

N-acetylcysteine Protects Mice from High Fat Diet-induced Metabolic Disorders

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ABSTRACT

Purpose To study the effects of N-acetylcysteine (NAC, C₅H₉NO₃S) on diet-induced obesity and obesity-related metabolic disorders.

Methods Six-week-old male C57BL/6 mice fed a chow or high-fat diet (HFD) were treated with NAC (2 g/L) in drinking water for 11 weeks. Its influences on body weight and food intake were manually measured, and influence on body composition were analyzed by magnetic resonance imaging. Glucose meter and ELISA were used to determine serum glucose and insulin levels, as well as lipid content in the liver. The effects of NAC treatment on mRNA levels of genes involved in inflammation, thermogenesis, and lipid metabolism in various tissues were determined by real time PCR.

Results NAC supplementation inhibited the increase of fat mass and the development of obesity when mice were fed an HFD. NAC treatment significantly lowered HFD-induced macrophage infiltration, and enhanced adiponectin gene expression, resulting in reduced hyperglycemia and hyperinsulinemia, and improvement of insulin resistance. NAC oral administration suppressed hepatic lipid accumulation, as evidenced by lower levels of triglyceride and cholesterol in the liver. The beneficial effects are associated with a decrease of hepatic *Pparγ* and its target gene expression, and an increase in the expression of genes responsible for lipid oxidation and activation of farnesoid X receptor.

Furthermore, NAC treatment also stimulates expression of thermogenic genes.

Conclusion These results provide direct proof of the protective potential of NAC against HFD-induced obesity and obesity-associated metabolic disorders.

KEY WORDS hepatic steatosis · inflammation · insulin resistance · N-acetylcysteine · obesity

ABBREVIATIONS

ANOVA	Analysis of variance
BAT	Brown adipose tissue
CD68	Cluster of differentiation 68
Cox2	Cyclooxygenase 2
Cpt1	Carnitine palmitoyltransferase 1
Cyp7a1	Cholesterol 7 alpha-hydroxylase
Dio2	Type II iodothyronine deiodinase
FXR	Farnesoid X receptor
Gapdh	Glyceraldehyde-3-Phosphate Dehydrogenase
GTT	Glucose tolerance test
H&E	Haematoxylin and eosin stain
HDL	High-density lipoprotein
HFD	High fat diet
Inos	Inducible nitric oxide synthase
ITT	Insulin tolerance test
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
Mcp1	Monocyte chemotactic protein 1
Mgat1	Monoacylglycerol O-acyltransferase 1
NADPH	Nicotinamide adenine dinucleotide phosphate
Pgc1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
Ppar	Peroxisome proliferator-activated receptor
RT-PCR	Real time PCR
SD	Standard deviation
Shp	Small heterodimer partner

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Tnf α	Tumor necrosis factor alpha
Ucp	Uncoupling Protein
WAT	White adipose tissue

INTRODUCTION

N-acetylcysteine (NAC, C₅H₉NO₃S) is a thiol compound that stimulates glutathione-S-transferase activity and promotes detoxification by providing sulfhydryl groups and acting as a scavenger of free radicals through direct interaction with reactive oxygen species (1,2). It is a precursor of L-cysteine and reduced glutathione (GSH), and acts as an antioxidant by restoring the pool of intracellular reduced GSH. NAC has been commonly used to treat paracetamol (acetaminophen) intoxication and cystic fibrosis (3,4). The mucolytic agent of NAC has been used for several decades to treat chronic obstructive pulmonary disease.

In addition to its antioxidant and anti-inflammation properties, there is a growing interest in the beneficial effects of NAC for treating metabolic disorders. NAC is capable of protecting pancreatic β cells and preserving insulin levels by enhancing pancreatic-duodenal homeobox-1 (PDX-1) expression, a major regulator of beta cell identity and function (5,6). NAC supplementation suppresses fructose and high sucrose diet-induced hyperglycemia and hyperinsulinemia, and improves peripheral insulin sensitivity (7–9). The beneficial effects of NAC on glucose metabolism were also reported in alloxan-induced diabetes in CD-1 mice and diabetic *db/db* mice (10,11). Administered in drinking water, NAC lowered plasma and liver triglyceride levels in *Balb/c* mice fed a saturated fat-enriched diet and significantly affected lipid metabolism (12). Yang *et al.* (13) reported that NAC administration partially reduces plasma triglycerides, total cholesterol, and LDL levels while increasing the HDL level in rats fed a high fat diet (HFD) for 4 weeks. Similar results were obtained in mice subjected to a high cholesterol diet and supplemented with NAC and sesame oil (14). Short-term NAC administration in drinking water prevented high-sucrose induced weight gain and dyslipidemia (15). Collectively, these studies show that NAC is capable of restoring dys-regulated glucose and lipid metabolism, suggesting its potential application in diet-induced obesity, which is closely associated with oxidative stress, inflammation, and metabolic disturbance (16).

By employing HFD-induced obesity as a model, we systematically examined the beneficial effects of NAC administration on preventing HFD-induced obesity and obesity-associated metabolic disorders including hypertrophy of adipocytes, fatty liver, and insulin resistance. Mechanistically, the beneficial effects observed are accompanied by suppression of HFD-induced inflammation, modulation of nuclear receptor-mediated lipid metabolism, and regulation of thermogenic gene expression. Our results provide new and critical evidence

in support of the application of NAC in the management of metabolic disorders.

MATERIALS AND METHODS

Materials

N-acetylcysteine (NAC, C₅H₉NO₃S) was purchased from Sigma (St. Louis, MO). RNeasy Tissue kit was from Qiagen (Valencia, CA). First-strand cDNA synthesis system was obtained from OriGene (Rockville, MD). PerfeCTa® SYBR® Green FastMix was acquired from Quanta BioSciences (Gaithersburg, MD). Merckodia Insulin ELISA kit was from Merckodia Developing Diagnostics (Winston Salem, NC). A TUREtrack glucose meter was purchased from Nipro Diagnostics Inc. (Fort Lauderdale, FL). Oil Red O solution was obtained from Electron Microscopy Science (Hatfield, PA). Cholesterol and triglyceride commercial kits were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). High-fat diet (60% kJ/fat) was purchased from Bio-serv (Frenchtown, NJ, catalog number S3282). C57BL/6 mice were purchased from the Charles River Laboratories (Wilmington, MA).

