REVIEW

Culprits or consequences: Understanding the metabolic dysregulation of muscle in diabetes

Colleen L O'Reilly, Selina Uranga, James D Fluckey

ORCID number: Colleen L O'Reilly 0000-0002-9948-9410; Selina Uranga 0000-0002-2250-6331; James D Fluckey 0000-0003-1231-0412.

Colleen L O'Reilly, Selina Uranga, James D Fluckey, Health and Kinesiology, Texas A&M University, TX 77843, United States

Corresponding author: James D Fluckey, PhD, Professor, Health and Kinesiology, Texas A&M University, TAMU 4243, College Station, TX 77843, United States. jfluckey@tamu.edu

Abstract

The prevalence of type 2 diabetes (T2D) continues to rise despite the amount of research dedicated to finding the culprits of this debilitating disease. Skeletal muscle is arguably the most important contributor to glucose disposal making it a clear target in insulin resistance and T2D research. Within skeletal muscle there is a clear link to metabolic dysregulation during the progression of T2D but the determination of culprits *vs* consequences of the disease has been elusive. Emerging evidence in skeletal muscle implicates influential cross talk between a key anabolic regulatory protein, the mammalian target of rapamycin (mTOR) and its associated complexes (mTORC1 and mTORC2), and the well-described canonical signaling for insulin-stimulated glucose uptake. This new understanding of cellular signaling crosstalk has blurred the lines of what is a culprit and what is a consequence with regard to insulin resistance. Here, we briefly review the most recent understanding of insulin signaling in skeletal muscle, and how anabolic responses favoring anabolism directly impact cellular glucose disposal. This review highlights key cross-over interactions between protein and glucose regulatory pathways and the implications this may have for the design of new therapeutic targets for the control of glucoregulatory function in skeletal muscle.

Key Words: Insulin resistance; Skeletal muscle; Mammalian target of rapamycin; Glucose uptake; Glucose regulation; Insulin signaling

Core Tip: The prevalence of type 2 diabetes (T2D) continues to rise despite the amount of research dedicated to finding the culprits of this debilitating disease. Within skeletal muscle there is a clear link to metabolic dysregulation during the progression of T2D but the determination of culprits *vs* consequences of the disease has been elusive. Emerging evidence in skeletal muscle implicates influential cross talk between the

mammalian target of rapamycin (mTOR) complexes (mTORC1 and mTORC2) during insulin stimulated glucose uptake. This review highlights interactions between protein and glucose regulatory pathways and the implications this may have for the control of glucoregulatory function in skeletal muscle.

INTRODUCTION

Globally, 462 million individuals are affected by type 2 diabetes (T2D) and it is ranked as the 9th leading cause of mortality[1]. The prevalence of diabetes over the past few decades has continued to rise with no sign of this changing[1]. T2D is characterized by insulin resistance and hyperglycemia and can lead to various other outcomes and comorbidities reducing quality of life in those effected. While the pathogenesis and progression of T2D is still widely debated, it is clear that a complex interplay between the pancreas and peripheral tissues is dependent for maintenance of glucose homeostasis. Peripheral tissues account for 80%-90% of glucose disposal[2,3] and of those tissues skeletal muscle is a large contributor to glucose disposal[4,5] and arguably the most important for glucose clearance[6,7]. Within skeletal muscle there is clear link to metabolic dysregulation during the progression of T2D, but the definition of causes *vs* consequences within the development of this disease is difficult. Identifying clear relationships, interactions and feedback loops within the insulin signaling cascade and other metabolic pathways in skeletal muscle is imperative to our understanding for the development, its progression and ultimately a cure for this disease. To that end, this review will present the canonical understanding of insulin signaling, the influential connections between mammalian target of rapamycin (mTOR) complexes (mTORC1 and mTORC2) and the current intertwined implications of these signaling paradigms in skeletal muscle metabolic dysregulation.

INSULIN SIGNALING

The insulin signaling cascade involves both glucoregulatory and anabolic processes which is outlined in Figure 1. Insulin responsive tissues have insulin receptors (IR) on the cell surface plasma membrane. These IR contain subunits where insulin can bind as well as residues that provide docking sites for downstream signaling molecules including the IR substrates (IRS). The two predominant insulin receptor substrates are IRS1 and IRS2 with similar sequences but specific signaling roles[8,9]. IRS1 appears to be the insulin receptor substrate protein whose primary responsibility is glucose regulation, including glucose transporter 4 (GLUT-4) translocation[8] with speculation that IRS2 is more involved with fatty acid metabolism, currently known to occur in adipose tissue[9]. IRS1 is a clear mediator of insulin signaling through a specific intermediate phosphatidylinositol 3 kinase (PI3K). Interaction of PI3K to IRS produces membrane phosphatidylinositol 3,4,5-triphosphates (PIP3) which is necessary for the recruitment and localization of Protein Kinase B, also known as AKT[10].

Upstream glucose related substrates

This serine/threonine kinase is part of the AGC protein family and is known for its diverse function in growth, survival, proliferation and most importantly substrate metabolism[11-13] AKT is often referred to as one molecule but actually comprises of three distinct isoforms (AKT1, AKT2, AKT3) , while all isoforms are present in skeletal muscle, AKT2 is the most prevalent[12], but varies from low to immeasurable amounts in skeletal muscle[14,15]. While defining the variation and overlap between the AKT isoforms is important and needed, it is beyond the scope of this review but what is known currently can be found in these reviews[12,16] It is important to note that AKT2 is expressed primarily in insulin responsive tissues like fat and skeletal muscle and is
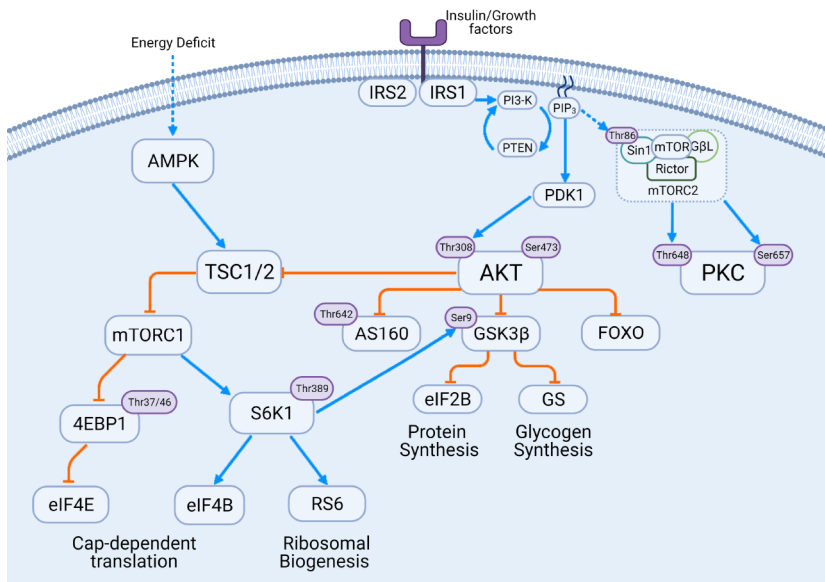
Figure 1 Insulin signaling cascade involving both glucoregulatory and anabolic pathways. Phosphorylation sites of interest indicated on figure. Blue arrows (→) indicate activation of the substrate, orange bars (⊥) indicate inhibitory action on the substrate. Figure created with BioRender.com. mTOR: Mammalian target of rapamycin; mTORC: mTOR complex; S6K1: S6 kinase beta-1; IRS: Insulin receptor substrates; PKC: Protein kinase C; AMPK: AMP-activated protein kinase; TSC: Tuberous sclerosis complex; GSK-3β: Glycogen synthase kinase 3β; PIP3: phosphatidylinositol 3,4,5-triphosphates.

the most abundant isoform in skeletal muscle[14,15,17,18]. AKT is as a critical regulator of insulin sensitive glucose uptake as well as anabolic signaling through mTORC1 making it a prime target in understanding metabolic dysregulation.

The upstream regulation of AKT, in its most simple iteration, appears to be very similar across isoforms. The two common phosphorylation sites of AKT are Ser473 (Ser474 in AKT2) and Thr308. The insulin receptors IRS1 and IRS2 will activate the PI3K-dependent conversion of PIP2 to PIP3, and PIP3 will recruit Pyruvate Dehydrogenase Kinase 1 (PDK1) and AKT to the membrane where colocalization will allow for phosphorylation at the Thr308 by PDK1[12,13]. Further, some evidence suggests that mitogen-activated protein kinase-associated protein 1 (mSin1) of the mTORC2 complex is brought to the membrane by PIP3 (binding with the pH domain) that promotes colocalization of mTORC2 to the membrane[19,20], which is the major kinase for the Ser473 phosphorylation site of AKT.

The regulation of mTORC2 activity by mSin1 phosphorylation is controversial. It has been proposed that PIP3) promotes mTORC2 activity directly[21,22]. Recent work has indicated a positive feedback loop between AKT and mTORC2 *via* phosphorylation of mSin1[23,24]. Those studies in adipocytes and Hela cells indicated that phosphorylation of mSin1 at Thr86 by AKT (*via* Thr308) increased mTORC2 activity and phosphorylation of AKT on Ser473[20,23]. This positive feedback loop provides an avenue for mTORC2 control *via* growth factors; however, the total impact of this feedback loop on mTORC2 activity and downstream substrates like AKT Ser473 is currently unknown. It is well established that PDK1 and mTORC2 are the major kinases involved upstream of AKT and that AKT is involved in a large scale, insulin sensitive pathway, but the distinct actions of these two phosphorylation sites are still not well understood.

There is also considerable debate over what the phosphorylation of specific AKT sites implicates for AKT activity and substrate specificity. Much of the early work in AKT reported a requirement of phosphorylation at Ser473 for full activation[25-28]. However, more recent work in platelets[29], HEK cells[27,30], and skeletal muscle[31, 32] demonstrated that not all downstream substrates are impacted by Ser473 phosphorylation. There is some evidence to support that these changes in activity and substrate *via* phosphorylation site may be isoform specific[33,34] but more work needs to be done in this area.

