

Research paper

High fat diet-induced obesity leads to depressive and anxiety-like behaviors in mice via AMPK/mTOR-mediated autophagy



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ABSTRACT

Depression is one of the most common mental illnesses in modern society. In recent years, several studies show that there are disturbances in lipid metabolism in depressed patients. High-fat diet may lead to anxiety and depression, but the mechanisms involved remain unclear. In our study, we found that 8 weeks of high-fat feeding effectively induced metabolic disorders, including obesity and hyperlipidemia in mice. Interestingly, the mice also showed depressive and anxiety-like behaviors. We further found activated microglia and astrocyte, increased neuroinflammation, decreased autophagy and BDNF levels in mice after high-fat feeding. Besides, high-fat feeding can also inhibit AMPK phosphorylation and induce mTOR phosphorylation. After treating with the mTOR inhibitor rapamycin, autophagy and BDNF levels were elevated. The number of activated microglia and astrocyte, and pro-inflammation levels were reduced. Besides, rapamycin can also reduce the body weight and serum lipid level in high fat feeding mice. Depressive and anxiety-like behaviors were also ameliorated to some extent after rapamycin treatment. In summary, these results suggest that high-fat diet-induced obesity may lead to depressive and anxiety-like behaviors in mice by inhibiting AMPK phosphorylation and promoting mTOR shift to phosphorylation to inhibit autophagy. Therefore, improving lipid metabolism or enhancing autophagy through the AMPK/mTOR pathway could be potential targets for the treatment of obesity depression.

1. Introduction

Depression is one of the most common mental illnesses in modern society (Monroe et al., 2019). It is characterized by persistent depressed mood with insomnia, loss of appetite, anxiety, motor agitation, hallucinations, and even suicidal behavior in some patients (Belvederi Murri et al., 2020; Monroe et al., 2019). The World Health Organization predicts that depression will surpass cardiovascular disease as the largest burden of disease worldwide by 2030 (Kupfer et al., 2016). Depression and anxiety are complex psychiatric disorders caused by multifactorial disorders in the organism, and their pathogenesis involves many aspects, such as hypothalamic-pituitary-adrenal axis (HPA axis) disorders, damage to the limbic system, neurotransmitter dysregulation, and neurotrophic deficiencies (Kokras et al., 2012). The hippocampus is a key brain region for learning, memory and emotional disorders, and the damage and protection of the hippocampus is an important factor for the

occurrence and recovery of depression and anxiety (Sheline et al., 2019). Besides, the hippocampus is the most researched brain region for depression. Recent epidemiological studies have found that individuals with obesity are at increased risk for developing mood disorders (O'Brien et al., 2017) such as major depression, the most common type of depression (Milaneschi et al., 2019). Whether depression and obesity are intrinsically linked and whether depression causes patients to become obese secondary to obesity or whether obesity leads to the development of depressive symptoms has been the unanswerable question.

Lipid metabolism levels are disturbed in obese depressed patients (Lasserre et al., 2017) and that there are differences in lipid metabolism levels such as blood lipids and fatty acids in obese depressed patients compared to healthy people (Oliveira et al., 2016). A high-fat diet allows people more susceptible to obesity. Excess saturated fatty foods can affect the body's homeostasis, and a diet rich in fatty foods can reduce hippocampus volume, impair cognitive functions including memory,

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psychomotor efficiency, human attention (Abbott et al., 2019), and more importantly increase their susceptibility to depression and anxiety (Zemdegs et al., 2016), but it has not been shown through what mechanism obesity causes depression to occur. A high-fat diet increases the intrinsic body fat content that can cause adipose tissue to secrete large amounts of inflammatory factors, and an abnormal increase in peripheral inflammatory factors is one of the major causes of insulin resistance in insulin target organs (Lee and Dixit, 2017). At the same time, immune activation due to an abnormal increase of inflammatory factors represents one of the pathogenic mechanisms of depression (Miller and Raison, 2016). Obesity can play a role in mediating neuroimmune and depression as an initiating mediator of the immune-inflammatory response in the brain (Saitiel and Olefsky, 2017).

