Malonyl-CoA and AMP-activated protein kinase (AMPK): possible links between insulin resistance in muscle and early endothelial cell damage in diabetes


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Abstract

Based on available evidence, we would propose the following. (i) Excesses of glucose and free fatty acids cause insulin resistance in skeletal muscle and damage to the endothelial cell by a similar mechanism. (ii) Key pathogenetic events in this mechanism very likely include increased fatty acid esterification, protein kinase C activation, an increase in oxidative stress (demonstrated to date in endothelium) and alterations in the inhibitor κB kinase/nuclear factor κB system. (iii) Activation of AMP-activated protein kinase (AMPK) inhibits all of these events and enhances insulin signalling in the endothelial cell. It also enhances insulin action in muscle; however, the mechanism by which it does so has not been well studied. (iv) The reported beneficial effects of exercise and metformin on cardiovascular disease and insulin resistance in humans could be related to the fact that they activate AMPK. (v) The comparative roles of AMPK in regulating metabolism, signalling and gene expression in muscle and endothelial cells warrant further study.

Introduction

Insulin resistance is defined as an impaired ability of insulin to produce its usual biological effects at a cellular, organ or whole-body level. In humans, it is characteristically associated with such disorders as obesity, Type 2 diabetes, essential hypertension, endogenous hypertriglyceridaemia and coagulation abnormalities [1,2]. In addition, in part because of the presence of these disorders and in part independently of them, insulin resistance is a risk factor for premature coronary heart disease [2]. Despite this observation, insulin resistance has been primarily studied in skeletal muscle and the basis for its independent relation to coronary artery disease is poorly understood.

In this brief review, we will examine data from our laboratories and others suggesting that common mechanisms could explain the insulin resistance in skeletal muscle and the early endothelial cell damage that is thought to initiate atherosclerotic vascular disease in diabetes. More specifically, the effects of excess glucose and fatty acids, such as are found in patients with diabetes, will be discussed. Excesses of these fuels alone and/or in combination have been shown to cause insulin resistance in skeletal muscle [3–5] and cell damage in cultured endothelium [6–10]. We will explore here the notions (i) that they produce these effects in both tissues by increasing fatty acid esterification and secondarily setting in motion a series of events that include protein kinase C (PKC) activation, increases in oxidative stress and activation of the inhibitor κB kinase (IKK)/nuclear factor κB (NFκB) system, and (2) that activation of the fuel-sensing enzyme AMP-activated protein kinase (AMPK) prevents these events from occurring.

Skeletal muscle: malonyl-CoA, fatty acid oxidation and esterification, and insulin resistance

In response to an increase in glucose availability, many cells, including those of skeletal muscle, inhibit the oxidation of fatty acids. In contrast, glucose deprivation or an increase in energy expenditure activates fatty acid oxidation. At a cellular level these events are regulated at least in part by changes in the activity of acetyl-CoA carboxylase (ACC), the enzyme that catalyses the synthesis of malonyl-CoA [11–13]. Malonyl-CoA is an allosteric inhibitor of carnitine palmitoyltransferase 1, which controls the transfer of long-chain fatty acyl-CoA from the cytosol into mitochondria where they are oxidized [13,14]. Thus, increases in malonyl-CoA concentration inhibit fatty acid oxidation and presumably lead to an increase in its esterification, and decreases in malonyl-CoA have the...
opposite effect. Numerous studies have shown that insulin resistance in skeletal muscle of rodents is associated with sustained elevations in the level of malonyl-CoA under a variety of conditions (Table 1) [15]. Where examined, the muscle of these rodents has also exhibited increases in diacylglycerol and triglyceride (triaclylglycerol) content and either PKC activity or the abundance of specific membrane-associated PKC isoforms [13]. Likewise, changes in PKC distribution have been observed in insulin-resistant, obese [16] and diabetic [17] humans.