Animals and Treatment

All procedures performed on mice were approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, Georgia. Six-week-old male C57BL/6 mice were housed under standard conditions with a 12 h light–dark cycle in 4 groups (Chow, Chow-NAC, HFD, HFD-NAC, 5 mice/per group). Mice fed an HFD or a regular chow had free access to the drinking water with/without NAC (2 g/L) for 11 weeks. Food consumption was determined by measuring the difference between the amount of food provided to each cage and the amount left at the end of the week throughout the experiment. Food intake per mouse was calculated based on the amount of food consumed divided by time and the number of mice per cage. Animals were weighed weekly and their body composition was determined using EchoMRI-100™ from Echo Medical Systems (Houston, TX).

Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)

Glucose and insulin tolerance tests were performed during weeks 10–11. For GTT, mice were injected intraperitoneally with glucose at 1.5 g/kg body weight after 6 h fasting. Blood samples were taken at designated time points (0, 30, 60, 90, 120 min), and the glucose concentrations were measured using a glucometer. For ITT, mice fasted for 4 h and blood glucose

levels were measured after an intraperitoneal injection of insulin (Humulin, from Eli Lilly) at 0.75 U/kg. Blood samples were collected from fasted mice and insulin level was measured using commercial assay kits according to the manufacturer's instruction. The same groups of animals were used for GTT and ITT with a time interval of 1 week.

Histochemical and Oil Red O Analysis

Mouse liver, epididymal, and subcutaneous white adipose as well as brown adipose tissues were collected at the end of the study, fixed in 10% formalin, and embedded in paraffin. H&E staining was performed with tissue sections at a thickness of 6 μm . Frozen hepatic sections (9 μm) were used for Oil Red O staining. Microscopic examination was performed and photographs were taken under a regular light microscope.

Hepatic Lipid Analysis

Hepatic lipid analysis was performed following the established procedure (17,18). Briefly, liver samples (100–200 mg) were freshly collected and homogenized in phosphate buffered saline (1 ml), mixed with 5 ml of an organic solvent (chloroform:methanol = 2:1, vol/vol) and incubated overnight at 4°C. The mixture was centrifuged and the organic phase collected. The collected fraction was dried and lipids were re-dissolved in 1% Triton X-100. The amounts of cholesterol and triglyceride were determined using a commercial kit following the manufacturers' instructions.

Gene Expression Analysis by Real Time PCR (RT-PCR)

Total RNA was isolated from the mouse liver, and white and brown adipose tissues using RNeasy kit or TRIZOL reagent. Two μg of total RNA were used for the first strand cDNA synthesis as recommended by the manufacturer. RT-PCR was performed using SYBR Green as an indicator on the ABI StepOne Plus Real-Time PCR system according to the recommend protocol. The PCR was carried out for 40 cycles at 95°C for 15 s and 60°C for 1 min. Fluorescence was read during the reaction, allowing a continuous monitoring of the amount of PCR product. The data were normalized to internal control *Gapdh* mRNA and analyzed using the $\Delta\Delta\text{Ct}$ method. The primer sequences employed are summarized in Supplemental Table S1.

Statistical Analysis

Statistical analysis was done by two-way ANOVA. All data are reported as mean \pm standard deviation (SD) with statistical significance set at $P < 0.05$.

RESULTS

NAC Administration Suppresses HFD-Induced Gain of Body Weight and Fat Mass

To evaluate the effect of NAC provided in drinking water on HFD-induced weight gain, we fed C57BL/6 mice chow or an HFD (60% kJ/fat), simultaneously providing drinking water with/without 2 g/L NAC for a total of 11 weeks. As expected, mice on an HFD rapidly gained weight, reaching 48 g at the end of 11 weeks compared to 30 g for animals fed regular chow (Fig. 1a). However, access to NAC containing water did not affect the growth rate of mice on regular chow, whereas the NAC treatment dramatically inhibited HFD-induced weight gain, exhibiting 15 g less than that of obese mice and a weight close to that of the control mice with a regular chow at the end of 11 weeks. The difference in body weight is recognized by animal appearance (Fig. 1b).

Body composition analysis revealed that the NAC treatment largely suppressed the gain of fat mass, with an approximately 55% reduction in fat mass compared to that of control animals, irrespective of mice with regular chow or HFD feeding (Fig. 1c). Moreover, difference in body weight is not correlated with calorie intake (Fig. 1d). Body fat reduction was further determined by weighing fat pads. The results in Fig. 2a show that the NAC treatment significantly blocked the increase of fat pads, including epididymal (EWAT) and subcutaneous (SubWAT) adipose tissues in HFD-fed mice by ~ 1.1 g and ~ 1.4 g, respectively. Accordingly, H&E staining of adipocytes demonstrate that NAC reduced the size of adipocytes in EWAT and SubWAT in mice fed an HFD; this was further confirmed by quantitative analysis using an imaging system (Fig. 2b-d). These results demonstrate that administration of NAC through drinking water blocks lipid accumulation in adipose tissue.

