

**Title: Adrenoceptor regulation of mTOR in muscle and adipose tissue**

*Running title:* Metabolic effects of mTOR in adrenoceptor signaling

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*Word count: 7256 words*

**Author contributions:**

All authors contributed to the writing of this review and provided critical feedback of the final manuscript

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bph.14616

## **Abstract:**

A vital role of adrenoceptors in metabolism and energy balance has been well-documented in heart, skeletal muscle, and adipose tissue. It has been only recently demonstrated, however, that activation of mechanistic/mammalian target of rapamycin (mTOR) makes a significant contribution to various metabolic and physiological responses to adrenoceptor agonists. mTOR exists as two distinct complexes named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), and has been shown to play a critical role in protein synthesis, cell proliferation, hypertrophy, mitochondrial function, and glucose uptake. This review will describe the physiological significance of mTORC1/2 as a novel paradigm of adrenoceptor signalling in heart, skeletal muscle and adipose tissue. Understanding the detailed signalling cascades of adrenoceptors and how they regulate physiological responses is important for identifying new therapeutic targets and identifying novel therapeutic interventions.

## **Keywords**

Adrenoceptor; mTOR; glucose uptake; GLUT4; skeletal muscle; cardiomyocyte

## **Abbreviations**

All nomenclature for drugs and molecular targets conforms to the BJP Concise Guide to Pharmacology (Alexander et al., 2017)

4E-BP1: eukaryotic translation initiation factor 4E binding protein-1

AMPK: 5'AMP-activated protein kinase

AR: adrenoceptor

AS160: Akt substrate of 160 kDa

BAT: brown adipose tissue

CHO-K1: Chinese hamster ovary-K1

DAG: diacylglycerol

DEPTOR: DEP domain-containing mTOR-interacting protein

eIF4E: eukaryotic translation initiation factor 4E

Erk1/2: extracellular signal-regulated kinase 1/2

ERR $\alpha$ : estrogen-related receptor  $\alpha$

GLUT4: glucose transporter 4

GPCR: G protein-coupled receptor

GRK: G protein receptor kinases

HIF1 $\alpha$ : hypoxia-induced factor 1 $\alpha$

hMADS: human multipotent adipose-derived stem

IGF1: insulin-like growth factor 1

IKK: inhibition of nuclear factor kappa-B kinase

IRS: insulin receptor substrate

LARP1: La-related protein 1

Lats2: large tumor suppressor kinase 2

MEK: mitogen-activated protein kinase kinase

MHC: myosin heavy chain

MLST8: mammalian lethal with SEC13 protein 8  
mSIN1: mammalian stress-activated protein kinase interacting protein 1  
Mst1: macrophage-stimulating 1  
mTOR: mechanistic/mammalian target of rapamycin  
mTORC1: mTOR complex 1  
mTORC2: mTOR complex 2  
NA: noradrenaline  
NRVM: neonatal rat ventricular myocytes  
PDK1: phosphoinositide-dependent kinase 1  
PGC-1 $\alpha$ : peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$   
PI3K: phosphatidylinositol 3-kinase  
PIP3: phosphatidylinositol 3,4,5 trisphosphate  
PIP3Ks: phosphatidylinositol-5-phosphate 4-kinases  
PKA: protein kinase A  
PKB: protein kinase B (Akt)  
PKC: protein kinase C  
PLC: phospholipase C  
PPAR $\alpha$ : peroxisome proliferator-activated receptor  $\alpha$   
PPAR- $\gamma$ : peroxisome proliferator-activated receptor  $\gamma$   
PRAS40: proline rich protein kinase B substrate of 40 kDa  
Protor 1/2: protein observed with Rictor 1 and 2  
Raptor: regulatory-associated protein of mTOR  
REPTOR: repressed by TOR  
Rheb: Ras homolog enriched in brain  
RhoA: Ras homolog gene family member A  
Rictor: rapamycin-insensitive companion of mTOR  
S6K1/2: ribosomal protein S6 kinase 1 and 2  
S6rp: S6 ribosomal protein  
SGK1: Serum and glucocorticoid-responsive kinase-1  
SREBP1/2: sterol regulatory element-binding protein 1 and 2  
Tel2: telomere maintenance 2  
TFEB: transcription factor EB  
TOP: terminal oligopyrimidine  
TSC1/2: tuberous sclerosis complex  
Ttll1: Tel2-interacting protein 1  
UCP1: uncoupling protein 1  
ULK1: Unc-51 like autophagy activating kinase  
UPS: ubiquitin-proteasome system  
WAT: white adipose tissue  
Xpln: exchange factor found in platelets, leukemic, and neuronal tissues

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Adrenoceptors:

<http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=4>

G protein-coupled receptor (GPCR):

<http://www.guidetopharmacology.org/GRAC/ReceptorFamiliesForward?type=GPCR>

Adrenaline:

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Noradrenaline:

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Isoprenaline:

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$\beta_1$ -AR:

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$\beta_2$ -AR:

<http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=29&familyId=4&familyType=GPCR>

$\beta_3$ -AR:

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Mechanistic target of rapamycin (mTOR):

<http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2109>

Phosphatidylinositol 3-kinase (PI3K):

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5' AMP-activated protein kinase (AMPK):

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Insulin: <http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5012>

Akt: <http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=285>

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Protein kinase C $\alpha$  (PKC $\alpha$ ):

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Wortmannin:

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LY294002:

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Akt inhibitor X:

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KU0063794:

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ICI118551:

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Dexamethasone:

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A61603: <http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=480>

Dabuzalgron:

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Rottlerin:

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uncoupling protein (UCP):

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Mirabegron:

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Torin-1:

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## Introduction

Adrenoceptors belong to the G protein-coupled receptor (GPCR) family, a conserved family of seven transmembrane receptors that is one of the largest protein classes to be targeted for drug therapy (Sriram and Insel, 2018). ARs are classified as  $\alpha$ - or  $\beta$ -ARs, based on differences in responses to various catecholamines such as adrenaline, noradrenaline, and isoprenaline.  $\alpha$ -ARs have been classified into 2 major families:  $\alpha_1$ - and  $\alpha_2$ -AR, and  $\beta$ -ARs are subdivided into  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ -ARs. All AR subtypes have common primary structures comprising one extracellular N-terminal domain, seven  $\alpha$ -helical transmembrane spanning regions, and one intracellular C-terminal tail. Recent studies have shown that  $\alpha_1$ -ARs and  $\beta_1$ -ARs in the heart,  $\beta_2$ -ARs in skeletal muscle, and  $\beta_3$ -ARs in brown adipose tissue can link to mechanistic target of rapamycin (mTOR), which plays a significant role in physiological and metabolic responses.

mTOR is an atypical serine/threonine kinase with a molecular weight of ~289kDa, belonging to the phosphatidylinositol 3-kinase (PI3K)-related kinase family. mTOR interacts with other molecular components to form two physically and functionally distinct complexes, namely mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). In mTORC1, the mTOR protein interacts with regulatory-associated protein of mTOR (Raptor) (Kim et al., 2002), proline rich protein kinase B (Akt) substrate of 40 kDa (PRAS40) (Sancak et al., 2007), mammalian lethal with SEC13 protein 8 (MLST8) (Kim et al., 2003), DEP domain-containing mTOR-interacting protein (DEPTOR) (Peterson et al., 2009), Tel two interacting protein 1 (Tti1) and telomere maintenance 2 (Tel2) (Kaizuka et al., 2010). On the other hand, mTORC2 comprises mTOR, the scaffold protein rapamycin-insensitive companion of mTOR (Rictor) (Sarbasov et al., 2004), mammalian stress-activated protein kinase interacting protein 1 (mSIN1) (Jacinto et al., 2006), protein observed with Rictor 1 and 2 (Protor 1, Protor 2) (Pearce et al., 2007), MLST8 (Kim et al., 2003), DEPTOR (Peterson et al., 2009), inhibitor of nuclear factor kappa-B kinase (IKK) (Xu et al., 2013), Sestrin 3 (Tao et al., 2015), exchange factor found in platelets, leukemic, and neuronal tissues (Xpln) (Khanna et al., 2013), tuberous sclerosis complex 2 (TSC2) (Huang et al., 2008), Tel2 and Tti1 (Kaizuka et al., 2010).

