The AMP-Activated Protein Kinase: Role in Regulation of Skeletal Muscle Metabolism and Insulin Sensitivity

Gregory R. Steinberg* and Sebastian Beck Jørgensen

St. Vincent's Institute of Medical Research and Department of Medicine, University of Melbourne, Melbourne, Australia

Abstract: Over the past decade, an epidemic of obesity has developed throughout the Western World. In recent years, significant interest has focused on the role of the AMP-activated protein kinase (AMPK) as a potential therapeutic target for the treatment of obesity and type 2 diabetes and is such the focus of this review. Specifically, the potential role of AMPK in skeletal muscle metabolism as it relates to the insulin sensitizing effects of exercise and the hormones, leptin, adiponectin, ciliary neurotrophic factor and interleukin-6 are discussed. We caution that despite the convincing associations between the activation of AMPK signalling and the restoration of insulin sensitivity, future studies in genetic models of AMPK deficiency or constitutive activation within skeletal muscle are needed to evaluate the quantitative role of AMPK and to validate whether strategies designed to activate skeletal muscle AMPK may be important for regulating whole-body insulin sensitivity.

Key Words: AMPK, obesity, fatty acid oxidation, glucose uptake, adiponectin, leptin, ciliary neurotrophic factor, metformin, exercise.

INTRODUCTION

Obesity and type 2 diabetes are causally linked through their association with the development of skeletal muscle insulin resistance. Over the past several years it has become increasingly apparent that elevated storage of lipids or lipid derivates within skeletal muscle is central to insulin resistance in this tissue [1]. Recent work has demonstrated that obesity and excess lipid accumulation within skeletal muscle can trigger the activation of a serine/threonine kinase cascade involving activation of protein kinase C (PKC) [2, 3], IKappaB kinase-β (IKK-β) [4, 5] and c-jun terminal amino kinase (JNK) [6]. AMP-activated protein kinase (AMPK) is pivotal in the regulation of skeletal muscle fatty acid- and glucose metabolism. AMPK activation results in increasing rates of fatty acid oxidation by phosphorylating acetyl-CoA carboxylase (ACC) leading to a reduced production of malonyl-CoA and increased long-chain fatty acyl CoA flux into the mitochondria *via* carnitine palmitoyl transferase-1 (CPT-1) [7]. Furthermore, activation of AMPK also increases translocation of the glucose transporter GLUT4 to the plasma membrane thus increasing muscle glucose uptake. This review discuses the role of AMPK in the regulation of skeletal muscle insulin sensitivity in obesity.

AMPK STRUCTURE AND REGULATION

The name AMPK was adopted in 1987 [8] but the enzyme was detected much earlier as a protein kinase contaminating preparations of ACC [9] and HMG CoA reductase [10], key regulatory enzymes in fatty acid and cholesterol synthesis, respectively. When the identity of AMPK was revealed [11] it was found to be a homolog of the yeast metabolic stress sensing kinase, snf1p kinase [12]. AMPK is present in all tissues as an αβγ heterotrimer and its expression is regulated by multiple genes encoding each of the subunits (α1, α2, β1, β2, γ1, γ2, γ3) each with tissue specific expression and activity [13]. In skeletal muscle, the majority of AMPK complexes contain both α2 and β2. Of these α2/β2 containing complexes, ~20% are associated with γ3 while the remaining are primarily associated with γ1 [14]. In contrast, in liver tissue the α1 and α2 isoform are equally expressed and β1 and γ1 are the major regulatory isoforms [15]. In skeletal muscle, the activity of AMPK is primarily dependent on phosphorylation of Thr172 in the activation loop of the α subunit by the upstream kinase LKB1 [16, 17]. Mice lacking LKB1 have substantially reduced levels of AMPKα2 activity and ACCβ phosphorylation (Fig. **1**) [18]. The β (β1 or β2) and the γ (γ1, γ2 or γ3) subunits are essential for optimum enzyme activity and participate in subcellular targeting and regulatory functions, respectively. The β subunit can be myristylated, which is believed to anchor AMPK to membranes, and contains an N-amylase-like domain targeting AMPK to glycogen [19, 20] as well as the αγ subunit-binding-sequence (SBS) (β186-270) responsible for the formation of the AMPK αβγ heterotrimer [21]. AMP further activates AMPK allosterically *via* the γ subunit, which contains four CBS sequence repeats that form two Bateman modules responsible for binding 2 moles of AMP or ATP where AMP binds with a considerable higher affinity than ATP [22, 23]. Binding of AMP facilitates phosphorylation of Thr172 by LKB1 [16, 17] and suppresses dephosphorylation by protein phosphatases PP2A and PP2C *in vitro* (Fig. **1**) [24]. Mutations in the AMPK γ subunits give rise to glycogen storage disease in both humans [25] and pigs [26], but interestingly, transgenic mice that harbour this mutation are protected from diet-induced insulin resistance - an effect believed to be mediated by increased lipid oxidation [27].

