Post-translational modifications: The signals at the intersection of exercise, glucose uptake and insulin sensitivity

Ben Stocks\(^1\) and Juleen R Zierath\(^{1,2}\)

\(^1\)Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen 2200, Denmark.

\(^2\)Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm 171 77, Sweden.

**Corresponding author and Reprint Requests:** Professor Juleen R Zierath, Department of Molecular Medicine and Surgery, Section for Integrative Physiology, Karolinska Institutet, Biomedicum C4. Solnavägen 9, SE 171 77 Stockholm, Sweden. Email: juleen.zierath@ki.se

**Financial Support:** This work was supported from the Swedish Research Council (Vetenskapsrådet) (2015-00165), the Knut and Alice Wallenberg Foundation (2018-0094), and the Novo Nordisk Foundation (NNF18CC0034900).

**Disclosure Summary:** The authors declare no conflicts of interest

© The Author(s) 2021. Published by Oxford University Press on behalf of the Endocrine Society. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
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 (e.g. 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 non-canonical 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.

Keywords: Exercise, glucose, insulin, phosphorylation, ubiquitination, acetylation
Introduction

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 two-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) (Figure 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 mechanism by which endurance exercise (inclusive of moderate-intensity continuous exercise, high-intensity exhaustive exercise, and high-intensity interval training; Figure 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 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 (i.e. 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 KO 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 (0-2h 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 post-exercise 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, whom 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 (HKII) abundance result 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 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, twelve 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 mechanism 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 are a wide range of post-translational modifications including phosphorylation, ubiquitination and ubiquitin-like modifications, various forms of acylation (e.g. 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 (Figure 2A). Upon insulin binding, the insulin receptor (INSR) undergoes autophosphorylation and subsequently
phosphorylates insulin responsive substrate 1 (IRS1), leading to phosphatidylinositol 3-kinase (PI3K) activation. PI3K activates protein kinase B (AKT), via 3-phosphoinositide-dependent protein kinase 1 (PDK1) and mammalian target of rapamyycin 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 TBC1 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 coflin (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’-AMP-activated protein kinase (AMPK), mitogen-activated protein kinases (MAPKs), protein kinase A (PKA), mTOR, ribosomal protein S6 kinase (p70S6K) and Ca2+/calmodulin-dependent protein kinases (CAMKs) (Figure 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 (i.e. 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 are 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 pathway (e.g. 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 (e.g. total phosphorylation of TBC1D4 versus phospho-site specific changes), the species, the exercise intensity, and/or the post-exercise 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 one 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 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 (Figure 3).

The importance of the AMPK-TBC1D1/TBC1D4 axes for increased glucose uptake and insulin sensitization has been demonstrated in the post-exercise 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 towards 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 three hours after contraction (130). Electroporation of non-phosphorylatable 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 post-exercise 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 PIKFYVE. 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 co-localization with endosomes (145). Inhibition of PIKFYVE reduces insulin, contraction, and 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 one-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 to the control leg (normal glycogen) (161). However, when the same degree of glycogen depletion was induced by dietary means (i.e. 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 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), as well as on a number of additional phospho-sites 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 three-step cascade; ubiquitin activation by E1 enzymes, conjugation by E2 enzymes and ligation by E3 ligases. In the ubiquitin-proteasome system the 26S proteasome recognizes, unfolds, and degrades short-lived proteins with K48-linked poly-ubiquitin chains. In addition, multiple other chains of ubiquitination and ubiquitin-like modifications occur, e.g. 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
(e.g. liver and skeletal muscle) inhibits insulin-stimulated glucose metabolism (174), mitochondrial function (177), and muscle mass (178). Using two-dimensional difference gel electrophoresis (2D DIGE)-mass spectrometry we have identified dysregulated ubiquitin-proteasome system proteins within primary myotubes from individuals with type 2 diabetes, such as the proteosomal 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 min cycling at 77-88% 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 (e.g. 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 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 re-interrogated 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) (Figure 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 (JOSD2) in cancer cells, which stabilizes these enzymes and promotes glycolysis (189). However, whether JOSD2 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.

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 PKA activation, with ubiquitination returning to baseline within 1-2 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 (e.g. 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-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 non-enzymatically 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.

A regulatory role for acetylation on various components of the insulin signaling cascade has been reported (198) (Figure 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 (i.e. transcription factors, co-activators 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 co-transcriptional 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 five 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).

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 (H3) Lys 36 is increased immediately after one 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 non-enzymatic 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 (MDH2) 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 (e.g. vesicle associated membrane protein 2 (VAMP2), TBC1D4, and ras-related protein rab14 (RAB14)), and related signaling cascades (e.g. AMPKα) (226). GLUT4 palmitoylation has subsequently been confirmed in skeletal muscle (232). Palmitoylation of GLUT4 at Cys 233 by palmitoyltransferase DHHC7 (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.
**ADP-ribosylation**

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). 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 (Figure 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 1,000 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 under-investigated post-translational modifications, as well as to provide multi-omic insights into the interplay between post-translational modifications, e.g. 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. Specifically, proteomic data into post-translational modifications following acute (phosphorylation and ubiquitination) or chronic (acetylation) exercise in humans currently arise from only three 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.

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. A complex network of signals converge and interact to regulate glucose metabolism and insulin sensitivity in response to exercise (Figure 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.
Acknowledgements: We thank members of the Zierath laboratory (Stephen P. Ashcroft and Amy M. Ehrlich) and colleagues within the Novo Nordisk Foundation Center for Basic Metabolic Research (Atul S. Deshmukh and Jeppe Kjærgaard Larsen) for their constructive feedback and careful reading of the manuscript. Figures were created with BioRender.
References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018;138:271-281.

2. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, Song X, Ren Y, Shan P-F. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Sci Rep.* 2020;10(1):14790.

3. Yang JJ, Yu D, Wen W, et al. Association of diabetes with all-cause and cause-specific mortality in Asia: A pooled analysis of more than 1 million participants. *JAMA Network Open.* 2019;2(4):e192696-e192696.

4. Talmud PJ, Hingorani AD, Cooper JA, Marmot MG, Brunner EJ, Kumari M, Kivimäki M, Humphries SE. Utility of genetic and non-genetic risk factors in prediction of type 2 diabetes: Whitehall II prospective cohort study. *BMJ.* 2010;340:b4838.

5. Hu FB, Li TY, Colditz GA, Willett WC, Manson JE. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA.* 2003;289(14):1785-1791.

6. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA.* 2003;289(1):76-79.

7. Murray CJL, Aravkin AY, Zheng P, et al. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. *The Lancet.* 2020;396(10258):1223-1249.

8. Thiebaud D, Jacot E, DeFronzo RA, Maeder E, Jequier E, Felber JP. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes.* 1982;31(11):957-963.

9. Zierath JR, He L, Gumà A, Odegoard Wahlström E, Klip A, Wallberg-Henriksson H. Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia.* 1996;39(10):1180-1189.

10. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes, *Diabetes Care.* 2009;32(Suppl 2):S157-S163.

11. Edinburgh RM, Bradley HE, Abdullah NF, Robinson SL, Chrzanowski-Smith OJ, Wallin JP, Joannisse S, Manolopoulos KN, Philp A, Hengst A, Chabowski A, Brodsky FM, Koumanov F, Betts JA, Thompson D, Wallis GA, Gonzalez JT. Lipid metabolism links nutrient-exercise timing to insulin sensitivity in men classified as overweight or obese. *J Clin Endocrinol Metab.* 2020;105(3):660-676.

12. Ivey FM, Ryan AS, Hafer-Macko CE, Goldberg AP, Macko RF. Treadmill aerobic training improves glucose tolerance and indices of insulin sensitivity in disabled stroke survivors: a preliminary report. *Stroke.* 2007;38(10):2752-2758.

13. Kirwan JP, Solomon TPJ, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab.* 2009;297(1):E151-E156.

14. Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA, Yannakoulia M, Chrousos GP, Sidossis LS. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism.* 2005;54(11):1472-1479.
15. Dela F, Larsen JJ, Mikines KJ, Ploug T, Petersen LN, Galbo H. Insulin-stimulated muscle glucose clearance in patients with NIDDM: Effects of one-legged physical training. *Diabetes*. 1995;44(9):1010-1020.

16. Coen PM, Tanner CJ, Helbling NL, Dubis GS, Hames KC, Xie H, Eid GM, Stefanovic-Racic M, Toledo FGS, Jakicic JM, Houmard JA, Goodpaster BH. Clinical trial demonstrates exercise following bariatric surgery improves insulin sensitivity. *J Clin Invest*. 2015;125(1):248-257.

17. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346(6):393-403.

18. Taheri S, Zaghoul H, Chagoury O, Elhadad S, Ahmed SH, El Khatib N, Amona RA, El Nahas K, Suleiman N, Alnaama A, Al-Hamaq A, Charlson M, Wells MT, Al-Abdulla S, Abou-Samra AB. Effect of intensive lifestyle intervention on bodyweight and glycaemia in early type 2 diabetes (DIADEM-I): an open-label, parallel-group, randomised controlled trial. *Lancet Diabetes Endocrinol*. 2020;8(6):477-489.

19. van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J Physiol*. 2001;536(Pt 1):295-304.