The implications of Ser473 phosphorylation *via* mTORC2 has been studied in various tissues. In mSin1 knockout mouse embryonic fibroblasts, a regulator of mTORC2 complex formation and stability, Forkhead box 01/03 (FOX01/3a) phosphorylation was inhibited but tuberous sclerosis complex 2 (TSC2) and glycogen synthase kinase 3 (GSK-3) phosphorylation was unaffected[35]. In adipose tissue[36] and liver[37], rapamycin insensitive companion of mTOR (RICTOR) knockouts demonstrated

tissue specific differences in mTORC2 substrate specificity. When mTORC2 inhibitors were applied in skeletal muscle, phosphorylation of AKT at Thr308 was unaffected and the downstream phosphorylation of TSC1/2, S6 kinase beta-1 (S6K1) and GSK-3β, all associated with protein synthesis and growth, were also unaffected by the reduction of Ser473 phosphorylation[32]. However AKT substrate of 160 kDa (AS160), an enzyme associated with GLUT-4 translocation and glucose disposal as well as proteins in the FOXO family associated with apoptosis were negatively affected by Ser473 reduction[32]. That work demonstrated that there is some demarcation of substrate specificity within AKT of skeletal muscle. It may also indicate phosphorylation of Thr308 focuses AKT kinase activity towards substrates involved with growth and phosphorylation of Ser473 focuses on substrates involved in glucose regulation and cell survival. Alternatively, substrates unaffected by inhibition or downregulation of mTORC2 phosphorylation of AKT at Ser473 may be phosphorylated by other proteins. For example GSK-3 can be phosphorylated at the same phosphorylation site that AKT does Ser9 by S6K[38] and protein kinase C (PKC)[39]. Despite the alternative theory there is evidence for at least some context-dependent substrate specificity towards AKT's downstream targets. As for whether the activity of AKT is dependent on Ser473 for full activation, a recent study in adipose tissue purports that AKT2 activity is reduced by about 50% for its substrates TSC2, PRAS40, FOX01/3a and AS160[40]. Taken together, there may be argument for some combination of Ser473 impacting substrate specificity and activity, but to our knowledge this has not been validated in skeletal muscle and would need more systematic study in both AKT1 and AKT2 to truly define this regulatory mechanism.

Downstream glucose related substrates
As previously mentioned AKT has various downstream substrates that make the action of this kinase quite diverse in cell function. These substrates include members of the mTOR complexes Pras40 and Sin1, Glucose uptake proteins AS160 and GSK-3, Protein synthesis related Tuberous sclerosis 2, and apoptotic signaling through the FOX0 family. This section will focus on signal transduction related to glucose uptake.

GLUT-4 is the predominant isoform of the GLUT family found in skeletal muscle, and one of insulin's primary metabolic roles is to promote the translocation of GLUT-4 to the surface membrane. AKT has been linked to downstream substrates that impact insulin-dependent GLUT-4 translocation including GSK-3[41] as well as AKT Substrate of 160kd (AS160)[31,42,43] making it a prime target for understanding glucose uptake. GSK-3β is a well-known inhibitor of glycogen synthase, but is also an inhibitor of eiF2B which is a potent regulator of protein synthesis. When GSK-3β is phosphorylated at Ser79 its activity is inhibited, which allows for the activation of both glycogen synthase and eiF2B. Interestingly GSK-3 has been linked to mTORC2 regulation *via* RICTOR phosphorylation at Ser1235 which interferes with mTORC2 binding to AKT[44] and Ser1695[45] which marks RICTOR for degradation. Also been linked to AS160 is a substrate of AKT that contains a Rab-GTPase activating protein and has been associated with regulating glucose transport. In basal conditions AS160 maintains GLUT-4 containing vesicles in the cytosol (intracellular) through its gap domain[46, 47], when insulin is applied AS160 is rapidly phosphorylated which disengages AS160 from the vesicles allowing them to move to the membrane for exocytosis. In skeletal muscle, like fat[43,48], AS160 is phosphorylated in response to insulin in a dose dependent manner[49] and insulin stimulation of GLUT-4 exocytosis is dependent on AS160 phosphorylation[48]. AS160 can be phosphorylated by other proteins including AMP-activated protein kinase (AMPK) making it part of both insulin dependent and insulin independent translocation of GLUT-4[31,50].

Anabolic signaling
AKT phosphorylates TSC2 at Thr1462 which regulates the tuberin-hamartin complex and it's activity[51-53]. Phosphorylation at this site releases the tuberin-hamartin complex inhibition of the mTORC1 complex and allows for downstream targets to be phosphorylated[51]. mTORC1 is a prolific kinase with multiple downstream substrates, but Ribosomal protein S6K1 and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) are arguably the most well-known downstream targets. 4E-BP1 is known as a translation repressor protein because it inhibits cap-dependent mRNA translation by binding to peptide-chain initiation factor eIF4E. Phosphorylation of 4E-BP1 disrupts the interaction of 4E-BP1 and eIF4E, releasing it so that it may participate in translation by chaperoning specific cap-dependent transcripts to the translation apparatus[54]. S6K1 is best known for its action on ribosomal protein S6 (S6) which is involved in the translational control of 5' oligopyrimidine tract (5'-TOP) mRNAs[55]. Phosphorylation of S6K1 at Thr389 is known to be critical for function of

the protein[55], as well as correlated with kinase activity *in vivo*[56]. The subsequent phosphorylation of S6 ribosomal protein correlates with increases in translation of cap-dependent proteins, that are necessary for the manufacture of ribosomal machinery and peptide-chain elongation factors necessary for mRNA translation[57,58]. The regulation of S6K1 activity is diverse but S6K1 activation has been shown to be elevated by hyperglycemia[59], hyperinsulinemia[60], and high fat diet in muscle and adipose tissue[61].

INSULIN SIGNALING AND DIABETES

It is generally agreed that glucose transport is the rate limiting step of glucose uptake, and the step most impacted by the progression of T2D. The consensus in diabetes research at large is that the translocation or trafficking of glucose transport molecules in skeletal muscle is impaired in T2D[43,62] but the culprit behind this impairment is still widely debated. In skeletal muscle GLUT-4 is the predominantly expressed isoform[63,64] and the localization of GLUT-4 has been confirmed with insulin[65], exercise[65,66] and hypoxia[67]. The first important finding with diabetes is that the limitation in glucose transport cannot be explained by production or maintenance of the GLUT itself, because total GLUT-4 protein is largely unchanged with diabetes[68-70]. This implies that the issue is not related to GLUT-4 expression, *per se*, but within the signaling cascades that assist in the translocation of GLUT-4 to the surface membranes.

As the initial step in the insulin signaling cascade, the insulin receptor was a primary target of research related to the breakdown of the glucoregulatory signals. While current data are conflicting on IR activity with some reporting impairment[62, 71,72] and others reporting normal activity[73-77], it appears that the important signaling 'defects' of T2D are further down the signal cascade. Signaling defects in IRS1 phosphorylation[73,77-79] and PI3K[73,77,78,80,81], activity are consistently found in the diabetic model. More controversial is the activity of AKT with studies reporting significant reductions of insulin stimulated AKT phosphorylation on Ser473 or Thr308[69,75,82,83]. While others report not impact of T2D on insulin dependent phosphorylation[80,81]. Downstream substrates of AKT have also been presented in the diabetic model with reduced glycogen synthase activity with protein levels of GSK-3 reported as being elevated which would inhibit GS activity[84]. Additionally, insulin dependent phosphorylation of AS160 has also been reported to be higher in T2D[42], despite the fact that AKT phosphorylation was not different in the same study.

Despite the continued exploration and detailed understanding of what the signaling cascades are doing during diabetes, there is still no consensus on where these dysfunctions are originating. Molecular mechanisms that underlie this dysfunction of glucoregulatory processes associated with T2D as outlined above have been studied extensively, but the interaction of glucoregulatory processes with those of protein metabolism (protein turnover) are still lacking, despite the evidence that the two processes may be dependent on one another.

It is well documented that muscle mass and strength decline with T2D[85,86] and contribute to a decline in quality life over time. Interestingly despite a loss in muscle mass, there appears to be an upregulation of protein synthesis and the anabolic signal cascade in diabetic muscle[87,88]. Previously, studies assessing anabolic responses [fractional synthesis rate (FSR)] in diabetic skeletal muscle have been inconsistent, ranging from decreased[89,90], to normal[91,92] but more recently increased FSR has been confirmed by our lab[87,88,93,94] and others[95,96]. In Fatty Zucker rats, a well-documented model for T2D, upregulated protein synthesis in specific muscle fractions and increased phosphorylation of S6K1 were observed despite an overall decrease in muscle mass. This upregulation of S6K1 appears to be linked to a loss of control of upstream mTOR activation. While the hyperactive mTOR activity may be a result of the maintained state of hyperinsulinemia with glucose intolerance, we suspect something much more sinister for the progression of diabetes.

Our recent studies have demonstrated that the constitutive activation of mTOR may be a result of suppressed DEPTOR expression in the diabetic state. DEPTOR is one of the mTOR associated binding partners that can be a part of either mTORC1 or mTORC2 and is a negative regulator of mTOR activity. Similar to several lines of cancer[97]. DEPTOR is substantially lower in obese subjects[87,88]. Since DEPTOR is still a fairly new discovery in the mTOR signaling cascade, the implications of low DEPTOR and the regulation of mTORC1 are still speculative but the low DEPTOR

appears to allow the downstream anabolic signals to go unchecked[98] which has implications for mRNA translation[99], as well as glucoregulatory signaling cascades. This is unbridled mTORC1 activity without concomitant muscle mass accretion is indicative of high protein turnover[88], where it may not be warranted or wanted. It is also an important bridge between mTORC1 and mTORC2 which will be discussed in a later section.

