The Role of AMPK in the Regulation of Appetite and Energy Homeostasis

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H I G H L I G H T S

> AMPK regulates the metabolic energy balance as well as governs appetite control.
> AMPK regulates cellular glucose uptake independently from insulin.
> Appetite-stimulating and suppressing agents act on AMPK to regulate nutritional intake and body weight control.

A B S T R A C T

Today, excessive nutrition and sedentary life have brought along many chronic diseases such as obesity and diabetes. As is known, obesity is a disorder of both energy metabolism and appetite regulation. In recent studies, it has been reported that 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) regulates the metabolic energy balance as well as governs appetite control. When AMPK is activated, anabolic reactions are inhibited, while catabolic reactions are activated to produce energy. In addition to many pharmacological drugs and nutritional supplements, exercise activates AMPK and enhances the translocation of the glucose transporter (GLUT4) protein, which provides insulin-independent cellular glucose uptake. AMPK is an essential intracellular sensor against obesity because when AMP and LKB1 activate AMPK, the use of body fat stores will be encouraged to produce energy. Appetite-stimulating and suppressing agents act on AMPK to regulate both food intake and body weight control. When appetite suppressors such as leptin, insulin, metformin, inhibit AMPK leucine, and berberine, the expression of orexigenic neuropeptides are decreased while the expression of anorexigenic neuropeptides is increased. Understanding the mechanisms controlled by the hypothalamic AMPK is crucial for developing effective nutritional strategies for the treatment of nutritional intake disorders such as obesity, diabetes mellitus, cardiovascular disease, hypertension and cancer.

Contents

1. Introduction

Obesity is a chronic health problem affecting children and adults, especially in developed and developing countries [1]. As is known, obesity is a disorder of both energy metabolism and appetite regulation [2]. The central nervous system and the endocrine system plays a critical role in regulating the energy balance by coordinating environmental and central signals to evaluate energy status and control nutritional behavior [3]. In recent studies, 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) has been reported to regulate the metabolic energy balance as well as regulate appetite control. Both food intake and energy consumption are regulated by activating and inhibiting the hypothalamic AMPK with pharmacological and endocrine system hormones [4]. In addition to its effects on the hypothalamus, AMPK also regulates energy metabolism throughout the body with the action of hormones and adipokines secreted from adipose tissue (Leptin, Adiponectin, and Omentin) [5]. In this review, the structure of AMPK and functions in carbohydrate, lipid, and protein metabolism are explained. Besides, how the appetite is regulated with the activation or inhibition of the hypothalamic AMPK by hormones is given in this review.

2. Structure and Function of AMPK

AMPK, a serine/threonine kinase enzyme, consists of the catalytic α subunit and the regulatory β and γ subunits, besides each subunit has multiple isoforms such as α1, α2, β1, β2, γ1, γ2, and γ3. These isoforms can also be in different combinations to create 12 different AMPKs [6]. AMPK regulates the intracellular energy requirement according to the AMP/ATP ratio (Figure 1). The maximum level of ATP in the cell indicates that the ATP/AMP ratio is also at the maximum level. Increasing AMP/ATP and ADP / ATP ratios inside the cell suggest that energy is used. Therefore, an increased AMP level stimulates AMPK, an energy sensor within the cell, and energy is produced through catabolic reactions from storage energy sources or dietary foods to provide [7, 8]. Hormones that control energy intake and consumption pathways in the hypothalamus [9] regulate AMPK. Activation of the AMPK begins with the phosphorylation of Thr172 in the α subunit of the AMPK. Phosphorylation of AMPK can also occur by AMP stimulating LKB1. Activation of AMPK occurs by stimulating CaMKKβ (Ca²⁺/calmodulin-dependent protein kinase β) when the intracellular calcium density increases [10]. The activation of AMPK by the AMP was first shown in 1980. Although this activation was described allosterically, it was stated that AMP-activated AMPK via Thr172 in the α subunit in 1990 [11]. In a study by Gowans et al. [12], it was reported that AMP-activated AMPK 10 fold higher than ADP. The relevance of AMP to α1 and α2 subunit in the catalytic region of AMPK is similar. Activated AMPK by AMP is 500 fold more active than its non-phosphorylated form [13]. It has been reported that the γ2-complex in the subunit of the AMPK is the most susceptible to activation by AMP, while the γ3-complex is the least sensitive [14]. Activation of the γ1-complex of AMPK by AMP is also provided by liver kinase B1 (LKB1), which is more effective than the γ2- and γ3-complexes. In addition, ADP's interest in the γ2-complex is higher than other γ

