Lipids in Health and Disease

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Deoxycholic acid ameliorates obesity and insulin resistance by enhancing lipolysis and thermogenesis

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Abstract

Background Bile acids are essential for energy metabolism. Deoxycholic acid (DCA) in particular is associated with metabolic disorders such as type 2 diabetes mellitus (T2DM) and obesity. However, the direct effects of DCA on metabolism and body composition have yet to be studied in depth.

Methods Targeted metabolomics analysis of human feces was performed. C57BL/6J mice fed a high-fat diet (HFD) were gavaged with DCA, and the effects were measured by metabolic tolerance tests and metabolic cages. Body composition was evaluated by echoMRI. To evaluate the beneficial function of DCA on thermogenesis and lipolysis, histological staining and qPCR were carried out.

Results There was negative correlation between fecal DCA levels and serum glucose levels, as well as the Homeostatic Model Assessment for Insulin Resistance (HOMA) index in humans. Our findings confirmed that DCA could ameliorate glucose metabolism and insulin sensitivity in mice fed with HFD. DCA supplementation alleviated HFD-induced obesity and decreased the fat mass significantly by promoting lipolysis. Moreover, DCA significantly enhanced energy expenditure and thermogenesis in brown adipose tissue in mice with obesity induced by HFD.

Conclusions Based on the results of our mouse model, DCA may have applications in alleviating obesity and its related metabolic disorders in humans.

Keywords DCA, Bile acid, Energy metabolism, Body composition

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Background

Obesity is a prevalent global problem in the worldwide nowadays [1]. Moreover, patients with overweight are more likely to develop diabetes, cardiovascular diseases and cancers [2]. Although multiple weight loss strategies can be implemented for weight management, their effectiveness is often limited due to undesirable side effects [3]. Therefore, there is a medical necessity that can satisfy the large population of obese patients.

The increasing trend in global obesity has been attributed to the imbalance between energy expenditure and food intake that causes the storage of redundant fat mass. Adipose tissues can play a major role in the regulation of systematic energy levels [4]. White adipose tissues (WAT) are responsible for the storage and release energy as fatty acids in answer to body demands, and BAT burn substrates in answer to various different stimuli in a process known as adaptive thermogenesis [5]. Adaptive thermogenesis of BAT and beige adipocytes is a central part of total body energy expenditure due to their abundant mitochondria and numerous uncoupling protein 1 (UCP1) [6–8]. In humans, there is a strong negative correlation between the levels of BAT activity and body mass, indicating that BAT is a key factor in human metabolism whose enhanced activity and thermogenesis may be one way to improve obesity [4, 9].

Bile acids have received considerable scholarly attention in recent years as they are not only fundamental substances for digestive function but also major signal molecules in the extrahepatic organ. Moreover, bile acid alterations are strongly associated with human health and various diseases, including cancers, metabolic disorders and cardiovascular diseases [10, 11]. The liver synthesizes bile acids, which are stored in the gall bladder and secreted into the gut, where they are essential for cholesterol metabolism. There are two main pathways for bile acid production. Chenodeoxycholic acid (CDCA) and cholic acid (CA) are the two main primary bile acids produced in humans via the classical pathway, utilizing the liver enzyme cholesterol 7a-hydroxylase (CYP7A1), and account for 75% of bile acid production. Through the alternative pathway, cholesterol is chiefly converted into CDCA by the enzymes sterol-27-hydroxylase (CYP27A1) and oxysterol 7a-hydroxylase (CYP7B1). Before entering into the intestine, bile acids are conjugated with glycine or a small amount of taurine in humans, and over 95% of bile acids are transported back to the liver with portal blood in the distal ileum through enterohepatic circulation. The gut microbiota deconjugates unabsorbed bile acids, converting them into secondary bile acids, including lithocholic acid (LCA) from CDCA and DCA from CA [12, 13].

Bile acids signaling can play a crucial role in metabolic homeostasis [13–15]. In particular, the secondary bile acid DCA is one of the most abundant bile acids in humans, and recent evidence suggests that the level of DCA is related to obesity [16–18]. Although alterations of DCA have been reported in metabolic diseases, the precise impacts of DCA on energy metabolism and body composition remain unknown. Therefore, further investigations are needed to elucidate the association between DCA and metabolism.

In this study, we explored the beneficial effectiveness of DCA on glucose metabolism and adiposity reduction by promoting lipolysis under HFD feeding. We also assessed the significance of DCA on energy expenditure and BAT activation. This research uncovered that DCA was vital for promoting energy metabolism and reshaping body composition.

Methods

Human subjects

A total of 14 patients with metabolic syndrome were enrolled from the First Affiliated Hospital of Xi'an Jiaotong University and observed over a period of 16 weeks. The only inclusion criteria were as listed: patients without severe hepatic diseases, severe diabetic complications gastrointestinal diseases, biliary tract diseases. 10 healthy controls also were included. The baseline characteristic of participants with metabolic syndrome and controls are listed in Table 1. Fecal samples from these patients were collected at week 0 and week 16, and stored at − 80 °C. The study adhered to the Declaration of Helsinki guidelines and was sanctioned by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All individuals involved gave their written informed consent.

Bile acid analysis

The fecal samples were dissolved and prepared using the precipitation method [19, 20]. First, 10 milligrams of feces was mixed with 100 µl of water at a homogenization speed of 3000 revolutions per minute (rpm) using a homogenizer. Subsequently, 10 µl of an internal standard solution and 300 µl of cold methanol with 0.01% ammonium hydroxide were introduced to enhance the extraction of bile acids (BAs). After mixing, the samples were kept at a low temperature of 4 °C for a period of 10 min, then vigorously agitated for 45 s using a vortex mixer. Finally, the mixtures were subjected to centrifugation at a high speed of 16,000 gravitational forces (g) for 10 min at a cold temperature of 4 °C [19, 20]. The concentrations of bile acid in the supernatants were then measured by a UPLC/Synapt G2-Si QTOF MS system (Waters Corp., Milford, MA) with an ESI source. Standards for all bile acids were used to identify the different bile acid metabolites detected by LC-MS.

