A Critically Swift Response: Insulin-Stimulated Potassium and Glucose Transport in Skeletal Muscle

Kevin Ho

It has been tempting to speculate that dietary glucose and potassium handling are coordinated in the post-prandial state as a result of common molecular mechanisms that evolved in the distant past to respond to frequent episodes of feeding. The increase in insulin secretion by pancreatic β cells mediated by ATP-sensitive K⁺ channels (1) after a meal results in rapid skeletal muscle K⁺ and glucose uptake. However, the observed correlation between two processes may be the outward manifestation of pathways that have evolved to operate in parallel triggered by an initiating event (e.g., ingested meal) and does not establish that both are mediated by a common pathway; cross-talk between pathways within a signaling network is as likely an explanation. Using the hyperinsulinemic-euglycemic insulin clamp technique, Nguyen et al. (2) show in this issue of CJASN that the reduced glucose uptake in skeletal muscle, characteristic of insulin resistance in patients with type 2 diabetes (3), is not associated with a parallel decrement in cellular potassium and phosphate uptake. Their work underscores limitations in our understanding regarding the relationship between insulin-mediated regulatory pathways for glucose and potassium handling both in health and in insulin-resistant states such as diabetes at a molecular level while at the same time providing further evidence that the pathways are discrete.

It is well established that the long-term maintenance of potassium homeostasis results from the intricate regulation of renal K⁺ excretion ultimately mediated in large part by inward rectifier ROMK channels in the aldosterone-sensitive distal nephron (4,5). However, the major extrarenal mechanism for achieving K⁺ homeostasis on a moment-to-moment basis is mediated through the transmembrane transport of extracellular K⁺ ions into skeletal muscle, the primary reservoir for dietary K⁺ and more than 70% of body K⁺ stores, approximately 2600 mmol, in exchange for intracellular Na⁺ ions by Na,K-ATPases that require the hydrolysis of Mg-ATP. Indeed, skeletal muscle contains one of the largest pools of Na,K-ATPase in the body with a calculated maximal transport capacity of 134 mmol/min (6). The x-ray crystal structure of Na,K-ATPase reveals a catalytic α-subunit and modulatory β-subunit and tissue-specific FXYD protein that alter enzyme affinities for Na⁺, K⁺, and ATP. After a meal, a rise in insulin with binding to its receptors increases Na,K-ATPase activity in skeletal muscle by increasing plasma membrane (sarcolemma, T tubule) pump expression possibly through translocation of the α/β heterodimeric Na,K-ATPase (α₁, α₂, β₁, and β₂ subunit isoforms) from intracellular stores by way of a phosphatidylinositol 3-kinase (PI3-K) and atypical protein kinase C-dependent pathway (7,8). Phosphorylation of Na,K-ATPase catalytic α-subunits by extracellular signal-regulated kinase 1 and 2 (ERK1/2) in response to insulin increases membrane abundance by regulating membrane trafficking (9). Furthermore, in skeletal muscle, insulin induces the phosphorylation of an inhibitory FXYD1 protein (phospholemman) that subsequently modulates its association with Na,K-ATPase and leads to increased pump function (7,10). Stimulation of increased Na⁺ affinity and enzyme activity also occurs in the presence of catecholamines through β₂-adrenergic receptors with phosphorylation of FXYD1 by protein kinase A (6).

Skeletal muscle also serves as the main site of insulin-stimulated glucose disposal, thus playing a major role in glucose homeostasis. At first glance, the action of insulin on glucose transport seems independent of its effect on skeletal Na,K-ATPase. Insulin initiates the translocation of the major glucose transporter responsible for glucose uptake into muscle and adipose tissue, GLUT4, to the plasma membrane (sarcolemma, T tubule) from an intracellular membrane storage compartment, GLUT4 storage vesicles. Na,K-ATPase and GLUT4 do not localize to the same intracellular vesicles. Basal sequestration of GLUT4 within intracellular membrane pools is the result of complex trafficking between endosomal, trans-Golgi network and “cycling” pools. In the absence of insulin stimulation, more than 95% of GLUT4 resides within intracellular membrane pools. Binding of insulin to its receptor stimulates a complex PI3-K–dependent signal transduction network involving insulin receptor substrate (IRS-1) and Akt/protein kinase B and its substrates—the negative regulators AS160 (Akt substrate of 160 kD) and TBC1D1—which regulate Rab8A and Rab14 GTPases involved in muscle cell membrane trafficking (8,11). Rab-GTPase–activating proteins AS160 and TBC1D1 inhibit GLUT4 translocation and glucose uptake. Akt (for insulin) and...
AMP-activated protein kinase (for aminomimidazole carboxamide ribonucleotide) mediate phosphorylation and inactivation of AS160 and TBC1D1, resulting in the loss of their inhibitory effects on Rab GTPases, thereby allowing GLUT4 translocation and glucose uptake. In addition to regulating GLUT4 translocation from intracellular storage pools, insulin increases the rate of both docking and fusion of GLUT4-laden vesicles with the plasma membrane by facilitating assembly of Rip11/Rab and exocyst complexes as well as soluble NSF attachment protein receptor (SNARE) complexes, respectively (11). In the insulin-stimulated state, intracellular glucose phosphorylated to glucose-6-phosphate by hexokinase II is directed mostly to glycolysis synthesis with the remainder to the oxidative pathway.