Supplementation of NAC in Drinking Water Inhibits HFD-induced Chronic Inflammation in Adipose Tissue

Macrophage infiltration in WAT is a major characteristic in HFD-induced obesity (19). We therefore performed real-time PCR analysis to determine the levels of adipose macrophages and inflammation. Results in Fig. 3a-c show that HFD-induced macrophage infiltration, as evidenced by increased mRNA levels of macrophage specific marker genes *Cd68*, *F4/80*, and *Cd11c*, by ~ 33.2 -, 22.3 -, and 40.6 -fold, respectively, over that of control animals fed regular chow. HFD also enhanced the *Mcp1* gene expression (Fig. 3d), which encodes the macrophage chemotactic factor. In contrast, induced expression of these genes is markedly suppressed by NAC treatment, suggesting the anti-inflammation effect of NAC on diet-induced obesity. In addition, NAC also inhibited HFD-induced expression of *gp91*^(phox), a subunit of NADPH oxidase

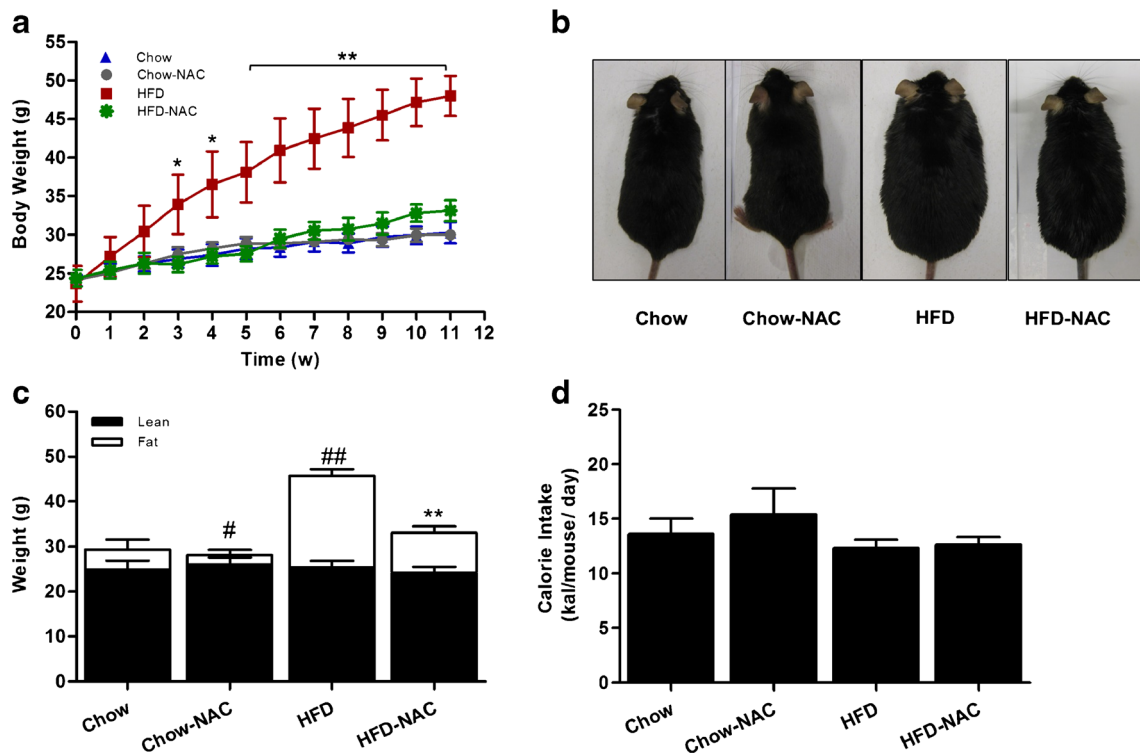


Fig. 1 NAC treatment protects mice from HFD-induced obesity. Eight-week-old C57BL/6 male mice were fed a chow or HFD with free access to drinking water with or without 2 g/L NAC for 11 weeks. **(a)** Time and body weight curve; **(b)** Photographs of control and treated mice at the end of experiment; **(c)** Fat mass and lean mass of different groups of animals at the end of 11-week feeding; **(d)** Average calories intake per mouse of different groups was calculated based on daily food (food-energy conversion: HFD, 1 g = 5.49 kcal; regular diet, 1 g = 3.46 kcal). Each data point represents the average \pm SD of 5 animals. # $P < 0.05$, ## $P < 0.01$ compared to chow fed animals; * $P < 0.05$, ** $P < 0.01$ compared to HFD fed animals.

involving in oxidative stress (Fig. 3e). Adiponectin mRNA level was significantly lower in HFD fed obese mice, whereas NAC evidently promoted adiponectin gene expression (Fig. 3f). To extend the investigation of the inhibitory role of NAC on inflammation, we exposed *in vitro* the RAW264.7 macrophage cell line to LPS in the presence of different amounts of NAC. As predicted, LPS dramatically induced expression of inflammatory factor genes including *iNos*, *Cox2*, *Mcp1*, and *Tnfa*, whereas NAC markedly inhibited LPS-induced gene expression in a dose-dependent manner (Supplemental Fig. 1). These data show that NAC is capable of suppressing macrophage activation and macrophage-mediated inflammation.

NAC Treatment Improved Obesity-associated Insulin Resistance

Adiposity and chronic inflammation are the major contributors to obesity-associated insulin resistance. To determine whether NAC treatment improves insulin sensitivity, we fasted mice for 6 h and performed intraperitoneal glucose tolerance tests. The results in Fig. 4a show that HFD feeding induced higher fasting glucose levels compared to that of animals fed a regular chow, and exhibited a much slower clearance rate of

injected glucose. NCA-treated mice exhibited a much higher clearance rate, which was better represented using the calculated area under the curve (AUC, Fig. 4b). NAC-treated mice also showed a rapid response to insulin administration (Fig. 4c). Accordingly, obesity-associated hyperinsulinemia was strikingly ameliorated by NAC treatment (8.3 $\mu\text{g/l}$ vs. 1.1 $\mu\text{g/l}$) (Fig. 4d). It is noteworthy that NAC treatment also enhanced insulin sensitivity in chow-fed mice (Fig. 4a-c). These results validate our prediction that NAC protects animals against obesity-associated insulin resistance.

