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Review Article

Role of AMP-activated protein kinase in adipose tissue metabolism and inflammation

Silvia BIJLAND[1], Sarah J. MANCINI[1] and Ian P. SALT

Institute of Cardiovascular & Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, U.K.

Abstract

AMPK (AMP-activated protein kinase) is a key regulator of cellular and whole-body energy balance. AMPK phosphorylates and regulates many proteins concerned with nutrient metabolism, largely acting to suppress anabolic ATP-consuming pathways while stimulating catabolic ATP-generating pathways. This has led to considerable interest in AMPK as a therapeutic target for the metabolic dysfunction observed in obesity and insulin resistance. The role of AMPK in skeletal muscle and the liver has been extensively studied, such that AMPK has been demonstrated to inhibit synthesis of fatty acids, cholesterol and isoprenoids, hepatic gluconeogenesis and translation while increasing fatty acid oxidation, muscle glucose transport, mitochondrial biogenesis and caloric intake. The role of AMPK in the other principal metabolic and insulin-sensitive tissue, adipose, remains poorly characterized in comparison, yet increasing evidence supports an important role for AMPK in adipose tissue function. Obesity is characterized by hypertrophy of adipocytes and the development of a chronic sub-clinical pro-inflammatory environment in adipose tissue, leading to increased infiltration of immune cells. This combination of dysfunctional hypertrophic adipocytes and a pro-inflammatory environment contributes to insulin resistance and the development of Type 2 diabetes. Exciting recent studies indicate that AMPK may not only influence metabolism in adipocytes, but also act to suppress this pro-inflammatory environment, such that targeting AMPK in adipose tissue may be desirable to normalize adipose dysfunction and inflammation. In the present review, we discuss the role of AMPK in adipose tissue, focussing on the regulation of carbohydrate and lipid metabolism, adipogenesis and pro-inflammatory pathways in physiological and pathophysiological conditions.

Key words: adipose, AMP-activated protein kinase (AMPK), diabetes, inflammation, obesity

INTRODUCTION

AMPK (AMP-activated protein kinase) is the downstream component of a protein kinase cascade that has a central role in the regulation of energy balance at both the cellular and whole-body level. As a consequence, AMPK activation is proposed to be a therapeutic target for the treatment of obesity and Type 2 diabetes [1–3]. In addition to the metabolic actions of AMPK, increasing evidence suggests that AMPK has anti-inflammatory actions, independent of its effects on carbohydrate and lipid metabolism [4]. Although the role of AMPK in liver and muscle is relatively well-characterized and has been reviewed extensively elsewhere [1–3], the role of AMPK in the other principal metabolic tissue, adipose, remains less well defined. The relative lack of data concerning the role of AMPK in adipose tissue may reflect the heterogeneity of adipose tissue and the marked difficulty in

Abbreviations: ACC, acetyl-CoA carboxylase; AICAR, 5′-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; aP2, adipocyte-specific fatty acid-binding protein; AS160, Akt substrate of 160 kDa; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; C/EBP, CCAAT/enhancer-binding protein; CaMKKβ, Ca^{2+}/calmodulin-dependent protein kinase kinase β; CBS, cystathionine-β-synthase; CGI-58, comparative gene identification-58; CPT1, carnitine palmitoyltransferase 1; DAG, diacylglycerol; FA, fatty acid; FAS, FA synthase; GLUT4, glucose transporter 4; HSL, hormone-sensitive lipase; IL, interleukin; IRS1, insulin-stimulated insulin receptor substrate 1; LKB1, liver kinase B1; LPL, lipoprotein lipase; MAG, monoacylglycerol; MCE, mitotic clonal expansion; MCP-1, monocyte chemoattractant protein-1; mTOR, mammalian target of rapamycin; NLRP3, nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3; p90RSK, p90 ribosomal S6 kinase; PDE3B, phosphodiesterase 3B; PI3K, phosphoinositide 3-kinase; PKA, cAMP-dependent protein kinase; PKB, protein kinase B; PPAR, peroxisome-proliferator-activated receptor; PGC, PPARγ co-activator; Rab-GAP, Rab GTPase-activating protein; SCAT, subcutaneous adipose tissue; SCD1, stearoyl-CoA desaturase 1; SGK1, serum- and glucocorticoid-induced protein kinase 1; siRNA, small interfering RNA; SREBP-1c, sterol-regulatory-element-binding protein 1c; TAG, triacylglycerol; TBC1D1, Tre-2/Bub2/Cdc16 domain family, member 1; TLR, Toll-like receptor; TNF-α, tumour necrosis factor-α; UCP1, uncoupling protein 1; VAT, visceral adipose tissue; WAT, white adipose tissue.

[1]**These authors contributed equally to this review.**

Correspondence: Dr Ian P. Salt (email ian.salt@glasgow.ac.uk).

using standard molecular biology techniques to specifically up- and down-regulate AMPK in terminally differentiated adipocytes when compared with skeletal muscle and the liver. Dysfunctional metabolism and a chronic sub-clinical pro-inflammatory environment are observed in adipose tissue of obese individuals, which contribute to the development of systemic insulin resistance and Type 2 diabetes [5]. Given the metabolic and anti-inflammatory actions of AMPK activation, understanding the role(s) of AMPK in adipose tissue is, therefore, of significant importance. The focus of the present review is to examine the evidence concerning the actions of AMPK in adipose tissue and how these actions may have an impact on the whole body in the physiological and pathophysiological setting.

ADIPOSE TISSUE PHYSIOLOGY

Adipose tissue composition and function

At the whole body level, adipose tissue is divided into SCAT (subcutaneous adipose tissue) and VAT (visceral adipose tissue) depots. Adipose tissue contains several cell types, including adipocytes, immune cells, fibroblasts and pre-adipocytes, which can differentiate into mature adipocytes. Furthermore, adipose tissue contains cells from the vascular and nervous systems [6]. The heterogeneity of adipose tissue is complicated further by the classification of adipose tissue as WAT (white adipose tissue) or BAT (brown adipose tissue). Adipocytes within WAT serve to store excess dietary calories as TAG [triacylglycerol (triglyceride)], whereas adipocytes within BAT allow energy dissipation by thermogenesis. In addition to its key role in lipid metabolism, WAT also has an endocrine action, secreting a large number of proteins, collectively termed adipocytokines, which influence systemic and local metabolism and inflammatory pathways. WAT represents the major proportion of adipose tissue in humans, although there is increasing evidence that BAT is present in significant quantities and co-exists with WAT within the same depot [6,7].

White adipocytes contain a single lipid droplet of TAG that is formed from the esterification of FAs (fatty acids) and glycerol 3-phosphate. FAs are obtained from circulating lipoproteins by LPL (lipoprotein lipase)-mediated lipolysis and are transported into the adipocyte or synthesized *de novo* by lipogenesis from non-lipid substrates such as glucose [8]. In the fasted state, or during prolonged physical activity, TAG in the lipid droplet is hydrolysed to FAs and glycerol, for use by other tissues for ATP generation or hepatic gluconeogenesis respectively. Insulin is the principal stimulus of TAG formation in white adipocytes, stimulating GLUT4 (glucose transporter 4)-mediated glucose transport and the activity of ACC (acetyl-CoA carboxylase), the rate-limiting step of FA synthesis from acetyl CoA [9]. In contrast, adrenergic stimulation of adipose tissue stimulates the formation of the second messenger cAMP, with subsequent activation of PKA (cAMP-dependent protein kinase) [8]. PKA phosphorylates and activates HSL (hormone-sensitive lipase), stimulating lipolysis. Insulin, via PKB (protein kinase B; also known as Akt)-mediated phosphorylation and activation of PDE3B (phosphodiesterase 3B), stimulates the breakdown of cAMP, thereby suppressing lipolysis [8].

Brown adipocytes contain multilocular lipid droplets and high numbers of mitochondria. The mitochondria in brown adipocytes characteristically express UCP1 (uncoupling protein 1), which uncouples ATP synthesis from substrate oxidation, allowing thermogenesis, yet the exact role of BAT in human energy balance remains uncertain [6,7].

