

# AMPK, mitochondria, and CVDs

Subjects: **Others**

Contributor: Shengnan Wu , Ming-Hui Zou

Adenosine monophosphate-activated protein kinase (AMPK) is in charge of numerous catabolic and anabolic signaling pathways to sustain appropriate intracellular adenosine triphosphate levels in response to energetic and/or cellular stress. In addition to its conventional roles as an intracellular energy switch or fuel gauge, emerging research has shown that AMPK is also a redox sensor and modulator, playing pivotal roles in maintaining cardiovascular processes and inhibiting disease progression. Pharmacological reagents, including statins, metformin, berberine, polyphenol, and resveratrol, all of which are widely used therapeutics for cardiovascular disorders, appear to deliver their protective/therapeutic effects partially via AMPK signaling modulation. The functions of AMPK during health and disease are far from clear. Accumulating studies have demonstrated crosstalk between AMPK and mitochondria, such as AMPK regulation of mitochondrial homeostasis and mitochondrial dysfunction causing abnormal AMPK activity. In this review, we begin with the description of AMPK structure and regulation, and then focus on the recent advances toward understanding how mitochondrial dysfunction controls AMPK and how AMPK, as a central mediator of the cellular response to energetic stress, maintains mitochondrial homeostasis. Finally, we systemically review how dysfunctional AMPK contributes to the initiation and progression of cardiovascular diseases via the impact on mitochondrial function.

AMPK

mitochondrial function

Cardiovascular diseases

## 1. Definition

5' AMP-activated protein kinase or AMPK or 5' adenosine monophosphate-activated protein kinase is an enzyme that plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low. It belongs to a highly conserved eukaryotic protein family and its orthologues are SNF1 in yeast, and SnRK1 in plants. It consists of three proteins (subunits) that together make a functional enzyme, conserved from yeast to humans. It is expressed in a number of tissues, including the liver, brain, and skeletal muscle. In response to binding AMP and ADP, the net effect of AMPK activation is stimulation of hepatic fatty acid oxidation, ketogenesis, stimulation of skeletal muscle fatty acid oxidation and glucose uptake, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipogenesis, inhibition of adipocyte lipolysis, and modulation of insulin secretion by pancreatic beta-cells.

## 2. Introduction

Cells constantly coordinate their metabolism to satisfy their energy needs and respond to the use of nutrients. Eukaryotes have developed a highly adaptive complex, the serine/threonine kinase adenosine monophosphate

(AMP)-activated protein kinase (AMPK), to sense low cellular adenosine triphosphate (ATP) levels [1]. Under conditions of insufficient energy, AMPK activates upon its binding with AMP or adenosine diphosphate (ADP) [1]. Once activated, AMPK redirects metabolism toward increased catabolism or decreased anabolism through the phosphorylation of key proteins across multiple pathways, including protein synthesis (e.g., mammalian target of rapamycin complex (1) [2][3], lipid homeostasis (e.g., acetyl coenzyme A carboxylase, ACC) [4][5], glucose metabolism (e.g., phosphofructokinase (2) [6], and mitochondrial homeostasis (e.g., peroxisome proliferator-activated receptor gamma coactivator (PGC) 1 alpha, PGC1α) [7]. Besides, AMPK can adjust intracellular metabolism in a prolonged way by aiming at transcriptional regulators (e.g., forkhead box O<sub>3</sub>) [8]. Overall, activated AMPK balances energy levels by raising ATP synthesis and/or reducing ATP consumption. Thanks to its core roles in intracellular metabolism, dysregulation of AMPK is prevalent in obesity, diabetes, cancer, and cardio-metabolic diseases. AMPK is a prospective pharmacological target [9][10][11][12][13][14][15], notably for treating type 2 diabetes [16][17][18].

## 3. AMPK Structure and Regulation

### 3.1. AMPK Structure

AMPK, a heterotrimeric complex, is composed of a catalytic  $\alpha$ -subunit and beta ( $\beta$ ) and gamma ( $\gamma$ ) regulatory subunits [19]. In humans, each subunit has multiple distinct isoforms encoded by different genes: the  $\alpha$ -subunit has two isoforms,  $\alpha 1$  and  $\alpha 2$ , encoded by gene *PRKAA1* and *PRKAA2*, respectively [20]; the  $\beta$ -subunit has two isoforms,  $\beta 1$  and  $\beta 2$ , encoded by gene *PRKAB1* and *PRKAB2*, respectively [21]; and the  $\gamma$ -subunit has three isoforms,  $\gamma 1$ ,  $\gamma 2$ , and  $\gamma 3$ , encoded by gene *PRKAG1*, *PRKAG2*, and *PRKAG3*, respectively [22]. Each AMPK complex is comprised of an  $\alpha$ -subunit, a  $\beta$ -subunit, and a  $\gamma$ -subunit, and all possible combinations produce 12 different AMPK complexes [23].

The  $\alpha$ -subunit contains the kinase domain and a critical residue (Thr174 in  $\alpha 1$ -subunit and Thr172 in  $\alpha 2$ -subunit, hereafter referred to as Thr172) that is phosphorylated by upstream kinases [1]. The  $\alpha$ -subunit is essential, and a double knockout (KO) of AMPK $\alpha 1$  and AMPK $\alpha 2$  in mice gives rise to embryonic lethality [24]. The  $\beta$ -subunit includes a carbohydrate-binding module that grants AMPK to bind to glycogen [25]. The  $\gamma$ -subunit allows AMPK to react to changes in the level of AMP, ADP, and ATP, as it includes four tandem cystathionine  $\beta$  synthase motifs that allow AMPK to bind adenine nucleotides [26].

