Effects of Lysine on Milk Fat Synthesis-Related Gene and Protein Expression in Dairy Cow Mammary Epithelial Cells (Postprint)

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Abstract

The present study aimed to investigate the effects of lysine (Lys) on the expression of genes and proteins related to milk fat synthesis in bovine mammary epithelial cells (BMECs), and to explore the mechanism by which Lys influences milk fat synthesis. Third-generation BMECs were randomly divided into 6 groups, with 6 replicates per group and 1 culture well per replicate. The Lys concentrations in the culture medium of each group were 0.5 (basal medium, control), 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L. After culturing at 37 °C with 5% CO2 for 48 h, the triglyceride (TAG) content and the expression levels of genes and proteins related to milk fat synthesis in BMECs were measured. The results showed that TAG content in BMECs (P=0.013) and the expression levels of fatty acid binding protein 3 (FABP3, P=0.001), lipoprotein lipase (LPL, P=0.096), fatty acid synthase (FASN, P=0.003), 1-acylglycerol-3-phosphate O-acyltransferase 6 (AGPAT6, P=0.038), and glycerol-3-phosphate acyltransferase (GPAM, P=0.022) genes exhibited concentration-dependent effects that were significant or tended toward significance. FABP3 gene expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups and LPL gene expression in the 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L groups were significantly higher than in the 0.5 mmol/L group (P<0.05); FASN gene expression was highest in the 2.0 mmol/L group, which was significantly higher than the 16.0 mmol/L group (P<0.05); stearoyl-CoA desaturase 1 (SCD1) gene expression in the 2.0 and 4.0 mmol/L groups was significantly higher than in the other groups (P<0.05); phosphatidic acid phosphatase 1 (LPIN1), butyrophilin subfamily 1 member 1 (BTN1A1), and xanthine dehydrogenase (XDH) gene expression in the 1.0, 2.0, 4.0, and 8.0 mmol/L groups were all significantly higher than in the 0.5 mmol/L group (P<0.05); peroxisome proliferator-activated receptor (PPAR) gene and protein expression in the 2.0 and 4.0 mmol/L groups were significantly higher than in the 0.5, 8.0, and 16.0 mmol/L groups (P<0.05); sterol regulatory element-binding protein 1 (SREBP1) gene expression in the 1.0, 2.0, and 4.0 mmol/L groups was significantly higher than in the other groups ($P<0.05$), and its protein expression level in the 1.0 mmol/L group was significantly higher than in the other groups ($P<0.05$). However, high concentrations of Lys inhibited AGPAT6 and GPAM gene expression. AGPAT6 gene expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups was significantly lower than in the 0.5 and 1.0 mmol/L groups ($P<0.05$), and GPAM gene expression in the 16.0 mmol/L group was significantly lower than in the 0.5, 1.0, 2.0, and 4.0 mmol/L groups ($P<0.05$). These results indicate that Lys has a significant promoting effect on milk fat synthesis in BMECs, but high concentrations of Lys inhibited the expression of genes related to milk fat synthesis. Under the conditions of this experiment, the appropriate concentration of Lys in the culture medium was 2.0–4.0 mmol/L.

Full Text

Effects of Lysine on Expressions of Genes and Proteins Involved in Milk Fat Synthesis in Bovine Mammary Epithelial Cells

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Abstract

This experiment was conducted to investigate the effects of lysine (Lys) on the expression of genes and proteins related to milk fat synthesis in bovine mammary epithelial cells (BMECs) and to explore the underlying mechanisms. Third-passage BMECs were randomly divided into six groups with six replicates per group and one well per replicate. The culture media contained Lys at concentrations of 0.5 (basal medium, control), 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L. After 48 hours of incubation at 37 °C and 5% CO , the triglyceride (TAG) content and the expression levels of genes and proteins involved in milk fat synthesis were measured. The results demonstrated that TAG content ($P=0.013$) and the expression of fatty acid-binding protein 3 (FABP3, $P=0.001$), lipoprotein lipase (LPL, $P=0.096$), fatty acid synthase (FASN, $P=0.003$), 1-acylglycerol-3-phosphate O-acyltransferase 6 (AGPAT6, $P=0.038$), and glycerol-3-phosphate acyltransferase (GPAM, $P=0.022$) showed significant or marginally significant dose-dependent responses to Lys. FABP3 expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups and LPL expression in the 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L groups were significantly higher than in the 0.5 mmol/L group ($P<0.05$). FASN expression peaked in the 2.0 mmol/L group, which was significantly higher than the 16.0 mmol/L group ($P<0.05$). Stearoyl-CoA desaturase 1 (SCD1) expression in the 2.0 and 4.0 mmol/L groups was significantly higher than in all other groups ($P<0.05$). The expression levels of phosphatidic acid phosphatase 1 (LPIN1), butyrophilin subfamily 1 member A1 (BTN1A1), and xanthine dehydrogenase