complexes [13]. AMPK regulates cell energy balance according to ADP/ATP and AMP/ATP ratios. When the intracellular ADP and AMP ratios are high, it indicates that the metabolism consumes energy. Therefore, catabolic reactions (glycolysis, fatty acid oxidation) are stimulated while anabolic reactions are inhibited (fatty acid, protein, and glycogen synthesis) to meet the energy requirement [15, 16]. AMPK is stimulated by AMP or inhibited by ATP according to energy requirement.
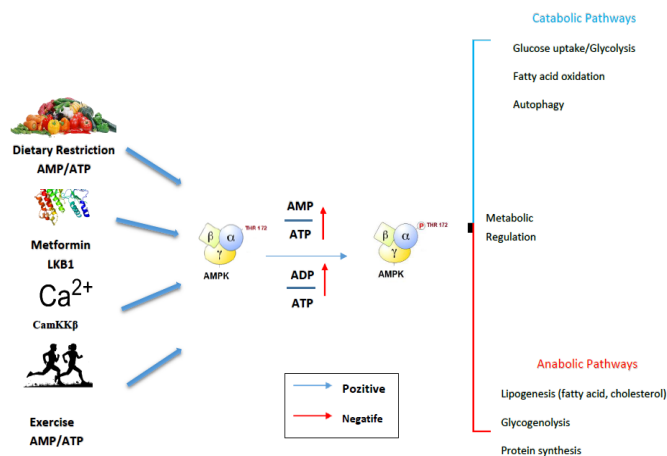

Figure 1 The Role of AMPK in the Regulation of Energy Homeostasis.

2.1. The Function of AMPK in Carbohydrate Metabolism

In carbohydrate metabolism, AMPK regulates cellular glucose uptake independently from insulin. AMPK enhances the translocation of glucose transporters GLUT1 (blood, brain, and kidney) and GLUT 4 (muscle and adipose tissue) and provides cellular glucose uptake. AICAR is one of the pharmacological activator of AMPK, stimulates the α2 subunit of AMPK and increases cellular glucose uptake [5]. In insulin-dependent cellular glucose uptake, TBC1D4 (AS160) is phosphorylated through a series of stimulation chains by phosphorylation of the insulin receptor (IRS1). With the inhibition of TBC1D4 by phosphorylation, the translocation of the glucose transporter protein (GLUT4) is increased, and insulin-dependent cellular glucose uptake occurs. Besides, AMPK inhibits TBC1D1 in the cell by phosphorylation independently from insulin and increases the translocation of GLUT4 [16–18]. AMPK activates glycolysis depending on the energy requirement. AMPK activates the phosphofructokinase 2 enzyme by phosphorylation. Fructose 2,6 biphosphate is synthesized from fructose 6 phosphate by activating the phosphofructokinase 2; thus, glycolysis is stimulated by activation of the phosphofructokinase 1 [19]. AMPK inhibits the glycogen synthase enzyme by phosphorylation depending on the energy requirement, and thereby glycogen synthesis is inhibited (glycogenesis) [16, 20]. Besides, the glycogen phosphorylase enzyme is activated by AMPK with phosphorylation, and glycogen breakdown begins to produce glucose for produce energy [19, 21]. AMPK inhibits gluconeogenesis in the liver according to the energy requirement [13].

