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Original Article

AMP-activated protein kinase (AMPK) in skeletal muscle during physical exercise

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Abstract
AMP-activated protein kinase (AMPK) is a phylogenetically conserved serine-threonine protein kinase that plays a major role as fuel-sensing enzyme.As such it stimulates the fatty acid oxidation, glucose uptake and ketogenesis with a concomitant inhibition of lipogenesis and a modulation of insulin secretion. AMPK is mainly expressed in liver, brain and skeletal muscle, tissues where the bioenergetic metabolism is relevant for the physiology of the entire organism. In addition, emerging evidences indicate that the dysregulation of AMPK functions can be associated to the pathogenesis of several diseases including type 2 diabetes and cancer. For these latter properties, AMPK functions turnover has also been considered as a target for specific therapeutic approaches.Considering the skeletal muscle physiology, during physical exercise,the energy demand increases compared to the resting state, and a large amount of ATP is hydrolyzed causing the increase of the AMP/ATP ratio. The increase of intracellular AMP concentration leads to the activation of AMPK thatmodulates the activity of the main regulatory enzymes involved in energy metabolism.The activation of AMPK triggers signaling pathways that lead to the stimulation of energy producing processes (substrates uptake and catabolic pathways)as well as to the inhibition of biosynthetic pathways that need energy expenditure. Moreover, the involvement of AMPK in mitochondrial biogenesis has also been proposed to be relevant in skeletal muscle tissue plasticity. In this mini reviewwe report of the main biochemical and functional properties of AMPK, its activation during physical exercise, focusingon the molecular and cellular effectsin muscle contraction.
Keywords: AMP-activated protein kinase (AMPK); physical exercise; energy metabolism; skeletal muscle.

Introduction

Skeletal muscle is a tissue not only responsible for human movement but it also affects the whole energy metabolism of the entire organism(Egan and Zierath, 2013). Moreover, the muscle tissue shows high plasticity for its ability to modify the number, the size, and the composition of its myofibers as well as to adapt the energy metabolism in response to physical exercise (PE) (Fluck, 2006).Several molecular and cellular mechanisms are involved in the adaptation of skeletal muscle to PE, including regulation of gene expression, protein synthesis and energy related metabolic pathways (Bassel-Duby and Olson, 2006). Since in skeletal muscle, the ATP turnover may increase up to 100-fold in response to PE, it is importantthat the ATP production and hydrolysis are maintained in balance. Among the intracellular component involved in the ATP concentration homeostasis, the AMP-activated protein kinase (AMPK) is a key regulatory enzyme that ensures this balance according to the cell energy needs (Gowens and Hardie, 2014; Garcia et al., 2017; Hardie, 2018).AMPK is activated upon the increase of AMP levels or coupled with decreased ATP concentration (Chen et al., 2003). Activated AMPK phosphorylates many target proteins, includingtranscription factors andkey metabolic enzymes causing either the activation of ATP-producing pathways, including the production of new mitochondria (Herzig et al., 2018) or the inhibition of ATP-consuming processes, thus restoring energy homeostasis (Kjøbsted et al., 2018).

Recently, it has been shown that AMPK can also sense the availability of glucose, the primary carbon source for most eukaryotic cells, via a mechanism independent of changes in AMP or ADP levels (Carling, 2019). This behaviour has also been related to cancer progression (Monteverde et al., 2015; Wang et al., 2016; Arcucci et al. 2014), via the involvement of metalloproteinase 9 (MMP-9) induction (Endo et al., 2018, Pagliara et al., 2014).

Drugs that modulate AMPK have great potential in the treatment of metabolic disorders such as obesity and Type 2 diabetes, and even in cancer (Daignan-Fornier et al., 2012; Kjøbsted et al., 2018). Indeed, some existing drugs such as metformin and aspirin, appear to work, in part, by activating AMPK. On the other hand, lifestyle behaviour including the use of naturally derived dietary supplements display a beneficial effect on

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cancer progression (Yuan et al., 2012; Tong et al., 2016; Margina et al., 2020) also addressing MMP-9 expression (Pagliara et al., 2019; Arcone et al., 2016).

