and quantity of carbohydrates and the reader is referred to other reviews for discussion of how to optimize carbohydrate availability during exercise (Hawley *et al.* 2011). At the fibre level, it is still debated whether the rate-limiting step *in vivo* is GLUT4-dependent transport across the plasma membrane or intracellular phosphorylation by hexokinase II. However, increased recruitment of GLUT4 from intracellular vesicular structures to the cell surface during acute muscle contraction/exercise is a well-described acute adaptation in both rodents and humans (for refs see Jessen & Goodyear, 2005; Rose & Richter, 2005) and a necessary contributor to increased skeletal muscle glucose uptake in exercising muscle since in mouse muscles where GLUT4 has been genetically ablated, contraction-induced glucose uptake is abrogated (Zisman *et al.* 2000). In addition, a contribution from an increase in GLUT4 intrinsic activity, which is clearly dissociable from GLUT4 translocation in some studies, cannot be discounted (Klip, 2009) although effects of exercise on GLUT4 intrinsic activity have not been rigorously demonstrated.

Overall, the GLUT4-translocation response to contraction has been proposed to involve feed-forward activation by sarcoplasmic reticulum (SR) Ca^{2+} release with subsequent fine-tuning by changes secondary to contraction (e.g. mechanical stretch, metabolism, redox-state). The feed forward proposition is supported by *ex vivo* rat muscle studies where caffeine-stimulated Ca^{2+} release from the SR was sufficient to elicit an increase in glucose transport in the absence of measurable increases in force development, nucleotide-status or activation of the AMP/ATP and ADP/ATP-sensitive AMP-activated protein kinase (AMPK) (Wright *et al.* 2004). However, whereas the original studies did not find changes in energy status or AMPK activation by sub-contraction threshold Ca^{2+} release, more recent studies have reported nucleotide-changes and AMPK activation using similar Ca^{2+} concentrations (Jensen *et al.* 2007; Raney & Turcotte, 2008; Egawa *et al.* 2009), questioning the usefulness of the caffeine-approach to isolate the Ca^{2+} response independently of energy turnover and other contraction-activated events. Furthermore, an old observation is that the glucose uptake response correlates excellently with the intensity of muscular work during both human exercise and in more reductionistic rodent muscle contraction models (Rose & Richter, 2005). Of particular interest, Ihlemann and coworkers, by adjusting the length of *ex vivo* stimulated rat muscles and as a consequence force production and metabolic stress, demonstrated that the glucose transport response correlates with the degree of tension development rather than stimulation frequency (Ihlemann *et al.* 2000). These studies were recently followed up by another approach where pharmacological inhibition of fast-twitch myosin II-dependent crossbridge cycling partially reduced electrically stimulated glucose transport in rat epitrochlearis muscle (Blair *et al.* 2009). Using a lower intensity tetanic stimulation protocol to minimize energy turnover by e.g. SERCA-dependent Ca^{2+} reuptake, we have data showing that the increase in glucose transport by electrical stimulation of mouse muscles *ex vivo* is fully prevented by myosin II inhibition despite normal Ca^{2+} activated phosphorylation events (T. E. Jensen, E. A. Richter, unpublished data). This suggests that, while some Ca^{2+} activated proteins provide necessary signals for contraction-stimulated glucose transport (Rose & Richter, 2005), Ca^{2+} *per se* is probably not sufficient to increase muscle glucose transport.

