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ORIGINAL ARTICLE

Allicin ameliorates obesity comorbid depressive-like behaviors: involvement of the oxidative stress, mitochondrial function, autophagy, insulin resistance and NOX/Nrf2 imbalance in mice

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Abstract

The increased prevalence of obesity has been a major medical and public health problem in the past decades. In obese status, insulin resistance and sustained oxidative stress damage might give rise to behavioral deficits. The anti-obesity and anti-oxidant effects of allicin have been previously reported in peripheral tissues. In the present study, the functions and mechanisms of allicin involved in the prevention of high-fat diet (HFD)-induced depressive-like behaviors were investigated to better understand the pharmacological activities of allicin. Obese mice (five weeks of age) were treated with allicin (50, 100, and 200 mg/kg) by gavage for 15 weeks and behavioral test (sucrose preference, open field, and tail suspension) were performed. Furthermore, markers of oxidative stress, mitochondrial function, autophagy, and insulin resistance were measured in the hippocampal tissue. Finally, the levels of NADPH oxidase (NOX2, NOX4) and the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway were evaluated in the hippocampus. The body weight, metabolic disorders, and depressive-like behaviors in obese mice were ameliorated by allicin. The depressive-like behaviors presented in the obese mice were accompanied by remarkably excessive reactive oxygen species (ROS) production and oxidative stress, damaged mitochondrial function, imbalanced autophagy, and enhanced insulin resistance in the hippocampus. We found that allicin improved the above undesirable effects in the obese mice. Furthermore, allicin significantly decreased NOX2 and NOX4 levels and activated the Nrf2 pathway. Allicin attenuated depressive-like behaviors triggered by long-term HFD consumption by inhibiting ROS production and oxidative stress, improving mitochondrial function, regulating autophagy, and reducing insulin resistance in the hippocampus via optimization of NOX/Nrf2 imbalance.

Keywords Obesity · Depressive-like behaviors · NADPH oxidase · Nrf2

Abbreviation

HFD	High fat diet
FER	Food efficiency ratio
SPT	Sucrose preference test
OFT	Open field test
TST	Tail suspension test
ROS	Reactive oxygen species
MDA	Malonaldehyde
SOD	Superoxide dismutase
CAT	Catalase

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GSH	Glutathione
GPx	Glutathion peroxidase
ATG 5	Autophagy-related protein 5
LC3B	Microtubule-associated protein light chain 3B
Nrf2	Nuclear factor erythroid 2-related factor 2
HO-1	Heme oxygenase-1
NOX	NADPH oxidase

Introduction

The prevalence of obesity, a serious health problem, has risen globally in the past three decades (Mandviwala et al. 2016). Obesity is associated with a number of chronic conditions, including type 2 diabetes mellitus, hypertension, cardiovascular diseases, and hyperlipidemia (Knight 2011; Bhaskaran et al. 2014). Recently, accumulating studies have suggested that obesity increases the risk of the onset of depressive symptoms (Mansur et al. 2015). Interestingly, oxidative stress is a pathological process common to both obesity and depression (Matsuda and Shimomura 2013; Zafir and Banu 2009).

When exposed to excess levels of reactive oxygen species (ROS) or lowered anti-oxidant defense, oxidative stress might take place, and when oxidative stress exceeds the capacity of cells to repair biomolecular oxidation, oxidative damage occurs. Excessive ROS production reduces the expression of genes that regulate the anti-oxidation system, such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx). Oxidative stress and lowered anti-oxidant defense injure cellular components and induce functional abnormalities, ultimately resulting in programmed cell death by apoptosis or necrosis (Nakamura et al. 2012; Lucca et al. 2009). Excessive fat intake and physical inactivity inducing obesity and chronic energy metabolism disorders may contribute to the increase in ROS production both in peripheral and brain tissue, especially in the prefrontal cortex and hippocampus (Bondia-Pons et al. 2012; Fernandez-Sanchez et al. 2011). The brain tissues in obese individuals are immersed in oxidative damage and accompanied by a decrease in the activities of anti-oxidant enzymes (Bonnard et al. 2008; Freeman et al. 2013; Ma et al. 2014). Furthermore, patients suffering from depression have been reported to exhibit enhanced oxidative damage and decreased anti-oxidant enzyme levels (Ng et al. 2008). Mice treated with glucocorticoid to induce depressive-like behaviors were accompanied by higher levels of ROS, both in vitro and in vivo, and lowered activities of various anti-oxidant enzymes. Chronic treatment with antidepressants reduced the levels of oxidative stress markers and increased those of endogenous anti-oxidants (Maes et al. 2011a; Maes et al. 2011b). Therefore, the reduction of oxidative stress might be beneficial for the treatment of both metabolic and psychiatric disorders.

Mitochondria are a key cellular target of oxidative stress that function as the primary producer of cellular energy and are involved in ROS generation, cell apoptosis, and calcium homeostasis. Obesity with elevated ROS levels may damage the mitochondrial respiratory chain and the activities of mitochondrial respiratory enzyme complexes, which reverse ROS generation and oxidative stress development (Fernandez-Sanchez et al. 2011; Bonnard et al. 2008; Freeman et al. 2013; Ma et al. 2014; Ng et al. 2008; Maes et al. 2011a; Maes et al. 2011b; Hernandez-Aguilera et al. 2013). In addition, in response to oxidative stress, the degree of autophagy is significantly elevated in order to remove damaged organelles related to oxidative stress (Lee et al. 2012). Autophagy is a persistent intracellular defense mechanism that protects cells against chronic stress or nutrient deprivation. Damaged organelles and aggregated proteins are degraded and engulfed by double-membraned autophagosomes to provide composition materials and energy for cells to survive from injury, apoptosis, and death (Liang et al. 2017). Oxidative stress is a crucial biological and cellular pathogenesis mechanism in obesity and depression, and improvements in its regulation may be beneficial for the treatment of metabolic disorders and depression (Natoli et al. 2018; Xu et al. 2014). NADPH oxidase (NOX) generates ROS and serves as a contributor to the pathology of chronic neurodegenerative disorders (Gao et al. 2012; Bedard and Krause 2007). Previously, because of the failure of clinical trials using ROS-scavenging drugs, researchers have shifted the therapeutic strategy from ROS scavenging to inhibition of ROS generation (Ma et al. 2017). Therefore, therapeutic pathways and targets that effectively inhibit NOX have become interesting topics of research. Additionally, nuclear factor erythroid 2-related factor 2 (Nrf2), a cellular regulator of oxidative stress, protects the brain from oxidative damage by elevating the expression of several anti-oxidants. Recent studies have shown that restoration of the redox balance between Nrf2 and NOX may be an effective therapeutic approach against neurodegenerative disorders (Liang 2018; Gan and Johnson 2014).

Allicin, a natural and biological component extracted from garlic, possesses pharmacological properties including anti-oxidation, neuroprotection, and anti-inflammation (Chung 2006; Chen et al. 2014; Abdel-Daim et al. 2017). Allicin has the capacity to permeate the blood-brain barrier and accumulate in the brain at high concentrations. However, whether allicin protects against obesity comorbid depressive-like behaviors and whether the protective effects are involved in oxidative stress, mitochondrial function, autophagy, insulin resistance, and NOX/Nrf2 imbalance need to be investigated. In the present study, we examined the anti-depressant effects of allicin in the context of obesity and further explored the mechanisms involved in the anti-oxidation and NOX/Nrf2 imbalance in the hippocampus.

