Effects of Citric Acid on Triglyceride and Metabolite Content in Mouse Adipocytes

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Abstract

This study aimed to investigate the effects of citric acid on triglyceride (TG) and its metabolite contents in mouse adipocytes. Mouse 3T3-L1 preadipocytes were induced into mature adipocytes as the experimental model. On day 14 of induction culture, cells were cultured in complete medium containing 0 (control group), 20 (experimental group I), 50 (experimental group II), or 200 mol/L citric acid (experimental group III), and cells were collected at 0, 36, and 72 h to detect the contents of key rate-limiting enzymes and metabolites in TG synthesis and catabolism. The results showed that: at 36 and 72 h, TG contents in experimental groups I, II, and III were significantly higher than that in the control group ($P < 0.05$); the contents of fructose-1,6-bisphosphate aldolase (FDA), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS) in all experimental groups were significantly or extremely significantly higher than those in the control group ($P < 0.05$ or $P < 0.01$); the contents of hormone-sensitive lipase (HSL), carnitine palmitoyltransferase 1 (CPT1), free fatty acid (FFA), and acetyl-CoA in all experimental groups were extremely significantly lower than those in the control group ($P < 0.01$). These results suggest that citric acid can promote TG synthesis and deposition in mouse adipocytes, significantly or extremely significantly increase the contents of FDA, ACC, and FAS products in TG anabolic metabolism, and extremely significantly decrease the contents of HSL, CPT1, FFA, and acetyl-CoA products in TG catabolic metabolism, with 20 mol/L being the optimal dose.

Full Text

Effects of Citric Acid on Contents of Triglyceride and Its Metabolic Intermediates in Mouse Adipocytes

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Abstract

This study investigated the effects of citric acid on triglyceride (TG) content and its metabolites in mouse adipocytes. Mouse 3T3-L1 preadipocytes were induced into mature adipocytes and used as the experimental model. On day 14 of induction, cells were cultured in complete medium supplemented with 0 (control group), 20 (test group I), 50 (test group II), or 200 mol/L citric acid (test group III). Cells were collected at 0, 36, and 72 hours to measure the contents of key rate-limiting enzymes and metabolites in TG synthesis and catabolism. The results showed that at 36 and 72 hours, TG content in all test groups was significantly higher than in the control group ($P < 0.05$). The contents of fructose-bisphosphate aldolase (FDA), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS) in test groups were significantly or extremely significantly higher than in the control group ($P < 0.05$ or $P < 0.01$). Conversely, the contents of hormone-sensitive lipase (HSL), carnitine palmitoyltransferase 1 (CPT1), free fatty acids (FFA), and acetyl-CoA in test groups were extremely significantly lower than in the control group ($P < 0.01$). These findings indicate that citric acid promotes TG synthesis and deposition in mouse adipocytes, significantly or extremely significantly increases the contents of FDA, ACC, and FAS in TG anabolism, and extremely significantly decreases the contents of HSL, CPT1, FFA, and acetyl-CoA in TG catabolism, with 20 mol/L being the optimal dose.

Keywords: citric acid; mouse adipocytes; triglyceride; lipid metabolism

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Intramuscular fat content and accumulation create the marbling pattern in muscle, which influences and determines meat quality. In animals, fat deposition occurs sequentially: first subcutaneous, then visceral, and finally intermuscular. This sequence necessitates regulation of lipid metabolism to maximize fat synthesis and deposition throughout the body, thereby achieving intermuscular fat deposition. As a key product of lipid metabolism, citric acid plays an important regulatory role. High concentrations of citric acid can activate ATP-citrate lyase, producing large amounts of acetyl-CoA, which together with citrate activates acetyl-CoA carboxylase to promote malonyl-CoA synthesis and consequently fatty acid synthesis [1]. Zhao [2] found that citrate, catalyzed by ATP-citrate lyase to produce oxaloacetate and acetyl-CoA, plays an important regulatory role in fungal lipid accumulation. Yang et al. [3] reported that citric acid promotes the tricarboxylic acid cycle in yeast, which directly affects lipid metabolism. Tian [4] demonstrated that citrate synthase gene is significantly correlated with beef fat traits, content, distribution, and quality. However, do-

mestic research on the effects of citric acid on adipocyte lipid metabolism is lacking, and the regulatory mechanisms remain to be elucidated. This study investigated the effects of different citric acid doses on mouse lipid metabolism to promote fat synthesis and deposition. Using enzyme-linked immunosorbent assay (ELISA) double-antibody sandwich method, we measured key rate-limiting enzymes and metabolites generated during fat synthesis and catabolism at the cellular and molecular level to elucidate the overall effects and regulatory mechanisms of citric acid on lipid metabolism, providing new insights and theoretical basis for regulating lipid metabolism and laying a foundation for improving meat quality through intramuscular fat deposition.

