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AMP-activated protein kinase and muscle insulin resistance

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1. ABSTRACT

The Metabolic Syndrome, which includes obesity and type 2 diabetes, is reaching alarming proportions. A key factor is insulin resistance, defined as a reduced ability of insulin to stimulate glucose utilization and storage. Compelling evidence links insulin resistance with an excess fatty acid supply over energy need, resulting in lipid accumulation in non-adipose tissues. The AMPK pathway plays a key role in sensing and regulating tissue energy metabolism, influencing fuel metabolism in tissues including muscle and liver. A number of its actions could improve muscle insulin sensitivity at least partly by increasing fatty acid oxidation and diminishing synthesis of malonyl CoA, glycerolipids, ceramide and other molecules linked to insulin resistance, although the extent of these effects, particularly in the human context, is uncertain. Secondly, its activation could bypass the metabolic block associated with insulin resistance. Thirdly, it is possible that a dysregulation of the AMPK pathway may itself contribute to the metabolic derangement associated with insulin resistance. These issues are important in considering the AMPK pathway as a therapeutic target in insulin resistant states.

2. INTRODUCTION

Insulin resistance, defined as a reduced potency of insulin action in its target tissues, particularly muscle and liver, is an early and persistent major perturbation in the Metabolic Syndrome. This syndrome, reaching alarming proportions in many societies (1), is a state of metabolic dysregulation characterized by insulin resistance, hyperinsulinemia, obesity (especially central obesity) and a predisposition to Type 2 Diabetes, dyslipidemia, hypertension, premature atherosclerosis and other diseases (2). There are now a number of theories as to what causes insulin resistance in the Metabolic Syndrome, but it is clear that abnormalities in lipid metabolism, particularly related to its accumulation in non-adipose tissues, (3-5) plays a key role. The AMPK pathway is believed to be important in the sensing and regulation of tissue energy metabolism and can influence lipid and glucose metabolism in a number of tissues including muscle and liver. While targets for AMPK action are diverse, an important consequence of AMPK action is the enhancement of fatty acid oxidation in muscle and liver, and by virtue of this there is interest in whether AMPK activation could oppose insulin resistance

particularly via reducing non-adipose tissue lipid accumulation. This scenario has been largely based on animal and *in vitro* investigation, but should it prove the case in humans, then AMPK activation would be an attractive target for the prevention and treatment of insulin resistance. On the flipside, it is also possible that there is a dysregulation of the AMPK pathway in insulin resistance states, contributing to the metabolic perturbation. The purpose of this review is to consider the evidence for and against these possibilities. While it is recognised that AMPK may have a regulatory function in many tissues, here we will particularly focus on AMPK and muscle insulin resistance.

3. ROLE OF LIPIDS IN THE PATHOGENESIS OF INSULIN RESISTANCE

Skeletal muscle and liver are quantitatively the most important organs for insulin action. Insulin resistance is characterised by an impairment of insulin's ability to enhance muscle glucose uptake and to suppress liver glycogenolysis and gluconeogenesis. Early studies by Randle and colleagues of substrate competition in heart muscle (6) led to enunciation of the glucose fatty acid (FA) cycle as a mechanism linking FAs to impaired glucose oxidation and glycolysis, but later studies pointed to other mechanisms of insulin resistance. Our own studies in rodents 15-20 years ago (3) linked insulin resistance to excess accumulation of muscle triglyceride, and similar accumulation in human insulin resistant states (eg obesity, T2D (7), acquired lipodystrophy (8) is now well-established using direct muscle biopsy or via non-invasive magnetic resonance spectroscopy (9). TG accumulation per se is now regarded mainly as a marker of excess tissue lipid supply, and it is believed that accumulation of metabolically active long chain acyl CoAs (LCACoAs) and other cytosolic lipid metabolites, such as ceramides and diacylglycerol (DAG), and attendant oxidative stress generated via lipid peroxidation are more likely to be causally related to insulin resistance (reviewed in (4, 5).

While more delineation is required, there are now plausible mechanisms whereby these metabolites could produce insulin resistance. These include activation of pathways and factors that lead to serine phosphorylation of insulin signalling proteins, (eg protein kinase C, reactive oxygen species, the nuclear factor kappaB (NFkappaB) pathway, mammalian target of rapamycin (mTOR) pathway, c-jun N-terminal kinase (JNK) and cytokines) and altered gene transcription (2). Abnormalities in one or more of the above metabolites and pathways have been found in many insulin resistant states, including obesity, inactivity, lipodystrophy, T2D, pre-diabetes and hypertension and Cushing's Syndrome (2). They have also been observed when insulin resistance is produced acutely (over several hours) by raising plasma FA levels by lipid emulsion infusion in humans (10) and experimental animals (11) and in rats infused with glucose for 5-24 hours (12, 13). The fact that these pathways seem associated with at least two of the major current theories of insulin resistance, namely via fatty acid metabolite generation and via activation of pro-inflammatory pathways (eg TNFalpha) (14), supports their collective importance. There is much current effort being directed at which of these factors might dominate in particular states of insulin resistance; for example the fact that knockout of the novel DAG-sensitive PKCtheta protects mice against dietary fat-induced insulin resistance points to the importance of the diacylglycerol-sensitive novel PKCs as a linking factor between FA accumulation and muscle insulin resistance (15). On the other hand, our own experiments involving direct activation of NFkappaB pathway in muscle, at a level which causes muscle fiber changes, does not generate insulin resistance (16).