SKELETAL MUSCLE AMPK EXPRESSION AND ACTIVITY IN OBESITY

In mammals, skeletal muscle is the primary tissue contributing to whole-body energy expenditure making it a primary target of therapies aimed at reducing excessive fat mass. Skeletal muscle from obese humans display suppressed rates of fatty acid oxidation [28-30] combined with increased rates of fatty acid uptake [31] and esterification [32]. This dysregulation of metabolism in resting muscle is alleged to be the principal suspect causing accumulation of detrimental intramuscular lipids and lipid derivates believed to be central in the development of skeletal muscle insulin resistance [1]. Therefore, due to the vital role that AMPK plays in regulating especially lipid metabolism (as reviewed in [33]), it has been hypothesized that defects in AMPK signalling may contribute to these abnormalities.

In both genetic and high-fat dietary models of rodent obesity, basal AMPK activity is modestly reduced in some [34, 35] but not all studies [36-38]. Similarly, we [39] have demonstrated in human muscle that AMPK protein expression and activity are unaltered with obesity while others have demonstrated modest reductions [40]. In human type 2 diabetic skeletal muscle AMPK expression and activity are unaltered relative to subjects with a similar body mass [41, 42]. The sensitivity of AMPK to allosteric activation by AMP [39] and LKB1 activity/expression [43] are also unchanged in obesity and in obese type 2 diabetic skeletal muscle, suggesting that the AMPK system is intact in obesity. In support of these findings, treatment of skeletal muscle with the AMPK activator 5-amino-imidazole--carboxamide-ribonucleoside (AICAR, which is converted to ZMP and acts as an AMP analogue to activate AMPK, Fig. **2**) has been demonstrated to increase AMPK activity to a similar degree in both obese and type 2 diabetic skeletal muscle from

*Address correspondence to this author at the St. Vincent's Institute, 9 Princes St., Fitzroy, Victoria, 3065, Australia; Tel: 61 3 9288-2480; Fax: 61 3 9416 2676; E-mail: gsteinberg@svi.edu.au

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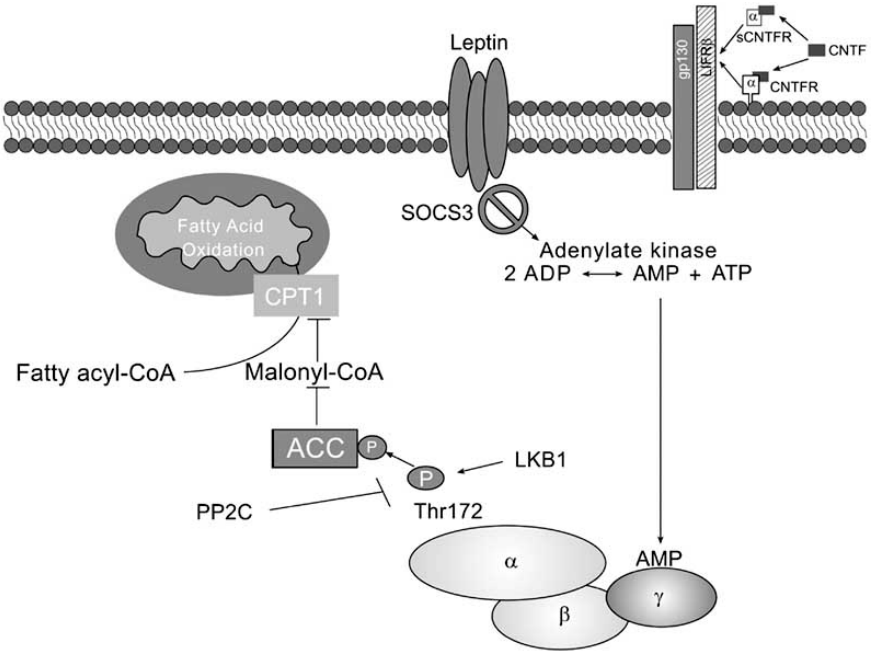


Fig. (1). Regulation of AMPK in obesity and effects on fatty acid oxidation highlighting the potential role of CNTF.

