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### **Symposium III: Brain signalling and onward communications**

## **Coupling nutrient sensing to metabolic homoeostasis: the role of the mammalian target of rapamycin complex 1 pathway**

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The mammalian target of rapamycin complex 1 (mTORC1) pathway is known to couple different environmental cues to the regulation of several energy-demanding functions within the cell, spanning from protein translation to mitochondrial activity. As a result, at the organism level, mTORC1 activity affects energy balance and general metabolic homoeostasis by modulating both the activity of neuronal populations that play key roles in the control of food intake and body weight, as well as by determining storage and use of fuel substrates in peripheral tissues. This review focuses on recent advances made in understanding the role of the mTORC1 pathway in the regulation of energy balance. More particularly, it aims at providing an overview of the status of knowledge regarding the mechanisms underlying the ability of certain amino acids, glucose and fatty acids, to affect mTORC1 activity and in turn illustrates how the mTORC1 pathway couples nutrient sensing to the hypothalamic regulation of the organisms' energy homoeostasis and to the control of intracellular metabolic processes, such as glucose uptake, protein and lipid biosynthesis. The evidence reviewed pinpoints the mTORC1 pathway as an integrator of the actions of nutrients on metabolic health and provides insight into the relevance of this intracellular pathway as a potential target for the therapy of metabolic diseases such as obesity and type-2 diabetes.

### **Mammalian target of rapamycin: S6 kinase 1: Nutrient sensing: Hypothalamus**

Multicellular organisms have to constantly adapt anabolic and catabolic processes to availability of nutrients in order to guarantee survival. In mammals, metabolic responses to nutrients, such as amino acids, glucose and fatty acids, are tightly coupled to the modulation of nutrient- and energy-sensing pathways, such as the mTOR (mammalian or mechanistic target of rapamycin) signalling cascade. At the cellular level, mTOR couples different environmental cues to the regulation of several, energy-demanding functions, spanning from translation to lipid biosynthesis. Consequently, at the organism level, mTOR critically affects energy balance and general metabolic

homoeostasis by modulating both the activity of neuronal populations that within the brain play key roles in the control of food intake and body weight, as well as energy storage and expenditure in peripheral tissues. As such, mTOR is implicated in diseases where metabolic homoeostasis is compromised, namely obesity, diabetes and cancer.

This review focuses on recent advances made in understanding the role of the mTOR pathway in the regulation of energy balance. More particularly, it will provide an overview of the status of knowledge regarding the ability of the mTOR pathway to couple nutrient sensing to the

**Abbreviations:** AMPK, AMP-activated protein kinase; BCAA, branched-chain amino acids; FAS, fatty acid synthase; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PI3 K, phosphoinositide 3-kinase; S6K1, S6 kinase 1; SREBP, sterol regulatory element-binding protein.

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regulation of food intake, body weight and peripheral metabolism. Finally, it will be discussed how dysregulation of the mTOR pathway might have relevance in obesity and metabolic disorders associated with it.

### Upstream and downstream of mammalian target of rapamycin complex 1

TOR is a highly conserved protein kinase present in almost all eukaryotic cells, which belongs to the phosphoinositide 3-kinase (PI3 K)-related protein kinases family. TOR was discovered through the use of genetic and biochemical approaches aimed at identifying the main intracellular target of the immunosuppressant rapamycin<sup>(1,2)</sup>. Its mammalian isoform (mTOR) is the catalytic subunit of two different complexes, called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which result from the coupling of mTOR with specific proteins within the cell. Rapamycin rapidly inhibits mTORC1 activity, and it is also able to inhibit mTORC2 when prolonged exposure to the macrolide occurs in certain cell lines and tissues<sup>(3)</sup>. Of the two complexes, mTORC1 is known to respond to nutrients and growth factors, and by doing so, it critically regulates proliferative, metabolic and cell survival responses<sup>(4,5)</sup>. Because of these roles and of mTORC1 implication in the regulation of the organismal metabolic homeostasis, mTORC1 is the main focus of this review.

To carry out its functions, mTORC1 needs to rapidly respond to levels of nutrients, energy, hormones and growth factors. Consequently, multiple intracellular pathways have evolved to control the activation of the mTORC1 signalling in response to different signals.

#### *Mammalian target of rapamycin complex 1, growth factors and hormones*

Recent studies have established that mTORC1 activity critically affects food intake, body weight and peripheral metabolism<sup>(6–8)</sup>.

