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Review

# Metabolic control by AMPK in white adipose tissue

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**White adipose tissue (WAT) plays an important role in the integration of whole-body metabolism by storing fat and mobilizing triacylglycerol when needed. The released free fatty acids can then be oxidized by other tissues to provide ATP. AMP-activated protein kinase (AMPK) is a key regulator of metabolic pathways, and can be targeted by a new generation of direct, small-molecule activators. AMPK activation in WAT inhibits insulin-stimulated lipogenesis and in some situations also inhibits insulin-stimulated glucose uptake, but AMPK-induced inhibition of β-adrenergic agonist-stimulated lipolysis might need to be re-evaluated *in vivo*. The lack of dramatic effects of AMPK activation on basal metabolism in WAT could be advantageous when treating type 2 diabetes with pharmacological pan-AMPK activators.**

## Highlights

In humans, AMP-activated protein kinase (AMPK) activity is reduced in white adipose tissue (WAT) of insulin-resistant individuals and increased by weight loss.

Adipocyte AMPK is activated by catecholamines and inhibited by insulin.

Allosteric drug and metabolite site activators potently activate adipocyte AMPK, but do not always inhibit lipolysis or glucose uptake, challenging former views of AMPK function in WAT.

AMPK activation suppresses insulin-stimulated lipogenesis in WAT.

AMPK inhibition could be important for the increase in acetyl-CoA carboxylase activity and stimulation of fatty acid synthesis by insulin.

## AMPK as a metabolic regulator and potential drug target in WAT

AMPK is a key regulator of metabolism that becomes activated mainly by a fall in intracellular adenylate charge, and is now a well-recognized drug target for treating diabetes. Comprehensive reviews in the literature on various aspects of AMPK, including structure/function, regulation and downstream targets [1,2], drug targeting to treat human diseases [3], and its role in liver [4], skeletal muscle [5], and brown adipose tissue (BAT) [6], can be found. AMPK in WAT has been reviewed [7,8], but not recently. The present review summarizes the current knowledge about the role of AMPK in WAT, focusing on metabolism, with emphasis on the control of **lipolysis** (see Glossary) and **lipogenesis**, including glucose uptake. The role of WAT in the development of insulin resistance is covered in Box 1. We consider effects of direct pharmacological AMPK activation in WAT by molecules that bind to the so-called **allosteric drug and metabolite (ADaM)** pocket (see Box 2 for more details on AMPK activators), along with hormones that are critical for the regulation of adipocyte metabolism, such as catecholamines that increase **cAMP**, as well as insulin. The role of AMPK in the regulation of fatty acid (FA) oxidation, adipogenesis, adipokine secretion and inflammation in WAT will not be described, as these areas have been covered previously and/or have not developed significantly in recent years. Due to space limitations, we apologize to authors whose work was not cited.

## Glucose and lipid metabolism in WAT

The main role of WAT is to store **triacylglycerol (TAG)** and release FAs that can be oxidized by other tissues for ATP production. Since the discovery of leptin, WAT is also regarded as an important endocrine organ, secreting adipokines that regulate, for example, appetite and insulin sensitivity in nonadipose tissues [9]. As described in more detail below, the main substrates for TAG synthesis are glucose and FAs (Figure 1). Glucose taken up from the circulation, facilitated by insulin [10], is metabolized via glycolysis to provide **glycerol 3-phosphate (Gro3P)**, but Gro3P can also be produced from noncarbohydrate substrates by the process of glyceroneogenesis [11]. FAs from the circulation are, together with Gro3P, incorporated into TAG by the esterification pathway. Adipocytes also synthesize FAs *de novo* from acetyl-CoA (Figure 1), especially in the well-fed

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Box 1. WAT dysfunction in relation to the development of insulin resistance and type 2 diabetes

Obesity is a major risk factor for type 2 diabetes and adipose tissue dysfunction is thought to be an underlying cause of the development of systemic insulin resistance; a hallmark of the disease. Exactly why obesity-associated insulin resistance develops is not understood, but altered adipokine secretion, low-grade inflammation and inefficient lipid storage, with ectopic lipid accumulation as a result, are likely to be involved. A number of alterations in glucose and lipid metabolism in adipocytes are indeed observed in obesity and insulin resistance, including increased basal lipolysis, and reduced insulin-induced glucose uptake and lipogenesis [13]. The increased basal lipolysis is thought to be a result of enlarged adipocytes and local inflammation [90]. Elevated lipolysis in turn results in increased delivery of FAs to skeletal muscle and liver – ultimately leading to ectopic lipid deposition and insulin resistance in these tissues [90]. Although glucose uptake in adipocytes is quantitatively not a major pathway for post-prandial glucose disposal [91], GLUT4 deficiency in mouse WAT is associated with impaired insulin sensitivity in liver and muscle [92]. Conversely, adipose specific overexpression of GLUT4 was shown to lead to reduced glycemia during starvation and enhanced glucose tolerance [93]. These positive effects of increased glucose uptake in WAT were proposed to be partly due to the induction of lipogenic genes in response to glucose [94]. Indeed, rates of lipogenesis in adipocytes, including *de novo* FA synthesis, have been shown to correlate positively with the degree of insulin sensitivity in obese individuals [95,96]. Moreover, specific types of *de novo* synthesized FAs, for example FA esters of hydroxy-FAs, were proposed to have favorable metabolic effects [95,96]. In summary, glucose uptake, FA synthesis and lipolysis in WAT are important for maintaining whole-body insulin sensitivity.

condition when carbohydrates are plentiful [12,13]. TAG synthesis is here referred to as lipogenesis and TAG formation from newly synthesized FAs is referred to as ***de novo* lipogenesis (DNL)**.

Upon energy demand, catecholamines such as adrenaline promote TAG hydrolysis by lipolytic enzymes, releasing free FAs and glycerol into the circulation. Indeed, during prolonged starvation, FAs are oxidized by skeletal muscle and other tissues/organs to provide ATP, and glycerol becomes an important substrate for liver gluconeogenesis [4]. The ability of adipocytes to store and release FAs in response to nutritional/hormonal status and energy demand underlies the importance of adipose tissue in the integration of whole-body metabolism. Early studies of metabolism in WAT were performed on rat epididymal adipose tissue [10,14], a visceral WAT (vWAT) depot, whereas human studies often used subcutaneous WAT (scWAT). When comparing results from various studies, one should bear in mind that metabolism, including responses to insulin and lipolytic agents, differs between WAT depots [15,16], and that there also are gender differences [17].

