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REVIEW ARTICLE

The role of AMPK activation in metabolic regulation, energy homeostasis and aging: A comprehensive overview

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Abstract

5′ Adenosine monophosphate-activated protein kinase (AMPK) is a metabolic sensor responsible for maintaining homeostasis, regulating metabolic control, monitoring energy status, and balancing energy production with consumption. The most important protein kinases that activate AMPK are liver kinase B1 and calcium-calmodulin-dependent protein kinase. AMPK significantly impacts physical performance by accelerating recovery periods and restoring energy stores through several key mechanisms. It regulates muscle glycogen content, ensuring readily available energy during physical exertion. In addition, a high number of mitochondria enable the utilization of fatty acids as an energy source, thereby improving endurance efforts. Furthermore, rich vascularization in the muscles enhances the delivery of nutrients and oxygen, optimizing performance and speeding up recovery. AMPK is also important for maintaining muscle homeostasis. It decreases insulin resistance, stimulates mitochondrial biogenesis, and exerts an antioxidant effect. As a regulator of energy homeostasis, AMPK plays a key role in linking cellular energy levels with the aging process. It acts as a modulator of cellular senescence and has the potential to extend life expectancy, particularly in the context of caloric restriction. By activating AMPK, caloric restriction (activated with a 30 – 70% reduction in nutrient intake) helps to maintain energy balance in cells, which may slow down aging and support healthier cellular function over time. In conclusion, the involvement of AMPK in metabolic regulation and maintenance makes it an important therapeutic target for the treatment and prevention of several diseases, both age-related and otherwise.

Keywords: AMPK; Oxidative stress; Physical effort; Exercise; Metabolism

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1. A short overview of AMPK

With advances in medicine, life expectancy has significantly increased. These advances are attributed to the emergence of the concept of personalized medicine and the development of new pharmacological agents, all aimed to improve quality of life, prolong life expectancy, and enhance physical condition. The purpose of the present review is to highlight the role of 5' adenosine monophosphate-activated protein kinase (AMPK) in certain processes (carbohydrate and lipid metabolism, autophagy, mitochondrial biogenesis, oxidative stress), and to explore how these mechanisms benefit athletes.

In 1987, researchers David Carling and Grahame Hardie hypothesized the existence of two protein kinases that inhibited enzymes involved in the *de novo* synthesis of fatty acids (acetyl-CoA carboxylase [ACC]) and cholesterol (hydroxymethylglutaryl-CoA reductase [HMG-CoA reductase]). Subsequent studies revealed that both kinases were, in fact, the same protein.[1,2] Studies on experimental animals have presented data confirming that physical exercise decreases the muscle concentration of malonyl-CoA formed during the ACC pathway, which contributes to more efficient utilization of energy substrates.[3] Under normal physiological conditions, malonyl-CoA inhibits carnitine palmitoyltransferase I, blocking the transport of fatty acids into the mitochondria where they would otherwise be oxidized. Consequently, a decrease in malonyl-CoA concentration promotes fatty acid transport into mitochondria, facilitating their use for ATP production.[4]

AMPK is a metabolic sensor responsible for maintaining homeostasis, metabolic control, and energy balance by monitoring energy status and matching energy production to its consumption. Following favorable metabolic outcomes after AMPK activation, it has been identified as an important therapeutic target.[5] Its activation is triggered when low energy levels are detected, indicated by reduced adenosine triphosphate (ATP) levels, which can occur during fasting, hypoxia, or when various toxic substances impact the electron transport chain (ETC) and inhibit oxidative phosphorylation (OXPHOS).[6,7]