Based on experiments using the AMP-mimetic aminoimidazole carboxamide ribonucleotide (AICAR), activation of AMPK appears sufficient to cause a partial increase in glucose transport in rodent fast-twitch muscle. In contrast, this response is lower in the mixed type I and II fibre mouse soleus and often absent in the type I fibre-dominated rat soleus despite activation of AMPK (Jørgensen *et al.* 2004; Wright *et al.* 2005). This does not seem to relate to differential expression of potential downstream mediators of GLUT4 translocation such as TBC1D1 and TBC1D4/AS160 in the rat (Castorena *et al.* 2011) but may relate to differential expression of AMPK β and γ subunits in different rodent muscles (Treebak *et al.* 2009). In humans, despite a lack of measurable changes in total AMPK phosphorylation early on during intense exercise, the $\alpha 2\beta 2\gamma 3$ containing subset of AMPK complexes are rapidly activated with exercise consistent with a role in promoting glucose transport (Birk & Wojtaszewski, 2006). A necessary role of AMPK for contraction-stimulated glucose transport is more controversial, with some studies reporting decreased glucose transport in AMPK deficient mouse models and others not, probably due to redundancy of signalling, differential contraction protocols, and transgenic manipulation strategies (Rose & Richter, 2005). Recently, conditional muscle-specific knockout of both β-AMPK regulatory subunits abolished AMPK activity and potently inhibited exercise-stimulated glucose uptake *in vivo* and contraction-stimulated glucose transport *ex vivo* (O'Neill *et al.* 2011). In parallel to AMPK, proposed to act through TBC1D1/4 (Cartee & Wojtaszewski, 2007;

Cartee & Funai, 2009) and eNOS (Lee-Young *et al.* 2009), a number of other pathways have been proposed to signal to increase glucose transport and may include LKB1 signalling through the AMPK-related kinase SNARK (Koh *et al.* 2010), and stretch-activated p38 MAPK (Chambers *et al.* 2009). In relation to the latter, it is, however, worth mentioning that at least one of the p38 MAPK inhibitors used by Chambers and co-workers, SB203580, has been shown interact with and inhibit GLUT4 directly (Antonescu *et al.* 2005; Ribe *et al.* 2005). Teasing out how AMPK and other signals blend to elicit a given level of increase in glucose transport in different muscle fibre types during exercise remains a challenging subject for future study.

Regulation of glycogen breakdown

Glycogenolysis is regulated by glycogen phosphorylase (GP), acting on the terminal α-1,4-glycosidic linked glucose residues, and debranching enzyme, targeting the α-1,6-branchpoints in the glycogen molecule (Roach, 2002). Most studies have focused on the regulation of GP, the activity of which is increased by allosteric binding of AMP or IMP and competed by ATP or glucose-6-phosphate (G-6-P). In addition, since GP requires inorganic phosphate to produce glucose-1-phosphate from glycogen, inorganic phosphate from ATP and creatine phosphate (CrP)-turnover has been speculated to limit GP activity at the substrate level (for refs see Hargreaves & Richter, 1988). Finally, high initial muscle glycogen concentration clearly augments net glycogen breakdown during contractions likely due to activation of GP by glycogen (Hespel & Richter, 1992). Apart from its allosteric and presumably substrate-level regulation, phosphorylation of GP on Ser14 by phosphorylase kinase (PK) increases the activity of GP measured *in vitro*. Classically, PK is thought to integrate local and systemic signals to promote glycogen breakdown by being activated initially by Ca^{2+} binding to the PK δ-subunit (identical to calmodulin), and then plasma adrenaline acting though a β2-adrenergic receptor–adenylate cyclase–PKA cascade (Hargreaves & Richter, 1988). In the absence of adrenaline stimulation, GP activity measured *in vitro* (reflecting its phosphorylation state) increases rapidly at the onset of contraction and then reverts towards resting activity within a few minutes despite continued contraction and therefore presence of Ca^{2+} transients (Richter *et al.* 1982). This is probably a result of dephosphorylation at Ser14 following the initial activation by Ca^{2+} although, to our knowledge, the mechanism behind this has not been studied in detail. With regards to the adrenergic stimulation of glycogenolysis, it is also worth noting that adrenaline-stimulated glycogen breakdown in incubated rat muscles is potently inhibited by the sodium–potassium pump inhibitor ouabain, suggesting a link to adrenaline-stimulated sodium-potassium pump activity (James *et al.* 1999). Whether this connection relates to local changes in e.g. nucleotides or K^+ is not clear. In humans, the evidence for adrenergic stimulation of glycogenolysis is not clear-cut, with some studies reporting increased glycogen use and GP activation with adrenaline infusion, while others do not (see e.g. Kjaer *et al.* 2000; Watt *et al.* 2001 and refs therein). As discussed by Watt and coworkers (2001), this may relate in part to the intensity of exercise, with allosteric regulation of GP playing a larger regulatory role with increasing intensity.

