

Emodin Improves Glucose and Lipid Metabolism Disorders in Obese Mice via Activating Brown Adipose Tissue and Inducing Browning of White Adipose Tissue

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Study protocol

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Abstract

Background: Obesity has become a worldwide health threat related to type 2 diabetes, hypertension, cardiovascular disease, etc. Activating brown adipocytes and inducing browning of white adipocytes has been proposed as a potential molecular target for obesity treatment. In the present study, we investigated the effects of emodin on browning in mice with high-fat diet (HFD) and explore its underlying pharmacological mechanisms.

Methods: The positive effects of emodin (40, 80 mg/kg/day, i.g. for 6 weeks) on lipid metabolism were evaluated in mice model of hyperlipidemia. Hyperlipidemia mice were induced by high-fat diet (60% of kilocalories from fat, 5.24 Kcal/kg) for 8 weeks. Body weight and food intake were monitored every week. After 6 weeks of treatment, fasting blood glucose, oral glucose tolerance, Lee's index, the ratio of fat weight to body weight, blood lipids, and adipose tissues morphology were assayed. Then uncoupling protein 1 (UCP1), CD36, fatty acid transporter 4 (FATP4), peroxisome proliferator activated receptor α (PPAR α) and prohibitin (PHB) protein of subcutaneous white adipose tissue (scWAT) and brown adipose tissue (BAT) were analyzed. In addition, the lipid metabolites in adipose tissues were analyzed by ultra-high-performance liquid chromatography with electrospray ionization tandem mass spectrometry.

Results: Emodin treatment decreased body weight gain, fasting blood glucose, Lee's index, the ratio of scWAT weight to body weight, and the levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) and Leptin in serum, and increased serum adiponectin content and improved glucose tolerance. Furthermore, emodin enhanced the expression of UCP1, CD36, FATP4, PPAR α and PHB protein in scWAT and BAT. Meanwhile, emodin can significantly up-regulated lipid levels in scWAT of mice fed with HFD such as PC(O-18:2/22:5), PE(O-18:1/18:2), PE(O-18:2/20:4), PE(O-20:1/20:5), Cer(d14:1/20:0) and SM(d18:0/23:0), and reduced the lipid levels such as PC(O-18:0/20:0), PE(O-18:2/22:2), PE(O-18:0/22:5). In addition, emodin significantly up-regulate lipid levels in BAT of mice fed with HFD such as PC(14:0/16:0), PC(16:0/16:1), PC(16:1/16:1), PC(15:1/18:3), PC(18:0/20:0), LysoPC(20:0), LysoPC(22:0) and LysoPC(22:1), and reduced the lipid levels PC(12:0/20:4) and PC(17:0/22:5).

Conclusions: These results indicated that hyperlipidemia could be alleviated by treatment of emodin via promoting browning of white adipose tissue. In addition, the disturbance of some small lipid metabolites in adipose also could be reversed by emodin.