To have such an impact, mTORC1 activity must be closely wired to the long-range communication signals that the body uses to coordinate the distribution of nutrients and the consequent tissue-specific functions relevant for the maintenance of energy balance.

Among those long-range communication signals, the hormones insulin and leptin play a central role<sup>(9,10)</sup>. They are known to act through the PI3 K/Akt pathway, which is among the intracellular cascades involved in determining the appetite-suppressing action of insulin and leptin in the hypothalamus<sup>(11,12)</sup>.

Binding of insulin to its receptor leads to activation of both PI3 K and Akt, which inhibits tuberous sclerosis protein 2, a negative regulator of mTORC1<sup>(13)</sup>. Interestingly, full activation of Akt requires mTORC2 activity, which is therefore posited upstream of mTORC1 and is relevant for mTORC1 functioning<sup>(14)</sup>.

However, growth factors can signal to mTORC1 through pathways other than the PI3 K/Akt cascade. For instance, evidence shows that growth factors can act onto mTORC1 through the extracellular signal regulated kinase 1/2, the Wnt pathway and the glycogen synthase kinase 3 $\beta$ <sup>(15,16)</sup>.

#### *Mammalian target of rapamycin complex 1 and cellular energy*

mTORC1 senses levels of energy, namely ATP, available in the cell. This is relevant, taking into account that during states of cellular energy depletion and stress cells have to be readily capable to turn off anabolic and energy-consuming pathways such as the mTORC1 signalling. When the AMP:ATP ratio increases, the AMP-activated protein kinase (AMPK), a master sensor of intracellular fuel status<sup>(17)</sup>, is activated and it inhibits mTORC1 by activating tuberous sclerosis protein 2<sup>(18)</sup> and inhibiting Raptor, a protein component of mTORC1<sup>(19)</sup>.

Consequently, mTORC1 activity is sensitive to any type of cellular stressor able to affect ATP levels, such as hypoxia, or stressors that critically affect cellular function, such as DNA damage (for review see<sup>(5)</sup>).

#### *Mammalian target of rapamycin complex 1 and nutrients*

mTORC1 signalling, as will be further detailed later, is a master regulator of protein synthesis and it is able to respond to the availability of amino acids, the building blocks of proteins. Amino acids are necessary to mTORC1 activity and their lack cannot be compensated for by other known mTORC1-activating stimuli<sup>(20)</sup>. Among the amino acids, branched-chained amino acids (BCAA) and in particular leucine, which is a key activator of mTORC1, are transported and sensed intracellularly<sup>(21)</sup>. In turn, availability of amino acids increases intracellular calcium levels, thus activating mTORC1 through the involvement of a calcium/calmodulin-dependent activation of the class III PI3 K hVPS34<sup>(22,23)</sup>. Importantly, it has also been shown that amino acids, by recruiting the Rag family of small GTPases, cause mTORC1 to shuttle to lysosomes, allowing it to respond to growth factors and to eventually regulate cell autophagy (for review see<sup>(5)</sup>). However, the intracellular amino acid sensor that directly couples amino acid-dependent signalling to mTORC1 is not known. Recently, it has been proposed that such a role might be played by the leucyl-tRNA synthetase, an enzyme catalysing the ligation of leucine to its cognate transfer RNA, which has been shown to work as mTORC1-associated protein required for amino acid-induced mTORC1 activation<sup>(24)</sup>.

Little is known on the intracellular mechanisms linking glucose availability to mTORC1 activation. Chemical inhibition of glycolysis suppresses mTORC1 activity<sup>(25)</sup> and low levels of glucose, by activating AMPK, also lead to mTORC1 inhibition. Conversely, mTORC1 promotes glucose uptake and flux through glycolysis, by activating the transcription and translation of the hypoxia-inducible factor 1 $\alpha$ <sup>(26)</sup>.

Similarly, little is known on the mechanisms engaged by fatty acids to modulate mTORC1 activity, although it has been shown that dietary lipids phosphorylate activate the mTORC1 downstream target S6 kinase 1 (S6K1) in a muscle cell line independently of amino acids availability<sup>(27)</sup>. On the other hand, mTORC1 activity critically regulates lipid synthesis and adipocyte differentiation (see later).