(XDH) in the 1.0, 2.0, 4.0, and 8.0 mmol/L groups were significantly higher than in the 0.5 mmol/L group ($P<0.05$). Both gene and protein expression of peroxisome proliferator-activated receptor (PPAR) were significantly higher in the 2.0 and 4.0 mmol/L groups compared to the 0.5, 8.0, and 16.0 mmol/L groups ($P<0.05$). Sterol regulatory element-binding protein 1 (SREBP1) gene expression was significantly higher in the 1.0, 2.0, and 4.0 mmol/L groups than in other groups ($P<0.05$), while its protein expression was significantly higher in the 1.0 mmol/L group than in all other groups ($P<0.05$). However, high concentrations of Lys inhibited AGPAT6 and GPAM gene expression. AGPAT6 expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups was significantly lower than in the 0.5 and 1.0 mmol/L groups ($P<0.05$), and GPAM expression in the 16.0 mmol/L group was significantly lower than in the 0.5, 1.0, 2.0, and 4.0 mmol/L groups ($P<0.05$). These findings indicate that Lys significantly promotes milk fat synthesis in BMECs, but high concentrations inhibit the expression of genes related to milk fat synthesis. Under the conditions of this experiment, the optimal Lys concentration in the culture medium was 2.0–4.0 mmol/L.

Keywords: dairy cow; bovine mammary epithelial cells; lysine; milk fat

Introduction

Milk fat is a major component of milk and one of the important indicators for evaluating milk quality. Amino acids (AA) are crucial precursors for milk protein synthesis, but they also influence milk fat synthesis and composition. Therefore, in-depth investigation of the effects and mechanisms of AA on milk fat synthesis is significant for regulating milk component synthesis and improving milk quality within the mammary gland. Lysine (Lys) is an essential amino acid for animals. Giallongo et al. observed that infusion of rumen-protected Lys (RP-Lys) in dairy cows promoted milk protein synthesis while also enhancing milk fat synthesis. Xu et al. found that feeding appropriate ratios of Lys and valine (Val) to lactating sows minimized backfat loss, suggesting that Lys and Val can influence milk fat synthesis. Han reported that arterial infusion of AA in dairy cows fed straw-based diets promoted the uptake of short-chain fatty acids in the mammary gland. These studies demonstrate that Lys affects milk fat synthesis to some extent. However, most domestic and international research has focused on the effects of ruminal or external pudendal artery infusion of Lys on milk protein and fat synthesis, with limited reports on using BMECs as a model to study the effects of different Lys concentrations on the expression of genes and proteins related to milk fat synthesis. The underlying mechanisms warrant further investigation. Therefore, this study employed BMECs as a model to investigate the effects of varying Lys concentrations on the expression of genes and proteins involved in milk fat synthesis, providing a theoretical foundation for elucidating the mechanisms of Lys action on milk fat synthesis in BMECs and offering guidance for regulating Lys nutrition levels to enhance dairy cow productivity.

1.1 Reagents and Instruments

Type II collagenase, DMEM/F12 medium, insulin-transferrin-selenium, fetal bovine serum (FBS), and 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco. Lys (L8662), hydrocortisone, epidermal growth factor, prolactin, Oil Red O, agarose, and rabbit anti-peroxisome proliferator-activated receptor (PPAR) antibody were obtained from Sigma (AV32880). Amphotericin B was from Amresco, cell culture antibiotics from Corning, phosphate-buffered saline (PBS) and Tris-buffered saline (TBS) from HyClone. RNAiso PLUS, PrimeScript RT Master Mix, and SYBR Premix Ex Taq™ II were from TaKaRa. Radioimmunoprecipitation assay (RIPA) lysis buffer, bicinchoninic acid (BCA) protein assay kit, Western primary antibody diluent, Western secondary antibody diluent, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) buffer, Western transfer buffer, and ECL chemiluminescent substrate were from Beyotime Biotechnology. Mouse anti-sterol regulatory element-binding protein 1 (SREBP1) antibody was from Abcam (ab3259), rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody from Proteintech (10494-1-AP), horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody from KPL (04-15-06), and HRP-conjugated goat anti-mouse secondary antibody from Tianjin Sungene Biotech (LK2003).