Full Text

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Figures

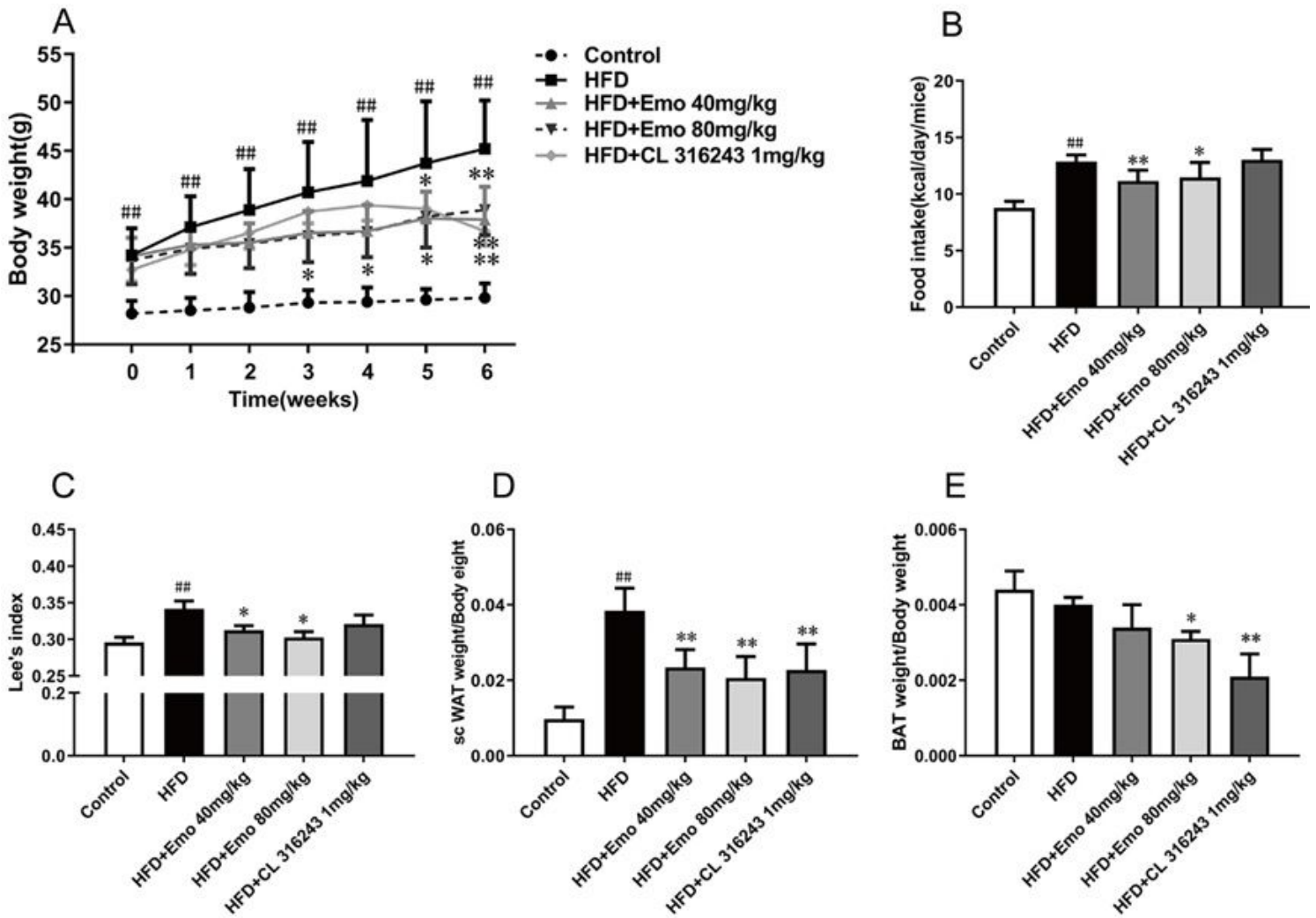


Figure 1

Effects of emodin on body weight, food intake and Lee's index in mice fed with HFD. HFD model mice were established by feeding with HFD (5.24Kcal/kg) for 8 weeks to induce obesity. Mice in both control and obesity model groups were given the same volume of 0.1% (w/v) CMC-Na. Emodin (40 or 80 mg/kg) was administrated to mice by oral gavage. Three days before the end of the experiment, CL 316243 (1mg/kg) was intraperitoneally injected. After 6 weeks treatment, the mice were subjected to different tests as indicated below. (A) Body weight and (B) food intake were recorded regularly during the treatment period. (C) Lee's index. (D) The ratio of scWAT/BW. (E) The ratio of BAT/BW. Data were shown as mean \pm SE, with n = 8. ##P < 0.01 vs. Control group, *P < 0.05, **P < 0.01 vs. HFD group.