Method

Animals and experimental design

5-week-old male C57 mice were purchased from Experimental Animal Center of China Three Gorges University. All animal were raised in an environment with temperature at 22 °C ± 2 °C, humidity at 55 ± 5%, and free access to food and water under a 12 h light/dark cycle. All animal experiments in the present study were approved by the Ethics Committee of the China Three Gorges University and in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985). The mice were randomly divided into the following five groups ($n = 10$/group): control (standard diet), obese model (high fat diet, Research Diets, D12492), obese+allicin (50, 100, 200 mg/kg). The allicin was treated by gavage at 8:00 each day. Normal mice and obese mice model were gavaged an equipotent volume of vehicle. After high fat diet uptake and allicin treatment for 15 weeks, the behavioral tests were preformed. Then, blood samples were collected after 12 h fasting periods. All the mice were sacrificed by CO_2, and their hippocampus were removed, and frozen at −80 °C for subsequently detection.

Reagents

Allicin was purchased from MeilunBio Co., Ltd. (MB5783, Meilun, China). The purity of allicin was 98%, as measured by high-performance liquid chromatography (HPLC). The doses of allicin were chosen based on the previous publications (Sheela 1992; Sheela et al. 1995; Kanth et al. 2008), which studies the protective effects of allicin on metabolic disease. Therefore, 50, 100, 200 mg/kg allicin were chosen for our present study. Allicin was dissolved in DMSO, subsequently diluted in saline for use. The final concentration of DMSO used in the solvent was less than 1 ‰ (*V*/V).

Sucrose preference test

The sucrose preference test was performed according to our previous reports (Deng et al. 2019). All the behavioral tests were all carried out in dark phase (18:00–22:00 p.m.). The sucrose preference test was performed first. All mice acclimatized to drink pure water and 1% sucrose solution for 2 days before the formal sucrose preference test. The bottles of sucrose and pure water were changed position daily to avoid place preference. The food and two bottles were deprived for 18 h on the third day. On the fourth day, the whole test lasted for 12 h during the dark phase (7:00 p.m.–7:00 a.m.). After the test, both the two bottles were weighed. The consume ratio of 1% sucrose solution was calculated by the equation: Sucrose preference (%) = sucrose intake / (sucrose intake + water intake) × 100%.

Open field test

The open field test was performed according to our previous reports (Jiang et al. 2018). The levels of mouse anxiety and locomotor activity were assessed by open field test (OFT). The open field test was performed 24 h after the SPT. Plexiglas box (100 × 100 × 40 cm) contained four open boxes and functioned as the open field. A virtual zone (20 cm × 20 cm) was delimited in the center of the open field. Each C57 mouse was placed in the center of the field and allowed to explore for 1 h under dim light. The number of entries and times spent in the centre of the open field were recorded. 70% ethanol was used to clean the equipment to remove the olfactory cues after each trial.

Tail suspension test

The tail suspension test was performed according to our previous reports (Deng et al. 2019). The test C57 mouse was individually suspended 60 cm above the floor with their immobility time recorded during a test period of 6 min. Adhesive tape was used to fasten the mice (1 cm from the tail tip). To eliminate olfactory interference, the trial chambers were wiped with 70% ethanol between two separated test sessions in tail suspension test.

Biochemical evaluation

The biochemical evaluation was performed according to the previous reports (Li et al. 2012). The hippocampus tissues were weighed and homogenized in the 50 mmol/l phosphate buffer (pH 7.0) containing 0.1 mmol/l EDTA on ice. The homogenate was centrifuged at 10,000×g for 10 min at 4 °C and then the supernatant was collected for biochemical tests. The protein concentration was determined using a microspectrophotometer (BioDropuLite, Britain).

The contents of ROS, MDA, protein carbonylation, activities of SOD, CAT, GSH, GPx, ATP production, and mitochondrial respiratory chain complexes (I, II, III, IV), in hippocampus of mice were determined using the commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. The concentration of corticoserone in the serum was measured by the Elisa kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions.

For the ROS staining in the brain, the tissue was frozen and then cut into a slice at 5 μm, which immediately incubating with DHE (5 μM) at 37 °C for 15 min. Then the sections were visualized with a fluorescence microscope via the red fluorescence intensity excited by green laser.

Immunostaining

The immunostaining was performed according to the previous reports (Xin et al. 2013). For the Nrf2, HO-1, NOX2, and NOX4 immunostaining, the paraffin blocks were sliced into 6 μm thickness sections, which were dewaxed in xylol, rehydrated, and rinsed in phosphate-buffered saline (PBS). The paraffin sections were immersed in 0.3% H_2O_2 for 30 min, followed by a serum block with 5% non-fat dry milk for 30 min. The sections were then incubated with primary polyclonal rabbit Nrf2 at 1:100 dilution, HO-1 at 1:100 dilution, NOX2 at 1:100 dilution, and NOX4 at 1:100 dilution (Proteintech, China) overnight at 4 °C. Subsequently, horse-radish peroxidase conjugated secondary antibodies (1:300) were used to incubated with the paraffin sections for 2 h at room temperature. Then, the paraffin sections were developed by diaminobenzidine (DAB) and restained with hematoxylin for 1 min. The light microscope was used to photograph the stained, in addition, the images were analyzed using IMAGE PRO PLUS software.

Real time quantitative PCR

In brief, total RNA from hippocampus was isolated using Trizol reagent (Invitrogen, USA). Reverse transcription kit was used to synthesis RNA into cDNA (TAKARA,). Quantitative PCR was conducted with the MX3005P system (Agilent, USA) using SYBR Green Master Mix (Invitrogen, USA). The primer pairs used for each gene were showed as follow. The relative mRNA expression was calculated by the eq. $2^{-\Delta\Delta CT}$ and normalized to β-actin. The primers (Invitrogen, Shanghai, China) showed as below:

β-actin: forward 5’-CCTCTATGCCAACACAGT-3′,
reverse:5′- AGCCACCAATCCACACAG-3′,
Nrf2: forward 5′- -3′, CAGCCATGACTGATTTAAGCAG
reverse 5′- -3’CAGCTGCTTGTTTTCGGTATTA
HO-1: forward 5′- -3′, TCCTTGTACCATATCTACACGG
reverse 5′- -3′, GAGACGCTTTACATAGTGCTGT
Nox 2: forward 5′- -3′, GACAGGAACCTCAC TTTCCATA
reverse 5′- -3’ TGAAGAGATGTGCAATTGTGTG
Nox 4: forward 5′- -3′, GAAAACCTTCCTGCTGTACA AC
reverse 5′- -3’ CTGCTTAAACACAATCCTAGGC

Western blot

The concentration of protein was quantified by micro-spectrophotometer (BioDropuLite, Britain). After quantified and denaturized, the samples were loaded and separated by 8%-12% SDS/PAGE gels and then transferred to PVDF membranes (0.45 μm, Millipore, USA). The primary antibodies used were, anti-Nrf 2 (1:500, Proteintech, China), anti-HO-1 (1:500, Proteintech, China), anti-NOX 2 (1:500, Proteintech, China), anti-NOX4 (1:500, Proteintech, China), anti-P-AKT (1:1000, Cell Signaling Technology, USA), anti-P-CREB (1:600, Cell Signaling Technology, USA), anti-BDNF (1:1000, Cell Signaling Technology, USA), anti-ATG5 (1:500, Cell Signaling Technology, USA), anti-LC3 (1:500, Cell Signaling Technology, USA), anti-Beclin1 (1:500, Cell Signaling Technology, USA). Then, the membranes were incubated with the horseradish peroxidase-conjugated secondary antibodies (1:3000) (Servicebio, Wuhan, China). The chemiluminescence system was used to detect the band (Clinx Science Instruments Co. China).

