

This Review is part of a thematic series on **AMP Kinase**, which includes the following articles:
AMP-Activated Protein Kinase in Metabolic Control and Insulin Signaling [2007;100:328–341]
AMP-Activated Protein Kinase in the Heart: Role During Health and Disease [2007;100:474–488]

Activation and Signaling by the AMP-Activated Protein Kinase in Endothelial Cells

Bruce Kemp, Guest Editor

Activation and Signaling by the AMP-Activated Protein Kinase in Endothelial Cells

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Abstract—The AMP-activated protein kinase (AMPK) was initially identified as the kinase that phosphorylates the 3-hydroxy 3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme for cholesterol biosynthesis. As the name suggests, the AMPK is activated by increased intracellular concentrations of AMP, and is generally described as a “metabolite-sensing kinase” and when activated initiates steps to conserve cellular energy. Although there is a strong link between the activity of the AMPK and metabolic control in muscle cells, the activity of the AMPK in endothelial cells can be regulated by stimuli that affect cellular ATP levels, such as hypoxia as well as by fluid shear stress, Ca^{2+} -elevating agonists, and hormones such as adiponectin. To date the AMPK in endothelial cells has been implicated in the regulation of fatty acid oxidation, small G protein activity and nitric oxide production as well as inflammation and angiogenesis. Moreover, there is evidence indicating that the activation of the AMPK may help to prevent the vascular complications associated with the metabolic syndrome. (*Circ Res.* 2009;105:114-127.)

Key Words: angiogenesis ■ nitric oxide synthase ■ atherosclerosis ■ 3-hydroxy-3-methylglutaryl coenzyme A ■ energy metabolism

The AMP-activated protein kinase (AMPK) is a heterotrimeric serine/threonine protein kinase consisting of the catalytic subunit (α) and 2 regulatory subunits (β and γ) that exist as multiple isoforms and splice variants, resulting in the generation of 12 possible heterotrimeric combinations. As its name suggests, the AMPK is activated in many different cell types by increased intracellular concentrations of AMP and is generally referred to as a “metabolite-sensing kinase.” Indeed, the AMPK is activated following heat shock, vigorous exercise, hypoxia/ischemia, and starvation and appears to be a metabolic master switch, phosphorylating key target proteins that control flux through metabolic pathways of hepatic ketogenesis, cholesterol synthesis, lipogenesis, triglyceride synthesis, adipocyte lipolysis, skeletal muscle fatty acid oxidation, and protein synthesis (reviewed recently^{1,2}). Although the complex picture regarding the regulation of AMPK activity is far from complete,

it seems safe to state that the AMPK determines whole body insulin sensitivity and may prevent insulin resistance, in part, by inhibiting pathways that antagonize insulin signaling. Through its effects on signaling, metabolism, and gene expression, the AMPK enhances insulin sensitivity and may reduce the risk of type 2 diabetes.³

The 2 previous review articles in this series have focused on the role of the AMPK in metabolic control and insulin signaling⁴ and in the heart.⁵ This review focuses on the role of the AMPK in vascular cells and the data implicating defects in AMPK signaling in the development of vascular disease.

Activation of the AMPK

Each subunit within the heterotrimeric AMPK complex has a distinct structure and function and their interaction is necessary for the modulation of kinase activity. The α subunits

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contain the catalytic domain of the enzyme; the β subunits provide a structural link between the other subunits and also contain a specialized sequence that binds to glycogen, whereas the γ subunits contain the binding sites that enable AMP to activate the complex. In its inactivated, nonphosphorylated state the kinase domain within the catalytic α subunit interacts with an autoinhibitory region in the same subunit. Alleviation of this intrinsic inhibition by interaction with the γ subunit is required for kinase activation, as is the phosphorylation of the α -subunit on Thr172 (reviewed elsewhere²). The latter phosphorylation step is necessary for the activation of the AMPK, but does not affect the interaction with either of the other subunits.⁶

There are 2 different α isoforms ($\alpha 1$ and $\alpha 2$) that are differentially expressed in different tissues. For example, whereas the AMP-insensitive $\alpha 1$ isoform predominates in adipose tissue and skeletal muscle, cardiomyocytes express much higher amounts of the AMPK $\alpha 2$.⁷ Interestingly, endothelial cells express both α subunits, although AMPK $\alpha 1$ is expressed at much higher levels than AMPK $\alpha 2$. Moreover, AMPK α subunit expression is highly variable and, at least in human umbilical vein endothelial cells, varies markedly from donor to donor. Unfortunately, many studies have attributed effects to the AMPK on the basis of data generated using pharmacological AMPK activators and the majority of studies in which the AMPK has been overexpressed or in which dominant-negative isoforms were used fail to specify the subunit of focus. This has made it difficult to piece together which pathways control the activation of which isoform and, more importantly, the physiological consequences of the activation of the $\alpha 1$ and $\alpha 2$ subunits in the vasculature.

The AMPK Kinases

Given that activity is clearly associated with the phosphorylation of the α subunit, it was initially assumed that an AMPK kinase (AMPKK) rather than the AMPK itself is activated by AMP. It now seems that AMP binds to the γ subunit, allosterically activating it, so that the α subunit can be phosphorylated at the same time as indirectly inhibiting dephosphorylation⁸ (Figure 1). Two major AMPK kinases have been identified to date, ie, the tumor suppressor gene product LKB1 and the Ca^{2+} /calmodulin-dependent protein kinase kinase β (CaMKK β).

The LKB1 serine/threonine kinase, a gene inactivated in the Peutz–Jeghers familial cancer syndrome,⁹ is essential for the activation of the AMPK by substances such as 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR). However, although the deletion of LKB1 prevents the activation of AMPK $\alpha 2$ in heart and skeletal muscle cells under normoxic as well as anoxic conditions, the deletion of LKB1 fails to affect the basal or stimulated activity of the AMPK $\alpha 1$.¹⁰ On the basis of such observations, it seems that LKB1 plays a major role in regulating the activity of the AMPK $\alpha 2$ subunit and that other, LKB1-independent, mechanisms regulate the activity of AMPK $\alpha 1$. Interestingly, although cells and animals expressing very low levels of LKB1 demonstrate a significantly reduced phosphorylation and activation of the AMPK $\alpha 2$, the activity of LKB1 is not always altered by stimuli that activate the AMPK.^{11,12} Rather,

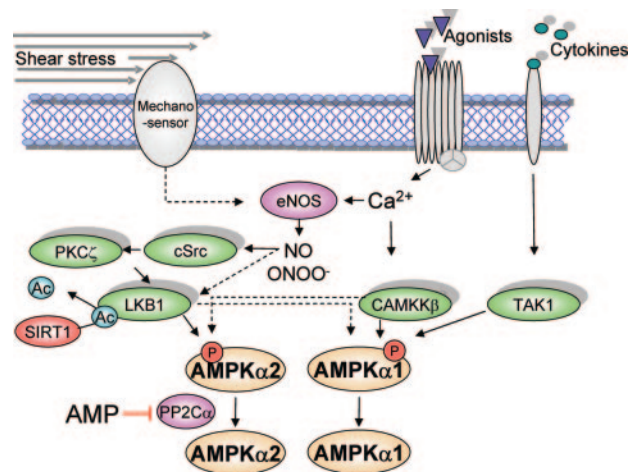


Figure 1. Mechanisms of AMPK activation in endothelial cells in response to shear stress, agonists of G protein-coupled receptors and cytokines. With the exception of the TAK1-mediated activation of AMPK, all of the pathways have been described in endothelial cells. ONOO⁻ indicates peroxynitrite; PP2C α , protein phosphatase 2C α .