Adipose tissue and obesity

The rising incidence of Type 2 diabetes is closely associated with the increased prevalence of obesity, particularly expansion of VAT rather than SCAT depots [10]. Chronic overfeeding leads to hypertrophy of adipocytes and the development of a chronic sub-clinical pro-inflammatory environment [5]. As a consequence, infiltration of adipose tissue with macrophages and other leucocytes occurs, which further exacerbates the pro-inflammatory environment in parallel with increasing insulin resistance of adipocytes [5]. The capacity of the dysfunctional adipocytes to store TAGs becomes impaired, leading to ectopic storage of lipid in tissues, including the liver and skeletal muscle [5]. Increased circulating adipose-derived pro-inflammatory cytokines, FAs and metabolites of this ectopic lipid subsequently impair insulin sensitivity in target tissues [5]. This may include cardiovascular tissues as a thin layer of adventitia-associated PVAT (perivascular adipose tissue) found in small arteries and arterioles also becomes dysfunctional in obesity and Type 2 diabetes [11]. Targeting WAT to reduce inflammation has therefore received interest as a potential therapeutic target to limit the development of insulin resistance and Type 2 diabetes. Furthermore, it has been hypothesized that targeting BAT to increase energy expenditure might be useful clinically for anti-obesity therapies [7].

AMPK

Structure and regulation

Since its initial characterization as a protein kinase activity activated by AMP [12], significant advances have been made in understanding the enzymology of AMPK and these have been reviewed recently elsewhere [2]. Briefly, AMPK is a heterotrimeric complex of catalytic α and regulatory β and γ subunits. Several subunit isoforms of each subunit ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$ and $\gamma 3$) have been identified in mammals, such that 12 theoretically possible complexes may be formed. The isoforms exhibit differential tissue expression and may influence subcellular localization of the resultant AMPK complex [13,14]. AMPK is subject to regulation by allosteric mechanisms and post-translational modifications, including phosphorylation, myristoylation and acetylation [1,2]. In addition, AMPKβ subunits contain a carbohydrate-binding domain that can interact with oligosaccharide components of glycogen, such that glycogen binding may inhibit AMPK activity [16].

AMPKγ subunits contain four CBS (cystathionine-β-synthase) domains in two tandem repeats, with each tandem repeat referred to as a Bateman domain [1]. Adenine nucleotides

bind to the Bateman domains, with three of the four potential binding sites occupied by nucleotides in the crystal structure of the regulatory core of AMPK [17]. Of these three sites, one binds AMP tightly, whereas the two remaining sites bind AMP, ADP or ATP in a competitive manner, thereby regulating AMPK activity. Binding of AMP or ADP promotes phosphorylation of Thr^{172} on the catalytic α subunit, required for full activation of AMPK (Figure 1), whereas binding of ATP competitively antagonizes allosteric activation and stimulation of Thr^{172} phosphorylation by AMP or ADP [1,2]. The different γ subunit isoforms exhibit different adenine nucleotide sensitivity, which may contribute to tissue-specific AMPK regulation [1,2]. As a consequence, increases in the AMP/ATP or ADP/ATP ratio allosterically activate AMPK and further promote activity due to increased Thr^{172} phosphorylation. However, the binding affinities for AMP, ADP or ATP at any given site are similar, whereas the cellular concentrations of ATP and ADP are considerably higher than AMP, such that it has been argued that the ADP/ATP ratio may be the more relevant physiological stimulus for AMPK (reviewed in [1,2]). Furthermore, it has been reasoned that the degree of activation due to allosteric activation by AMP or ADP is modest compared with the effect of phosphorylation at Thr^{172}, such that the effect of allosteric activation may be small *in vivo* [1]. Therefore the regulation of AMPK by adenine nucleotides warrants further research, particularly to determine the relationship between AMPK activity and the cellular/subcellular concentrations of free ATP, ADP and AMP.

Two AMPK kinases have been identified that mediate phosphorylation of the AMPK α catalytic subunit at Thr^{172}, namely LKB1 (liver kinase B1) and CaMKKβ (Ca^{2+}/calmodulin-dependent protein kinase kinase β) (Figure 1). Changes in adenine nucleotide concentrations do not directly regulate LKB1 or CaMKKβ, and it has been demonstrated that AMP and ADP binding to the AMPKγ subunit inhibits dephosphorylation of Thr^{172} in the presence of constitutive LKB1 activity (Figure 1) [18]. Increased intracellular Ca^{2+} concentrations are therefore able to activate AMPK independent of changes in adenine nucleotide ratios in cells expressing CaMKKβ (Figure 1) [19,20].

In addition to phosphorylation, AMPKβ subunits are N-terminally myristoylated, and AMPKα subunits are acetylated on lysine residues by p300 acetyltransferase [1,21]. H_2O_2 has also been reported to activate AMPK via oxidative modification of α subunit cysteine residues, yet the physiological relevance of these modifications has yet to be fully characterized [1,22].

Activation and physiological role

AMPK is activated under conditions in which cellular energy demands are increased or when fuel availability is decreased, due to reduced intracellular ATP and/or increased AMP levels [1,2]. As a consequence, physiological stimuli that activate AMPK include exercise in muscle, ischaemia, glucose deprivation, heat shock and hypoxia [1,2,23]. Activated AMPK subsequently phosphorylates key proteins concerned with the regulation of carbohydrate and lipid metabolism, resulting in inhibition of ATP-consuming anabolic pathways, including FA synthesis, cholesterol and isoprenoid synthesis, hepatic gluconeogenesis and mTOR (mammalian target of rapamycin)-mediated protein translation. Concurrently, AMPK activation stimulates ATP production by increasing FA oxidation, muscle glucose transport, mitochondrial biogenesis and caloric intake [1,2,23]. Thus AMPK maintains cellular energy stores and regulates whole-body energy balance, leading to the hypothesis that stimulation of AMPK in the metabolic tissues is a useful target for therapies to treat obesity, insulin resistance and Type 2 diabetes [1,2,23]. In support of this, the widely used hypoglycaemic biguanide drug metformin and the thiazolidinedione class of anti-diabetic drugs stimulate AMPK, although it remains uncertain to what extent AMPK activation underlies their clinical action [24–27]. Recently, salicylate has been demonstrated to stimulate AMPK [28]. Furthermore, salicylate-stimulated FA oxidation was absent in AMPK$\beta 1^{-/-}$ mice, indicating that the effects of salicylate on lipid metabolism *in vivo* are mediated by AMPK [28].

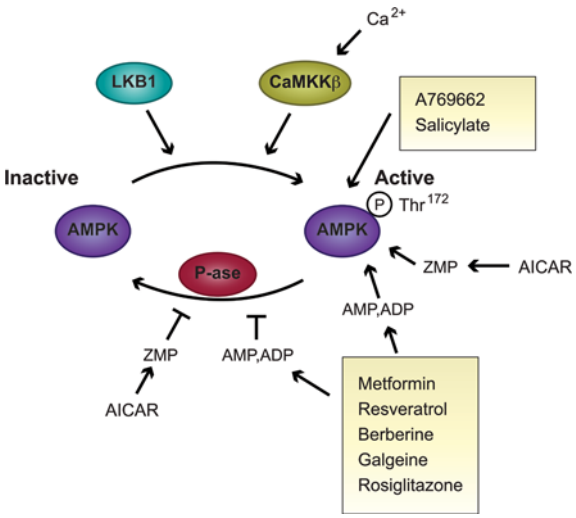

Figure 1 Regulation of AMPK

AMPK is activated by phosphorylation of the α catalytic subunit at Thr^{172} by LKB1 or CaMKKβ. Increased AMP or ADP concentrations (relative to ATP) bind to the regulatory γ subunit, allosterically activating AMPK and inhibiting dephosphorylation of Thr^{172} by an as yet uncharacterized protein phosphatase (P-ase). Neither LKB1 nor CaMKKβ are regulated directly by adenine nucleotides. Increased intracellular Ca^{2+} stimulates CaMKKβ-mediated AMPK activation independent of changes in adenine nucleotide ratios. The antidiabetic drugs metformin and rosiglitazone (thiazolidinedione) increase AMP/ATP or ADP/ATP concentrations, thereby activating AMPK. The plant-derived compounds resveratrol, galgeine and berberine also activate AMPK by this mechanism. AICAR is phosphorylated to the nucleotide ZMP, which mimics AMP, thereby activating AMPK without altering adenine nucleotide ratios. A769662 and salicylate are reported to activate AMPK complexes containing the $\beta 1$ regulatory subunit directly.