### 3.2. Regulation of AMPK

#### 3.2.1. AMPK is Activated by AMP/ADP and Inhibited by ATP

AMPK is activated by energy stress and/or cellular stress in response to increased ATP consumption (e.g., exercise, cell proliferation, and anabolism) or decreased ATP production (e.g., low glucose levels, oxidative stress, and hypoxia), which are sensed as low ratios of ATP-to-AMP/ADP. Binding of AMP, and to a lesser degree ADP, to the  $\gamma$ -subunit stimulates AMPK activity via three mechanisms [27][28][29]. First, AMP has been hypothesized to

promote the phosphorylation of Thr172 by directly stimulating the activity of the upstream kinase, or by an allosteric mechanism that would render AMPK a suitable substrate for the upstream kinase [30][31][32]. Liver kinase B 1 (LKB1) [32] and calcium/calmodulin-dependent protein kinase kinase (CAMKK) 2 [33][34] can be employed as the two main mammalian upstream molecules that phosphorylate AMPK. Second, AMP protects Thr172 against dephosphorylation by phosphatases [28][35][36][37]. Third, AMP causes the allosteric activation of AMPK that has already been phosphorylated on Thr172. Activation loop phosphorylation increases AMPK activity by about 100-fold, while allosteric regulation changes AMPK activity up to tenfold in mammalian cells and about twofold in recombinant, bacterially produced AMPK [26][28][38][39]. Lastly, allosteric AMPK activation can also occur in non-phosphorylated AMPK, with even higher fold activation than phosphorylated AMPK [40].

### 3.2.2. Regulation of AMPK by Reactive Oxygen Species (ROS)

ROS are plenty of free radicals and chemically reactive molecules derived from oxygen molecule [41]. Most ROS are generated as byproducts of mitochondrial electron transportation from the reduction of molecular oxygen to produce superoxide [41]. The sequential reduction of superoxide gives rise to the generation of various ROS that includes hydroxyl radical, hydrogen peroxide, and hydroxyl ion [41]. The actions of ROS can be both beneficial and detrimental [41]. The beneficial aspects of ROS at physiological levels are usually related to their effects in cell survival signaling cascades [41]. Excessive production of ROS, usually termed oxidative stress, are caused by increased production and/or insufficient presence of endogenous antioxidants [41]. Oxidative stress is considered detrimental because of its damage to fats, deoxyribonucleic acid, and proteins in a cell, which can lead to numerous diseases such as diabetes, cancer, neurodegenerative disease, and cardiovascular disease (CVD) [41]. Recent lines of evidence have demonstrated AMPK responsiveness to imbalanced redox status, thus bringing new insights into the networks of redox-stimulated signals upstream of AMPK (Table 1) [42][43]. For instance, Trolox, a vitamin E analog and antioxidant, reduces cellular ROS levels [44]. The addition of Trolox to mouse embryonic fibroblasts (MEFs) resulted in basal AMPK activity reduction, as evidenced by the decreased phosphor-AMPK $\alpha$  at Thr172 [44].

**Table 1.** The type of reactive oxygen species (ROS) that activates adenosine monophosphate-activated protein kinase (AMPK). ATP, adenosine triphosphate; ADP, adenosine diphosphate; BAEC, bovine aortic endothelial cell; MEF, mouse embryonic fibroblast; VSMC, vascular smooth muscle cell; HUVEC, human umbilical vein endothelial cell; NOS, nitric oxide synthase; LKB1, liver kinase B 1; CAMKK2, calcium/calmodulin-dependent protein kinase kinase; PDK1, pyruvate dehydrogenase kinase 1; CRAC, calcium release-activated calcium.

Stimuli	ROS	Cell Types	Animal Strains	Mechanisms	Refs
N/A	Hydrogen peroxide	H4IIEC3	N/A	PKCz-LKB1	[45]

N/A	Hydrogen peroxide	HeLa	N/A	CaMKK	<a href="#">[45]</a>
Berberine	Mitochondrial ROS, peroxynitrite	BAEC	N/A	LKB1	<a href="#">[46]</a>
N/A	Hydrogen peroxide	C2C12	N/A	ATP-to-ADP ratio	<a href="#">[47]</a>
N/A	Hydrogen peroxide	NIH-3T3	N/A	AMP-to-ATP ratio	<a href="#">[48]</a>
N/A	Hydrogen peroxide	HEK293	N/A	ADP-to-ATP ratio	<a href="#">[49]</a>
N/A	NO, peroxynitrite	N/A	nNOS <sup>-/-</sup> mice	N/A	<a href="#">[50]</a>
N/A	Hydrogen peroxide	HEK293	C3Ga.Cg-Cat B/J mice	S-glutathionylation of Cys299 and Cys304 on the AMPK $\alpha$ subunit	<a href="#">[51]</a>
N/A	Mitochondrial ROS	MEF	N/A	N/A	<a href="#">[44]</a>
Trolox	Physiological ROS	MEF	N/A	N/A	<a href="#">[44]</a>
Thromboxane receptor	Hydrogen peroxide	VSMC	N/A	LKB1	<a href="#">[52]</a>