While remarkable progress has been made in understanding the role of mTORC1, the contributions of mTORC2 are less well-understood. Collectively, many studies have demonstrated that mTORC1 plays a vital role in the regulation of cellular homeostasis, growth and response to stress. mTORC1 activated under nutrient-replete conditions promotes protein synthesis by several complementary mechanisms. Firstly, mTORC1 activates the ribosomal protein S6 kinase 1 and 2 (S6K1/2), which in turn activates the protein translation process (Laplante and Sabatini, 2013; Saxton and Sabatini, 2017). In parallel, mTORC1 inhibits eukaryotic translation initiation factor 4E (eIF4E)-binding protein-1 (4E-BP1) and thus allows the formation of the eIF4F complex that triggers cap-dependent translation (Kennedy and Lamming, 2016; Laplante and Sabatini, 2013; Saxton and Sabatini, 2017). Finally mTORC1 boosts translation by phosphorylation and consequent inactivation of the target La-related protein 1 (LARP1) (Fonseca et al., 2015). When active, LARP1 represses translation of terminal oligopyrimidine (5'TOP) mRNAs that encode ribosomal proteins and positive regulators of translation.

mTORC1 modulates cell metabolism, as it increases glycolysis by promoting transcription and translation of hypoxia-induced factor 1 $\alpha$  (HIF1 $\alpha$ ) (Hudson et al., 2002). It also activates the transcription factor sterol regulatory element-binding protein 1 and 2 (SREBP1/2) which promotes lipogenesis (Kennedy and Lamming, 2016; Laplante and Sabatini, 2013; Saxton and Sabatini, 2017). Further, mTORC1 plays a role in mitochondrial biogenesis through peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ )-mediated activation of the transcription factor Ying-Yang 1 (Cunningham et al., 2007; Laplante and Sabatini, 2012). When cells are subjected to stress or nutrient starvation, they undergo a regulated catabolic process termed autophagy. Another well-characterised role of mTORC1 is the inhibition of autophagy under nutrient replete conditions. mTORC1 phosphorylates Unc-51 like autophagy activating kinase (ULK1), preventing its activation via 5'AMP-activated protein kinase (AMPK), which in turn inhibits autophagy (Kim et al., 2011). The phosphorylation and nuclear translocation of the transcription factor EB (TFEB), which regulates the expression of proteins governing autophagy and lysosomal biogenesis, is inhibited by mTORC1 (Settembre et al., 2012). Recent studies have demonstrated that mTORC1 also contributes to protein turnover via the ubiquitin-proteasome system (UPS). Acute inhibition of mTORC1 increases proteasome-dependent proteolysis (Rousseau and Bertolotti, 2016; Zhao et al., 2015a). Interestingly, long-term activation of mTORC1 in mouse embryonic fibroblasts due to

deletion of inhibitory Tsc2 also increases proteasome activity (Zhang et al., 2014). This finding was replicated in a mouse model of neuronal Tsc2 deletion, and in liver of wild type mice subject to fasting then 6 h refeeding. The authors suggest that longer-term activation of proteasomal pathways by mTORC1 is an adaptive response that supports protein synthesis by replenishing the cellular amino acid pool (Zhang et al., 2014). Two further mTORC1 targets have been identified, (i) the  $\gamma$  isoform of phosphatidylinositol-5-phosphate 4-kinase (PIPK) maintains basal mTORC1 signalling during starvation (Mackey et al., 2014), and (ii) REPTOR is a downstream effector of TORC1 in *Drosophila melanogaster* (Tiebe et al., 2015). When TORC1 phosphorylates REPTOR, it leads to cytoplasmic retention, in contrast, upon inhibition of TORC1, REPTOR is dephosphorylated, translocates into the nucleus and activates transcription of target genes involved in energy homeostasis and cellular survival under conditions of nutrient starvation (Tiebe et al., 2015).

Compared to mTORC1, studies and knowledge of mTORC2 regulation and function have lagged behind. One well-characterised role of mTORC2 is its response to growth factors and insulin via PI3K-dependent mechanisms (Gan et al., 2011). mTORC2 directly phosphorylates Akt at Ser473, which is facilitated by prior phosphorylation of Thr308 by phosphoinositide-dependent kinase 1 (PDK1), as part of the insulin cascade (Sarbasov et al., 2005). mTORC2 can modulate protein kinase C $\alpha$  (PKC $\alpha$ ) activity and thereby play a role in remodelling of the actin cytoskeleton (Sarbasov et al., 2004). Similarly, a study by Jacinto and co-workers (Jacinto et al., 2004) demonstrated that mTORC2 regulates cell polarity and cytoskeletal organisation through the regulation of PKC $\alpha$  and Ras homolog gene family member A (RhoA). mTORC2 has also been demonstrated to regulate other PKC family members, including protein kinase C $\delta$  (PKC $\delta$ ) (Gan et al., 2012) and protein kinase C $\zeta$  (PKC $\zeta$ ) (Li and Gao, 2014). Hydrophobic motif phosphorylation and activation of PKC $\delta$  plays a vital role in fibroblast migration and pulmonary fibrosis development (Gan et al., 2012) whereas mTORC2 modulation of PKC $\zeta$  activity is involved in organisation of the actin cytoskeleton (Li and Gao, 2014). Sciarretta *et al* (Sciarretta et al., 2015) conducted a study showing that mTORC2 negatively regulates the activity of MST1, as disruption of Rictor/mTORC2 leads to a significant activation of MST1. This marked MST1 activation promotes cardiac dilation, cardiac dysfunction, impaired cardiac growth and adaptation in response to pressure overload.

While mTORC1 and mTORC2 both have distinct functions, there is evidence that these two complexes are interconnected. S6K1, downstream of mTORC1, directly phosphorylates rictor of mTORC2 and promotes a negative regulatory effect on the mTORC2-dependent phosphorylation of Akt Ser473 (Dibble et al., 2009). mTORC2-activated Akt, in contrast, enhances mTORC1 activity through the inactivation of tuberous sclerosis complex (TSC1/2), a complex that inhibits mTORC1 via GTPase-activating protein activity toward Ras homolog enriched in brain (Rheb) (Dibble et al., 2012).

We will discuss the manner in which current knowledge of mTOR relates to recent studies demonstrating that adrenoceptor agonists increase activation of mTORC1-mediated cell growth, and also mTORC2-mediated glucose uptake and cell survival *in vivo* and *in vitro* (Olsen et al., 2014; Sato et al., 2014a; Sato et al., 2018). We have focused this review on the interplay between ARs and mTOR in skeletal and cardiac muscle as well as adipose tissue, in light of our own expertise and the need to assimilate considerable information that is now available for these tissues. However, given the ubiquitous expression of mTOR and its partner proteins, as well as widespread expression of different AR subtypes, it is highly likely that AR-mTOR pathways are important in additional cell types. For example there are a number of studies linking activation of hippocampal  $\beta$ -ARs with mTOR-dependent increases in protein translation (Connor et al., 2011; Gelinas et al., 2007). These mechanisms are critical for long-term potentiation and memory consolidation.

### **Role of $\beta_2$ -AR mediated mTOR activation in skeletal muscle**

Skeletal muscle comprises up to 50% of total body mass, consumes a significant proportion of metabolic fuel, and has a major role in whole body metabolic homeostasis, being responsible for 75% of insulin-mediated glucose uptake and utilisation in the fed state. There is evidence showing that the sympathetic nervous system promotes glucose uptake in active skeletal muscle (for example during exercise, fight or flight responses), which results primarily from noradrenaline (NA) release from adrenergic nerve terminals, acting on  $\beta$ -ARs at the cell surface (Nonogaki, 2000). Skeletal muscle expresses abundant  $\beta$ -ARs that are predominantly  $\beta_2$ -ARs, with 7–10%  $\beta_1$ -ARs and no detectable  $\beta_3$ -ARs (Nevzorova et al., 2002). Stimulation with the  $\beta$ -AR agonist isoprenaline promotes glucose uptake in L6 myoblasts and myotubes, and intact skeletal muscle *in vitro* and *in vivo* (Nevzorova et al., 2006; Sato et al., 2014a). Notably, isoprenaline increases glucose uptake to a greater extent than insulin *in vivo* in wild type mice, but not in  $\beta_1\beta_2$ -AR knockout mice (Sato et al., 2014a),

consistent with another study showing that mice lacking all three  $\beta$ -ARs display glucose intolerance (Asensio et al., 2005).