Several agents have been used experimentally to activate AMPK. AICAR (5′-aminoimidazole-4-carboxamide ribonucleoside) is a nucleoside that has been used widely to activate AMPK in intact cells, tissues and animals. After transport of AICAR into cells, it is phosphorylated to the nucleotide ZMP, which mimics AMP, thereby activating AMPK without altering adenine nucleotide ratios (Figure 1), although a number of AMPK-independent effects of AICAR have been identified [27,29]. In addition to metformin and thiazolidinediones such as rosiglitazone mentioned above, several plant-derived compounds

including galgeine, berberine and resveratrol activate AMPK in intact cells by altering adenine nucleotide ratios (Figure 1) [30]. Furthermore, the lipid-lowering statin drugs, and several adipocytokines and hormones including leptin, adiponectin and ghrelin, have been demonstrated to activate AMPK in certain tissues, yet the mechanism(s) by which they activate AMPK remain poorly characterized [31–34]. The thienopyridone A769662 is a recently characterized specific activator of AMPK, which stimulates complexes containing the β1, but not β2, regulatory subunit in an AMP-independent manner (Figure 1), demonstrating that selective manipulation of AMPK complexes containing specific subunit isoforms is possible [35,36]. Intriguingly, the mode of action of salicylate is related to that of A769662, which similarly only stimulates AMPK complexes containing the β1 non-catalytic subunit and does not alter adenine nucleotide concentrations (Figure 1) [28].

Expression and activation in adipose tissue

AMPKα1 has been reported to be the principal catalytic subunit isoform expressed in human SCAT and mouse BAT, as assessed by AMPK activity in immunoprecipitates using isoform-specific anti-AMPKα subunit antibodies [37,38]. Using similar techniques, AMPK α1 has also been reported to be the dominant isoform in isolated epididymal rat adipocytes and cultured 3T3-L1 adipocytes [39,40]. As these assays assess total cellular/tissue AMPK activity, important roles for AMPK complexes containing the α2 subunit in specific subcellular compartments cannot be excluded, particularly as down-regulation of AMPKα2 in cells where it is only a minor isoform can have marked effects [41]. As A769662 and salicylate have been reported to stimulate only AMPK complexes containing the β1 subunit isoform, the observations that A769662 activated AMPK in 3T3-L1 adipocytes and salicylate activated AMPK in mouse WAT indicate that adipose tissue and adipocytes express the β1 subunit isoform [28,42], observations that are supported by the modest reduction in AMPK Thr^{172} and ACC phosphorylation reported in the adipose tissue of AMPK$\beta1^{-/-}$ mice [43]. As the reduction in AMPK phosphorylation was not substantial, this may suggest that WAT expresses significant levels of AMPKβ2, yet no effect on WAT AMPK activity was observed in AMPK$\beta2^{-/-}$ mice [44], indicating that AMPKβ1 may be the principal β isoform in WAT. There is little published information concerning the AMPK subunit isoform expression in the other cell types within WAT or BAT, yet AMPKα1 is reported to be the principal catalytic isoform expressed in human endothelial cells [41] and mouse macrophages [45], which also respond to A769662 or express AMPKβ1 [46,47].

Several physiological stimuli have been reported to activate adipose AMPK, including fasting in mouse and rat WAT [40,48], cold exposure in mouse WAT [38], and exercise in human SCAT and rat epididymal adipose tissue [49,50]. In contrast, androgens have been reported to reduce AMPK activity in mouse WAT [51]. Cold exposure has also been reported to stimulate AMPK in BAT from rats and mice [38,52]. Endogenous stimuli such as β-adrenergic stimulators, HDLs (high-density lipoproteins), homocysteine and eicosapentaenoic acid have also been reported to stimulate AMPK activity in isolated rodent adipocytes or cultured 3T3-L1 adipocytes [40,53–56], whereas ghrelin and testosterone have been reported to inhibit AMPK activity [57,58]. The adipocytokine adiponectin, secreted by adipose tissue, has also been reported to activate adipocyte AMPK [59], illustrating the potential autocrine regulation of adipocyte AMPK activity. Similarly, WAT of mice with an adipose-specific overexpression of the leptin receptor exhibited increased AMPK Thr^{172} phosphorylation, suggesting leptin may activate adipocyte AMPK in an autocrine manner [60]. Finally, metformin has been demonstrated to increase AMPK activity in WAT from mice and volunteers with Type 2 diabetes in addition to cultured 3T3-L1 adipocytes [61,62]. It is therefore apparent that a number of stimuli may regulate adipose AMPK under physiological and pathophysiological conditions.

EFFECT OF AMPK ON ADIPOCYTE CARBOHYDRATE METABOLISM

Glucose transport

In striated muscle and adipocytes, insulin stimulates glucose uptake, mediated by translocation of vesicles containing GLUT4 to the plasma membrane (Figure 2). The increased influx of glucose provides a substrate for glycogen synthesis in skeletal muscle and TAG synthesis in adipocytes. In skeletal muscle, AMPK activation has been demonstrated to further increase both basal and insulin-stimulated glucose uptake by increasing GLUT4 translocation [63–69]. The effect of AMPK activation on glucose uptake in adipocytes, however, is less clear. In 3T3-L1 adipocytes, AICAR increased basal [39,70] and inhibited insulin-stimulated glucose uptake without affecting early steps in the insulin signalling pathway [39,61]. On the other hand, in isolated rat adipocytes, AICAR inhibited both basal and insulin-stimulated glucose uptake [71,72]. In contrast, AICAR-stimulated glucose uptake was not affected by infection of 3T3-L1 adipocytes with adenoviruses expressing a dominant-negative mutant AMPK, despite complete suppression of AMPK activation [70]. Therefore the involvement of AMPK in the effect of AICAR on basal glucose uptake is uncertain. Despite this, most published evidence supports inhibition of insulin-stimulated glucose uptake by AMPK in adipocytes without affecting early steps in insulin signalling, including IRS1 (insulin-stimulated insulin receptor substrate 1) tyrosine phosphorylation [39,73], PI3K (phosphoinositide 3-kinase) recruitment to IRS1 [39] or PKB activity/phosphorylation [39,61,72,73].

In contrast, treatment of rat adipocytes with globular adiponectin rapidly activated AMPK and increased both basal and insulin-stimulated glucose uptake in a manner sensitive to two different inhibitors of AMPK, araA and compound C, suggesting involvement of AMPK in the stimulatory effect of adiponectin on glucose uptake [73]. Compound C has been used widely as an inhibitor of AMPK, yet it inhibits several other protein kinases with similar or greater potency *in vitro* [74–76]. Similarly AraA, which is metabolized into ara-ATP, a competitive inhibitor for AMPK [77], has also been reported to inhibit adenylate cyclase, reduce cAMP production and stimulate the mitogenic ERK (extracellular-signal-regulated kinase) protein kinases [78].

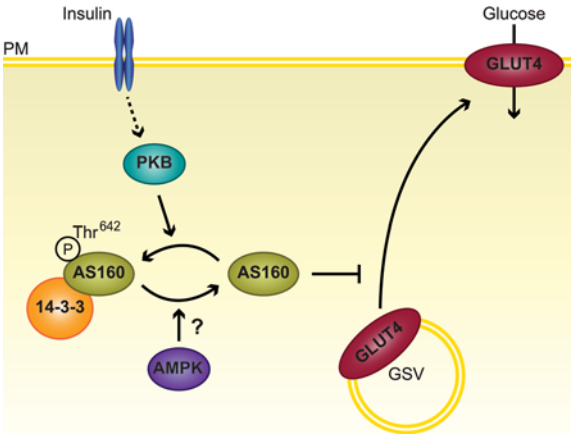

Figure 2 Regulation of insulin-stimulated GLUT4 translocation in adipocytes by AMPK

Insulin binding to the insulin receptor results in activation of PKB which in turn phosphorylates AS160 at Thr^{642}. This enhances 14-3-3 binding to AS160 and inhibits AS160 Rab-GAP activity, permitting GLUT4 to translocate from GLUT4 storage vesicles (GSV) to the plasma membrane (PM). The substantial increase in GLUT4 at the plasma membrane increases glucose transport. Activated AMPK has been reported to reduce insulin-stimulated Thr^{642} phosphorylation and 14-3-3 binding to AS160, although the mechanism by which this occurs remains uncertain.