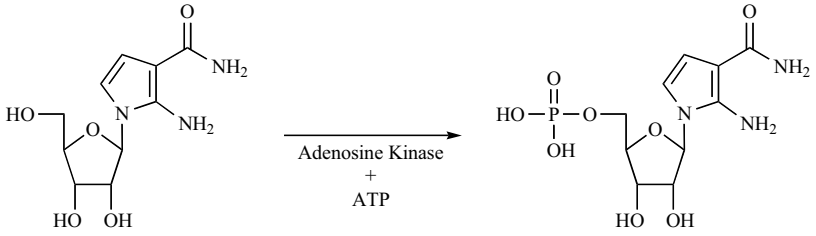
rodents [38, 44, 45] and humans [39, 46-48] relative to lean controls.

THE REGULATION OF AMPK IN SKELETAL MUSCLE BY CONTRACTION AND EXERCISE

Skeletal muscle is a highly dynamic tissue which is exposed to large changes in energy demand in response to exercise (>100 fold from rest to exercise) and it seems reasonable to speculate that AMPK may play an important role as a metabolic safe guard in this tissue as previously reviewed [49]. It is well documented that exercise is both preventative for - and improves symptoms associated with the metabolic syndrome [50, 51]. These effects may be attributed to the activation of AMPK signalling as there is tight coupling between exercise intensity and AMPK activity [52-57]. The activation of AMPK by exercise is greatly enhanced when muscle glycogen (a principle substrate of contracting skeletal muscle) is reduced by a "relief of inhibition mechanism" although the causal relationship is currently incompletely understood [54, 58, 59]. While LKB1 phophorylates and activates the entire phylogenetically related AMPK enzyme family [60] only AMPK is activated by exercise [61]. Importantly, in mice lacking skeletal muscle LKB1, contraction completely fails to activate AMPK α2 [18]. The importance of AMPK in the maintenance of energy charge during contraction has been highlighted by studies in AMPK α2 null mice and in LKB1 deficient mice which have reduced AMPK signalling in response to exercise/contraction correlating with an elevated AMP:ATP ratio relative to wild-type controls [18, 62]. Muscle specific AMPK dominant negative mice also have reduced exercise tolerance during voluntary running but it is unknown whether this is due principally to effects in skeletal muscle or secondary effects due to reduced AMPK in the heart [63]. Taken together, these studies suggest that AMPK is critical for the maintenance of skeletal muscle energy balance during exercise. Importantly, the effects of contraction on AMPK signalling are maintained in human type 2 diabetes [42] and rodent obesity [34] suggesting that pharmacological agents that target the activation of AMPK may have valuable effects on the regulation of glucose uptake, glycogen metabolism, fatty acid metabolism or mitochondrial function as will be discussed below.

AMPK, EXERCISE AND MUSCLE GLUCOSE UPTAKE

During exercise and/or skeletal muscle contraction glucose uptake is increased, an effect which is independent of the proximal part of the insulin signalling pathway (for review see [49]). The role of AMPK in regulating exercise stimulated glucose uptake has been



AICAR or Acadesine
(5-Aminoimidazole-4-Carboxamide-1-β-D-Ribofuranoside)

AICAR-monophosphate
(5-Aminoimidazole-4-Carboxamide-1-β-D-Ribofuranozyl 5'-Monophosphate)

Fig. (2). Structure of 5-aminoimidazole--carboxamide-ribonucleoside (AICAR) (CID: 65110).

an area of considerable interest in recent years due to the therapeutic potential of stimulating glucose uptake *via* alternative pathways in insulin resistant skeletal muscle. Many studies have examined the role of AMPK in mediating glucose uptake utilizing the pharmacological AMPK activator AICAR in resting (non contracting) skeletal muscle. These studies found that AICAR rapidly increased glucose uptake [64-67] at least in part by increasing GLUT4 translocation to the plasma membrane [68, 69] but not to T-tubules [70]. Similarly, studies in skeletal muscle cells have shown that a constitutively active AMPK mutation increased glucose uptake while a dominant negative AMPK inhibited AICAR-stimulated uptake [71]. Earlier studies have indicated that AICAR [67, 72], but not contraction [73], stimulated glucose uptake may be dependent on nitric oxide synthase (NOS), although these data are equivocal [74]. More recent observations using several transgenic mice strains with impaired AMPK signalling suggest that AS160, a Rab GTPase-activating protein, may provide a mechanism linking AMPK signalling to glucose uptake [75, 76].