Insulin stimulates skeletal muscle glucose uptake by activating signalling steps that increase the translocation of GLUTs from intracellular vesicles to the cell surface. Following insulin-mediated increases in PI3K activity, phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>) recruits PDK1 and inactive Akt to the plasma membrane via N-terminal PH domains, facilitating Akt phosphorylation at Thr308 by PDK1. In parallel mTORC2 is phosphorylated *via* unknown mechanisms. A conformational change in Akt associated with phosphorylation of Thr308 enables mTORC2 to phosphorylate Akt at Ser473, leading to full activation. Akt promotes subsequent phosphorylation of the Rab GTPase-activating protein AS160 at Thr642, which is critical for insulin-increased GLUT4 translocation (Figure 1). Our previous studies showed that isoprenaline-stimulated glucose uptake in L6 muscle cells was markedly reduced by the PI3K inhibitors PI-103, wortmannin, and LY294002 (Sato et al., 2014a), suggesting that insulin receptor and  $\beta_2$ -AR mediated glucose uptake may share a common signalling pathway. Unlike responses we observed to insulin, however, there was no Akt phosphorylation at Thr308 or Ser473, or AS160 phosphorylation at Thr642 upon isoprenaline treatment, nor any increase in PIP<sub>3</sub> levels, and glucose uptake was not inhibited by Akt inhibitor X (Nevzorova et al., 2002; Nevzorova et al., 2006; Sato et al., 2014a). Earlier studies demonstrated that PI-103 and other widely-used PI3K inhibitors including wortmannin and LY294002 have substantial affinity for related kinases including mTOR (Brunn et al., 1996; Knight et al., 2006). It is thus clearly important to consider the involvement of mTOR as well as PI3K when interpreting inhibitory effects of LY294002, wortmannin or PI-103 on downstream signalling outputs. In light of this we found that the highly specific mTOR inhibitor KU0063794 (Sato et al., 2014a) inhibited both isoprenaline and insulin-stimulated glucose uptake indicating that mTOR is involved in adrenergic stimulated glucose uptake. The combined results show that the pathways shared by insulin and isoprenaline overlap at a more downstream point leading to mTOR activation, and the  $\beta_2$ -AR associated pathway does not include PI3K or Akt. siRNA knockdown of mTORC2 (rictor), but not mTORC1 (raptor), markedly inhibits both insulin-mediated and  $\beta_2$ -AR mediated glucose uptake (Sato et al., 2014a). In addition, in muscle lacking rictor, insulin-stimulated Akt phosphorylation at Ser473 and AS160 at Thr642 are dramatically decreased, and muscle-specific rictor knockout mice display glucose intolerance and decreased insulin-stimulated glucose uptake (Kumar et al., 2008). This confirms mTORC2 as a key regulator of glucose uptake in skeletal muscle.

Confirming this we found that KU0063794 also inhibits  $\beta_2$ -AR mediated skeletal muscle glucose uptake *ex vivo* and *in vivo* (Sato et al., 2014a).

The  $\beta_2$ -AR couples primarily to G $\alpha$ s proteins, activating adenylyl cyclase to increase intracellular cAMP levels, resulting in protein kinase A (PKA) activation.  $\beta_2$ -AR stimulation can also cause cellular effects independently of this classical cAMP-PKA pathway. After agonist stimulation, the  $\beta_2$ -AR is rapidly phosphorylated by G protein receptor kinases (GRKs), allowing recruitment of  $\beta$ -arrestins (which uncouple the receptor from its G $\alpha$  protein partners), receptor internalization, and activation of  $\beta$ -arrestin-mediated signalling pathways (Tobin et al., 2008). The signalling effectors linking the  $\beta_2$ -AR with activation of mTORC2 are still unknown but may be downstream of PKA as the selective PKA inhibitor PKI decreases isoprenaline-induced mTORC2 phosphorylation, and 8-bromo-cAMP increases mTORC2 phosphorylation (Sato et al., 2014a). Interestingly,  $\beta_2$ -AR stimulated glucose uptake is only partly dependent on cAMP (Nevzorova et al., 2002; Nevzorova et al., 2006; Sato et al., 2014a), suggesting contributions from alternative effectors that are cAMP-independent. Involvement of GRK2 in  $\beta_2$ -AR mediated glucose homeostasis has been suggested as one possible mechanism (Dehvari et al., 2012). Chinese hamster ovary-K1 (CHO-K1) cells stably expressing the human glucose transporter 4 (GLUT4) carrying an exofacial c-Myc epitope (CHO-GLUT4myc) were transfected with wild type or a truncated  $\beta_2$ -AR lacking the entire C-terminal tail, or co-transfected with wild type  $\beta_2$ -AR and  $\beta$ ARKct, which sequesters G $\beta\gamma$  subunits required for GRK2 recruitment to the plasma membrane. Cells expressing wild type  $\beta_2$ -AR plus  $\beta$ ARKct, or the truncated receptor alone, showed markedly reduced isoprenaline-stimulated glucose uptake compared with cells expressing the wild type  $\beta_2$ -AR only. In addition, CHO-GLUT4myc cells expressing a kinase-dead GRK2 K220R mutant displayed significantly decreased GLUT4 translocation to the cell surface (Dehvari et al., 2012). Collectively, our studies indicate the potential role of GRK2 and PKA as upstream kinases of mTORC2 following activation of  $\beta_2$ -AR.

$\beta_2$ -AR mediated glucose uptake is blocked by GLUT inhibitors and by pretreatment with GLUT4 siRNA (Sato et al., 2014a). Type 2 diabetes is closely associated with defects in insulin signalling mechanisms involving insulin receptor substrates (Chakrabarti et al., 2013), PI3K activity and Akt phosphorylation (Cusi et al., 2000), but  $\beta_2$ -ARs expressed in skeletal muscle could bypass these defects through mTORC2-mediated regulation of

GLUT4 trafficking, providing a compensatory pathway following loss of insulin sensitivity (Sato et al., 2014a; Sato et al., 2014b). This is of particular interest considering that  $\beta_2$ -AR expression is unaltered in skeletal muscle from diabetic patients (Frederiksen et al., 2008).

Aside from being important for glucose uptake,  $\beta_2$ -AR stimulated cAMP accumulation can have long-term effects on muscle phenotype (Pearen et al., 2009). Chronic stimulation of skeletal muscle  $\beta_2$ -ARs utilizing agonists such as clenbuterol, fenoterol and formoterol can activate anabolic signalling pathways, leading to increased muscle mass and force-producing capacity (Lynch and Ryall, 2008) The anabolic and anti-catabolic processes in response to  $\beta_2$ -AR agonists occur via protein translation and synthesis mediated by the Akt-mTOR-S6 kinase signalling axis (Hagg et al., 2016)(Figure 1). Chronic stimulation of  $\beta_2$ -ARs increases the transcription of peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ), which is associated with the suppression of myostatin, and these effects are blocked by ICI118,551, a highly selective  $\beta_2$ -AR antagonist (Jesinkey et al., 2014). Treatment of mice with formoterol stimulates small but significant increases in the phosphorylation of Akt and mTOR in gastrocnemius muscle after 8 hours, differing in time-frame from more acute measurements of Akt / mTOR phosphorylation and glucose uptake (10 mins – 2h) (Sato et al., 2014a). Dexamethasone-induced muscular atrophy and slow-to-fast myosin heavy chain (MHC) isoform transition is antagonized by the  $\beta_2$ -AR agonist clenbuterol, which stimulates Akt and mTORC1 activity, and insulin-like growth factor 1 (IGF1) expression (Jesinkey et al., 2014). These finding could potentially provide a new basis for a pharmacological approach to target mTOR for the treatment of conditions involving muscle loss.

### **The role of adrenoceptor-mediated mTOR activation in heart**

#### $\alpha_1$ -ARs and mTOR in the heart

Cardiac function is tightly regulated via both  $\alpha_1$ - and  $\beta$ -ARs, due to release of noradrenaline from sympathetic nerve terminals innervating the heart, and by circulating adrenaline released from the adrenal gland in response to danger or stress.  $\beta$ -ARs comprise roughly 90% of total cardiac ARs, and  $\alpha_1$ -ARs account for the remaining 10%. In heart failure, unlike  $\beta_1$ -ARs,  $\alpha_1$ -ARs are not downregulated and may therefore play an enhanced role in regulating cardiac contractility (Skomedal et al., 1997). Although all three  $\alpha_1$ -AR subtype mRNAs are detected in heart of mice and rats, cardiomyocytes express only the  $\alpha_{1A}$ - and  $\alpha_{1B}$ - subtypes

(O'Connell et al., 2003) while  $\alpha_{1D}$ -ARs are confined to the coronary vasculature (McCloskey et al., 2003; O'Connell et al., 2003). Due to the putative enhanced role in heart failure,  $\alpha_1$ -AR function and signalling are therefore of particular interest.

