AMP kinase and malonyl-CoA: Targets for therapy of the metabolic syndrome

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AMP KINASE AND MALONYL-COA: TARGETS FOR THERAPY OF THE METABOLIC SYNDROME

Neil Ruderman and Marc Prentki‡*

Patients with the metabolic syndrome are characterized by insulin resistance, obesity and a predisposition to hypertension, dyslipidaemia, pancreatic β-cell dysfunction, type 2 diabetes and premature atherosclerosis. Here we review the hypothesis that a common feature linking these multiple abnormalities is dysregulation of the AMP-activated protein kinase (AMPK)/malonyl-CoA fuel-sensing and signalling network. It is proposed that such dysregulation leads to alterations in cellular fatty-acid metabolism that in turn cause ectopic lipid accumulation, cellular dysfunction and ultimately disease. Evidence is also presented that factors that activate AMP kinase and/or reduce malonyl-CoA levels might reverse these abnormalities or prevent them from occurring.

METABOLIC SYNDROME
A state of metabolic dysregulation characterized by insulin resistance, hyperinsulinaemia, central obesity and a predisposition to type 2 diabetes, dyslipidaemia, hypertension, premature atherosclerosis and other diseases.

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Patients with the METABOLIC SYNDROME are typically INSULIN RESISTANT, and demonstrate impaired pancreatic β-cell function and a predisposition to hypertension, dyslipidaemia, type 2 diabetes mellitus (T2DM, or non-insulin-dependent diabetes) and premature atherosclerotic vascular disease[1–3]. In addition, they are usually overtly obese or have more subtle manifestations of increased adiposity, such as an increase in visceral fat[4], and they can also have a decreased capacity for exercise[5]. The precise reason that so many abnormalities occur in people with the metabolic syndrome remains to be determined; however, an increasing body of evidence indicates that a common factor could be a dysregulation of cellular lipid metabolism in several tissues that becomes increasingly manifest in the presence of a high caloric intake and/or inactivity[6–9]. As such, insulin resistance in muscle and liver of patients with T2DM, and in many non-diabetic individuals, correlates closely with an excess of intracellular triglyceride (TG) in these tissues[10–13], as well as with an increase in abdominal fat[2,4]. In addition, premature atherosclerosis, which is a common occurrence in people with T2DM and the metabolic syndrome, is often associated with hypertriglyceridaemia. Finally, abnormally large accumulations of TG in the pancreatic β-cell, liver and muscle have been shown to precede the onset of hyperglycaemia and insulin resistance in the ZUCKER DIABETIC FATTY RAT (ZDF)[14], and dietary restriction[15] or troglitazone therapy[16] (see below), both of which diminish this lipid accumulation, prevent the development of diabetes[7,17].

The increases in cellular TG observed in tissues of humans and experimental animals with insulin resistance and other components of the metabolic syndrome could be related to increases in the uptake of free fatty acids (FFA) from plasma, an enhanced rate of *de novo* fatty acid synthesis, and/or a dysregulation of intracellular lipid partitioning in which fatty acid oxidation is impaired and its esterification enhanced[6,7,9]. As recently reviewed[18,19], when one or more of these events occur, an accumulation of non-β-oxidation products of fatty acid metabolism, such as long-chain fatty acyl-co-enzyme A (FA-CoA) and/or diacylglyerol (DAG), could set in motion a series of events including protein kinase C (PKC) activation, increases in ceramide synthesis *de novo*, oxidative stress, and activation of inhibitor of nuclear factor-κB (IκB) kinase (IKKβ)/nuclear factor-κB (NF-κB) system (FIG. 1), all of which could lead to insulin resistance and, in some tissues, mitochondrial damage and apoptosis. The increase in tissue TG is generally viewed as a marker of insulin resistance and cellular dysfunction, rather than as a causal factor[20], although by providing the cell with an additional source

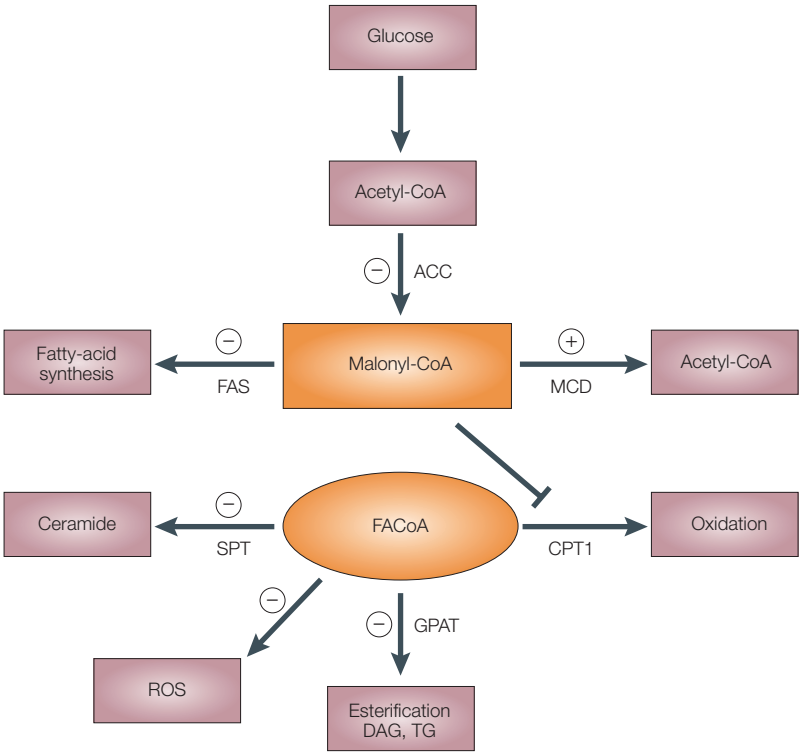

Figure 1 | **Regulation of malonyl-CoA and cytosolic FA-CoA by AMPK.** By inhibiting CPT1, malonyl-CoA, which is derived from glucose, diminishes FA-CoA entrance into mitochondria where they are oxidized, thereby making more cytosolic FA-CoA available for TG, DAG and ceramide synthesis and possibly for lipid peroxidation. AMPK could inhibit these events and increase fatty acid oxidation, acutely by phosphorylating or otherwise inhibiting ACC and GPAT and activating MCD, and chronically by diminishing the expression of SREBP1c and activating PGC1α and PPARα (not shown). The basis for its ability to diminish oxidant stress is not known. Whether AMPK activation results in enhancement or inhibition of a process is denoted in the figure by plus and minus signs, respectively. ROS, reactive oxygen species.

of FFA, it could play a pathogenic role. In this review, we will examine the hypothesis that dysregulation of the AMP-activated protein kinase (AMPK)/malonyl-coenzyme A (malonyl-CoA) fuel-sensing and signalling network is a key factor in causing these events, as well as a target for their therapy (see BOX 1 for background on AMPK and its regulation).

The AMPK/malonyl-CoA signalling network

Malonyl-CoA. Malonyl-CoA is both an intermediate in the *de novo* synthesis of fatty acids and an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT1), the enzyme that controls the transfer of long-chain FA-CoA molecules from the cytosol to the mitochondria where they are oxidized[7,21]. By virtue of the latter effect, malonyl-CoA is a key physiological regulator of cellular fatty acid oxidation and lipid partitioning (FIG. 1). Prentki and Corkey[22–24] were the first to propose that malonyl-CoA, besides its metabolic actions, acts as a coupling factor and signal of plenty that controls insulin secretion (see section on pancreatic β-cells). They also suggested that an excessive accumulation of malonyl-CoA could cause β-cell toxicity, by virtue of its effects on lipid partitioning[9]. Studies in skeletal muscle, which established that the concentration of malonyl-CoA responds acutely to changes in both fuel availability and energy expenditure[25], provided the initial experimental evidence linking dysregulation of malonyl-CoA to insulin resistance[6,26]. Recent studies indicate that malonyl-CoA has important additional signalling actions, particularly in the brain where it can act as a signal of plenty that reduces food intake[27]. The regulation of malonyl-CoA at a cellular level is described in BOX 2 and FIG. 2.

