Regulation of nutrient uptake by AMP-activated protein kinase

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Abstract

AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is a key regulator of energy balance at both the cellular and whole-body level. AMPK acts to stimulate ATP production and reduce ATP consumption when cellular ATP levels fall, thereby normalizing energy balance. Given the central role of AMPK in cellular carbohydrate and lipid metabolism, AMPK activation has been proposed to be a therapeutic target for conditions associated with dysfunctional nutrient metabolism including obesity, type 2 diabetes, hepatic steatosis, cardiovascular diseases and cancer. One way by which increased ATP production can be achieved is by increasing the supply of nutrient substrates. In the 1990s, AMPK activation was demonstrated to stimulate glucose uptake in striated muscle, thereby improving substrate supply for ATP production. Subsequently AMPK activation was postulated to underlie the increase in glucose uptake that occurs during muscle contraction. More recently, however, several lines of evidence have demonstrated that AMPK activation is unlikely to be required for contraction-mediated glucose uptake. Furthermore, despite the importance of AMPK in cellular and whole-body metabolism, far fewer studies have investigated either the role of AMPK in glucose uptake by non-muscle tissues or whether AMPK regulates the uptake of fatty acids. In the present review, we discuss the role of AMPK in nutrient uptake by tissues, focusing on glucose uptake out with muscle and fatty acid uptake.

1. Introduction

1.1   AMPK

AMP-activated protein kinase (AMPK) was first identified as an activity that inhibited two metabolic enzymes, acetyl CoA carboxylase (ACC) and HMG CoA reductase (HMGR), which regulate FA (fatty acid) and isoprenoid/sterol synthesis respectively [1,2]. Over the subsequent decades, it has become apparent that AMPK acts as a cellular energy sensor, stimulating ATP production and inhibiting ATP utilization when ATP concentrations fall. This fundamental role of AMPK in the regulation of nutrient metabolism has led to its proposal as a therapeutic target for conditions of

dysfunctional metabolism, including type 2 diabetes, insulin resistance, obesity and hepatic steatosis [3,4]. Furthermore, AMPK activation is associated with anti-inflammatory, anti-atherosclerotic and anti-tumorigenic actions which have highlighted its potential as a therapeutic target for proinflammatory disorders, cardiovascular diseases and cancer [5-7].

1.2 Structure and regulation of AMPK

AMPK is a heterotrimer of an α catalytic subunit containing a serine/threonine protein kinase domain complexed with β and γ regulatory subunits. AMPK belongs to the CAMKL (CAMK-like) family of protein kinases [8] and is conserved in virtually all eukaryotes [9]. In mammals, there are several isoforms of each subunit (α1, α2, β1, β2, γ1, γ2, γ3), such that 12 different heterotrimers may exist [10].

AMPK was initially named due to its allosteric activation by AMP [2]. There is a substantial increase in AMP concentrations when ATP utilization increases or ATP production falls due to adenylate kinase. AMP (or ADP) bind to CBS (cystathionine β-synthase) motifs on AMPKγ, allosterically activating kinase activity, whereas ATP binding to one of these CBS motifs inhibits AMPK activity (Figure 1) [11,12]. Consequently, AMPK is activated by an increase in the AMP:ATP ratio, which will occur under physiological conditions including hypoxia, hypoglycaemia and ischaemia, due to the limited supply of nutrients and/or oxygen limiting ATP synthesis [3-7]. Binding of AMP at the CBS motifs also promotes phosphorylation of the AMPKα catalytic subunit at Thr172 by LKB1 (liver kinase B1), which further activates AMPK (Figure 1) [13-16], and protects Thr172 from dephosphorylation by protein phosphatases [17]. Three other mechanisms have been identified by which AMPK may be activated independent of changes in AMP/ATP.

Firstly, AMPKα can be phosphorylated at Thr172 by Ca^{2+}/calmodulin-dependent kinase kinase-2 (CaMKK2) (Figure 1) [18-20]. Signals that increase intracellular Ca^{2+} concentrations, such as VEGF, acetylcholine and thrombin therefore activate AMPK in cells that express CaMKK2 [21-23]. Secondly, synthetic molecules including A-769662, compound 991 and the xenobiotic salicylate can bind to a different allosteric site (termed the ADaM site) between the β and α subunits, activating AMPK [24]. Very recently, long-chain fatty acyl-CoA esters have been found to bind AMPK at this

site, and therefore represent an endogenous ADaM site ligand (Figure 1) [25]. Finally, glucose starvation in certain cells can also activate AMPK by an AMP-independent mechanism involving translocation of LKB1 complexed with the adaptor Axin1 to a pool of AMPK at the lysosomal membrane surface [26]. In this mechanism, the glucose concentration is sensed by the glycolytic enzyme aldolase [27]. For more detail of AMPK enzymology see the following recent reviews [9,28,29].

A number of other mechanisms have been reported to regulate AMPK, including inhibitory phosphorylation of AMPKα1/α2 at Ser487/491 by kinases including PKA (protein kinase A), Akt and protein kinase C isoforms [30-33]. Phosphorylation of AMPKβ1 at Ser108 greatly improves the binding affinity of ADaM site ligands [34] and has been reported to be phosphorylated by the AMPK substrate ULK1 (Unc-51 like autophagy activating kinase), so may represent a positive feedback mechanism [35]. Phosphorylation at Ser108 also requires myristylation of AMPKβ1 [35], which may also contribute to targeting of AMPK to mitochondria [36]. AMPKβ isoforms contain glycogen binding domains, which have been reported to stabilize AMPK *in vivo* [37,38], yet whether glycogen regulates AMPK under physiological circumstances remains uncertain. Finally, AMPK isoforms display tissue-specific distribution [10,39,40], which may influence subcellular localization and substrate specificity of AMPK [41-43]. The regulation and functional consequences of AMPK heterotrimer localization remains relatively poorly-characterised and has been recently reviewed [44].

1.3   AMPK activators

In addition to physiological activation of AMPK by nutrient depletion, hypoxia, ischaemia and increased intracellular Ca^{2+}, several small molecule activators have been utilized experimentally to imply a role for AMPK. The first of these to be used widely was the nucleoside AICAR (5-aminoimidazole-4-carboxamide ribonucleoside, which is phosphorylated by cells to the active ribonucleotide (also known as ZMP) [45,46]. ZMP mimics the effect of AMP, binding to the CBS motifs on AMPKγ, allosterically activating AMPK and promoting activating phosphorylation of AMPKα at Thr172 [47]. AICAR therefore does not display any selectivity for AMPK

heterotrimers containing specific subunit isoforms. As ZMP is an endogenous intermediate in the purine biosynthesis pathway, levels can also be increased by the immunosuppressant drug methotrexate, which inhibits the conversion of ZMP to inosine monophosphate [48]. However, numerous AMPK-independent actions of AICAR have been reported [49-53], likely due to ZMP also mimicking the effects of AMP on other AMP-sensitive enzymes [54,55]. Subsequently other AMPK activators that mimic AMP or act as allosteric activators binding to AMPKγ have been developed, including compound 2 and activator 3 [56,57]. Intriguingly, compound 2 displayed selectivity for activation of AMPK heterotrimers containing the α1 catalytic isoform [58].

A large number of small molecules have also been demonstrated to indirectly activate AMPK by increasing the cellular AMP/ATP ratio. These include the hypoglycaemic drugs metformin, canagliflozin and the thiazolidinediones, 2-deoxyglucose and xenobiotics including berberine and resveratrol. These compounds either increase AMP/ATP via inhibition of glycolysis or complex I of the mitochondrial respiratory chain [59,60].

Selective allosteric activators of AMPK have been developed that bind to the ADaM site and thereby activate AMPK independent of AMP/ATP. The first of these activators, A-769662, was first reported in 2006 [61] and has since been used widely to examine the role of AMPK experimentally. Salicylate was subsequently also found to activate AMPK by binding directly to the ADaM site [62]. More recently, several other ADaM site-binding activators have been reported, including 991 [63], MT 63-78 [64], MK-8722 [65], PF06409577 [66] and SC4 [67]. Activators that bind at the ADaM site exhibit isoform selectivity, likely due to variations in amino acid sequence of AMPKα and AMPKβ isoforms, between which the ADaM site lies [28,63]. Although the selectivity of these compounds varies, all of them bind more tightly to heterotrimers containing AMPKβ1 relative to complexes containing AMPKβ2 [28]. As AMPKβ2 is the principal AMPKβ isoform in striated muscle [40], these compounds provide the potential to selectively activate AMPK in non-muscle tissues. It should be noted that AMPK-independent effects of A-769662 are increasingly being reported [68-71], and off-target actions of the other ADaM site activators may yet be demonstrated.

Another small molecule, O304, has been reported to activate AMPK by an additional mechanism, protecting phospho-AMPKα Thr172 from dephosphorylation without any allosteric activation of AMPK [72], yet where O304 binds AMPK has yet to be reported. The plethora of AMPK activators, their modes of action and therapeutic potential have been recently reviewed [28].