A series of knockout mouse studies indicate that neither  $\alpha_{1A}$ - nor  $\alpha_{1B}$ -AR subtypes are required for basal contractile function (O'Connell et al., 2003; Vecchione et al., 2002). However, cardiomyocyte-specific over-expression of the  $\alpha_{1A}$ -AR enhances basal contractile function (Lin et al., 2001) and reduces adverse remodelling following pressure overload (Du et al., 2004; Du et al., 2006). These results are consistent with an *in vitro* study by Mohl *et al* (Mohl et al., 2011), identifying an  $\alpha_{1A}$ -AR mediated signalling pathway that increases calcium entry and cardiomyocyte contractility. In contrast, over-expression of the  $\alpha_{1B}$ -AR causes depressed contractile function and pathologic remodelling in the heart (Lemire et al., 2001; Wang et al., 2000). The capacity of  $\alpha_{1A}$ -ARs to increase contractile function may have important compensatory roles in the failing heart.

In addition to maintaining myocyte contractility, activation of  $\alpha_1$ -ARs promotes glucose uptake (Shi et al., 2016), receptor-mediated preconditioning, cardiac hypertrophy, and inhibition of cardiomyocyte apoptosis (Jensen et al., 2011; O'Connell et al., 2014).  $\alpha_1$ -ARs are expressed in human myocardium, and are not down-regulated in heart failure (Jensen et al., 2009), and blockade of  $\alpha_1$ -ARs worsens heart failure (Dhaliwal et al., 2009; Jensen et al., 2011). In murine cardiac myocytes that express endogenous  $\alpha_{1A}$  and  $\alpha_{1B}$ -ARs, long-term agonist treatment increases  $\alpha_{1A}$ -AR abundance without desensitisation of inotropic effects, while increased stimulation or expression of the  $\alpha_{1A}$ -AR but not the  $\alpha_{1B}$ -AR *in vivo* limits global cardiac remodelling and reduces mortality from heart failure (Du et al., 2006; Rorabaugh et al., 2005). In a transgenic rat model that over-expresses the cardiomyocyte  $\alpha_{1A}$ -AR, animals are protected from heart failure by increased angiogenesis associated with secretion of vascular endothelial growth factor from cardiomyocytes (Zhao et al., 2015b).

*In vitro* and *in vivo* studies have indicated that  $\alpha_1$ -AR stimulation reduces cardiomyocyte cell death. Hypoxia-, serum starvation- and isoprenaline-induced apoptosis can be inhibited by exposure of cardiomyocytes to phenylephrine, a non-selective  $\alpha_1$ -AR agonist. This phenylephrine cytoprotective effect was blocked by phentolamine and prazosin (Iwai-Kanai et al., 1999). Cardiomyocytes from  $\alpha_{1A}/\alpha_{1B}$ -AR knockout mice display significantly

increased necrosis and apoptosis when subject to toxic stimuli such as doxorubicin or H<sub>2</sub>O<sub>2</sub> (Huang et al., 2007; O'Connell et al., 2006), and this sensitivity can be reduced by re-expression of  $\alpha_{1A}$ -ARs but not  $\alpha_{1B}$ -ARs (Huang et al., 2007). The chemotherapeutic agent doxorubicin produces cardiotoxic effects in patients and in animal models. In mice, long-term *in vivo* infusion of the  $\alpha_{1A}$ -AR agonist A61603 protects cardiomyocytes against apoptosis and reduces adverse ventricular remodelling and myocardial fibrosis following doxorubicin treatment, thereby improving cardiac function (Chan, 2008; Montgomery et al., 2017). These protective effects of A61603 are not observed in  $\alpha_{1A}$ -AR knockout mice. Another study showed that dabuzalgron, an orally-available, selective  $\alpha_{1A}$ -AR agonist also increases survival and preserves fractional shortening in wild type but not in  $\alpha_{1A}$ -AR knockout mice (Beak et al., 2017). All of these studies indicate that  $\alpha_1$ -AR could be an important target in the failing heart.

The non-selective  $\alpha_1$ -AR agonist phenylephrine is a well-known hypertrophic agent in the heart, and has been linked to activation of the mTORC1 target S6K1 (Boluyt et al., 1997). Treatment of neonatal rat ventricular myocytes (NRVMs) with phenylephrine stimulated the activity of S6K1, increased protein synthesis, and produced a 50% increase in cardiomyocyte area. Phenylephrine-induced S6K1 activity and hypertrophy were significantly reduced by the mTORC1 inhibitor rapamycin, and by the PI3K inhibitor LY294002, however the authors acknowledge that compounds such as LY294002 affect other PI3K-related kinases (Boluyt et al., 1997). As outlined in the skeletal muscle section of this review, PI3K inhibitors including wortmannin and LY294002 have substantial activity at mTOR (Brunn et al., 1996; Knight et al., 2006). Thus studies in which LY294002 is used as a sole PI3K inhibitor should be regarded with caution. Taken together, these results suggest that phenylephrine activates S6K1 and promotes cardiomyocyte hypertrophy *via* mTORC1 and possibly PI3K. We have shown recently that treatment of NRVMs with the highly selective  $\alpha_{1A}$ -AR agonist A61603 increases S6RP phosphorylation, a downstream target of mTORC1 and S6K1, and this is inhibited by rapamycin. NRVM hypertrophy observed in response to A61603 was prevented by the mTOR inhibitor KU0063794, which blocks the phosphorylation and activation of both mTORC1 and mTORC2 (Sato et al., 2018). It is thus clear that  $\alpha_1$ -ARs stimulate mTORC1 and that this could be an important player in the ability of  $\alpha_1$ -ARs to protect the heart.

Phenylephrine stimulates activation of S6K1 and phosphorylation of 4E-BP1 in adult cardiomyocytes (Wang and Proud, 2002). The latter protein interacts with eukaryotic translation initiation factor 4E (eIF4E) and represses translation. Phosphorylation of 4E-BP1 results in its dissociation from eIF4E and activation of mRNA translation. The response to phenylephrine was blocked by MEK inhibitors, and adenoviral expression of constitutively-active MEK caused activation of S6K1, phosphorylation of 4E-BP1 and activation of protein synthesis in a rapamycin-sensitive manner. This study provides insight into a signalling pathway involving Ras, MEK and mTOR (Wang and Proud, 2002). Phenylephrine also activates S6K2 in adult rat ventricular cardiomyocytes. Both MEK1/2 inhibitors and rapamycin abolished phenylephrine-induced activation of S6K2, and the expression of constitutively-active MEK1 activated S6K2. This indicates that MEK/Erk1/2 in combination with mTOR signalling plays a role in regulating phenylephrine-induced S6K2 activation (Wang et al., 2001).

Although the classic  $\alpha_1$ -AR signalling pathway includes  $\text{Ca}^{2+}$ -dependent PKC, phenylephrine also regulates S6K1/2 and 4E-BP1 (downstream substrates of mTORC1) leading to protein synthesis in a  $\text{Ca}^{2+}$ -independent PKC manner in adult cardiomyocytes (Wang et al., 2003). The classical  $\text{Ca}^{2+}$ -dependent PKC $\alpha$  and the  $\text{Ca}^{2+}$ -independent PKC $\delta$  and PKC $\epsilon$  are readily detected in adult cardiomyocytes (Puceat et al., 1994; Steinberg et al., 1995). In addition,  $\text{Ca}^{2+}$ -independent PKC is also required for the phenylephrine-induced ERK1/2 activation demonstrated by the significantly reduced Erk1/2 activation in the presence of the broad-spectrum PKC inhibitor BIM I (Toullec et al., 1991). Rottlerin (Gschwendt et al., 1994), a selective inhibitor of PKC $\delta$  almost completely inhibited the phenylephrine-induced Erk1/2 phosphorylation, while Gö6979 (Martiny-Baron et al., 1993), an inhibitor of  $\text{Ca}^{2+}$  dependent PKC has no obvious effect on Erk1/2 activation. Furthermore, Rottlerin prevented phenylephrine-induced S6K activation whereas Gö6979 had no apparent effects. Phosphorylation of 4E-BP1 was also inhibited by Rottlerin in a similar manner (Wang et al., 2003). These data suggest  $\text{Ca}^{2+}$ -independent PKC isoforms play a vital role in  $\alpha_1$ -AR mediated mTOR signalling in adult cardiomyocytes.

