EMBO reports

review

The multifaceted role of mTORC1 in the control of lipid metabolism

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The mechanistic target of rapamycin is a protein kinase that, as part of the mechanistic target of rapamycin complex 1 (mTORC1), senses both local nutrients and, through insulin signalling, systemic nutrients to control a myriad of cellular processes. Although roles for mTORC1 in promoting protein synthesis and inhibiting autophagy in response to nutrients have been well established, it is emerging as a central regulator of lipid homeostasis. Here, we discuss the growing genetic and pharmacological evidence demonstrating the functional importance of its signalling in controlling mammalian lipid metabolism, including lipid synthesis, oxidation, transport, storage and lipolysis, as well as adipocyte differentiation and function. Defining the role of mTORC1 signalling in these metabolic processes is crucial to understanding the pathophysiology of obesity and its relationship to complex diseases, including diabetes and cancer.

Keywords: adipocytes; Akt; insulin; liver; mTOR

EMBO *reports* (2013) **14,** 242–251; published online 12 February 2013;
doi:10.1038/embor.2013.5

See the Glossary for abbreviations used in this article.

Introduction

Of the four main classes of biological macromolecule, our understanding of the molecular mechanisms by which cellular signalling pathways regulate lipid metabolism has lagged behind that of carbohydrates, proteins and nucleic acids. However, lipids are crucially important both structurally and functionally in all living organisms. An obvious reason for this dependence is the lipid makeup of the plasma membrane and many subcellular organelles. Moreover, lipids act as signalling molecules on both a cellular, for example phosphoinositides, and organismal, for example steroid hormones, scale. Lipids are also used for energy storage, primarily as triacylglycerides in adipocytes, and as an alternative to glucose for catabolic metabolism. Despite the dependence of living organisms on lipids, we know little about how lipid homeostasis is controlled by the intricate network of cellular signalling pathways that sense cellular growth conditions. As detailed in this review, the mechanistic target of rapamycin (mTOR) protein kinase has emerged as a crucial link between cellular and systemic growth signals and the regulation of lipid metabolism.

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Received 6 November 2012; accepted 16 January 2013; published online 12 February 2013

mTOR is an evolutionarily conserved serine/threonine kinase that exists within two functionally distinct protein complexes, the mechanistic target of rapamycin complexes 1 (mTORC1) and 2 (mTORC2). mTORC1 senses and integrates a diverse array of cellular signals, with mTOR kinase activity within the complex being influenced by a variety of nutrients—for example, amino acids, glucose and oxygen, cellular energy levels, such as ATP, and many secreted growth factors, cytokines and hormones, including insulin. All of these signals require the Ras-related small G protein Rheb, which on GTP-loading is an essential upstream activator of mTORC1 [1]. Many of the signals that regulate mTORC1 do so by altering the GTP-binding status of Rheb through activation or inhibition of a GTPase-activating protein complex, comprised of TSC1, TSC2 and TBC1D7—the TSC–TBC complex [2]. For instance, insulin, IGF1 and other growth factors inhibit the complex to activate Rheb and mTORC1 through Akt-mediated phosphorylation of TSC2 [3,4]. By contrast, a decrease in cellular ATP, such as the decrease that occurs during glucose depletion, activates the complex to inhibit Rheb and mTORC1, at least in part, through the action of AMPK (Fig 1; [5–7]). On activation, mTORC1 directly phosphorylates S6K1 and S6K2, 4E-BP1 and 4E-BP2, and a growing number of other downstream targets [8]. Whilst the overall effects of mTORC1 signalling differ in cells and tissues, it has an evolutionarily conserved role in promoting anabolic cell growth and inhibiting the catabolic process of autophagy. On the other hand, mTORC2 seems to be regulated primarily by growth factor signalling and phosphorylates a conserved hydrophobic motif in the protein kinases Akt, SGK and some isoforms of PKC, thereby increasing their kinase activity [9]. Through these targets, and probably through others, mTORC2 signalling is believed to promote cell survival, proliferation, metabolism and changes in the actin cytoskeleton. The two mTOR complexes can be distinguished from one another by their differential sensitivity to rapamycin, an allosteric and partial inhibitor of mTOR (Sidebar A).

Many studies in cell and mouse models, combined with preclinical and clinical data on mTOR inhibitors, have revealed a pivotal role for mTOR—particularly within mTORC1—in controlling lipid homeostasis in many settings, both physiological and pathological. We review this evidence below, with a focus on the key aspects of lipid synthesis, storage and mobilization. The emerging picture is that, through a variety of molecular mechanisms, mTORC1 signalling promotes processes to synthesize and store lipids, whilst inhibiting those leading to lipid consumption (Fig 1).

Sidebar A | mTORC1 versus mTORC2 and the differential effects of mTOR inhibitors

In studying the mechanistic target of rapamycin (mTOR) signalling network, or interpreting the mTOR literature, it is crucial to understand some of the basic complexities of mTOR signalling and inhibition. The mechanistic target of rapamycin complex 1 (mTORC1) is composed of the core essential components mTOR, mTOR-associated protein, LST8 homologue (mLST8) and the regulatory-associated protein of mTOR (Raptor), whereas mTORC2 is composed of mTOR, mLST8, SAPK-interacting protein 1 (SIN1) and the Raptor-independent companion of mTOR (Rictor). Although these complexes are functionally distinct, they can have an influence on each other's activity. For instance, as mTORC2 stimulates an increase in Akt activity [84], it might influence its downstream signalling from mTORC1. On the other hand, several negative feedback mechanisms are triggered by mTORC1 activation, which influences mTORC2 activity, including one leading to direct phosphorylation of Rictor within mTORC2 by ribosomal S6 kinase 1 (S6K1) downstream from mTORC1 [85,86]. Regarding mTOR inhibitors, the widely used rapamycin and its many analogues, which on interaction with the ubiquitous protein FK506 binding protein of 12 kDa (FKBP12) binds to an allosteric site amino terminal to the mTOR kinase domain—the FKBP12-rapamycin binding domain—only has access to mTOR within mTORC1. However, it is evident that in both cell culture and mice, prolonged exposure to rapamycin can block the assembly of mTORC2 by sequestering uncomplexed mTOR [82,87]. Therefore, although rapamycin is specific to mTORC1 for acute inhibition and generally leads to an increase in upstream signalling from mTORC2 and Akt by blocking negative feedback mechanisms, one must consider that the observed effects of long-term rapamycin treatment might be due to loss of mTORC2 in some experimental systems, which affects the many processes downstream from Akt. Also, the development of mTOR kinase domain inhibitors, which completely block mTOR within both complexes, has revealed that rapamycin only partly inhibits mTORC1 activity. Whilst the nature of this differential sensitivity is unknown, rapamycin strongly affects the phosphorylation of some mTORC1 targets (for example, S6K1) but only modestly inhibits other targets (for example, eIF4E-binding protein 1; [88]).

