Peroxisome Proliferator-Activated Receptor Regulates Ketogenesis in the Rumen of Young Ruminants and Its Mechanism: Postprint

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Abstract

A well-developed rumen is essential for young ruminants, being critical not only for their own health but also for the expression of their production performance in adulthood. In newborn young ruminants, the rumen lacks ketogenic function; however, with increasing age, rumen morphology and function gradually mature, thereby acquiring this capability. Ketogenesis represents a key factor in rumen maturation, with -hydroxybutyrate (BHBA) considered a marker of rumen developmental maturity. Over the past decade, numerous scholars have conducted extensive research on factors influencing rumen ketogenesis, revealing that peroxisome proliferator-activated receptors (PPARs) are crucial for rumen ketogenesis and epithelial cell proliferation, and that the transcription factor PPARs can influence the expression of the key ketogenic enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). Nevertheless, current understanding of the molecular mechanisms underlying PPARs regulation of rumen ketogenesis remains very limited; therefore, this review summarizes the research progress on PPARs regulation of rumen development in young ruminants.

Full Text

Peroxisome Proliferator-Activated Receptors: Regulation of Ketogenesis in the Rumen of Young Ruminants and Its Mechanisms

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Abstract: A well-developed rumen is critical for young ruminants, affecting not only their health but also their future production performance as adults. Newborn ruminants lack ketogenic function in the rumen, but as age increases, rumen morphology and function gradually mature, eventually acquiring this capability. Ketogenesis is a key factor in rumen development and maturation, with -hydroxybutyric acid (BHBA) considered a marker of rumen development. Over the past decade, numerous studies have investigated factors affecting rumen ketogenesis, revealing that peroxisome proliferator-activated receptors (PPARs) play important roles in rumen ketogenesis and epithelial cell proliferation. The transcription factor PPARs can influence expression of 3-hydroxy-3-methylglutaryl coenzyme A synthase 2 (HMGCS2), a key enzyme in ketogenesis. However, current understanding of the molecular mechanisms by which PPARs regulate rumen ketogenesis remains limited. This review summarizes research progress on PPARs regulation of rumen development in young ruminants.

Keywords: peroxisome proliferator-activated receptors; young ruminants; rumen; ketogenesis; molecular mechanism

Rumen functional development is essential for young ruminants, and ketogenesis is a critical factor promoting rumen maturation. PPARs are members of the ligand-activated nuclear transcription factor superfamily that, in monogastric animals, primarily participate in mitochondrial fatty acid oxidation and energy metabolism. Only a few studies have reported on PPARs' role in ketogenesis, identifying partial target genes such as HMGCS2 and cholesterol acyltransferase 1 (ACAT1). Research in mice has shown that butyrate can activate PPARs, though whether butyrate activates PPARs in the ruminant rumen remains unknown. We hypothesize that ruminal butyrate likely acts as a ligand to activate PPARs and thereby regulate downstream target gene expression. In monogastric animals, limited studies have reported epigenetic regulation of PPARs gene expression; for example, low methylation of the PPAR- promoter region in mice increased PPAR- and target gene mRNA expression. Butyrate can cause epigenetic changes, leading us to speculate that ruminal butyrate may induce epigenetic modifications of PPARs, subsequently affecting their expression. Nevertheless, understanding of the epigenetic mechanisms affecting PPARs expression remains very limited. This review summarizes research progress on PPARs regulation of rumen ketogenesis in young ruminants, aiming to provide clues for further exploration of the molecular mechanisms and to deepen understanding of rumen development for establishing optimal nutritional strategies.

1 Developmental Characteristics of Rumen Ketogenesis

Rumen functional development is key to ensuring healthy growth of ruminants after weaning. Previous studies have shown that lamb rumen development occurs in three stages: non-ruminant phase (1–3 weeks), transition phase (4–8 weeks), and ruminant phase (after 9 weeks). Newborn ruminants primarily survive on digested milk, lacking adult rumen function, with glucose serving as the

main energy source. Through continuous environmental exposure, they gradually transition to digesting plant-based feed, accompanied by rumen changes including increased keratinization of the rumen wall and enhanced absorption of volatile fatty acids (VFA) by rumen epithelial cells for energy metabolism. From 2 days to 6 months of age, glucose utilization decreases by approximately 90%, while rumen epithelial cell keratinization, VFA metabolism, and utilization of butyrate and lactate increase progressively, along with the efficiency of butyrate conversion to ketone bodies.