Caution should therefore be exercised in interpreting studies that have used compound C and araA to implicate a role for AMPK.

In adipocytes, impaired glucose uptake is reflected by a reduction of glycerol 3-phosphate production [79], such that inhibition of glucose transport by AMPK is supported by the observation that AICAR impaired the incorporation of glucose into lipids in isolated rat adipocytes [71,80].

AS160 (Akt substrate of 160 kDa)

Insulin-stimulated trafficking of GLUT4 is regulated by two members of the Rab-GAP (Rab GTPase-activating protein) family, TBC1D1 [TBC (Tre-2/Bub2/Cdc16) domain family, member 1)] and AS160 (also known as TBC1D4) (Figure 2) [81]. In muscle, PKB phosphorylates AS160, promoting its interaction with 14-3-3 proteins, resulting in inactivation of the Rab-GAP activity and stimulation of GLUT4 trafficking. Similarly, AMPK is reported to phosphorylate TBC1D1, thereby stimulating GLUT4 trafficking in muscle. In adipocytes TBC1D1 does not appear to play a role in GLUT4 trafficking since its expression is very low in 3T3-L1 adipocytes and undetectable in WAT from mice [82]. As AS160 plays an essential role in GLUT4 trafficking in adipocytes [83], it could be reasoned that the differential effects of AMPK activation on insulin-stimulated glucose uptake in adipocytes compared with muscle reflect the expression of TBC1D1 and AS160 in muscle, but AS160 alone in adipocytes (Figure 2). Indeed, in primary rat adipocytes, inhibition of insulin-stimulated glucose uptake by AICAR was accompanied by a reduction in phosphorylation of AS160 at the PKB site Thr^{651} (equivalent to Thr^{642} in humans), the interaction of AS160 with 14-3-3 and less translocation of GLUT4 to the plasma membrane (Figure 2) [72]. Moreover, that study shows that infection of rat adipocytes with adenoviruses expressing a kinase-dead AMPKα1 mutant completely prevented the effect of AICAR on inhibition of insulin-stimulated AS160 Thr^{651} phosphorylation and glucose uptake, despite only partial inhibition of AICAR-induced AMPK activation [72].

AS160 contains multiple phosphorylation sites and PKB phosphorylates Ser^{318}, Ser^{588} and Thr^{642} and, to a lesser extent, Ser^{570} and Ser^{751} of human AS160 *in vitro*. AMPK on the other hand phosphorylates Ser^{588} and Ser^{570} to a similar degree as PKB, yet Thr^{642} is a poor substrate of AMPK compared with PKB, which is also reflected in less 14-3-3 binding to AS160 compared with PKB [84]. In 3T3-L1 adipocytes, insulin stimulated phosphorylation of AS160 at Ser^{318}, Ser^{341}, Ser^{570}, Ser^{588}, Thr^{642}, Ser^{666} and Ser^{751}. However, treatment with the AMPK activators AICAR, A769662 and phenformin did not phosphorylate AS160 at any of these sites and did not promote 14-3-3 binding [85,86]. This suggests that AMPK does not phosphorylate AS160 at the insulin-regulated sites in adipocytes, yet no studies to date have examined the effect of AMPK activation on site-specific AS160 phosphorylation other than Thr^{642} in the presence of insulin.

The mechanism underlying this potential inhibition of AS160 phosphorylation by AMPK is therefore uncertain. AS160 Thr^{642} (Thr^{651}) is phosphorylated by p90RSK (p90 ribosomal S6 kinase) and SGK1 (serum- and glucocorticoid-induced protein kinase 1) in addition to PKB *in vitro* [84]. Although PKB is widely thought to be the kinase responsible for phosphorylation at Thr^{642} in response to insulin, PKB-stimulated phosphorylation of GSK3 (glycogen synthase kinase 3) was not affected under conditions in which AMPK activation inhibited insulin-stimulated Thr^{642} phosphorylation [72]. It might be possible that AMPK prevents the specific interaction of PKB with AS160, thereby lowering AS160 phosphorylation. Alternatively, AMPK may activate a phosphatase that dephosphorylates AS160 specifically at Thr^{651}. A third alternative is that AMPK directly phosphorylates AS160 and makes it a poorer substrate for PKB phosphorylation, although this seems unlikely given the lack of effect on many phosphorylation sites as described above. Finally, AMPK might reduce SGK1- or p90RSK-stimulated Thr^{642} phosphorylation, as both kinases have been reported to be sensitive to insulin [86,87].

Long-term AMPK activation and glucose transport

Less is known about the long-term effects of AMPK activation in adipose tissue. 3T3-L1 adipocytes treated with metformin for 24–48 h inhibited the fold-stimulation of glucose transport by insulin due to higher basal uptake [61]. Similarly, human SCAT and VAT pre-adipocyte-derived adipocytes incubated for 24 h with metformin had an increase in glucose uptake [88]. Furthermore, human SCAT pre-adipocyte-derived adipocytes exhibited enhanced insulin-stimulated glucose uptake after metformin treatment [88]. It has been reported that AMPK activation stimulates GLUT4 expression in muscle [89–92] and metformin treatment for 5 days in rainbow trout increased WAT GLUT4 mRNA levels [93], as did prolonged incubation of human SCAT with AICAR *ex vivo* [94]. In contrast, GLUT4 protein levels were unaffected by prolonged AICAR or metformin treatment of 3T3-L1 adipocytes or in adipose biopsies from volunteers with Type 2 diabetes treated with metformin for 10 weeks compared with sulfonylurea treatment [61]. In conclusion, the majority of evidence

supports inhibition of insulin-stimulated glucose metabolism by AMPK activation in adipocytes, but more studies are necessary to fully understand the molecular mechanisms underlying the effect of AMPK.

ROLE OF AMPK IN LIPOLYSIS

Regulation of lipolysis

Lipid droplets in adipocytes are tightly regulated specialized organelles [95]. In lipolysis, TAG is hydrolysed to glycerol and FAs by the successive action of ATGL (adipose triglyceride lipase; also known as desnutrin), which converts TAG into DAG (diacylglycerol), HSL, which converts DAG into MAG (monoacylglycerol) and MAG lipase, which converts MAG into FAs and glycerol (Figure 3). In the fasted state, noradrenergic stimulation activates PKA, which phosphorylates and recruits the lipid droplet scaffolding protein perilipin and HSL to the lipid droplet to stimulate lipolysis [95]. As mentioned above, insulin, via PKB-mediated phosphorylation and activation of PDE3B, stimulates the breakdown of cAMP, thereby suppressing lipolysis.

HSL is phosphorylated by PKA at Ser^{563}, which increases its intrinsic activity, and Ser^{659} and Ser^{660}, which are involved in the translocation of HSL from the cytosol to the lipid droplet (Figure 3) [96]. In the absence of cAMP, perilipin is associated with the protein CGI-58 (comparative gene identification-58). Upon perilipin phosphorylation by PKA, CGI-58 is released and interacts with ATGL, stimulating lipase activity [97]. *In vitro*, PKA has recently been reported to phosphorylate ATGL at various sites including Ser^{404} (Figure 3) [98], in contrast with an earlier study [99]. Both fasting and exercise increased ATGL phosphorylation at Ser^{406} (equivalent to human Ser^{404}) in mouse epididymal adipose tissue [98]. Furthermore, expression of an ATGL Ser^{406}Ala mutant in both HEK (human embryonic kidney)-293 [100] and COS1 [98] cells has been reported to reduce lipolysis, whereas in 293FT cells TAG breakdown was not affected [101]. Reduced forskolin-stimulated lipolysis has also been reported in adipocytes derived from embryonic fibroblasts of $ATGL^{-/-}$ mice infected with retroviruses expressing Ser^{406}Ala mutant ATGL compared with those infected with wild-type ATGL, suggesting that Ser^{404} phosphorylation is important for increased ATGL activity in adipocytes [98].