## AMPK expression and regulation in WAT

### AMPK isoform expression in white adipocytes from different species

A strategy for tissue-specific activation of AMPK is the development of compounds that preferentially activate particular AMPK trimers. Existing ADaM site activators have (varying) preference for targeting AMPKβ1-containing complexes. Detailed knowledge about protein expression and contribution of different isoforms to AMPK activity in metabolically relevant tissues is essential for drug targeting. Several studies have shown that AMPKα1 is the predominant catalytic subunit isoform in white adipocytes [18–20].

The expression pattern of the regulatory AMPK β-subunit and the contribution of AMPKβ1 and AMPKβ2 to AMPK activity in adipocytes has recently been described [21,22]. Based on western blotting using a pan-AMPKβ antibody, as well as quantitative and isoform-specific immunoprecipitation/kinase assays, it was shown that AMPKβ1 is predominant in rat and 3T3-L1 adipocytes, while in primary mouse and primary human adipocytes, AMPKβ1 and AMPKβ2 were expressed and contributed more or less equally to total AMPK activity [21,22]. It was also shown that the AMPKβ1/AMPKβ2 ratio in adipocytes from humans was similar and around 1 over a large range of body mass index (BMI) values [22]. In contrast, another study concluded that AMPKβ2-associated AMPK activity was predominant in both 3T3-L1, rodent and human adipocytes [19].

## Glossary

**Acetyl-CoA carboxylase (ACC):** enzyme that converts acetyl-CoA to malonyl-CoA, a key control point in the synthesis of fatty acids.
**AKT substrate of 160 kDa (AS160):** Rab GTPase activating protein (GAP), whose inactivation participates in insulin-induced glucose uptake. AS160 is also known as TBC1D4.
**Allosteric drug and metabolite (ADaM) site:** pocket located between the small lobe of the AMPK α-subunit kinase domain and the AMPK β-subunit CBM, to which a novel class of direct activators bind.
**5-Aminoimidazole-4-carboxamide riboside (AICAR):** first AMPK activator to be identified and widely used before the discovery of ADaM site activators. It is converted to the AMP analogue ZMP in cells.
**cAMP:** second messenger that activates PKA and mediates diverse effects of hormones (e.g., glucagon and catecholamines).
***De novo* lipogenesis (DNL):** synthesis of TAG from newly synthesized FAs.
**Glycerol 3-phosphate (Gro3P):** three-carbon metabolite produced via glycolysis to which FAs are esterified to form TAG.
**Hormone-sensitive lipase (HSL):** enzyme whose activation mediates catecholamine-induced hydrolysis of TAG.
**Lipogenesis:** formation (synthesis) of lipid (fat). It is the biochemical process by which acetyl-CoA is converted to TAG and thus includes (1) *de novo* FA synthesis and (2) TAG synthesis (esterification).
**Lipolysis:** process by which TAGs are hydrolyzed to FAs and glycerol.
**Protein kinase A (PKA):** kinase activated by cAMP that phosphorylates a wide range of proteins involved in the regulation of metabolism.
**Protein kinase B (PKB)/Akt:** kinase that mediates many of the metabolic effects of insulin.
**Triacylglycerol (TAG):** neutral lipid storage molecule found in adipose tissues.

## Box 2. AMPK structure, regulation and pharmacological activation

AMPK is a conserved Ser/Thr protein kinase, whose main role is to sense changes in intracellular adenylate charge [2]. AMPK is activated via an increase in AMP:ATP ratio, switching on ATP-generating processes and shutting down ATP-consuming pathways. AMPK is a heterotrimer consisting of a catalytic α-subunit (α1 or α2), a regulatory β-subunit (β1 or β2) and a regulatory γ-subunit (γ1, γ2, or γ3) [2] (Figure I). AMPK activation requires phosphorylation of AMPKα Thr172 by upstream kinases, mainly liver kinase B1 (LKB1) [2]. The AMPK γ-subunits bind AMP, while the β-subunits contain a carbohydrate-binding module (CBM) [2]. A rise in AMP activates AMPK via three mechanisms: (i) allosteric stimulation of AMPK activity; (ii) promotion of AMPKα Thr172 phosphorylation; (iii) inhibition of AMPKα Thr172 dephosphorylation [2]. A rise in ADP can also lead to AMPK activation by promoting and preventing AMPKα Thr172 dephosphorylation, without inducing allosteric stimulation.

Other phosphorylation sites control AMPK (Figure I). Rat AMPKα1 Ser485 (Ser496 in the human protein) is phosphorylated by AKT/PKB antagonizing AMPK activation by LKB1 [39,40]. The equivalent residue in AMPKα2, Ser491, is not targeted by AKT/PKB [1] and represents an autophosphorylation site [1]. AMPKα1 Ser485 can also be phosphorylated by PKA and PKC [1]. AMPKβ1 Ser108 is an autophosphorylation site [97] in the CBM that stabilizes the ADaM pocket [98] and is required for allosteric AMPK activation by A-769622 [99] (see below).

AMPK is a recognized drug target for treating type 2 diabetes [2,3]. A summary of the structures and action of indirect and direct AMPK activators can be found in the Tables of [3]. AICAR, the first pharmacological AMPK activator, is a prodrug converted to the AMP analog ZMP in cells [100]. A novel class of direct activators was discovered that bind to the ADaM pocket, located between the small lobe of the AMPK α-subunit kinase domain and the AMPK β-subunit CBM [98]. A-769662 [101,102] and compound 991 [98] both allosterically increase AMPK activity and inhibit AMPKα Thr172 dephosphorylation by binding to this site. A-769662 and 991 specifically activate AMPKβ1-containing heterotrimers [98,103]. More recently, pan-β AMPK direct activators, such as SC4, MK-8722 and PF-793, have been developed [3] that, along with AICAR, A-769662 and 991, activate AMPK in WAT [21,57]. Furthermore, long-chain (LC)-FA-CoA, was recently proposed to directly activate AMPK by binding to the ADaM site [32].