Rumen ketogenesis primarily refers to the process where butyrate generates acetyl-CoA and subsequently converts to ketone bodies acetoacetate and BHBA. 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase is the rate-limiting enzyme in ketogenesis, existing in two forms: HMGCS1 and HMGCS2. Since rumen ketogenesis occurs mainly in mitochondria, HMGCS2 plays the dominant role. Recent studies have found that both HMGCS2 and HMGCS1 participate in rumen ketogenesis, with HMGCS2 expression regulated by PPARs, highlighting the important regulatory role of PPARs in rumen ketogenesis.

2 Structure and Function of PPARs

PPARs are ligand-activated nuclear transcription factors comprising three members: PPAR- , PPAR- , and PPAR- , belonging to the receptor superfamily. PPARs contain four functional domains divided into six regions (A–F) for research convenience. Domains A and B contain serine residues that can be phosphorylated by mitogen-activated protein kinase (MAPK), affecting PPARs activity. Domain C is the DNA-binding domain (DBD) through which PPARs bind to response elements to regulate gene transcription. Domain D is a transcriptional activity regulatory domain that modulates PPARs activity by binding nuclear cofactors. Domains E and F constitute the ligand-binding domain [Figure 1: see original paper].

PPARs first bind to ligands, then form heterodimers with retinoid X receptor (RXR), altering covalent bond properties and recruiting various coactivators. These complexes recognize and bind to peroxisome proliferator response elements (PPRE) in target gene promoters to exert regulatory effects [Figure 2: see original paper]. PPRE consists of a hexanucleotide repeat (AGGTCA).

Extensive research has investigated PPARs functions in humans and monogastric animals. The three PPAR subtypes show different tissue distributions and physiological functions. PPAR- , the first studied subtype in mammals, is highly expressed in liver, heart, and kidney, primarily participating in mitochondrial fatty acid transport, oxidation, energy metabolism, and oxidative stress. PPAR- is highly expressed in skeletal cells and mainly involved in fatty acid metabolism. PPAR- also regulates rumen epithelial proliferation and differentiation, with significantly higher expression in rumen than PPAR- , suggesting a potentially more important role in regulating rumen epithelial cell proliferation that requires further verification. In human cancer research, PPAR- expression

is significantly elevated in renal cell carcinoma compared to normal cells, indicating a potential role in cell proliferation regulation. PPAR- is also expressed in ruminant rumen at higher levels than PPAR- , though its role in regulating epithelial cell proliferation remains to be investigated.

Recently, PPARs functions in ruminants have gained attention, particularly in lipid metabolism. Fatty acids and their derivatives can serve as PPAR ligands to activate them. PPARs play crucial roles in lipid and carbohydrate metabolism, promoting adipocyte proliferation upon binding specific ligands. PPAR- research in ruminants is relatively limited, showing high expression in kidney and liver but low expression in rumen. Studies in goats 15 years ago found PPAR- involvement in hepatic fatty acid oxidation, and subsequent research demonstrated that PPAR- regulates fatty acid metabolism by controlling carnitine palmitoyltransferase 1A (CPT1A) expression. While PPAR- may also participate in fatty acid oxidation, this requires further verification. PPAR- is the most studied in ruminants, being highly expressed in adipose tissue and associated with adipogenesis and long-chain fatty acid oxidation.

3 Molecular Mechanisms of PPARs Regulation of Rumen Ketogenesis

During rumen development, ketone body production increases continuously. Activated PPARs regulate downstream target gene expression, promoting rumen ketogenesis and epithelial cell proliferation and differentiation. Recent gene expression profiling studies have identified numerous differentially expressed genes accompanying accelerated rumen papillae growth. The promoter regions of HMGCS2, ACAT1, and fatty acid binding protein 3 (FABP3) contain PPRE response elements, making their expression subject to PPAR- regulation. Thus, PPAR- plays a vital regulatory role in rumen development.

Kinoshita et al. found that VFA can affect rumen ketogenesis and ketogenic gene expression, leading Penner et al. to propose that VFA influence PPAR- expression to regulate ketogenesis during the rumen' s transition from glucose to butyrate utilization. Connor et al. identified PPAR- as associated with rumen epithelial cell proliferation and differentiation while exploring rumen developmental mechanisms. Additionally, fibroblast growth factor 21 (FGF21), another PPAR- target gene, plays an important role in energy metabolism. Mouse studies showed that PPAR- agonist treatment increased FGF21 expression, which in turn elevated hepatic CPT1A and HMGCS2 expression to promote ketogenesis. However, whether PPAR- can induce FGF21 expression in young ruminant rumen and whether FGF21 promotes HMGCS2 expression to enhance rumen ketogenesis remain unknown.