4. THE EXPANDING ROLE OF THE AMPK PATHWAY

AMPK has been described as a metabolic fuel gauge, responding to changes in cellular energy state and initiating appropriate responses. For example, during exercise or ischaemia/hypoxia, an increase in the AMP/ATP ratio will allosterically activate AMPK making it more susceptible to phosphorylation and activation by upstream AMPK kinases, such as the tumour suppressor LKB1 (17-19). Intriguingly, it now appears that there is a significant hormonal influence on AMPK phosphorylation and activation in peripheral tissues; hormones such as leptin, adiponectin, glucagon, interleukin-6 (IL-6) and catecholamines (17, 20) can increase AMPK activation, whereas it is decreased in liver by resistin (21) and ghrelin (22). This growing list of endogenous regulators of AMPK supports its likely physiological importance. In stressed conditions (i.e. contracting muscle, some energy-deprived tissues) the role of AMPK activation is to restore energy balance by enhancing processes that will result in ATP generation (e.g. muscle FA oxidation and glucose transport or glycolysis) and inhibiting others that use up ATP but are not acutely necessary for survival (e.g. protein and lipid synthesis, see reference (18)). The role of hormonally-induced changes in AMPK activation is less clear, although it is most likely playing a regulatory role in cellular fuel metabolism and inter-organ fuel homeostasis. This role is multifaceted and recent work suggests that AMPK plays a key role in governing both insulin secretion and the hypothalamic regulation of food intake and possibly glucose homeostasis (19).

5. THE AMPK PATHWAY AND ITS ROLE REGULATING LIPID METABOLISM

In the Introduction we postulated a role of AMPK in lipid metabolism and how it might influence insulin action. To elaborate, AMPK activation has the potential to alter activity of a number of enzymes, leading to increased oxidation of LCACoAs and decreased availability for the synthesis of TG (Figure 1), DAG and ceramide. Two actions lead to a decrease in malonyl CoA, firstly AMPK phosphorylates and inhibits acetyl CoA carboxylase (ACC), diminishing its ability to synthesize malonyl CoA, and secondly by phosphorylating and activating malonyl CoA decarboxylase (MCD), it increases malonyl CoA degradation (23, 24). The decrease in malonyl CoA resulting from these dual effects promotes FA oxidation,