Autophagy, as a fundamental response to stress in eukaryotic cells, is involved in a variety of cellular activities to maintain dynamic homeostasis under stressful conditions (Nikoletopoulou et al., 2017). It mainly participates in the process of intracellular material turnover, allowing some damaged proteins or organelles and intracellular pathogens to be degraded by lysosomes for recycling, allowing the normal metabolism and function of the cell to be maintained, thus maintaining the normal operation of the organism (Sciarretta et al., 2018). Autophagy not only regulates lipid metabolism, improves insulin resistance, and reduces oxidative stress, but also protects nerve cells by mitigating nerve cell damage (Deretic, 2021; Nabavi et al., 2018). Excessive accumulation of lipids leads to lipoatrophy, which causes inflammatory oxidative stress and cellular damage, and autophagy also protects cells from damage by limiting inflammation and oxidative stress (Morel et al., 2017; Nabavi et al., 2018). The formation of autophagy is associated with a number of autophagy-related proteins. For example, Beclin1 is an essential molecule for autophagosome formation, and the expression level of Beclin1 tends to be elevated during autophagy (Chen et al., 2019). Microtubule-associated protein light chain 3 (LC3) is a marker molecule of autophagosomes, and mature LC3 is referred to as LC3-I, while phosphatidylethanolamine-bound LC3-I is referred to as LC3-II. Because LC3-II is consistently associated with autophagosomes, increased LC3-II is commonly used as a marker of autophagic activation. p62 is a classical substrate of autophagy, and increased p62 indicates an increase in autophagic response to intracellular misfolded or damaged proteins, and increased expression levels of ATG7 also suggest that autophagy is activated (Hirano et al., 2016). mTOR is a key regulatory molecule in the autophagic process (Winden et al., 2018). Under physiological conditions, mTOR activation can block autophagy. Activation of mTOR can be inhibited by phosphorylation of AMPK, which is one of the classical pathways of autophagic signaling (Huang et al., 2018).

In this study, we produced excessive obesity in mice by high-fat diet and investigated the changes of lipid metabolism, inflammation, and autophagy in excessive obesity. Our results showed that a high-fat diet caused depressive and anxiety-like behaviors in mice and led to disruption of lipid metabolism, an increase of inflammation, mTOR phosphorylation, and reduced autophagy level. And these phenotypes were partially reversed by treating with mTOR inhibitor rapamycin. Collectively, these results suggest a close relationship between obesity, hyperlipidemia and neurological disorders.

2. Material and methods

2.1. Experimental animals and diets

Male C57BL/6 mice (7–8 weeks old, about 20 g) were obtained from Laboratory conditions without specific pathogens, under 12 h of light / the dark cycle, food and water are freely available at the appropriate temperature ($22 \pm 2^\circ\text{C}$) and 60% humidity. All animal experiments were conducted following the requirements of the Experimental Animal Committee, and the ethics of the Laboratory Animal Center were obtained in the advance license. The mice were randomly divided into four groups, the first group was fed with normal diet (ND), the second group

was fed with high-fat diet (HFD), the third group was given an intraperitoneal injection of rapamycin (2 mg/kg, every day) after one month of high-fat diet (HFD-Rapamycin), the fourth group was given an intraperitoneal injection of saline (containing 1% DMSO, every day) after one month of high-fat diet as the control of the drug administration group (HFD-Saline), and the drug administration was continued during the period of high-fat chow. After 8 weeks, each group of mice was tested for depressive and anxiety-like behaviors.

2.2. Behavioral tests

2.2.1. Open field test (OFT)

The OFT is a behavioral test used to systematically assess anxiety responses and exploration of emotions or new environments in mice (Takase et al., 2016). The open-field apparatus (96 × 96 × 50 cm) was divided into 9 equal squares. Firstly, place the mice in the center area and let them acclimate to the environment for 2 min. Then record the total moving distance, average speed, threading times, and staying time in the center within 3 min. After each mouse experiment, the floor surface and the inner wall of the OFT device were thoroughly cleaned with 75% ethanol to eliminate any signs of olfactory cues, and the device was dried between each test.