In contrast there are no specific inhibitors of AMPK. Compound C (dorsomorphin) is used widely to imply AMPK-dependence experimentally, yet this shows minimal selectivity for AMPK [73]. More recently, SBI-0206965 has been reported to selectively inhibit AMPK, yet this is also an inhibitor of ULK1/2 [74] and has also been demonstrated to have effects likely to be independent of AMPK and ULK1/2 in skeletal muscle [75]. Although many published reports claim a role for AMPK when using these AMPK activators and inhibitors, their lack of specificity demonstrate that AMPK-dependence of effects should also be assessed using genetic approaches.

1.4   Physiological role of AMPK

As mentioned previously, AMPK was first identified as an activity that inhibited ACC and HMGR, suppressing FA and isoprenoid/sterol synthesis respectively which has been confirmed in genetic knock in mice with Ser/Ala mutations at the AMPK sites [76,77]. Indeed, as AMPK is the only kinase known to phosphorylate ACC1 at Ser79 (Ser80 in humans), this is the most common biomarker of AMPK activation utilized in intact cells. More than 100 AMPK targets have now been validated [28] and AMPK has been demonstrated to inhibit many other anabolic pathways including glycogen and nucleotide synthesis [78,79] and causes cell cycle arrest by mechanisms that remain uncertain [80,81]. AMPK tightly regulates protein synthesis, inhibiting mTORC1-mediated translation, protein elongation via elongation factor-2-kinase and ribosomal RNA synthesis [82,83], Inhibition of these pathways conserve ATP, whereas AMPK has also been demonstrated to stimulate numerous catabolic pathways, thereby generating ATP. AMPK phosphorylates 6-phosphofructo-2-kinase isoforms, thereby promoting glycolysis in certain tissues [84,85]. In addition, inhibition of mitochondrial ACC2 by AMPK reduces concentrations of malonyl CoA. As malonyl CoA is an inhibitor of FA-CoA (fatty acyl CoA) entry into the mitochondrion, AMPK activation stimulates FA oxidation [86,87]. All of these actions

of AMPK reinforce its role as a sensor of cellular energy charge, acting to suppress ATP utilisation and stimulate ATP synthesis.

AMPK activation also causes long term adaptations to reduced energy charge, promoting oxidative metabolism by increasing mitochondrial biogenesis [88]. Concomitantly, AMPK improves the quality/efficiency of mitochondria by promoting mitophagy via phosphorylation of ULK1 [89] and increasing mitochondrial fission [90,91]. Many other target pathways of AMPK have been described and have been reviewed in greater detail elsewhere [4-7,28]. The distinct actions of AMPK in different metabolic tissues have also demonstrated that AMPK acts in a coordinated fashion as a whole body energy sensor, stimulating feeding, reduced nutrient storage and increased nutrient oxidation when indicated by cellular energy levels and hormonal signals [4,5,28].

Regulated nutrient uptake by cells is critical to maintain homeostasis and is dysfunctional in diabetes mellitus, where insufficient insulin leads to a lack of glucose uptake by muscle and adipose tissue, contributing to hyperglycaemia [92]. Furthermore, nutrient excess or inappropriate nutrient storage characterise obesity and hepatic steatosis, which contribute to type 2 diabetes [4,93]. Given the importance of AMPK activation in increasing ATP synthesis, it is perhaps unsurprising that AMPK has been demonstrated to regulate nutrient uptake, yet both the mechanisms by which this occurs and the physiological relevance of this remain incompletely understood and are discussed in more detail in this review.

2. Regulation of glucose uptake by AMPK

2.1 Glucose transporter proteins

Two principal families of glucose transporters have been identified in mammals, the sodium–glucose linked transporters (SGLTs) and facilitated diffusion glucose transporters (GLUTs). SGLT1 and SGLT2 rely on the sodium gradient to drive glucose absorption in the small intestine and reabsorption in the kidney by a secondary active process [94]. On the other hand, GLUT family members transport glucose down it's concentration gradient of which GLUT1-4 are the best characterised [95-97]. GLUT-mediated glucose uptake is mostly regulated by the

levels of transporter at the plasma membrane, yet the activities of transporters at the plasma membrane may also be regulated under certain circumstances.

GLUT1 is ubiquitously expressed, has a high affinity for glucose and is thought to be responsible for the basal rate of glucose uptake in many cell types whilst also providing a rate limiting barrier for glucose supply to the brain at the blood brain barrier [97]. GLUT2 is the predominant GLUT isoform in hepatocytes and small intestine, with a uniquely low affinity for glucose which allows for glucose uptake to alter with the changes in plasma glucose concentration that occur during feeding and fasting [97,98]. GLUT2 is also the major glucose transporter in rodent pancreatic β-cells and has a functional role in glucose uptake by human pancreatic β-cells [99]. GLUT3 is most abundant in the central nervous system and has the highest affinity for glucose thus ensuring efficient glucose uptake independent of the plasma level of glucose [97].

GLUT4 is highly expressed in skeletal muscle, adipocytes and cardiomyocytes where it is sequestered intracellularly into specialized GLUT4 storage vesicles (GSVs) under basal conditions [92,100-102]. Insulin stimulates GLUT4 translocation to the plasma membrane, increasing glucose uptake and contributing to postprandial glucose disposal for storage as glycogen or triglyceride in muscle and adipose tissue respectively. Muscle contraction also stimulates GLUT4 translocation by an insulin-independent mechanism. Once the stimulus is removed, GLUT4 is internalized and returns to its intracellular vesicular localisation [92,102]. It should be noted that GLUT1 levels at the plasma membrane can also be regulated by insulin in these tissues, although this occurs independent of specialised GSVs [103,104]. Trafficking of GLUT4 in response to insulin and contraction is discussed in further detail later in this review.

2.2 Insulin-stimulated glucose uptake in skeletal muscle and adipose tissue

Tissue specific deletion of GLUT4 in either skeletal muscle or adipose tissue results in insulin resistance, glucose intolerance and dysfunctional metabolism demonstrating that GLUT4 is a key determinant of glucose homeostasis [105,106]. Insulin-mediated GLUT4 translocation requires phosphatidylinositol 3-kinase (PI3K)-mediated activation of the serine/threonine kinase Akt (also known as protein kinase

B) [92,107-109]. Binding of insulin to its receptor leads to the recruitment and tyrosine phosphorylation of insulin receptor substrate proteins (IRSs) [110]. Phosphorylated IRS binds to and activates PI3K, leading to the production of phosphatidylinositol 3,4,5 trisphosphate (PIP3) in the plasma membrane which in turn activates PDK1 (phosphoinositide-dependent protein kinase 1), which phosphorylates Akt Thr308 [111]. This causes a conformational change in Akt, allowing target of rapamycin complex 2 (mTORC2) to phosphorylate Akt at a second site, Ser473, fully activating Akt (Figure 2) [112]. The discovery of AS160 (Akt substrate of 160 kDa, also known as TBC1D4) and the related protein TBC1D1 as downstream effectors of Akt have greatly enhanced our understanding of the link between insulin signalling and GSV trafficking [92,113,114]. AS160 and TBC1D1 are Rab-GAPs (Rab GTPase activating proteins) that maintain Rab small GTPases in a GDP-bound inactive form [115]. Upon phosphorylation by Akt, the Rab-GAP activity is inhibited, resulting in the activation of Rabs required for the recruitment of GSVs to the plasma membrane [116]. Rab10 and Rab14 are likely to be functional targets of AS160 that mediate insulin-stimulated GLUT4 translocation in adipocytes [117-119], whereas Rab8a and Rab13 fulfil a similar role in muscle cells [120,121]. Studies in mice lacking AS160 have demonstrated that it is critical for insulin-stimulated glucose uptake in muscle [122,123]. In contrast, studies in TBC1D1-deficient rodents have provided conflicting evidence concerning the role of TBC1D1 in insulin-stimulated glucose uptake. In TBC1D1-deficient mice, impaired insulin-stimulated glucose uptake was observed in isolated glycolytic muscles, but not oxidative muscles, likely a consequence of reduced GLUT4 expression in glycolytic muscle [124,125]. In contrast, transfection of mouse muscle with wild type or mutant TBC1D1 unable to be phosphorylated by Akt had no effect on insulin-stimulated glucose uptake in mouse muscle [126]. Furthermore, in muscle of TBC1D1 deficient rats, insulin-stimulated trafficking of GLUT4 and levels of GLUT4 expression were unaltered, indicating a species-specific effect on GLUT4 levels [127]. Taken together, it is likely that AS160, rather than TBC1D1 is necessary for insulin-stimulated glucose uptake in muscle.

Other small GTPases may also regulate insulin-stimulated GLUT4 trafficking, including RalA and the RalA GAP complex (RGC1/2) in adipocytes (Figure 2) [128,129]. In parallel to Akt signalling via AS160, insulin also exerts distal effects on

GSV trafficking including activation of the small GTPase Rac1 (Ras-related C3 botulinum toxin substrate 1), which is reported to contribute to GLUT4 translocation in muscle cells [130,131]. Additionally, PI3K-independent activation of the small GTPase TC10 by insulin has been proposed to contribute to GLUT4 translocation specifically in adipocytes [132], yet the roles of these other GTPases in insulin-stimulated glucose uptake in an *in vivo* setting remains unclear. A brief scheme of the signalling underlying insulin-stimulated GLUT4 translocation is shown in Figure 2. Multiple other proteins have been demonstrated to regulate the trafficking and formation of GSVs in response to insulin and have been recently reviewed elsewhere [102,133].