20. Goldstein MS, Mullick V, Huddlestun B, Levine R. Action of muscular work on the transfer of sugars across cell barriers: Comparison with action of insulin. *Am J Physiol*. 1953;173(2):212-216.

21. Huycke EJ, Kruhoffer P. Effects of insulin and muscular exercise upon the uptake of hexoses by muscle cells. *Acta Physiol Scand*. 1955;34(2-3):232-249.

22. Reichard GA, Issekutz B, Kimbel P, Putnam RC, Hochella NJ, Weinhouse S. Blood glucose metabolism in man during muscular work. *J Appl Physiol*. 1961;16(6):1001-1005.

23. Ploug T, Galbo H, Richter EA. Increased muscle glucose uptake during contractions: no need for insulin. *Am J Physiol*. 1984;247(6 Pt 1):E726-731.

24. Wojtaszewski JF, Higaki Y, Hirshman MF, Michael MD, Dufresne SD, Kahn CR, Goodyear LJ. Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. *J Clin Invest*. 1999;104(9):1257-1264.

25. Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am J Physiol*. 1988;254(3 Pt 1):E248-259.

26. Devlin JT, Horton ES. Effects of prior high-intensity exercise on glucose metabolism in normal and insulin-resistant men. *Diabetes*. 1985;34(10):973-979.

27. Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI, Shulman RG. Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *J Appl Physiol*. 1994;76(1):104-111.

28. Birnbaum MJ. Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell*. 1989;57(2):305-315.

29. Charron MJ, Brosius FC, 3rd, Alper SL, Lodish HF. A glucose transport protein expressed predominately in insulin-responsive tissues. *Proc Natl Acad Sci U S A*. 1989;86(8):2535-2539.

30. James DE, Strube M, Mueckler M. Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature*. 1989;338(6210):83-87.

31. Ryder JW, Kawano Y, Chibalin AV, Rincón J, Tsao TS, Stenbit AE, Combatsiaris T, Yang J, Holman GD, Charron MJ, Zierath JR. In vitro analysis of the glucose-
transport system in GLUT4-null skeletal muscle. *Biochem J.* 1999;342 (Pt 2):321-328.

32. Ryder JW, Yang J, Galuska D, Rincon J, Bjornholm M, Krook A, Lund S, Pedersen O, Wallberg-Henriksson H, Zierath JR, Holman GD. Use of a novel impermeable biotinylated photolabeling reagent to assess insulin- and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. *Diabetes.* 2000;49(4):647-654.

33. Koistinen HA, Galuska D, Chibalin AV, Yang J, Zierath JR, Holman GD, Wallberg-Henriksson H. 5-Amino-Imidazole Carboxamide Riboside increases glucose transport and cell-surface GLUT4 content in skeletal muscle from subjects with type 2 diabetes. *Diabetes.* 2003;52(5):1066-1072.

34. Karlsson HK, Chibalin AV, Koistinen HA, Yang J, Koumanov F, Wallberg-Henriksson H, Zierath JR, Holman GD. Kinetics of GLUT4 trafficking in rat and human skeletal muscle. *Diabetes.* 2009;58(4):847-854.

35. Furuzano S, Kubota T, Taura J, Konishi M, Naito A, Tsutsui M, Karasawa H, Kubota N, Kadowaki T. A xanthene derivative, DS20060511, attenuates glucose intolerance by inducing skeletal muscle-specific GLUT4 translocation in mice. *Commun Biol.* 2021;4(1):994.

36. Ploug T, Ralston E. Anatomy of glucose transporters in skeletal muscle. Effects of insulin and contractions. *Adv Exp Med Biol.* 1998;441:17-26.

37. Andersen P, Saltin B. Maximal perfusion of skeletal muscle in man. *J Physiol.* 1985;366:233-249.

38. Kennedy JW, Hirshman MF, Gervino EV, Ocel JV, Hoenig SJ, Aronson D, Goodyear LJ, Horton ES. Acute exercise induces GLUT4 translocation in skeletal muscle of normal subjects and subjects with type 2 diabetes. *Diabetes.* 1999;48(5):1192-1197.

39. Kristiansen S, Hargreaves M, Richter EA. Exercise-induced increase in glucose transport, GLUT-4, and VAMP-2 in plasma membrane from human muscle. *Am J Physiol Endocrinol Metab.* 1996;270(1):E197-E201.

40. Kristiansen S, Hargreaves M, Richter EA. Progressive increase in glucose transport and GLUT-4 in human sarcolemmal vesicles during moderate exercise. *Am J Physiol.* 1997;272(3 Pt 1):E385-389.

41. Lauritzen HPMM, Galbo H, Toyoda T, Goodyear LJ. Kinetics of contraction-induced GLUT4 translocation in skeletal muscle fibers from living mice. *Diabetes.* 2010;59(9):2134-2144.

42. Goodyear LJ, Hirshman MF, King PA, Horton ED, Thompson CM, Horton ES. Skeletal muscle plasma membrane glucose transport and glucose transporters after exercise. *J Appl Physiol.* 1990;68(1):193-198.

43. Hansen PA, Nolte LA, Chen MM, Holloszy JO. Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. *J Appl Physiol.* 1998;85(4):1218-1222.

44. Thorell A, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JFP, Dufresne SD, Horton ES, Ljungqvist O, Goodyear LJ. Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. *Am J Physiol Endocrinol Metab.* 1999;277(4):E733-E741.

45. Fazakerley DJ, Holman GD, Marley A, James DE, Stöckli J, Coster ACF. Kinetic evidence for unique regulation of GLUT4 trafficking by insulin and AMP-activated protein kinase activators in L6 myotubes. *J Biol Chem.* 2010;285(3):1653-1660.

46. Yang J, Holman GD. Insulin and contraction stimulate exocytosis, but increased AMP-activated protein kinase activity resulting from oxidative metabolism stress
slows endocytosis of GLUT4 in cardiomyocytes. *J Biol Chem.* 2005;280(6):4070-4078.

47. Ryder JW, Kawano Y, Galuska D, Fahlman R, Wallberg-Henriksson H, Charron MJ, Zierath JR. Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT4-deficient mice. *FASEB J.* 1999;13(15):2246-2256.

48. Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB, Wojtaszewski JFP, Hirshman MF, Virkamaki A, Goodyear LJ, Kahn CR, Kahn BB. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med.* 2000;6(8):924-928.

49. Fueger PT, Li CY, Ayala JE, Shearer J, Bracy DP, Charron MJ, Rottman JN, Wasserman DH. Glucose kinetics and exercise tolerance in mice lacking the GLUT4 glucose transporter. *J Physiol.* 2007;582(2):801-812.

50. Martin IK, Katz A, Wahren J. Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am J Physiol.* 1995;269(3 Pt 1):E583-590.

51. Bogardus C, Thuillez P, Ravussin E, Vasquez B, Narimiga M, Azhar S. Effect of muscle glycogen depletion on in vivo insulin action in man. *J Clin Invest.* 1983;72(5):1605-1610.

52. Steenberg DE, Jørgensen NB, Birk JB, Sjøberg KA, Kiens B, Richter EA, Wojtaszewski JFP. Exercise training reduces the insulin-sensitizing effect of a single bout of exercise in human skeletal muscle. *J Physiol.* 2019;597(1):89-103.

53. Richter EA, Mikines KJ, Galbo H, Kiens B. Effect of exercise on insulin action in human skeletal muscle. *J Appl Physiol.* 1989;66(2):876-885.

54. Wojtaszewski JF, Hansen BF, Gade, Kiens B, Markuns JF, Goodyear LJ, Richter EA. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. *Diabetes.* 2000;49(3):325-331.

55. Wojtaszewski JF, Hansen BF, Kiens B, Richter EA. Insulin signaling in human skeletal muscle: time course and effect of exercise. *Diabetes.* 1997;46(11):1775-1781.

56. Frøsig C, Sajan MP, Maarbjerg SJ, Brandt N, Roepstorff C, Wojtaszewski JFP, Kiens B, Farese RV, Richter EA. Exercise improves phosphatidylinositol-3,4,5-trisphosphate responsiveness of atypical protein kinase C and interacts with insulin signalling to peptide elongation in human skeletal muscle. *J Physiol.* 2007;582(Pt 3):1289-1301.

57. Heath GW, Gavin JR, 3rd, Hinderliter JM, Hagberg JM, Bloomfield SA, Holloszy JO. Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *J Appl Physiol.* 1983;55(2):512-517.

58. McConell GK, Sjøberg KA, Ceutz F, Gliemann L, Nyberg M, Hellsten Y, Frøsig C, Kiens B, Wojtaszewski JFP, Richter EA. Insulin-induced membrane permeability to glucose in human muscles at rest and following exercise. *J Physiol.* 2020;598(2):303-315.

59. Sjøberg KA, Frøsig C, Kjøbsted R, Sylow L, Kleinert M, Betik AC, Shaw CS, Kiens B, Wojtaszewski JFP, Rattigan S, Richter EA, McConell GK. Exercise increases human skeletal muscle insulin sensitivity via coordinated increases in microvascular perfusion and molecular signaling. *Diabetes.* 2017;66(6):1501-1510.