CONNECTING ANABOLISM TO INSULIN RESISTANCE

A relatively recent but important discovery in the connection of anabolic and glucoregulatory signaling paths is an inhibitory pathway that directly links S6K1 to IRS1. IRS1 can be serine phosphorylated through many pathways including c-Jun NH2-terminal kinase, IkB kinase, PKC, and S6K1[100,101]. It is now known that the insulin receptor contains multiple phosphorylation sites[102] and even in a basal state it is highly phosphorylated[103]. Ser/Thr phosphorylation of IRS-1 has been linked to the degradation of IRS1 itself and the downstream signaling needed for glucose uptake. While the patterns and requirements of these phosphorylation's for the downstream signal disruption are still undefined it has been clearly demonstrated that chronic exposure of cells to insulin results in degradation of IRS-1 protein[104-106]. It was later found that AKT mediated the Ser/Thr phosphorylation of IRS-1 and that this was inhibited by rapamycin[107]. More specifically IRS1 phosphorylation at Ser307 and Ser636/639 were observed in moments of increased mTORC1 activation and this increase was absent in mice that were S6K1 deficient[61]. In support of this constitutive activation of S6K1 lead to IRS1 phosphorylation and degradation as well as inhibition of IRS-1 transcription[108,109]. It is now a well-supported conclusion that IRS1 phosphorylation by S6K1 (Figure 2), decreases insulin signaling through the insulin receptor substrate[61,100,103,110,111]. This critical role is highlighted in the elevated levels of activation in liver adipose and muscle of obese animals[61,87,88,112] and is further supported by S6K1 deficient mice being protected against diet-induced obesity and insulin resistance[61]. This clearly links mTORC1 and more specifically S6K1 to the general insulin signaling cascade making it a target molecule for alteration of insulin signaling.

While we are gaining perspective in the current literature about the interaction between mTORC1 signaling for protein synthesis and the disruption of insulin signaling for glucose disposal in skeletal muscle, far less is known about how the two mTOR complexes interact in this process. While the S6K1 connection to IRS1 is now fairly accepted, S6K1 also appears to have a role in the cross-talk between the two mTOR complexes that is not yet well defined but thought to play a role in insulin resistance. To date, very little is known about the regulation of mTORC2[113] despite its role in phosphorylation of AKT at Ser473. The role of AKT and its regulation through Ser473, both upstream and downstream is still quite controversial in the literature as discussed earlier in section 2.1 AKT/protein kinase B (PKB), despite its being a widely used marker of AKT activity[25-27]. The downstream targets of AKT include various substrates involved in glucose uptake so the choice of this important intermediate as a marker seems obvious; however, the interpretation of what phosphorylation of AKT at Ser473 truly implies remains ambiguous.

The mTORC2 complex is best known for its involvement in cell survival but is known to phosphorylate AKT through Ser473[25,114-117] as well as the PKC family [40,116-119]. This complex is composed of binding partners mSin1, DEPTOR, Protor1, mLST8 and RICTOR. While all of these binding partners play roles in mTORC2 activity, the RICTOR has currently demarcated mTORC2's role in signal transduction [25]. RICTOR aids in localization of mTOR to the plasma membrane as well as the binding of mSin1 to the mTORC2 complex[19], making it an important binding partner worthy of the interest it has received. While mTORC2 has been established as the kinase responsible for phosphorylation of AKT at Ser473 the mechanism behind this phosphorylation is controversial. Two binding partners, RICTOR and Sin1, have been established as important regulators of mTORC2 complex activity, and of interest is that both of these binding partners appear to be regulated by S6K1. RICTOR is prone to phosphorylation[114,120,121] and that phosphorylation may impact downstream targets like AKT, as indicated by phosphorylation at Ser473[115,122].

Work by others indicated that the muscle-specific deletion of RICTOR led to decreased Ser473 phosphorylation of AKT and was accompanied by reduced phosphorylation of AS160 at Thr642 and overall glucose intolerance[123]. That work lead to speculation that regulation of RICTOR through phosphorylation was responsible for
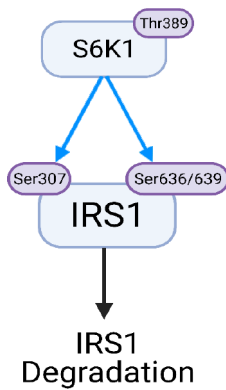
Figure 2 Downstream mammalian target of rapamycin complex 1 substrate S6 kinase beta-1 phosphorylation of insulin receptor substrates 1 at Ser307 and Ser636/639 leads to insulin receptor substrates 1 degradation. Blue arrows (→) indicate activation of the substrate, black arrow (→) indicates degradative pathway. Figure created with BioRender.com. S6K1: S6 kinase beta-1; IRS: Insulin receptor substrates.

the increases or decreases in Ser473 phosphorylation[115,122], and the concomitant responses of insulin-stimulated glucose homeostasis. Others determined that the phosphorylation of RICTOR at thr1135 (Figure 3) was responsible for inhibition of kinase activity toward AKT at Ser473[119,122,124,125]. Phosphorylation of RICTOR at Thr1135 was sensitive to both growth factors and rapamycin[124] and was the direct target, established through silencing and pharmacology, of S6K1[119]. Although the evidence connecting S6K1 to RICTOR regulation is compelling, the functional consequences of this phosphorylation are controversial. Some studies have indicated that this phosphorylation is a direct regulator of mTORC2 activity towards AKT[119,122], while others report no alteration in mTORC2 activity[124,125]. It must be noted that different experimental models were used across these studies, so it is possible that some of the differences observed were due to the differences in genetic models used to arrive at those conclusions. Despite those discrepancies, the S6K1-RICTOR interaction further supports the concept of crosstalk between the insulin glucoregulatory and protein synthesis pathways, as implicated by data demonstrating that mTORC1 regulation is important for Ser473 regulation. With mTORC1 and S6K1 activity being upregulated with diabetes, this connection to the insulin signaling pathways and the direct control mTORC1 may be critically important for further understanding of the metabolic dysregulation of T2D.

RESISTANCE EXERCISE

Exercise and physical activity are effective, low cost interventions for insulin resistance and T2D[126,127]. The benefits of aerobic exercise on glucose tolerance are well established[128-132] and the improvements are independent of improvements in general condition[132]. However many people with T2D are overweight and/or obese, have mobility issues and other neuropathies making aerobic-type exercises difficult to accomplish[133,134]. Resistance exercise has been proposed as a more feasible activity when aerobic exercise is inaccessible and there is a growing body of evidence to support that this form of exercise can be beneficial with regard to glucose tolerance [135,136]. Much of this work attributes the glucoregulatory improvements following resistance training are due to increased muscle mass[2,137,138] which may or may not be applicable to T2D. Additionally, acute resistance exercise appears to increase insulin clearance without a change in glucose tolerance[139], which was originally attributed to increases in insulin sensitivity *via* receptor number or a greater liver or tissue clearance following exercise.

It is often speculated that insulin-resistant skeletal muscle is desensitized or 'resistant' to the anabolic actions of exercise[88,140,141], making it difficult to achieve gains in muscle mass. Given the aforementioned hyperactivation of mTOR with insulin resistance, the current theory is that the 'anabolic resistance' observed with diabetes/obesity may really be due to an "anabolic ceiling" in skeletal muscle that has been achieved in the hyper-insulinemic state. In healthy tissue. resistance exercise is a potent stimulator of rates of protein synthesis in muscle and repeated bouts of resistance exercise lead to skeletal muscle hypertrophy[142]. It has also been established that insulin is a necessary component in elevated protein synthesis rates after re-
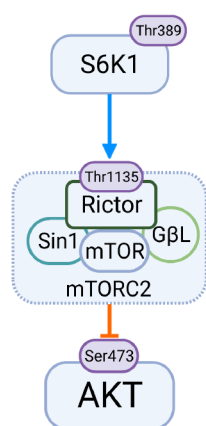
Figure 3 Downstream mammalian target of rapamycin complex 1 substrate S6 kinase beta-1 is the primary kinase responsible for phosphorylation of the mammalian target of rapamycin complex 2 component Rictor at Thr1135 which has been implicated in phos-**phorylation of AKT at Ser473.** Blue arrow (→) indicates activation of the substrate, orange bar (⊥) indicates inhibitory action on the substrate. Figure created with BioRender.com. mTOR: Mammalian target of rapamycin; mTORC: mTOR complex; S6K1: S6 kinase beta-1.

sistance exercise and it is the combination of resistance exercise and insulin that causes this modulation[143,144]. This effect of insulin appears to be through a rapamycin sensitive pathway[145-148] at least in healthy unperturbed tissue, but en-gaging in a moderate to high intensity exercise bouts involving eccentric muscle actions lead to a transiently-reduced capacity of insulin to elevate glucose uptake[149,150]. The mechanisms behind this alteration are still not well defined, but speculation includes a diminished capacity for glycogen synthesis and reductions in GLUT-4 protein which may be fiber type specific[150]. Further, as noted above, there are circumstances where the activation of protein anabolism requires S6K1 activation, which may feedback on upstream signals that impair glucose uptake by insulin[61,87,88,111]. More work is warranted to better define these mechanisms.

Aside from insulin sensitivity, there are benefits to regular exercise, whether it is of an aerobic or anaerobic nature. It is important to note here that there are insulin independent pathways that trigger glucose uptake that are directly related to skeletal muscle contraction. This pathway is triggered by muscle contraction and involves a distinct subset of GLUT-4[66,151-153]. These pathways can involve nitric oxide[154] and activation of AMPK[155,156] as well as cytosolic calcium[130] but these effects are distinct and additive to those of insulin mediated glucose uptake[2,157-159]. Probably most important for T2D research is that these contraction mediated glucose pathways are not only present in T2D but are fully functional[160,161].

Interestingly, in insulin resistant muscle there seems to be a difference in the control of muscle protein synthesis. It appears that in tissue where the upstream activators of the mTORC1 pathway are impaired there are alterations to the use in protein synthesis. Unlike their lean counterparts obese Zucker rats administered insulin had augmented rates of muscle protein synthesis and that these actions persisted in the presence of rapamycin[94]. This suggest that the rapamycin sensitive mTORC1 pathway is not responsible for the increased muscle protein synthesis rates observed.