Based on the above studies demonstrating positive associations between muscle contraction, AMPK activity and glucose uptake, it was somewhat surprising that in transgenic AMPK dominant negative mice [77, 78] and AMPK α2 null animals [79] that *ex vivo* contraction-stimulated glucose uptake was either normal [78, 79] or only moderately [77] reduced. In contrast, muscle specific LKB1 deficient mice which lack the ability of AMPK to be phosphorylated/activated display an impaired ability to stimulate both contraction and AICAR-stimulated glucose uptake, suggesting that LKB1 may be upstream of other important regulators of glucose uptake in addition to AMPK [18]. Even though all of the above models have some degree of residual AMPK signalling, these findings collectively point towards a system depending on more than one signalling pathway and alternative pathways could be regulated by calcium sensitive molecules such as CaMK and PKC isoforms (for review see [80])

It is well documented that a single exercise bout is capable of improving skeletal muscle insulin sensitivity in patients with type 2 diabetes long after AMPK signalling has returned to resting levels [81]. This may mean, that if AMPK is implicated in improving skeletal muscle insulin sensitivity the mechanism must be preserved after AMPK signalling has ceased [82]. One potentially important mechanism involves the phosphorylation by AMPK of the insulin receptor substrate-1 (IRS-1) at Ser789 which in turn increases IRS-1 associated PI-3 kinase in C2C12 muscle cells [83]. However, it still remains unknown whether AMPK phosphorylates IRS-1 at Ser789 in differentiated human skeletal muscle and if this interaction leads to improved muscle insulin sensitivity *in vivo*. A more distal step in the insulin-dependent regulation of glucose transport involves AMPK serine phosphorylation of AS160 [84] While AS160 was initially shown to be essential in regulating insulin dependent GLUT4 trafficking and glucose uptake in adipocytes [84, 85] subsequent studies showing thatAS160 serine phosphorylation was also increased with insulin and contraction in muscle [86]. This suggested that AS160 is a point of convergence between AMPK, and insulin-signalling pathways that may result in additive effects on muscle glucose uptake [75, 76, 86].

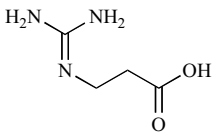
A second mechanism by which AMPK may increase glucose uptake involves the observation that a single exercise bout enhances GLUT4 mRNA and protein expression in human muscle [87]. In support of this concept, we recently demonstrated translocation of AMPK α2 to the nucleus with exercise in skeletal muscle [88, 89], and increased phosphorylation of myocyte enhancer factor (MEF) -2 an important factor regulating GLUT4 gene expression [90]. Studies in rodent skeletal muscle also support this concept as chronic pharmacological activation of AMPK by AICAR increases GLUT4 protein expression, similar to exercise training, an effect not observed in AMPKα2 null mice [91-94]. The infection of rodent [71] or primary human [88] skeletal muscle cells with a constitutively active AMPK adenovirus also increases GLUT4 expression while a dominant negative AMPK inhibits the effects of AICAR [71]. Taken together these data support the concept that AMPK is a positive regulator of GLUT4 gene transcription and expression. It should be noted that the regulation of GLUT4 expression in muscle is complex and that in addition to AMPK is also dependent on calcium sensitive pathways. Indeed, studies of cultured rodent muscle cells and incubated muscles suggest that CaMK isoforms regulate GLUT4 protein expression *via* MEF2 [96]. Indeed, recent data by us [62] and others [97] in AMPKα2 null mice and AMPK dominant negative mice, respectively, indicate that AMPK is not obligatory for the effects of exercise on GLUT4 expression. The mechanisms by which AMPK activation increases GLUT4 expression is not entirely clear but an important role of MEF2A, MEF2D and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) has been demonstrated following AMPK activation by either exercise [98, 99] or AICAR treatment [62, 96]. Future studies examining direct effects of AMPK on transcription factors regulating GLUT-4 transcription are warranted.