We recently assessed in normal adult men the cellular events associated with the impaired ability of insulin to stimulate glucose uptake into muscle when plasma free (non-esterified) fatty acid (FFA) levels are elevated by infusing fat and heparin during a euglycaemic/hyperinsulinaemic clamp. Prior studies had shown that the concentration of malonyl-CoA is increased in both human and rat muscle during the clamp [18]. After 6 h of the fat infusion (but not after 2 h), we observed impaired insulin-stimulated glucose utilization, 3-fold increases in diacylglycerol mass and membrane-associated PKC activity and a selective translocation of associated PKC isoforms [13]. Likewise, changes in PKC distribution have been observed in insulin-resistant, obese [16] and diabetic [17] humans.

Table 1 | Lipid metabolites and PKC in muscle of insulin-resistant rodents

<table>
<thead>
<tr>
<th>Model</th>
<th>Plasma</th>
<th>Muscle</th>
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<tbody>
<tr>
<td></td>
<td>Insulin</td>
<td>Glucose</td>
</tr>
<tr>
<td>KKAy mouse</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>la/la rat</td>
<td>+</td>
<td>0/+</td>
</tr>
<tr>
<td>Glucose-infused rat</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fat-fed rat</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GK rat</td>
<td>0/+</td>
<td>+</td>
</tr>
<tr>
<td>Denervated rat</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fat-glucose infused rat</td>
<td>ND</td>
<td>ND</td>
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</tbody>
</table>

Compiled from data from the author’s laboratory, and the laboratories of Farese, Turinsky, Kraegen, Biden and Schulman. TG, triglycerides; DAG, diacylglycerol; LCFA CoA, long-chain fatty acyl-CoA. An increase in concentration is indicated by +. ND, not done. Recent studies suggest that alterations in the distribution of PKC and/or PKCα occur in the muscle of most of these rodents.

discussed elsewhere [19], the insulin resistance could result acutely from direct effects of PKC or IKKβ on events in the insulin signalling cascade and chronically from NFκB-mediated expression of various genes [e.g. tumour necrosis factor α (TNFα)]. Another potential player in this scheme are reactive oxygen species (ROS), which in large amounts have been shown to cause insulin resistance in adipose and other tissues [24]. In this context, activation of PKC by glucose [7–9] and fatty acids [8] has been implicated in ROS generation in some cells including endothelium, and ROS in turn have been implicated in NFκB activation [25], as has PKC [26,27].

The importance of esterified lipid deposition in insulin resistance has been highlighted by recent observations showing a close correlation between intracellular triglyceride content and an impaired ability of insulin to exert its usual biological effects in muscle and liver in vivo [14]. In the lipodystrophic mouse (i.e. with no adipose tissue), an extreme example of such fat deposition, very marked insulin resistance in vivo was reversed when the increased liver and muscle triglyceride content of these animals was diminished following adipose tissue implantation [28]. Likewise, a similar decrease in insulin resistance has been observed in lipodystrophic humans, when their lipid stores are diminished by an infusion of leptin [29], which has also been reported to increase AMPK activity in skeletal muscle [30]. We would speculate that these correlations between triglyceride stores and insulin resistance reflect the influence of FFA derived from these stores on diacylglycerol synthesis and/or ROS generation.

AMPK

AMPK is activated in skeletal muscle by an increase in energy expenditure (exercise) and by fuel deprivation and hypoxia [12,13]. When activated, AMPK phosphorylates and inhibits ACC and phosphorylates and activates malonyl-CoA decarboxylase, leading to a decrease in the concentration of malonyl-CoA and an increase in fatty acid oxidation [31]. In addition, it decreases fatty acid incorporation into
glycerolipids, either secondary to its effect on fatty acid oxidation or by virtue of the fact that in some tissues it phosphor-ylates and inhibits sn-glycerophosphate acyltransferase, the first committed enzyme in diacylglycerol and triglyceride synthesis [31,32]. In this context, it is of interest that in fast-fed rats a single injection of 5-amino-4-imidazolecarboxamide riboside (AICAR) [33] or prior exercise [22], both of which transiently increase AMPK activity, cause sustained decreases in malonyl-CoA and increase insulin-stimulated glucose uptake in muscle 24 h later. AICAR has also been shown to increase insulin-stimulated glucose uptake by the muscle of control rats [33,34]; however, malonyl-CoA and PKC were not assayed in these studies.