Lipogenesis

The regulation of *de novo* sterol and fatty acid synthesis by signalling pathways, especially insulin signalling, has garnered intense interest. Unlike most terminally differentiated cells, hepatocytes and adipocytes synthesize significant amounts of lipid *de novo* through pathways in which cytosolic acetyl-CoA, derived from glucose or amino acid catabolism, is used to form the hydrophobic carbon backbone of lipids. Acetyl-CoA is either committed to sterol and isoprenoid biosynthesis through the action of HMG-CoA synthase or to fatty acid biosynthesis through acetyl-CoA carboxylase. Both the sterol and fatty acid synthesis branches comprise many steps requiring many specific enzymes. Importantly, the SREBPs are transcription factors that stimulate the expression of genes encoding nearly all of these lipogenic enzymes [10]. The three SREBP isoforms, encoded by two genes, are produced as inactive transmembrane proteins at the endoplasmic reticulum (Fig 2). Under conditions of abundant sterols, full-length SREBP, through its sterol-sensing binding partner SCAP, is retained in the endoplasmic reticulum by the INSIG proteins [11]. Depletion of intracellular sterols results in release of the SREBP–SCAP complex from Insig and their transport to the Golgi apparatus, in which two proteolytic cleavage events by the site-specific proteases S1P and S2P liberate the active amino-terminus of SREBP. This fragment then enters the nucleus and

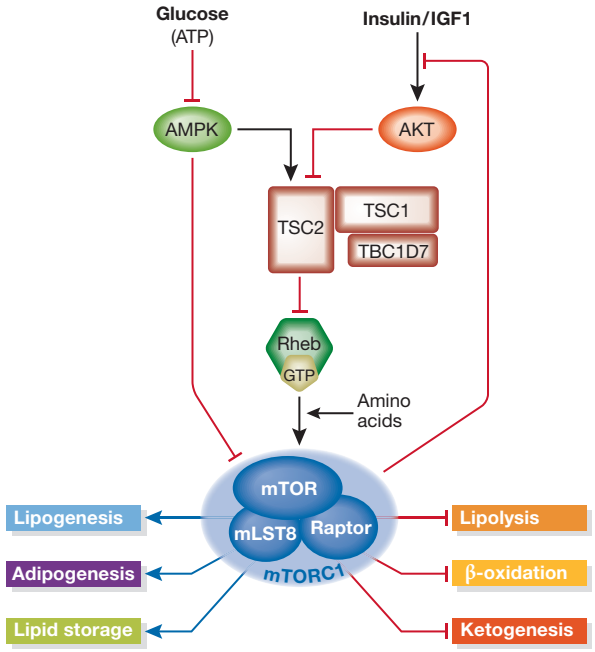

Fig 1 | Upstream regulation from the mTORC1 and its downstream functions related to lipid metabolism. The presence of amino acids is required for the activation of mTORC1 by GTP-bound Rheb. Upstream from Rheb, the TSC–TBC complex receives signals about systemic and local nutrient and energy availability, in part through AMPK and Akt. These signals either activate or inhibit the ability of the TSC–TBC complex to act as a GAP for Rheb, thereby inhibiting or activating mTORC1, respectively. Activated mTORC1 leads to enhanced phosphorylation of IRS1, which serves as negative feedback to dampen the insulin response. mTORC1 has many roles in regulating lipid metabolism, including the promotion of lipid synthesis and storage and inhibition of lipid release and consumption, which are detailed in the text. AMPK, adenosine monophosphate-activated protein kinase; GAP, GTPase-activating protein; IRS1, insulin receptor substrate 1; IGF1, insulin-like growth factor 1; mTORC1, mechanistic target of rapamycin complex 1; Raptor, regulatory-associated protein of mTOR; TSC, tuberous sclerosis complex; TBC, Tre-2/Bub2/Cdc16 domain-containing protein.

induces transcription from SREs within target genes. SREBP1a and 1c are products of alternative splicing of the *SREBF1* gene and have been primarily implicated in the control of genes involved in fatty acid synthesis, although SREBP1a is thought to activate most SRE-containing genes [12]. SREBP2 is encoded by *SREBF2* and is believed to have a more important role in the transcription of steroidogenic genes, including those involved in cholesterol synthesis in the liver [13,14]. Although the SREBPs preferentially activate transcription of different sets of genes, there is substantial overlap between the targets of the SREBP isoforms and the tissue specificity of these preferences, which has not been fully established. Importantly, independent studies have identified the SREBPs as major transcriptional effectors of mTORC1 signalling and have demonstrated that mTORC1 activation promotes lipogenesis through this family of transcription factors [15,16].

mTORC1 signalling promotes SREBP activation and lipogenesis in response to both physiological and genetic stimuli. In primary rodent hepatocytes and the intact liver, insulin or feeding has been shown to increase the expression of the major liver isoform

Glossary

4E-BP1/2	eIF4E-binding protein 1/2
AMPK	adenosine monophosphate-activated protein kinase
ATG5/7	autophagy-related 5/7
ATGL	adipose triglyceride lipase
C/EBP	CCAAT/enhancer-binding protein
CPT1	carnitine palmitoyltransferase 1
DAG	diacylglycerol
eIF4E	eukaryotic translation initiation factor 4E
GSK3	glycogen synthase kinase 3
HSL	hormone-sensitive lipase
IDL	intermediate density lipoprotein
IGF1	insulin-like growth factor 1
Insig	insulin-induced gene
LDL	low density lipoprotein
LDLR	LDL receptor
lipin 1	phosphatidate phosphatase LPIN1
LPL	lipoprotein lipase
LST8	lethal with SEC13 protein 8
MAG	monoacylglycerol
MEF	mouse embryonic fibroblast
N-CoR1	nuclear receptor co-repressor 1
PCSK9	proprotein convertase subtilisin/kexin type 9
PKA/C	protein kinase A/C
PPARα/γ	peroxisome proliferator-activated receptor α/γ
Raptor	regulatory-associated protein of mTOR
Rictor	Raptor-independent companion of mTOR
S6K1/2	ribosomal S6 kinase 1/2
SCAP	SREBP cleavage-activating protein
SCD	stearoyl-CoA desaturase
SGK	serum and glucocorticoid regulated kinase
shRNA	short hairpin RNA
siRNA	small interfering RNA
SRE	sterol response element
SREBP	sterol regulatory element-binding protein
TAG	triacylglycerol
TBC1D7	TBC1 domain family, member 7
TCA	tricarboxylic/citric acid
TSC1/2	tuberous sclerosis complex 1/2
VLDL	very low density lipoprotein