2.3 Contraction-stimulated glucose uptake in skeletal muscle

Contraction increases glucose uptake in skeletal and cardiac muscle [134-138]. Once exercise/contraction ceases, muscle glucose uptake returns slowly to basal levels [136,139] and during the recovery period insulin sensitivity is increased [136,137]. It is clear that contraction-stimulated glucose transport is mediated by GLUT4 translocation from intracellular compartments, phenocopying the effects of insulin, yet the initial signalling pathways utilised by insulin and contraction are different [92,105,134,140-142]. Contraction-stimulated GLUT4 translocation is independent of IRS-PI3K-Akt signalling, yet has been proposed to involve TBC1D1 from experiments using TBC1D1-deficient mice and rats [124,143]. Despite this, the signalling pathways mediating GLUT4 translocation remain elusive.

Several potential mediators of contraction-stimulated glucose uptake have been proposed, including Ca^{2+}, AMPK (see section 2.4), NO, reactive oxygen species (ROS) and Rac1 [136,137]. In particular, NO synthesis has been demonstrated to be required for increased skeletal muscle glucose uptake during contraction since inhibition of NO synthesis attenuates contraction-mediated glucose uptake in skeletal muscle [144,145]. Furthermore, pharmacological inhibition or genetic ablation of Rac1 impaired increased glucose uptake following exercise [146]. As Rac1 is also implicated in insulin-stimulated glucose uptake [130,131], this suggests that Rac1 activation might be one distal convergence point between insulin- and contraction-activated signalling pathways, although Rac1 has been reported to be

unnecessary for contraction-mediated increases in insulin sensitivity following exercise [147]. In addition, capillary recruitment in muscle during contraction increases the supply of glucose for transport, thereby increasing glucose uptake *in vivo* [136,137]. Given that contraction mimics the effects of insulin yet occurs independently of insulin signalling, the signalling underlying contraction-stimulated glucose uptake is an area of significant interest for therapies to normalise blood glucose in people with type 2 diabetes or insulin resistance.

2.4 The role of AMPK in muscle glucose uptake

During the late 1990s, muscle contraction either due to exercise or electrical stimulation was shown to increase AMPK activity. At the same time AICAR was demonstrated to increase glucose uptake in muscle [87,148,149]. AICAR was subsequently shown to increase GLUT4 translocation and glucose uptake in the heart [150]. Excitingly, AS160 and TBC1D1 were found to be AMPK substrates, mimicking the Akt-mediated phosphorylation in response to insulin, leading to GLUT4 translocation and concomitant glucose uptake (Figure 3) [151-153]. Using purified proteins, AMPK preferentially phosphorylates AS160 at Ser588, rather than the principal Akt site, Thr642 *in vitro*, whereas both Akt and AMPK phosphorylate Thr596 of TBC1D1 *in vitro* [154,155]. Muscle-specific AMPKβ1β2 knockout mice, which exhibited no activation of AMPK during exercise, also exhibited impaired contraction-stimulated glucose uptake [156]. These findings led to the widespread belief that AMPK underlies the effects of contraction on glucose uptake through phosphorylation and inhibition of TBC1D1 (or AS160). However, this notion has been challenged in other mouse models with muscle-specific AMPK downregulation where contraction-stimulated glucose uptake was unaltered [157,158]. This evidence has been reviewed recently clearly indicating that AMPK activation is not required for contraction-mediated glucose uptake during exercise [159]. However, as *ex vivo* muscle glucose uptake in muscle-specific AMPKα1α2 knockout mice was attenuated after but not during exercise, AMPK activation may still be important for maintaining muscle glucose uptake after contraction, potentially via increased phosphorylation of TBC1D1 [158,160]. Therefore, although AMPK activation does increase muscle glucose uptake, likely via phosphorylation of TBC1D1 [161] it is not responsible for

the increased glucose uptake during contraction – indeed, AMPK activation may not occur during contraction in many physiological settings.

Over the last 5 years, phosphoproteomic analysis of exercise-regulated phosphosites in human skeletal muscle in conjunction with phosphoproteomic data from a rat muscle cell line has allowed the validation of several new AMPK substrates [162,163]. Of these novel substrates, AMPK-mediated phosphorylation of stromal interaction molecule 1 (STIM1) has been identified, thereby reducing its SOCE (store-operated Ca^{2+} entry) activity (Figure 3) [163]. As Ca^{2+} is also involved in contraction mediated glucose uptake, whether this newly described AMPK-STIM1 regulatory axis is implicated in post exercise metabolic adaptions remains to be explored.

Given the potential importance of AMPK in sensitising muscle to insulin after exercise, further research is required to understand the mechanisms by which this occurs. Similarly, most studies have investigated the effects of AMPK in relatively short-term exercise models, so whether AMPK has any role in more prolonged exercise-stimulated glucose uptake cannot be dismissed. Indeed, prolonged stimulation with AICAR for 18 h was demonstrated to increase expression of GLUT4 in murine muscle [164], reported to be due to phosphorylation of histone deacetylase-5 (HDAC5) [165] and/or GLUT4 enhancer factor (Figure 3) [166]. Finally, as AMPK has been demonstrated to increase NO synthesis by a variety of mechanisms [6], it is intriguing that NO synthesis has been reported to influence muscle glucose uptake by direct effects on GLUT4 translocation and also improving capillary recruitment [145]. It should be noted however, that inhibition of NO synthase had no effect on capillary recruitment or glucose uptake in rat muscle electrically stimulated *in situ* [167].

In type 2 diabetes, insulin-independent contraction-mediated muscle glucose uptake is unimpaired, therefore providing a therapeutic target for improving glycaemia. Despite AMPK being unlikely to be the mediator underlying contraction-stimulated glucose uptake, the stimulatory effects of AMPK on muscle glucose uptake (in addition to its roles in other metabolic tissues) therefore make it a therapeutic target for type 2 diabetes. For example, the activator O304 which stimulates glucose uptake in mice reduced insulin resistance and improved fasting glucose levels in people with type 2 diabetes [72].

2.5 The role of AMPK in white adipocyte glucose uptake

White adipose tissue (WAT), stores excess nutrients as triglycerides, and defects in adipocyte glucose transport are associated with the development of insulin resistance and type 2 diabetes in rodents and humans [106,168]. Despite the wealth of data concerning the role of AMPK in muscle glucose uptake, the role of AMPK in adipose tissue is far less well characterised. Initial studies in 3T3-L1 adipocytes and isolated rodent adipocytes demonstrated that incubation with AICAR inhibited insulin-stimulated glucose uptake independent of insulin-stimulated Akt activity [169,170]. Given this, it is surprising that AICAR was also reported to reduce insulin-stimulated phosphorylation of AS160 at the Akt site, Thr642 in rat adipocytes [171]. Furthermore, in that study, infection of the adipocytes with adenoviruses expressing a kinase dead AMPKα1 mutant normalised the inhibition of insulin-stimulated AS160 phosphorylation and glucose uptake, indicating the effect of AICAR is AMPK-dependent [171]. Despite purified AMPK being able to phosphorylate AS160 at Thr642 *in vitro* [154], activation of AMPK with AICAR had no effect at this site in adipocytes [171]. These data indicate that AMPK activation inhibits insulin-stimulated glucose uptake in adipocytes, the opposite effect to AMPK activation in muscle. This may reflect the different AMPK isoforms expressed in adipocytes and muscle or the different GLUT4 trafficking machinery employed by either cell type in response to insulin. It should also be noted that only one study has provided evidence that the effect of AICAR in adipocytes is AMPK-dependent [171]. It could be argued however, that from a physiological perspective, it would be in keeping for AMPK activation during ATP depletion to generate ATP by increasing glucose uptake in muscle and inhibit insulin-stimulated glucose uptake in adipocytes, which would largely be used for the ATP-consuming synthesis of FAs and triglycerides.