2.2. The Function of AMPK in Lipid Metabolism

Exercise and skeletal muscle contraction provide activation of AMPK, and thus beta-oxidation of fats begins to produce

ATP while lipid synthesis is inhibited in the liver. AMPK increases the translocation of CD36 proteins located in the plasma membrane that enables the transport of long-chain fatty acids to mitochondria [22]. The activation of AMP inhibits Acetyl-CoA carboxylase 2 (ACC2) and 3-Hydroxy-3-Methylglutaryl CoA (HMG-CoA) enzymes, and thereby lipid, and cholesterol synthesis are inhibited [16]. AMPK regulates the sterol regulating protein SREBP-1. SREBP-1, a transcription factor, regulates the transcription of enzymes responsible for the synthesis of cholesterol, fatty acids, triacylglycerol (TAG), and phospholipids [23]. AMPK decreases the transcription of fatty acid synthase enzymes by inhibiting gene expression of SREBP-1, thereby preventing lipogenesis in both the liver and adipose tissue [24]. According to the energy requirement, AMPK inhibits the glycerol 3 phosphate acyltransferase enzyme (GPAT), which is involved in the synthesis of TAG [25]. In exercise and other cellular stress situations, the ACC enzyme is inhibited by AMPK with phosphorylation according to the ATP requirement. Thus, the synthesis of fatty acids in the cytosol is inhibited. ACC increases malonyl-CoA production in the synthesis mechanism of fatty acids. Increased malonyl-CoA due to obesity inhibits carnitine palmitoyl-CoA transferase-1 (CPT-1) enzyme, which enables the entry of fatty acids into mitochondria beta-oxidation of fatty acids is inhibited. CPT-1 is activated with the inhibition of ACC by AMPK [26]. The accumulation of fatty acids in the liver and other tissues causes insulin resistance and insulin resistance-related metabolic diseases. Lipid oxidation increases by the inhibition of ACC and lipid stores reduce in muscle and liver. AMPK enhances the translocation of the lipoprotein lipase enzyme located on the endothelial surface in the heart and promoting the entry of fatty acids into the heart muscle cells. AMPK increases the CD36 translocation by the phosphorylation of AS160 and provides the absorption of fatty acids into cells. ACC is inhibited with the phosphorylation by AMPK, and beta-oxidation of fatty acids begins in the heart [27]. As can be seen, AMPK is involved in the regulation of enzymes involved in glucose metabolism and cardiac fatty acid metabolism. AMPK stimulates fatty acid metabolism by providing phosphorylation of peroxisome proliferator-activated receptor-gamma coactivator and forkhead fox O , which are involved in long-term regulation of cardiac energetic homeostasis [28]. AMPK inhibits protein synthesis by inhibiting rapamycin (mTOR), thereby abnormal growth (cardiac hypertrophy) of the heart muscle fibers is prevented [29].

It has been reported that reducing the expression of orexigenic neuropeptides Y (NPY) by the activation of the fatty acid synthesis inhibitör (C75) is contributing to weight loss [30]. In peripheral tissues, C75 stimulates carnitine palmitoyltransferase-1 (CPT-1) and provides the transport and oxidation of fatty acids to mitochondria. Besides, C75 prevents the binding of malonyl-CoA to the β-ketoacyl synthase during the synthesis of fatty acids, and thus the synthesis of long-chain fatty acids is inhibited [31]. When energy stores and fatty acid synthesis are high, malonyl-CoA inhibits CPT-1, and the breakdown of fatty acids is blocked. Conversely, when the energy levels are low, the inhibition on CPT-1 is eliminated as the malonyl-CoA level decreases, thereby destroying the mitochondria's fatty acids to create energy. In the hypothalamus, the activation of the AMPK is decreased by suppressing the phosphorylation of the alpha subunit of the AMPK, and the appetite is suppressed [32].

Alpha-lipoic acid, known as an antioxidant, inhibits the activity of the hypothalamic AMPK and reduces food consumption. Besides, α-lipoic acid inhibits fatty acid synthesis by increasing the activity of AMPK in peripheral tissues, thus promoting beta-oxidation of fatty acids to produce ATP [31]. Thyroid hormones in the hypothalamus increase AMPK and acetyl CoA carboxylase enzymes [33].

3.　The Role of Hypothalamic AMPK in the Regulation of Appetite

In addition to regulating body weight and energy balance in peripheral tissues, AMPK acts as a food sensory in the hypothalamus. AMPK is expressed in some regions of the brain to control the nutrient intake and neuroendocrine functions (Figure 2). Appetite stimulating and suppressive agents act on AMPK, providing both nutrient intake and body weight control [4].

