Exercise-stimulated glucose uptake — regulation and implications for glycaemic control

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Abstract | Skeletal muscle extracts glucose from the blood to maintain demand for carbohydrates as an energy source during exercise. Such uptake involves complex molecular signalling processes that are distinct from those activated by insulin. Exercise-stimulated glucose uptake is preserved in insulin-resistant muscle, emphasizing exercise as a therapeutic cornerstone among patients with metabolic diseases such as diabetes mellitus. Exercise increases uptake of glucose by up to 50-fold through the simultaneous stimulation of three key steps: delivery, transport across the muscle membrane and intracellular flux through metabolic processes (glycolysis and glucose oxidation). The available data suggest that no single signal transduction pathway can fully account for the regulation of any of these key steps, owing to redundancy in the signalling pathways that mediate glucose uptake to ensure maintenance of muscle energy supply during physical activity. Here, we review the molecular mechanisms that regulate the movement of glucose from the capillary bed into the muscle cell and discuss what is known about their integrated regulation during exercise. Novel developments within the field of mass spectrometry-based proteomics indicate that the known regulators of glucose uptake are only the tip of the iceberg. Consequently, many exciting discoveries clearly lie ahead.

The major fuel sources for muscle during exercise are carbohydrates and lipids. Lipid stores are large and potentially inexhaustible; however, stores of carbohydrate are limited, comprising 300–500 g of glycogen in skeletal muscle, 60–100 g of glycogen in the liver and 4–5 g of glucose circulating in the blood in individuals at rest[1].

The acute metabolic and mechanical demands of exercise require the coordinated regulation of diverse signalling pathways, which together form a complex and highly flexible network that elicits a range of rapid cellular homeostatic adaptations, including increasing glucose uptake. To date, no single signalling pathway has been demonstrated to fully account for any one of the three key steps involved in exercise-mediated glucose uptake (delivery, transport and metabolism). This failure to identify a master signal transduction pathway probably reflects the complexity and partial redundancy of an evolutionarily well-conserved system to ensure sufficient glucose delivery to the working muscle. Evidence to support the enormous intricacy of the exercise-stimulated signalling network includes data from our collaborative study of global protein phosphorylation status in biopsies taken from human skeletal muscle[2]. Approximately 1,000 phosphorylation sites were significantly regulated in response to an intense session of bicycling, of which >90% were not previously known to be exercise-responsive[2]. These findings suggest that only a small fraction of the molecules regulating exercise-stimulated processes such as glucose uptake are involved in glucose uptake by muscle.

This Review will examine the molecular mechanisms that regulate glucose uptake from the blood into the muscle during exercise. To date, no studies have systematically and conclusively linked the signalling processes regulating glucose delivery, transport and metabolism together to fully understand the regulation of exercise-stimulated glucose uptake by skeletal muscle. However, some important signalling pathways have been elucidated. For example, roles have been proposed for the 5′-AMP-activated protein kinase (AMPK), the calcium/calmodulin-dependent kinases and several stretch-sensitive proteins in glucose uptake[3] and we will discuss the roles of these molecules and potential candidate molecules in the regulation of glucose uptake during exercise. Other aspects of muscle substrate metabolism during exercise have been reviewed elsewhere[4,5].

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Key points

- Exercise-stimulated signal transduction can restore glucose metabolism in insulin-resistant muscle through both acute activation of glucose transport and by improving insulin sensitivity for up to 48 hours after exercise
- Glucose is a major fuel source during exercise and glucose uptake by skeletal muscle can increase by up to 50-fold during bouts of exercise
- In excess of 1,000 phosphorylation sites in human skeletal muscle are regulated by exercise, which suggests that many regulators of muscle glucose uptake have yet to be discovered
- Regulation of exercise-stimulated glucose uptake by skeletal muscle requires three major steps (delivery, transport and intramyocellular metabolism), any of which could be rate-limiting during various exercise conditions
- Intensity and duration of exercise are key determinants of glucose uptake by skeletal muscle
- Exercise-stimulated glucose transport is regulated by two major pathways that sense either alterations in the intracellular metabolic milieu (probably mediated by AMPK) or mechanical stress (partly mediated by RAC1)

Exercise-stimulated glucose uptake

A historical perspective

During the 1960s–1980s, exercise-stimulated glucose uptake by muscle was quantified using various techniques in human participants[6]. This work led to the recognition that intensity and duration of exercise are key determinants of muscle glucose uptake. Subsequent studies that measured substrate turnover among endurance-trained cyclists across escalating intensities of exercise indicated that glucose utilization contributed 10–18% to whole-body energy turnover[7,8]. Blood glucose accounted for up to 40% of all oxidative metabolism that occurred during prolonged exercise once muscle glycogen stores had been depleted[9–11]. By contrast, glycogen represents the major fuel source during intense sessions of short-term exercise[12].

Treatment of insulin-resistant states

Exercise-stimulated glucose uptake by muscle occurs independently of insulin signal transduction[13–15]. This feature makes exercise an excellent nonpharmacological method by which to decrease hyperglycaemia in insulin-resistant states, including obesity and type 2 diabetes mellitus[16]. The effects of exercise on insulin action are summarized in BOX 1 and FIG. 1.

In addition to exerting acute effects on glucose uptake, exercise promotes a short-term increase in insulin sensitivity after cessation of physical exertion[17–19]. Such insulin sensitization can last for 48 h after a 60-min session of moderate ergometer cycling among healthy volunteers[20] and for ≥15 h among patients with type 2 diabetes mellitus[21]. Indeed, the evidence strongly suggests that regular exercise improves glycaemic control and insulin action among both obese patients and those with type 2 diabetes mellitus[22–25]. The observed effects of exercise can be superior to those exerted by the widely prescribed anti-diabetic agent metformin[26]. Consequently, increased understanding of the intricate and adaptable (yet tightly regulated) system that mediates uptake and disposal of glucose in skeletal muscle is of great pharmacological potential.

Regulation of glucose uptake by muscle

As noted earlier, glucose uptake into the muscle during exercise is governed by three tightly regulated processes: delivery, transport across the muscle-cell surface and intramyocellular metabolism. For an overview of the most important studies investigating delivery, transport and intramyocellular metabolism, please see TABLES 1–3 respectively. All three processes must be increased in a coordinated fashion for efficient glucose uptake to occur. With the techniques currently available, it can be difficult to determine which process is rate-limiting in any particular situation.

Glucose delivery

Increased glucose delivery to the working muscle during exercise occurs primarily through a rise in blood flow that is proportional to the level of exercise intensity. In a resting young adult, the mean blood flow in one leg is ~300–500 ml/min (~0.03–0.05 l/min/kg)[17]. During ergometer cycle exercise, blood flow increases to 5–6 l/min per leg at moderate exercise intensities[27] and by up to 9–10 l/min during intense exercise at 100% of maximal oxygen uptake (VO_2max)[28]. The changes in blood flow reflect a combination of increased cardiac output, local dilatation of resistance vessels in the active muscles and mechanical effects of muscle contraction on the veins to increase venous return[29].

Muscle blood flow is tightly coupled to the metabolic demands of contractions[30,31]. Given that glucose uptake in the muscle is also dependent on metabolic demands, coupling of blood flow and oxygen consumption ensures adequate delivery of glucose to the muscle during exercise. The relationship between muscle blood flow and oxygen use suggests that one or more signals from the contracting muscles are the main factors responsible for increased muscle perfusion and vasodilation in the contracting muscles. The hunt for these signals has not yet provided definitive answers and it is likely that some degree of redundancy exists among vasodilators[32]. These molecules can be released from contracting skeletal muscle, vascular endothelial cells, or red blood cells; potential candidates that might couple metabolism to vasodilation include nitric oxide, adenosine, ADP and ATP released during exercise[29,32]. The release of ATP from skeletal muscle could also increase glucose uptake by muscle[33]. Therefore, ATP might be an important coordinator of exercise-stimulated muscle blood flow and glucose uptake[29].

Capillary recruitment and microvascular delivery. The increase in bulk muscle blood flow observed during exercise is accompanied by a substantial elevation in capillary recruitment within the muscle that expands the surface area available for delivery of glucose and oxygen. Contrast-enhanced ultrasonographic imaging shows marked increases in both microvascular perfusion and blood volume (an index of muscle capillary recruitment) during exercise[34,35]. Increased muscle perfusion maintains interstitial glucose concentrations close to the venous plasma glucose concentration during exercise of increasing intensity[36]. This adaptation ensures

Box 1 | **Exercise as a nonpharmacological treatment for insulin resistance**

The phenomenon of exercise-induced improvement in insulin sensitivity is somewhat surprising because insulin and exercise use distinct signalling pathways to stimulate glucose uptake[45,201,202].

The mechanisms underlying the cross-talk between insulin and exercise have remained elusive; however, convergence points between insulin-stimulated and exercise-stimulated signalling have been discovered. These convergence points involve signalling molecules such as the GTPase-activating proteins TBC1 domain family member 4 (TBC1D4)[108,203] and RAC1 (REFS 78,79), which are responsive to both insulin and exercise. However, *Rac1* knockout mice can increase their insulin sensitivity post exercise, suggesting that RAC1 does not mediate this effect[204]. Conversely, TBC1D4's regulator, AMPK, was recently implicated in the insulin-sensitizing effects of exercise[205]. Exercise can counteract insulin resistance and improve hyperglycaemia in at least three ways:

- Stimulation of insulin-independent signalling pathways increases glucose uptake in muscle.
- Improved insulin-stimulated glucose uptake occurs in the hours following an acute period of exercise.
- Insulin sensitivity and responsiveness become elevated in the exercise-trained state.

Consequently, identifying proteins responsible for both the acute increase in insulin-independent glucose uptake and elevated insulin-stimulated glucose uptake following acute exercise as well as exercise training has important therapeutic implications for patients with type 2 diabetes mellitus or other diseases causing insulin resistance.

maintenance of the extracellular glucose concentration during exercise. Direct evidence for the importance of muscle perfusion during contractions was provided by studies of isolated perfused rat hindlimb[37–39]. In this model, increased glucose uptake was coupled to increasing levels of perfusion at a fixed rate of muscle contraction; however, reduced blood flow can apparently be compensated for by other mechanisms. Glucose uptake was greatly enhanced in mice with global deletion of endothelial nitric oxide synthase (eNOS), a model characterized phenotypically by decreased glucose delivery to working muscles owing to decreased nitric oxide production and blood flow[40]. Consequently, blood flow might not be the major mechanism regulating glucose delivery during exercise (TABLE 1).

Plasma glucose concentration. Delivery of glucose can also be regulated through changes in the plasma concentration of glucose. The concentration of plasma glucose eliciting half maximal glucose uptake (K_m) during exercise is ~10 mM in humans[41]. Therefore, changes in plasma glucose concentrations below the K_m value, typically around the physiological level of ~5 mM, translate almost linearly into changes in muscle glucose uptake. A practical example that takes advantage of this relationship is when plasma glucose levels are increased during exercise by ingesting glucose, leading to increased glucose utilization compared to when glucose concentrations are lower[42] (TABLE 1).

Glucose transport across the muscle membrane

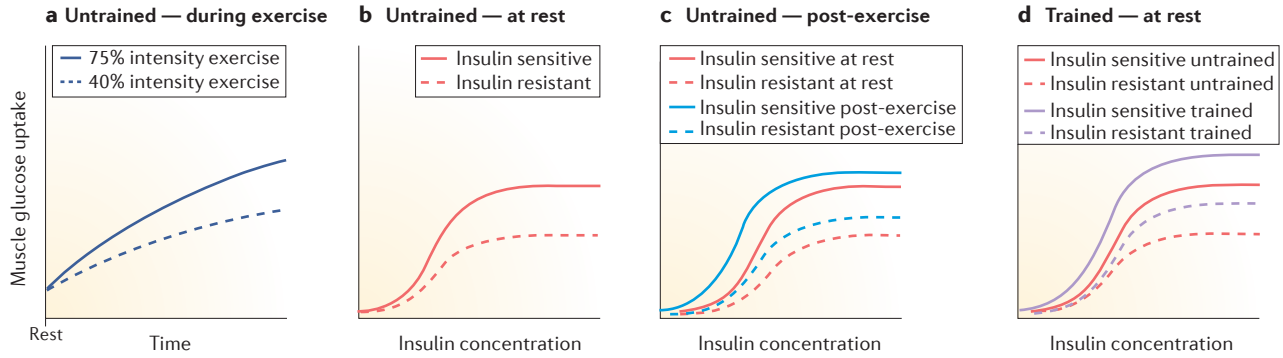
Translocation of GLUT4. Controlling permeability of the muscle surface membrane to glucose is another key component in the regulation of glucose uptake. Increased permeability during exercise depends on insertion of the highly conserved glucose transporter (GLUT4) into the plasma membrane and t-tubules[43] (FIG. 2). Evidence from studies in both rodent[44–48] and human[49–51] muscle indicates that muscle contraction promotes translocation of GLUT4 to the sarcolemma and t-tubules.

Studies strongly suggest that GLUT4 is essential for exercise-induced glucose uptake. Contraction-induced and exercise-induced glucose uptake was almost abolished in mouse muscles where expression of the gene encoding GLUT4 was ablated[52,53]. Despite an essential function for GLUT4, the signalling mechanisms and networks that regulate the mobility of this molecule during exercise remain to be fully elucidated (TABLE 2). Parallel studies of how insulin regulates GLUT4 translocation in adipocytes and muscle cell culture have revealed that the underlying mechanisms are amazingly complex[54]. This suggests that our current understanding of exercise-stimulated GLUT4 translocation is probably over-simplistic. Also, given that hundreds of proteins are acutely regulated during exercise[2], many of which are known to regulate intracellular vesicle movement, it is highly probable that some of these proteins participate in the regulation of GLUT4 translocation.

Despite this complexity, several mechanisms have already been identified and certain pathways excluded. In particular, exercise-stimulated glucose transport does not rely on the proximal part of the insulin signalling pathway, which comprises the insulin receptor, insulin receptor substrate and Akt[6,14,55]. Exercise-stimulated glucose uptake, therefore, remains intact in humans under states of insulin resistance[56], and constitutes an important alternative pathway to increase glucose uptake in muscle (BOX 1; FIG. 1).

