
Regular Review

AMPK-mediated regulation of lipid metabolism by phosphorylation

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Summary  AMP-activated protein kinase (AMPK) is a metabolic sensor in mammals that is activated when ATP levels in the cell decrease. AMPK is a heterotrimeric protein that comprises 3 subunits, each of which has multiple phosphorylation sites that play critical roles in the regulation of either anabolism or catabolism by directly phosphorylating proteins or modulating gene transcription in multiple pathways, such as synthesis, oxidation and lipolysis of lipid. Research focused on the phosphorylation sites that are involved in lipid metabolism will lead to a better recognition of the role of AMPK in therapeutics for several common diseases. In this review, close attention is paid to the recent research on the structure, and multisite phosphorylation of AMPK subunits, as well as AMPK regulation of lipid metabolism via phosphorylation of related molecules.

Key words  AMP-activated protein kinase (AMPK), lipid metabolism, phosphorylation, fatty acid, review
1. INTRODUCTION

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that emerges as a sensor of cellular energy.\textsuperscript{1)} The compromised cellular energy status gives rise to an increase in the ratio of cellular AMP:ATP that activates AMPK and that AMPK also promotes its own $\alpha$ subunit phosphorylation at Thr172 through upstream kinases. In 1973, it was observed that AMPK activity was related to hydroxy-methylglutaryl coenzyme A reductase (HMGCR) and acetyl-CoA carboxylase (ACC), which are key modulators for cholesterol and fatty acid synthesis.\textsuperscript{2, 3)} In eukaryotes such as mammals, AMPK is believed to act as a key master switch that modulates lipid metabolism by directly phosphorylating proteins or modulating gene transcription in specific tissues such as the liver, fat and muscle.

2. AMPK SUBUNITS

AMPK is a heterotrimeric protein kinase that is composed of $\alpha$ (isoforms $\alpha1$ and $\alpha2$), $\beta$ (isoforms $\beta1$and $\beta2$) and $\gamma$ (isoforms $\gamma1$, $\gamma2$ and $\gamma3$) subunits. The twelve different heterotrimeric holoenzymes consist from the combination of 2 alpha, 2 beta and 3 gamma subunits. The human $\alpha1$ isoform (559 amino acids, 64 kDa) and $\alpha2$ isoform (552 amino acids, 62 kDa) are similar, each having an N-terminal kinase domain (KD), an autoinhibitory domain (AID, residues 302-381), two regulatory-subunit-interacting motifs (RIM) and a C-terminal $\beta/\gamma$ subunits binding domain. One tyrosine residue, 11 threonine residues and 14 serine residues within the $\alpha1$ isoform have been identified, while three threonine residues and nine serine residues within the $\alpha2$ isoform can be phosphorylated. Phosphorylation produces different effects on enzyme activity depending on the residue that is phosphorylated. AMPK is stimulated when it is phosphorylated at a conserved key threonine-172 residue in the
N-terminal regions of the α2 subunit (α2 threonine-172, equivalent to α1 threonine-183), while the C-terminal domain is required to form complexes with the other two subunits. The β1 isoform consists of 270 amino acids (30 kDa), including one myristoylated glycine residue, as well as two tyrosine residue, five threonine residues and 15 serine residues that can be phosphorylated. The β2 isoform consists of 272 amino acids (30 kDa), of which one tyrosine residue, two threonine residues and 13 serine residues can be phosphorylated.

The β1 regulatory isoform has a carbohydrate-binding module (CBM, 96 amino acids) that mediates binding to glycogen, and a C-terminal module that acts as a scaffold to allow binding of the α and γ subunits. Post-translational modification of the β1 regulatory isoform is critical for its regulatory function. Removal of the myristoylated residue results in an increase in AMPK activity and has an effect on its redistribution from intracellular membrane to cytosol. AMPK activity also increases when the β1 isoform is phosphorylated. Moreover, it has been demonstrated that lysine262-sumoylation of the AMPK β2 isoform also causes activation of AMPK.