60. Knudsen JR, Steenberg DE, Hingst JR, Hodgson LR, Henriquez-Olguin C, Li Z, Kiens B, Richter EA, Wojtaszewski JFP, Verkade P, Jensen TE. Prior exercise in humans redistributes intramuscular GLUT4 and enhances insulin-stimulated sarcolemmal and endosomal GLUT4 translocation. *Mol Metab.* 2020;39:100998.

61. Young DA, Wallberg-Henriksson H, Sleeper MD, Holloszy JO. Reversal of the exercise-induced increase in muscle permeability to glucose. *Am J Physiol.* 1987;253(4 Pt 1):E331-335.
62. Cartee GD, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson H, Holloszy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab*. 1989;256(4):E494-E499.

63. Host HH, Hansen PA, Nolte LA, Chen MM, Holloszy JO. Glycogen supercompensation masks the effect of a training-induced increase in GLUT-4 on muscle glucose transport. *J Appl Physiol*. 1998;85(1):133-138.

64. Kawanaka K, Han D-H, Nolte LA, Hansen PA, Nakatani A, Holloszy JO. Decreased insulin-stimulated GLUT-4 translocation in glycogen-supercompensated muscles of exercised rats. *Am J Physiol Endocrinol Metab*. 1999;276(5):E907-E912.

65. Kawanaka K, Nolte LA, Han D-H, Hansen PA, Holloszy JO. Mechanisms underlying impaired GLUT-4 translocation in glycogen-supercompensated muscles of exercised rats. *Am J Physiol Endocrinol Metab*. 2000;279(6):E1311-E1318.

66. Nielsen JN, Visser J, Wojtaszewski JF, Haller RG, Begum N, Richter EA. Decreased insulin action in skeletal muscle from patients with McArdle's disease. *Am J Physiol Endocrinol Metab*. 2002;282(6):E1267-E1275.

67. Meex RCR, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Pieliex E, van de Weijer T, Sels J-P, Schrauwen P, Hesselink MKC. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes*. 2010;59(3):572-579.

68. O’Gorman DJ, Karlsson HK, McQuaid S, Yousif O, Rahman Y, Gasparro D, Glund S, Chibalin AV, Zierath JR, Nolan JJ. Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes. *Diabetologia*. 2006;49(12):2983-2992.

69. Andersen P, Henriksson J. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *J Physiol*. 1977;270(3):677-690.

70. Frosig C, Rose AJ, Treebak JT, Kiens B, Richter EA, Wojtaszewski JF. Effects of endurance exercise training on insulin signaling in human skeletal muscle: interactions at the level of phosphatidylinositol 3-kinase, Akt, and AS160. *Diabetes*. 2007;56(8):2093-2102.

71. Daugaard JR, Nielsen JN, Kristiansen S, Andersen JL, Hargreaves M, Richter EA. Fiber type-specific expression of GLUT4 in human skeletal muscle: influence of exercise training. *Diabetes*. 2000;49(7):1092-1095.

72. Dela F, Handberg A, Mikines KJ, Vinten J, Galbo H. GLUT 4 and insulin receptor binding and kinase activity in trained human muscle. *J Physiol*. 1993;469:615-624.

73. Gulve EA, Spina RJ. Effect of 7-10 days of cycle ergometer exercise on skeletal muscle GLUT-4 protein content. *J Appl Physiol*. 1995;79(5):1562-1566.

74. Houmard JA, Shinebarger MH, Dolan PL, Leggett-Frazier N, Bruner RK, McCammon MR, Israel RG, Dohm GL. Exercise training increases GLUT-4 protein concentration in previously sedentary middle-aged men. *Am J Physiol*. 1993;264(6 Pt 1):E896-901.

75. Phillips SM, Han XX, Green HI, Bonen A. Increments in skeletal muscle GLUT-1 and GLUT-4 after endurance training in humans. *Am J Physiol*. 1996;270(3 Pt 1):E456-462.

76. Granata C, Oliveira RS, Little JP, Renner K, Bishop DJ. Training intensity modulates changes in PGC-1alpha and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle. *FASEB J*. 2016;30(2):959-970.
77. Granata C, Oliveira RS, Little JP, Renner K, Bishop DJ. Mitochondrial adaptations to high-volume exercise training are rapidly reversed after a reduction in training volume in human skeletal muscle. *FASEB J*. 2016;30(10):3413-3423.

78. Granata C, Oliveira RSF, Little JP, Renner K, Bishop DJ. Sprint-interval but not continuous exercise increases PGC-1α protein content and p53 phosphorylation in nuclear fractions of human skeletal muscle. *Sci Rep*. 2017;7:44227.

79. Meinild Lundby AK, Jacobs RA, Gehrig S, de Leur J, Hauser M, Bonne TC, Flück D, Dandanell S, Kirk N, Kaech A, Ziegler U, Larsen S, Lundby C. Exercise training increases skeletal muscle mitochondrial volume density by enlargement of existing mitochondria and not de novo biogenesis. *Acta physiologica (Oxford, England)*. 2018;222(1):e12905.

80. Montero D, Diaz-Canestro C, Lundby C. Endurance training and VO2max: Role of maximal cardiac output and oxygen extraction. *Med Sci Sports Exerc*. 2015;47(10):2024-2033.

81. Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, Weibel ER. Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol*. 1985;59(2):320-327.

82. Jacobs RA, Flück D, Bonne TC, Bürgi S, Christensen PM, Toigo M, Lundby C. Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. *J Appl Physiol*. 2013;115(6):785-793.

83. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*. 2002;51(10):2944-2950.

84. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science*. 2003;300(5622):1140-1142.

85. Daemen S, Gemmink A, Brouwers B, Meex RCR, Huntjens PR, Schaart G, Moonen-Kornips E, Jørgensen J, Hoeks J, Schrauwen P, Hesselink MKC. Distinct lipid droplet characteristics and distribution unmask the apparent contradiction of the athlete’s paradox. *Mol Metab*. 2018;17:71-81.

86. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab*. 2001;86(12):5755-5761.

87. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia*. 1999;42(1):113-116.

88. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*. 1997;46(6):983-988.

89. White MF, Kahn CR. Insulin action at a molecular level – 100 years of progress. *Mol Metab*. 2021;52:101304.

90. Olsen JV, Mann M. Status of large-scale analysis of post-translational modifications by mass spectrometry. *Mol Cell Proteomics*. 2013;12(12):3444-3452.

91. Sharma K, D’Souza Rochelle CJ, Tyanova S, Schaab C, Wiśniewski Jacek R, Cox J, Mann M. Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. *Cell Rep*. 2014;8(5):1583-1594.

92. Bouzakri K, Zachrisson A, Al-Khalili L, Zhang BB, Koistinen HA, Krook A, Zierath JR. siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-
2/Akt1 in glucose and lipid metabolism in human skeletal muscle. *Cell Metab.* 2006;4(1):89-96.

93. Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, Klip A. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol Cell Biol.* 1999;19(6):4008-4018.

94. Sylow L, Kleinert M, Pehmøller C, Prats C, Chiu TT, Klip A, Richter EA, Jensen TE. Akt and Rac1 signaling are jointly required for insulin-stimulated glucose uptake in skeletal muscle and downregulated in insulin resistance. *Cell Signal.* 2014;26(2):323-331.

95. Ueda S, Kataoka T, Satoh T. Activation of the small GTPase Rac1 by a specific guanine-nucleotide-exchange factor suffices to induce glucose uptake into skeletal-muscle cells. *Biol Cell.* 2008;100(11):645-657.

96. JeBailey L, Wanono O, Niu W, Roessler J, Rudich A, Klip A. Ceramide- and oxidant-induced insulin resistance involve loss of insulin-dependent Rac-activation and actin remodeling in muscle cells. *Diabetes.* 2007;56(2):394-403.

97. Vichaiwong K, Purohit S, An D, Toyoda T, Jessen N, Hirshman MF, Goodyear LJ. Contraction regulates site-specific phosphorylation of TBC1D1 in skeletal muscle. *Biochem J.* 2010;431(2):311-320.

98. Roach WG, Chavez JA, Mîinea CP, Lienhard GE. Substrate specificity and effect on GLUT4 translocation of the Rab GTPase-activating protein Tbc1d1. *Biochem J.* 2007;403(2):353-358.

99. Chavez JA, Roach WG, Keller SR, Lane WS, Lienhard GE. Inhibition of GLUT4 translocation by Tbc1d1, a Rab GTPase-activating protein abundant in skeletal muscle, is partially relieved by AMP-activated protein kinase activation. *J Biol Chem.* 2008;283(14):9187-9195.

100. Thong FS, Bilan PJ, Klip A. The Rab GTPase-activating protein AS160 integrates Akt, protein kinase C, and AMP-activated protein kinase signals regulating GLUT4 traffic. *Diabetes.* 2007;56(2):414-423.

101. Kane S, Sano H, Liu SC, Asara JM, Lane WS, Garner CC, Lienhard GE. A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. *J Biol Chem.* 2002;277(25):22115-22118.

102. Raun SH, Ali M, Kjøbsted R, Møller LLV, Federspiel MA, Richter EA, Jensen TE, Sylow L. Rac1 muscle knockout exacerbates the detrimental effect of high-fat diet on insulin-stimulated muscle glucose uptake independently of Akt. *J Physiol.* 2018;596(12):2283-2299.