Statistical analysis

The data were present as mean ± SD. The statistical significances were assessed by one-way ANOVA followed by post hoc LSD test. Differences were considered significant at $P < 0.05$.

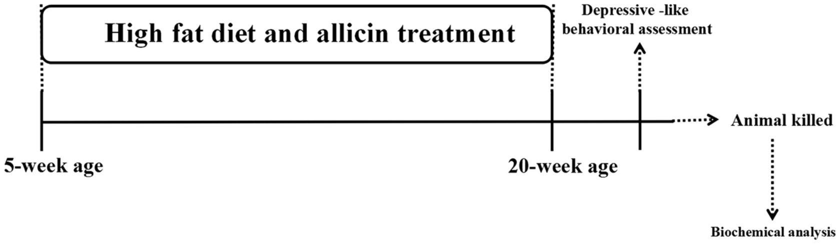
Result

Allicin improves abdominal obesity and metabolic disorders on obese mice

The experimental design is illustrated in Fig. 1. After five-week-old mice were fed a high-fat diet (HFD) for 15 weeks, they exhibited remarkable obesity and metabolic disorders, as shown in Figs. 2 and 3. The body weights and Lee indexes of obese mice were obviously larger than those of control mice. Similarly, the food efficiency ratio (FER) of obese mice was higher than that control mice. Allicin treatment for 15 weeks significantly decreased the body weights [$F(4, 45) = 25.567$, $p < 0.05$], Lee indexes [$F(4, 45) = 2.861$, $p < 0.05$], and FER of obese mice and showed no inhibitory effect on food consumption.

Considering that allicin reduced obesity, the basal metabolic indexes were also detected to assess whether allicin could protect against metabolic disorders. Compared with the control group, obese mice exhibited higher plasma levels of fasting glucose, fasting insulin, triglyceride (TG), and total cholesterol (TC), as well as damaged glucose tolerance. Of interest, allicin significantly attenuated the levels of all metabolic indicators in obese mice. The data were subjected to a

Fig. 1 The experimental arrangement of the present study



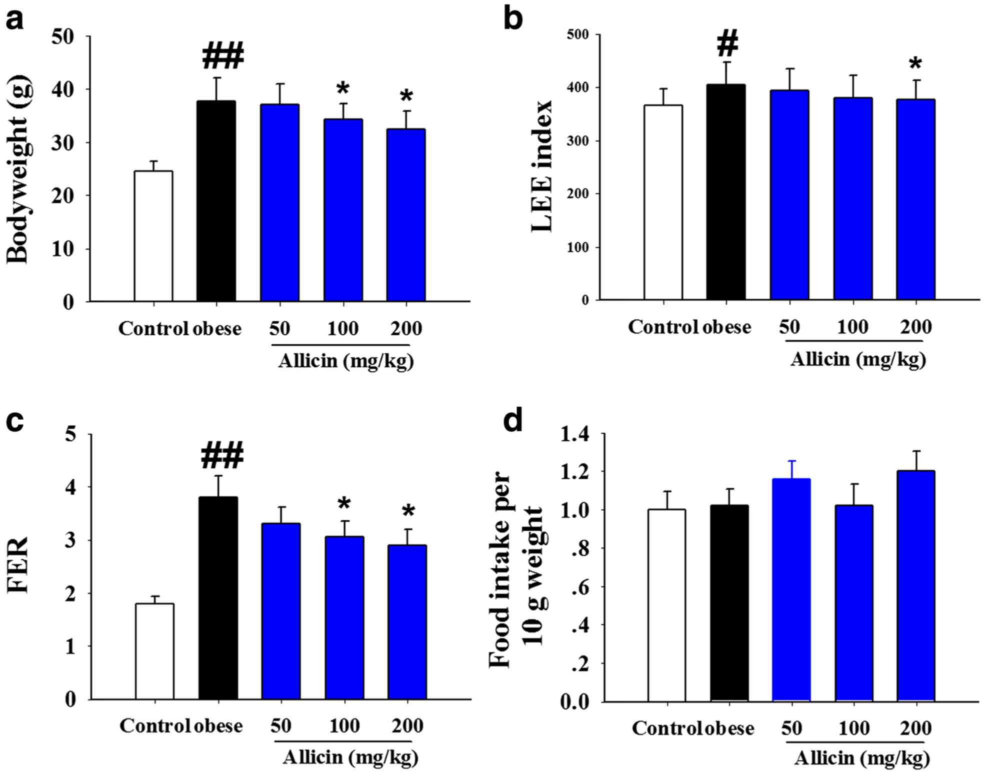
one-way analysis of variance (ANOVA) with allicin treatment as the factor and revealed the significant effect of allicin [fasting glucose: F(4, 45) = 44.297, $p < 0.05$; fasting insulin: F(4, 45) = 33.086, $p < 0.01$; area under curve of oral glucose tolerance test: F(4, 45) = 53.873, p < 0.01; TG: F(4, 45) = 5.213, p < 0.05; TC: F(4, 45) = 70.552, p < 0.01]. The results indicated that allicin ameliorated HFD-induced obesity and metabolic disorders.

Effects of allicin on depressive-like behaviors in obese mice

To investigate the behavioral changes in the obese mice and the protective effects of allicin, the sucrose preference test (SPT), open field test (OFT), and tail suspension test (TST) as classical experiments were performed. As shown in Fig. 4, compared with control mice, the obese mice showed substantial reductions in the ratio of sucrose water consumption (Fig. 4a) and mobility times in TST (Fig. 4b), indicating the appearance of behavioral despair. Furthermore, the results of OFT showed that HFD consumption significantly reduced the entry number (Fig. 4c) and time spent in the center of the open field (Fig. 4d) compared with those exhibited by the control mice. Interestingly, allicin treatment for 15 weeks reversed the behavioral defects in the obese mice by elevating sucrose consumption, mobility time in TST, and entry number and time spent in the center of the open field in OFT. One-way ANOVA revealed the significant effects of allicin on the behavioral tasks in obese mice [SPT: F(4, 45) = 107.54, $p < 0.05$; TST: F(4, 45) = 120.996, $p < 0.01$; entry number in the open field: F(4, 45) = 49.623, p < 0.01; time spent in the center of the open field: F(4, 45) = 120.996, p < 0.05].

In addition, there was a remarkable increase in corticosterone level in the serum of obese mice compared with that in control mice (Fig. 3f). Allicin supplement induced a significant decrease on the corticosterone content in obese mice, as demonstrated by one-way ANOVA [F(4, 45) = 17.146, p < 0.01].