of SREBP (SREBP1c) and its targets, and to promote *de novo* lipid synthesis in a manner that is sensitive to rapamycin [17–19]. Insulin activates mTORC1 through a pathway involving the Akt-mediated phosphorylation and inhibition of TSC2, within a complex with TSC1 and TBC1D7 [2–4]. Expression of constitutively active Akt or loss of either TSC1 or TSC2, both of which result in insulin-independent activation of mTORC1 signalling, stimulates the global expression of SREBP1 and SREBP2 targets and drives lipogenesis through mTORC1 [15,16]. These latter studies found that mTORC1 signalling promotes accumulation of the processed, mature form of SREBP1, which resides in the nucleus to induce its own expression and that of genes involved in both steroid and fatty acid biosynthesis. In exploring the molecular mechanism of this regulation, it was found that S6K1 is required downstream from mTORC1 to stimulate the increase in levels of active SREBP1, expression of SREBP1 and SREBP2 targets, and *de novo* lipogenesis in TSC2-deficient cells [15]. SREBP1 regulation in this setting is independent of the effects on the proteasomal degradation of its active form, suggesting that S6K1 promotes the processing of SREBP1. Consistent with these findings, S6K1 has been found to promote the activation of hepatic SREBP1c by having an effect on its processing [20,21], and to affect the processing of SREBP2 in a hepatocellular carcinoma cell line [22]. mTORC1 signalling has also been suggested to increase SREBP1 activation in an S6K1-dependent manner in cultured myotubes [23].

Genetic mouse models have demonstrated that mTORC1 activation is essential, but not sufficient, to stimulate hepatic SREBP1c and its lipogenic targets in response to feeding [18,24]. Mice lacking mTORC1 in their liver, through liver-specific *Raptor* knockout, fail to induce SREBP1c and lipogenesis [24], and have reduced levels of both liver triglycerides and circulating cholesterol on a 'Western' diet [25]. However, characterization of mice with a liver-specific knockout of *Tsc1* (LTsc1KO) and constitutive activation of mTORC1, which is independent of insulin and feeding, revealed that mTORC1 signalling, although essential, is not capable of activating SREBP1c and hepatic lipid synthesis on its own [18]. In fact, these mice were found on two independent strain backgrounds to be resistant to the development of both age- and diet-induced hepatic steatosis due to decreased SREBP1c activation [18,26]. These seemingly paradoxical findings are the result of a strong feedback attenuation of Akt signalling that accompanies loss of function of the TSC1–TSC2 complex in all settings [27]. A crucial role for Akt signalling in the induction of SREBP1c and lipogenesis in the liver has been established through rodent models [28–30], and this has been extended by using mice with liver-specific *Rictor* knockout, which results in the loss of mTORC2 activity and its activating phosphorylation of Akt [31]. Consistent with the essential nature of Akt signalling to hepatic SREBP1c, a restoration of Akt activity in LTsc1KO hepatocytes restores SREBP1c activation and lipogenesis [18]. Whilst many mTORC1-independent pathways might function in parallel downstream from Akt to help to promote the activation of hepatic SREBP1c, including GSK3 inhibition [32], data from the LTsc1KO mice suggest that one pathway involves the repression of an isoform of the SREBP inhibitor Insig, Insig2a, which is only expressed in the liver [18]. A liver-specific mechanism is also consistent with the fact that mTORC1 activation alone is sufficient to promote SREBP activation and lipogenesis in other settings, even in the absence of Akt signalling [15].

The molecular mechanism by which S6K1 promotes SREBP processing is unknown, and it is clear from additional studies that S6K1 is not the only direct target downstream from mTORC1 involved in SREBP isoform regulation, which might vary by cellular context. For instance, siRNA knockdown of the mRNA cap-binding protein eIF4E, which is normally activated by mTORC1 signalling through the phosphorylation and release of its inhibitory binding partner 4E-BP1, decreases overall levels of SREBP1 and its canonical target SCD in breast cancer cell lines [33]. The potential involvement of 4E-BP1 regulation by mTORC1 in some cells might explain the resistance of SREBP1 or SREBP2 activation to rapamycin in specific settings [22,34]. The resistance of some mTORC1 targets to rapamycin (Sidebar A) is an important consideration when examining the role of mTORC1 signalling in any aspect of lipid metabolism. Another direct target of mTORC1 that, as with 4E-BP1, is partly resistant to rapamycin for its regulation is the phosphatidic acid phosphatase lipin 1, which has also been implicated in SREBP regulation [25,35]. Lipin 1 seems to have a role in the remodelling of the nuclear lamina, which is inhibited by mTORC1-mediated phosphorylation of many residues on this enzyme. Lipin 1 phosphorylation also coincides