Within a given fibre, glycogen particles have been proposed to be present in at least three distinct subcellular locations, with ~80% between the myofibrils in close vicinity to the SR and mitochondria and two smaller compartments located within the myofibrils and underneath the sarcolemma contributing ~10% each (Nielsen *et al.* 2011; Prats *et al.* 2011). The detailed roles of these different glycogen compartments to muscle contraction–metabolism remain to be uncovered but the various glycogen pools are differentially depleted and supercompensated by different kinds of exercise and training (for refs and discussion, see Prats *et al.* 2009; Nielsen *et al.* 2011). In relation to fatigue, the emptying of intramyofibrillar glycogen correlates somewhat with lower SR Ca^{2+} release by 4-chloro-*m*-cresol *in vitro* ($r^2 = 0.23$) (for ref see Nielsen *et al.* 2011), suggesting a potential contribution to the unexplained relation between fatigue and low glycogen. It would be interesting to examine if e.g. most of the ~20% depletion of muscle glycogen with 30 s of all-out bicycle sprint exercise (Birk & Wojtaszewski, 2006) preferentially stems from intramyofibrillar glycogen. The regulation of glucose transport and glycogen turnover in working muscle is summarized in Fig. 1.

Regulation of glycogen synthesis

Glycogen synthase (GS) catalyses the rate-limiting incorporation of UDP-glucose via α-1,4-glycosidic linkages into the growing glycogen polymer, with branching enzyme catalysing formation of α-1,6-branchpoints (Roach, 2002). Counterintuitively, this UTP-requiring anabolic glycogen synthase is not only stimulated by insulin but also by exercise although unchanged or inhibited GS activity at high intensity has been described (for refs see Nielsen & Wojtaszewski, 2004). In stark contrast to GP regulation, the regulation of GS by post-translational modifications is quite complex, with at least nine phosphorylation sites targeted by multiple kinases (Nielsen & Wojtaszewski, 2004; Jensen & Lai, 2009). The dephosphorylated state of GS, in particular

at sites 2, 2a, 3a and 3b, increases GS activity *in vitro*. This dephoshorylation is catalysed by protein phosphatase 1 (PP1), which is also the phosphatase for GP and PK. At least one glycogen-binding protein, G_M, targets PP1 to glycogen and has been shown in mice to be required for exercise-stimulated GS activation (Aschenbach *et al.* 2001). It is tempting to speculate that the co-localization between PP1–G_M and glycogen regulatory enzymes like GS is linked to the well described inverse correlation between *in vitro* GS activity and glycogen content in muscle (Danforth, 1965; Nielsen *et al.* 2001). Worth mentioning, PP1 is also known to be regulated by phosphorylation of endogenous inhibitors of PP1 like inhibitor-1 and -2, both of which are expressed in skeletal muscle (for refs see Nicolaou *et al.* 2009). In addition, GS activity shows a partial resistance to phosphatase treatment *in vitro*, suggesting that other described covalent modifications of GS such as glycolysation (Parker *et al.* 2003) may contribute to GS regulation in skeletal muscle. *In vivo*, allosteric activation by G-6-P is probably an all-important point of regulation. This is evidenced by recent data in mice where muscle-specific replacement of wild-type GS with a G-6-P insensitive mutant GS protein potently reduced insulin and prevents AICAR-stimulated glycogen synthesis (Bouskila *et al.* 2010; Hunter *et al.* 2011), suggesting that sensing of G-6-P from transported glucose is required for most of the stimulation of glycogen synthesis. Interestingly, the stimulatory effect of AICAR on glycogen synthesis occurred despite the fact that direct AMPK-dependent phosphorylation of GS at sites 2+2a causes a moderate reduction of GS activity *in vitro* (Jorgensen *et al.* 2004), arguing that allosteric regulation can override covalent regulation, at least on sites 2+2a.