One key player that may have an impact on muscle protein synthesis in response to insulin is a serine/threonine kinase called PKC. PKC has long been considered as a regulatory contributor during mRNA translation in a number of tissues[162,163] but more recently specific isoforms of PKC have been implicated in the regulation of glucose uptake. Specifically, the conventional family of PKCs (α, β, γ) lead to atte-nuated insulin receptor tyrosine kinase and PI3K activity[164,165] which leads to reduced glucose disposal. It has been discovered that in diabetic tissue, when insulin complexes with its receptor PKC is activated which then impairs downstream insulin signal[93]. This phenomenon is not observed in muscle from lean humans who have normal glucose response, mirroring the observed changes in insulin induced protein synthesis not present in lean counterparts[94]. Additionally inhibition of PKC activity through pharmacology has been demonstrated to partially restore signal transduction and glucose disposal in otherwise insulin resistant muscle[164,166]

The regulation of PKC, like many of the enzymes related to insulin signal trans-duction and glucose uptake is complex. It is known that PKCα is a downstream substrate of mTORC2 at both its turn motif (Thr638) and is hydrophobic motif (Ser657) both of which are required for PKCα stability[40,116-119]. Deletion of RICTOR, abo-

lishes phosphorylation of the hydrophobic motif of PKCα[114,115] and deletion of either RICTOR or Sin1 dramatically reduces PKCα protein content[117], implicating that RICTOR, a component of mTORC2, plays a role in PKC activation much like it does for the activation of AKT at Ser473. This draws mTORC2 further into the complex crosstalk that impacts insulin signaling and provides a feasible opportunity for mTORC2 to assist in the bypass of normal insulin signaling with the upregulation of PKC. It is important to note that PKC activation does not rely on mTORC2 however because it can also be activated by Diacylglycerol[117] which would be high in the obese state.

CONCLUSION

Dysregulation of mTOR signaling is a key player in the development of many disease states including diabetes. While decades of research have been dedicated to understanding the insulin signaling cascade, many aspects of its regulation and control remain elusive. It is becoming clear that crosstalk between the two mTOR complexes is adding considerable complexity by impacting both hormone-mediated glucose uptake and the underlying pathogenesis of this disease. This emerging evidence now blurs their roles and responsibilities of fixtures in protein homeostasis. Research in this area has focused on specific culprits in the glucoregulatory pathway that are thought to cause the manifestation of the disease, but with all of the newly emerging anabolic/glucoregulatory cross talk that are involved with the manifestation of this disease, it is possible that the factors once viewed as culprits for this disease may actually be the consequence of anabolic/glucoregulatory cross talk. These recent findings offer exciting new targets for the control of insulin resistance.

REFERENCES

1 **Khan MAB**, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health* 2020; **10**: 107-111 [PMID: 32175717 DOI: 10.2991/jegh.k.191028.001]

2 **DeFronzo RA**, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981; **30**: 1000-1007 [PMID: 7030826 DOI: 10.2337/diab.30.12.1000]

3 **DeFronzo RA**, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest* 1985; **76**: 149-155 [PMID: 3894418 DOI: 10.1172/JCI111938]

4 **Issa-Chergui B**, Guttmann RD, Seemayer TA, Kelley VE, Colle E. The effect of diet on the spontaneous insulin dependent diabetic syndrome in the rat. *Diabetes Res* 1988; **9**: 81-86 [PMID: 3073033]

5 **Moore MC**, Cherrington AD, Wasserman DH. Regulation of hepatic and peripheral glucose disposal. *Best Pract Res Clin Endocrinol Metab* 2003; **17**: 343-364 [PMID: 12962690 DOI: 10.1016/s1521-690x(03)00036-8]

6 **Sato M**, Dehvari N, Oberg AI, Dallner OS, Sandström AL, Olsen JM, Csikasz RI, Summers RJ, Hutchinson DS, Bengtsson T. Improving type 2 diabetes through a distinct adrenergic signaling pathway involving mTORC2 that mediates glucose uptake in skeletal muscle. *Diabetes* 2014; **63**: 4115-4129 [PMID: 25008179 DOI: 10.2337/db13-1860]

7 **Ren JM**, Marshall BA, Gulve EA, Gao J, Johnson DW, Holloszy JO, Mueckler M. Evidence from transgenic mice that glucose transport is rate-limiting for glycogen deposition and glycolysis in skeletal muscle. *J Biol Chem* 1993; **268**: 16113-16115 [PMID: 8344895 DOI: 10.1016/S0021-9258(19)85395-4]

8 **Huang C**, Thirone AC, Huang X, Klip A. Differential contribution of insulin receptor substrates 1 versus 2 to insulin signaling and glucose uptake in l6 myotubes. *J Biol Chem* 2005; **280**: 19426-19435 [PMID: 15764603 DOI: 10.1074/jbc.M412317200]

9 **Bouzakri K**, Zachrisson A, Al-Khalili L, Zhang BB, Koistinen HA, Krook A, Zierath JR. siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-2/Akt1 in glucose and lipid metabolism in human skeletal muscle. *Cell Metab* 2006; **4**: 89-96 [PMID: 16814735 DOI: 10.1016/j.cmet.2006.04.008]

10 **Franke TF**, Kaplan DR, Cantley LC, Toker A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science* 1997; **275**: 665-668 [PMID: 9005852 DOI: 10.1126/science.275.5300.665]

11 **Zhang Z**, Liu H, Liu J. Akt activation: A potential strategy to ameliorate insulin resistance. *Diabetes Res Clin Pract* 2019; **156**: 107092 [PMID: 29111280 DOI: 10.1016/j.diabres.2017.10.004]

12 **Gonzalez E**, McGraw TE. The Akt kinases: isoform specificity in metabolism and cancer. *Cell Cycle* 2009; **8**: 2502-2508 [PMID: 19597332 DOI: 10.4161/cc.8.16.9335]

13   **Manning BD**, Toker A. AKT/PKB Signaling: Navigating the Network. *Cell* 2017; **169**: 381-405 [PMID: 28431241 DOI: 10.1016/j.cell.2017.04.001]

14   **Cho H**, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J Biol Chem* 2001; **276**: 38349-38352 [PMID: 11533044 DOI: 10.1074/jbc.C100462200]

15   **Garofalo RS**, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, Coskran T, Black SC, Brees DJ, Wicks JR, McNeish JD, Coleman KG. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. *J Clin Invest* 2003; **112**: 197-208 [PMID: 12843127 DOI: 10.1172/JCI16885]

16   **Schultze SM**, Jensen J, Hemmings BA, Tschopp O, Niessen M. Promiscuous affairs of PKB/AKT isoforms in metabolism. *Arch Physiol Biochem* 2011; **117**: 70-77 [PMID: 21214427 DOI: 10.3109/13813455.2010.539236]

17   **Cleasby ME**, Reinten TA, Cooney GJ, James DE, Kraegen EW. Functional studies of Akt isoform specificity in skeletal muscle in vivo; maintained insulin sensitivity despite reduced insulin receptor substrate-1 expression. *Mol Endocrinol* 2007; **21**: 215-228 [PMID: 17021050 DOI: 10.1210/me.2006-0154]

18   **Jaiswal N**, Gavin MG, Quinn WJ 3rd, Luongo TS, Gelfer RG, Baur JA, Titchenell PM. The role of skeletal muscle Akt in the regulation of muscle mass and glucose homeostasis. *Mol Metab* 2019; **28**: 1-13 [PMID: 31444134 DOI: 10.1016/j.molmet.2019.08.001]

19   **Yuan HX**, Guan KL. The SIN1-PH Domain Connects mTORC2 to PI3K. *Cancer Discov* 2015; **5**: 1127-1129 [PMID: 26526694 DOI: 10.1158/2159-8290.CD-15-1125]

20   **Yang Q**, Inoki K, Ikenoue T, Guan KL. Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. *Genes Dev* 2006; **20**: 2820-2832 [PMID: 17043309 DOI: 10.1101/gad.1461206]

21   **Liu P**, Gan W, Chin YR, Ogura K, Guo J, Zhang J, Wang B, Blenis J, Cantley LC, Toker A, Su B, Wei W. PtdIns(3,4,5)P3-Dependent Activation of the mTORC2 Kinase Complex. *Cancer Discov* 2015; **5**: 1194-1209 [PMID: 26293922 DOI: 10.1158/2159-8290.CD-15-0460]

22   **Gan X**, Wang J, Su B, Wu D. Evidence for direct activation of mTORC2 kinase activity by phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* 2011; **286**: 10998-11002 [PMID: 21310961 DOI: 10.1074/jbc.M110.195016]

23   **Yang G**, Murashige DS, Humphrey SJ, James DE. A Positive Feedback Loop between Akt and mTORC2 *via* SIN1 Phosphorylation. *Cell Rep* 2015; **12**: 937-943 [PMID: 26235620 DOI: 10.1016/j.celrep.2015.07.016]

24   **Humphrey SJ**, Yang G, Yang P, Fazakerley DJ, Stöckli J, Yang JY, James DE. Dynamic adipocyte phosphoproteome reveals that Akt directly regulates mTORC2. *Cell Metab* 2013; **17**: 1009-1020 [PMID: 23684622 DOI: 10.1016/j.cmet.2013.04.010]

25   **Sarbassov DD**, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; **307**: 1098-1101 [PMID: 15718470 DOI: 10.1126/science.1106148]

26   **Alessi DR**, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 1996; **15**: 6541-6551 [PMID: 8978681 DOI: 10.1002/j.1460-2075.1996.tb01045.x]

27   **Scheid MP**, Marignani PA, Woodgett JR. Multiple phosphoinositide 3-kinase-dependent steps in activation of protein kinase B. *Mol Cell Biol* 2002; **22**: 6247-6260 [PMID: 12167717 DOI: 10.1128/MCB.22.17.6247-6260.2002]

28   **Hemmings BA**. Akt signaling: linking membrane events to life and death decisions. *Science* 1997; **275**: 628-630 [PMID: 9019819 DOI: 10.1126/science.275.5300.628]

29   **Moore SF**, Hunter RW, Hers I. mTORC2 Protein-mediated Protein Kinase B (Akt) Serine 473 Phosphorylation Is Not Required for Akt1 Activity in Human Platelets∗. *J Biol Chem* 2011; **286**: 24553-24560 [PMID: 21592956 DOI: 10.1074/jbc.m110.202341]

30   **Ananthanarayanan B**, Fosbrink M, Rahdar M, Zhang J. Live-cell molecular analysis of Akt activation reveals roles for activation loop phosphorylation. *J Biol Chem* 2007; **282**: 36634-36641 [PMID: 17928291 DOI: 10.1074/jbc.M706227200]