it appears that LKB1 is constitutively active and increases in cellular AMP levels promote the phosphorylation of the AMPK on Thr172 by decreasing its dephosphorylation by the phosphatase protein phosphatase 2C.⁸

In endothelial cells, however, stimuli such as fluid shear stress,¹³ peroxynitrite,¹⁴ and the antidiabetic drug metformin¹⁵ have been reported to elicit the phosphorylation and increase the activity of LKB1. In the cases of peroxynitrite and metformin, this phenomenon has been linked to the phosphorylation of LKB1 by protein kinase (PK) ζ ; a step that results in its nuclear export.^{14,15} Of particular interest is that the activity of LKB1 can be modified by acetylation, as well as by phosphorylation, and that the protein/histone deacetylase sirtuin (SIRT)1 targets LKB1. Indeed, SIRT1 overexpression decreases the lysine acetylation of LKB1, which induces its nuclear export and association with the coactivator STRAD, events that increase AMPK activity.¹⁶ Similarly, resveratrol, a polyphenol and SIRT1 activator has been shown to increase AMPK activity in HEPG2 cells, as well as in muscle cells.^{16,17} Although polyphenols can affect endothelial cell function and gene expression,¹⁸ a link among resveratrol, LKB1, and the AMPK has yet to be demonstrated.

Unlike LKB1, the activity of CaMKK β is subject to regulation within the cell, and is increased in response to signals that increase intracellular Ca^{2+} . Therefore, stimuli such as bradykinin and thrombin activate the AMPK $\alpha 1$ as a downstream consequence of increased CaMKK β activity without the need for a parallel increase in AMP levels.^{19,20}

An additional candidate AMPKK is the transforming growth factor β -activated kinase (TAK1), a member of the mitogen-activated protein kinase (MAPK) kinase family that can also phosphorylate the α subunit of the AMPK.²¹ Cardiac-specific inactivation of TAK1 results in an $\approx 80\%$ decrease in AMPK activation; however, this step also decreased the activity of LKB1, indicating that TAK1 may interact with the LKB/AMPK pathway at different levels.²²

Although TAK1 is expressed in endothelial cells and has been linked to the nuclear translocation of the p65 subunit of nuclear factor (NF)- κ B,²³ no link between TAK1 and the regulation of endothelial cell AMPK activity has yet been established.

Intracellular Localization

There are distinct differences in the intracellular localization and activation of the AMPK; the complex containing the α 1 subunit is reported to be exclusively cytosolic, whereas the activation of the AMPK α 2 subunit can result in its nuclear translocation, presumably to regulate gene expression.²⁴ The β subunit has also been reported to be important for intracellular localization, and whereas the myristoylation of the β 1 subunit is thought to target the kinase complex to the Golgi apparatus, as well as to regulate enzyme activity, its phosphorylation on different residues has been linked with kinase inhibition, as well as nuclear translocation of the subunit.²⁵

Is the AMPK an Energy-Sensing Kinase in Endothelial Cells?

Although the link between cellular metabolism and AMPK activation has been repeatedly demonstrated in tissues such as skeletal and cardiac muscle,⁵ the situation is not so straightforward in endothelial cells, and the precise role played by the AMPK in endothelial cell metabolism remains incompletely understood. Although there are a number of excellent AMPK-activating stimuli, such as glucose deprivation and hypoxia, that could potentially impact on the AMP/ATP ratio, it is unclear whether or not changes in AMP levels really represent an endothelial metabolic switch. However, despite the fact that the contribution of glucose oxidation to endothelial cell ATP production is reportedly minimal, glucose deprivation elicits the same changes in AMPK and acetyl coenzyme (Co)A carboxylase (ACC) activity, as well as malonyl coenzyme (Co)A levels and palmitate oxidation, that are observed in response to cell stimulation with AICAR.²⁶

It is, however, generally considered unlikely that an increase in AMP levels can account for the rapid activation (within 10 seconds) of the AMPK in response to endothelial cell stimulation with Ca²⁺-elevating receptor agonists, because endothelial cells have been proposed to exist in a more or less permanent state of “metabolic hypoxia” by virtue of the inhibitory effects of constitutively produced nitric oxide (NO) on cell respiration.²⁷ Thus, whereas there are some situations in which activation of the AMPK is reported to depend on an increase in the ADP/ATP ratio and on LKB1 (eg, following cell stimulation with rosiglitazone²⁸), the activation of the AMPK by Ca²⁺-elevating agonists such as bradykinin^{29,30} and thrombin²⁰ can be attributed to the activity of an AMPKK such as CaMKK β rather than to changes in AMP levels.

Exactly how the AMPK is activated in endothelial cells exposed to shear stress is unclear but the mechanism involved appears to be distinct from that leading to the activation of other kinases, such as Akt.^{31,32} Indeed, the downregulation of platelet endothelial cell adhesion molecule (PECAM)-1 significantly attenuates the shear stress-stimulated phosphorylation of Akt as well as the endothelial NO synthase (eNOS)

without compromising the phosphorylation and activation of the AMPK.³¹ Assuming that it is possible to translate the relationship between LKB1 and AMPK from skeletal muscle to endothelial cells, it is tempting to suggest that Ca²⁺-elevating agonists activate the AMPK α 1, whereas stimuli such as shear stress preferably activate AMPK α 2. This suggestion is supported by data obtained using a combination of LKB1 immunoprecipitation and the *in vitro* phosphorylation of a GST-AMPK construct, indicating that shear stress can indeed activate LKB1.¹³ It remains to be determined whether the downregulation of LKB1 prevents the shear stress-induced activation of the AMPK and whether or not changes in the activity of protein phosphatase 2C contribute to this phenomenon.

Consequences of AMPK Activation

One of the difficulties in attributing specific effects to AMPK activation is the simple fact that the kinase markedly influences the activation of other signaling cascades, in particular Akt and possibly also PKA. Moreover, experiments in endothelial cells in which adenoviruses are used to overexpress the AMPK or AMPK mutants, the virus alone can result in marked changes in kinase phosphorylation and activity (B.F. and I.F., unpublished observations, 2008), making data particularly difficult to interpret. Other viruses eg, the human cytomegalovirus are reported to exert a similar effect in fibroblasts³³ and even in plants geminivirus infection has been reported to activate AMPK-related kinases.³⁴

Genetically modified animals are now available and the phenotyping of these animals is in progress in many different laboratories. However, double AMPK α 1^{-/-} α 2^{-/-} animals are not viable and to what extent one subunit can compensate for the deletion of another in conventional knockout models is presently unclear. The phenotype also seems to be strain dependent as the knockout of the α 1 subunit on the C57BL/6 background is problematic.³⁵ The development of tissue-specific conditional AMPK knockout mice should help to clear up the role of the AMPK in the vasculature but these models have not been investigated extensively to date.