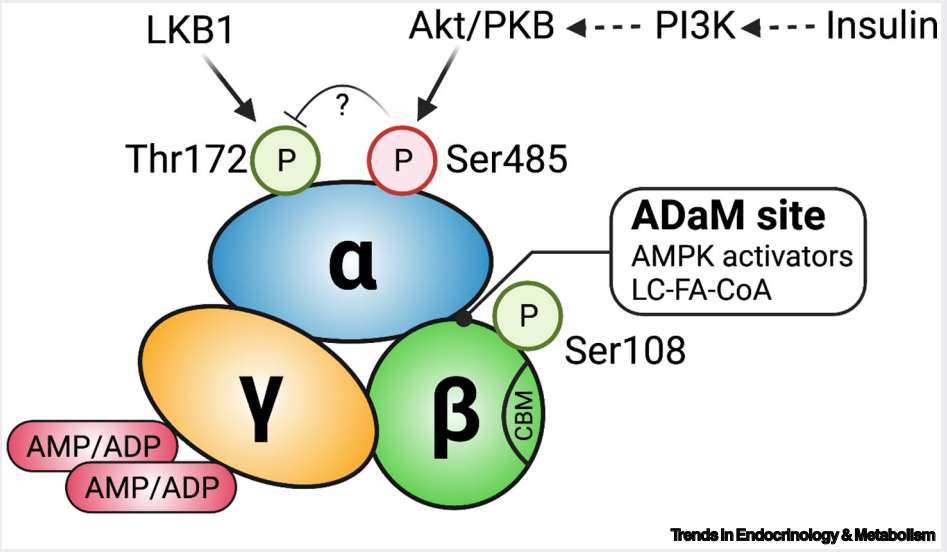



Figure I. Structural and functional features of AMPK. AMPK is a heterotrimeric protein kinase consisting of a catalytic AMPKα-, and its two regulatory, AMPKβ- and AMPKγ-subunits. Phosphorylation of the T-loop AMPKα Thr172 residue, mainly by LKB1, leads to dramatic AMPK activation, whereas insulin-induced, PI3K-dependent AMPKα1 Ser485 phosphorylation has been proposed to inhibit AMPK activity. Net phosphorylation of AMPKα Thr172 is promoted by AMP or ADP, binding to sites on the AMPKγ-subunit. AMP also allosterically stimulates AMPK. The AMPKβ-subunit contains a CBM, that forms part of the ADaM pocket for binding pharmacological direct AMPK activators as well as LC-FA-CoA. Activation of AMPK via the ADaM site is largely dependent on the phosphorylation of AMPKβ1 Ser108. Abbreviations: ADaM, allosteric drug and metabolite; AMPK, AMP-activated protein kinase; CBM, carbohydrate-binding module; FA, fatty acid; LKB1, liver kinase B1; PI3K, phosphatidylinositol-3 kinase.

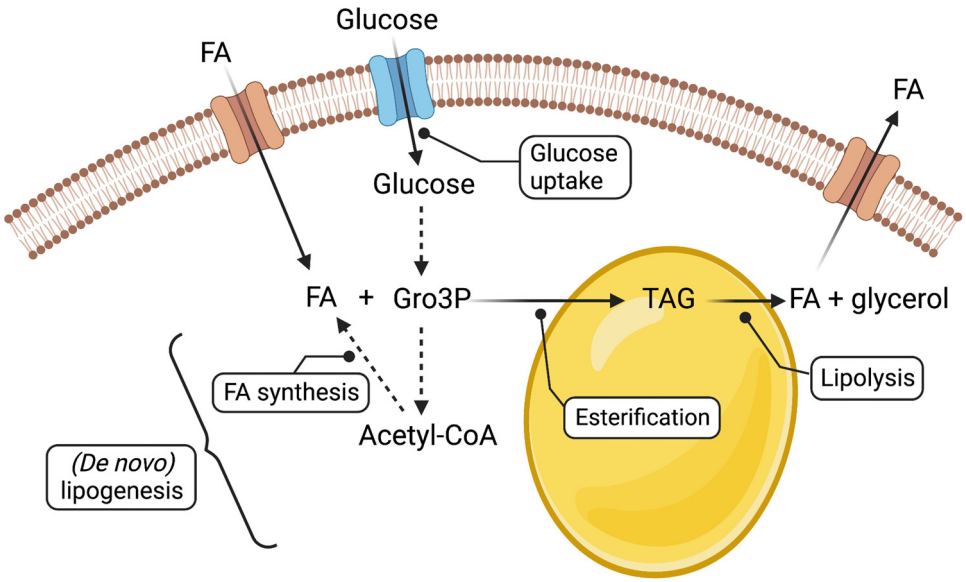



Figure 1. Simplified overview of glucose and lipid metabolism in adipocytes. The main role of adipose tissue is to store TAG and release FAs to provide ATP via their β-oxidation in other tissues when required. TAG is mainly formed by the esterification of glucose-derived Gro3P with FAs taken up from the circulation or synthesized *de novo*. TAG synthesis (lipogenesis) from such newly synthesized FAs is referred to as *de novo* lipogenesis in this review. Abbreviations: FA, fatty acid; Gro3P, glycerol 3-phosphate; TAG, triacylglycerol.

AMPK γ-subunit isoform expression in adipose tissue, and in general, has so far proven to be challenging to study, due to the existence of several different splice variants and the lack of specific antibodies. However, mRNA analyses point towards AMPKγ1 and AMPKγ2 being present in adipocytes [23]. AMPKγ3 expression appears to be restricted to skeletal muscle [23].

## Control of AMPK activity by hormones in WAT

Adipocyte AMPK activity is acutely controlled by the most important hormones regulating WAT metabolism, namely insulin and catecholamines (see Figure I in Box 2 and Figure 2A). An early observation was that AMPK in adipocytes becomes activated in response to exercise [24,25] and starvation [20] in humans and/or rats, suggesting that AMPK might respond to stress hormones and cAMP elevation. Indeed, numerous studies have demonstrated AMPK activation in adipocytes stimulated with cAMP-elevating agents, including forskolin, cAMP analogs and β-adrenergic receptor agonists, such as adrenaline and isoproterenol [18,20,24,26–29]. These studies were mainly performed in 3T3-L1 and rat adipocytes; however, in human adipocytes, AMPK was activated by incubation with the lipolytic agents isoproterenol or atrial natriuretic peptide (ANP), pointing to similar regulation in humans and rodents [30]. The mechanisms underlying cAMP-dependent activation of AMPK have been proposed to involve phosphodiesterase (PDE) 4, exchange protein activated by cAMP 1 (Epac1), **protein kinase A (PKA)**, as well as increased lipolysis [26,31]. As a consequence of the stimulation of lipolysis, the intracellular AMP:ATP ratio becomes elevated due to increased re-esterification of a proportion of released FAs [26], leading to AMPK activation (Figure 2A). Indeed, the re-esterification of FAs involves LC-FA-CoA synthetase, which produces AMP. Inhibition of the enzyme by triacsin C blunted the rise in AMP:ATP ratio and AMPK activation in response to isoproterenol treatment [26]. In addition, AMPK activation as a result of increased lipolysis could be due to increased LC-FA-CoAs, since these are thought to directly activate AMPK by binding to the ADaM site (see Figure I in Box 2 and Figure 2A) [32].