Fig. 2 The effects of allicin on the (**a**) bodyweight, (**b**) LEE index, (**c**) FER, (**d**) food intake per 10 g bodyweight. Data are expressed as mean ± SD, $n = 10$. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, versus the obese group



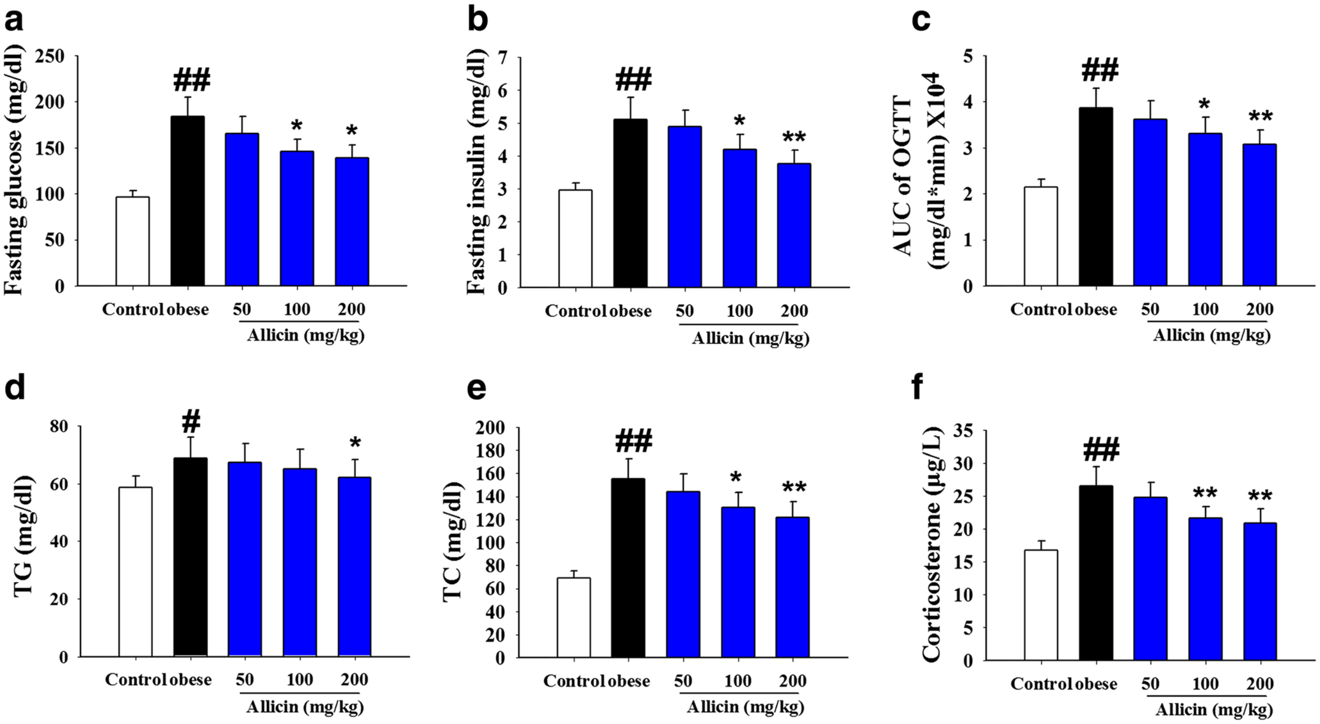
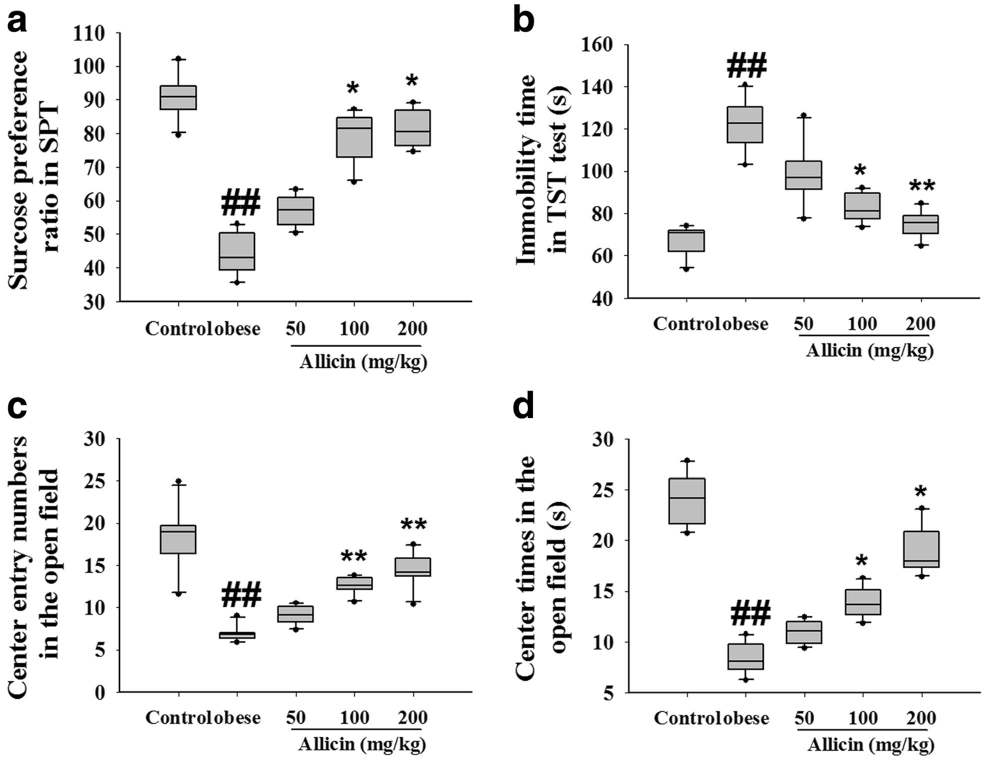


Fig. 3 The effects of allicin on the metabolic indexes of obese mice. Treatment of allicin significantly improved (**a**) fasting glucose, (**b**) fasting insulin, (**c**) AUC of OGTT, (d) TG, (**e**) TC, (**f**) corticosterone. Data are expressed as mean ± SD, n = 10. #p < 0.05, ##$p < 0.01$, versus the control group, *p < 0.05, **p < 0.01, versus the obese group

The present data confirmed that long-term HFD consumption evidently induced depressive-like behaviors, which were successfully ameliorated by allicin. Treatment at 200 mg/kg showed remarkable beneficial effects and thus, this dose was selected to investigate the potential mechanisms of allicin.

Fig. 4 The effects of allicin on the behavioral tasks. Treatment of allicin significantly improved (**a**) sucrose preference ratio, (**b**) tail suspension test, (**c**) center entry numbers in the open field, (**d**) times spent in the center of the open field. Data are expressed as mean ± SD, n = 10. ##p < 0.01, versus the control group, *p < 0.05, **p < 0.01, versus the obese group