NAC Administration Blocks Lipid Accumulation in the Liver by Modulating Nuclear Receptor-mediated Lipid Metabolism

Consumption of an HFD leads to fatty liver disease. Results in Fig. 5a show that liver weight in HFD-fed mice was 1.7-fold higher than that of animals fed a chow food. NAC treatment reduced liver weight in mice fed either a chow or an HFD. H&E-stained liver sections showed normal liver structure of mice on chow with or without NAC treatment. However, there was extensive hepatocyte vacuolation in animals fed an HFD (Fig. 5c), reflecting intrahepatic fat accumulation. In contrast, NAC

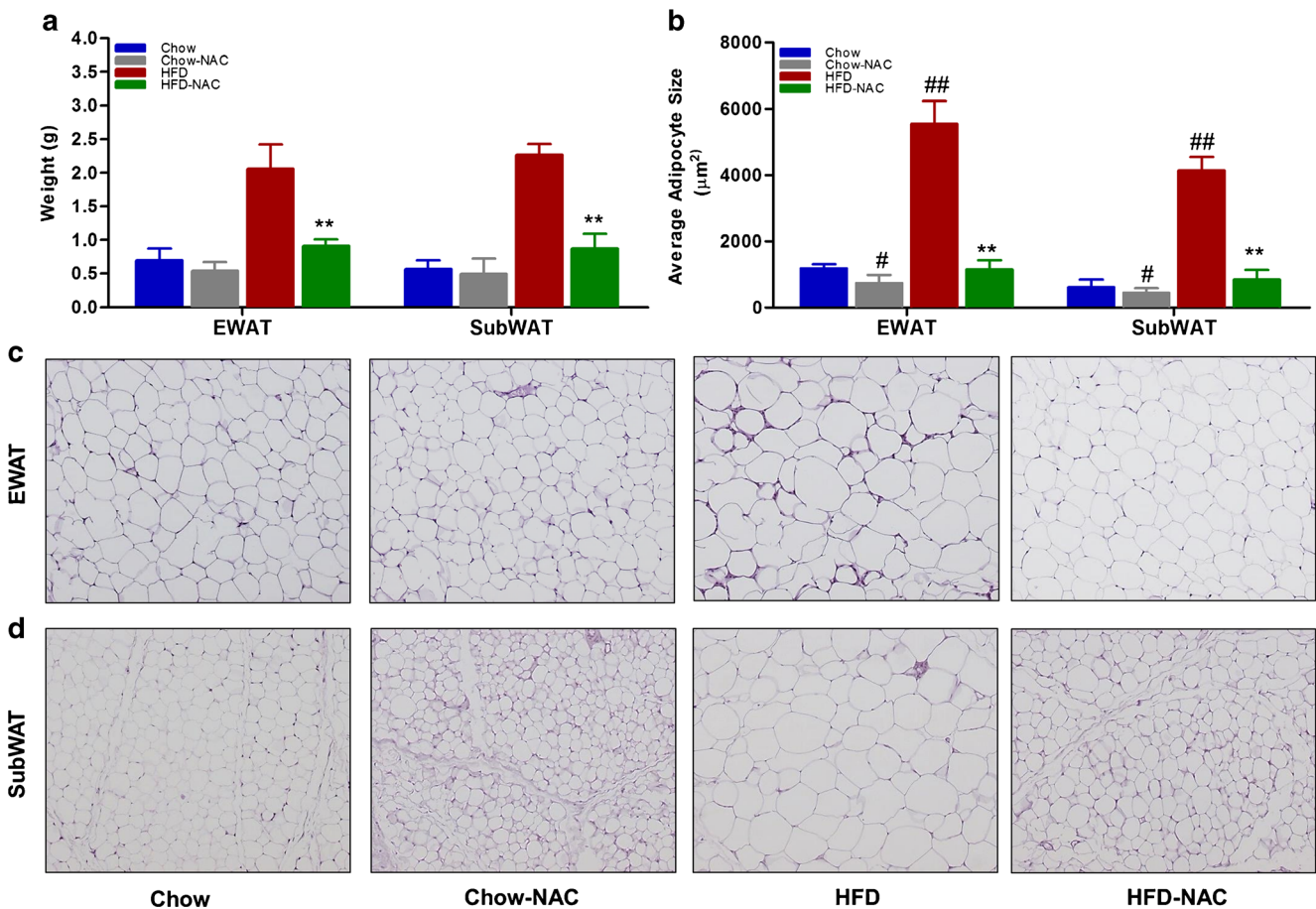


Fig. 2 NAC treatment reduces fat mass and average size of adipocytes. Mice were sacrificed at the end of 11-week treatment and the epididymal and subcutaneous adipose pads were collected. Adipose tissues were fixed in 10% neutrally buffered formalin and H&E staining was performed to show the morphology of adipose tissues. **(a)** Weight of epididymal (EWAT) and subcutaneous (SubWAT) adipose tissues; **(b)** Average size of adipocytes calculated from 200 adipocytes from 5 separate slides; **(c)** and **(d)** Representative images of H&E staining of EWAT and SubWAT (100 \times). ## $P < 0.01$ compared to chow fed animals; # $P < 0.05$, ** $P < 0.01$ compared to HFD fed animals ($n = 5$).