WAT from obese rodents and humans has been demonstrated to have a lower AMPK activity [172-175], begging the question as to whether impaired AMPK activity in adipose tissue contributes to insulin sensitivity. What then happens to indices of insulin sensitivity in rodents with downregulated AMPK activity? Adipocytes contain AMPK complexes primarily composed of AMPKα1 [169,176], yet the contributions of AMPKβ isoforms remain uncertain as although AMPKβ1 is the predominant isoform expressed in adipocytes [176,177], heterotrimers containing AMPKβ2 may account

for the majority of total cellular AMPK activity [176]. Mice with global deletions of AMPKα1 or AMPKβ1 exhibited smaller adipocytes, reduced fat mass and were normoglycemic [178,179]. Conversely, germline deletion of AMPKβ2 was associated with hyperglycaemia and susceptibility to obesity-induced adiposity and insulin resistance [180]. Likewise, adiposity and adipocyte hypertrophy have been observed in mice lacking AMPKα2 [181]. Whether the distinctive metabolic phenotypes observed in global AMPK subunit knockout mice were associated with increased, decreased, or unaltered adipocyte glucose uptake has not been examined. More recently, contrasting data were reported in mice with adipocyte-specific abrogation of AMPK. Mice with adipocyte-specific deletion of both AMPK α1 and α2 isoforms had impaired glucose tolerance and defects in insulin sensitivity [182]. Similarly, mice with adipocyte-specific knockout of both AMPKβ1 and β2 were reported to develop hyperglycaemia and insulin resistance when challenged with high fat diet likely due to compromised brown adipose tissue (BAT) and WAT function [183]. However, others have reported normal fasting blood glucose concentration, glucose tolerance and insulin sensitivity in adipocyte-specific AMPKα1α2 knockout mice as well as a lowered fat mass phenotype [184,185]. The reasons for these differences are uncertain but likely due to different age, mice strain or cre-lox models used. Despite these differences, only one study has examined adipose tissue glucose uptake in mice with downregulated AMPK, where mice with adipocyte-specific deletion of AMPKβ1β2, exhibited unaltered insulin-stimulated glucose uptake by gonadal WAT *in vivo*, suggesting that adipocyte AMPK does not regulate glucose uptake [183].

Stimulation of cultured adipocytes with the indirect AMPK activators metformin and resveratrol have provided conflicting results, with metformin reported to increase basal and insulin-stimulated glucose uptake in 3T3-L1 adipocytes and human preadipocyte-derived adipocytes [186,187] or have no effect in human adipocytes [188], whereas resveratrol has been reported to attenuate insulin-stimulated glucose uptake in insulin-resistant 3T3-L1 adipocytes and human adipocytes, likely through interfering with Akt phosphorylation [189,190]. As AICAR, metformin and resveratrol all influence other signalling pathways and metabolic enzymes independent of AMPK, these differential actions and the lack of data in adipocytes from knockout models demonstrate the need for data obtained using more selective AMPK activators in adipocytes and direct measurement of glucose uptake in adipocytes

isolated from knockout mice. The AMPKβ1-selective activators A-769662 and 991 have been demonstrated to activate AMPK in 3T3-L1 adipocytes and human adipocytes [177,191], so it will be informative to assess the effects of these activators on glucose uptake in future studies. Finally, as with muscle, phosphoproteomic analyses in adipocytes would be useful to identify AMPK substrates concerned with the regulation of GLUT4 trafficking. A chemical genetic screen employed to determine AMPK substrates in hepatocytes identified several proteins involved in vesicle trafficking and cytoskeleton organisation, including Gapex-5, which contains both a Rab GTPase activating domain and Rab5 guanine nucleotide exchange factor domain [192]. Further analysis of this in 3T3-L1 adipocytes indicated that Gapex5 knockdown reduced insulin-stimulated glucose uptake at sub-maximal insulin concentrations [192], yet it remains unknown whether Gapex5 mediates any effects of AMPK on adipocyte glucose uptake. The potential mechanisms by which AMPK influences adipocyte glucose uptake are shown in Figure 4.

2.6 The role of AMPK in brown adipocyte glucose uptake

In contrast to WAT, BAT primarily functions to expend energy by non-shivering thermogenesis and may improve caloric balance and whole-body energy homeostasis [193,194]. Brown adipocytes contain abundant mitochondria and express high levels of uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP production in favour of heat production [193,194]. As oxidation of glucose is not coupled to ATP production, BAT relies on glycolysis for ATP production and there is significant interest in "browning" of adipose tissue as a therapeutic goal for normalising metabolic dysfunction in obesity and its complications [3].

Two principal stimuli for glucose uptake in BAT have been described. As in muscle and WAT, insulin-mediated PI3K-Akt activation markedly increases BAT glucose uptake in a manner likely to involve GLUT4-dependent manner [195,196]. Alternatively, stimulation of β-adrenoreceptors in response to cold exposure or direct (nor)adrenaline action increases glucose uptake by increasing GLUT1 activity at the plasma membrane [197]. β-adrenergic stimulation has been reported to increase

GLUT1-mediated glucose uptake in brown adipocytes by mechanisms including a PKA-mediated increase in GLUT1 levels [198] and mTORC2-dependent translocation of GLUT1 to the plasma membrane, potentially mediated by Akt [199,200]. However, a more recent study has reported that inhibition of PI3K had no effect on glucose uptake indicating Akt is not involved [201].

AMPK activation is undoubtedly important in the differentiation of brown adipocytes, particularly with respect to its stimulation of mitochondrial function and biogenesis [3], yet its potential role in BAT glucose uptake remains poorly defined. Both cold exposure and β-adrenoreceptor stimulation has been demonstrated to activate AMPK in rodent BAT and isolated brown adipocytes [202,203]. It has been reported that AICAR and the β-adrenoreceptor agonist isoprenaline increased basal glucose uptake in primary brown adipocytes in a manner sensitive to AraA (adenine 9-β-D-arabinofuranoside) [202], which has been demonstrated to inhibit AMPK in some cell types [204]. More recently, BAT isolated from mice with adipocyte-specific ablation of AMPKβ1β2 displayed reduced insulin-stimulated Akt phosphorylation and decreased insulin-stimulated glucose uptake [183]. Therefore, as in WAT, AMPK activation may suppress insulin-stimulated glucose uptake in BAT (Figure 4). There are no definitive studies examining the effects of adipocyte-specific AMPK ablation on BAT glucose uptake in response to β-adrenoreceptor stimulation. Therefore, it is important that future studies assess whether BAT glucose uptake is regulated by AMPK *in vivo*.

2.7 AMPK and glucose uptake in non-metabolic tissues

Hypoglycaemia and hypoxia have been demonstrated to increase GLUT1 expression and glucose uptake in multiple cell types [97,205-207]. Similarly, GLUT3 is upregulated in response to hypoxia and ATP depletion [208,209]. Furthermore, as decreased oxygen availability and nutrient deprivation occurs in tumour microenvironments, increased GLUT1 expression is observed in a number of cancer cells and it is central for cancer cell growth [210-212]. Cancer cells, while adapting to hypoxia, alter their metabolism from oxidative phosphorylation to aerobic glycolysis, a phenomenon known as Warburg effect [213]. This is mainly determined by HIF-1α (hypoxia induced factor-1α) that acts as a key regulatory transcription factor responsible for glycolytic genes including GLUT1 [214,215].

Unsurprisingly, given that hypoxia, hypoglycaemia and ATP depletion stimulate AMPK, the role of AMPK in the upregulation of GLUT1 has been investigated. AMPK activation was demonstrated to stimulate GLUT1 activity at the plasma membrane of a rat liver epithelial cell line [216]. The mechanisms by which AMPK increases GLUT1 activity at the membrane has been demonstrated to involve thioredoxin-interacting protein (TXNIP), which induces GLUT1 retention in the cytoplasm. AMPK directly phosphorylates and inhibits TXNIP, thereby increasing GLUT1 translocation and subsequent glucose uptake (Figure 4) [217]. Indeed, TXNIP may also influence GLUT4 trafficking as Akt phosphorylates TXNIP at the same site as AMPK and TXNIP knockout mice exhibit higher levels of GLUT4 at the cell surface [218]. The stimulation of GLUT1-mediated glucose uptake by AMPK may therefore improve cancer survival [219], although AMPK also acts as suppressor of cancer cell proliferation and tumour formation through negatively regulating Warburg metabolism [220]. These opposing effects of AMPK in tumorigenesis and survival may suggest that AMPK acts as a tumour suppressor, yet once cancer occurs, AMPK promotes tumour survival [81]. It is important therefore, that should AMPK activation be utilised as a therapy for cancer, that these cancer-promoting and anti-tumourigenic actions are taken into account.

3 Regulation of fatty acid uptake by AMPK

3.1 Mechanisms of fatty acid uptake

FAs are a major source of energy for many tissues in the fasted state and circulating levels are regulated by the rate of i) lipid synthesis in liver and adipose tissue and ii) metabolism in muscle, liver and adipose tissue [221]. In adipose tissue, circulating FAs are taken up in the postprandial state to be esterified as triglycerides [222]. In the heart, approximately 60-70% of ATP is generated by oxidation of FAs [223], whereas in skeletal muscle, oxidation of FAs is the principal source of ATP in the fasted state and during low to moderate intensity exercise [224]. Therefore, as with glucose utilisation, any increased uptake of FAs would be used for ATP synthesis in muscle, whereas in adipocytes the FAs would be stored as triglycerides.

The mechanism by which FA is transported into cells has been the subject of considerable debate and evidence for two mechanisms has been reported. In the first mechanism, FA are proposed to enter cells via extremely rapid, passive flip-flop diffusion across the lipid bilayer membrane followed by a slow dissociation into the cytosol [225,226]. The second mechanism suggests that FA uptake is a protein-mediated process in which several proteins have been implicated including CD36/SR-B2 and FA transporter proteins (FATPs) [227]. In cells that utilise significant quantities of FAs, such as skeletal muscle, heart and adipose tissue, protein-mediated FA transport has been proposed to dominate [228]. Once FAs enter the cell, they are rapidly esterified to fatty acyl CoA (FA-CoA), thereby preventing efflux of FA and allowing utilisation of the FA-CoA for mitochondrial oxidation and ATP production, or esterification to triglyceride [229]. The putative FA transporter proteins are discussed in more detail in sections 3.2 and 3.3.