Calcium sensitive signalling. Muscle contraction is initiated by an increased concentration of intracellular calcium (Ca^{2+}). This process rapidly activates signal transduction proteins with the ability to sense levels of Ca^{2+}, several of which are proposed to regulate contraction-stimulated glucose transport (for example, CaMKK, CaMKII and nPKC isoforms)[3,57]. Substantial alterations in the phosphorylation status of several Ca^{2+}-sensing proteins have been described in human muscle following exercise[2].

For many years, release of Ca^{2+} from the ryanodine receptors in the terminal cisternae of the sarcoplasmic reticulum during excitation–contraction coupling was believed to mediate glucose transport because increased intracellular Ca^{2+} concentration induced by caffeine was associated with increased glucose uptake in muscle[58–60]. However, complete dissociation between stimulation of glucose transport and a marker of ryanodine-receptor-mediated Ca^{2+} release (phosphorylation of Eef2 at Thr57) was observed in incubated mouse muscles[61]. In this study, pharmacologically and electrically stimulated contraction-induced glucose transport was strongly inhibited by pharmacological blockade of cross-bridge cycling despite normal levels of EEF2 Thr57 phosphorylation[61]. Furthermore, passive stretching combined with pharmacological activation of AMPK mimicked contraction-stimulated glucose transport without altering EEF2 Thr57 phosphorylation status[61]. Consequently, ryanodine-receptor-mediated



	a Untrained — during exercise	b Untrained — at rest	c Untrained — post-exercise	d Trained — at rest
Delivery	↑ Microvascular recruitment and blood flow	↓ Microvascular recruitment in insulin resistance	↑ Microvascular recruitment	↑ Capillarization
Transport	↑ GLUT4 translocation	↓ GLUT4 translocation by insulin in insulin resistance	↑ GLUT4 translocation by insulin	↑ GLUT4 expression and translocation
Metabolism	↑ Glycolysis ↑ Oxidative phosphorylation	↓ HK expression in insulin resistance	↑ Glycogen synthesis	↑ HK and mitochondrial expression

Figure 1 | **Exercise enhances insulin sensitivity. a** | Exercise exerts acute effects on glucose uptake in muscle. As exercise-stimulated glucose uptake occurs independently of the effects of insulin, muscle contraction is a potent stimulator of glucose uptake in both healthy and insulin-resistant muscle[51,206,207]. **b** | Diseases related to obesity, inactivity and age, such as type 2 diabetes mellitus[208], are strongly linked to the development of insulin resistance within the skeletal muscle. **c** | The sensitivity of muscle to insulin is increased for up to 48 h following a single bout of exercise[17,20]. **d** | Long-term exercise training also improves the effect of insulin on glucose uptake in muscle[24,209]. Exercise can, therefore, circumvent dysfunctional insulin signalling and offers a potent nonpharmacological tool to augment glucose uptake in insulin-resistant muscle to normalize glycaemic control[16] GLUT4, glucose transporter type 4; HK, hexokinase.

Ca^{2+} release from the sarcoplasmic reticulum is unlikely to be either necessary or sufficient to increase glucose transport in response to *ex vivo* muscle contraction.

By contrast, introduction of a CaMKII inhibitory peptide into mouse tibialis anterior muscles suggested that CaMKII might be necessary for contraction-stimulated glucose transport[62]. Autophoshorylation of CaMKII at Thr287, which is indicative of its activation by Ca^{2+}–calmodulin, was increased by mild mechanical stress *ex vivo*[60], a stimulus known to promote extracellular Ca^{2+} entry. Activation of CaMKII might, therefore, involve activation of cell-surface Ca^{2+} channels to increase glucose transport[63]. Furthermore, the stimulation of CaMKII and glucose transport during muscle contraction in isolated mouse gastrocnemius fibres was proposed to involve a complex signalling cascade that required entry of extracellular Ca^{2+} and release of intracellular Ca^{2+} by cyclic ADP ribose (cADPR) and nicotinic acid adenine nucleotide phosphate (NAADP) as second messengers[64]. These nucleotide second messengers are thought to cause ryanodine-receptor-dependent Ca^{2+} release from the sarcoplasmic reticulum and lysosomal Ca^{2+} release by the two pore channels and/or ryanodine receptor 1, respectively[65,66].

If the ryanodine receptor is indeed the source of Ca^{2+} during nucleotide stimulation, it raises the question as to how this process might differ from depolarization-induced Ca^{2+} release from junctional sarcoplasmic reticulum by the ryanodine receptor. One possibility is that ryanodine receptors residing outside the junctional sarcoplasmic reticulum have a distinct role in Ca^{2+} signalling, similar to the ryanodine receptor signalling functions found in many non-muscle cell types[67]. Activation of AMPK in rodent muscle does not activate CaMKII[59] nor increase intracellular Ca^{2+} concentration[68,69]. Nonetheless, stimulation of glucose transport by the mitochondrial respiratory chain inhibitor rotenone seems to require cADPR and/or NAADP[64] and AMPK[70]. This finding suggests that in addition to functioning as activators of CaMKII, increased cADPR and/or NAADP might also be a downstream requirement for AMPK-dependent glucose transport.

In summary, although depolarization-induced sarcoplasmic reticulum Ca^{2+} release in response to contraction does not seem to regulate glucose transport directly, there is some evidence to suggest that other Ca^{2+} sources, as well as Ca^{2+}-dependent signalling proteins, could contribute to regulation of glucose transport during contraction (FIG. 2).

Mechanical stress. Little consideration has been given to the contribution of mechanically transduced signals in the regulation of glucose uptake. However, it is becoming increasingly apparent that mechanical stress is a potent stimulus to increase glucose transport in muscle independent of insulin[61,71–74] (FIG. 2; TABLE 2).

Apart from being sufficient to stimulate glucose transport, signalling via mechanical stress is also necessary for optimal levels of contraction-induced glucose transport. *Ex vivo* contraction-stimulated glucose transport

Table 1 | **Role of signalling pathways in glucose delivery**

Parameter	Experimental model	Observations
Glucose delivery and flow	Rat hind limbs perfused with a varied flow in the presence of electrically stimulated contractions	When flow was increased, combined with constant contraction stimuli, disappearance of glucose increased by 2–4 fold[37–39]
	Whole-body eNos KO mouse	Exercise-stimulated glucose uptake increased 3-fold in VL and gastrocnemius muscle compared with WT mice[40]
Glucose concentration	Highly exercise-trained human participants ingested a high amount of carbohydrate every 15 min during a 2-h period of exercise	Glucose uptake was higher (96 g) when participants ingested carbohydrate than when they ingested water (60 g)[42]

KO, knockout, VL, vastus lateralis; WT, wild type.

was reduced when tension development was inhibited by either pharmacological or mechanical methods[61,74–76]. These findings strongly suggest that signal transduction in response to mechanical stress is required to elicit an optimal glucose transport response. One study reported that *N*-benzyl-*p*-toluene sulphonamide (an inhibitor of myosin–actin interaction and therefore contractile force) did not affect contraction-stimulated glucose uptake in mouse extensor digitorum longus (EDL) muscles[77]. However, this result was probably related to the intense *ex vivo* contraction regimen used in the study because the active-force production rapidly approaches zero during intense electrical stimulation[61,77], and so inhibiting the contraction machinery has little effect on mechanical stress.

The combination of passive stretch-stimulation with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR)-induced stimulation of AMPK promoted an additive effect on glucose transport during co-stimulation with insulin but not muscle contraction[61]. This observation suggests that passive stretch and AICAR recruit GLUT4 transporters from the same pool as contraction but from a different pool than recruited by insulin. Passive-stretch signalling probably works in parallel to AMPK, as passive stretch does not increase AMPK activity and muscle-specific overexpression of a dominant-negative kinase-dead form of AMPK does not inhibit stretch-stimulated glucose transport[61,71]. The well-documented link between mechanical stress and glucose transport in muscle raises the possibility that many stretch-activated pathways might be involved in regulation of exercise-stimulated glucose uptake. One potential candidate is the Rho family GTPase RAC1. This molecule is activated by passive stretch and is necessary for both insulin-stimulated and contraction-stimulated glucose transport in mice, but not AICAR-stimulated glucose transport[78–80]. The downstream target of RAC1, p21-activated kinase, (PAK), is also regulated by exercise[2], and PAK activation has been reported in contraction-stimulated rodent muscle[81]. Such findings suggest that this pathway is activated during exercise in both rodents and humans. Both pharmacological inhibition of RAC1 and knockout of the gene encoding this protein reduced stretch-induced glucose transport by 30–40%[74]. Inhibition of RAC1 reduced contraction-stimulated glucose transport in the muscles producing tension but not in muscles left loosely hanging or incubated with myosin heavy chain inhibitors to block tension development[74]. RAC1 is, therefore, likely to be a necessary component of the mechanical stress stimuli to contraction-stimulated glucose transport (FIG. 2). Mechanical stress can be sensed by several proteins located at the plasma membrane, including the dystrophin–glycoprotein complex, ion channels, integrins and focal adhesions, all of which have been reported to associate with and activate RAC1 in various cell types[82–84].

At least three mechanisms could link RAC1to GLUT4 translocation and glucose transport in response to mechanical stress during exercise. First, RAC1 regulates remodelling of the actin cytoskeleton. Pharmacological induction of actin depolymerization reduces glucose transport stimulated by passive stretch, contraction and insulin[74,79,85], However, investigating the impact of RAC1-deficiency on the cortical actin cytoskeleton consisting of β-actin and γ-actin in skeletal muscle is challenging, because these actin isoforms are expressed at low levels relative to contractile α-actin[86]. Further investigation is required to firmly link RAC1 to the actin cytoskeleton in mature muscle. Second, RAC1 might facilitate GLUT4 translocation in skeletal muscle via activation of another GTPase Ras-related protein (RALA), which is suggested to mediate RAC1-dependent GLUT4 translocation in response to insulin[87,88]. Whether RALA is activated by muscle contraction or passive-stretch stimulation remains to be investigated. Finally, RAC1 is both a subunit of the superoxide-producing NADPH oxidase 2 (NOX2) complex and required for its activation[89], which has been proposed to regulate muscle glucose transport[90].

Numerous other signalling proteins, including p38 MAPK, ERK and CaMKII, are activated by mechanical stress and remain candidates for regulation of glucose transport. Indeed, inhibition of RAC1 only partially blocks glucose transport stimulated by passive stretch[74], suggesting the existence of other stretch signals.

Metabolic stress and AMPK. The activity of AMPK in muscle increases substantially during contraction and exercise[91]; however, whether AMPK is necessary for the regulation of glucose transport remains unclear. Some, but not all, studies of *Ampk*-transgenic and -deficient mouse muscles have observed a reduction in contraction-stimulated glucose transport *ex vivo* or in exercise-stimulated glucose uptake *in vivo* (TABLE 2). The variable reduction in contraction-stimulated glucose transport observed in some *Ampk*-deficient and

Table 2 | **Role of signalling pathways in glucose transport across the sarcolemma and t-tubuli**

Parameter	Experimental model	Observations
Calmodulin	Calmodulin inhibitors (trifluoperazine or W-7) used in mouse	~50–60% inhibition of electrically-induced contraction-stimulated glucose transport in incubated soleus muscle[210]
CaMKII	CaMKII inhibitors (KN62 and KN93) used in rat	~50% reduction in electrically-induced glucose uptake in epitrochlearis muscle[59]
	KN62 and KN93 used in mouse	~50% decreased electrically-induced contraction-stimulated glucose uptake in incubated soleus and EDL muscles[211]
	Electroporation of a CaMKII inhibitor in mouse	~30% decreased *in situ* electrically-induced contraction-stimulated glucose uptake in TA muscle[62]
	Isolated single muscle fibres (mouse)	Ca^{2+} second messengers cADPR and NAADP required for glucose uptake in response to electrically-induced contraction via CaMKII[64]
LKB1	*Lkb1* KO mouse	• ~50–60% decreased electrically-induced contraction-stimulated glucose uptake *in situ* and *in vitro* in EDL, soleus and TA muscle[212,213] • No difference in glucose uptake induced by treadmill running in EDL and quadriceps muscles[214]
AMPK	Muscle α2 *Ampk* KD mouse	• ~50% and ~70% reduction in *ex vivo* and *in situ* electrically-induced contraction-stimulated glucose uptake, respectively[215] • ~50% reduced glucose transport in response to electrically-induced submaximal contraction in EDL[216] • No effect on glucose uptake induced by treadmill exercise in quadriceps and gastrocnemius muscle[217] • Treadmill-exercise-stimulated glucose clearance reduced by ~60% in gastrocnemius muscle[218] • *In situ* electrically-induced contraction-stimulated glucose uptake reduced by ~60% in gastrocnemius–soleus–plantaris muscle[96]
	Whole-body α1 or α2 *Ampk* KO mouse	No effect on electrically-induced contraction-stimulated glucose uptake in soleus and EDL muscle[219]
	Ampk γ3 KO mouse	No effect on electrically-induced contraction-stimulated glucose transport in EDL muscle[101]
	Muscle β1 and β2 *Ampk* KO mouse	~90% and ~50% reduced treadmill-exercise-stimulated glucose uptake in soleus and EDL, respectively[94]
	Muscle α1 and α2 *Ampk* KO mouse	• Normal levels of contraction-stimulated glucose transport, except from the soleus muscle of male mice (~40% reduction)[93] • Normal levels of glucose uptake during treadmill exercise, except from the soleus (~30% increase)[220]
PKC	Use of a PKC inhibitor (calphostin C) in rat	• ~50–75% reduced electrically-induced contraction-stimulated glucose uptake in fast-twitch muscles (but not slow-twitch muscles)[221] • ~50% reduced electrically-induced contraction-stimulated glucose transport in soleus muscle[210]
	Whole-body *Pkcα* KO mouse	No effect on electrically-induced contraction-stimulated glucose uptake in EDL muscle[222]
TBC1D4 and TBC1D1	Electroporation of non-phosphorylatable mutant *Tbc1d4* (4P) in mouse	~60–70% decreased electrically-induced contraction-stimulated glucose uptake in TA muscle[108]
	Electroporation of mutant *Tbc1d4* (protein product unable to bind calmodulin) in mouse	~40% reduction in electrically-induced contraction-stimulated glucose uptake in TA muscle[223]
	Electroporation of non-phosphorylatable *Tbc1d1* mutants (S231, T499, S621, S660 and S700) in mouse	~20–35% reduced electrically-induced contraction-stimulated glucose uptake in TA muscle[224,225]
	Tbc1d1 KO mouse	Treadmill-exercise-stimulated glucose uptake reduced by ~30% in white, but not red, quadriceps muscle, with ~40% reduced levels of GLUT4[111]
RAC1	Muscle-specific *Rac1* KO mouse	• ~20% and ~40% decreased electrically-induced contraction-stimulated glucose uptake in soleus and EDL, respectively[79] • ~50–100% reduced running-stimulated glucose uptake in skeletal muscle[80]
GLUT4	*Glut4* whole body null mouse	• Swimming-induced glucose uptake blunted in EDL muscle[53] • Electrically-induced contraction-stimulated glucose uptake decreased by ~80% in triceps muscle[52]
	Muscle-specific *Glut4* KO mouse	Decreased running-stimulated glucose uptake in soleus and gastrocnemius muscle (~90%)[226]
Mechanical stress	Loosely hanging muscles in mouse	Contraction-stimulated glucose transport reduced by ~20–40% in non-force producing muscles[74,75]
	Inhibition of cross-bridge formation using ATPase inhibitors (BTS and blebbistatin) in mouse	• Contraction-induced glucose transport reduced by ~20–50% in non-force producing muscles[61,74] • Contraction-stimulated (high intensity) glucose transport unaffected[77]