Glucose oxidase	Hydrogen peroxide	HEK293, HeLa	N/A	AMP-to-ATP ratio	<a href="#">[53]</a>
N/A	Peroxynitrite	BAEC	N/A	N/A	<a href="#">[54]</a>
Sodium nitroprusside	NO	HUVEC	N/A	sGC-Ca <sup>2+</sup> -CAMKK2 axis	<a href="#">[55]</a>
Hypoxia-reoxygenation	Peroxynitrite	BAEC	N/A	cSRC-PI <sub>3</sub> K-PDK1	<a href="#">[56]</a>
Hypoxia	Mitochondrial ROS	AMPK $\alpha 1^{-/-} 2^{-/-}$ MEF	N/A	LKB1	<a href="#">[57]</a>
Hypoxia	Cellular ROS	143B, LKB1 <sup>-/-</sup> MEF	N/A	CRAC-CAMKK2	<a href="#">[58]</a>
2-Deoxy-d-glucose	Mitochondrial ROS	BAEC	N/A	N/A	<a href="#">[59]</a>
Metformin	RNS	BAEC	eNOS <sup>-/-</sup> mice	cSRC-PI <sub>3</sub> K-LKB1 axis	<a href="#">[60]</a>

### Hydrogen Peroxide and AMPK Activation

AMPK is activated by hydrogen peroxide in various cell types. In H4IIEC3 cells, exogenous hydrogen peroxide transiently increased the level of phosphor-AMPK by LKB1 signaling, while exogenous hydrogen peroxide promoted AMPK phosphorylation in HeLa cells by CaMKK signaling. Thromboxane A2 receptor (TPr) agonists increased the expression of nicotinamide adenine dinucleotide phosphate oxidase (NOX), thereby promoting the formation of superoxide in vascular smooth muscle cells (VSMCs). Exposure of cultured rat VSMCs to either TPr agonists or hydrogen peroxide led to AMPK activation in a time- and dose-dependent manner, as supported by the increased phosphor-AMPK at Thr172 and its downstream enzyme phosphor-ACC at Ser79 [\[61\]](#). TPr agonists-activated AMPK was mediated by hydrogen peroxide, as demonstrated by the results showing overexpression of

catalase (CAT) abolished Tpr agonists-induced AMPK activation [61]. In the meantime, LKB1 was activated by either TPr agonists or hydrogen peroxide [61]. Further, dominant negative mutation of LKB1 abolished both TPr agonists and hydrogen peroxide-stimulated AMPK activation, which suggested that hydrogen peroxide mediated TPr-triggered AMPK activation by LKB1 signaling [61]. These results collectively suggest that AMPK activation by hydrogen peroxide occurs through activation of either LKB1 or CaMKK, depending on the cell type.

ROS, in excess, can provoke energy stress and metabolic failure by direct oxidizing and inactivating enzymes of the tricarboxylic acid cycle, electron transport chain (ETC), and glycolysis [62][63]. It is well established that the ROS-induced energy stress strongly activates AMPK, which is likely the main mechanism of ROS-mediated AMPK activation. For instance, exposure of exogenous hydrogen peroxide to C2C12 cells led to a dose-dependent increase in AMPK activity by decreasing the ATP-to-ADP ratio. Similarly, in NIH-3T3 cells, exogenous hydrogen peroxide transiently activated AMPK in a dose-dependent manner, which was associated with an increased AMP-to-ATP ratio. Further, using HEK293 cells expressing AMPK $\gamma$ 2 R531G, an AMP-insensitive AMPK complex, AMPK cannot be activated by hydrogen peroxide. These findings suggest that the target for hydrogen peroxide may not be AMPK itself, but components of the ETC, leading to a secondary effect on AMPK through changes to ADP, AMP, and ATP.

Alternatively, some studies showed that ROS directly activated AMPK independent of changes in ADP, AMP, and ATP. Exogenous or glucose oxidase-generated hydrogen peroxide induced direct S-glutathionylation on the AMPK $\alpha$  subunit at Cys299 and Cys304, which contributes to AMPK activation. However, this view has been challenged with the fact showing that replacement of redox-sensitive cysteine residues with insensitive alanine residues does not affect hydrogen peroxide-induced AMPK activation.

### Reactive Nitrogen Species (RNS) and AMPK Activation

Nitric oxide (NO) is produced by nitric oxide synthase (NOS) from L-arginine in the cell. RNS is produced beginning with the interaction between NO and superoxide to generate peroxynitrite. The sequential reaction of peroxynitrite with other molecules generates additional species of RNS, including dinitrogen trioxide and nitrogen dioxide, as well as other types of chemically reactive molecules. ROS broadly include NO and its derivative RNS, both of which have been implicated in AMPK activation.

Compared with wild-type (WT) mice, neuronal NOS KO (nNOS<sup>-/-</sup>) mice showed both reduction of stroke damage and AMPK activation, which suggests a possible link between NO and AMPK activity in the context of stroke. In the studies using cultured bovine aortic endothelial cells (BAECs), peroxynitrite significantly activated AMPK, as supported by increased phosphor-AMPK at Thr172 as well as increased phosphor-ACC, a downstream target of AMPK, at Ser79. In endothelial cells (ECs), studies showed that NO endogenously activated AMPK via elevating Ca<sup>2+</sup> levels by the activation of soluble guanylyl cyclase. Further, NO-triggered Ca<sup>2+</sup> increase causes AMPK activation through CaMKK2 signaling.

Exposure of ECs to chemically synthesized peroxynitrite acutely and significantly increased phosphorylation of AMPK and its downstream target ACC without affecting cellular AMP. Hypoxia-reoxygenation (H/R) increased both AMPK and ACC phosphorylation in cultured BAECs, which was prevented by either inhibition of NOS or overexpression of SOD, suggesting a role of peroxynitrite formed during H/R. These results indicate that H/R via peroxynitrite activates AMPK in ECs. It is debatable whether LKB1 participates in AMPK activation under hypoxia condition. Some studies showed that LKB1<sup>-/-</sup> MEFs under hypoxia for 30 min could not increase ACC phosphorylation, while LKB1<sup>-/-</sup> MEFs under hypoxia for 2 h activated AMPK through CaMKK2, indicating that LKB1-induced AMPK activation occurs in a context-dependent manner.