AMPK exists as a heterotrimeric complex, consisting of a catalytic α subunit (α_1, α_2) encoded by *PRKAA1*; regulatory β subunits (β_1, β_2) encoded by *PRKAB1* and *PRKAB2*; and γ subunit (γ_1, γ_2, γ_3) encoded by *PRKAG1*, *PRKAG2,* and *PRKAG3*. These subunits are encoded in the genomes of all cells, suggesting that the AMPK heterotrimer arose very early in the evolution of eukaryotic cells.[8,9] One of the most important event in this evolution was the endosymbiotic capture of aerobic bacteria, which gave rise to mitochondria. After this event, the newly formed organism developed new abilities to convert adenosine diphosphate (ADP) into ATP and established signaling pathways to monitor ATP availability. In evolved organisms, AMPK's role in ATP regulation, mitochondrial biogenesis, mitophagy, and fission underscores its significance as a link between mitochondria (aerobic bacteria) and the host cell (anaerobic bacteria).[10-13] While AMPK initially served a primordial role in energy regulation, it has evolved to also regulate the energy balance of the whole organism through hormone-mediated responses in the hypothalamus.[3,14] The adaptability of the eukaryotic cell ensures cellular homeostasis and survival under adverse conditions. In athletes, AMPK activation may confer advantages by promoting mitochondrial gene expression and increasing mitochondria numbers. One of these regulators of multiple mitochondrial genes is peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which, in muscle, promotes the conversion of type IIb muscle fibers to type I and type IIa fibers, both characterized by increased mitochondrial content.[15,16]

The γ subunit of AMPK contains two Bateman domains, which serve as binding sites for adenosine monophosphate (AMP) and ATP. Therefore, when the cell does not detect an energy deficiency, ATP molecules bind to the appropriate site on the enzyme, maintaining it in an inactive state. Conversely, if the AMP/ATP ratio exceeds unity, AMP molecules bind to the Bateman subunit, triggering the activation of the enzyme through three mechanisms:

- Allosteric activation;
- Stimulation of AMPK activating proteins, which phosphorylate the α catalytic subunit;
- Preventing dephosphorylation as a result of AMP binding to the γ subunit which extends the phosphorylation of the α subunit.

The primary protein kinases that activate AMPK are liver kinase B1 (LKB1) and calcium-calmodulin-dependent protein kinase α and β (CaMKK).[17,18] In response to energy-related stress, such as an energy deficit or depletion, skeletal muscle shows increased LKB1 activity. Conversely, the absence of this protein is associated with reduced physical endurance. CaMKK exists in two isoforms, α and β, which are 70% similar. These isoforms become active in the presence of Ca^{2+} and calmodulin and are capable of phosphorylating AMPK in the brain, endothelium, lymphocytes, and striated muscles. Given that the enzyme has multiple subunits with multiple isoforms, it can be inferred that AMPK also exists in multiple forms, as shown in Table 1. Among them, the $\alpha_2\beta_2\gamma_3$ isoform is activated under conditions of short-term physical exercise, while the $\alpha_2\beta_2\gamma_1$ isoform is present in long-term effort.[2]

2. AMPK activators and physical activity

For most compounds that activate AMPK, the activation occurs by inhibiting the ETC, leading to an increase in ADP and/or AMP levels. Another important aspect to consider is the functional differences between AMPK isoforms.

A study of transgenic mice deficient in AMPKα_2 showed that in the absence of this enzyme, the contractility of the heart is impaired, indicating that the absence of the α_2 isoform is correlated with reduced physical capacity. In contrast, mice injected with 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR, a known AMPK modulator) showed improved running capacity, enhanced endurance, increased oxygen consumption, and decreased

Table 1. Activation of the AMPK enzyme complex depending on the intensity and duration of physical exercise

Type of exercise	High intensity, short duration	Moderate intensity, medium duration	Low intensity, long duration
AMPK activator	LKB1		CaMKK
AMPK complex α subunit	$\alpha_2\beta\gamma$		$\alpha_1\beta\gamma$
Activated AMPK isoforms	$\alpha_2\beta_2\gamma_3$	$\alpha_2\beta2\gamma_1$	$\alpha_1\beta_2\gamma_1$

Abbreviations: AMPK: 5' Adenosine monophosphate-activated protein kinase; LKB1: Liver kinase B1; CaMKK: Calcium-calmodulin-dependent protein kinase.

body mass. These effects are due to the phosphorylation and activation of target genes under the control of AMPK, such as transcription factors and co-activators of the p53 gene, cyclic-AMP response element-binding protein, mammalian target of rapamycin (mTOR), and PGC-1α pathways. However, there is also evidence showing a protective role for AMPK in cancer cells, which are susceptible to oxidative stress. These events occur because the LKB1-AMPK system helps maintain cellular homeostasis under stress.[7] Preclinical studies suggest that combinations of agents that activate AMPK produce stronger therapeutic effects. Thus, by targeting AMPK, the *de novo* synthesis of fatty acids, a hallmark of several cancers, can be prevented. A study by O'Brien *et al.*,[19] suggests that the combination of metformin and aspirin decreases the survival rate of prostate and lung cancer cells.

