Changes in lipid metabolism-related biochemical indicators and gene expression in mammary gland tissue of hyperlipidemic dairy cows (Post-print)

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Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate changes in lipid metabolism-related biochemical indicators and gene expression in mammary tissue of hyperlipidemic dairy cows. Twelve Holstein dairy cows were selected, and major biochemical indicators in serum were measured using an automatic biochemical analyzer. Cows were divided into normal and hyperlipidemic groups based on whether serum triglyceride (TG) and total cholesterol (TC) levels were elevated. Mammary tissue samples were collected for histopathological sectioning and Oil Red O staining to observe pathomorphological changes and lipid droplet accumulation. Total RNA was extracted from mammary tissue, and the relative expression levels of lipid metabolism-related genes were detected using quantitative real-time PCR (qRT-PCR). The results showed: 1) In the hyperlipidemic group, the contents of lipid metabolism indicators TC, TG, and very low-density lipoprotein (VLDL) in mammary tissue were significantly increased ($P<0.05$), while high-density lipoprotein cholesterol (HDL-C) showed a decreasing trend and low-density lipoprotein cholesterol (LDL-C) showed an increasing trend, but the differences were not significant ($P>0.05$). 2) Pathological observation revealed that alveolar epithelial cells in mammary tissue of hyperlipidemic cows were shed, alveolar walls were significantly thickened, cells were swollen, some cells had increased lipid droplets, nuclear chromatin was condensed and marginalized, and cell outlines were unclear. Oil Red O staining showed that in the normal group, mammary tissue cell nuclei appeared distinctly blue with fewer red areas mainly concentrated at cell edges and only small amounts of lipid droplet aggregation, whereas hyperlipidemic group mammary tissue had large red areas, increased density of interlobular connective tissue, and mammary epithelium showed lipid droplets fusing to form large vacuolated adipocytes. 3) Compared

with the normal group, the relative expression levels of key endogenous synthesis enzyme genes acetyl-CoA carboxylase (ACC1) and activation-related genes acetyl-CoA synthetase 2 (ACSS2), long-chain acyl-CoA synthetase 1 (ACSL1) were significantly increased ($P<0.05$), while the relative expression levels of uptake and transport key genes fatty acid binding protein 3 (FABP3), cluster of differentiation 36 (CD36), lipoprotein lipase (LPL), desaturase key genes stearoyl-CoA desaturase (SCD), fatty acid desaturase 1 (FADS1), and key gene for milk lipid synthesis acylglycerol phosphate acyltransferase 6 (AGPAT6) were significantly decreased ($P<0.05$). In conclusion, hyperlipidemia promotes elevated blood lipid levels in dairy cows, causes obvious pathological changes in mammary tissue, and simultaneously induces significant changes in the expression of mammary lipid metabolism-related genes.

Full Text

Changes in Lipid Metabolism-Related Biochemical Indexes and Gene Expression in Mammary Tissue of Hyperlipidemic Dairy Cows

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Abstract

This study investigated alterations in lipid metabolism-related biochemical indexes and gene expression patterns in the mammary tissue of hyperlipidemic dairy cows. Twelve Holstein cows were selected, and their serum biochemical profiles were analyzed using an automatic biochemical analyzer. Based on serum triglyceride (TG) and total cholesterol (TC) levels, the animals were classified into normal and hyperlipidemic groups. Mammary tissue samples were collected for histopathological sectioning and Oil Red O staining to observe pathomorphological changes and lipid droplet accumulation. Total RNA was extracted from mammary tissue, and real-time quantitative PCR (qRT-PCR) was employed to quantify the relative expression of lipid metabolism-related genes. The results revealed: (1) In hyperlipidemic cows, mammary tissue levels of TC, TG, and very low-density lipoprotein (VLDL) were significantly elevated ($P<0.05$), while high-density lipoprotein cholesterol (HDL-C) showed a decreasing trend and low-density lipoprotein cholesterol (LDL-C) an increasing trend, though these latter differences were not statistically significant ($P>0.05$). (2) Histopathological examination revealed detachment of mammary alveolar epithelial cells, marked thickening of alveolar walls, cellular swelling, increased lipid droplets in some cells, chromatin condensation and marginalization, and indistinct cellular outlines in the hyperlipidemic group. Oil Red O staining showed