AMPK action. Hardie and Carling[28] first proposed that the AMPK cascade is a fundamental component of the response of cells to stresses that deplete ATP and threaten their viability[29]. They suggested that it acts as a 'fuel gauge', such that when it detects a 'low fuel' situation it protects the cell by regulating processes that generate and utilize ATP. In keeping with this logic, activation of AMPK leads to the phosphorylation of a number of target molecules that result in, among other things, increases in fatty acid oxidation, muscle glucose transport, and cardiac glycolysis (to generate more ATP), and inhibition of synthetic processes and ion channels not acutely necessary for survival (to conserve ATP) (FIG. 3). On the other hand, instances in which AMPK activity is altered independent of changes in cellular energy state have been increasingly reported[30–33] and, as already noted, a recently identified AMPKK is the tumour suppressor STK11 (also known as LKB1)[34,158]. As such, the role of AMPK in the cell might not be as simple as originally conceived.

Acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) were the first enzymes shown to be AMPK targets[28]; however, other molecules are being identified at a rapid rate (FIGS 1,4). For instance, AMPK might phosphorylate and activate malonyl-CoA decarboxylase (MCD), an enzyme involved in malonyl-CoA turnover[35], and it acutely inhibits glycerol-3-phosphate acyltransferase (GPAT), the first committed enzyme in glycerolipid synthesis[36]. In addition, AMPK suppresses the synthesis of ACC, fatty acid synthase (FAS), GPAT and other enzymes of lipid biogenesis in many tissues by inhibiting the generation of the transcriptional factor sterol-regulatory-element-binding transcription factor 1 (SREBP1c)[37]. The net effect of AMPK activation is therefore to increase the oxidation of fatty acids (at least in part by lowering the concentration of malonyl-CoA) and to decrease their esterification and use in other non-β-oxidative pathways (FIGS 1,4). The relevance of these and other actions of AMPK to its ability to diminish insulin resistance and lipotoxicity will be discussed in the next section.

Longer-term effects of AMPK activation at the level of transcription have also been described. Winder *et al.*[38] were the first to report that treatment of intact rats with 5-aminoimidazole-4-carboxamide riboside (AICAR), like exercise, increases the expression of a wide variety of proteins in muscle, including the glucose transporter GLUT4 and several mitochondrial oxidative enzymes. AICAR is the most widely used pharmacological activator of AMPK, although it has some actions that are not mediated by AMPK[39], and for this reason data obtained

INSULIN RESISTANCE
A state in which insulin at a physiological concentration does not exert its usual biological effect. In some instances, the effect of insulin on certain processes (for example, glycogen synthesis) can be impaired, whereas its effect on others (for example, diacylglycerol synthesis) can be normal or even enhanced.

ZUCKER DIABETIC FATTY RAT (ZDF) rat. A rodent with both a mutant, functionally deficient leptin receptor and a genetic defect that predisposes it to diabetes, as it becomes obese and lipid accumulates in the pancreatic β-cell.

Box 1 | **AMPK and its regulation**

AMPK is a heterotrimer containing α-, β- and γ-subunits, each of which has at least two isoforms. The α-subunit contains the catalytic site; the β-subunit, a glycogen-binding domain; and the γ-subunit the AMP-binding site. All three subunits are necessary for full activity[29,157]. In general, AMP kinase (AMPK) is found in the cytosol of a cell; however, the α2 isoform of the enzyme is also present in the nucleus[157]. Decreases in the energy state of a cell, as reflected by increases in the ratio of AMP/ATP, activate AMPK by a number of mechanisms, including covalent modification due to phosphorylation of its catalytic subunit on Thr172 by an AMPK kinase (AMPKK)[157] (FIG. 3). Until recently, it was believed that this occurred as a result of activation of the AMPKK by an increase in the AMP/ATP ratio[28]. However, recent studies from the laboratories of Hardie and Alessi[158], and Carling and Carlson[34], failed to demonstrate such an increase in AMPKK activity in response to various AMPK activators. Rather, their results indicate that AMP binds to AMPK, and that this makes it more susceptible to phosphorylation by AMPKK. Interestingly, the first AMPKK that has been identified is LKB1 (REFS 34,158), a tumour suppressor that is mutated in humans with Peutz–Jegher syndrome, a disorder associated with an increased risk of developing carcinomas of the colon, stomach and pancreas.

AMPK can be phosphorylated and activated in various tissues by hormones that act through G_Q receptors[157] and by adiponectin[96,97], leptin[115], α- and β-adrenoceptor agonists[100,115,159] and such pharmacological agents as metformin[32,33,123] and the thiazolidinediones (TZDs)[32,120] (FIG. 3). AMPK might also be regulated by cellular glycogen content, possibly as a consequence of AMPK binding to glycogen via its β-subunit[157].

with this agent have to be confirmed by other means. Exercise, pharmacological agents and molecular biological approaches have been used for this purpose (see below). AICAR treatment increases the expression of genes encoding uncoupling protein-3 (UCP3)[3], UCP2 (REF. 41) and GLUT4 in muscle[42]. It has a similar effect on peroxisome proliferative-activated receptor PPARα in the pancreatic β-cell (M.P. *et al.*, unpublished data), and it decreases the expression of genes for FAS, ACC and phosphoenolpyruvate carboxykinase in liver[37]. In addition, treatment with AICAR results in the phosphorylation and inhibition of the transcriptional co-activator p300 (REF. 43) and diminishes the expression of SREBP1c[37], forkhead box O1α[44] and hepatocyte nuclear factor 4α[45]. In common with exercise[46], AICAR induces the expression of the master thermogenic regulator PPARγ co-activator 1α (PGC1α) in muscle[47] and probably other tissues (N.R., unpublished data) (FIG. 4). As illustrated in FIG. 4 and discussed in the next section, we believe that it is by virtue of both these long-term and short-term (phosphorylation) effects of AMPK, some mediated by malonyl CoA and others independent of it, that AMPK protects against insulin resistance and cellular dysfunction.

Box 2 | **Regulation of malonyl-CoA**

The concentration of malonyl-CoA in rat skeletal muscle is acutely regulated by changes in energy expenditure (that is, exercise and inactivity) and fuel availability (glucose excess or deprivation) in keeping with the need of the muscle cell to generate ATP from fatty acid oxidation[25]. Similar changes have been shown to occur in muscle of humans and experimental animals *in vivo*[6,151,152] and in the pancreatic β-cell[9], liver[59,153] and vascular endothelium[30]. In most of these cells, the concentration of malonyl-CoA is determined by the relative activities of the enzymes that regulate its synthesis and degradation. These include the two isoforms of acetyl CoA carboxylase (ACC), ACC1 and ACC2, which, respectively, are thought to generate the malonyl-CoA used for *de novo* fatty acid synthesis and CPT1 inhibition, and malonyl-CoA decarboxylase (MCD). In addition, in cells capable of a high rate of *de novo* fatty acid synthesis, the activity of fatty acid synthase (FAS) could contribute to its turnover (FIG. 1, upper half). As exemplified by skeletal muscle and the pancreatic β-cell, ACC activity is regulated acutely by changes in the cytosolic concentration of citrate, which is both an allosteric activator of ACC and a precursor of its substrate cytosolic acetyl-CoA[6,9,25], and by changes in the activity of the fuel-sensing enzyme AMPK[154,155] (FIG. 2). The concentration of citrate in the cytosol is elevated acutely when the cell has a fuel surfeit (excess glucose) or its energy expenditure is diminished (in muscle by inactivity). This causes the concentration of citrate in the mitochondrial matrix to increase and the citrate in excess of the needs of the Krebs cycle moves to the cytosol[6]. In contrast, AMPK, which is activated by increases in cellular energy expenditure, fuel deprivation and various hormones and pharmacological agents, both phosphorylates and acutely inhibits the two isoforms of ACC and diminishes their synthesis at the level of transcription (see below). Recent studies suggest that the activity of MCD is concurrently increased in situations in which AMPK is activated[99] (FIG. 1); however, this has not been a universal finding[156].