AMPK EXERCISE AND FATTY ACID OXIDATION

During contraction and exercise, the activation of AMPK in skeletal muscle increases the phosphorylation of acetyl CoA carboxylase β (ACCβ) at Ser222 which results in reductions in malonyl-CoA [64, 100-104]. Reductions in malonyl-CoA in turn increases CPT1-mediated transport of long-chain fatty-acids into the mitochondria matrix for β-oxidation [105-110]. Despite findings that AICAR-induced AMPK activity increases muscle fatty acid oxidation [64] and that AMPK activation directly phosphorylates ACCβ and reduces malonyl-CoA, there exists no direct evidence demonstrating a critical role of AMPK or ACCβ phosphorylation in the regulation of exercise stimulated fatty acid oxidation. Indeed, recent studies by Raney and colleagues [111, 112] imply the importance of alternative pathways as there is significant upregulation of fatty acid oxidation in the absence of AMPK activity during low intensity muscle contraction. Similarly, Smith *et al*. [113] have illustrated additive effects of contraction and AICAR on fatty acid oxidation suggesting parallel pathways may be important for regulating fatty acid oxidation during contraction. Therefore, based on findings discussed previously demonstrating that AMPK is not critical for contraction stimulated glucose uptake, similar studies examining fatty acid metabolism are fundamentally important to establish a causative role of AMPK in regulating exercise-stimulated fatty acid oxidation.

AMPK, EXERCISE AND MITOCHONDRIAL BIOGENESIS

Aging is associated with increased insulin resistance and reductions in muscle oxidative capacity [114]. Insulin resistant offspring of patients with type 2 diabetes also display impaired mitochondrial content and activity, which is associated with dysregulation of intramyocellular fatty acid metabolism and adiponection signalling [115]. It has been suggested that lifestyle changes, especially physical activity, may reverse these trends. For instance, exercise-training increases peak oxygen uptake, content of mitochondriogenic transcriptional modulators as well as mitochondrial enzymes and density in skeletal muscle of both aged individuals and type 2 diabetic subjects in addition to healthy young controls [49]. Winder *et al*. [92] were the first to demonstrate the potential role of AMPK in mediating mitochondrial biogenesis as chronic AICAR treatment was found to increase mitochondrial enzymes to a similar degree as endurance exercise training in rat muscle. This effect was later confirmed by Bergeron *et al*. [116] who activated AMPK chronically using beta-guanidinopropionic acid (β-GPA) (Fig. **3**), a creatine analog, that reduces the intramuscular ATP: AMP ratio and phosphocreatine concentrations. This relationship was proven causal as β-GPA failed to induce mitochondrial biogenesis in AMPK dominant negative mice [117]. In line with these observations, we have



β-GPA or β-guanadinopropionic acid
(3-(diaminomethylideneamino)propanoic acid)

Fig. (3). Structure of beta-guanidinopropionic acid (β-GPA) (CID: 67701).

recently demonstrated that untrained muscle from AMPK α2 null mice has decreased expression of PGC-1α, hexokinase II (HKII) and several mitochondrial proteins [62, 94]. Interestingly despite differences basally, exercise training with impaired AMPK signalling does not effect exercise-induced mitochondrial protein expression [94] suggesting that while under resting conditions AMPK is critical for mitochondrial biogenesis during exercise increases in intramyocellular calcium may be sufficient [93, 96, 118].

THE ROLE OF HORMONES IN REGULATING SKELETAL MUSCLE AMPK

One of the most exciting developments in the field of AMPK biology has been the finding that AMPK is regulated not only by exercise but also hormonally thereby vastly increasing the potential role of the kinase in regulating energy metabolism.

LEPTIN

Over a decade ago, seminal studies identified the protein product of the *ob* gene, leptin, [119] and demonstrated that recombinant leptin injection in *ob/ob* mice restored euglyceamia independent of, and before, appreciable weight-loss, suggesting a role of leptin in regulating skeletal muscle insulin sensitivity [120-123]. Leptin signalling is mediated by short and long leptin receptor isoforms, both of which are expressed in skeletal muscle [124]. In rodents, leptin acutely [125] and chronically [126] stimulates skeletal muscle fatty acid oxidation, resulting in reduced intramuscular triacylglycerol storage [125, 127, 128]. The acute [129] and chronic [130] effects of leptin on fatty acid metabolism are attributed to the activation of AMPK, which stimulates fatty acid oxidation by phosphorylation and inhibition of ACC. Leptin directly activates AMPK, stimulating fatty acid oxidation acutely in skeletal muscle by altering the AMP:ATP ratio [129]. The chronic effects of leptin on AMPK are mediated by the central nervous system stimulation of α-adrenergic signalling [129]. Although leptin has pronounced effects in rodents fed a high carbohydrate chow-diet [129] and rodents with lipodystrophy [131], we and others have demonstrated the presence of leptin resistance following high-fat feeding [127, 132]. Similarly, human obesity is characterized by elevated levels of circulating leptin [133] and we have demonstrated a blunting of leptin's effects on fatty acid oxidation in skeletal muscle from obese humans suggesting the presence of skeletal muscle leptin resistance [39]. Importantly we have recently demonstrated that AMPK signalling by AICAR is not downregulated in skeletal muscle from obese individuals suggesting that factors upstream of AMPK may contribute to the development of leptin resistance [39].