3.2 CD36/SR-B2

CD36/SR-B2 is a multifunctional membrane receptor belonging to the class B scavenger receptor family, able to bind modified LDL and facilitate FA uptake in adipocytes and muscle [230-232]. CD36/SR-B2 likely facilitates flip-flop of FA across the plasma membrane and localises to caveolae, where its binding to caveolin may further facilitate FA uptake [138,233,234]. People with homozygous mutations of CD36/SR-B2 exhibited markedly reduced FA uptake in adipose tissue and muscle when FA concentrations were low, yet CD36/SR-B2 was crucial for cardiac FA uptake regardless of the plasma FA concentration [235]. Supporting this, CD36/SR-B2-deficient mice also had impaired FA uptake in adipose tissue and muscle FA uptake [236,237]. CD36/SR-B2 is found in both intracellular compartments and the sarcolemma in cardiomyocytes. Furthermore, subcellular fractionation experiments suggest these overlap in part with GLUT4 localisation and that CD36/SR-B2 traffics to the cell surface in response to insulin and contraction [138]. Stimulation of FA uptake in response to insulin, contraction and the potential role of AMPK are discussed in sections 3.4 and 3.5.

3.3 FATPs

FATPs are a family of six isoforms that exhibit tissue-specific expression. In the metabolic tissues, adipose tissue and muscle express FATP1 and FATP4, heart expresses FATP1 and FATP6, whereas liver expresses FATP2 and FATP5 [238]. The roles of the different FATP isoforms in FA transport have been studied using overexpression or knockout models in cell lines and mice. Cardiomyocyte-specific overexpression of FATP1 increased FA uptake 4-fold, with mice showing evidence of lipotoxic cardiomyopathy within 3 months [239]. It should be noted, however, that FATP6 is likely to be the predominant FATP isoform in cardiomyocytes [240]. Similarly, muscle-specific overexpression of FATP1 increased FA transport [241]. Silencing of FATP1 in 3T3-L1 adipocytes reduced insulin-stimulated FA uptake [242] and mice with a global deletion of FATP1 did not exhibit any change in adiposity yet were protected from high fat diet-induced insulin resistance and accumulation of intramuscular FA, further indicating a key role for FATP1 in regulating muscle FA uptake [243].

As with FATP1, overexpression of FATP4 enhanced FA uptake in cultured cells [244] and transfection of FATP4 into skeletal muscle increased FA uptake [245]. Mice with an adipose-specific deletion of FATP4 have been reported to exhibit increased weight gain and adipocyte hypertrophy, consistent with increased FA uptake [246]. Importantly, FATPs have acyl-CoA synthetase (ACS) activity, and therefore facilitate FA transport, at least in part, by coupling diffusion of FA with esterification to FA-CoA [238,247].

3.4 Regulation of fatty acid uptake by insulin and contraction

Early studies examining regulated FA uptake reported that contraction and insulin increase translocation of CD36/SR-B2 in heart and skeletal muscle in a manner analogous to that of GLUT4 [248-252]. Furthermore, insulin was reported to stimulate translocation of FATP1 and FATP4 in skeletal muscle [253,254], yet in cardiac myocytes insulin had no effect on FATP1 translocation [252]. CD36/SR-B2 is thought to account for 50-70% of FA uptake in cardiac myocytes, suggesting it is the predominant transport system [255]. CD36/SR-B2 trafficking has been proposed to share many features with that of GLUT4 trafficking in response to insulin and contraction in muscle and the heart [138]. Specifically, insulin has been reported to

stimulate CD36/SR-B2 plasma membrane recruitment via AS160 and Rab8a in cardiomyocytes [256] and TBC1D1 knockdown (mimicking inhibition by insulin) increased cell surface CD36/SR-B2 levels in L6 skeletal muscle cells [257]. Very recently, increased FA uptake was observed in muscles from mice deficient in TBC1D1 or AS160. In the same study, levels of FATP4, but not CD36/SR-B2 were increased in both TBC1D1 and AS160 knockout models and combined knockdown of FATP4 with TBC1D1 or AS160 in C2C12 myotubes abrogated the increase in FA uptake [258]. These recent data suggest that FATP4 may be an important mediator of FA uptake in response to signalling via AS160/TBC1D1 in muscle.

In 3T3-L1 adipocytes, insulin has been reported to stimulate translocation of FATP1 [253], and knockdown studies in 3T3-L1 cells demonstrated that FATP1 knockdown abolished insulin-stimulated FA uptake, whereas knockdown of FATP4 had no effect on FA uptake [259]. In support of this, insulin was recently reported to stimulate FATP1 translocation in adipocytes via a mechanism involving mTORC1 [260]. In that study, insulin stimulation of S6K1 via mTORC1 phosphorylated glutamyl-prolyl-tRNA synthetase (EPRS), a protein demonstrated by interaction screening to bind to and translocate FATP1 to the plasma membrane [260]. Despite this, as previously mentioned, CD36/SR-B2-deficient mice also had impaired FA uptake in adipose tissue [236,237], suggesting CD36/SR-B2 is also important for adipocyte FA uptake. Importantly, the very concept that insulin is a physiologically relevant stimulus for FA uptake in muscle has been challenged, as the response of CD36/SR-B2 and FATP1 in rodent muscle is transient and also does not match the timing of the postprandial rise in insulin concentrations [261].

In brown adipocytes, stimuli that regulate FA uptake are poorly characterised. Both CD36/SR-B2 and FATP1 have been shown to be required for brown adipose tissue function [262-264], and β-adrenoreceptor stimulation has been reported to increase FA uptake in isolated brown adipocytes and a BAT-derived cell line [262,264]. Furthermore, a lipid increased during cold exposure has been demonstrated to stimulate FA uptake into brown adipocytes by promoting the translocation of FATP1 and CD36/SR-B2 [265]. The role of insulin in the regulation of FA uptake in BAT has yet to be characterised, however.

3.5 Regulation of fatty acid uptake by AMPK

The first indication that AMPK may regulate FA uptake utilised AICAR and the ATP synthase inhibitor, oligomycin, which were demonstrated to stimulate FA uptake in cardiac myocytes, likely mediated by CD36/SR-B2 [251]. These effects of AICAR and oligomycin were later shown to be dependent on AMPKα2 [266]. Furthermore, AICAR had no effect on FATP1 translocation and prolonged stimulation of cardiac myocytes with AICAR increased cellular levels of CD36/SR-B2 [252,267]. Similarly, in contracting skeletal muscle, AICAR stimulated FA uptake and plasma membrane CD36/SR-B2 levels, an effect demonstrated to be AMPK-dependent whereas prolonged stimulation of L6 cells with AICAR stimulated CD36/SR-B2 expression [268-270]. Further evidence that AMPK regulates CD36/SR-B2 levels in muscle has been obtained in knockout mice, where CD36/SR-B2 levels were reduced in muscle-specific AMPK knockout mice [271] and fasting-stimulated levels of CD36/SR-B2 mRNA were reduced in mice lacking AMPKγ3, the predominant AMPKγ isoform in skeletal muscle [272]. Conversely, overexpression of a R225Q gain of function AMPKγ3 mutant was associated with increased CD36/SR-B2 expression during fasting [272]. In liver and intestine, AMPK-dependent stimulation of CD36/SR-B2 levels has also been shown, arguing that prolonged AMPK activation upregulates CD36/SR-B2 levels in many tissues [273,274].

What is the mechanism by which AMPK regulates CD36/SR-B2? Knockdown of AS160 increased cell surface levels of CD36/SR-B2 and prevented AICAR from further stimulating CD36/SR-B2 translocation in a cardiac myocyte cell line [256]. In the same study, knockdown of the AS160 target Rab8a or overexpression of a phosphorylation-deficient AS160, which will increase Rab GTP loading and activity also prevented AICAR-stimulated CD36/SR-B2 translocation, arguing that AMPK-mediated phosphorylation and inactivation of AS160 with subsequent activation of Rab8a underlies the stimulation of CD36/SR-B2 translocation by AICAR (Figure 5), although the AMPK-dependence of the AICAR effect was not addressed in that study [256]. In contrast, TBC1D1 overexpression in skeletal muscle was shown to only subtly reduce AICAR-stimulated FA oxidation without changing CD36/SR-B2 levels, suggesting TBC1D1 does not regulate the stimulation of CD36/SR-B2 levels by AMPK [275].

No published reports indicate that AMPK influences FATP1 translocation, with one study showing that AICAR has no effect on FATP1 localisation in cardiac myocytes [252]. Indeed, given insulin-stimulated FATP1 translocation in adipocytes has been reported to occur via mTORC1-mediated phosphorylation of S6K [260], this is surprising, as AMPK activation will inhibit mTORC1 and therefore should suppress insulin-stimulated FA uptake by this mechanism (Figure 5).