103. Sylow L, Jensen TE, Kleinert M, Højlund K, Kiens B, Wojtaszewski J, Prats C, Schjerling P, Richter EA. Rac1 signaling is required for insulin-stimulated glucose uptake and is dysregulated in insulin-resistant murine and human skeletal muscle. *Diabetes.* 2013;62(6):1865-1875.

104. Ueda S, Kitazawa S, Ishida K, Nishikawa Y, Matsui M, Matsumoto H, Aoki T, Nozaki S, Takeda T, Tamori Y, Aiba A, Kahn CR, Kataoka T, Satoh T. Crucial role of the small GTPase Rac1 in insulin-stimulated translocation of glucose transporter 4 to the mouse skeletal muscle sarcolemma. *FASEB J.* 2010;24(7):2254-2261.

105. Chiu TT, Patel N, Shaw AE, Bamburg JR, Klip A. Arp2/3- and cofilin-coordinated actin dynamics is required for insulin-mediated GLUT4 translocation to the surface of muscle cells. *Mol Biol Cell.* 2010;21(20):3529-3539.

106. Tong P, Khayat ZA, Huang C, Patel N, Ueyama A, Klip A. Insulin-induced cortical actin remodeling promotes GLUT4 insertion at muscle cell membrane ruffles. *J Clin Investig.* 2001;108(3):371-381.
107. Nelson ME, Parker BL, Burchfield JG, Hoffman NJ, Needham EJ, Cooke KC, Naim T, Sylow L, Ling NX, Francis D, Norris DM, Chaudhuri R, Oakhill JS, Richter EA, Lynch GS, Stöckli J, James DE. Phosphoproteomics reveals conserved exercise-stimulated signaling and AMPK regulation of store-operated calcium entry. *EMBO J*. 2019;38(24):e102578.

108. Hoffman NJ, Parker BL, Chaudhuri R, Fisher-Wellman KH, Kleinert M, Humphrey SJ, Yang P, Holliday M, Trefely S, Fazakerley DJ, Stockli J, Burchfield JG, Jensen TE, Jothi R, Kiens B, Wojtaszewski JF, Richter EA, James DE. Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates. *Cell Metab*. 2015;22(5):922-935.

109. Maier G, Delezie J, Westermark PO, Santos G, Ritz D, Handschin C. Transcriptional proteomic and phosphoproteomic underpinnings of daily exercise performance and zeitgeber activity of training in mouse muscle. *J Physiol*. 2021;Jun 18. doi: 10.1113/JP281535. Epub ahead of print. PMID: 34142717.

110. Howlett KF, Sakamoto K, Hirshman MF, Aschenbach WG, Dow M, White MF, Goodyear LJ. Insulin signaling after exercise in insulin receptor substrate-2-deficient mice. *Diabetes*. 2002;51(2):479-483.

111. Koval JA, Maezono K, Patti ME, Pendergrass M, DeFronzo RA, Mandarino LJ. Effects of exercise and insulin on insulin signaling proteins in human skeletal muscle. *Med Sci Sports Exerc*. 1999;31(7):998-1004.

112. Zhou Q, Dohm GL. Treadmill running increases phosphatidylinositol 3-kinase activity in rat skeletal muscle. *Biochem Biophys Res Commun*. 1997;236(3):647-650.

113. Whitehead JP, Soos MA, Aslesen R, ORahilly S, Jensen J. Contraction inhibits insulin-stimulated insulin receptor substrate-1/2-associated phosphoinositide 3-kinase activity, but not protein kinase B activation or glucose uptake, in rat muscle. *Biochem J*. 2000;349 Pt 3(Pt 3):775-781.

114. Yeh J-I, Gulve EA, Rameh L, Birnbaum MJ. The effects of wortmannin on rat skeletal muscle: Dissociation of signaling pathways for insulin- and contraction-activated hexose transport. *J Biol Chem*. 1995;270(5):2107-2111.

115. Funai K, Schweitzer GG, Castorena CM, Kanzaki M, Cartee GD. In vivo exercise followed by in vitro contraction additively elevates subsequent insulin-stimulated glucose transport by rat skeletal muscle. *Am J Physiol Endocrinol Metab*. 2010;298(5):E999-1010.

116. Goodyear LJ, Giorgino F, Balon TW, Condorelli G, Smith RJ. Effects of contractile activity on tyrosine phosphoproteins and PI 3-kinase activity in rat skeletal muscle. *Am J Physiol*. 1995;268(5 Pt 1):E987-995.

117. Treebak JT, Froisig C, Pehmøller C, Chen S, Maarbjerg SJ, Brandt N, MacKintosh C, Zierath JR, Hardie DG, Kiens B, Richter EA, Pilegaard H, Wojtaszewski JFP. Potential role of TBC1D4 in enhanced post-exercise insulin action in human skeletal muscle. *Diabetologia*. 2009;52(5):891-900.

118. Treebak JT, Glund S, Deshmukh A, Klein DK, Long YC, Jensen TE, Jorgensen SB, Viollet B, Andersson L, Neumann D, Wallimann T, Richter EA, Chibalin AV, Zierath JR, Wojtaszewski JF. AMPK-mediated AS160 phosphorylation in skeletal muscle is dependent on AMPK catalytic and regulatory subunits. *Diabetes*. 2006;55(7):2051-2058.

119. Kramer HF, Witzczak CA, Fujii N, Jessen N, Taylor EB, Arnolds DE, Sakamoto K, Hirshman MF, Goodyear LJ. Distinct signals regulate AS160 phosphorylation in response to insulin, AICAR, and contraction in mouse skeletal muscle. *Diabetes*. 2006;55(7):2067-2076.
120. Treebak JT, Birk JB, Rose AJ, Kiens B, Richter EA, Wojtaszewski JF. AS160 phosphorylation is associated with activation of alpha2beta2gamma1- but not alpha2beta2gamma3-AMPK trimeric complex in skeletal muscle during exercise in humans. Am J Physiol Endocrinol Metab. 2007;292(3):E715-E722.

121. Schweitzer GG, Arias EB, Cartee GD. Sustained postexercise increases in AS160 Thr642 and Ser588 phosphorylation in skeletal muscle without sustained increases in kinase phosphorylation. J Appl Physiol. 2012;113(12):1852-1861.

122. Arias EB, Kim J, Funai K, Cartee GD. Prior exercise increases phosphorylation of Akt substrate of 160 kDa (AS160) in rat skeletal muscle. Am J Physiol Endocrinol Metab. 2007;292(4):E1191-E1200.

123. Kramer HF, Witczak CA, Taylor EB, Fujii N, Hirshman MF, Goodyear LJ. AS160 regulates Insulin- and Contraction-stimulated Glucose Uptake in Mouse Skeletal Muscle. J Biol Chem. 2006;281(42):31478-31485.

124. Hawley SA, Davison M, Davies SP, Beri RK, Carling D, Hardie DG. Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. J Biol Chem. 1996;271(44):27879-27887.

125. Oakhill JS, Chen ZP, Scott JW, Steel R, Castelli LA, Ling N, Macaulay SL, Kemp BE. beta-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). Proc Natl Acad Sci U S A. 2010;107(45):19237-19241.

126. Birk JB, Wojtaszewski JF. Predominant alpha2beta2gamma3 AMPK activation during exercise in human skeletal muscle. J Physiol. 2006;577(Pt 3):1021-1032.

127. Fujii N, Hayashi T, Hirshman MF, Smith JT, Habinowski SA, Kaijser L, Mu J, Ljungqvist O, Birnbaum MJ, Witters LA, Thorell A, Goodyear LJ. Exercise induces isoform-specific increase in 5'-AMP-activated protein kinase activity in human skeletal muscle. Biochem Biophys Res Commun. 2000;273(3):1150-1155.

128. Stocks B, Dent JR, Ogden HB, Zemp M, Philp A. Post-exercise skeletal muscle signaling responses to moderate-to-high-intensity steady-state exercise in the fed or fasted state. Am J Physiol Endocrinol Metab. 2019;316(2):E230-E238.

129. Taylor EB, An D, Kramer HF, Yu H, Fujii NL, Roeckl KSC, Bowles N, Hirshman MF, Xie J, Feener EP, Goodyear LJ. Discovery of TBC1D1 as an insulin-, AICAR-, and contraction-stimulated signaling nexus in mouse skeletal muscle. J Biol Chem. 2008;283(15):9787-9796.

130. Kjøbsted R, Chadt A, Jørgensen NO, Kido K, Larsen JK, de Wendt C, Al-Hasani H, Wojtaszewski JFP. TBC1D4 is necessary for enhancing muscle insulin sensitivity in response to AICAR and contraction. Diabetes. 2019;68(9):1756-1766.

131. Pehmoller C, Brandt N, Birk JB, Høeg LD, Sjøberg KA, Goodyear LJ, Kiens B, Richter EA, Wojtaszewski JF. Exercise alleviates lipid-induced insulin resistance in human skeletal muscle-signaling interaction at the level of TBC1 domain family member 4. Diabetes. 2012;61(11):2743-2752.

132. Mu J, Brozinick JT, Jr., Valladares O, Bucan M, Birnbaum MJ. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell. 2001;7(5):1085-1094.