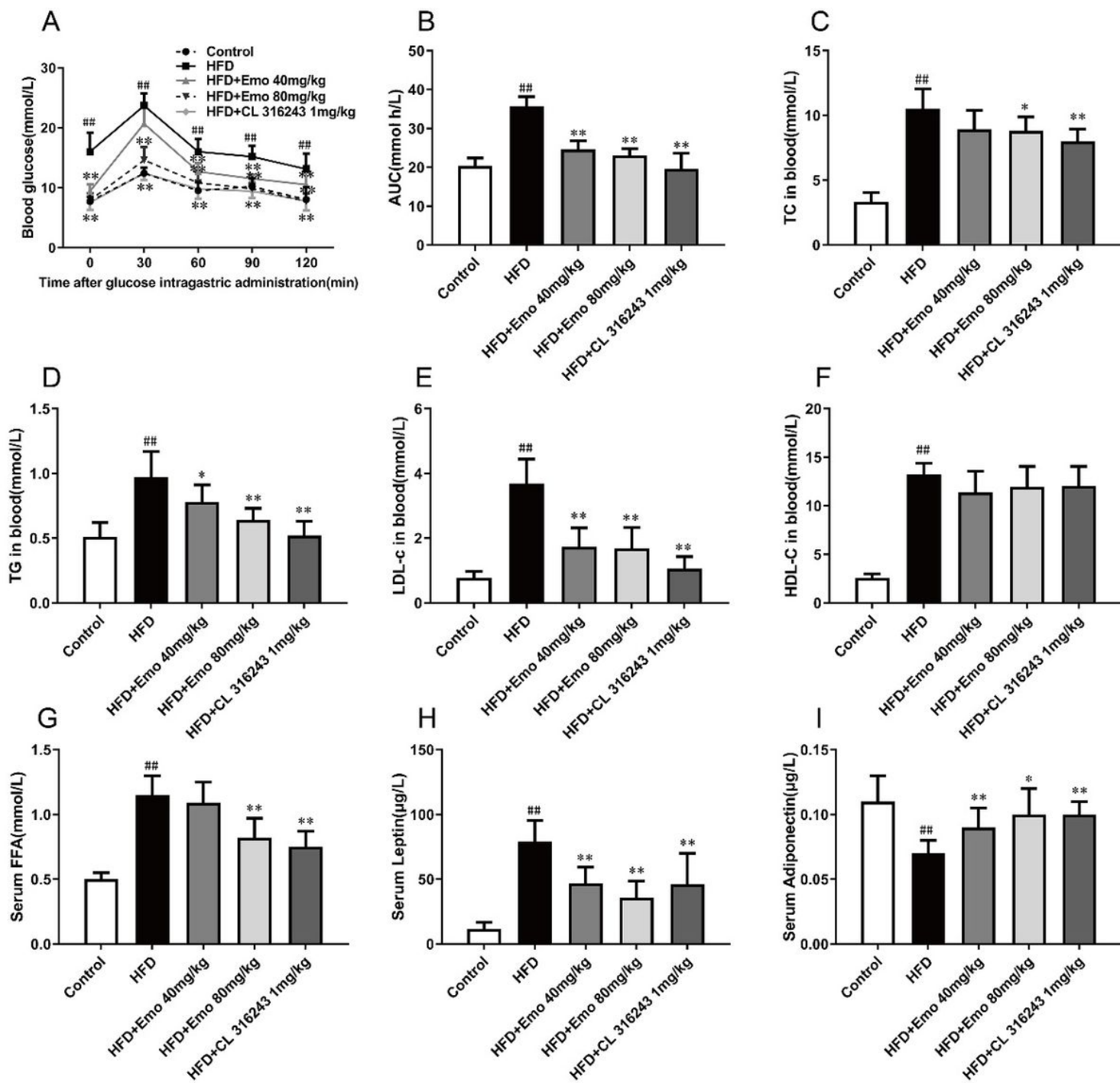


Figure 2

Effect of emodin on blood glucose in mice fed with HFD. (A) gavage glucose tolerance test (GTT). The mice were fasted for 12 h, then 2 g/kg glucose was fed by oral gavage. Glucose levels were tested at regular intervals of 0, 30, 60, 90, and 120 min. (B) Quantification of AUC from the GTT in (B). (C-F) Serum TC, TG, LDL-c, HDL-c content. (G) Serum FFA content. (H) Serum LEP content. (I) Serum ADPN content. Data were shown as mean \pm SE, with $n = 8$. $##P < 0.01$ vs. Control group, $*P < 0.05$, $**P < 0.01$ vs. HFD group.