31   **Kramer HF**, Witczak CA, Fujii N, Jessen N, Taylor EB, Arnolds DE, Sakamoto K, Hirshman MF, Goodyear LJ. Distinct signals regulate AS160 phosphorylation in response to insulin, AICAR, and contraction in mouse skeletal muscle. *Diabetes* 2006; **55**: 2067-2076 [PMID: 16804077 DOI: 10.2337/db06-0150]

32   **Kumar N**, Afeyan R, Sheppard S, Harms B, Lauffenburger DA. Quantitative analysis of Akt phosphorylation and activity in response to EGF and insulin treatment. *Biochem Biophys Res Commun* 2007; **354**: 14-20 [PMID: 17214972 DOI: 10.1016/j.bbrc.2006.12.188]

33   **Cozzone D**, Fröjdö S, Disse E, Debard C, Laville M, Pirola L, Vidal H. Isoform-specific defects of insulin stimulation of Akt/protein kinase B (PKB) in skeletal muscle cells from type 2 diabetic patients. *Diabetologia* 2008; **51**: 512-521 [PMID: 18204829 DOI: 10.1007/s00125-007-0913-8]

34   **Brozinick JT Jr**, Roberts BR, Dohm GL. Defective signaling through Akt-2 and -3 but not Akt-1 in insulin-resistant human skeletal muscle: potential role in insulin resistance. *Diabetes* 2003; **52**: 935-941 [PMID: 12663464 DOI: 10.2337/diabetes.52.4.935]

35   **Jacinto E**, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 2006; **127**: 125-137 [PMID: 16962653 DOI: 10.1016/j.cell.2006.08.033]

36   **Tang Y**, Wallace M, Sanchez-Gurmaches J, Hsiao WY, Li H, Lee PL, Vernia S, Metallo CM, Guertin DA. Adipose tissue mTORC2 regulates ChREBP-driven de novo lipogenesis and hepatic glucose metabolism. *Nat Commun* 2016; **7**: 11365 [PMID: 27098609 DOI: 10.1038/ncomms11365]

37   **Hagiwara A**, Cornu M, Cybulski N, Polak P, Betz C, Trapani F, Terracciano L, Heim MH, Rüegg MA, Hall MN. Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. *Cell Metab* 2012; **15**: 725-738 [PMID: 22521878 DOI: 10.1016/j.cmet.2012.03.015]

38   **Zhang HH**, Lipovsky AI, Dibble CC, Sahin M, Manning BD. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. *Mol Cell* 2006; **24**: 185-197 [PMID: 17052453 DOI: 10.1016/j.molcel.2006.09.019]

39   **Wang Q**, Zhou Y, Evers BM. Neurotensin phosphorylates GSK-3alpha/beta through the activation of PKC in human colon cancer cells. *Neoplasia* 2006; **8**: 781-787 [PMID: 16984735 DOI: 10.1593/neo.06259]

40   **Facchinetti V**, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ, Sessa WC, Qin J, Zhang P, Su B, Jacinto E. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J* 2008; **27**: 1932-1943 [PMID: 18566586 DOI: 10.1038/emboj.2008.120]

41   **Cross DA**, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995; **378**: 785-789 [PMID: 8524413 DOI: 10.1038/378785a0]

42   **Karlsson HK**, Zierath JR, Kane S, Krook A, Lienhard GE, Wallberg-Henriksson H. Insulin-stimulated phosphorylation of the Akt substrate AS160 is impaired in skeletal muscle of type 2 diabetic subjects. *Diabetes* 2005; **54**: 1692-1697 [PMID: 15919790 DOI: 10.2337/diabetes.54.6.1692]

43   **Sano H**, Kane S, Sano E, Mîinea CP, Asara JM, Lane WS, Garner CW, Lienhard GE. Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. *J Biol Chem* 2003; **278**: 14599-14602 [PMID: 12637568 DOI: 10.1074/jbc.C300063200]

44   **Chen CH**, Shaikenov T, Peterson TR, Aimbetov R, Bissenbaev AK, Lee SW, Wu J, Lin HK, Sarbassov dos D. ER stress inhibits mTORC2 and Akt signaling through GSK-3β-mediated phosphorylation of rictor. *Sci Signal* 2011; **4**: ra10 [PMID: 21343617 DOI: 10.1126/scisignal.2001731]

45   **Koo J**, Wu X, Mao Z, Khuri FR, Sun SY. Rictor Undergoes Glycogen Synthase Kinase 3 (GSK3)-dependent, FBXW7-mediated Ubiquitination and Proteasomal Degradation. *J Biol Chem* 2015; **290**: 14120-14129 [PMID: 25897075 DOI: 10.1074/jbc.M114.633057]

46   **Eguez L**, Lee A, Chavez JA, Miinea CP, Kane S, Lienhard GE, McGraw TE. Full intracellular retention of GLUT4 requires AS160 Rab GTPase activating protein. *Cell Metab* 2005; **2**: 263-272 [PMID: 16213228 DOI: 10.1016/j.cmet.2005.09.005]

47   **Larance M**, Ramm G, Stöckli J, van Dam EM, Winata S, Wasinger V, Simpson F, Graham M, Junutula JR, Guilhaus M, James DE. Characterization of the role of the Rab GTPase-activating protein AS160 in insulin-regulated GLUT4 trafficking. *J Biol Chem* 2005; **280**: 37803-37813 [PMID: 16154996 DOI: 10.1074/jbc.M503897200]

48   **Zeigerer A**, McBrayer MK, McGraw TE. Insulin stimulation of GLUT4 exocytosis, but not its inhibition of endocytosis, is dependent on RabGAP AS160. *Mol Biol Cell* 2004; **15**: 4406-4415 [PMID: 15254270 DOI: 10.1091/mbc.e04-04-0333]

49   **Bruss MD**, Arias EB, Lienhard GE, Cartee GD. Increased phosphorylation of Akt substrate of 160 kDa (AS160) in rat skeletal muscle in response to insulin or contractile activity. *Diabetes* 2005; **54**: 41-50 [PMID: 15616009 DOI: 10.2337/diabetes.54.1.41]

50   **Kramer HF**, Witczak CA, Taylor EB, Fujii N, Hirshman MF, Goodyear LJ. AS160 regulates insulin- and contraction-stimulated glucose uptake in mouse skeletal muscle. *J Biol Chem* 2006; **281**: 31478-31485 [PMID: 16935857 DOI: 10.1074/jbc.M605461200]

51   **Manning BD**, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 2002; **10**: 151-162 [PMID: 12150915 DOI: 10.1016/s1097-2765(02)00568-3]

52   **Aicher LD**, Campbell JS, Yeung RS. Tuberin phosphorylation regulates its interaction with hamartin. Two proteins involved in tuberous sclerosis. *J Biol Chem* 2001; **276**: 21017-21021 [PMID: 11290735 DOI: 10.1074/jbc.C100136200]

53   **Dan HC**, Sun M, Yang L, Feldman RI, Sui XM, Ou CC, Nellist M, Yeung RS, Halley DJ, Nicosia SV, Pledger WJ, Cheng JQ. Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J Biol Chem* 2002; **277**: 35364-35370 [PMID: 12167664 DOI: 10.1074/jbc.M205838200]

54   **Pause A**, Belsham GJ, Gingras AC, Donzé O, Lin TA, Lawrence JC Jr, Sonenberg N. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. *Nature* 1994; **371**: 762-767 [PMID: 7935836 DOI: 10.1038/371762a0]

55   **Pullen N**, Thomas G. The modular phosphorylation and activation of p70s6k. *FEBS Lett* 1997; **410**: 78-82 [PMID: 9247127 DOI: 10.1016/s0014-5793(97)00323-2]

56   **Weng QP**, Kozlowski M, Belham C, Zhang A, Comb MJ, Avruch J. Regulation of the p70 S6 kinase by phosphorylation in vivo. Analysis using site-specific anti-phosphopeptide antibodies. *J Biol Chem* 1998; **273**: 16621-16629 [PMID: 9632736 DOI: 10.1074/jbc.273.26.16621]

57   **Peterson RT**, Schreiber SL. Translation control: connecting mitogens and the ribosome. *Curr Biol* 1998; **8**: R248-R250 [PMID: 9545190 DOI: 10.1016/s0960-9822(98)70152-6]

58   **Jefferies HB**, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. *EMBO J* 1997; **16**: 3693-3704 [PMID: 9218810 DOI: 10.1093/emboj/16.12.3693]

59   **Tzatsos A**, Kandror KV. Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling *via* raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. *Mol Cell Biol* 2006; **26**: 63-76 [PMID: 16354680 DOI: 10.1128/MCB.26.1.63-76.2006]

60   **Ueno M**, Carvalheira JB, Tambascia RC, Bezerra RM, Amaral ME, Carneiro EM, Folli F, Franchini KG, Saad MJ. Regulation of insulin signalling by hyperinsulinaemia: role of IRS-1/2 serine phosphorylation and the mTOR/p70 S6K pathway. *Diabetologia* 2005; **48**: 506-518 [PMID: 15692808 DOI: 10.1007/s00125-004-1662-6]

61   **Um SH**, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004; **431**: 200-205 [PMID: 15306821 DOI: 10.1038/nature02866]

62   **Maegawa H**, Shigeta Y, Egawa K, Kobayashi M. Impaired autophosphorylation of insulin receptors from abdominal skeletal muscles in nonobese subjects with NIDDM. *Diabetes* 1991; **40**: 815-819 [PMID: 1647993 DOI: 10.2337/diab.40.7.815]

63   **Birnbaum MJ**. Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell* 1989; **57**: 305-315 [PMID: 2649253 DOI: 10.1016/0092-8674(89)90968-9]

64   **Fukumoto H**, Kayano T, Buse JB, Edwards Y, Pilch PF, Bell GI, Seino S. Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. *J Biol Chem* 1989; **264**: 7776-7779 [PMID: 2656669 DOI: 10.1016/S0021-9258(18)83106-4]

65   **Thorell A**, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JF, Dufresne SD, Horton ES, Ljungqvist O, Goodyear LJ. Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. *Am J Physiol* 1999; **277**: E733-E741 [PMID: 10516134 DOI: 10.1152/ajpendo.1999.277.4.E733]