133. Lefort N, St-Amand E, Morasse S, Côté CH, Marette A. The alpha-subunit of AMPK is essential for submaximal contraction-mediated glucose transport in skeletal muscle in vitro. Am J Physiol Endocrinol Metab. 2008;295(6):E1447-1454.

134. Lee-Young RS, Griffie SR, Lynes SE, Bracy DP, Ayala JE, McGuinness OP, Wasserman DH. Skeletal muscle AMP-activated protein kinase is essential for the metabolic response to exercise in vivo. J Biol Chem. 2009;284(36):23925-23934.
135. Abbott MJ, Bogachus LD, Turcotte LP. AMPKa2 deficiency uncovers time dependency in the regulation of contraction-induced palmitate and glucose uptake in mouse muscle. *J Appl Physiol*. 2011;111(1):125-134.

136. O’Neill HM, Maarbjergh SJ, Crane JD, Jeppesen J, Jorgensen SB, Schertzer JD, Shyroka O, Kiens B, van Denderen BJ, Tarnopolsky MA, Kemp BE, Richter EA, Steinberg GR. AMP-activated protein kinase (AMPK) beta1beta2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proc Natl Acad Sci U S A*. 2011;108(38):16092-16097.

137. Kjøbsted R, Munk-Hansen N, Birk JB, Foretz M, Viollet B, Bjornholm M, Zierath JR, Treebak JT, Wojtaszewski JF. Enhanced muscle insulin sensitivity after contraction/exercise is mediated by AMPK. *Diabetes*. 2017;66(3):598-612.

138. Crawford SA, Costford SR, Aguer C, Thomas SC, deKemp RA, DaSilva JN, Lafontaine D, Kendall M, Dent R, Beanlands RS, McPherson R, Harper ME. Naturally occurring R225W mutation of the gene encoding AMP-activated protein kinase (AMPK)gamma3 results in increased oxidative capacity and glucose uptake in human primary myotubes. *Diabetologia*. 2010;53(9):1986-1997.

139. Stöckli J, Meoli CC, Hoffman NJ, Fazakerley DJ, Pant H, Cleasby ME, Ma X, Kleinert M, Brandon AE, Lopez JA, Cooney GJ, James DE. The RabGAP TBC1D1 plays a central role in exercise-regulated glucose metabolism in skeletal muscle. *Diabetes*. 2015;64(6):1914-1922.

140. Kjøbsted R, Roll JLW, Jørgensen NO, Birk JB, Foretz M, Virollet B, Chadt A, Al-Hasani H, Wojtaszewski JFP. AMPK and TBC1D1 regulate muscle glucose uptake after, but not during, exercise and contraction. *Diabetes*. 2019;68(7):1427-1440.

141. An D, Toyoda T, Taylor EB, Yu H, Fujiit M, Hirshman MF, Goodyear LJ. TBC1D1 regulates insulin- and contraction-induced glucose transport in mouse skeletal muscle. *Diabetes*. 2010;59(6):1358-1365.

142. Fentz J, Kjobsted R, Birk JB, Jordy AB, Jeppesen J, Thorsen K, Schjerling P, Kiens B, Jessen N, Virollet B, Wojtaszewski JF. AMPKalpha is critical for enhancing skeletal muscle fatty acid utilization during in vivo exercise in mice. *FASEB J*. 2015;29(5):1725-1738.

143. Wojtaszewski JFP, Mourtzakis M, Hillig T, Saltin B, Pilegaard H. Dissociation of AMPK activity and ACCβ phosphorylation in human muscle during prolonged exercise. *Biochem Biophys Res Commun*. 2002;298(3):309-316.

144. McConell GK, Lee-Young RS, Chen Z-P, Stepto NK, Huynh NN, Stephens TJ, Canny BJ, Kemp BE. Short-term exercise training in humans reduces AMPK signalling during prolonged exercise independent of muscle glycogen. *J Physiol*. 2005;568(2):665-676.

145. Liu Y, Lai YC, Hill EV, Tyteca D, Carpentier S, Ingvaldsen A, Vertommen D, Lantier L, Foretz M, Dequiedt F, Courtoy PJ, Erneux C, Virollet B, Shepherd PR, Tavare J, Jensen J, Rider MH. Phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) is an AMPK target participating in contraction-stimulated glucose uptake in skeletal muscle. *Biochem J*. 2013;455(2):195-206.

146. Ikonomov OC, Sbrissa D, Delvecchio K, Feng HZ, Cartee GD, Jin JP, Shisheva A. Muscle-specific Pikfyve gene disruption causes glucose intolerance, insulin resistance, adiposity, and hyperinsulinemia but not muscle fiber-type switching. *Am J Physiol Endocrinol Metab*. 2013;305(1):E119-131.

147. Bartlett JD, Louhelainen J, Iqbal Z, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP. Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: Implications for mitochondrial biogenesis. *Am J Physiol Regul Integr Comp Physiol*. 2013;304(6):R450-R458.
148. Lai YC, Zarrinpashneh E, Jensen J. Additive effect of contraction and insulin on glucose uptake and glycogen synthase in muscle with different glycogen contents. *J Appl Physiol.* 2010;108(5):1106-1115.

149. Philp A, MacKenzie MG, Belew MY, Towler MC, Corstorphine A, Papalamprou A, Hardie DG, Baar K. Glycogen content regulates peroxisome proliferator activated receptor-δ (PPAR-δ) activity in rat skeletal muscle. *PLoS One.* 2013;8(10):e77200.

150. Steinberg GR, Watt MJ, McGee SL, Chan S, Hargreaves M, Febbraio MA, Stapleton D, Kemp BE. Reduced glycogen availability is associated with increased AMPKalpha2 activity, nuclear AMPKalpha2 protein abundance, and GLUT4 mRNA expression in contracting human skeletal muscle. *Appl Physiol Nut Metab.* 2006;31(3):302-312.

151. Wojtaszewski JF, MacDonald C, Nielsen JN, Hellsten Y, Hardie DG, Kemp BE, Kiens B, Richter EA. Regulation of 5’AMP-activated protein kinase activity and substrate utilization in exercising human skeletal muscle. *Am J Physiol Endocrinol Metab.* 2003;284(4):E813-E822.

152. Yeo WK, McGee SL, Carey AL, Paton CD, Garnham AP, Hargreaves M, Hawley JA. Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen. *Exp Physiol.* 2010;95(2):351-358.

153. Derave W, Ai H, Ihlemann J, Witters LA, Kristiansen S, Richter EA, Ploug T. Dissociation of AMPK activated protein kinase activation and glucose transport in contracting slow-twitch muscle. *Diabetes.* 2000;49(8):1281-1287.

154. Derave W, Lund S, Holman GD, Wojtaszewski J, Pedersen O, Richter EA. Contraction-stimulated muscle glucose transport and GLUT-4 surface content are dependent on glycogen content. *Am J Physiol.* 1999;277(6):E1103-E1110.

155. Hudson ER, Pan DA, James J, Luceoq JM, Hawley SA, Green KA, Baba O, Terashima T, Hardie DG. A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. *Curr Biol.* 2003;13(10):861-866.

156. McBride A, Ghilagaber S, Nikolaev A, Hardie DG. The glycogen-binding domain on the AMPK beta subunit allows the kinase to act as a glycogen sensor. *Cell Metab.* 2009;9(1):23-34.

157. Polekchina G, Gupta A, Michell BJ, van Denderen B, Murthy S, Feil SC, Jennings IG, Campbell DJ, Witters LA, Parker MW, Kemp BE, Stapleton D. AMPK beta subunit targets metabolic stress sensing to glycogen. *Curr Biol.* 2003;13(10):867-871.

158. Oligschläger Y, Migliancico M, Chanda D, Scholz R, Thali RF, Tuerk R, Stapleton DI, Gooley PR, Neumann D. The recruitment of AMPK-activated protein kinase to glycogen is regulated by autophosphorylation. *J Biol Chem.* 2015;290(18):11175-11178.

159. Oligschläger Y, Migliancico M, Dahlmans V, Rubio-Villena C, Chanda D, Garcia-Gimeno MA, Coumans WA, Liu Y, Voncken JW, Luiken JJ, Glatz JF, Sanz P, Neumann D. The interaction between AMPKbeta2 and the PP1-targeting subunit R6 is dynamically regulated by intracellular glycogen content. *Biochem J.* 2016;473(7):937-947.

160. Hespel P, Richter EA. Glucose uptake and transport in contracting, perfused rat muscle with different pre-contraction glycogen concentrations. *J Physiol.* 1990;427(1):347-359.