The γ1 regulatory isoform consists of 331 amino acids (38 kDa) including one lysine residue that can be acetylated, as well as two threonine residues and six serine residues that can be phosphorylated. The γ2 isoform consists of 569 amino acids (63 kDa) with one tyrosine residue, four threonine residues and 20 serine residues that can be phosphorylated. The γ3 isoform consists of 489 amino acids (54 kDa), of which only one residue, a serine at position 65, can be phosphorylated.

In the γ subunit, four tandem cystathionine-β-synthase sequences (CBS motifs) consist of one or more copies of a conserved domain of~60 amino acids forming two α helices and
three β strands.9) CBS domains are numbered as CBS1 to CBS4, which are involved in AMP or ATP binding.10) CBS1 (amino acids 43-103) and CBS3 (amino acids 198-260) bind ATP, ADP or AMP in a competitive manner depending on the cellular energy status.10) CBS2 (amino acids 125-187) does not bind to a nucleotide but is closely related to ACC phosphorylation.11, 12) CBS4 (amino acids 272-329) strongly binds to AMP and may play a structural role.10, 12) When ATP levels decreased in the cell, AMP or ADP production relatively increased. And then AMP or ADP can bind to the CBS1 or CBS3 of γ subunit, resulting in a conformational change that activates AMPK through phosphorylation at Thr172 in α subunit.13) AMPK facilitates the generation of additional ATP by promoting catabolic pathways and inhibiting anabolic pathways, thus acting as a precise indicator of the cellular energy status (Fig.1, TABLE1).

3. MULTISITE PHOSPHORYLATION OF AMPK SUBUNITS

3.1. α and β Subunit Phosphorylation One site in the α subunit (Thr172) is the crucial for AMPK activation via phosphorylation by upstream kinases, which may include liver kinase B1 (LKB1), calmodulin-dependent kinase kinase (CaMKK), and transforming growth factor (TGF)-β-activated kinase-1 (TAK1).14-16) The studies demonstrated that LKB1, in complex with two accessory subunits (STRADα and MO25α), was required for AMPK activation in response to increased AMP, which may promote AMPK phosphorylation or inhibit AMPK dephosphorylation.17, 18) AMPK activity is also promoted by other AMPK kinases in cells with the lack of LKB1 expression. An increase in intracellular Ca^{2+} activates CaMKK, which plays a physiological role in phosphorylating and activating AMPK.16) The third AMPK kinase, TAK1, is a mediation in TGFβ signaling and it stimulated AMPK
phosphorylation in mammalian cells.\textsuperscript{19)}

Besides the active site Thr\textsubscript{172}, a number of sites also play indispensable roles in AMPK activity. One study reported that cyclic AMP (cAMP)-dependent protein kinase (PKA) phosphorylated AMPK\textsubscript{a1} at Ser\textsubscript{173}, resulting in its inhibition.\textsuperscript{20)} Multiple additional phosphorylation sites in the \(\alpha\) subunit have also been studied.\textsuperscript{21)} The \(\alpha\textsubscript{1}\text{Ser485}\) residue is involved in negative regulation of AMPK by cAMP-PKA.\textsuperscript{22)} In additional, phosphorylation of AMPK\(\alpha\) Ser\textsubscript{485}/491, when stimulated with insulin, has been shown to lead to a decrease in AMPK activity.\textsuperscript{23)} AMPK activity is also regulated by many metabolic stresses that disrupt the ratio of AMP:ATP, including mitochondrial poisons (thiazolidinediones, biguanides, and resveratrol), glycolysis inhibitors (2-deoxyglucose), exercise, hypoxia, and adiponectin.\textsuperscript{24, 25)} Moreover, AMPK can be activated in response to other agents. 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) directly activates AMPK through binding to the \(\gamma\) subunit.\textsuperscript{26)} Resistin decreases phosphorylation of LKB1, and subsequently decreases phosphorylation of AMPK.\textsuperscript{27)} The \(\alpha/\beta\)-adrenergic stimulation may lead to AMPK activation by stimulating the upstream signaling of AMPK. Intracellular \(\beta\)-adrenergic signaling pathways, via a G protein activate the downstream enzyme, PKA,\textsuperscript{28)} while the \(\alpha\)-adrenergic agonists activate \(\text{Ca}^{2+}\) signaling in rat cells.\textsuperscript{29)} Additionally, like AMP, a small-molecule activator of AMPK, A769662, directly activated AMPK by allosteric activation and inhibition of dephosphorylation of Thr\textsubscript{172}. However, A769662 activated AMPK harboring a mutation within the \(\gamma\) subunit that counteracted activation by AMP. And the mutation of Ser\textsubscript{108} in the region of the \(\beta1\) subunit termed CBM almost completely neutralized activation of AMPK by A769662, while only partially impairing AMP activation.
These provided evidence that the mechanisms of activation by AMP and A769662 are different. Surprisingly, an ancient drug, salicylate, was also found to directly activate AMPK by binding the same site as A769662. Both salicylate and A769662 need the CBM of AMPKβ1 and its phosphorylation at Ser108 to activate AMPK without increasing cellular ADP:ATP ratio, indicating an AMP independent mechanism (Fig. 2). Besides Ser108, Ser24/25, and Ser182 can be also partially phosphorylated in the AMPKβ1 subunit. The mutants of Ser24/25 and Ser182 do not disrupt AMPK activity, but results in the nuclear redistribution of the holoenzyme (TABLE2).