Insulin resistance and cellular dysfunction

Muscle. Insulin resistance is defined as an impaired ability of insulin, at a physiological concentration, to exert its usual biological effects. It is present in most T2DM patients and their non-diabetic, first-degree relatives, as well as non-diabetic individuals with central or generalized obesity[1,2]. In 1963, Randle, on the basis of studies of glucose use by a perfused heart preparation, proposed that insulin resistance, in diabetic and obese rats, could be related to increases in plasma FFA levels and enhanced fat oxidation (the so-called glucose–fatty acid cycle)[48]. However, many investigators were unable to repeat these findings and their relevance to skeletal muscle was unclear for many years[49].

In 1991, Boden *et al.*[50], using humans undergoing a EUGLYCAEMIC–HYPERINSULINAEMIC CLAMP as a model, demonstrated inhibition of glucose uptake in leg muscle when plasma FFA levels were increased for 4–6 hours by infusing a lipid emulsion. The mechanism responsible for the insulin resistance was not established. However, in studies carried out concurrently in another model of insulin resistance — DENERVATED rat muscle — we observed that impaired insulin action is associated with increases in DAG content and protein kinase C (PKC) activity[51]. Furthermore, despite a near total inhibition of the ability of insulin to stimulate glucose incorporation into glycogen, its ability to stimulate glucose incorporation into DAG and other glycerolipids was increased several-fold, indicating an intracellular dichotomy in insulin action. Later studies provided an explanation by revealing that a marked increase in the concentration of malonyl-CoA occurred 6–8 hours after denervation[25] and that it preceded the impairment in insulin action[52]. Collectively, these and other findings[6] indicated that impaired insulin action is linked to malonyl-CoA accumulation and a redirection of glucose and fat metabolism to lipid esterification processes (FIG. 1), rather than to enhanced oxidation of fatty acids, as initially proposed by Randle.

Increases in DAG, malonyl-CoA and TG content, and alterations in PKC activity or distribution in skeletal muscle, have subsequently been reported in a wide variety of insulin-resistant rodents[6,18] and, in some instances, humans[53,54] (TABLE 1). Where studied, abnormalities in insulin signalling, including serine phosphorylation of IRS-1 and impaired activation of

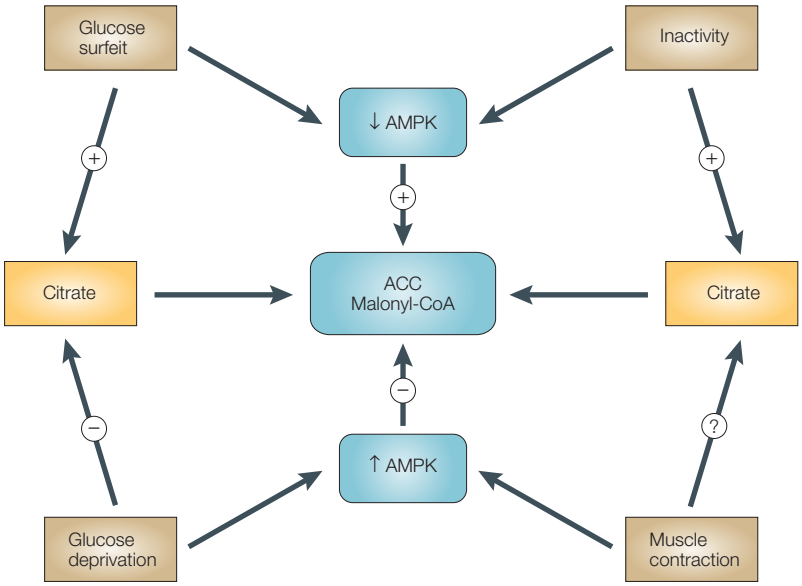

Figure 2 | **Regulation of ACC and, secondarily, the concentration of malonyl-CoA by AMPK and cytosolic citrate.** AMPK activation, such as occurs in many tissues during exercise or glucose deprivation, phosphorylates ACC and inhibits its activity. Conversely, a sustained excess of glucose, and possibly inactivity, decrease AMPK phosphorylation and activity and cause ACC activation. In muscle, the pancreatic β-cell, and probably in other cells, glucose availability also determines the concentration of cytosolic citrate, an allosteric activator of ACC and a precursor of its substrate, cytosolic acetyl-CoA. Such changes in citrate occur rapidly (min) and may be responsible for early changes in malonyl-CoA concentration and for sustained changes in malonyl-CoA under conditions in which assayable AMPK activity is not altered. ACC, acetyl-CoA carboxylase; AMPK, AMP kinase.

EUGLYCAEMIC–HYPERINSULINAEMIC CLAMP
A widely used technique to assess whole-body (and tissue) insulin sensitivity in humans and experimental animals.

DENERVATED MUSCLE
Muscle with a nerve supply that has been severed or made non-functional by other means.

ANAPLEROTIC
Reactions that lead to the net synthesis of Krebs (citric acid) cycle intermediates, for example, the carboxylation of pyruvate to form oxaloacetate.

MALONYL-CoA/FA-CoA HYPOTHESIS
A theory that proposes that a sustained increase in the concentrations of malonyl-CoA, and secondarily long-chain fatty acid-CoA (FA-CoA), lead to insulin resistance and cellular dysfunction in many tissues. The theory also proposes that a transient stimulation of malonyl-CoA/FA-CoA signalling is involved in glucose- and fatty-acid-stimulated insulin release by the pancreatic β-cell.

phosphatidylinositol 3-kinase and AKT, have also been observed[55]. Similar alterations in DAG and PKC have been described in muscle of humans in whom insulin resistance was produced by infusing lipid for 6 hours during a euglycaemic–hyperinsulinaemic clamp to raise plasma FFA levels[54]. A noteworthy finding in this study was a decrease in the abundance of the NF-κB inhibitor IκB-α, suggesting activation of the IKKβ/NF-κB system (FIG. 4). The importance of the IKKβ/NF-κB system to the development of insulin resistance in muscle and other tissues remains to be determined; however, studies by Shoelson and co-workers linking activation of IKKβ to the pathogenesis of insulin resistance in muscle of fat-fed and obese rodents[56] and humans with T2DM[57], as well as studies in cultured endothelial cells incubated with FFA or tumour-necrosis factor-α (TNF-α)[18], indicate that it very probably plays a key role. Interestingly, despite the presence of a moderate increase in intra-myocellular TG, trained athletes are not insulin resistant; indeed, the sensitivity of their muscle to insulin is greater than that of sedentary individuals, possibly related to an enhanced ability to oxidize fatty acids[58]. To our knowledge, malonyl-CoA, DAG, PKC and the IKKβ/NF-κB system have not been compared in trained and untrained individuals.

