Effects of Ferulic Acid on Adipose Deposition and Fatty Acid Composition of Abdominal Fat in ob/ob Mice: Postprint

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Abstract

This study aimed to investigate the effects of ferulic acid on fat deposition and abdominal fatty acid composition in ob/ob mice. Thirty male ob/ob mice at 5 weeks of age were randomly divided into three groups (n=10) and fed experimental diets supplemented with 0 (control group), 0.25%, and 0.50% ferulic acid in the basal diet for a 9-week experimental period. The results demonstrated that, compared with the control group, dietary supplementation with 0.25% and 0.50% ferulic acid significantly reduced total weight gain and abdominal fat percentage ($P<0.05$), significantly decreased serum triglyceride levels as well as hepatic triglyceride and total cholesterol contents ($P<0.05$), attenuated hepatic lipid droplet accumulation, significantly lowered the contents of palmitoleic acid and oleic acid in abdominal fat ($P<0.05$), and significantly decreased the fatty acid saturation indices of palmitoleic acid/palmitic acid and oleic acid/stearic acid in abdominal fat ($P<0.05$). It was concluded that ferulic acid can inhibit fat deposition in ob/ob mice, improve the fatty acid composition of abdominal fat, and exhibits notable weight-reducing and lipid-lowering effects.

Full Text

Effects of Ferulic Acid on Lipid Deposition and Fatty Acid Composition in Abdominal Lipid of ob/ob Mice

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Abstract: This experiment was conducted to investigate the effects of ferulic acid (FA) on lipid deposition and fatty acid composition in abdominal lipid of ob/ob mice. Thirty male ob/ob mice at 5 weeks of age were randomly divided into three groups (n=10) and fed experimental diets supplemented with 0 (control), 0.25%, or 0.50% FA for 9 weeks. The results demonstrated that dietary supplementation with 0.25% and 0.50% FA significantly reduced total weight gain and abdominal fat percentage ($P<0.05$), significantly decreased serum triglyceride levels as well as hepatic triglyceride and total cholesterol levels ($P<0.05$), attenuated hepatic lipid droplet accumulation, significantly suppressed the contents of palmitoleic acid and oleic acid in abdominal adipose tissue ($P<0.05$), and significantly reduced the fatty acid desaturation indexes of palmitoleic acid/palmitic acid and oleic acid/stearic acid ($P<0.05$). It is concluded that FA can inhibit lipid deposition and improve the fatty acid composition of abdominal adipose tissue in ob/ob mice, exhibiting pronounced weight-reducing and lipid-lowering effects.

Keywords: ferulic acid; ob/ob mice; obesity; lipid deposition; fatty acid composition

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Ferulic acid (FA), chemically known as 4-hydroxy-3-methoxycinnamic acid, is a phenolic compound widely distributed in traditional Chinese medicines, cereals, vegetables, and fruits. It is particularly abundant in cereal bran, with concentrations reaching 1.252 g/kg in whole wheat flour and as high as 2.317 g/kg in corn meal. Polyphenolic compounds such as apple polyphenols, tea polyphenols, and grape seed polyphenols generally exhibit strong antioxidant properties and exert regulatory effects on hyperlipidemia and diabetes. FA also demonstrates potent antioxidant activity and has therapeutic potential against Type II diabetes, hypertension, and cardiovascular diseases. Obesity is characterized by excessive fat accumulation in the body and represents a major risk factor for Type II diabetes, cardiovascular disease, and other metabolic disorders. Various polyphenols and their derivatives, including coffee polyphenols rich in FA, hop polyphenols, gallic acid, epigallocatechin, and epigallocatechin gallate, have been shown to inhibit fat accumulation. Son et al. reported that dietary supplementation with FA or its esterified form, γ-oryzanol, significantly suppressed weight gain in high-fat diet-induced obese mice, reducing total weight gain by 47.50% and 26.67%, respectively. Senaphan et al. demonstrated that oral administration of 30 or 60 mg/kg BW FA to rats ameliorated high-fat, high-carbohydrate diet-induced metabolic syndrome, reducing serum triglyceride (TG) and total cholesterol (TC) levels while decreasing body weight. However, these studies focused primarily on body weight and blood lipid parameters rather than systematic investigation of FA' s effects on lipid deposition and metabolism in obese organisms. The ob/ob mouse, characterized by leptin gene deficiency, develops severe obesity during youth and exhibits obesity-related metabolic disturbances including hyperlipidemia and hepatic lipid deposition. This study employed male ob/ob mice to investigate the effects of dietary FA supplementation at different con-

centrations (0.25% or 0.50%) on lipid deposition and fatty acid composition in abdominal adipose tissue, aiming to provide scientific evidence for the potential efficacy of FA in obesity prevention and treatment.