Early in the development of type 2 diabetes, insulin sensitivity markedly declines, but the progression from normal glucose tolerance to glucose intolerance is initially blunted by compensatory hyperinsulinemia in the face of insulin resistance. Impaired skeletal muscle glycogen synthase activity leading to reduced glycogen synthesis as well as impaired glucose transport and phosphorylation are early metabolic defects resulting in reduced glucose uptake and glucose intolerance (3,12). Skeletal muscle molecular defects include decreased insulin-stimulated IRS-1 tyrosine phosphorylation and PI3-K activity associated with insulin–insulin receptor binding, as well as reductions in GLUT4 gene expression, insulin stimulation of GLUT4 translocation (13), GLUT4 membrane density in slow muscle fibers (14), and insulin-mediated PI3-K activation and GLUT4 translocation in the T-tubule system, especially when compared with the sarcolemma (15). Insulin resistance is also associated with a marked decrease in both adipose cell GLUT4 gene expression and the intracellular membrane pool of GLUT4. Using euglycemic insulin clamp, Pendergrass et al. (16) recently showed a marked reduction in forearm glucose uptake, in vivo trans-membrane glucose transport, and glucose phosphorylation in lean and obese individuals with type 2 diabetes.

By comparison, controversy characterizes studies of skeletal Na,K-ATPase activity and expression (protein, gene) in hyperinsulinemic and insulin-resistant states and in diabetes (17). Reductions in skeletal muscle Na,K-ATPase $[^{3}H]$ouabain binding, a measure of Na,K-ATPase content, in ob/ob mice and in untreated streptozotocin- or partial pancreatectomy-induced diabetic rats were associated with unaltered or reduced enzyme activity, whereas exogenous insulin upregulated Na,K-ATPase content. In contrast, others reported increased skeletal muscle $\alpha_1$- and $\alpha_2$-subunit expression and $\alpha_2$-subunit mRNA expression but with unaltered or reduced pump activity in the same diabetic rat model. In humans, Na,K-ATPase skeletal muscle $[^{3}H]$ouabain binding was increased in treated patients with type 1 or type 2 diabetes compared with control subjects; a linear correlation between muscle Na,K-ATPase content and plasma insulin concentration was observed in patients with type 2 diabetes (18).

In studies of both glucose and potassium transport processes in skeletal muscle, efforts so far have been directed largely at understanding the relationship between both processes at a physiologic level. Impaired skeletal muscle glucose uptake in high-fat diet, insulin-resistant Wistar rats was observed to be associated with reduced plasma membrane Na,K-ATPase $[^{3}H]$ouabain binding and activity, increased muscle $\alpha_1$-subunit and FXYD1 expression, but reduced $\alpha_2$- (major muscle isoform) and $\beta_1$-subunit expression (19). However, while insulin-stimulated glucose uptake was also reduced in rats on a similar diet supplemented with potassium, Choi et al. (20) also reported unchanged insulin-stimulated K$^+$ uptake in rats with the same K$^+$ intake on either a high-fat or a control diet. In a study of identical twins discordant for type 2 diabetes and healthy control subjects, insulin-stimulated glucose uptake rates were reduced to the greatest extent in twins with diabetes and least in control subjects compared with twins without diabetes, whereas no differences were observed in plasma K$^+$ reduction (15%) among the three groups or between muscle K$^+$/Na$^+$ ratios in twins with type 2 diabetes and control subjects. Consistent with animal studies, muscle Na,K-ATPase $[^{3}H]$ouabain binding was lowest in patients with type 2 diabetes compared with control subjects; twins without diabetes were intermediate (21). Conversely, potassium deprivation studies in rats have revealed a selective insulin resistance in muscle reducing cellular K$^+$ uptake associated with reduced Na,K-ATPase $[^{3}H]$ouabain binding and $\alpha_2$-subunit abundance while maintaining normal insulin-sensitive glucose uptake (22). Taken together, these observations are consistent with self-regulating insulin-stimulated glucose and K$^+$ transport pathways within a complex signaling network but do not establish a necessary interdependence.

Thus far, evidence at a molecular and cellular level does not strongly support the idea that insulin-stimulated glucose and potassium uptake by skeletal muscle are interdependent, mutually coordinated processes, although the notion of cross-talk between the regulatory pathways at a molecular level remains intriguing. The latter possibility is suggested by the work of Taniguchi et al. (8), who identified the insulin receptor–insulin receptor substrate proteins PI3-K and AKT/protein kinase B as critical “nodes” for mediating cross-talk between other pathways within the complex insulin-signaling network. This has clinical relevance given that diabetes is frequently associated with hyperkalemia (hyporeninemic hypoaldosteronism, impaired renal K$^+$ excretion, pharmacologic agents blocking the renin-angiotensin-aldosterone system). Moreover, diabetic nephropathy is the major cause of chronic kidney disease accounting for close to one half of patients with ESRD. In the face of clinically significant hyperkalemia, the skeletal muscle Na,K-ATPase pool can rapidly reduce plasma K$^+$ content (6). Thus, insulin remains first-line therapy in the acute treatment of hyperkalemia in individuals with and without diabetes and with or without renal function.