Metabolism

The role of the AMPK in regulating energy homeostasis is related to its effects on mitochondrial biogenesis and function, as well as on glucose and lipid metabolism. These aspects have recently been reviewed in detail elsewhere.³⁶ In the endothelium, it is becoming clear that the AMPK plays a central role in regulating mitochondrial function and biogenesis and thus determining sensitivity to oxidative stresses.³⁷⁻³⁹

Glucose Metabolism

AMPK α 1^{-/-} mice have a reportedly normal glucose homeostasis and respond to AICAR in much the same way as wild-type mice. AMPK α 2^{-/-} mice, on the other hand, demonstrate modest insulin resistance and impaired glucose tolerance and are insensitive to the hypoglycemic effects of AICAR.^{40,41} The AMPK is not only activated by glucose withdrawal and hypoxia, which would be expected to increase AMP levels, but also by sustained hyperglycemia.⁴²

High concentrations of extracellular glucose impair insulin-stimulated glucose utilization and glycogen synthesis in human and rat skeletal muscles, a phenomenon referred to clinically as glucose toxicity. It seems that a similar process takes place in endothelial cells and that sustained exposure to glucose also inhibits fatty acid oxidation and elicits insulin resistance as well as apoptosis.³⁷ These effects could be prevented by activating the AMPK with AICAR or a constitutively active AMPK mutant. Thus, on the basis of the available evidence, it seems that excesses of glucose and free fatty acids cause insulin resistance in skeletal muscle and damage to endothelial cells by similar mechanisms and that AMPK activation can, at least partially, counteract these effects.

Lipid Metabolism

The fact that the first functions attributed to the AMPK were the phosphorylation and thereby inhibition of the ACC,⁴³ as well as of the 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, the rate-limiting enzyme for cholesterol biosynthesis,⁴⁴ highlight the importance of the kinase in the regulation of metabolism. The availability of AMPK $\alpha 1^{-/-}$ and AMPK $\alpha 2^{-/-}$ mice have helped to clarify the roles of the AMPK $\alpha 1$ and $\alpha 2$ in whole body as well as cellular metabolism (reviewed elsewhere³⁵).

In the liver, the AMPK $\alpha 1$ subunit seems to play the most dominant role in the regulation of the HMG CoA reductase; at least this is inferred from the observation that cholesterol levels are normal in AMPK $\alpha 2^{-/-}$ mice.³⁵ However, it is likely that both isoforms can phosphorylate and inactivate the enzyme. The effects on triglyceride metabolism are more pronounced with the $\alpha 2^{-/-}$ animals demonstrating attenuated phosphorylation (and therefore higher activity) of the ACC. In keeping with the importance of the ACC for generating malonyl CoA, a critical fatty acid precursor, the liver-specific deletion of the AMPK $\alpha 2$ leads to elevated circulating triglyceride levels and decreased ketone body levels⁴⁵; exactly the opposite occurs when a constitutively active AMPK is overexpressed in the liver.⁴⁶ Although hypertriglyceridemia would be expected to affect vascular function, to date there is no information available regarding vascular regulation in AMPK $\alpha 2^{-/-}$ mice.

In human endothelial cells, AICAR-induced AMPK activation decreases ACC activity and elicits a 2-fold increase in fatty acid oxidation.⁴⁷ In addition, the bradykinin-induced activation of the AMPK increases palmitate oxidation, indicating that AMPK activation may decrease endothelial cell lipotoxicity mediated by accumulation of free fatty acids, an early event in the pathogenesis of atherosclerosis.²⁹

HMG CoA Reductase

Although hepatic HMG CoA reductase activity is probably the most important for determining whole body cholesterol levels, it is often overlooked that all cells express this enzyme and that it eventually determines the activity of guanosine triphosphate (GTP)-binding proteins, such as Rho, Ras, and Rac, whose membrane localization and function are dependent on isoprenylation.⁴⁸ It follows therefore that the activity of the HMG CoA reductase in endothelial cells should affect the activity of small G proteins and associated downstream

signaling cascades such as extracellular signal-regulated kinase (ERK)1/2, as well as NADPH oxidases. The importance of interfering in the mevalonate pathway has been highlighted by numerous studies aimed at determining the cellular mechanisms underlying the pleiotropic effects of HMG CoA reductase inhibitors (statins). Consistent with the known effects of statin treatment, the activation of the AMPK, either by the overexpression of a constitutively active AMPK mutant or by exposing endothelial cells to fluid shear stress, regulates HMG CoA reductase activity and Ras-dependent signaling in endothelial cells via short- and long-term mechanisms. These involve the phosphorylation of the reductase by AMPK, the AMPK-dependent phosphorylation and degradation of forkhead transcription factor (FoxO)1a, and decrease in HMG CoA reductase expression, respectively.⁴⁹ The relevance of this mechanism could also be demonstrated in vivo as voluntary running decreased the expression of the HMG CoA reductase in the femoral artery and the bradykinin-induced Ras-dependent vasodilatation was attenuated to a similar extent as in mice treated with cerivastatin.⁴⁹ Whether or not Rho and Ras are regulated in a similar manner following AMPK activation is not known; however, the AMPK may affect G protein signaling at different levels. For example, it was recently reported that knockdown of the AMPK $\alpha 1$ subunit in static cultures of endothelial cells abrogated the vascular endothelial growth factor (VEGF) and sphingosine 1-phosphate (S1P)-stimulated activation of Akt and Rac1. Conversely, small interfering RNA-mediated knockdown of Rac1 decreased the agonist-mediated phosphorylation of AMPK substrates without affecting that of AMPK, implicating Rac1 activation (rather than inhibition) as a molecular link between AMPK and Akt in agonist-mediated cell responses.⁵⁰

In most of the cell types analyzed, AMPK activation results in inhibition of protein synthesis and decreased proliferation as a consequence of the inhibition of mTOR (mammalian target or rapamycin), a critical regulator of protein translation. From studies in nonvascular cell types, this effect may be related to the AMPK-mediated phosphorylation of the tuberous sclerosis complex (TSC)1/TSC2 tumor suppressor complex and subsequent activation of the GTPase activator protein that converts the mTOR-activating small G-protein RhebGTP to the inactive RhebGDP.⁵¹ Recent data indicate that the activation of the AMPK does not result in a general decrease in endothelial cell protein synthesis as silencing of the AMPK $\alpha 1$ in human endothelial cells decreases the expression of several genes involved in antioxidant defense. The mechanisms underlying the AMPK-related changes in expression were linked to peroxisome proliferator-activated receptor (PPAR) γ coactivator (PGC)-1 α , cAMP response element binding protein, and FoxO3a.³⁹

In cardiac myocytes and fibroblasts,⁵² as well as in vascular smooth muscle cells,⁵³ the pharmacological activation or overexpression of AMPK mutants exerts a pronounced antiproliferative effect that has yet to be attributed to mTOR. Only one study has addressed this signaling pathway in endothelial cells and found that the shear stress-induced activation of AMPK counteracted the activation of Akt and resulted in transient activation of the p70S6 kinase, the