In monogastric animals, synthetic WY-14643, L165041, and TZD are specific ligands for PPAR- , PPAR- , and PPAR- respectively, activating PPARs to exert gene regulatory functions. In ruminants, endogenous PPAR ligands may exist. Ruminal glucose and fermentation-produced long-chain fatty acids (LCFA), pro-

pionate, and butyrate could potentially serve as specific PPAR ligands to activate them and regulate target gene expression, promoting rumen ketogenesis and papillae development [Figure 3: see original paper], though this requires verification. Whether LCFA are true PPAR ligands in ruminants remains uncertain, as mouse studies indicate that hepatic LCFA exert transcriptional regulation by activating other transcription factors such as hepatic nuclear factor-4 (HNF4) and liver X receptor (LXR) in addition to PPARs. Furthermore, estrogen-related receptors (ESRRA) can directly activate PPAR- gene expression. Connor et al. demonstrated in calves that activated ESRRA further activates PPAR- to regulate rumen ketogenesis. PPAR- and PPAR- are regulators of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1), which in turn regulates ESRRA. Therefore, PPAR- and PPAR- activate ESRRA through interaction with PGC-1 [Figure 3: see original paper]. Elucidating the interrelationships among PPAR, ESRRA, and PGC-1 is crucial for understanding PPAR' s role in rumen ketogenesis.

4 Epigenetic Regulatory Mechanisms of PPARs Gene Expression

Epigenetics refers to molecular mechanisms causing phenotypic and gene expression changes through modifications beyond DNA sequence, including DNA methylation, histone modification, non-coding RNA, and chromatin remodeling. Mouse studies have shown that butyrate can activate PPAR- , while PPAR- can be silenced or activated through DNA hyper- or hypomethylation. High methylation of PPAR- and CPT1A promoter regions was observed in mice fed fructose, reducing their expression, whereas betaine supplementation decreased PPAR- promoter methylation and upregulated PPAR- and target gene expression. Similarly, low PPAR- promoter methylation in rats increased PPAR- expression and hepatic fatty acid -oxidation. Studies in humans and mice have demonstrated that lipid metabolism-related gene expression is affected by DNA methylation. Methionine supplementation in dairy cow diets caused global DNA hypomethylation and specific PPAR- promoter hypermethylation, upregulating PPAR- expression. Therefore, nutritional regulation of young ruminants can affect lipid metabolism and ketogenesis-related genes through DNA methylation. DNA methylation levels influence PPARs activation and subsequent target gene expression, and butyrate plays an important role in rumen development. We hypothesize that butyrate regulates rumen PPARs DNA methylation, altering PPARs and target gene expression and consequently rumen ketogenesis [Figure 4: see original paper].

Histone modification-mediated chromatin remodeling plays an important role in eukaryotic gene expression regulation. Histone acetylation is catalyzed by histone acetylases (HATs) and histone deacetylases (HDAC). Histone acetylation activates gene transcription, while deacetylation inhibits it. Studies have shown that butyrate causes histone hyperacetylation, acting as an HDAC inhibitor. Therefore, ruminal butyrate likely affects PPARs transcription as an

HDAC inhibitor, influencing rumen ketogenesis and development [Figure 4: see original paper], though this requires verification. Currently, research on epigenetic mechanisms in rumen development is scarce, and no studies have reported on epigenetic mechanisms regulating PPARs expression in the rumen.

5 Summary

Ketogenesis marks rumen maturation, and PPARs are important transcriptional regulators controlling ketogenesis and rumen development. Currently, only partial enzymes related to ketogenesis and regulated by PPARs have been identified. Mouse studies have shown that butyrate can activate PPARs, though whether butyrate activates PPARs in the ruminant rumen remains unreported. The pathways through which PPARs regulate rumen ketogenesis and the factors activating PPARs are unclear. The proposed molecular mechanism involves ruminal glucose and fermentation-produced VFA (especially propionate and butyrate) acting as ligands to activate PPARs, thereby regulating target gene expression. VFA (especially butyrate) affect rumen DNA methylation levels, regulating PPARs and related target gene expression, while butyrate acts as an HDAC inhibitor to influence PPARs transcription and consequently rumen ketogenesis and development. However, the pathways of PPARs regulation of rumen ketogenesis and factors activating PPARs remain unclear. The relative expression levels of the three PPAR subtypes in the ruminant rumen require further investigation, and the relative functional importance among the three subtypes warrants significant exploration.

References

[1] Hu Y, Xu J, Liu YH, et al. Research progress on hypolipidemic effects of PPAR agonists [J]. Journal of China Pharmaceutical University, 2016, 47(1): 118-124.

