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

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### 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

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**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



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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.

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## INTRODUCTION

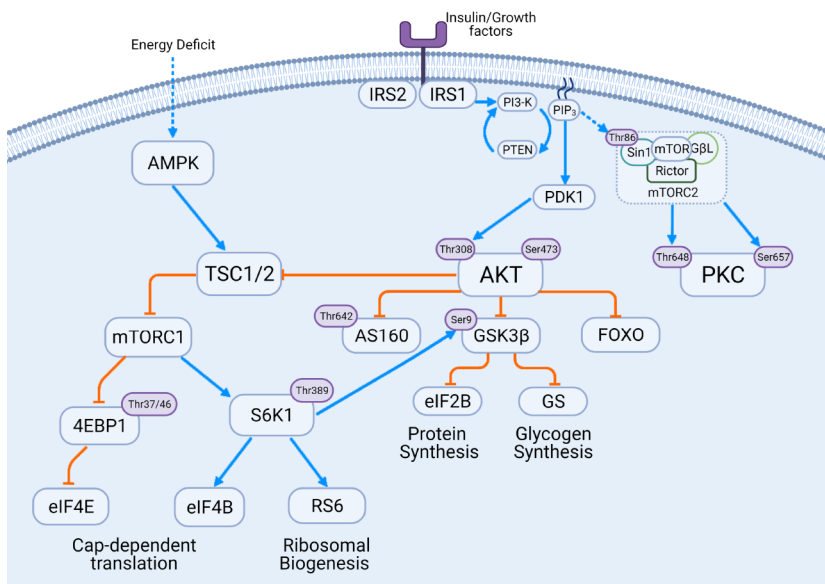
Globally, 462 million individuals are affected by type 2 diabetes (T2D) and it is ranked as the 9<sup>th</sup> leading cause of mortality[1]. The prevalence of diabetes over the past few decades has continued to rise with no sign of this changing[1]. T2D is characterized by insulin resistance and hyperglycemia 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-3 $\beta$ , 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, FOXO1/3a 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 FOXO 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-3 $\beta$  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-3 $\beta$  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 tuberlin-hamartin complex and it's activity[51-53]. Phosphorylation at this site releases the tuberlin-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.

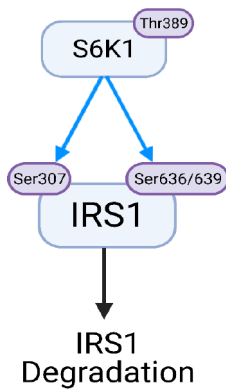
## CONNECTING ANABOLISM TO INSULIN RESISTANCE

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, I $\kappa$ B 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



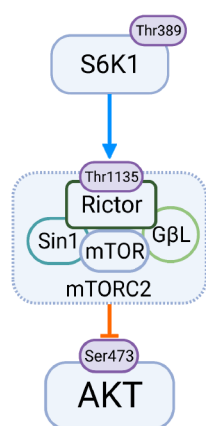
**Figure 2 Downstream mammalian target of rapamycin complex 1 substrate S6 kinase beta-1 phosphorylation of insulin receptor substrates 1 at Ser307 and Ser636/639 leads to insulin receptor substrates 1 degradation.** Blue arrows (→) indicate activation of the substrate, black arrow (→) indicates degradative 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 phosphorylation 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 ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) lead to attenuated 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 transduction and glucose uptake is complex. It is known that PKC $\alpha$  is a downstream substrate of mTORC2 at both its turn motif (Thr638) and is hydrophobic motif (Ser657) both of which are required for PKC $\alpha$  stability[40,116-119]. Deletion of RICTOR, abo-

lishes phosphorylation of the hydrophobic motif of PKC $\alpha$ [114,115] and deletion of either RICTOR or Sin1 dramatically reduces PKC $\alpha$  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.

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