To date, most studies of AMPK autophosphorylation sites have focused the β-subunits. Autophosphorylation of Ser24/25, Ser108, Ser96, Ser101 and Thr148 within the β subunit have been reported. Autophosphorylation of Thr148 in AMPKβ1/β2 leads to a loss of carbohydrate binding ability. As a switch, it plays a role in regulating the subcellular localization of AMPK. Moreover, AMPKα Ser485/491, which was confirmed by using recombinant protein, was also autophosphorylated to limits AMPK activation in response to energy depletion or other regulators. The functional roles of additional AMPK autophosphorylation sites, such as α1Thr477, α1Ser349, β1Thr158, and β1Thr80, requires further investigation.

3.2. γ Subunit Phosphorylation Phosphorylation of the γ subunit has rarely been reported. One study, which explored the effects of an R225Q mutation in the AMPKγ3 subunit, showed increased basal AMPK phosphorylation that was accompanied by a reduction in sensitivity to AMP. The effects of this mutation on insulin resistance and dietary-induced triacylglycerol (TG) accumulation were explored in AMPKγ3R225Q
transgenic mice. However, the effect of the R225Q mutation on AMPK activity was inadequate to remit obesity and insulin resistance in mice with leptin deficiency. Leptin insufficiency leads to the lack of central leptin signaling, and the metabolic benefit from AMPK$\gamma3$ R225Q mutation was overridden.\textsuperscript{39}\textsuperscript{) Recently, an additional study observed that an AMPK$\gamma2$ R299Q mutation in mice caused obesity and reduced insulin secretion, which may provide new insights to drug targets.\textsuperscript{40}\textsuperscript{)}