Table 1 Baseline characteristic of participants with metabolic syndrome and controls

Characteristics	Patients with metabolic syndrome ($n=14$)	Control participants ($n=14$)	*P* value
Gender, n (%)			
Male	8 (57.1%)	7 (50%)	ns
Female	6 (42.9%)	7 (50%)	ns
Age (y)	52.43 ± 11.15	51.57 ± 6.48	ns
BMI (kg/m^2)	26.54 ± 3.23	24.41 ± 3.36	ns
SBP (mmHg)	135.21 ± 14.96	121.21 ± 12.87	0.013
DBP (mmHg)	85.07 ± 10.67	74.50 ± 18.63	ns
Hypertension (%)	64.3%	35.7%	ns
Diabetes (%)	21.4%	14.3%	ns
Smoking (%)	28.6%	35.7%	ns
Drinking (%)	28.6%	0%	0.049
HbA1c (%)	5.95 ± 1.43	5.49 ± 0.25	ns
FPG (mmol/L)	6.35 ± 3.25	5.08 ± 0.47	ns
AST (U/L)	24.07 ± 11.17	24.14 ± 11.60	ns
ALT (U/L)	34.21 ± 28.24	33.71 ± 21.15	ns
TG (mmol/L)	1.99 ± 0.86	1.77 ± 0.99	ns
TC (mmol/L)	5.66 ± 1.02	4.95 ± 1.06	ns
LDL-c (mmol/L)	3.85 ± 0.93	3.05 ± 0.69	0.023
HDL-c (mmol/L)	1.22 ± 0.33	1.06 ± 0.25	ns
HOMA-IR	2.59 ± 1.15	2.53 ± 1.77	ns
Previous medication			
Aspirin (%)	7.1%	14.3%	ns
ACEI/ARB (%)	21.4%	14.3%	ns
CCB (%)	21.4%	15.4%	ns
Metformin (%)	14.3%	7.1%	ns
DCA (nmol/L)	2492.83 ± 666.24	1162.31 ± 310.64	0.012

Animals

All animal experiments complied with the guidelines of Xi'an Jiaotong University's Animal Experimental Ethics Committee. The animal protocol number is XJTUAE2023-462. Male C57BL/6J mice, aged 8 weeks, were acquired from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed in an SPF environment. All mice performed GTT and ITT after intervention. Four mice were chosen randomly from each group to be placed in metabolic cages. With all experiments done, all mice were euthanized by exsanguination under isoflurane after 12 h of fasting. All tissues and serum samples were then fixed or stored at − 80℃ as necessary for further experimentation.

In a HFD feeding study, all mice were randomized into three groups: (1) control group fed with a low fat diet obtained from Research Diets (D12450J, consisting of 10%kcal fat, 20%kcal protein, 70%kcal carbohydrate), (2) HFD group mice fed with a HFD from Research Diets (D12492, consisting of 60%kcal fat, 20%kcal protein, 20%kcal carbohydrate), (3) HFD plus DCA 50 mg/kg/d by gavage group also fed with the same HFD from Research Diets (D12492, consisting of 60%kcal fat, 20%kcal protein, 20%kcal carbohydrate). All HFD-fed mice were gavaged with 50 mg/kg/d of DCA or vehicle once a day during the last 4 weeks.

Metabolic tolerance tests

After 8 h of fasting, all mice received glucose tolerance tests (GTTs). Blood glucose levels were measured at the time of 0, 15, 30, 60, 90 and 120 min after 2 g/kg glucose intraperitoneal injection.

For the insulin tolerance tests (ITTs), blood glucose levels were measured at the time of 0, 20, 40, 60 and 80 min after 0.6 U/kg insulin intraperitoneal injection after 2 h fasting.

Metabolic cage and body composition

Indirect calorimetry including energy expenditure, RER，locomotor activity and food intake was measured using automatic metabolic cages (Sable promethion core system, USA) in which mice were individually monitored for 2 days after acclimatizing for 1 day. Data were collected every 5 min. We included the full data of the calorimetric analysis in the supplemental data 1.

Body composition was measured in conscious mice using an EchoMRI-100 analyzer (EchoMRI-100, ACQSYS 2019).

Real-time quantitative PCR

An RNA Extraction Kit (Takara, 9767, Japan) was used to isolate RNA from the frozen BAT and inguinal white adipose tissue (iWAT), and PrimeScript™ RT Master Mix (Takara, RR036A, Japan) was used to synthesize cDNA, which was analyzed with FastStart Essential DNA Green Master (Roche, Cat. No. 06402712001, Switzerland). All primers used in RT-qPCR are showed in Table 2 and all involved genes were normalized to β-Actin.

Staining and immunohistochemistry

Sections of 4% paraformaldehyde-fixed paraffin-embedded adipose tissue were ready for haematoxylin and eosin (H&E) staining using standard protocols. BAT and epididymal adipose tissues (eWAT) were stained for uncoupling protein-1 (UCP-1, 1/4000; Ab10983; Abcam). The average adipocyte size (μm [2]) was quantified for each mouse using AdipoCount software [21]. The lipid droplet content and Immunohistochemistry (IHC) quantification were measured by ImageJ.

Statistics

Spearman's rank correlation coefficients were utilized to assess the relationship between DCA and various parameters, including insulin levels, blood glucose concentrations, and the HOMA index. Data are expressed as mean values with standard error of the mean (SEM). For comparisons between two groups, Student's t-test was

Table 2 Primers used for RT-qPCR

Gene name	Forward primers	Reverse primers
β-actin	ACCTGACAGACTACCTCATGAAGA	TCATGGATGCCACAG-GATTCCATA
Dio2	CTTCCTCCTAGATGCCTACAAAC	CGAGGCATAATTGT-TACCTGATTC
Ucp1	GCCTCTACGACTCAGTCCAA	CATTAG-ATTAGGGGTCGTCC
Cpt1b	ACTAACTATGTGAGTGACTGG	TGGCATAATAGTT-GCTGTTC
Pgc1a	TCCTCTTCAAGATCCTGTTAC	CACATA-CAAGGGAGAATTGC
Atgl	TGACCATCTGCCTTCCAGA	TGTAGGTGGCG-CAAGACA
Hsl	GCGCTGGAGGAGTGTTTTT	CCGCTCTCCAGTT-GAACC
Pparγ	GCATGGTGCCTTCGCTGA	TGGCATCTCTGTGT-CAACCATG
Lpl	GGGAGTTTGGCTCCAGAGTTT	TGT-GTCTTCAGGGGTCCT-TAG
Ap2	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAA-CACATTCC
Fasn	GACCTTCATGGACACAATGCT	ATACCACCAGAGAC-CGTTATG
Scd1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAAT-GTTCTTGTCGT
Plin1	CAATCAACGAGAGAGCCCG	CGTTTTGTCCCTTCT-CACGC

employed, and one-way ANOVA coupled with Tukey's post-hoc analysis was applied for three-group comparisons. Normality of the data distribution was assessed using the Shapiro-Wilk test and ANCOVA analysis was conducted to determine the differences on EE controlling for lean mass [22, 23]. Statistical significance was set at $p < 0.05$ for all two-tailed tests. GraphPad Prism version 8.0 software was to carry out all of the above calculations.

