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Review

Review

Post-translational Modifications: The Signals at the Intersection of Exercise, Glucose Uptake, and Insulin Sensitivity

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Abbreviations: ACC, acetyl-CoA carboxylase; ADP, adenosine diphosphate; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AKT, protein kinase B; ALDOA, fructose biphosphate aldolase a; AMP, adenosine monophosphate; AMPK, 5′-adenosine monophosphate–activated protein kinase; ARP, actin-related protein; CAMK, calmodulin-dependent protein kinase; CAMKK, calmodulin-dependent protein kinase kinase; cAMP, cyclic AMP; CBP, cyclic AMP-responsive element binding protein; CoA, coenzyme A; CrAT, carnitine acetyltransferase; CREB, cyclic AMP-responsive element binding protein; DHHC7, Zinc finger DHHC-type containing 7; E1 enzymes, ubiquitin-activating enzymes; E2 enzymes, ubiquitin-conjugating enzymes; E3 ligases, ubiquitin-ligases; ENO3, beta-enolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT4, glucose transporter type 4; GS, glycogen synthase; GSK, glycogen synthase kinase; HDAC, histone deacetylase; INSR, insulin receptor; IRAP, leucyl-cistinyl aminopeptidase; IRS1, insulin receptor substrate 1; KEAP1, Kelch-like ECH-associated protein 1; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; MARylation, mono-ADP ribosylation; MEF2, myocyte enhancer factor 2; MG53, tripartite motif-containing protein 72; MKRN1, makorin-1; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NRF2, nuclear factor erythroid 2-related factor 2; p300, E1A-binding protein p300; p70 S6K, ribosomal protein S6 kinase; PARylation, poly-ADP ribosylation; PARP, poly-ADP ribose polymerase; PDH, pyruvate dehydrogenase; PGAM, Phospoglycerate mutase; PGC1α, Peroxisome proliferator activated receptor gamma coactivator 1 alpha; PI3K, Phosphatidylinositol 3-kinase; PIKFYVE, Phosphoinositide kinase, FYVE-type zinc finger containing; PKA, protein kinase A; PSMA, proteasomal subunit alpha; PSMB, proteasomal subunit beta; PSMC5, 26S proteasome regulatry subunit 8; PSMD11, 26S proteasome ATPase regulatory subunit 11; RAC1, ras-related protein Rac1; SIRT, Sirtuin; TBC1D1, TBC1 domain family member 1; TBC1D4, TBC1 domain family member 4; TNKS, tankyrase.

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Abstract

Diabetes is a global epidemic, of which type 2 diabetes makes up the majority of cases. Nonetheless, for some individuals, type 2 diabetes is eminently preventable and treatable via lifestyle interventions. Glucose uptake into skeletal muscle increases during and in recovery from exercise, with exercise effective at controlling glucose homeostasis in individuals with type 2 diabetes. Furthermore, acute and chronic exercise sensitizes skeletal muscle to insulin. A complex network of signals converge and interact to regulate glucose metabolism and insulin sensitivity in response to exercise. Numerous forms of post-translational modifications

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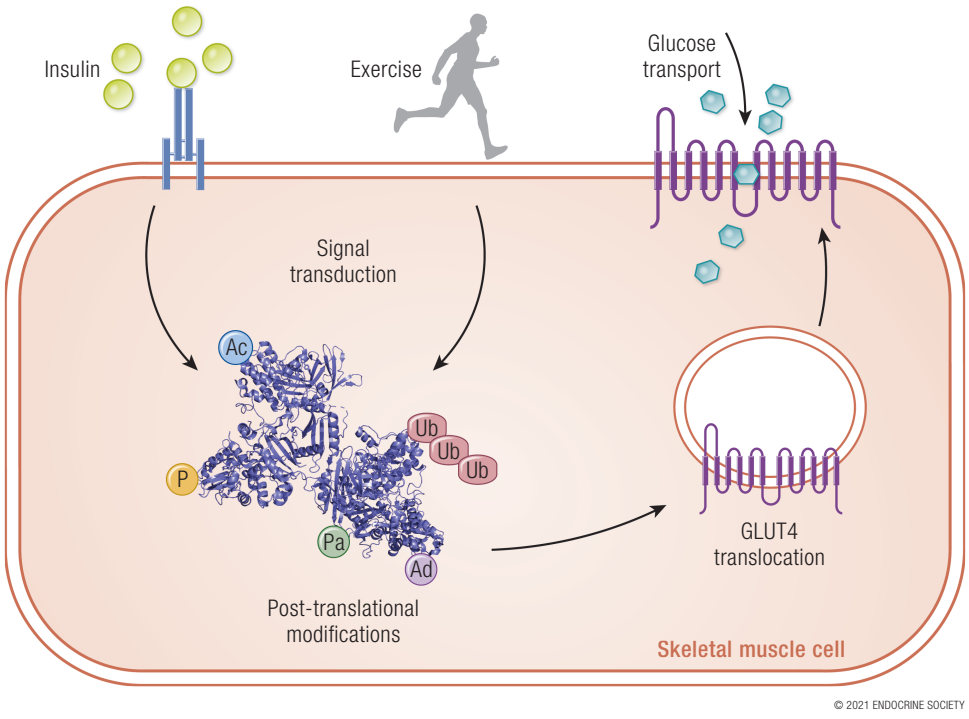
(eg, phosphorylation, ubiquitination, acetylation, ribosylation, and more) are regulated by exercise. Here we review the current state of the art of the role of post-translational modifications in transducing exercise-induced signals to modulate glucose uptake and insulin sensitivity within skeletal muscle. Furthermore, we consider emerging evidence for noncanonical signaling in the control of glucose homeostasis and the potential for regulation by exercise. While exercise is clearly an effective intervention to reduce glycemia and improve insulin sensitivity, the insulin- and exercise-sensitive signaling networks orchestrating this biology are not fully clarified. Elucidation of the complex proteome-wide interactions between post-translational modifications and the associated functional implications will identify mechanisms by which exercise regulates glucose homeostasis and insulin sensitivity. In doing so, this knowledge should illuminate novel therapeutic targets to enhance insulin sensitivity for the clinical management of type 2 diabetes.

Key Words: exercise, glucose, insulin, phosphorylation, ubiquitination, acetylation

Graphical Abstract



ESSENTIAL POINTS

- Exercise training improves insulin sensitivity and glycemic control in type 2 diabetes.
- Insulin and exercise regulate a range of phospho-signaling pathways to control insulin sensitivity, glucose uptake, and metabolism.
- Exercise- and insulin-sensitive signals converge on the Rab GTPase-activating proteins TBC1 domain family member 1 (TBC1D1) and 4 (TBC1D4; aka AS160) to facilitate GLUT4 translocation and glucose uptake.
- Exercise regulates the ubiquitination or ubiquitin-like modification of glycolytic enzymes in skeletal muscle.
- Proteins involved in the regulation of skeletal muscle glucose metabolism are acetylated in response to insulin stimulation or exercise training.
- Insulin and exercise regulate the ADP ribosylome and thereby regulate energy metabolism, glucose homeostasis, and insulin sensitivity.
- A deeper understanding of the wider regulatory signals that control glucose homeostasis may lead to the development of novel therapeutic strategies to improve insulin sensitivity in type 2 diabetes.

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Diabetes is a global epidemic, affecting approximately 451 million adults worldwide (9% of the adult population), of which approximately 90% of the cases are type 2 diabetes. This figure is rising and is predicted to continue to do so in the subsequent decades (1, 2). People with diabetes have an almost 2-fold increase in all-cause mortality, including increased risk of death from renal disease, liver disorders, cardiovascular disease, infectious diseases, multiple forms of cancer, and mental health disorders (3). These data indicate the urgent need to develop and implement effective strategies to prevent and treat type 2 diabetes.

Type 2 diabetes pathogenesis is multifaceted. Alongside a genetic component (4), type 2 diabetes has a number of lifestyle-related risk factors, including inactivity, overeating, and being overweight or obese (5-7). In terms of pathophysiology, type 2 diabetes is characterized by altered whole-body and tissue-specific metabolism, hyperglycemia, hyperinsulinemia, and peripheral insulin resistance. Insulin resistance in metabolically active tissues, including skeletal muscle, adipose tissue, and liver, impairs glucose disposal, which, alongside reduced insulin-mediated suppression of hepatic glucose production, results in hyperglycemia. As skeletal muscle is the predominant site for insulin-stimulated glucose disposal (8), insulin resistance in this tissue is critical to the development of type 2 diabetes (9, 10). Thus, interventions targeting skeletal muscle are effective at opposing type 2 diabetes pathogenesis.

Physical activity and exercise training improve insulin sensitivity and glycemic control in a range of populations, including those with obesity, prediabetes, or type 2 diabetes (11-16) (Fig. 1A). Indeed, lifestyle interventions that include exercise are effective at reducing the incidence of type 2 diabetes in individuals with elevated plasma glucose (17). Furthermore, diet and exercise intervention can lead to disease remission in a substantial proportion of the type 2 diabetic cases (18). For the purposes of this review, we will focus on the mechanisms by which endurance exercise (ie, moderate-intensity continuous exercise, high-intensity exhaustive exercise, and high-intensity interval training Fig. 1B) regulates glucose uptake and insulin sensitivity.