**Endothelial cells**

**The malonyl-CoA fuel-sensing and signalling mechanism**

Until recently most investigators believed endothelial cells obtained the great majority of their energy from glycolysis and that fatty acid oxidation was of limited importance [35]. In keeping with an earlier study suggesting otherwise [36], we found that high rates of fatty acid oxidation are demonstrable in human umbilical vein endothelial cells (HUVEC) when they are provided with carnitine [37]. Indeed, we were able to demonstrate that when these cells were deprived of glucose for several hours (i.e. no ATP derived from glycolysis), a 3-fold increase in AMPK activity and fatty acid oxidation occurred, and the concentration of malonyl-CoA was diminished by 80%. Even more striking, no decrease in ATP content was observed, indicating that the cells could maintain their energy state when fatty acids were essentially their sole fuel. Overall these findings suggest that a malonyl-CoA fuel-sensing mechanism, similar to that of muscle, operates in the endothelial cell and that it is regulated by AMPK.

**Hyperglycaemia, FFA and endothelial cell damage**

In later studies, we observed that incubation of HUVEC in a hyperglycaemic (30 mM glucose) medium for 72 h led to an increase in apoptosis (Tunel staining) that was preceded at 24 h by an elevated concentration of malonyl-CoA, inhibition of fatty acid oxidation, increased diacylglycerol synthesis, an increase in caspase 3 activity and an impaired ability of insulin at a physiological concentration (150 μ-units/ml = 10^−9 M) to activate Akt [9]. AMPK activity was not diminished at this time; however, the addition to the incubation medium of the AMPK activator AICAR both increased AMPK activity several-fold and completely prevented all of the abnormalities observed at 24 h. Likewise, infection of the HUVEC with a constitutively active AMPK-adenoviral vector for 24 h prevented the increase in caspase 3 activity. When the HUVEC were incubated in media containing a normal concentration of glucose (6 mM), but excess fatty acid (palmitate, 0.2–0.4 mM), an even greater increase in apoptosis was observed, and this too was inhibited by AICAR. A hypothetical common mechanism to explain how hyperglycaemia and/or excess FFA lead to both insulin resistance in skeletal muscle and endothelial cell damage in diabetes is shown in Figure 1.

**Other factors contributing to endothelial cell damage**

A substantial body of research has implicated inflammation in the pathogenesis of the early endothelial cell damage that eventually leads to atherosclerosis [38,39]. In keeping

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**Figure 1** Hypothetical common mechanism to explain how hyperglycaemia and/or excess FFA lead to both insulin resistance in skeletal muscle and endothelial cell damage in diabetes

Available data suggest that AMPK activation affects this scheme at multiple sites. See text for details. ICAM, intercellular cell adhesion molecule; RAGE, receptor for advanced glycosylation end products; DAG, diacylglycerol.
with this finding, incubation of endothelial cells with excess glucose has been shown to lead to increases in cell Ca^{2+} and reactive O₂ species [6–8] and NFκB activation as assessed by an increase in its translocation to the nucleus [6,7]. A similar effect of fatty acids on ROS production has been observed [8], and where studied it appeared to be PKC-dependent [7,8].

**Effects of AMPK**

In keeping with these earlier reports, we found that incubation with a hyperglycaemic medium increased DCHF fluorescence (cellular H₂O₂) within 24 h, and that incubation with 0.4 mM palmitate increased both DCHF fluorescence, to a substantially greater extent than did glucose, and lipid peroxides (8-epi-PGF₂α) [40]. Furthermore, the increase in ROS caused by both fuels was inhibited by AICAR. In contrast to the earlier studies [6,7], we found only a modest decrease in inhibitor αB degradation and no change in either NFκB translocation or transactivation (NFκB-mediated gene expression) after 16–24 h incubation in a hyperglycaemic medium, but we observed large alterations in all of these parameters in cells incubated with palmitate [10].

Incubation with AICAR, and in a few studies infection with an constitutively active AMPK-adenoviral vector, prevented the increases in NFκB translocation and transactivation [10].