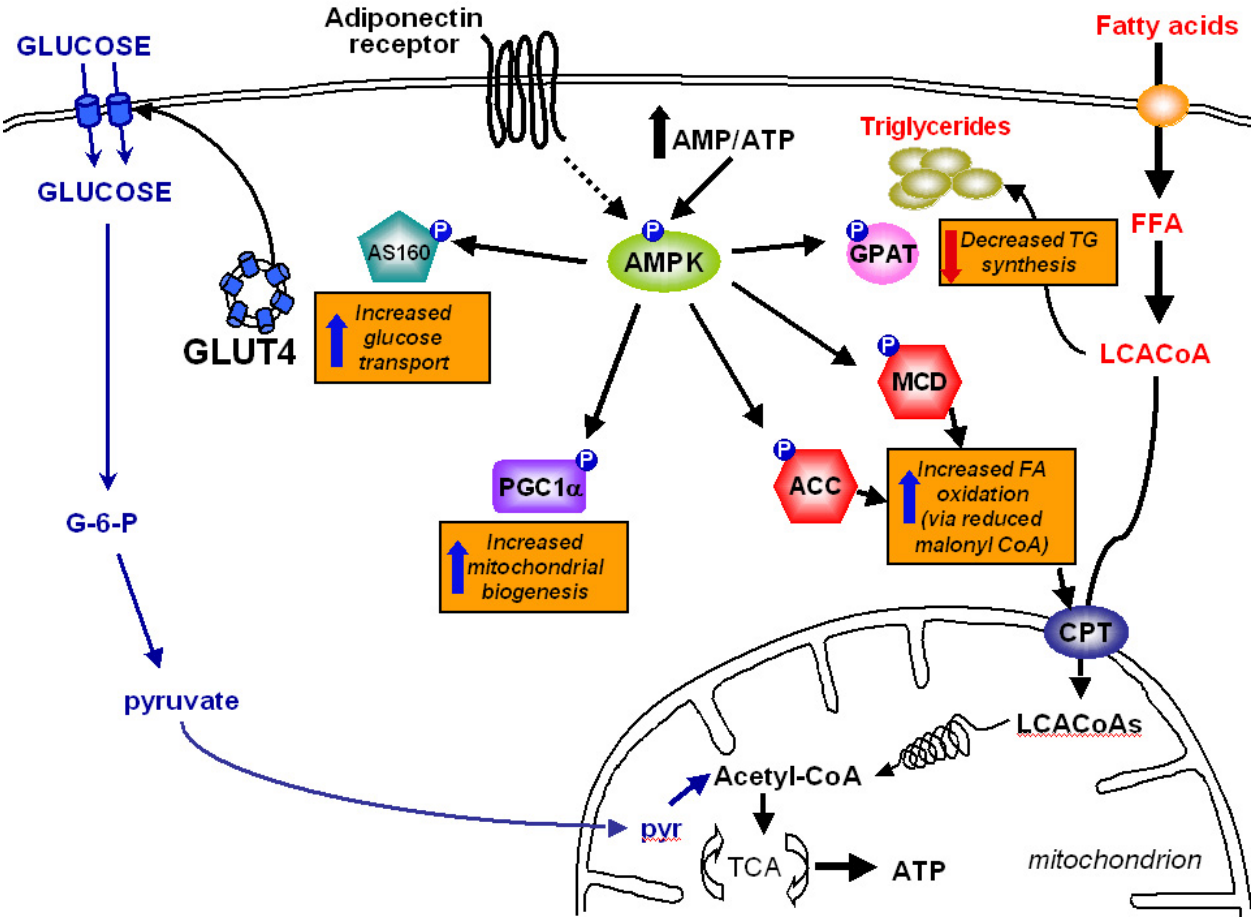

Figure 1. AMPK Activation and Lipid and Glucose Metabolism in Muscle. AMPK activity may be increased by an altered energy nucleotide ratio (eg during contraction) or by hormonal (eg adiponectin) action. The diagram highlights some of the protein targets for AMPK discussed in the text and their resultant metabolic effects. These actions could be expected to lessen the impact of insulin resistance by counteracting triglyceride accumulation (and by so doing improving insulin sensitivity believed to be associated with cytosolic lipid metabolites such as ceramides and DAGs). In addition increased AMPK activity can directly increase glucose transport

since malonyl CoA is an inhibitor of carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme that controls the transfer of cytosolic LCACoAs into mitochondria for oxidation (25-27). Co-incident with these acute changes in FA oxidation, AMPK inhibits FA and glycerolipid synthesis in liver and other tissues by reducing the abundance and secondarily the activities of ACC, FA synthase and sn-glycerol-3-phosphate acyltransferase, at least in part by suppressing the expression of the transcriptional activator sterol regulatory-element-binding protein-1 (SREBP-1c) (28, 29). AMPK activation also decreases ceramide synthesis (30), FA-induced NFkappaB activation (31), and mTOR activity (19), all of which have been implicated in the generation of insulin resistance. Taken together these are quite desirable actions with the potential of ameliorating insulin resistance, however because they have mainly been derived from *in vitro* and animal-based findings, their relevance to human physiology needs further study.

There is a paucity of evidence showing a direct link between AMPK activation and increased fatty acid oxidation in human skeletal muscle, although there are some relevant reports. Steinberg *et al.* (32) demonstrated that AICAR increased rates of FA oxidation to a similar extent in isolated muscle strips obtained from both lean and obese subjects. Studies in primary human muscle cells obtained from lean individuals have also shown that activation of AMPK by AICAR stimulates FA oxidation (33, 34). This effect is maintained in both obese and obese type 2 diabetic myotubes (33, 34). A major factor contributing to muscle cytosolic deposition of triglyceride and other lipid derived metabolites is an impaired ability to utilise fat as a fuel source, with muscle from obese and insulin resistant humans having a reduced capacity to oxidize FAs (35). Recently, it has been shown that malonyl CoA levels are increased in skeletal muscle from insulin resistant obese and type 2 diabetic subjects, which could be explained by an increase in ACC and decreased AMPK