Cellular Mechanisms of Exercise-induced Glucose Uptake and Insulin Sensitivity in Skeletal Muscle

Acute Exercise

Exercise causes a large increase in energy utilization (19). Carbohydrates, in the form of plasma glucose and skeletal muscle glycogen, are predominant fuel sources in

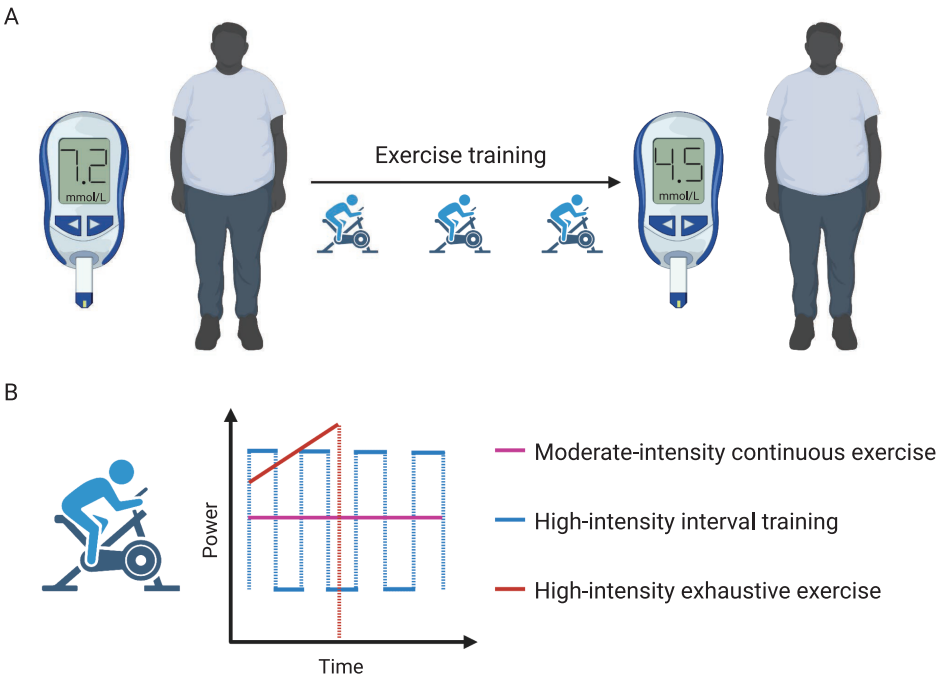

Figure 1. Exercise training, insulin sensitivity, and glycemic control in type 2 diabetes. (A) Repeated endurance exercise training improves insulin sensitivity and glycemic control, often leading to type 2 diabetes remission. (B) Endurance exercise training is an umbrella term for various forms of exercise that lead to improvements in aerobic capacity. For example, endurance exercise encompasses moderate-intensity continuous exercise, in which a constant load is maintained for an extended period of time (ie, 50-70% Wmax for >30 minutes), high-intensity interval training, in which periods of high (ie, >90% Wmax), and low (ie, <50% Wmax) intensities are alternated for numerous intervals, and high-intensity exhaustive exercise, in which high-intensity exercise (ie, >80% Wmax) is performed to exhaustion.

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moderate- to high-intensity exercise (19). Thus, acute exercise increases glucose uptake into skeletal muscle during exercise in an insulin-independent manner and post exercise via both insulin-dependent and -independent mechanisms to replenish skeletal muscle glycogen stores (20-27). The acute increase in skeletal muscle glucose uptake occurs through the modulation of activity and subcellular localization of a number of signaling proteins, enzymes, and transporters. Therefore, the focus of this review will be on these signals.

The discovery of glucose transporter type 4 (GLUT4) as an insulin- and contraction-responsive glucose transporter (28-35) has formed the basis for our understanding of glucose uptake in skeletal muscle. In unstimulated muscle (ie, at rest and without insulin stimulation) GLUT4 is predominantly sequestered intracellularly (36) and glucose delivery and transport are rate-limiting factors to glucose uptake. However, upon the commencement of exercise, elevated skeletal muscle blood flow (37) and translocation of GLUT4 to the sarcolemma and T-tubules (38-44) removes these barriers and increases glucose uptake. Exercise-induced signaling cascades (discussed in subsequent sections) likely increase the rate of exocytosis and decrease the rate of endocytosis of GLUT4 vesicles (45, 46), although direct data in relation to skeletal muscle contraction are missing. Nonetheless, we have demonstrated the importance of GLUT4 for exercise-induced insulin-independent glucose uptake, showing that GLUT4 knockout mice have substantially reduced ex vivo glucose uptake following swimming exercise and during in vitro muscle contraction (47). These data have subsequently been recapitulated in GLUT4 muscle-specific knockout mice (48) and in vivo (49). Importantly, and in contrast to insulin-stimulated conditions (9), exercise-induced glucose uptake and GLUT4 translocation is not impaired in type 2 diabetes (38, 50), making exercise an effective glucose-lowering intervention in patients with type 2 diabetes.

Exercise also acutely increases insulin sensitivity. One bout of endurance exercise increases insulin-stimulated glucose disposal and skeletal muscle glucose uptake during the gold standard hyperinsulinemic–euglycemic clamp for at least 48 hours (25, 51-56). Furthermore, acute exercise improves glucose tolerance in response to the more physiological oral glucose tolerance test (57). Prior exercise increases insulin-stimulated glucose uptake by augmenting insulin-induced skeletal muscle perfusion (58, 59) and glucose transport capacity of the myocyte (38-43, 60). In the initial hours after exercise (0-2 hours post exercise), the insulin sensitizing effects of acute exercise on the myocyte are likely explained by increased GLUT4 content on the plasma membrane (38-43), with subsequent exposure to insulin slowing the rate of GLUT4 internalization (61). However, in the absence of insulin, plasma membrane GLUT4 content levels return to baseline within ~2 hours of recovery (42). Thereafter, prior exercise enhances the translocation of GLUT4 in response to insulin (43), suggesting that exercise primes the internal pool of GLUT4 for insulin-action. Indeed, exercise results in the redistribution of GLUT4 into insulin-responsive GLUT4 storage vesicles, which can more readily be activated by insulin (60).

The insulin-sensitizing effects of exercise on skeletal muscle are influenced by nutritional status. Carbohydrate refeeding abolishes the postexercise increased insulin-stimulated glucose uptake in humans and rats (51, 62-65), an effect that is unrelated to caloric intake in rats (62-64). Indeed, carbohydrate refeeding ablates the increase in insulin-stimulated GLUT4 translocation 18 hours after exercise in rats (64). The increased insulin sensitivity with carbohydrate deprivation is associated with prolonged depletion of skeletal muscle glycogen content, which is rapidly restored during carbohydrate feeding (51, 62-64). Furthermore, humans with the glycogen storage disease McCardle's syndrome, who cannot breakdown glycogen and have elevated skeletal muscle glycogen content, have impaired insulin-stimulated glucose uptake (66). These data indicate that carbohydrate availability can influence acute exercise-induced insulin sensitivity.

Chronic Exercise Training

Endurance exercise training improves whole-body and skeletal muscle insulin sensitivity (11-16, 67, 68). In skeletal muscle, enhanced insulin sensitivity following exercise training is underpinned by enhancements in glucose delivery and the capacity to uptake, utilize, and store glucose as glycogen. Increased capillary density of skeletal muscle following exercise training ensures improved nutrient supply (69), while augmented GLUT4 and hexokinase 2 abundance results in an increased capacity for glucose uptake and conversion to glucose-6-phosphate for utilization or storage as glycogen (68, 70-75). Furthermore, increased mitochondrial volume and respiratory capacity with exercise training increases the capacity for energy metabolism (67, 76-82), which potentially plays a role in promoting insulin sensitivity (67, 83, 84). An additional metabolic outcome of exercise training is an altered storage of intramyocellular lipids (85). Intramyocellular lipid content and lipid droplet size negatively correlate with insulin sensitivity (85-88). However, intriguingly, both athletes and individuals with type 2 diabetes have elevated intramuscular lipid accumulation, despite being on opposite ends of the insulin sensitivity continuum (86). An explanation for this apparent paradox lies in how these lipids are stored within skeletal muscle of

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individuals who are either exercise-trained or type 2 diabetic. Individuals with type 2 diabetes predominantly store lipids in large subsarcolemmal droplets in type II fibers, while athletes store lipids in small intramyofibrillar droplets close to the mitochondria of type I fibers (85). Encouragingly, 12 weeks of combined exercise training (endurance and resistance) can reduce lipid droplet size in individuals with type 2 diabetes (85). Thus, these data suggest that localization and size of lipid droplets are sensitive to physical activity level and can contribute to insulin sensitivity.

Overall, exercise increases glucose uptake and insulin sensitivity of skeletal muscle and these effects are primarily mediated by increased GLUT4 translocation. In subsequent sections, mechanisms by which intracellular signaling converge on GLUT4 to induce glucose uptake and sensitize skeletal muscle to insulin are discussed.

Post-translational Modifications at the Intersection of Exercise and Insulin Sensitivity

Post-translational modifications refer to reversible or irreversible chemical alterations to a protein that occur after translation. There is a wide range of post-translational modifications including phosphorylation, ubiquitination and ubiquitin-like modifications, various forms of acylation (eg, acetylation, succinylation, malonylation, and palmitoylation), ribosylation, and many more. Post-translational modifications are crucial in controlling the function of proteins, by regulating conformation, localization, stability, complexing, and activity. Therefore, post-translational modifications represent major intracellular (and likely extracellular) signals.

