Regulatory Mechanisms of the AMP-Activated Protein Kinase Signaling Pathway in the Pathogenesis of Ketosis in Dairy Cows: Postprint

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Abstract

Dairy cow ketosis is a common nutritional metabolic disease in periparturient dairy cows, causing huge losses to the dairy industry. The regulatory mechanism of the AMP-activated protein kinase signaling pathway in the occurrence and development of dairy cow ketosis. During ketosis, related energy metabolism hormones also undergo significant changes, mainly glucagon and insulin. AMP-activated protein kinase is considered the energy sensor of the body, and some energy metabolism hormones can cause changes in its activity. This article discusses the mechanism of action of glucagon and insulin on the AMP-activated protein kinase signaling pathway, aiming to provide theoretical support for future research on dairy cow ketosis.

Full Text

Adenosine Monophosphate-Activated Protein Kinase Signal Transduction Pathways: Regulatory Mechanisms in the Occurrence and Development of Dairy Cow Ketosis

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Abstract: Dairy cow ketosis is a common nutritional and metabolic disease during the perinatal period that has caused tremendous economic losses to

the dairy industry. During ketosis, energy metabolism-related hormones undergo significant changes, primarily involving glucagon and insulin. Adenosine monophosphate-activated protein kinase (AMPK) is considered a cellular energy sensor, and certain energy metabolic hormones can induce changes in its activity. This review discusses the mechanisms through which glucagon and insulin act on AMPK signal transduction pathways, aiming to provide theoretical support for further research on dairy cow ketosis.

Keywords: glucagon; insulin; adenosine monophosphate-activated protein kinase; dairy cow ketosis

Dairy cow ketosis is a common nutritional and metabolic disease in periparturient cows, particularly prevalent in those experiencing negative energy balance during early lactation [1-2]. The condition often leads to symptoms such as loss of appetite and depression, and in severe cases can cause neurological dysfunction. It frequently precipitates other metabolic diseases including fatty liver, abomasal displacement, retained placenta, and parturient paresis [3], resulting in substantial economic losses to the dairy industry. During negative energy balance, extensive fat mobilization occurs, leading to hepatic lipid metabolism disorders [1,4]. When metabolic disturbances such as oxidative stress or hypoglycemia arise, AMPK becomes phosphorylated and activated, suppressing anabolic pathways for protein, lipid, and glycogen synthesis while enhancing metabolic pathways for glucose transport, fatty acid oxidation, and glycolysis [5-6].

1. AMPK Signal Transduction Pathway

The liver serves as the central hub for lipid metabolism in animals, and hepatic lipid metabolism disorders represent a primary cause of energy metabolic diseases including ketosis, fatty liver, and insulin resistance [7]. AMPK is an evolutionarily conserved serine/threonine protein kinase composed of catalytic α subunits and regulatory β and γ subunits. It participates in multiple cellular metabolic pathways to adapt to energy fluctuations and plays a crucial role in regulating cellular and whole-body energy homeostasis, earning its designation as a cellular energy sensor [8]. AMPK is widely expressed in skeletal muscle, liver, pancreas, and adipose tissue, where it regulates metabolic pathways and influences lipid metabolism through phosphorylation of target proteins [9]. In hepatic lipid metabolism, AMPK serves a central role [9]. Studies in rats have demonstrated that AMPK activation reduces the activity of β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) synthase, acetyl-CoA carboxylase (ACC), and glycerol-3-phosphate acyltransferase, thereby inhibiting cholesterol, fatty acid, and triglyceride (TG) synthesis while accelerating fatty acid oxidation [10]. AMPK regulates lipid metabolism through sterol regulatory element-binding protein-1c (SREBP-1c), phosphorylated carbohydrate-responsive element-binding protein (ChREBP), and peroxisome proliferator-activated receptor (PPAR)α [5,11-12].

SREBP-1c is a transcription factor highly expressed primarily in the liver that promotes lipid synthesis and transport by regulating expression of enzymes involved in fatty acid and TG synthesis and transport [5]. Mice with hepatic overexpression of SREBP-1c exhibit TG accumulation, increased fatty acid synthesis rates, and elevated expression of related enzymes [5]. ChREBP is another major transcription factor regulating hepatic lipid metabolism that cooperates with SREBP-1c to coordinately regulate hepatic lipid metabolism [13]. Under normal dietary conditions, ChREBP knockout mice show significantly decreased mRNA levels of ATP-citrate lyase, ACC1, and fatty acid synthase in the liver compared to control mice, ultimately leading to reduced hepatic fatty acid synthesis [13]. PPARα, a PPAR isoform, is predominantly expressed in tissues rich in mitochondria and β-oxidation activity, such as liver and heart [11]. Animal studies have shown that PPARα activation through ligand binding enhances transcription of enzymes and genes related to lipid metabolism, including carnitine palmitoyltransferase I and II, acyl-CoA oxidase, and HMG-CoA synthase, thereby strengthening the liver' s capacity to oxidize fatty acids [11].