In relation to the recently re-emphasized distinct subcellular depots of muscle glycogen, GS seems located to different compartments depending on its phosphorylation state. Hence, GS phosphorylation on site 1b, presumably by adrenaline-activated PKA, is located intramyofibrillarly following roughly 2 h of exhaustive human knee-extension exercise, while GS phosphorylation on the AMPK sites 2+2a is located with subsarcolemmal and intermyofibrillar glycogen depots (Prats *et al.* 2009). The details of how GS re-localizes to these compartments are not clear but have been suggested to depend on the actin cytoskeleton in as much as GS and GP assemble with β-actin, but not γ-actin, into spherical structures after glycogen-depleting electrical rabbit tibialis anterior muscle stimulation (Prats *et al.* 2005).

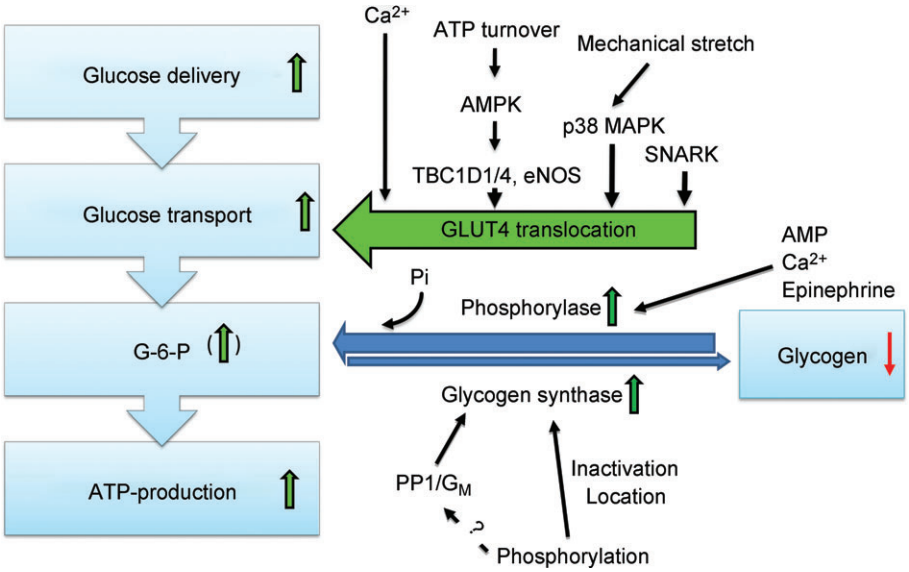

Figure 1. Glucose utilization in the working muscle is increased through increased delivery and uptake of plasma glucose and increased glycogenolysis
Transport of glucose across the sarcolemma and T-tubular membranes is determined by the amount of contraction- and insulin-responsive glucose transporter 4 (GLUT4) proteins in the outer membrane. This magnitude of glucose transport response with contraction correlates with work intensity with evidence suggesting the involvement of kinases like AMPK, p38 MAPK and SNARK whereas Ca^{2+} activated proteins are probably required but likely to be insufficient to stimulate glucose transport. Allosteric and covalent regulation increases both glycogen mobilization by glycogen phosphorylase (GP) and resynthesis by glycogen synthase (GS) simultaneously during exercise by altering enzyme activity and/or location. GP may also be regulated by the availability of its substrates glycogen and inorganic phosphate (P_i). Depending on the work intensity and duration, glucose-6-phosphate (G-6-P), an important allosteric inhibitor of GP and stimulator of GS, may increase.