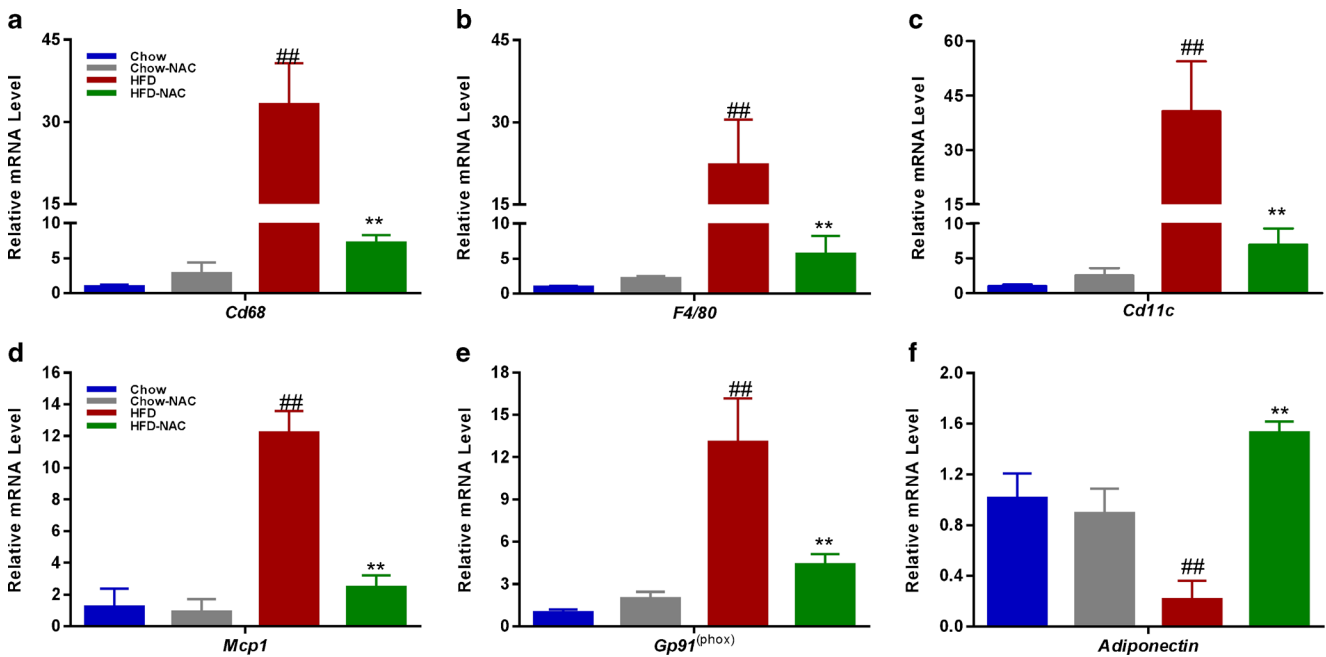
treatment efficiently blocked lipid accumulation in hepatocytes, which was evidenced by Oil Red O staining of liver sections (Fig. 5d). Blockage of hepatic lipid accumulation by NAC was further confirmed by measurements of hepatic triglyceride and cholesterol levels (Fig. 5b). Taken together, these results demonstrate that NAC treatment is capable of blocking hepatic lipid accumulation.

To further understand how NAC treatment affects hepatic lipid metabolism, we performed RT-PCR to evaluate the effect of NAC treatment on mRNA levels of genes responsible for lipid homeostasis. The PPAR γ -mediated pathway is responsible for lipid storage (20). As shown in Fig. 6a-d, HFD significantly enhanced mRNA levels of hepatic *Ppar1* and *Ppar2* by 2.7- and 4.8-fold, respectively, compared to that of chow-fed animals. In contrast, NAC treatment significantly inhibited *Ppar* expression, suppressing the induction of its target genes, including *Cd36* (84%) and monoacylglycerol O-acyltransferase 1 (*Mgat1*, 98%). Moreover, NAC treatment also reduced *Cyp7a1* gene expression but enhanced *Shp* expression. Both *Cyp7a1* and *Shp* are target genes of the farnesoid

X receptor (FXR). NAC treatment also increased mRNA levels of genes involved in fatty acid metabolism including carnitine palmitoyltransferase I (*Cpt1a*, *Cpt1b*).

NAC Treatment Increases Expression of the Genes Involved in Energy Expenditure in HFD-fed Mice

H&E-stained brown adipose tissue (BAT) show that HFD feeding increased fat deposition in brown adipocytes, resulting in BAT whitening. NAC treatment, however, dramatically inhibited adipocyte expansion in both chow- and HFD-fed mice (Fig. 7a). Since BAT plays an important role in thermogenesis, we examined the changes of thermogenic gene expression in BAT to assess the potential effect of NAC treatment on BAT activity. NAC treatment significantly enhanced the transcriptional levels of the genes that are critical for cellular thermogenesis, including *Dio2*, *Pgc-1 α* , *Pgc-1 β* , *Cidea*, and *Ucp3*. (Fig. 7b), suggesting that NAC treatment influences energy expenditure in BAT.

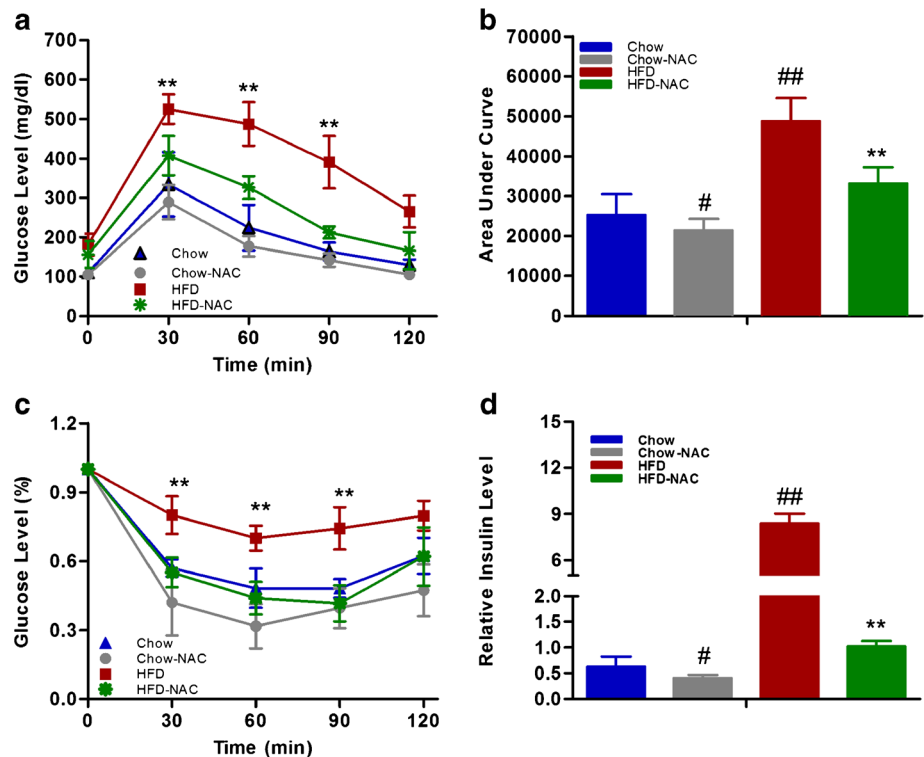


DISCUSSION

In this study we demonstrate that NAC supplementation greatly inhibited HFD-induced gain of body weight and fat

mass (Figs. 1 and 2). NAC treatment significantly curbed HFD-induced macrophage infiltration and enhanced the adiponectin gene expression, resulting in improvement of hyperglycemia, hyperinsulinemia, and insulin resistance (Figs. 3

Fig. 4 NAC treatment improves hyperinsulinemia and hyperglycemia of animals fed an HFD. (a) Glucose tolerance test. Animals were fasted for 6 h and injected (*i.p.*) with glucose (1.5 g/kg) and blood glucose levels were determined. (b) Area under the curve from (a). (c) Insulin tolerance test. Animals were fasted for 4 h followed by injection (*i.p.*) of insulin (0.75U/kg) and blood glucose levels were determined. (d) Serum insulin levels at the end of the 11-week feeding with or without NAC treatment. Each data point represents the mean \pm SD ($n = 5$). * $P < 0.05$, ** $P < 0.01$ compared to HFD-fed animals.