2.1. GLN and Dairy Cow Ketosis

During ketosis, endocrine hormones involved in energy regulation undergo significant changes, primarily insulin and glucagon (GLN) [7]. Ketotic cows exhibit decreased blood insulin content and significantly elevated GLN levels [7]. GLN is a polypeptide hormone secreted by pancreatic α-cells, composed of 29 amino acid residues that primarily acts on the liver to promote glycogenolysis, inhibit glycogen synthesis, and stimulate gluconeogenesis, glucose utilization, and lipolysis [12]. Consequently, GLN is crucial for energy metabolism in ruminants. Research on GLN' s role in lipid metabolism has increased in recent years. Bobe et al. [14] demonstrated that injecting GLN (15 mg/d) into cows with moderate fatty liver 2-3 weeks postpartum could alleviate fever caused by fatty liver and reduce the risk of mastitis. They also noted that subcutaneous GLN injection was more effective and without side effects when environmental temperatures were below 35°C.

2.2. GLN and AMPK Signal Transduction Pathway

Increased GLN and cAMP can inactivate HMG-CoA reductase and ACC, both of which are targets of AMPK [15-16]. Stimulating rat hepatocytes with GLN leads to ACC phosphorylation and inactivation. Initial studies attributed this to direct inhibition by cAMP-dependent protein kinase, but further investigation revealed it to be mediated by AMPK [16]. Therefore, either increased GLN content or GLN elevation induced by conditional changes can lead to hepatic AMPK activation [17]. The relationship between GLN and the AMPK signal transduction pathway is illustrated in Figure 1 [Figure 1: see original paper].

To better understand GLN' s physiological effects, it is essential to recognize that GLN is secreted from the pancreas into the portal vein, which is the liver'

s primary blood supply vessel [18]. While this provides an effective route for GLN to act on the liver, it also creates experimental challenges because most GLN is utilized by the liver, and systemic blood measurements do not reflect portal vein GLN concentrations [18]. GLN increases hepatic glucose output, fatty acid oxidation, amino acid metabolism, and urea production [7]. Recent studies indicate that elevated GLN in vivo can increase the AMP/ATP ratio and activate AMPK, and that GLN receptor signaling is essential for AMPK activation during starvation and exercise [19].

GLN increases and AMPK becomes activated when oxygen uptake and fat oxidation increase [20]. The relationship between the tricarboxylic acid (TCA) cycle and gluconeogenesis corresponds to ATP production and consumption. Increased TCA cycle activity and gluconeogenesis cause hepatic ATP consumption, similar to the AMP/ATP ratio increases observed during exercise and starvation [21]. Activated fatty acid oxidation and amino acid gluconeogenesis also consume ATP. Research shows that gluconeogenesis from amino acids and fatty acids consumes 6 and 4 moles of ATP per mole of glucose produced, respectively; when lactate, alanine, pyruvate, and oleic acid enter the TCA cycle, AMP levels increase [22]. Thus, GLN signaling can activate AMPK by increasing hepatic AMP content.

The increased AMPK phosphorylation resulting from elevated AMP is attributed to the action of liver kinase B1 (LKB1) [23]. GLN can also increase cytosolic calcium ion (Ca^{2+}) content, thereby activating calcium/calmodulin-dependent protein kinase kinase α/β and ultimately phosphorylating AMPK [24]. Berglund et al. [21] demonstrated that in wild-type mice, 18 hours of starvation or exhaustive exercise increased the AMP/ATP ratio by 5- and 10-fold, respectively, whereas these changes did not occur in glucagon receptor-deficient mouse models. Additionally, using a hyperglucagonemic-euglycemic clamp technique can increase circulating GLN without causing hyperglycemia or hyperinsulinemia.

During starvation, hepatic AMPK activation and ACC inactivation exhibit a zonal distribution pattern [25]. Tissues near the portal vein have stronger capacities for gluconeogenesis, urea production, β-oxidation, and ketogenesis, corresponding to hormone and substrate concentrations in the liver [26]. Under starvation conditions, AMPK activation is concentrated near the portal vein, consistent with higher GLN concentrations in this region [27]. Prolonged voluntary and forced exercise also leads to ACC inactivation, decreased malonyl-CoA content, increased β-hydroxybutyrate (BHBA) content, and AMPK activation [28]. GLN-mediated AMPK activation rapidly suppresses hepatic de novo lipogenesis and promotes fatty acid oxidation by inhibiting ACC, thereby regulating malonyl-CoA levels, reducing lipogenic carbon substrates, and relieving inhibition of carnitine palmitoyltransferase I (CPT I) [29]. Similarly, GLN inhibits SREBP-1c expression, which is a master regulator of hepatic lipogenesis [30]. Li et al. [5] showed that AMPK phosphorylation reduces SREBP-1c activity, while hepatocytes lacking AMPK$ $1 exhibit enhanced TG synthesis and re-

duced fatty acid oxidation capacity. Therefore, it can be inferred that GLN inhibits SREBP-1c activity through AMPK.