Table 2 (cont.) | **Role of signalling pathways in glucose transport across the sarcolemma and t-tubuli**

Parameter	Experimental model	Observations
Autophagy	*Bcl2*-AAA mutant, *Becn1*$^{+/-}$ and hypomorphic *Atg16l1* mouse (prevented autophagy activation)	Exercise-induced GLUT4 translocation and glucose transport impaired[119]
Nitric oxide	Pharmacological NOS inhibitors (L-NMMA and NAC) in mouse	• Basal glucose uptake reduced by ~30% and glucose uptake following *in situ* electrically-induced contraction almost completely abolished in EDL[131] • Contraction-stimulated glucose transport inhibited by ~30% in EDL and by ~15% in soleus (only affected by NAC in this muscle)[127]
	Use of L-NMMA in human	~30% reduced glucose uptake in response to bicycling exercise[133,134]
	Pharmacological NOS inhibitor (L-NAME) in rat	*In situ* electrically-induced contraction reduced by ~20% in lower leg muscles[132]
	nNos or *eNos* KO mouse	Normal levels of contraction-stimulated glucose transport but increased exercise-stimulated glucose transport[135,227]
Reactive oxygen species	Treatment of isolated mouse skeletal muscle with NAC (as an antioxidant)	Contraction-stimulated glucose transport reduced by ~50% in EDL[71,142]
Actin cytoskeleton	Depolymerization of actin in mouse by latrunculin B	Contraction-stimulated glucose transport reduced by ~20–30% in soleus and EDL[79]
SNARK	Overexpression of an inactive mutant SNARK in TA muscle and a whole-body *Snark* KO mouse	~20–30% reduction in electrically-induced *in situ* contraction-stimulated glucose uptake in TA[212]
Neuregulin	ErbB4 (downstream target of Neuregulin)-blocking antibody injected into rat muscle	Glucose uptake following *in situ* electrically-induced contraction almost abolished in soleus and reduced by ~20% in EDL[188]

BTS, *N*-benzyl-*p*-toluene sulfonamide; EDL, extensor digitorum longus; KD, kinase dead; KO, knockout; TA, tibialis anterior.

transgenic mouse models might be related to secondary effects on muscle mitochondrial function and muscle ultrastructure[92–94]. By contrast, compelling evidence suggests that isolated activation of the AMPK complex, which contains the α2 catalytic subunit in combination with the regulatory β2 and γ3 subunits, is both necessary and sufficient to increase glucose transport in response to various AMPK activators in resting mouse[3,5,6] and human[95] muscle.

A delay in reaching maximal glucose transport has been observed in *Ampk*-deficient mice. Mice overexpressing a kinase-dead inactive version of AMPK in muscle exhibited lowered contraction-stimulated glucose uptake at early time points (up to 15 min) but not at a later time point (20 min) when compared to wild-type mice[96]. Mechanistically, this delay might involve AMPK acting as a brake on endocytosis of GLUT4 vesicles, whereas contraction predominantly stimulates exocytosis of GLUT4, as shown in cardiomyocytes[97]. The AMPKα2β2γ3 complex is rapidly and potently activated by exercise in human skeletal muscle[98]. By contrast, the AMPKα2β2γ1 complex is activated only during prolonged exercise[99]. Given that activation of the AMPKα2β2γ3 complex seems to be sufficient to increase glucose transport[100,101], we propose that AMPK does in fact contribute to contraction-stimulated glucose transport in healthy muscle (FIG. 2).

Evidence from people with a naturally occurring activating mutation in the γ3 subunit (Arg225Trp) also supports a role for AMPK in glucose uptake. Affected individuals exhibit increased AMPK activity and an increase in skeletal muscle glycogen levels of ~90%[102]. These changes are accompanied by a doubling in basal glucose uptake in primary myotubes and a trend towards augmented exercise-stimulated glucose uptake compared to control individuals[103], indicating that regulation of glucose uptake by AMPK in human muscle is evolutionally conserved. However, human studies also suggest that expression of AMPK γ3 is decreased following exercise training[104]. Furthermore, the overall AMPK activity in muscle (primarily owing to AMPKα2β2γ3 activity)[105] is much less during exercise taken after a period of exercise training than during exercise taken before training, whereas the increase in glucose uptake observed during acute exercise was only marginally decreased in the trained state[106]. This finding is consistent with the notion that AMPK is not the sole regulator of glucose uptake in human skeletal muscle during exercise.

AMPK has also been linked to at least two vesicle trafficking control mechanisms; namely, regulation of Rab and generation of phosphatidylinositol 3,5-bisphosphate ($PtdIns(3,5)P_2$) in the control of GLUT4 translocation. The Rab GTPase-activating proteins TBC1D1 and TBC1D4 are thought to mediate the effects of AMPK on GLUT4 translocation and glucose transport[107,108]. In mice, TBC1D1 is highly expressed in muscles dominated by type II fibres, such as EDL, whereas TBC1D4 is enriched in muscles dominated by type I fibres, such as the soleus[109]. Knockout of *Tbc1d1*, but not *Tbc1d4*, abolished AICAR-stimulated glucose transport in incubated mouse EDL muscles, whereas knockout of *Tbc1d4*, but not *Tbc1d1*, prevented AICAR-stimulated glucose transport in soleus[110,111]. Glucose uptake stimulated by treadmill exercise was reduced in white, but not red, quadriceps muscle in the *Tbc1d1* knockout mice[111], suggesting a role for this molecule in type II fibres. However, the abundance of TBC1D4 is not tightly coupled to fibre type in all species, particularly in rat[112] and human[113]

Table 3 | **Role of signalling pathways in intracellular glucose metabolism**

Parameter	Experimental model	Observations
Hexokinase-2	Muscle-specific overexpression of *Hk2* in mouse	Increased exercise-induced glucose uptake in gastrocnemius (~30%) and in VL (~60%), but not in soleus and diaphragm[172,228,229]
	Partial *Hk2* KO mouse (heterozygous)	Exercise-stimulated glucose uptake reduced by 70% in soleus muscle, but not in gastrocnemius or VL[230]
Glycogen content	Perfused rat hind limb, pre-conditioned by exercise and diet, resulting in low or high levels of glycogen	Basal and contraction-stimulated glucose uptake and cell-surface labelling of GLUT4 reduced by 50% reduced in gastrocnemius and plantaris, but not soleus, in response to high levels of glycogen[158,159]
	Healthy human volunteers	GLUT4 translocation and glucose uptake highest late in a bicycle ergometer exercise (when glycogen levels are lowered)[50]
	Patients with McArdle disease (glycogen phosphorylase deficiency)	Higher exercise-induced glucose utilization than recorded among matched control individuals[231]
	Patients with gain-of-function R225W mutation AMPKγ3 (increased glycogen)	Increased basal (2-fold) glucose uptake in human primary myotubes and a trend towards increased exercise-stimulated glucose uptake in quadriceps muscle[102,103]

KO, knockout; VL, vastus lateralis

muscle. Studies in which TBC1D1 or TBC1D4 protein expression have been altered are difficult to interpret owing to the consistent finding of reduced GLUT4 expression in such knockout models[111,114]. Evidence from humans to support a role for TBC1D4 in the regulation of glucose uptake has come from the small and historically isolated founder population of Greenland. This population carries a high frequency of a dysfunctional muscle-specific *TBC1D4* splice variant[115], essentially providing a human muscle-specific knockout model for TBC1D4. Affected individuals display markedly reduced glucose uptake by muscle in response to insulin. This finding suggests that the studies of this protein conducted in rodents are relevant to humans, although the effect of the mutation has not yet been analysed with regards to exercise-stimulated glucose uptake.

At present, it is unclear which specific Rab proteins might be regulated by AMPK downstream of TBC1D1 and TBC1D4. RAB8A, RAB13 and RAB14 seem to act downstream of TBC1D4 in insulin-stimulated GLUT4 translocation in a rat myoblast cell line[116]. These molecules are, therefore, potential candidates for regulation by contraction. Production of PtdIns(3,5)P_2 by PIKFYVE, a FYVE finger-containing phosphoinositide kinase has been linked to insulin-stimulated GLUT4 translocation; AMPK can phosphorylate PIKFYVE and presumably regulate its intracellular localization but not its activity[117]. Inhibition and knockdown of PIKFYVE in a mouse myoblast cell line markedly reduced AICAR-stimulated glucose transport[117]. A muscle-specific knockout mouse lacking PIKFYVE protein displays reduced insulin-stimulated glucose transport in both soleus and EDL muscles[118]; however, AICAR-stimulated and contraction-stimulated glucose transport remain to be analyzed in this model.

Exercise-stimulated AMPK activation and glucose uptake was markedly reduced in mice with mutations of genes encoding autophagy proteins, specifically those expressing non-phosphorylatable *Bcl-2* mutants, heterozygous *Beclin1* and hypomorphic *Atg16l1* (REF. 119). The disturbed AMPK activation could reflect dependency on the lysosomal surface Ragulator-complex protein LAMTOR for exercise-stimulated AMPK activation[120]. However, a follow-up study using inducible muscle-specific *Atg7* knockout mice reported AMPK activation during exercise similar to controls[121]. The AMPK-phosphorylated autophagy-initiating kinases Unc-51-like (ULK) 1 and ULK 2 have several non-autophagy roles. Those roles include regulation of protein trafficking from the endoplasmic reticulum to the Golgi via phosphorylation of Sec16A[122]. This has been linked to GLUT4 translocation in an adipocyte cell line[123], and stimulation of glycolysis by phosphorylation of several glycolytic enzymes[124]. Whether autophagy-related signalling and membrane structures play a role in AMPK activation and regulation of glucose uptake remains unclear at present.

Interestingly, AMPK might play a more prominent role in the regulation of glucose uptake in the post-exercise period than during exercise. One study has implicated AMPK in the partitioning of carbohydrate and lipid via regulation of pyruvate dehydrogenase 1 (PDH 1) activity following exercise in mice[125].

Nitric oxide. Production of nitric oxide through the activity of NOS in rodent muscle is increased by exercise[126] and electrically-induced contraction[127,128]. Production is also increased by mechanical stretching in primary cultures of muscle cells[129]. In rodent muscle, nNOS is probably the main isoform activated by exercise or contraction as its expression in EDL muscle is threefold greater than that of the other muscle isoform (eNOS)[130]. Furthermore, nitric-oxide-dependent production of cGMP during contraction was abolished in the EDL muscle of a *Nos* (but not an *eNos*) knockout mouse[130]. A role for nitric oxide in muscle glucose uptake has been proposed because pharmacological inhibition of NOS activity with L-NMMA (N(G)-monomethyl-l-arginine) decreased glucose transport following rat muscle contraction *ex vivo*[131], electrically-induced