Despite decades of research into post-translational modifications and their regulatory role, the comprehensive understanding of this biochemistry is still evolving. The sheer number of different modifications and the likelihood of multiple modifications on each protein give rise to an exponential number of permutations for modifications on even a single protein. Indeed, multiple modifications on a protein likely cooperate to govern protein function, while a coordinated regulation of modifications in a network of proteins is required to regulate any given biological process. Nonetheless, the functional relevance of a considerable number of site-specific post-translational modifications are known within intracellular signaling pathways, including insulin signaling (89). Furthermore, advances in modification-specific proteomic technologies are rapidly advancing the mapping and cartography of various post-translational modifications on an "omics" scale (90).

Phosphorylation

Phosphorylation is the reversible addition of a phosphoryl group (PO_3^{2-}) to amino acids, principally serine, threonine, and tyrosine. Phosphorylation is the most well-characterized post-translational modification within intracellular signaling, particularly in response to insulin. Phosphorylation is pervasive across the proteome with an estimated 75% of proteins reported to be phosphorylated (91).

Signals transduced by phosphorylation are critical to insulin action (Fig. 2A). Upon insulin binding, the insulin receptor (INSR) undergoes autophosphorylation and subsequently phosphorylates insulin receptor substrate 1 (IRS1), leading to phosphatidylinositol 3-kinase (PI3K) activation. PI3K activates protein kinase B (AKT), via 3-phosphoinositide-dependent protein kinase 1 and mammalian target of rapamycin complex 2 (mTORC2), and ras-related protein Rac1 (RAC1), which transmit parallel signals to affect GLUT4 translocation (92-96). AKT phosphorylates the Rab GTPase-activating proteins Tre-2, Bub2, and Cdc16 (TBC)1 domain family member 1 (TBC1D1) and 4 (TBC1D4; aka AS160), which relieve their inhibitory action on GLUT4 translocating Rab GTPases (97-101). In concert, PI3K activation of RAC1 orchestrates actin remodeling at the plasma membrane via actin-related protein 2 (ARP2) and 3 (ARP3) and cofilin (94-96, 102-106).

Acute exercise influences a substantial proportion of the phosphoproteome (107-109), with exhaustive high-intensity cycling regulating approximately 10% of the phosphoproteome within human skeletal muscle (108). Pathway enrichment analysis within this exercise-induced human skeletal muscle phosphoproteome has identified various phosphorylation pathways that were regulated by exercise, including those of the canonical exercise response kinases 5′-adenosine monophosphate–activated protein kinase (AMPK), mitogen-activated protein kinases (MAPKs), protein kinase A (PKA), mTOR, ribosomal protein S6 kinase (p70S6K) and Ca^{2+}/calmodulin-dependent protein kinases (CAMKs) (Fig. 2B), as well as pathways related to insulin signaling such as INSR, PI3K, AKT, and Rho signaling pathways (108). Indeed, the endurance exercise/contraction-induced regulation of these pathways are typically highly conserved across different species (ie, human, rat, and mouse) (107).

For the most part, exercise alone does not influence the activity of proximal proteins within the insulin signaling cascade (24, 44, 110-112), although phosphorylation of IRS1 at Ser 36, Ser 374, Ser 560, Ser 629, and Ser 1100 is increased following an exhaustive bout of high-intensity cycling (108). Nonetheless, insulin receptor knockout or inhibition of PI3K does not impair glucose uptake during exercise or contraction (24, 113, 114). Thus, alternative mechanisms must regulate contraction-induced insulin-independent glucose uptake. However, the insulin-induced activity of proteins within the proximal insulin signaling

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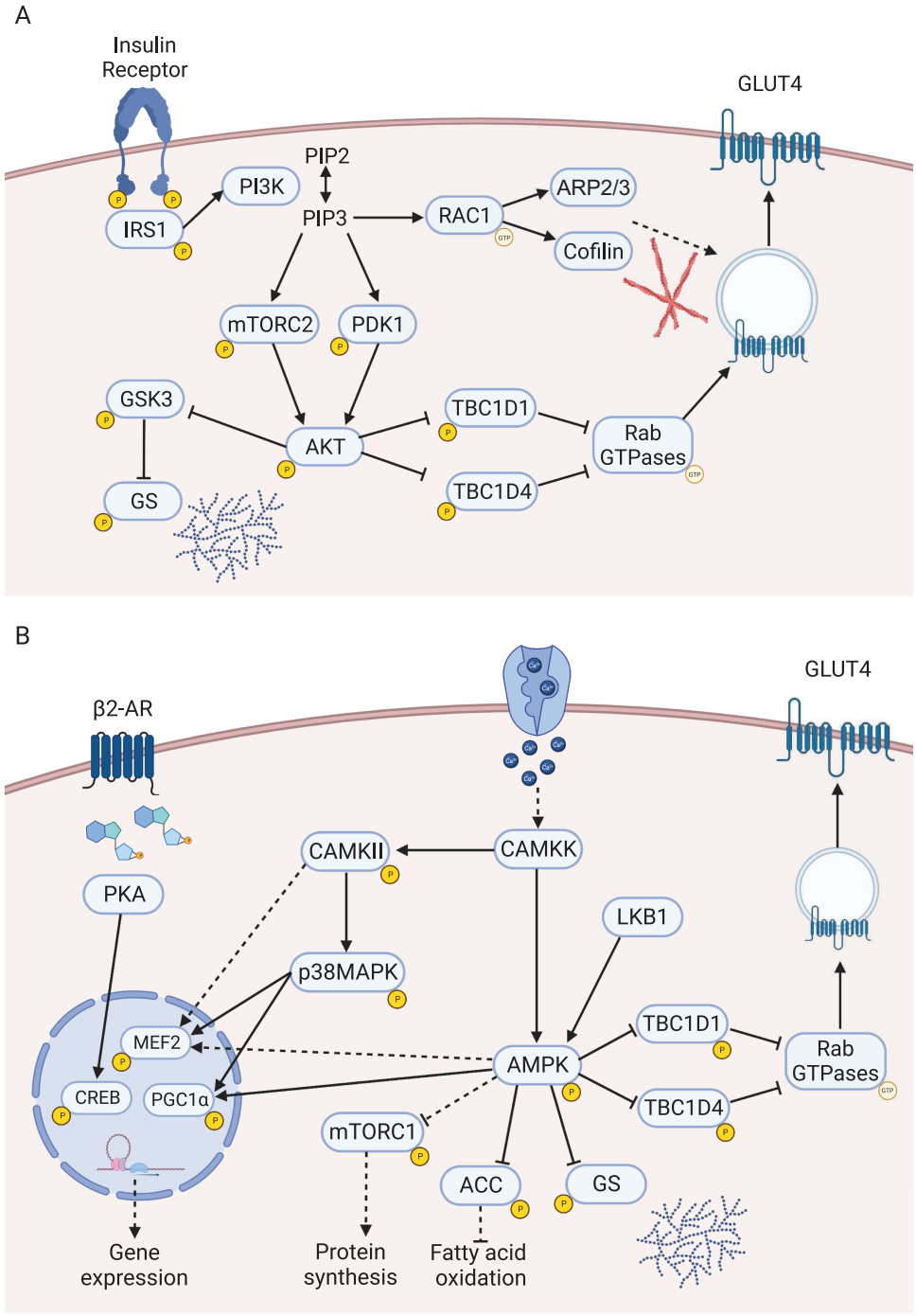

Figure 2. Insulin- and exercise-sensitive phospho-signaling in skeletal muscle. (A) Insulin stimulation induces autophosphorylation on the insulin receptor, which results in the recruitment and phosphorylation of IRS1, leading to PI3K activation (92). PI3K indirectly activates AKT via mTORC2 and PDK1 (92-94). AKT phosphorylates TBC1D1 and TBC1D4, which prevents their inhibition of the GLUT4 translocating Rab GTPases (97-101). Furthermore, AKT phosphorylates GSK3 leading to the activation of glycogen synthase (GS) and synthesis of glycogen. PI3K also activates RAC1, which promotes GLUT4 translocation via ARP2/3- and cofilin-mediated actin remodeling (95, 96). (B) Exercise regulates a range of phospho-signaling pathways. AMPK is phosphorylated and activated by CAMKK and liver kinase B1 (LKB1) (170, 255-259). AMPK orchestrates a shift towards catabolic processes: GLUT4 translocation is promoted via phosphorylation ofTBC1D1 andTBC1D4 (97-99, 119, 123, 129), net glycogen breakdown via inhibition of GS (260), fatty acid oxidation via inhibition of acetyl-CoA carboxylase (ACC) (261), and inhibition of protein synthesis through negative regulation of mTORC1 activity (262). AMPK also promotes the expression of metabolic genes, including GLUT4, through myocyte enhancer factor 2 (MEF2) and peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC1α) activation (202, 263, 264), which can also be activated via CAMKII and p38MAPK (265-267). Accumulation of cyclic AMP (cAMP) activates PKA, which phosphorylates cyclic AMP-responsive element binding protein (CREB) and promotes transcription of metabolic genes (268, 269).

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pathway (eg, PI3K and AKT) can be augmented following exercise in vivo (24, 44, 110, 112), although this is a far from consistent finding (54, 55, 113, 115, 116). Indeed, augmented proximal insulin signaling would be consistent with increased skeletal muscle blood flow and insulin delivery post exercise (58, 59).