As AMPK stimulates FA oxidation via inhibition of ACC and effects on mitochondrial activity [5,6,28], increased FA uptake may reflect changes in flux driven by these actions of AMPK rather than FA uptake by cells. In addition, reports that both FATP1 and CD36/SR-B2 may contribute to activation of AMPK in response to FAs or the adipocytokine adiponectin make the regulation of FA uptake by AMPK complex [263,276,277]. This activation of AMPK by FA may be a result of the recently demonstrated allosteric activation of AMPK by FA-CoA [25].

Taken together, these data consistently indicate that AMPK increases CD36/SR-B2 translocation and expression, yet the mechanisms remain obscure. AS160 provides a potential link that coordinates glucose and FA uptake in response to AMPK activation in muscle. Whether AMPK regulates the FATP isoforms remains barely characterised, however. Whether AMPK underlies contraction-stimulated CD36/SR-B2 translocation and FA uptake has been addressed in mice with reduced AMPK activity, where neither FA uptake nor CD36/SR-B2 translocation were altered [278]. These data argue that, like GLUT4 translocation in muscle [159], AMPK activation does stimulate CD36/SR-B2-mediated FA uptake yet it is not required for increased FA uptake during muscle contraction, where AMPK activation may not occur.

4. Hepatic steatosis – a therapeutic target for AMPK activation

Due to the actions of AMPK on carbohydrate and lipid metabolism, increasing FA oxidation and reducing lipogenesis, AMPK activation has been proposed as a therapeutic target for conditions where there is nutrient overload. NAFLD/NASH (non-alcoholic fatty liver disease/non-alcoholic steatohepatitis) are characterised by nutrient overload leading to hepatic steatosis, progressing to fibrosis and liver injury. Hepatic steatosis represents ectopic lipid accumulation in the liver and is tightly associated with obesity, insulin resistance and type 2 diabetes [4,279]. Lipids

accumulate in the liver either due to *de novo* lipogenesis (DNL) from carbohydrates or increased FA uptake and triglyceride synthesis. Conversely, increased hepatic FA oxidation will reduce steatosis [4,279].

In DNL, glucose-derived pyruvate is converted to acetyl CoA in the mitochondria by pyruvate dehydrogenase, and then after conversion to citrate is shuttled across the mitochondrial membranes to the cytoplasm, where it is converted back to acetyl CoA by ATP citrate lyase. Acetyl CoA is then utilised to generate FAs in the cytoplasm by ACC and FA synthase (Figure 6) [280]. Dietary fructose is more effective in stimulating DNL than glucose and recent evidence suggests this involves conversion of fructose to acetate by the gut microbiome [281].

Given that AMPK-mediated inhibition of ACC reduces FA synthesis from acetyl CoA and increases FA oxidation [76], AMPK activators have been and are currently being trialled as potential therapeutics for NAFLD/NASH [282]. There are many other actions of AMPK that would potentially improve NAFLD/NASH, including phosphorylation and stabilisation of Insig (insulin-induced gene)-1, which regulates the processing of the key lipogenic transcription factor, sterol response element binding protein (SREBP) [283]. Indeed, AMPK has also been reported to phosphorylate SREBP directly, inhibiting DNL (Figure 6) [284]. In addition, AMPK-mediated suppression of inflammation [285], and improvement of mitochondrial function would also improve NAFLD/NASH, and the mechanisms by which AMPK might act to improve hepatic steatosis have been recently reviewed [4,286,287].

How then would the effects of AMPK activation on nutrient transport impact on NAFLD? Global AMPK activation would potentially increase muscle glucose and FA uptake, thereby reducing glucose available for DNL and FA for triglyceride synthesis in the liver. Furthermore, despite the experimental evidence indicating AMPK may inhibit insulin-stimulated glucose uptake in adipocytes, the glucose would be unlikely to be stored inappropriately in the liver as AMPK activation suppresses lipogenesis and increases oxidation of nutrients.

5. Concluding remarks

As described in this review, it is clear that AMPK stimulates GLUT4 trafficking and glucose uptake in muscle, whilst stimulating GLUT1 expression and glucose uptake

in other cell types. Furthermore, multiple lines of evidence indicate that AMPK stimulates expression of CD36/SR-B2, which may contribute to fatty acid uptake in a number of tissues. Many questions remain, however, concerning the mechanisms by which AMPK acts to regulate nutrient uptake. In muscle, the physiological circumstances under which AMPK is activated have not been clearly defined, and similarly whether AMPK impacts GLUT4 trafficking events downstream of AS160/TBC1D1 phosphorylation is uncertain. Given the importance of insulin-stimulated muscle glucose uptake in maintaining glucose homeostasis, AMPK activation remains an important therapeutic target that would permit insulin-independent glucose uptake to maintain glycaemia in people who are insulin resistant or have type 2 diabetes. Further understanding the mechanisms by which AMPK acts in GLUT4 trafficking is therefore critical as it may highlight other novel targets for therapies. Furthermore, small molecule activators of AMPK that target complexes containing AMPKα2 and AMPKβ2, the dominant isoforms expressed in skeletal muscle, would be useful to extend our understanding in muscle, rather than the ADaM site activators which are more selective for complexes containing AMPKβ1.

Another important area of AMPK research that requires resolution is the role of AMPK in adipocyte glucose uptake – as described in this review far less work has been published addressing this. Indeed, although adipocytes have a very similar molecular machinery for GLUT4 translocation to that of muscle, AMPK activation appears either to have no effect or suppress insulin-stimulated glucose uptake. Phosphoproteomic analyses to assess potential AMPK targets in adipocytes would be highly useful in this regard as would direct measurement of glucose uptake in adipocytes isolated from genetic models where AMPK has been down- or upregulated. Also, whether/how AMPK influences BAT nutrient uptake has been barely examined to date.

Finally, whether AMPK plays any role in regulated FA uptake has yet to be definitively answered. Intriguing data suggesting AMPK-dependent regulation of CD36/SR-B2 trafficking has been reported, and it is an attractive hypothesis that glucose and fatty acid uptake are regulated in a coordinated fashion by a master metabolic sensor such as AMPK. Indeed, given the role of AMPK in amino acid metabolism via actions on mTORC1, it would also be useful to assess whether

AMPK regulates any of the numerous amino acid transport systems in different tissues.

In conclusion, despite more than 20 years research concerning AMPK and nutrient uptake, it is only certain that AMPK can stimulate glucose uptake via GLUT4 in muscle and GLUT1 expression in some other tissues. Further research to better define the mechanisms by which AMPK regulates nutrient uptake in the other metabolic tissues is critical given the therapeutic potential of AMPK activation in diseases associated with metabolic dysfunction.