66   **Douen AG**, Ramlal T, Rastogi S, Bilan PJ, Cartee GD, Vranic M, Holloszy JO, Klip A. Exercise induces recruitment of the "insulin-responsive glucose transporter". Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. *J Biol Chem* 1990; **265**: 13427-13430 [PMID: 2199436 DOI: 10.1016/S0021-9258(18)77362-6]

67   **Ryder JW**, Yang J, Galuska D, Rincón J, Björnholm M, Krook A, Lund S, Pedersen O, Wallberg-Henriksson H, Zierath JR, Holman GD. Use of a novel impermeable biotinylated photolabeling reagent to assess insulin- and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. *Diabetes* 2000; **49**: 647-654 [PMID: 10871204 DOI: 10.2337/diabetes.49.4.647]

68   **Garvey WT**, Maianu L, Hancock JA, Golichowski AM, Baron A. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. *Diabetes* 1992; **41**: 465-475 [PMID: 1535055 DOI: 10.2337/diab.41.4.465]

69   **Pedersen O**, Bak JF, Andersen PH, Lund S, Moller DE, Flier JS, Kahn BB. Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. *Diabetes* 1990; **39**: 865-870 [PMID: 2354749 DOI: 10.2337/diab.39.7.865]

70   **Ciaraldi TP**, Kong AP, Chu NV, Kim DD, Baxi S, Loviscach M, Plodkowski R, Reitz R, Caulfield M, Mudaliar S, Henry RR. Regulation of glucose transport and insulin signaling by troglitazone or metformin in adipose tissue of type 2 diabetic subjects. *Diabetes* 2002; **51**: 30-36 [PMID: 11756319 DOI: 10.2337/diabetes.51.1.30]

71   **Arner P**, Pollare T, Lithell H, Livingston JN. Defective insulin receptor tyrosine kinase in human skeletal muscle in obesity and type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1987; **30**: 437-440 [PMID: 2824266 DOI: 10.1007/BF00292549]

72   **Nolan JJ**, Freidenberg G, Henry R, Reichart D, Olefsky JM. Role of human skeletal muscle insulin receptor kinase in the in vivo insulin resistance of noninsulin-dependent diabetes mellitus and obesity. *J Clin Endocrinol Metab* 1994; **78**: 471-477 [PMID: 8106637 DOI: 10.1210/jcem.78.2.8106637]

73   **Krook A**, Björnholm M, Galuska D, Jiang XJ, Fahlman R, Myers MG Jr, Wallberg-Henriksson H, Zierath JR. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes* 2000; **49**: 284-292 [PMID: 10868945 DOI: 10.2337/diabetes.49.2.284]

74   **Klein HH**, Vestergaard H, Kotzke G, Pedersen O. Elevation of serum insulin concentration during euglycemic hyperinsulinemic clamp studies leads to similar activation of insulin receptor kinase in skeletal muscle of subjects with and without NIDDM. *Diabetes* 1995; **44**: 1310-1317 [PMID: 7589829 DOI: 10.2337/diab.44.11.1310]

75   **Meyer MM**, Levin K, Grimmsmann T, Beck-Nielsen H, Klein HH. Insulin signalling in skeletal muscle of subjects with or without Type II-diabetes and first degree relatives of patients with the disease. *Diabetologia* 2002; **45**: 813-822 [PMID: 12107725 DOI: 10.1007/s00125-002-0830-9]

76   **Caro JF**, Sinha MK, Raju SM, Ittoop O, Pories WJ, Flickinger EG, Meelheim D, Dohm GL. Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. *J Clin Invest* 1987; **79**: 1330-1337 [PMID: 3033021 DOI: 10.1172/JCI112958]

77   **Kim YB**, Kotani K, Ciaraldi TP, Henry RR, Kahn BB. Insulin-stimulated protein kinase C lambda/zeta activity is reduced in skeletal muscle of humans with obesity and type 2 diabetes:

reversal with weight reduction. *Diabetes* 2003; **52**: 1935-1942 [PMID: 12882908 DOI: 10.2337/diabetes.52.8.1935]

78   **Cusi K**, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 2000; **105**: 311-320 [PMID: 10675357 DOI: 10.1172/JCI7535]

79   **Bouzakri K**, Roques M, Gual P, Espinosa S, Guebre-Egziabher F, Riou JP, Laville M, Le Marchand-Brustel Y, Tanti JF, Vidal H. Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes. *Diabetes* 2003; **52**: 1319-1325 [PMID: 12765939 DOI: 10.2337/diabetes.52.6.1319]

80   **Beeson M**, Sajan MP, Dizon M, Grebenev D, Gomez-Daspet J, Miura A, Kanoh Y, Powe J, Bandyopadhyay G, Standaert ML, Farese RV. Activation of protein kinase C-zeta by insulin and phosphatidylinositol-3,4,5-(PO4)3 is defective in muscle in type 2 diabetes and impaired glucose tolerance: amelioration by rosiglitazone and exercise. *Diabetes* 2003; **52**: 1926-1934 [PMID: 12882907 DOI: 10.2337/diabetes.52.8.1926]

81   **Kim YB**, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB. Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. *J Clin Invest* 1999; **104**: 733-741 [PMID: 10491408 DOI: 10.1172/JCI6928]

82   **Krook A**, Roth RA, Jiang XJ, Zierath JR, Wallberg-Henriksson H. Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects. *Diabetes* 1998; **47**: 1281-1286 [PMID: 9703329 DOI: 10.2337/diab.47.8.1281]

83   **Prada PO**, Coelho MS, Zecchin HG, Dolnikoff MS, Gasparetti AL, Furukawa LN, Saad MJ, Heimann JC. Low salt intake modulates insulin signaling, JNK activity and IRS-1ser307 phosphorylation in rat tissues. *J Endocrinol* 2005; **185**: 429-437 [PMID: 15930169 DOI: 10.1677/joe.1.06028]

84   **Nikoulina SE**, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. *Diabetes* 2000; **49**: 263-271 [PMID: 10868943 DOI: 10.2337/diabetes.49.2.263]

85   **Park SW**, Goodpaster BH, Strotmeyer ES, de Rekeneire N, Harris TB, Schwartz AV, Tylavsky FA, Newman AB. Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. *Diabetes* 2006; **55**: 1813-1818 [PMID: 16731847 DOI: 10.2337/db05-1183]

86   **Kim KS**, Park KS, Kim MJ, Kim SK, Cho YW, Park SW. Type 2 diabetes is associated with low muscle mass in older adults. *Geriatr Gerontol Int* 2014; **14** Suppl 1: 115-121 [PMID: 24450569 DOI: 10.1111/ggi.12189]

87   **Nilsson MI**, Greene NP, Dobson JP, Wiggs MP, Gasier HG, Macias BR, Shimkus KL, Fluckey JD. Insulin resistance syndrome blunts the mitochondrial anabolic response following resistance exercise. *Am J Physiol Endocrinol Metab* 2010; **299**: E466-E474 [PMID: 20606077 DOI: 10.1152/ajpendo.00118.2010]

88   **Nilsson MI**, Dobson JP, Greene NP, Wiggs MP, Shimkus KL, Wudeck EV, Davis AR, Laureano ML, Fluckey JD. Abnormal protein turnover and anabolic resistance to exercise in sarcopenic obesity. *FASEB J* 2013; **27**: 3905-3916 [PMID: 23804240 DOI: 10.1096/fj.12-224006]

89   **Durschlag RP**, Layman DK. Skeletal muscle growth in lean and obese Zucker rats. *Growth* 1983; **47**: 282-291 [PMID: 6196256]

90   **Guillet C**, Delcourt I, Rance M, Giraudet C, Walrand S, Bedu M, Duche P, Boirie Y. Changes in basal and insulin and amino acid response of whole body and skeletal muscle proteins in obese men. *J Clin Endocrinol Metab* 2009; **94**: 3044-3050 [PMID: 19470633 DOI: 10.1210/jc.2008-2216]

91   **Lobley GE**, Webster AJ, Reeds PJ. Protein synthesis in lean and obese Zucker rats. *Proc Nutr Soc* 1978; **37**: 20A [PMID: 662845]

92   **Halvatsiotis P**, Short KR, Bigelow M, Nair KS. Synthesis rate of muscle proteins, muscle functions, and amino acid kinetics in type 2 diabetes. *Diabetes* 2002; **51**: 2395-2404 [PMID: 12145150 DOI: 10.2337/diabetes.51.8.2395]

93   **Fluckey JD**, Cortright RN, Tapscott E, Koves T, Smith L, Pohnert S, Dohm GL. Active involvement of PKC for insulin-mediated rates of muscle protein synthesis in Zucker rats. *Am J Physiol Endocrinol Metab* 2004; **286**: E753-E758 [PMID: 14693507 DOI: 10.1152/ajpendo.00155.2003]

94   **Fluckey JD**, Pohnert SC, Boyd SG, Cortright RN, Trappe TA, Dohm GL. Insulin stimulation of muscle protein synthesis in obese Zucker rats is not *via* a rapamycin-sensitive pathway. *Am J Physiol Endocrinol Metab* 2000; **279**: E182-E187 [PMID: 10893338 DOI: 10.1152/ajpendo.2000.279.1.E182]

95   **Bell JA**, Volpi E, Fujita S, Cadenas JG, Sheffield-Moore M, Rasmussen BB. Skeletal muscle protein anabolic response to increased energy and insulin is preserved in poorly controlled type 2 diabetes. *J Nutr* 2006; **136**: 1249-1255 [PMID: 16614412 DOI: 10.1093/jn/136.5.1249]

96   **She P**, Olson KC, Kadota Y, Inukai A, Shimomura Y, Hoppel CL, Adams SH, Kawamata Y, Matsumoto H, Sakai R, Lang CH, Lynch CJ. Leucine and protein metabolism in obese Zucker rats. *PLoS One* 2013; **8**: e59443 [PMID: 23527196 DOI: 10.1371/journal.pone.0059443]

97   **Peterson TR**, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS, Sabatini DM. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for

their survival. *Cell* 2009; **137**: 873-886 [PMID: 19446321 DOI: 10.1016/j.cell.2009.03.046]