4. AMPK-MEDIATED REGULATION OF PROTEINS INVOLVED IN LIPID METABOLISM

In liver and adipose tissue, surplus energy is stored as TG by the fatty acid pathway. Fatty acids can be oxidized to provide energy during times of energy deficiency. When fatty acids are absorbed across the plasma membrane, they are activated to fatty acyl-CoA, which can either be stored or oxidized. The genes that encode ACC1, fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD)1, which are targeted by the transcription factor sterol regulatory element binding protein 1c (SREBP1c), are lipogenic enzymes that promote the \textit{de novo} synthesis of cytoplasmic fatty acids. ACC1 can catalyse the carboxylation of acetyl-CoA to malonyl-CoA, which is the main substrate of FASN. FASN catalyzes the \textit{de novo} synthesis of long-chain fatty acids in the cytosol through the condensation of acyl-CoA and malonyl-CoA. FASN causes increased synthesis of saturated fatty acids, which are converted into monounsaturated fatty acids by SCD1. The final step of this process is TG synthesis from long-chain fatty acids. Mitochondrial glycerol-3-phosphate acyltransferase (GPAT) lies on the mitochondrial outer membrane, which channels acyl-CoAs towards the synthetic pathways. Unlike hormone-sensitive lipase (HSL), mitochondrial GPAT plays a
pivotal role in preventing fatty acids from undergoing β-oxidation by converting acyl-CoA into lysophosphatidate. Adipose triglyceride lipase (ATGL) and HSL are the key enzymes acting successively on lipolysis. When lipolysis occurs, TG can be hydrolysed into glycerol and fatty acids that can be transferred back to the blood. Sequentially, fatty acids can be oxidized to provide energy. Malonyl-CoA is a vital regulatory factor of fatty acid oxidation in liver and it is a potent inhibitor of carnitine acyltransferase I (CPT1), which is allosterically suppressed to reduce the β-oxidation of fatty acid in mitochondria. The inhibition of ACC2 causes the reduced malonyl-CoA, sequentially promotes CPT1, leading to the increased transport of acyl-CoA to mitochondria and the β-oxidation. Recent reports have suggested that AMPK modulates lipid synthesis, lipolysis, and fatty acid oxidation through phosphorylation of key substrates, as discussed below (Fig. 3).

4.1. ACC Nearly 4 decades ago, AMPK regulation of ACC activity was first observed. ACC is an important site of regulation within the fatty acid synthesis and oxidation pathways, as it can catalyse the carboxylation of acetyl-CoA to malonyl-CoA during the synthesis of fatty acids or allosterically inhibit CPT-1, a key enzyme for β-oxidation. ACC consists of both ACC1 and ACC2 isoforms. Data have accumulated that show ACC1 is involved in the regulation of fatty acid synthesis, unlike ACC2, which has a 146-amino acid N-terminal region that directs its localization in mitochondria to inhibit the oxidation of fatty acids. Malonyl-CoA synthesized by ACC2 allosterically inhibits CPT1 to reduce β-oxidation in mitochondria. When purified from lactating rat mammary glands or rat liver, ACC was observed to be phosphorylated by purified AMPK at three sites: Ser79, Ser1200 and Ser1215. Moreover, Ser79 was observed to be responsible for the inactivation of ACC1 by AMPK,
after which phospho-ACC1 Ser79 became a universal indicator of AMPK activity.\textsuperscript{42, 43} In ACC2, Ser219 is the equivalent of Ser79 in ACC1 because of the N-terminal extension. ACC2 activity is inhibited by the phosphorylation of Ser219 by AMPK. It was shown that a lower malonyl-CoA level that was accompanied by increased fatty acid oxidation was associated with decreased ACC2 activity in mouse skeletal muscle.\textsuperscript{44} Moreover, studies on the fruit fly \textit{Drosophila melanogaster} showed that AMPK increased the phosphorylation of Ser-93 in ACC.\textsuperscript{45} The identification of sites of AMPK phosphorylation in human ACC1 (Ser80 and Ser1216) and yeast ACC (Ser1157) have enhanced our understanding of ACC regulation by phosphorylation. Phosphorylation of Ser80 in human ACC1 and Ser1157 in yeast ACC have been reported to stabilize inactive ACC conformations by facilitating the dissociation of the biotin carboxylase domain dimer and stabilizing the monomeric form of the biotin carboxylase domain, which repressed the catalytic activity of ACC.\textsuperscript{46}

\section*{4.2. SREBP}

Consistent with the above-mentioned study, activation of AMPK\textsubscript{\textalpha} was observed to reduce TG accumulation in the hepatocytes,\textsuperscript{47} where ACC1,\textsuperscript{48} a downstream substrate of AMPK, inhibited fatty acid synthesis. The genes that encode ACC1 and other lipogenic enzymes, such as FASN, and SCD1, are targeted by SREBP1c,\textsuperscript{49} a member of the SREBP family.