Results

1. Fecal DCA levels are negatively associated with HOMA index in humans.

To test whether a potential correlation between glucose metabolism and fecal DCA species levels existed, we recruited 14 patients from our metabolic cohort and performed fecal bile acids profile tests at 0 and 16 weeks. The baseline information and fecal DCA levels of the patients with metabolic syndrome and healthy controls were listed in the Table 1. These results showed that DCA was negatively correlated with insulin, blood glucose levels and HOMA index (Fig. 1A-C).

2. DCA-fed mice are resistant to HFD-induced obesity.

Firstly, we evaluated the effect of daily gavage administration of DCA in a HFD-fed mice for 4 weeks and found that there was a significant decrease in body weight after DCA supplementation compared to HFD-fed mice (Fig. 2A). Additionally, we also performed echoMRI to quantify the body composition. Compared to the HFD-fed mice, DCA treatment decreased the fat mass percentage while increasing lean mass percentage significantly (Fig. 2B). We furthermore examined the histology and weight of tissues in order to assess the regulation of energy metabolism (Fig. 2C-D). Figure 2D revealed that there was a steady decrease in the weight of both eWAT and iWAT for DCA-treated mice but no statistically significant difference in BAT, liver and quadriceps. Overall, these results suggested that DCA treatment significantly attenuated HFD-induced obesity.

3. DCA decreases adipocyte size and promotes lipolysis.

To determine the reason for the observed decrease in adipose tissues, we evaluated the contribution of

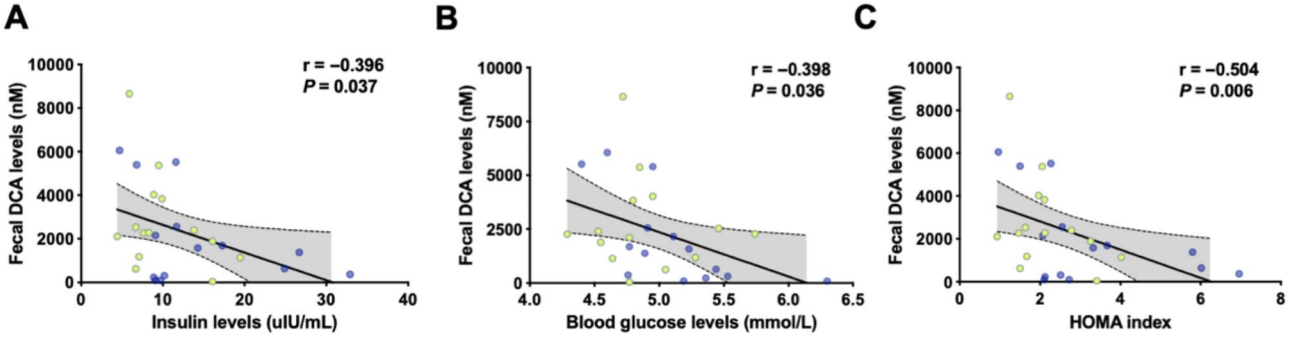

Fig. 1 Fecal DCA levels are negatively associated with serum insulin levels, glucose levels and HOMA index in humans. Scatter plots of fasting blood glucose levels and fecal concentrations of DCA levels in humans at week 0 (purple), 16 (light yellow). (**A**-**C**) Correlation between DCA with insulin, blood glucose levels and HOMA index respectively. The r value indicate the Spearman correlation coefficients, and P value indicates the statistical significance