Exercise and contraction per se increase the phosphorylation of TBC1D4 on multiple sites (Ser 318, Ser 341, Ser 588, Ser 600, Thr 642, Ser 704, and Ser 751) (107, 108, 117-122), although decreased phosphorylation of sites on TBC1D4 (Ser 318, Ser 597, Ser 666, and Thr 642) has also been reported immediately post exercise (107, 108). These apparent inconsistencies may be reflective of differences in the methodology of measurement (eg, total phosphorylation of TBC1D4 vs phospho-site–specific changes), the species, the exercise intensity, and/or the postexercise timepoint investigated. For example, although a time course analysis has yet to be performed within a single investigation in humans, phosphorylation of TBC1D4 on Ser 318 and Thr 642 decreases immediately after high-intensity exhaustive cycling (108), while it is increased 4 hours after 1 hour of single-legged kicking at 80% of peak power output (117), indicating a possible temporal regulation. In addition to phosphorylation by AKT, TBC1D4 is also a target of AMPK (119, 123). AMPK is activated during exercise via phosphorylation of the catalytic α-subunit on Thr 172 (107, 108, 120, 124-128), subsequently phosphorylating TBC1D4. AMPK also phosphorylates TBC1D1, another

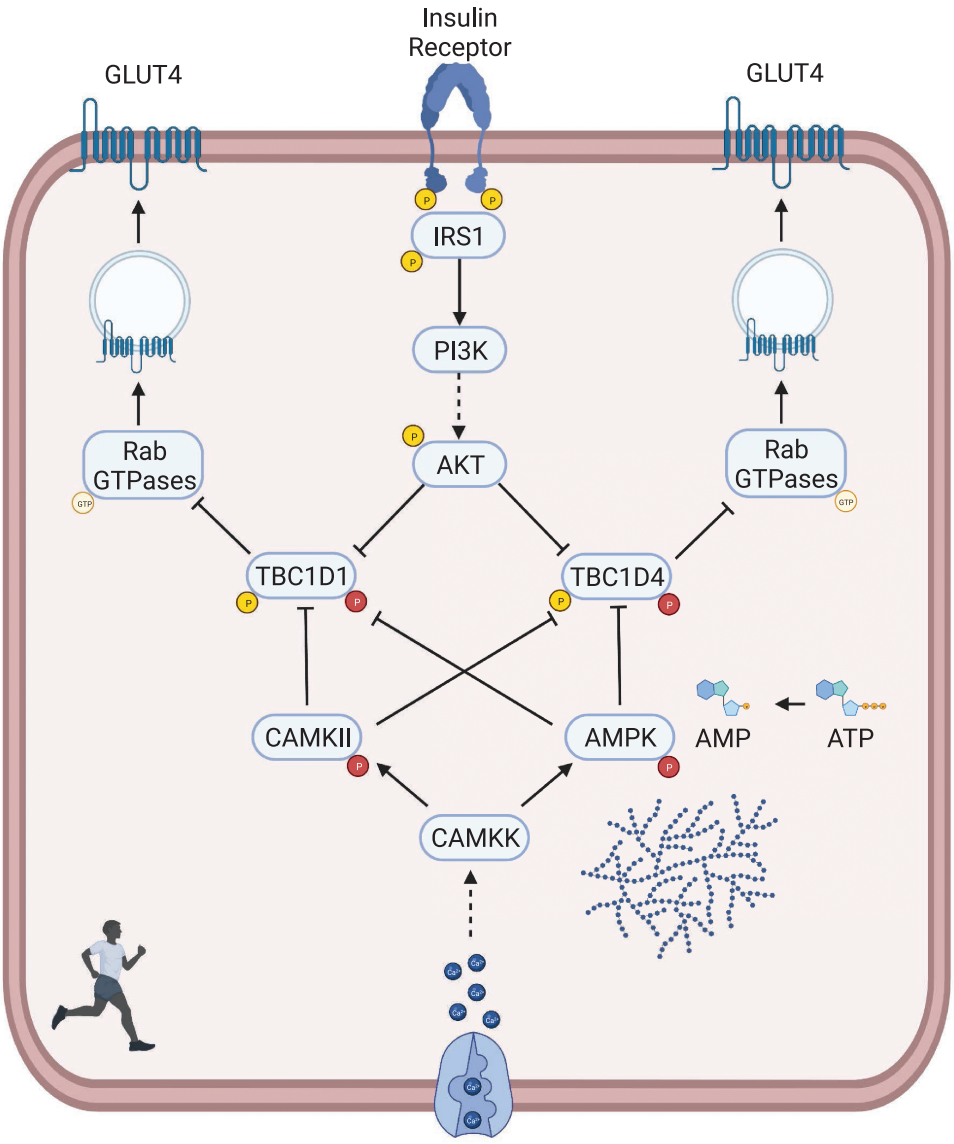

Figure 3. Exercise- and insulin-sensitive signals converge on TBC1D1 and TBC1D4 to facilitate GLUT4 translocation. Exercise- and insulin-signaling cascades converge on TBC1D1 and TBC1D4. Insulin promotes AKT-mediated phosphorylation of TBC1D1 and TBC1D4, while AMPK and CAMKII mediate the exercise-induced phosphorylation of TBC1D1 and TBC1D4 on independent and overlapping sites to those targeted by AKT. Yellow phospho-sites represent insulin-induced phosphorylation. Red phospho-sites represent exercise-induced phosphorylation.

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Rab inhibiting enzyme (97-99, 129), with the phosphorylation of TBC1D1 increased at Ser 108, Ser 159, Ser 231, Ser 301, Ser 614, Ser 660, and Ser 700 during exercise and contraction (97, 107, 108, 130). Phosphorylation of TBC1D1 and TBC1D4 persist for hours after exercise (115, 117, 121), potentially priming these proteins for further inhibition by insulin, thereby augmenting Rab GTPase activity. Indeed, insulin-induced TBC1D1 and TBC1D4 phosphorylation is augmented in the hours following an acute bout of exercise (115, 117, 121, 122, 131). Thus, TBC1D1 and TBC1D4 may represent a convergence point for insulin- and exercise-induced signals to promote GLUT4 translocation and glucose uptake (Fig. 3).

The importance of the AMPK–TBC1D1/TBC1D4 axes for increased glucose uptake and insulin sensitization has been demonstrated in the postexercise period. Knockout of AMPK isoforms impairs elevated glucose uptake following contraction and reduces the insulin-sensitizing effects of prior exercise and contraction (132-137). Furthermore, humans harboring a rare AMPK-activating mutation (R225W) display a trend toward elevated exercise-induced glucose uptake during a period that spanned exercise and recovery (138). Knockout of TBC1D1 reduces skeletal muscle glucose uptake following exercise (139, 140), while TBC1D4 is required for exercise to enhance skeletal muscle insulin sensitivity 3 hours after contraction (130). Electroporation of nonphosphorylatable TBC1D1/TBC1D4 mutants also impair contraction-stimulated glucose uptake (97, 123, 141). However, glucose uptake during contraction and exercise is normal in AMPKα1α2 and TBC1D1 knockout mice (140, 142). Furthermore, exercise-induced glucose uptake precedes the activation of AMPK in humans (143), while moderate-intensity exercise increases whole-body glucose disposal in the absence of AMPK activation after short-term exercise training in humans (144). Together, these data indicate that while AMPK and TBC1D1/TBC1D4 are critical in the regulation of glucose uptake and insulin sensitivity in the postexercise period, these signaling axes are not required for the increase in glucose uptake during contraction and exercise. However, whether these data point towards a limited involvement of AMPK in glucose uptake during exercise or a substantial redundancy with other pathways within this physiological system remains up for debate.

An additional target of AMPK is the phosphoinositide kinase, FYVE-type zinc finger containing (PIKFYVE) protein. Phosphorylation of PIKFYVE increases during ex vivo contraction, which occurs alongside the translocation of PIKFYVE to intracellular membranes (145). Although specific phosphorylation site(s) and upstream kinase(s) remain to be elucidated with respect to contraction, AMPK phosphorylates PIKFYVE on Ser 307, which promotes PIKFYVE colocalization with endosomes (145). Inhibition of PIKFYVE reduces insulin, contraction, and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR)-stimulated glucose uptake in vitro (145), while skeletal muscle-specific knockdown of PIKFYVE reduces insulin-stimulated glucose uptake in soleus and extensor digitorum longus muscles (146). Nonetheless, the specific mechanism of PIKFYVE-mediated glucose uptake remains unclear. Furthermore, the phosphorylation of Ser 307 remains unchanged following endurance exercise in humans and mice (107, 108). Thus, the relevance of PIKFYVE to exercise-induced glucose uptake in vivo remains elusive.