AMPK and lipolysis

Several studies report an anti-lipolytic effect of AMPK [40,102,103], whereas others suggest AMPK stimulates lipolysis [104,105]. Furthermore, exercise has been reported to activate AMPK in rat adipose tissue in a β-adrenergic-receptor-mediated fashion and β-adrenergic-stimulated lipolysis was sensitive to compound C, suggesting that AMPK is necessary for adrenaline-induced lipolysis [105]. It is important, however, to note that the FAs liberated during lipolysis can directly be re-esterified, thereby consuming ATP. As re-esterification of FAs is positively correlated with the rate of lipolysis [106–108], an increase in lipolysis may indirectly activate AMPK as a result of an increase in the AMP/ATP ratio [109]. This increase in AMPK activity could subsequently inhibit lipolysis, which is supported by the observation that mice lacking AMPKα1 have smaller adipocytes with higher basal and β-adrenergic-stimulated lipolysis rates [40]. Importantly, activation of AMPK by AICAR has been reported to initially inhibit lipolysis both in isolated rat adipocytes and rats *in vivo* as reflected by a decrease in serum FAs, yet prolonged AICAR treatment increased lipolysis, which the authors attributed to an increase in ATGL content as well as activity, since HSL activity was lowered [80]. On the other hand, A769662 and salicylate have been reported to reduce fasting plasma FA concentrations in an AMPKβ1-dependent manner, which may be a result of reduced adipose lipolysis, supporting a role for AMPK in inhibiting lipolysis *in vivo*. [28].

In adipocytes isolated from human SCAT, both biguanides and thiazolidinediones inhibited lipolysis in a compound C-sensitive manner, accompanied by activation of AMPK [110]. Furthermore, metformin lowered lipolysis in human SCAT from obese subjects *in vivo* [111]. However, in isolated rat adipocytes, metformin has been reported to decrease cellular cAMP levels as well as the activity of PKA, suggesting the effect of metformin on lipolysis may be mediated by inhibition of PKA rather than AMPK activation [112].

AMPK regulation of ATGL and HSL?

Conflicting data have been published concerning the role of AMPK in the regulation of HSL. AMPK has been reported to phosphorylate HSL on Ser^{565}, preventing PKA-stimulated activation of HSL (Figure 3) [102,103,113]. Furthermore, *ex vivo* AMPK activation in human SCAT-derived adipocytes was demonstrated to reduce HSL translocation to the lipid droplet in association with a compound C-sensitive inhibition of lipolysis [110]. In contrast, mutation of Ser^{565} to alanine has been reported to prevent the translocation of HSL to the lipid droplet in 3T3-L1 adipocytes, suggesting Ser^{565} phosphorylation does not inhibit lipolysis and expression of a dominant-negative AMPK mutant had no effect on HSL Ser^{565} phosphorylation under basal or β-adrenergic-stimulated conditions, calling into question AMPK regulation of this site [114,115].

Intriguingly, ATGL Ser^{404} is an AMPK consensus site and this site can be phosphorylated by AMPK *in vitro* [98,100]. In support of this, HEK-293 cells and 3T3-L1ΔCAR adipocytes stimulated with AICAR have been reported to exhibit increased ATGL phosphorylation at Ser^{406}, which was suppressed by compound C [100]. Furthermore, expression of Ser406Ala mutant ATGL completely prevented AICAR-induced lipolysis, whereas injection of mice with AICAR increased serum FA levels, which was not seen in adipose-specific ATGL-knockout mice, supporting an ATGL-mediated pro-lipolytic action of AMPK [100]. More recently it was reported that *ex vivo* AICAR stimulation did not stimulate ATGL Ser^{404} phosphorylation or lipolysis in SCAT from obese subjects [98].

Taken together, these findings suggest that AMPK may phosphorylate ATGL and HSL to regulate lipolysis (Figure 3), but conclusions are difficult to draw given the lack of studies using tools to manipulate AMPK specifically. Furthermore, the overall effect of AMPK activation on lipolysis is still controversial, and the duration and mode of AMPK activation may be of particular importance.

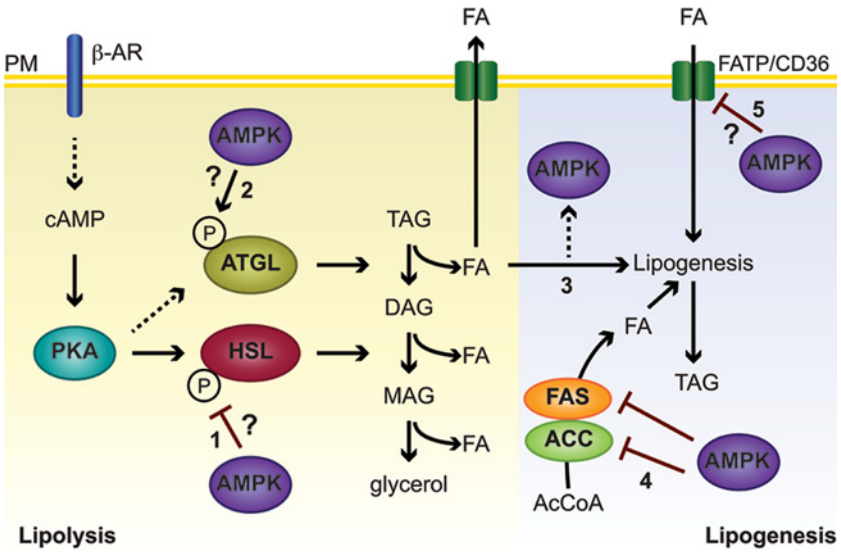

Figure 3 **Regulation of adipocyte lipid metabolism by AMPK**

Lipolysis is stimulated by β-adrenergic receptor (β-AR)-mediated stimulation of cAMP production, resulting in PKA activation. PKA phosphorylates HSL and ATGL, increasing lipolysis, although whether ATGL phosphorylation by PKA *in vivo* remains uncertain. AMPK activation has been reported to influence lipolysis by: (1) phosphorylation of HSL at Ser^{565}, thereby preventing PKA-mediated HSL phosphorylation at Ser^{563} and subsequent lipolysis; (2) phosphorylation and activation of ATGL, thereby stimulating lipolysis. ATP-consumption during re-esterification of FAs after lipolysis may also activate AMPK (3), such that regulation of lipolysis by AMPK is likely to be dependent on the duration and mode of AMPK activation. AMPK phosphorylates and inactivates ACC in adipocytes, reducing FA synthesis, and may inhibit the expression of ACC and FAS genes (4). It has also been suggested that AMPK activity inhibits FA oxidation due to reduced FA transport (5) mediated by FA transporters, including CD36/FATP; however, this remains to be examined in adipocytes.

AMPK REGULATION OF LIPOGENESIS

Early studies in isolated rat adipocytes reported that AICAR inhibited lipogenesis and stimulated phosphorylation of ACC, the key regulated step in FA synthesis and FA oxidation (Figure 3) [102]. ACC catalyses the synthesis of malonyl-CoA from acetyl-CoA and is inhibited by AMPK-mediated phosphorylation at Ser^{79} on ACC1 or the orthologous site Ser^{212} on ACC2. ACC1 is mainly expressed in lipogenic tissues such as WAT, and isolated rat adipocytes infected with adenoviruses expressing constitutively active mutant AMPK showed increased phosphorylation of ACC1 Ser^{79}, whereas infection with dominant-negative mutant AMPK inhibited AICAR-stimulated phosphorylation of Ser^{79}, indicating that ACC is certainly regulated by AMPK in adipocytes [40]. This is supported further by the observation that both fasting and exercise stimulate AMPK in rat adipose tissue with a concomitant reduction in malonyl-CoA, which may reflect inhibition of ACC and increased malonyl-CoA decarboxylase activity [40,50,116,117].

Prolonged activation of AMPK by leptin or AICAR in rat adipose tissue [80,118] and metformin in human adipose tissue [61] increased ACC phosphorylation and lowered the expression of lipogenic genes including ACC. In addition, adipocytes from AMPK$\beta 1^{-/-}$ mice had increased lipogenesis in adipose explants [43], suggesting a specific role for complexes containing this subunit isoform in attenuating lipogenesis. Lipogenic gene expression is under the control of the SREBP-1c (sterol-regulatory-element-binding protein-1c) transcription factor. In liver, AMPK has been reported to phosphorylate and inactivate SREBP-1c, thereby decreasing the expression of lipogenic genes including ACC1, FAS (FA synthase) and SCD1 (stearoyl-CoA desaturase 1) [119]. There is, however, a dearth of studies examining AMPK regulation of SREBP-1c in adipose tissue. In 3T3-L1 preadipocytes, AICAR inhibited the expression of SREBP-1c, yet AICAR treatment for 6 weeks was without effect on SREBP-1c expression in epididymal WAT [120]. Taken together, these results indicate that AMPK activation in adipose tissue is linked to decreased lipid storage by lowering TAG synthesis (Figure 3), but the role of SREBP-1c-regulated lipogenic gene expression in the action of AMPK in adipose tissue is yet to be fully addressed. It is clear that more specific strategies to down- or up-regulate AMPK in adipocytes are required to assess the role of AMPK in both lipogenesis and lipolysis.