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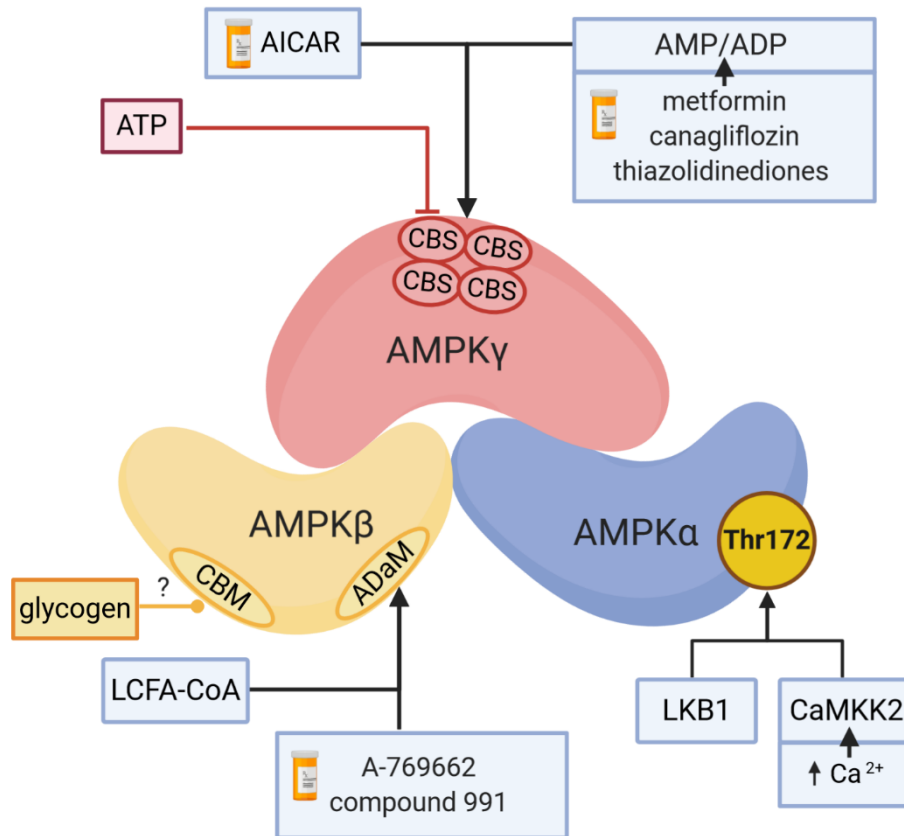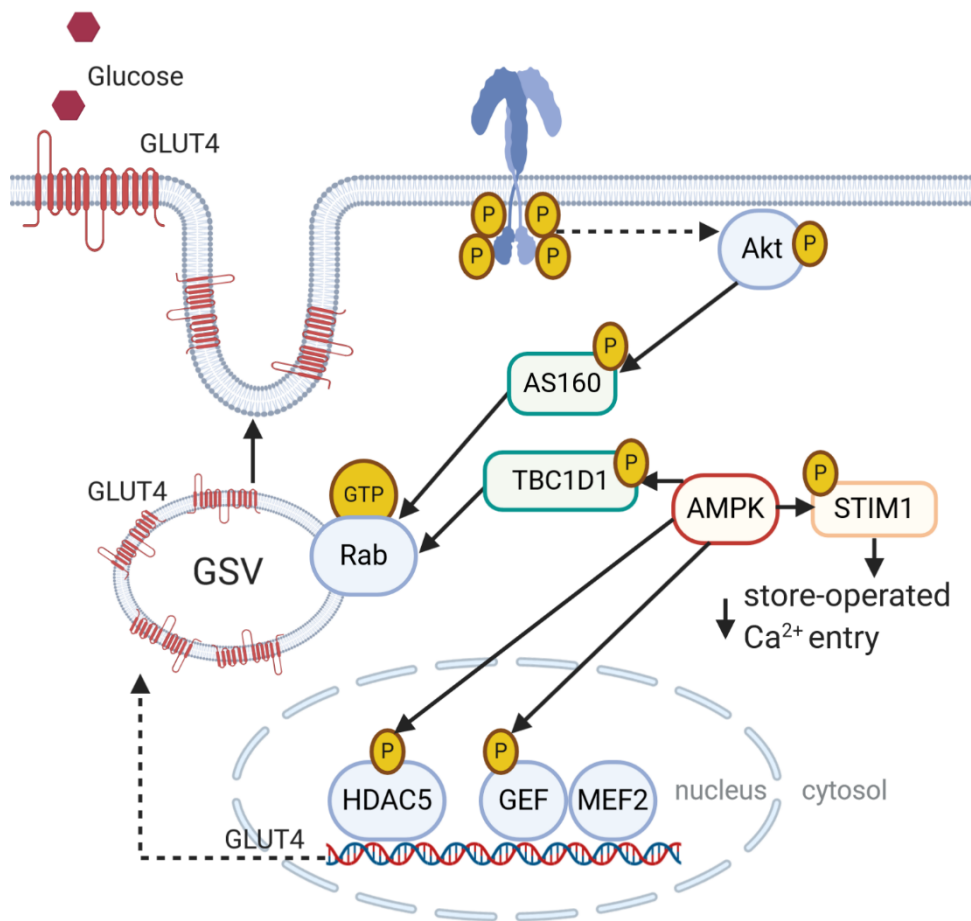
Figure 1: Regulation of AMPK

AMPK is a heterotrimer of an α catalytic subunit complexed with β and γ regulatory subunits. AMP (or ADP) bind to CBS motifs on AMPKγ, allosterically activating kinase activity, promoting phosphorylation of AMPKα Thr172 by LKB1 and protects Thr172 from dephosphorylation by protein phosphatases, which further activates AMPK. ATP binding to one of these CBS motifs inhibits AMPK activity. AICAR is phosphorylated to ZMP, which mimics the effect of AMP. AMPKα Thr172 can also be phosphorylated by CaMKK2 independent of AMP levels in response to increased intracellular Ca^{2+} concentrations. Long-chain fatty acyl-CoA esters (LCFA-CoA) and synthetic molecules such as A-769662 and compound 991 bind to the ADaM site between the β and α subunits and allosterically activate AMPK, also promoting Thr172 phosphorylation. AMPKβ contains a carbohydrate binding domain (CBM), yet whether glycogen regulates AMPK under physiological circumstances remains uncertain. Created with BioRender.com.
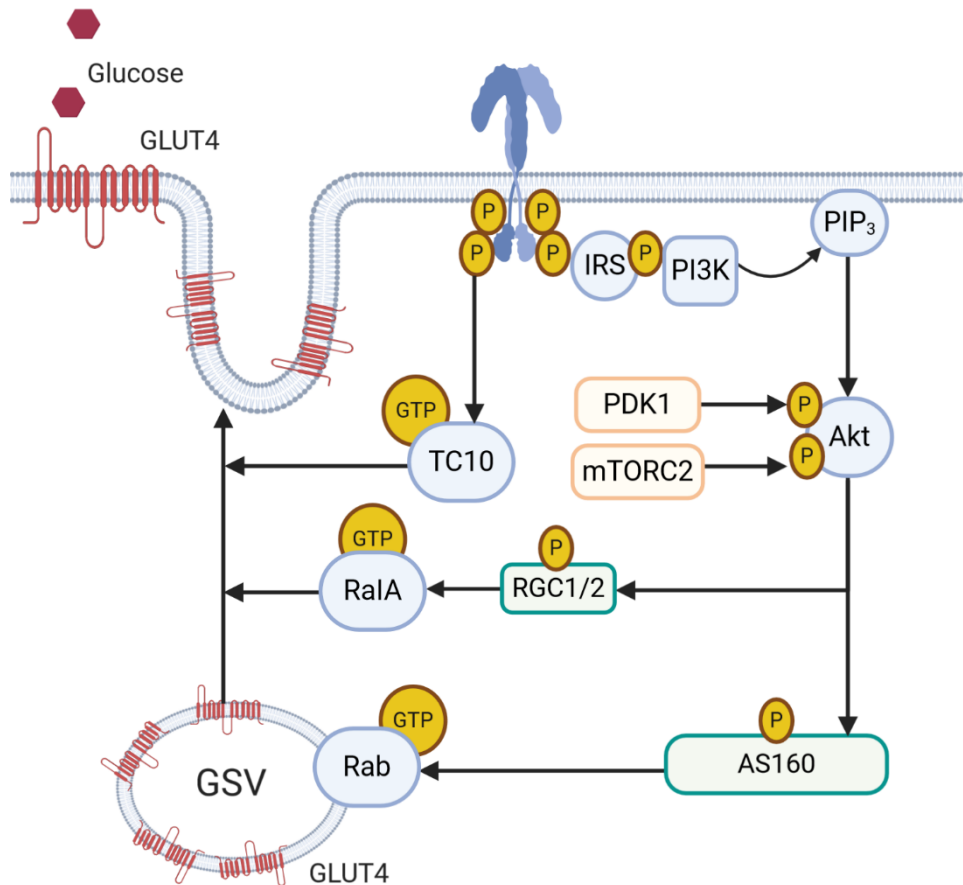
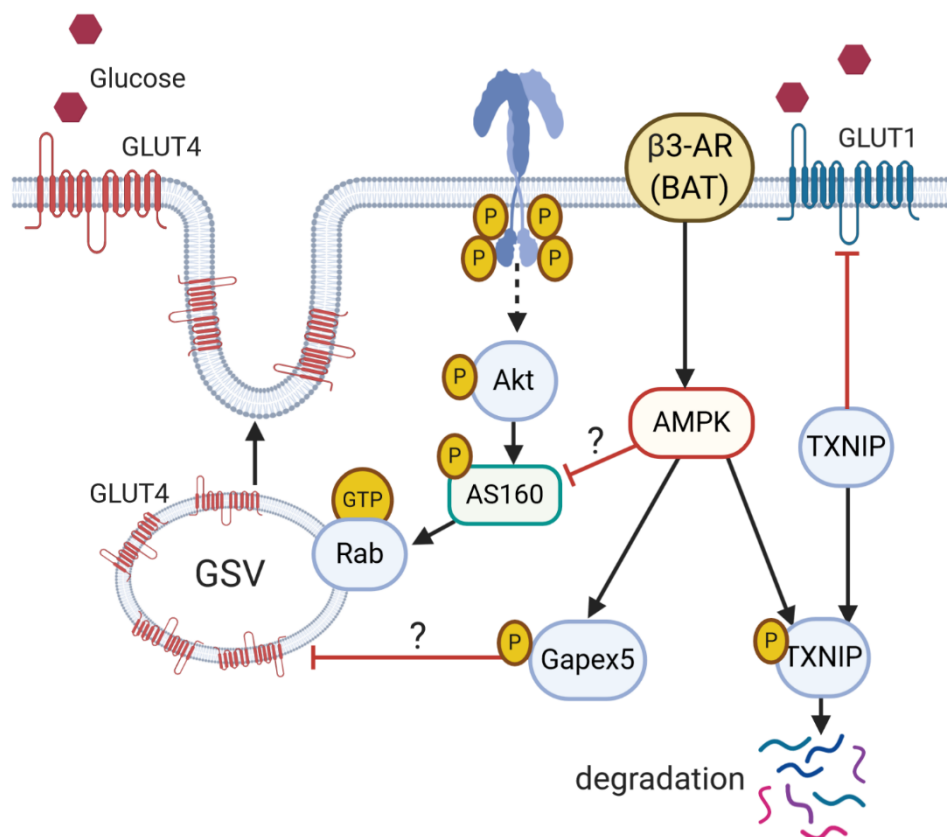**Figure 2: Insulin-stimulated glucose uptake.**

Binding of insulin to its receptor leads to autophosphorylation of the cytoplasmic tyrosine kinase domains, recruitment and phosphorylation of IRS proteins which then bind to and activates PI3K, leading to the production of PIP3 in the plasma membrane. PIP_3 activates PDK1 which phosphorylates Akt Thr308, allowing mTORC2 to phosphorylate Akt at Ser473, fully activating Akt. Akt phosphorylates and inactivates AS160, resulting in the activation of Rab small GTPases required for the recruitment of GSVs containing GLUT4 to the plasma membrane Akt also phosphorylates RGC1/2, leading to increased RalA activity, contributing to GSV trafficking. The insulin receptor, independent of Akt, also stimulates the small GTPase TC10, further contributing to trafficking of GSVs in adipocytes. Created with BioRender.com.