The role of AMPK deficiency per se in causing insulin resistance, and AMPK activation in treating and preventing it, have received little attention; however, a spate of recent studies suggest that these possibilities need to be investigated. For example, in rats infused with glucose for as little as 5 hours, we have found that the development of insulin resistance in both skeletal muscle and liver is associated temporally with a decrease in AMPK phosphorylation and activity, as well as increases in DAG content and PKC activity[59,60] (TABLE 1). Conversely, various drugs and hormones that activate AMPK in muscle, including metformin, thiazolidinediones (TZDs), adiponectin and leptin, have been shown to diminish insulin resistance in experimental animals and humans (see TABLE 2 and section on the AMPK/malonyl-CoA network as a therapeutic target). In addition, the administration of AICAR 24 hours before a euglycaemic–hyperinsulinaemic clamp[61], as well as exercise[62], have been shown to diminish insulin resistance and lower malonyl-CoA levels in muscle of rats with diet-induced obesity (DIO). In contrast to these findings, studies in genetically manipulated mice have not supported the notion that a lack of AMPK per se in muscle tissue leads to insulin resistance: insulin signalling was found to be normal in the muscle of mice expressing a muscle-specific, dominant-negative AMPK[63], and insulin-stimulated glucose uptake in muscle was not diminished in mice deficient in the α1 or α2 isoform of AMPK[64], although some systemic insulin resistance was observed. In the latter studies, a compensatory increase in the α1-AMPK isoform in muscle of α2-AMPK deficient mice might have contributed to the findings[64]. Studies of lipid metabolites, PKC and other factors linked to insulin resistance have not yet been reported in these mice.

Pancreatic islets. Insulin resistance in muscle and liver does not initially result in hyperglycaemia because it is usually compensated for by hyperinsulinaemia, due to increases in the number and/or volume of pancreatic β-cells and augmentation of their function[65]. What seems clear is that T2DM develops only when the insulin resistance is accompanied by a failure of the β-cell to compensate[19,66,67]. The pathogenesis of β-cell failure is not well understood; however, studies of animal models[68] and human autopsy specimens[69] indicate that both dysfunction of β-cells and a reduction in their mass is involved. This failure of the β-cell to cope seems to be genetically determined, because it does not occur in all humans or experimental animals with insulin resistance.

We and others have adduced much evidence in favour of a model that links β-cell nutrient sensing to insulin secretion via an ANAPLEROTIC/lipid signalling pathway in which malonyl-CoA acts as a coupling factor[9,19,70]. The MALONYL-COA/FA-COA HYPOTHESIS, as it relates to the pancreatic β-cell, has been challenged by data showing unimpaired secretion of insulin in β-cells overexpressing MCD in the cytoplasm[71]. However, this apparent discrepancy has been reconciled by a recent study demonstrating the importance of the supply of exogenous fatty acids for lipid signalling in the β-cell[165]. MCD overexpression curtailed glucose-induced insulin release only in the presence of fatty acids[72]. Many predictions of the 'malonyl CoA/FA-CoA' model have been verified. For instance, glucose stimulation of the β-cell increases anaplerosis, and causes rapid increases in citrate and malonyl-CoA levels that precede insulin secretion[23,73]. Such actions of glucose on malonyl-CoA and lipid

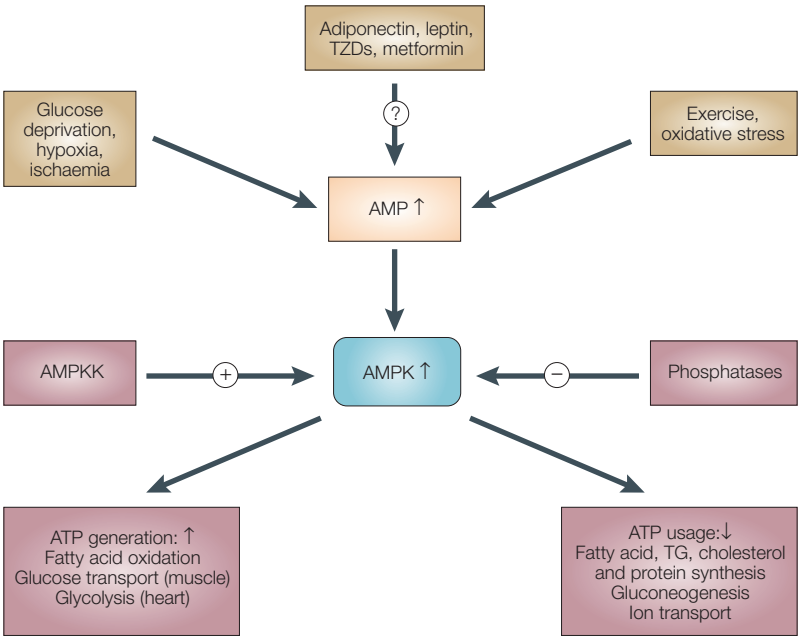

Figure 3 | **AMPK activation and its effects on cellular energy state.** AMPK activation concurrently leads to an increase in cellular processes that generate ATP and a decrease in processes that use ATP, but are not immediately necessary for cell survival. AMPK activation has been attributed to ATP depletion that leads to changes in the AMP/ATP ratio. Recent studies suggest this may be due to the effects of AMP on AMPK that make it more susceptible to phosphorylation by an AMPKK[34,158] (see text for details). Whether leptin and adiponectin, and pharmacological agents such as the thiazolidinediones activate AMPK by altering the energy state of a cell, as does exercise is uncertain. Metformin appears to activate AMPK without altering the AMP/ATP ratio. The precise role of phosphatases in this scheme has received little attention (adapted in part from REF. 29). AMPK, AMPK kinase; TZD, thiazolidinediones.

partitioning might, in part, be mediated by changes in AMPK activity, because elevations in glucose concentration increase the ATP/AMP ratio and reduce AMPK activity in the β-cell, whereas diminished concentrations of glucose have the opposite effect[74,75]. The AMPK/malonyl-CoA system is therefore operative in the β-cell and it is acutely influenced by glucose.

Long-term actions of glucose on β-cell lipid metabolism, possibly mediated by a decrease in AMPK activity (FIGS 2,4), are also prominent. Chronically elevated glucose markedly enhances β-cell ACC and FAS gene expression[76], probably as a result of induction of SREBP1c[39]. Likewise, in the β-cell line INS-1 it causes a sustained enhancement of citrate and malonyl-CoA formation, an almost total inhibition of fat oxidation and a pronounced increase in phospholipid and TG deposition[77], much as it seems to do in skeletal muscle and liver[59]. Chronic exposure of rat islets to an elevated glucose concentration also decreases the expression of PPARα[72], a transcriptional regulator that controls the expression of numerous genes of fat oxidation and metabolism including CPT1. Finally, the transcription factor SREBP1c, whose induction is associated with a coordinate increase in lipogenic genes in many cells, is induced by an elevated glucose concentration in MIN6 cells[78] and cultured human islets (E. Joly and M.P., unpublished data), and is overexpressed in islets from hyperglycaemic ZDF rats[8]. As such, both short and long-term exposure of β-cells to a surfeit of glucose dramatically influences lipid metabolism by diminishing fatty acid oxidation and enhancing fatty acid esterification and the conversion of glucose carbons into lipids.

Chronic increases in the concentration of glucose and saturated FFA cause dysfunction and damage to the β-cell, and ultimately result in apoptosis[79], an effect similar to that which they have in endothelium[18,80]. A detailed account of the β-cell GLUCOLIPOTOXICITY HYPOTHESIS and the role of the AMPK/malonyl-CoA signalling network in its causation is illustrated in BOX 3. Much of our understanding of the β-cell decompensation phenomenon has come from longitudinal studies in the ZDF rat model[8]. β-cell loss precedes overt hyperglycaemia in the ZDF model and, once diabetes is established, chronic hyperglycaemia further accelerates β-cell destruction[8]. What remains uncertain at present is whether the early phase of β-cell apoptosis is due to the elevated lipids alone (lipotoxicity) or to the combination of elevated lipids in conjunction with post-prandial hyperglycaemia (glucolipotoxicity). The recent demonstration that even moderately elevated concentrations of glucose synergize with saturated FFAs to cause β-cell apoptosis[79] favours the latter possibility. Finally, the applicability of these findings to humans is highlighted by the results of the US Type 2 Diabetes Prevention Trial (DPP)[81] and the TRIPOD study[82], in which metformin and troglitazone, respectively, delayed or prevented the development of diabetes in people with impaired glucose tolerance.