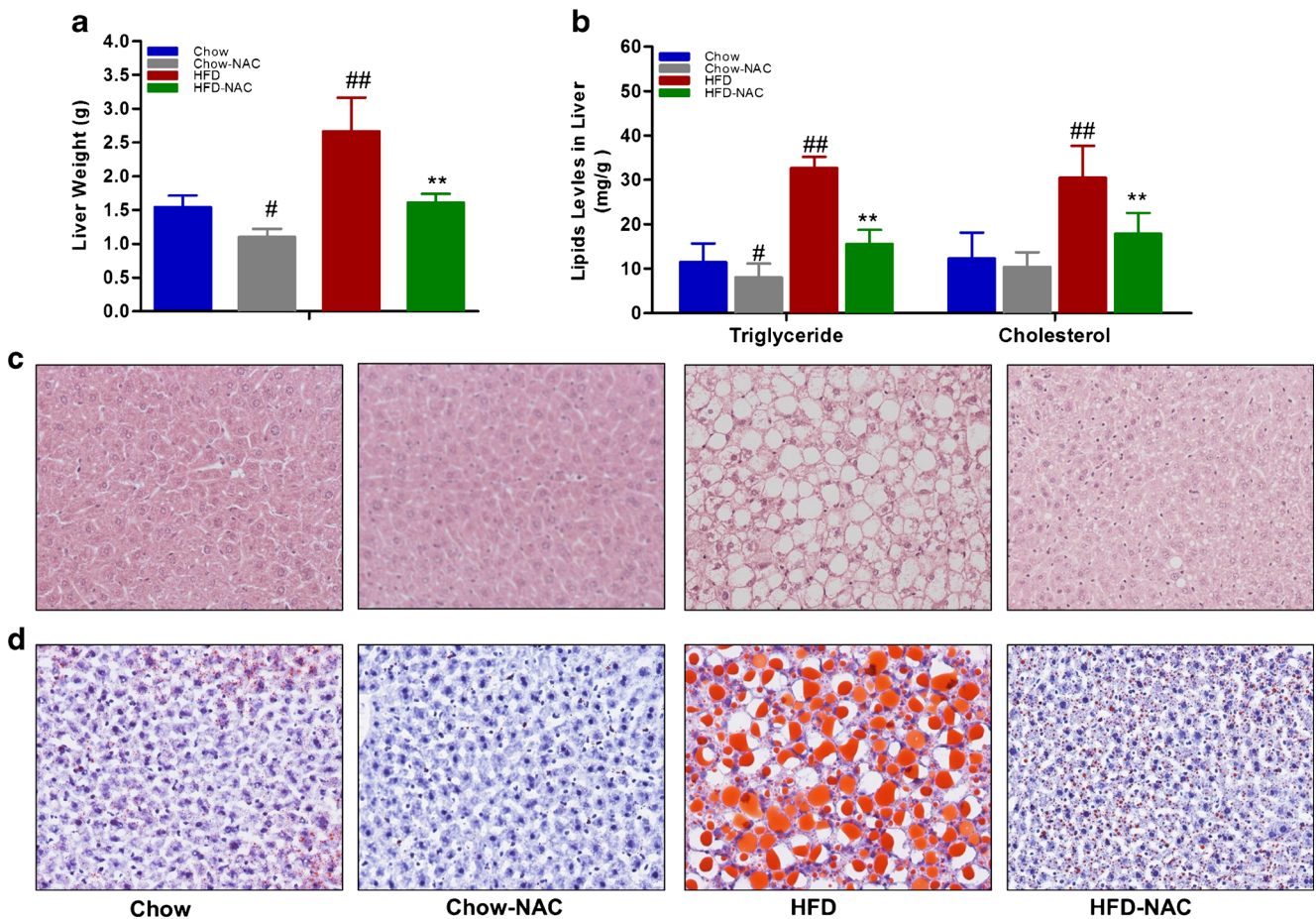


Fig. 5 NAC treatment inhibits lipid accumulation in the liver. At the end of the 11-week feeding, mice were sacrificed and liver samples were collected. **(a)** Liver weight of animals with or without NAC treatment; **(b)** Hepatic total triglyceride and cholesterol levels; **(c)** Photographs of liver sections with H&E staining (200 \times); **(d)** Lipid distribution in the liver revealed by Oil Red O staining (200 \times). # $P < 0.05$, ## $P < 0.01$ compared to chow fed animals; * $P < 0.05$, ** $P < 0.01$ compared to HFD fed animals ($n = 5$).

and 4). NAC treatment almost entirely blocked lipid accumulation in the liver, which was evidenced by lower levels of triglyceride and cholesterol in the liver (Fig. 5). The beneficial effects are associated with expression suppression of hepatic *Ppar γ* and its target genes, and an increase in mRNA levels of genes responsible for lipid oxidation and FXR activation (Fig. 6). Furthermore, NAC treatment stimulates thermogenic gene expression (Fig. 7). These results provide direct evidence to support the use of NAC supplementation as an effective means of blocking HFD-induced metabolic disorders.