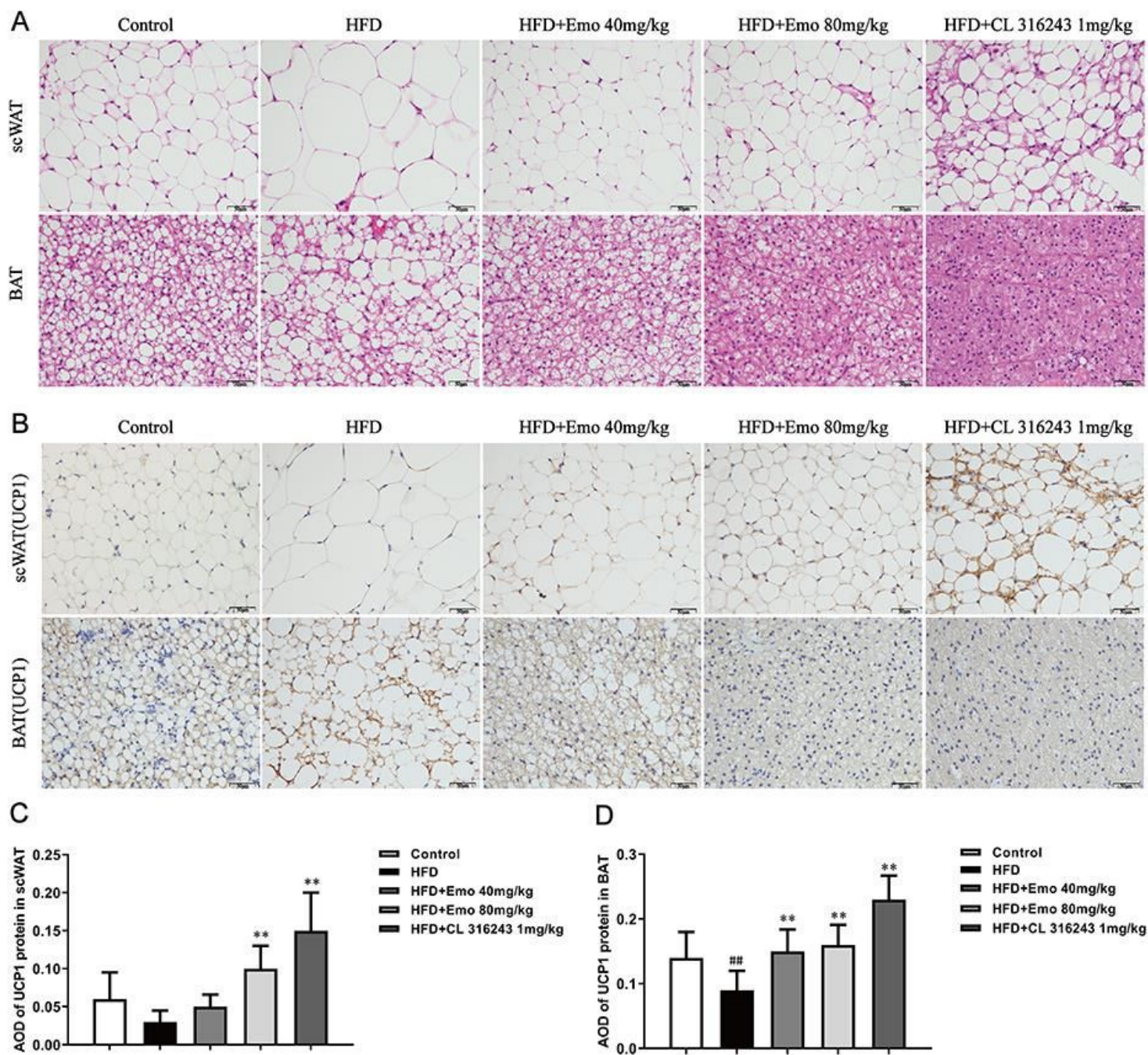


Figure 3

Effect of emodin on the morphology and function of scWAT and BAT. Three scWAT and BAT samples were randomly selected for each group for H&E staining and immunohistochemical staining (n=3/group). The following antibodies and concentrations were used in the IHC assay: UCP1 antibody (1:500, Abcam). Images were captured with a microscope (Leica, German). Images were captured with a microscope (Leica, German) with a ruler 50 μ m. At least three photos were taken from different areas of each section. (A) scWAT and BAT HE staining. (B) scWAT and BAT immunohistochemical staining. (C) Expression analysis of UCP1 protein in scWAT. (C) Expression analysis of UCP1 protein in BAT. Data were shown as mean \pm SE, with n =3. ##P < 0.01 vs. Control group, *P < 0.05, **P < 0.01 vs. HFD group.