Skeletal muscle contraction and energysystems

In the cell, adenosine triphosphate (ATP)is consumed for energy-requiring anabolic processes, such as biosynthesis of macromolecules and for mechanical work, during physical exercise in the skeletal muscle. Muscle contraction needs ATP hydrolysis with the production of adenosine diphosphate (ADP), phosphate ions (Pi) and the concomitant release of Energy (Fig. 1 a). Then, in the recovery phase, the ADP and Pi are reconverted into ATP mainly by the electron chain transport and oxidative phosphorylation (figure 1b) a mitochondrialprocess in the eukaryotic cells. Under rest conditions, the balancegenerated by reactions that produce ATP.

a) ATP $\Longrightarrow$ ADP + P_i+ Energy(mechanic) Muscle contraction

b) ADP + P_i+ Energy (metabolic)ATP $\Longrightarrow$ Catabolic pathways

Figure 1: a) ATP hydrolysis by myosin ATPase during skeletal muscle contraction; b) ATP synthesis, mainly by ATP synthase during the electron chain transport and oxidative phosphorylation.

Up today, three major energy systems have been described for ATP synthesis, that are based on different mechanisms depending on exercise intensity and duration: a) fosfagen system: b) glycolitic system and c) mitochondrial respiration.

a) PCr + ADP □ Cr + ATP (*creatine kinase*)
ADP + ADP □ ATP + AMP (*adenylate kinase*)
b) ADP + Pi □ ATP (substrate level phosphorylation: *pyruvate and phosphoglycerate kinase)*
c) ADP + Pi □ ATP (mitochondrial oxidative phosphorylation)

Figure 2. Main skeletal muscle ATP production systems

Therefore, in these energy production systems, adenosine monophosphate (AMP) in mainly produced by the adenylate kinase, that catalyses the reversible conversion of the two ADP molecules in ATP and AMP (Figure 2 a).

AMPK: anenergy-sensing enzyme

AMPK is a highly conserved heterotrimeric enzymecomplex (E.C. 2.7.11.31) in all eukaryotic organisms, from the yeast to the man, that consists of the three distinct components, a catalytic (α)and two regulatory (β and γ) subunits.The 3D structure of the human AMPK derived for the atomic coordinates deposited in the RCS Protein data Bank (Xiao et al., 2013) is reported in Fig. 3.

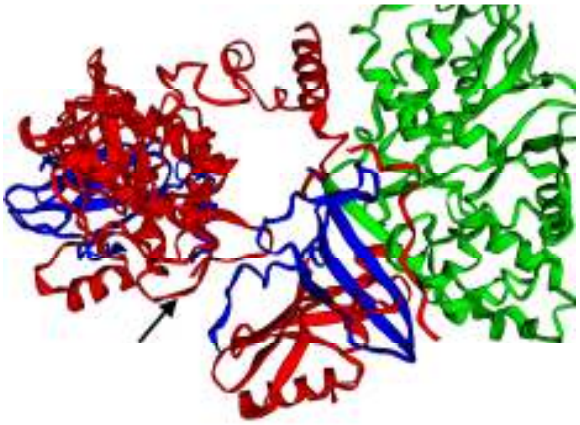

Figure 3. 3D model of human AMPK. The 3D atomic coordinates of the AMPK heterotrimeric structure (PDB accession 4CFE) have been used to visualize the model by the Molecular Display Wizard "EzMol", freely available on the web (http://www.sbg.bio.ic.ac.uk/~ezmol/).
The ribbon display of the secondary elements of the α, β, and γ subunits are coloured in red, blue, and green, respectively. The black arrow indicates the position of Thr172 phosphorilation site.