[2] Jin P, Chen BL, Yang YY. Regulation of PPAR /PGC-1 signaling pathway on energy metabolism in doxorubicin-injured h9c2 cardiomyocytes [J]. Journal of Clinical Cardiology, 2016, 32(4): 388-391.

[3] Naeem A. Effect of age and dietary plane of nutrition on transcript profiles of ruminal tissue of Holstein calves [D]. Ph.D. Thesis. Urbana: University of Illinois Urbana-Champaign, 2012.

[4] Wang WM, Chng L, Li FD, et al. Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs [J]. Scientific Reports, 2016, 6: 32479.

[5] Kinoshita M, Suzuki Y, Saito Y. Butyrate reduces colonic paracellular permeability by enhancing PPAR activation [J]. Biochemical and Biophysical Research Communications, 2002, 293(2): 827-831.

[6] Wang LJ, Chen L, Tan YZ, et al. Betaine supplement alleviates hepatic triglyceride accumulation of apolipoprotein E deficient mice via reducing methylation of peroxisomal proliferator-activated receptor alpha promoter [J]. Lipids in Health and Disease, 2013, 12(1): 34.

[7] Jia JZ, Li XP, Beauchemin KA, et al. Rumen development process in goats as affected by supplemental feeding v. grazing: Age-related anatomic development, functional achievement and microbial colonisation [J]. British Journal of Nutrition, 2015, 113(6): 888–900.

[8] Gergiadi A, Kersten S. Mechanisms of gene regulation by fatty acids [J]. Advances in Nutrition, 2012, 3(2): 127–134.

[9] Lin SC. Structural and functional studies of nuclear receptor PPARs family and novel agonists [D]. Ph.D. Thesis. Xiamen: Xiamen University, 2013.

[10] Waku T, Shiraki T, Yama T, et al. Structural insight into PPARgamma activation through covalent modification with endogenous fatty acids [J]. Journal of Molecular Biology, 2009, 385(1): 188–199.

[11] Ferré P. The biology of peroxisome proliferator-activated receptors: Relationship with lipid metabolism and insulin sensitivity [J]. Diabetes, 2004, 53(1S): 43–50.

[12] Yang YW, Yuan J. Research progress on antioxidant stress effects of PPAR [J]. Journal of Southeast University (Medical Science Edition), 2016, 35(1): 147–150.

[13] Naeem A, Drackley JK, Stamey J, et al. Role of metabolic and cellular proliferation genes in ruminal development in response to enhanced plane of nutrition in neonatal Holstein calves [J]. Journal of Dairy Science, 2012, 95(4): 1807–1820.

[14] Cllet N, Théleyre S, Rageul J, et al. PPAR is functionally expressed in clear cell renal cell carcinoma [J]. International Journal of Oncology, 2011, 38(3): 851–857.

[15] Binaz M, Chen SW, Khan MJ, et al. Functional role of PPARs in ruminants: potential targets for fine-tuning metabolism during growth and lactation [J]. PPAR Research, 2013, 2013: 684159.

[16] Pyper SR, Navin V, Yu ST, et al. PPAR : energy combustion, hypolipidemia, inflammation and cancer [J]. Nuclear Receptor Signaling, 2010, 8: e002.

[17] Wang L, Na W, Wang YX, et al. Expression characteristics of chicken PPAR gene and its effect on adipocyte proliferation and differentiation [J]. Hereditas, 2012, 34(4): 454–464.

[18] Cappn GD, Liu RCM, Frame SR, et al. Effects of the rat hepatic peroxisome proliferator, wyeth 14,643, on lactating goat [J]. Drug and Chemical Toxicology, 2002, 25(3): 255–266.

[19] Schlegel G, Keller J, Hirche F, et al. Expression of genes involved in hepatic carnitine synthesis and uptake in dairy cows in the transition period and at different stages of lactation [J]. BMC Veterinary Research, 2012, 8: 28.

[20] Brennan KM, Michal JJ, Ramsey JJ, et al. Body weight loss in beef cows: I. The effect of increased -oxidation on messenger ribonucleic acid levels of uncoupling proteins two and three and peroxisome proliferator-activated receptor in skeletal muscle [J]. Journal of Animal Science, 2009, 87(9): 2860–2866.

[21] García-Rojas P, Antaramian A, González-Dávalos L, et al. Induction of peroxisomal proliferator-activated receptor gamma and peroxisomal proliferator-activated receptor gamma coactivator 1 by unsaturated fatty acids, retinoic acid, and carotenoids in preadipocytes obtained from bovine white adipose tissue [J]. Journal of Animal Science, 2010, 88(5): 1801–1808.