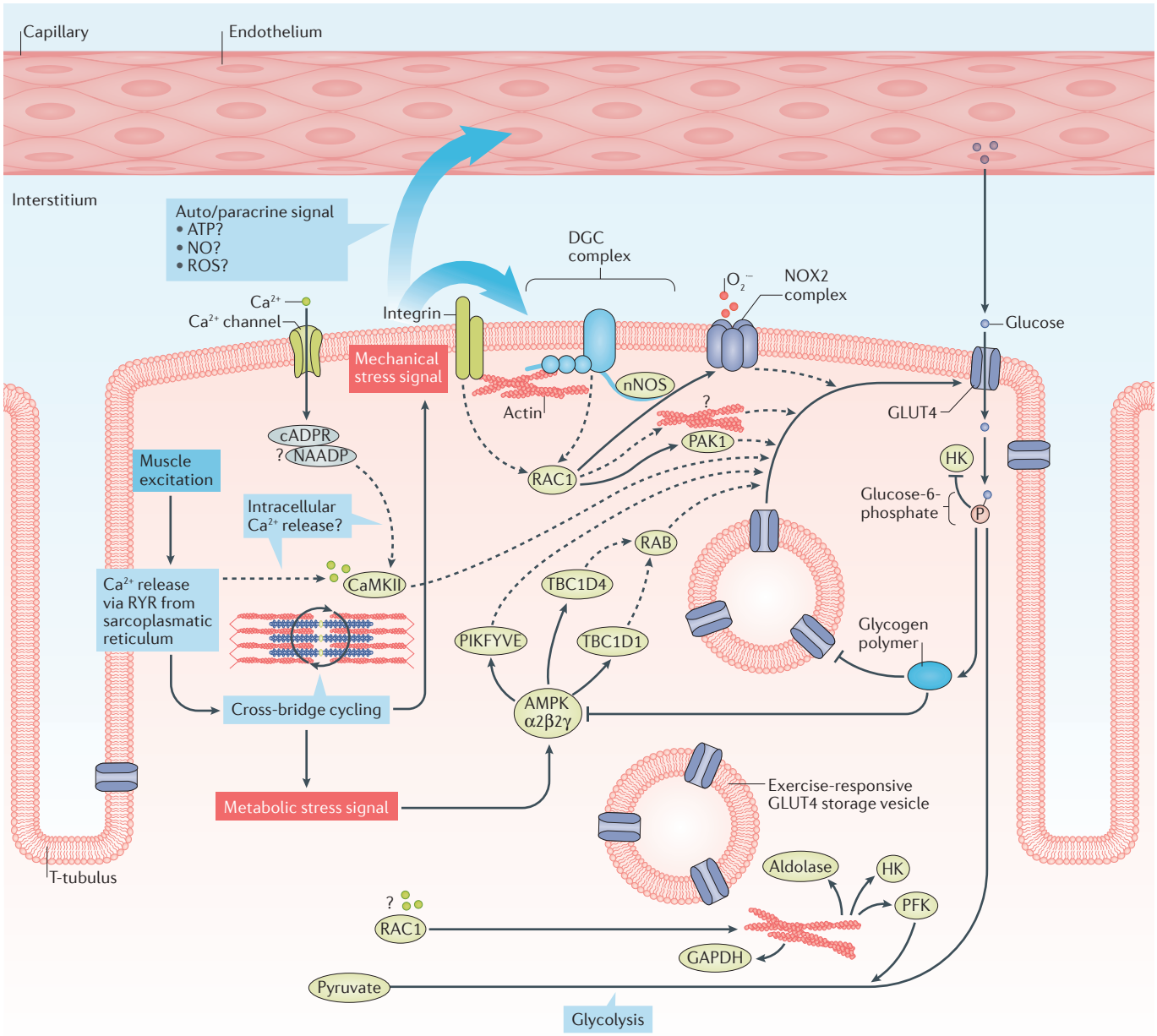


Figure 2 | **Molecular mechanisms of exercise-regulated glucose uptake by skeletal muscle.** In this working hypothesis, exercise promotes glucose uptake by increasing its delivery, transport and intracellular metabolism. Paracrine release of ATP, nitric oxide (NO) and reactive oxygen species (ROS) from working muscle might, in concert with extramuscular signals, increase microvascular blood flow during exercise to maintain a stable interstitial glucose concentration. Simultaneously, multiple intracellular signalling pathways become activated and promote glucose transporter type 4 (GLUT4) translocation to the cell surface, facilitating glucose entry into the muscle fibres. Muscle contraction occurs due to ATP-consuming cyclic attachment and de-attachment of myosin to actin filaments (cross-bridge cycling). ATP turnover increases the AMP concentration and so activates the 5′-AMP-activated protein kinase (AMPK) pathway. Contraction-induced mechanical stress activates p21-Rac1 (RAC1) through ill-defined mechanisms, possibly downstream of stretch sensitive molecules in the plasma membrane, such as integrins and the dystrophin glycoprotein complex (DGC). The role of different Ca^{2+} sources in contraction-stimulated glucose uptake is also unclear. Glucose within the muscle fibre is phosphorylated by hexokinase (HK) to glucose-6-phosphate, which either enters glycolysis or is incorporated into glycogen. Translocation of HK to mitochondria and release of glycolytic enzymes from F-actin increases their activity and might be regulated by Ca^{2+} and RAC1. Intracellular metabolism of glucose ensures the maintenance of a high extracellular-to-intracellular glucose diffusion gradient and low glucose-6-phosphate concentration to avoid allosteric inhibition of HK. CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cADPR, ADP-ribosyl cyclase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NAADP, nicotinic acid adenine dinucleotide phosphate; nNOS, nitric oxide synthase, brain; NOX2, NADPH oxidase 2; PAK1, serine/threonine-protein kinase PAK1; PFK, phosphofructokinase; PIKFYVE, 1-phosphatidylinositol 3-phosphate 5-kinase; RYR, ryanodine receptor; TBC1D1, TBC1 domain family member 1; TBC1D4, TBC1 domain family member 4.

contraction in mice *ex vivo*[127], *in situ* contraction in anaesthetized rats[132], and during bicycle exercise in humans[133,134]. By contrast, other studies found no effect of NOS inhibitors on glucose uptake in mouse, rat and human muscle[135–138].

The involvement of nitric oxide in the regulation of glucose transport has been challenged by studies of *nNos* knockout mice. These mice displayed similar contraction-stimulated glucose transport in EDL muscle *ex vivo*[139] and increased glucose uptake during treadmill exercise compared with wild-type mice. Importantly, the enzyme activity of NOS was completely abolished in EDL muscle of *nNos* knockout mice, indicating that other isoforms of NOS do not compensate to produce nitric oxide[139]. Furthermore, L-NMMA reduced contraction-stimulated glucose transport in the muscles of *nNos* KO mice, suggesting off-target effects of this inhibitor. The data evaluating the role of intramuscular nitric oxide as a regulator of glucose transport in muscle during exercise are conflicting. The possibility exists that *ex vivo* effects of NOS inhibitors in isolated, incubated contraction-stimulated muscle are due to off target effects.

Reactive oxygen species. Production of reactive oxygen species (ROS) is elevated by muscle contraction in isolated muscle[140–142] and following high-intensity and prolonged exercise[143–145]. Although the mitochondria constitute a major source of cellular ROS under basal conditions, membrane-localized superoxide production by NADPH oxidase-2 (NOX2) is a major source of ROS during muscle contraction[146,147] (FIG. 2). The activity of NOX2 increases in response to both muscle contraction and passive stretch in mouse muscle[148,149]. Given that both stretch-stimulated and contraction-stimulated glucose transport *ex vivo* is reduced in the presence of antioxidants, ROS production might be required for efficient regulation of glucose transport[71,142]. However, these *ex vivo* findings could not be recapitulated *in vivo* as infusion of the antioxidant *N*-acetyl-L-cysteine (NAC) did not affect glucose uptake during contractions of rat hind limb muscle *in situ*[150] or during exercise in humans[151]. This discrepancy might relate to increased electrical-stimulation intensity *ex vivo*, to suboptimal NAC concentrations *in vivo*, or it could reflect the fact that the rate-limiting step for glucose uptake measurements *in vivo* is different than that *ex vivo*.

AMPK and RAC1 exert opposing effects on the regulation of NOX2 activity[152,153]. RAC1 positively regulates NOX2 activity in cardiac muscle[153], whereas AMPK seems to inhibit NOX2 activity in various non-muscle cell types[152]. Nevertheless, AMPK and mechanical stress (probably mediated by RAC1) are additive regulators of contraction-stimulated glucose transport[61].

One study reported that NOX2 subunit expression and NOX2-dependent ROS production were increased by high-fat feeding in wild-type mice. In contrast, whole-body NOX2 knockout mice were protected from insulin resistance induced by a high-fat diet, as measured by Akt Ser473 phosphorylation in muscle[154]. These data suggest that NOX2 is a negative regulator of muscle glucose uptake. This apparent contradiction might relate to the dosage and duration of ROS stimulation, with glucose transport being stimulated by intermittent low-level ROS exposure (muscle contraction) but inhibited by chronic high-level exposure (for example, a high-fat diet).

Intracellular glucose metabolism

The intracellular metabolic state of the muscle is essential for maintaining the high glucose concentration gradient across the membrane that is required for facilitated diffusion of glucose into the working muscle.

Glycogen content. During exercise, muscle preferentially utilizes glycogen stores to provide glucose for ATP generation; therefore, the rate of muscle glucose uptake from the blood is inversely related to muscle glycogen content[155,156] (FIG. 3). During a prolonged session of exercise, glucose uptake becomes increasingly important[11,157]. Rat muscles with high initial glycogen levels display reduced contraction-induced GLUT4 translocation and glucose transport compared with those muscles characterized by low initial glycogen levels[158,159]. Similarly, translocation of GLUT4 in human muscle peaks late in exercise when the glycogen levels are low[50]. Glycogen content per se might diminish permeability of the muscle to glucose but this hypothesis is difficult to test directly because glycogen manipulation studies require confounding diet or exercise activity regimens. The findings of transgenic mouse studies in which glycogen content and glucose transport responsiveness are clearly dissociated suggest that the association is indirect[101,160,161]. However, these data are difficult to generalize to humans given that the glycogen concentration in mouse muscle is 10–20 times lower than that in human muscle.

High glycogen content correlates with low AMPK activity[155,162]. As AMPK activity also increases with time and/or intensity of exercise[163–165], AMPK might represent the mechanistic link between glycogen and translocation of GLUT4. Binding of AMPK to glycogen[166,167] could sequester this enzyme to prevent its full activation by upstream regulators, although this hypothesis requires further investigation. Indeed, the physiological relevance of AMPK binding to glycogen in skeletal muscle has been questioned[168]. Patients with McArdle disease have elevated glycogen levels owing to a loss-of-function mutation in the glycogen breakdown pathway. Affected individuals also exhibit increased exercise-induced blood glucose utilization and AMPK activity[162,169], suggesting that a high concentration of glycogen in the muscle is insufficient by itself to inhibit glucose uptake during exercise. However, absence of glycogen breakdown creates a severe energy crisis in the muscles of patients with McArdle disease, which leads to elevated AMPK activation (and probably other aspects of glucose uptake). Consequently, it is difficult to evaluate the role of glycogen levels per se on glucose uptake in these patients. Furthermore, as discussed above, AMPK might not participate in the regulation of glucose uptake during all exercise conditions.

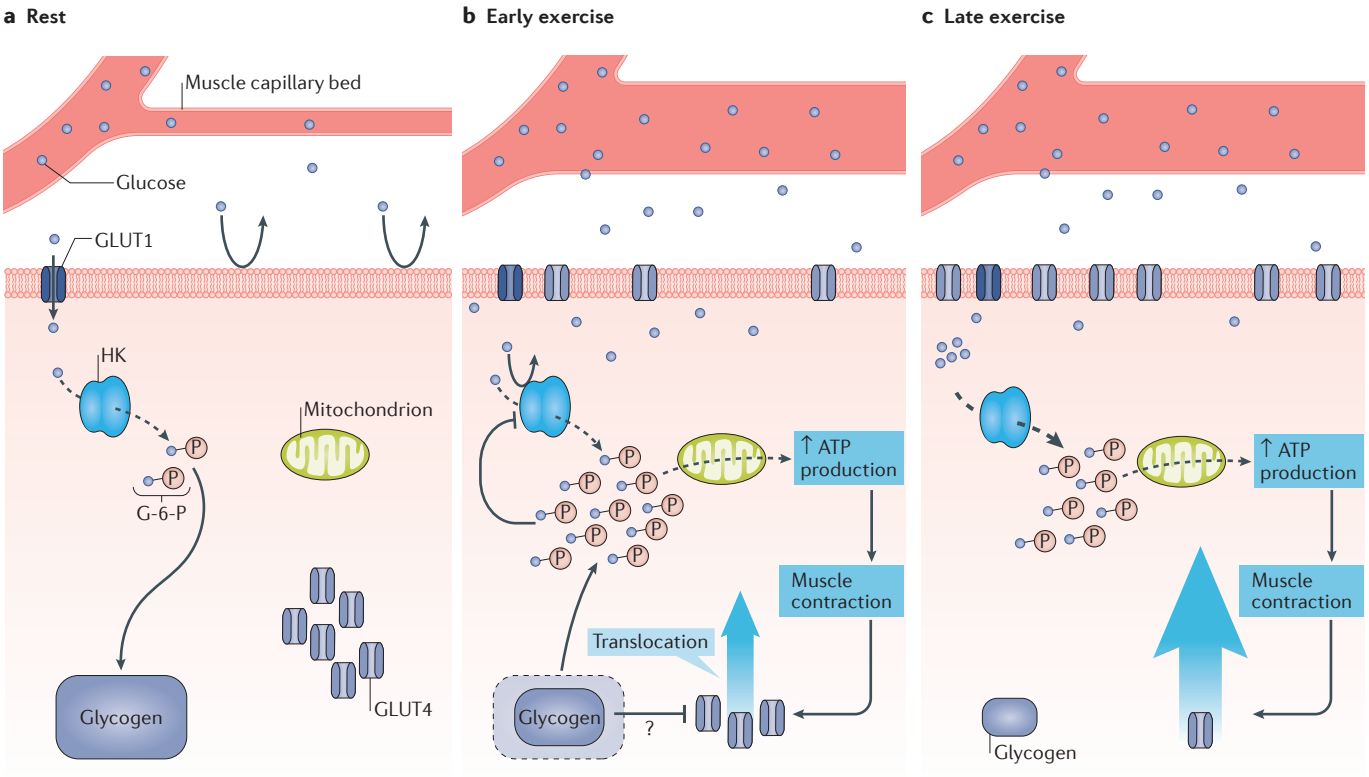


Figure 3 | **An integrated view of exercise-stimulated glucose uptake. a** | Requirements for glucose are minimal when muscle is at rest; therefore, glucose delivery is low. Glucose transport is also reduced owing to low permeability, a function controlled by the absolute number of glucose transporters in the plasma membrane. Any glucose that does enter the muscle is phosphorylated by hexokinase (HK), and then either stored as glycogen or metabolized to generate ATP via glycolysis and mitochondrial oxidative phosphorylation. **b** | At the onset of exercise, muscle preferentially uses glycogen to supply glucose for the generation of ATP to meet increased metabolic demands. This process leads to accumulation of glucose 6-phosphate (G-6-P), which blunts the activity of HK and so decreases glucose phosphorylation. Free glucose then accumulates in the muscle, which decreases the gradient required for glucose entry and thus overall transport rates. Glycogen might also inhibit translocation of glucose transporter type 4 (GLUT4) to the plasma membrane. Although both delivery and transport of glucose are increased, blood glucose is spared at the expense of the internal glucose stores. **c** | Glycogen is depleted during continued exercise at a rate dependent on exercise intensity. Inhibition of HK activity is alleviated and muscle permeability to glucose increased. The glucose supply gradually shifts from glycogen to blood glucose uptake to generate the required amount of ATP.

Intracellular phosphorylation of glucose. Glucose phosphorylation is another intramuscular metabolic mechanism that influences glucose uptake. Hexokinase phosphorylates glucose to yield glucose-6-phosphate. As muscle has negligible glucose-6-phosphatase activity, phosphorylation irreversibly traps glucose in the muscle. This process is vital to preserve the high-to-low free glucose gradient for facilitated diffusion across the muscle membrane.