predominantly blue nuclei with minimal red staining confined to cell peripheries in the normal group, indicating sparse lipid droplet aggregation. In contrast, the hyperlipidemic group exhibited extensive red-stained areas, increased interlobular connective tissue density, and fused lipid droplets forming large vacuolated adipocyte-like structures. (3) Compared with the normal group, the hyperlipidemic group showed significantly upregulated expression of key genes involved in endogenous fatty acid synthesis (acetyl-CoA carboxylase, ACC1) and activation (acetyl-CoA synthetase 2, ACSS2; long-chain acyl-CoA synthetase 1, ACSL1) ($P<0.05$), while genes critical for fatty acid uptake and transport (fatty acid-binding protein 3, FABP3; cluster of differentiation 36, CD36; lipoprotein lipase, LPL), desaturation (stearoyl-CoA desaturase, SCD; fatty acid desaturase 1, FADS1), and milk fat esterification (acylglycerol-3-phosphate acyltransferase 6, AGPAT6) were significantly downregulated ($P<0.05$). These findings demonstrate that hyperlipidemia elevates blood lipid levels, induces distinct pathological changes in mammary tissue, and alters the expression of lipid metabolism-related genes in dairy cows.

Keywords: hyperlipidemia; dairy cows; biochemical indexes; mammary lipid metabolism; gene expression

Introduction

The mammary gland is a specialized organ in dairy cows with an exceptionally rich blood supply. Mammary epithelial cells absorb precursor substances from blood and convert them into lactose, fat, and protein, functioning as a crucial "biological factory." A comprehensive understanding of mammary metabolism patterns is essential for the rational regulation of nutrient synthesis and secretion in the mammary gland. Fatty acids constitute a vital component of mammary nutrients, with over 95% of milk fat existing as triglycerides (TG), while the remainder comprises phospholipids, cholesterol esters, diglycerides, monoglycerides, and free fatty acids. During lactation, the metabolic demands of pregnancy and milk production frequently lead to negative energy balance (NEB) in early lactation, which directly causes hypoglycemia and significantly elevates glucagon levels. This hormonal shift promotes lipolysis, resulting in increased non-esterified fatty acid (NEFA) concentrations and elevated reactive oxygen species (ROS) levels. When excessive NEFA influx into the liver surpasses hepatic oxidative capacity, incomplete oxidation generates ketone bodies (-hydroxybutyrate, acetoacetate, and acetone), triggering ketosis and fatty liver disease and further promoting nutritional metabolic disorders. Numerous studies have confirmed that such metabolic diseases are associated with energy metabolism disturbances that impair immune function and inflammatory responses, increasing disease susceptibility. Hyperlipidemia is characterized by abnormal plasma lipid metabolism, manifesting as elevated TC, TG, and LDL-C levels alongside decreased HDL-C. With rising incidence rates of hyperlipidemia and related disorders, elucidating the molecular mechanisms of hyperlipidemia pathogenesis is crucial for developing clinical treatments. This study

employed automatic biochemical analysis, hematoxylin-eosin (HE) and Oil Red O staining for histopathological examination, and qRT-PCR to investigate lipid metabolism-related gene expression, aiming to reveal the pathogenesis of hyperlipidemia in dairy cows and provide a theoretical basis for understanding milk quality decline associated with this condition.

1. Materials and Methods

1.1 Experimental Animals and Feeding Twelve Holstein cows were selected from a dairy farm in Anhui Province based on blood lipid metabolism profiles. Six cows with normal serum parameters and six with hyperlipidemia were chosen, matched for age, parity, expected calving date, and physiological status. All cows were fed total mixed rations (TMR) formulated according to nutritional requirements. Diet composition and nutrient levels are presented in .

1.2 Blood and Tissue Sample Collection Five milliliters of non-anticoagulated blood were collected from the tail vein of each cow after overnight fasting. Samples were allowed to clot at 4°C for 30 minutes, then centrifuged at 3,500 rpm for 10 minutes. Serum was harvested, aliquoted into 2 mL tubes, and stored at -20°C for biochemical analysis. Based on the serum parameters shown in , six cows with elevated TG and TC levels were assigned to the hyperlipidemic group, while six cows with normal values served as the control group.