While mTORC1 plays an important role in cardiomyocyte hypertrophy, there is convincing evidence that mTORC2 promotes cardiomyocyte development and survival (Gonzalez-Teran et al., 2016; Shende et al., 2016; Xu and Brink, 2016). For example, mice with

cardiomyocyte-specific knockdown of rictor and thus disruption of mTORC2 display abnormalities by the age of 6 months, including cardiac dilation, fibrosis and exacerbated heart failure in response to pressure overload (Sciarretta et al., 2015; Yano et al., 2014). Following ischemic preconditioning, activation of mTORC2 promotes cardiomyocyte survival in part by suppressing activity of the kinase Mst1 (Lats2), a key component of the Hippo pathway that promotes apoptosis and inhibits cell growth (Sciarretta et al., 2015; Yano et al., 2014). Importantly, cardiomyocytes that are rictor-deficient or over-express Mst1 display increased cell death. In the study by Shende et al (2016), tamoxifen-inducible cardiomyocyte-specific rictor knockdown was used to allow normal cardiac development. Mice in which Cre recombinase expression was induced at 4 or 10 weeks of age displayed normal cardiac size and echocardiography up to 44 weeks after tamoxifen treatment, but transverse aortic constriction and resultant pressure overload caused more pronounced cardiac dysfunction than in wild type mice, indicating the importance of mTORC2 in failing heart (Shende et al., 2016; Volkers et al., 2013).

Cardiac  $\alpha_1$ -ARs have been linked with mTOR in exerting cardioprotective effects. Serum and glucocorticoid-responsive kinase-1 (SGK1) is a downstream substrate of mTORC2 (Garcia-Martinez and Alessi, 2008) that regulates cardiomyocyte survival and hypertrophy in response to the non-selective  $\alpha_1$ -AR agonist phenylephrine, both *in vivo* and *in vitro* (Aoyama et al., 2005). Cardiomyocytes infected with an adenoviral vector encoding constitutively active SGK1 show reduced apoptosis after serum- or oxygen-deprivation, and increased [<sup>3</sup>H]-leucine incorporation in response to phenylephrine, while expression of kinase-dead SGK1 increases apoptosis. SGK1 has also been placed downstream of PI3K (Park et al., 1999), though again inhibition of mTOR may have confounded the interpretation of these experiments involving the use of LY294002 as a PI3K inhibitor.

We have demonstrated that noradrenaline and the  $\alpha_{1A}$ -AR agonist A61603 increase glucose uptake in NRVMs by parallel activation of 5'AMP-activated protein kinase (AMPK) and mTORC2, but do not promote phosphorylation of Akt at Thr308 or Ser473 (Sato et al., 2018). The lack of Akt phosphorylation mirrors similar findings by (Wang et al., 2001), who demonstrated using adult cardiomyocytes that phenylephrine does not produce Akt phosphorylation at Ser473, and that adenoviral expression of a dominant-negative Akt mutant fails to block activation of S6K2 by phenylephrine. We found that the mTORC1/2 inhibitor

KU0063794 partially reduced  $\alpha_{1A}$ -AR and insulin-stimulated glucose uptake in cardiomyocytes, whereas the mTORC1 inhibitor rapamycin had no effect. A61603 stimulated the phosphorylation of mTOR at Ser2448 and Ser2481. Overall, the data suggest that  $\alpha_{1A}$ -ARs stimulate mTORC2 to increase glucose uptake and mTORC1 to promote protein synthesis and hypertrophy in NRVMs (Sato et al., 2018) (Figure 2) but the detailed mechanism whereby  $\alpha_{1A}$ -ARs activate mTORC2 is still not known.

### *$\beta$ -ARs and mTOR in the heart*

Both  $\beta_1$ - and  $\beta_2$ -ARs are expressed in the mammalian heart, though in isolated cardiomyocytes the  $\beta_1$ -AR is the predominant subtype (Buxton and Brunton, 1985). In human heart, the abundance of  $\beta_1$ -AR protein in cardiomyocytes is 68 – 80 fmol/mg protein, and it decreases to 30–41 fmol/mg protein in failing hearts (Bristow et al., 1993; Morisco et al., 2008). Both  $\beta_1$ - and  $\beta_2$ -subtypes are present in large coronary arteries (Young et al., 1990), while the primary subtype found in fibroblasts and on the small vessel endothelium is the  $\beta_2$ -AR (Freissmuth et al., 1986; Zhou and Pu, 2016).

Activation of  $\beta$ -ARs plays an important role in the regulation of cardiovascular function, including positive inotropic and chronotropic effects (Bristow et al., 1993; Brodde, 1991). Noradrenaline exerts its effects on the heart nearly exclusively via  $\beta_1$ -ARs (Kaumann et al., 1989). Thus, under normal physiological conditions,  $\beta_1$ -ARs are the predominant cardiac ARs responsible for regulation of heart rate and contractility.  $\beta_1$ -ARs activate the canonical Gas-adenylate cyclase-cAMP-PKA signalling cascade. In cardiomyocytes, the activation of PKA promotes phosphorylation of multiple proteins that increase calcium mobilization primarily from the sarcoplasmic reticulum, and to a lesser extent from the extracellular milieu, leading to increased rates of contraction and relaxation, and increased force of contraction (Sirenko et al., 2014) (Figure 2). In the early stages of heart failure, cardiac output is increased *via* overstimulation of  $\beta_1$ -ARs as a compensatory mechanism for the insufficient blood and oxygen supply (Brodde, 1993), but this leads to longer-term structural damage, including ventricular remodelling, cardiomyocyte apoptosis and fibrosis, and cardiac hypertrophy (Engelhardt et al., 1999; O'Connor et al., 1999). In addition, recent evidence has shown that  $\beta_1$ -ARs decrease myocardial autophagy that maintains cellular homeostasis (Wang et al., 2015; Wang et al., 2013). Inhibition of autophagy causes the accumulation of denatured proteins and damaged organelles, contributing to cardiac dysfunction (Magnusson

et al., 1994), and up-regulation of autophagy by the mTORC1 inhibitor rapamycin can improve impaired cardiac function (Wang et al., 2015). The  $\beta_1$ -AR mediated inhibition of autophagy occurs via PKA phosphorylation of Ser<sup>12</sup> in the autophagy-related protein LC3 (Kroemer et al., 1997). mTORC1 is over-active in the early stages of heart failure, and plays a role in the  $\beta_1$ -AR mediated inhibition of autophagy (Wang et al., 2015) (Figure 2).

Cardiac  $\beta_1$ -ARs become desensitised and down-regulated as heart failure progresses to end-stage dilated cardiomyopathy (Bohm et al., 1988). Desensitisation is related in part to an increased abundance and activity of GRK2, the predominant GRK subtype in the heart (Cannavo et al., 2013). Phosphorylation of  $\beta_1$ -ARs by GRK2 leads to increased interaction with  $\beta$ -arrestin, thereby promoting receptor internalization and degradation (Rockman et al., 2002) (Figure 2).  $\beta_2$ -ARs, on the other hand, are pleiotropic receptors that couple to Gas, Gai/o and G $\beta\gamma$  (Evans et al., 2010; Xiao et al., 1999). In the healthy human heart  $\beta_2$ -ARs preferentially couple to Gas proteins, whereas in pathological states involving high circulating catecholamine levels and high expression levels of cardiac Gai/o proteins during congestive heart failure,  $\beta_2$ -ARs switch to Gai/o signalling (Brown and Harding, 1992; Woo et al., 2015). Activation of the  $\beta_2$ -AR-Gai/o pathway inhibits cAMP production and protects cardiomyocytes from the pro-apoptotic effects of excessive  $\beta_1$ -AR stimulation (Chesley et al., 2000; Zhu et al., 2001).  $\beta_2$ -AR-Gai/o signalling also activates Akt which is known to be activated by PI3K and mTORC2 (Figure 2). The Akt signalling cascade is known to promote protein synthesis and glucose uptake in cardiomyocytes (Chesley et al., 2000).