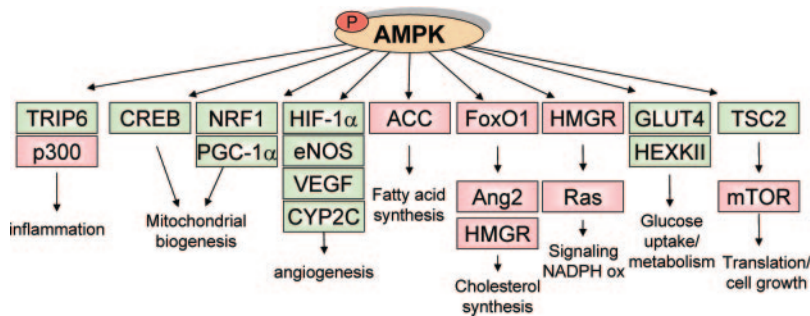


Figure 2. Targets of the AMPK and physiological consequences of AMPK activation. In green are proteins that are functionally activated by AMPK; red are inhibited. Ang2 indicates angiotensin II; CREB, cAMP response element binding protein; CYP2C, cytochrome P450 2C; GLUT4, glucose transporter 4; HEXKII, hexokinase II; HMGR, 3-hydroxy 3-methylglutaryl CoA reductase; mTOR, mammalian target of rapamycin; NADPH ox: NADPH oxidase; NRF1, nuclear respiratory factor 1; TRIP6, thyroid receptor-interacting protein 6; TSC2, tuberous sclerosis complex 2.

mTOR target, to attenuate cell cycle progression. In contrast, AMPK inhibition promoted endothelial cell cycle progression by decreasing the cell population in the G₀/G₁ phase and increasing the numbers of those in the S and G₂/M phases.⁵⁴ How this antiproliferative consequence of AMPK activation can be reconciled with its angiogenic effects (see below) remains to be determined.

AMPK and Gene Expression

Several transcription factors and transcriptional coactivators are reported to be AMPK targets, at least *in vitro* (Figure 2). Functional importance for AMPK-mediated phosphorylation has been demonstrated for hypoxia-inducible factor (HIF)-1 α , FoxO1 α , and PGC-1 α .

Hypoxia-Inducible Factor-1 α

Because the AMPK is activated by hypoxia and has been implicated in angiogenesis, it seems logical to suggest that a link may exist between the kinase and HIF-1. Indeed, hypoxia stimulates the nuclear translocation of the AMPK α 2 subunit,⁵⁵ and AMPK inhibitors prevent the induction of HIF-1 and VEGF by low O₂ tension.⁵⁵ This link was initially confirmed in prostate cancer cells in which the blockade of AMPK activity significantly attenuated hypoxia-induced responses including HIF-1 expression, secretion of VEGF, glucose uptake, and HIF-1–dependent reporter gene expression.⁵⁶ Of note is that although AMPK-stimulated HIF-1 activity via a signaling pathway independent of phosphatidylinositol 3-kinase (PI3K)/Akt, AMPK activation alone was not sufficient to stimulate HIF-1 transcriptional activity.⁵⁶ The AMPK-dependent regulation of HIF is clearly of relevance for processes such as angiogenesis (see below).

Forkhead Transcription Factor

FoxO1 α is a well-described target of Akt; however, in human endothelial cells, the phosphorylation of FoxO1 α following the initiation of fluid shear stress was shown to depend on the activity of the AMPK and to be unaffected by a dominant-negative Akt mutant. As a consequence of its phosphorylation by AMPK α 2, FoxO1 α was exported from the nucleus and presumably degraded as its overall expression was decreased.⁵⁷ Two FoxO1 α -regulated endothelial genes whose expression was decreased as a result were angiotensin-2⁵⁷ and the HMG CoA reductase.⁴⁹ There appear to be differences in the effects of AMPK activation *in vitro* and *in vivo*, because although AMPK α 2 activation has been reported to downregulate FoxO1, -3, and -4 mRNA expression in cultured myotubes, the opposite was observed in skeletal muscle

following injection of AICAR, indicating a more complicated regulation involving other cellular pathways.⁵⁸

Both AMPK α subunits have been linked with FoxO transcription factors and FoxO3 α protein and mRNA are reported to be reduced in AMPK α 1-deficient cells without any apparent effect on FoxO1 α .³⁹ In HEK293 cells, the AMPK α 2 directly phosphorylates mammalian FoxO3 on six regulatory sites, which increases transcriptional activity and promotes resistance to oxidative stress, tumor suppression, and longevity.⁵⁹ Such observations fit well with reports in *Caenorhabditis elegans*, in which the regulation of FoxO by AMPK has been linked with lifespan extension induced by dietary restriction.⁶⁰ It remains to be determined whether or not the AMPK plays a role in endothelial cell senescence.

PGC-1 α

The AMPK also phosphorylates PGC-1 α a transcriptional coactivator that is crucial for the coordinated expression of the nuclear encoded mitochondrial proteins.⁶¹ Mutation of the appropriate sites decreases the AICAR-stimulated transcription of mitochondrial proteins.⁶² Little is known about this relationship in endothelial cells, although AICAR- or metformin-induced AMPK activation was reported to prevent H₂O₂-induced cell damage by PGC-1 α , stabilizing mitochondria, and increasing mitochondrial biogenesis, thus attenuating the activation of the c-Jun N-terminal kinase (JNK).³⁸

Nuclear Factor κ B

There are numerous reports of an attenuated activation of NF- κ B following AMPK activation in different cell types, including endothelial cells.^{63–66} Indeed, AMPK activation with AICAR or overexpression with a constitutively active AMPK prevent the palmitate- or tumor necrosis factor- α -induced activation of NF- κ B and increase in vascular cell adhesion molecule (VCAM)-1 mRNA expression.⁶³ Moreover, the adiponectin-stimulated activation of the AMPK was linked to the downregulation of NF- κ B and a decrease in the secretion of C-reactive protein from human aortic endothelial cells.⁶⁷ Exactly how the AMPK modifies NF- κ B activity is not entirely clear as the effects on DNA binding are relatively weak, and a strong nuclear translocation of the active NF- κ B complex has yet to be described. It is tempting to speculate, however, that the acetylation of the NF- κ B by p300⁶⁸ is involved in this response.

Epigenetic Regulation

There are several reports linking AMPK activation with alterations in gene expression via the phosphorylation and modulation of histone acetylases and deacetylases (HDACs)

including SIRT1.⁶⁹ The functional consequence of the AMPK-mediated phosphorylation of HDAC5, for example, is its association with 14-3-3 proteins, nuclear export, and an attenuated interaction of HDAC5 with the myocyte enhancer factor 2A. This reduces the presence of HDAC5 at the GLUT4 promoter and increases GLUT4 gene expression in myotubes via an increased activity of myocyte enhancer factor 2A.⁷⁰ Given the importance of histone acetylases and HDACs for endothelial functions, including eNOS expression⁷¹ and angiogenesis,⁷² and the fact that the AMPK is involved in the regulation of many of the same pathways, a closer look for a possible link between AMPK activity and acetylation/deacetylation is warranted. Another transcriptional coactivator targeted by the AMPK is thyroid receptor interacting protein 6, which acts as a coactivator for activator protein-1 and NF- κ B.⁷³ AMPK α 2 overexpression significantly increases the transcriptional activation of NF- κ B; however, whether or not such a mechanism also exists in vascular cells remains to be demonstrated.