### *Downstream of mammalian target of rapamycin complex 1: regulation of protein synthesis and beyond*

mTORC1 regulates protein synthesis and translation by coordinating upstream inputs coming from growth factors, intracellular energy status and nutrient availability. In particular, mTORC1 phosphorylation of S6K1 and eukaryotic initiation factor 4E-binding proteins is the rate-limiting step for translation. S6K1 in turn phosphorylates a number of downstream substrates including the S6 ribosomal protein (S6) and leads to increased mRNA biogenesis and cap-dependent translation and elongation<sup>(4,28)</sup>. The phosphorylation status of S6K1, S6 and 4E-binding proteins is often used as marker of mTORC1 activation in cells and tissues. And of relevance, in case of chronic activation of the pathway, S6K1 exerts an inhibitory role on insulin-dependent PI3 K/Akt activation through the inhibition of insulin receptor substrate proteins. Such negative feedback has been proposed to favour cellular insulin desensitisation and resistance<sup>(29)</sup>.

Although mTORC1 stimulates protein synthesis, a process that requires high cellular energy levels, it also inhibits autophagy, a controlled self-degradation process, which provides energy substrates in times these are low (for review see<sup>(5)</sup>).

In addition, mTORC1 regulates several metabolic pathways through transcription. For instance, mTORC1 activity drives adipocyte differentiation and lipid accumulation by up-regulating the transcription factor PPAR $\gamma$ , probably through the activation of the sterol regulatory element-binding protein (SREBP). Conversely, rapamycin inhibits PPAR $\gamma$  and impairs adipocytes differentiation (for review see<sup>(30)</sup>). The mTOR kinase also phosphorylates the signal transducer and activator of transcription 3, a transcription factor critically implicated in the actions of leptin on energy balance regulation<sup>(31)</sup>. Interestingly, mTOR-dependent phosphorylation of signal transducer and activator of transcription 3 leads to its translocation in the mitochondria, with consequent increase of oxidative phosphorylation<sup>(32)</sup>. In fact, mTORC1 activity promotes mitochondria biogenesis and enhances mitochondrial respiration also by positively regulating the activity of the transcription factor PPAR $\gamma$  coactivator 1 $\alpha$ <sup>(33)</sup>. Accordingly, deletion of the mTORC1 protein Raptor in the muscle causes defective mitochondrial biogenesis and decreased oxidative capacity<sup>(34)</sup>.

Fig. 1 summarises the earlier-mentioned pathways involved in nutrient sensing and their relationship with mTORC1. For a more in-depth description of the molecular composition of mTORC1 and mTORC2 as well as of the growing number of intracellular pathways identified upstream and downstream of mTORC1, the reader should refer to recently published reviews focusing on these aspects<sup>(5,28,35)</sup>.

### **Mammalian target of rapamycin complex 1 signalling in the regulation of energy balance and metabolism**

#### *Mammalian target of rapamycin complex 1 pathway and amino acids sensing*

Within the last decade, the implication of the mTORC1 pathway in determining the anabolic effect of BCAA and

leucine in particular has been extensively studied in skeletal muscle. Indeed, among the BCAA, leucine is the most effective in inducing protein synthesis in this tissue by stimulating the mTORC1 pathway<sup>(36)</sup>.