Major instruments included a CO incubator (Forma-311, Thermo), biosafety cabinet (MSC-Advantage, Thermo), inverted microscope (Olympus), microplate reader (Synergy H4 BioTek), cell counter (Cytorecon, GE), real-time PCR system (ABI-7500, ABI), and protein electrophoresis apparatus, transfer apparatus, and imaging system (BIO-RAD).

1.2 Primary BMECs Culture

BMECs were isolated using collagenase digestion. Mammary tissue was collected from healthy mid-lactation Holstein cows aged 3–5 years at a halal slaughterhouse in Hohhot, Inner Mongolia. The superficial layer was removed, and approximately 1 cm^3 of deep tissue was placed in cold PBS containing 3× antibiotics at 4 °C. In a laminar flow hood, the tissue was washed sequentially with 3× PBS, 75% ethanol, and 1× PBS. After removing the surface layer, acinar-rich tissue was minced into a paste. An equal volume of 0.5% Type II collagenase solution was added, and the tissue was digested for 1 hour at 37 °C and 5% CO . The digest was filtered through an 80-mesh screen, and the cell filtrate was centrifuged at 179×g for 5 minutes. The supernatant was discarded, and the cells were washed with PBS and centrifuged at 179×g for 3 minutes; this washing step was repeated twice. Complete medium was added, and cells were resuspended and seeded into 25 cm^2 culture flasks, then incubated at 37 °C and 5% CO . When primary cells reached 80–90% confluence, they were purified and passaged using 0.25% trypsin/EDTA.

1.3 Experimental Design

Third-passage BMECs were seeded onto different culture plates at the required density and cultured at 37 °C and 5% CO for 24 hours. A single-factor randomized design was employed. The DMEM/F12 medium used in the experiment contained 0.5 mmol/L Lys. Based on the research of Li and Gao, and confirmed by MTT assay for cell proliferation, Lys concentrations were set at 0.5 (basal medium, control), 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L, with six replicates per group and one well per replicate. When cell confluence reached 80–90%, the medium was replaced with starvation medium. After 12 hours, cells were switched to media with different Lys concentrations and cultured for 48 hours at 37 °C and 5% CO .

1.4 Test Indicators and Methods

1.4.1 Cell Viability and TAG Content Cell viability was assessed using the MTT assay, with relative growth rate (RGR) calculated from absorbance at 490 nm (OD) using the formula: RGR (%) = (OD of treatment group / OD of control group) × 100. TAG content was measured following the method of Ramírez-Zacarías et al. Cells were seeded in 24-well plates at 5×10 cells/mL. After culture, the medium was removed, and cells were washed twice with PBS. Each well was fixed with 0.2 mL of 4% paraformaldehyde for 1 hour, then washed twice with PBS. Cells were stained with 0.5 mL of Oil Red O working solution for 2 hours in the dark, washed three times with PBS, air-dried, and extracted with 0.3 mL isopropanol for 30 minutes. TAG content was determined by measuring absorbance at 510 nm (OD) using a microplate reader.