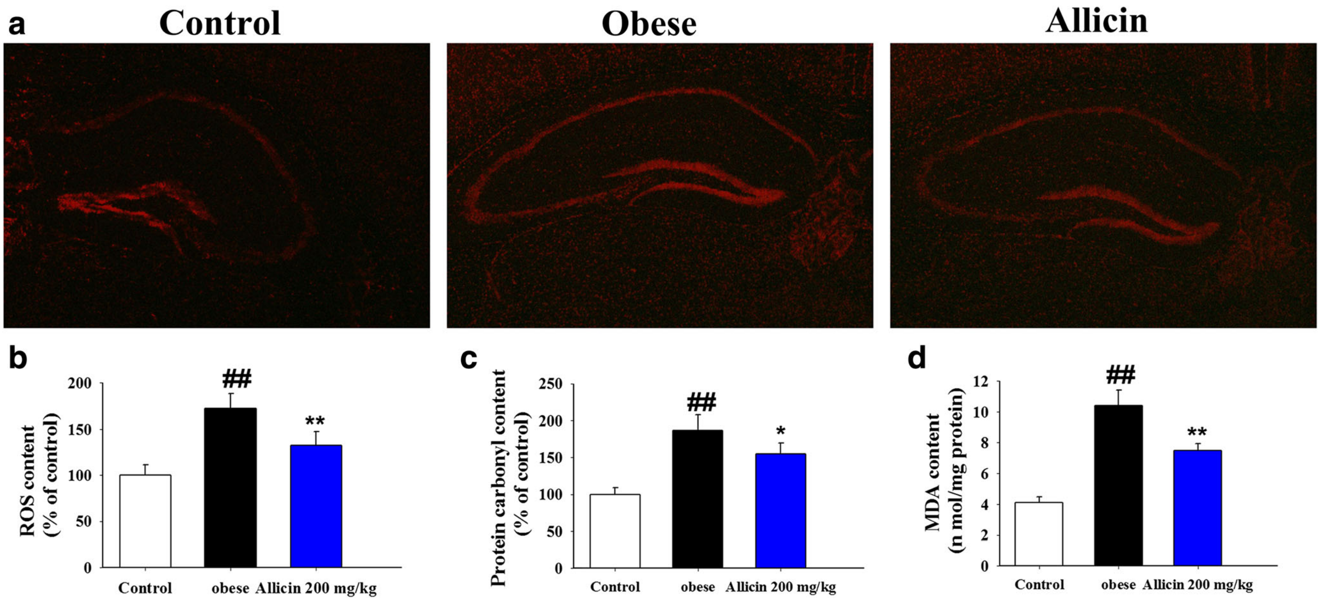
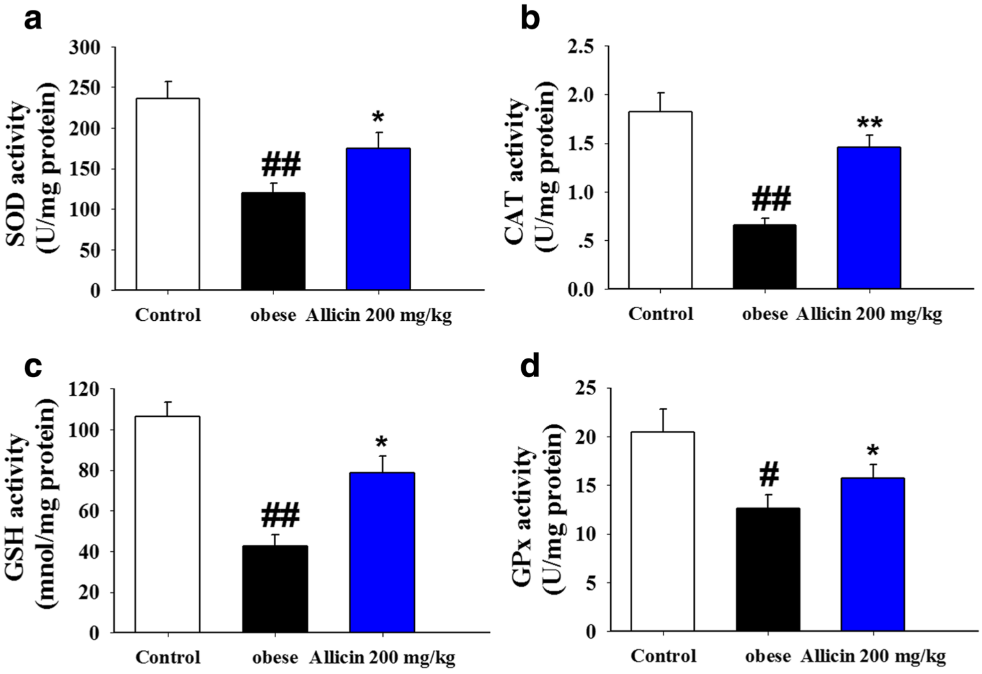


Fig. 5 The effects of allicin on oxidative stress in the hippocampus of the obese mice. Treatment of allicin significantly decreased (**a**) ROS staining, content of (**b**) ROS (**c**) MDA (D) Protein carbonyl production. Data are expressed as mean ± SD, *n* = 8. ##p < 0.01, versus the control group, *p < 0.05, **p < 0.01, versus the obese group

Effects of allicin on oxidative stress markers in the hippocampus of obese mice

As shown in Fig. 5, a significantly elevated level of ROS was observed in the hippocampus of obese mice compared with that of control mice. ROS over-production was inclined to induce lipid peroxidation and oxidative stress. Thus, we examined the end products of lipid and protein peroxidation. The obese mice showed significantly higher levels of malondialdehyde (MDA) and protein carbonylation in the hippocampus than those of control mice. In turn, ROS over-production, elevated MDA levels, and increased protein carbonylation triggered by HFD consumption were inhibited by allicin treatment. Furthermore, the activities of the anti-oxidant enzymes SOD, CAT, GSH, and GPx were significantly downregulated in the hippocampus of obese mice (Fig. 6), and treatment with allicin reversed these effects. These results indicated that allicin possessed beneficial effects in ameliorating oxidative stress-induced injury through elevating the activity of the anti-oxidant enzymes SOD, CAT, GSH, and GPx

Fig. 6 The effects of allicin on activities of antioxidant enzymes in the hippocampus of the obese mice. Treatment of allicin significantly increased (**a**) SOD (**b**) CAT (**c**) GSH (D) GPx activities. Data are expressed as mean ± SD, n = 8. #p < 0.05, ##p < 0.01, versus the control group, *p < 0.05, **p < 0.01, versus the obese group



and inhibiting ROS over-production, MDA activity, and protein carbonylation in the hippocampus of obese mice.

Effects of allicin on mitochondrial function in the hippocampus of obese mice

Oxidative stress could injure the mitochondrial respiratory chain and mitochondrial function. Therefore, adenosine triphosphate (ATP) production and the activities of mitochondrial respiratory chain complexes as an indication of mitochondrial function were determined. As illustrated in Fig. 7, obese mice showed a remarkable decline in ATP production and the activities of complexes I, II, III, and IV in the hippocampus, suggesting that mitochondrial dysfunction occurred. Interestingly, all of these were effectively restored by allicin treatment. Taken together, our results indicated that allicin possessed protective effects on mitochondrial function in the hippocampus of obese mice.

Effects of allicin on autophagy biomarkers in the hippocampus of obese mice

Considering that autophagy was involved in clearing damaged biomacromolecules and organelles injured by oxidative stress, the protein expression of autophagy markers was determined in the hippocampus. As shown in Fig. 8, HFD caused a significant increase in the protein expression of autophagy biomarkers in the hippocampus, and these altered protein levels were restored by allicin. Collectively, these findings revealed that allicin repaired HFD-stimulated autophagy imbalance in the hippocampus.

Effects of allicin on insulin resistance in the hippocampus of obese mice

Previous studies and our results both indicated that HFD-fed mice exerted dramatic systemic insulin resistance. To evaluate whether the insulin signaling pathway was abnormal in the hippocampus of obese mice and the effects of allicin therein, we analyzed the activation of the protein kinase B/cyclic-AMP response element binding/brain-derived neurotrophic factor (AKT/CREB/BDNF) pathway in the hippocampus. As shown in Fig. 9, the phosphorylation of AKT (Ser473) and that of CREB (Ser133) were both down-regulated in the hippocampus of obese mice and effectively up-regulated after allicin treatment. As a downstream target of the AKT/CREB/BDNF pathway, BDNF expression decreased after HFD feeding but was substantially restored by allicin. All in all, our results demonstrated that allicin significantly lowered insulin resistance and corrected aberrant insulin signaling triggered by HFD in the hippocampus.