**Activation of AMPK by oxidant stress**

Interestingly, in addition to the activation of AMPK attenuating the generation of ROS caused by elevated glucose and fatty acids, we have obtained evidence that oxidants themselves can activate AMPK. For instance, we have found that incubation with H₂O₂ leads to large increases in AMPK activity in HUVEC (J. Keaney and Y. Ido, unpublished work). Recent studies also indicate that other oxidants, in particular peroxynitrite, are involved in apoptosis and adhesion-molecule expression in endothelial cells exposed to a high concentration of glucose [41], due at least in part to generation of ROS by endothelial nitric oxide synthase [42]. In testing direct effects of peroxynitrite on endothelial cells, we observed phosphorylation of ACC and AMPK, indicating activation of AMPK. Furthermore, the phosphorylation of nitric oxide synthase (a direct downstream event mediated by AMPK), as well as an increase in its catalytic activity, were prevented by expressing dominant negative AMPK [43]. These findings suggest that oxidants stimulate AMPK, and that this could contribute to its activation in response to metabolic and possibly other stresses in the endothelial cell.

**TNFα-induced NFκB activation**

In keeping with the prior work of others, we found that the proinflammatory cytokine TNFα rapidly increased the translocation, DNA binding and transactivation of NFκB in HUVEC [44]. In addition, the expression of a number of NFκB-mediated genes was subsequently increased, including those for intercellular cell adhesion molecule (ICAM), receptor for advanced glycosylation end products (RAGE) and TNFα itself, all of which are increased in damaged endothelium. Interestingly, incubation with AICAR prevented these alterations in gene expression even though it failed to prevent the changes in NFκB translocation and DNA binding. This suggests that AMPK has a direct effect on the nucleus. In keeping with such a notion, TNFα-induced increases in NFκB acetylation in COS cells were blocked by transfection with constitutively active AMPK. Preliminary studies suggest that phosphorylation of the transcriptional co-activator p300 by AMPK accounted for this action [44]. Irrespective of the precise mechanism, these findings indicate that AMPK may block the adverse effects of hyperglycaemia and excess FFA by more than one mechanism.

**Other considerations**

Other theories put forth to explain both insulin resistance and the complications of diabetes have implicated hexosamines [45], advanced glycosylation end products [7] and lipotoxicity [46]. Of these, the lipotoxicity theory, with its emphasis on glyceroipid accumulation, apoptosis and the protective effect of leptin, most closely resembles the malonyl-CoA/AMPK hypothesis. It seems likely that research examining these two theories will ultimately become complementary.

**Clinical implications**

The findings reviewed here raise the possibility that AMPK activation could be a useful target for the prevention and treatment of disorders associated with insulin resistance, including atherosclerotic vascular disease [47]. Indirect evidence from human studies supports this notion. Thus exercise has long been known to increase insulin sensitivity in skeletal muscle [48] and, when performed regularly, it is associated with a decreased risk of heart attack [49]. Although the latter effect is generally attributed to a decrease in risk factors for coronary heart disease, such as dyslipidaemia and hypertension, the possibility that AMPK activation in the endothelium plays a role requires exploration. In this context, we have recently observed that 30 min after a treadmill run, AMPK activity is substantially increased in rat liver and adipose tissue, indicating that the exercise-induced changes in this enzyme are not confined to muscle [31].

A second line of evidence relates to findings with the widely used anti-diabetic agent metformin. Metformin has long been known to lower blood glucose levels in patients with Type 2 diabetes by increasing insulin sensitivity. In 1998, the United Kingdom Prospective Diabetes Study (UKPDS) [50] reported that in contrast to other anti-diabetic therapies (e.g. insulin or sulphonylureas) metformin diminishes the incidence of cardiovascular disease when used as a monotherapy in obese patients with Type 2 diabetes. In the past year, Zhou et al. [51] demonstrated that metformin activates AMPK and causes AMPK-mediated changes in gene expression in rat liver cells. In addition, Goodyear and her co-workers [52] have found...
References