In vitro studies on cardiomyocyte cell lines reveal translocation of the fatty acid translocase transporter (FAT/CD36) to the cell membrane with increased fatty acid uptake and oxidation rates. Other studies state that when muscle glycogen stores are low, fatty acid oxidation becomes more intense. Activation of AMPK during physical effort inhibits triglyceride synthesis by suppressing glycerol-3-phosphate acyltransferase activity.[4,20,21] Moreover, AMPK prevents the incorporation of palmitic acid into ceramides, lipids involved in the generation of reactive oxygen species (ROS). AMPK is also involved in glucose uptake through the TBC1D1 protein, facilitating translocation of the glucose transporter GLUT4. It inhibits glycogen synthetase, an enzyme that catalyzes an energy-consuming anabolic process.[22,23] Glycogen is broken down to glucose by glycogen phosphorylase. While it was originally thought that AMPK regulates both glycogen phosphorylase and glycogen synthase, studies show that only glycogen synthesis is affected. Specifically, glycogen synthesis is regulated through phosphorylation of glycogen synthase by the α2 isoform of AMPK. In skeletal muscles, endurance training activates AMPK, which favors glycogen synthesis by increasing glucose uptake and its conversion to glucose-6-phosphate, an allosteric activator of glycogen synthase. This activation can offset the inhibitory effects of AMPK on glycogen synthase. During feeding after a period of fasting, insulin activates AMPK, which in turn influences genes involved in gluconeogenesis, including cyclic AMP-regulated transcriptional co-activator 2 (CRTC2) and class IIA histone deacetylases (HDACs).[24,25] This activation can benefit athletes by enabling more efficient use of energy reserves and improving their replenishment during rest and recovery periods.

Given the role in the regulation of energy consumption and maintenance of carbohydrate and lipid metabolism homeostasis,[26,27] as well as its involvement in mitochondrial biogenesis,[28] AMPK is considered a therapeutic target not only in metabolic diseases (diabetes, obesity) but also in other pathologies. Thus, there are studies in the literature that highlight the role of AMPK in suppressing tumorigenesis.[29,30] In addition, AMPK activation induces G1 cycle arrest, an effect associated with p53 activation, followed by cell cycle inhibition through p21. It is also important to note that AMPK has the ability to protect tumor cells against cytotoxic medication and hypoxic conditions.[5]

Another important role of AMPK is its crucial role in autophagy processes, which rely on the catabolic activity of lysosomes. In this context, AMPK regulates the activity of two proteins involved in the initiation of autophagy, such as Unc-51-like autophagy-activating kinase (ULK1) and the phosphatidylinositol 3-kinase catalytic subunit type 3 (PI3KC3/VPS34).[31,32]

Last but not least, the role of AMPK in redox homeostasis and its involvement in increasing antioxidant activity should be highlighted.[13] Analysis of a study published in 2008 during the Beijing Olympic Games demonstrated that AICAR significantly enhanced sports performance by increasing the physical endurance capacity of mice, even in the absence of prior physical training. Following these findings, the World Anti-Doping Agency (WADA) was notified, leading to the prohibition of AMPK activators, such as AICAR and their inclusion in the list of "hormones and metabolic modulators". This raises the question on whether all AMPK activators should be included in the list of prohibited substances or is the improvement of sports performance is only correlated with certain activators of the enzyme.[2]

From the perspective of physical exercise, AMPK influences performance, accelerates recovery, and restores

energy reserves through several mechanisms. These include the presence of glycogen in muscles, a high number of mitochondria that facilitate the utilization of fatty acids as an energy source, and extensive vascularization to ensure efficient nutrient and oxygen delivery during physical activity.[33] In addition, the use of AMPK modulators decreases insulin resistance in skeletal muscles and allows glucose utilization as an energy substrate by translocating the GLUT4 to the cell membrane. In terms of regenerative and recovery capacity, AMPK also plays a role in responding to muscle inflammatory processes. During the first phase, M1 macrophages secrete pro-inflammatory cytokines that result in ROS, which are necessary for muscle recovery. Subsequently, the recruitment of M2 macrophages is necessary to combat inflammation. Muscle regeneration involves the migration, proliferation, and fusion of myoblasts, as well as their interaction with immune cells, to form myotubules. Combating the inflammatory process is extremely important, as disruptions can impair the regeneration capacity of the skeletal muscles.[34] In degenerative myopathies, an inflammatory response is commonly observed, similar to the condition seen in muscle tissues deficient in AMPK. Both AICAR and metformin have shown anti-inflammatory effects, demonstrating potential therapeutic benefits in these conditions.[35-37]