The endothelial cell. Endothelial cell dysfunction, as manifest by impaired vascular relaxation or increases in circulating vascular cell adhesion molecules (for example, VCAM1), is present in patients with T2DM and the metabolic syndrome[83,84], and is thought to be one component of the inflammatory process that initiates atherogenesis[83]. That AMPK dysregulation (that is, an absolute decrease or a failure to increase its activity) could contribute to this process was first suggested by studies showing that AMPK activation can increase nitric oxide (NO) synthase (eNOS) phosphorylation and activity in endothelium[85]. More compelling evidence was obtained in cultured endothelial cells incubated in a high-glucose medium. As reported by Ido *et al.*[80], we found that apoptosis, quantified by TUNEL staining, was increased after 72 hours when these cells were incubated with 30 mM versus 5 mM glucose, in agreement with earlier reports. After 24 hours of incubation, DAG synthesis, malonyl-CoA concentration and the activity of caspase 3 (an enzyme in the apoptotic cascade) were already increased; fatty-acid oxidation and mitochondrial membrane potential were diminished; and the ability of insulin to activate Akt was decreased by 50% (that is, insulin resistance was observed). All of these changes were prevented by incubating the cells with the AMPK activator AICAR, and where studied by overexpressing a constitutively active AMPK[80]. Importantly, we observed similar changes, and even greater increases, in oxidant stress, NF-κB expression and VCAM1 expression in endothelial cells incubated with the fatty acid palmitate[18,86]; here too the effects were inhibited by AMPK activation.

GLUCOLIPOTOXICITY HYPOTHESIS
A theory proposing that elevated glucose, in particular post-prandial hyperglycemia, and fatty acids synergize in causing β-cell death and multiple tissue defects.

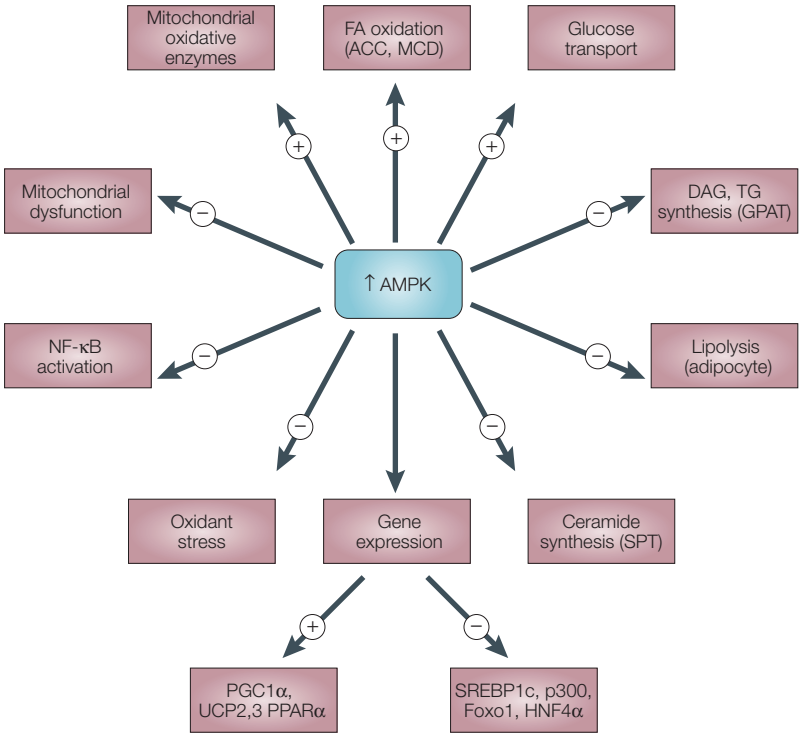

Figure 4 | **Effects of AMPK activation on events that could account for its ability to diminish lipid accumulation, cell dysfunction and insulin resistance.** In addition to altering the activity of key enzymes of lipid metabolism by phosphorylation and regulation of their synthesis (FIG. 1), AMPK activation alters many other events. It increases the expression of PPARα, PGC1α and the uncoupling proteins UCP2 and UCP3, and it suppresses the expression of the transcription factor SREBP1c, HNF4α and the transcriptional co-activator p300. AMPK has also been shown to inhibit NF-κB mediated gene expression and to decrease oxidant stress in endothelial cells incubated with fatty acids. Whether it has similar effects in other cells, and if so, under what conditions, remains to be determined. (See text for details). Whether AMPK activation results in enhancement or inhibition of process is denoted in the figure by plus and minus signs, respectively. ACC, acetyl-CoA carboxylase; AMPK, AMP kinase; DAG, diacylglycerol; GPAT, glycerol-3-phosphate acyltransferase; HNF4α, hepatocyte nuclear factor 4α; MCD, malonyl-CoA decarboxylase; NF-κB; nuclear factor-κB; PGC1α, PPARγ co-activator 1α; PPARα, peroxisome proliferative-activated receptor-α; SPT, serine palmitoyltransferase; SREBP1, sterol-regulatory-element-binding transcription factor 1; TG, triglyceride; UCP, uncoupling protein.

Whether the changes produced by AMPK activation in these investigations are attributable solely to its effects on fatty acid metabolism remains to be determined. In particular, a more direct effect of AMPK, and specifically its α2 subunit, on NF-κB transactivation in the nucleus has been described. Preliminary studies indicate that this could involve the phosphorylation of the transcriptional co-activator p300 (REF. 18). Whatever the mechanism, these findings and the reported benefits of exercise, metformin, TZDs and adiponectin in preventing or attenuating atherosclerosis *in vivo* (see section on AMPK) strongly suggest a protective action of AMPK on the endothelium.

Liver. Events similar to those described in muscle, the pancreatic β-cell and endothelium seem to occur in liver in insulin-resistant states. Excess TG deposition in liver, sometimes referred to as non-alcoholic fatty liver disease (NAFLD), is a very common disorder affecting as much as 20% of the US population[87,88]. Furthermore, a significant percentage (roughly 10%) of patients with NAFLD later develop non-alcoholic steatotic hepatitis (NASH), a disorder characterized by mitochondrial dysfunction, increases in oxidant stress, cell cytokines, inflammation[89], a predisposition to cirrhosis (roughly 20% of patients with NASH), and, less commonly, hepatocellular carcinoma[87,88]. As recently reviewed, NAFLD and NASH are predominately found in individuals with T2DM, obesity and other components of the metabolic syndrome, and nearly all patients with these disorders are insulin resistant[87,88,90].

Although the association between hepatic lipid deposition and insulin resistance has been clearly demonstrated in humans[13], relatively few studies have examined the role of dysregulation of lipid metabolism in causing insulin resistance in liver. A recent study[91] has described increases in PKC-δ (in liver, similar to that noted in human muscle[54], when plasma FFA levels are increased during a euglycaemic–hyperinsulinaemic clamp. Likewise, we have found that increases in the concentrations of DAG, TG and malonyl-CoA and a decrease in AMPK activity temporally (at 5 hours) paralleled the development of insulin resistance in liver of rats infused with glucose[59]. Unger *et al.*[8] have noted marked lipid accumulation in liver of the leptin-receptor-deficient ZDF rat. Finally, increases in PKC activity or altered PKC distribution, together with increased lipid accumulation have been noted in liver of massively obese insulin-resistant humans[53].