NO- and RNS-dependent activation of AMPK is probably related to the ability of NO and RNS to suppress mitochondrial ATP production by modulating the ETC [64]. In fact, NO at nanomolar concentration can form a nitrosyl-heme complex to selectively and reversibly inhibit cytochrome c oxidase [65]. RNS at higher concentration can induce cysteine S-nitrosylation or tyrosine nitration in almost all the complexes of mitochondrial ETC [66][67].

### 3.2.3. AMPK is a Key Modulator for Maintaining Redox Homeostasis

AMPK is not only a sensor of redox signal, but also plays an anti-oxidative role once activated (Table 2). AMPK can diminish the superoxide production derived from either mitochondria or nicotinamide adenine dinucleotide phosphate oxidase (NOX). Further, AMPK also can suppress oxidative stress by upregulation of antioxidant gene expression.

**Table 2.** Activation of AMPK suppresses oxidative stress. AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; WT, wild-type; SD, Sprague Dawley; SOD, superoxide dismutase; CAT, catalase; UCP, uncoupling protein; NOX, nicotinamide adenine dinucleotide phosphate oxidase; PGC, peroxisome proliferator-activated receptor gamma coactivator.

Stimuli	ROS	Cell Types	Animal Strains	Mechanisms	Refs
A-769662	Mitochondrial ROS	MEF	N/A	AMPK-PGC1 $\alpha$ -CAT/SOD1/SOD2/UCP2 axis	[51]
Salicylate, AICAR	Mitochondrial ROS	RAEC, EA.hy926	WT SD rats	AMPK axis	[68]

Miglitol	N/A	bEnd.3	N/A	AMPK-eNOS axis	[69]
N/A	Intracellular ROS	HUVEC	AMPK $\alpha$ 2 <sup>-/-</sup> mice	AMPK-NOX axis	[70]
Metformin, AICAR	Mitochondrial ROS	HUVEC	N/A	AMPK-PGC1 $\alpha$ -MnSOD axis	[71]
Metformin, AICAR	Superoxide	HUVEC	AMPK $\alpha$ 2 <sup>-/-</sup> mice	AMPK-UCP2 axis	[72]
Metformin	Oxidative stress	N/A	WT BALB/c mice	AMPK-PGC1/SOD1 axis	[73]

### AMPK Suppresses ROS

Mitochondrial ROS is increased by many atherosclerosis stimuli, including hyperglycemia, triglycerides, and oxidized low-density lipoprotein (oxLDL) [74][75][76]. One study reported that pharmacological activation of AMPK by either salicylate or 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) regulated mitochondrial morphology and ameliorated endothelial dysfunction through suppression of mitochondrial ROS-associated endoplasmic reticulum (ER) stress and the subsequent activation of the thioredoxin-interacting protein-associated NACHT, LRR and PYD domains-containing protein 3 inflammasome. Miglitol protected against endothelial cell damage from oxidative stress via inhibition of apoptosis and mitochondrial superoxide production by activation of the AMPK–eNOS axis.

Under basal conditions, in addition to mitochondrial ETC, NOX is another source of ROS production in ECs [77]. Inflammation-triggered endothelium activation generally gives rise to a large scale of ROS production that is mainly attributed to membrane-bound and/or cytosolic enzymes such as NOX [78]. Further, NOX-induced ROS production can enhance ROS production from other sources such as mitochondrial ETC and xanthine oxidase (XO) [77][79][80]. Analysis of AMPK $\alpha$ 2 KO ECs and AMPK  $\alpha$ 2-silenced human umbilical vein endothelial cells (HUVECs) indicated that suppression of AMPK could increase NOX expression, NOX-mediated superoxide production, 26S proteasome activity, inhibitory subunit of nuclear factor kappa B $\alpha$  degradation, and nuclear translocation of nuclear factor kappa B. These studies indicated that AMPK $\alpha$ 2 functions as a physiological suppressor of NOX and ROS production in ECs, in which way AMPK maintains the nonatherogenic and non-inflammatory phenotype of ECs.

### AMPK Increases Antioxidant Potentials



The continuously produced ROS from mitochondrial ETC can be eliminated by antioxidant systems. It has been demonstrated that AMPK activation limited mitochondrial ROS generation in MEFs through PGC1 $\alpha$ -dependent antioxidant response . In this study, the potent AMPK activator A-769662 promoted an AMPK-dependent increase in several antioxidant genes, including *CAT*, superoxide dismutase (*SOD*)1, *SOD*2, and uncoupling protein 2 (*UCP*2). Moreover, a study using control or PGC1 $\alpha$ -deficient MEFs with A-769662 showed that antioxidant gene expression was dependent on PGC1 $\alpha$  expression. Similarly, in HUVECs, metformin normalized hyperglycemia-induced mitochondrial ROS production by inducing manganese SOD and promoting mitochondrial biogenesis through the activation of the AMPK–PGC1 $\alpha$  pathway . AMPK activation by either metformin or AICAR in HUVECs could suppress superoxide production by increasing the expression of the antioxidant gene *UCP*2 . Further, AMPK activation by metformin lowered the grade of acute lung injury and acute respiratory distress syndrome via the inhibition of oxidative stress by upregulating *SOD*1 and PGC1 $\alpha$ , thus providing potential value for treating such conditions in clinic .