2.2.2. Elevated-plus maze (EPM)

The EPM can be used to assess anxiety-like behavior (Sharma and Fulton, 2013). The instrument consists of a white elevated plus maze and four arms. The EPM device is a white labyrinth, 60 cm above the ground, with two open arms (30 × 5 cm), two closed arms (30 × 5 × 15 cm), and a central area (5 × 5 cm). The mice were placed in the central area facing the outstretched arm and allowed to explore for five minutes while a video-tracking system recorded their behavior. Several variables were analyzed including the number of times the mouse entered the open arm within 5 min and the time spent in the open arm. After each experiment, spray the bottom of the open field with 75% ethanol and wipe it dry with a clean rag to prevent the residual odor of the previous animal from affecting this experiment.

2.2.3. Sucrose preference test (SPT)

The sucrose preference test is a method used to assess depressive states by detecting the animal's response to natural rewards (Yamada et al., 2011). Two identical drinking bottles were placed on all mice cages. One contains 1% sucrose and the other contains tap water. Keeping the weight of the two bottles as consistent as possible for 24 h. The positions of the two bottles were interchanged every 6 h to avoid the influence of position preference on the measurement results. After training, all mice fasted for 12 h before the start of SPT. Then after the end of SPT, the intake of sucrose and water is measured. The sucrose preference rate is expressed as the percentage of sucrose intake/sucrose intake plus pure water intake. The sucrose preference rate was positively correlated with the pleasure of mice. The larger the value, the more normal mice; the smaller the value, the more severe the depression-like symptoms of mice.

2.2.4. Forced swim test (FST)

The forced swimming test was a highly reliable experiment for evaluating depression-like behavior (Yamada et al., 2011). Place mice individually in a transparent glass cylinder (25 cm in diameter) filled with 30 cm deep water (maintained at $24 \pm 1^\circ\text{C}$). The mice swam in the glass tank for 6 min, and the cumulative immobility time was recorded for 4 min afterward. The immobility time was defined as the time when the mice stopped struggling in the water and were floating, or only had small limb movements to keep their head floating above the water surface. The duration of immobility in the water reflects the degree of depression of the mice, and the longer the immobility, the more depressed the mice are. During the experiment, a proper light source and soft light should be maintained in the room.

2.2.5. Tail suspension test (TST)

Depression was measured by comparing the time the animals remained stationary after their tails were suspended (Takase et al., 2016). Mice suspended by tape from the tip of their tails at about 1 cm above the ground for about 40 cm exhibit escape-like behavior and then become immobile. After an initial period of struggle, the mice adapt to immobility, which resembles a state of despair and mental depression. The test period is 6 min, and the cumulative immobility (the mice give up the active struggle and their carcasses hang without twisting) is recorded for the next 4 min, the longer the immobility, the more depressed they are.

2.3. Determination of serum lipid content

After the behavior measurement was completed, the blood was collected by removing mouse eyeballs and placed in EP tubes. The blood was left standing at room temperature for 1–2 h, then placed in a refrigerated centrifuge, centrifuged at 3500 rpm for 15 min. The supernatant was taken and stored at 4°C for later use or –80°C. Lipid profiles (total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were assessed using commercially available assay kits (A110-1, A111-1, A112-1, A113-1, Nanjing Jiancheng Institute of Bioengineering, Nanjing, China), and were determined according to the kit instructions.

2.4. ELISA analysis of pro-inflammatory cytokines

The serum samples stored at –80°C were thawed and tested. The mean absorbance of each group of repeating standard and sample was determined. Serum levels of inflammatory cytokines IL-1 β , and TNF- α were measured using a commercial enzyme-linked immunoassay kit (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's protocol.