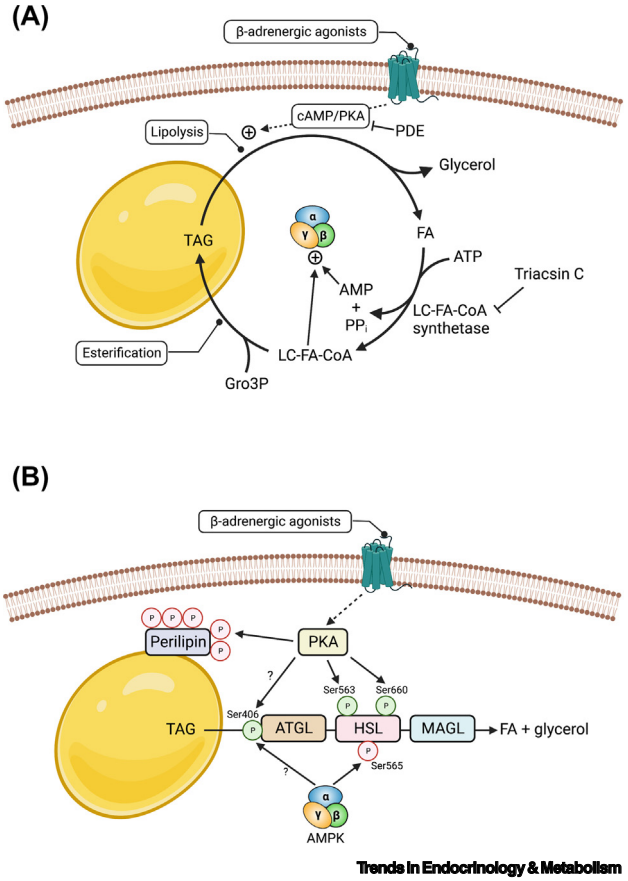



Figure 2. Control of lipolysis by AMPK and PKA. (A) β-Adrenergic receptor agonists and other cAMP-elevating agents, such as PDE inhibitors, are known to activate AMPK. This is thought to be mediated by increased cycling of FAs produced via cAMP/PKA activation and lipolysis. A proportion of released FAs are converted to LC-FA-CoA, an ATP-consuming reaction catalyzed by LC-FA-CoA synthetase. LC-FA-CoAs can then be re-esterified with Gro3P to form TAG. The rise in AMP and LC-FA-CoA resulting from the LC-FA-CoA synthetase reaction is thought to mediate AMPK activation in response to elevated cAMP, supported by experiments using the LC-FA-CoA synthetase inhibitor triacsin C. (B) PKA activation in response to β-adrenergic receptor agonists promotes lipolysis by phosphorylating perilipin – causing its dissociation from the lipid droplet, allowing lipases, such as ATGL, HSL, and MAGL, to access TAG. PKA is well known to activate HSL via phosphorylation at several sites including Ser563 and Ser660. ATGL can be phosphorylated by PKA at Ser406 but its functional relevance is debated. AMPK was also reported to phosphorylate ATGL Ser406. AMPK phosphorylates HSL on Ser565, a site thought to inhibit HSL activation by PKA. AMPK has been proposed to stimulate basal lipolysis, but to inhibit cAMP-induced lipolysis. Abbreviations: AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; FA, fatty acid; Gro3P, glycerol 3-phosphate; HSL, hormone-sensitive lipase; LC, long chain; MAGL, monoacylglycerol lipase; PDE, phosphodiesterase; PKA, protein kinase A; TAG, triacylglycerol.

Interestingly, incubation of rat adipocytes with palmitate was shown to lead to AMPK activation, independently of a rise in AMP:ATP ratio [33], and this might have been due to increased intracellular palmitoyl-CoA levels. Other hormones that would be expected to activate AMPK in WAT by stimulating lipolysis are noradrenaline, glucagon, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), and growth hormone [34].

In contrast, early studies on hepatoma cells showed that insulin inhibited AMPK [35]. More recently, insulin-induced AMPK inhibition was seen in adipocytes and WAT from rats and humans, both under basal and catecholamine-stimulated conditions [27,31,36,37]. As discussed in more detail below, inhibition of AMPK by insulin might mediate the stimulatory effect of insulin on FA synthesis, as inhibition of AMPK is associated with reduced **acetyl-CoA carboxylase (ACC)** phosphorylation [31,36]. Insulin has also been shown to inhibit AMPK in other important insulin-sensitive cells and tissues, such as hepatocytes, myotubes, skeletal muscle [38] and heart [39]. The regulation of AMPK by insulin in adipocytes was shown to be dependent on AKT/**Protein kinase B (PKB)**, and associated with increased phosphorylation of AMPKα1 Ser485 (see Figure I in Box 2) [31,36]. Several studies have shown that phosphorylation of this site is linked to reduced AMPKα1 Thr172 phosphorylation and AMPK inhibition [31,36,38–40]

and it was taken for granted that increased AMPKα1 Ser485 phosphorylation was the cause of the lowering of AMPK activity by insulin. However, by expressing an AMPKα1 Ser485Ala mutant in rat adipocytes, it was recently shown that AMPKα1 Ser485 phosphorylation is dispensable for the inhibition of AMPK by insulin [36]. Also, marked insulin-induced AMPKα1 Ser485 phosphorylation was observed in the absence of changes in AMPKα1 Thr172 or AMPK activity in 3T3-L1 fibroblasts and human adipocytes [36,41]. We believe that the insulin-induced inhibition of AMPK in adipocytes might be mediated by other mechanisms such as changes in nucleotide levels and indeed, the intracellular AMP:ATP ratio was shown to be reduced by insulin in rat adipocytes [36].

### AMPK expression and activity in human WAT – links to insulin resistance and diabetes

Studies in humans revealed decreased AMPK activity, measured as reduced AMPKα Thr172 phosphorylation, in both scWAT and vWAT from individuals with severe obesity and insulin resistance, compared to BMI-matched insulin sensitive individuals [42]. Moreover, it was shown that AMPKα Thr172 phosphorylation in scWAT was significantly increased after bariatric surgery [37,43]. Increased AMPKα Thr172 phosphorylation was associated with increased ACC phosphorylation and reduced malonyl-CoA levels [37,43]. Collectively, these findings indicate that increased AMPK activity might lead to lower *de novo* FA synthesis in subjects who have undergone bariatric surgery. It was also observed that the ability of insulin to inhibit AMPK, potentially through increased AMPKα1 Ser485 phosphorylation, was restored after surgery [37], such that *de novo* FA synthesis in the presence of insulin might be unchanged, or even increased. The studies performed so far have indicated that AMPK activity and its regulation by insulin is altered in obesity, which might have consequences for the control of FA synthesis. The mechanisms underlying these alterations include potential dysregulation of upstream AMPK kinases, but whether the observed alterations are due to changes in AMPK activity in adipocytes or in other cells present in the tissue, are not known. A recent study in fact found no correlation between BMI and AMPKα Thr172 phosphorylation/*in vitro* AMPK activity in adipocytes isolated from human scWAT [22]. This indicates that the previously observed changes in AMPK activity after bariatric surgery were probably attributable to AMPK in nonadipose cells. Alternatively, the previously observed increase in AMPK activity following bariatric surgery could be linked to the *loss* of weight, rather than to body weight *per se*.