AMPK REGULATION OF FA OXIDATION

Malonyl-CoA is not only a precursor for FA synthesis, but also regulates FA oxidation by inhibiting CPT1 (carnitine palmitoyltransferase 1), the rate-limiting enzyme of FA entry into the mitochondrion for subsequent oxidation. As AMPK-mediated phosphorylation and inactivation of ACC reduces malonyl-CoA concentrations, inhibition of CPT1 is relieved and AMPK stimulates FA oxidation in many tissues [1–3]. Despite this, acute stimulation of isolated rat adipocytes with AICAR has been reported to inhibit FA oxidation, which is suggested to be due to reduced FA uptake [71]. AMPK activation has recently been

reported to stimulate the translocation of the FA transporter CD36 to the plasma membrane in an AS160-mediated manner in cardiomyocytes [121]. As CD36 and GLUT4 both undergo similar trafficking routes [122], it is tempting to speculate that AMPK activation inhibits CD36 translocation to the plasma membrane in an analogous fashion to GLUT4 in adipocytes, thereby providing a mechanism for reduced FA uptake through AMPK activation (Figure 3). To our knowledge there have been no studies on the effect of AMPK on FA transporters including CD36 in adipocytes.

In contrast with the rapid inhibition of FA oxidation by AICAR [71], sustained activation of AMPK stimulated FA oxidation [80,123] and increased mitochondrial content in adipocytes [118,124]. In support of this, sustained AICAR stimulation increased WAT expression of PPAR (peroxisome-proliferator-activated receptor)α, PPARδ and PGC (PPARγ co-activator)-1α, all of which influence the expression of enzymes involved in FA oxidation and mitochondrial biogenesis [80,100]. These structural changes in adipocytes after prolonged AICAR administration were accompanied by a reduction in body fat mass. However, it is important to note that whole-body energy expenditure was increased after sustained treatment with AICAR, as AMPK activation also increases the oxidative capacity of liver and muscle [125,126]. As with lipolysis, the role of AMPK in the regulation of WAT FA oxidation may therefore be dependent on the duration and mode of AMPK stimulation, whereby AMPK activation rapidly inhibits FA oxidation, potentially due to effects on FA transport, yet prolonged AMPK activation stimulates FA oxidation.

AMPK AND ADIPOGENESIS

Differentiation of pre-adipocytes involves a highly regulated transcriptional cascade induced by hormones, including insulin and glucocorticoids, and is characterized by expression of FAS and aP2 (adipocyte-specific FA-binding protein) in addition to increased expression of the PPAR and C/EBP (CCAAT/enhancer-binding protein) transcription factors [127]. The process begins with re-entry of growth-arrested pre-adipocytes into the cell cycle, where they undergo several rounds of mitosis. This initial phase is known as MCE (mitotic clonal expansion) and is accompanied by the transient expression of C/EBPβ and C/EBPδ. These transcription factors subsequently stimulate transcription of PPARγ, which in turn can activate C/EBPα. PPARγ and C/EBPα exist in a positive-feedback loop to propagate differentiation and induction of late adipogenic genes, including aP2 and FAS, in the terminal differentiation phase [128].

Proliferation and adipogenesis are anabolic processes that multiple lines of evidence suggest are inhibited by AMPK. Activation of AMPK has been proposed to suppress proliferation in a variety of cell types in different ways. Most recently, studies in cell lines using AICAR or metformin have proposed AMPK inhibits proliferation via inhibition of mTOR [1,129]. Stimulation with AICAR, A769662 and infection with viruses expressing a constitutively active mutant AMPK have been reported to inhibit endothelial cell proliferation via elevation of p21 and p27 expression [130], and AICAR has been demonstrated to block growth of the HepG2 cell line via phosphorylation of p53, all leading to cell-cycle arrest [131]. The exact mechanism by which AMPK inhibits proliferation remains to be fully elucidated, particularly in pre-adipocytes of WAT, and may differ depending on the type of cell.

AMPK activation has been suggested to inhibit adipogenesis via the MCE phase, with reduced expression of C/EBPβ (which is essential for initiation of the adipogenic transcriptional cascade), and subsequent inhibition of PPARγ, C/EBPα and late adipogenic markers such as FAS, aP2 and SREBP-1c [132]. It has been demonstrated in 3T3-L1 pre-adipocytes that A769662 inhibited the accumulation of lipid droplets and reduced the expression of C/EBPα and PPARγ, as well as the earlier adipogenic transcription factors C/EBPβ and C/EBPδ [42]. Interestingly, A769662 was shown to maintain C/EBPδ expression, where it would normally decline [42]. AICAR treatment blocked adipogenesis at the MCE phase and reduced expression of C/EBPα, PPARγ SREBP-1c and aP2 [120,132]. Recently, novel synthetic compounds which are structurally similar to resveratrol have been identified which have been proposed to activate AMPK indirectly [133]. As with other established AMPK activators, they have been found to inhibit differentiation during the MCE phase and reduce expression of several adipogenic markers, including PPARγ, C/EBPα, FAS and aP2 [133].

Although these studies all indicate that AMPK acts at the early proliferation phase to inhibit adipogenesis, the exact mechanism by which AMPK produces these effects is still unclear. It has recently been suggested that AICAR may inhibit adipocyte differentiation via modulation of the WNT/β-catenin pathway [134]. In that study, AICAR stimulation during adipogenesis of 3T3-L1 adipocytes led to an increase in β-catenin expression and nuclear accumulation concomitant with the significant reduction in expression of adipogenic genes; this was ablated with siRNA (small interfering RNA)-mediated knockdown of β-catenin, providing a possible mechanism by which AMPK inhibits adipogenesis [134]. Furthermore, AMPK activation has been proposed to maintain the expression of Pref-1 (pre-adipocyte factor-1), which inhibits adipogenesis [135].

An increase in adipose tissue mass can be due to an increase in adipocyte size (hypertrophy), an increase in adipocyte number (hyperplasia) or both. Studies using mice in which catalytic subunit isoforms of AMPK have been knocked out have been used to examine the role of AMPK in changes in adipose tissue mass. AMPKα2-deficient mice fed on a high-fat diet exhibited increased adipose tissue mass compared with wild-type littermates, resulting from adipocyte hypertrophy, with no change in cell number or adipogenic marker expression [136]. Similar observations were made in a recent study which demonstrated AMPKα1-deficient mice fed on a high-fat diet also had increased adiposity compared with their wild-type littermates, and that this was the result of adipocyte hypertrophy [137]. Caution has to be taken when interpreting these findings as this may reflect ablation of AMPK-mediated increased FA oxidation and a concomitant decrease in lipogenesis. In addition, the animal models utilized in these studies have a global knockout of either AMPKα

isoform, such that the observed effects may not be a direct effect of reduced AMPK activity in adipose or adipocytes. There is a need for adipose-specific knockouts of AMPK isoforms to help address this.

REGULATION OF ADIPOSE ADIPOCYTOKINES AND INFLAMMATION BY AMPK

As mentioned above, WAT has an endocrine function in addition to its key role in metabolism, secreting a large number of adipocytokines, which influence systemic and local metabolism in addition to inflammatory pathways. Dysfunctional metabolism and a chronic sub-clinical pro-inflammatory environment are observed in the WAT of obese individuals, and this pro-inflammatory environment contributes to the development of systemic insulin resistance and Type 2 diabetes [5].

Adipocytokines

Proteins secreted by WAT that influence metabolism and inflammation include leptin, adiponectin, MCP-1 (monocyte chemoattractant protein-1), TNF-α (tumour necrosis factor-α), IL (interleukin)-1β and IL-6. Plasma leptin levels are positively correlated with an increase in total body fat mass as a result of more leptin being released from large hypertrophic adipocytes compared with smaller adipocytes [138]. Leptin acts at neurons within the hypothalamus to regulate satiety, but also exerts anti-diabetic actions independent of its effect on body mass [139]. Adiponectin is a protein secreted exclusively from adipose tissue [140], with plasma levels correlating negatively with obesity [141]. Adiponectin acts to improve peripheral insulin sensitivity and has systemic anti-inflammatory effects [142]. MCP-1, TNF-α, IL-1β and IL-6 are pro-inflammatory chemokines or cytokines secreted by WAT and are increased in concentration upon obesity [143].