## 4. Perspectives

It is well-accepted that AMPK activation plays protective roles across diverse CVDs. AMPK has emerged as a guardian of mitochondria because almost all mitochondrial insults can activate AMPK. However, the interplay within the AMPK–mitochondrial axis during the initiation and progression of CVDs is poorly investigated. Limited evidence has focused on mitochondrial dynamics, mitochondrial biogenesis, and mitophagy, the three common functions of mitochondria that have been studied by cell biologists. Far beyond our current knowledge are the structural and functional changes to mitochondria that may be amplified under disease conditions. Therefore, it would be advantageous for investigators in the field of CVDs to focus on observing, defining, and clarifying novel characteristics and properties of mitochondria, thus contributing to both cell biology and advancing disease treatments.

## References

1. Hardie, D.G.; Ross, F.A.; Hawley, S.A. AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* 2012, 13, 251–262.
2. Gwinn, D.M.; Shackelford, D.B.; Egan, D.F.; Mihaylova, M.M.; Mery, A.; Vasquez, D.S.; Turk, B.E.; Shaw, R.J. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* 2008, 30, 214–226.
3. Inoki, K.; Zhu, T.; Guan, K.L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003, 115, 577–590.
4. Carling, D.; Zammit, V.A.; Hardie, D.G. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Lett.* 1987, 223, 217–222.

5. Munday, M.R.; Campbell, D.G.; Carling, D.; Hardie, D.G. Identification by amino acid sequencing of three major regulatory phosphorylation sites on rat acetyl-CoA carboxylase. *Eur. J. Biochem.* 1988, 175, 331–338.
6. Marsin, A.S.; Bertrand, L.; Rider, M.H.; Deprez, J.; Beauloye, C.; Vincent, M.F.; Van den Berghe, G.; Carling, D.; Hue, L. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. *Curr. Biol.* 2000, 10, 1247–1255.
7. Jager, S.; Handschin, C.; St-Pierre, J.; Spiegelman, B.M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 $\alpha$ . *Proc. Natl. Acad. Sci. USA* 2007, 104, 12017–12022.
8. Greer, E.L.; Oskoui, P.R.; Banko, M.R.; Maniar, J.M.; Gygi, M.P.; Gygi, S.P.; Brunet, A. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *J. Biol. Chem.* 2007, 282, 30107–30119.
9. Yuan, H.X.; Xiong, Y.; Guan, K.L. Nutrient sensing, metabolism, and cell growth control. *Mol. Cell* 2013, 49, 379–387.
10. Garcia, D.; Shaw, R.J. AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol. Cell* 2017, 66, 789–800.
11. Steinberg, G.R.; Kemp, B.E. AMPK in health and disease. *Physiol. Rev.* 2009, 89, 1025–1078.
12. Hardie, D.G. AMPK: A target for drugs and natural products with effects on both diabetes and cancer. *Diabetes* 2013, 62, 2164–2172.
13. Hardie, D.G. Targeting an energy sensor to treat diabetes. *Science* 2017, 357, 455–456.
14. Hardie, D.G.; Ross, F.A.; Hawley, S.A. AMP-activated protein kinase: A target for drugs both ancient and modern. *Chem. Biol.* 2012, 19, 1222–1236.
15. Guigas, B.; Viollet, B. Targeting AMPK: From ancient drugs to new small-molecule activators. *Exp. Suppl.* 2016, 107, 327–350.
16. Cokorinos, E.C.; Delmore, J.; Reyes, A.R.; Albuquerque, B.; Kjobsted, R.; Jorgensen, N.O.; Tran, J.L.; Jatkar, A.; Cialdea, K.; Esquejo, R.M. et al. Activation of skeletal muscle ampk promotes glucose disposal and glucose lowering in non-human primates and mice. *Cell Metab.* 2017, 25, 1147–1159.e10.
17. Myers, R.W.; Guan, H.P.; Ehrhart, J.; Petrov, A.; Prahalada, S.; Tozzo, E.; Yang, X.; Kurtz, M.M.; Trujillo, M.; Gonzalez Trotter, D. et al. Systemic pan-AMPK activator MK-8722 improves glucose homeostasis but induces cardiac hypertrophy. *Science* 2017, 357, 507–511.
18. Steneberg, P.; Lindahl, E.; Dahl, U.; Lidh, E.; Straseviciene, J.; Backlund, F.; Kjellkvist, E.; Berggren, E.; Lundberg, I.; Bergqvist, I. et al. PAN-AMPK activator O304 improves glucose