2.5. Western blotting

After the behavioral test was completed, the mice were first anesthetized and then the animals were killed by decapitation and the brain tissues were dissected to remove the hippocampus. The half hippocampus tissue was homogenized in a homogenizer containing 400 μ l of lysate and 10 μ l of PMSF. After dissolving on ice for 30 min, the sample was centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatant was separated. The protein concentration was determined using a BCA protein detection kit (Rockford Pierce Biotechnology Company, USA). The tissue lysate was subjected to SDS-PAGE (Bio-Rad) and transferred to a polyvinylidene fluoride (PVDF) membrane. Sealed with 5% skimmed milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) at room temperature for 1 h and rinsed, then mixed with different primary antibodies NLRP1 (1:1000; Abcam, ab3683), NLRP3 (1:800; Abcam, ab214185), ASC (1:1000; Affinity, DF6304), TNF- α (1:1000; Service, GB13188-1), BDNF (1:1000; Affinity, DF6387), IL-1 β (1:1000; Bioss, bs-0812R), Atg7 (1:1000; CST, 2631S), P62/SQSTM1 (1:1000; Affinity, AF5384), Beclin1 (1:1000; Affinity, AF5128), LC3 (1:1000; CST, 3868S), AMPK (1:1000; Affinity, AF6423), p-AMPK (1:1000; Affinity, AF3423), mTOR (1:1000; Affinity, AF6308), p-mTOR (1:1000; Affinity, AF3309), β -actin (1:5000; Affinity, AF7081) combined and incubated overnight in a refrigerator shaker at 4°C. The next day, the membrane was washed three times with TBST, and at room temperature, the membrane incubate with the corresponding secondary antibody (1:10000; Affinity, S002) in TBST containing 1% skim milk for 1 h, and then wash three more times with TBST at room temperature, bands were visualized using a Chemi Doc TMMP Imaging System (Bio-Rad; Hercules, CA, USA) and analyzed with Gel Pro Analysis software.

2.6. Immunofluorescent staining

Immunofluorescence analysis of paraffin-embedded brain tissue sections (4 μ m). Briefly, paraffin sections were dewaxed to water and then heated to 121°C for 10 min with 0.01 mol/l sodium citrate buffer (pH 6.0) for antigen extraction. After natural cooling, sections were washed with PBS and incubated in 3% H₂O₂ for 10 min to quench endogenous peroxidase activity. After PBS washing, sections were incubated with a blocking buffer for 30 min at room temperature. Primary antibodies were incubated overnight at 4°C. Nuclei were stained with DAPI (10 μ g/ml) for 3 min. Blocked slices were washed with PBS and shaken dry, images were obtained using an electron microscope (Leica, Germany) and evaluated with ImageJ software (NIH, Bethesda, MD, USA).

2.7. Statistical analysis

All data were analyzed using statistical software Prism 7.0 for Windows (GraphPad Software, USA). Data are expressed as mean \pm SEM. Statistical comparisons between the experimental group and the control group were performed using a one-way analysis of variance or Student *t*-test. *p* < 0.05 was considered statistically significant.

3. Results

3.1. The high-fat diet led to excessive obesity and hyperlipidemia in mice

As shown in Fig. 1B, after 8 weeks of high-fat feeding, mice gained more body weight than ND group mice ($t_{18} = 11.01$; *p* = 0.0001; Fig. 1B). Besides, an increase in levels of TG ($t_{34} = 2.042$; *p* = 0.049; Fig. 1C), TC ($t_{34} = 2.211$; *p* = 0.0339; Fig. 1D), and LDL-C ($t_{34} = 2.723$; *p* = 0.0101; Fig. 1E) was also found by lipid levels test, while leaving HDL-C ($t_{34} = 1.476$; *p* = 0.1491; Fig. 1F) unchanged. These results suggested that a high-fat diet can cause excessive obesity and hyperlipidemia in mice.

3.2. HFD showed depressive and anxiety-like behaviors in mice

To investigate the association between behavioral phenotypes and a high-fat diet in mice, a series of behavioral tests were performed. Firstly, mice were examined for spontaneous movements in the open field. Compared to ND group, HFD group mice showed significant reduced total distance ($t_{39} = 3.312$; *p* = 0.02; Fig. 2B), mean speed ($t_{39} = 3.312$; *p* = 0.02; Fig. 2D), and threading times ($t_{39} = 3.492$; *p* = 0.0012; Fig. 2C), which indicating that a high-fat diet has an effect on motor function in mice. Besides, mice fed a high-fat diet also spent less time in the central region than mice fed a normal diet ($t_{39} = 2.891$; *p* = 0.0062; Fig. 2E). In EPM, compared to ND group, HFD group mice showed significantly fewer trips to the open arm ($t_{15} = 3.843$; *p* = 0.0016; Fig. 2H) and less dwell time ($t_{15} = 5.085$; *p* = 0.001; Fig. 2G), suggesting that the high-fat diet induced anxiety-like behavior in mice. Next, we performed SPT and FST, and found the rate of sugar-water preference was significantly lower in HFD group mice ($t_{22} = 2.257$; *p* = 0.0343; Fig. 2J), while the immobility time in FST remained unchanged between each group ($t_{39} = 1.779$; *p* = 0.083; Fig. 2I). Although there was no significant difference in forced swimming test between the two groups, the rate of sugar-water preference was significantly reduced in the HFD mice, which indicates HFD group mice are depressive. Together, these results indicated that a high-fat diet can lead to depressive and anxiety-like behaviors in mice.