Pro-inflammatory adipocytokines and obesity

Obesity is associated with the low-grade inflammation of WAT and increased necrosis/apoptosis of adipocytes [144]. Pro-inflammatory cytokines signal via distinct pathways, with TNF-α and IL-1β signalling via the pro-inflammatory transcription factor NF-κB (nuclear factor κB) and MAPKs (mitogen-activated protein kinases), whereas IL-6 signals via a JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway [4]. These pathways culminate in the secretion of chemokines, including MCP-1, recruiting macrophages to the site of inflammation [145,146]. Studies in genetically obese (*ob*/*ob*) and high-fat-diet-induced obese mice have suggested the presence of other chemokine receptors and ligands, including MIP-1 (macrophage inflammatory protein-1), RANTES (regulated and normal T-cell expressed and secreted) and MCP-2, are also up-regulated in obesity [147], demonstrating the complexity of the pro-inflammatory profile of obese adipose tissue. Migration of macrophages into adipose tissue is also stimulated by necrotic adipocytes [144], nutrient excess (increased FA) (Figure 4) [148] or a combination of these. Interplay between the different cell types within adipose tissue, particularly between adipocytes and macrophages, is central to the development of inflammation within the tissue. Several genetic studies have demonstrated the importance of macrophages in the development of obesity-related inflammation and insulin resistance [149–152]. Macrophages have been reported to aggregate and form crown-like structures surrounding necrotic adipocytes (Figure 4) [143,153,154]. Macrophages can also be recruited and rendered pro-inflammatory by the presence of saturated FAs (as is often the case in obesity) which activate the TLR (Toll-like receptor) family [155]. Indeed, mice with haematopoietic deletion of TLRs are largely protected from obesity-induced inflammation and insulin resistance [156]. Following migration into adipose tissue in response to one or more of the factors detailed above, macrophages then undergo a shift in their polarity, switching from an anti-inflammatory 'alternatively activated' M2 state to a 'classically activated' pro-inflammatory M1 state (Figure 4) [5]. Conversely, adiponectin has been reported to switch adipose macrophages into the anti-inflammatory M2 state [142]. Macrophages can also induce inflammatory responses via activation of the inflammasome, the best-characterized of which is NLRP3 (nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3) inflammasome. Activation of the NLRP3 inflammasome leads to maturation and secretion of pro-inflammatory cytokines IL-1β and IL-18, and as a result has been linked to obesity-associated inflammation and the development of insulin resistance and Type 2 diabetes [157].

AMPK inhibits inflammation in adipose tissue

There have been a number of studies suggesting AMPK is anti-inflammatory in different tissues and cell types including those that constitute adipose such as adipocytes, endothelial cells and macrophages [4]. Various studies have reported that activation of AMPK can inhibit the synthesis of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 in macrophages [45,47,158,159] and IL-6 and IL-8 in adipocytes [94]. Conversely, AMPK activation has been reported to up-regulate the expression of the anti-inflammatory cytokine IL-10 in macrophages [47,159]. In human SCAT cultured *ex vivo*, AICAR was reported to reduce TNF-α and IL-6 secretion (Figure 4) [37]. Berberine, which activates AMPK and inhibits pro-inflammatory signalling in isolated macrophages, has also been reported to inhibit cytokine expression in adipose tissue of obese *db*/*db* mice, yet the AMPK-dependence of this has not been demonstrated [158]. A very recent study has shown that activation of the NLRP3 inflammasome is markedly increased in myeloid cells from patients with Type 2 diabetes, and found that treatment with metformin appeared to reduce maturation and secretion of IL-1β and IL-18 in monocyte-derived macrophages. This was suggested to occur in an AMPK-dependent manner; however, the mechanism remains to be elucidated [160].

Recent studies have utilized AMPK-deficient mice to confirm the anti-inflammatory role of AMPK. It has been reported that high-fat-diet-fed AMPK$\alpha 1^{-/-}$ mice had an increased level of infiltration of pro-inflammatory macrophages and exhibited an elevation in IL-6, IL-1β and TNF-α at the mRNA level in adipose tissue, as well as significantly higher levels of circulating cytokines [137]. Furthermore, increased production of IL-1β and TNF-α (but not IL-6) were observed in AMPKα1-deficient

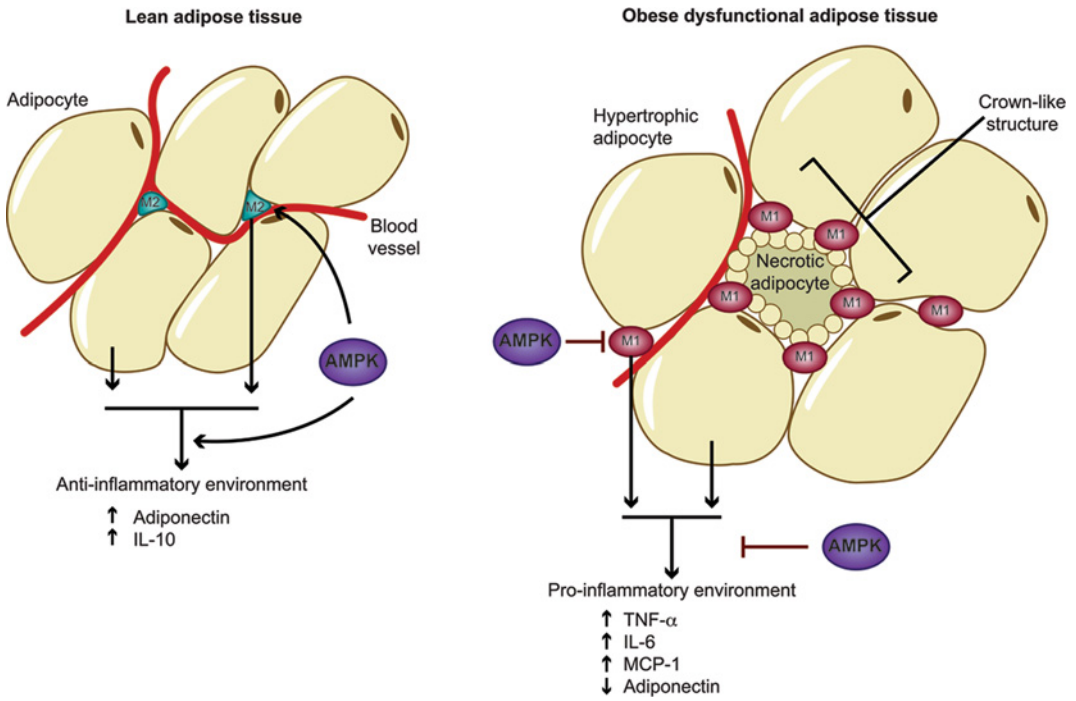

Figure 4 **Anti-inflammatory actions of AMPK in adipose tissue**
In lean adipose tissue, small adipocytes and alternatively activated macrophages secrete adipocytokines such as IL-10 and adiponectin, which maintain an anti-inflammatory environment. Production of these adipocytokines is promoted by activated AMPK. Excess calorie intake leads to the development of a pro-inflammatory environment within obese adipose tissue. Adipocytes enlarge, becoming hypertrophic and often necrotic, and there is an increased infiltration of macrophages. The pro-inflammatory environment drives macrophage polarization towards the classically activated M1 state, and they tend to accumulate around necrotic adipocytes, forming crown-like structures. The secretory products of the macrophages and hypertrophic adipocytes further exacerbate this inflammation. Adipose tissue inflammation is linked to obesity-related insulin resistance and Type 2 diabetes. Activation of AMPK can inhibit production of pro-inflammatory cytokines and chemokines, increase adiponectin secretion and inhibit macrophage recruitment and M1 polarization, thereby potentially reducing insulin resistance.

macrophages stimulated with the TLR4 activator lipopolysaccharide, and these macrophages displayed increased activity of pro-inflammatory signalling pathways compared with wild-type macrophages [137]. Stable knockdown of AMPKα1 in 3T3-L1 adipocytes supported these findings, with higher mRNA levels of the pro-inflammatory cytokines TNF-α and IL-1β as well as MCP-1 in response to FA treatment than control 3T3-L1 cells [137]. Similarly, a study utilizing obese AMPK$\beta 1^{-/-}$ mice reported that AMPK activation is crucial for the regulation of macrophage polarization, as bone-marrow-derived macrophages from mice lacking the β1 subunit had reduced levels of AMPK activation and elevated JNK (c-Jun N-terminal kinase) phosphorylation in response to pro-inflammatory stimuli [47]. Interestingly, however, the ability of AMPK to suppress palmitate-stimulated JNK phosphorylation appears to be blocked by inhibition of FA oxidation [47].