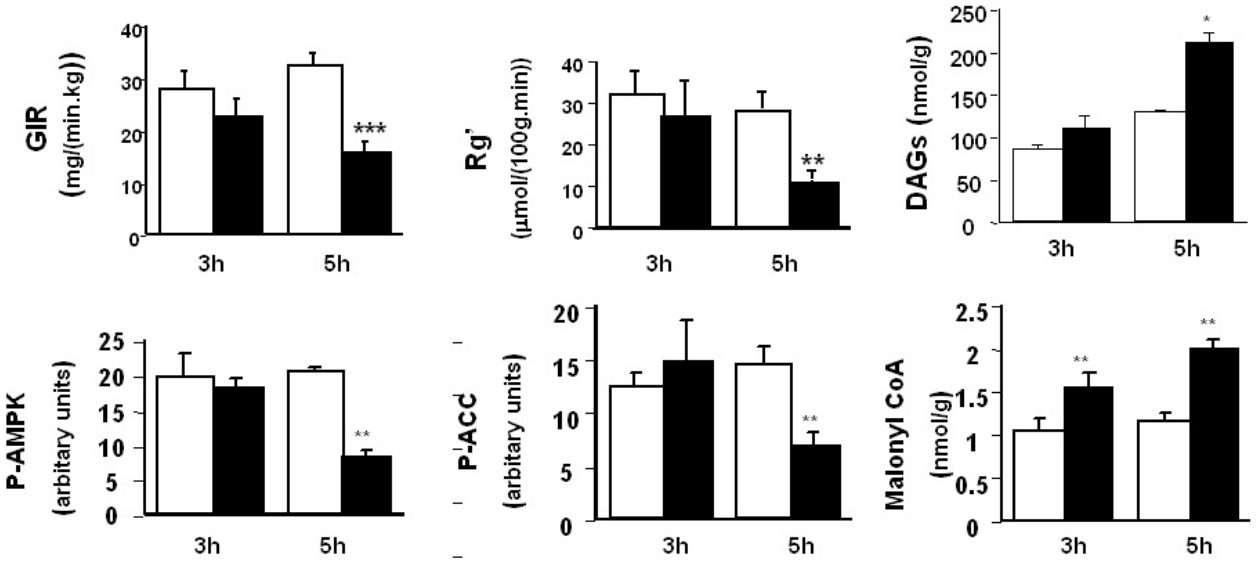

Figure 2. Responses during a glucose clamp performed in rats after a prior glucose (solid histogram) or saline (open histogram) infusion for 3 or 5h as indicated. Whole body response (glucose infusion rate GIR) is shown. All other responses are from red quadriceps (RQ) muscle. After 5h glucose infusion there is whole body and muscle insulin resistance (indicated by reduced clamp GIR and muscle glucose uptake Rg'). This is associated with increased muscle DAGs and malonyl CoA, and with reduced AMPK activity as indicated by reduced phosphorylated AMPK and ACC. Data from ref 13.

activity (36). These findings could account for a reduction in CPT1 activity (37) and subsequent decrement in muscle FA oxidation observed in obesity and insulin resistant states. We recently directly demonstrated that small increases in muscle CPT1 activity can direct lipids away from storage and into oxidation, stressing the importance of this regulatory step in opposing cytosolic lipid accumulation (25).

Other effects of AMPK activation on mitochondria are consistent with an action to increase capacity to oxidize FA in skeletal muscle and liver. An example is the ability of AMPK to increase the abundance of peroxisome proliferation-activated receptor (PPAR)gamma coactivator 1alpha (PGC1alpha), which via its effects on nuclear response factor 1, enhances the expression of genes regulating mitochondrial biogenesis and oxidative phosphorylation in skeletal muscle (38) (Figure 1). In addition AMPK activation promotes expression of uncoupling proteins 2 and 3 (39), lessens superoxide generation by mitochondria (40), and increases the expression of MCD and CPT1 (41), via effects on PPAR alpha. These latter actions would be expected to enhance mitochondrial FA uptake.

6. IS THE AMPK PATHWAY DYSREGULATED IN INSULIN RESISTANT STATES?

From the preceding argument it is clear that AMPK has a number of actions which could potentially alter insulin sensitivity. The question therefore arises as to whether the AMPK pathway may be dysregulated in insulin resistant states and be a significant contributing factor to defective insulin action.

6.1. Animal Evidence

Data from a number of animal models point to a link between insulin resistance and decreased AMPK activity. Decreased AMPK activity has been found in tissue of ob/ob mice and fa/fa and ZDF rats (42), in liver and adipose tissue of the Dahl-salt sensitive rat, a mildly insulin-resistant rodent with hypertriglyceridemia (43); and in skeletal muscle and adipose tissue of the IL6 knockout (KO) mouse (44), a rodent which becomes obese, glucose intolerant and hypertriglyceridemic as it ages (45). Furthermore, insulin resistance produced in otherwise normal rats by a glucose infusion is associated with a decrease in AMPK activity and an increase in DAG content in both muscle and liver (13)) (Figure 2). In another study it was reported that high fat feeding–induced insulin resistance was accompanied by impaired muscle AMPKalpha expression and activity (46). These rodent studies are consistent with a hypothesis (17) that a common feature underlying the metabolic syndrome could be a decrease in AMPK activity or an increase in malonyl CoA. As elaborated by Ruderman and Saha in a later review (2) however, it remains a conundrum as to why genetic manipulations to knock down alpha1 or alpha2 AMPK activity (47) or to knock out both isoforms in muscle (48) apparently do not produce insulin resistance in rodent muscle. There may be reasons for this, such as a partial redundancy and compensatory increase in the remaining alpha subunit when the alternate subunit is knocked out. Alternatively chronically low muscle glycogen levels in the double knockout (Birnbaum, M, personal communication) may compensate for insulin insensitivity when it is acutely assessed. There could also be other reasons but all are speculative without further investigation of these models. Of particular interest would be the status of muscle lipid

metabolism in these animals but we are unaware of this data.

The question also arises as to what are the metabolic factors leading to AMPK inhibition in the metabolic syndrome. Mechanisms linking lipids to AMPK inhibition are not clear, but it has been suggested that an increased accumulation of lipid in cells may reduce AMPK activity, since LCACoAs have been shown to inhibit AMPK kinase activity *in vitro* (49). In addition it was recently reported that prolonged incubation of endothelial cells with palmitate led to inhibition of AMPK activation via a ceramide-dependent activation of protein phosphatase 2A (PP2A) (50). Inhibition of AMPK together with increased PP2A activity in aortas of mice fed palmitate-rich diets points to the possible *in vivo* relevance of the findings at least regarding the endothelium, and further studies in other tissues are warranted. Another finding suggests a lipid-AMPK link in muscle (although precise mechanisms are not established); several days administration of the TZD rosiglitazone to high fat fed rats, known to reduce cytosolic lipid accumulation (51) can also potentiate acute AICAR stimulation of muscle AMPK activity (52).

Two recent studies (53, 54) examining glucoregulation and insulin action in relation to AMPK activity in aging rats provide support for dysregulation of AMPK as a contributing factor in insulin resistance associated with aging. In the first from Shulman's group (54), it was shown that both exercise and AICAR are less efficient in activating AMPK in aged versus young rats, and that chronic AMPK activation by beta-guanidinopropionic acid resulted in less mitochondrial biogenesis in the aged rats. A second study (53) assessed *in vitro* insulin-stimulated glucose uptake and AMPK activity in muscle excised from young and aged rats. Reduced AMPKalpha activity accompanied insulin resistance and reduced GLUT4 expression.