Among the benefits of physical exercise, sports, and movement are reduced incidence of cardiovascular diseases, cancer, neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's), and other neurological conditions. Neurogenesis in the hippocampal region and increased neuronal plasticity are associated with improved memory and cognitive abilities. However, a direct link between these benefits and muscle metabolism has not been demonstrated.[38] To mimic the effects of physical activity, compounds have been developed and extracted that activate regulatory pathways and stimulate genes involved in skeletal muscle remodeling.[39-41] However, from an ethical perspective, this raises the question of whether such modulators of physical movement might open the door to controlled forms of sports doping.

AICAR is known to promote angiogenesis and vascularization by inducing vascular endothelial growth factor gene expression, similar to the effects of physical exercise.[42,43] In doses of 500 mg/kg administered to small, young, and adult rodents for 1 and 2 weeks, respectively, AICAR improved spatial memory and coordination. These effects are unlikely to result from direct central effects, as AICAR is poorly permeable to the blood-brain barrier. Notably, treatments longer than 14 days in young mice do not provide any neuronal benefits.[44] However, central effects have been observed in the hippocampal dentate gyrus, where increased levels of brain-derived neurotrophic factor (BDNF) were found following short-term treatment.[45]

A hypothesis suggesting that chronic treatment does not provide benefits supports the idea that prolonged use increases cytokine levels, leading to the onset of an inflammatory process followed by oxidative stress. Based on these observations, it can be stated that AICAR improves physical effort parameters, but only with short-term use. Like AICAR, metformin activates AMPK through an indirect mechanism by blocking complex I of the respiratory chain, which results in ATP depletion and an increase in AMP levels, with the possibility of similar effects.[46]

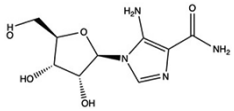
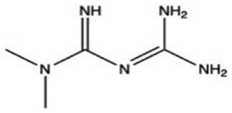
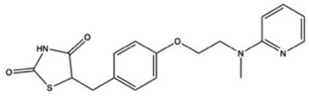
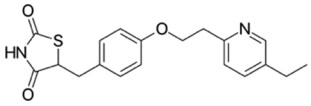
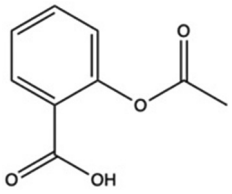
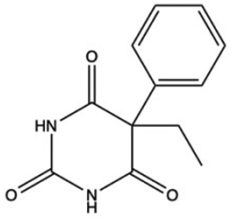
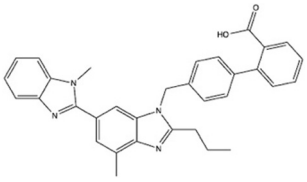
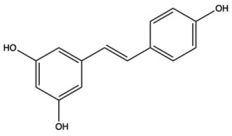
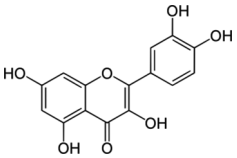
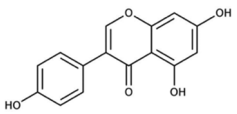
In conclusion, AMPK is extremely important for the regulation of muscle homeostasis with extension to other types of cells and tissues by decreasing insulin resistance, stimulating mitochondrial biogenesis, and exerting an antioxidant effect.[28,33,47,48] Table 2 lists the main molecules known to activate AMPK and their mechanism of activation.