Several isoforms have been described for the three subunits (α1, α2, β1, β2, γ1, γ 2, γ3) providing many possible combinations of the assembly of the heterotrimer (Hardie et al., 2018).The αsubunit contains the kinase catalytic domainthat induces AMPK activation, followingthe reversible phosphorylation on its Thr172 residue, (Hawley et al., 1996). The β subunit containsaglycogen binding domain (GBM)as well as anα and γbinding domain (Hudson et al., 2003). The GBM domain, interacting with glycogen,mediates the interaction between glycogen synthetase and AMPK (Hudeson et al., 2003).The γ subunit is involved in the direct bindingof adenosine nucleotides (Scott et al., 2004), and its N-terminal region has four identical domains repeated in tandem called CBS (cystathionine-beta-synthetase) or Bateman domains (Bateman, 1997) each of them binding AMP or ATP in a mutually exclusive way (Kahn et al., 2005).These mechanisms ensure a sensitive response to any variation in AMP concentration. Moreover, since AMP activation is antagonized by high concentrations of ATP, this mechanism also allows a response to variations in either AMP or ATP ratio within the cell.

The regulation of AMPK activation occurs by different mechanisms that involve: 1) an allosteric activation control by AMP; 2) a covalent modification through phosphorylation of itsThr-172 by several kinases

such as LKB1,Tak1 (transforming growth factors (TGF)-β activated kinase), and aCa^{2+}/kinase dependent kinase (CaMKK), all interacting directly with the α subunit of AMPK (Hawley S. et al., 1996); 3) other small activating molecules such as 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranosyl 5'-monophosphate (AICAR) a natural metabolic intermediate of purine biosynthesis (Daignan-Fornier et al., 2012).In addition, indirect AMPK activators have also been reported, including metformin, dinitrophenol and rotenone, acting by increasing the AMP concentration.Inactivation of AMPK is induced by ATP and de-phosphorylation by specific phosphatase;evenglycogen can inhibit AMPK, through its binding to the β subunit (Kjøbsted et al., 2018).

AMPK metabolic functions in physical exercise

High intensity physical exercise can be considered as a metabolic stress, that through the increase of AMP/ATP ratio in the skeletal muscle leads to the AMPK activation(Kjøbsted et al., 2018).

The activation of AMPK, due to this energy depletion condition, will allow the restore of ATP levels by activating catabolic processes such as of carbohydrates and fatty acids oxidation.

Up to now, more than 60 target proteins for AMPK have been reported and most of them have been identified as metabolic enzymes. AMPK activates glycolysis, promotes uptake of fatty acids into the cells and their mitochondrial oxidation, and enhances the expression of oxidative enzyme within mitochondria (TCA cycle)(Kjøbsted et al., 2018). AMPK seems to play an important role in the oxidation of free fatty acids (FFA), increasing the uptake of FFA in the mitochondria of skeletal muscle. This effect is accomplished by the inhibition of the enzyme acetyl-CoA carboxylase (ACC), through the reversible phosphorylation of its serine-79 residue.Some other studies suggest that AMPK can reduce the levels of Malonyl-CoA also by the phosphorylation and activation of Malonyl-CoA decarboxylase (MCD) (Saha et al., 2000; Park et al., 2002). These phenomena leads to theinhibition of fatty acids biosynthesis through the reduction of intracellular levels of malonyl-CoA; on the other hand, the lack of inhibition of carnitine palmitoyltransferase 1 will be able to guarantee the physiological amount of activated fatty acids (Winder et al., 1996; Hardie et al., 2006; Lim et al., 2010) the prerequisite for their mitochondrial oxidation.

Moreover, studies performed in genetically modified mice, in which the skeletal-muscle specific AMPK isoforms were deleted (mdKO) demonstrated an impaired exercise capacity and that AMPK regulates the stimulation of mitochondria biogenesis, fatty acid oxidation and glucose up-take(Steinberg et al. 2010; Lantier et al., 2014; Hardle, 2017).