1.1 Materials and Instruments

FA standard (powder, purity $ $99%) was purchased from Shanghai Jingchun Biochemical Technology Co., Ltd. TG and TC assay kits were obtained from Nanjing Jiancheng Bioengineering Institute, fatty acid standards from Beijing Puxi Technology Co., Ltd., and all other routine reagents were of analytical grade. Major instruments included a Synergy™ HT microplate reader (BioTek, USA), Haier ultra-low temperature freezer (Qingdao Haier Special Appliance Co., Ltd.), Centrifuge-5810R high-speed refrigerated centrifuge (Eppendorf, Germany), Roche biochemical analyzer (Roche, Switzerland), Agilent 7890A gas chromatograph with flame ionization detector (FID) equipped with a DB-23 column (Agilent, USA), KD-BM biological tissue embedding machine, KD-3368AM microtome, and KEDEE biological tissue staining machine (Jinhua Kedi Instrument Equipment Co., Ltd.).

1.2 Experimental Animals and Diets

Thirty 5-week-old male ob/ob mice with similar body weights were purchased from Beijing Huafukang Biotechnology Co., Ltd. as specific pathogen-free (SPF) animals. The basal diet for ob/ob mice was provided by Nantong Trophic Animal Feed High-tech Co., Ltd. and formulated according to AIN-93M standard, with composition and nutrient levels shown in Table 1 . Mice were housed in SPF animal facilities (Academy of State Administration of Grain, Beijing) using individual ventilated cages (IVC) under controlled temperature (23$±$2°C), 50% relative humidity, and a 12 h/12 h light-dark cycle. Pair-feeding was implemented with ad libitum water access during a 1-week acclimation period.

1.3 Experimental Design and Sample Collection

The 30 ob/ob mice were randomly assigned to three groups (n=10), with each mouse housed separately. The control group (CON) received the basal diet, while the low-dose FA group (L-FA) and high-dose FA group (H-FA) received diets supplemented with 0.25% and 0.50% FA, respectively. Body weight and weekly feed intake were measured throughout the 9-week experimental period after a 12-hour fast.

Prior to sampling, mice were fasted for 12 hours and final body weights were recorded. Blood samples were collected via orbital enucleation, and serum was separated and stored at -80°C for subsequent analysis. Following blood collection, mice were euthanized by cervical dislocation, and liver and abdominal white adipose tissue (WAT) were dissected and weighed for calculation of liver index and abdominal fat percentage. Liver tissue sections were fixed in 10% formalin, while remaining liver and WAT samples were frozen at -80°C until

analysis. Liver index (%) = (liver weight/body weight) × 100; Abdominal fat percentage (%) = (abdominal WAT weight/body weight) × 100.

1.4 Biochemical and Histological Analyses

Serum TG, TC, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using a biochemical analyzer. Hepatic TG and TC levels were determined by enzyme-linked immunosorbent assay using commercial kits according to the manufacturer' s instructions. For histopathological examination, liver tissues fixed for 24 hours were embedded, sectioned (5 m thickness), stained with hematoxylin-eosin (HE), and observed under optical microscopy at 10$×$25 magnification to evaluate lipid droplet size and density. Fatty acid composition of adipose tissue was analyzed according to GB/T 9695.7-2008. Lipids were extracted and methylated using the boron trifluoride method (GB/T 17367-2008), and fatty acid composition was determined by gas chromatography (GB/T 17377-2008). The analysis focused on palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1) contents (mass percentage), as well as the desaturation indexes C16:1/C16:0 and C18:1/C18:0.

1.5 Statistical Analysis

Data are presented as means ± standard deviation. One-way ANOVA was performed using SAS 9.0 statistical software, with $P<0.05$ considered statistically significant.

2.1 Effects of FA on Body Weight, Total Weight Gain, and Feed Intake

As shown in Figure 1 [Figure 1: see original paper], all groups exhibited stable weight gain during the initial period. No significant differences were observed among groups during the first four weeks ($P>0.05$). From weeks 5-6, weight gain in both L-FA and H-FA groups began to plateau. From week 7 until the end of the experiment, body weights in L-FA and H-FA groups were significantly lower than in the CON group ($P<0.05$). Table 2 reveals that total feed intake did not differ significantly among groups ($P>0.05$), while total weight gain in L-FA and H-FA groups was significantly reduced compared to CON ($P<0.05$).

2.2 Effects of FA on Liver Index and Abdominal Fat Percentage

Table 3 shows that liver weight and liver index did not differ significantly among groups ($P>0.05$), although both FA-supplemented groups exhibited a trend toward reduced liver weight ($P<0.10$). Dietary supplementation with 0.25% or 0.50% FA significantly decreased abdominal WAT weight and abdominal fat percentage compared to CON ($P<0.05$).

2.3 Effects of FA on Serum Lipid Profiles

As presented in Table 4 , FA supplementation at 0.25% or 0.50% did not significantly affect serum TC or LDL-C levels (P>0.05). However, serum TG levels were significantly reduced in both L-FA and H-FA groups compared to CON (P<0.05). Serum HDL-C levels were significantly elevated only in the H-FA group (P<0.05), with no significant difference observed between L-FA and CON groups (P>0.05).