3. Unraveling aging: The interplay between AMPK, caloric restriction and autophagy

One of the most interesting and bold hypotheses, proven by evidence in some studies, is the "free radical theory", which suggests that the uncontrolled generation of free radicals is the main driving force of biological aging. Following ROS accumulation, the senescence process accelerates due to the loss of mitochondrial integrity, resulting from increased membrane permeability.[74-76] In addition, factors such as increased adipose tissue and metabolic imbalances trigger inflammatory processes, leading to mitochondrial lesions, which favors the acceleration of the senescence rate.[77-79]

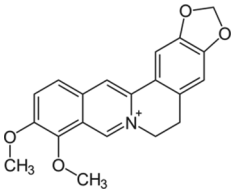
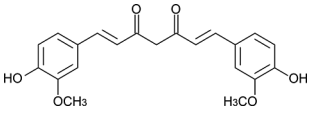
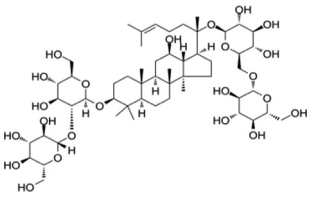
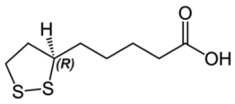
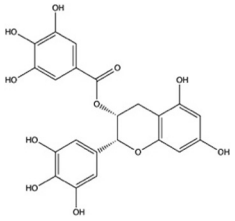
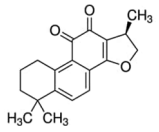
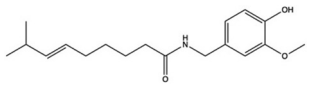
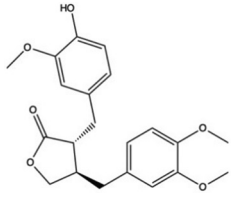
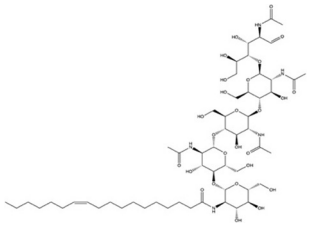
As aging is an inevitable (temporary) process that is characterized by the loss of tissue functionality and an increased risk of age-related diseases, the use of AMPK modulators is an alternative. Studies suggest the activation of AMPK is associated with increased life expectancy by maintaining cellular homeostasis, enhancing stress resistance, and promoting apoptosis and autophagy. Thus, activation of AMPK in *Caenorhabditis elegans* and rodents has been shown to extend lifespan, with numerous studies in *C. elegans* suggesting a possible link between AMPK activation and caloric restriction.[80] Irregularities in mitochondrial dynamics with abnormal mitochondrial morphology are key characteristics of aging and play a significant role in the development of various age-related neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease.[81-83] In an animal model of Parkinson's disease induced in mice, treatment with metformin (500 mg/kg) for 21 days increased the level of BDNF and reduced oxidative stress in the substantia

Table 2. AMPK activators, mechanism of action, and impact on exercise capacity

Compound	Chemical structure	AMPK activation mechanism	References
AICAR		ZMP binding to the γ-subunit of AMPK	5
Metformin		Respiratory chain blockage (complex I) Increase in AMP/ATP ratio	49
Rosiglitazone		Respiratory chain blockage Increase in AMP/ATP ratio	50
Pioglitazone		Respiratory chain blockage Increase in AMP/ATP ratio	51
Salicylates		Allosteric activator (AMPK binding, β1 subunit)	52
Phenobarbital		Respiratory chain blockage Increase in AMP/ATP ratio	53
Telmisartan		PPARγ-dependent mechanism	54
		Increase in AMP/ATP ratio (action on mitochondrial F1F0-ATPase/ATP synthase)	55
Quercetin		Increase in AMP/ATP ratio (action on mitochondrial F1F0-ATPase/ATP synthase)	56,57
Genistein		Increase in AMP/ATP ratio (action on mitochondrial F1F0-ATPase/ATP synthase)	57,58

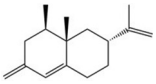
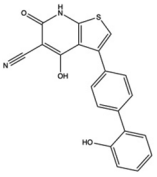
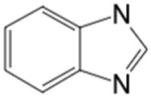
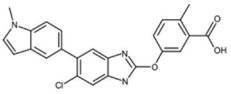
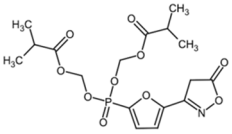
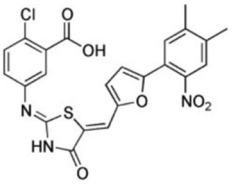
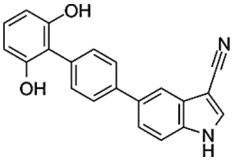
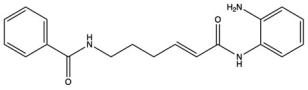
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Table 2. ***(Continued)***

