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# Unraveling the actions of AMP-activated protein kinase in metabolic diseases: Systemic to molecular insights

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ABSTRACT

AMP-activated protein kinase (AMPK) plays a critical role both in sensing and regulating cellular energy state. In experimental animals, its activation has been shown to reduce the risk of obesity and diabetes-related co-morbidities such as insulin resistance, the metabolic syndrome and atherosclerotic cardiovascular disease. However, in humans, AMPK activation alone often does not completely resolve these conditions. Thus, an improved understanding of AMPK action and regulation in metabolic and other diseases is needed. Herein, we provide a brief description of the enzymatic regulation of AMPK and review its role in maintaining energy homeostasis. We then discuss tissue-specific actions of AMPK that become distorted during such conditions as obesity, type 2 diabetes and certain cancers. Finally, we explore recent findings regarding the interactions of AMPK with mammalian target of rapamycin complex 1 and the lysosome and discuss how changes in these relationships during overnutrition may lead to AMPK dysfunction. A more thorough understanding of AMPK's molecular interactions during diseases of overnutrition may provide key insights for the development of AMPK-based combinatorial treatments for metabolic disease.



## 1. Introduction

Escalating rates of obesity and its associated co-morbidities highlight the critical need for an improved understanding of energy metabolism during disease [1]. AMP-activated protein kinase (AMPK) is an enzyme that responds to decreases in cellular energy state by stimulating processes that generate ATP such as fatty acid oxidation and glucose transport, and inhibiting others that consume ATP such as fatty acid and protein synthesis [2]. AMPK also counteracts cellular abnormalities that commonly occur during obesity and diabetes such as endoplasmic reticulum (ER) stress, oxidative stress and inflammation. Evidence from experimental animals with obesity and/or diabetes and obese humans indicates that AMPK may become inhibited or dysfunctional, yet the mechanisms responsible for this are poorly understood [3]. What is clear is that the consequences of dysfunctional AMPK include an increased risk of insulin resistance, hypertension and cardiovascular disease (CVD) and possibly a predilection to certain cancers [4–6]. Finally, several

*Abbreviations:* ACC, acetyl CoA carboxylase; AMPK, AMP-activated protein kinase; AS160, Akt substrate of 160 kDa; ER, endoplasmic reticulum; GSK3β, glycogen synthase kinase 3β; HAECs, human aortic endothelial cells; LKB1, liver kinase B1; MEF, mouse embryonic fibroblast; NOS, nitric oxide synthase; mTORC1, mammalian target of rapamycin complex 1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; PKC, protein kinase C; Rheb, Ras homolog enriched in brain; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element binding protein-1c; TBC1D1, TBC1 domain family member 1; TFEB, transcription factor EB; TSC, tuberous sclerosis complex; ULK1, Unc-51-like kinase 1.

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endogenous factors have been identified that could contribute to such AMPK dysregulation. They include high concentrations of glucose, fatty acids such as palmitate, and glucocorticoids and a decreased concentration of adiponectin; the same factors that predispose individuals to metabolic diseases [3,7]. However, the mechanisms by which AMPK is dysregulated are not clear and may vary among these factors and between different types of cells.

Conversely, AMPK is activated in multiple tissues by physical exercise, which has long been known to increase insulin sensitivity and decrease adiposity, hypertension, atherosclerotic CVD and certain cancers [8,9]. In addition, highly-prescribed medications such as metformin and statins that have shown some efficacy in treating these conditions are, among their other actions, AMPK activators at least in certain tissues. Intriguingly these drugs do not completely ameliorate disease risk in all patients [10]. We propose that this could be attributed to metabolic disease induced-modifications of the pathways these drugs normally activate that make them less efficacious, as may be the case with AMPK activators.

We will briefly discuss how AMPK is regulated enzymatically and during exercise and review its regulation in different tissues during obesity, type 2 diabetes and cancer, metabolic conditions associated with chronic overnutrition. We will then explore recent findings describing its molecular interactions with mammalian target of rapamycin complex 1 (mTORC1) and the lysosome and discuss how alterations of these relationships during overnutrition may contribute to AMPK dysregulation.