AMPK may provide the link between glycogen content and glucose uptake into skeletal muscle. Exercise-induced AMPK activity and Thr 172 phosphorylation correlate with glycogen content in skeletal muscle (147-154). Indeed, AMPK physically binds to glycogen via its β-subunit (155-159). Autophosphorylation of AMPKβ on Thr 148, which lies in the carbohydrate binding domain, releases the AMPK complex from glycogen (157-159), increasing free AMPK and enhancing the activating phosphorylation of Thr 172 on the catalytic α-subunit. Glucose uptake, GLUT4 translocation, and AMPK activity are elevated when contraction is commenced with low glycogen in murine fast-twitch muscle (153, 154, 160). However, contraction increases glucose uptake independently of glycogen content and AMPK activation in slow-twitch muscle (153, 154). Therefore, while AMPK activation and glycogen content may regulate glucose uptake in fast-twitch muscle, AMPK is not the only regulator of exercise- or contraction-induced glucose uptake in skeletal muscle. The picture regarding glycogen content and exercise-induced glucose uptake is further complicated when considering data from human studies. When exercise was commenced with low skeletal muscle glycogen content, induced by 1-legged glycogen-depleting exercise performed the previous evening (16 hours prior), glucose uptake during exercise was enhanced in the prior exercised leg (low glycogen) compared with the control leg (normal glycogen) (161). However, when the same degree of glycogen depletion was induced by dietary means (ie, no prior exercise), leg glucose uptake during the first 90 minutes of exercise was not different between high and low carbohydrate (glycogen) trials. In fact, glucose uptake was reduced in the low carbohydrate trial (low muscle glycogen) after 120 minutes of exercise, likely owing to the low systemic glucose availability (161). However, the effects of skeletal muscle glycogen concentration are difficult to isolate from the confounding effects of prior exercise and dietary manipulation, including altered circulating metabolites and hormones. Therefore, the influence of glycogen on exercise-induced glucose uptake remains controversial.

Contraction of skeletal muscle is initiated by Ca^{2+} release from the sarcoplasmic reticulum. In addition to initiating

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contraction, elevated Ca^{2+} concentrations are sensed by CAMKs. The transduction of signals through this cascade are mediated by phosphorylation. Exercise increases the autophosphorylation of CAMKII on Thr 286, which is highly correlated with CAMKII activity in human skeletal muscle (162-164), and on a number of additional phosphosites on various CAMKII subunits (107, 108). The activation of CAMKII may be necessary for contraction-induced glucose uptake. Inhibition of CAMKII reduces contraction-induced glucose uptake and GLUT4 translocation (165-167). Furthermore, inhibition of calmodulin and Ca^{2+}/calmodulin-dependent protein kinase kinase (CAMKK), an upstream kinase of CAMKII, impairs contraction-induced glucose uptake (168, 169), an effect that may be partially mediated by reduced CAMKK phosphorylation of AMPK (166, 170). CAMKII- and AMPK-mediated glucose uptake are independent but not additive, suggesting a downstream convergence of these pathways (167). Indeed, similarly to AMPK, knockdown of CAMKII reduces the contraction-induced phosphorylation of TBC1D1 on Ser 231 and TBC1D4 on Thr 642 (167), providing a putative mechanism for CAMKII-mediated GLUT4 translocation and glucose uptake.

Ubiquitination and Ubiquitin-like Modifications

Protein turnover is critical for quality control and cellular homeostasis. Degradation is under the control of autophagy and the ubiquitin-proteasome system, both via ubiquitin signaling. Ubiquitin is a small 8.6 kDa protein that can be attached to proteins, principally via lysine residues. Protein ubiquitination is regulated by a 3-step cascade; ubiquitin activation by ubiquitin-activating (E1) enzymes, conjugation by ubiquitin-conjugating (E2) enzymes and ligation by ubiquitin-ligases (E3 ligases). In the ubiquitin-proteasome system the 26S proteasome recognizes, unfolds, and degrades short-lived proteins with K48-linked polyubiquitin chains. In addition, multiple other chains of ubiquitination and ubiquitin-like modifications occur, such as NEDDylation, allowing for a diversity of signals including enzyme activation and sub-cellular localization. Thus, ubiquitination is a major post-translational modification in the control of cellular homeostasis.

Dysregulation of the ubiquitin–proteasome system is implicated in the pathogenesis of many diseases (171), including type 2 diabetes (172-175). Aberrant ubiquitination in beta-cells impairs insulin secretion (176), while dysregulated ubiquitin–proteasome system activity in peripheral tissues (eg, liver and skeletal muscle) inhibits insulin-stimulated glucose metabolism (174), mitochondrial function (177), and muscle mass (178). Using 2-dimensional difference gel electrophoresis mass spectrometry we have identified dysregulated ubiquitin–proteasome system proteins within primary myotubes from individuals with type 2 diabetes, such as the proteasomal subunits alpha 1 (PSMA1), alpha 6 (PSMA6), and beta 2 (PSMB2) (179). Furthermore, siRNA silencing of PSMA1, PSMA6, and PSMB2 and proteasomal inhibition in human primary myotubes impaired basal and insulin-stimulated glucose incorporation into glycogen, recapitulating the diabetic phenotype (179). Indeed, there is growing evidence for the regulatory role of ubiquitination in the insulin signaling pathway (172). For example, INSR is ubiquitinated by multiple E3 ligases resulting in a diversity of signals mediating receptor internalization as well as proteasomal and lysosomal degradation (174, 180-182). Consequently, the metabolic role of several ligases and deubiquitinases has been investigated. For example, skeletal muscle overexpression of tripartite motif-containing protein 72 (MG53) induces insulin resistance via the degradation of INSR and IRS1 (174), while kelch-like ECH-associated protein 1 (KEAP1) knockdown stabilizes nuclear factor erythroid 2–related factor 2 (NRF2) and activates the NRF2 gene program, opposing the development of type 2 diabetes (175).

Acute endurance exercise alters proteasome activity and the skeletal muscle ubiquitinome (183, 184). Exhaustive high-intensity cycling activates the 26S proteasome through PKA-mediated phosphorylation of 26S proteasome non-ATPase regulatory subunit 11 (PSMD11) on Ser 14, which leads to a reduction in global ubiquitination (108, 183, 184) as damaged proteins are degraded. CAMKII can also regulate proteasomal activity via phosphorylation of 26S proteasome regulatory subunit 8 (PSMC5), albeit in HEK293T cells (185), providing an additional hypothetical avenue of contraction-regulated proteasomal activation. Furthermore, these data indicate the interplay between phosphorylation and ubiquitination in controlling protein degradation.

The effect of high-intensity exercise (~10 minutes of cycling at 77% to 88% Wmax to exhaustion) on ubiquitination and ubiquitin-like modifications in skeletal muscle was investigated by enrichment of K-GG (lysine-glycine/glycine)–modified peptides followed by mass spectrometry (184). During digestion by trypsin, ubiquitin and ubiquitin-like modifications are cleaved at their initial arginine, leaving a K-GG remnant on the modified peptide, which can be enriched by immunoprecipitation and detected by mass spectrometry (186). This poses a unique challenge when studying ubiquitination and ubiquitin-like modifications in this manner as the specific type of modification (eg, ubiquitin, NEDD8, or ISG15) cannot be discriminated, nor can chain length or branching be determined. Of the 1536 quantified K-GG-modified sites, 391 were regulated immediately after exercise (275 downregulated and 116 upregulated), with all sites returning to pre-exercise levels

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within 2 hours of recovery (184). Ubiquitin itself displayed site-specific regulation following exercise, with K-GG motifs decreased on K6, K11, K29, K48, and K63, while K27 increased, suggesting that exercise may differentially regulate specific ubiquitin chains (184).

Enriched within the proteins with regulated K-GG sites were proteins related to glycolysis (184). To illuminate how ubiquitin and ubiquitin-like modifications may regulate glucose homeostasis we reinterrogated this dataset with a specific focus on glucose metabolism and insulin signaling. INSR is ubiquitinated on Ser 1057 in human skeletal muscle, although this was not quantified sufficiently to assess the effect of exercise on this modification. The glycolytic proteins with exercise-regulated ubiquitination included fructose biphosphate aldolase a (ALDOA), beta-enolase (ENO3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate mutase 1 (PGAM1), and phosphoglycerate mutase 1 (PGAM2) (Fig. 4). Of the regulated sites, ubiquitination was typically downregulated on these proteins, pointing towards a stabilization of glycolytic enzymes during high-intensity exercise. Aldolase also plays a regulatory role in GLUT4 translocation (187). Aldolase interacts with GLUT4 in 3T3L1 adipocytes, regulating the interaction between GLUT4 and filamentous actin and consequently insulin-stimulated GLUT4 exocytosis (187). Furthermore, calcium promotes the binding of aldolase to the cytoskeleton in skeletal muscle (188). Deubiquitination of glycolytic enzymes including ALDOA and GAPDH is mediated by joshepin-2 in cancer cells, which stabilizes these enzymes and promotes glycolysis (189). However, whether joshepin-2 is responsible for the deubiquitination of glycolytic enzymes during exercise remains unknown. Future investigations should delineate the specific ubiquitin and/or ubiquitin-like modifications on glycolytic enzymes that are regulated by exercise, as well as the ligases and/or deubiquitinating enzymes that mediate these effects.