Compound	Chemical structure	AMPK activation mechanism	References
Berberine		Respiratory chain blockage (complex I) Increase in AMP/ATP ratio	59
Curcumine		Increase in AMP/ATP ratio (action on mitochondrial F1F0-ATPase/ATP synthase)	60
Ginsenoside Rb1		Increase in AMP/ATP ratio (unknown mechanism)	61
α-lipoic acid		Unknown mechanism	62
EGCG		Respiratory chain blockage. Increase AMP level	63
Cryptotanshinone		Increase ROS (unknown mechanism)	64
Capsaicin		Not specified	65
Arctigenin		LKB1/CaMKK	66
Chitooligosaccharides		Not specified	67

(Cont'd...)

Table 2. ***(Continued)***

Compound	Chemical structure	AMPK activation mechanism	References
Nootkatone		Not specified	68
Thienopyridone (A-769662)		Allosteric activator (AMPK binding, β1 subunit)	69
Benzimidazole (Compound 911)		Allosteric activator (AMPK binding, β1 subunit)	5
Compound 991		Not specified (AMPK binding, β1 subunit)	70
Compound-13		Allosteric activator (AMPK binding, α1 subunit)	71
PT-1		Allosteric activator (AMPK binding, γ1 subunit)	72
MT 63-78		Allosteric activator (AMPK binding, β1 subunit)	73
R118		Respiratory chain blockage	69

Abbreviations: AICAR: 5-Aminoimidazole-4-carboxamide ribonucleoside; ZMP: 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate; AMPK: 5' adenosine monophosphate-activated protein kinase; EGCG: Epigallocatechin-3-gallate; LKB1: Liver kinase B1; CaMKK: Calcium-calmodulin-dependent protein kinase; ROS: Reactive oxidative species; AMP: Adenosine monophosphate; ATP: Adenosine triphosphate.

nigra pars compacta (SNpc), a region involved in the development of specific symptoms.[84] However, long-term treatment has been shown to negatively influence intellectual capacities, with the exact mechanism remaining unknown, though interference with vitamin B_{12} absorption is a likely factor.[85-87] Mitochondrial dynamics regulate many key cellular functions, including metabolic plasticity, mitochondrial turnover, and organelle communication, but the mechanisms by which these functions are affected by perturbations of mitochondrial dynamics during aging remain unclear. However, the appearance of swollen and fragmented mitochondria has been reported across all species studied, suggesting that mitochondrial fragmentation is a senescence-promoting factor.[88-90]

AMPK, as a regulator of energy homeostasis, links energy levels with the rate of aging and can be considered a modulator of senescence and lifespan extension, particularly through mechanisms such as caloric restriction (activated with a 30 – 70% reduction in the initial amount nutrient

consumed). Another interesting observation is the low level of leptin following metformin treatment, which may explain its anorexic effect. This effect could be due to the sensitization of the hypothalamus to leptin, potentially through an increase in the number of specific receptors in this region. The hypothalamus plays a crucial role in regulating energy balance, making it a key region for this effect.[91] Regarding the anti-inflammatory effect of metformin, various studies show a positive effect, following a reduction in cytokines such as interleukin-6, tumor necrosis factor-α, nuclear factor-kappa B, but this remains a hypothesis at the research stage.[91-93]

A mechanism for eliminating aged cellular organelles or damaged proteins can be achieved through autophagy. This process can be activated through the administration of exogenous agents, such as rapamycin, resveratrol, nicotinamide derivatives (Vitamin B_3), metformin, urolithin A or spermidine, compounds thought to delay aging, and even extend lifespan.[94,95]

Instead, the body has several defense and preservation mechanisms, with autophagy, which represents a process of "purification". This involves recycling outdated or damaged protein structures, including cellular organelles, by forming autophagosomes, which later fuse with lysosomes.[96,97]

Autophagy is the cell's survival mechanism, regulated by various signaling pathways, with the AMPK-mTOR system being one of the most important.