1.4.2 Expression of Genes Related to Milk Fat Synthesis in BMECs Cells were seeded in 6-well plates at 2×10 cells/mL. After culture, total RNA was extracted using the Trizol method. RNA purity and concentration were assessed using a microplate reader; an OD /OD ratio of 1.8–2.2 indicated good purity. Integrity was verified by 2% agarose gel electrophoresis. Total RNA was reverse-transcribed to cDNA using the PrimeScript RT Master Mix kit in a 10 µL reaction. Gene expression was quantified using the SYBR Premix Ex Taq™ II kit in a 20 µL reaction. GAPDH served as the housekeeping gene for relative quantification of milk fat synthesis-related genes including fatty acid synthase (FASN), acetyl-CoA carboxylase (ACACA), PPAR , SREBP1, stearoyl-CoA desaturase 1 (SCD1), FABP3, LPL, AGPAT6, GPAM, LPIN1, BTN1A1, and XDH. Primer sequences are listed in Table 1 . The qPCR program consisted of initial denaturation at 95 °C for 30 seconds, followed by 40 cycles of 95 °C for 5 seconds and 60 °C for 34 seconds, then 95 °C for 20 seconds. A melting curve was generated with 51 cycles of 95 °C for 5 seconds, 60 °C for 30 seconds, and 95 °C for 15 seconds. Relative gene expression was calculated using the 2 ΔΔCt method.

1.4.3 Protein Expression of PPAR and SREBP1 in BMECs Protein expression was determined by Western blot using GAPDH as the internal control. Cells were seeded in 25 cm^2 flasks at 1×10 cells/mL. After culture, the supernatant was removed, and cells were washed twice with PBS. Each flask was lysed with 250 µL RIPA buffer containing 0.1% phenylmethylsulfonyl fluoride (PMSF) for 5 minutes at 4 °C. Cells were scraped, collected, and centrifuged at 15,455×g for 10 minutes at 4 °C. The supernatant was used for protein expression analysis. Protein concentration was measured using the BCA method. For each sample, 60 µg of protein was mixed with 5× loading buffer at a 4:1 ratio, heated at 100 °C for 5 minutes for denaturation, and separated by electrophoresis at 80 V for 30 minutes through the stacking gel and 120 V for 120 minutes through the resolving gel. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes (100 V, 4 °C, 50 minutes). Membranes were washed three times with TBST for 5 minutes each, blocked for 1 hour at room temperature on a shaker, and washed again three times with TBST for 5 minutes each. Membranes were incubated overnight at 4 °C with rabbit anti-PPAR primary antibody (1:250 dilution) or mouse anti-SREBP1 primary antibody (1:50 dilution). After incubation, membranes were washed three times with TBST for 5 minutes each, then incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:1000 dilution) or goat anti-mouse secondary antibody (1:500 dilution) for 1 hour at room temperature on a shaker. Membranes were washed three times with TBST for 10 minutes each, developed using an ECL kit, and imaged with a protein imaging system. Grayscale values were analyzed using Quantity One software. Protein expression levels of PPAR and SREBP1 were expressed as the ratio of each group' s grayscale value to that of the 0.5 mmol/L group.

1.5 Data Processing

All data were organized using Excel and analyzed for significance using SAS 9.0 software (ANOVA). Linear and quadratic regression analyses were performed using the regression procedure. Differences or regression relationships were considered significant at $P<0.05$, marginally significant at 0.05 $P<0.10$, and non-significant at $P>0.10$.

Results

2.1 Effects of Lys on BMECs Viability, TAG Content, and Expression of Genes Related to Milk Fat Synthesis

As shown in Table 2 , RGR decreased significantly with increasing Lys concentration in a linear manner ($P<0.001$), while TAG content increased significantly in a quadratic pattern ($P=0.013$). FABP3 and LPL gene expression increased significantly and marginally significantly in a linear manner, respectively ($P=0.001$ and $P=0.096$). The RGR in the 4.0, 8.0, and 16.0 mmol/L groups was significantly lower than in the 0.5, 1.0, and 2.0 mmol/L groups ($P<0.05$). FABP3 expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups was significantly higher than in the 0.5 and 1.0 mmol/L groups ($P<0.05$). LPL expression in the 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L groups was significantly higher than in the 0.5 mmol/L group ($P<0.05$). FASN expression showed a significant quadratic increase with Lys concentration ($P=0.003$), peaking in the 2.0 mmol/L group, which was significantly higher than the 16.0 mmol/L group ($P<0.05$). SCD1 expression in the 2.0 and 4.0 mmol/L groups was significantly higher than in all other groups ($P<0.05$). PPAR gene expression in the 2.0 and 4.0 mmol/L groups was significantly higher than in the 0.5, 8.0, and 16.0 mmol/L groups ($P<0.05$). SREBP1 gene expression in the 1.0, 2.0, and 4.0 mmol/L groups was significantly higher than in all other groups ($P<0.05$). AGPAT6 and GPAM expression decreased significantly in a linear manner with increasing Lys concentration ($P=0.038$ and $P=0.022$). AGPAT6 expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups was significantly lower than in the 0.5 and 1.0 mmol/L groups ($P<0.05$). GPAM expression in the 16.0 mmol/L group was significantly lower than in the 0.5, 1.0, 2.0, and 4.0 mmol/L groups ($P<0.05$). LPIN1 expression was higher in the 1.0, 2.0, 4.0, and 8.0 mmol/L groups, significantly exceeding the 0.5 mmol/L group ($P<0.05$). BTN1A1 and XDH expression were also higher in the 1.0, 2.0, 4.0, and 8.0 mmol/L groups, significantly higher than in all other groups ($P<0.05$).