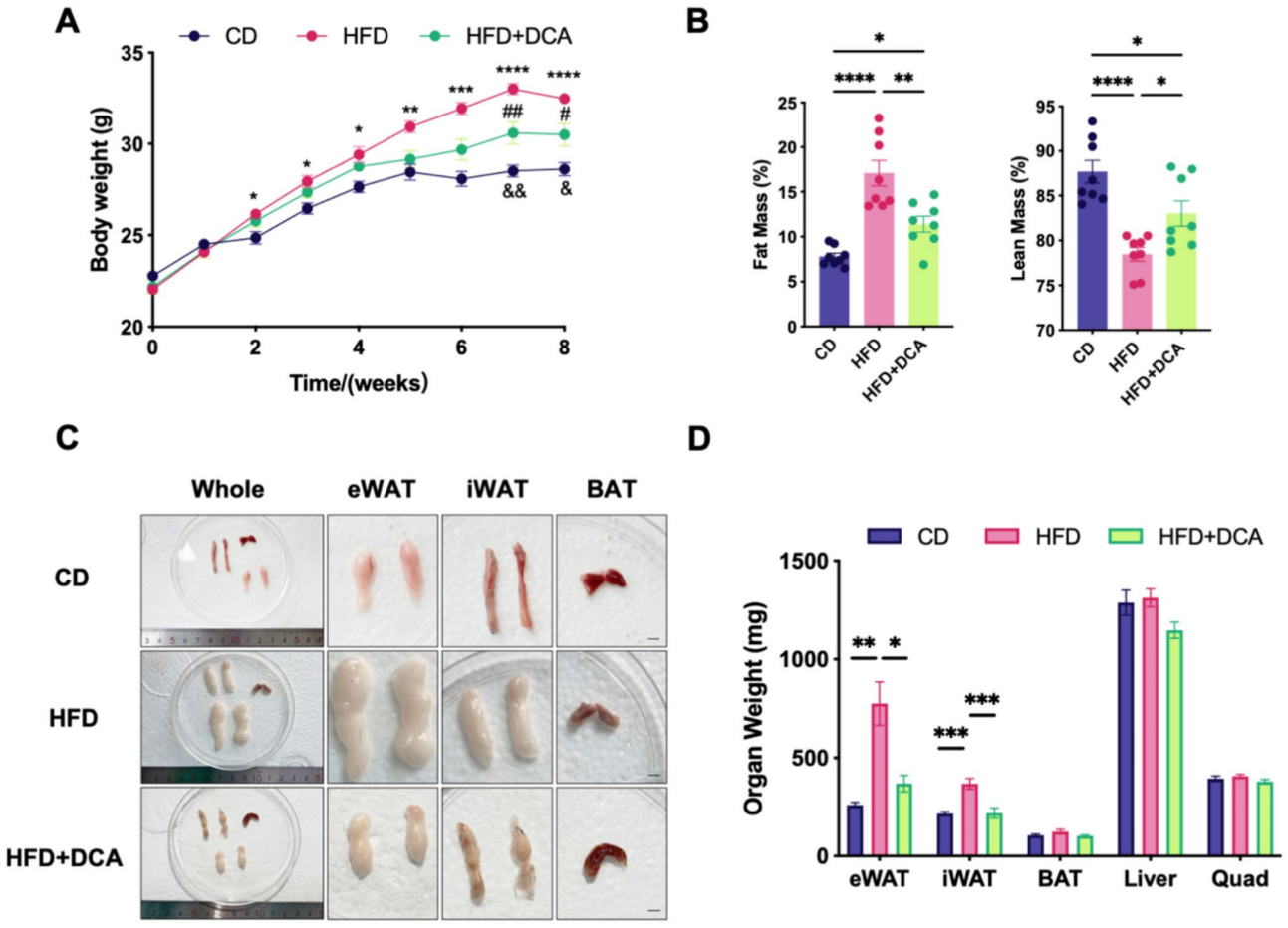

Fig. 2 DCA-fed mice are resistant to HFD-induced obesity. Mice were gavaged with DCA(50 mg/kg/d) and vehicle under HFD feeding. (**A-B**) The body weight and body composition of CD, HFD and DCA-fed HFD mice ($n = 8, 8, 8$). (**C-D**) Representative photographs of fat pads and quantification in CD, HFD and DCA-fed HFD mice ($n = 8, 8, 8$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ for the HFD group compared to the CD group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for the HFD group compared to the DCA-fed HFD group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ for the DCA-fed HFD mice compared to the CD group. Scale bar: 1 cm

adipocyte size or adipocyte number as a result of differentiation, or both. Firstly, we measured the adipocyte size in adipose tissues under HFD feeding. Histology staining displayed an overview of adipocyte size in WAT and BAT (Fig. 3A). As shown in Fig. 3B, the results of histology analysis indicated a significant decrease in adipocyte diameters in WAT and lipid droplet area in BAT of DCA-treated HFD mice compared to HFD-fed mice. In order to reveal the potential mechanism of DCA supplementation in inducing smaller adipocytes, we also quantified the mRNA expressions of lipolysis, adipocyte differentiation and lipogenesis related genes. There was an increase in the mRNA expression of lipolysis genes (*Atgl*, *Hsl*) in the eWAT of DCA-treated mice under HFD feeding compared with mice only on HFD (Fig. 3C), although the mRNA expressions of lipogenesis (*Fasn*, *Scd1* and *Lpin1*) and adipocyte differentiation (*Pparγ*, *Lpl* and *Ap2*) was found no change between the two groups (Fig. 3D).

4. DCA increases the thermogenic capacity of brown adipose tissue.

Given the evidence for the increased lipolysis in WAT, we next assessed the function of DCA on thermogenic functions in details. The biomarkers of brown adipose activation genes in BAT were quantified by qPCR. The expressions of *Dio2*, *Ucp1* and *Pgc1α* in BAT were significantly upregulated in DCA-fed mice compared to control mice under HFD feeding (Fig. 4A). In addition, we observed the upregulating expression of UCP1 in BAT by immunohistochemical staining (IHC) (Fig. 4B-C). These results revealed that DCA can activate BAT thermogenesis by upregulating related genes.

5. DCA treatment ameliorates glucose metabolism and insulin sensitivity.

For further assessing the impact of DCA on glucose metabolism, GTT and ITT metabolic tolerance tests