The hypothalamus is a region of the brain that regulates food intake and energy. The hypothalamus consists of different cores or areas, including curved (ARC, curved core), paraventricular (PVN, paraventricular core), ventromedial (VMH, ventromedial core), dorsomedial (DMN, dorsomedial core), and lateral hypothalamic area (LHA). These areas are responsible for the expression of anorexic and anorexigenic neuropeptides that regulate food intake and energy expenditures. AgRP (Agouti-related protein) and NPY (neuropeptide Y) are the orexigenic neuropeptides, while CART (Cocaine- and amphetamine-regulated transcript) is an anorexigenic neuropeptide. LHA produces concentrating melanin hormone and orexigenic neuropeptides, as well as anorexigenic neuropeptide CART [34, 35].

Changes in the modulation of the hypothalamic AMPK alter the expression of anorexigenic neuropeptides (NPY and AgRP) and anorexigenic neuropeptides (POMC and CART) in the ARC nucleus. Since AMPK has been shown to play a key role in controlling food intake and body weight regulation, it has been reported to be an important therapeutic protein kinase in the treatment of energy homeostasis disorders, metabolic syndrome and other related diseases such as cancer-related anorexia [36, 37]. AMPK regulates food intake and control of the hypothalamus-centered energy expenditure [35]. The hypothalamus regulates neuropeptides' synthesis and controls food intake or energy balance in response to changes in environmental signals such as glucose and hormones. If energy intake exceeds energy consumption, the expression of anorexic neuropeptides such as AgRP and NPY decreases, while the expression of anorexigenic neuropeptides such as CART and POMC increases [38].

The expression of NPY, POMC, and AgRP, which are orexigenic and anorexigenic genes in the hypothalamus, is regulated by AMPK according to the state of hunger and satiety. Over-expression of AMPK in the mediobasal hypothalamus (toughness center) suppresses the mRNA expression of NPY and AgRP in ARC. Excessive expression of AMPK in the lateral hypothalamus (fasting center) stimulates the desire for food intake by increasing the expression of NPY and AgRP. As can be seen, the hypothalamus is a control center that regulates both hunger and satiety [37].
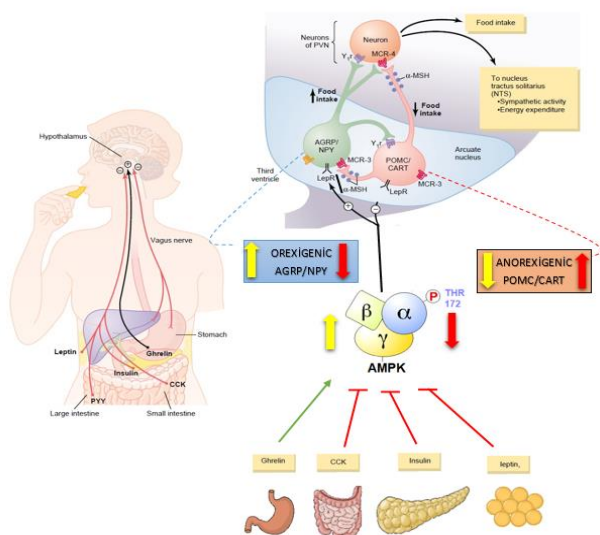
Figure 2 The Role of AMPK in the Regulation of Appetite

3.1. Effects of Hormones on Hypothalamic AMPK

The most important activators of hypothalamic AMPK are ghrelin, low glucose, adiponectin, hypoglycemia, glucocorticoids, thyroid hormones, cannabinoids, and low temperature, while glucose, leptin, insulin, alpha-lipoic acid and metformin are among the most important inhibitors [4]. Adipokines such as leptin and ghrelin are hormones that control food intake in the hypothalamus. Leptin regulates appetite, food intake, energy consumption, and neuroendocrine functions [39]. Since leptin inhibits AMPK in the hypothalamus, it regulates body weight. It reduces food intake and provides weight control [37], while ghrelin activates AMPK and increases food intake [4]. As a result, low leptin and high ghrelin levels in the body increase the activation of AMPK in the hypothalamus, resulting in increased appetite [37].