2.2 Effects of Lys on PPAR and SREBP1 Protein Expression in BMECs

As shown in Table 3 and Figure 1 [Figure 1: see original paper], PPAR protein expression in the 1.0, 2.0, and 4.0 mmol/L groups was significantly higher than in all other groups ($P<0.05$). SREBP1 protein expression in the 1.0 mmol/L group was significantly higher than in all other groups ($P<0.05$).

Discussion

Milk fat is a crucial indicator of milk quality, with approximately 98% of milk fat composed of TAG. Medium- and short-chain fatty acids (SMCFA) account for over two-thirds of milk fat content. About 50% of C16:0 fatty acid and nearly all C4:0–C14:0 fatty acids in bovine milk are synthesized de novo in the mammary gland from precursors such as acetate, -hydroxybutyrate, and propionate. TAG is synthesized on the surface of rough endoplasmic reticulum membranes and accumulates as lipid droplets, which are then secreted from the cell after being enveloped by the plasma membrane. Therefore, TAG content and lipid droplet accumulation in BMECs directly reflect milk fat synthesis. Amino acids not only affect milk protein synthesis but also influence milk fat synthesis. Li reported that essential amino acids significantly promoted milk protein synthesis in BMECs and may regulate milk fat synthesis through SREBP1 and PPAR . However, the effects of Lys on TAG synthesis in BMECs and its underlying mechanisms remain unreported. Our results showed that TAG content increased significantly in a quadratic pattern with increasing Lys concentration, indicating a dose-dependent relationship, though the mechanism remains unclear and requires further investigation.

Genes involved in de novo synthesis, long-chain fatty acid (LCFA) uptake and transport, and lipid droplet formation all affect TAG synthesis. The primary genes for LCFA uptake in mammalian tissues are LPL and FABP3. LPL catalyzes the hydrolysis of TAG into fatty acids and glycerol for energy supply and storage. FABP3 is involved in intracellular LCFA transport, shuttling LCFA from the cell membrane to sites of TAG and phospholipid synthesis. Zhao et al. demonstrated that appropriate methionine concentrations significantly promoted FABP3 and LPL expression in BMECs, suggesting that methionine enhances LCFA uptake and transport. Our results showed that FABP3 expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups and LPL expression in the 1.0, 2.0, 4.0, 8.0, and 16.0 mmol/L groups were significantly higher than in the 0.5 mmol/L group, with both genes showing significant or marginally significant linear increases with Lys concentration. This suggests that Lys may promote LCFA uptake and transport in BMECs, indicating a dose-dependent effect on milk fat synthesis.

FASN is a multifunctional enzyme system that participates in the entire fatty acid synthesis process and represents a key gene in the de novo synthesis of milk fatty acids in dairy cows. The synthesis of SMCFA (C4–C16) in lactating cow mammary glands is regulated by FASN-encoded proteins, which catalyze the conversion of acetyl-CoA and malonyl-CoA into LCFA. Stearoyl-CoA desaturase (SCD) is a primary Δ9-desaturase and the rate-limiting enzyme for monounsaturated fatty acid synthesis in cells. Li et al. found that supplementation with essential amino acid mixtures containing Lys upregulated LPIN1, FASN, and SCD expression in BMECs. Our results demonstrated that Lys promoted FASN expression in a significant dose-dependent manner, peaking at 2.0 mmol/L, which was significantly higher than the 16.0 mmol/L group. Lys also promoted SCD1 expression, with the 2.0 and 4.0 mmol/L groups significantly higher than other groups. These findings partially explain the promoting effect of Lys on milk fat synthesis.