161. Steensberg A, van Hall G, Keller C, Osada T, Schjerling P, Pedersen BK, Saltin B, Febbraio MA. Muscle glycogen content and glucose uptake during exercise in humans: influence of prior exercise and dietary manipulation. *J Physiol.* 2002;541(Pt 1):273-281.
162. Rose AJ, Hargreaves M. Exercise increases Ca2+-calmodulin-dependent protein kinase II activity in human skeletal muscle. *J Physiol*. 2003;553(Pt 1):303-309.
163. Rose AJ, Kiens B, Richter EA. Ca2+-calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. *J Physiol*. 2006;574(Pt 3):889-903.
164. Combes A, Dekerle J, Webborn N, Watt P, Bougault V, Daussin FN. Exercise-induced metabolic fluctuations influence AMPK, p38-MAPK and CaMKII phosphorylation in human skeletal muscle. *Physiol Rep*. 2015;3(9):e12462.
165. Witczak CA, Jessen N, Warro DM, Toyoda T, Fuji N, Anderson ME, Hirshman MF, Goodyear LJ. CaMKII regulates contraction- but not insulin-induced glucose uptake in mouse skeletal muscle. *Am J Physiol Endocrinol Metab*. 2010;298(6):E1150-1160.
166. Park D-R, Park K-H, Kim B-J, Yoon C-S, Kim U-H. Exercise ameliorates insulin resistance via Ca2+ signals distinct from those of insulin for GLUT4 translocation in skeletal muscles. *Diabetes*. 2015;64(4):1224-1234.
167. Li Z, Yue Y, Hu F, Zhang C, Ma X, Li N, Qiu L, Fu M, Chen L, Yao Z, Bilan PJ, Klip A, Niu W. Electrical pulse stimulation induces GLUT4 translocation in C(2)C(12) myotubes that depends on Rab8A, Rab13, and Rab14. *Am J Physiol Endocrinol Metab*. 2018;314(5):E478-e493.
168. Wright DC, Hucker KA, Holloszy JO, Han DH. Ca2+ and AMPK both mediate stimulation of glucose transport by muscle contractions. *Diabetes*. 2004;53(2):330-335.
169. Ihlemann J, Galbo H, Ploug T. Calphostin C is an inhibitor of contraction, but not insulin-stimulated glucose transport, in skeletal muscle. *Acta Physiol Scand*. 1999;167(1):69-75.
170. Jensen TE, Rose AJ, Jorgensen SB, Brandt N, Schjerling P, Wojtaszewski JF, Richter EA. Possible CaMKK-dependent regulation of AMPK phosphorylation and glucose uptake at the onset of mild tetanic skeletal muscle contraction. *Am J Physiol Endocrinol Metab*. 2007;292(5):E1308-E1317.
171. Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. *Nat Med*. 2014;20(11):1242-1253.
172. Balaji V, Pokrzywa W, Hoppe T. Ubiquitylation pathways in insulin signaling and organismal homeostasis. *Bioessays*. 2018;40(5):e1700223.
173. Yang XD, Xiang DX, Yang YY. Role of E3 ubiquitin ligases in insulin resistance. *Diabetes Obes Metab*. 2016;18(8):747-754.
174. Song R, Peng W, Zhang Y, Lv F, Wu HK, Guo J, Cao Y, Pi Y, Zhang X, Jin L, Zhang M, Jiang P, Liu F, Meng S, Zhang X, Jiang P, Cao CM, Xiao RP. Central role of E3 ubiquitin ligase MG53 in insulin resistance and metabolic disorders. *Nature*. 2013;494(7437):375-379.
175. Uruno A, Furusawa Y, Yagishita Y, Fukutomi T, Muramatsu H, Negishi T, Sugawara A, Kensler TW, Yamamoto M. The Keap1-Nrf2 system prevents onset of diabetes mellitus. *Mol Cell Biol*. 2013;33(15):2996-3010.
176. Kawaguchi M, Minami K, Nagashima K, Seino S. Essential role of ubiquitin-proteasome system in normal regulation of insulin secretion. *J Biol Chem*. 2006;281(19):13015-13020.
177. Yamada T, Murata D, Adachi Y, Itoh K, Kameoka S, Igarashi A, Kato T, Araki Y, Huganir RL, Dawson TM, Yangawa T, Okamoto K, Iijima M, Sessaki H. Mitochondrial stasis reveals p62-mediated ubiquitination in parkin-independent mitophagy and mitigates nonalcoholic fatty liver disease. *Cell Metab*. 2018;28(4):588-604.e585.
178. Price SR, Bailey JL, Wang X, Jurkovitz C, England BK, Ding X, Phillips LS, Mitch WE. Muscle wasting in insulinopenic rats results from activation of the ATP-
dependent, ubiquitin-proteasome proteolytic pathway by a mechanism including gene transcription. *J Clin Invest.* 1996;98(8):1703-1708.

179. Al-Khalili L, de Castro Barbosa T, Ostling J, Massart J, Cuesta PG, Osler ME, Katayama M, Nyström AC, Oscarsson J, Zierath JR. Proteasome inhibition in skeletal muscle cells unmasks metabolic derangements in type 2 diabetes. *Am J Physiol Cell Physiol.* 2014;307(9):C774-787.

180. Nagarajan A, Petersen MC, Nasiri AR, Butrico G, Fung A, Ruan HB, Kursawe R, Caprio S, Thibodeau J, Bourgeois-Daigneault MC, Sun L, Gao G, Bhanot S, Jurczak MJ, Green MR, Shulman GI, Wajapeeyee N. MARCH1 regulates insulin sensitivity by controlling cell surface insulin receptor levels. *Nat Commun.* 2016;7:12639.

181. Ahn BH, Kim HS, Song S, Lee IH, Liu J, Vassilopoulos A, Deng CX, Finkel T. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. *Proc Natl Acad Sci U S A.* 2008;105(38):14447-14452.

182. VerPlank JJS, Lokireddy S, Zhao J, Goldberg AL. 26S Proteasomes are rapidly activated by diverse hormones and physiological states that raise cAMP and cause Rpn6 phosphorylation. *Proc Natl Acad Sci U S A.* 2019;116(10):4228-4237.

183. Djakovic SN, Schwarz LA, Barylko B, DeMartino GN, Patrick GN. Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. *J Biol Chem.* 2009;284(39):26655-26665.

184. Chen-Zion M, Lilling G, Beittner R. The dual effects of Ca2+ on binding of the glycolytic enzymes, phosphofructokinase and aldolase, to muscle cytoskeleton. *Biochem Med Metab Biol.* 1993;49(2):173-181.

185. Krassikova L, Zhang B, Nagarajan D, Queiroz AL, Kacal M, Samakidis E, Vakifahmetoglu-Norberg H, Norberg E. The deubiquitinase JOSD2 is a positive regulator of glucose metabolism. *Cell Death Differ.* 2021;28(3):1091-1109.

186. Dagon Y, Hur E, Zheng B, Wellenstein K, Cantley Lewis C, Kahn Barbara B. p70S6 kinase phosphorylates AMPK on serine 491 to mediate leptin's effect on food intake. *Cell Metab.* 2012;16(1):104-112.
193. Hawley Simon A, Ross Fiona A, Gowan Graeme J, Tibarewal P, Leslie Nicholas R, Hardie DG. Phosphorylation by Akt within the ST loop of AMPK-α1 down-regulates its activation in tumour cells. *Biochem J.* 2014;459(2):275-287.

194. Lundby A, Lage K, Weinert BT, Bekker-Jensen DB, Secher A, Skovgaard T, Kelstrup CD, Dmytryiev A, Choudhary C, Lundby C, Olsen JV. Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns. *Cell Rep.* 2012;2(2):419-431.

195. Hostrup M, Lemminger AK, Stocks B, Gonzalez-Franquesa A, Larsen JK, Thomassen M, Weinert B, Bangsbo J, Deshmukh AS. High-intensity interval training remodels the proteome and acetylome of human skeletal muscle. *bioRxiv.* 2021:2021.2002.2010.430602.

196. Overmyer KA, Evans CR, Qi NR, Minogue CE, Carson JJ, Chermside-Scabbo CJ, Koch LG, Britton SL, Pagliarini DJ, Coon JJ, Burant CF. Maximal oxidative capacity during exercise is associated with skeletal muscle fuel selection and dynamic changes in mitochondrial protein acetylation. *Cell Metab.* 2015;21(3):468-478.

197. Williams AS, Koves TR, Davidson MT, Crown SB, Fisher-Wellman KH, Torres MJ, Draper JA, Narowski TM, Slentz DH, Lantier L, Wasserman DH, Grimsrud PA, Muoio DM. Disruption of acetyl-lysine turnover in muscle mitochondria promotes insulin resistance and redox stress without overt respiratory dysfunction. *Cell Metab.* 2020;31(1):131-147.e111.

198. LaBarge S, Migdal C, Schenk S. Is acetylation a metabolic rheostat that regulates skeletal muscle insulin action? *Mol Cells.* 2015;38(4):297-303.

199. Sun C, Zhou J. Trichostatin A improves insulin stimulated glucose utilization and insulin signaling transduction through the repression of HDAC2. *Biochem Pharmacol.* 2008;76(1):120-127.

200. Sundaresan NR, Pillai VB, Wolfeger D, Samant S, Vasudevan P, Parekh V, Raghuraman H, Cunningham JM, Gupta M, Gupta MP. The deacetylase SIRT1 promotes membrane localization and activation of Akt and PDK1 during tumorigenesis and cardiac hypertrophy. *Sci Signal.* 2011;4(182):ra46.

201. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell.* 2007;128(4):707-719.

202. McGee SL, van Denderen BJ, Howlett KF, Mollica J, Schertzer JD, Kemp BE, Hargreaves M. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes.* 2008;57(4):860-867.