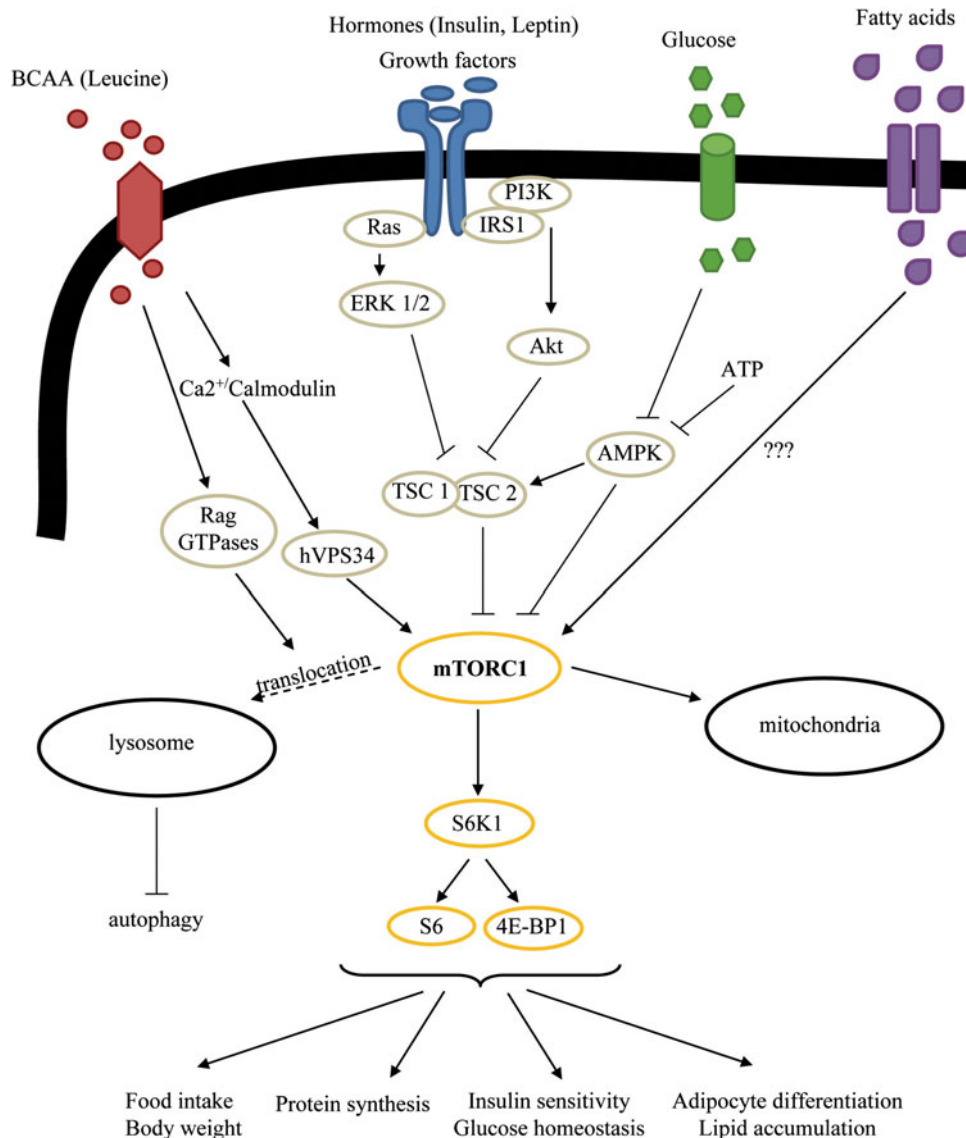
Several studies have also investigated BCAA and leucine ability to help preserve muscle mass and demonstrated their beneficial metabolic and clinical effects under different pathological conditions characterised by substantial loss of muscle, such as cancer and uraemia<sup>(37)</sup>. A lot of information is actually available on the safety and use of leucine as a supplement in the clinical setting, to help preserve muscle mass during wasting syndromes, as well as an anabolic enhancer consumed by athletes and body builders in the attempt to increase their muscle mass<sup>(38,39)</sup>. However, only very recent evidence has linked leucine and mTORC1 activity to energy balance regulation.

In 2006, we were the first to describe that leucine could acutely decrease food intake and body weight in lean rats when administered intracerebroventricularly within the brain<sup>(6)</sup>. This effect was specific for leucine, since other BCAA such as valine did not affect food intake under the same conditions, and it was dependent on the activation of mTORC1 and its downstream targets S6K1 and S6 in the hypothalamus<sup>(6)</sup>. The latter is one of the main brain structures involved in the regulation of energy balance<sup>(9,40)</sup>.

Similar to leucine, the appetite-suppressant action of the adipocyte-derived hormone leptin, one of the major peripheral signals studied so far in the context of food intake and body weight regulation<sup>(41)</sup>, depends on the activation of the hypothalamic mTORC1 signalling<sup>(6,7)</sup>, thus suggesting that this pathway is an essential intracellular integrator of the actions of both nutrients and hormones on the central circuits regulating energy balance.

Other animal studies have subsequently described the appetite-suppressant action of leucine, by administering the amino acid either directly in the brain, or peripherally, as a dietary supplement. These reports further confirmed that leucine-induced decrease in food intake is dependent on hypothalamic mTORC1 activity and associated with the inhibition of AMPK<sup>(8,42,43)</sup>, an intracellular energy sensor known to inhibit mTORC1<sup>(4)</sup>. Furthermore, intracerebroventricularly administration of leucine decreases gene expression levels of the appetite-inducing neuropeptides, Agouti-related protein and neuropeptide Y, while increasing mRNA levels of the appetite-suppressant precursor polypeptide pro-opio-melanocortin within the arcuate nucleus of the hypothalamus<sup>(6,8,42)</sup>.