Glycogen resynthesis post-exercise – the role of increased insulin-stimulated glucose uptake

Mechanistically, while increased microvascular recruitment may play a role in insulin sensitization *in vivo* by increasing glucose delivery (for refs see Wojtaszewski & Richter, 2006), prior contraction also sensitizes glucose transport and GLUT4 translocation *ex vivo* independently of the capillary network (Fisher *et al.* 2002; Geiger *et al.* 2005), suggesting that part of insulin sensitization by prior exercise stems from an effect on GLUT4-mediated glucose transport. It is worth noting, however, that if exercise involves muscle damaging eccentric components, then insulin sensitivity may in fact be decreased in the days after exercise due to decreased GLUT4 expression and impaired insulin signalling (for refs see Maarbjerg *et al.* 2011).

What might be the molecular mechanisms behind the exercise effect on insulin-stimulated glucose transport when no muscle damage is induced? One study has shown that AMPK activation in incubated rat epitrochlearis by AICAR or hypoxia, in conjunction with one or more unknown serum proteins >10 kDa (Gao *et al.* 1994), can increase submaximal insulin-stimulated glucose transport *ex vivo* 3.5 h after removal of the AICAR stimulus (Fisher *et al.* 2002). Importantly, this occurred without a measurable potentiation of proximal steps of insulin signalling like PI3K activity and Akt phosphorylation, consistent with previous observations in humans (Wojtaszewski & Richter, 2006). This effect could speculatively be relayed by downstream phosphorylation of TBC1D4, an emerging regulator of GLUT4 trafficking, which shows an increase lasting many hours post-exercise at certain residues including known AMPK sites in rats and humans (Sakamoto & Holman, 2008; Maarbjerg *et al.* 2011). Low muscle glycogen content correlates with high AMPK activity (Jorgensen *et al.* 2004) and glycogen has been shown to directly bind and inactivate AMPK through the carbohydrate-binding domain of the AMPK β-subunit (McBride *et al.* 2009). This makes it tempting to speculate about a connection between the release of AMPK from glycogen during exercise and the ensuing increase in insulin sensitivity. Supporting a regulatory role of glycogen is the finding that the increased post-exercise insulin sensitivity correlates significantly with the amount of glycogen broken down during the preceding exercise bout ($r^2 = 0.53$; Richter *et al.* 2001). However, if a serum-factor is required for contraction to cause insulin sensitization *ex vivo* (Gao *et al.* 1994) but not for contraction-stimulated glycogen breakdown, then the relationship between contraction-stimulated glycogen use and insulin sensitivity is probably non-causal.

Both exercise and the protein synthesis inhibitor anisomycin acutely increased p38 MAPK activation in incubated rat soleus and epitrochlearis and increased submaximal insulin-stimulated glucose transport 3 h after