### **The role of $\beta$ -ARs and mTOR in adipose tissue**

There are two types of adipose tissue with distinct physiological functions: white adipose tissue (WAT) that stores chemical energy as triacylglycerol, and brown adipose tissue (BAT) that releases chemical energy as heat (thermogenesis). BAT is responsible for sympathetically mediated non-shivering thermogenesis in mammals, and is activated by members of the AR family (Cannon and Nedergaard, 2004). In addition, many groups have described the existence of brown adipocytes in depots thought to be primarily WAT, both in animal models and in humans (Petrovic et al., 2010; Wu et al., 2012). These cells differ from prototypical BAT found in rodents or human infants, and have been termed “brite” (brown in white) or “beige” adipocytes (Petrovic et al., 2010; Wu et al., 2012). The appearance of brite adipocytes *per se* is insufficient to promote increased energy

expenditure, as these cells must also be activated by environmental, hormonal or pharmacological stimuli such as drugs acting at GPCRs (Merlin et al., 2016). The expression of ARs in brown, white and brite adipocytes and their contribution to adipocyte function is described in detail in an accompanying review (Evans et al.). We will focus here on the interplay between AR signalling and the role of mTOR complexes in adipocyte browning and glucose metabolism.

### *$\beta$ -ARs and mTOR in white adipose tissue*

When nutrients are plentiful, insulin is released from the pancreas and stimulates the uptake of glucose and fatty acids by adipose tissue, where they are stored as triacylglycerol forming lipid droplets. Insulin signalling in adipocytes is mediated by the PI3K–Akt–mTOR pathway, producing anabolic effects including cell growth and inhibition of lipolysis (Chakrabarti et al., 2013). During periods of fasting or stress, catecholamines are released by the sympathetic nervous system to activate  $\beta$ -ARs. Stimulation of the  $\beta_3$ -ARs in WAT activates adenylyl cyclase, leading to increased cAMP levels and PKA activity. PKA phosphorylates and regulates several important targets in adipocytes, including hormone-sensitive lipase and the lipid droplet-associated perilipins, which collectively promote triglyceride hydrolysis and liberation of free fatty acids (Granneman and Moore, 2008)(Figure 3).

Two studies have suggested that adrenoceptor-stimulated lipolysis inactivates mTOR in WAT (Mullins et al., 2014; Scott and Lawrence, 1998). Mullins and colleagues demonstrated that  $\beta$ -AR-mediated lipolysis suppresses glucose uptake because lipolysis causes both mTORC1 and mTORC2 complexes to dissociate (Mullins et al., 2014) (Figure 3). This is in agreement with the proposal that in white adipocytes, cAMP indirectly prevents activation of mTOR, since there is a decrease in p70S6K, a downstream target of mTORC1 (Scott and Lawrence, 1998). Conversely, there are new studies indicating that stimulation of  $\beta_3$ -ARs in WAT does not inhibit mTOR complexes but instead activates mTORC1 through PKA (Liu et al., 2016), resulting in browning of WAT depots. This variance in results might be due to the fact that  $\beta$ -AR stimulation interacts differently with mTOR in different WAT depots. Nonetheless, these results suggest that  $\beta$ -AR regulation of mTOR could have an important role in WAT function.

### *β-ARs and mTOR in brown adipose tissue*

Binding of noradrenaline to BAT β-ARs activates intracellular signalling cascades leading to increased expression of uncoupling protein 1 (UCP1), and breakdown of triglycerides to free fatty acids that activate UCP1 in the inner mitochondrial membrane (Figure 3). Activated UCP1 collapses the proton gradient that drives ATP synthesis and energy storage, thus β-AR signalling increases mitochondrial respiration and non-shivering thermogenesis (Cannon and Nedergaard, 2004). The metabolic capacity of BAT potentially allows it to influence whole-body energy homeostasis. For instance, BAT has been shown to play an important role in the regulation of glucose homeostasis and insulin secretion (Guerra et al., 2001). Cold exposure of animals increases glucose uptake into BAT due to activation of the sympathetic nervous system (Shibata et al., 1989; Shimizu et al., 1991), and this response is mimicked by administration of β-AR agonists *in vivo* (Liu et al., 1994; Olsen et al., 2014). Mouse brown adipocytes cultured *in vitro* also display increased glucose uptake upon treatment with β-AR agonists (Chernogubova et al., 2005; Dallner et al., 2006; Merlin et al., 2018; Olsen et al., 2014). While a role for β<sub>3</sub>-AR mediated glucose uptake in rodents is well-established, the contribution of β<sub>3</sub>-ARs in human adipose tissue is less clear. It has been demonstrated, however, that cold exposure increases <sup>18</sup>F-2deoxyglucose uptake in human BAT depots, and this effect can be mimicked by administration of the β<sub>3</sub>-AR agonist mirabegron that is used clinically for overactive bladder (Baskin et al., 2018; Cypess et al., 2015).

There is strong evidence that glucose uptake in response to β<sub>3</sub>-AR agonists occurs via a Gas-cAMP-PKA pathway, based on the use of pharmacological inhibitors (Chernogubova et al., 2004; Olsen et al., 2014). In addition, 8-bromo-cAMP and upstream activation of Gas by cholera toxin increase glucose uptake in primary brown adipocytes (Chernogubova et al., 2004; Olsen et al., 2014). Other mechanisms involved in β<sub>3</sub>-AR mediated glucose uptake include localisation of the β<sub>3</sub>-AR in lipid-rich microenvironments in the plasma membrane (Sato et al., 2012), conventional and novel protein kinase C (PKC) isoforms (Chernogubova et al., 2004), and AMPK (Hutchinson et al., 2005; Inokuma et al., 2005). As demonstrated in skeletal muscle, mTORC2 plays a pivotal role in adipocyte glucose uptake stimulated by β-AR agonists as well as insulin.

The contributions of mTORC1 and mTORC2 have been examined in mice with specific ablation of raptor or rictor in all adipocytes, as these cells express Cre recombinase under control of the adiponectin promoter (Kumar et al., 2010; Polak et al., 2008). Ablation of raptor (mTORC1) in adipose tissue increases mitochondrial uncoupling, but has no effect on insulin-mediated Akt phosphorylation or glucose tolerance profiles in chow-fed mice (Polak et al., 2008). In contrast, adipocytes isolated from mice with fat-specific ablation of rictor (mTORC2) display reduced insulin-stimulated Akt Ser473 phosphorylation, GLUT4 translocation to the cell surface and glucose uptake, and these mice have impaired glucose tolerance profiles *in vivo* (Kumar et al., 2010). These studies indicate that like in skeletal muscle, the mTORC2 complex is involved in glucose homeostasis in adipocytes.

We have demonstrated using brown adipocytes that mTORC2 is involved in  $\beta_3$ -AR mediated glucose uptake (Olsen et al., 2014). Overall inhibition of mTOR by Torin-1 or KU0063794 reduces glucose uptake, but two lines of evidence demonstrate the involvement of mTORC2 rather than mTORC1: (i) 24-hour but not 2-hour rapamycin treatment attenuates  $\beta_3$ -AR mediated glucose uptake (rapamycin acutely inhibits mTORC1, whereas long-term treatment prevents mTORC2 assembly), and (ii) siRNA against rictor, but not raptor, reduces glucose uptake by  $\beta_3$ -ARs (Mohl et al., 2011; Olsen et al., 2014). In brown adipocytes,  $\beta_3$ -AR mediated glucose uptake depends on *de novo* synthesis and translocation of GLUT1 (Dallner et al., 2006), which are both cAMP-dependent (Figure 3). mTORC2 is specifically involved in the translocation of newly synthesized GLUT1 to the plasma membrane, but is not required for *de novo* synthesis of GLUT1 (Olsen et al., 2014). In brown adipocyte cultures, inhibition of PI3K by compound 15e, or of Akt by inhibitor X reduced insulin- but not isoprenaline-stimulated glucose uptake. Akt was phosphorylated at Thr308 and Ser473 in response to insulin but not isoprenaline (Olsen et al., 2014).