It has been further shown that the acute intrahypothalamic administration of leucine or its metabolite alpha-ketoisocaproic acid in lean mice or rats reduces meal size and meal number by engaging a forebrain–hindbrain neurocircuit that activates pro-opio-melanocortin-producing neurons in the arcuate nucleus of the hypothalamus, oxytocin neurons in the paraventricular nucleus and satiety effector neurons in the brainstem<sup>(43)</sup>. Direct modulation of hypothalamic circuits by leucine might be physiologically relevant for the control of food intake, since levels of leucine in the cerebrospinal fluid are directly proportional to the level of the amino acid in the blood, which changes in relation to the quantity of leucine present in the meal<sup>(43)</sup>. Interestingly, published animal studies have shown that the



**Fig. 1.** (colour online) The mammalian target of rapamycin complex 1 (mTORC1) pathway can be activated by hormones, growth factors and nutrients. Various upstream kinases [e.g. extracellular signal-regulated kinases1/2 (ERK1/2), phosphoinositide 3-kinase (PI3 K/Akt), AMP-activated protein kinase (AMPK)] that converge on the tuberous sclerosis complex (TSC1/TSC2), regulate mTORC1 activity. High-glucose levels inhibit AMPK, whereas low-glucose and low-ATP levels lead to the AMPK-dependent activation of TSC2, which inhibits mTORC1 signalling. AMPK also directly inhibits mTORC1 by phosphorylating Raptor, a component of mTORC1. Hormones such as insulin signal to mTORC1 through PI3 K and Akt, which inactivates TSC2 to prevent TSC2 inhibition of mTORC1. Activation of mTORC1 leads to phosphorylation of its downstream targets ribosomal protein S6 kinase (S6K1), eukaryotic initiation factor 4E-binding protein (4E-BP) and ribosomal protein S6 (S6). Prolonged or constitutive activation of mTORC1/S6K1 signalling attenuates insulin-dependent PI3 K/Akt activity by inhibiting insulin receptor substrate (IRS) proteins. Amino acids increase mTORC1 signalling by favouring Rag protein action on mTORC1 and its translocation to the lysosome, a phenomenon that leads to autophagy inhibition, and by inducing a  $\text{Ca}^{2+}$ /calmodulin-mediated activation of the class III PI3 K human vacuolar protein sorting-34 (hVPS34). Certain fatty acids have been shown to activate mTORC1 downstream targets, but actual mechanisms of actions are currently unknown. Once activated the mTORC1 pathway affects the hypothalamic control of energy balance and regulates protein synthesis, whole body glucose metabolism, insulin sensitivity, lipogenesis and adipogenesis. BCAA, branched-chain amino acid.

administration of an increased amount of leucine directly through the diet can be equally feasible and efficient. In fact, a leucine-enriched standard diet is as effective in acutely

reducing food intake in lean rats as a high-protein diet, when the leucine addition was equivalent to the amount of casein used in the high-protein diet<sup>(8)</sup>.



Apart from its direct action on hypothalamic circuits, leucine might also modulate peripheral hormonal signals, which are in turn able to affect the activity of neurons controlling food intake and body weight. Indeed, it has been shown that the post-prandial rise of circulating leptin depends in part on the presence of leucine in the ingested food. Conversely, a leucine-deficient meal reduces by almost 40% the meal-related stimulation of leptin secretion in rats<sup>(44)</sup>. Leucine-induced leptin secretion is associated to increased mTORC1 activity in the adipose tissue, which is actually required to increase plasma leptin, since administration of the mTOR inhibitor rapamycin inhibits the leptin peak<sup>(44)</sup>. Thus, it is possible that part of the appetite-suppressant effect of leucine might depend on an increase in peripheral leptin levels and subsequent action of this hormone on neural circuits regulating food intake.

This set of initial observations has led to a series of intervention studies evaluating whether chronic leucine supplementation could be beneficial in models of diet-induced obesity.