203. Hong S, Zhou W, Fang B, Lu W, Loro E, Damle M, Ding G, Jager J, Zhang S, Zhang Y, Feng D, Chu Q, Dill BD, Molina H, Khurana TS, Rabinowitz JD, Lazar MA, Sun Z. Dissociation of muscle insulin sensitivity from exercise endurance in mice by HDAC3 depletion. *Nat Med.* 2017;23(2):223-234.

204. Song S, Wen Y, Tong H, Loro E, Gong Y, Liu J, Hong S, Li L, Khurana TS, Chu M, Sun Z. The HDAC3 enzymatic activity regulates skeletal muscle fuel metabolism. *J Mol Cell Biol.* 2019;11(2):133-143.

205. Gerhart-Hines Z, Rodgers JT, Bare O, Liner C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *EMBO J.* 2007;26(7):1913-1923.

206. Schenk S, McCurdy CE, Philip A, Chen MZ, Holliday MJ, Bandyopadhyay GK, Osborn O, Baar K, Olefsky JM. Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. *J Clin Invest.* 2011;121(11):4281-4288.

207. White AT, Philip A, Fridolfsson HN, Schilling JM, Murphy AN, Hamilton DL, McCurdy CE, Patel HH, Schenk S. High-fat diet-induced impairment of skeletal
muscle insulin sensitivity is not prevented by SIRT1 overexpression. *Am J Physiol Endocrinol Metab.* 2014;307(9):E764-E772.

208. Kang JH, Park JE, Dagoon J, Masson SWC, Merry TL, Bremner SN, Dent JR, Schenk S. Sirtuin 1 is not required for contraction-stimulated glucose uptake in mouse skeletal muscle. *J Appl Physiol.* 2021;130(6):1893-1902.

209. Philip A, Chen A, Lan D, Meyer GA, Murphy AN, Knapp AE, Olfert IM, McCurdy CE, Marcotte GR, Hogan MC, Baar K, Schenk S. Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) deacetylation following endurance exercise. *J Biol Chem.* 2011;286(35):30561-30570.

210. Dent JR, Martins VF, Svensson K, LaBarge SA, Schlenk NC, Esparza MC, Buckner EH, Meyer GA, Hamilton DL, Schenk S, Philip A. Muscle-specific knockout of general control of amino acid synthesis 5 (GCN5) does not enhance basal or endurance exercise-induced mitochondrial adaptation. *Mol Metab.* 2017;6(12):1574-1584.

211. Martins VF, Dent JR, Svensson K, Tahvili SA, Begur M, Lakkaraju S, Buckner EH, LaBarge SA, Hetrick B, McCurdy CE, Schenk S. Germline or inducible knockout of p300 or CBP in skeletal muscle does not alter insulin sensitivity. *Am J Physiol Endocrinol Metab.* 2019;316(6):E1024-E1035.

212. LaBarge SA, Migdal CW, Buckner EH, Okuno H, Gertsman I, Stocks B, Barshop BA, Nalbandian SR, Philip A, McCurdy CE, Schenk S. p300 is not required for metabolic adaptation to endurance exercise training. *FASEB J.* 2016;30(4):1623-1633.

213. Svensson K, LaBarge SA, Sathe A, Martins VF, Tahvili SA, Cunliffe JM, Sasik R, Mahata SK, Meyer GA, Philip A, David LL, Ward SR, McCurdy CE, Aslan JE, Schenk S. p300 and cAMP response element-binding protein-binding protein in skeletal muscle homeostasis, contractile function, and survival. *J Cachexia Sarcopenia Muscle.* 2020;11(2):464-477.

214. Muoio DM, Noland RC, Kovalik J-P, Seiler SE, Davies MN, DeBalsi KL, Ilkayeva OR, Stevens RD, Kheterpal I, Zhang J, Covington JD, Bajpeyi S, Ravussin E, Kraus W, Koves TR, Mynatt RL. Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. *Cell Metab.* 2012;15(5):764-777.

215. Hirschey Matthew D, Shimazu T, Jing E, Grueter Carrie A, Collins Amy M, Aouizerat B, Stančáková A, Goetzman E, Lam Maggie M, Schwer B, Stevens Robert D, Muehlbauer Michael J, Kakar S, Bass Nathan M, Kuusisto J, Laakso M, Alt Frederick W, Newgard Christopher B, Farese Robert V, Kahn CR, Verdin E. SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol Cell.* 2011;44(2):177-190.

216. Lantier L, Williams AS, Williams IM, Yang KK, Bracy DP, Goelzer M, James FD, Giúd S, Wasserman DH. SIRT3 is crucial for maintaining skeletal muscle insulin action and protects against severe insulin resistance in high-fat-fed mice. *Diabetes.* 2015;64(9):3081-3092.

217. Jing E, O'Neill BT, Rardin MJ, Kleinridders A, Ilkayeva OR, Ussar S, Bain JR, Lee KY, Verdin EM, Newgard CB, Gibson BW, Kahn CR. Sirt3 regulates metabolic flexibility of skeletal muscle through reversible enzymatic deacetylation. *Diabetes.* 2013;62(10):3404-3417.

218. Constantin Teodosiu D, Constantin D, Stephens F, Laithwaite D, Greenhaff PL. The role of FOXO and PPAR transcription factors in diet-mediated inhibition of PDC activation and carbohydrate oxidation during exercise in humans and the role of
pharmacological activation of PDC in overriding these changes. *Diabetes*. 2012;61(5):1017-1024.

219. Stocks B, Ashcroft SP, Joanisse S, Dansereau LC, Koay YC, Elhassan YS, Lavery GG, Quek L-E, O’Sullivan JF, Philp AM, Wallis GA, Philp A. Nicotinamide riboside supplementation does not alter whole-body or skeletal muscle metabolic responses to a single bout of endurance exercise. *J Physiol*. 2021;599(5):1513-1531.

220. Cantó C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J. Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab*. 2010;11(3):213-219.

221. Henriksson J, Katz A, Sahlin K. Redox state changes in human skeletal muscle after isometric contraction. *J Physiol*. 1986;380:441-451.

222. Sahlin K. NADH in human skeletal muscle during short-term intense exercise. *Pflugers Arch*. 1985;403(2):193-196.

223. Sahlin K, Katz A, Henriksson J. Redox state and lactate accumulation in human skeletal muscle during dynamic exercise. *Biochem J*. 1987;245(2):551-556.

224. McGee SL, Fairlie E, Garnham AP, Hargreaves M. Exercise-induced histone modifications in human skeletal muscle. *J Physiol*. 2009;587(Pt 24):5951-5958.

225. Weinert BT, Moustafa T, Iesmantavicius V, Zechner R, Choudhary C. Analysis of acetylation stoichiometry suggests that SIRT3 repairs nonenzymatic acetylation lesions. *EMBO J*. 2015;34(21):2620-2632.

226. Ren W, Jhala US, Du K. Proteomic analysis of protein palmitoylation in adipocytes. *Adipocyte*. 2013;2(1):17-28.

227. Hirschev MD, Zhao Y. Metabolic regulation by lysine malonylation, succinylation, and glutarylation. *Mol Cell Proteomics*. 2015;14(9):2308-2315.

228. Bandopadhayay GK, Yu JG, Ofrecio J, Olefsky JM. Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis: thiazolidinedione treatment reverses these defects. *Diabetes*. 2006;55(8):2277-2285.

229. Odland LM, Howlett RA, Heigenhauser GJF, Hultman E, Spriet LL. Skeletal muscle malonyl-CoA content at the onset of exercise at varying power outputs in humans. *Am J Physiol Endocrinol Metab*. 1998;274(6):E1080-E1085.

230. Odland LM, Heigenhauser GJ, Lopaschuk GD, Spriet LL. Human skeletal muscle malonyl-CoA at rest and during prolonged submaximal exercise. *Am J Physiol*. 1996;270(3 Pt 1):E541-544.

231. Zhao Z, Lee Y-J, Kim S-K, Kim H-J, Shim W-S, Ahn C-W, Lee H-C, Cha B-S, Ma ZA. Rosiglitazone and fenofibrate improve insulin sensitivity of pre-diabetic OLETF rats by reducing malonyl-CoA levels in the liver and skeletal muscle. *Life Sci*. 2009;84(19):688-695.

232. Du K, Murakami S, Sun Y, Kilpatrick CL, Luscher B. DHHC7 palmitoylates glucose transporter 4 (Glut4) and regulates Glut4 membrane translocation. *J Biol Chem*. 2017;292(7):2979-2991.

233. Ren W, Sun Y, Du K. Glut4 palmitoylation at Cys223 plays a critical role in Glut4 membrane trafficking. *Biochem Biophys Res Commun*. 2015;460(3):709-714.

234. Bai P, Canto C, Brunyanszki A, Huber A, Szanto M, Cen Y, Yamamoto H, Houten SM, Kiss B, Oudart H, Gergely P, Menissier-de Murcia J, Schreiber V, Sauve AA, Auwerx J. PARP-2 regulates SIRT1 expression and whole-body energy expenditure. *Cell Metab*. 2011;13(4):450-460.

235. Bai P, Canto C, Oudart H, Brunyanszki A, Cen Y, Thomas C, Yamamoto H, Huber A, Kiss B, Houtkooper RH, Schoonjans K, Schreiber V, Sauve AA, Menissier-de...
Murcia J, Auwerx J. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* 2011;13(4):461-468.