SREBPs comprise a family of transcription factors with three isoforms (SREBP1\textsubscript{a}, SREBP1\textsubscript{c} and SREBP2), which modulate the expression of enzymes involved in the synthesis of fatty acids, TG, phospholipids, and cholesterol.\textsuperscript{50} As a key transcription factor, SREBP1c directly promotes the expression of over 30 genes that facilitate fatty acid uptake and TG synthesis.\textsuperscript{51} SREBPs can be modified by phosphorylation to regulate their stability.
A number of post-translational modifications of SREBP1, including phosphorylation at Thr426, Ser430 or Ser410, have been shown to be related to SREBP1c activity. Li Y et al. showed that SREBP1c was directly phosphorylated by AMPK and associated with reduction of nuclear SREBP1c. AMPK was shown to phosphorylate SREBP1c at Ser372, suppressing the proteolytic cleavage of precursor SREBP1c into mature SREBP1c, leading to the suppression of hepatic steatosis in diet-induced insulin-resistant mice. In addition to direct regulation, AMPK can further reduce hepatic lipid contents by suppressing SREBP1c expression through decreasing the activity of mammalian target of rapamycin complex (mTORC), an important mediator for the regulation of cellular metabolism and growth that may also promote SREBP-dependent fatty acid synthesis. mTORC1-dependent activation of SREBP1c is thought to accelerate fatty acid synthesis by cleavage of the SREBP1c molecule. mTORC2 is also required for the coordinated regulation of insulin-induced glycolysis and lipogenesis by glucokinase and SREBP1c. Moreover, activation of AMPK may also suppress the transcriptional activity of downstream targets, such as liver X receptors (LXRs), which is an upstream transcription factor that increase SREBP1c activity to promote fatty acid synthesis. AMPK suppresses SREBP1c expression through these targets, reducing FASN expression, and subsequently inhibiting lipid synthesis.

SREBP2 is the primary transcriptional regulator in cholesterol metabolism, and preferentially activates the expression of 12 enzymes involved in cholesterol biosynthesis. Short-term chronic activation of AMPK led to a 1.6-fold increase of SREBP2 mRNA in the transgenic liver that selectively expressed constitutively active AMPK, while SREBP2
mRNA levels fell by 70% in white adipose tissue. In addition, AMPK decreased the nuclear translocation of SREBP2 in hepatic cells. A recent research study observed that AMPK suppressed SREBP2 activity by direct phosphorylation, which impeded the accumulation of nuclear SREBP2, repressed the expression of its downstream targets, and ameliorated dyslipidemia. In another study, an immunoprecipitation and immunoblot analysis with specific antibodies showed that AMPK phosphorylated threonine residues of the SREBP2 precursor and nuclear forms in HepG2 cells, LO2 cells, and in TSH receptor knockout mice. Future research will require that the specific AMPK-induced phosphorylation sites in SREBP2 be identified. Moreover, Wang XH et al. observed that the intracellular cholesterol was increased and high levels of SREBP2 and HMGCR mRNA were present in a time-dependent manner in steatotic hepatocytes, suggesting that the increased intracellular cholesterol was probably a result of the upregulation of SREBP2 and its target genes. HMGCR is the most important target of SREBP2. It was suggested that AMPK activation decreased cleavage of SREBP2 in diet-induced insulin-resistant LDLR−/− mice that were treated with the synthetic polyphenol S17834 and reduced the expression of the downstream targets, such as HMGCR. Another study also suggested that AICAR-induced activation of AMPK suppressed the expression of the genes encoding HMGCR and HMGCS, which was associated with reduced expression of SREBP2.

4.3. GPAT TG synthesis is inhibited by pre-treatment with AICAR, an AMPK activator, which could decrease the incorporation of fatty acids into TG. GPAT, which exists in microsomal and the mitochondrial isoforms, catalyses the formation of lysophosphatidic acid from long chain acyl-CoA and glycerol-3-phosphate, and it is the rate-limiting enzyme that
catalyses the first and critical step in TG synthesis.\cite{68} GPAT activity and TG biosynthesis in the liver have been inhibited by AMPK activation.