Zhang and colleagues have reported that leucine supplementation (administered in water in sufficient quantity to double the circulating levels of the amino acid), reduces diet-induced body weight gain and adiposity in mice in a food-intake-independent manner, while improving both insulin sensitivity and dyslipidemia<sup>(45)</sup>. Weight-independent improvements in glycemic control after leucine supplementation were also described in a monogenic mouse model of obesity and in a polygenic model of type-2 diabetes<sup>(46)</sup>. Increased energy expenditure, associated to an improvement of mitochondrial oxidative function in the skeletal muscle and reduced inflammation in the white adipose tissue might explain the beneficial effects of leucine supplementation on body weight and glucose metabolism<sup>(45–47)</sup>. These changes were also associated with an increase in mTORC1 activity in the liver, skeletal muscle and white fat<sup>(47)</sup>. However, not all studies have reported an effect on body weight<sup>(47,48)</sup> or relevant improvement in glucose metabolism<sup>(48)</sup>. Nevertheless, and in agreement with the suggested beneficial role of higher leucine levels on the regulation of adiposity, mice lacking the mitochondrial branched-chain aminotransferase, which catalyses the first step of BCAA metabolism, have elevated plasma BCAA, decreased adiposity and body weight, improved insulin sensitivity and increased energy expenditure, which results from futile cycles due to increased protein degradation and synthesis associated with increased mTORC1 activity in skeletal muscle<sup>(49)</sup>. Accordingly, a recent cross-sectional epidemiological investigation has reported that a higher BCAA intake is actually associated with a lower prevalence of overweight and obesity in otherwise healthy middle-aged adults<sup>(50)</sup>.

Whilst it must also be mentioned that recent studies in human subjects have linked high circulating levels of BCAA to insulin resistance and diabetes<sup>(51,52)</sup>, it is unclear from these studies whether elevated BCAA levels are the cause or consequence of the observed metabolic phenotypes.

Finally, BCAA supplementation has been shown to increase the lifespan in mice<sup>(53)</sup>. This effect was associated with an increase in mitochondrial biogenesis in muscle,

through increased endothelial nitric oxide synthase and mTORC1 activity, which favoured increased physical endurance<sup>(53)</sup>. Seemingly in contrast to these findings, other studies have reported that reduced mTORC1 signalling underlies life span extension by energy restriction, since both chronic rapamycin administration and S6K1 deletion increase life span, an effect observed prevalently or only in female mice<sup>(54,55)</sup>. Thus, the apparent divergent evidence available so far overall suggests that the role of mTORC1 signalling in energy restriction and lifespan modulation might be more complex than what was initially thought, and likely tissue-specific.

#### *Mammalian target of rapamycin complex 1 pathway and glucose sensing*

Like amino acids, glucose is also able to activate the mTORC1 pathway in cells and its action on this signalling cascade as well as the relationship between mTORC1 activity and glucose uptake and metabolism have been mostly studied in the skeletal muscle and endocrine pancreas. It is known, for instance, that *in vitro* exposure to 2-deoxyglucose, a non-metabolisable glucose analogue, which impedes glucose utilisation, rapidly inhibits mTORC1 signalling<sup>(25)</sup>. Conversely, rapamycin-mediated inhibition of mTORC1 activity in muscle cells reduces glucose utilisation while increasing fatty acid oxidation<sup>(56)</sup>, thus suggesting that mTORC1 activity determines fuel substrate use. Instead, high glucose levels *in vitro* favour intracellular lipid accumulation and adipogenesis through the mTORC1-dependent activation of SREBP (for review see<sup>(30,57)</sup>).

Glucose also has a permissive role in promoting insulin-dependent regulation of mTORC1 targets, such as 4E-binding proteins 1<sup>(58)</sup>, whereas mTORC1 activity leads to increased expression of glucose transporters in several cell types, thus further favouring glucose uptake<sup>(59)</sup>.

In pancreatic islets, glucose requires a functional mTORC1 signalling to enhance  $\beta$ -cell growth and proliferation<sup>(60)</sup>, a phenomenon relevant for the appropriate, compensatory response of the islets to chronic nutrient overload and increased insulin demand during type-2 diabetes. In fact, it has been shown that rapamycin administration inhibits  $\beta$ -cell proliferation, induces  $\beta$ -cell apoptosis and causes fulminant diabetes in obese animal models<sup>(61,62)</sup>. Although genetic models with specific over-activity of mTORC1 in  $\beta$  cells, due to deletion of tuberous sclerosis protein 2 (an inhibitor of mTORC1) or liver kinase B1 (an activator of AMPK), have increased  $\beta$ -cell mass and improved glucose tolerance<sup>(63,64)</sup>.