Mohamed JS, Hajira A, Pardo PS, Boriek AM. MicroRNA-149 inhibits PARP-2 and promotes mitochondrial biogenesis via SIRT-1/PGC-1alpha network in skeletal muscle. *Diabetes.* 2014;63(5):1546-1559.

Pirinen E, Canto C, Jo YS, Morato L, Zhang H, Menzies KJ, Williams EG, Mouchiroud L, Moullan N, Hagberg C, Li W, Timmers S, Imhof R, Verbeek J, Pujol A, van Loon B, Viscomi C, Zeviani M, Schrauwen P, Sauve AA, Schoonjans K, Auwerx J. Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. *Cell Metab.* 2014;19(6):1034-1041.

Leutert M, Menzel S, Braren R, Rissiek B, Hopp A-K, Nowak K, Bisceglie L, Gehrig P, Li H, Zolkiewska A, Koch-Nolte F, Hottiger MO. Proteomic characterization of the heart and skeletal muscle reveals widespread arginine ADP-ribosylation by the ARTC1 ectoenzyme. *Cell Rep.* 2018;24(7):1916-1929.e1915.

Hendriks IA, Larsen SC, Nielsen ML. An advanced strategy for comprehensive profiling of ADP-ribosylation sites using mass spectrometry-based proteomics. *Mol Cell Proteomics.* 2019;18(5):1010-1026.

Pezzolesi MG, Nam M, Nagase T, Klupa T, Dunn JS, Mlynarski WM, Rich SS, Warram JH, Krolewski AS. Examination of candidate chromosomal regions for type 2 diabetes reveals a susceptibility locus on human chromosome 8p23.1. *Diabetes.* 2004;53(2):486-491.

Scherag A, Dina C, Hinney A, et al. Two new Loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and german study groups. *PLoS Genet.* 2010;6(4):e1000916.

Zhong L, Ding Y, Bandyopadhyay G, Waaler J, Börjeson E, Smith S, Zhang M, Phillips SA, Mahooti S, Mahata SK, Shao J, Krauss S, Chi N-W. The PARylation activity of tankyrase in adipose tissue modulates systemic glucose metabolism in mice. *Diabetologia.* 2016;59(3):582-591.

Wang H, Kuusela S, Rinnankoski-Tuikka R, Dumont V, Bouslama R, Ramadan UA, Waaler J, Linden A-M, Chi N-W, Krauss S, Pirinen E, Lehtonen S. Tankyrase inhibition ameliorates lipid disorder via suppression of PGC-1α PARylation in db/db mice. *Int J Obes.* 2020;44(8):1691-1702.

Chi N-W, Lodish HF. Tankyrase is a golgi-associated mitogen-activated protein kinase substrate that interacts with IRAP in GLUT4 vesicles. *J Biol Chem.* 2000;275(49):38437-38444.

Yeh T-YJ, Sbodio II, Tsun Z-Y, Luo B, Chi N-W. Insulin-stimulated exocytosis of GLUT4 is enhanced by IRAP and its partner tankyrase. *Bioch J.* 2007;402(2):279-290.

Mohamed JS, Wilson JC, Myers MJ, Sisson KJ, Alway SE. Dysregulation of SIRT-1 in aging mice increases skeletal muscle fatigue by a PARP-1-dependent mechanism. *Aging (Albany NY).* 2014;6(10):820-834.

Stocks B, Ashcroft SP, Joanisse S, Elhassan YS, Lavery GG, Dansereau LC, Philp AM, Wallis GA, Philp A. Nicotinamide Riboside supplementation does not alter whole-body or skeletal muscle metabolic responses to a single bout of endurance exercise. *bioRxiv.* 2020:2020.2006.2023.143446.

Cobleyn JN, Sakellariou GK, Murray S, Waldron S, Gregson W, Burniston JG, Morton JP, Iwanejko LA, Close GL. Lifelong endurance training attenuates age-related genotoxic stress in human skeletal muscle. *Longev Healthspan.* 2013;2:11.
249. Deshmukh AS, Murgia M, Nagaraj N, Treebak JT, Cox J, Mann M. Deep proteomics of mouse skeletal muscle enables quantitation of protein isoforms, metabolic pathways, and transcription factors. *Mol Cell Proteomics*. 2015;14(4):841-853.

250. Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nature Methods*. 2020;17(1):41-44.

251. Bekker-Jensen DB, Martínez-Val A, Steigerwald S, Rüther P, Fort KL, Arrey TN, Harder A, Makarov A, Olsen JV. A compact quadrupole-orbitrap mass spectrometer with FAIMS interface improves proteome coverage in short LC gradients. *Mol Cell Proteomics*. 2020;19(4):716-729.

252. Hansen FM, Tanzer MC, Brüning F, Bluda I, Stafford C, Schulman BA, Robles MS, Karayel O, Mann M. Data-independent acquisition method for ubiquitinome analysis reveals regulation of circadian biology. *Nat Commun*. 2021;12(1):254.

253. Humphrey SJ, Karayel O, James DE, Mann M. High-throughput and high-sensitivity phosphoproteomics with the EasyPhos platform. *Nat Protoc*. 2018;13(9):1897-1916.

254. Ludwig C, Gillet L, Rosenberger G, Amon S, Collins BC, Aebersold R. Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial. *Mol Syst Biol*. 2018;14(8):e8126.

255. Abbott MJ, Edelman AM, Turcotte LP. CaMKK is an upstream signal of AMP-activated protein kinase in regulation of substrate metabolism in contracting skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. 2009;297(6):R1724-R1732.

256. Hawley SA, Pan DA, Mustard KJ, Ross L, Bain J, Edelman AM, Frenquelli BG, Hardie DG. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab*. 2005;2(1):9-19.

257. Hurley RL, Anderson KA, Franzone JM, Kemp BE, Means AR, Witters LA. The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. *J Biol Chem*. 2005;280(32):29060-29066.

258. Sakamoto K, McCarthy A, Smith D, Green KA, Grahame Hardie D, Ashworth A, Alessi DR. Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J*. 2005;24(10):1810-1820.

259. Thomson DM, Porter BB, Tall JH, Kim HJ, Barrow JR, Winder WW. Skeletal muscle and heart LKB1 deficiency causes decreased voluntary running and reduced muscle mitochondrial marker enzyme expression in mice. *Am J Physiol Endocrinol Metab*. 2007;292(1):E196-E202.

260. Jørgensen SB, Nielsen JN, Birk JB, Olsen GS, Viollet B, Andreelli F, Schjerling P, Vaulont S, Hardie DG, Hansen BF, Richter EA, Wojtaszewski JFP. The α2–5′AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. *Diabetes*. 2004;53(12):3074-3081.

261. Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB. Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. *J Biol Chem*. 1997;272(20):13255-13261.

262. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell*. 2003;115(5):577-590.

263. Ojuka EO, Jones TE, Nolte LA, Chen M, Wamhoff BR, Sturek M, Holloszy JO. Regulation of GLUT4 biogenesis in muscle: evidence for involvement of AMPK and Ca(2+). *Am J Physiol Endocrinol Metab*. 2002;282(5):E1008-1013.

264. Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A*. 2007;104(29):12017-12022.
265. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. *Mol Cell*. 2001;8(5):971-982.

266. McGee SL, Hargreaves M. Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. *Diabetes*. 2004;53(5):1208-1214.

267. Zhao M, New L, Kravchenko VV, Kato Y, Gram H, di Padova F, Olson EN, Ulevitch RJ, Han J. Regulation of the MEF2 family of transcription factors by p38. *Mol Cell Biol*. 1999;19(1):21-30.

268. Mehrani H, Storey KB. Control of glycogenolysis and effects of exercise on phosphorylase kinase and cAMP-dependent protein kinase in rainbow trout organs. *Biochem Cell Biol*. 1993;71(11-12):501-506.

269. Ray Hamidie RD, Yamada T, Ishizawa R, Saito Y, Masuda K. Curcumin treatment enhances the effect of exercise on mitochondrial biogenesis in skeletal muscle by increasing cAMP levels. *Metabolism*. 2015;64(10):1334-1347.
Figure legends

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 (i.e. 50-70% Wmax for >30 minutes), high-intensity interval training, in which periods of high (i.e. >90% Wmax) and low (i.e. <50% Wmax) intensities are alternated for numerous intervals, and high-intensity exhaustive exercise, in which high-intensity exercise (i.e. >80% Wmax) is performed to exhaustion.

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 ARP1/2- 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 of TBC1D1 and TBC1D4 (97-99,119,123,129), net glycogen breakdown via inhibition of glycogen synthase (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 AMPK (cAMP) activates PKA, which phosphorylates cyclic AMP-responsive element-binding protein (CREB) and promotes transcription of metabolic genes (268,269).

**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.

**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. Yellow ubiquitin-sites represent K-GG remnants downregulated by exercise. Red ubiquitin-sites represent K-GG remnants upregulated by exercise.

**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.

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.

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.
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.
Figure 1

A

Exercise training

B

- Moderate-intensity continuous exercise
- High-intensity interval training
- High-intensity exhaustive exercise
Figure 6