A potential mediator of leptin resistance is the suppressor of cytokine signalling 3 (SOCS3). SOCS3 is a member of a family of proteins (SOCS1-SOCS7, and CIS) in which their central SH2 domains bind to phosphotyrosine residues in cytokine receptors [134]. Initial studies by Bjorbaek *et al*. [135] demonstrated the inhibition of leptin signalling *via* the signal transducer and activator of transcription (STAT)-3 in hypothalamic nuclei was mediated by SOCS3 binding of Tyr985 of the leptin receptor [136, 137]. Both SOCS3 hypothalamic specific null mice [138] and SOCS3 mice with haploinsufficiency [139] have enhanced leptin sensitivity and are resistant to diet-induced obesity. In skeletal muscle, we have demonstrated that SOCS3 is up-regulated following high-fat feeding [140], an effect that is associated with skeletal muscle leptin resistance as demonstrated by the blunted ability of leptin to stimulate palmitate oxidation and AMPKα2 activity [48, 141]. The overexpression of SOCS3 *via* adenovirus-mediated infection in skeletal muscle cells, to a similar degree as observed in skeletal muscle of mice fed a high-fat diet, or in obese humans, inhibited leptin but not AICAR activation of AMPK α2 activity and ACCβ phosphorylation [48]. These data demonstrate that SOCS3 inhibits leptin activation of AMPK and suggest that the down-regulation of leptin signalling in skeletal muscle may contribute to the aberrant regulation of fatty acid metabolism observed in obesity.

CILIARY NEUROTROPHIC FACTOR (CNTF)

Unfortunately, from a pharmacological perspective, activators of AMPK such as AICAR, which are needed at mM concentrations to induce maximal effects on fatty acid oxidation, are not effective therapies to bypass leptin resistance *in vivo*. Therefore there is a specific need for the development of therapeutics that are effective at nM-pM concentrations. To address this, we examined the role of ciliary neurotrophic factor (CNTF), a 22 kDa IL-6 related cytokine, that was serendipitously found during clinical trials for the treatment of amyotrophic lateral sclerosis to induce severe anorexia and weight loss [142]. While CNTF-induced weight loss was initially attributed to a cachectic response, subsequent studies in diet-induced [143] and genetic (*ob/ob*, $MC4R^{-/-}$) obesity [144, 145] demonstrated that low doses of CNTF and the CNTF homologue, Axokine® ($CNTF_{AX15}$), induced weight loss without causing the typical deleterious effects of other related cytokines. Indeed, recent clinical trials demonstrated the efficacy and safety of $CNTF_{AX15}$, as prolonged treatment in obese human subjects induced a modest decrease in body weight without adverse side effects [146]. The weight loss effects of CNTF were initially attributed primarily to reductions in food intake, mediated by hypothalamic STAT3 phosphorylation and hypothalamic neurogenesis [147, 148], but since some effect of CNTF on weight loss is still present in calorically matched mice we hypothesized that CNTF may have a potentially important role in skeletal muscle. We have recently demonstrated that CNTF increases fatty acid oxidation in isolated skeletal muscle, effects that are associated with activation of AMPK and are mediated by signalling *via* CNTF receptor-α or the IL-6 receptor and inhibited by the expression of a dominant negative AMPK [37]. The administration of CNTF, at doses demonstrated to induce weight loss, increased AMPK activity, palmitate oxidation and stimulated the expression of mitochondrial oxidative genes in skeletal muscle while improving insulin stimulated glucose uptake. Further, this effect was maintained in leptin resistant high-fat fed mice which exhibited elevated SOCS3. Acutely, the stimulatory effect of CNTF on fatty acid oxidation reversed fatty acid induced insulin resistance, an effect observed both *in vivo* and *in vitro* and was inhibited in cells infected with a dominant negative AMPK, demonstrating that AMPK signalling is essential for the insulin sensitising effects of CNTF on skeletal muscle. Taken together, these data indicate that CNTF improves insulin sensitivity and induces weight loss by enhancing skeletal muscle fatty acid oxidation, a process that appears to be mediated by the activation of AMPK.