There is, therefore, an increasing body of evidence that links dysregulation of lipid metabolism with impaired insulin action and cellular dysfunction in liver, as well as in muscle, pancreatic β-cells and endothelium. Furthermore, abnormalities in PKC, oxidant stress and the IKKβ/NF-κB system also seem to be common events in these tissues[8,18,89,92]. The role of the AMPK/malonyl-CoA network in the pathogenesis and treatment of NAFLD/NASH remains to be established; however, its importance is strongly indicated by recent successes in treating these disorders with therapies that activate AMPK. These include diet and exercise[88], leptin[93], adiponectin[94], metformin and TZDs[88,95] (see section on therapy).

Adipocytes. Until recently, the contribution of the adipocyte to insulin resistance has principally been linked to its role as a source of FFA entering the circulation. However, the adipocyte almost certainly also participates in the regulation of insulin action by virtue of the fact it releases adiponectin and leptin, both of which can activate AMPK[96,97]. AMPK itself is also present in the adipocyte[98] and, as in muscle and liver, its activity is increased twofold in the rat 30 minutes after a bout of treadmill exercise[99]. In addition, activation of AMPK in fat cells by other factors, including isoproterenol[100] and adiponectin[101], has been described. Evidence that dysregulation of the AMPK/malonyl-CoA system and the enzymes it regulates in the adipocyte and central nervous system (CNS) could contribute to the pathogenesis of obesity and be a target for its treatment has been reviewed elsewhere[102].

Table 1 | **Metabolic and enzymatic changes in muscle of insulin-resistant organisms**

Model	TG	DAG	Malonyl-CoA	PKC activity	Activated IKKβ/NF-κB	AMPK activity
fa/fa rat	(+)	(+)	(+)	(+)	ND	(–)
Glucose-infused rat	(+)	(+)	(+)	(+)	ND	(–)
Fat-fed rat	(+)	(+)	(+/–)	(+)	(+)	ND
Fat-infused humans	(+)	(+)	(+)	(+)	(+)	ND
Obese insulin-resistant humans	(+)	nd	nd	(+)	ND	ND

Data are from the laboratories of one of the authors (N.R.) and those of Turinsky, Kraegen, Caro, Biden, and Shulman. Many of these changes have also been demonstrated in liver of insulin resistant obese humans, as well as fat-fed and glucose-infused rats and fa/fa rats. Studies, primarily *in vitro*, also suggest that similar events occur in the pancreatic β-cell and cultured vascular endothelium. (Adapted from REF. 18). AMPK, AMP kinase; DAG, diacylglycerol; IKK, inhibitor of NF-κB kinase; PKC, protein kinase C; ND, not determined; TG, triglyceride.

AMPK/malonyl-CoA as a therapeutic target

As already noted, a number of pharmacological agents and hormones, as well as exercise, activate AMPK, improve insulin sensitivity and diminish ectopic lipid accumulation in tissues of experimental animals and in humans. Where assessed, they were also shown to improve β-cell function and attenuate a number of disease processes that could be attributable to dysregulation of the AMPK/malonyl-CoA signalling network. Although definitive proof that their beneficial effects are AMPK-mediated is still lacking, the fact that they all activate AMPK and have somewhat similar clinical and biological actions is striking (TABLE 2).

Exercise and caloric reduction. The prototypical AMPK activator listed in TABLE 2 is exercise, which activates AMPK, lowers the concentration of malonyl-CoA and alters intracellular lipid metabolism in muscle, liver, adipose tissue[99] and possibly other organs. A single bout of exercise increases insulin sensitivity in muscle of humans and experimental animals[62,103], as does physical training[2]. In addition, regular physical activity has been associated with a decreased incidence of both myocardial infarction (coronary heart disease) and T2DM[104], and it has proven efficacious in at least some patients in treating NAFLD/NASH[87,88]. By itself[105], and in concert with diet therapy[81,106], regular exercise has also been demonstrated to delay or prevent progression from impaired glucose tolerance to overt T2DM in several large studies. Finally, regular exercise improves glycaemic control in many people with T2DM and it diminishes multiple risk factors for atherosclerosis both in patients with T2DM and the metabolic syndrome, whereas inactivity causes insulin resistance and increases the risk of coronary heart disease[2,104].

In general, caloric reduction in obese or overweight humans and experimental animals diminishes insulin resistance and improves many of the disorders associated with the metabolic syndrome[8,88]. Its effect on AMPK is less clear; however, we have observed that assayable AMPK activity is much lower in the liver of normal fed than starved rats (A.K. Saha, N.R., unpublished data). Whether a similar phenomenon occurs in obese or insulin-resistant rodents is not known, although it is noteworthy that we have found diminished AMPK activity in tissues of the fa/fa rat and ob/ob mouse (X. Yu, R. Unger, N.R. *et al.*, unpublished data).

Adiponectin and leptin. The adipokine adiponectin (also referred to as ACRP30) activates AMPK and diminishes ACC activity in liver[96], and its globular subunit (g-adiponectin) has similar actions in skeletal muscle[97]. To what extent activation of AMPK accounts for the biological actions of adiponectin is unclear; however, in rodents adiponectin administration decreases adiposity, increases insulin sensitivity and muscle FFA oxidation[107] and, like other AMPK activators (for example, AICAR)[55], it diminishes hepatic glucose production[108]. Furthermore, genetically knocking out adiponectin causes glucose intolerance and insulin resistance[109], whereas overexpression of both full-length[110] and g-adiponectin[111] attenuates the severity of

Table 2 | **Effect of factors that increase AMPK and/or malonyl-CoA**

Factor	Muscle insulin resistance	Pancreatic β-cell dysfunction cell	Endothelial dysfunction	Coronary heart disease	NAFLD/NASH syndrome
Exercise	(–)	ND	(–)	(–)	(–)
Calorie/weight reduction	(–)	(–)	(–)	(–)	(–)
Adiponectin	(–)	(–)	(–)	(–)	(–)
Leptin	(–)	(–)	(–)	ND	(–)
AICAR	(–)	(–)	(–)	ND	ND
Metformin	(–)	(–)	(–)	(–)	(–)
TZDs	(–)	(–)	(–)	(–)	(–)

Where studied, these factors also alter ectopic lipid deposition in keeping with their effects on AMPK. Inactivity, caloric excess (glucose) and deficiencies of leptin or adiponectin where studied have shown to have opposite effects. (–), decrease; AICAR, 5-aminoimidazole-4-carboxamide riboside; AMPK, AMP kinase; NAFLD/NASH, non-alcoholic fatty liver disease/non-alcoholic steatotic hepatitis; ND, not determined; TZDs, thiazolidinediones.