Following blood collection, cows were euthanized by jugular exsanguination. The mammary gland was exposed via midline abdominal incision, and tissue samples were collected. Portions were rinsed with physiological saline and immediately fixed in 10% neutral formalin for HE and Oil Red O staining, while remaining tissue was snap-frozen in liquid nitrogen and stored at -80°C for gene expression analysis.

1.3 Biochemical Analysis of Lipid Metabolism in Mammary Tissue One hundred milligrams of mammary tissue were ground in liquid nitrogen. To the powdered tissue, 5.01 mL of heptane-isopropanol-Tween 20 extraction solution (3.00:2.00:0.01) was added, followed by centrifugation at 1,800×g for 10 minutes at 4°C. The supernatant was collected for determination of TC, TG, HDL-C, LDL-C, and VLDL levels using an automatic biochemical analyzer.

1.4 HE Staining of Mammary Tissue Fixed mammary tissue samples were rinsed in running water for 24 hours, then dehydrated through a graded ethanol series (70%, 80%, 90%, 95%, and 100%, one hour each). After xylene clearing and paraffin embedding, 5 m sections were cut, mounted, and baked. Sections were deparaffinized in xylene, rehydrated through descending ethanol concentrations (100%, 95%, 80%, 70%, five minutes each), stained with hematoxylin, differentiated in 70% hydrochloric acid alcohol, and counterstained with

eosin. Following dehydration through an ascending ethanol series (70% for 1-3 minutes, 80% for 1-3 minutes, 95% for 3 minutes, 100% for 5 minutes), sections were cleared in xylene and mounted with neutral resin for microscopic examination of pathological changes.

1.5 Oil Red O Staining of Mammary Tissue Saturated Oil Red O stock solution was diluted 3:2 with distilled water, mixed, allowed to stand at room temperature for 5-10 minutes, and filtered. Frozen mammary tissue samples stored at -80°C were transferred to -20°C for 30 minutes, then sectioned at 5-8 m thickness using a cryostat with OCT embedding medium. After air-drying, sections were fixed in formaldehyde-calcium solution for 10 minutes, washed thoroughly with distilled water, rinsed in 60% isopropanol, stained with Oil Red O solution for 10 minutes, differentiated in 60% isopropanol until stromal structures were clear, washed with distilled water, counterstained with Mayer' s hematoxylin, washed again, and mounted in glycerin gelatin for microscopic examination.

1.6 qRT-PCR Detection of Lipid Metabolism-Related Genes Total RNA was extracted from 100 mg of frozen mammary tissue ground in liquid nitrogen using Trizol Reagent. Complementary DNA was synthesized using an Invitrogen reverse transcription kit and stored at -20°C. Primers for lipid metabolism-related genes were designed using Primer 5.0 software based on bovine sequences from GenBank, with -actin as the internal reference. Relative gene expression was quantified by qRT-PCR and calculated using the 2^-ΔΔCt method. Primer sequences are listed in .

1.7 Statistical Analysis Data were analyzed using SPSS 20.0 software. Student' s t-test and Duncan' s multiple range test were used for intergroup comparisons. Significance was set at $P<0.05$ and high significance at $P<0.01$. All data are expressed as mean±standard deviation (SD).

2. Results

2.1 Biochemical Indexes of Lipid Metabolism in Mammary Tissue As shown in , mammary tissue levels of TC, TG, and VLDL were significantly higher in the hyperlipidemic group compared with the normal group ($P<0.05$). HDL-C showed a decreasing trend while LDL-C showed an increasing trend, but neither difference was statistically significant ($P>0.05$).

2.2 HE Staining Results HE staining revealed intact alveolar structures with neatly arranged cells and clear boundaries in the normal group ([Figure 1: see original paper]A, B). In contrast, the hyperlipidemic group ([Figure 1: see original paper]C, D) showed detached alveolar epithelial cells, markedly thickened alveolar walls, congestion, cellular swelling, increased lipid droplets, chromatin condensation and marginalization, and indistinct cell outlines.