## Lipolytic control by AMPK in WAT

Lipolysis involves the sequential hydrolysis of TAGs to free FAs and glycerol. Adipose triglyceride lipase (ATGL) first hydrolyses TAG to diacylglycerol (DAG), which is then further hydrolyzed by **hormone-sensitive lipase (HSL)** to monoacylglycerol before final hydrolysis by monoacylglycerol lipase (MAGL) [44] (Figure 2B). By inducing cAMP/PKA signaling, catecholamines increase lipolysis via several mechanisms, the most well-known being phosphorylation-induced HSL activation [44]. PKA also phosphorylates perilipin (PLIN) 1, causing its dissociation from lipid droplets [44]. Potential regulation of ATGL by phosphorylation is controversial, but both PKA and AMPK have been proposed to phosphorylate and activate the enzyme [45,46]. Insulin has a well-known antilipolytic effect in WAT. AKT/PKB-induced phosphorylation and activation of PDE3B by insulin leads to a lowering of cAMP levels and reduced PKA activity [47]. However, phosphorylation of PDE3B by AKT/PKB might not be required for the effect of insulin to suppress lipolysis [48].

A prevailing view until recently was that AMPK activation inhibits adipocyte lipolysis. This was largely based on **5-aminoimidazole-4-carboxamide riboside (AICAR)**-induced inhibitory effects of AMPK activation on basal as well as β-adrenergic agonist-stimulated lipolysis in primary rat adipocytes [20,49,50]. AICAR treatment was proposed to reduce HSL activity via AMPK-mediated phosphorylation of HSL Ser565, hindering PKA from phosphorylating the activating HSL Ser563 and HSL Ser660 sites [20,29,51] (Figure 2B). AMPK activation also inhibited HSL

translocation to the lipid droplet [20]. However, in 3T3-L1 adipocytes, it was shown that if PKA stimulation and increased HSL Ser563 and HSL Ser660 phosphorylation preceded AICAR treatment and increased HSL Ser565 phosphorylation, HSL phosphorylation at Ser660 could be maintained, in contrast to HSL Ser563 phosphorylation [25]. This suggests a major role for HSL Ser660 phosphorylation in the regulation of HSL activity, which could overcome AMPK-induced HSL inhibition. Moreover, decreased basal as well as decreased β-agonist-stimulated lipolysis in response to AICAR was shown in human adipocytes [52]. In human adipocytes, treatment with biguanides such as metformin or thiazolidinediones also activated AMPK, associated with an inhibition of isoproterenol/ANP-stimulated lipolysis, effects that were opposed by the AMPK inhibitor compound C [30]. In contrast to the many reported antilipolytic effects of AMPK activation, a study in 3T3-L1 adipocytes claimed that AMPK was required for maximal lipolysis [28]. Furthermore, chronic AICAR treatment was proposed to change adipocyte lipid metabolism from lipid storage towards ATP production by inducing FA release and oxidation [53,54], proposed to be due to increased expression of ATGL and PLIN1.

An *in vivo* study on inducible adipocyte specific AMPKα1/α2-deficient mice indicated defective HSL Ser565 phosphorylation and higher isoproterenol-induced HSL Ser563 and HSL Ser660 phosphorylation, increasing isoproterenol-stimulated lipolysis [29]. In the same study, ATGL was proposed to be an AMPK target and reduced ATGL Ser406 phosphorylation was seen as a result of adipose AMPK deficiency along with reduced TAG hydrolase activity, lowering basal lipolysis. In addition, adipocyte FA oxidation and oxygen consumption were increased in AMPKα1/α2-deficient mice [29]. These observations are in line with the lean phenotype of these mice and the importance of ATGL in controlling basal lipolysis [29,45,55]. This study [29] was the first to use the A-769662 direct AMPK activator on adipocytes, indicating stimulatory and inhibitory effects of AMPK activation on basal and isoproterenol-stimulated lipolysis, respectively. Using inducible adipocyte specific AMPKβ1/β2 knockout (KO) mice, no changes in basal or catecholamine-induced FA/glycerol release or HSL activating phosphorylation were reported in white adipocytes isolated from these mice [56]. Interestingly, the antilipolytic effect of AICAR was preserved in the absence of AMPKβ1/β2, suggesting off-target, AMPK-independent inhibition of lipolysis by AICAR [56]. An important difference between these two contrasting studies is that gonadal adipocytes were prepared from AMPKα1/α2 deficient mice [29], whereas subcutaneous adipocytes from AMPKβ1/β2 KO mice were used [56].

A more recent study focused on effects of AMPK activation by ADaM site activators A-769662 and 991 on lipolysis in human adipocytes compared with effects on rat and mouse adipocytes [21]. Although treatment with A-769622 inhibited catecholamine-stimulated lipolysis in mouse adipocytes, and slightly increased basal lipolysis, it did not affect isoproterenol-stimulated lipolysis in human or rat adipocytes. The potent activator 991 affected neither basal nor isoproterenol-stimulated lipolysis in rat or human adipocytes. Treatment with 991 increased HSL Ser565 and reduced HSL Ser563 phosphorylation, but at the same time, phosphorylation at HSL Ser660 increased. The latter could explain why overall, HSL activity and lipolysis were unaffected as a result of AMPK activation by 991. However, in rat adipocytes incubated with a combination of suboptimal concentrations of SC4 and AICAR to achieve robust and specific AMPK activation, noradrenaline-stimulated lipolysis was modestly decreased, accompanied by decreased HSL Ser563 phosphorylation, without affecting HSL Ser565 phosphorylation or basal lipolysis [57]. The fact that adipocytes from AMPKα1 KO mice were significantly smaller would suggest that basal AMPK activity somehow impedes lipolysis [20]. However, data on novel ADaM site activators reveal that in rat and human adipocytes, the changes in HSL phosphorylation are such that the outcome of AMPK activation is not antilipolytic [21]. Based on the current evidence, our view is that AMPK activity/activation does not have a major impact on basal and catecholamine-stimulated TAG hydrolysis, but more *in vivo*

measurements using direct AMPK activators, combined with specific genetic AMPK deletion in WAT, are needed to clarify the long-standing anti-lipolytic role of AMPK in WAT (see Concluding remarks and perspectives).