Leptin, a hormone secreted from adipocytes, regulates nutritional intake, energy consumption, and control of body weight as well as neuroendocrine functions [40]. Leptin increases fatty acid oxidation [41], glucose uptake [42] in peripheral tissues, and prevents lipid accumulation in the tissues [41]. Lipotoxicity occurs in tissues in the disorder of leptin function [43]. Leptin activates AMPK by stimulating the α2 catalytic sub (α2 AMPK) unit of AMPK in skeletal muscles. Leptin inhibits the ACC2 by activating AMPK and provides fatty acid oxidation in peripheral tissues. Also, AMPK inhibits lipogenesis in the liver. In obesity and Type2 diabetes patients, leptin resistance occurs as fat accumulation increases in lean tissues. AMPK activators reduce both leptin resistance and insulin resistance. Adiponectin is a hormone secreted from adipocytes, which increases AMPK activation and increases fatty acid oxidation and glucose uptake [44]. Besides, it inhibits gluconeogenesis [45].

According to recent studies, AICAR inhibits leptin and increases AMPK activity, thus increasing food intake [46]. The hormone ghrelin secreted from the stomach stimulates AMPK in the hypothalamus and increases appetite. Ghrelin reduces the activation of AMPK in the liver and adipose tissue and thus inhibits the catabolic reactions [47, 48]. AMPK works opposite in the hypothalamus, and other tissues are thought to be tissue-specific for enzymes that

phosphorylate or inhibit phosphorylation in these tissues [47, 48].

Ghrelin can activate NPY by increasing the Ca^{+2} signal. Ca^{+2} regulates the CaMKKb activation, which is one of the activators of AMPK. This mechanism supports the activity of ghrelin on AMPK [49, 50]. Exogenous and endogenous cannabinoids increase appetite in the hypothalamus through the cannabinoid receptor type 1 [51]. However, it supports fat synthesis by inhibiting AMPK in the liver and adipose tissue. As can be seen, the opposite stimulation of both mechanisms by cannabinoids causes weight gain [48].

Cortisols known as glucocorticoids increase the appetite by activating AMPK in the hypothalamus, and similar symptoms may occur in this case, as in Cushing's syndrome. In a study on rats, it was reported that AMPK decreased when sucrose was given with cortisol, while AMPK increased when given with salt [52]. Unlike adiponectin's, leptin activates AMPK in both peripheral tissues and the hypothalamus.

Adiponectin secreted from fat white cells activate the AMPK in peripheral tissues and provide oxidation of fatty acids. Also, adiponectin can enter the cerebrospinal fluid in trimer and tetramer forms in the hypothalamus. In the case of fasting, adiponectin increases food intake by activating AMPK. In a study on mice, it was observed that food intake decreased due to the decrease in phosphorylation of AMPK in adiponectin deficiency. In peripheral tissues, AMPK activation decreases due to the decline in adiponectin and causes obesity due to resistance against the breakdown of dietary fats [53].

Insulin is a hormone that reduces appetite in the hypothalamus while providing glucose uptake to the cells. Insulin decreases food intake in the hypothalamus by inhibiting AMPK activity. In insulin deficiency, nutrient intake is encouraged as activation of the hypothalamic AMPK increases [37]. The level of AMPK controlled by insulin and adipokines varies depending on the need for food intake. In the postprandial state, decreasing the level of AMPK causes reducing the expression of AgRP and NPY (orexigenic) neuropeptides, while increasing the expression of CART and POMC (anorexigenic) neuropeptides. In the case of hunger, the level of CART and POMC decreases with the increase of the level of AMPK in the hypothalamus, while the expression of AgRP and NPY increases [37]. Both glucose and insulin inhibit the activity of AMPK in the hypothalamus [13].

Both aging and a long-term high-fat diet cause oxidative stress and inflammation in the hypothalamus, causing continuous activation of the hypothalamic AMPK. Obesity increases with the desire for food intake by the activation of AMPK [54]. Inflammation due to high-fat nutrition and aging in the hypothalamus develops both insulin resistance and leptin resistance. Therefore, since food intake and energy balance change due to inflammation, hyperphagia, obesity, and Type 2 diabetes occur in individuals [55].