Conclusion

In this mini review we describe the properties of AMPK, a key enzyme activated during muscle contraction,following ATP level depletion and increase of AMP concentration. Activated AMPK acts as a key enzyme able to switch the metabolism from anabolism to catabolismthus suggesting AMPK as therapeutic target for metabolic disorders including cancer.Indeed, AMPK functions demonstrate its key role in response to the high energy demands for the skeletal muscle contraction that occurs during physical exercise. In addition, AMPK acts as a key enzyme able to switch the metabolism from the anabolic to the catabolic pathway thus suggesting AMPK as therapeutic target for several human diseases (Richter and Ruderman, 2009; Sang-Min, 2016) and metabolic disorders, such as metabolic syndrome and type 2 diabetes (Kjøbsted et al., 2016; Ferrari et al., 2019; Grahame et al., 2019). Furthermore, AMPK activation during skeletal muscle contraction represents one of the major mechanisms triggering the beneficial effects induced by sport, physical exercise, as well as an appropriate nutritional lifestyle, on human health.

References

Arcone R, Palma M, Pagliara V, Graziani G, Masullo M, Nardone G (2016) Green tea polyphenols affect invasiveness of human gastric MKN-28 cells by inhibition of LPS or TNF-α induced Matrix Metalloproteinase-9/2, *Biochimie Open* 3, 56–63.

Arcucci A, Ruocco MR, Albano F, Granato G, Romano V, Corso G, Bancone C, De Vendittis E, Della Corte A, Montagnani S (2014)Analysis of extracellular superoxide dismutase and Akt in ascending aortic aneurysm with tricuspid or bicuspid aortic valve, *Eur. J. Histochem.* 58, 200–206.

Bassel-Duby R, Olson EN (2006) Signaling pathways in skeletal muscle remodeling, *Annu. Rev. Biochem. 75, 19–37.*

Birk JB, Wojtaszewski JF (2006) Predominant α2/β2/γ3 AMPK activation during exercise in human skeletal muscle, *J. Physiol.* 577, 1021–1032.

Carling D (2017) AMPKsignalling in health and disease, *Curr. Opin. Cell Biol.* 45, 31–37.

Chen ZP, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, Kemp BE, McConell GK. (2003) Effect of exercise intensity on skeletal muscle AMPK signaling in humans, *Diabetes* 5, 2205–2212.

Daignan-Fornier B, Pinson B (2012) 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranosyl 5'-Monophosphate (AICAR), a Highly Conserved Purine Intermediate with Multiple Effects,*Metabolites*, 2, 292–302.

Daval M, Foufelle F, Ferre' P (2006) Functions of AMP-activated protein kinase in adipose tissue, *J. Physiol.* 574, 55–62.

Egan B, Zierath JR (2013) Exercise metabolism and the molecular regulation of skeletal muscle adaptation,*Cell Metab.* 1,162–184.

Endo H, Owada S, Inagaki Y, Shida Y, Tatemichi M (2018) Glucose starvation induces LKB1-AMPK-mediated MMP-9 expression in cancer cells, *Sci. Rep.* 8, Art. n. 10122.

Ferrari F, Bock PM, Motta MT, Helal L (2019) Biochemical and Molecular Mechanisms of Glucose Uptake Stimulated by Physical Exercise in Insulin Resistance State: Role of Inflammation, *Arch. Bras. Cardiol.* 113, Epub Oct 21.

Flück M (2006)Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli, *J. Exp. Biol. 209, 2239–2248.*

Garcia D, Shaw RJ (2017) AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance, *Mol. Cell.* 66, 789–800.

Gowans GJ, Hardie DG (2014) AMPK: a cellular energy sensor primarily regulated by AMP, *Biochem. Soc. Trans.* 42, 71–75.

Grahame DH (2014) AMP-activated protein kinase: a key regulator of energy balance with many roles in human disease, *J. Intern. Med.* 276, 543–559.

Hardie DG (2018) Keeping the home fires burning: AMP-activated protein kinase, *J. R. Soc. Interface* 138, 20170774.

Herzig S, Reuben JS (2018) AMPK: guardian of metabolism and mitochondrial homeostasis, *Nat. Rev. Mol. Cell. Biol.* 19, 121–135.

Hudson ER, Pan DA, James J, Lucocq JM, Hawley SA, Green KA, Baba O, Terashima T, Hardie DG (2003) A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias, *Curr. Biol.* 13, 861–866.