## AMPK and the control of glucose uptake in WAT

As in skeletal muscle, insulin stimulates glucose uptake in adipocytes by promoting GLUT4-containing vesicle translocation to the plasma membrane [58] (Figure 3A). GLUT4 vesicle trafficking depends on activation of GTPase Ras-related in brain (Rab) proteins [59], located in the vesicle membrane, which in turn requires inactivation of the Rab GTPase activating protein (GAP) **AKT substrate of 160 kDa (AS160)**, also known as TBC1D4 [60] (Figure 3A). In response to insulin-induced AKT/PKB activation, AS160 becomes phosphorylated at multiple sites, accompanied by its GAP inactivation [60]. The exact mechanism by which AS160 is inactivated by insulin is not fully understood, but might involve association of AS160 with the scaffold protein 14-3-3, proposed to be mediated by insulin-induced AS160 Ser341 and Thr642 phosphorylation via AKT/PKB [60].

AMPK activation in skeletal muscle increases glucose uptake independently of insulin and its role in mediating the effect of contraction-induced glucose uptake has been extensively studied [5]. However, the role of AMPK in controlling glucose uptake in WAT has received far less attention, and until recently was mainly studied by using AICAR in rat or 3T3-L1 adipocytes. The conclusions drawn from these early studies was that AMPK activation by AICAR leads to a marked reduction in insulin-stimulated glucose uptake in both rat and 3T3-L1 adipocytes, but that the effect on basal glucose uptake varied, either increasing in 3T3-L1 adipocytes or going down in rat adipocytes [61–64]. The mechanism by which AICAR reduces insulin-stimulated glucose uptake is still unclear. In AICAR-treated rat adipocytes, reduced AS160 Thr642 phosphorylation was seen along with impaired GLUT4 translocation to the plasma membrane [65]. This effect of AICAR was abolished by expression of a kinase-inactive AMPKα1 mutant [65]. However, another study failed to detect clear changes in AS160 Thr642 phosphorylation or its association with 14-3-3 proteins due to AICAR treatment [66]. By using AMPK KO mouse models, as well as novel and more specific AMPK activators, it has become clear that some effects of AICAR previously attributed to AMPK activation are in fact AMPK independent. A recent study compared effects of AICAR and ADaM site activators A-769662 and 991 in several different adipocyte models, including isolated rat and human primary adipocytes. While AICAR and A-769662 were found to inhibit basal and insulin-stimulated glucose uptake in all models tested, the more potent and specific activator, 991, was without effect on basal glucose uptake, despite AMPK activation [66]. A transgenic mouse model expressing AMPKβ1 Ser108Ala, in which AMPK activation was essentially insensitive to ADaM site activators, was exploited to explore whether the effect of A-769662 on glucose uptake was due to AMPK activation. Strikingly, although AMPK was not activated by A-769662 in adipocytes from Ser108Ala mice, the inhibitory effect of A-769662 on glucose uptake was maintained [66]. The data suggest that A-769662 and AICAR, inhibit glucose uptake through AMPK-independent mechanisms and that AMPK activation *per se* does not reduce this process. In future studies it would be interesting to assess glucose uptake, and the effects of AICAR and A-769662, in adipocytes from adipose tissue specific AMPKα1/α2 [67] or AMPKβ1/β2 KO mice [56]. Moreover, additional ADaM site activators such as MK-8722 should be tested for effects on glucose uptake [68,69]. In a study where AICAR and SC4 were combined to activate AMPK in incubated mouse epididymal fat pads, basal glucose uptake was unaffected, whereas insulin-stimulated glucose uptake was abrogated [57]. These results are in agreement with the lack of effect of 991 on basal glucose uptake, but differ from findings in rat and human adipocytes where no effect of 991 on insulin-stimulated glucose uptake was seen [66]. Moreover, the effect of combined SC4 and AICAR treatment to decrease insulin-stimulated glucose uptake was still