Less explored is the relationship between glucose sensing and mTORC1 signalling in the hypothalamic regulation of energy balance. However, a recently published study has shown that hypothalamic mTORC1 activation by glucose mediates activation of hypoxia-inducible factor, which in turn regulates glucose-dependent food intake and body weight by increasing expression of the anorexiogenic pro-opio-melanocortin peptide<sup>(65)</sup>. Genetic deletion of hypoxia-inducible factor in pro-opio-melanocortin neurons, which are glucose responsive and are known to regulate whole-body glucose homeostasis<sup>(66)</sup>, impairs

hypothalamic glucose sensing and favours the development of obesity, while overexpression of hypoxia-inducible factor in the mediobasal hypothalamus decreases body weight gain during exposure to a high-fat diet, mainly by reducing food intake in treated mice<sup>(65)</sup>.

#### *Mammalian target of rapamycin complex 1 pathway and fatty acids sensing*

As previously mentioned, very little is known on the ability of lipids to activate the mTORC1 pathway in cells. Although, both oleic and palmitoleic acid drive phosphorylation of S6K1 in muscle cells independently of amino acid availability<sup>(27)</sup>. Moreover, palmitate exposure increases insulin-stimulated S6K1 phosphorylation in L6 myotubes, while AMPK activation abolishes lipid-induced mTORC1 activation<sup>(67)</sup>. Consequently, excess of fatty acids drives insulin resistance *in vitro* in hepatocytes and muscle cells through overstimulation of mTORC1 signalling<sup>(68,69)</sup>.

On the other hand, studies published over the past few years show that mTORC1 signalling is a critical regulator of lipid biosynthesis, an essential mechanism guaranteeing the production of lipids then used by the cells to constitute membranes, as precursors of other cellular constituents or as signalling molecules (for review see<sup>(30)</sup>). Insulin treatment or constitutive Akt activation rapidly induces nuclear accumulation of SREBP-1 and the expression of lipogenic genes, such as acetyl-CoA carboxylase, fatty acid synthase (FAS) and stearoyl-CoA desaturase 1. mTORC1 activity is required for SREBP-1 recruitment [reviewed in<sup>(30)</sup>]. Dysregulation of such mechanism due to mTORC1 overactivity might favour the development of non-alcoholic fatty liver disease, the most common liver disease found in obesity and type-2 diabetes (for review see<sup>(30)</sup>). mTORC1 activity also induces adipogenesis in the adipose tissue through the activation of PPAR $\gamma$ , its co-activator lipin1 and SREBP-1 (for review see<sup>(30)</sup>).

Interestingly, mice lacking S6K1 and therefore characterised by a defective mTORC1 signalling, have reduced adipose tissue mass and are resistant to diet-induced obesity<sup>(29)</sup>. This seems due to the fact that S6K1 is required for the commitment of embryonic stem cells to adipocyte progenitors, while being dispensable for terminal adipocyte differentiation<sup>(70)</sup>.

Exposure to high-saturated fat diets results in a resistance to the actions of both leptin and insulin at the level of the hypothalamus (for review see<sup>(71)</sup>). However, the role of fatty acid metabolism in the hypothalamic regulation of energy balance, and its reciprocal relationship with glucose metabolism has only recently begun to be investigated.

Neurons derive most of their ATP from the oxidation of glucose rather than lipids<sup>(72)</sup>. Nevertheless, the use of FAS inhibitors and of other pharmacological or genetic tools able to modulate different enzymes of the FAS pathway in the hypothalamus suggests that this pathway integrates signals from nutrients and nutrient-derived hormones and therefore affects energy balance regulation<sup>(73–75)</sup>.

In the hypothalamus, AMPK inhibits fatty acid synthesis by inhibiting acetyl-CoA carboxylase, the enzyme controlling the conversion of acetyl-CoA to malonyl-CoA, an

intermediate of fatty acid synthesis, which is considered to work as a fuel-sensing signal<sup>(73,74)</sup>. The gastric hormone ghrelin increases food intake by driving hypothalamic AMPK activity and consequently increasing fatty acid oxidation in this brain area<sup>(76)</sup>. Conversely, leptin decreases food intake by inhibiting AMPK activity<sup>(77)</sup>, and increasing hypothalamic acetyl-CoA carboxylase activity and malonyl-CoA levels<sup>(78)</sup>.