Effects of allicin on NOX/Nrf2 balance in the hippocampus of obese mice

The physiological functions of NOX2 and NOX4 are main contributors to ROS production. Moreover, the Nrf2/heme oxygenase 1 (HO-1) pathway is a major protective cellular defense mechanism against oxidative stress. Therefore, we questioned whether HFD feeding induced an imbalance of NOX/Nrf2 in the hippocampus (Fig. 10). Using quantitative polymerase chain reaction, western blot, and immunohistochemistry, we found that the mRNA and protein expressions of NOX2 and NOX4 were up-regulated, while Nrf2/HO-1 signaling was down-regulated in the hippocampus of obese mice. However, allicin treatment reduced NOX2/NOX4 expression and increased Nrf2/HO-1 levels, suggesting that the anti-depressant effects of allicin in obese mice were correlated with the regulation of NOX/Nrf2 imbalance.

Discussion

Our present study demonstrated for the first time the protective effects of allicin against HFD-induced depressive-like behaviors, and the underlying mechanisms were investigated

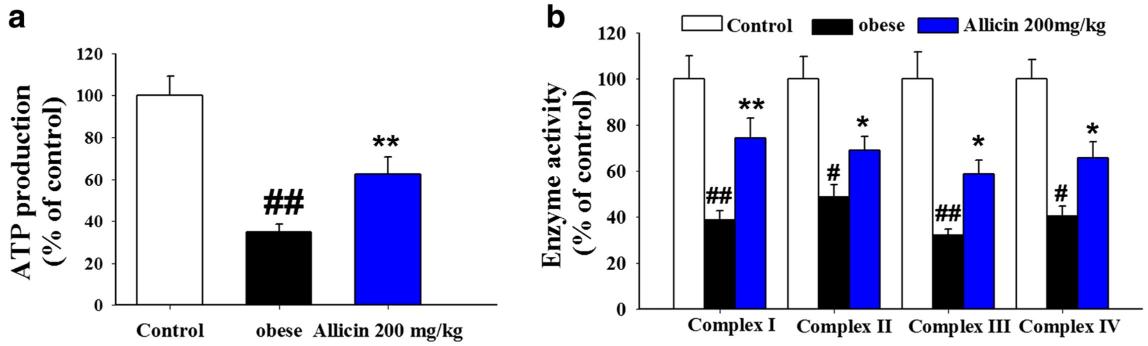
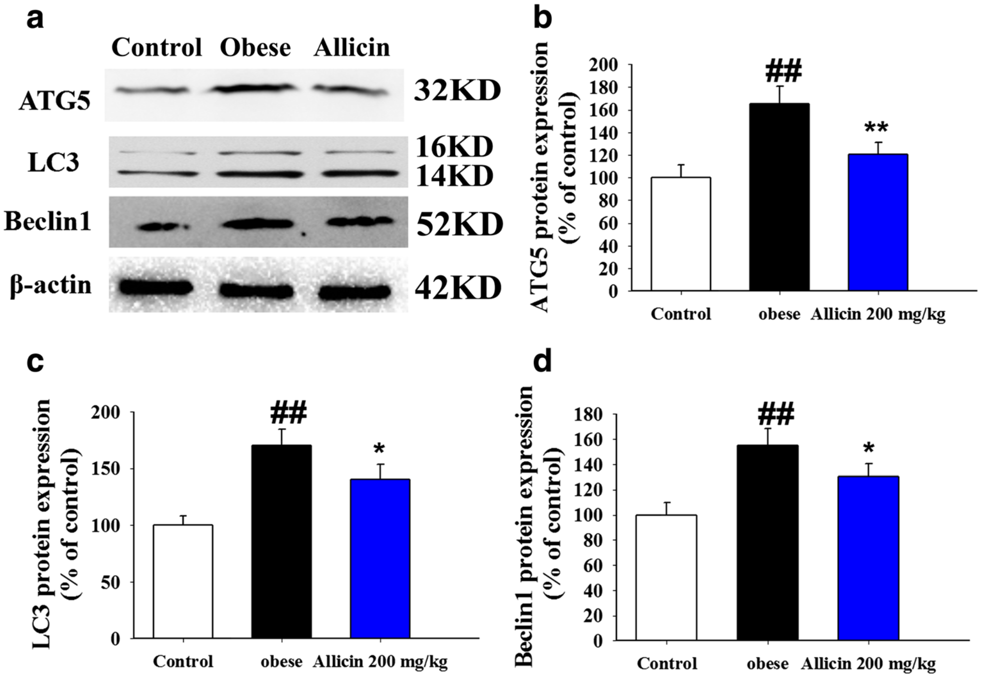


Fig. 7 The effects of allicin on mitochondrial function in hippocampus of the obese mice. Treatment of allicin significantly increased production of (A) ATP and activities of (B) complex I, II, III, IV. Data are expressed as mean ± SD, n = 8. #$p < 0.05$, ##$p < 0.01$, versus the control group, *$p < 0.05$, **$p < 0.01$, versus the obese group

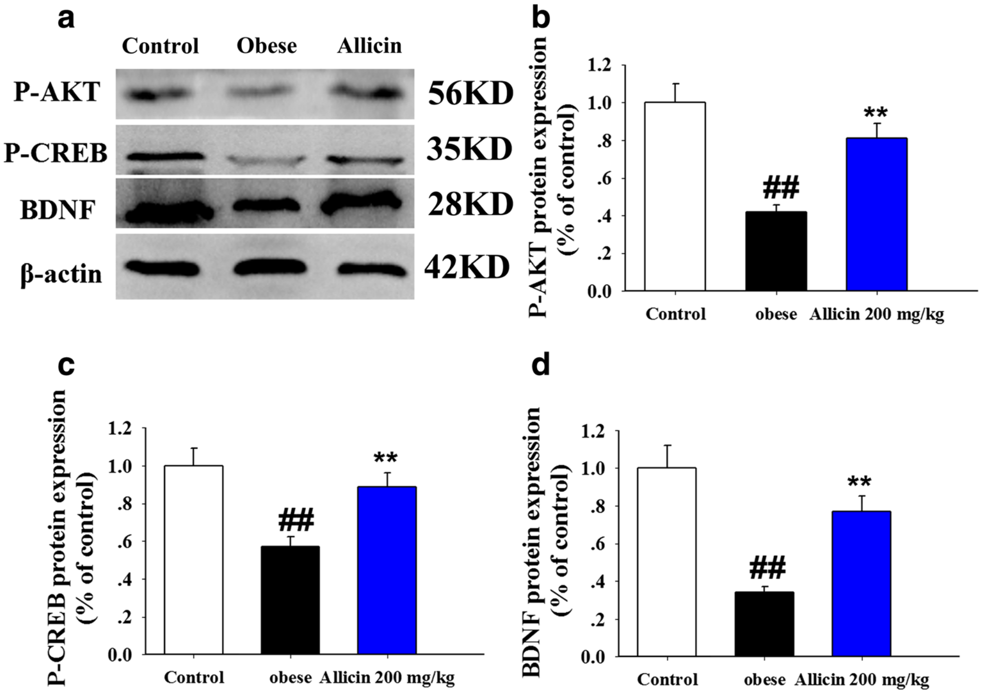
Fig. 8 The effects of allicin on autophagy markers in the hippocampus of obese mice. Treatment of allicin significantly decreased protein expressions of (**a**) ATG5 (**b**) LC3 (**c**) Beclin1. Data are expressed as mean ± SD, *n* = 4. #p < 0.05, ##p < 0.01, versus the control group, *p < 0.05, **p < 0.01, versus the obese group



with respect to anti-oxidation. First, we provided evidence supporting that allicin remarkably ameliorated abdominal obesity, hyperglycemia, hyperinsulinemia, hyperlipidemia, systematic insulin resistance, and brain oxidative stress triggered by HFD. Correspondingly, allicin attenuated depressive-like behaviors in obese mice. Moreover, the comprehensive beneficial effects on the behaviors of obese mice generated by allicin were mediated by reduced oxidative stress, alleviated mitochondrial injury, improved autophagy balance, and lowered insulin resistance in the hippocampus.