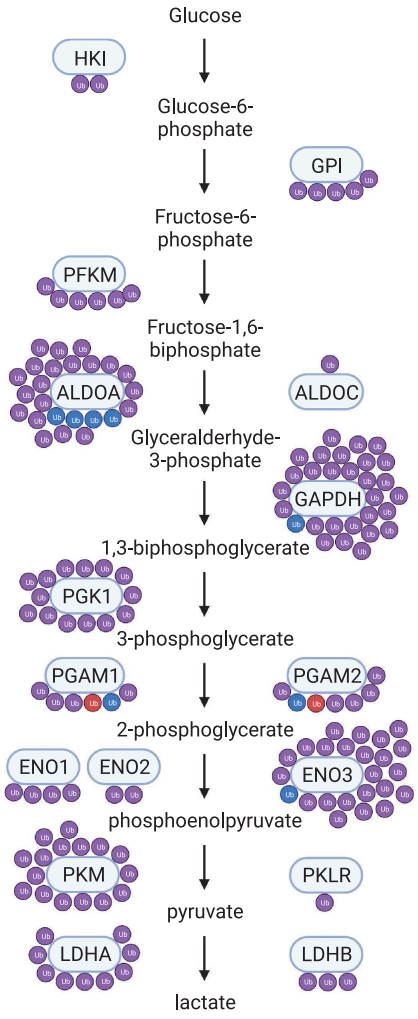

Figure 4. Exercise regulates the ubiquitination or ubiquitin-like modification of glycolytic enzymes in skeletal muscle. Glycolytic enzymes are enriched within proteins with K-GG remnants (ubiquitin and ubiquitin-like modifications) regulated by exercise (184). K-GG remnants on ALDOA, GAPDH, PGAM1, PGAM2, and ENO3 are regulated by exercise. Blue ubiquitin sites represent K-GG remnants downregulated by exercise. Red ubiquitin sites represent K-GG remnants upregulated by exercise.

AMPKα2 is ubiquitinated by the E3 ligases MG53 and E3 ubiquitin-protein ligase makorin-1 (MKRN1) leading to degradation (190, 191). Ubiquitination of AMPKα is increased in skeletal muscle of obese mice, while high glucose availability induces MG53-mediated degradation of AMPKα (190). Stabilization of AMPK via knockout of MKRN1 increases glucose uptake in mouse embryonic fibroblasts, while MKRN1 knockout mice display resistance to high fat diet–induced metabolic syndrome (191). Phosphorylation of AMPKα2 on Ser 491, a site of autophosphorylation and the target site of p70S6K1 (192, 193), is required for the interaction between AMPK and MG53, and the subsequent ubiquitin-mediated degradation of AMPKα2 (190). Indeed, phosphorylation of AMPKα2 on Ser 491 is inhibitory in biological systems (190, 192). Interestingly, acute endurance exercise and contraction increases phosphorylation of AMPKα2 on Ser 491 (107-109), advancing the hypothesis that AMPKα2 is ubiquitinated and degraded during exercise. Despite this, the AMPK complex was still activated following exercise (as assessed by the downstream phosphorylation of target proteins) (108), suggesting that Ser 491 either has additional functions or that activating phosphorylation (Thr 172) outweighs inhibitory phosphorylation (Ser 491) on AMPK during exercise.

Evidence is emerging for an interplay between phosphorylation, ubiquitination, and NEDDylation during exercise (184). Global NEDDylation increases and ubiquitination decreases in response to exercise and forskolin-stimulated

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PKA activation, with ubiquitination returning to baseline within 1 to 2 hours. However, when NEDDylation is inhibited, ubiquitination remains depressed following forskolin treatment, indicating that NEDDylation is required for the activation of E3 ligases to promote ubiquitination and continued flux through the ubiquitin–proteasome system (184). Nonetheless, whether and indeed how this interplay may influence insulin sensitivity remains unclear.

Acylation

Acylation is the process of adding an acyl group to a compound (eg, a protein), of which there are various forms, including succinylation, malonylation, palmitoylation, and more. The most well-studied form of protein acylation is acetylation. Acetylation is the reversible addition of an acetyl group from acetyl-coenzyme A (CoA) to an amino acid, the best characterized being lysine. Acetyltransferases catalyze the transfer of acetyl groups to the ε-amino acid side chain of lysine, while deacetylases remove them. Alternatively, acetylation can occur nonenzymatically from acetyl-CoA. Initially identified on histones, acetylation is pervasive across the proteome. In skeletal muscle, mitochondrial proteins, in particular those of the tricarboxylic cycle and the electron transport chain, make up the majority of acetylated proteins and also show elevated acetylation stoichiometry (194, 195). Furthermore, acetylation is sensitive to the cellular energetic state (196, 197), potentially linking metabolic flux to protein function and enzyme activity.

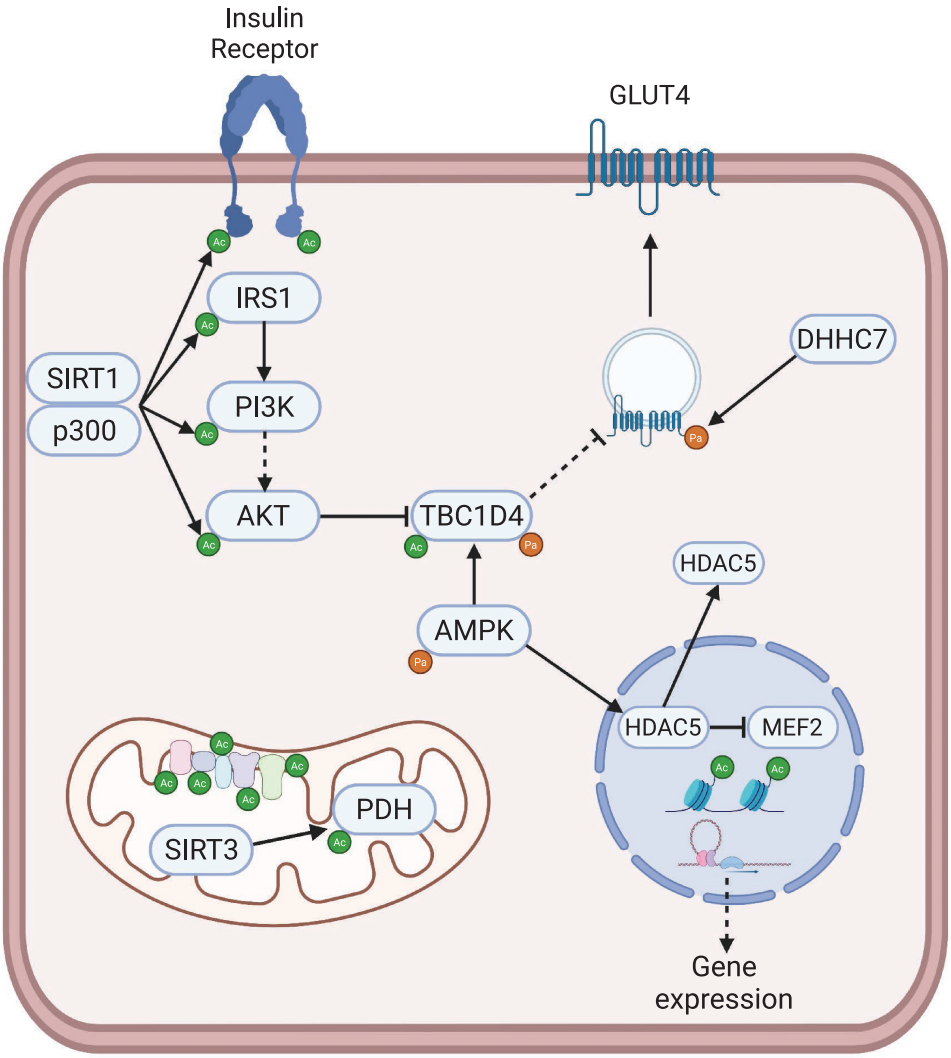

Figure 5. Acetylation and palmitoylation in insulin signaling, GLUT4 translocation, and mitochondria. Acetylation and palmitoylation are apparent on numerous proteins in the insulin signaling cascade. Palmitoylation of GLUT4 by the palmitoyltransferase DHHC7 is critical for insulin-stimulated membrane translocation in adipocytes. Mitochondrial proteins are highly acetylated in skeletal muscle. Endurance exercise increases mitochondrial protein acetylation, including PDH, which the mitochondrial deacetylase SIRT3 opposes. Acetylation of PDH may regulate the flexibility between glucose and fatty acid oxidation. Exercise also increases histone acetylation, concomitant with the nuclear export of HDAC5, which may play a role in MEF2 activation and GLUT4 transcription.

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A regulatory role for acetylation on various components of the insulin signaling cascade has been reported (198) (Fig. 5). For example, the class I and II histone deacetylase inhibitor trichostatin A increases insulin-stimulated glucose uptake and glycogen synthesis in C2C12 cells via increased phosphorylation of INSR Tyr 1146, AKT Ser 473, and glycogen synthase kinase-3 beta (GSK3β) Ser 9 (199). Furthermore, insulin reduces the acetylation of AKT in skeletal muscle of fasted, but not fed, mice (200). However, acetylated peptides from proteins within the insulin signaling pathways have mostly been undetectable via mass spectrometry-based proteomics performed in rat and human skeletal muscle (194, 195), which might reflect technical challenges in their enrichment or their low abundance/absence. Conversely, proteins involved in glycolysis are abundantly acetylated within skeletal muscle (194, 195).

