Effects of Short-term Feed Restriction on Lipid Metabolism in the Adipose Tissue of Growing Meat Rabbits: Postprint

Authors: Zhang Bin, Liu Lei, royal city, Liu Hanzhong, Yu Zhiju, Li Fuchang

Date: 2018-12-25T00:00:00+00:00

Abstract

This study aimed to investigate the effects of short-term feed restriction on lipid metabolism-related genes and associated signaling pathways in adipose tissue of growing meat rabbits, and to elucidate the regulatory mechanisms of energy homeostasis in growing meat rabbits. Forty 40-day-old Ira meat rabbits with similar body weight were selected and randomly divided into 2 groups: a control group (ad libitum feeding) and a feed restriction group (feeding level was approximately 70% of that of the control group), with 20 replicates per group and 1 rabbit per replicate; the experiment lasted for 5 d. The results showed that: 1) Compared with the control group, short-term feed restriction significantly reduced the daily weight gain of growing meat rabbits (P<0.05), and tended to decrease total fat deposition (0.050.05). 2) Compared with the control group, short-term feed restriction significantly decreased the gene expression of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and lipoprotein lipase (LPL) (P<0.05), but significantly increased the gene expression of peroxisome proliferator-activated receptors (PPAR , PPAR), carnitine palmitoyltransferases (CPT1, CPT2), and G protein-coupled receptor (GPR41) (P<0.05), with no significant effect on the gene expression of hormone-sensitive lipase (HSL) and G protein-coupled receptor (GPR43) (P>0.05). 3) Compared with the control group, short-term feed restriction had no significant effect on triglyceride (TG) concentration or phosphorylated AMP-activated protein kinase (AMPK) protein expression level in adipose tissue (P>0.05). In conclusion, short-term feed restriction can inhibit fatty acid synthesis and promote fatty acid oxidation in adipose tissue of growing meat rabbits; PPAR and GPR41 signaling may be involved in the regulation of energy homeostasis in adipose tissue of growing meat rabbits.

Full Text

Effects of Short-Term Feed Restriction on Lipid Metabolism in Adipose Tissue of Growing Meat Rabbits

Bin Zhang[1], Lei Liu[1]*, Cheng Wang[2], Hanzhong Liu[3], Zhiju Yu[3], Fuchang Li[1]

[1]Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, College of Animal Science and Technology, Shandong Agricultural University, Tai' an 271018, China
[2]Shandong Jianyuan Biotechnology Co., Ltd., Tai' an 271000, China
[3]Sichuan Academy of Grassland Sciences, Chengdu 610091, China

Abstract

This study investigated the effects of short-term feed restriction on lipid metabolism-related genes and signaling pathways in adipose tissue of growing meat rabbits to elucidate the regulatory mechanisms of energy homeostasis. Forty 40-day-old Ira rabbits with similar body weight were randomly divided into two groups: a control group (ad libitum feeding) and a feed restriction group (fed approximately 70% of the control intake), with 20 replicates per group (one rabbit per replicate). The experiment lasted 5 days. The results showed that: (1) Compared with the control group, short-term feed restriction significantly reduced daily weight gain ($P<0.05$) and tended to decrease total fat deposition ($0.05<P<0.10$), while having no significant effect on shoulder or perirenal fat deposition ($P>0.05$). (2) Short-term feed restriction significantly downregulated the expression of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and lipoprotein lipase (LPL) genes ($P<0.05$), but significantly upregulated the expression of peroxisome proliferator-activated receptors (PPAR , PPAR), carnitine palmitoyltransferases (CPT1, CPT2), and G protein-coupled receptor 41 (GPR41) ($P<0.05$). No significant effects were observed on hormone-sensitive lipase (HSL) or GPR43 gene expression ($P>0.05$). (3) Feed restriction did not significantly affect triglyceride (TG) concentration or phosphorylated AMP-activated protein kinase (AMPK) protein expression in adipose tissue ($P>0.05$). In conclusion, short-term feed restriction inhibited fatty acid synthesis while promoting fatty acid oxidation in adipose tissue of growing meat rabbits, with PPAR and GPR41 signaling potentially participating in the regulation of energy homeostasis.