6.2. Human Evidence

In contrast to the animal data, investigations examining the expression and activity of AMPK in skeletal muscle from obese and type 2 diabetic individuals has yielded conflicting results. Supporting a possible dysregulation was the demonstration that AMPK activity was reduced in skeletal muscle from obese and type 2 diabetic subjects (36). AMPK alpha1 activity has also been reported to be reduced in obesity (55), and AMPK activation in response to adiponectin is impaired in cultured skeletal muscle cells grown from tissues of obese and obese diabetic patients (33). Furthermore it was reported recently that obese and T2D subjects had attenuated exercise activation of AMPK and phosphorylation of the putative downstream target, AS160 (56). In contrast other studies suggest normal functioning of the AMPK pathway in human obesity and T2D. For example AMPK mRNA and protein expression and AMPK alpha1 and alpha2 activities have been shown to be similar in skeletal muscle obtained from lean and obese subjects (32). In addition, AMPK isoform expression and activity has been reported to be normal in T2D patients compared to healthy subjects with a similar body mass (57-59). These latter findings suggest that the AMPK function is intact in skeletal muscle of obese and type 2 diabetic subjects. Indeed, AICAR treatment has been shown to activate AMPK to the same extent in skeletal muscle of lean, obese and T2D subjects (32, 58). At the moment reasons for these conflicting results are not clear, although it may be that reduced AMPK activity is associated with increased adiposity rather than with the T2D state per se. Thus comparisons between healthy and T2D subjects where BMI is matched may not show differences in AMPK activity (57-59). Nevertheless this would not explain all findings (32). It is hoped that some of these issues can be clarified in the near future. In particular one possibility is that while there may be no intrinsic defect in AMPK signalling in obesity or T2D (and hence responses to external activators such as AICAR appear normal) it is possible that there is dysregulation of endogenous hormonal regulation (eg via adiponectin). This requires further investigation.

It remains to be investigated also whether or not AMPK dysregulation occurs in tissues other than muscle (i.e liver and adipose tissue) in human insulin resistant states. Possibly AMPK dysfunction is an early event that can be masked by secondary changes, such as an increase in intracellular FA levels. In support of such a notion, increased FA has been reported to increase the AMP/ATP ratio and activate AMPK in cultured rodent hepatocytes (60).

7. PHYSIOLOGICAL ACTIVATORS OF AMPK IN INSULIN RESISTANT STATES

7.1. Exercise and Muscle Glucose and Fatty Acid Uptake

Exercise is recognised as an important component in the treatment of obesity and T2D. Exercise rapidly increases whole body energy metabolism by up to 20-fold, increasing requirements for metabolic substrates in the working muscle. An acute bout of exercise increases glucose disposal and FA oxidation in contracting muscle. Despite the therapeutic effects of exercise on glycemic control in patients with type 2 diabetes, the molecular mechanisms mediating these metabolic responses are not well understood. AMPK has emerged as a key signalling molecule in regulating exercise-induced changes in glucose and FA metabolism (61, 62). In healthy humans, an acute bout of exercise activates AMPK in an isoform and intensity dependent manner (63,64). During low- to moderate-intensity exercise, complexes containing AMPK alpha2 but not AMPK alpha1 have increased activity (63,65). Furthermore, during high-intensity exercise there is still little activation of AMPK alpha1 complexes (66). However, following a 30 s bicycle sprint, at power outputs three- to four-fold higher than at VO_2max, the activity of complexes containing both AMPK alpha1 and alpha2 isoforms are increased (67). The ability of exercise to activate AMPK was reported to be maintained in skeletal muscle from lean type 2 diabetic patients (57). However, more recent data suggests that exercise-induced increase in AMPK activity is indeed attenuated in both obese non-diabetic and obese type 2 diabetic subjects (56), again suggesting that dysregulation of the AMPK pathway may

be more associated with obesity rather than with T2D per se.

While the role of upstream stimuli that activate AMPK are now reasonably well-defined (18), the signaling mechanisms downstream of AMPK which regulate muscle glucose uptake are not so well understood. Recent studies have revealed that the Akt substrate of 160 kDa (AS160) is a downstream target of Akt and plays an important role in regulating insulin-stimulated glucose uptake (68, 70). Like insulin, exercise and activation of AMPK by AICAR causes phosphorylation of AS160 in muscle (69). Thus, it appears that AMPK induced increases in muscle glucose uptake may be mediated via AS160 and this represents a point of convergence connecting insulin- and contraction-stimulated glucose transport (Figure 1). Recently, AS160 phosphorylation was shown to be blunted in skeletal muscle from obese type 2 diabetic patients following moderate intensity exercise (56). Taken together with the findings that contraction-induced AMPK activation is attenuated, it has been suggested that these individuals may need to exercise at a higher intensity to achieve the same activation of AMPK as in lean subjects (56).