1. Materials and Methods

1.1 Experimental Design

The experiment was conducted from October 2016 to September 2017 at the Xinjiang Key Laboratory of Meat & Milk Production Herbivore Nutrition. A single-factor design was employed with different citric acid doses added to cell culture medium. Test groups received 20 (test group I), 50 (test group II), or 200 mol/L citric acid (test group III), while the control group (CK) received no citric acid. Cells and culture medium were collected at 0, 36, and 72 hours for testing. Each group had three parallel samples, with each sample repeated three times.

1.2 Experimental Materials

Mouse preadipocytes (3T3-L1 cells) were purchased from Shanghai Saili Biotechnology Co. Citric acid (CA), DMEM high-glucose medium, fetal bovine serum (FBS), ethylenediaminetetraacetic acid (EDTA), phosphate-buffered saline (PBS), penicillin-streptomycin, 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), and insulin (INS) were purchased from GIBCO (USA). All ELISA kits were produced by Shanghai Meixuan Biotechnology Co.

1.3 Experimental Procedures

1.3.1 Reagent Preparation Complete medium consisted of 89% DMEM high-glucose medium, 10% FBS, and 1% penicillin-streptomycin solution. Induction solution I was prepared by adding 0.5 mmol/L IBMX, 1 mol/L DEX, and 5 mg/L INS to complete medium. Induction solution II was prepared by adding 5 mg/L INS to complete medium.

1.3.2 Cell Culture Following the method of Guo et al. [5], mouse preadipocytes were cultured in complete medium with 10% FBS at 37°C with 5% CO in saturated humidity. When cells reached approximately 80% confluence, they were passaged into culture plates. At 80% confluence, induction was initiated with induction solution I for 2 days, followed by induction solution II for 2 days. On day 14 after changing to complete medium, cells were cultured

with complete medium containing citric acid at the designed doses. Cells were collected at 0, 36, and 72 hours for subsequent detection.

1.3.3 Cell Morphology Observation Cell morphology was observed using an optical microscope after oil red O staining.

1.3.4 Determination of Metabolites and Enzyme Contents All metabolite and enzyme contents were determined using the double-antibody sandwich method described by Yan et al. [6]. Collected cells were frozen and sent to Shanghai Saili Biotechnology Co. for detection.

1.4 Statistical Analysis

Data are expressed as means ± standard deviation (SD). One-way ANOVA was performed using SPSS 22.0, followed by LSD multiple comparison tests.

2. Results

2.1 Effects of Citric Acid on Lipid Droplet Morphology in Mouse Adipocytes

Lipid droplet morphology in mouse adipocytes under different citric acid concentrations is shown in [Figure 1: see original paper] (a: control group; b: test group I; c: test group II; d: test group III).

Fig. 1 Effects of citric acid on lipid droplet morphology of mouse adipocytes (72 hours, oil red O staining, 400×)

2.2 Effects of Citric Acid on Key Rate-Limiting Enzyme Contents in TG Synthesis

As shown in , at 36 and 72 hours, FDA content in test group I was significantly higher than in the control group ($P < 0.05$), while FDA content in test groups II and III was extremely significantly higher than in the control group ($P < 0.01$). No significant differences were observed between test groups and the control group at 0, 36, or 72 hours in the contents of fructose-6-phosphate kinase (6-PFK), glucose-6-phosphate dehydrogenase (G6PD), pyruvate dehydrogenase E1 (PDHE1), dihydrolipoamide acetyltransferase (DLAT), or DGAT2 ($P > 0.05$). At 36 and 72 hours, the contents of dihydrolipoamide dehydrogenase (DLD), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS) in all test groups were extremely significantly higher than in the control group ($P < 0.01$).

Table 1 Effects of citric acid on key rate-limiting enzyme contents in TG synthesis of mouse adipocytes

Note: In the same column, values with different capital letter superscripts indicate extremely significant difference ($P < 0.01$), different small letter super-

scripts indicate significant difference ($P < 0.05$), and same or no letter superscripts indicate no significant difference ($P > 0.05$). The same applies below.