ADIPONECTIN

Adiponectin is secreted exclusively from adipose tissue and is an abundant plasma protein that is reduced with obesity and in patients with type 2 diabetes [149]. Structurally, adiponectin is related to the complement 1q family and contains a carboxyl-terminal globular domain and an amino-terminal collagenous domain [150]. Globular adiponectin (gAD) treatment has been shown to reverse skeletal muscle insulin resistance in models of genetic and diet-induced obesity [151-155]. These effects are attributed to the activation of AMPK [156, 157] by adiponectin receptor 1 (AdipoR1) [155, 158]. Two reports [159, 160] in human skeletal muscle and a recent study in primary myotubes [161] suggest that skeletal muscle

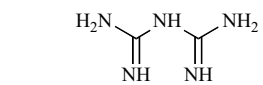
contains abundant levels of both AdipoR1 and AdipoR2. We recently investigated the effects of adiponectin on AMPK signalling in cultured skeletal muscle from lean, obese and obese type 2 diabetic skeletal muscle and found that surprisingly, obese subjects display a blunted activation of AMPK in response to adiponectin treatment an effect that is exasperated in obese individuals with type 2 diabetes [43]. Importantly, we demonstrated that this defect was not attributed to reduced adiponectin receptor expression, suggesting that post-receptor defects may contribute to suppressed adiponectin signalling in skeletal muscle from obese subjects, alike to the development of leptin resistance, a finding that was corroborated by Bruce *et al*. [162]. A recent study has shown that chronic activation of AMPK by adiponectin is a significant regulator of muscle mitochondrial biogenesis which suggests that a consequence of reduced adiponectin signalling may be impaired lipid oxidation and increased intramuscular lipid storage [163]. Taken together, these studies suggested that the combined effect of reduced adiponectin production [149, 164, 165] and reduced sensitivity [43, 162] may be important factors contributing to the suppressed rates of fatty acid oxidation observed with obesity.

INTERLEUKIN-6 (IL-6)

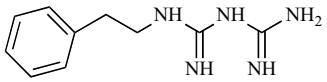
Obesity and type 2 diabetes are associated with a chronic inflammatory response that is characterized by abnormal production of the cytokines IL-6, resistin and tumour necrosis factor-α [166]. While there is compelling evidence for the negative effects of both resistin and TNF-α on insulin sensitivity, at least in cell culture and rodent studies, the role of IL-6 in the aetiology of insulin resistance is not fully understood. While IL-6 leads to hepatic insulin resistance, in rodents, its role in skeletal muscle and adipose tissue is equivocal and has been reported to either enhance [167, 168] or suppress [169] insulin stimulated glucose transport in myotubes or adipocytes. Several studies [170, 171] have reported that IL-6 can increase skeletal muscle fatty acid oxidation, an effect that is associated with the activation of AMPK [172]. Supporting these observations, we have recently demonstrated that IL-6 infusion enhances the glucose rate of disappearance during a hyper-insulinemic euglycaemic clamp in humans suggesting enhanced muscle glucose uptake [173]. We moreover examined the effects of IL-6 in myotubes and found that the activation of AMPK by IL-6 is essential for increases in fatty acid oxidation and both basal and insulin stimulated glucose uptake, as infection of cells with a dominant negative AMPK inhibits these effects [173]. The mechanism by which IL-6 increases AMPK and enhances insulin stimulated glucose uptake are presently not understood but will be critical for our understanding of the mechanisms mediating IL-6 signalling in skeletal muscle.