Keywords: growing meat rabbit; short-term feed restriction; lipid metabolism; gene expression; signaling pathway

Introduction

Feed restriction influences numerous physiological and metabolic processes in animals. In humans, caloric restriction extends lifespan, reduces disease incidence, and modulates energy metabolism and immune responses. In young animals, feed restriction improves meat or carcass quality, promotes gastrointestinal emptying, enhances nutrient digestibility, and controls obesity to prevent reproductive problems associated with excessive fat deposition. Growing meat rabbits have thin intestinal walls that are susceptible to damage, particularly during the post-weaning period when insufficient digestive enzyme secretion readily leads to digestive disorders. Ad libitum feeding often causes severe intestinal problems in these animals. Previous research demonstrated that one week of moderate feed restriction (approximately 85% of ad libitum intake) significantly promoted small intestinal villus and crypt development in growing rabbits. Gidenne et al. found that mild feed restriction could reduce morbidity and mortality rates in growing rabbits. Furthermore, appropriate feed restriction improves intestinal physiological status, stimulates coprophagy, reduces dietary transit rate through the digestive tract, and enhances cecal fermentation. Feed restriction is commonly employed in rabbit production to improve intestinal health and modify fat metabolism and deposition, thereby altering meat quality. However, the underlying mechanisms through which feed restriction affects lipid metabolism in growing meat rabbits remain unclear.

As herbivores, rabbits rely heavily on adipose tissue for lipid metabolism. Adipose tissue serves not only as the primary site for fat storage but also plays a crucial role in fatty acid synthesis. Several key enzymes regulate this process: hormone-sensitive lipase (HSL) is the rate-limiting enzyme for lipolysis, catalyzing triglyceride hydrolysis into glycerol and free fatty acids to meet metabolic demands. Fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) are critical enzymes for fatty acid synthesis, while carnitine palmitoyltransferase (CPT) is the rate-limiting enzyme for long-chain fatty acid oxidation. Lipoprotein lipase (LPL) hydrolyzes triglycerides in very low-density lipoproteins and chylomicrons. Additionally, lipid metabolism is regulated by signaling pathways including peroxisome proliferator-activated receptors (PPARs), G protein-coupled receptors (GPR41/43), and AMP-activated protein kinase (AMPK). This study aimed to investigate how short-term feed restriction affects lipid metabolism-related genes and signaling pathways in adipose tissue of growing meat rabbits to clarify the regulatory mechanisms involved.

Materials and Methods

1.1 Experimental Design and Animal Management Forty 40-day-old Ira meat rabbits with similar body weight [(1510±10) g] were randomly allocated to two groups: a control group (ad libitum feeding) and a feed restriction group (fed approximately 70% of the control intake). Each group comprised 20

replicates with one rabbit per replicate. The experiment lasted 5 days, with feed restriction levels and duration based on previous studies. The composition and nutrient levels of the experimental diet are presented in . Rabbits were housed individually in cages under natural lighting and ventilation with free access to water.

1.2 Growth Performance Measurement and Sample Collection Daily feed intake and body weight were recorded throughout the trial. At the end of the experiment, rabbits were euthanized by cervical dislocation. Shoulder and perirenal adipose tissues were dissected and weighed, then immediately snap-frozen in liquid nitrogen and stored at -80°C.

1.3 Gene Expression Analysis Total RNA was extracted from adipose tissue using the guanidine thiocyanate method. RNA quality and concentration were assessed by agarose gel electrophoresis and spectrophotometry following previously described procedures. Reverse transcription and quantitative real-time PCR were performed using TaKaRa RNA PCR kits according to the manufacturer' s instructions. Primer sequences for target genes are listed in . Gene expression was quantified using the 2-ΔΔCt method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene.

1.4 Determination of Triglyceride Concentration and Protein Expression Triglyceride concentration in adipose tissue was measured using a commercial kit from Nanjing Jiancheng Bioengineering Institute with a microplate reader (SpectraMax iD3, USA). For protein expression analysis, adipose tissue was lysed and total protein concentration determined using a BCA protein assay kit (Kangwei Century Biotechnology, Beijing). Samples were denatured at 100°C for 5 minutes, then 20 L was loaded onto polyacrylamide gels for electrophoretic separation at 20 mA. Proteins were transferred to nitrocellulose membranes at 4°C and 100 V, blocked for 1 hour at room temperature, incubated with primary and secondary antibodies, and visualized using ECL reagent. Images were captured with a gel imaging system (Vilber, France) and quantified using Fusion software.

1.5 Statistical Analysis Data were analyzed by one-way ANOVA using SAS 8.0 software. When significant differences were detected ($P<0.05$), Duncan' s multiple range test was applied. Results are expressed as means ± SEM, with $P<0.05$ considered statistically significant.

Results

2.1 Effects of Short-Term Feed Restriction on Growth Performance As shown in , short-term feed restriction significantly reduced daily weight gain

(P<0.05) and tended to decrease total fat deposition (0.05<P<0.10), while having no significant effect on shoulder or perirenal fat deposition (P>0.05).