Alternate pathways that stimulate skeletal muscle glucose uptake independently of insulin signalling could have the potential to counteract effects of insulin resistance associated with obesity and type 2 diabetes via bypassing defective insulin signalling. Exercise increases muscle glucose uptake in an insulin independent manner, and as AMPK is activated during muscle contraction, it has been proposed to play an important role in regulating exercise-induced muscle glucose uptake. At this stage the molecular mechanisms regulating this response have not been fully elucidated.

Studies examining the role of AMPK in mediating glucose uptake in human skeletal muscle have mainly utilised the AMPK activator AICAR. In isolated muscle strips obtained from lean subjects, AICAR has been shown to increase glucose transport and GLUT4 translocation to a similar degree as that observed with insulin-stimulation (58). Importantly, AICAR also increased glucose uptake and sarcolemmal GLUT4 content in muscle strips obtained from subjects with type 2 diabetes, providing evidence that the AMPK pathway can be activated in insulin-resistant skeletal muscle (58). In addition, it has recently been demonstrated that an AICAR infusion increases muscle glucose uptake in men with type 2 diabetes (70), although the stimulation of glucose uptake was not as great as that observed in healthy young men (70). Whole body glucose disposal during a euglycemic, hyperinsulinemic clamp was also increased with AICAR infusion (70). Interestingly in this study, the increase in whole body glucose disposal and muscle glucose uptake in response to AICAR administration appeared independent of AMPK activation (70).

7.2. Adipokine regulation of muscle AMPK Activity

It has recently been shown that a number of cytokines secreted from adipose tissue, referred to as adipokines, play a key role in regulating skeletal muscle FA metabolism and insulin sensitivity by virtue of their ability to activate AMPK.

7.2.1. Leptin

AMPK-mediated increases in FA oxidation and decreases in lipid synthesis are likely to contribute to the ability of both leptin, the protein product of the *ob* gene (71) (72) and adiponectin (73) to reduce insulin resistance in skeletal muscle and liver in experimental animals. These actions are additional to the central actions of leptin to modulate appetite. Recently it has been demonstrated that leptin has efficacy in improving insulin action and reducing TG content in muscle and liver of patients with lipodystrophy, although the role of AMPK activation in mediating this was not studied (74). Leptin is increased in the circulation of obese humans (75) which suggests that central and/or peripheral leptin resistance develops. There is a blunting of leptin's effects on fatty acid oxidation in muscle isolated from obese humans, providing direct evidence for the presence of leptin resistance in skeletal muscle (72). As AMPK signalling is maintained in muscle from obese individuals in response to AICAR, this indicates that factors upstream of AMPK are likely to be involved in the development of leptin resistance in obesity (72) (32).

7.2.2. Adiponectin

Adiponectin is an adipose-derived hormone that appears to play an important role in regulating energy homeostasis and insulin sensitivity. Plasma levels of adiponectin are suppressed in obesity and type 2 diabetes (76,77). Adiponectin has been shown to reduce lipid accumulation in the muscle of normal and genetically obese mice (78) as well as to diminish hepatic lipid accumulation (78, 79) and to diminish insulin resistance in fat-fed mice (80). In humans, an inverse relationship between plasma adiponectin levels and propensity to T2D (2) and coronary heart disease has been described (81). The adiponectin protein comprises a globular head and collagenous tail and circulates in the form of various multerimeric complexes. In addition, many studies have used a cleaved form of the adiponectin protein, comprising only the globular head. Adiponectin interacts with cell surface receptors denoted AdipoR1 and R2 (82). In muscle, the predominant receptor isoform is AdipoR1 which is reported to have a high affinity for the globular form of adiponectin (82). AdipoR2 is reported to interact most strongly with full-length adiponectin and is highly expressed in the liver (82. Preparations containing only the globular head of the adiponectin molecule can interact with AdipoR1 in skeletal muscle to induce adiponectin's metabolic effects. In conjunction with lower circulating adiponectin in the obese and diabetic condition, it is also possible that there is an impaired peripheral response. In support of this, treatment with the globular head of adiponectin has been shown to activate AMPK and phosphorylate ACC causing an increase in fatty acid oxidation in muscle from lean subjects, but these effects are blunted in muscle from obese individuals (33,55). In addition, globular adiponectin has been shown to increase glucose uptake in both lean and obese skeletal muscle and the combined exposure of insulin and adiponectin resulted in an additive effect on glucose

uptake, but this effect was significantly reduced in obese skeletal muscle (55). Importantly, Chen *et al.* (33) were able to show that the blunted metabolic effects of adiponectin in obesity was not due to a change in adiponectin receptor gene expression, suggesting that the impaired response to adiponectin lies downstream from its receptor. Taken together, these findings indicate the possible development of adiponectin resistance in obesity. It should be noted that the data for adiponectin are confounded somewhat by the fact that it circulates in multiple forms and the nature of the oligomer or its cleavage product that acts at the cell surface is unclear. Like leptin, evidence that adiponectin regulates AMPK in humans as it does in experimental animals is still lacking.