- homeostasis and microvascular perfusion in mice and type 2 diabetes patients. *JCI Insight* 2018, 3.
19. Yan, Y.; Zhou, X.E.; Xu, H.E.; Melcher, K. Structure and physiological regulation of AMPK. *Int. J. Mol. Sci.* 2018, 19.
  20. Stapleton, D.; Mitchelhill, K.I.; Gao, G.; Widmer, J.; Michell, B.J.; Teh, T.; House, C.M.; Fernandez, C.S.; Cox, T.; Witters, L.A. et al. Mammalian AMP-activated protein kinase subfamily. *J. Biol. Chem.* 1996, 271, 611–614.
  21. Thornton, C.; Snowden, M.A.; Carling, D. Identification of a novel AMP-activated protein kinase beta subunit isoform that is highly expressed in skeletal muscle. *J. Biol. Chem.* 1998, 273, 12443–12450.
  22. Cheung, P.C.; Salt, I.P.; Davies, S.P.; Hardie, D.G.; Carling, D. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. *Biochem. J.* 2000, 346 Pt 3, 659–669.
  23. Ross, F.A.; MacKintosh, C.; Hardie, D.G. AMP-activated protein kinase: A cellular energy sensor that comes in 12 flavours. *FEBS J.* 2016, 283, 2987–3001.
  24. Viollet, B.; Athea, Y.; Mounier, R.; Guigas, B.; Zarrinpashneh, E.; Horman, S.; Lantier, L.; Hebrard, S.; Devin-Leclerc, J.; Beauloye, C. et al. AMPK: Lessons from transgenic and knockout animals. *Front. Biosci. (Landmark Ed)* 2009, 14, 19–44.
  25. Hudson, E.R.; Pan, D.A.; James, J.; Lucocq, J.M.; Hawley, S.A.; Green, K.A.; Baba, O.; Terashima, T.; Hardie, D.G. A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. *Curr. Biol.* 2003, 13, 861–866.
  26. Xiao, B.; Heath, R.; Saiu, P.; Leiper, F.C.; Leone, P.; Jing, C.; Walker, P.A.; Haire, L.; Eccleston, J.F.; Davis, C.T. et al. Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature* 2007, 449, 496–500.
  27. Hardie, D.G.; Carling, D.; Gamblin, S.J. AMP-activated protein kinase: Also regulated by ADP? *Trends Biochem. Sci.* 2011, 36, 470–477.
  28. Gowans, G.J.; Hawley, S.A.; Ross, F.A.; Hardie, D.G. AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. *Cell Metab.* 2013, 18, 556–566.
  29. Ross, F.A.; Jensen, T.E.; Hardie, D.G. Differential regulation by AMP and ADP of AMPK complexes containing different gamma subunit isoforms. *Biochem. J.* 2016, 473, 189–199.
  30. Hawley, S.A.; Boudeau, J.; Reid, J.L.; Mustard, K.J.; Udd, L.; Makela, T.P.; Alessi, D.R.; Hardie, D.G. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta

are upstream kinases in the AMP-activated protein kinase cascade. *J. Biol.* 2003, 2, 28.

31. Shaw, R.J.; Kosmatka, M.; Bardeesy, N.; Hurley, R.L.; Witters, L.A.; DePinho, R.A.; Cantley, L.C. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc. Natl. Acad. Sci. USA* 2004, 101, 3329–3335.
32. Woods, A.; Johnstone, S.R.; Dickerson, K.; Leiper, F.C.; Fryer, L.G.; Neumann, D.; Schlattner, U.; Wallimann, T.; Carlson, M.; Carling, D. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol.* 2003, 13, 2004–2008.
33. Fujiwara, Y.; Kawaguchi, Y.; Fujimoto, T.; Kanayama, N.; Magari, M.; Tokumitsu, H. Differential AMP-activated Protein Kinase (AMPK) Recognition mechanism of Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase isoforms. *J. Biol. Chem.* 2016, 291, 13802–13808.
34. Hawley, S.A.; Pan, D.A.; Mustard, K.J.; Ross, L.; Bain, J.; Edelman, A.M.; Frenguelli, B.G.; Hardie, D.G. Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell Metab.* 2005, 2, 9–19.
35. Xiao, B.; Sanders, M.J.; Underwood, E.; Heath, R.; Mayer, F.V.; Carmena, D.; Jing, C.; Walker, P.A.; Eccleston, J.F.; Haire, L.F. et al. Structure of mammalian AMPK and its regulation by ADP. *Nature* 2011, 472, 230–233.
36. Garcia-Haro, L.; Garcia-Gimeno, M.A.; Neumann, D.; Beullens, M.; Bollen, M.; Sanz, P. The PP1-R6 protein phosphatase holoenzyme is involved in the glucose-induced dephosphorylation and inactivation of AMP-activated protein kinase, a key regulator of insulin secretion, in MIN6 beta cells. *FASEB J.* 2010, 24, 5080–5091.
37. Joseph, B.K.; Liu, H.Y.; Francisco, J.; Pandya, D.; Donigan, M.; Gallo-Ebert, C.; Giordano, C.; Bata, A.; Nickels, J.T., Jr. Inhibition of AMP kinase by the protein phosphatase 2a heterotrimer, PP2A<sup>pp2r2d</sup>. *J. Biol. Chem.* 2015, 290, 10588–10598.
38. Sanders, M.J.; Ali, Z.S.; Hegarty, B.D.; Heath, R.; Snowden, M.A.; Carling, D. Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family. *J. Biol. Chem.* 2007, 282, 32539–32548.
39. Davies, S.P.; Helps, N.R.; Cohen, P.T.; Hardie, D.G. 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. *FEBS Lett.* 1995, 377, 421–425.
40. Willows, R.; Sanders, M.J.; Xiao, B.; Patel, B.R.; Martin, S.R.; Read, J.; Wilson, J.R.; Hubbard, J.; Gamblin, S.J.; Carling, D. Phosphorylation of AMPK by upstream kinases is required for activity in mammalian cells. *Biochem. J.* 2017, 474, 3059–3073.
41. Alfadda, A.A.; Sallam, R.M. Reactive oxygen species in health and disease. *J. Biomed. Biotechnol.* 2012, 2012, 936486.