2.2 Effects of Short-Term Feed Restriction on Gene and Protein Expression in Adipose Tissue Short-term feed restriction significantly decreased the expression of FAS, ACC, and LPL genes (P<0.05), while significantly increasing the expression of PPAR , PPAR , CPT1, CPT2, and GPR41 (P<0.05). No significant effects were observed on HSL or GPR43 expression (P>0.05) [Figure 1: see original paper]. Additionally, feed restriction did not significantly affect triglyceride concentration or phosphorylated AMPK protein expression in adipose tissue (P>0.05) [Figure 2: see original paper].

Discussion

Body fat deposition is a complex physiological and biochemical process influenced by breed, growth stage, and nutritional status. The extent of fat deposition depends on fatty acid synthesis, degradation, transport, adipocyte differentiation, and lipid mobilization. HSL is the key enzyme regulating lipolysis, primarily expressed in adipose tissue where it hydrolyzes triglycerides into glycerol and fatty acids to meet metabolic demands. In this study, short-term feed restriction did not significantly alter HSL gene expression or triglyceride concentration, suggesting that lipolysis in adipose tissue was not substantially modified.

LPL is synthesized and secreted by adipose tissue into the bloodstream, where it hydrolyzes triglycerides in chylomicrons and very low-density lipoproteins. LPL activity is closely associated with lipid metabolism and obesity, with elevated white adipose tissue LPL activity promoting lipid storage. We found that short-term feed restriction significantly reduced LPL gene expression in adipose tissue, contrasting with findings in poultry skeletal muscle where feed restriction increased LPL expression. This suggests that during feed restriction, triglycerides derived from circulating lipoproteins are preferentially directed to skeletal muscle for energy production rather than to adipose tissue for storage.

CPT, located on the mitochondrial outer membrane, transports long-chain fatty acids into mitochondria for oxidation. The observed increase in CPT1 and CPT2 expression aligns with previous research, indicating enhanced fatty acid oxidation and increased energy supply from fatty acids. FAS catalyzes the synthesis of fatty acids from acetyl-CoA and malonyl-CoA, while ACC catalyzes the initial rate-limiting step of fatty acid synthesis. The significant downregulation of FAS and ACC expression indicates reduced de novo fatty acid synthesis. Collectively, these results demonstrate that short-term feed restriction suppresses fatty acid synthesis while promoting -oxidation in adipose tissue, concurrently reducing lipid uptake from circulating lipoproteins.

PPARs are crucial transcription factors regulating lipid metabolism. PPAR and

PPAR belong to the PPAR superfamily and modulate fatty acid oxidation and uptake through mitochondrial and peroxisomal -oxidation pathways. PPAR is a ligand-activated nuclear transcription factor that stimulates adipocyte differentiation. The increased PPAR transcription observed in this study suggests enhanced adipocyte differentiation during feed restriction. PPAR activation upregulates transcription of lipid metabolism-related enzymes and genes, including CPT, acyl-CoA synthetase, and acyl-CoA oxidase, thereby increasing fatty acid oxidation capacity. The consistent upregulation of PPAR , CPT1, and CPT2 indicates that PPAR likely participates in regulating fatty acid oxidation during feed restriction.

GPR41 and GPR43 are the only known specific short-chain fatty acid receptors, playing important roles in lipid metabolism, immune responses, and intestinal nutrient absorption. Activation of these receptors promotes leptin secretion from adipose tissue, and GPR41 activation specifically increases sympathetic nervous system activity, heart rate, and postprandial energy expenditure. Previous studies detected substantial GPR41 and GPR43 expression in rabbit adipose tissue, and our finding that feed restriction increased GPR41 but not GPR43 expression suggests GPR41 plays a significant role in rabbit energy metabolism. GPR41 activation may upregulate PPAR transcription to modulate adipocyte differentiation, potentially explaining the increased PPAR expression observed during feed restriction.

AMPK is a critical intracellular energy sensor that regulates energy production and consumption according to cellular energy status. In lipid metabolism, AMPK activation inhibits fatty acid synthesis in bovine mammary epithelial cells and downregulates PPAR expression in 3T3-L1 cells. Activated AMPK suppresses cholesterol, diglyceride, triglyceride, and fatty acid synthesis while reducing ACC activity and enhancing fatty acid oxidation. However, we observed no significant change in AMPK phosphorylation levels in adipose tissue, consistent with findings in liver but contrasting with results in skeletal muscle where feed restriction significantly increased AMPK phosphorylation. These discrepancies suggest that AMPK responses to dietary energy status vary among different tissues.

Conclusion

Short-term feed restriction significantly increased the expression of CPT1, CPT2, and PPAR genes while decreasing FAS, ACC, and LPL expression in adipose tissue of growing meat rabbits, indicating suppressed fatty acid synthesis, enhanced fatty acid oxidation, and increased adipocyte differentiation. Furthermore, PPAR and GPR41 signaling pathways may participate in regulating energy homeostasis in adipose tissue of growing meat rabbits.

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