COMPOUNDS TARGETING AMPK FOR THE TREATMENT OF TYPE 2 DIABETES

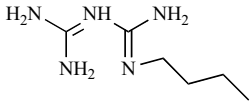
Metformin, one of the most commonly used drugs for the treatment and prevention of type 2 diabetes, is an oral biguanidine (Fig. **4**) that improves insulin sensitivity and reduces plasma glucose and lipids in patients with type 2 diabetes [50]. Although biguanidines became available for diabetes therapy in the 1950s the mechanism by which they improved insulin sensitivity remained elusive until findings demonstrated that AMPK was activated within skeletal muscle and liver [174, 175]. Importantly, the effects of metformin on AMPK signalling are preserved in skeletal muscle of subjects with type 2 diabetes [175]. The mechanism by which metformin activates AMPK is still largely unknown [176, 177]. However, metformin is known to inhibit respiratory chain complex I, leading to an inhibition of mitochondrial respiration [178-180] and accumulation of reactive nitrogen species. This accumulation activates the c-Src/PI3K pathway which might generate a metabolite or other molecules inside the cell to promote AMPK activation by the LKB1 complex [180]. Future studies are required to examine this possibility. Interestingly, metformin reduces inflammatory



Metformin
(1-diaminomethylidene-3,3-dimethyl-guanidine)



Phenformin
(2-N'-phenethylcarbamimidoyl-guanidine)

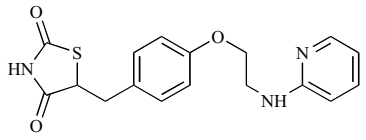


Buformin
(1-butyl-imidodicarbonimidic-diamide)

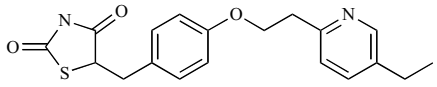
Fig. (4). Structure of the bi guanidines metformin (CID: 14219), phenformin (CID: 8249) and buformin (CID: 2468).

markers such as TNFα [181] and C-reactive protein [182] effects that are likely mediated by AMPK inhibition of NFκB transcription [183] suggesting a secondary mechanism by which AMPK may improve insulin sensitivity. In addition, metformin also induces modest weight loss [184], an effect primarily mediated by selective loss of adipose tissue stores which could be attributed to the activation of AMPK in skeletal muscle and enhanced fatty acid oxidation [185]. Importantly, Shaw et al. [5] have demonstrated that the insulin sensitizing effects of metformin are entirely dependent on LKB1 in liver therefore the physiological significance of metformin action in skeletal muscle may be questionable.

Another class of insulin sensitizing agents is the thiazolidinediones which target the PPARγ nuclear receptor in several tissues (TZD, Fig. **5**) [186]. TZDs activate AMPK in skeletal muscle cells by increasing cellular adenine nucleotide levels despite the relatively



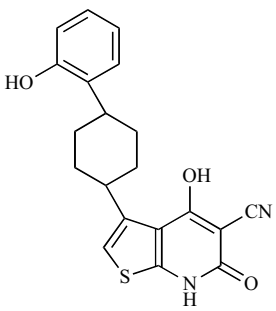
Rosiglitazone
(5-[[4-[2-(methyl-pyridin-2-yl-amino)ethoxy]phenyl]methyl]thiazolidine-2,4-dione)



Pioglitazone
(5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]thiazolidine-2,4-dione)

Fig. (5). Structure of rosiglitazone (CID: 77999) and pioglitazone (CID:4829).

low expression of PPARγ receptor expression in this tissue [176]. *In vivo*, chronic treatment with TZDs increases skeletal muscle and cardiac AMPK activity but it is difficult to discern whether this effect is direct or due to the potent effects of these agents in stimulating adiponectin production, which could also contribute to the observed increase in AMPK activity [187, 188]. Unlike TZDs and metformin which appear to activate AMPK in both muscle and liver the recently identified small molecule activator of AMPK, A-769662 (Fig. **6**), appears to be selective in it's activation of only liver AMPK since it is not taken up by muscle [189].



A-769662
(Nonnucleoside thienopyridone)

Fig. (6). Structure of small molecule AMPK activator A-769662 [187].

CONCLUSIONS

The rapid rise in the rates of obesity is an important health issue for the developed world. In subjects that are obese or suffer from type 2 diabetes, the activity of AMPK may be reduced due to inflammation [190] and the upregulation of SOCS3 [37, 48, 140]. Importantly, interventions that reverse insulin resistance such as exercise, metformin and CNTF activate skeletal muscle AMPK in obesity to a degree comparable with healthy muscles (Fig. **1**). Future studies in genetic models of AMPK deficiency or activation within skeletal muscle are needed to evaluate the quantitative role of skeletal muscle AMPK in reversing obesity and the metabolic syndrome and to determine whether therapeutic strategies that activate skeletal muscle AMPK may indeed be important for regulating whole-body insulin sensitivity.

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