98   **Shimkus KL**, Wudeck EV, Shirazi-Fard Y, Nilsson MI, Greene NP, Hogan HA, Fluckey JD. DEPTOR Expression Correlates with Muscle Protein Synthesis. *Int J Exerc Sci* 2013; 7 [DOI: 10.1249/01.mss.0000494112.29472.01]

99   **Deaver JW**, López SM, Ryan PJ, Nghiem PP, Riechman SE, Fluckey JD. Regulation of cellular anabolism by mTOR: Or how I learned to stop worrying and love translation. *Sports Health* 2020 [DOI: 10.1016/j.smhs.2020.11.003]

100   **Tremblay F**, Brûlé S, Hee Um S, Li Y, Masuda K, Roden M, Sun XJ, Krebs M, Polakiewicz RD, Thomas G, Marette A. Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. *Proc Natl Acad Sci USA* 2007; **104**: 14056-14061 [PMID: 17709744 DOI: 10.1073/pnas.0706517104]

101   **Gual P**, Le Marchand-Brustel Y, Tanti JF. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 2005; **87**: 99-109 [PMID: 15733744 DOI: 10.1016/j.biochi.2004.10.019]

102   **Sun XJ**, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, Cahill DA, Goldstein BJ, White MF. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 1991; **352**: 73-77 [PMID: 1648180 DOI: 10.1038/352073a0]

103   **Haruta T**, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, Olefsky JM, Kobayashi M. A rapamycin-sensitive pathway down-regulates insulin signaling *via* phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol* 2000; **14**: 783-794 [PMID: 10847581 DOI: 10.1210/mend.14.6.0446]

104   **Rice KM**, Turnbow MA, Garner CW. Insulin stimulates the degradation of IRS-1 in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 1993; **190**: 961-967 [PMID: 8382493 DOI: 10.1006/bbrc.1993.1143]

105   **Smith LK**, Vlahos CJ, Reddy KK, Falck JR, Garner CW. Wortmannin and LY294002 inhibit the insulin-induced down-regulation of IRS-1 in 3T3-L1 adipocytes. *Mol Cell Endocrinol* 1995; **113**: 73-81 [PMID: 8674815 DOI: 10.1016/0303-7207(95)03622-e]

106   **Smith LK**, Rice KM, Garner CW. The insulin-induced down-regulation of IRS-1 in 3T3-L1 adipocytes is mediated by a calcium-dependent thiol protease. *Mol Cell Endocrinol* 1996; **122**: 81-92 [PMID: 8898350 DOI: 10.1016/0303-7207(96)03875-0]

107   **Li J**, DeFea K, Roth RA. Modulation of insulin receptor substrate-1 tyrosine phosphorylation by an Akt/phosphatidylinositol 3-kinase pathway. *J Biol Chem* 1999; **274**: 9351-9356 [PMID: 10092613 DOI: 10.1074/jbc.274.14.9351]

108   **Shah OJ**, Wang Z, Hunter T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. *Curr Biol* 2004; **14**: 1650-1656 [PMID: 15380067 DOI: 10.1016/j.cub.2004.08.026]

109   **Harrington LS**, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR, Gout I, Downes CP, Lamb RF. The TSC1-2 tumor suppressor controls insulin-PI3K signaling *via* regulation of IRS proteins. *J Cell Biol* 2004; **166**: 213-223 [PMID: 15249583 DOI: 10.1083/jcb.200403069]

110   **Takano A**, Usui I, Haruta T, Kawahara J, Uno T, Iwata M, Kobayashi M. Mammalian target of rapamycin pathway regulates insulin signaling *via* subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. *Mol Cell Biol* 2001; **21**: 5050-5062 [PMID: 11438661 DOI: 10.1128/MCB.21.15.5050-5062.2001]

111   **Barbour LA**, McCurdy CE, Hernandez TL, Friedman JE. Chronically increased S6K1 is associated with impaired IRS1 signaling in skeletal muscle of GDM women with impaired glucose tolerance postpartum. *J Clin Endocrinol Metab* 2011; **96**: 1431-1441 [PMID: 21289241 DOI: 10.1210/jc.2010-2116]

112   **Khamzina L**, Veilleux A, Bergeron S, Marette A. Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. *Endocrinology* 2005; **146**: 1473-1481 [PMID: 15604215 DOI: 10.1210/en.2004-0921]

113   **Fu W**, Hall MN. Regulation of mTORC2 Signaling. *Genes (Basel)* 2020; **11** [PMID: 32899613 DOI: 10.3390/genes11091045]

114   **Sarbassov DD**, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 2004; **14**: 1296-1302 [PMID: 15268862 DOI: 10.1016/j.cub.2004.06.054]

115   **Guertin DA**, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev Cell* 2006; **11**: 859-871 [PMID: 17141160 DOI: 10.1016/j.devcel.2006.10.007]

116   **García-Martínez JM**, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J* 2008; **416**: 375-385 [PMID: 18925875 DOI: 10.1042/BJ20081668]

117   **Ikenoue T**, Inoki K, Yang Q, Zhou X, Guan KL. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. *EMBO J* 2008; **27**: 1919-1931 [PMID: 18566587 DOI: 10.1038/emboj.2008.119]

118   **Hresko RC**, Mueckler M. mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1

adipocytes. *J Biol Chem* 2005; **280**: 40406-40416 [PMID: 16221682 DOI: 10.1074/jbc.M508361200]

119   **Dibble CC**, Asara JM, Manning BD. Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1. *Mol Cell Biol* 2009; **29**: 5657-5670 [PMID: 19720745 DOI: 10.1128/MCB.00735-09]

120   **Jacinto E**, Loewith R, Schmidt A, Lin S, Rüegg MA, Hall A, Hall MN. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 2004; **6**: 1122-1128 [PMID: 15467718 DOI: 10.1038/ncb1183]

121   **Frias MA**, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, Sabatini DM. mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s. *Curr Biol* 2006; **16**: 1865-1870 [PMID: 16919458 DOI: 10.1016/j.cub.2006.08.001]

122   **Julien LA**, Carriere A, Moreau J, Roux PP. mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling. *Mol Cell Biol* 2010; **30**: 908-921 [PMID: 19995915 DOI: 10.1128/MCB.00601-09]

123   **Kumar A**, Harris TE, Keller SR, Choi KM, Magnuson MA, Lawrence JC Jr. Muscle-specific deletion of rictor impairs insulin-stimulated glucose transport and enhances Basal glycogen synthase activity. *Mol Cell Biol* 2008; **28**: 61-70 [PMID: 17967879 DOI: 10.1128/MCB.01405-07]

124   **Treins C**, Warne PH, Magnuson MA, Pende M, Downward J. Rictor is a novel target of p70 S6 kinase-1. *Oncogene* 2010; **29**: 1003-1016 [PMID: 19935711 DOI: 10.1038/onc.2009.401]

125   **Boulbes D**, Chen CH, Shaikenov T, Agarwal NK, Peterson TR, Addona TA, Keshishian H, Carr SA, Magnuson MA, Sabatini DM, Sarbassov dos D. Rictor phosphorylation on the Thr-1135 site does not require mammalian target of rapamycin complex 2. *Mol Cancer Res* 2010; **8**: 896-906 [PMID: 20501647 DOI: 10.1158/1541-7786.MCR-09-0409]

126   **Laaksonen DE**, Lakka HM, Niskanen LK, Kaplan GA, Salonen JT, Lakka TA. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. *Am J Epidemiol* 2002; **156**: 1070-1077 [PMID: 12446265 DOI: 10.1093/aje/kwf145]

127   **Lakka TA**, Laaksonen DE. Physical activity in prevention and treatment of the metabolic syndrome. *Appl Physiol Nutr Metab* 2007; **32**: 76-88 [PMID: 17332786 DOI: 10.1139/h06-113]

128   **Schwaab B**, Kafsack F, Markmann E, Schütt M. Effects of aerobic and anaerobic exercise on glucose tolerance in patients with coronary heart disease and type 2 diabetes mellitus. *Cardiovasc Endocrinol Metab* 2020; **9**: 3-8 [PMID: 32104785 DOI: 10.1097/XCE.0000000000000188]

129   **de Mello MA**, de Souza CT, Braga LR, dos Santos JW, Ribeiro IA, Gobatto CA. Glucose tolerance and insulin action in monosodium glutamate (MSG) obese exercise-trained rats. *Physiol Chem Phys Med NMR* 2001; **33**: 63-71 [PMID: 11758736]

130   **Holloszy JO**, Schultz J, Kusnierkiewicz J, Hagberg JM, Ehsani AA. Effects of exercise on glucose tolerance and insulin resistance. Brief review and some preliminary results. *Acta Med Scand Suppl* 1986; **711**: 55-65 [PMID: 3535414 DOI: 10.1111/j.0954-6820.1986.tb08932.x]

131   **Mikines KJ**, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am J Physiol* 1988; **254**: E248-E259 [PMID: 3126668 DOI: 10.1152/ajpendo.1988.254.3.E248]

132   **Mikines KJ**, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of training on the dose-response relationship for insulin action in men. *J Appl Physiol (1985)* 1989; **66**: 695-703 [PMID: 2651385 DOI: 10.1152/jappl.1989.66.2.695]

133   **Linke SE**, Gallo LC, Norman GJ. Attrition and adherence rates of sustained vs. intermittent exercise interventions. *Ann Behav Med* 2011; **42**: 197-209 [PMID: 21604068 DOI: 10.1007/s12160-011-9279-8]

134   **Yang Z**, Scott CA, Mao C, Tang J, Farmer AJ. Resistance exercise versus aerobic exercise for type 2 diabetes: a systematic review and meta-analysis. *Sports Med* 2014; **44**: 487-499 [PMID: 24297743 DOI: 10.1007/s40279-013-0128-8]

135   **Irvine L**, Barton GR, Gasper AV, Murray N, Clark A, Scarpello T, Sampson M. Cost-effectiveness of a lifestyle intervention in preventing Type 2 diabetes. *Int J Technol Assess Health Care* 2011; **27**: 275-282 [PMID: 22004767 DOI: 10.1017/S0266462311000365]

136   **Kelley GA**, Kelley KS, Hootman JM, Jones DL. Exercise and Health-Related Quality of Life in Older Community-Dwelling Adults: A Meta-Analysis of Randomized Controlled Trials. *J Appl Gerontol* 2009; **28**: 369-394 [DOI: 10.1177/0733464808327456]