Kjøbsted R, Hingst JR, Fentz J, Foretz M, Sanz MN, Pehmøller C, Shum M, Marette A, Mounier R, Treebak JT, Wojtaszewski JFP, Viollet B, Lantier L (2018) AMPK in skeletal muscle function and metabolism, *FASEB J.* 32, 1741–1777.

Kjøbsted R, Pedersen AJT, Hingst JR, Sabaratnam R, Birk JB, Kristensen JM, Højlund K, Wojtaszewski JFP (2016) Intact Regulation of the AMPK Signaling Network in Response to Exercise and Insulin in Skeletal Muscle of Male Patients With Type 2 Diabetes: Illumination of AMPK Activation in Recovery From Exercise, *Diabetes* 65,1219–1230.

Lantier L, Fentz J, Mounier R et al. (2014) AMPK controls exercise endurance, mitochondrial oxidative capacity, and skeletal muscle integrity,*FASEB J.* 28, 3211–3224.

Margină D, Ungurianu A, Purdel C, Tsoukalas D, Sarandi E, Thanasoula M, Tekos F, Mesnage R, Kouretas D, Tsatsakis A (2020) Chronic Inflammation in the Context of Everyday Life: Dietary Changes as Mitigating Factors, *Int. J. Environ. Res. Public Health* 17, Art. n. 4135.

Monteverde T, Muthalagu N, Port J, Murphy DJ (2015) Evidence ofcancer-promoting roles forAMPKand relatedkinases, *FEBS J.* 282, 658–671.

Pagliara V, Adornetto A, Mammì M, Masullo M, Sarnataro D, Pietropaolo C, Arcone R (2014) Protease Nexin-1 affects the migration and invasion of C6 glioma cells through the regulation of urokinase Plasminogen Activator and Matrix Metalloproteinase-9/2, *Biochim. Biophys. Acta* 1843, 2631–2644.

Pagliara V, Nasso R, Di Donato P, Finore I, Poli A, Masullo M, Arcone R (2019) Lemon Peel Polyphenol Extract Reduces Interleukin-6-Induced Cell Migration, Invasiveness, and Matrix Metalloproteinase-9/2 Expression in Human Gastric Adenocarcinoma MKN-28 and AGS Cell Lines*Biomolecules* 9, Art. n. 833.

Richter EA, Ruderman NB (2009) AMPK and the biochemistry of exercise: Implications for human health and disease, *Biochem. J.* 418, 261–275.

Sang-Min J (2016) Regulation and function of AMPK in physiology and diseases, *Exp. And Mol. Med.* 48, e245.

Scott JW, Hawley SA, Green KA, Anis M, Stewart G, Scullion GA, Norman DG, Hardie DG (2004) CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations, *J. Clin. Invest. 113, 274–284.*

Steinberg GR, O'Neill HM, Dzamko NL, et al. (2010) Whole body deletion of AMP-activated protein kinase {beta}2 reduces muscle AMPK activity and exercise capacity, *J. Biol. Chem*. 285, 37198–37209.

Tong W, Wang Q, Sun D, Suo J (2016) Curcumin suppresses colon cancer cell invasion via AMPK-induced inhibition of NF-κB, uPA activator and MMP9, *Oncol. Lett.* 12, 4139-4146.

Wang Z, Wang N, Liu P, Xie X (2016) AMPKandCancer, *Exp. Suppl.*, 107, 203–226.

Xiao B, Sanders MJ, Carmena D, Bright NJ, Haire LF, Underwood E Patel BR, Heath RB, Walker PA, Hallen S, Giordanetto F, Martin SR, Carling D, Gamblin SJ (2013) Structural Basis of Ampk Regulation by Small Molecule Activators, *Nat. Commun.* 4, Art. n. 3017

Yuan Y, Xue X, Guo RB, Sun XL, Hu G (2012) Resveratrol enhances the antitumor effects of temozolomide in glioblastoma via ROS-dependent AMPK-TSC-mTOR signaling pathway, *CNS Neurosci. Ther.* 18, 536–546.