Glucose-6-phosphate is an allosteric inhibitor of hexokinase. At the onset of exercise, particularly that of high intensity, increased rates of glycogenolysis yield high levels of glucose-6-phosphate, which in turn inhibit the activity of hexokinase. The glucose gradient becomes impaired as evidenced by elevated intramuscular levels of free glucose[50,157] (FIG. 3). When glycogen stores decrease during prolonged exercise, the intramuscular glucose-6-phosphate levels decrease, relieving the inhibition of hexokinase[50,157]. In concert with a concomitant increase in GLUT4 translocation to the sarcolemma and t-tubuli[50], this drop in intramuscular glucose-6-phosphate likely explains the increased rates of glucose uptake observed late in exercise. Hexokinase-2 has been proposed to bind directly to GLUT4, inhibiting intrinsic GLUT4 activity[170]. Late in exercise, it is possible that hexokinase dissociates from GLUT4, resulting in activation of both molecules, although this mechanism remains to be demonstrated.

Overexpression of hexokinase-2, but not GLUT4, in transgenic mouse models augments exercise-stimulated glucose uptake[171]. This finding indicates that hexokinase-mediated glucose phosphorylation is the rate-limiting step for glucose utilization during exercise in mice[172–174]. Whether this observation can be extrapolated to humans is uncertain; however, it is clear that intramuscular metabolic cues are important in the overall exercise-controlled glucose uptake system. These metabolic cues are highlighted in patients with markedly impaired muscle glycogenolysis (McArdle disease). Affected individuals exhibited lowered intracellular

glucose-6-phosphate levels and increased HK activity, which probably contributes to higher exercise-induced glucose utilization than is observed among matched controls[162,169], despite their high muscle glycogen levels.

Some studies have emphasized the importance of the subcellular location of hexokinase to its *in vivo* activity. Skeletal muscle expresses both the hexokinase-1 and hexokinase-2 isoforms[175]. Hexokinase-2 is the predominant isoform in rodent muscle[176]. By contrast, hexokinase-1 and hexokinase-2 protein content is equally distributed between human muscle fibre types[177,178]. Hexokinase-1 seems to be permanently bound to the mitochondria, which might efficiently couple cytosolic glycolysis with the mitochondrial tricarboxylic acid cycle[179]. The subcellular distribution of hexokinase-2 is complex. This isoform can bind to the mitochondria, where it also promotes glycolysis; however, when located in the cytosol it chiefly promotes glycogen synthesis[179]. Dissociation of hexokinase-2 from the mitochondria is facilitated by glucose-6-phosphate. AKT-mediated phosphorylation of hexokinase-2 reduces the sensitivity of this isoform to glucose-6-phosphate, which leads to increased mitochondrial binding of hexokinase-2[180]. Exercise-related kinases might also potentially signal to hexokinase-2, impacting its subcellular distribution to fine-tune enzyme activity in accord with exercise intensity and duration. However, this hypothesis remains to be tested.

Glycolysis. Patients lacking the glycolytic enzyme phosphofructokinase in muscle (Tarui disease) are exercise intolerant because muscle glycolysis, glycogenolysis and glucose uptake are impaired[169,181]. The regulation of glycolysis is highly complex as it comprises many steps and enzymes. Some data suggest that the glycolysis enzymes are interconnected with components of the GLUT4 translocation machinery. Hexokinase seems to be able to bind to GLUT4 directly, modulating the intrinsic activity of this glucose transporter[170]. Other enzymes of the glycolysis pathway (phosphofructokinase and aldolase) physically associate with the actin cytoskeleton[182,183]. Stimuli such as calcium or insulin cause dissociation of these enzymes from the actin cytoskeleton, which increases their enzymatic activity[182,183] and thereby increase glycolysis. In addition, aldolase has been proposed as a scaffolding protein, linking GLUT4 to the actin cytoskeleton to facilitate its translocation to the cell surface[184]. This association is noteworthy given the evidence discussed in the previous section that implicates RAC1 and the actin cytoskeleton as vital components in glucose uptake regulation via GLUT4. How and whether these findings are relevant for exercise-induced glucose uptake remains to be determined. Nevertheless, it is likely that GLUT4 translocation (FIG. 2) and muscle metabolism (for example, glycolysis; FIG. 3) are not two independent processes, but more likely two completely interlaced processes, during exercise (FIG. 3).

Autocrine and paracrine signals

The most important physiological stimuli of glucose transport in skeletal muscle are traditionally thought to be insulin and exercise but data are rapidly emerging on novel muscle-secreted autocrine and/or paracrine regulators of signalling that increase glucose transport[185]. These potential regulators include IL-13 (REF. 186), irisin[187], neuregulin[188] and urocortin-3 (REF. 189); however, they require further rigorous testing before they can be assigned a role in exercise metabolism.

The myokine IL-6 was suggested to be of importance for muscle glucose uptake during exercise owing to observations in humans where leg release of IL-6 correlated with AMPKα2 activity[190] and plasma IL-6 concentration positively correlated with leg glucose uptake during graded exercise[191]. Furthermore, IL-6 can activate AMPK and increase glucose transport in myotubes[192]. However, *Il6* knockout mice display normal levels (or only a minor reduction) of exercise-stimulated glucose uptake, substrate utilization and running capacity, indicating that IL-6 is not necessary for exercise-induced muscle glucose uptake in this model[193,194].

Electrical stimulation of isolated primary rat myotubes and flexor digitorum brevis (FDB) muscle fibres causes release of ATP and exogenous ATP stimulates glucose transport into mouse FDB muscle fibres[33,195]. ATP release in muscle has been suggested to be sensitive to mechanical stress[196]. Furthermore, ATP release increases in the muscle interstitium following human endurance exercise[197] to stimulate a diverse range of physiological responses in skeletal muscle, including blood flow and glucose delivery[198]. In relation to regulation of glucose transport across the muscle fibre surface membranes, ATP and insulin seem to stimulate this process to a similar extent in rat primary myotubes[33] but it is unclear whether these stimuli have an additive effect on glucose transport. It is, however, puzzling that ATP signalling to glucose transport in rat primary myotubes seems to be dependent on at least two molecules (PI3K and AKT) that are required for insulin, but not contraction-stimulated, glucose transport[15,61,199,200]. Establishing whether these stimuli are additive and consequently whether ATP stimulation mobilizes the contraction and/or insulin pool of GLUT4 vesicles to increase glucose transport is therefore important. ATP can also increase NOX2-dependent ROS production via P2Y1 purinergic receptors in electrically stimulated mouse flexor digitorum brevis single fibres[149]. Further studies are needed to clarify the importance of ATP and purinergic receptor signalling in contraction-stimulated glucose transport.

Conclusions

Proteomic analysis of phosphorylation sites has indicated that the molecular mechanisms underpinning exercise-stimulated glucose uptake are likely to be complex, with many factors still to be identified[2]. Incontrovertible evidence supports the highly conserved GLUT4 glucose transporter as an essential regulator of glucose transport across the muscle plasma membrane. However, no single molecular pathway has yet been found to account for all exercise-stimulated glucose transport into muscle. This fact likely reflects some degree of redundancy in the signalling pathways that mediate glucose uptake as evolutionary pressures would have promoted the development of

Box 2 | **Unresolved issues**

- How does the time and intensity of muscle contraction and exercise influence the relative contribution of metabolic and mechanical stress signals to increase the glucose-transport response *ex vivo* and *in vivo*?
- Does reorganization and/or redistribution of intracellular glucose metabolism proteins (for example, hexokinase and glycolytic enzymes) contribute to the regulation of glucose uptake *in vivo*?
- What factors, other than RAC1, contribute to glucose uptake activated by mechanical stress *ex vivo* and *in vivo*?
- What are the upstream regulators and downstream mediators of AMPK and RAC1? How do these regulatory molecules interact with both known and yet to be identified intramuscular and autocrine and/or paracrine regulators of glucose transport?
- How can the mechanisms underlying exercise-stimulated glucose uptake be exploited to treat insulin-resistant states, such as type 2 diabetes mellitus, and what are the optimal pharmacological targets?

parallel pathways to ensure the maintenance of muscle energy supply during physical activity. Metabolic and mechanical stress have been established as potentially key intracellular stimuli for glucose transport across the sarcolemma. Furthermore, major components of these stimuli have been identified, including AMPK acting downstream of the turnover of ATP, and RAC1 acting downstream of tension development.

Future studies should test the extent to which combined AMPK and RAC1 inhibition disrupts exercise and/or contraction-stimulated glucose uptake. In addition, the interplay between glucose delivery, transmembrane glucose transport and intramyocellular glucose metabolism remains to be fully elucidated (BOX 2). Clearly, many exciting discoveries lie ahead in this field and the development of novel techniques will advance our ability to look below the tip of the iceberg to understand the complexity of the signalling network regulating glucose uptake during exercise. Enhanced mechanistic understanding of how exercise regulates glucose uptake in muscle will provide a major step forward in harnessing the immense therapeutic potential of exercise as a lifestyle intervention for metabolic disease.

1. Wasserman, D. H. Four grams of glucose. *Am. J. Physiol. Endocrinol. Metab.* **296**, E11–E21 (2009).
2. Hoffman, N. J. *et al.* Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates. *Cell Metab.* **22**, 922–935 (2015).
3. Jensen, T. E., Angin, Y., Sylow, L. & Richter, E. A. Is contraction-stimulated glucose transport feedforward regulated by Ca^{2+}? *Exp. Physiol.* **99**, 1562–1568 (2014).
4. Jordy, A. B. & Kiens, B. Regulation of exercise-induced lipid metabolism in skeletal muscle. *Exp. Physiol.* **99**, 1586–1592 (2014).
5. Jensen, T. E. & Richter, E. A. Regulation of glucose and glycogen metabolism during and after exercise. *J. Physiol.* **590**, 1069–1076 (2012).
6. Richter, E. A. & Hargreaves, M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol. Rev.* **93**, 993–1017 (2013).
7. van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. & Wagenmakers, A. J. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J. Physiol.* **536** (Pt. 1), 295–304 (2001).
8. Romijn, J. A. *et al.* Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* **265**, E380–E391 (1993).
9. Ahlborg, G., Felig, P., Hagenfeldt, L., Hendler, R. & Wahren, J. Substrate turnover during prolonged exercise in man. Splanchinc and leg metabolism of glucose, free fatty acids and amino acids. *J. Clin. Invest.* **53**, 1080–1090 (1974).
10. Coyle, E. F. *et al.* Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. *J. Appl. Physiol.* **55**, 230–235 (1983).
11. Wahren, J., Felig, P., Ahlborg, G. & Jorfeldt, L. Glucose metabolism during leg exercise in man. *J. Clin. Invest.* **50**, 2715–2725 (1971).
12. Katz, A., Broberg, S., Sahlin, K. & Wahren, J. Leg glucose uptake during maximal dynamic exercise in humans. *Am. J. Physiol.* **251**, E65–E70 (1986).
13. Ploug, T., Galbo, H. & Richter, E. A. Increased muscle glucose uptake during contractions: no need for insulin. *Am. J. Physiol.* **247**, E726–E731 (1984).
14. Wojtaszewski, J. F. *et al.* Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. *J. Clin. Invest.* **104**, 1257–1264 (1999).
15. Sakamoto, K. *et al.* Role of Akt2 in contraction-stimulated cell signaling and glucose uptake in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **291**, E1031–E1037 (2006).
16. Minuk, H. L. *et al.* Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. *Am. J. Physiol.* **240**, E458–E464 (1981).
17. Richter, E. A., Mikines, K. J., Galbo, H. & Kiens, B. Effect of exercise on insulin action in human skeletal muscle. *J. Appl. Physiol.* **66**, 876–885 (1989).
18. Richter, E. A., Garetto, L. P., Goodman, M. N. & Ruderman, N. B. Muscle glucose metabolism following exercise in the rat. *J. Clin. Invest.* **69**, 785–793 (1982).
19. Bogardus, C. *et al.* Effect of muscle glycogen depletion on *in vivo* insulin action in man. *J. Clin. Invest.* **72**, 1605–1610 (1983).
20. Mikines, K., Sonne, B., Farrell, P., Tronier, B. & Galbo, H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am. J. Physiol.* **254**, E248–E259 (1988).
21. Devlin, J. & Horton, E. Effects of prior high-intensity exercise on glucose metabolism in normal and insulin-resistant men. *Diabetes* **34**, 973–979 (1985).
22. Boule, N. G., Haddad, E., Kenny, G. P., Wells, G. A. & Sigal, R. J. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA* **286**, 1218–1227 (2001).
23. Fatone, C. *et al.* Two weekly sessions of combined aerobic and resistance exercise are sufficient to provide beneficial effects in subjects with type 2 diabetes mellitus and metabolic syndrome. *J. Endocrinol. Invest.* **33**, 489–495 (2010).
24. Dela, F., Mikines, K. J., von Linstow, M., Secher, N. H. & Galbo, H. Effect of training on insulin-mediated glucose uptake in human muscle. *Am. J. Physiol.* **263**, E1134–E1143 (1992).
25. Dela, F. *et al.* Insulin-stimulated muscle glucose clearance in patients with NIDDM. Effects of one-legged physical training. *Diabetes* **44**, 1010–1020 (1995).
26. Knowler, W. C. *et al.* Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* **346**, 393–403 (2002).
27. Kjaer, M., Kiens, B., Hargreaves, M. & Richter, E. A. Influence of active muscle mass on glucose homeostasis during exercise in humans. *J. Appl. Physiol.* **71**, 552–557 (1991).
28. Calbet, J. A. *et al.* Central and peripheral hemodynamics in exercising humans: leg versus arm exercise. *Scand. J. Med. Sci. Sports* **25** (Suppl. 4), 144–157 (2015).
29. Joyner, M. J. & Casey, D. P. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol. Rev.* **95**, 549–601 (2015).
30. Mackie, B. G. & Terjung, R. L. Influence of training on blood flow to different skeletal muscle fiber types. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **55**, 1072–1078 (1983).
31. Laughlin, M. H. & Armstrong, R. B. Muscular blood flow distribution patterns as a function of running speed in rats. *Am. J. Physiol.* **243**, H296–H306 (1982).
32. Hellsten, Y., Nyberg, M., Jensen, L. G. & Mortensen, S. P. Vasodilator interactions in skeletal muscle blood flow regulation. *J. Physiol.* **590**, 6297–6305 (2012).
33. Osorio-Fuentealba, C. *et al.* Electrical stimuli release ATP to increase GLUT4 translocation and glucose uptake via PI3Kγ-Akt-AS160 in skeletal muscle cells. *Diabetes* **62**, 1519–1526 (2013).
34. Vincent, M. A. *et al.* Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am. J. Physiol. Endocrinol. Metab.* **290**, E1191–E1197 (2006).
35. Sjoberg, K. A., Rattigan, S., Hiscock, N., Richter, E. A. & Kiens, B. A new method to study changes in microvascular blood volume in muscle and adipose tissue: real-time imaging in humans and rat. *Am. J. Physiol. Heart Circ. Physiol.* **301**, H450–H458 (2011).
36. MacLean, D. A., Bangsbo, J. & Saltin, B. Muscle interstitial glucose and lactate levels during dynamic exercise in humans determined by microdialysis. *J. Appl. Physiol.* **87**, 1483–1490 (1999).
37. Hespel, P., Vergauwen, L., Vandenberghe, K. & Richter, E. A. Important role of insulin and flow in stimulating glucose uptake in contracting skeletal muscle. *Diabetes* **44**, 210–215 (1995).
38. Schultz, T. A. *et al.* Glucose delivery — a clarification of its role in regulating glucose uptake in rat skeletal muscle. *Life Sci.* **20**, 733–736 (1977).
39. Schultz, T. A., Lewis, S. B., Westbie, D. K., Wallin, J. D. & Gerich, J. E. Glucose delivery: a modulator of glucose uptake in contracting skeletal muscle. *Am. J. Physiol.* **233**, E514–E518 (1977).
40. Lee-Young, R. S. *et al.* Endothelial nitric oxide synthase is central to skeletal muscle metabolic regulation and enzymatic signaling during exercise *in vivo*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **298**, R1399–R1408 (2010).
41. Richter, E. A. in *Handbook of Physiology. Section 12: Exercise: Regulation and Integration of Multiple Systems* (eds Rowell, L. B. & Shepherd, J. T.) (Oxford Univ. Press, 1996).
42. McConell, G., Fabris, S., Proietto, J. & Hargreaves, M. Effect of carbohydrate ingestion on glucose kinetics during exercise. *J. Appl. Physiol.* **77**, 1537–1541 (1994).
43. Thorens, B. & Mueckler, M. Glucose transporters in the 21st Century. *Am. J. Physiol. Endocrinol. Metab.* **298**, E141–E145 (2010).
44. Lauritzen, H. P., Galbo, H., Toyoda, T. & Goodyear, L. J. Kinetics of contraction-induced GLUT4 translocation in skeletal muscle fibers from living mice. *Diabetes* **59**, 2134–2144 (2010).