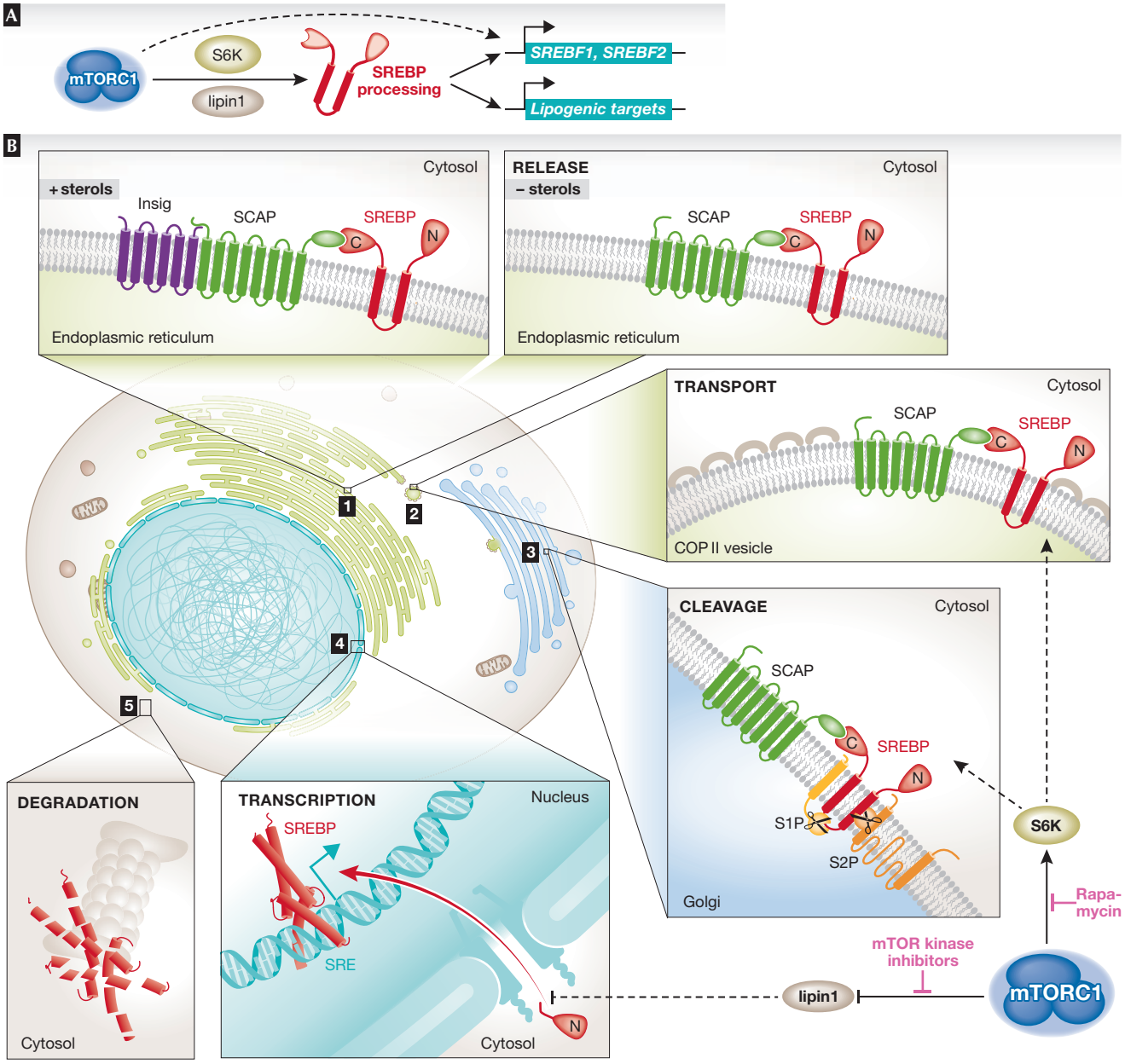

Fig 2 | The complex steps leading to SREBP activation and input from mTORC1 signalling. (**A**) SREBP processing and activation is regulated by mTORC1 through S6K and lipin 1 leading to the transcriptional induction of the *SREBF1* and *SREBF2* genes, encoding SREBP1 and SREBP2, respectively, and genes encoding many lipogenic enzymes involved in both fatty acid and sterol synthesis. The mTORC1-mediated transcriptional activation of *SREBF1* could result from either autoregulation by SREBP1 or from an unknown parallel pathway downstream from mTORC1. (**B**) In the presence of sterols, SREBP resides in the endoplasmic reticulum bound to SCAP and the Insig proteins. When sterols become scarce SCAP undergoes a conformational change, which releases the SCAP–SREBP complex from the Insig, allowing its transport from the endoplasmic reticulum to the Golgi apparatus through COPII vesicles. Once in the Golgi, SREBP comes into contact with two site-specific proteases. S1P cleaves the luminal loop of SREBP and S2P cleaves the amino-terminal transmembrane region of SREBP, which releases the N-terminal region of SREBP containing the DNA-binding and -transactivating domains. The NLS-containing processed form of SREBP enters the nucleus to activate transcription of genes containing SREs in their promoters. Finally, the processed form of SREBP is unstable and subject to proteasome-mediated degradation. In some settings, SREBP processing has been found to require S6K1 downstream from mTORC1 and is therefore sensitive to rapamycin. However, the nuclear shuttling of SREBP has been found to require lipin 1 downstream from mTORC1, the phosphorylation of which is largely resistant to rapamycin but sensitive to mTOR kinase domain inhibitors (Sidebar A). The precise molecular mechanisms by which either of these two mTORC1 targets regulates SREBP activation are unknown. COPII, coatamer protein II; Insig, insulin-induced gene; lipin 1, phosphatidate phosphatase LPIN1; mTORC1, mechanistic target of rapamycin complex 1; NLS, nuclear localization signal; S1/2P, site 1/2 protease; S6K1, ribosomal S6 kinase 1; SCAP, SREBP cleavage-activating protein; SRE, sterol response element; SREBP1/2, sterol regulatory element-binding protein 1/2.