7.2.3. Interleukin-6

Interleukin-6 (IL-6) is a proinflammatory cytokine that activates AMPK and is thought to modify insulin sensitivity. Some reports suggest that elevated levels of IL-6 in plasma and/or adipose tissue would have a negative effect on metabolism; for example its levels in plasma are increased in people at risk for T2D and coronary heart disease (83). However plasma IL-6 levels increase during exercise, with muscle being the major source (84). Based on this fact, it is difficult to believe that IL-6 would impair glucose uptake given that it is upregulated in contracting muscle when the requirement for glucose uptake is augmented (84). Indeed, acute administration of IL-6 did not impair glucose homeostasis in healthy humans (85). Moreover, IL-6 infusion increased glucose disposal during a hyperinsulinemic euglycemic clamp in healthy individuals. In order to study the direct effects of IL-6 on muscle glucose metabolism, Glund *et al.* (86) incubated muscle strips obtained from healthy men with IL-6. Treatment with IL-6 increased glucose transport, glucose incorporation into glycogen and glucose oxidation concomitant with an increase in AMPK phosphorylation (86). IL-6 infusion has also been shown to stimulate lipolysis and rates of whole body fat oxidation in humans (87), suggesting that IL-6 is a regulator of FA metabolism. In fact recent studies have shown that IL-6 stimulates FA oxidation in primary human muscle cells and this effect is mediated via an AMPK-dependent mechanism (88). Finally support for IL6 having a positive effect on metabolism comes from knockout studies in mice: at 3months of age IL-6 KO mice are AMPK deficient (44) and later at 9 months they exhibit a metabolic-syndrome like phenotype with obesity, glucose intolerance and dyslipidemia (89). In summary the reason for the somewhat paradoxical findings regarding IL-6 needs resolution, and the extent to which the metabolic responses to IL-6 are maintained in muscle from insulin resistant humans needs to be established.

8. IS AMPK A GOOD THERAPEUTIC TARGET FOR INSULIN RESISTANCE?

Following an original suggestion that AMPK activation may be a useful target for treating T2D (61) there is now a considerable body of literature supporting this concept. AICAR administration *in vivo* has been shown to increase insulin sensitivity in muscle and liver of high-fat fed rats (90) and to prevent the development of T2D and diminishes ectopic lipid deposition in liver and muscle of the ZDF rat (42). *In vitro* studies of muscle demonstrated enhanced insulin-mediated glucose transport after AICAR administration (91, 92). Moreover the findings that the commonly used insulin-sensitizing anti-diabetic drugs metformin (28, 93) and thiazolidinediones (TZDs) (43, 94) were AMPK activators added further support to the concept that AMPK activators could be a therapeutic option for the treatment of insulin resistance (11, 43, 95). Metformin is one of the most commonly used drugs for the treatment of type 2 diabetes, reducing blood glucose concentrations by suppressing hepatic glucose production (96) and increasing glucose disposal into skeletal muscle (93). The molecular mechanism by which metformin improved glycemic control was not well understood until it was shown that AMPK was activated in skeletal muscle of type 2 diabetic patients following treatment with metformin (93). The increase in AMPK activity was suggested to be due to a change in the energy status of muscle as ATP and phosphocreatine levels were reduced after metformin treatment (93) possibly as a result of inhibition of complex I in the mitochondrial respiratory chain (97). Based on *in vitro* studies (98), it is likely that AMPK activation is the major mediator of beneficial actions of metformin in the liver.

Thiazolidinediones (TZDs), another class of insulin sensitizing drugs, not only increase AMPK activity in type 2 diabetic patients, but also increase the phosphorylation of ACC resulting in a reduction in malonyl CoA content, ultimately causing a repartitioning of FAs away from storage toward oxidation (36). The mechanism by which TZDs activate AMPK and thus in turn influence muscle lipid metabolism is likely to be principally mediated by enhanced production of adiponectin from adipose tissue (99) although more direct mechanisms may be involved (100)

The list of compounds which can activate AMPK and improve insulin sensitivity continues to expand. Berberine was recently identified as a component of traditional Chinese medicine with, amongst other actions, the ability to activate AMPK and enhance insulin sensitivity (101-103). It is likely that berberine acts in a similar fashion to metformin and can inhibit complex I of the mitochondrial respiratory chain (104) thus indirectly activating AMPK via alteration of the energy nucleotides. Increasing fatty acid oxidation by inhibiting electron transport in the mitochondria may seem contradictory. However, partial inhibition of complex I of the electron transport chain could lead to an increased reliance on FADH-linked electron transfer that is largely coupled to fatty acid beta oxidation, bypassing complex I of the electron transport chain and having less efficiency than NADH-linked electron transfer. It is therefore quite possible that inhibition of complex I would be associated with increased fatty acid oxidation (facilitated by AMPK activation) and fat loss as a result of the increased reliance on less efficient FADH-linked electron transport to support oxidative phosphorylation.

Recently another class of compounds, the triterpenoids, isolated from bitter melon, were found to be highly potent AMPK activators with antidiabetic actions, although mechanisms of action are not yet clear (105). Another compound that can activate AMPK is beta-guanidinopropionic acid (beta-GPA) (38) which appears to act by depleting creatine phosphate storage. Like AICAR, beta-GPA and its derivatives have been shown to improve insulin sensitivity and to promote weight loss in diabetic animals (106).

An exciting recent development was the identification of the thienopyridones (eg A-769662) as small molecule direct activators of AMPK (107, 108). A-769662 had a number of desirable effects such as substantial lowering of plasma glucose and liver triglyceride in ob/ob mice. A major part of the mechanism seemed to be modulation of hepatic gluconeogenic gene expression, and while the drug could be shown to have effects in *in vitro* muscle preparations, its tissue distribution favoured a predominantly hepatic action (107). Availability of direct selective AMPK activators will clarify problems of relating responses of other "indirect" activators such as AICAR, metformin and the TZDs to specific effects of AMPK.