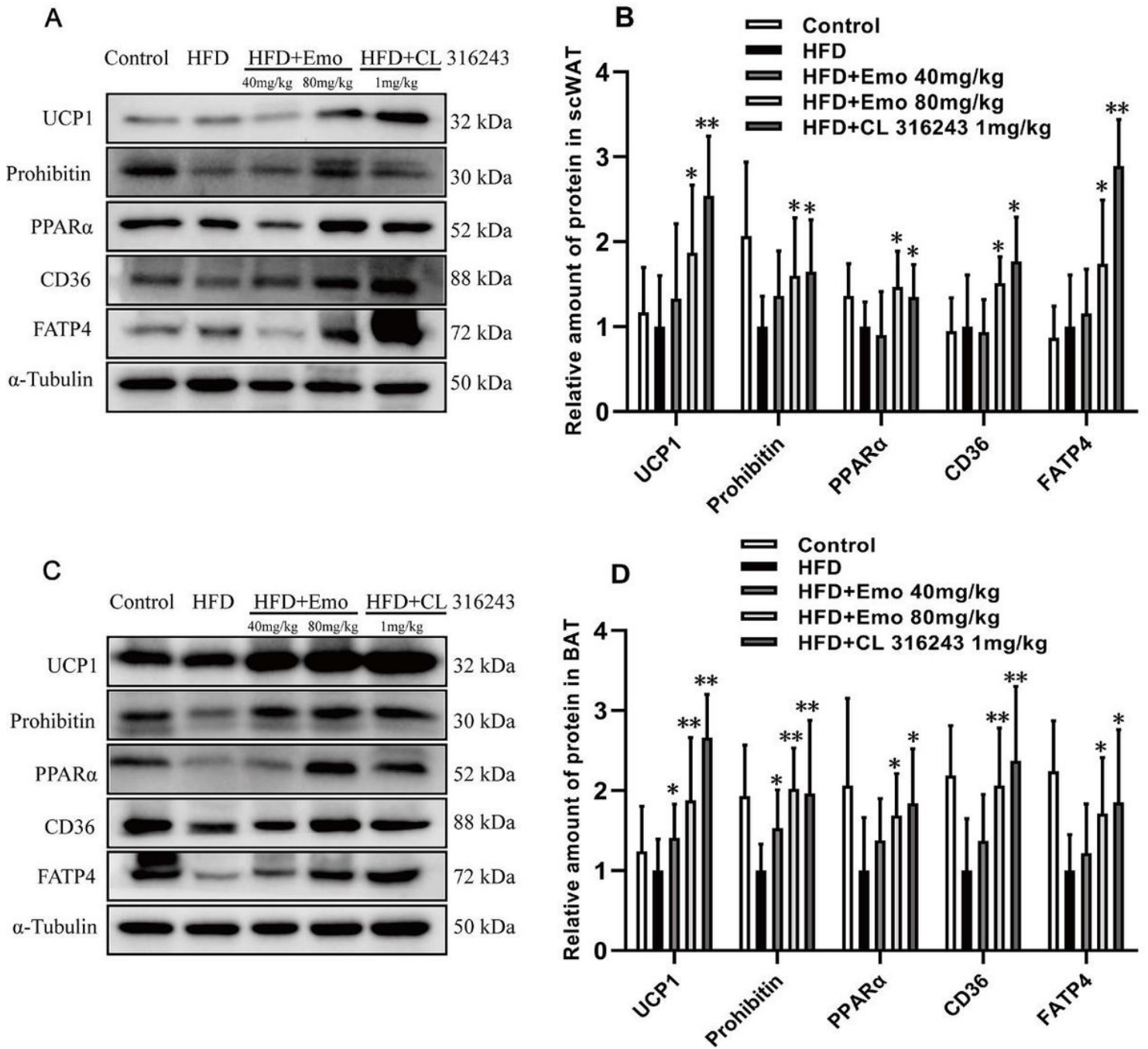


Figure 4

Effect of emodin on protein level of adipose tissue. Three scWAT and BAT samples were randomly selected from each group and immunoblotted with UCP1, Prohibitin, PPAR α , CD36 and FATP4 antibodies (n=3/group). (A) Western blot results of UCP1, Prohibitin, PPAR α , CD36 and 704 FATP4 proteins in scWAT. (B) Expression analysis of UCP1, Prohibitin, PPAR α , CD36 and FATP4 protein in scWAT. (C) Western blot results of UCP1, Prohibitin, PPAR α , CD36 and FATP4 proteins 706 in BAT. (D) Expression analysis of UCP1, Prohibitin, PPAR α , CD36 and FATP4 protein in BAT. Data were shown as mean \pm SE, with n =3. *P < 0.05, **P < 0.01 vs. HFD group.