45. Ploug, T., van Deurs, B., Ai, H., Cushman, S. W. & Ralston, E. Analysis of GLUT4 distribution in whole skeletal mu scle fibers: identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J. Cell Biol.* **142**, 1429–1446 (1998).
46. Lemieux, K., Han, X. X., Dombrowski, L., Bonen, A. & Marette, A. The transferrin receptor defines two distinct contraction-responsive GLUT4 vesicle populations in skeletal muscle. *Diabetes* **49**, 183–189 (2000).
47. Roy, D. & Marette, A. Exercise induces the translocation of GLUT4 to transverse tubules from an intracellular pool in rat skeletal muscle. *Biochem. Biophys. Res. Commun.* **223**, 147–152 (1996).
48. Douen, A. *et al.* Exercise induces recruitment of the "insulin-responsive glucose transporter". *J. Biol. Chem.* **265**, 13427–13430 (1990).
49. Kristiansen, S., Hargreaves, M. & Richter, E. A. Exercise-induced increase in glucose transport, GLUT4, and VAMP-2 in plasma membrane from human muscle. *Am. J. Physiol.* **270**, E197–E201 (1996).
50. Kristiansen, S., Hargreaves, M. & Richter, E. A. Progressive increase in glucose transport and GLUT-4 in human sarcolemmal vesicles during moderate exercise. *Am. J. Physiol.* **272**, E385–E389 (1997).
51. Kennedy, J. W. *et al.* Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes* **48**, 1192–1197 (1999).
52. Zisman, A. *et al.* Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat. Med.* **6**, 924–928 (2000).
53. Ryder, J. W. *et al.* Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT4-deficient mice. *FASEB J.* **13**, 2246–2256 (1999).
54. Klip, A., Sun, Y., Chiu, T. T. & Foley, K. P. Signal transduction meets vesicle traffic: the software and hardware of GLUT4 translocation. *Am. J. Physiol. Cell. Physiol.* **306**, C879–C886 (2014).
55. Cho, H. *et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). *Science* **292**, 1728–1731 (2001).
56. Martin, I. K., Katz, A. & Wahren, J. Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am. J. Physiol.* **269** (3 Pt. 1), E583–E590 (1995).
57. Rose, A. J., Kiens, B. & Richter, E. A. Ca^{2+}–calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. *J. Physiol.* **574** (Pt. 3), 889–903 (2006).
58. Youn, J. H., Gulve, E. A. & Holloszy, J. O. Calcium stimulates glucose transport in skeletal muscle by a pathway independent of contraction. *Am. J. Physiol.* **260**, C555–C561 (1991).
59. Wright, D. C., Hucker, K. A., Holloszy, J. O. & Han, D. H. Ca^{2+} and AMPK both mediate stimulation of glucose transport by muscle contractions. *Diabetes* **53**, 330–335 (2004).
60. Jensen, T. E., Rose, A. J., Hellsten, Y., Wojtaszewski, J. F. & Richter, E. A. Caffeine-induced Ca^{2+} release increases AMPK-dependent glucose uptake in rodent soleus muscle. *Am. J. Physiol. Endocrinol. Metab.* **293**, E286–E292 (2007).
61. Jensen, T. E. *et al.* Contraction-stimulated glucose transport in muscle is controlled by AMPK and mechanical stress but not sarcoplasmatic reticulum Ca^{2+} release. *Mol. Metab.* **3**, 742–753 (2014).
62. Witczak, C. A. *et al.* CaMKII regulates contraction- but not insulin-induced glucose uptake in mouse skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **298**, E1150–E1160 (2010).
63. Kappel, V. D., Zanatta, L., Postal, B. G. & Silva, F. R. Rutin potentiates calcium uptake via voltage-dependent calcium channel associated with stimulation of glucose uptake in skeletal muscle. *Arch. Biochem. Biophys.* **532**, 55–60 (2013).
64. Park, D. R., Park, K. H., Kim, B. J., Yoon, C. S. & Kim, U. H. Exercise ameliorates insulin resistance via Ca^{2+} signals distinct from those of insulin for GLUT4 translocation in skeletal muscles. *Diabetes* **64**, 1224–1234 (2015).
65. Lee, H. C. Cyclic ADP-ribose and NAADP: fraternal twin messengers for calcium signaling. *Sci. China Life Sci.* **54**, 699–711 (2011).
66. Guse, A. H. & Wolf, I. M. Ca^{2+} microdomains, NAADP and type 1 ryanodine receptor in cell activation. *Biochim. Biophys. Acta* **1863** (6 Pt B), 1379–1384 (2016).
67. Berridge, M. J., Lipp, P. & Bootman, M. D. The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **1**, 11–21 (2000).
68. Lanner, J. T. *et al.* AICAR prevents heat-induced sudden death in RyR1 mutant mice independent of AMPK activation. *Nat. Med.* **18**, 244–251 (2012).
69. Terada, S., Muraoka, I. & Tabata, I. Changes in $[Ca^{2+}]_i$ induced by several glucose transport-enhancing stimuli in rat epitrochlearis muscle. *J. Appl. Physiol.* **94**, 1813–1820 (2003).
70. Fujii, N. *et al.* AMP-activated protein kinase α2 activity is not essential for contraction- and hyperosmolarity-induced glucose transport in skeletal muscle. *J. Biol. Chem.* **280**, 39033–39041 (2005).
71. Chambers, M. A., Moylan, J. S., Smith, J. D., Goodyear, L. J. & Reid, M. B. Stretch-stimulated glucose uptake in skeletal muscle is mediated by reactive oxygen species and p38 MAP-kinase. *J. Physiol.* **587**, 3363–3373 (2009).
72. Shoji, S. Effects of stretch and starvation on glucose uptake of rat soleus and extensor digitorum longus muscles. *Muscle Nerve* **9**, 144–147 (1986).
73. Sakamoto, K., Aschenbach, W. G., Hirshman, M. F. & Goodyear, L. J. Akt signaling in skeletal muscle: regulation by exercise and passive stretch. *Am. J. Physiol. Endocrinol. Metab.* **285**, E1081–E1088 (2003).
74. Sylow, L., Moller, L. L., Kleinert, M., Richter, E. A. & Jensen, T. E. Stretch-stimulated glucose transport in skeletal muscle is regulated by Rac1. *J. Physiol.* **593**, 645–656 (2015).
75. Ihlemann, J., Ploug, T., Hellsten, Y. & Galbo, H. Effect of tension on contraction-induced glucose transport in rat skeletal muscle. *Am. J. Physiol.* **277**, E208–E214 (1999).
76. Blair, D. R., Funai, K., Schweitzer, G. G. & Cartee, G. D. A myosin II ATPase inhibitor reduces force production, glucose transport, and phosphorylation of AMPK and TBC1D1 in electrically stimulated rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **296**, E993–E1002 (2009).
77. Sandstrom, M. E., Zhang, S. J., Westerblad, H. & Katz, A. Mechanical load plays little role in contraction-mediated glucose transport in mouse skeletal muscle. *J. Physiol.* **579**, 527–534 (2007).
78. Sylow, L. *et al.* Rac1 signaling is required for insulin-stimulated glucose uptake and is dysregulated in insulin-resistant murine and human skeletal muscle. *Diabetes* **62**, 1865–1875 (2013).
79. Sylow, L. *et al.* Rac1 is a novel regulator of contraction-stimulated glucose uptake in skeletal muscle. *Diabetes* **62**, 1139–1151 (2013).
80. Sylow, L. *et al.* Rac1 governs exercise-stimulated glucose uptake in skeletal muscle through regulation of GLUT4 translocation in mice. *J. Physiol.* **594**, 4997–5008 (2016).
81. Zhou, Y., Jiang, D., Thomason, D. B. & Jarrett, H. W. Laminin-induced activation of Rac1 and JNKp46 is initiated by Src family kinases and mimics the effects of skeletal muscle contraction. *Biochemistry* **46**, 14907–14916 (2007).
82. Oak, S. A., Zhou, Y. W. & Jarrett, H. W. Skeletal muscle signaling pathway through the dystrophin glycoprotein complex and Rac1. *J. Biol. Chem.* **278**, 39287–39295 (2003).
83. Kitajima, N. *et al.* TRPC3-mediated Ca^{2+} influx contributes to Rac1-mediated production of reactive oxygen species in MLP-deficient mouse hearts. *Biochem. Biophys. Res. Commun.* **409**, 108–113 (2011).
84. Huveneers, S. & Danen, E. H. Adhesion signaling — crosstalk between integrins, Src and Rho. *J. Cell Sci.* **122**, 1059–1069 (2009).
85. Brozinick, J. T. Jr, Hawkins, E. D., Strawbridge, A. B. & Elmendorf, J. S. Disruption of cortical actin in skeletal muscle demonstrates an essential role of the cytoskeleton in glucose transporter 4 translocation in insulin-sensitive tissues. *J. Biol. Chem.* **279**, 40699–40706 (2004).
86. Tondeleir, D., Vandamme, D., Vandekerckhove, J., Ampe, C. & Lambrechts, A. Actin isoform expression patterns during mammalian development and in pathology: insights from mouse models. *Cell. Motil. Cytoskeleton* **66**, 798–815 (2009).
87. Nozaki, S., Ueda, S., Takenaka, N., Kataoka, T. & Satoh, T. Role of RalA downstream of Rac1 in insulin-dependent glucose uptake in muscle cells. *Cell. Signal.* **24**, 2111–2117 (2012).
88. Takenaka, N. *et al.* Role for RalA downstream of Rac1 in skeletal muscle insulin signalling. *Biochem. J.* **469**, 445–454 (2015).
89. Panday, A., Sahoo, M. K., Osorio, D. & Batra, S. NADPH oxidases: an overview from structure to innate immunity-associated pathologies. *Cell. Mol. Immunol.* **12**, 5–23 (2015).
90. Espinosa, A., Henriquez-Olguin, C. & Jaimovich, E. Reactive oxygen species and calcium signals in skeletal muscle: a crosstalk involved in both normal signaling and disease. *Cell Calcium* **60**, 172–179 (2016).
91. Richter, E. A. & Ruderman, N. B. AMPK and the biochemistry of exercise: implications for human health and disease. *Biochem. J.* **418**, 261–275 (2009).
92. Thomas, M. M. *et al.* Muscle-specific AMPK β1β2-null mice display a myopathy due to loss of capillary density in nonpostural muscles. *FASEB J.* **28**, 2098–2107 (2014).
93. Lantier, L. *et al.* AMPK controls exercise endurance, mitochondrial oxidative capacity, and skeletal muscle integrity. *FASEB J.* **28**, 3211–3224 (2014).
94. O'Neill, H. M. *et al.* AMP-activated protein kinase (AMPK) β1β2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proc. Natl Acad. Sci. USA* **108**, 16092–16097 (2011).
95. Koistinen, H. A. *et al.* 5-Amino-imidazole carboxamide riboside increases glucose transport and cell-surface GLUT4 content in skeletal muscle from subjects with type 2 diabetes. *Diabetes* **52**, 1066–1072 (2003).
96. Abbott, M. J., Bogachus, L. D. & Turcotte, L. P. AMPKα2 deficiency uncovers time-dependency in the regulation of contraction-induced palmitate and glucose uptake in mouse muscle. *J. Appl. Physiol.* **111**, 125–134 (2011).
97. Yang, J. & Holman, G. D. 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.* **280**, 4070–4078 (2005).
98. Birk, J. B. & Wojtaszewski, J. F. Predominant α2/β2/γ3 AMPK activation during exercise in human skeletal muscle. *J. Physiol.* **577**, 1021–1032 (2006).
99. Treebak, J. T. *et al.* AS160 phosphorylation is associated with activation of α2β2γ1- but not α2β2γ3-AMPK trimeric complex in skeletal muscle during exercise in humans. *Am. J. Physiol. Endocrinol. Metab.* **292**, E715–E722 (2007).
100. Jørgensen, S. B. *et al.* Knockout of the α2 but not α1 5′-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1-β-4-ribifuranoside but not contraction-induced glucose uptake in skeletal muscle. *J. Biol. Chem.* **279**, 1070–1079 (2004).
101. Barnes, B. R. *et al.* The 5′-AMP-activated protein kinase γ3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. *J. Biol. Chem.* **279**, 38441–38447 (2004).
102. Costford, S. R. *et al.* Gain-of-function R225W mutation in human AMPKγ_3 causing increased glycogen and decreased triglyceride in skeletal muscle. *PLoS ONE* **2**, e903 (2007).
103. Crawford, S. A. *et al.* Naturally occurring R225W mutation of the gene encoding AMP-activated protein kinase (AMPKγ_3) results in increased oxidative capacity and glucose uptake in human primary myotubes. *Diabetologia* **53**, 1986–1997 (2010).
104. Frosig, C., Jorgensen, S. B., Hardie, D. G., Richter, E. A. & Wojtaszewski, J. F. 5′-AMP-activated protein kinase activity and protein expression are regulated by endurance training in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **286**, E411–E417 (2004).
105. Mortensen, B. *et al.* Effect of birth weight and 12 weeks of exercise training on exercise-induced AMPK signaling in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **304**, E1379–E1390 (2013).
106. McConell, G. K. *et al.* Short-term exercise training in humans reduces AMPK signalling during prolonged exercise independent of muscle glycogen. *J. Physiol.* **568**, 665–676 (2005).
107. Taylor, E. B. *et al.* Discovery of TBC1D1 as an insulin-, AICAR-, and contraction-stimulated signaling nexus in mouse skeletal muscle. *J. Biol. Chem.* **283**, 9787–9796 (2008).
108. Kramer, H. F. *et al.* AS160 regulates insulin- and contraction-stimulated glucose uptake in mouse skeletal muscle. *J. Biol. Chem.* **281**, 31478–31485 (2006).