Box 3 | **The glucolipotoxicity hypothesis**

According to the glucolipotoxicity hypothesis[9,19], in the presence of both high glucose and FFA concentrations, cytosolic fatty acyl-CoA (FA-CoA) increases because the ability of fatty acids to increase their oxidation is diminished due to decreased PPARα activation, enhanced SREBP1c expression, reduced AMP kinase (AMPK) activity and increased malonyl-CoA levels caused by the elevation in glucose. This, in turn, results in an increase in FA-CoA partitioning toward potentially toxic cellular products, including possibly diacylglycerol (DAG), ceramide and lipid peroxides. The nature of the downstream events that lead to β-cell death is not entirely clear; however, increases in oxidant stress and the production of nitric oxide could be involved[8,140,160]. An emerging body of evidence suggests that changes in malonyl-CoA–AMPK signalling is important in the pathogenesis of both β-cell glucolipotoxicity and type 2 diabetes mellitus (T2DM). Metformin and AICAR, agents that activate AMPK and thereby favour fatty acid β-oxidation, prevent glucolipotoxicity-induced apoptosis in INS832/13 cells[79]; overexpression of a constitutively active form of SREBP1c in β-cells leads to activation of fatty acid synthase gene expression, an accumulation of triglyceride (TG) and a profound inhibition of glucose induced insulin secretion[78,161,162], all of which are partially reversed by treatment with AICAR; metformin inhibits lipotoxicity-induced insulin secretory dysfunction in isolated human islets[163]; and last, in the Zucker diabetic fatty (ZDF) rat, a rodent with a mutated, non-functioning leptin receptor (Ob-Rb)[8], overexpression of the Ob-Rb gene in islet tissue *in vitro* allowed leptin, which activates AMPK[115], to reverse the diabetogenic phenotype, reduce TG stores and inhibit FFA-induced apoptosis[8]. Likewise, feeding ZDF rats the thiazolidinedione, troglitazone, a PPARγ agonist that seems to activate AMPK[32,120], has a nearly identical effect[16], as does treatment with metformin[164] and AICAR (X. Yu, R. Unger, N.R., manuscript in preparation).

atherosclerosis in apolipoprotein-E-deficient mice. Adiponectin has also been shown to diminish TNF-α-induced NF-κB activation in cultured endothelial cells[112], much as does AICAR and expression of a constitutively active AMPK-adenovirus construct[113]. In keeping with these findings, low levels of circulating adiponectin in humans are associated with obesity, T2DM and a predisposition to diabetes independent of adiposity and insulin resistance[108]. In addition, polymorphisms of the adiponectin gene are associated with the metabolic syndrome in some populations and a predisposition to T2DM in others[108,114]. A recent study has also demonstrated that adiponectin administration diminishes hepatic lipid accumulation in ob/ob mice with NAFLD[94].

Leptin activates AMPK in rat skeletal muscle both *in vivo* and *in vitro*, with the former effect in large part due to a CNS-mediated increase in sympathetic nervous system activity[115]. It is not known whether leptin activates AMPK either directly or indirectly in other tissues. It also remains to be determined whether the accumulation of lipid in the pancreatic β-cell, liver, heart and skeletal muscle and the development of diabetes and cardiomyopathy in the leptin-receptor-deficient ZDF rat[8] are related to a decrease in AMPK activity. Studies showing that AMPK activity is low in the liver of these and other leptin-receptor-deficient rats (X. Wu, R. Unger, N.R. *et al.*, unpublished data) are consistent with this possibility, as is the observation that treatments that activate AMPK, such as AICAR administration (X. Wu *et al.*, unpublished data), diminish ectopic lipid deposition in these animals.

Thiazolidinediones and metformin. The administration of the TZD troglitazone, a PPARγ agonist, diminishes lipid deposition in multiple tissues, including the pancreatic β-cell and heart, and prevents the development of diabetes and lipotoxic cardiomyopathy in the ZDF rat[8,16]. In humans, TZDs diminish insulin resistance[116] and lipid accumulation in the liver[117], and they seem to be useful in the treatment of NASH[88] and possibly in the prevention of T2DM[82]. Recent studies indicate that in humans TZDs also diminish endothelial cell dysfunction and vascular inflammation[118], lower serum interleukin-6 (IL-6) and C-reactive protein[119] and diminish carotid intima-media thickening[118]. TZDs have been reported to activate AMPK in cultured cells[32], and in rat liver and adipose tissue *in vivo*[120]. Increases in insulin sensitivity caused by TZDs in T2DM humans and experimental animals are associated with increases in adiponectin[108,121]. Whether this increase in adiponectin and/or a direct cellular action of TZDs accounts for its ability to increase AMPK *in vivo* remains to be determined. Another issue requiring study is whether the action of PPARα agonists — which, similarly to the TZDs, increase fatty acid oxidation and diminish insulin resistance[122] — are AMPK mediated.

Metformin, in common with the TZDs, is an insulin-sensitizing agent that is widely used in the treatment of T2DM; it has also shown some benefit in patients with NAFLD/NASH[88]. In addition, in the DPP trial metformin delayed or prevented the development of T2DM in people with impaired glucose tolerance, as did diet and exercise[81]. Recent evidence indicates that metformin too can act by increasing AMPK activity. For example, at concentrations observed clinically in the portal vein it increases AMPK activity in rat liver and muscle[123]. Likewise, increases in AMPK activity have been observed in the muscle of diabetic patients treated with this agent[124] and in various cultured cells[32,33]. Perhaps most intriguingly, in the UK prospective diabetes study[125], obese individuals treated with metformin were found to have a lower incidence of cardiovascular disease than did patients with comparable glycaemic control who were treated with insulin or sulphonylureas. It has also been found that metformin prevents the apoptosis caused by elevated glucose and FFA levels in cultured pancreatic β-cells[79]. Such an action of metformin, by diminishing β-cell failure, could have accounted for its ability to delay the transition from impaired tolerance to overt diabetes in the T2DM prevention (DPP) trial.

Additional implications

Until this point, the effects of AMPK on preventing insulin resistance and cellular dysfunction and damage have been viewed principally in relation to its actions on intracellular lipid metabolism. In recent years, several other mechanisms have been proposed to explain how insulin resistance and cellular dysfunction develop in the setting of T2DM and the metabolic syndrome. In the following sections we will discuss briefly whether they too can be linked to the AMPK/malonyl-CoA network.

Oxidant stress. A common denominator in many tissues in which insulin resistance and/or cellular dysfunction is present could be an increase in oxidant stress (that is, a persistent imbalance between the generation of reactive oxygen species (ROS) and antioxidant defenses)[126]. For

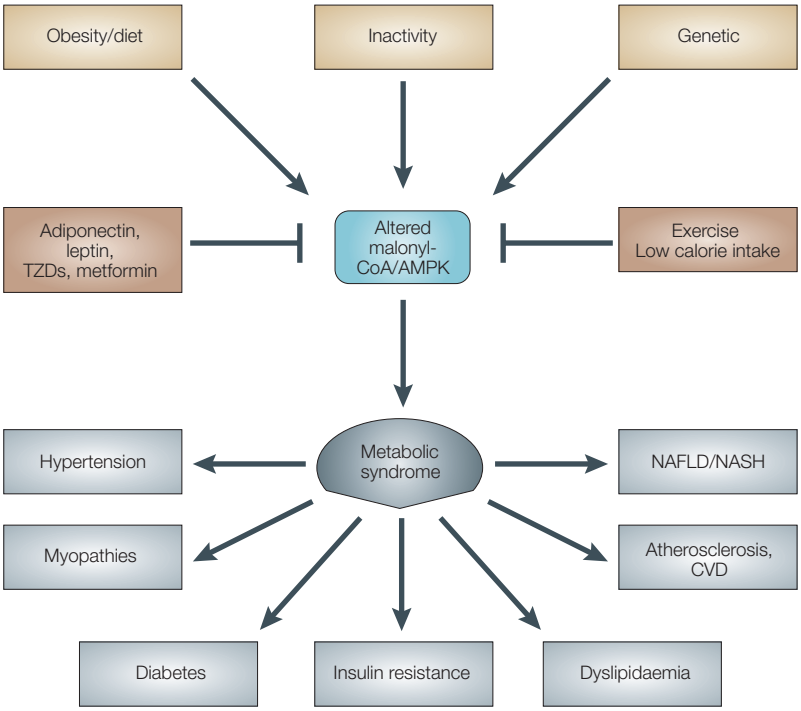

Figure 5 | **Links between the AMPK/malonyl-CoA fuel-sensing and signalling network and the metabolic syndrome.**

instance, oxidant stress has been implicated in endothelial cell damage caused by both hyperglycaemia and an excess of FFA[86,92,127] and in the pathogenesis of various diabetic complications[126], β-cell failure[66], NASH[88,90] and insulin resistance in muscle and fat[126]. AMPK activation by AICAR diminishes the net release of the peroxidized lipid 8-keto PGF2α from endothelial cells incubated with palmitate, although the mechanism by which it does this, and its relevance to AMPK action in other tissues, is not known[86]. Interestingly, increases in oxidative stress have also been shown to activate AMPK[18]. Whether a failure of oxidant stress to promote AMPK activation contributes to cell dysfunction in some situations has not, to our knowledge, been studied.