## 2. AMPK: Structure and Regulation

AMPK is a heterotrimer consisting of a catalytic subunit (α) and two regulatory subunits (β and γ) (Fig. 1). The existence of two α, two β and three γ subunit isoforms creates a diversity of trimeric complexes which likely contributes to the similar, but not always identical, actions of AMPK in different tissues of a single organism. Recent structural analyses have confirmed decades of experimental findings indicating that AMP, ADP and ATP regulate AMPK, as extensively reviewed by Hardie [2]. The γ subunit contains 4 CBS domains, 1 of which binds AMP constitutively, and two others on which AMP can displace ATP. When the AMP/ATP ratio is high (e.g. during glucose deprivation, hypoxia or exercise), AMP displaces ATP at these two sites (Fig. 1). In addition to this allosteric activation which moderately (2–10-fold) activates the enzyme, binding of AMP increases the susceptibility of the α subunit to phosphorylation at Thr172 by upstream kinases and decreases its accessibility to phosphatases. ADP can also activate AMPK by decreasing the accessibility of phosphorylated Thr172 to phosphatases [2]. Phosphorylation of Thr172 is not required for activity, but increases it nearly 100-fold, making it a more potent modulator of activity than allosteric activation alone. This has led to the use of p-AMPK$^{Thr172}$ as a surrogate indicator for AMPK activity. Depending upon the context, there are at least three protein kinases that may phosphorylate Thr172 and activate AMPK (Fig. 1). There are also many pharmaceutical and naturally-occurring activators of AMPK that operate through a variety of mechanisms, as previously reviewed [2]. Conversely, AMPK activity may be inhibited by several factors such as glycogen synthase kinase 3β (GSK3β), Akt [2] and possibly other molecules including protein kinase C (PKC) (Coughlan et al., unpublished) (Fig. 1).

## 3. AMPK in Exercise

It has long been established that regular exercise is beneficial for maintaining optimal health. Many studies have shown that it reduces the risk of developing type 2 diabetes, CVD and certain cancers [8,9]. Studies in both humans and experimental animals suggest that such benefits are related to its ability to decrease insulin resistance, obesity and ectopic lipid deposition, and increase muscle capillary density, as previously reviewed [3,11,12]. Despite this, the cellular mechanisms responsible for these effects are incompletely understood.

One important insight into the cellular mechanisms by which exercise acts was the discovery in rats that both treadmill running and electrical stimulation of the hindlimb skeletal muscle activate AMPK [13]. Activation of AMPK and its upstream kinase LKB1 in skeletal muscle during exercise has been extensively confirmed in experimental animals and in humans [11,14]. In this context, it has been hypothesized that activation of AMPK increases muscle glucose uptake and mitochondrial biogenesis, and decreases protein synthesis [11]. Whether all of these processes directly rely on AMPK remains to be determined. Data from muscle-specific AMPK knock-out mice currently suggest that AMPK is necessary for exercise-induced glucose uptake and efficient mitochondrial biogenesis [15], but the involvement of factors that may be independent of AMPK (e.g. ERK1/2, PKC) in fatty acid oxidation in muscle (as is suggested by some animal and human studies [8]) remains to be clearly established [16].

Given the important role of skeletal muscle in glucose disposal during exercise, dysfunction of skeletal muscle AMPK could impact the development of diabetes, other metabolic syndrome-associated disorders and insulin resistance. There is also a more comprehensive physiological benefit of exercise that involves a systemic response of AMPK. Experimental models have shown that exercise activates AMPK in adipose tissue, heart, liver, pancreas and aortic endothelium, as well as muscle and that this may contribute to its many benefits [17–21]. Interestingly, exercise-induced activation of AMPK is attenuated in patients with obesity or type 2 diabetes compared to healthy adults, suggesting that chronic metabolic disease may suppress AMPK activity and/or result in AMPK dysfunction [22]. In this context, whether the decrease in AMPK activity is a marker of the metabolic disease or a causative factor remains to be determined.

## 4. AMPK Dysfunction in Specific Tissues and in Cancer

### 4.1. *Tissue-Specific Mechanisms of AMPK Action*

#### 4.1.1. *Skeletal Muscle*

During exercise, AMPK in skeletal muscle is activated which in turn leads to the phosphorylation and activation of Akt