In accordance with the initial characterization of acetylation on histones, this process also plays a regulatory role in transcription. Acetylation of histones can regulate chromatin conformation and therefore accessibility of regulatory factors (ie, transcription factors, coactivators and repressors) to DNA (201). As such, acetylation is likely to be involved in the transcriptional control of insulin sensitivity and the adaptive responses to exercise training. For example, dissociation of histone deacetylase 5 (HDAC5) from the transcription factor myocyte enhancer factor 2 (MEF2) and the MEF2 binding domain of the GLUT4 gene promoter induces GLUT4 transcription in human primary myotubes, an effect that is regulated by AMPK-mediated phosphorylation of HDAC5 (202). In vivo, skeletal muscle–specific knockout of histone deacetylase 3 (HDAC3) and loss of HDAC3 deacetylase activity impairs insulin sensitivity, although paradoxically this occurs alongside an increase in exercise capacity (203, 204). Furthermore, the deacetylase activity of sirtuin 1 (SIRT1), which has been proposed as a central regulator of mitochondrial biogenesis in skeletal muscle via its ability to regulate PGC1α cotranscriptional activity (205), is required for the insulin sensitizing effects of calorie restriction (206). Conversely, overexpression of SIRT1 does not prevent high-fat diet induced insulin resistance (207), while SIRT1 is also dispensable for contraction-induced glucose uptake (208) and the mitochondrial adaptations to exercise training (209). Metabolism and the development of insulin resistance are also predominantly unaffected by the individual knockout of nuclear-localized acetyltransferases. For example, skeletal muscle-specific deletion of general control of amino acid synthesis protein 5 (GCN5), which opposes SIRT1 by transferring an acetyl moiety to PGC1α, does not influence the metabolic adaptations to endurance exercise training (210). Furthermore, skeletal muscle-specific knockout of E1A binding protein p300 (p300) or CREB-binding protein (CBP) do not influence skeletal muscle insulin sensitivity (211), while p300 is not required for the metabolic adaptations to endurance exercise training (212). However, skeletal muscle-specific double knockout of p300 and CBP is lethal even when induced in adult mice (213), demonstrating both the importance of acetylation and the inherent redundancy within these systems. The series of studies identifying no discernible phenotype with individual knockout of p300 and CBP, but lethality with double knockout (211-213) also serve to highlight the experimental difficulty in probing these complex physiological systems.

Evidence for a role of acetylation in regulating skeletal muscle glucose metabolism and insulin sensitivity in vivo predominantly come from knockout and transgenic mouse models in which hyperacetylation of mitochondrial proteins are induced. Elevations in skeletal muscle acetylation can be achieved by individual and combined knockout of proteins controlling acetyl-CoA buffering (carnitine acetyltransferase; CrAT) and mitochondrial lysine acetylation (sirtuin 3; SIRT3) (197). Indeed, mice with skeletal muscle hyperacetylation display increased susceptibility to diet-induced insulin resistance (197, 214-216). However, this effect is independent of insulin signaling and GLUT4 translocation (216). The defect may lie at the level of glucose oxidation, which is reduced in skeletal muscle of SIRT3 knockout mice (217). Indeed, pyruvate dehydrogenase E1 component subunit alpha (PDH E1a) is hyperacetylated on Lys 336 and its enzyme activity is suppressed in SIRT3 knockout mice (217). Furthermore, pyruvate-linked respiration is impaired in CrAT knockout mice (214). By catalyzing the reaction of pyruvate to acetyl-CoA, PDH provides the link between glycolysis and the tricarboxylic acid cycle, and therefore the flexibility between carbohydrate and fat metabolism (218). Given that the product of PDH activity is acetyl-CoA, it is probably unsurprising that acetylation can mediate a negative feedback loop, preventing excess acetyl-CoA production from glucose metabolism.

In spite of mitochondrial acetylation being linked to insulin resistance (197, 214-216), we have shown that 5 weeks of high-intensity interval training increases acetylation within human skeletal muscle, predominantly within the mitochondria (195). Furthermore, increased acetylation of PDH E1a on Lys 336 was within the top 5 most regulated acetyl sites with exercise training (195). As exercise training increases insulin sensitivity (11-16), elevated acetylation within skeletal muscle is unlikely to cause insulin resistance. Rather, physiological hyperacetylation of PDH E1a may play a role in the increased preference for fatty acid oxidation following exercise training, which likely opposes the development of insulin resistance. Indeed, mitochondrial hyperacetylation increases the capacity for mitochondrial fatty acid metabolism (197).

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In contrast to chronic exercise in humans, acute exercise in rodents decreases mitochondrial acetylation in skeletal muscle, particularly targets of the mitochondrial deacetylase SIRT3 (196). Whether acute exercise regulates the acetylome in human skeletal muscle remains to be determined. However, targeted analysis of individual acetyl sites suggests that, in contrast to rodents (196, 209), SIRT1 and SIRT3 are not activated during moderate-intensity endurance exercise in humans, nor is pan-acetylation regulated by acute exercise (219). Sirtuins are an NAD^+-dependent class of protein deacetylases. The discrepancy in exercise-induced sirtuin activation between rodents and humans likely arises from differences in NAD^+ flux during exercise. In rodents, NAD^+ increases during swimming exercise (220), while skeletal muscle NAD^+ content remains unchanged during exercise in humans (219, 221-223). However, acetylation of histone 3 Lys 36 is increased immediately after 1 hour of moderate-to-high intensity cycling, which occurred concomitantly with a reduced nuclear abundance of HDAC4 and HDAC5, the latter of which was ubiquitinated by exercise (224). Further investigation into the skeletal muscle acetylome following acute exercise in humans is warranted, particularly as acetylation is regulated by numerous NAD^+-dependent and -independent deacetylases and acetyltransferases, as well as via nonenzymatic mechanisms, of which many are yet to be investigated during exercise in human skeletal muscle.

One major consideration when assessing the contribution of protein acetylation to metabolic control is that acetylation stoichiometry is very low, even on mitochondrial proteins. In the liver, median acetyl stoichiometry is 0.05%, increasing to 0.11% in mitochondria (225). Thus, whether a relatively modest reduction or a chronic doubling (median fold change) in acetylation is sufficient to alter metabolism remains questionable. Nonetheless, physiological levels of mitochondrial malate dehydrogenase acetylation negatively correlate with its enzyme activity (196), indicating that acetylation can regulate the activity of individual metabolic enzymes in vivo.

In addition to acetylation, various other forms of acylation can modify lysine residues on proteins. Proteins can be post-translationally modified by succinylation, malonylation, glutarylation, and palmitoylation (226, 227). Like acetyl-CoA, the acyl substrates for these modifications (succinyl-CoA, malonyl-CoA, glutaryl-CoA, and palmitoyl-CoA) can be regulated by cellular energy status, exercise, insulin, and type 2 diabetes (228-231). As many of these modifications are directly related to fatty acid availability and oxidation, acylation is likely to be substantially disturbed in conditions of excess nutrient supply such as in obesity and type 2 diabetes.

The functional role of palmitoylation in the regulation of insulin-sensitive proteins has been explored, predominantly in adipocytes. Palmitoylation of cysteine residues occurs via reversible transfer of palmitate by palmitoyltransferases and removal by protein palmitoyl thioesterases. Palmitoylation adds a hydrophobic moiety to a protein that serves as a lipid anchor, aiding the membrane localization of proteins, thus it is of considerable interest in the context of glucose transport. Proteomic and targeted analyses of palmitoylated proteins in 3T3L1 adipocytes revealed palmitoylation of GLUT4, proteins involved in GLUT4 translocation (eg, vesicle associated membrane protein 2, TBC1D4, and ras-related protein rab14), and related signaling cascades (eg, AMPKα) (226). GLUT4 palmitoylation has subsequently been confirmed in skeletal muscle (232). Palmitoylation of GLUT4 at Cys 233 by palmitoyltransferase zinc finger DHHC-type containing 7 (DHHC7) is critical for insulin-stimulated membrane translocation (232, 233). Indeed, silencing of DHHC7 or serine substitution of Cys 233 (that cannot undergo palmitoylation) ablates GLUT4 translocation in 3T3L1 and primary adipocytes (232, 233). While it is currently unclear whether this mechanism translates to skeletal muscle, the regulation of skeletal muscle palmitoylation in response to insulin and exercise should be of considerable interest owing to its demonstrated role in GLUT4 translocation in adipocytes.

Adenosine Diphosphate Ribosylation

Adenosine diphosphate (ADP) ribosylation is a post-translational modification in which ADP ribose moieties are cleaved from NAD^+ and covalently transferred to proteins either as mono-ADP-ribosylation (MARylation) or poly-ADP-ribosylation (PARylation). Poly-ADP ribose polymerases (PARP) 1 and 2 are enzymes whose nuclear poly-ADP-ribosylation activity regulate energy metabolism, glucose homeostasis and insulin sensitivity (234-237).

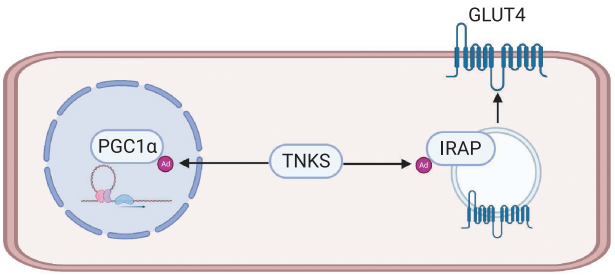

Figure 6. ADP ribosylation via the TNKS family of PARPs may regulate metabolism via GLUT4 translocation and inhibition PGC1α. TNKS ADP-ribosylates the GLUT4 storage vehicle-related protein IRAP and inhibition of TNKS PARP activity impairs GLUT4 translocation in adipocytes. Conversely, TNKS impairs skeletal muscle oxidative metabolism via PGC1α PARylation and degradation.