109. Pehmoller, C. *et al.* Genetic disruption of AMPK signaling abolishes both contraction- and insulin-stimulated TBC1D1 phosphorylation and 14-3-3 binding in mouse skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **297**, E665–E675 (2009).
110. Chadt, A. *et al.* Deletion of both Rab-GTPase-activating proteins TBC14KO and TBC1D4 in mice eliminates insulin- and AICAR-stimulated glucose transport. *Diabetes* **64**, 746–775 (2015).
111. Stockli, J. *et al.* The RabGAP TBC1D1 plays a central role in exercise-regulated glucose metabolism in skeletal muscle. *Diabetes* **64**, 1914–1922 (2015).
112. Castorena, C. M., Mackrell, J. G., Bogan, J. S., Kanzaki, M. & Cartee, G. D. Clustering of GLUT4, TUG, and RUVBL2 protein levels correlate with myosin heavy chain isoform pattern in skeletal muscles, but AS160 and TBC1D1 levels do not. *J. Appl. Physiol.* **111**, 1106–1117 (2011).
113. Kristensen, D. E. *et al.* Human muscle fibre type-specific regulation of AMPK and downstream targets by exercise. *J. Physiol.* **593**, 2053–2069 (2015).
114. Szekeres, F. *et al.* The Rab-GTPase-activating protein TBC1D1 regulates skeletal muscle glucose metabolism. *Am. J. Physiol. Endocrinol. Metab.* **303**, E524–E533 (2012).
115. Moltke, I. *et al.* A common Greenlandic TBC1D4 variant confers muscle insulin resistance and type 2 diabetes. *Nature* **512**, 190–193 (2014).
116. Sun, Y., Bilan, P. J., Liu, Z. & Klip, A. Rab8A and Rab13 are activated by insulin and regulate GLUT4 translocation in muscle cells. *Proc. Natl Acad. Sci. USA* **107**, 19909–19914 (2010).
117. Liu, Y. *et al.* Phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) is an AMPK target participating in contraction-stimulated glucose uptake in skeletal muscle. *Biochem. J.* **455**, 195–206 (2013).
118. Ikonomov, O. C. *et al.* Muscle-specific *Pikfyve* gene disruption causes glucose intolerance, insulin resistance, adiposity, and hyperinsulinemia but not muscle fiber-type switching. *Am. J. Physiol. Endocrinol. Metab.* **305**, E119–E131 (2013).
119. He, C. *et al.* Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature* **481**, 511–515 (2012).
120. Zhang, C. S. *et al.* The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism. *Cell Metab.* **20**, 526–540 (2014).
121. Lo, V. F., Carnio, S., Vainshtein, A. & Sandri, M. Autophagy is not required to sustain exercise and PRKAA1/AMPK activity but is important to prevent mitochondrial damage during physical activity. *Autophagy* **10**, 1883–1894 (2014).
122. Joo, J. H. *et al.* The noncanonical role of ULK/ATG1 in ER-to-Golgi trafficking is essential for cellular homeostasis. *Mol. Cell* **62**, 491–506 (2016).
123. Bruno, J., Brumfield, A., Chaudhary, N., Iaea, D. & McGraw, T. E. SEC16A is a RAB10 effector required for insulin-stimulated GLUT4 trafficking in adipocytes. *J. Cell Biol.* **214**, 61–76 (2016).
124. Li, T. Y. *et al.* ULK1/2 constitute a bifurcate node controlling glucose metabolic fluxes in addition to autophagy. *Mol. Cell* **62**, 359–370 (2016).
125. Fritzen, A. M. *et al.* 5′-AMP activated protein kinase α2 controls substrate metabolism during post-exercise recovery via regulation of pyruvate dehydrogenase kinase 4. *J. Physiol.* **593**, 4765–4780 (2015).
126. Roberts, C. K., Barnard, R. J., Jasman, A. & Balon, T. W. Acute exercise increases nitric oxide synthase activity in skeletal muscle. *Am. J. Physiol.* **277** (2 Pt. 1), E390–E394 (1999).
127. Merry, T. L., Steinberg, G. R., Lynch, G. S. & McConell, G. K. Skeletal muscle glucose uptake during contraction is regulated by nitric oxide and ROS independently of AMPK. *Am. J. Physiol. Endocrinol. Metab.* **298**, E577–E585 (2010).
128. Pye, D., Palomero, J., Kabayo, T. & Jackson, M. J. Real-time measurement of nitric oxide in single mature mouse skeletal muscle fibres during contractions. *J. Physiol.* **581** (Pt. 1), 309–318 (2007).
129. Wozniak, A. C. & Anderson, J. E. The dynamics of the nitric oxide release-transient from stretched muscle cells. *Int. J. Biochem. Cell Biol.* **41**, 625–631 (2009).
130. Lau, K. S. *et al.* nNOS and eNOS modulate cGMP formation and vascular response in contracting fast-twitch skeletal muscle. *Physiol. Genom.* **2**, 21–27 (2000).
131. Balon, T. W. & Nadler, J. L. Evidence that nitric oxide increases glucose transport in skeletal muscle. *J. Appl. Physiol.* **82**, 359–363 (1997).
132. Ross, R. M., Wadley, G. D., Clark, M. G., Rattigan, S. & McConell, G. K. Local nitric oxide synthase inhibition reduces skeletal muscle glucose uptake but not capillary blood flow during *in situ* muscle contraction in rats. *Diabetes* **56**, 2885–2892 (2007).
133. Kingwell, B. A., Formosa, M., Muhlmann, M., Bradley, S. J. & McConell, G. K. Nitric oxide synthase inhibition reduces glucose uptake during exercise in individuals with type 2 diabetes more than in control subjects. *Diabetes* **51**, 2572–2580 (2002).
134. Bradley, S. J., Kingwell, B. A. & McConell, G. K. Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans. *Diabetes* **48**, 1815–1821 (1999).
135. Hong, Y. H. *et al.* No effect of NOS inhibition on skeletal muscle glucose uptake during *in situ* hindlimb contraction in healthy and diabetic Sprague-Dawley rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **308**, R862–R871 (2015).
136. Etgen, G. J. Jr, Fryburg, D. A. & Gibbs, E. M. Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent pathway. *Diabetes* **46**, 1915–1919 (1997).
137. Higaki, Y., Hirshman, M. F., Fujii, N. & Goodyear, L. J. Nitric oxide increases glucose uptake through a mechanism that is distinct from the insulin and contraction pathways in rat skeletal muscle. *Diabetes* **50**, 241–247 (2001).
138. Heinonen, I. *et al.* Effect of nitric oxide synthase inhibition on the exchange of glucose and fatty acids in human skeletal muscle. *Nutr. Metab. (Lond.)* **10**, 43 (2013).
139. Hong, Y. H. *et al.* Glucose uptake during contraction in isolated skeletal muscles from neuronal nitric oxide synthase μ knockout mice. *J. Appl. Physiol.* **118**, 1113–1121 (2015).
140. Pattwell, D. M., McArdle, A., Morgan, J. E., Patridge, T. A. & Jackson, M. J. Release of reactive oxygen and nitrogen species from contracting skeletal muscle cells. *Free Radic. Biol. Med.* **37**, 1064–1072 (2004).
141. Reid, M. B. *et al.* Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue *in vitro*. *J. Appl. Physiol.* **73**, 1797–1804 (1992).
142. Sandstrom, M. E. *et al.* Role of reactive oxygen species in contraction-mediated glucose transport in mouse skeletal muscle. *J. Physiol.* **575**, 251–262 (2006).
143. Sen, C. K., Rankinen, T., Vaisanen, S. & Rauramaa, R. Oxidative stress after human exercise: effect of *N*-acetylcysteine supplementation. *J. Appl. Physiol.* **76**, 2570–2577 (1994).
144. Gomez-Cabrera, M. C. *et al.* Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J. Physiol.* **567**, 113–120 (2005).
145. Svensson, M. B. *et al.* Adaptive stress response of glutathione and uric acid metabolism in man following controlled exercise and diet. *Acta Physiol. Scand.* **176**, 43–56 (2002).
146. Jackson, M. J. Redox regulation of muscle adaptations to contractile activity and aging. *J. Appl. Physiol.* **119**, 163–171 (2015).
147. Ward, C. W., Prosser, B. L. & Lederer, W. J. Mechanical stretch-induced activation of ROS/RNS signaling in striated muscle. *Antioxid. Redox Signal.* **20**, 929–936 (2014).
148. Pal, R., Basu, T. P., Li, S., Minard, C. & Rodney, G. G. Real-time imaging of NADPH oxidase activity in living cells using a novel fluorescent protein reporter. *PLoS ONE* **8**, e63989 (2013).
149. Diaz-Vegas, A. *et al.* ROS production via P2Y1-PKC-NOX2 is triggered by extracellular ATP after electrical stimulation of skeletal muscle cells. *PLoS ONE* **10**, e0129882 (2015).
150. Merry, T. L., Dywer, R. M., Bradley, E. A., Rattigan, S. & McConell, G. K. Local hindlimb antioxidant infusion does not affect muscle glucose uptake during *in situ* contractions in rat. *J. Appl. Physiol.* **108**, 1275–1283 (2010).
151. Merry, T. L. *et al. N*-Acetylcysteine infusion does not affect glucose disposal during prolonged moderate-intensity exercise in humans. *J. Physiol.* **588**, 1623–1634 (2010).
152. Song, P. & Zou, M. H. Regulation of NAD(P)H oxidases by AMPK in cardiovascular systems. *Free Radic. Biol. Med.* **52**, 1607–1619 (2012).
153. Hordijk, P. L. Regulation of NADPH oxidases: the role of Rac proteins. *Circ. Res.* **98**, 453–462 (2006).
154. Souto Padron de, F. A. *et al.* Nox2 mediates skeletal muscle insulin resistance induced by a high fat diet. *J. Biol. Chem.* **290**, 13427–13439 (2015).
155. Wojtaszewski, J. F., Jorgensen, S. B., Hellsten, Y., Hardie, D. G. & Richter, E. A. Glycogen-dependent effects of 5-aminoimidazole-4-carboxamide (AICA)-riboside on AMP-activated protein kinase and glycogen synthase activities in rat skeletal muscle. *Diabetes* **51**, 284–292 (2002).
156. Roepstorff, C., Vistisen, B., Roepstorff, K. & Kiens, B. Regulation of plasma long-chain fatty acid oxidation in relation to uptake in human skeletal muscle during exercise. *Am. J. Physiol. Endocrinol. Metab.* **287**, E696–E705 (2004).
157. Katz, A., Sahlin, K. & Broberg, S. Regulation of glucose utilization in human skeletal muscle during moderate dynamic exercise. *Am. J. Physiol.* **260**, E411–E415 (1991).
158. Derave, W. *et al.* Contraction-stimulated muscle glucose transport and GLUT-4 surface content are dependent on glycogen content. *Am. J. Physiol.* **277**, E1103–E1110 (1999).
159. Hespel, P. & Richter, E. A. Glucose uptake and transport in contracting, perfused rat muscle with different pre-contraction glycogen concentrations. *J. Physiol.* **427**, 347–359 (1990).
160. Fogt, D. L. *et al.* Effect of glycogen synthase overexpression on insulin-stimulated muscle glucose uptake and storage. *Am. J. Physiol. Endocrinol. Metab.* **286**, E363–E369 (2004).
161. Xirouchaki, C. E. *et al.* Impaired glucose metabolism and exercise capacity with muscle-specific glycogen synthase 1 (gys1) deletion in adult mice. *Mol. Metab.* **5**, 221–232 (2016).
162. Nielsen, J. N. *et al.* Role of 5′AMP-activated protein kinase in glycogen synthase activity and glucose utilization: insights from patients with McArdle's disease. *J. Physiol.* **541**, 979–989 (2002).
163. Wojtaszewski, J. F., Nielsen, P., Hansen, B. F., Richter, E. A. & Kiens, B. Isoform-specific and exercise intensity-dependent activation of 5′-AMP-activated protein kinase in human skeletal muscle. *J. Physiol.* **528**, 221–226 (2000).
164. Fujii, N. *et al.* Exercise induces isoform-specific increase in 5′AMP-activated protein kinase activity in human skeletal muscle. *Biochem. Biophys. Res. Commun.* **273**, 1150–1155 (2000).
165. Chen, Z. P. *et al.* Effect of exercise intensity on skeletal muscle AMPK signaling in humans. *Diabetes* **52**, 2205–2212 (2003).
166. Polekhina, G. *et al.* AMPK β subunit targets metabolic stress sensing to glycogen. *Curr. Biol.* **13**, 867–871 (2003).
167. Oligschlaeger, Y. *et al.* The recruitment of AMP-activated protein kinase to glycogen is regulated by autophosphorylation. *J. Biol. Chem.* **290**, 11715–11728 (2015).
168. Xu, H. *et al.* When phosphorylated at Thr148, the β2 subunit of AMP activated kinase does not associate with glycogen in skeletal muscle. *Am. J. Physiol. Cell Physiol.* **311**, C35–C42 (2016).
169. Haller, R. G. & Vissing, J. No spontaneous second wind in muscle phosphofructokinase deficiency. *Neurology* **62**, 82–86 (2004).
170. Zaid, H., Talior-Volodarsky, I., Antonescu, C., Liu, Z. & Klip, A. GAPDH binds GLUT4 reciprocally to hexokinase-II and regulates glucose transport activity. *Biochem. J.* **419**, 475–484 (2009).
171. Fueger, P. T., Bracy, D. P., Malabanan, C. M., Pencek, R. R. & Wasserman, D. H. Distributed control of glucose uptake by working muscles of conscious mice: roles of transport and phosphorylation. *Am. J. Physiol. Endocrinol. Metab.* **286**, E77–E84 (2004).
172. Fueger, P. T. *et al.* Control of exercise-stimulated muscle glucose uptake by GLUT4 is dependent on glucose phosphorylation capacity in the conscious mouse. *J. Biol. Chem.* **279**, 50956–50961 (2004).
173. Fueger, P. T. *et al.* Hexokinase II protein content is a determinant of exercise endurance capacity in the mouse. *J. Physiol.* **566**, 533–541 (2005).
174. Fueger, P. T. *et al.* Control of muscle glucose uptake: test of the rate-limiting step paradigm in conscious, unrestrained mice. *J. Physiol.* **562**, 925–935 (2005).
175. Katzen, H. M. & Schimke, R. T. Multiple forms of hexokinase in the rat: tissue distribution, age dependency, and properties. *Proc. Natl Acad. Sci. USA* **54**, 1218–1225 (1965).
176. Grossbard, L. & Schimke, R. T. Multiple hexokinases of rat tissues: purification and comparison of soluble forms. *J. Biol. Chem.* **241**, 3546–3560 (1966).