Furthermore, GPAM, AGPAT6, and LPIN1 catalyze TAG synthesis and are key enzymes in milk fat synthesis. GPAM catalyzes the binding of acyl-CoA to the sn-1 position of glycerol-3-phosphate to form lysophosphatidic acid. AGPAT6 catalyzes the binding of a second acyl-CoA to the sn-2 position to form phosphatidic acid. LPIN transfers phosphate groups to convert phosphatidic acid to diacylglycerol. Studies show that knockout of AGPAT6 in lactating mice abolishes milk fat synthesis. Our results revealed that Lys had significant dose-dependent effects on GPAM and AGPAT6 expression, with increasing Lys concentrations inhibiting their expression. AGPAT6 expression in the 2.0, 4.0, 8.0, and 16.0 mmol/L groups was significantly lower than in the 0.5 and 1.0 mmol/L groups, while GPAM expression in the 16.0 mmol/L group was significantly lower than in the 0.5, 1.0, 2.0, and 4.0 mmol/L groups. Concurrently, Lys significantly promoted LPIN1 expression, with higher levels observed in the 1.0, 2.0, 4.0, and 8.0 mmol/L groups. These results indicate that high Lys concentrations inhibit the expression of genes related to milk fat synthesis, further explaining the dose-dependent effect of Lys on milk fat synthesis.

BTN1A1 and XDH are major proteins regulating lipid droplet formation. Sterol regulatory element-binding proteins (SREBP) belong to a family of nuclear transcription factors, while peroxisome proliferator-activated receptors (PPAR) are ligand-activated receptors in the nuclear hormone receptor family. PPAR can regulate SREBP1 expression, and genes such as LPL and ACACA are target genes of PPAR . Kadegowda et al. reported that treatment of BMECs with PPAR activators upregulated ACACA, FASN, SREBF1, SCD, and LPIN1 expression. Our results showed that BTN1A1 and XDH expression were higher in the 1.0, 2.0, 4.0, and 8.0 mmol/L groups. Both gene and protein expression of PPAR were significantly higher in the 2.0 and 4.0 mmol/L groups compared to the 0.5, 8.0, and 16.0 mmol/L groups. SREBP1 gene expression was higher in the 1.0, 2.0, and 4.0 mmol/L groups, while protein expression was higher in the 1.0 and 2.0 mmol/L groups. These findings further explain the promoting effect of Lys on milk fat synthesis from the perspectives of transcription factors and lipid droplet formation. Although FABP3, LPL, FASN, GPAM, and AGPAT6 are target genes of PPAR and SREBP1, this study only examined the protein expression of PPAR and SREBP1. Future studies should investigate the protein expression of FABP3, LPL, FASN, GPAM, and AGPAT6 to better elucidate the mechanisms of Lys action on milk fat synthesis.

Sheng reported that rapamycin inhibition of the mTOR signaling pathway significantly decreased SREBP1, PPAR , ACACA, and SCD1 expression. Soliman et al. obtained similar results, showing that rapamycin inhibited SREBP1 and its target genes ACACA, FASN, and SCD1 in human mammary explants, demonstrating that the mTOR signaling pathway regulates milk fat synthesis through transcription factors SREBP1 and PPAR . Whether Lys indirectly regulates milk fat synthesis through the mTOR signaling pathway remains unreported, and this experiment did not examine mTOR pathway-related genes, necessitating further investigation.

Considering the results on fatty acid uptake and transport, de novo synthesis, TAG synthesis, and lipid droplet formation, Lys demonstrates a dose-dependent promoting effect on milk fat synthesis in BMECs, with an optimal concentration of 2.0–4.0 mmol/L. However, research on Lys supplementation and its regulatory mechanisms in dairy cow milk fat synthesis is limited, and our findings require further validation in vivo. Therefore, additional in-depth studies are warranted.

Conclusions

1. Lys significantly promotes milk fat synthesis in BMECs, but high concentrations inhibit the expression of genes related to milk fat synthesis.
2. Under the conditions of this experiment, the optimal Lys concentration in the culture medium is 2.0–4.0 mmol/L.

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