42. Xie, Z.; Dong, Y.; Zhang, M.; Cui, M.Z.; Cohen, R.A.; Riek, U.; Neumann, D.; Schlattner, U.; Zou, M.H. Activation of protein kinase C zeta by peroxynitrite regulates LKB1-dependent AMP-activated protein kinase in cultured endothelial cells. *J. Biol. Chem.* 2006, 281, 6366–6375.
43. Wu, Y.; Song, P.; Zhang, W.; Liu, J.; Dai, X.; Liu, Z.; Lu, Q.; Ouyang, C.; Xie, Z.; Zhao, Z. et al. Activation of AMPK $\alpha$ 2 in adipocytes is essential for nicotine-induced insulin resistance in vivo. *Nat. Med.* 2015, 21, 373–382.
44. Rabinovitch, R.C.; Samborska, B.; Faubert, B.; Ma, E.H.; Gravel, S.P.; Andrzejewski, S.; Raissi, T.C.; Pause, A.; St-Pierre, J.; Jones, R.G. AMPK maintains cellular metabolic homeostasis through regulation of mitochondrial reactive oxygen species. *Cell Rep.* 2017, 21, 1–9.
45. Liangpunsakul, S.; Wou, S.E.; Zeng, Y.; Ross, R.A.; Jayaram, H.N.; Crabb, D.W. Effect of ethanol on hydrogen peroxide-induced AMPK phosphorylation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2008, 295, G1173–G1181.
46. Han, Y.; Wang, Q.; Song, P.; Zhu, Y.; Zou, M.H. Redox regulation of the AMP-activated protein kinase. *PLoS ONE* 2010, 5, e15420.
47. Hinchey, E.C.; Gruszczyn, A.V.; Willows, R.; Navaratnam, N.; Hall, A.R.; Bates, G.; Bright, T.P.; Krieg, T.; Carling, D.; Murphy, M.P. Mitochondria-derived ROS activate AMP-activated protein kinase (AMPK) indirectly. *J. Biol. Chem.* 2018, 293, 17208–17217.
48. Choi, S.L.; Kim, S.J.; Lee, K.T.; Kim, J.; Mu, J.; Birnbaum, M.J.; Soo Kim, S.; Ha, J. The regulation of AMP-activated protein kinase by H<sub>2</sub>O<sub>2</sub>. *Biochem. Biophys. Res. Commun.* 2001, 287, 92–97.
49. Hawley, S.A.; Ross, F.A.; Chevtzoff, C.; Green, K.A.; Evans, A.; Fogarty, S.; Towler, M.C.; Brown, L.J.; Ogunbayo, O.A.; Evans, A.M. et al. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab.* 2010, 11, 554–565.
50. McCullough, L.D.; Zeng, Z.; Li, H.; Landree, L.E.; McFadden, J.; Ronnett, G.V. Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. *J. Biol. Chem.* 2005, 280, 20493–20502.
51. Zmijewski, J.W.; Banerjee, S.; Bae, H.; Friggeri, A.; Lazarowski, E.R.; Abraham, E. Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. *J. Biol. Chem.* 2010, 285, 33154–33164.
52. Koupparis, A.J.; Jeremy, J.Y.; Muzaffar, S.; Persad, R.; Shukla, N. Sildenafil inhibits the formation of superoxide and the expression of gp47 NAD[P]H oxidase induced by the thromboxane A<sub>2</sub> mimetic, U46619, in corpus cavernosal smooth muscle cells. *BJU Int.* 2005, 96, 423–427.
53. Auciello, F.R.; Ross, F.A.; Ikematsu, N.; Hardie, D.G. Oxidative stress activates AMPK in cultured cells primarily by increasing cellular AMP and/or ADP. *FEBS Lett.* 2014, 588, 3361–3366.

54. Zou, M.H.; Hou, X.Y.; Shi, C.M.; Nagata, D.; Walsh, K.; Cohen, R.A. Modulation by peroxynitrite of Akt- and AMP-activated kinase-dependent Ser1179 phosphorylation of endothelial nitric oxide synthase. *J. Biol. Chem.* 2002, 277, 32552–32557.
55. Zhang, J.; Xie, Z.; Dong, Y.; Wang, S.; Liu, C.; Zou, M.H. Identification of nitric oxide as an endogenous activator of the AMP-activated protein kinase in vascular endothelial cells. *J. Biol. Chem.* 2008, 283, 27452–27461.
56. Zou, M.H.; Hou, X.Y.; Shi, C.M.; Kirkpatrick, S.; Liu, F.; Goldman, M.H.; Cohen, R.A. Withdrawal: Activation of 5'-AMP-activated kinase is mediated through c-Src and phosphoinositide 3-kinase activity during hypoxia-reoxygenation of bovine aortic endothelial cells: Role of peroxynitrite. *J. Biol. Chem.* 2019, 294, 18016.
57. Emerling, B.M.; Weinberg, F.; Snyder, C.; Burgess, Z.; Mutlu, G.M.; Viollet, B.; Budinger, G.R.; Chandel, N.S. Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. *Free Radic. Biol. Med.* 2009, 46, 1386–1391.
58. Mungai, P.T.; Waypa, G.B.; Jairaman, A.; Prakriya, M.; Dokic, D.; Ball, M.K.; Schumacker, P.T. Hypoxia triggers AMPK activation through reactive oxygen species-mediated activation of calcium release-activated calcium channels. *Mol. Cell. Biol.* 2011, 31, 3531–3545.
59. Wang, Q.; Liang, B.; Shirwany, N.A.; Zou, M.H. 2-Deoxy-D-glucose treatment of endothelial cells induces autophagy by reactive oxygen species-mediated activation of the AMP-activated protein kinase. *PLoS ONE* 2011, 6, e17234.
60. Zou, M.H.; Kirkpatrick, S.S.; Davis, B.J.; Nelson, J.S.; Wiles, W.G.t.; Schlattner, U.; Neumann, D.; Brownlee, M.; Freeman, M.B.; Goldman, M.H. Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. *J. Biol. Chem.* 2004, 279, 43940–43951.
61. Zhang, M.; Dong, Y.; Xu, J.; Xie, Z.; Wu, Y.; Song, P.; Guzman, M.; Wu, J.; Zou, M.H. Thromboxane receptor activates the AMP-activated protein kinase in vascular smooth muscle cells via hydrogen peroxide. *Circ. Res.* 2008, 102, 328–337.
62. Avery, S.V. Molecular targets of oxidative stress. *Biochem. J.* 2011, 434, 201–210.
63. Cecarini, V.; Gee, J.; Fioretti, E.; Amici, M.; Angeletti, M.; Eleuteri, A.M.; Keller, J.N. Protein oxidation and cellular homeostasis: Emphasis on metabolism. *Biochim. Biophys. Acta* 2007, 1773, 93–104.
64. Piantadosi, C.A. Regulation of mitochondrial processes by protein S-nitrosylation. *Biochim. Biophys. Acta* 2012, 1820, 712–721.
65. Sarti, P.; Forte, E.; Giuffre, A.; Mastronicola, D.; Magnifico, M.C.; Arese, M. The chemical interplay between nitric oxide and mitochondrial cytochrome c oxidase: Reactions, effectors and pathophysiology. *Int. J. Cell Biol.* 2012, 2012, 571067.