Approximately half of the changes in gene expression can be ascribed to changes in mRNA decay rates rather than transcription and degradation can be regulated by the binding of proteins to RNA. One of the RNA-binding proteins known to be a target of the AMPK is HuR.^{74,75} Nuclear shuttling of HuR has been proposed to be related to the concomitant AMPK-dependent activation of p300 (an acetyl transferase) and phosphorylation of importin α 1, which promotes the nuclear import of HuR.⁷⁴ The consequence of the latter step is a decrease in RNA stability and reduced protein expression. Whether or not the AMPK-mediated phosphorylation of HuR contributes to alterations in vascular expression of the soluble guanylyl cyclase⁷⁶ or vascular cell senescence⁷⁷ remains to be addressed.

Crosstalk Between AMPK and Akt

In rat neonatal cardiomyocytes Akt negatively influences AMPK activity,⁷⁸ and AMPK activation is blunted by the overexpression of a constitutively active Akt mutant.⁷⁹ Mechanistically, this inhibition has been attributed to the direct phosphorylation of the AMPK α 1 subunit on Ser485/491, by Akt, which negatively interferes with the LKB1-mediated phosphorylation of Thr172.⁸⁰ A similar mechanism has been described in HEK293 cells,⁸¹ as well as in follicle-stimulating hormone-treated granulosa cells,⁸² and may be active in endothelial cells as the H₂O₂-stimulated activation of AMPK is significantly increased after pharmacological inhibition of Akt.⁸³

Numerous stimuli elicit the parallel activation of both the AMPK and Akt in endothelial cells including shear stress,^{31,54} VEGF,⁸⁴ and adiponectin⁸⁵ to name just a few. The influence of AMPK on Akt-mediated signaling events in endothelial cells appears to depend on the metabolic conditions. For example, under hypoxic but not normoxic conditions, a dominant-negative AMPK mutant abolished the VEGF-induced, Akt-mediated phosphorylation of eNOS, as well as the VEGF-induced tube formation in Matrigel.⁸⁴ A similar phenomenon was observed in vivo in carp in response to anoxia,⁸⁶ and studies addressing the response to laminar flow in cultured endothelial cells also indicate that the AMPK can

negatively regulate Akt, resulting in the transient activation of p70S6 kinase.⁵⁴ On the other hand, a dominant-negative AMPK mutant was reported to inhibit Akt phosphorylation in adiponectin-treated endothelial cells, although a dominant-negative Akt mutant failed to affect AMPK activation, allowing the authors to conclude that the activation of the AMPK must lie upstream from that of Akt.⁸⁵ A similar conclusion was reached in studies in human aortic endothelial cells in which the small interfering RNA-mediated knock-down of AMPK α 1 abolished the leptin-induced phosphorylation of Akt and eNOS activation⁸⁷ and in cardiac myocytes in which resveratrol stimulated the activation of AMPK and inhibition of Akt.⁸⁸ Also in HEK293 cells, AMPK activation was found to inhibit cell survival by binding to and phosphorylation of the insulin receptor substrate-1, which in turn inhibited PI3K/Akt signaling, thus suppressing the mitochondrial membrane potential and promoting apoptosis.⁸¹

How can the AMPK affect Akt signaling? One possibility is via Rac1 (see above),⁵⁰ another is the regulation of Akt activity by other proteins such as the Akt binding protein TRIB3, a mammalian tribbles homolog also known as TRB3/NIPK.^{89,90} TRIB3 expression can be increased by several stimuli including starvation,⁸⁹ PPAR- α activation,⁹⁰ and chronic alcohol ingestion,⁹¹ and, in all of the conditions listed, is linked to insulin insensitivity. TRIB3 is also expressed in endothelial cells and human umbilical vein endothelial cells carrying different *TRIB3* genotypes (QQ, QR, or RR) show a distinctly different sensitivity to insulin. Specifically, cells expressing the *TRIB3* R84 (RR) variant demonstrate an attenuated insulin-induced NO production in vitro compared to the QQ-expressing cells. No data are available from AMPK α knockout animals, but mice lacking LKB1 in skeletal muscle demonstrated enhanced insulin sensitivity and increased insulin-stimulated Akt phosphorylation and a more than 80% decrease in muscle expression of *TRIB3*.⁹²

AMPK and Vascular Redox Balance

Oxidative stress and a shift in the cellular redox balance have been linked with the endothelial dysfunction that is characteristic of the early stages of cardiovascular disease. Endothelial dysfunction is characterized by a decreased bioavailability of endothelium-derived NO. The latter is paradoxically accompanied by an increase in the expression of eNOS, together with the elevated production of oxygen-derived free radicals that together lead to the generation of more damaging species such as peroxynitrite.⁹³ From a large number of recent studies, it seems that there is an intricate balance between the AMPK and the enzymes and signaling pathways that determine endothelial redox balance. For example, the AMPK can inhibit the formation of reactive oxygen species (ROS) by the NADPH oxidase and stimulate NO production by eNOS. Moreover, AMPK activation suppresses JNK activation,³⁸ NF- κ B-mediated transcription,⁶⁶ as well as E-selectin⁵⁷ and VCAM-1⁶³ expression, in endothelial cells exposed to H₂O₂, tumor necrosis factor- α , or fatty acids and, as a consequence, attenuates monocyte adhesion to the endothelium.⁹⁴ Recently, silencing of the AMPK α 1 in human endothelial cells was reported to decrease the expression of some genes (eg, manganese superoxide dismutase, catalase, γ

glutamylcysteine synthase, and thioredoxin) involved in antioxidant defense, but interestingly not of others including the heme oxygenase.³⁹

NADPH Oxidase

The most direct evidence for a link between the AMPK and NADPH oxidase comes from studies reporting that the treatment of neutrophils with AICAR or AMP suppressed the production of superoxide anions (O_2^-) stimulated by phorbol esters or fMLP.⁹⁵ In addition, the AMPK has been suggested to phosphorylate the p47^{phox} subunit (much in the same way as Akt) and thus prevent its translocation to the plasma membrane.⁹⁵ In neutrophils, AICAR also reduced PMA-dependent H_2O_2 release and induced the phosphorylation of JNK, p38 MAPK, and ERK1/2.