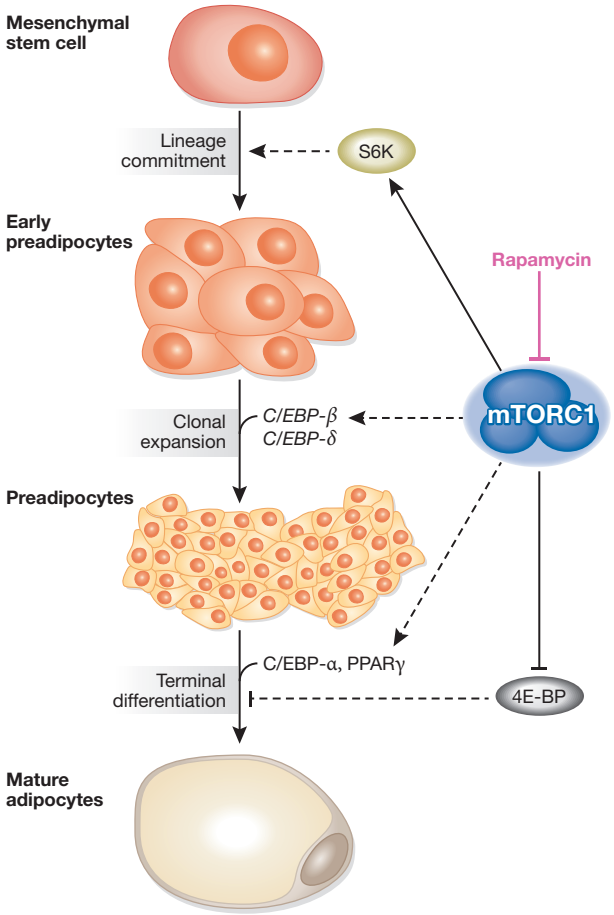

Fig 3 | mTORC1 signalling has been implicated in promoting the three main steps of adipogenesis. Adipogenesis consists of the differentiation of a mesenchymal stem cell to a mature adipocyte, which makes up a significant part of adipose tissue in which energy is stored as lipids. The commitment of the mesenchymal stem cells to the adipocyte lineage is the first step of adipogenesis and is facilitated by S6K1 activity. C/EBP-β and -δ are the primary drivers of clonal expansion, which is crucial for preadipocyte maturation, and the former has been suggested to be activated by mTORC1 signalling. The terminal differentiation of preadipocytes to mature adipocytes is mediated by PPARγ and C/EBP-α. mTORC1 promotes this final step through both its inhibition of 4E-BP and its activation of PPARγ through a poorly understood mechanism. Although the precise molecular mechanisms have yet to be defined, rapamycin blocks adipogenesis. 4E-BP, eIF4E-binding protein; C/EBP-α/β/δ, CCAAT/enhancer-binding protein-α/β/δ; mTORC1, mechanistic target of rapamycin complex 1; PPARγ, peroxisome proliferator-activated receptor γ; S6K1, ribosomal S6 kinase 1.

with an increase in the levels of processed, nuclear SREBP1 and SREBP2, and the expression of SREBP targets. Although the phosphatidic acid phosphatase activity of lipin 1 was shown to be important for its inhibitory effect on nuclear SREBP levels [35], the molecular mechanism and tissue specificity of this regulation, as with S6K1 and 4E-BP1, remains unknown. Finally, it is clear that mTORC1 signalling also increases the transcript levels of SREBP1 and SREBP2 in cell culture models [15], and SREBP1c in both rodent hepatocytes and the intact liver in response to insulin or feeding [18–21]. This mTORC1-dependent transcriptional response leads to an increase in full-length SREBP isoforms that accompany the increased processing and activation of SREBP. However, it remains unclear whether this transcriptional effect is simply a result of autoregulation by processed SREBPs at the *SREBF1* or *SREBF2* promoter or a parallel pathway independent from the effects of mTORC1 on SREBP processing (Fig 2). Both *SREBF1* and *SREBF2* contain a characterized SRE in their promoters [36,37]. In cell culture models, exogenous expression of processed SREBP1a stimulates the expression of endogenous SREBP1 and SREBP2 transcripts in a manner that is no longer sensitive to rapamycin, suggesting that the transcriptional effects of mTORC1 signalling on SREBP expression are upstream from processed SREBP [15]. However, elegant studies with a transgenic version of SREBP1c in rats suggest that the role of mTORC1 in SREBP1c processing and gene expression is separable [21]. More studies are needed to understand the many inputs of mTORC1 signalling, especially *in vivo*, into the regulation of SREBP isoforms.

Adipogenesis

Adipocytes are specialized mesenchymal cells that either store lipids as energy reserves (white adipose tissue) or burn lipids through oxidation to generate heat (brown adipose tissue). Pharmacological and genetic studies have demonstrated that the differentiation of mesenchymal stem cells into mature adipocytes—adipogenesis—requires mTOR signalling (Fig 3). Rapamycin treatment has been reported to reduce adipogenesis in a variety of cell culture models. Rapamycin seems to block the early determination step in brown adipocyte differentiation, in which a mesenchymal stem cell commits to becoming a preadipocyte [38]. Similarly, rapamycin treatment or shRNA-mediated knockdown of *S6K1* in embryoid bodies hinders their commitment to preadipocytes [39]. However, much of our knowledge of adipogenesis comes from cell culture models of preadipocytes after lineage commitment and also from MEFs, and has therefore been focused on the later steps of white adipose differentiation. Treatment of preadipocytes with rapamycin leads to a marked decrease in adipocyte differentiation [40–44]. mTOR has been implicated in hormonal induction of clonal expansion, which is an initial step of differentiation that occurs through the action of two C/EBP family transcription factors, C/EBP-β and -δ. Overall levels of C/EBP-β have been found to decrease on rapamycin treatment, which corresponds with a repression of clonal expansion of preadipocytes [41]. However, rapamycin has also been shown to inhibit preadipocyte differentiation after clonal expansion, thereby ruling out the anti-proliferative effects of rapamycin as its primary mode of inhibiting adipogenesis [42–44].

Several genetic models have further supported a crucial role for mTORC1 activation in terminal adipocyte differentiation, in which it seems to be both necessary and sufficient. For instance, MEFs lacking TSC1 or TSC2, which have sustained, insulin-independent activation of mTORC1 signalling, have an mTORC1-dependent enhanced capacity to differentiate into adipocytes despite these cells being severely resistant to insulin, a major adipogenic factor [45]. Reciprocally, TSC2-deficient MEFs that express a phosphorylation site mutant of TSC2, which blocks the ability of mTORC1 to be activated by insulin and Akt signalling, show reduced adipogenesis [45]. The enhanced adipogenesis in mesenchymal cells lacking the TSC tumour suppressors probably explains the common development of adipocyte-rich renal angiomyolipomas in patients with TSC [46]. Consistent with an essential role

for mTORC1, RNA interference knockdown of *Raptor* also blocks adipogenesis in preadipocytes [47]. Downstream from mTORC1, genetic evidence suggests a role for both S6K and 4E-BP in the control of adipogenesis. The involvement of S6K in the commitment of stem cells to preadipocytes was reinforced by the reduced size of this progenitor cell population in *S6K1* knockout mice and a defect in the capacity of embryonic stem cells from these mice to commit to the adipocyte lineage [39]. Reciprocally, *4E-BP1/2* double-knockout MEFs show enhanced differentiation towards adipocytes [48], suggesting that the ability of mTORC1 to both activate S6K and inhibit 4E-BP contributes to its role in promoting adipogenesis. Interestingly, the *S6K1* knockout mice have a lean phenotype on both normal and high-fat diets [39,49], whereas the *4E-BP1/2* double-knockout mice are more sensitive to diet-induced obesity than their wild-type counterparts [48]. However, the differences in adiposity in these systemic mouse models probably reflect many effects of mTORC1 signalling on lipid synthesis and mobilization, discussed elsewhere in this review, in addition to its role in promoting the development of adipose deposits.