177. Ritov, V. B. & Kelley, D. E. Hexokinase isozyme distribution in human skeletal muscle. *Diabetes* **50**, 1253–1262 (2001).
178. Albers, P. H. *et al.* Human muscle fiber type-specific insulin signaling: impact of obesity and type 2 diabetes. *Diabetes* **64**, 485–497 (2015).
179. John, S., Weiss, J. N. & Ribalet, B. Subcellular localization of hexokinases I and II directs the metabolic fate of glucose. *PLoS ONE* **6**, e17674 (2011).
180. Roberts, D. J., Tan-Sah, V. P., Smith, J. M. & Miyamoto, S. Akt phosphorylates HK-II at Thr-473 and increases mitochondrial HK-II association to protect cardiomyocytes. *J. Biol. Chem.* **288**, 23798–23806 (2013).
181. Yamada, Y. *et al.* Low glucose-1, 6-bisphosphate and high fructose-2, 6-bisphosphate concentrations in muscles of patients with glycogenosis types VII and V. *Biochem. Biophys. Res. Commun.* **176**, 7–10 (1991).
182. Hu, H. *et al.* Phosphoinositide 3-kinase regulates glycolysis through mobilization of aldolase from the actin cytoskeleton. *Cell* **164**, 433–446 (2016).
183. Chenzion, M., Lilling, G. & Beitner, R. The dual effects of Ca^{2+} on binding of the glycolytic enzymes, phosphofructokinase and aldolase, to muscle cytoskeleton. *Biochem. Med. Metab. Biol.* **49**, 173–181 (1993).
184. Kao, A. W., Noda, Y., Johnson, J. H., Pessin, J. E. & Saltiel, A. R. Aldolase mediates the association of F-actin with the insulin-responsive glucose transporter GLUT4. *J. Biol. Chem.* **274**, 17742–17747 (1999).
185. Pedersen, B. K. & Febbraio, M. A. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat. Rev. Endocrinol.* **8**, 45–465 (2012).
186. Jiang, L. Q. *et al.* Autocrine role of interleukin-13 on skeletal muscle glucose metabolism in type 2 diabetic patients involves microRNA let-7. *Am. J. Physiol. Endocrinol. Metab.* **305**, E1359–E1366 (2013).
187. Lee, H. J. *et al.* Irisin, a novel myokine, regulates glucose uptake in skeletal muscle cells via AMPK. *Mol. Endocrinol.* **29**, 873–881 (2015).
188. Canto, C. *et al.* Neuregulins mediate calcium-induced glucose transport during muscle contraction. *J. Biol. Chem.* **281**, 21690–21697 (2006).
189. Roustit, M. M., Vaughan, J. M., Jamieson, P. M. & Cleasby, M. E. Urocortin 3 activates AMPK and AKT pathways and enhances glucose disposal in rat skeletal muscle. *J. Endocrinol.* **223**, 143–154 (2014).
190. MacDonald, C., Wojtaszewski, J. F., Pedersen, B. K., Kiens, B. & Richter, E. A. Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity. *J. Appl. Physiol.* **295**, 2273–2277 (2003).
191. Helge, J. W. *et al.* The effect of graded exercise on IL-6 release and glucose uptake in human skeletal muscle. *J. Physiol.* **546**, 299–305 (2003).
192. Carey, A. L. *et al.* Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation *in vitro* via AMP-activated protein kinase. *Diabetes* **55**, 2688–2697 (2006).
193. O'Neill, H. M. *et al.* IL-6 is not essential for exercise-induced increases in glucose uptake. *J. Appl. Physiol.* **114**, 1151–1157 (2013).
194. Benrick, A., Wallenius, V. & Asterholm, I. W. Interleukin-6 mediates exercise-induced increase in insulin sensitivity in mice. *Exp. Physiol.* **97**, 1224–1235 (2012).
195. Buvinic, S. *et al.* ATP released by electrical stimuli elicits calcium transients and gene expression in skeletal muscle. *J. Biol. Chem.* **284**, 34490–34505 (2009).
196. Taguchi, T., Kozaki, Y., Katanosaka, K. & Mizumura, K. Compression-induced ATP release from rat skeletal muscle with and without lengthening contraction. *Neurosci. Lett.* **434**, 277–281 (2008).
197. Mortensen, S. P., Gonzalez-Alonso, J., Nielsen, J. J., Saltin, B. & Hellsten, Y. Muscle interstitial ATP and norepinephrine concentrations in the human leg during exercise and ATP infusion. *J. Appl. Physiol.* **107**, 1757–1762 (2009).
198. Casas, M., Buvinic, S. & Jaimovich, E. ATP signaling in skeletal muscle: from fiber plasticity to regulation of metabolism. *Exerc. Sport Sci. Rev.* **42**, 110–116 (2014).
199. Sylow, L. *et al.* Akt and Rac1 signaling are jointly required for insulin-stimulated glucose uptake in skeletal muscle and downregulated in insulin resistance. *Cell. Signal.* **26**, 323–331 (2014).
200. Lee, A. D., Hansen, P. A. & Holloszy, J. O. Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. *FEBS Lett.* **361**, 51–54 (1995).
201. Ploug, T., Galbo, H., Vinten, J., Jørgensen, M. & Richter, E. A. Kinetics of glucose transport in rat muscle: effects of insulin and contractions. *Am. J. Physiol.* **253**, E12–E20 (1987).
202. Lund, S., Holman, G. D., Schmitz, O. & Pedersen, O. Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc. Natl Acad. Sci. USA* **92**, 5817–5821 (1995).
203. Treebak, J. T. *et al.* Acute exercise and physiological insulin induce distinct phosphorylation signatures on TBC1D1 and TBC1D4 proteins in human skeletal muscle. *J. Physiol.* **592**, 351–375 (2014).
204. Sylow, L. *et al.* Rac1 in muscle is dispensable for improved insulin action after exercise in mice. *Endocrinology* **157**, 3009–3015 (2016).
205. Kjobsted, R. *et al.* Prior AICAR stimulation increases insulin sensitivity in mouse skeletal muscle in an AMPK-dependent manner. *Diabetes* **64**, 2042–2055 (2014).
206. Martin, I. K., Katz, A. & Wahren, J. Enhanced leg glucose uptake and normal hepatic glucose output during exercise in patients with NIDDM [abstract]. *Diabetes* **42** (Suppl. 1), 107A (1993).
207. Wallberg-Henriksson, H. & Holloszy, J. Activation of glucose transport in diabetic muscle: responses to contraction and insulin. *Am. J. Physiol.* **249**, C233–C237 (1985).
208. DeFronzo, R. A. & Tripathy, D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* **32** (Suppl. 2), 157–163 (2009).
209. Mikines, K., Sonne, B., Farrell, P., Tronier, B. & Galbo, H. Effect of training on the dose-response relationship for insulin action in men. *J. Appl. Physiol.* **66**, 695–703 (1989).
210. 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.* **167**, 69–75 (1999).
211. Jensen, T. E. *et al.* Possible CaMKK-dependent regulation of AMPK phosphorylation and glucose uptake at the onset of mild tetanic skeletal muscle contraction. *Am. J. Physiol. Endocrinol. Metab.* **292**, E1308–1317 (2007).
212. Koh, H. J. *et al.* Sucrose nonfermenting AMPK-related kinase (SNARK) mediates contraction-stimulated glucose transport in mouse skeletal muscle. *Proc. Natl Acad. Sci. USA* **107**, 15541–15546 (2010).
213. Sakamoto, K. *et al.* Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J.* **24**, 1810–1820 (2005).
214. Jeppesen, J. *et al.* LKB1 regulates lipid oxidation during exercise independently of AMPK. *Diabetes* **62**, 1490–1499 (2013).
215. Mu, J., Brozinick, J. T. Jr, Valladares, O., Bucan, M. & Birnbaum, M. J. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol. Cell* **7**, 1085–1094 (2001).
216. Lefort, N., St-Amand, E., Morasse, S., Cote, C. H. & Marette, A. The α-subunit of AMPK is essential for submaximal contraction-mediated glucose transport in skeletal muscle *in vitro*. *Am. J. Physiol. Endocrinol. Metab.* **295**, E1447–E1454 (2008).
217. Maarbjerg, S. J. *et al.* Genetic impairment of α2-AMPK signaling does not reduce muscle glucose uptake during treadmill exercise in mice. *Am. J. Physiol. Endocrinol. Metab.* **297**, E924–E934 (2009).
218. Lee-Young, R. S. *et al.* Skeletal muscle AMP-activated protein kinase is essential for the metabolic response to exercise *in vivo*. *J. Biol. Chem.* **284**, 23925–23934 (2009).
219. Jorgensen, S. B. *et al.* Knockout of the α2 but not α1 5′-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranosidebut not contraction-induced glucose uptake in skeletal muscle. *J. Biol. Chem.* **279**, 1070–1079 (2004).
220. Fentz, J. *et al.* AMPKα is critical for enhancing skeletal muscle fatty acid utilization during *in vivo* exercise in mice. *FASEB J.* **29**, 1725–1738 (2015).
221. Wojtaszewski, J. F., Laustsen, J. L. & Richter, E. A. Contraction- and hypoxia-stimulated glucose transport in skeletal muscle is affected differently by wortmannin. Evidence for different signalling mechanisms. *Biochim. Biophys. Acta* **1340**, 396–404 (1998).
222. Jensen, T. E., Maarbjerg, S. J., Rose, A. J., Leitges, M. & Richter, E. A. Knockout of the predominant conventional PKC isoform, PKCα, in mouse skeletal muscle does not affect contraction-stimulated glucose uptake. *Am. J. Physiol. Endocrinol. Metab.* **297**, E340–E348 (2009).
223. Kramer, H. F. *et al.* Calmodulin-binding domain of AS160 regulates contraction- but not insulin-stimulated glucose uptake in skeletal muscle. *Diabetes* **56**, 2854–2862 (2007).
224. Vichaiwong, K. *et al.* Contraction regulates site-specific phosphorylation of TBC1D1 in skeletal muscle. *Biochem. J.* **431**, 311–320 (2010).
225. An, D. *et al.* TBC1D1 regulates insulin- and contraction-induced glucose transport in mouse skeletal muscle. *Diabetes* **59**, 1358–1365 (2010).
226. Fueger, P. T. *et al.* Glucose kinetics and exercise tolerance in mice lacking the GLUT4 glucose transporter. *J. Physiol.* **582**, 801–812 (2007).
227. Hong, Y. H., Yang, C., Betik, A. C., Lee-Young, R. S. & McConell, G. K. Skeletal muscle glucose uptake during treadmill exercise in neuronal nitric oxide synthase-μ knockout mice. *Am. J. Physiol. Endocrinol. Metab.* **310**, E838–E845 (2016).
228. Fueger, P. T. *et al.* Hexokinase II overexpression improves exercise-stimulated but not insulin-stimulated muscle glucose uptake in high-fat-fed C57BL/6J mice. *Diabetes* **53**, 306–314 (2014).
229. Halseth, A. E., Bracy, D. P. & Wasserman, D. H. Overexpression of hexokinase II increases insulinand exercise-stimulated muscle glucose uptake *in vivo*. *Am. J. Physiol.* **276**, E70–E77 (1999).
230. Fueger, P. T. *et al.* Hexokinase II partial knockout impairs exercise-stimulated glucose uptake in oxidative muscles of mice. *Am. J. Physiol. Endocrinol. Metab.* **285**, E958–E963 (2003).
231. Nielsen, J. N. *et al.* Decreased insulin action in skeletal muscle from patients with McArdle's disease. *Am. J. Physiol. Endocrinol. Metab.* **282**, E1267–E1275 (2002).

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Competing interests statement

The authors declare no competing interests.