The exposure of cultured human endothelial cells to high concentrations of glucose (10 mmol/L) results in the generation of ROS, an effect that can be attenuated by AMPK-activating agents such as rosiglitazone.⁹⁶ Indeed, AICAR prevents the glucose-induced formation of ROS, and the downregulation of AMPK α 1 renders rosiglitazone inactive against glucose-induced ROS. There are other possible mechanisms by which the AMPK could affect vascular O_2^- levels. For example, AMPK activation in endothelial cells increases the expression of manganese superoxide dismutase⁹⁷ and results in a decrease in HMG CoA reductase activity and the inhibition of the small G proteins.⁴⁹ The latter mechanism has been demonstrated for Ras and a similar effect on Rac-1 would certainly be expected to decrease endothelial ROS generation by Nox2.⁹⁸ However, although the latter may seem a logical extrapolation of much of the available literature (reviewed elsewhere⁹⁹), recent reports indicate that AMPK activity is required for Rac activation in endothelial cells as AMPK α 1 downregulation abolished its VEGF-induced activation.⁵⁰ The same authors have reported that a similar response can be elicited by statins and found that simvastatin elicits an increase rather than a decrease in Rac activity and ROS production.^{100,101} Because the latter response was dependent on NO (ie, not observed in eNOS^{-/-} mice), the source of the ROS is presumably the mitochondria and most probably related to the generation of peroxynitrite.

Another redox-regulatory function attributed to the AMPK is its ability to prevent the tyrosine nitration and inhibition of the prostacyclin synthase in endothelial cells exposed to high concentrations of glucose.¹⁰² This effect also seems linked to the inhibition of endothelial cell O_2^- production and is reported to involve the downstream activation of the p38 MAPK and subsequent upregulation of mitochondrial uncoupling protein-2.¹⁰² The latter observations are of particular interest given that the basic role of uncoupling proteins is to prevent oxidative tissue injury by reducing oxidative stress,¹⁰³ a function that also seems to be applicable to endothelial cells.¹⁰⁴ Such a mechanism does not seem to be restricted to cultured cells as AMPK activation with AICAR markedly increased uncoupling protein-2 expression and reduced both O_2^- and prostacyclin synthase nitration in diabetic wild-type but not in diabetic AMPK α 2-deficient mice.¹⁰²

Angiotensin II has been reported to attenuate the phosphorylation of the AMPK without affecting its expression,¹⁰⁵ and it is tempting to speculate that the partial inactivation of the AMPK underlies some of the effects of angiotensin II on vascular remodeling. Certainly, AMPK inhibits the hyperplasia and hypertrophy of vascular tissues as well as angiotensin II-mediated vascular smooth muscle and cardiomyocyte proliferation.^{53,105} Moreover, AICAR decreases neointimal hyperplasia in the rat femoral artery denudation model an effect at least partly been attributed to the inhibition of ERK1/2.⁵³ Although a cause and effect relationship regarding the angiotensin II-induced activation of the NADPH oxidase and AICAR-stimulated activation of the AMPK seems plausible, this relationship needs to be investigated in detail in the appropriate mouse models.

Endothelial Nitric Oxide Synthase

The AMPK is the only kinase identified to date that can potentially phosphorylate eNOS on more than one site, ie, on Ser1177¹⁰⁶ and Ser633,¹³ which are both activating sites in the reductase domain and on Thr495 which is an inhibitory site in the CaM-binding domain of the enzyme.^{106,107}

There have been numerous reports of the AMPK-dependent phosphorylation of eNOS (on Ser1177) following endothelial cell stimulation with agents such as VEGF,¹⁰⁸ PPAR agonists,²⁸ AICAR,¹⁰⁹ metformin,^{110,111} and adiponectin.¹¹² However, the effects are generally weak and much less impressive than the stimulation seen in response to hypoxia,⁸⁴ shear stress,^{31,49,57} and thrombin,¹¹³ which also result in AMPK activation. It should be borne in mind, however, that a number of stimuli, such as pentobarbital and glucose deprivation, which are excellent AMPK activators in endothelial cells,¹¹⁴ have absolutely no effect on eNOS phosphorylation or NO output (B.F. and I.F., unpublished observations, 2008). Whether or not this apparent conflict can be explained by the specific α subunit activated by a given stimuli, or to crosstalk between the AMPK and other eNOS-activating kinases remains to be clarified. Phosphorylation may not be the only way in which AMPK activation can affect eNOS activity as a dominant-negative AMPK mutant was found to decrease eNOS activity and NO production despite being unable to prevent estradiol-induced changes in eNOS phosphorylation on Ser1177 or Thr495. Rather, the reduction in NO production was attributed to an impaired association of eNOS with heat shock protein 90.¹¹⁵

Although a clear link has been established between AMPK and activation of eNOS in endothelial cell cultures the agonist (acetylcholine)-induced relaxation of conductance and resistance arteries is normal in AMPK α 1^{-/-},³⁸ as well as in AMPK α 2^{-/-} mice (B.F. and I.F., unpublished observations, 2009). Such findings may indicate that the AMPK is unlikely to play a major role in the regulation of eNOS under normal conditions in which Akt, PKA and CaMKII would be expected to dominate the regulation of eNOS. Clearly, a more thorough assessment of vascular function in animals lacking the α 1 and α 2 subunits is required to determine the consequences for flow-induced vasodilatation and endothelial cell activation by the compounds (eg, VEGF, hypoxia, PPAR-agonists, and adiponectin) shown to activate eNOS in vitro.

To add to the present confusion regarding the interactions between the AMPK and eNOS, some groups have been able to demonstrate that the activation of the AMPK by several stimuli (fluid shear stress, calcium ionophore, bradykinin) is sensitive to NOS inhibition and thus seems to be NO-dependent.^{30,49} Certainly, the shear stress-induced phosphorylation and activation of the AMPK are markedly attenuated in endothelial cells from eNOS^{-/-} mice,⁴⁹ and the bradykinin-stimulated activation of the AMPK is greater in aortae from wild-type than from eNOS^{-/-} mice.³⁰ Inhibition of either the soluble guanylyl cyclase or CaMKK β abolished the NO-induced AMPK activation, indicating that the activation of the AMPK is guanylyl cyclase-mediated and Ca²⁺-dependent. However, whether or not protein kinase G can phosphorylate the AMPK is presently unknown. Such data imply that a positive feedback relationship would exist between the AMPK and eNOS. It will be particularly interesting to determine whether the NO-induced activation of the AMPK depends on global or on localized (eg, mitochondrial) NO production.²⁷

Vascular Consequences of AMPK Activation/Inhibition

Angiogenesis

Sustained activation of the AMPK induces apoptosis in several cell types, including pancreatic β cells, and can be counteracted by the activation of mTOR by Akt.¹¹⁶ In endothelial cells, the overexpression of either of the α subunits does not induce apoptosis; on the contrary, the AMPK α 1 was shown to inhibit anoxia-induced apoptosis¹¹⁷ and be required for the caffeine-induced enhancement of reendothelialization in a mouse carotid artery injury model.¹¹⁸ On the whole, the activation of the AMPK by basic fibroblast growth factor,⁸⁴ adiponectin,⁸⁵ hypoxia,⁸⁴ and VEGF^{84,114} seems to be crucial for angiogenesis. Indeed, dominant-negative AMPK mutants suppressed both endothelial cell migration to VEGF and in vitro differentiation into tube-like structures under hypoxic conditions.⁸⁴

