AMPK as a direct sensor of long-chain fatty acyl–CoA esters

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Nutrient sensing

AMPK as a direct sensor of long chain fatty acyl-CoA esters

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AMPK is a crucial sensor of the cellular energetic state and is also activated during glucose starvation. A new study reports that AMPK is activated by interaction with long-chain fatty acid-CoA esters (LCFA-CoAs), which appear to be the long-sought endogenous AMPK ligands that bind to the Allosteric Drug and Metabolite (ADaM) site.

The AMP-activated protein kinase (AMPK), which occurs in essentially all eukaryotes as heterotrimeric complexes comprising catalytic α subunits and regulatory β and γ subunits, is best known as a sensor of cellular energy status that is also activated during glucose starvation[1]. AMPK senses energy and nutrient status by competitive binding of AMP, ADP or ATP at multiple nucleotide-binding sites on the γ subunit and via an indirect, nucleotide-independent mechanism involving sensing of the glucose metabolite fructose 1,6-bisphosphate by the glycolytic enzyme aldolase[1]. Once switched on, AMPK restores energy balance and adjusts cellular metabolism by phosphorylating numerous downstream targets[2]. In this issue of Nature Metabolism, Pinkosky et al[3] report that AMPK is also a direct sensor of nutrients, by providing evidence that long-chain fatty acid-CoA esters (LCFA-CoAs) are the long-sought naturally-occurring ligands that activate AMPK by binding the so-called "ADaM" site.

Since AMPK activation was already known to cause a switch away from anabolism and nutrient storage and towards catabolism instead, small molecule activators of AMPK were proposed as potential treatments for disorders of energy balance such as obesity and Type 2 diabetes over 20 years ago[4]. These considerations led to high-throughput screens aimed at identifying novel compounds that allosterically activated AMPK. First to be described was A-769662, which had poor oral availability and was selective for AMPK complexes containing the β1 isoform[5], but these efforts culminated in the development of MK-8722 and PF-739, which activate both β1- and (less potently) β2-complexes, and have beneficial effects on metabolism in mouse and non-human primate models of obesity and diabetes when administered orally[6,7]. The exact binding site for this

class of activator was identified by crystallography to be a hydrophobic cleft between the β subunit carbohydrate-binding module (β-CBM) and the N-lobe of the α subunit kinase domain[7]; since this site lies between two subunits of the heterotrimer, it is unique to AMPK. However, a conundrum was that all compounds known to bind this site were synthetic molecules (apart from salicylate, a plant product[8]), and no ligands that occur naturally in mammals had been found. This site was therefore considered a type of "orphan receptor", although it was suspected that a natural metabolite ligand exists, hence its speculative designation as the "Allosteric Drug and Metabolite" (ADaM) site[9].

To "de-orphanize" this site, Pinkosky et al[3] started with an educated guess that the mystery ligands might be LCFA-CoA esters, based in part on much earlier reports (e.g.[10]). They found that micromolar concentrations of LCFA-CoAs containing saturated or mono-unsaturated fatty acids of 12 carbons or more all activated AMPK, whereas the corresponding free acids or carnitine esters did not. Like A-769662, palmityl-CoA (C16) only activated β1-complexes, and β-CBM mutations that perturb the ADaM site, but not γ subunit mutations affecting the nucleotide-binding sites, eliminated or reduced LCFA-CoA-induced AMPK activation. Although the authors did not succeed in obtaining crystal structures of AMPK with LFCA-CoAs, they modelled the binding of palmityl-CoA, which suggested that the fatty acid could bind in the well-defined ADaM site cleft, while the adenine moiety of CoA bound in a pocket in the β-CBM not utilized by the synthetic activators.

In cells or tissues subject to energy stress or glucose starvation, mitochondrial oxidation of LCFAs can represent a crucial source of ATP. Activation of AMPK by LCFA-CoAs, derived either from external sources or breakdown of triglycerides stored in lipid droplets, would represent a type of feed-forward activation triggering enhanced LCFA oxidation via phosphorylation of acetyl-CoA carboxylases (ACC1/ACC2), with consequent relief of malonyl-CoA inhibition of carnitine:palmitoyl-CoA transferase-1 (CPT1) to promote LCFA uptake into mitochondria (Fig. 1). Consistent with this model, Pinkosky et al[3] found that incubation of mouse hepatocytes with LCFAs promoted phosphorylation of ACC1/ACC2 at the AMPK sites (S79/S221), while oral administration of Intralipid (an emulsion containing triglycerides and phospholipids) *in vivo* promoted fat oxidation in wild type mice, but not in knock-in mice in which S79 and S221 had been mutated.

These results are exciting because they appear to have solved a long-standing conundrum about the physiological role of the ADaM site, and also suggest for the first time that AMPK is a direct nutrient sensor. However, some important questions remain. Firstly, which pathways downstream of AMPK other than LCFA oxidation (e.g. LCFA synthesis, mitochondrial biogenesis, autophagy?) are also modulated by LCFA-CoAs? Secondly, are there other natural metabolites that activate AMPK by binding the ADaM site, particularly for β2-complexes that appear to be resistant to LCFA-CoAs? Thirdly, since AMP does not allosterically activate AMPK orthologs from all species, how well-conserved is this effect of LCFA-CoAs across eukaryotes?

The author has no conflict of interest.