A recent study has also shown that mice lacking rictor in adipose tissue are hypothermic, show increased susceptibility to cold, and have impairment of cold-induced glucose uptake and glycolysis (Albert et al., 2016). This study indicates that mTORC2 plays a central role in adipose tissue metabolism and translocation of GLUT 1/4 *in vitro* and *in vivo*. Interestingly, the GLUT 1/4 content in the plasma membrane of brown adipocytes was not altered by cold exposure in that study. Also in contrast to our previous findings (Olsen et al., 2014), both immortalised mouse brown adipocytes treated with 1  $\mu$ M noradrenaline for 5 min, and native BAT from wild type mice treated for 30 min *in vivo* with 1 mg/kg

noradrenaline, showed phosphorylation of Akt at Ser473, known to be downstream of mTORC2. There is no clear explanation for the disparity with our brown adipocytes; however, an emerging view is that adipose depots display considerable heterogeneity in cell composition (Shinoda et al., 2015). This would account for differences between *in vivo* and *in vitro* data, and may also be consistent with phenotypic differences between primary brown adipocyte cultures that are representative of the starting population of stromal vascular pre-adipocytes, and immortalised adipocytes that have been selected for the presence of plasmid encoding SV40 T antigen (Klein et al., 2002), and therefore represent only a small subset of the starting cell population. In addition, noradrenaline may activate the  $\alpha_2$ -ARs present in BAT or brown adipocytes, promoting signalling via a G $\alpha$ i/o-G $\beta$  $\gamma$ -PI3K-PDK1-Akt axis. In immortalized human multipotent adipose-derived stem (hMADS) brown adipocytes treated with low concentrations of isoprenaline, glucose uptake is blocked by the mTOR inhibitor KU0063794, as seen in mouse brown adipocyte primary cultures (Olsen et al., 2014). It would be interesting to determine whether hMADS cells display Akt phosphorylation at Ser473 in response to isoprenaline treatment.

#### *mTORC1 mediates browning of brite adipocytes.*

In addition to BAT, there is increasing evidence for the existence of brown adipocytes in depots thought to be primarily WAT, both in animal models and in humans (Petrovic et al., 2010). These cells differ from prototypical BAT found in rodents or human infants, and have been termed “brite” (brown in white) or “beige” adipocytes. Two studies indicate that brite adipocytes contribute significantly to whole body energy expenditure: mouse models that have increased brite adipocytes in WAT are protected from diet-induced obesity (Seale et al., 2011), and browning of WAT contributes to non-shivering adaptive thermogenesis in the absence of classical brown adipocytes (Schulz et al., 2013). Our *in vitro* results show that stimulation of the  $\beta_3$ -AR increases glucose uptake in brown and brite adipocytes, but not white adipocytes, in contrast to insulin which increases glucose uptake in all three adipocyte cultures (Merlin et al., 2018).

Separate studies have shown that the  $\beta$ -AR-cAMP-PKA pathway can lead to mTORC1 activation (Figure 3) and is necessary for the induction of adipose tissue browning and BAT development (Liu et al., 2016). In addition, wild type mice treated with the mTORC1 inhibitor rapamycin or mice with adipocyte-specific deletion of raptor are cold-intolerant,

and show impaired expression of UCP1 and other mitochondrial components in iWAT, suggesting that there may be a role for mTORC1 even in the early development of iWAT brite adipocytes (Liu et al., 2016; Tran et al., 2016). Several downstream target genes of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) and estrogen-related receptor  $\alpha$  (ERR $\alpha$ ) are similarly under the control of mTORC1. PPAR $\alpha$  is a master nuclear receptor for fatty acid  $\beta$ -oxidation, and PPAR $\alpha$  has been shown to participate in UCP1 expression either directly or indirectly through ERR $\alpha$  (Morganstein et al., 2010). Therefore, mTORC1 appears to have an important role in the catabolic process of adipose tissue browning and the dissipation of chemical energy by thermogenesis.

### **Conclusions**

This review has summarised the evidence for metabolic and survival roles of AR-mTOR signalling in heart, skeletal muscle and brown/brite adipocytes.  $\alpha_{1A}$ -ARs mediate glucose uptake and cardioprotection via mTOR in the failing heart. In skeletal muscle,  $\beta_2$ -ARs facilitate protein synthesis and glucose uptake via mTORC1 and mTORC2, respectively. Type 2 diabetes is associated with defects in insulin signalling components including insulin receptor substrate, PI3K, and Akt, causing impaired glucose uptake. These defects can be bypassed by the skeletal muscle  $\beta_2$ -AR-mTORC2 pathway which is independent of insulin signalling. Likewise, adipose  $\beta$ -ARs play a significant role in lipolysis in WAT, and increase glucose uptake in BAT which can contribute significantly to whole body energy expenditure. mTORC1 also plays a role in browning of brite adipocytes. The capacity of key GPCRs to modulate physiological responses through mTOR activation represents a novel paradigm that holds great potential in the identification of drug targets for treating a range of metabolic disorders.

### Competing interest statement

Prof. Tore Bengtsson own stocks in the following pharmaceutical companies: Sigrid Therapeutics AB, Atrogi AB and Glucox Biotechnology AB. Dr Dana Hutchinson owns stocks in Glucox Biotechnology AB, and is a scientific advisor for Atrogi AB.

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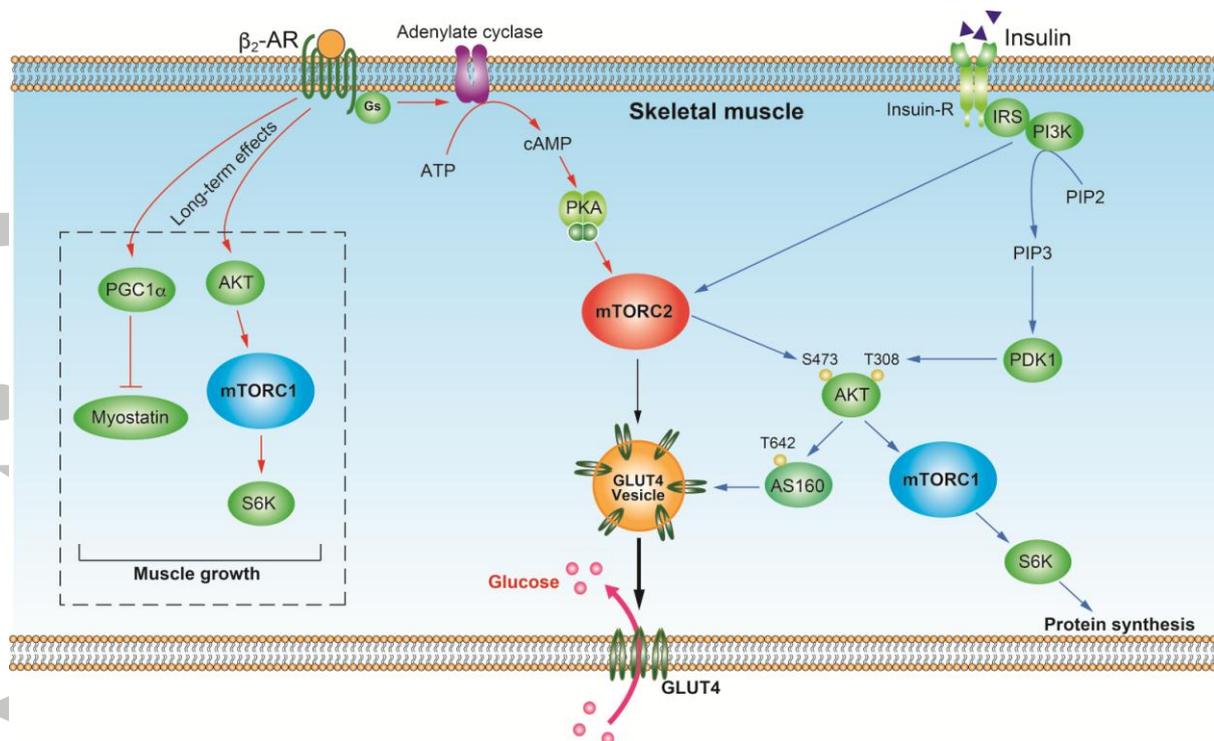
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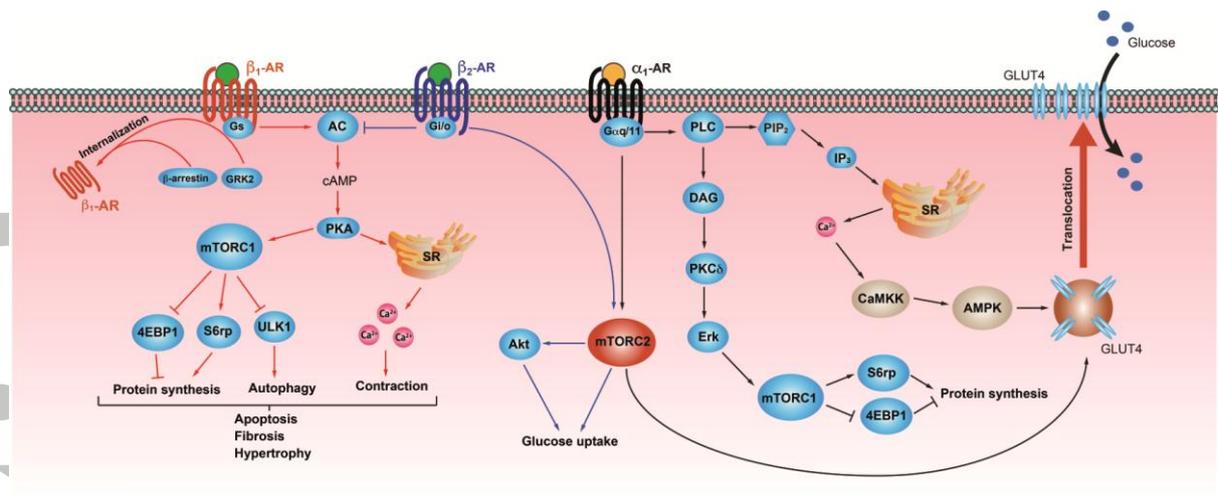
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**Figure 1: Proposed mechanisms for  $\beta_2$ -AR-mediated mTOR signalling in skeletal muscle**