Studies in mice with hepatic AMPK$ 2$de f iciencyoroverexpressionhavedemonstratedthatAMPKactivationsu$ *mediatedhepaticAMPK* $2 overexpression decreased plasma TG content and increased BHBA levels [31].

Both voluntary and forced prolonged exercise enhance GLN action and activate AMPK, which correlates with improved fatty liver induced by high-fat diet in mice [19]. Intense and prolonged exercise increases hepatic AMP/ATP ratio and activates AMPK in a manner dependent on GLN receptor signaling and phosphoenolpyruvate carboxykinase content [21]. Improvements in fatty liver are accompanied by increased AMP/ATP ratio, AMPK activation, and transcription and translation of PPARα and fibroblast growth factor 21 (FGF21) [19]. In adipocytes, FGF21 increases AMPK activation, NAD^+/NADH ratio, and oxygen consumption; in mitochondria, FGF21 function depends primarily on peroxisome proliferator-activated receptor gamma coactivator 1α, LKB1, AMPK, and sirtuin [33]. In the liver, FGF21 mediates the long-term effects of GLN. An intact GLN-AMPK signaling network may be crucial for recovery from liver diseases. Therefore, it can be hypothesized that hepatic lipid metabolism disorders in dairy cow ketosis may be related to changes in the hepatic AMPK signaling pathway induced by hyperglucagonemia.

3. INS and Dairy Cow Ketosis and AMPK Signal Transduction Pathway

When cows develop ketosis, another energy metabolic hormone, insulin (INS), shows decreased levels [13]. INS is a protein hormone secreted by pancreatic β-cells and is the only hormone in the body that lowers blood glucose, primarily acting on the liver to promote glycogen, fat, and protein synthesis.

INS and AMPK signaling pathways jointly maintain organ homeostasis through overlapping at key signaling nodes [34]. Imbalances between INS and AMPK signaling pathways can be detected in many pathological states, such as diabetes, obesity, and nutritional deficiency [5]. Studies have demonstrated that INS can reduce hepatic AMPK activity. Pre-treatment with INS enables serine/threonine protein kinase B (Akt) to phosphorylate serine 485 (Ser485) on AMPK$ 1$andserine$491(Ser491)onAMPK$ 2$, whilereducingphosphorylationofthreonine$172(Thr172)onAM1$ [35]. As previously mentioned, AMPK inhibits fat synthesis and promotes fat oxidation, and can be rapidly activated by increased cAMP, GLN, and epinephrine to inactivate ACC. In contrast, INS reduces AMPK activity while increasing ACC activity [36]. The specific mechanism of INS-mediated ACC activation remains unclear but may result from both covalent and allosteric modifications [37].

GLN and AMPK jointly reduce SREBP-1c expression and activation, with GLN intermittently stimulating AMPK activation. For example, regular exercise can regulate oxidative stress, create anti-lipogenic zones in the liver, activate

PPARα, and inhibit mTOR complex I and SREBP-1c [30]. Conversely, INS enhances SREBP-1c coding, transcription, and target gene expression [38]. Under normal or insulin-resistant conditions, INS mediates hepatic fat synthesis through control of SREBP-1c [39]. AMPK regulation is not singular but functions as a complex network comprising multiple metabolic pathways and regulatory signals. The interrelationship between GLN, INS, and AMPK signal transduction pathway regulation is illustrated in Figure 1.

4. Dairy Cow Ketosis and AMPK Signal Transduction Pathway

Dairy cow ketosis is characterized by high non-esterified fatty acids, high BHBA, and hypoglycemia, reflecting negative energy balance and systemic metabolic disorders. The AMPK signal transduction pathway plays a crucial role in regulating glycolysis, glucose transport, and lipid metabolism. Researchers have cultured bovine hepatocytes and added BHBA and AMPK inhibitor (Compound C, Cpd C) to examine BHBA' s role in the AMPK signaling pathway. Results showed that when BHBA concentration reached 1.2 mmol, the AMPK signaling pathway was activated, and SREBP-1c and its target gene expression decreased. In groups without Cpd C, PPARα, ChREBP, and their target gene expression increased significantly, indicating that BHBA can activate the AMPK signal transduction pathway and regulate AMPK-related lipid metabolism genes [40]. Mahmoudi et al. [41] demonstrated that a mutation in the 3' -untranslated region of AMPK$ 1$ $was associated with significantly increased serum BHBA levels, suggesting that the AMPK$ 1 gene plays an important role in ketogenesis.

5. Summary and Outlook

The development of dairy cow ketosis is a highly complex process. Although numerous studies on cow ketosis have been published, most have focused on prevention and treatment, while the molecular mechanisms underlying its occurrence remain unclear. In recent years, the AMPK signaling pathway has gradually become a hotspot in life sciences research. However, most related reports have concentrated on mice and humans, with limited literature available on ruminants, particularly dairy cows. Therefore, investigating how GLN and INS regulate key enzymes, genes, and non-coding RNA expression in the AMPK signal transduction pathway during ketosis development will be of great significance.

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