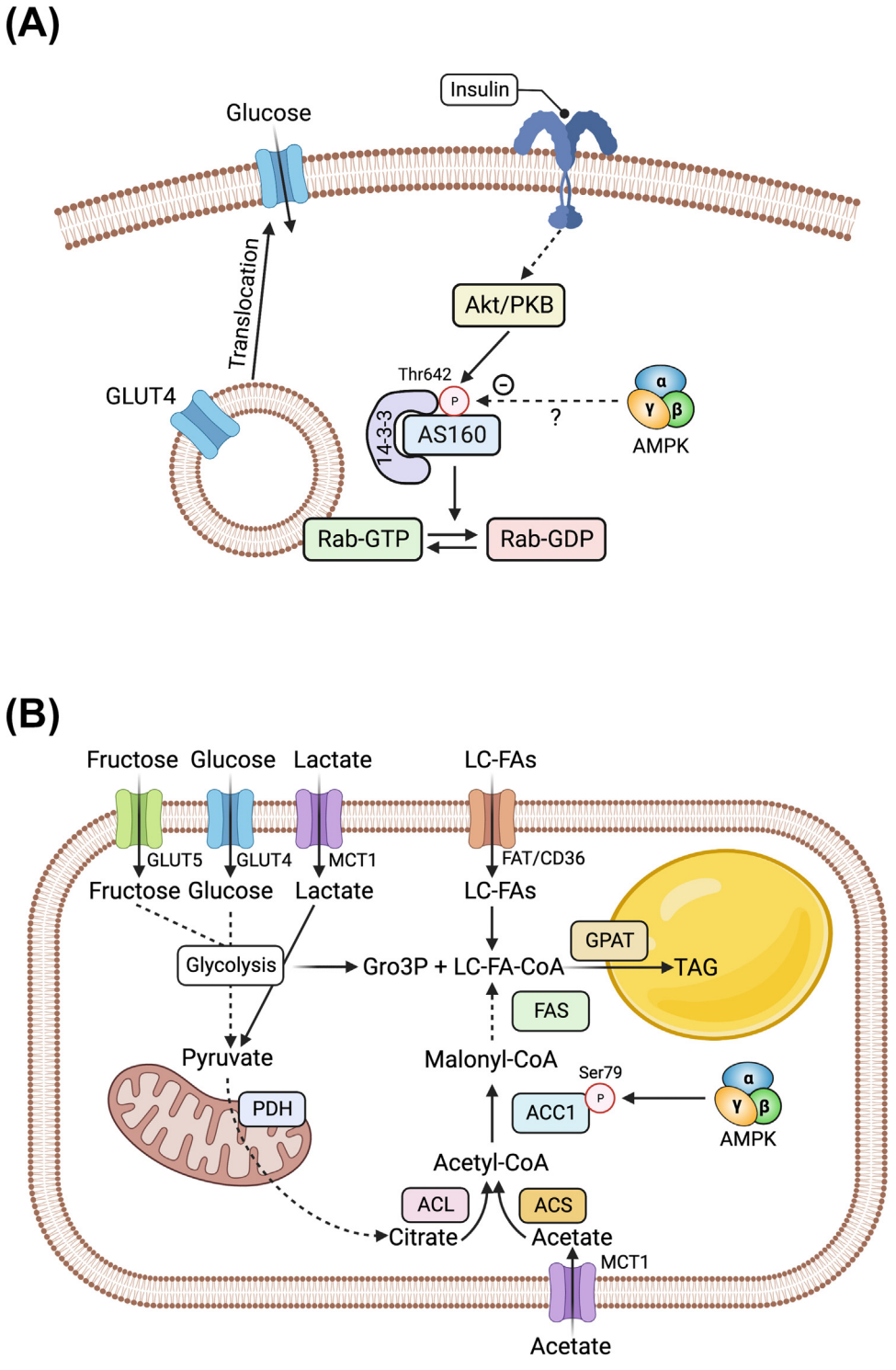



Figure 3. Control of glucose uptake and lipogenesis by AMPK. (A) Insulin-induced glucose uptake is mediated by increased translocation of GLUT4-containing vesicles to the plasma membrane, a process requiring Rab proteins to be in their

*(Figure legend continued at the bottom of the next page.)*

observed in fat pads from AMPKα1 KO mice, suggesting the effect was partly AMPKα1-independent. We believe that AMPK-induced inhibition of insulin-stimulated glucose uptake could be important, but *in vivo* experiments, for example by [$^{18}$F]-fluorodeoxyglucose positron emission tomography imaging, perhaps exploiting AMPKβ1 Ser108Ala mice, are needed to confirm this effect.

## AMPK, insulin, and regulation of lipogenesis in WAT

FAs are synthesized *de novo*, mainly from glucose, but fructose or lactate [11] can also serve as substrates for DNL (Figure 3B). *De novo* FA synthesis is a relatively minor pathway in adipocytes [70]. Nevertheless, *de novo* FA synthesis is important during high carbohydrate intake and could be involved in the production of FA-derived signaling molecules that improve insulin sensitivity [12,71]. A key control point in FA synthesis is the conversion of acetyl-CoA to malonyl-CoA, catalyzed by ACC1/2 [72] (Figure 3B). In addition to phosphorylation-induced inactivation of ACC1/2 at Ser79/Ser212 (numbering refers to the mouse proteins) by AMPK, ACC activity is allosterically stimulated by citrate [73]. The final enzyme, FA synthase (FAS), catalyzes the synthesis of palmitate from malonyl-CoA (Figure 3B). Insulin, by increasing intracellular glucose, would stimulate FA synthesis in the long-term by promoting the activation of carbohydrate response element-binding protein (ChREBP), a transcription factor activated by glucose that induces the expression glycolytic, FA and TAG synthesis genes [74]. In the short-term, insulin was also shown to increase the incorporation of radioactive acetate into total lipid – a technique that measures FA synthesis where acetate bypasses pyruvate dehydrogenase (PDH) and ATP-citrate lyase (ACL) [36,75] (Figure 3B).

As mentioned earlier, although insulin has long been known to acutely stimulate FA synthesis (both from glucose and acetate) [75], the exact mechanisms have not been fully elucidated. Many reports have shown that insulin induces ACC Ser79 dephosphorylation [31,36,76,77], explaining the old observation of ACC activation by insulin [78]. However, it is also possible that insulin activates ACC by increasing citrate, secondary to the stimulation of glucose uptake and glycolysis. This is supported by the fact that, in fat pads from mice expressing non-phosphorylatable Ser79Ala/Ser212Ala ACC1/2, the stimulatory effect of insulin on acetate incorporation into total lipid was only partly ablated [77]. However, since glucose was present, this could also have been attributed to the effect of insulin to stimulate Gro3P production and esterification. It would be of interest to investigate in more detail the effect of insulin on the incorporation of acetate specifically into FAs in adipocytes isolated from Ser79Ala/Ser212Ala ACC1/2 knock-in mice. Another interesting area that deserves attention is the precise mechanism(s) whereby

GTP-bound, active form. In the absence of insulin, the Rab-GAP AS160 keeps Rabs in an inactive state. Upon insulin stimulation, AS160 is phosphorylated by AKT/PKB, inducing 14-3-3-binding and preventing AS160 from inhibiting Rabs. AMPK activation has been proposed to inhibit insulin-induced glucose uptake, possibly associated with reduced AS160 phosphorylation. (B) TAG synthesis requires Gro3P for the esterification of FAs entering from the circulation via the FAT/CD36 transporter or synthesized *de novo*. Gro3P can be provided by glycolysis from carbohydrate substrates such as glucose and fructose or can be made from lactate by glyceroneogenesis (not shown). Glucose is transported by GLUT4, fructose mainly enters the adipocyte via GLUT5, while lactate and acetate enter via the MCT1. Glucose, fructose and lactate can be converted to pyruvate. The product of the PDH reaction is acetyl-CoA, which then condenses with oxaloacetate to yield citrate. Citrate is subsequently cleaved to acetyl-CoA for *de novo* FA synthesis, and TAG synthesis from such newly synthesized FAs is referred to as DNL. Acetate can directly form acetyl-CoA via ACS, bypassing PDH and ACL, but DNL from acetate requires provision of Gro3P. Cytosolic acetyl-CoA synthetase is highly expressed in subcutaneous and abdominal human adipose tissues (https://genevisible.com/tissues/HS/Gene Symbol/ACSS2). The main control enzyme inactivated by AMPK is ACC1, explaining how AMPK activation inhibits DNL; however, other AMPK target proteins in the pathway cannot be excluded. Abbreviations: ACC, acetyl-CoA carboxylase; ACS, acetyl-CoA synthetase; AKT/PKB, protein kinase B; AMPK, AMP-activated protein kinase; AS160, AKT substrate of 160 kDa; DNL, *de novo* lipogenesis; FA, fatty acid; Gro3P, glycerol 3-phosphate; MCT1, monocarboxylate transporter-1; PDH, pyruvate dehydrogenase; TAG, triacylglycerol.

insulin reduces ACC phosphorylation (see below). As mentioned previously, the inhibition of AMPK by insulin has been reported to occur both in rodent and human adipocytes [31,36,37].