In conclusion, a central question poses itself from physiologic observations in individuals and animals with and without diabetes that so far suggest that the independent regulation of glucose and potassium transport occurs and that this takes place within the context of a complex insulin-stimulated network triggered by a common event: the binding of insulin to the insulin receptor tyrosine kinase. With insulin stimulation, we ask, “What are the molecular...
differences and mechanisms that maintain the observed uptake of potassium by the Na,K-ATPase in skeletal muscle in the diabetic state (e.g., in the short-term postprandial state) while GLUT4-mediated glucose uptake is simultaneously impaired? “Do these mechanisms that allow for a differential regulation of these transport processes provide additional insight into the defects characterizing skeletal muscle glucose handling in humans with diabetes while suggesting potential new therapeutic avenues in the treatment of diabetes?” With the question thus stated, we then ask how much of the answer lies within the negative feedback loop and suppressor pathways (8) modulating the insulin-stimulated signal transduction network, as well as in the spatial distribution of these pathways (e.g., sarcolemma versus T-tubule) in muscle fibers. Future research, for instance, might be directed at a consideration of the suppressor roles and negative feedback mediated by the following mechanisms: Differential roles of PI3-K regulatory subunits, phosphorylation of specific IRS-1 serine residues by IRS kinases (e.g., ERK, C-Jun-N-terminal kinase) reported in states of insulin resistance, protein tyrosine phosphatases, other phosphatases including phospholipid phosphatases, suppressor proteins (e.g., suppressor of cytokine signaling proteins), altered IRS protein expression (decreased in insulin resistance), intracellular compartmentalization of signaling pathways, or differential alterations in the membrane trafficking and translocation of GLUT4 and Na,K-ATPase. New techniques such as in vivo genetic constructs in knockout mice, the muscle-specific expression of tagged proteins in transgenic animals, RNAi, and intravital imaging of green fluorescence protein– or exofacial epitope–tagged transport proteins using confocal microscopy made possible by gene gun transient transfec-

---

**Figure 1.** Insulin-stimulated glucose and potassium transport in skeletal muscle. The figure summarizes the combined major signaling pathways and components involved in insulin-stimulated glucose and potassium cellular uptake by the facilitative glucose transporter GLUT4 and the Na,K-ATPase pump, respectively, in skeletal muscle. Negative regulatory pathways are not shown for clarity. Insulin binding to the insulin receptor, IR, a receptor tyrosine kinase consisting of a tetramer of extracellular α-subunits and intracellular β-subunits activates β-subunit kinase activity and transphosphorylation leading to phosphorylation of the insulin receptor substrate protein IRS-1. IRS proteins are characterized by pleckstrin-homology and phosphotyrosine-binding domains that enable the binding of IRS-1 to PI3-K, a dimer of catalytic subunits, phosphorylation of specific IRS-1 serine residues by IRS kinases (e.g., ERK, C-Jun-N-terminal kinase) or K⁺/H⁺-subunits and intracellular Rab8A and Rab14, leading to activation of Akt/protein kinase B via the 3-phosphoinositide–dependent protein kinase 1 (PDK1). Akt/protein kinase B inhibits the Rab-GTPase–activating protein AS160, resulting in activation of Rab small GTPases required for translocation of GLUT4 from GLUT4 storage vesicles (GSVs) enriched in IRAP and the vesicle-associated SNARE protein VAMP-2. Docking and fusion of GSVs with the plasma membrane is regulated by insulin. GLUT4 and Na,K-ATPase do not co-localize to the same skeletal muscle intracellular membrane vesicles. Insulin increases Na,K-ATPase activity and plasma membrane expression through a PI3-K, atypical protein kinase C (aPKC), and ERK1/2 mitogen-activated protein kinase pathway, although the pathway is less well defined. ERK1/2 activation occurs through aPKC in a MEK1/2 kinase–dependent manner. ERK1/2 kinase phosphorylation of the Na,K-ATPase α-subunit promotes membrane expression and translocation of Na,K-ATPase from an intracellular membrane pool to the sarcolemma possibly in part by retarding clathrin-dependent endocytosis. The regulatory protein FXYD1, or phospholemman, belongs to a family of proteins characterized by an FXYD sequence that associates with Na,K-ATPase and modulates its affinities for Na⁺ or K⁺ or its Iₘₚₓ. FXYD1 suppresses Na,K-ATPase activity by reducing its Na⁺ affinity or altering its Iₘₚₓ. Phosphorylation of FXYD1 by protein kinase A or protein kinase C modifies its interaction with Na,K-ATPase.
tion methods (23) will likely provide invaluable tools in elucidating the involved signaling pathways (Figure 1).

Disclosures

None.

References


See related article, “Comparison of Insulin Action on Glucose versus Potassium Uptake in Humans” on pages 1533–1539.