3.2. Pharmaceutical Drugs and Nutritional Supplements in Regulation of Hypothalamic AMPK

Recent studies have shown that different nutrients regulate the nutritional status by controlling the hypothalamic AMPK

independently from the leptin. Understanding the mechanisms by which foods control hypothalamic AMPK activity is crucial for developing effective dietary strategies for the treatment of food-related disorders such as anorexia and obesity [56].

3.2.1. Metformin

Today, synthetic drugs such as metformin and thiazolidinediones are used in the treatment of Type 2 diabetes. These medications reduce blood glucose levels and inflammation due to Type 2 diabetes [57, 58]. Metformin, an AMPK activator, has a large number of potential antiatherosclerotic effects, including blocking the attachment of inflammatory cells to the blood vessel endothelium, reducing lipid accumulation, the proliferation of inflammatory cells caused by oxidized lipids, increase and stimulation of cellular antioxidant defenses [59]. Besides, metformin, an anti-diabetic drug, is known to activate AMPK in liver and muscle tissues. In the hypothalamus, metformin has the opposite effect and inhibits the phosphorylation of AMPK. Thus, reducing the mRNA expression of NPY reduces food intake and ensures a low blood glucose level. In contrast, it has been reported that metformin does not affect gene expression of POMC [60, 61].

3.2.2. Leucine

In recent studies, high protein diets have been shown to increase satiety compared to low protein diets [62]. High protein meals or certain amino acids in diets rich in branched-chain amino acids have been shown to affect the central nervous system, leading to reduced energy intake [63] mTOR complex 1 (mTORC1) regulates nutrient intake and energy expenditure in the central nervous system [64]. When the level of mTORC1 increases in peripheral tissues, the leptin hormone's signals increase the level of AMPK, are disrupted, and food intake increases [65]. Leucine also suppresses food intake by decreasing NPY expression in the central nervous system [63]. Leucine also increases protein synthesis in peripheral tissues by stimulating the mTOR pathway [66].

3.2.3. Conjugated Linoleic Acid and Alpha Lipoic Acid

Conjugated linoleic acid (CLA), an omega 6 fatty acid found in meat and dairy products, inhibits AMPK, and reduces orexigenic neuropeptides such as NPY and AgRP [67]. Alpha-lipoic acid, a fatty acid found in the kidney, heart, liver, spinach, and broccoli, activates AMPK in the muscles and increases GLUT4 protein synthesis, thereby regulating the blood glucose level. Alpha-lipoic acid also inhibits hypothalamic AMPK and reduces orexigenic neuropeptides such as NPY and AgRP [68].

3.2.4. Berberine

Studies have reported that the berberine crosses the blood-brain barrier and reaches the brain. The inhibition of hypothalamic AMPK by berberine decreases the expression of AgRP while increasing the expression of POMC. Thus, the desire for food intake is reduced by berberine. In the peripheral tissues, the ACC enzyme is inhibited with activating the AMPK by berberine, and thereby fatty acid oxidation is initiated [69].

4. Conclusion

Today, excessive nutrition and sedentary life have brought along many chronic diseases such as obesity and diabetes. As is known, obesity is a disorder of both energy metabolism and appetite regulation. In recent studies, it has been reported that AMPK regulates the metabolic energy balance as well as governs appetite control. When AMPK is activated, anabolic reactions are inhibited, while catabolic reactions are activated to produce energy. It is also known that exercise activates AMPK, enhances the translocation of the glucose transporter (GLUT4) protein, and provides insulin-independent cellular glucose uptake. When AMP and LKB1 activate AMPK, the use of body fat stores will be encouraged to produce energy. AMPK plays a role in regulating food intake and control of the hypothalamus-centered energy expenditure. Appetite-stimulating and suppressing agents act on AMPK to regulate both nutritional intake and body weight control positively. When AMPK is inhibited by appetite suppressors such as leptin, insulin, metformin, leucine, and berberine, the expression of orexigenic neuropeptides is decreased while the expression of anorexigenic neuropeptides is increased. Understanding the mechanisms controlled by the hypothalamic AMPK is crucial for developing effective nutritional strategies for the treatment of nutritional intake disorders such as obesity, diabetes mellitus, cardiovascular disease, hypertension and cancer.

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