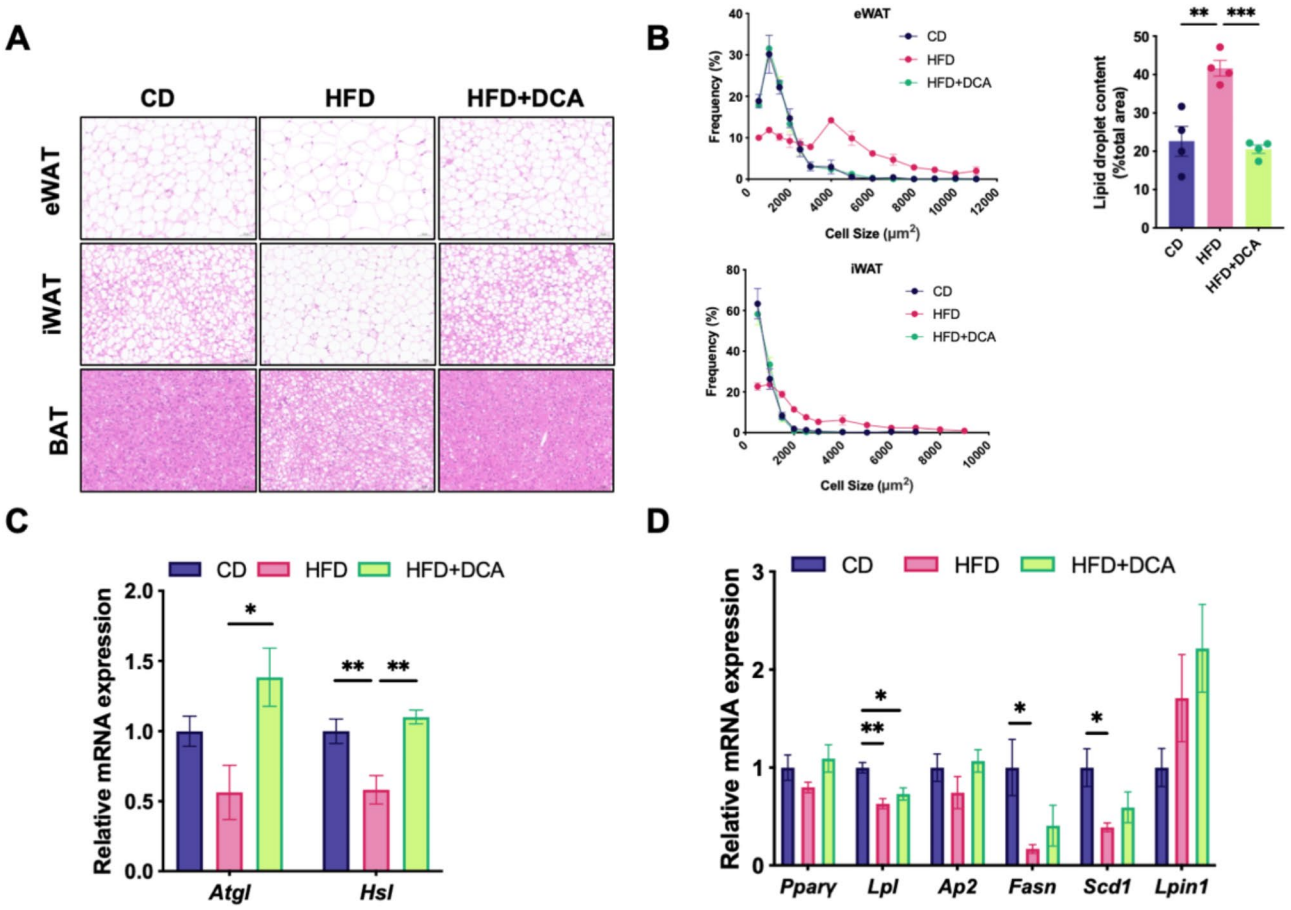

Fig. 3 DCA treatment decreases adipocyte size and promotes lipolysis. (**A-B**) Representative photographs of eWAT, iWAT and BAT in H&E staining and quantification of lipid droplet content of BAT ($n = 4, 4, 4$) and WAT adipocyte area ($n = 4, 4, 4$). (**C**) Relative mRNA expression of lipolysis genes in eWAT under HFD feeding ($n = 5, 5, 5$). (**D**) Relative mRNA expression of adipocyte differentiation (*Ppary*, *Lpl* and *Ap2*) and lipogenesis (*Fasn*, *Scd1* and *Lpin1*) in eWAT under HFD feeding ($n = 5, 5, 5$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

were conducted. As shown in Fig. 5A-C, DCA significantly ameliorated glucose tolerance and insulin sensitivity in DCA-treated mice under HFD feeding. These results showed that DCA enhanced glucose metabolism under HFD feeding.

6. DCA treatment enhances energy expenditure.

Adiposity is often accompanied with the alterations in energy metabolism. In order to explore the mechanism underlying the observed improvement of glucose metabolism and obesity, we used the metabolic cages to monitor the metabolic rates. Like the there was no significant difference in food intake between the HFD-fed mice and the DCA-fed HFD mice, but there was an elevation in the daily locomotor activity in the latter group (Fig. 6A-B). No changeable respiratory exchange ratio (RER) or fat oxidation rates were observed in DCA-fed mice under HFD feeding compared to the HFD-fed mice (Fig. 6C-F). Additionally, ANCOVA analysis with lean mass as a covariate showed no change in EE during the day and full day but indicated a significant increase in the DCA-fed mice at night (Fig. 6G). In summary, DCA markedly accelerated energy expenditure rates under HFD feeding.

Discussion

Several studies have shown that DCA is associated with obesity and metabolic disorders, but there is limited research focused on the direct effect of DCA on metabolism. Moreover, the mechanisms underlying the regulation of energy homeostasis by DCA are still unknown [24]. In this study, we found a negative correlation between the fecal DCA levels and insulin, blood glucose and HOMA index in humans. We also found that DCA prevented HFD-induced obesity and fat mass gain in mice by triggering lipolysis and adipocyte thermogenesis. What's more, DCA treatment evidently improved glucose metabolism and insulin resistance under HFD feeding. In summary, DCA promoted fat and carbohydrate metabolism without distinction under obese conditions.

DCA accounts for one of the most abundant bile acids in both humans and mice, and it plays a critical role in