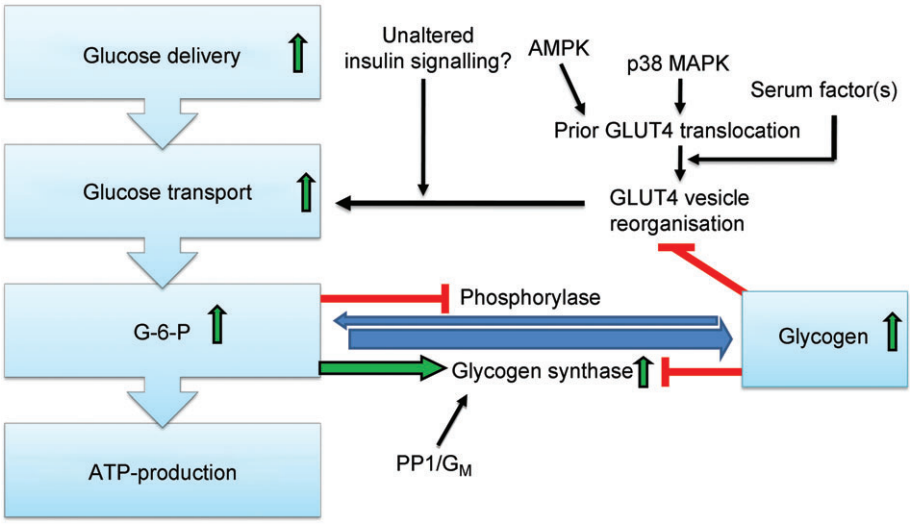

Figure 2. Augmented glycogen resynthesis post-exercise is explained to a large part by sensitization of the insulin-stimulated glucose transport response and glycogen synthase activation
Although some signalling proteins display a prolonged increase in phosphorylation for many hours after exercise, perhaps contributing to insulin sensitization, many insulin-signalling endpoints seem unaffected by prior contraction. Mobilization of GLUT4 during contraction may cause a subsequent sorting into a more insulin-responsive pool or position. Insulin sensitization may require permissive input from one or more unidentified serum factors. Increased amounts of transported glucose are converted to glucose-6-phosphate (G-6-P) which allosterically increases glycogen synthase and inhibits phosphorylase activity, respectively. High glycogen shows a correlation with decreased insulin-stimulated glucose transport and glycogen synthase inactivation but whether this is a causal relationship remains unclear.

cessation of either stimulus (Geiger *et al.* 2005). The effect of anisomycin was prevented by the selective p38 MAPK inhibitor SB202190, whereas the exercise-effect was not, suggesting that contraction could utilize redundant signalling pathways to increase insulin sensitivity after exercise. Interestingly, resting p38 MAPK phosphorylation 3 h post-exercise has been observed to remain 50% higher in previously exercised leg muscles compared to controls (Thong *et al.* 2003).

Studies in incubated rat muscles have indicated that any glucose transport-increasing stimulus including insulin itself may enhance insulin-stimulated glucose transport some hours after stimulation, possibly by re-location of GLUT4 to a more easily recruitable pool, thereby allowing a larger GLUT4 mobilization by an unaltered insulin signal (Geiger *et al.* 2006). Worth mentioning, this attractive hypothesis is not supported by a recent study showing no insulin-sensitizing effect of doing sequential insulin clamps in humans (Lucidi *et al.* 2010). However, muscle insulin sensitization of prior stimulation may have been overridden by the anti-hypoglycaemic hormonal and lipolytic responses that are activated in the period between the two clamps. A direct examination of the hypothesized altered location of insulin-responsive GLUT4 pools by insulin-sensitizing stimuli in rat and human muscle will be needed to directly test this hypothesis. The effects of prior exercise on insulin-stimulated glucose transport and glycogen resynthesis are recapitulated in Fig. 2.

Conclusion

Carbohydrates in the form of plasma glucose and muscle glycogen are important fuels during exercise. The increase in muscle glucose uptake during exercise is dependent upon the delivery of glucose (capillary perfusion and plasma glucose concentration) and the permeability of the muscle membrane to glucose. The latter is regulated by a plethora of molecular signalling thought to include calcium, stretch and energy stress signalling and probably others. Muscle glycogen is utilized as a function of exercise intensity and duration and is controlled by the activity of the enzyme glycogen phosphorylase as well as the concentration of both of its substrates (glycogen and inorganic phosphate). In the post-exercise recovery period, muscle glucose uptake displays an increased sensitivity to insulin in this way increasing glucose uptake after a meal in the muscles that have performed the exercise and therefore are in need of rebuilding their glycogen stores. Whereas the molecular mechanisms involved in post-exercise increased insulin sensitivity are not fully understood, they could involve repackaging of the GLUT4 vesicles in more easily recruitable pools post-exercise. Furthermore, exercise-induced phosphorylation of proteins such as TBC1D4 and p38 MAPK, which remain phosphorylated for hours after exercise, may contribute to insulin-sensitization.

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