The molecular mechanisms by which mTORC1 and its downstream targets stimulate adipocyte differentiation have yet to be fully defined. The temporal activation of two transcription factors, C/EBP-α and PPARγ—the master regulator of terminal adipocyte differentiation—is responsible for inducing the final stages of differentiation [50]. mTORC1 signalling has been shown to increase PPARγ transcript and protein levels, as well as its transactivating activity [45,47,51,52], albeit through unknown mechanisms. Cell culture experiments have suggested that regulation of the final differentiation steps is primarily independent of S6K and is probably dependent on 4E-BP inhibition downstream from mTORC1 [40,48]. However, a study has indicated that PPARγ activation can also be suppressed by hyperactive mTORC1 signalling through its negative feedback effects on insulin signalling [53]. These findings indicate that there are probably mTORC1-dependent and -independent inputs into PPARγ activation and adipocyte differentiation downstream from insulin signalling, with more *in vivo* experiments needed.

Lipolysis

In addition to its role in stimulating lipogenesis through SREBP, mTORC1 signalling is believed to promote the storage of fatty acids in lipid stores by inhibiting lipolysis. Neutral lipids, in the form of MAG, DAG and TAG inside the cell are subject to lipolysis to mobilize free fatty acids for energy production or remodelling into new lipid species, including specific membrane and signalling lipids. Patients treated with rapamycin frequently have dyslipidaemia, one facet of which is elevated levels of plasma free fatty acids, which could reflect an increase in lipolysis in adipose tissue [54,55]. Mice treated with rapamycin show a reduction in adipocyte size and overall adiposity, and rapamycin stimulates lipolysis in cultured adipocytes [56–58]. Genetic manipulations of mTORC1 signalling in several mouse models have reinforced the link between mTORC1 activation and an inhibition of lipolysis. The adipose tissue of *4E-BP1/2* double-knockout mice shows decreased lipolysis [48], and *S6K1* knockout mice are leaner with elevated rates of lipolysis [49]. However, mice with adipose-specific *Raptor* knockout, whilst also lean with reduced adiposity, do not show an obvious increase in lipolysis [47]. This suggests that the lipolysis phenotypes observed in the whole-body *4E-BP* and *S6K1* knockout models could be due to systemic effects rather than those intrinsic to the adipocyte. Interestingly, adipose-specific *Atg7* knockout mice that have a defect in autophagy, show decreased adipocyte lipolysis [59], suggesting that the inhibitory effects of mTORC1 on lipolysis could be, at least in part, through its attenuation of autophagy.

Although the molecular mechanisms of lipolytic regulation by mTOR are not fully understood, mTORC1 signalling has been found to influence three distinct lipases: ATGL, HSL and LPL [60]. In adipocytes, ATGL catalyses the lipolysis of TAGs to DAGs within lipid droplets. HSL then converts the DAGs to MAGs. In 3T3-L1 adipocytes, mTORC1 suppression increases the transcription of ATGL, which parallels the enhanced lipolysis induced by rapamycin or siRNA knockdown of *Raptor* [57]. The phosphorylation of HSL at Ser563, an established PKA site, is associated with an increase in its lipase activity. A decrease in HSL phosphorylation correlates with mTORC1 activation and the diminished release of free fatty acids [58]. However, as with ATGL transcriptional suppression, how mTORC1 signalling negatively affects HSL phosphorylation on this PKA site is unknown. Similarly to mTORC1 inhibition, adipocyte-specific *Rictor* knockout also leads to the phosphorylation of HSL at Ser563 [61]. In addition to adipocyte lipolysis, mTORC1 has been implicated in the control of the extracellular lipase LPL. LPL is a water-soluble lipase present in plasma, as well as on the surface of endothelial cells, primarily in muscle and adipose tissue. It hydrolyses TAG in circulating VLDL to promote conversion to IDL and LDL, which facilitates the uptake of lipoprotein into tissues [62]. Systemic rapamycin treatment has been found to decrease LPL activity in mouse adipose tissue, and mouse and human plasma, albeit through an unknown mechanism [63,64]. The collective studies in patients treated with rapamycin and a variety of cell and mouse models suggest that mTORC1 activation, which occurs in metabolic tissues after feeding, promotes the synthesis and storage of lipids. By contrast, mTORC1 inhibition, such as during fasting, stimulates lipolysis and the release of free fatty acids into the circulation.

β-oxidation and ketogenesis

Consistent with the inhibition of mTORC1 signalling promoting fatty acid release and consumption, there is growing evidence that mTORC1 suppresses the β-oxidation of fatty acids for energy or ketogenesis. Rapamycin has been found to increase β-oxidation in rat hepatocytes and this has been attributed to increased expression of β-oxidation enzymes, including long-chain acyl-CoA dehydrogenase and carnitine acyltransferase [17,65]. This effect of rapamycin could be due to the induction of autophagy, which seems to promote the β-oxidation of fatty acids from TAGs in hepatocytes [66]. However, genetic evidence suggests that autophagy has inhibitory effects on β-oxidation in adipose tissue [59,67]. Mice with whole-body knockout of *S6K1* seem to have enhanced β-oxidation, as evidenced by increased levels of CPT1 transcript in isolated adipocytes [49]. Consistent with mTORC1 signalling attenuating β-oxidation, myoblasts isolated from *S6K1/S6K2* double-knockout mice also show enhanced β-oxidation of fatty acids [68]. However, this phenotype was attributed to indirect effects from energy stress and AMPK activation in this setting. As with the *S6K1* knockout and the *S6K1/S6K2* double-knockout mice, mice with adipose-specific *Raptor* knockout are lean with adipocytes that show increased mitochondrial uncoupling, which could allow them to burn lipids rapidly without generating ATP [47,49,68]. Paradoxically, mTORC1 activation has also