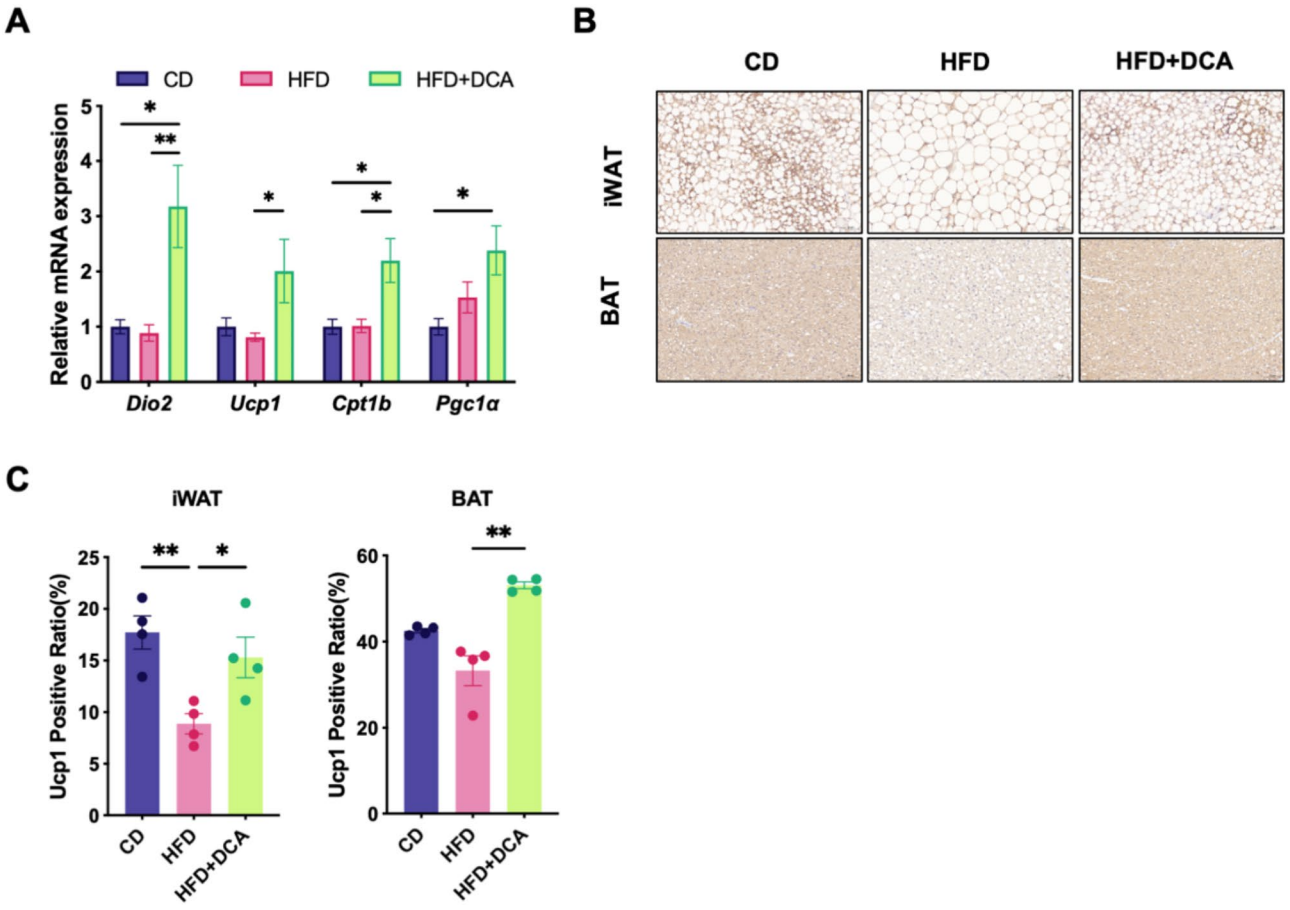

Fig. 4 DCA increases the thermogenic capacity of brown adipose tissue. (**A**) Relative mRNA expressions of thermogenic-related genes in BAT ($n = 5, 5, 5$) under HFD feeding. (**B-C**) UCP1 protein expression in iWAT and BAT under HFD feeding and densitometric quantitation of UCP1from IHC staining ($n = 4, 4, 4$). $^{*}P < 0.05$, $^{**}P < 0.01$

energy metabolism [25]. DCA is a potent agonist of TGR5 receptor and a modest agonist of FXR receptor. Increased activation of TGR5 has a crucial role in metabolism homeostasis and inducing thermogenesis in BAT [13]. It's also reported that INT-767, a dual agonist of both FXR and TGR5, can stimulate GLP-1 secretion, indicating that TGR5 may coordinate with FXR to stimulate GLP-1 secretion. As an incretion hormone, GLP-1 can improve insulin secretion and glucose homeostasis [26].

In our search for a clinical relationship between fecal DCA levels and glucose metabolism, we first tried to evaluate the correlation between fecal DCA levels and serum glucose levels or insulin sensitivity. Several studies have suggested that DCA levels decrease in T2DM patients or obese people [27]. Interestingly, increased levels of DCA have been reported to be associated with obesity and T2DM, which is contrary to another previous study [17, 28]. DCA may also ameliorate metabolic disorders in NASH mice models, although, Karolina et al. demonstrated the harmful effect of DCA on glucose metabolism in short-term HFD-fed mice [29, 30]. As very little has been reported regarding the question of the direct impact of DCA on metabolism, to fill the gap, we investigated the function of DCA in mice and found that DCA improved glucose metabolism and insulin sensitivity under HFD feeding. These results further support the idea that DCA has direct metabolic benefits.

Adipose tissue mass is mainly determined by the balance between fat synthesis and fat catabolism. Increasing lipolysis serves as fuels for *Ucp-1* mediated thermogenesis in BAT and iWAT [31]. Therefore, promoting lipolysis and adipocyte thermogenesis have emerged as powerful and effective therapeutic strategies for alleviating obesity and its complications [32]. To assess the critical role of DCA in fat metabolism, we used an obese mouse model under HFD feeding and found that DCA could protect against HFD-induced obesity. Moreover, the weights of eWAT and iWAT were decreased in DCA-fed HFD mice. Histological analysis further showed that DCA significantly decreased adipocyte diameter in eWAT and iWAT, and lipid droplet area in BAT from HFD-fed mice. In addition, ATGL is an important determinant of hydrolyzing triglycerides into diglycerides, which are further