137   **Miller WJ**, Sherman WM, Ivy JL. Effect of strength training on glucose tolerance and post-glucose insulin response. *Med Sci Sports Exerc* 1984; **16**: 539-543 [PMID: 6392812 DOI: 10.1249/00005768-198412000-00003]

138   **Ibañez J**, Izquierdo M, Argüelles I, Forga L, Larrión JL, García-Unciti M, Idoate F, Gorostiaga EM. Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. *Diabetes Care* 2005; **28**: 662-667 [PMID: 15735205 DOI: 10.2337/diacare.28.3.662]

139   **Fluckey JD**, Hickey MS, Brambrink JK, Hart KK, Alexander K, Craig BW. Effects of resistance exercise on glucose tolerance in normal and glucose-intolerant subjects. *J Appl Physiol (1985)* 1994; **77**: 1087-1092 [PMID: 7836108 DOI: 10.1152/jappl.1994.77.3.1087]

140   **De Filippis E**, Alvarez G, Berria R, Cusi K, Everman S, Meyer C, Mandarino LJ. Insulin-resistant muscle is exercise resistant: evidence for reduced response of nuclear-encoded mitochondrial genes to exercise. *Am J Physiol Endocrinol Metab* 2008; **294**: E607-E614 [PMID: 18182465 DOI:

10.1152/ajpendo.00729.2007]

141   **Katta A**, Kakarla S, Wu M, Paturi S, Gadde MK, Arvapalli R, Kolli M, Rice KM, Blough ER. Altered regulation of contraction-induced Akt/mTOR/p70S6k pathway signaling in skeletal muscle of the obese Zucker rat. *Exp Diabetes Res* 2009; **2009**: 384683 [PMID: 20368999 DOI: 10.1155/2009/384683]

142   **Wong TS**, Booth FW. Skeletal muscle enlargement with weight-lifting exercise by rats. *J Appl Physiol (1985)* 1988; **65**: 950-954 [PMID: 2459101 DOI: 10.1152/jappl.1988.65.2.950]

143   **Fluckey JD**, Vary TC, Jefferson LS, Farrell PA. Augmented insulin action on rates of protein synthesis after resistance exercise in rats. *Am J Physiol* 1996; **270**: E313-E319 [PMID: 8779954 DOI: 10.1152/ajpendo.1996.270.2.E313]

144   **Fluckey JD**, Vary TC, Jefferson LS, Evans WJ, Farrell PA. Insulin stimulation of protein synthesis in rat skeletal muscle following resistance exercise is maintained with advancing age. *J Gerontol A Biol Sci Med Sci* 1996; **51**: B323-B330 [PMID: 8808980 DOI: 10.1093/gerona/51a.5.b323]

145   **Dardevet D**, Sornet C, Vary T, Grizard J. Phosphatidylinositol 3-kinase and p70 s6 kinase participate in the regulation of protein turnover in skeletal muscle by insulin and insulin-like growth factor I. *Endocrinology* 1996; **137**: 4087-4094 [PMID: 8828461 DOI: 10.1210/endo.137.10.8828461]

146   **Graves LM**, Bornfeldt KE, Argast GM, Krebs EG, Kong X, Lin TA, Lawrence JC Jr. cAMP- and rapamycin-sensitive regulation of the association of eukaryotic initiation factor 4E and the translational regulator PHAS-I in aortic smooth muscle cells. *Proc Natl Acad Sci USA* 1995; **92**: 7222-7226 [PMID: 7638171 DOI: 10.1073/pnas.92.16.7222]

147   **Lin TA**, Kong X, Saltiel AR, Blackshear PJ, Lawrence JC Jr. Control of PHAS-I by insulin in 3T3-L1 adipocytes. Synthesis, degradation, and phosphorylation by a rapamycin-sensitive and mitogen-activated protein kinase-independent pathway. *J Biol Chem* 1995; **270**: 18531-18538 [PMID: 7629182 DOI: 10.1074/jbc.270.31.18531]

148   **von Manteuffel SR**, Gingras AC, Ming XF, Sonenberg N, Thomas G. 4E-BP1 phosphorylation is mediated by the FRAP-p70s6k pathway and is independent of mitogen-activated protein kinase. *Proc Natl Acad Sci USA* 1996; **93**: 4076-4080 [PMID: 8633019 DOI: 10.1073/pnas.93.9.4076]

149   **Kirwan JP**, Hickner RC, Yarasheski KE, Kohrt WM, Wiethop BV, Holloszy JO. Eccentric exercise induces transient insulin resistance in healthy individuals. *J Appl Physiol (1985)* 1992; **72**: 2197-2202 [PMID: 1629073 DOI: 10.1152/jappl.1992.72.6.2197]

150   **Fluckey JD**, Ploug T, Galbo H. Attenuated insulin action on glucose uptake and transport in muscle following resistance exercise in rats. *Acta Physiol Scand* 1999; **167**: 77-82 [PMID: 10519980 DOI: 10.1046/j.1365-201x.1999.00592.x]

151   **Nesher R**, Karl IE, Kipnis DM. Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *Am J Physiol* 1985; **249**: C226-C232 [PMID: 3898861 DOI: 10.1152/ajpcell.1985.249.3.C226]

152   **Ploug T**, Galbo H, Richter EA. Increased muscle glucose uptake during contractions: no need for insulin. *Am J Physiol* 1984; **247**: E726-E731 [PMID: 6391198 DOI: 10.1152/ajpendo.1984.247.6.E726]

153   **Richter EA**, Ploug T, Galbo H. Increased muscle glucose uptake after exercise. No need for insulin during exercise. *Diabetes* 1985; **34**: 1041-1048 [PMID: 3899806 DOI: 10.2337/diab.34.10.1041]

154   **Higaki Y**, Hirshman MF, Fujii N, Goodyear LJ. Nitric oxide increases glucose uptake through a mechanism that is distinct from the insulin and contraction pathways in rat skeletal muscle. *Diabetes* 2001; **50**: 241-247 [PMID: 11272132 DOI: 10.2337/diabetes.50.2.241]

155   **Lefort N**, St-Amand E, Morasse S, Côté CH, Marette A. The alpha-subunit of AMPK is essential for submaximal contraction-mediated glucose transport in skeletal muscle in vitro. *Am J Physiol Endocrinol Metab* 2008; **295**: E1447-E1454 [PMID: 18812461 DOI: 10.1152/ajpendo.90362.2008]

156   **Winder WW**, Hardie DG. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol* 1996; **270**: E299-E304 [PMID: 8779952 DOI: 10.1152/ajpendo.1996.270.2.E299]

157   **Ploug T**, Galbo H, Vinten J, Jørgensen M, Richter EA. Kinetics of glucose transport in rat muscle: effects of insulin and contractions. *Am J Physiol* 1987; **253**: E12-E20 [PMID: 3300362 DOI: 10.1152/ajpendo.1987.253.1.E12]

158   **Dela F**, Mikines KJ, Larsen JJ, Galbo H. Glucose clearance in aged trained skeletal muscle during maximal insulin with superimposed exercise. *J Appl Physiol (1985)* 1999; **87**: 2059-2067 [PMID: 10601150 DOI: 10.1152/jappl.1999.87.6.2059]

159   **Christ-Roberts CY**, Pratipanawatr T, Pratipanawatr W, Berria R, Belfort R, Mandarino LJ. Increased insulin receptor signaling and glycogen synthase activity contribute to the synergistic effect of exercise on insulin action. *J Appl Physiol (1985)* 2003; **95**: 2519-2529 [PMID: 12909611 DOI: 10.1152/japplphysiol.00605.2003]

160   **King PA**, Betts JJ, Horton ED, Horton ES. Exercise, unlike insulin, promotes glucose transporter translocation in obese Zucker rat muscle. *Am J Physiol* 1993; **265**: R447-R452 [PMID: 8368400 DOI: 10.1152/ajpregu.1993.265.2.R447]

161   **Brozinick JT Jr**, Etgen GJ Jr, Yaspelkis BB 3rd, Ivy JL. Contraction-activated glucose uptake is normal in insulin-resistant muscle of the obese Zucker rat. *J Appl Physiol (1985)* 1992; **73**: 382-387 [PMID: 1506395 DOI: 10.1152/jappl.1992.73.1.382]

162   **Angenstein F**, Evans AM, Settlage RE, Moran ST, Ling SC, Klintsova AY, Shabanowitz J, Hunt DF, Greenough WT. A receptor for activated C kinase is part of messenger ribonucleoprotein

complexes associated with polyA-mRNAs in neurons. *J Neurosci* 2002; **22**: 8827-8837 [PMID: 12388589 DOI: 10.1523/JNEUROSCI.22-20-08827.2002]

163   **Shih SC**, Mullen A, Abrams K, Mukhopadhyay D, Claffey KP. Role of protein kinase C isoforms in phorbol ester-induced vascular endothelial growth factor expression in human glioblastoma cells. *J Biol Chem* 1999; **274**: 15407-15414 [PMID: 10336429 DOI: 10.1074/jbc.274.22.15407]

164   **Itani SI**, Pories WJ, Macdonald KG, Dohm GL. Increased protein kinase C theta in skeletal muscle of diabetic patients. *Metabolism* 2001; **50**: 553-557 [PMID: 11319716 DOI: 10.1053/meta.2001.22512]

165   **Kanoh Y**, Sajan MP, Bandyopadhyay G, Miura A, Standaert ML, Farese RV. Defective activation of atypical protein kinase C zeta and lambda by insulin and phosphatidylinositol-3,4,5-(PO4)(3) in skeletal muscle of rats following high-fat feeding and streptozotocin-induced diabetes. *Endocrinology* 2003; **144**: 947-954 [PMID: 12586772 DOI: 10.1210/en.2002-221017]

166   **Cortright RN**, Azevedo JL Jr, Zhou Q, Sinha M, Pories WJ, Itani SI, Dohm GL. Protein kinase C modulates insulin action in human skeletal muscle. *Am J Physiol Endocrinol Metab* 2000; **278**: E553-E562 [PMID: 10710511 DOI: 10.1152/ajpendo.2000.278.3.E553]