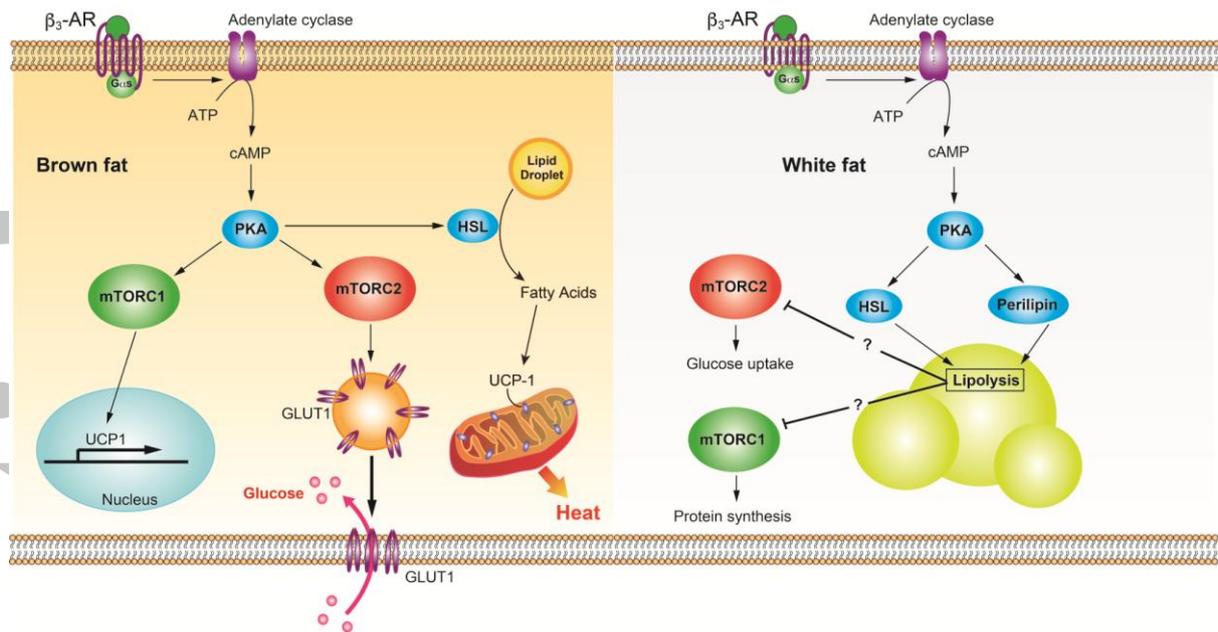
In skeletal muscle, activation of the  $\beta_2$ -AR coupled to  $G_{\alpha s}$  stimulates adenylate cyclase, leading to cAMP accumulation. cAMP activates PKA which then phosphorylates mTORC2. The phosphorylated mTORC2 and GRK2 stimulates translocation of GLUT4 vesicles from the cytosol to the plasma membrane, leading to increased glucose uptake. The long-term stimulation of  $\beta_2$ -AR causes activation of the Akt-mTORC1-S6K pathway and inhibition of myostatin production by PGC1 $\alpha$ , which contributes to muscle growth. Insulin binds to the insulin receptor (Insulin-R), resulting in activation of PI3K. PI3K then increases levels of PIP3, which activates PDK1 to phosphorylate Akt at Thr308. PI3K also activates mTORC2 which phosphorylates Akt at Ser473. Fully activated Akt phosphorylates AS160 to promote GLUT4 translocation to the plasma membrane leading to increased glucose uptake. Akt also phosphorylates mTORC1 and thereby promotes protein synthesis.



**Figure 2: Proposed mechanisms for  $\alpha_1$ -AR and  $\beta$ -AR mediated mTOR signalling in heart**

Activation of  $\alpha_1$ -ARs results in increased levels of cytosolic  $\text{Ca}^{2+}$  through a classical pathway involving  $\text{G}\alpha\text{q}/11$ , phospholipase C (PLC), phosphatidylinositol biphosphate ( $\text{PIP}_2$ ), and inositol trisphosphate ( $\text{IP}_3$ ). Release of intracellular  $\text{Ca}^{2+}$  activates CaMKK and AMPK pathways.  $\alpha_1$ -ARs also activate mTORC2 via unknown mechanisms. AMPK and mTORC2 both play significant roles in GLUT4 translocation to the plasma membrane, resulting in increased glucose uptake.  $\alpha_1$ -ARs promote mTORC1 activation via diacylglycerol (DAG),  $\text{PKC}\delta$ , and Erk1/2, leading to increased activation of S6rp and 4EBP1, which promote protein translation.

$\beta_1$ - and  $\beta_2$ -AR both couple to  $\text{G}_s$ , whereas  $\beta_2$ -ARs switch coupling to  $\text{G}_i/o$  in pathological states such as heart failure. Overstimulation of  $\beta_1$ -ARs increases  $\text{Ca}^{2+}$  mobilization and mTORC1 activation leading to increased protein synthesis and inhibition of autophagy, resulting in apoptosis, fibrosis, and hypertrophy. In the later stages of heart failure, GRK2 overexpression results in adrenoceptor phosphorylation and interaction with  $\beta$ -arrestin, thereby promoting receptor internalization. Activation of the  $\beta_2$ -AR- $\text{G}_i/o$  pathway inhibits cAMP production and counteracts the pro-apoptotic effects of excessive  $\beta_1$ -AR stimulation.  $\beta_2$ -AR exerts glucose uptake through mTORC2-Akt activation.



**Figure 3: mTOR signalling pathways stimulated by  $\beta_3$ -ARs in BAT and WAT**

Stimulation of  $\beta_3$ -ARs increases the production of cAMP via G $\alpha_s$ -adenylate cyclase and enhances activation of PKA in BAT and WAT. In brown adipocytes, PKA promotes the transcription and translation of UCP1 via mTORC1. The increased abundance and activation of UCP1 in mitochondria by free fatty acids promotes thermogenesis. GLUT1 translocation is increased due to mTORC2 activation, leading to increased glucose uptake. In white adipocytes,  $\beta_3$ -AR mediated PKA activation leads to phosphorylation of hormone-sensitive lipase (HSL) and perilipin. Phosphorylated perilipin undergoes a conformational change and interacts with lipid droplet HSL, which then hydrolyses the stored triglycerides into fatty acids. Lipolytic products may inhibit mTORC1 mediated protein synthesis and mTORC2 mediated glucose uptake in white adipocytes.