GPAT has four dominating isoforms (GPAT1-GPAT4). GPAT1 and GPAT2 lie in the outer mitochondrial membrane, and the other two are the isoforms of endoplasmic reticulum. Among them, three (in addition to GPAT1) are suppressed by N-ethylmaleimide (NEM), which is a significant contributor to TG synthesis. All four isoforms take part in the glycerolipid biosynthetic pathway, working in coordination for TG synthesis. One study showed that the overexpression of mitochondrial GPAT, which is primarily expressed in the liver, led to a dramatic 80% reduction in fatty acid oxidation and a significant increase in hepatic diacylglycerol and phospholipid biosynthesis, resulting in a significant increase in intracellular TG synthesis.\cite{69} In hepatocytes, AMPK-mediated phosphorylation of mitochondrial GPAT leads to its inactivation.\cite{70} Following exercise, GPAT activity appears to be inhibited by an increase in AMPK activity\cite{70, 71} by the administration of AICAR in the liver and epididymal fat, but not in muscle.\cite{72} A very low activity of GPAT in muscle was also observed in another study,\cite{70} in which the effects of AICAR or AMPK on muscle GPAT activities in this tissue could not be directly tested, although TG synthesis was substantially inhibited. Therefore, the liver is the primary organ for GPAT regulation with respect to intracellular TG biosynthesis. Moreover, Muoio DM et al. suggested that when cellular ATP levels were compromised, inactivation of mitochondrial GPAT, mediated by AMPK, served to conserve energy by suppressing TG synthesis and eliminating the direct competitor of CPT1 for acyl-CoA substrates, which may potentiate fatty acid entry into the mitochondria.\cite{70} GPAT gene expression was also suppressed in the presence of an AMPK inhibitor, compound C,
which subsequently reduced TG accumulation.73)

4.4. HSL There are three stages occurring in the TG lipolysis, accompanying with key enzymes acting on different steps. ATGL catalyzes the hydrolysis of TG to generate diacylglycerol (DG). HSL exhibits as a DG hydrolase by primary converting DG to monoacylglycerol (MAG). Finally MAG is hydrolyzed to release glycerol. Among them, fatty acid is liberated at each stage.

HSL is considered as a rate-limiting enzyme in TG hydrolysis, and is the major lipase that regulates catecholamine- and natriuretic peptide-induced lipolysis. Experiments have suggested that PKA phosphorylates HSL at Ser563 and Ser660 to activate HSL for lipolysis in adipose tissue, which is essential for HSL translocation to lipid droplets in response to lipolytic stimuli. Additionally, AICAR-induced AMPK could phosphorylate HSL at Ser565 to inhibit HSL Ser660 and Ser563 phosphorylation, reducing HSL activity and significantly suppressing lipolysis in adipocytes.74, 75) These data demonstrate that AMPK inhibits HSL by phosphorylation, that phosphorylated HSL is unable to translocate to lipid droplets in AMPKα1 knock-out mice and in primary rodent adipocytes and in 3T3-L1 adipocytes by using pharmacological activators or with adenovirus that constitutively expresses active or dominant negative forms of AMPK.76) However, the role of AMPK in the regulation of TG lipolysis in adipocytes was controversial.76, 77) AMPK may phosphorylate Ser406 of ATGL to stimulate TG hydrolase activity and activate lipolysis.78) AMPK can promote lipolysis under basal conditions by increasing desnutrin/ATGL phosphorylation. Nevertheless, under stimulated conditions, AMPK decreases HSL activity in adipose tissue, leading to an inhibition of lipolysis, but free fatty acid release increased.79) Moreover, one study reported
that HSL Ser563 phosphorylation, activity and lipolysis were not inhibited by AMPK activation during exercise.\textsuperscript{80) However, activated AMPK was shown to be involved in suppressing the activity of HSL, overriding β-adrenergic stimulation of TG lipolysis.\textsuperscript{81) This discrepancy may be associated with tissue-specific function of AMPK at different conditions.}