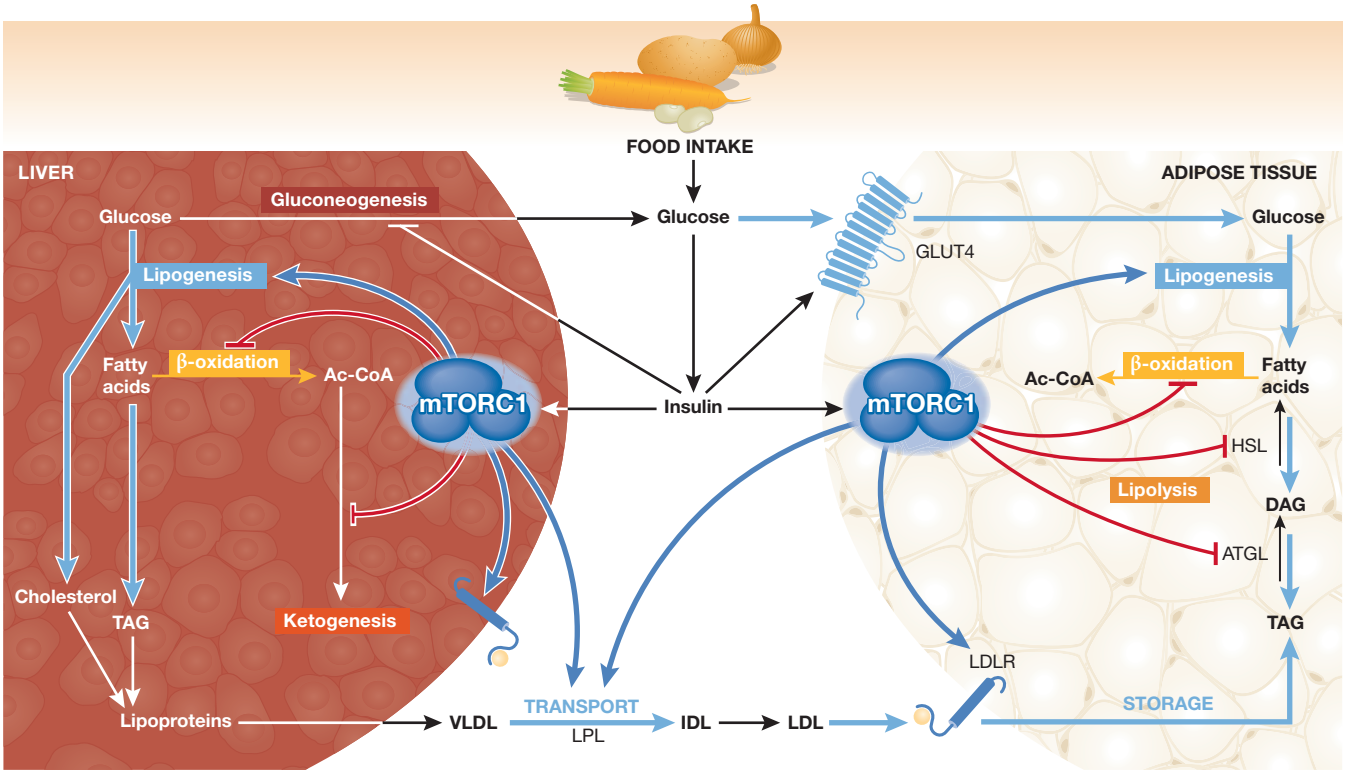

Fig 4 | The increase in insulin levels after a meal alters hepatic and adipose lipid metabolism, at least in part, through mTORC1 signalling (a working model). In the liver, mTORC1 promotes lipid synthesis through SREBP1c activation. In addition, mTORC1 signalling blocks lipid catabolism by blocking β-oxidation and ketogenesis in the liver. Consequently, mTORC1 activation in the liver promotes the synthesis of TAGs and perhaps cholesterol, which are incorporated into VLDL for transport to peripheral tissues. Evidence suggests that mTORC1 signalling positively influences LPL activity, which promotes lipid delivery to peripheral tissues by hydrolysing VLDL to IDL, which is then converted to LDL. Lipoprotein-bound TAGs are taken up by tissues, including adipocytes, through the LDLR. Both the expression and stability of LDLR, at least in the liver, are probably promoted by mTORC1 activation. In response to insulin, mTORC1 has been suggested to inhibit lipolysis in adipocytes by downregulating ATGL and HSL. Therefore, the systemic effects of postprandial mTORC1 activation are to promote the flux of carbon from glucose towards TAG storage in adipose tissue. See text for details regarding the evidence underlying this model. Ac-COA, acetyl-CoA; ATGL, adipose triglyceride lipase; DAG, diacylglycerol; GLUT4, glucose transporter type 4; HSL, hormone-sensitive lipase; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDLR, LDL receptor; LPL, lipoprotein lipase; mTORC1, mechanistic target of rapamycin complex 1; SREBP1c, sterol regulatory element-binding protein 1c; TAG, triacylglycerol; VLDL, very low density lipoprotein.

been linked to increased mitochondrial biogenesis in some settings [69]. This could explain the decrease in oxidative capacity of muscle [69–71] and Jurkat T cells [72] after the inhibition or complete loss of mTORC1 signalling. However, further studies are needed to determine how the observed changes in mitochondrial gene expression and oxygen consumption in these settings influence the β-oxidation of fatty acids. The collective data suggest that mTORC1 signalling inhibits fatty acid oxidation, whilst also promoting mitochondrial biogenesis in some settings.

The acetyl-CoA released from β-oxidation can either enter the TCA cycle or, under fasting conditions in the liver, be converted to ketone bodies. Genetic evidence suggests that mTORC1 signalling in the liver, which is respectively inhibited and activated by fasting and feeding, suppresses ketogenesis [73]. Mice with L*Tsc1*KO that show sustained mTORC1 signalling under fasting have a defect in ketogenesis, whereas mice with liver-specific *Raptor* knockout show an increase in fasting-induced ketogenesis. mTORC1 seems to suppress the expression of ketogenic enzymes through its regulation of N-CoR1 and PPARα [73], by a mechanism probably dependent on S6K2 [74]. These inhibitory effects on PPARα and its transcriptional targets could also explain the negative regulation of fatty acid oxidation by mTORC1. The repression of β-oxidation and ketogenesis by mTORC1 probably acts together with its stimulation of lipogenesis, further promoting the flux of acetyl-CoA towards lipid synthesis and storage.

Lipid transport

Several lines of evidence suggest a role for mTORC1 signalling in the control of lipid mobilization and transport. As stated above, patients treated with mTORC1 inhibitors suffer frequently from a dyslipidaemia consisting of hypertriglyceridaemia and hypercholesterolaemia, as well as increased levels of plasma free fatty acids [55]. The source of the elevated circulating lipids in these patients is unknown. However, TAG and cholesterol transport out of the liver involves their packaging into apolipoprotein complexes, and plasma levels of both apolipoprotein B-100 and apolipoprotein C-III have been found to be increased in patients treated with rapamycin [54]. A study in guinea pigs revealed that the increase in circulating TAGs observed in the response to rapamycin correlates with an increase in VLDL, the primary mode of