There are three types of autophagy described:
- Macro-autophagy
- Micro-autophagy
- Chaperone protein-mediated autophagy

In macro-autophagy, the autophagosome represents the catalytic unit. It is formed from an insulating membrane in which a small part of the cytoplasm, soluble molecules, and cell organelles are included. The next step involves fusion with lysosomes to form autolysosomal units, where the degradation of internal contents take place.

Micro-autophagy, on the other hand, occurs through the engulfment of small components by the lysosome. Thus, significant amounts of cytosolic material, including protein molecules are incorporated into this complex.

The third type of autophagy, chaperone protein-mediated autophagy, does not involve membrane reorganization. Instead, cytosolic proteins are selectively recognized and translocated to the lysosomal membrane by the chaperone protein Hsc7 together with its co-chaperones. The unfolded proteins are then transported to the lysosome through a multimeric complex.[98]

Experimental models in *C. elegans*, *Drosophila*, and rodents have demonstrated that mTOR growth factor inhibitors or caloric restriction led to similar results, significantly increasing the life expectancy of those organisms.[99,100] In contrast, continuous mTORC1 blockade can lead to the onset of cardiomyopathy and rapid progression to heart failure, hence impairing life expectancy.[101]

Thus, the autophagic process is crucial for promoting health and longevity. Disruptions in this mechanism can contribute to the development of age-related diseases.[102-104]

Substances known to be longevity promoters are divided into three categories according to their mode of action:
- Those that have demonstrated anti-aging effect but lack conclusive evidence for this ability;
- Those suggested to prolong youth by preventing or delaying the onset or progression of age-related diseases, though their influence on the aging process itself has not been proven;
- Those that reverse the aging mechanism, at least under certain conditions, thereby prolonging youth;

4. AMPK at the hypothalamus level

AMPK in the central nervous system is known to control nutrient intake and restore energy balance which is activated under pathophysiological conditions. The neuroprotective effect of metformin is thought to result from increased neuronal sensitivity to insulin, as poor insulin signaling in the brain is associated with an increased risk of neurodegenerative diseases.[105] AMPK integrates peripheral signals, such as metabolites and hormones, through neural networks. Following the integration of signals at the level of the hypothalamus, it causes the sensation of hunger, the production of heat (thermogenesis) and energy by mobilizing brown and white adipose tissue.

Studies have identified specific hypothalamic nuclei where AMPK is expressed, including the arcuate, dorsomedial, paraventricular, ventromedial, and lateral hypothalamic nuclei. Unlike AMPK in the rest of the body, hypothalamic AMPK activation increases food intake and causes weight gain, while its inhibition reduces appetite and weight.[3]

Using genetic models, it was shown that by blocking the dominant negative isoform of hypothalamic AMPKα decreases the messenger RNA (mRNA) expression of orexigenic neuropeptides, such as agouti-related protein (AgRP) and neuropeptide Y (NPY). Conversely, increased expression of the active isoform of AMPKα increases AgRP and NPY synthesis in the arcuate nucleus.[106] New data also indicate that AMPK modulates autophagy, thereby controlling the production of both NPY and proopiomelanocortin (POMC).[107] Both orexigenic and anorexigenic hormones control appetite through their

influence on AMPK activity. Anorexigenic hormones, such as leptin, glucagon-like peptide (GLP-1), estradiol, insulin, and ciliary neurotrophic factor (CNTF), inhibit AMPK activity, while orexigenic hormones, including ghrelin, adiponectin, glucocorticoids, cannabinoids, and AgRP, stimulate it.[108] AMPK is also involved in regulating thermogenesis in brown adipose tissue through its influence on the sympathetic nervous system, which in turn activates to increase heat production, thereby contributing to body temperature regulation and energy expenditure. Estradiol centrally binds to α-type estrogen receptors, selectively decreasing AMPK activity in the ventromedial nucleus, which increases the thermogenic capacity of brown adipose tissue. Variations in estradiol levels during the estrogenic cycle and pregnancy further modulate the AMPK pathway, establishing its important role for in energy balance and thermoregulation.[109]