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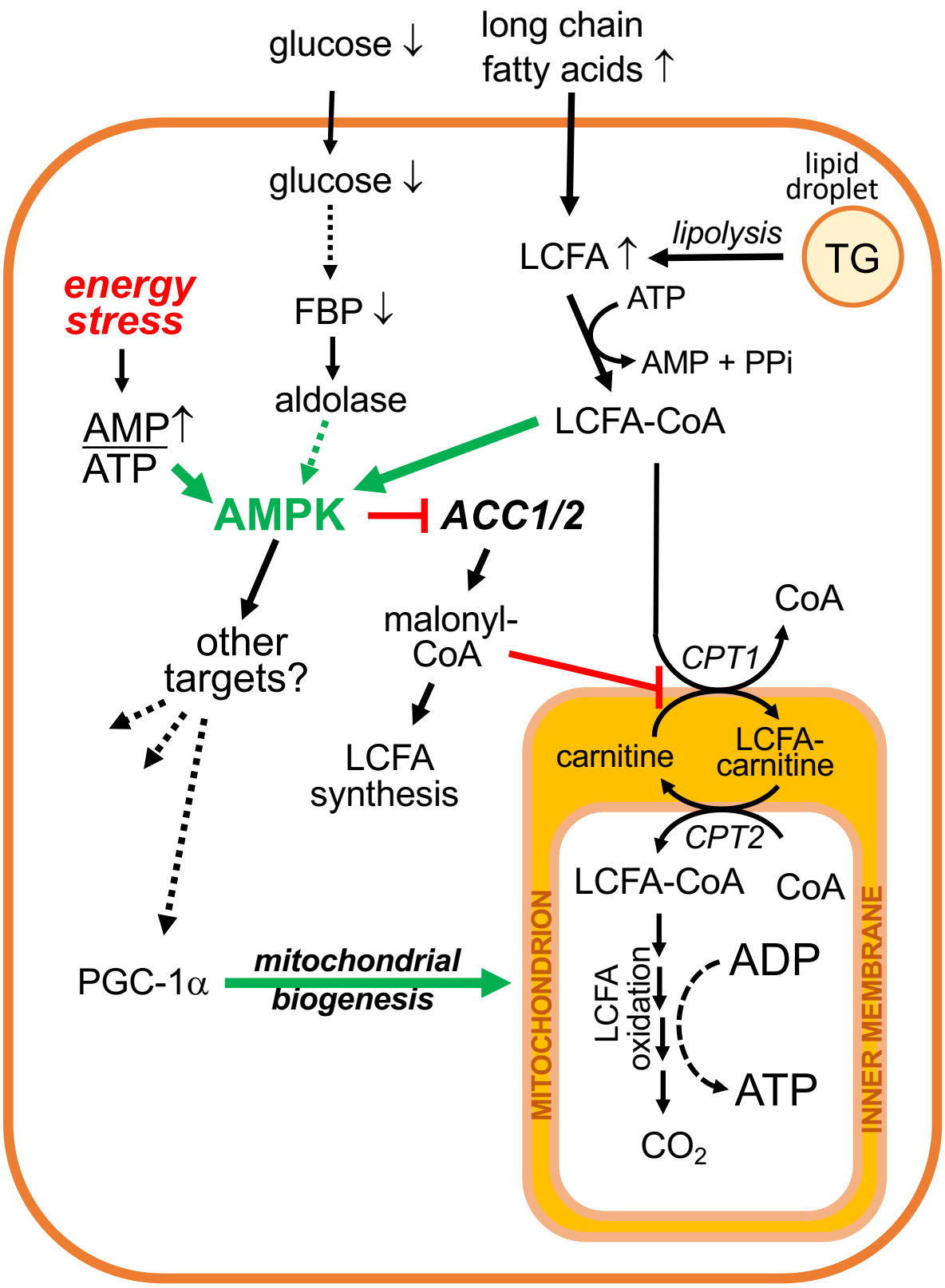
FIGURE LEGEND:

Figure 1: Activation of AMPK by long chain fatty acid (LCFA)-CoA esters and other stimuli. AMPK can be activated by energy stress, by glucose starvation or by the newly found binding of LCFA-CoAs to the ADaM site[3]. AMPK then phosphorylates and inactivates ACC1 and/or ACC2, leading to decreases in their product malonyl-CoA, relieving inhibition of LCFA uptake into mitochondria via the CPT1/CPT2 system. Once inside mitochondria, LCFAs are oxidized to generate large quantities of ATP. Phosphorylation of ACC1/ACC2 by AMPK would also be expected to inhibit LCFA synthesis, while AMPK may phosphorylate other targets with additional effects on metabolism, such as up-regulation of PGC-1α leading to mitochondrial biogenesis, which would further promote LCFA oxidation in the longer term. KEY: ACC1/2, acetyl-CoA carboxylase-1/-2; CPT1/2, carnitine:palmityl-CoA transferase-1/-2; FBP, fructose-1,6-bisphosphate; TG, triglyceride; dashed arrows indicate multiple steps and/or indirect effects.

Figure 1


glucose ↓
long chain fatty acids ↑
glucose ↓
lipid droplet
TG
lipolysis
LCFA ↑
energy stress
FBP ↓
ATP
AMP + PPi
aldolase
LCFA-CoA
AMP↑ / ATP
AMPK
ACC1/2
malonyl-CoA
CoA
other targets?
CPT1
carnitine
LCFA-carnitine
LCFA synthesis
CPT2
LCFA-CoA
CoA
ADP
LCFA oxidation
ATP
CO2
PGC-1α
mitochondrial biogenesis
MITOCHONDRION
INNER MEMBRANE