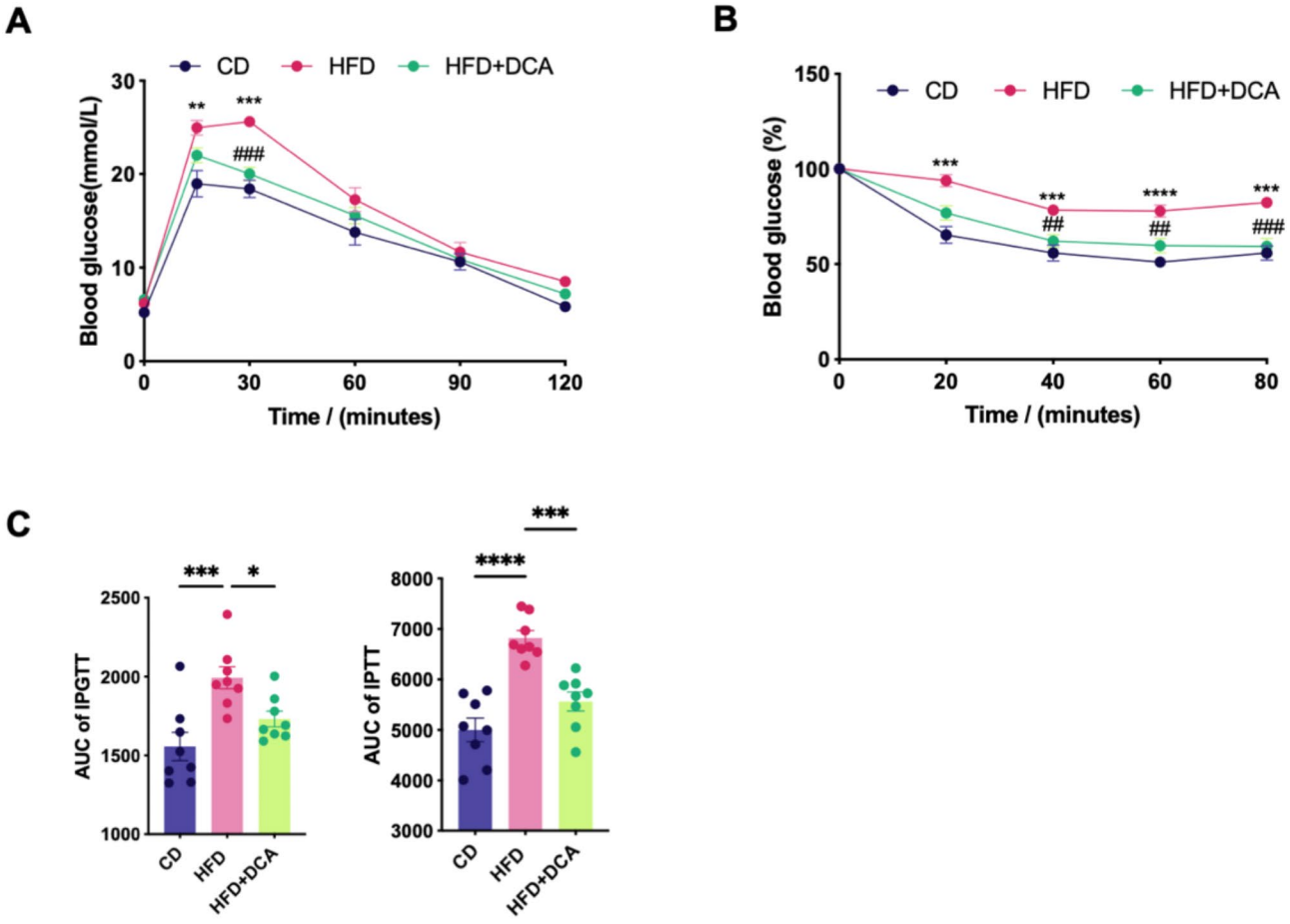

Fig. 5 DCA ameliorates glucose metabolism and insulin sensitivity. (**A-B**) Blood glucose levels of GTT ($n = 8, 8, 8$) and ITT ($n = 8, 8, 8$) under HFD feeding. (**C**) AUC of GTT and ITT. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; $^{\#\#\#}P < 0.001$, for the DCA-fed HFD group compared to the HFD group

catalyzed by HSL into monoglycerides and glycerols [33, 34]. Our results showed that DCA was prone to decreasing adipocyte diameters by promoting lipolysis activation but not by increasing adipocyte differentiation and lipogenesis in eWAT.

Previous studies do not paint a clear picture of the function of DCA supplementation on energy metabolism is unclear. In our study, it was surprising that DCA treatment enhanced glucose metabolism and had the anti-obesity effects. One possible explanation for this result may have been higher energy expenditure or respiratory exchange ratio changes, and we therefore used metabolic cages to validate our initial results. Interestingly, we still found that DCA indeed accelerated energy expenditure without changing RER, indicating that DCA had no partial effect on fatty acid or carbohydrate oxidation but promoted both of them. One indicator of increasing fatty acid oxidation is BAT activation, due to demand for intercellular fatty acids instead of total cholesterol-derived fatty acids. DCA treatment decreased the size of WAT and lipid droplets in BAT, and induced mRNA expressions of thermogenesis genes in BAT as well, which we confirmed by IHC staining.

Conclusions

In summary, our study provides the evidence that DCA supplementation can alleviate metabolic disorders caused by obesity. As shown in Fig. 7, DCA may protect against HFD-induced obesity and improve glucose tolerance by enhancing lipolysis and thermogenesis via upregulating the expression of *Atgl*, *Hsl* and *Ucp1*. Based on these results, DCA may have therapeutic value for metabolic disorders and weight loss strategies as a new target.

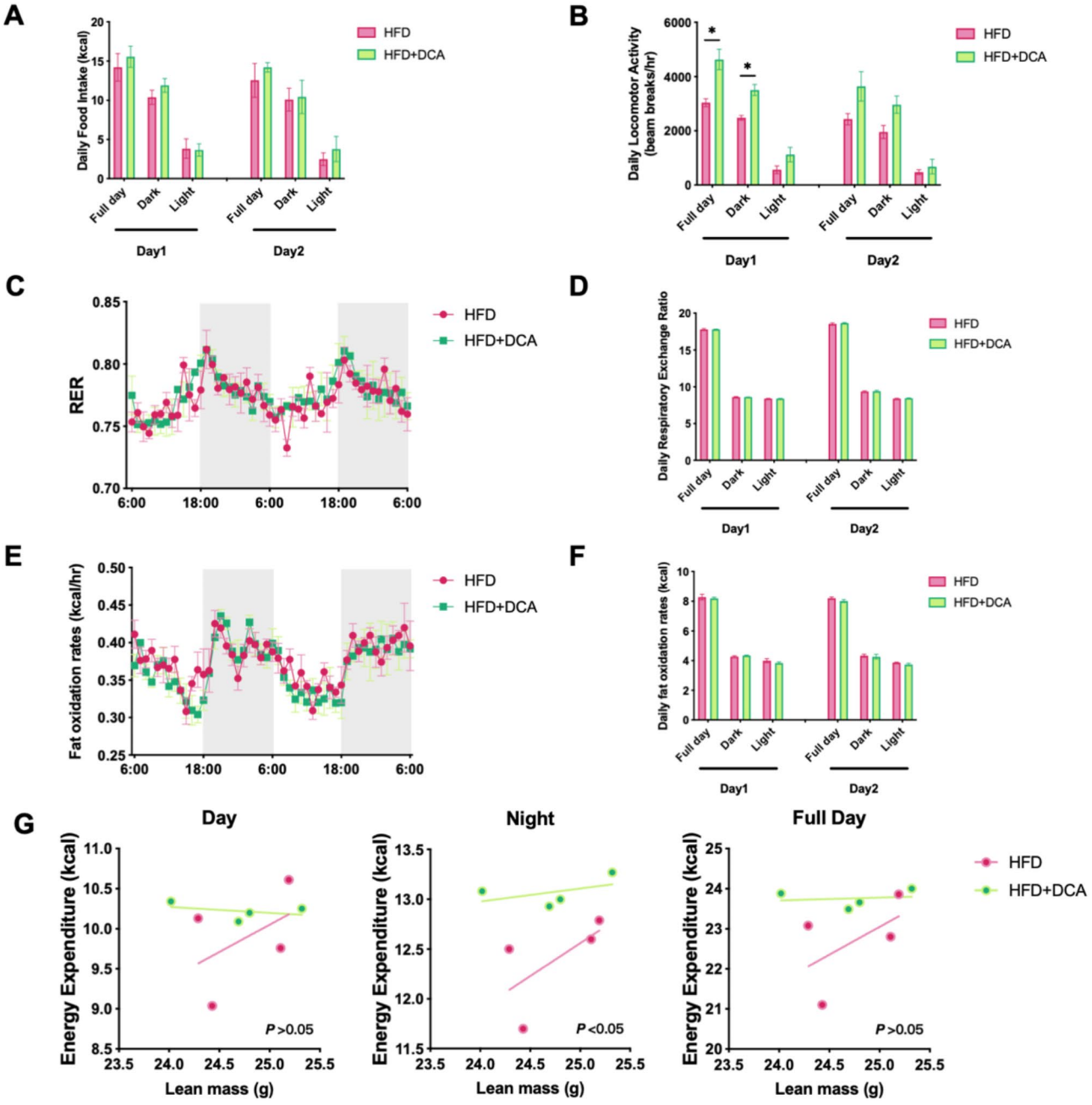

Fig. 6 DCA treatment enhances energy expenditure but had no effect on RER. Mice were housed in fully automated metabolic cages under HFD ($n = 4$, 4) feeding. (**A-F**) Daily food intake, daily locomotor activity, respiratory exchange ratio, daily respiratory exchange ratio, fat oxidation and daily fat oxidation were monitored for 2 days under HFD feeding. (**G**) Comparison of linear analyses of unnormalized energy expenditure and lean mass during day, night and full day respectively between DCA-fed mice under HFD feeding compared to the HFD-fed mice. *$P < 0.05$, **$P < 0.01$