66. Almeida, A.; Ciudad, P.; Delgado-Esteban, M.; Fernandez, E.; Garcia-Nogales, P.; Bolanos, J.P. Inhibition of mitochondrial respiration by nitric oxide: Its role in glucose metabolism and neuroprotection. *J. Neurosci. Res.* 2005, 79, 166–171.
67. Moncada, S.; Bolanos, J.P. Nitric oxide, cell bioenergetics and neurodegeneration. *J. Neurochem.* 2006, 97, 1676–1689.
68. Li, J.; Wang, Y.; Wang, Y.; Wen, X.; Ma, X.N.; Chen, W.; Huang, F.; Kou, J.; Qi, L.W.; Liu, B. et al. Pharmacological activation of AMPK prevents Drp1-mediated mitochondrial fission and alleviates endoplasmic reticulum stress-associated endothelial dysfunction. *J. Mol. Cell. Cardiol.* 2015, 86, 62–74.
69. Aoki, C.; Suzuki, K.; Yanagi, K.; Satoh, H.; Niitani, M.; Aso, Y. Miglitol, an anti-diabetic drug, inhibits oxidative stress-induced apoptosis and mitochondrial ROS over-production in endothelial cells by enhancement of AMP-activated protein kinase. *J. Pharmacol. Sci.* 2012, 120, 121–128.
70. Wang, S.; Zhang, M.; Liang, B.; Xu, J.; Xie, Z.; Liu, C.; Viollet, B.; Yan, D.; Zou, M.H. AMPK $\alpha$ 2 deletion causes aberrant expression and activation of NAD(P)H oxidase and consequent endothelial dysfunction in vivo: Role of 26S proteasomes. *Circ. Res.* 2010, 106, 1117–1128.
71. Kukidome, D.; Nishikawa, T.; Sonoda, K.; Imoto, K.; Fujisawa, K.; Yano, M.; Motoshima, H.; Taguchi, T.; Matsumura, T.; Araki, E. Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes* 2006, 55, 120–127.
72. Xie, Z.; Zhang, J.; Wu, J.; Viollet, B.; Zou, M.H. Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. *Diabetes* 2008, 57, 3222–3230.
73. Wang, G.; Song, Y.; Feng, W.; Liu, L.; Zhu, Y.; Xie, X.; Pan, Y.; Ke, R.; Li, S.; Li, F. et al. Activation of AMPK attenuates LPS-induced acute lung injury by upregulation of PGC1 $\alpha$  and SOD1. *Exp. Ther. Med.* 2016, 12, 1551–1555.
74. Cho, Y.E.; Basu, A.; Dai, A.; Heldak, M.; Makino, A. Coronary endothelial dysfunction and mitochondrial reactive oxygen species in type 2 diabetic mice. *Am. J. Physiol. Cell Physiol.* 2013, 305, C1033–1040.
75. Matsumoto, S.; Gotoh, N.; Hishinuma, S.; Abe, Y.; Shimizu, Y.; Katano, Y.; Ishihata, A. The role of hypertriglyceridemia in the development of atherosclerosis and endothelial dysfunction. *Nutrients* 2014, 6, 1236–1250.
76. Zmijewski, J.W.; Moellering, D.R.; Le Goffe, C.; Landar, A.; Ramachandran, A.; Darley-Usmar, V.M. Oxidized LDL induces mitochondrially associated reactive oxygen/nitrogen species formation in endothelial cells. *Am. J. Physiol. Heart Circ. Physiol.* 2005, 289, H852–H861.

77. Shen, H.M.; Liu, Z.G. JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. *Free Radic. Biol. Med.* 2006, 40, 928–939.
78. Ray, R.; Shah, A.M. NADPH oxidase and endothelial cell function. *Clin. Sci. (Lond)* 2005, 109, 217–226.
79. Cai, H. NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. *Circ. Res.* 2005, 96, 818–822.
80. Zinkevich, N.S.; Gutterman, D.D. ROS-induced ROS release in vascular biology: Redox-redox signaling. *Am. J. Physiol. Heart Circ. Physiol.* 2011, 301, H647–H653.

---

Retrieved from <https://encyclopedia.pub/entry/history/show/3188>