[22] Sharma S, Sun XT, Rafikov R, et al. PPAR-gamma regulates carnitine homeostasis and mitochondrial function in a lamb model of increased pulmonary blood flow [J]. PLoS One, 2012, 7(9): e41555.

[23] Penner GB, Steele MA, Aschenbach JR, et al. Ruminant Nutrition Symposium: Molecular adaptation of ruminal epithelia to highly fermentable diets [J]. Journal of Animal Science, 2011, 89(4): 1108–1119.

[24] Connor EE, Baldwin RL, Walker MP, et al. Transcriptional regulators transforming growth factor- 1 and estrogen-related receptor- identified as putative mediators of calf rumen epithelial tissue development and function during weaning [J]. Journal of Dairy Science, 2014, 97(7): 4193–4207.

[25] Lin ZF, Pan XB, Wu F, et al. Fibroblast growth factor 21 prevents atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in mice [J]. Circulation, 2015, 131(21): 1861–1871.

[26] Kine BN, Spicer C, Millns P, et al. Systemic administration of WY-14643, a selective synthetic agonist of peroxisome proliferator activator receptor-alpha, alters spinal neuronal firing in a rodent model of neuropathic pain [J]. Scandinavian Journal of Pain, 2015, 9: 42–48.

[27] Shen GJ, An P, Nie YQ, et al. Effects of PPAR- / and its agonist L165041 on liver tumorigenesis in mice [J]. Journal of Gastroenterology and Hepatology, 2015, 24(11): 1350–1353.

[28] Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential [J]. Nature Medicine, 2013, 19(10): 1252–1263.

[29] Ruby MA, Goldenson B, Rasanu G, et al. VLDL hydrolysis by LPL activates PPAR- through generation of unbound fatty acids [J]. Journal of Lipid Research, 2010, 51(8): 2275–2281.

[30] Dengler F, Rackwitz R, Benesch F, et al. Both butyrate incubation and hypoxia upregulate genes involved in the ruminal transport of SCFA and their

metabolites [J]. Journal of Animal Physiology and Animal Nutrition, 2015, 99(2): 379–390.

[31] Khan SA, Heuvel JP. Reviews: current topics—role of nuclear receptors in the regulation of expression of dietary fatty acids (review) [J]. Journal of Nutritional Biochemistry, 2003, 14(10): 554–567.

[32] Huss JM, Torra IP, Staels B, et al. Estrogen-related receptor alpha directs peroxisome proliferator-activated receptor alpha signaling in the transcriptional control of energy metabolism in cardiac and skeletal muscle [J]. Molecular and Cellular Biology, 2004, 24(20): 9079–9092.

[33] Sha D, Liu Y, Liu XJ, et al. PGC-1 -regulated mitochondrial biogenesis and function in myotubes is mediated by NRF-1 and ERR [J]. Mitochondrion, 2010, 10(5): 516–527.

[34] Ding Y, Xu C, Wu JH, et al. Research progress in epigenetics [J]. Scientia Sinica (Vitae), 2017, 47(1): 3–15.

[35] Tang D, Zhou YJ, Li YY, et al. Demethylation effect of curcumin on peroxisome proliferator-activated receptor- in steatotic hepatocytes [J]. Guangdong Medical Journal, 2014, 35(2): 175–179.

[36] Kirchner H, Sinha I, Gao H, et al. Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and diabetic patients [J]. Molecular Metabolism, 2016, 5(3): 171–183.

[37] Hashi K, Munetsuna E, Yamada H, et al. High fructose consumption induces DNA methylation at PPAR and CPT1A promoter regions in the rat liver [J]. Biochemical & Biophysical Research Communications, 2015, 468(1/2): 185–189.

[38] Srivastava J, Jacometo CB, Zhu Z, et al. Hepatic global DNA and peroxisome proliferator-activated receptor alpha promoter methylation are altered in peripartal dairy cows fed rumen-protected methionine [J]. Journal of Dairy Science, 2016, 99(1): 234–244.

[39] Wang J, Zhang XY, Hao CL, et al. Research progress on histone deacetylase inhibitors [J]. Progress in Modern Biomedicine, 2014, 14(14): 2783–2785.

[40] Myzak MC, Dashwood RH. Histone deacetylases as targets for dietary cancer preventive agents: lessons learned with butyrate, diallyl disulfide, and sulforaphane [J]. Current Drug Targets, 2006, 7(4): 443–452.

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