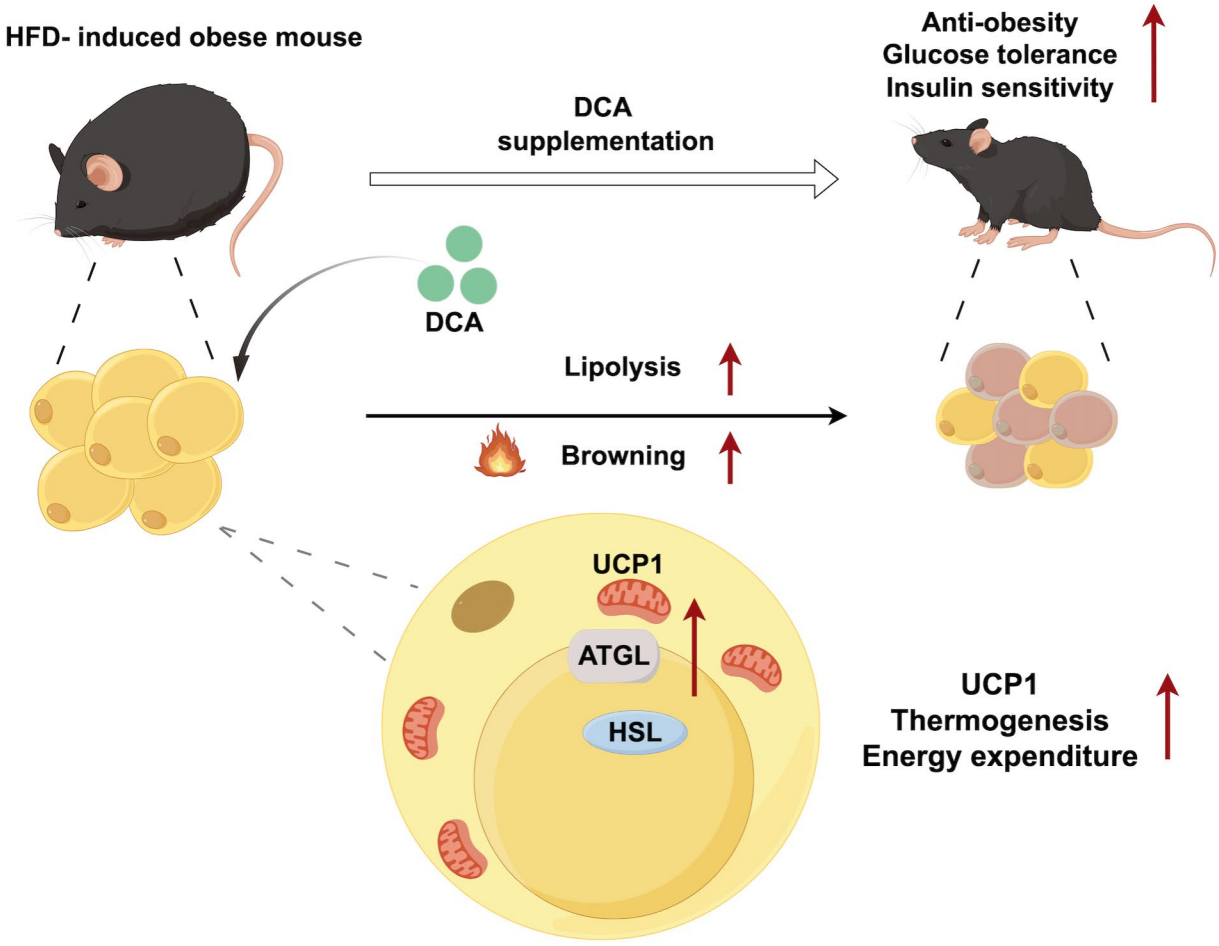

Fig. 7 Graphic abstract of the mechanism underlying the beneficial effects of DCA. DCA protects against HFD-induced obesity and improves glucose tolerance by enhancing lipolysis and thermogenesis via upregulating the expression of *Atgl*, *Hsl* and *Ucp1*

Abbreviations

DCA	Deoxycholic acid
T2DM	Type 2 diabetes mellitus
CD	Chow diet
HFD	High-fat diet
HOMA	Homeostatic Model Assessment for Insulin Resistance
BAT	Brown adipose tissues
WAT	White adipose tissues
UCP1	Uncoupling protein 1
CDCA	Chenodeoxycholic acid
CA	Cholic acid
CYP7A1	7a-hydroxylase
CYP27A1	Sterol-27-hydroxylase
CYP7B1	Oxysterol 7a-hydroxylase
LCA	Lithocholic acid
GTT	Glucose tolerance test
ITT	Insulin tolerance test
iWAT	Inguinal white adipose tissue
eWAT	Epididymal adipose tissues
EE	Energy expenditure

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-025-02485-x.

Supplementary Material 1

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Figure 7 was created by Figdraw. The authors thank AiMi Academic Services (www.aimieditor.com) for English language editing and review services.

Author contributions
GT and JL designed experiments. GT, YL, XG, GM and PL performed the experiments. GT, YW, YZ and JZ collected and analyzed data. GT drafted the manuscript. JL, YW, YW, YL and ZM revised the manuscript. The final manuscript was approved by all the authors. GT, ZM and YL did the revision work.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All patients was sanctioned by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All individuals involved gave their written informed consent. All animal experiments comply with the guidelines of

Xi'an Jiaotong University's Animal Experimental Ethics Committee. The animal protocol number is XJTUAE2023-462.

Competing interests
The authors declare no competing interests.

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