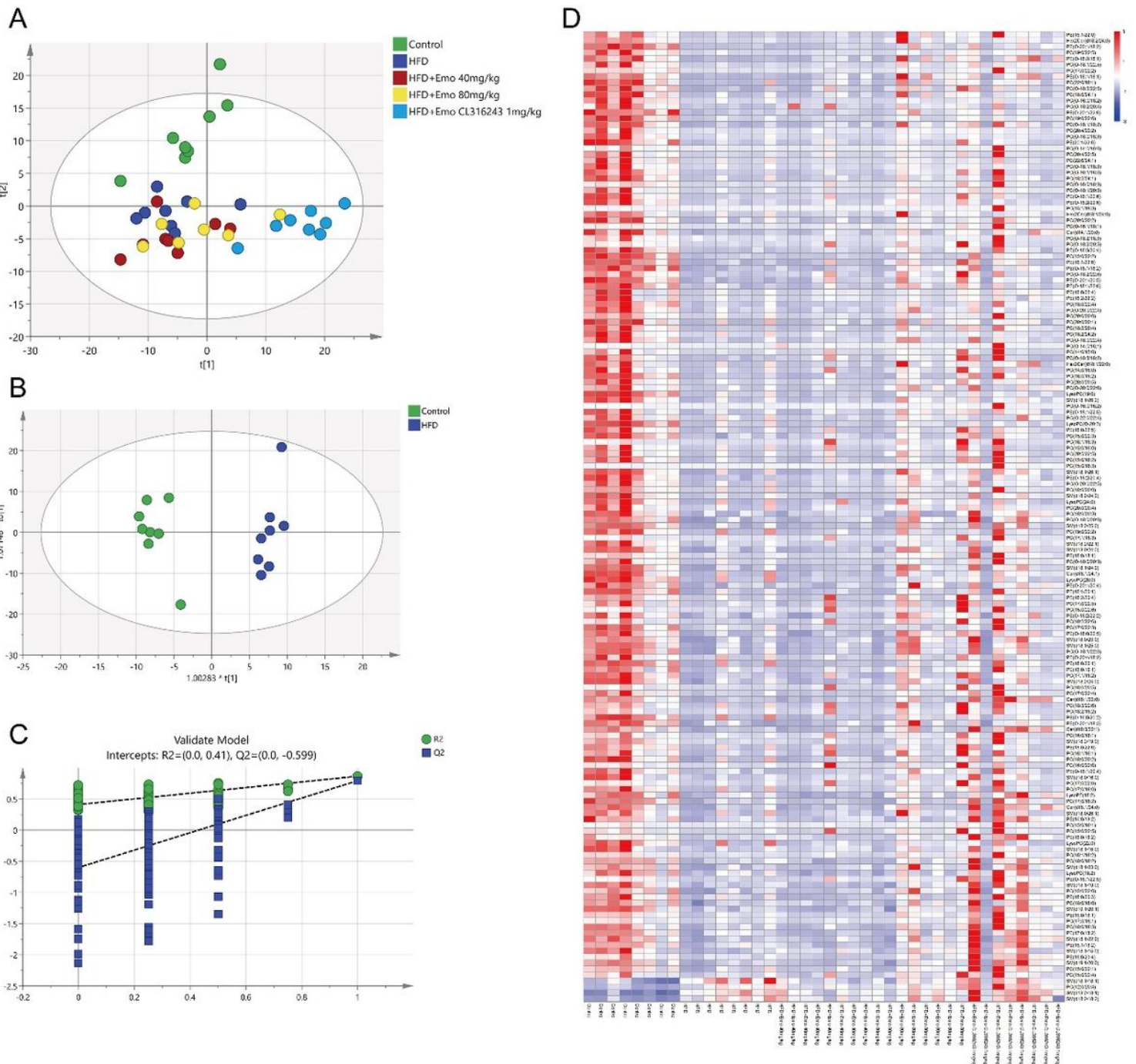


Figure 5

Effect of emodin on the types of phospholipids in scWAT of obese mice. (A) The PCA scores plot of five groups. (B) The orthogonal projection to latent structures discriminant analysis (OPLS-DA) scores scatter plot obtained from the diabetic cardiomyopathy (DCM) group vs control group. (C) Validation plot for OPLS-DA model. (D) The heat map showed the difference of lipid metabolism in scWAT of mice in normal diet group, HFD group, emodin (40 mg/kg, 80 mg/kg) treatment group and CL 316243 treatment group (n=8/group). Only metabolites with VIP >1 and P <0.05 were selected in heat map. Each column represents a sample. Each row represents the amount of lipid differential metabolites in different samples. Red represents the up-regulation of metabolites. The deeper the red represent the more

metabolite content. Blue represents the down-regulation of metabolites. The deeper the blue represent the less metabolite content. Phosphatidylcholine (PC); Phosphatidylethanolamine (PE); Sphingolipid (SM); Ceramides (Cer); Lyso-phosphatidylcholine (LPC); Lyso-phosphatidylethanolamine (LPE).