Early studies revealed that incubation of hepatocytes with AICAR led to AMPK activation together with an inhibition of FA synthesis and decreased ACC activity [79]. Subsequently, AMPK was also implicated in the control of adipocyte FA synthesis, reflected by decreased ACC activity in response to AICAR, exercise or expression of constitutively active AMPK [20,50,64,80]. The likely role of AMPK in the control of adipocyte FA synthesis was reinforced by the fact that ACC1 Ser79 is the main inactivating phosphorylation site and AMPK is the only known protein kinase that specifically phosphorylates this site [81]. AICAR was shown to profoundly inhibit lipogenesis, measured by the incorporation of glucose into total lipid as well as specifically into FAs [50]. However, this effect of AICAR could have been due to inhibition of glucose uptake or AMPK-independent off-target effects. More recently, A-769662 treatment was shown to inhibit insulin-induced incorporation of radioactive acetate into total lipid in rat adipocytes [36]. While this method bypasses the production of acetyl-CoA via glycolysis, it still largely relies on glucose uptake and glycolysis to furnish Gro3P for TAG synthesis. Thus, the effect of A-769662 could potentially be due to inhibition of glucose uptake. In incubated mouse fat pads, AMPK activation by combining SC4 and AICAR inhibited insulin-stimulated lipogenesis from radioactive acetate and this effect was lost both in AMPKα1 KO mice and in ACC1/2 Ser79Ala/Ser212Ala knock-in mice, indicating AMPKα1 dependency and pinpointing ACC as the main AMPK target [57].

Another control point in *de novo* FA synthesis is PDH, activated by insulin-induced dephosphorylation of PDH E1α Ser293 and Ser300. However, in WAT, neither basal PDH Ser293 and Ser300 phosphorylation nor insulin-induced dephosphorylation at these sites were affected by AMPK activation [57]. ACL is a major insulin-sensitive phosphoprotein in WAT phosphorylated at Ser455 by AKT/PKB, but its phosphorylation is somewhat an enigma as changes in activity have been difficult to detect. In WAT, insulin-induced ACL Ser455 phosphorylation was slightly reduced by AMPK activation [57].

Adrenaline treatment was shown to lead to ACC inactivation in WAT [82]. As mentioned above, PKA and AMPK become activated by catecholamines in WAT, but surprisingly, adrenaline had no effect on basal FA synthesis in incubated fat pads [14]. Catecholamines also cause inactivation of key enzymes of the esterification pathway, such as *sn*-glycerol-3-phosphate-*O*-acyltransferase (GPAT) [83], but precise mechanisms remain to be elucidated. In mouse epididymal fat pads, incubation with a combination of SC4 and AICAR did not affect palmitate esterification or GPAT activity [57], possibly ruling out effects of AMPK activation on FA transport and esterification, even though AMPK has been implicated in phosphorylation-induced inactivation of liver mitochondrial GPAT [84] and microsomal GPAT3 Ser68 was found to be targeted by AMPK in a study on skeletal muscle [85].

## Concluding remarks and perspectives

The outstanding questions (see Outstanding questions) address how the field might develop in the future, what challenges remain and how they might be overcome, as well as what the prospects are for translational applications. The general picture from this review is that the new generation ADaM site direct AMPK activators do not seem to have dramatic effects on basal WAT metabolism. This might in fact be desirable if activators that target specific complexes in specific tissues cannot be conceived. In that case, pan-AMPK activators, that target all AMPK complexes across all tissues, would need to be used, for example to achieve AMPK activation in skeletal muscle. The fact that AMPK activation seems to be without effect on basal glucose uptake in WAT is perhaps not surprising. In skeletal muscle, glucose is either stored as glycogen or used

### Outstanding questions

Are proteins regulated by AMPK in skeletal muscle also controlled by AMPK in WAT, such as proteins involved in the GLUT4/FA translocation machinery?

Does AMPK mediate effects of adrenaline and other β-agonists on adipocyte metabolism, including glucose uptake and FA esterification?

Taking advantage of AMPKα1 KO mice, could MS analyses of effects of small-molecule AMPK activators alongside β-adrenergic agonists on the phosphoproteome of WAT provide new mechanisms for controlling WAT metabolism?

Does inhibition of AMPK by insulin mediate effects of insulin on DNL?

Could further studies pinpoint precise mechanisms underlying the effect of AMPK activation on lipogenesis, including the role of ACC phosphorylation, and how insulin inhibits AMPK?

Does AMPK contribute to the control of insulin-sensitizing FA esters of hydroxy-FAs?

Could high-resolution MS provide absolute quantification of AMPK α-, β- and γ-subunit expression to determine if there are differences across WAT depots in both humans and rodents?

What could *in vivo* measurements tell us about effects of AMPK activation/deletion on WAT metabolism?

From a translational perspective, would direct or indirect (for example metformin treatment) AMPK activation have beneficial or undesirable effects on WAT metabolism *in vivo*?

for ATP production; whereas, in WAT, glucose is the preferred substrate for DNL, and since AMPK activation inhibits ATP consumption, one would not expect WAT glucose transport to be increased by AMPK activation. However, AMPK might somehow control fatty acid uptake via FAT/CD36 translocation in WAT (Figure 3B), by analogy with the situation in cardiac myocytes [86] and skeletal muscle [87]. High basal AMPK activity seen in WAT could potentially explain the modest effects of AMPK activators, but might be an artefact of isolated adipocyte and fat pad incubations. Clearly, more *in vivo* studies are needed to assess the effects of pharmacological/genetic AMPK activation and genetic AMPK deletion in WAT *in situ*. Measurements of metabolic fluxes using radioactive tracers and stable isotope-labeled substates [88], along with MS-based metabolomics would be ideal approaches. Another important outstanding question is the relative expression of the AMPK subunits in different WAT depots, which could in turn shed light on differences in metabolism and contrasting findings reported regarding the effect of AMPK activators in the different WAT depots.

Catecholamine action in WAT might involve AMPK as well as PKA, for example to inactivate the enzymes of the esterification pathway. A phosphoproteomics study of the insulin signaling network in 3T3-L1 adipocytes has been reported [89], but surprisingly there have been no in-depth MS-based phosphoproteomic analyses of AMPK activation in WAT.

Lastly, we believe that an important AMPK-dependent effect of AMPK activators in adipocytes is the inhibition of insulin-stimulated lipogenesis. Since FA synthesis and insulin-sensitizing FA-derived lipids are reduced in obesity and insulin resistance, increased knowledge of how these processes are regulated, including the role of AMPK, is critical. Importantly, the effect of direct AMPK activation on total lipogenesis and/or FA synthesis in human adipocytes has not been investigated.

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## Declaration of interests

No interests are declared.

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