Despite the above encouraging data suggesting the desirability of enhancement of AMPK activity as a target for ameliorating muscle insulin resistance, there are some confounding and unresolved issues. Firstly while it is clear that pharmacological enhancement of AMPK activity can enhance muscle fatty acid oxidation, it is unclear as to the extent if any that total energy expenditure is also enhanced, particularly over the longer term. Even though increased AMPK activity may facilitate various steps in the muscle fatty acid utilisation pathway, the overall rate of fatty acid oxidation may be principally dictated by the energy needs of the muscle. Whereas it is possible to increase energy demand by muscle (eg by exercise or uncoupling agents), this has not been shown for AMPK activators in sedentary muscle, and increased energy expenditure may turn out to be a requirement to significantly lessen cytosolic lipid intermediates thought to be mediators of insulin resistance. Another confounding issue arises from the recent work of Koves *et al* (109), suggesting that a major cause of muscle insulin resistance is related to incomplete fat oxidation and mitochondrial stress resulting from rates of mitochondrial beta oxidation exceeding the capacity of the TCA cycle. If this is correct then it is possible that AMPK activators, via increasing mitochondrial entry of acyl CoAs, could worsen rather than lessen insulin resistance in resting muscle. There is currently a need to resolve these issues.

9. OTHER THERAPUTIC TARGETS IN THE AMPK-MALONYL COA NETWORK

Malonyl CoA, independent of AMPK may be a target for treating insulin resistance. For example, knockout mice deficient in ACC2 (the form of ACC that generates the malonyl CoA which regulates CPT1) have higher FA oxidation rates in muscle and liver cells and are protected against obesity and diabetes when fed a high-fat diet (110, 111). The latter study from Shulman's group suggested that this protective effect may be directly related to reductions in tissue cytosolic lipids, namely DAG and LCACoAs. Consistently, Harwood and colleagues (112) have reported that a pharmacological ACC inhibitor increases fat oxidation in the rat and protects it from becoming obese and insulin resistant when fed a high-fat diet. Finally, overexpression of MCD in liver has been shown to lower its content of malonyl CoA and TG and to improve insulin sensitivity in rats fed a high-fat diet (113). It is still not resolved as to whether drugs aimed at increasing FA oxidation need to be targeted at both muscle and liver to be effective at counteracting insulin resistance and weight gain. Beneficial systemic effects have been obtained in insulin resistant mouse models with overexpression of liver-specific constitutively active AMPKalpha2 (114), and as reviewed above the liver-directed AMPK activator A-769662 has beneficial effects at the whole body level. On the other hand studies from Shulman's group suggest that ACC2 inhibition using liver-specific antisense oligonucleotide inhibitors (115) was not as effective at reducing weight gain in high fat fed mice as was global ACC2 inhibition (111). It may be that to be effective total energy expenditure rather than just fat oxidation needs to be increased, as occurred in the ACC2 $^{-/-}$ animals (111).

10. CONCLUSIONS

In summary there is now considerable evidence to suggest that the AMPK-malonyl CoA network has a significant influence on insulin sensitivity. This evidence includes rodent studies in which various factors that increase AMPK activity and reduce malonyl CoA also enhance insulin sensitivity. Such factors include TZDs, metformin, AICAR, exercise, and hormones. Furthermore, a number of animal models have been described in which decreased AMPK activity and increased malonyl CoA accompanies or precedes insulin resistance or associated disorders. Finally, there are plausible mechanisms that link increased AMPK or reduced malonyl CoA to enhanced insulin sensitivity; in particular, increased fat oxidation in muscle and liver reduces cytosolic levels of LCACoAs, ceramides, DAGs and lipid peroxidation products, all of which have been implicated in the pathogenesis of insulin resistance.

There are nonetheless a number of key issues that currently require resolution. For example it is not clear whether changes in AMPK and malonyl CoA are causal factors for insulin resistance and cellular dysfunction or simply epiphenomena. Secondly there is a need for more quantitative studies of muscle lipid fluxes and fates in states of excess lipid availability to determine where metabolic blocks predominate. In addition relevance of some findings in rodent models to humans needs further clarification. Although exercise, TZDs and metformin increase insulin sensitivity in humans it has not yet been demonstrated that this is a result of their actions on the AMPK-malonyl CoA network. Whereas in many rodent models, clear cut decreases in AMPK activity and/or

increases in malonyl CoA are associated with insulin resistance, such a link has not yet been demonstrated in most human studies. Finally, more studies are needed in mice in which AMPK and malonyl CoA are genetically manipulated. To date, genetic manipulations that reduce AMPK activity in mice have not caused the abnormalities seen in insulin resistance when AMPK and malonyl CoA are spontaneously altered, although the presence of increased adiposity and diminished physical activity in some of these mice suggest that they might appear at a later time. Notwithstanding the above issues, there are exciting prospects for achieving therapeutic manipulation of the AMPK-malonyl CoA network and both AMPK activators and ACC inhibitors are in development. Given current interest, it is expected that our knowledge of AMPK biology and optimal strategies for targeting AMPK-malonyl CoA network for therapeutic purposes will expand considerably in the near future.

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