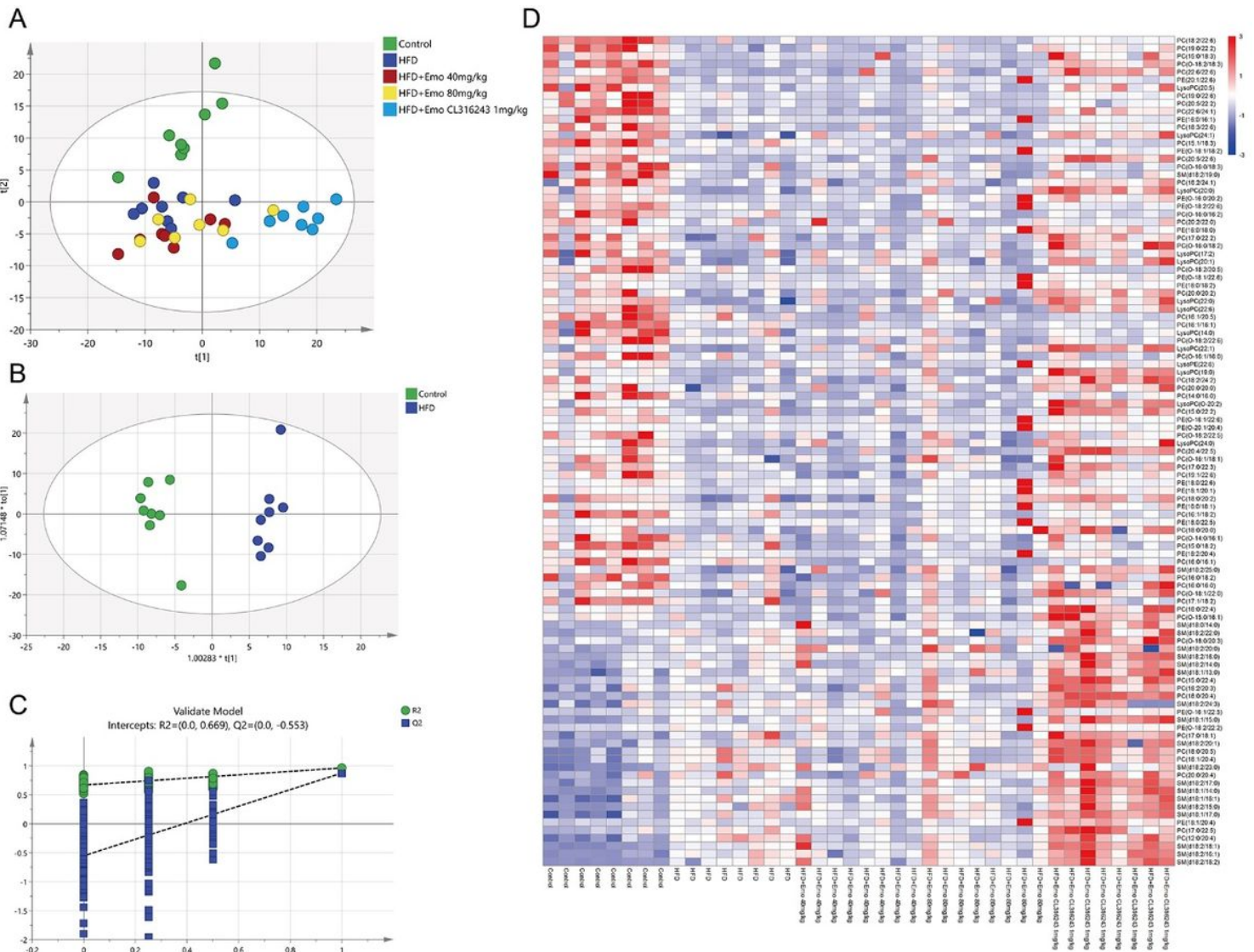


Figure 6

Effect of emodin on the types of phospholipids in BAT of obese mice. (A) The PCA scores plot of five groups. (B) The orthogonal projection to latent structures discriminant analysis (OPLS-DA) scores scatter plot obtained from the diabetic cardiomyopathy (DCM) group vs control group. (C) Validation plot for OPLS-DA model. (D) The heat map showed the difference of lipid metabolism in BAT of mice in normal diet group, HFD group, emodin (40 mg/kg, 80 mg/kg) treatment group and CL 316243 treatment group (n=8/group). Only metabolites with VIP >1 and P<0.05 were selected in heat map. Each column represents a sample. Each row represents the amount 733 of lipid differential metabolites in different samples. Red represents the up-regulation of metabolites. The deeper the red represent the more metabolite content. Blue represents the down-regulation of metabolites. The deeper the blue represent the less metabolite content. Phosphatidylcholine (PC); Phosphatidylethanolamine (PE); Sphingolipid (SM); Ceramides (Cer); Lyso-phosphatidylcholine (LPC); Lyso-phosphatidylethanolamine (LPE).