TAG export from the liver [75]. In cultured hepatocytes, the ability of insulin to repress the expression of both apolipoprotein B and apolipoprotein A-5 is sensitive to rapamycin, suggesting that the increase in apolipoproteins observed on rapamycin treatment *in vivo* might be due to direct effects on hepatocytes [76,77]. How mTORC1 negatively regulates the expression or protein levels of specific apolipoproteins is unknown and could be secondary to changes in apolipoprotein uptake or degradation. Conversely, mTORC1 signalling seems to upregulate LDLR, which facilitates the uptake of cholesterol-rich LDL from the plasma into the liver and peripheral tissues. *LDLR* gene expression is controlled by SREBP [78] and would, therefore, be predicted to be stimulated by insulin in an mTORC1-dependent manner. In addition, mTORC1 signalling downstream from the insulin receptor in the liver has been found to repress the expression of PCSK9, a known negative regulator of LDLR protein levels [79]. Consequently, rapamycin treatment decreases LDLR levels in a PCSK9-dependent manner, thereby reducing LDL uptake and increasing its circulating levels. Combined with the rapamycin-stimulated increase in lipolysis and apolipoprotein levels, these effects on the LDLR suggest a mechanistic basis for the dyslipidaemia observed in patients treated with mTORC1 inhibitors.

mTORC1 in physiology, obesity and diabetes

The global effects of the mTORC1-mediated regulation of lipid metabolism detailed above are predicted to promote the systemic flux of carbon into lipids and their storage as TAGs within adipose tissue (Fig 4). The postprandial increase in both glucose and insulin stimulates the acute activation of mTORC1 within metabolic tissues, in which mTORC1 has contextual roles in controlling lipid metabolism. In the liver, and probably in adipose tissue, mTORC1 activation induces lipogenesis. At the same time, mTORC1 probably blocks the β-oxidation of fatty acids in the liver, adipose, and perhaps muscle, instead promoting the use and storage of glucose in these tissues. TAGs and cholesterol produced in the liver facilitate the packaging and release of VLDL into circulation. mTORC1 signalling might enhance uptake of lipids by peripheral tissues through the activation of LPL, which hydrolyses VLDL to IDL, and an increase in the levels of LDLR. In adipose tissue, the insulin-stimulated activation of mTORC1 is predicted to contribute to the inhibition of lipolysis, further promoting the storage of TAGs, either mobilized from the liver or produced *de novo* within the adipocytes.

Whilst mTORC1 is activated transiently within metabolic tissues by normal feeding, conditions of nutrient overload and obesity can lead to chronically elevated mTORC1 signalling in these tissues [49,80]. The mechanism by which obesity leads to hyperactivation of mTORC1 is unknown but happens probably through a combination of hyperglycaemia and hyperinsulinaemia under these conditions. Furthermore, evidence suggests that increased circulating levels of branch-chain amino acids, which are known to activate mTORC1, correlates with the development of obesity and insulin resistance [81]. In addition to potentially exacerbating obesity by further promoting lipid storage in adipose depots, chronic mTORC1 activation under such conditions is believed to contribute to the development of insulin resistance, which frequently accompanies obesity. Increased mTORC1 signalling can trigger several distinct feedback mechanisms, which in a cell-autonomous manner, dampens the cellular response to insulin. The *in vivo* contribution of these feedback mechanisms to insulin resistance is well illustrated by loss- and gain-of-function mouse models of mTORC1 signalling. For instance, *S6K1* knockout mice have enhanced peripheral insulin sensitivity [49], whereas mice with L*Tsc1*KO show hepatic insulin resistance with greatly reduced Akt signalling [18]. Therefore, under conditions of obesity, mTORC1 activation in metabolic tissues probably both perpetuates obesity and promotes insulin resistance, thereby expediting the progression to type II diabetes.

The fundamental role of mTORC1 in regulating whole-body lipid homeostasis, paired with its frequent upregulation in obesity and type 2 diabetes, suggests that mTOR inhibitors might offer some therapeutic benefit in metabolic diseases. In theory, mTORC1-specific inhibitors should suppress lipid synthesis and promote lipolysis and lipid catabolism, in addition to blocking mTORC1-dependent feedback mechanisms to resensitize tissues to insulin. However, important caveats arise from the use of mTORC1 inhibitors to combat obesity and diabetes. First, prolonged treatment with rapamycin disrupts mTORC2 and therefore Akt activation downstream from the insulin receptor, further exacerbating the insulin-resistant phenotype (Sidebar A; [82]). Second, patients treated with rapamycin frequently have increased levels of circulating TAGs, cholesterol and free fatty acids [55]. Therefore, whilst rapamycin treatment might help mobilize lipids and deplete fat stores, lipid clearance offers an additional pathological challenge. Targeting mTORC1 signalling indirectly might offer a more promising avenue. AMPK is a potent negative regulator of mTORC1, blocking its function through phosphorylation of both the TSC–TBC complex [2,5] and Raptor [6]. Therefore, mTORC1 signalling is blocked on activation of AMPK, which is stimulated by a large variety of natural and synthetic compounds, including metformin, resveratrol and aspirin [83]. Importantly, metformin is the most widely prescribed anti-diabetes drug in the world. Whether any of the beneficial metabolic effects of metformin are attributed to its inhibition of mTORC1 signalling is one of several important outstanding questions (Sidebar B).

Sidebar B | In need of answers

(i) What are the molecular mechanisms by which mTORC1 regulates SREBP1 and SREBP2?
(ii) Which lipid species are most influenced by the activation state of mTORC1 signalling?
(iii) Does mTORC1 stimulate the synthesis of membrane lipids in addition to storage lipids?
(iv) How do lipids influence mTORC1 signalling?
(v) How does mTORC1 become dysregulated under conditions of obesity?
(vi) Does mTORC1 inhibition contribute to the effects of AMPK-activating compounds on cellular and systemic metabolism?
(vii) What is the role of mTORC1 activation in the common lipogenic phenotype of cancer cells?
(viii) How is lipid metabolism differentially regulated by mTORC1 in different tissues?

ACKNOWLEDGEMENTS

We apologize to our colleagues whose work we were not able to cover in this review due to space constraints. Research in the Manning laboratory related to the subject of this review was supported by a predoctoral training grant DGE-1144152 from the National Science Foundation (S.J.H.R.) and by National Institutes of Health grants R01-CA122617 and P01-CA120964, Department of Defense grants TS093033 and TS110065, a Sanofi Innovation Award and grants from the American Diabetes Association and Ellison Medical Foundation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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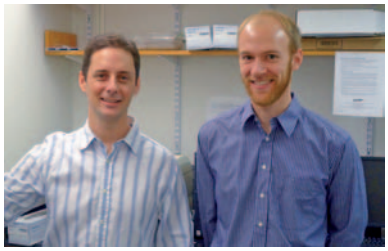

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