Previous studies have shown that HFD feeding elevates production of reactive oxygen species in adipose tissue and boosts macrophage infiltration and inflammation (19,21,22), while chronic inflammation is closely associated with metabolic disorders (23). We have shown that macrophage elimination by clodronate liposomes (24) or overexpression of superoxide dismutase 3 gene (25) is capable of maintaining redox homeostasis and blocking HFD-induced obesity. Herein, consistent with its antioxidant and anti-inflammation properties, NAC treatment notably inhibited HFD-induced oxidative stress and

chronic inflammation in adipose tissue, which was evidenced by suppression of NADPH oxidase subunit *Gp91^{phox}*, macrophage chemokine *Mcp1*, and macrophage marker gene expression. In addition, we demonstrate that NAC treatment significantly reduced LPS-induced expression of inflammatory factor genes, including *iNos*, *Cox2*, *Tnfa* in RAW264.7 macrophage cells *in vitro*. In line with our results, Calzadilla *et al.* also demonstrated that NAC inhibited ROS levels and reduced adipocyte differentiation by inhibition of *C/EBP β* and *Ppar γ* gene expression and fat accumulation using 3 T3-L1 pre-adipocytes (26). Mechanically, NAC suppressed the proteasome activity (27) of NF κ B kinases (28), thereby inhibiting activation of NF- κ B, a master regulator for inflammation.

The beneficial effects of NAC in modulating glucose homeostasis are primarily achieved by its activity in suppressing inflammation. Growing evidence demonstrates that HFD-induced lipid overload in adipose tissue, liver, and muscle leads to an increased stress response, stimulating recruitment of immune cells and activation of inflammatory pathways. Consequently, chronic inflammation blunts insulin action in

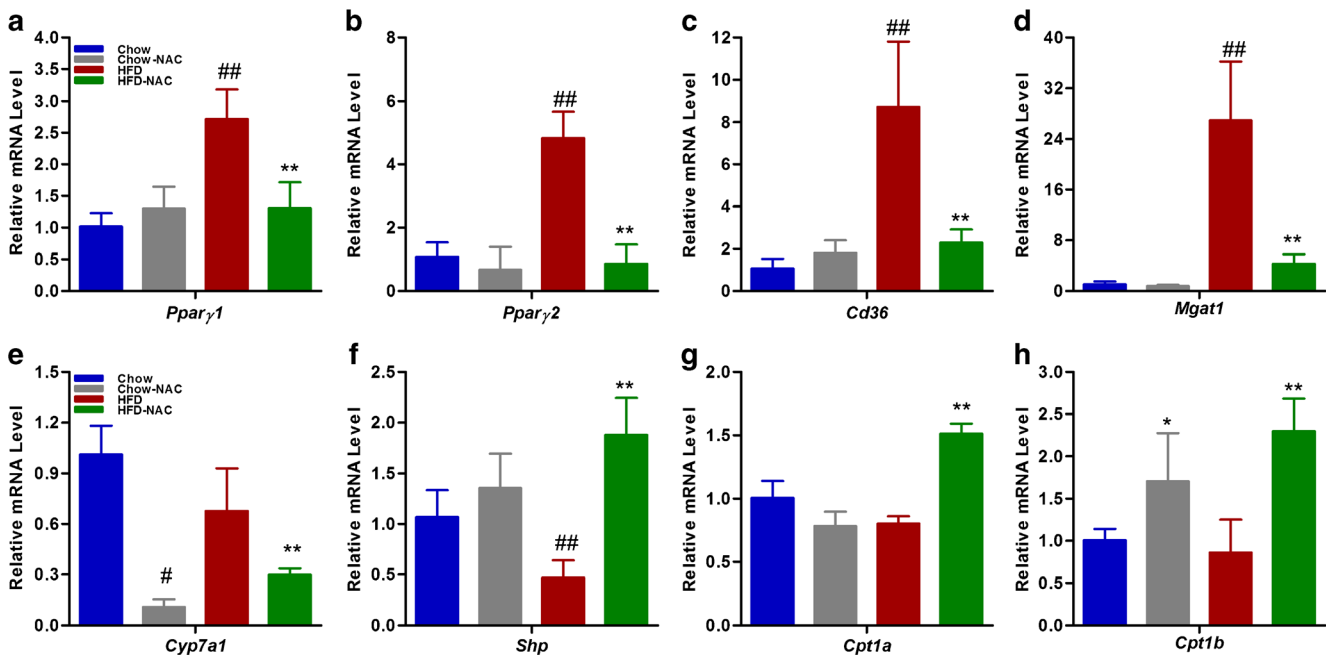


Fig. 6 Effect of NAC treatment on expression of genes involved in regulation of hepatic lipid storage and metabolism. Mice were sacrificed at the end of NAC treatment and liver samples were harvested, and total RNA was extracted. RT-PCR analysis was performed to evaluate relative mRNA levels of selected genes. (a) *Pparγ1*; (b) *Pparγ2* and its target genes; (c) *Cd36*; (d) *Mgat1*; (e) *Cyp7a1*; (f) *Shp*; (g) *Cpt1a*; and (h) *Cpt1b*. Each data point represents the mean \pm SD, ($n = 4$). # $P < 0.05$, ## $P < 0.01$ compared to chow fed animals; * $P < 0.05$, ** $P < 0.01$ compared to HFD fed animals.

stressed tissues by inhibiting insulin signal transduction and causing cellular insulin resistance (29,30). NAC directly or indirectly removes diet-induced ROS production, alleviating the inflammatory reaction, thus resulting in improved insulin sensitivity. In addition, our data also shows that NAC elevates

adiponectin expression. Adiponectin is an adipocytokine secreted from adipocytes (31). Previous studies show that adiponectin expression and its plasma concentration were inversely related to the development of obesity (32). Adiponectin-deficient mice developed diet-induced glucose