2.3 Effects of Citric Acid on Key Rate-Limiting Enzyme Contents in TG Catabolism

As shown in , at 36 and 72 hours, HSL content in all test groups was extremely significantly lower than in the control group ($P < 0.01$), with no significant differences among test groups ($P > 0.05$). No significant differences were observed in triglyceride hydrolase (TGH) content between test groups and the control group at 0, 36, or 72 hours ($P > 0.05$). At 0 hour, no significant difference was found in carnitine palmitoyltransferase 1 (CPT1) content between test groups and the control group ($P > 0.05$). However, at 36 and 72 hours, CPT1 content in all test groups was extremely significantly lower than in the control group ($P < 0.01$), with no significant differences among test groups ($P > 0.05$).

Table 2 Effects of citric acid on key rate-limiting enzyme contents in TG catabolism of mouse adipocytes

2.4 Effects of Citric Acid on TG Metabolite Contents

As shown in , at 0 hour, no significant differences were observed in TG content between test groups and the control group ($P > 0.05$). However, at 36 and 72 hours, TG content in all test groups was significantly higher than in the control group ($P < 0.05$). No significant differences were found in diglyceride (DG) content between test groups and the control group at any time point ($P > 0.05$). At 36 and 72 hours, free fatty acid (FFA) content in all test groups was extremely significantly lower than in the control group ($P < 0.01$), with no significant differences among test groups ($P > 0.05$). At 0 hour, no significant difference was observed in propionic acid (PA) content between test groups and the control group ($P > 0.05$), but at 36 and 72 hours, PA content in all test groups was significantly higher than in the control group ($P < 0.05$). At 36 and 72 hours, acetyl-CoA content in all test groups was extremely significantly lower than in the control group ($P < 0.01$), with no significant differences among test groups ($P > 0.05$).

Table 3 Effect of citric acid on the contents of TG metabolites of mouse adipocytes

3. Discussion

3.1 Effects of Citric Acid on Key Rate-Limiting Enzyme Contents in TG Synthesis

6-PFK, G6PD, and FDA are key rate-limiting enzymes in glycolysis during lipid metabolism that catalyze glucose metabolism to pyruvate. In this study, although 6-PFK and G6PD contents in citric acid-treated groups were higher than in the control group, the differences were not significant. However, at

36 and 72 hours, FDA content in test group I was significantly higher than in the control group, while test groups II and III showed extremely significant increases. Khu et al. [7] found that increased G6PD and FDA contents in glucose metabolism can promote cellular glucose utilization and increase fat synthesis and deposition. Men et al. [8] also confirmed that elevated FDA expression significantly increases TG synthesis and deposition, consistent with our results. This indicates that citric acid at different doses can promote glycolysis in mouse adipocytes, providing more substrates for TG synthesis, which is further supported by the significantly higher PA content in all test groups.

PDHE1, DLAT, and DLD are collectively known as the pyruvate dehydrogenase complex, a key enzyme system that allows pyruvate to enter mitochondria for decarboxylation to produce the lipogenic substrate acetyl-CoA. DLAT and DLD play major regulatory roles in this complex, modulating its activity through phosphorylation and dephosphorylation. In this study, different citric acid doses had little effect on PDHE1 and DLAT contents, but DLD content was extremely significantly increased at 36 and 72 hours. Cheng et al. [9] found that increased pyruvate dehydrogenase complex activity enhances fat synthesis, while its inhibition reduces fat synthesis, similar to our results. This suggests that citric acid can increase pyruvate dehydrogenase complex activity, promoting pyruvate metabolism and providing more acetyl-CoA for TG synthesis.

ACC is a crucial rate-limiting enzyme for fatty acid synthesis from acetyl-CoA in cells, and citrate is its activator that catalyzes acetyl-CoA to malonyl-CoA for fat synthesis. In this study, different citric acid doses extremely significantly increased ACC content without significant differences among test groups. Mao et al. [10] and Barber et al. [11] confirmed that increased ACC activity promotes cellular TG synthesis and deposition, consistent with our findings.

FAS is a multi-enzyme complex that catalyzes de novo fatty acid synthesis. Our results showed that different citric acid doses extremely significantly increased FAS content without significant differences among test groups. Dentin et al. [12] demonstrated that FAS activity directly controls the rate of fat synthesis, and its increased gene expression significantly enhances TG deposition, fully supporting our results.