De novo *synthesis of ceramide.* Ceramide, and particularly ceramide synthesized *de novo*, has been linked to insulin resistance[128] and to apoptosis[8,129,130] in a variety of cells. The first committed step in *de novo* ceramide synthesis, the esterification of the fatty acids palmitate or stearate to serine, is catalysed by serine palmitoyl transferase (SPT), an enzyme inhibited in cells in which AMPK is activated with AICAR[129] (E. Chou, Y. Ido and N.R., unpublished data). AICAR inhibits palmitate-induced apoptosis in both astrocytes[129] and bovine retinal pericytes[130], at least in part by suppressing *de novo* ceramide synthesis. Although increases in ceramide mass have not been shown to accompany the insulin resistance induced in skeletal muscle by raising plasma FFA in humans[54,55,131], apoptosis of pancreatic β-cells[8] and retinal pericytes induced by palmitate[130] are both associated with increases in ceramide mass and are prevented by AMPK activation and ceramide synthesis inhibitors. Therefore, whether AMPK activation improves the function of certain cell types, in part through effects on *de novo* ceramide synthesis, requires further study.

Other hyperglycaemia-induced mechanisms. Hyperglycaemia has also been reported to alter cell function by increasing advanced glycosylation end product (AGE) formation, the polyol pathway and hexosamine biosynthesis[92,132]. Furthermore, overexpression of glutamine-fructose amidotransferase, the first committed enzyme in the hexosamine pathway, causes a metabolic syndrome in mice[133]. It will clearly be of interest to examine whether the AMPK/malonyl-CoA network and these other hyperglycaemia-induced mechanisms interact with each other.

Mitochondrial dysfunction. An increasing body of evidence has linked mitochondrial dysfunction to the insulin resistance and increase in intra-myocellular lipid associated with both T2DM[134–136] and ageing[137]. Such mitochondrial abnormalities could explain the long-held observation that patients with T2DM often have a decrease in VO_2 MAX that is apparently only partially corrected by physical training[138]. Mitochondrial dysfunction has also been observed in the liver of subjects with NASH[139], in the pancreatic β-cells of ZDF rats[8], in β-cells chronically treated with elevated FFA[140] and in endothelial cells incubated for 24 hours at high glucose concentration[86]. AMPK activation has been linked to mitochondrial biogenesis via its action on PGC1α[47,141], and, as noted earlier, AICAR preserves mitochondrial function in HUVEC cells incubated in a high-glucose medium[80]. Whether mitochondrial dysfunction is the cause or the consequence of the lipid accumulation observed in older individuals[137] or patients with T2DM[136] remains to be determined. Whichever is the primary event, it will be of interest to determine whether therapies aimed at AMPK activation prevent or reverse the mitochondrial dysfunction in these individuals.

Lipodystrophy. Perhaps the most compelling argument for a link between ectopic lipid deposition and insulin resistance is the presence of severe insulin resistance in both humans and experimental animals with full or partial lipodystrophy (that is, lack of adipose tissue)[142]. Humans with both congenital lipodystrophy and lipodystrophy related to protease treatment for AIDS have severe insulin resistance, impaired glucose tolerance and large accumulations of lipid in liver, muscle and, in some instances, visceral fat[108]. Furthermore, a similar pattern has been observed in transgenic mice with lipodystrophy, and in these animals implantation of normal adipose tissue reversed both the ectopic lipid accumulation and the insulin resistance[143]. To our knowledge, there is no direct evidence indicating that dysregulation of the AMPK/malonyl-CoA network plays a role in the pathogenesis of the ectopic lipid deposition in lipodystrophy. On the other hand, the finding of low levels of leptin and adiponectin in the serum of patients with lipodystrophies[14], and recent studies in which beneficial effects of

VO_2 MAX
The amount of O_2 consumed by an individual when performing aerobic exercise at maximal capacity.

leptin[145,146] and TZDs[147] have been reported in humans, suggest it could be a target for their therapy. Studies in which adiponectin treatment reversed insulin resistance in lipoatrophic rodents[148] also support this notion.

Concluding remarks

Several manifestations of the metabolic syndrome and T2DM — including insulin resistance in skeletal muscle and liver, hypertension, dysfunction of endothelium and the pancreatic β-cell, and the development of NAFLD/NASH — are associated with alterations in intracellular lipid metabolism that, in predisposed individuals, seem to lead to cellular dysfunction and, in some instances, irreversible damage (FIG. 5). We have proposed that a common causal factor could be dysregulation of the AMPK/malonyl-CoA fuel-sensing and signalling network due to either a decrease in AMPK activity and/or a failure of AMPK activity to increase in response to stress. Alternatively, increased malonyl-CoA levels could result from primary alterations in one or more of the enzymes responsible for its turnover (that is, ACC, MCD or FAS). In support of this notion, we have found decreased AMPK activity and/or increased malonyl-CoA levels in insulin-resistant tissues of rodents in a wide variety of conditions. In addition, where studied, treatment with AICAR and therapies that activate AMPK, including exercise, leptin, adiponectin, metformin and TZDs, have been useful in treating insulin resistance and disorders associated with the metabolic syndrome in humans and experimental animals (TABLE 2).

It must be emphasized that although the evidence in support of our hypothesis is increasingly compelling, it is mainly correlative and many questions remain to be answered. They include the following:

What is the precise nature of the lipid abnormality that ultimately leads to cellular dysfunction and damage? As already discussed, it is unlikely to be TG accumulation itself. Available evidence indicates that increases in cytosolic FA-CoA, DAG, ceramide, ROS derived from lipid peroxidation, protein acylation or some combination of these factors could be involved; however, the role of each remains to be proven.

Is the apparent protective effect of AMPK activation solely due to its action on lipid metabolism? We believe this is unlikely, because AMPK also exerts numerous effects directly on the nucleus, alters ion channels and causes other changes that are not readily explained by alterations in lipid metabolism.

Does a link exist between the AMPK/malonyl-CoA network and other pathways and mechanisms that have been implicated in the pathogenesis of insulin resistance and β-cell dysfunction? In particular, studies relating AMPK to mitochondrial dysfunction, the hexosamine biosynthetic pathway, *de novo* synthesis of ceramide and the generation of pro-inflammatory cytokines and ROS are needed.

What is the contribution of elevated cellular malonyl-CoA levels, independent of changes in AMPK activity, to tissue alterations associated with the metabolic syndrome? The observation that a deletion mutation of the ACC_2 isoform by itself leads to decreases in obesity and ectopic lipid accumulation and an increase in insulin sensitivity[149] strongly indicates that this possibility needs to be considered[70].

Finally, do we need to revise our thinking about the biological role of AMPK? The classic view[28,150] that AMPK is a fuel-sensing enzyme that enables a cell to respond to a decrease in its energy state has proven extremely useful in explaining the response to such stresses as ischaemia, fuel deprivation and, in muscle, contraction. As reviewed here, an increasing body of evidence suggests that AMPK has additional roles. In this regard, its regulation by endocrine as well as autocrine factors, its possible involvement in glucose/insulin-regulated gene expression and the recent discovery that at least one AMPK kinase is a tumour suppressor are noteworthy. One would predict that during the next few years our understanding of the logic of AMPK in governing biological processes will expand considerably.

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Competing interests statement

The authors declare that they have no competing financial interests.

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