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However, as PARPs are NAD^+ consumers, much of the metabolic adaptations resulting from altered PARP activity have been attributed to modulation of sirtuin activity, particularly SIRT1 (234-237), rather than ADP ribosylation per se. Nonetheless, ADP ribosylation is pervasive across the proteome and in various cellular compartments, with ADP ribosylation detected on proteins from various metabolic pathways within skeletal muscle (238, 239). Tankyrases (TNKSs) are a predominantly cytosolic family of PARP enzymes that may more directly regulate metabolism (Fig. 6). In humans, the *TNKS* gene, which encodes TNKS1, lies within a susceptibility locus for type 2 diabetes (240), and *TNKS* gene variants are associated with early-onset obesity (241). Oral dosing with the TNKS inhibitor G007-LK improves glucose tolerance and insulin sensitivity independently of body and fat mass in mice fed a high-fat diet (242). In obese diabetic *db/db* mice, G007-LK reduces body mass gain, fat mass and hepatic steatosis, which occurred alongside increased mitochondrial protein content and fatty acid oxidation in skeletal muscle (243). TNKS1/2 interacts with, and PARylates, PGC1α in skeletal muscle, which was speculated to mediate the effects on energy metabolism (243). In adipocytes, TNKS interacts with leucyl-cistinyl aminopeptidase (IRAP) on GLUT4 storage vesicles, and insulin induces MAPK-mediated phosphorylation of TNKS that increases the PARylation of IRAP (244). Furthermore, knockdown and inhibition of TNKS impairs GLUT4 translocation (245). Thus, PARylation, and in particular TNKS, can regulate metabolism and insulin sensitivity.

To date, investigations into the effect of exercise on ADP ribosylation have primarily focused upon PARylation and PARP1 activation. In skeletal muscle, global PARylation increases following electrically evoked isometric contractions in mice (246), whereas autoPARylation of PARP1 is unaffected by moderate-intensity cycling in humans (247). PARP1 protein content increases following an acute bout of high-intensity interval training in young healthy males (248), and displays a trend to increase following moderate intensity exercise in a similar cohort (247). Conversely, in older individuals high-intensity interval training acutely decreases PARP1 protein content (248). Thus, while some studies have investigated PARylation in response to exercise this remains an immature field. Future studies should take advantage of emerging proteomic technologies to investigate how insulin and exercise regulate the ADP ribosylome (238, 239).

Future Directions

Despite the wealth of data collected regarding insulin signal transduction, and the interplay with exercise-sensitive cascades, the understanding of the signals that mediate exercise-induced glucose uptake and insulin sensitization is limited. Indeed, the identity of the signals that mediate glucose uptake during exercise remain unclear. Furthermore, the knowledge of signals transduced from insulin and exercise via post-translational modifications, aside from phosphorylation, is sparse. Future research efforts should be directed at decoding the diversity of ubiquitin and ubiquitin-like signals on metabolic enzymes, transporters, and signaling proteins. In addition, how fluxes in acyl-metabolites regulate protein acylation and consequently metabolism during exercise is of considerable interest, particularly in the context of palmitoylation and GLUT4 translocation. Furthermore, while the functional implications of canonical post-translational modifications in insulin- and exercise-sensitive signaling cascades are typically well characterized (for example phosphorylation on AKT Thr 308 and Ser 473, AMPK Thr172, and CAMKII Thr 286), little is known about the majority of insulin- and exercise-sensitive post-translational modifications. Exercise alone regulates over 1000 phospho-sites, nearly 400 ubiquitin-sites, and nearly 300 acetyl-sites (108, 184, 195). To truly understand how insulin and exercise induce glucose uptake in skeletal muscle, it is important to expand the understanding of the functional roles of post-translational modifications over and above canonical sites. Indeed, an understanding of the wider regulatory signals that control glucose homeostasis may lead to the development of new therapeutic targets.

Technical advances are enabling proteome-wide investigations of post-translational modifications (90). Nonetheless, skeletal muscle remains a challenging tissue for proteomic analyses, with the high dynamic range of protein abundance within skeletal muscle compromising quantification depth (249). However, advancing mass spectrometry technologies, emerging new acquisition methods, improving quantification algorithms, and the increased availability of effective enrichment techniques for a swathe of post-translational modifications (250-254) are collectively providing a powerful platform to study diverse signaling cascades and their responses to physiological stimuli on an "omics" scale (108, 184, 195-197, 238). Future studies should leverage these approaches to investigate the skeletal muscle landscape of previously underinvestigated post-translational modifications, as well as to provide multi-omic insights into the interplay between post-translational modifications, such as between phosphorylation and ubiquitination.

Owing to the nascent nature of techniques to study proteome-wide post-translational modifications, the wealth of data related to global changes in post-translational modifications in response to exercise stems from only a small number of studies (108, 184, 195, 196). This represents a limitation in terms of the diversity of the participants and the range of exercise interventions studied.

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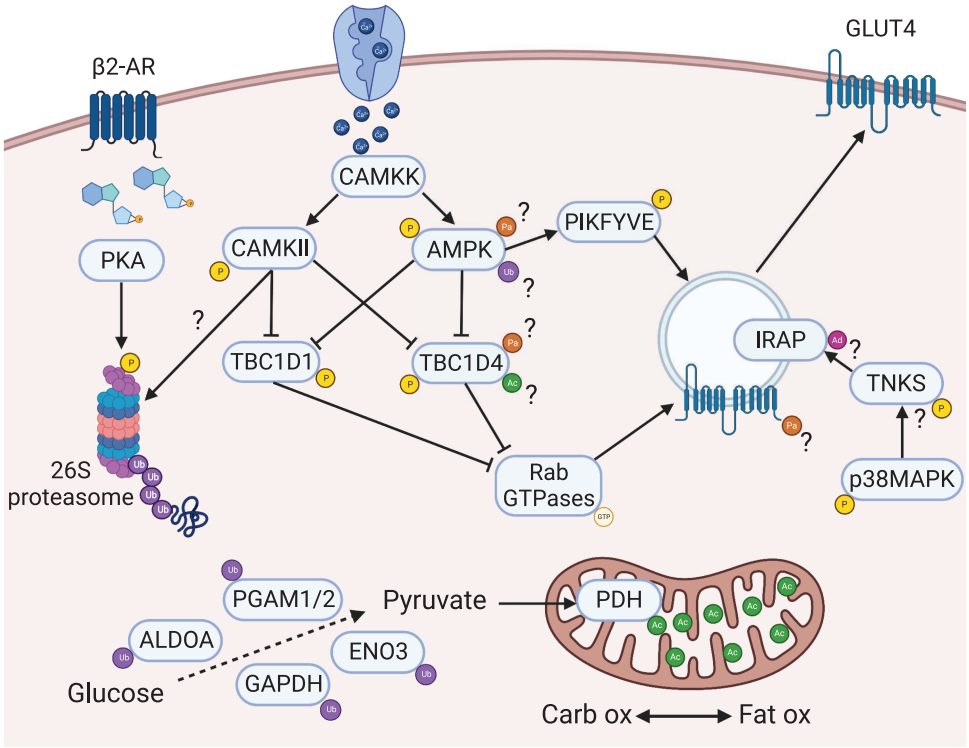

Figure 7. Complex signals interact within exercising skeletal muscle to regulate insulin sensitivity, glucose uptake, and metabolism. Numerous post-translational modifications interact to control glucose homeostasis. Phospho-signaling emanating from CAMKK, AMPK, and CAMKII regulate GLUT4 translocation via TBC1D1/TBC1D4 and PIKFYVE. Palmitoylation and ADP-ribosylation of GLUT4 and IRAP, respectively, may also contribute to GLUT4 translocation. Ubiquitination and/or ubiquitin-like modification of enzymes within the glycolytic pathway are decreased during exercise, while the 26S proteasome is activated via phosphorylation of proteasomal subunits by PKA. Endurance exercise training induces mitochondrial hyperacetylation, including acetylation of PDH E1A, which may facilitate elevated fatty acid oxidation. (Ac), acetylation; (Ad), ADP ribosylation; (P), phosphorylation; (Pa), palmitoylation; (Ub), ubiquitination and ubiquitin-like modifications; (GTP), GTP-bound protein.?; –, unconfirmed in exercising/contracting skeletal muscle.

Specifically, proteomic data into post-translational modifications following acute (phosphorylation and ubiquitination) or chronic (acetylation) exercise in humans currently arise from only 3 studies and a small number of male participants (108, 184, 195). Furthermore, the proteome-wide modifications that result from exercise other than acute high-intensity exhaustive cycling (108, 184) or chronic high-intensity interval training (195) remain unknown. Future studies should expand the mapping of exercise-induced post-translational modifications by investigating a range of exercise modalities, intensities, durations, and intermittency of exercise, as well as individuals of different ages, sex, race, and at various stages of metabolic disease development.

A complex network of signals converge and interact to regulate glucose metabolism and insulin sensitivity in response to exercise (Fig. 7). Indeed, exercise regulates thousands of post-translational modifications on hundreds of proteins, though the functional relevance of such modifications is not always known. The field is poised to decode how numerous forms of post-translational modifications confer exercise-induced signals to modulate glucose uptake and insulin sensitivity. Further mapping of the proteomic and proteome-wide post-translational modification landscape will provide additional clarity into mechanisms orchestrating the development of peripheral insulin resistance and the adaptive responses to exercise.

Conclusions

Exercise and skeletal muscle have a central role in promoting whole-body insulin sensitivity and opposing the development of type 2 diabetes. Exercise increases glucose uptake and insulin sensitivity through a range of mechanisms, though principally via GLUT4 translocation.

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