3.2 Effects of Citric Acid on Key Rate-Limiting Enzyme Contents in TG Catabolism

TG exists in a dynamic balance between synthesis and catabolism. HSL plays a decisive role in fat decomposition as the most critical rate-limiting enzyme. In this study, HSL content in all citric acid-treated groups was extremely significantly lower than in the control group, with no significant differences among test groups. This is consistent with findings by Lorente-Cebrián et al. [13] and Chong et al. [14], indicating that citric acid can extremely significantly reduce HSL content and activity, thereby inhibiting intracellular TG decomposition.

TGH is present in the cytoplasm, lipid droplets, and cell membrane, and is an

important lipase catalyzing TG hydrolysis. Cornaciu et al. [15], Chakrabarti et al. [16], and Serr et al. [17] found that TGH expression inhibition promotes TG deposition. Yuan [18] reported that high-fat nutrition levels can inhibit fat deposition but do not significantly affect TGH expression or content, consistent with our results. This suggests that citric acid primarily inhibits TG decomposition by suppressing HSL synthesis rather than affecting TGH content, thereby increasing TG deposition in adipocytes.

CPT1 is the key rate-limiting enzyme that transports activated fatty acids (acyl-CoA) into mitochondria for oxidative decomposition. Our results showed that CPT1 content in all citric acid-treated groups was extremely significantly lower than in the control group at 36 and 72 hours, with no significant differences among test groups. Dong [19] and Abu-Elheiga et al. [20] confirmed that inhibiting CPT1 expression and synthesis can suppress fat oxidation and increase fat deposition, consistent with our findings. This demonstrates that citric acid can inhibit CPT1 content in adipocytes, suppress fatty acid -oxidation, and thereby increase TG deposition.

3.3 Effects of Citric Acid on Key Metabolite Contents in TG Metabolism

In this study, TG content in all citric acid-treated groups was significantly higher than in the control group at 36 and 72 hours, with no significant differences among test groups, indicating that an appropriate dose of citric acid can promote TG synthesis and deposition in mouse adipocytes, with 20 mol/L being optimal. This occurs because: first, citric acid acts as an activator catalyzing acetyl-CoA to malonyl-CoA during fatty acid synthesis—more malonyl-CoA leads to more fatty acid and TG synthesis; second, citric acid participates in the citrate-pyruvate cycle, where glucose-derived pyruvate enters mitochondria and is converted to acetyl-CoA, which combines with citrate to cross the mitochondrial membrane into the cytosol for fatty acid and TG synthesis; third, as a key intermediate in glucose metabolism, citrate itself can be catalyzed by various enzymes to generate acetyl-CoA for fatty acid synthesis. Tong [21] reported that increased citrate synthase expression and citrate content can enhance fatty acid and fat synthesis, similar to our results.

DG and FFA are both TG synthesis precursors and catabolic products. In this study, FFA content in all citric acid-treated groups was extremely significantly lower than in the control group, consistent with Yang et al. [22] who significantly reduced FFA content by inhibiting TG decomposition with niacin. Since citric acid significantly reduced TG hydrolase HSL content, FFA content was consequently decreased.

PA is a key intermediate in lipid metabolism that, together with citrate, forms the citrate-pyruvate cycle to transport the lipogenic substrate acetyl-CoA. In this study, PA content in all test groups was higher than in the control group. Wang et al. [23] found that excess glucose induces expression of glucokinase,

pyruvate kinase, ACC, and FAS, thereby promoting fat synthesis.

Acetyl-CoA is the most critical intermediate in lipid metabolism, produced from glucose catabolism for fat anabolism. In this study, acetyl-CoA content in all test groups was extremely significantly lower than in the control group. Although our results show that citric acid promoted DLAT and DLD contents in the pyruvate dehydrogenase complex, enhancing pyruvate metabolism to generate more acetyl-CoA, the reduced acetyl-CoA content suggests that added citric acid better activated ACC, channeling more glycolysis-derived acetyl-CoA into fat synthesis. Wang et al. [24] found that increased ACC expression significantly promoted TG synthesis in goose adipocytes while reducing intracellular acetyl-CoA content, consistent with our results.

4. Conclusions

1. Citric acid promotes TG synthesis and inhibits TG catabolism in mouse adipocytes, increasing fat content and deposition, with 20 mol/L being the optimal dose.
2. Citric acid significantly or extremely significantly increases the contents of key rate-limiting enzymes FDA, ACC, and FAS in TG anabolism, while extremely significantly inhibiting the contents of key rate-limiting enzymes HSL and CPT1 in TG catabolism.
3. Citric acid significantly increases the content of the intermediate metabolite PA, while extremely significantly decreasing the contents of FFA and acetyl-CoA.

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