Obesity and mood disorders have frequently been cross-correlated. Population-based studies have revealed that overweight and obesity increased the risk of the onset of depressive symptoms (Dong et al. 2004; Zhao et al. 2009). Furthermore, researchers have proposed that co-occurrence of obesity and depressive symptoms might be considered a

Fig. 9 The effects of allicin insulin signaling pathway in the hippocampus of obese mice. (**a**-**d**) Protein expressions of P-AKT, CREB and BDNF. Data are expressed as mean ± SD, n = 4. #p < 0.05, ##p < 0.01, versus the control group, **p < 0.01, versus the obese group



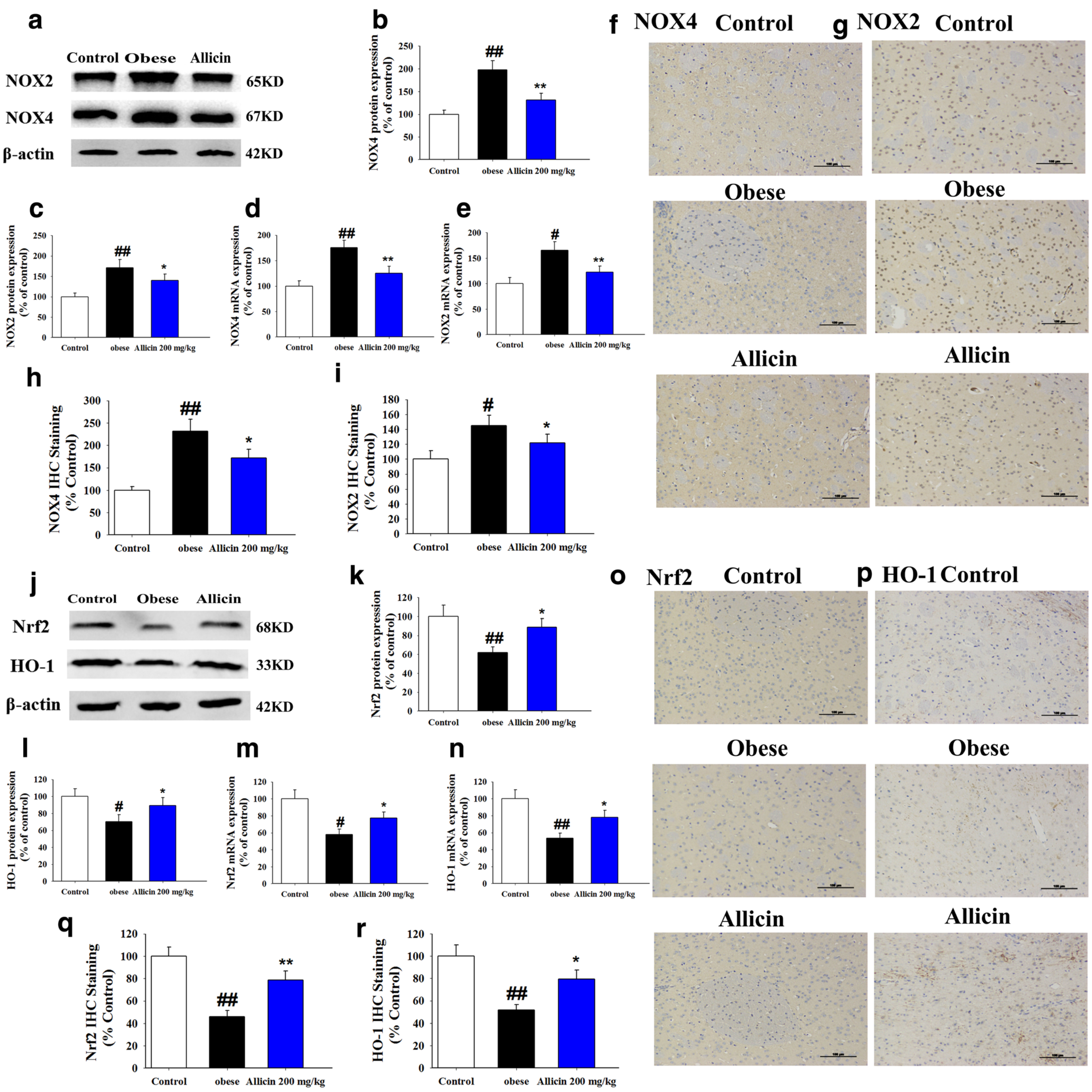


Fig. 10 The effects of allicin on NADPH oxidase expressions and Nrf2/HO-1 signaling pathway in hippocampus of obese mice. (**a**-c) Protein expressions of NOX2 and NOX4. (**d**-**e**) mRNA expressions of NOX2 and NOX4. (F-I) Immunohistochemistry staining and analysis of NOX2 (**f**, **h**) and NOX4 (**g**, **i**). (**j**-**l**) Protein expressions of Nrf2 and HO-1. (**m**-**n**) mRNA expressions of Nrf2 and HO-1. (**o**-**r**) Immunohistochemistry staining and analysis of Nrf2 (**o**, **q**) and HO-1 (**p**, **r**). Data are expressed as mean ± SD, n = 4. #p < 0.05, ##p < 0.01, versus the control group, *p < 0.05, **p < 0.01, versus the obese group

distinct illness subtype known as metabolic mood syndrome (McIntyre et al. 2007; Vogelzangs et al. 2011; Levitan et al. 2012). In animal studies, Yamada et al. showed that mice exposed to long-term HFD exhibited symptoms of depression (Yamada et al. 2011). The results of our study are consistent with those in recent studies, showing that a 15-week HFD induced depressive-like behaviors as demonstrated by three behavioral tasks: sucrose preference, open field, and tail suspension tests. We also observed that increased corticosterone levels followed chronic HFD intake. More interestingly, in addition to attenuating abdominal obesity, preventing metabolic disorders, and inhibiting brain oxidative stress, allicin dramatically ameliorated the behavioral defects in obese mice. Numerous studies have demonstrated that allicin treatment attenuated obesity, insulin resistance, lipid profiles, and oxidative damage in HFD-induced obese mice (Dong et al. 2014;

Kim et al. 2013). Here we extend the knowledge of allicin to its neuroprotective effects against mood disorders in the context of metabolic disturbances.