2.3 Oil Red O Staining Results Oil Red O staining of normal group tissue ([Figure 2: see original paper]A, B) showed predominantly blue nuclei with minimal red staining localized to cell peripheries, indicating sparse lipid droplet accumulation. The hyperlipidemic group ([Figure 2: see original paper]C, D) exhibited extensive red-stained areas with deeper coloration, increased interlobular connective tissue density, and fused lipid droplets forming large vacuolated adipocyte-like structures with blurred cell boundaries and marginalized chromatin.

2.4 qRT-PCR Results As presented in , the hyperlipidemic group showed significantly upregulated expression of genes involved in fatty acid synthesis (ACC1) and activation (ACSS2, ACSL1) ($P<0.05$). Conversely, genes critical for fatty acid uptake and transport (FABP3, CD36, LPL), desaturation (SCD, FADS1), and milk fat esterification (AGPAT6) were significantly downregulated ($P<0.05$). Expression of regulatory genes (SREBP-1c, PPAR) and other esterification-related genes (GPAM, LPIN1, DGAT1) showed decreasing trends but without statistical significance ($P>0.05$).

3. Discussion

Hyperlipidemia is a metabolic disorder characterized by elevated blood lipid levels, typically manifesting as increased serum TC and TG, decreased HDL, and often leading to obesity, hypertension, and diabetes. This study comprehensively investigated serum biochemical changes and mammary lipid metabolism gene expression in hyperlipidemic dairy cows through biochemical assays, histopathological observation, and gene expression analysis. The observed trends of decreased insulin and HDL-C alongside increased TC and LDL-C in hyperlipidemic cows align with previous reports. Blood parameters serve as primary indicators for monitoring herd health and predicting disease risk. In this study, cows were classified into normal and hyperlipidemic groups based on differences in insulin and lipid metabolism indices.

Histopathological examination revealed that normal mammary tissue maintained intact alveolar cell structures with orderly cellular arrangement and clear boundaries, whereas hyperlipidemic tissue exhibited epithelial cell detachment, thickened alveolar walls, congestion, swelling, increased lipid droplets, chromatin condensation, and marginalization. These findings are consistent with previous observations of HE-stained adipose-rich organs. Oil Red O staining specifically stains adipocyte triglycerides red, with staining intensity and area correlating with fat content. Normal tissue showed minimal red staining, while hyperlipidemic tissue displayed extensive red areas, increased connective tissue density, and fused lipid droplets forming large vacuolated structures, corroborating earlier studies.

qRT-PCR analysis revealed significantly reduced expression of genes involved in fatty acid uptake (FABP3, CD36), desaturation (SCD, FADS1), and esterification (AGPAT6), while activation genes (ACSS2, ACSL1) were upregulated.

These results partially align with previous studies showing decreased expression of CD36, AGPAT6, and DGAT1 in bovine mammary tissue. However, gene expression is influenced by multiple complex factors, and the interrelationships among these expression changes and their clinical significance require further investigation.

Milk fat synthesis occurs in three main stages: fatty acid synthesis, TG synthesis, and lipid droplet formation. TG is stored in adipose tissue and transported in blood as water-soluble lipoproteins. TG measurement primarily reflects lipid metabolism status, while cholesterol bound to apolipoproteins exists as soluble lipoproteins. Abnormal cholesterol metabolism can lead to cholesterol accumulation and dyslipidemia. Milk fat synthesis involves multiple transcription factors and functional genes, with sterol regulatory element-binding proteins (SREBP) and PPAR identified as key regulators that initiate downstream gene expression. Investigating these expression patterns enhances our understanding of lactation mechanisms.

This study examined a limited set of lipid metabolism-related genes. Future research should expand the gene panel and incorporate immunohistochemistry, Western blotting, and other molecular techniques to provide a more comprehensive understanding of lipid metabolism patterns in hyperlipidemic dairy cows.

4. Conclusion

Hyperlipidemia in dairy cows causes extensive lipid droplet accumulation in mammary tissue, vacuolar degeneration of mammary epithelial cells, and characteristic pathological changes including chromatin condensation and marginalization. Additionally, hyperlipidemia disrupts biochemical indexes of lipid metabolism and alters the expression of related genes in mammary tissue, potentially through mechanisms involving enhanced mammary fatty acid synthesis.

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Note: Figure translations are in progress. See original paper for figures.