2.4 Effects of FA on Hepatic Lipid Levels

Table 5 demonstrates that compared to CON, dietary FA at 0.25% and 0.50% reduced hepatic TG levels by 50.00% and 39.29%, respectively, and significantly decreased hepatic TC levels by 39.62% and 43.72% (P<0.05).

2.5 Hepatic Histopathological Analysis

Figure 2 [Figure 2: see original paper] illustrates that at 10$×$25 magnification, liver sections from CON mice displayed large, dense lipid droplets (Figure 2-A), whereas L-FA mice showed markedly reduced lipid droplets with most being smaller than those in CON (Figure 2-B). H-FA mice also exhibited significant improvements in lipid droplet size and density compared to CON (Figure 2-C).

2.6 Effects of FA on Abdominal Fatty Acid Composition and Desaturation Index

Table 6 shows that FA did not significantly affect C16:0 or C18:0 contents (P>0.05) but significantly suppressed C16:1 and C18:1 contents (P<0.05). The desaturation indexes C16:1/C16:0 and C18:1/C18:0 were significantly lower in both L-FA and H-FA groups compared to CON (P<0.05), with H-FA showing an even greater reduction in C18:1/C18:0 than L-FA (P<0.05).

Discussion

This study investigated whether dietary FA supplementation could ameliorate weight gain, lipid deposition, and metabolism in ob/ob mice through pair-feeding to ensure no significant differences in absolute total feed intake among groups. The average daily FA intake was 0 mg/d for CON, 7.6 mg/d for L-FA, and 15.5 mg/d for H-FA, establishing the intended dosage gradient. The results showed that L-FA and H-FA reduced total weight gain by 23.01% and 19.4%, respectively, compared to CON. These findings align with Son et al., who reported a 26.67% reduction in weight gain with 0.50% FA supplementation, but demonstrate that lower FA doses can also be effective. Similarly, 0.25% FA reduced fat mass by 24.66% and abdominal fat percentage by 18.58%, while H-FA reduced these parameters by 21.97% and 16.17%, respectively. Although liver index did not differ significantly among groups, both FA doses reduced

liver weight by 15.4%, suggesting that FA' s inhibitory effects on lipid deposition may primarily target adipose tissue. Relative to CON, hepatic TG levels decreased by 50.00% and 39.29% in L-FA and H-FA groups, respectively, while TC levels declined by 39.62% and 43.72%, consistent with the histological evidence of reduced hepatic lipid accumulation. These findings indicate that while 0.25% and 0.50% FA did not significantly reduce liver weight, they effectively inhibited hepatic lipid accumulation. Wang et al. reported that oral FA reduced hepatic TC but only tended to decrease TG in high-fat diet-induced obese rats, possibly due to differences in absorption efficiency between orally administered and diet-incorporated FA.

HDL-C promotes reverse cholesterol transport from serum. In this study, H-FA significantly elevated serum HDL-C without affecting serum TC, contrasting with Marimuthu et al., who found that oral FA increased HDL-C by 74.56% while decreasing serum TC by 35.37% in metabolic syndrome rats. These discrepancies may stem from differences in FA dosage and administration route. Serum TG levels in L-FA and H-FA groups were reduced by 10.56% and 6.11%, respectively, consistent with Marimuthu' s report that FA injection lowered serum and hepatic TG in hyperlipidemic rats.

Jeyakumar et al. observed elevated desaturation indexes C16:1/C16:0 and C18:1/C18:0 in diet-induced obese rats but did not examine FA' s effects on fatty acid composition. In the present study, L-FA significantly reduced these desaturation indexes by 15.59% and 8.09%, respectively, while H-FA decreased them by 20.18% and 13.62%. FA did not significantly affect C16:0 or C18:0 contents but reduced C16:1 and C18:1 levels, thereby lowering the desaturation indexes. Stearoyl-CoA desaturase (SCD) is an endoplasmic reticulum enzyme that catalyzes unsaturated fatty acid synthesis. SCD-deficient ob/ob mice exhibit reduced C16:1 and C18:1 contents and lower desaturation indexes. Therefore, FA may inhibit adipogenesis in ob/ob mice by downregulating SCD expression and reducing unsaturated fatty acid synthesis.

Under the conditions of this study, 0.25% FA was slightly more effective than 0.50% FA in improving total weight gain, abdominal fat, and serum and hepatic TG levels, whereas 0.50% FA showed better efficacy in reducing serum and hepatic TC levels. Thus, 0.25% FA appears optimal for weight and lipid reduction, while 0.50% may be more suitable for cholesterol lowering. Since phenolic acids like FA are abundant in daily diets—approaching 0.25% in coarse grains such as corn—these findings encourage increased consumption of FA-rich grains like corn, millet, and brown rice to enhance intake of these beneficial phytochemicals for obesity prevention.

In conclusion, dietary supplementation with 0.25% and 0.50% FA effectively inhibited weight gain, reduced adipose tissue mass and abdominal fat percentage, decreased blood and hepatic lipid levels, and ameliorated hepatic lipid accumulation in ob/ob mice. A dosage of 0.25% FA is recommended for weight and lipid reduction, while 0.50% may be more appropriate for cholesterol reduction.

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