It has been shown that activation of AMPK drives anti-inflammatory polarization of macrophages to M2 (Figure 4), whereas inhibition of AMPKα expression by RNA interference or transfection with a dominant-negative form of AMPK significantly enhanced mRNA and protein expression of TNF-α and IL-6 [159]. Furthermore, PGC-1β and oxidative metabolism has been reported to be important in priming macrophages for alternative activation and attenuates pro-inflammatory signalling, suggesting resident M2 macrophages are reliant upon FA metabolism and AMPK activity to maintain the anti-inflammatory phenotype [159,161]. Conversely, macrophages from AMPK$\beta 1^{-/-}$ mice were found to have reduced mitochondrial content, consistent with their M1 activation and reduced rates of FA oxidation [47]. There is evidence to suggest that mature adipocytes may modulate macrophage polarization via secretion of lipid mediators which activate AMPK in macrophages and impair their polarization to a pro-inflammatory phenotype [162].

Adipocytokines regulated by AMPK

As described above, AMPK activation has been reported to suppress inflammatory responses in adipose tissue from obese subjects [37,94,158,163]. Adiponectin and leptin levels may also be regulated by AMPK as high-fat-diet-fed AMPK$\beta 1^{-/-}$ mice displayed significantly less adiponectin and increased leptin concentrations relative to chow-fed or wild-type littermates [47]. In addition, AICAR increased adiponectin gene expression in human SCAT cultured *ex vivo* [37]. These findings suggest that AMPK activation is partially able to override obesity-induced dysregulation of adiponectin and leptin concentrations. However, AICAR and metformin have also been reported to suppress adiponectin expression in 3T3-L1 adipocytes [164].

AMPK regulation by adipocytokines

Adipocytes express adiponectin receptors, indicating that adiponectin can act in an autocrine fashion [165]. Adiponectin stimulates AMPK activity in several tissues by a mechanism that has yet to be fully characterized, but adiponectin also engages many other signalling pathways and has AMPK-independent effects [142]. A few studies have suggested that AMPK itself can be regulated by pro-inflammatory cytokines in different cell types, for example TNF-α has been reported to activate AMPK in various cell lines including endothelial and kidney cell lines [166]. Conversely, it has been reported to suppress AMPK activity in myotubes and muscle [167]. IL-6 has similarly been reported to have a varied influence on AMPK activity in different cell types, with evidence suggesting IL-6 stimulates AMPK in muscle, with suppressed AMPK activity being reported in IL-6-deficient mice [168,169], yet in endothelial cells IL-6 has been proposed to inhibit AMPK activity [170]. In a recent study, IL-6 was found to increase phosphorylation of ACC and AMPK in cultured eWAT (epididymal WAT) and was independent of any increase in lipolysis [171]. It is therefore apparent that certain adipocytokines may regulate AMPK activity; however, this is appears to be cell-type-specific and further investigation is required, particularly to elucidate the effect of adipocytokines on AMPK in adipose tissue.

AMPK AND BROWN ADIPOSE

As mentioned above, brown adipocytes contain high numbers of mitochondria and characteristically express UCP1, which allows thermogenesis, yet the exact role of BAT in human energy balance remains uncertain [6,7]. Cold exposure has been reported to stimulate AMPK in BAT from rats and mice [38,52], and both AICAR and β_3-adrenergic stimulation increase AMPK activity and glucose transport in cultured mouse brown adipocytes in an araA-sensitive manner [172,173]. These findings have been extended in mice, indicating that adrenergic nerves stimulate AMPK in BAT *in vivo* [174]. Furthermore, AMPK activity increases during brown adipocyte differentiation, and siRNA targeted to AMPK inhibited differentiation into mature brown adipocytes, suggesting AMPK promotes differentiation into thermogenic BAT [175]. Indeed, prolonged treatment of mice with AICAR increased the extent of BAT within WAT deposits in mice [175]. These studies suggest that AMPK may play a role in differentiation into FA-oxidizing BAT, leading to greater energy expenditure, yet AMPK$\alpha 1^{-/-}$ mice showed no alteration in cold tolerance or acute non-shivering thermogenesis, although a compensatory increase in AMPKα2 expression may explain this lack of effect [176].

REGULATION OF AMPK IN HUMAN ADIPOSE

In adipocytes isolated from the SCAT of normal-to-moderately overweight women, the thiazolidinedione and biguanide antidiabetic drugs have been demonstrated to activate AMPK and inhibit lipolysis in a compound C-sensitive manner *ex vivo* [110]. Similarly, cultured SCAT explants from similar individuals stimulated with AICAR exhibited increased adiponectin and GLUT4 expression and reduced TNF-α, IL-8 and IL-6 secretion [37,94]. Few studies have examined human adipose AMPK activity *in vivo* however. Of these, exercise has been reported to activate AMPK in SCAT from healthy individuals [49], yet this may depend on the degree of exercise, as a further study has indicated that SCAT AMPK activity is unaffected [177]. AMPK expression and activity has been reported to be lower in VAT compared with SCAT of morbidly obese individuals [178,179]. Furthermore, when this morbidly obese group was divided into insulin-resistant and insulin-sensitive subgroups, AMPK activity was reduced in the adipose tissue of the insulin-resistant obese volunteers compared with BMI (body mass index)-matched controls, suggesting an association in WAT between AMPK activity and insulin sensitivity [179,180]. Intriguingly, AMPK activity is also reported to be decreased in VAT from individuals with Cushing's syndrome, which is also associated with insulin resistance [181]. Finally, metformin was recently shown to increase SCAT AMPK activity of individuals with Type 2 diabetes in a randomized glycaemia-controlled cross-over study [61]. Although these clinical studies have been on small numbers of people, these findings do suggest that improving AMPK activity in adipose tissue in people with insulin resistance may benefit adipose function and that exercise and metformin may be able to achieve this. However, it has yet to be proven whether the activation of AMPK observed with exercise, metformin or in more insulin-sensitive individuals represents increased AMPK activity in adipocytes or the other constituent cells of adipose.

CONCLUSIONS

The studies of AMPK function in adipose published to date are largely based on isolated rodent adipocytes or cells that have been differentiated into adipocytes *in vitro*. As these cells are intractable to molecular biology techniques that would allow specific up- or down-regulation of AMPK, most studies have relied on less specific methods of transiently activating or inhibiting AMPK. Similarly, data obtained in animals where one isoform of AMPK has been genetically deleted have revealed interesting potential actions of AMPK in adipose tissue, but these may represent secondary indirect effects mediated by down-regulation of AMPK in other tissues, particularly the liver and muscles. There is, therefore, an urgent need for animals in which AMPK is specifically ablated in adipocytes to confirm the actions of AMPK in adipose. The heterogeneity of adipose tissue in terms of constituent cell types and different depots increase the challenge of determining the function of AMPK in adipose tissue. The exciting new observations concerning the role of BAT in humans is another area in which the role of AMPK should be explored. The few investigations of AMPK activity in human adipose have demonstrated that AMPK activation in adipose tissue is feasible in man and that this may have beneficial consequences attenuating adipose dysfunction and inflammation. As activators, such as A769662 and salicylate, that target specific subunit isoforms of AMPK are

already used in the laboratory, it is feasible to design activators of AMPK that are targeted to certain tissues, given the differential tissue distribution of AMPK subunit isoforms. It is clear, therefore, that AMPK regulates carbohydrate and lipid metabolism in adipose tissue and suppresses pro-inflammatory signalling that contributes to adipose tissue dysfunction and insulin resistance. More studies that specifically manipulate AMPK activity in the constituent cells of adipose tissue are required to further characterize its role.

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