5. AMPK and the endocannabinoid system

The metabolic effects associated with excess glucocorticoids are correlated with steps controlled by AMPK. As previously mentioned, activation of hypothalamic AMPK increases appetite.[110] In addition, there appears to be a link between AMPK and metabolic effects involving the endocannabinoid system.[111] These effects of the cannabinoid system are mediated by cannabinoid type 1 (CB1) receptors, which are also expressed in the hypothalamus.[112]

Activation of hypothalamic CB1 receptors stimulates appetite.[113] Concurrently, glucocorticoids induce changes at the hypothalamic level that are correlated with the increase in endocannabinoid content, suggesting an interdependence between endocannabinoids, glucocorticoids, AMPK, and appetite regulation. However, the effects of glucocorticoids and cannabinoids on AMPK can vary depending on tissue type. For example, in the hypothalamus (stimulation) and adipose tissue (inhibition), the effects are the same, whereas in the liver and heart, they differ.[114] In the heart, cardiac AMPK activation by cannabinoids appears to have cardioprotective effects, whereas reduced CB1 receptors expression is associated with negative effects.[115] The content of endocannabinoids at the hypothalamic level varies with caloric intake. During fasting, the concentration of endocannabinoids increases, and they decrease during feeding periods, mediating both the anorexigenic effects of leptin and orexigenic effects of ghrelin.[116,117]

Caloric restriction has additional implications, particularly in modulating nociception (perception of pain). Caloric restriction has been shown to modulate pain sensitivity, potentially reducing pain perception and influencing the body's response to injury or inflammation. Thus, reduced caloric intake is correlated with pain relief, as shown in clinical studies on patients with rheumatoid arthritis or fibromyalgia.[118] In addition, this pain relief correlates with increased levels of endocannabinoid mediators. Specifically, caloric restriction considerably increases the levels of anandamide (AEA) and 2-arachydonoyl glycerol (2-AG) in certain areas of the brain.[116] Through the same mechanism, activation of AMPK by caloric restriction increasing endocannabinoid levels is proposed to reduce nociception.[119] However, studies suggest conflicting effects of the endocannabinoid system on AMPK activity. Findings indicate a reduction in AMPK activity following endocannabinoid system activation.[120,121] Furthermore, a gradual and moderate reduction in caloric intake is more effective to activate these mechanisms, because the sudden and drastic caloric restriction can lead to unwanted effects, such as malnutrition. Intermittent fasting has been shown in both human and animal studies to increase levels of endocannabinoid mediators.[122]

In contrast to the previously mentioned approach, prolonged periods of moderate caloric restriction correlate with reduced AEA and 2-AG levels.[123] The observed analgesic effect and increased 2-AG level are correlated with low leptin level and/or decreased leptin sensitivity.[124]

Finally, the antinociceptive response mediated by the endocannabinoid system during caloric restriction is attributed to an increase in the number of CB1 receptors.[125]

6. Conclusion

Due to its central role in metabolic processes, AMPK represents an important therapeutic target for the treatment and prevention of several diseases, including age-related conditions. However, questions remain regarding its mechanism of action, activation pathways, and its diverse roles throughout the body. A multitude of studies consider AMPK as a metabolic "switch" that is inactive under sufficient energy supply and activated when energy sources are limited. AMPK appears to coordinate heart metabolism through protein interactions to generate targeted effects, with the goal of restoring cellular homeostasis. Systemic activation of AMPK, as discussed earlier, can influence the level of the hypothalamus and food consumption. In addition, the existence of multiple AMPK isoforms adds complexity, as the activation of AMPK may benefit one organ while disadvantaging another. For example, a gain-in-function mutation in the γ subunit is correlated with greater glycogen storage in skeletal muscles (γ_3) and in the heart (γ_2). This can lead to glycogen storage cardiomyopathy, cardiac hypertrophy, and arrhythmias, which are disadvantages for non-athletes, especially athletes. It is important to note that the activation

of AMPK by some compounds does not improve sports performance, as the outcomes depend on various factors, such as the type of physical activity (aerobic or anaerobic) and its intensity.

Future studies are essential for evaluating the impacts of AMPK-activating substances on sports performance, with particular attention to their mechanism of action and physiological outcomes. An additional critical consideration is the development of isoform-specific AMPK activators to ensure targeted effects and minimize potential adverse consequences. Until such advancements are achieved, regulatory frameworks, including "The Prohibited List" remain pertinent and subject to ongoing updates.

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