In addition, we have recently shown that intracerebroventricular administration of inhibitors of the FAS pathway decrease food intake via an mTORC1-dependent mechanism<sup>(79)</sup>. FAS inhibitors possibly suppress food ingestion by increasing hypothalamic glucose utilisation<sup>(75)</sup>. Thus, during ketosis, when neurons use ketone bodies in preference to glucose, central administration of FAS inhibitors has no effect on food intake or hypothalamic mTORC1 activity<sup>(75,79)</sup>. This therefore suggests that a regulatory loop exists between fatty acid metabolism and mTORC1 signalling, such that inhibition of FAS drives mTORC1 activity depending on glucose availability and/or utilisation<sup>(79)</sup>.

#### **Mammalian target of rapamycin complex 1 signalling as therapeutic target for metabolic disease?**

Exposure to a high-fat diet and development of diet-induced obesity have been associated with activation of the mTORC1 pathway in peripheral tissues, like the liver and the muscle<sup>(80)</sup> and prolonged activity of the pathway *in vitro* leads to desensitisation of target cells to the action of insulin (for review see<sup>(35)</sup>), thus favouring insulin resistance.

Owing to this evidence, it was suggested that pharmacological inhibition of mTORC1 would have improved insulin sensitivity and peripheral glucose metabolism. Instead, administration of rapamycin worsens glucose metabolism and diabetes in obese animals and causes dyslipidemia<sup>(61)</sup>. These data could be explained taking into account that mTORC1 signalling is critical for the cellular adaptation to nutrient overload and increased insulin demand during diet-induced obesity. Furthermore to complicate the picture even more, very recent findings actually suggest that mTORC2 disruption contributes to rapamycin-induced insulin resistance *in vivo*<sup>(81)</sup>. Furthermore, it has been also shown that chronic exposure to high-fat diets and obesity are actually associated with decreased protein synthesis and mTORC1 activity in skeletal muscle<sup>(82)</sup> as well as with impaired activation of skeletal muscle protein synthesis in response to nutrient ingestion<sup>(83)</sup>. These reported discrepancies in the activity of the pathway in peripheral tissues under high-fat diet exposure might be due to different factors, including the point in time when the pathway was analysed, the length of exposure to the diet and the type of diet used, just to cite a few.

Exposure to high-fat diets also alters the activity of hypothalamic circuits regulating energy balance. This in turn favours hyperphagia, body weight gain and altered whole-body glucose metabolism. Hypothalamic mTORC1 activity is down-regulated by the consumption of high-fat diets and such down-regulation might precipitate the onset

of molecular resistance in the hypothalamus to the action of nutrients and hormones, such as insulin and leptin, which become unable to regulate food intake and body weight<sup>(7,43,84)</sup>.

Thus, considering the evidence provided so far about the effects of high-fat, obesogenic diets on the modulation of mTORC1 signalling in peripheral tissues and in the hypothalamus, it would seem counterproductive to broadly inhibit the pathway for the treatment of obesity and its associated metabolic alterations and definitely further work is needed to identify molecular determinants of the action of nutrients on mTORC1 activity and to better define the context- and cell-dependent function of mTORC1 itself, in the hope to determine specific components of the pathway that might be eligible targets for the therapy of obesity and diabetes.

### Conclusions

The mTORC1 pathway is responsible for the appropriate coupling of intracellular responses to availability of nutrients and it importantly participates in several aspects of energy balance regulation, spanning from the intake of energy to their storage and use.

The evidence reviewed here provides further understanding of how different types of nutrients affect mTORC1 activity and of the role of the mTORC1 pathway in modulating other intracellular signals involved in the control of glucose and lipid metabolism.

The seemingly opposite functions played by mTORC1 signalling in the hypothalamus, where it has a catabolic action by reducing food intake in response to nutrients and hormones, and peripheral tissues, where it exerts an anabolic action by increasing glucose uptake, lipid biosynthesis and adipogenesis, essentially reconcile with the pathway being a downstream target of insulin.

However, exact mechanisms through which the mTORC1 signalling exerts its functions in different tissues and how changes in its activity actually result in behavioural and metabolic changes *in vivo* need to be further investigated.

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