**Figure 3: Regulation of muscle glucose uptake by AMPK.**

AMPK phosphorylates and inactivates TBC1D1, mimicking the effect of insulin via Akt and AS160, leading to GLUT4 translocation. Furthermore, AMPK-mediated phosphorylation of STIM1 reduces store-operated Ca^{2+} entry. AMPK activation is also associated with increased transcription of GLUT4 in muscle, mediated by phosphorylation of HDAC5 and GEF, the latter of which is associated with the transcription factor MEF2 (myocyte enhancer factor 2). Created with BioRender.com.

Figure 4: Regulation of glucose uptake by AMPK in non-myocytes.

AMPK activation may impair insulin-stimulated phosphorylation of AS160 at Thr642 in adipocytes, and phosphorylates Gapex5, although the role of this in insulin-stimulated GLUT4 translocation remains uncertain. AMPK-mediated phosphorylation of TXNIP stimulates its degradation and alleviates its inhibition of GLUT1 activity. In BAT, β3-adrenoreceptor (β3-AR) stimulation leads to AMPK activation. Created with BioRender.com.
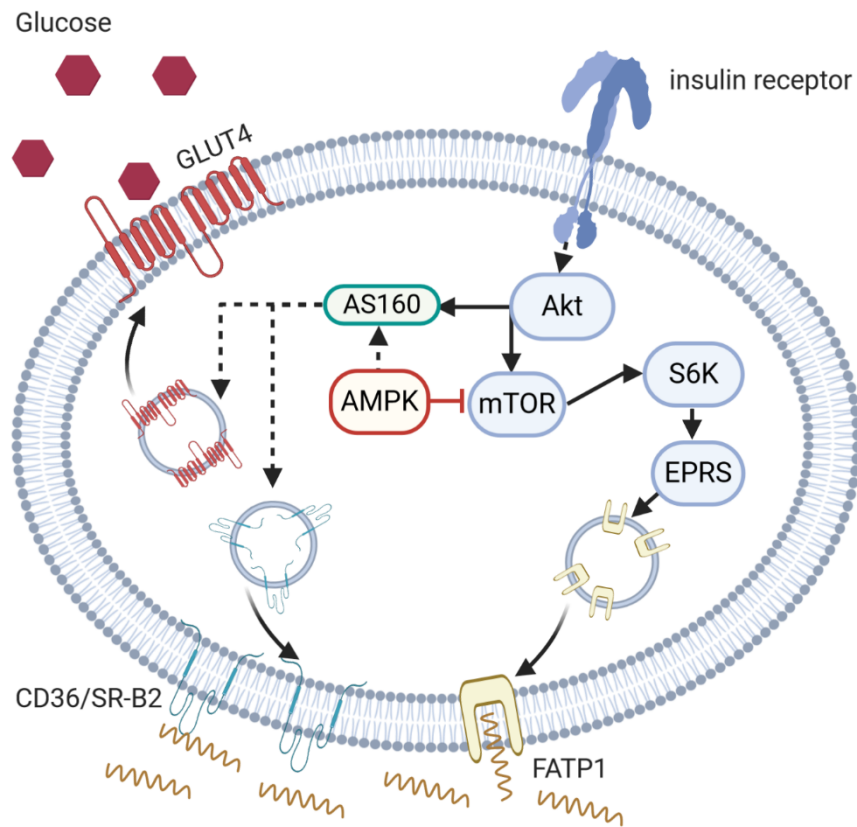
Figure 5: Regulation of fatty acid uptake by AMPK.

In myocytes, insulin stimulates translocation of CD36/SR-B2 in parallel with GLUT4, with both likely mediated by Akt-mediated phosphorylation of AS160/TBC1D1. AMPK phosphorylates and inactivates AS160/TBC1D1, mimicking the effect of insulin on CD36/SR-B2 translocation. Insulin stimulates FATP1 translocation in adipocytes via phosphorylation of EPRS. EPRS is phosphorylated by S6K, which is, in turn, phosphorylated and activated by the Akt substrate mTOR. AMPK would be predicted to suppress insulin-stimulated FATP1 translocation by this mechanism, as AMPK inhibits mTORC1 activity, yet whether AMPK regulates FATP1 translocation in response to insulin has not been examined. Created with BioRender.com.
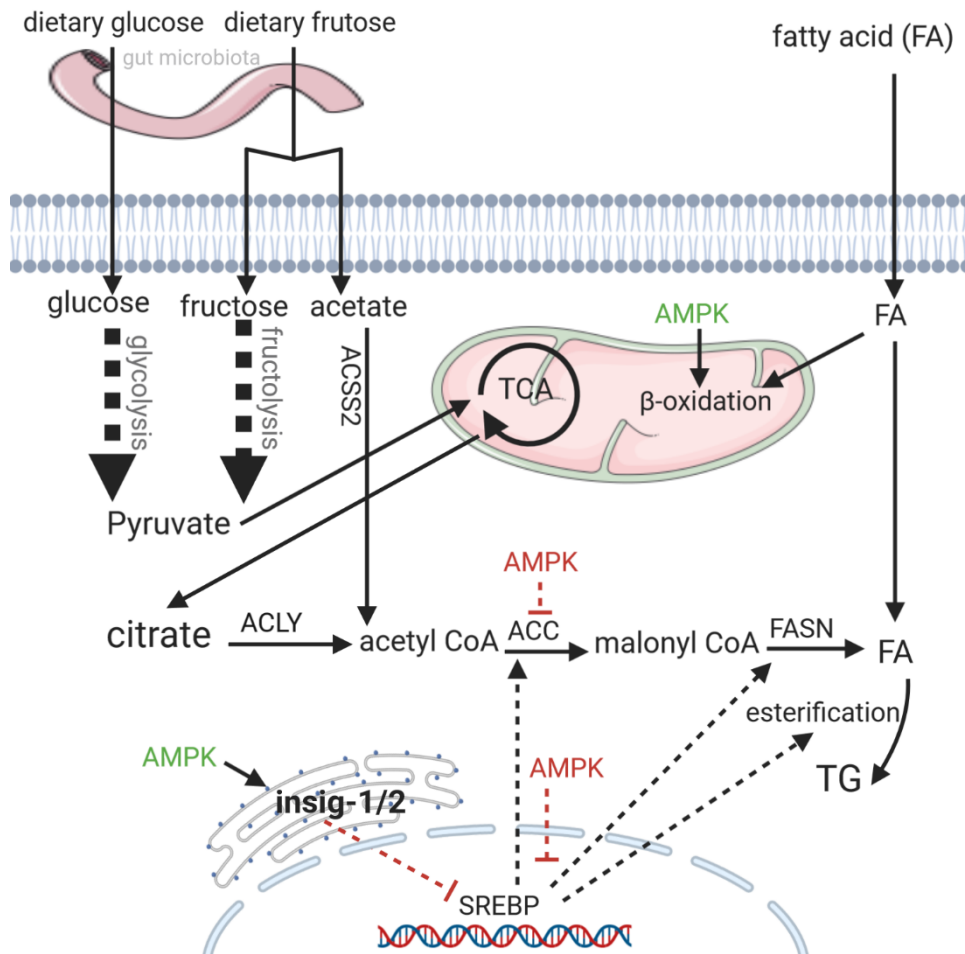
Figure 6: Regulation of lipogenesis by AMPK.

In hepatocytes, triglycerides can be generated from circulating FAs, or by DNL from glucose, fructose or acetate. Glucose/fructose-derived pyruvate is converted to acetyl CoA in the mitochondria by pyruvate dehydrogenase, and then after conversion to citrate is shuttled across the mitochondrial membranes to the cytoplasm, where it is converted back to acetyl CoA by ACLY. Circulating acetate is converted to acetyl CoA by acetyl-CoA synthetase 2 (ACSS2). Acetyl CoA is then utilised to generate malonyl CoA in the cytoplasm by ACC and then FAs by FASN. AMPK antagonises DNL by stimulating β-oxidation (FA oxidation) and inhibiting FA synthesis via phosphorylation and inhibition of ACC. AMPK also -phosphorylates and stabilises Insig1/2, leading to suppression of SREBP processing. AMPK also phosphorylates and inhibits SREBP directly. Created with BioRender.com.