4.5. HMGCR HMGCR is a key rate-limiting enzyme in the cholesterol biosynthetic pathway. Because of the involvement of cholesterol in the development of atherosclerosis and cardiovascular disease, regulating HMGCR has been a subject of great interest. Its activity may be regulated by the equilibrium between enzyme phosphorylation and dephosphorylation, where phosphorylation inactivates the enzyme. HMGCR phosphorylation, which occurs at Ser871 in rodents and Ser872 in humans, is of crucial importance in regulating hepatic cholesterol biosynthesis. In addition to protein kinase C (PKC) and a Ca\textsuperscript{2+}/calmodulin-dependent protein kinase, AMPK is an important HMGCR kinase in the rat liver and copurifies with ACC kinase activity. When phosphorylated at Thr172, AMPK is activated and can phosphorylate HMGCR, leading to its inactivity. Recently, Zhang X et al.\textsuperscript{82) observed that AMPK activation of HMGCR was inhibited by HMGCR phosphorylation at Ser872, establishing a potential mechanism for hypercholesterolemia.

5. CONCLUSIONS

It is well known that AMPK plays a vital role in the development and treatment of diabetes, obesity, and metabolic syndromes with respect to the above-mentioned effects of AMPK on lipid metabolism. In this review, we explored several aspects of AMPK, such as its structure, multisite phosphorylation of subunits and its regulation of the phosphorylation of key molecules involved in lipid metabolism. Furthermore, studies on the potential
mechanisms of AMPK-mediated modulation of lipid metabolism are required for exploration in greater detail. The information will tremendously help in the design of drugs to treat obesity and metabolic syndromes.

**Conflict of Interest** The authors declare no conflict of interest.
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Fig. 1. Domain structure of AMP-activated protein kinase (AMPK) subunit. The important phosphorylation sites in AMPK subunits are indicated. The positions of nucleotide binding to residues are shown within γ subunit. AID, autoinhibitory domain; RIM, regulatory-subunit-interacting motifs; CBM, carbohydrate-binding module; CBS, cystathionineβsynthase repeats. (Color figure can be accessed in the online version.)
Fig. 2. AMP-activated protein kinase (AMPK) phosphorylation is regulated not only by the upstream kinases (LKB1, CaMKK, and TAK1), but also by many metabolic stresses that interfere with AMP:ATP ratio or other mechanisms. The phosphorylation at αSer173, αSer485/491, and βSer108 also regulate AMPK activity. LKB1, liver kinase B1; CaMKK, calmodulin-dependent kinase kinase; TGFβ, transforming growth factor β; TAK1, TGF-β-activated kinase-1; PKA, cAMP-dependent protein kinase; AKT, protein kinase B; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide. Activating interactions are marked with arrows and inhibitory connections with stoppers.
Fig. 3. AMP-activated protein kinase (AMPK) plays critical roles in the regulation of either anabolism or catabolism by directly phosphorylating proteins or modulating gene transcription in multiple pathways, such as synthesis, oxidation and lipolysis of lipid. The phosphorylation sites of the related lipogenic molecules are mentioned in the present review. TG, triacylglycerol; DG, diacylglycerol; MAG, monoacylglycerol; FA, fatty acid; ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; SREBP, sterol regulatory element binding protein; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; ATGL, adipose triglyceride lipase; HMGCR, hydroxy-methylglutaryl coenzyme A reductase; mTORC, mammalian target of rapamycin complex; LXR, liver X receptor. Activating interactions are marked with green arrows and
inhibitory connections with red stoppers. The color circles/boxes indicated whether that general process is activated (green) or inhibited (red) by AMPK. The dotted box indicated potentially ambiguous regulation. AMPK-mediated phosphorylation events were in yellow. Transcriptional regulators are denoted by an asterisk. Descending arrows indicated ACC2 inhibition and malonyl-CoA reduction; ascending arrows depicted CPT1 activation and the increase of β-oxidation. (Color figure can be accessed in the online version.)
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LKB1, liver kinase B1; CaMKK, calmodulin-dependent kinase kinase; TAK1, transforming growth factor (TGF)-β-activated kinase-1; PKA, cAMP-dependent protein kinase; AKT, protein kinase B;