The beneficial effects of allicin in the co-occurrence of obesity and depressive symptoms were successfully observed in our present study and the mechanisms involved therein were subsequently investigated. Oxidative stress might be the primary contributor to the shared and common pathogenesis of obesity and depression. On the one hand, excess ROS and oxidative stress were present in obesity, which in turn render mitochondria vulnerable to oxidative damage and dysfunction (Kroller-Schon et al. 2014; Daiber et al. 2017). Moreover, ROS generated within the mitochondria subsequently targeted various components of the electron transport chain (in particular, complexes I and III), resulting in a vicious forward feed cycle of enhanced ROS production, severe ATP depletion, and ultimately neuron death. On the other hand, the insulin signaling pathway could be inhibited by increased ROS, resulting in insulin resistance (Furukawa et al. 2004). Meta-analysis revealed that insulin resistance is correlated with various brain disorders, including depression (Kan et al. 2013). Furthermore, the adjunctive use of insulin sensitizers exerted markedly beneficial effects in depression therapy (Zong et al. 2018; Zhao et al. 2017). Because of the low anti-oxidant capacity and non-replicative nature of neuronal cells, brain tissues are particularly sensitive to oxidative stress, vulnerable to oxidative damage, and liable to neuron death. In line with this knowledge, our present study showed obvious oxidative stress in the hippocampus of obese mice, as displayed by the conspicuous increase in the levels of ROS, MDA, and protein carbonylation and decreased activities of anti-oxidant enzymes such as SOD, CAT, GSH, and GPx. Allicin remarkably inhibited ROS generation, attenuated oxidative stress, and elevated the activities of anti-oxidant enzymes in the hippocampal region, suggesting that it might target oxidative stress in the development of obesity comorbid depressive-like behaviors. We also showed that chronic HFD feeding triggered a decrease in ATP production and the activities of mitochondrial respiratory enzyme complexes, suggesting that mitochondrial dysfunction occurred during HFD-induced stress. The present study indicated that allicin protected the hippocampus of obese mice from oxidative damage and improved mitochondrial functions. Additionally, recent evidences have reported that oxidative stress was intimately associated with insulin resistance via regulation of AKT signaling (Pipatpiboon et al. 2012). The P-AKT/P-CREB/BDNF signaling pathway plays an important role in the regulation of hippocampal function and thus, the activity of this pathway was examined in the hippocampus of obese mice. In accordance with these investigations, obese mice in our study exhibited suppressed insulin signaling activity, suggesting that hippocampal functions might be defected by the phenomenon of insulin resistance. However, allicin restored these hippocampal changes, manifesting that allicin lowered hippocampal insulin resistance via up-regulation of the P-AKT/P-CREB/BDNF signaling pathway in obese mice.

Oxidative stress could lead to autophagy (Cao et al. 2009; Li et al. 2015). ROS over-production, which participates in the pathological mechanisms of depression, is an important initiator of autophagy. Anti-depressants could inhibit oxidative stress and may also affect the autophagic system. Rapamycin, an inhibitor of mammalian target of rapamycin, exerts anti-depressive effects in depressed mice by maintaining essential neuron autophagy, suggesting that autophagy plays a significant part in the pathophysiological process of depression and is critically involved in the anti-depressant action. Therefore, autophagy is a crucial biological and cellular pathogenesis mechanism of depression, and improved regulation of autophagy could benefit depression treatment (Xu et al. 2014). Previous studies have suggested that the disturbance in autophagy balance was associated with several neurodegenerative and mood disorders (Keller and Lunemann 2017; Yin et al. 2017). Many anti-depressants facilitate autophagy flux and induce the expression of autophagy markers (Gassen et al. 2015; Jia and Le 2015). In agreement with previous studies, we found that the protein expressions of autophagy-associated genes ATG5, LC3, and Beclin 1 were up-regulated in the hippocampus of obese mice, which suggested that oxidative stress resulted in abnormal autophagy and impaired the autophagic system. The present study indicated that abnormal autophagy was improved by allicin treatment.

The consequence of chronic stress is the accumulation of oxidative stress in the brain (Zafir and Banu 2009). Oxidative stress is caused by ROS production and results in lipid peroxidation, protein degradation, and DNA damage (Ma et al. 2017). NOX is reported to work as a major generator of ROS and a primary contributor in stress-induced production of oxidative stress (Seo et al. 2012). Therefore, NOX inhibition could attenuate ROS production and subsequently reduce depressive-like behaviors. We thus investigated whether NOX was involved in the progression of obesity comorbid depression and showed that the mRNA and protein expressions of NOX2 and NOX4 obviously increased in obese animals, whereas allicin reduced them. In addition, mounting evidence has reported the cross-talk between NOX and Nrf2, and NOX/Nrf2 redox balance serves as an effective therapeutic strategy against brain damage and disorders (Chandran et al. 2017; Dai et al. 2018). Thus, the mRNA and protein expressions of Nrf2/HO-1 were detected in the hippocampus of obese mice. Allicin treatment increased the mRNA and protein expressions of Nrf2/HO-1 and corrected the NOX/Nrf2 imbalance triggered by HFD.

Allicin, rich in sulfur-containing amino acids, has almost no toxicity and has been used as a popular traditional Chinese medical agent for centuries. Allicin possesses a broad range of health-promoting properties, such as anti-inflammation, antihypertension, anti-microbial, and anti-oxidation (Hosseini and Hosseinzadeh 2015). Accumulating research has suggested that allicin treatment exhibited anti-diabetic, hypotensive, and hypolipidemic effects (Ali et al. 2000). In the present study, allicin obviously decreased systemic obesity, lowered insulin resistance, and reduced lipid levels in obese mice. It is also reported that allicin could pass the blood-brain barrier and accumulate at high levels in the brain to carry out neuroprotective functions (Chung 2006; Chauhan 2003). Importantly, allicin-treated animals exhibited a significant increase in sucrose water consumption, mobility time spent in the TST, and entry number and time spent in the center of the open field in the OFT. These signs indicated that allicin ameliorated obesity comorbid depressive behaviors and alleviated brain insulin resistance by enhancing P-AKT/P-CREB/BDNF pathway activation. Compared to ROS scavenging, allicin may be more successful in attenuating oxidative stress and thus may become a novel therapeutic strategy in place of ROS inhibition (Ma et al. 2017).

In addition, there was a limitation in the present study that we have not measure the inflammation. Recent studies showed that low-grade chronic inflammation is an important factor in the pathogenesis of obesity in humans and rodent animal models (Li et al. 2009; Jiang et al. 2017; Saltiel and Olefsky 2017). Adipose tissue have been considered as an important endocrine organ modulate energy expenditure and glucose homeostasis (Stolarczyk 2017). Adipose tissue is composed of brown and white tissue. Brown adipose tissue functioned as an producer for non-shivering thermogenesis and energy dissipation that can serve to protect against diet-induced obesity. White adipose tissue is responsible for the secretion of adipokines, especially pro-inflammatory factor, to modulate inflammation under the obesity status (Kwon and Pessin 2013). Inflammation in adipose tissues is an significant mechanism to induce insulin resistance that is the primary pathogenesis of obesity (Grillo et al. 2019). In obese humans and rodent models, the peripheral insulin resistance is enhanced by the higher expression of pro-inflammatory adipokines. The major pro-inflammatory factors secreted from adipose tissue are IL-6 and TNF-α. The secretion levels of IL-6 and TNF-α from adipose tissue is increased in obese patients and animal model, and participates in the development of insulin resistance (Kern et al. 2018). In addition, another two major adipokine, termed adiponectin and leptin, also play an important role in metabolism. Adiponectin is highly expressed by adipocytes with potent anti-inflammatory properties. However, the level of adiponectin is decreased in obese humans and animal models. The pro-inflammatory factors such as TNF-α and IL-6 suppress the expression of adiponectin in adipocytes (Kern et al. 2018). All these adipokines regulate inflammation involved in the insulin resistance, which is the main approach for brain injury in metabolic disturbance. In our follow-up work, we prepared to focus on the crosstalk between the adipose tissue and brain, start with inflammation. Adipose tissue derived pro-inflammatory adipokines functioned as the key connections to joint the adipose tissue and brain.

On the basis of these results, we demonstrated that allicin reduced depressive-like behaviors in the context of obesity. We speculated that the mechanisms might be attributed to the inhibition of ROS production, subsequently attenuating oxidative stress, mitochondrial damage, autophagy, and insulin resistance. Therefore, allicin could be recommended as a possible effective candidate for the prevention and therapy of obesity comorbid depression.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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