There is also a clear link between AMPK activation and VEGF expression/release and in many cells the kinase seems to be critical for the expression of VEGF under oxygen- and glucose-deprived conditions.^{56,119} A similar AMPK-dependent increase in VEGF expression has been demonstrated in prostrate carcinoma cells treated with different carcinogenic metals and attributed to several different HIF-1 α -dependent and -independent mechanisms.¹²⁰ In this context, it is interesting to note the proposal that abnormal AMPK activation in the brains of double mutant amyloid precursor protein/presenilin 2 mice could underlie the vascular dysfunction (elevated levels of VEGF and increased brain endothelial cell division but abnormally low brain vessel density) observed in this model of Alzheimer's disease.¹²¹ Whether or not the AMPK affects the angiogenic potential of circulating progenitor cells is unclear, but the treatment of endothelial cell progenitor cells with AICAR is reported to increase their ability to form capillary-like tubes in vitro via an NO-dependent mechanism.¹²²

Given that the AMPK is activated by nutrient deprivation, it is not surprising that the kinase is active in growing tumors.¹²³ Activation of the kinase seems to be crucial to tumor growth as the introduction of AMPK antisense RNA expression vectors into pancreas cancer cell lines significantly diminished their tolerance to glucose deprivation. Moreover, the stable transfection of AMPK antisense into these cancer cells inhibited tumor growth in nude mice.¹²³ Such effects can be, at least partly, attributed to the modulation of VEGF expression by the AMPK. Indeed, glucose deprivation in cancer cells results in the activation of the AMPK and in the increased expression of VEGF via a HIF-1-independent increase in VEGF mRNA stability, which was sensitive to AMPK inhibition.¹¹⁹ Few studies have compared the consequences of α 1 and α 2 activation, but in human glioblastoma cells, it seems that the hypoxia-induced activation and nuclear translocation of the AMPK α 2 subunit is responsible for the increase in VEGF mRNA.⁵⁵

A similar increase in VEGF expression was recorded in muscle cells treated with AICAR, which accelerated angiogenic repair in response to ischemic injury in the mouse hindlimb and was reported to involve the AMPK-dependent activation of p38 MAPK.¹²⁴ However, although the AMPK was found to regulate basal VEGF expression and capillarization in murine skeletal muscle, it was not necessary for exercise-induced angiogenesis.¹²⁵ However, a recent study reported that a AMPK/PGC-1 α -dependent mechanism is essential for the exercise-induced up regulation of skeletal muscle VEGF expression and for a training-induced prevention of an age associated decline in VEGF protein content.¹²⁶ Because the latter investigation was performed using PGC-1 α mice rather than AMPK α ^{-/-}, it is not possible to exclude that PGC-1 α -dependent but AMPK-independent mechanisms contributed to the response observed.

Endothelial Function/Dysfunction and Atherosclerosis

The role of the AMPK in the regulation of vascular tone is presently controversial, because although AMPK has been linked to the phosphorylation of eNOS in cultured endothelial cells, no obvious defect in NO-mediated relaxation is evident in arteries from AMPK α 1^{-/-} or AMPK α 2^{-/-} mice (see above). That the activation of the AMPK in vivo is of benefit to cardiovascular function is assumed on the basis of observations that metformin improves vasodilator function^{127,128} and can prevent the progression of heart failure.¹²⁹ However, whether these effects are dependent on eNOS activation or the modulation of other endothelium-dependent vasodilator/vasoconstrictor pathways are affected is presently unknown. The latter is certainly possible because metformin treatment decreases the production of an endothelium-derived vasoconstrictor prostanoid in a rat model of type 2 diabetes.¹³⁰

Endothelium-dependent relaxation, however, is not exclusively regulated by NO and on the basis of the available literature it is tempting to speculate a link between the AMPK and relaxation linked to the generation of epoxyeicosatrienoic acids by cytochrome P450 epoxygenases. Indeed, LKB1 and the AMPK can be activated by stimulation of the constitutive androstane receptor and pregnane X receptor, using pheno-

barbital, which is a classic cytochrome P450 inducer.¹³¹ This response, however, is not observed in mice lacking the hepatic expression of both the $\alpha 1$ and $\alpha 2$ subunits.¹³² Although phenobarbital has recently been reported to induce cytochrome P450 2C8 expression by an AMPK $\alpha 2$ -dependent mechanism in cultured endothelial cells,¹¹⁴ no information is presently available regarding the characterization of epoxyeicosatrienoic acid-mediated/NO-independent vasodilatation in the AMPK $\alpha^{-/-}$ mice.

Relaxation, in general, is largely determined by the vascular smooth muscle, not the endothelium, and agents such as AICAR¹³³ and metformin^{110,111} are reported to relax isolated arterial preparations. However, although the AICAR induced relaxation of isolated arteries was normal in arteries from AMPK $\alpha 2^{-/-}$ mice and not observed in arteries from AMPK $\alpha 1^{-/-}$ mice, responses to AICAR were NO- and endothelium-independent and have been attributed to the activation of the AMPK in vascular smooth muscle cells.¹³³ Similarly, relaxation induced by metformin can also be detected in arteries treated with NOS inhibitors.¹³⁴ There seem to be species- and vessel-associated differences in sensitivity to chemical AMPK activation because, in contrast to the studies focusing on rat or murine aorta, porcine carotid artery smooth muscle AMPK was found to be insensitive to AICAR and metformin, even though it could be activated by metabolic stress induced by anoxia and 2-deoxyglucose.¹³⁵ AMPK activation by the latter means was associated with a rapid and pronounced reduction in endothelin-induced force and reduced phosphorylation of Akt and ERK1/2. Taken together, these results suggest that AMPK activators can induce relaxation in by a direct action on smooth muscle cells. Such findings fit well with the report that the AMPK can phosphorylate and inactivate smooth muscle myosin light chain kinase and thus could attenuate the tonic phase of smooth muscle contraction.¹³⁶

From the link between the AMPK and redox balance, as well as NF- κ B activity, it follows that there is likely to be a link between the AMPK (or rather a decrease in its activity) and the development of endothelial dysfunction. Indeed, the endothelial dysfunction initiated by stimuli such as hyperglycemia can be improved by AMPK activation, as can lipotoxicity induced by substances such as palmitate (see above).¹³⁷ Exercise is also able to improve endothelial function^{138,139} and because AMPK is stimulated by exercise (at least in skeletal muscle but potentially also in liver and adipose tissue¹⁴⁰), it is tempting to attribute the beneficial effects of exercise to the kinase. Few studies have addressed this link directly, but in mice, voluntary exercise attenuated the vascular expression of the HMG CoA reductase (shown to be AMPK-dependent), as well as the vasodilator response to the Ras-activating agonist bradykinin, albeit to a lesser extent than the HMG CoA reductase inhibitor cerivastatin.⁴⁹