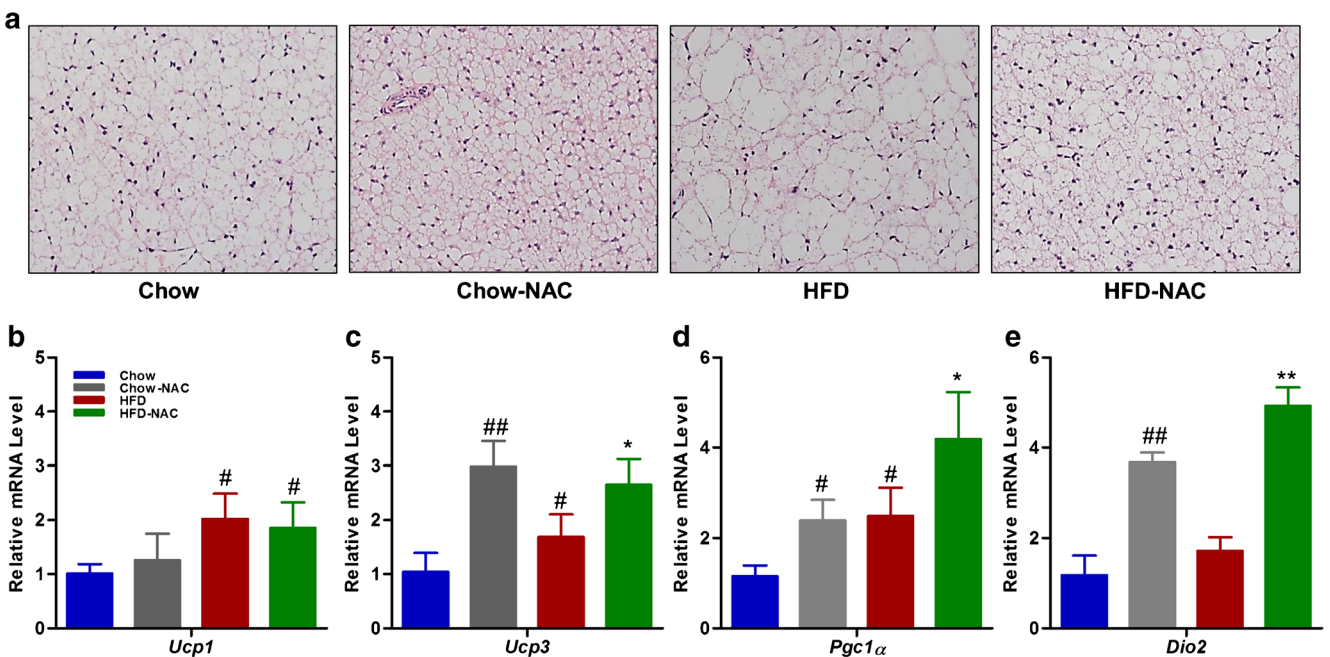


Fig. 7 Effect of NAC treatment on thermogenesis. Animals were sacrificed at the end of 11-week treatment and BAT was collected and total RNA extracted. (a) Photographs of BAT section with H&E staining (200 \times); Relative mRNA levels of (b) *Ucp1*; (c) *Ucp3*; (d) *Pgc1α*, and (e) *Dio2*. Each data point represents the mean \pm SD, ($n = 4$). # $P < 0.05$, ## $P < 0.01$ compared to chow fed animals; * $P < 0.05$, ** $P < 0.01$ compared to HFD fed animals.

intolerance and insulin resistance (33,34). In contrast, overexpression of adiponectin in mice leads to improved insulin sensitivity and glucose tolerance (35). These studies demonstrate the important role of adiponectin in maintaining glucose homeostasis. In line with these reports, results in Fig. 3f show a significant reduction of adiponectin expression in obese mice, concordant with insulin resistance in these mice. NAC treatment elevated adiponectin gene expression, suggesting its contribution to the improved insulin sensitivity.

Results in Fig. 5 show that NAC treatment inhibited diet-induced lipid accumulation in the liver, evidenced by H&E and Oil Red O staining of liver sections. The effect is in part related to the suppression of hepatic PPAR γ -mediated fat storage. HFD stimulated both *Ppar1* and *Ppar2* gene expression in the liver, concordant with HFD-induced hepatic lipid accumulation (Fig. 6a-b). Blockage of HFD-induced expression of *Ppar* and its target genes reduces lipid storage. Moreover, NAC treatment also reduced *Cyp7a1* gene expression but increased *Shp* transcript level. Both are the target genes of FXR, indicating that the potential effect of NAC is related to activation of the nuclear receptor FXR, which is an endogenous sensor for bile acids, and plays an important role in cholesterol and lipid metabolism. Our previous study demonstrated that activation of FXR by GW4064 significantly curbed HFD-induced hepatic steatosis by markedly reducing expression of lipid transporter *Cd36* gene (17). Therefore, induction of expression of FXR target genes facilitates the suppression of hepatic steatosis. In addition, previous studies also show that oxidative stress can lead to triglyceride accumulation in hepatocytes through up-regulation of lipogenic transcription factors and enzymes involved in fatty acid synthesis (36). Therefore, effective prevention of diet-induced hepatic oxidative stress by NAC may also reduce de novo lipid synthesis.

Furthermore, we observed that NAC treatment markedly inhibited lipid accumulation in BAT, even in chow-fed mice, according to the average size and the density of vacuole type structures in the H&E-stained BAT sections. BAT is known for its role in thermogenesis (37). Bartelt *et al.* (38) reported that increased BAT activity enhanced triglyceride-rich lipoprotein metabolism in mice, suggesting its beneficial role in mobilizing lipids utilization. Our real-time PCR analysis shows NAC treatment enhanced thermogenic gene expression, including *Ucp3*, *Pgc1 α* , and *Dio2*, suggesting that NAC treatment may enhance energy expenditure. Concordant with our results, Novelli *et al.* (15) showed that NAC is able to inhibit carbohydrate oxidation and enhance fat oxidation in rats fed a high-sucrose diet, boosting whole body energy expenditure.

Taken together, our current study demonstrates that supplement of NAC in drinking water is capable of suppressing inflammation, modulating glucose and lipid metabolism, and stimulating thermogenic gene expression in HFD-fed mice, consequently preventing mice from diet-induced obesity and

obesity-associated liver steatosis and insulin resistance. These results provide direct evidence to support that NAC supplementation can be considered as an effective means in blocking diet-induced obesity and associated complications.

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