In addition to its effects on endothelial function and cell adhesion molecule expression, the AMPK influences a number of pathways (eg, the proliferation of vascular smooth muscle cells,¹⁴¹) that should support antiatherosclerotic mechanisms. Indeed, the activation of mTOR by oxidized LDL is involved in smooth muscle cell proliferation, and AMPK activation by resveratrol was reported to block the

activation of the PI3K/Akt/mTOR/p70S6K pathway, thus inhibiting both the DNA synthesis and proliferation of SMC.¹⁴² Moreover, the reduction in neointimal formation observed in rats treated with β -lapachone, a potent antitumor agent that stimulates NAD(P)H:quinone oxidoreductase activity, was attributed to the LKB1-dependent activation of the AMPK.¹⁴³

The contribution of inflammatory cells to atherogenesis is well documented; however, although hypoxia stimulates the AMPK in activated monocytes,¹⁴⁴ and AMPK activation attenuates the lipopolysaccharide-induced expression of inducible NOS,¹⁴⁵ regulates macropinocytosis,¹⁴⁶ and reduces migration to stromal cell-derived factor,¹⁴⁷ surprisingly little is known about the control and consequences of AMPK activation in monocytes/macrophages. In a study not directed at atherosclerosis, it could be demonstrated that AMPK signaling is a potential potent counter regulator of inflammatory signaling pathways. For example, stimulation of macrophages with the antiinflammatory cytokines interleukin-10 and transforming growth factor- β , resulted in the rapid phosphorylation of AMPK, whereas stimulation of macrophages with lipopolysaccharide resulted in AMPK dephosphorylation and inactivation.¹⁴⁸ On the basis of the data presented, the authors of the latter study suggested that the AMPK directs signaling in macrophages in a manner that suppresses proinflammatory responses and promote macrophage polarization to an antiinflammatory functional (M2) phenotype. In addition, the alleviation of edema in the carrageenan-induced rat paw model stimulated by nicotine was attributed to the activation of the AMPK in macrophages.¹⁴⁹

Cardiovascular Disease Therapy and the AMPK

AMPK dysregulation has been linked with obesity and the vascular consequences of prediabetes/metabolic syndrome, and many of the positive effects of the adipokine adiponectin are linked to the activation of the AMPK. It has even been proposed that the vascular consequences of the hypo-adiponectinemia associated with atherosclerosis and diabetes may be circumvented by agents that increase AMPK activity.¹⁵⁰ A number of compounds are reported to activate the AMPK in endothelial cells and improve endothelium-dependent relaxation, including the selective inhibitor of phosphodiesterase 3 cilostazol,^{65,151} fenofibrate,^{64,152,153} and rosiglitazone.²⁸ Polyphenols such as resveratrol also increase phosphorylation of AMPK¹⁵⁴ and improve the survival of mice on a high-calorie diet.¹⁵⁵ Also, the treatment of type 1 diabetic LDL receptor-deficient mice with a synthetic polyphenol prevented the diabetes-induced decrease in AMPK and ACC phosphorylation, as well as the lipid accumulation in the liver and the acceleration of aortic lesion development.¹⁵⁴

Activation of the AMPK by the statins has been proposed to contribute to the pleiotropic effects of this class of compounds. The mechanisms involved are presently unclear because activation of the AMPK is linked to the phosphorylation and inhibition of the HMG CoA reductase⁴⁹ and also to increased vascular eNOS expression.^{156,157} Although data on

AMP levels are not available, both the in vitro incubation of cultured endothelial cells and the in vivo treatment of mice with simvastatin stimulate the phosphorylation of both LKB1 and AMPK α .^{100,101} Whereas the former study attributed these effects to the eNOS-dependent generation of reactive nitrogen species and the subsequent activation of PKC ζ and nuclear export of LKB1,¹⁰⁰ a more recent report suggests that CaMKK β may be upstream of LKB1, because its downregulation prevented the statin induced phosphorylation of LKB1 and AMPK, as well as Rac activation.¹⁰¹ The latter implies that complicated interrelationships exist between the different AMPK kinases.

Whether or not other commonly prescribed cardiovascular therapeutic agents also target the AMPK is unknown, but the fact that bradykinin stimulates the AMPK to increase endothelial cell fatty acid oxidation²⁹ makes it tempting to speculate that inhibitors of the kininase II/ angiotensin-converting enzyme are able to elicit comparable effects. There is circumstantial evidence supporting this hypothesis, because captopril treatment increased fatty acid oxidation and glycolysis in hearts from ob/ob mice at the same time as normalizing AMPK phosphorylation.¹⁵⁸

Metformin is among the most commonly used therapeutic drugs for patients with type 2 diabetes. It has been shown that metformin not only reduces hyperglycemia and improves insulin sensitivity but also that it has vasculoprotective effects that are largely independent of its well-known antihyperglycemic action.¹⁵⁹ For example, metformin reduces the development of atherosclerotic lesions in animal models, and clinical studies have shown it to reduce carotid intima-media thickness (reviewed elsewhere¹⁶⁰). The antiatherogenic effects of metformin include reductions in insulin resistance, hyperinsulinemia, and obesity, but despite the large number of studies published, it remains unclear what mechanisms underlie its vasculoprotective actions. Certainly, metformin is able to activate the AMPK in endothelial cells,^{110,111} as well as in vascular smooth muscle cells,¹⁶¹ and in many cases, the actions of metformin can be mimicked by the AMPK activator AICAR. For example, in mesenteric arteries from a rat model of type 2 diabetes the NO and prostacyclin-independent (ie, endothelium-derived hyperpolarizing factor-mediated) relaxation is impaired, whereas endothelium-dependent contraction is enhanced. Metformin and AICAR are able to redress this balance and to decrease oxidative stress without altering the expression of eNOS or cyclooxygenase-1 but by increasing the expression of cyclooxygenase-2 protein.¹³⁰ Because vascular complications are the leading cause of death in subjects with diabetes, a therapeutic approach that activates the AMPK should be able to improve glycemic control at the same time as maintaining or restoring endothelial function.

Outlook

Although the AMPK pathway is traditionally thought of as an intracellular fuel gauge and regulator of metabolism, recent evidence indicates that it may also be important for the maintenance of endothelial function and to redress the disturbed redox balance associated with vascular disease. Certainly, the AMPK can influence a number of signaling

casades that would be expected to result in antiatherosclerotic effects, such as an improved NO bioavailability, attenuated free radical generation, and the activation of angiogenic factors. Approaches aimed at increasing vascular AMPK activity would therefore be expected to have a potential therapeutic benefit; however, the agents presently recognized to do this (eg, metformin) seem to act indirectly and exhibit a number of unpleasant side effects. There have been attempts to generate alternative AMPK-activating agents, eg, the thienopyridone A769662,¹⁶² which activates the AMPK by a mechanism involving the β and γ subunits.¹⁶³ However, it is important to note that this compound may have potentially limiting side effects and has been reported to inhibit the function of the 26S proteasome by an AMPK-independent mechanism, leading to cell cycle arrest in mouse embryonic fibroblasts in vitro.¹⁶⁴ Despite this observation, treatment of ob/ob mice A769662 lowered plasma glucose, reduced body weight gain, and significantly decreased both plasma and liver triglyceride levels.¹⁶² It will be interesting to determine whether or not A769662 can affect vascular function and homeostasis.

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None.

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