3.3. High-fat diet activates microglia and astrocytes and induces neuroinflammation in mice

Increased inflammation and reduced BDNF were reported in the pathogenesis of depression (Jin et al., 2019; Kishi et al., 2017). To

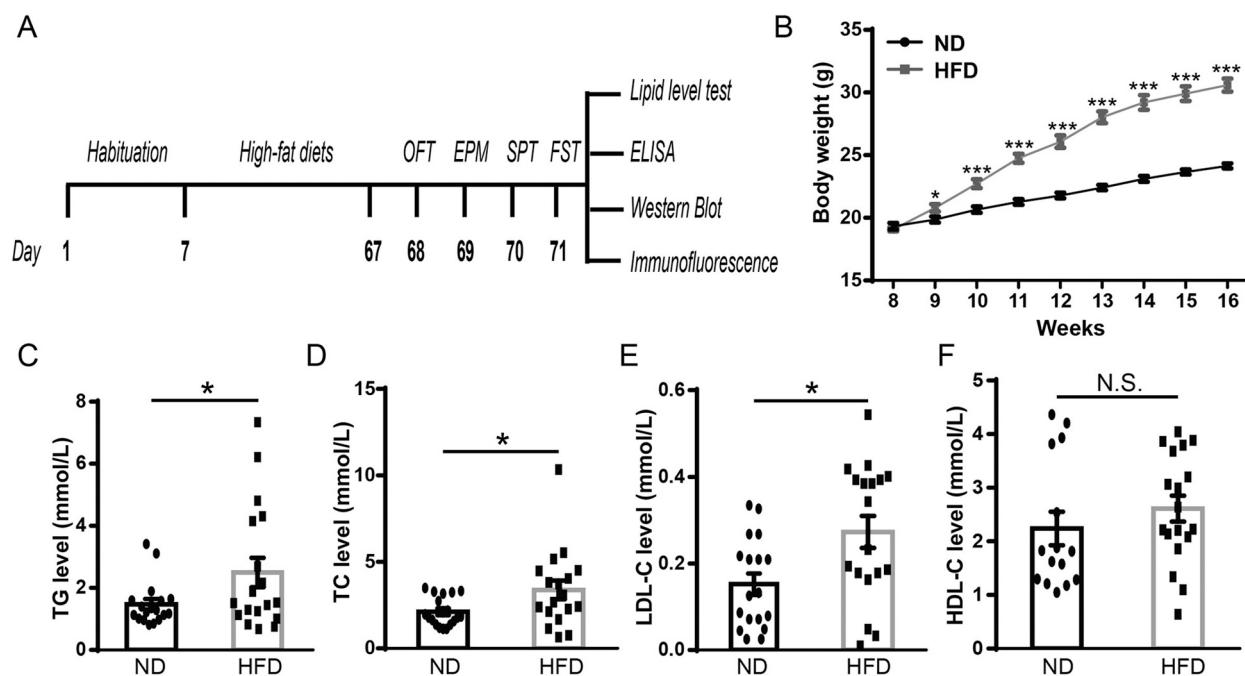


Fig. 1. Study the timeline diagram of the design. In the experiments, the mice were allowed to acclimate to their environment for 7 days, then after 8 weeks of high-fat feeding, behavioral tests of OFT, EPM, SPT, and FST were performed sequentially. After the behavioral tests were completed, tissues and sera were extracted for tests such as western blot, lipid level test, immunofluorescent staining, and ELISA (A). Changes in body weight (ND, $n = 10$ mice; HFD, $n = 10$ mice) (B). Levels of TG, TC, LDL-C, and HDL-C in serum (ND, $n = 18$ mice; HFD, $n = 18$ mice) (C-F). Data are represented as mean \pm SEM. * $p < 0.05$; ** $p < 0.001$; N.S., not significant.

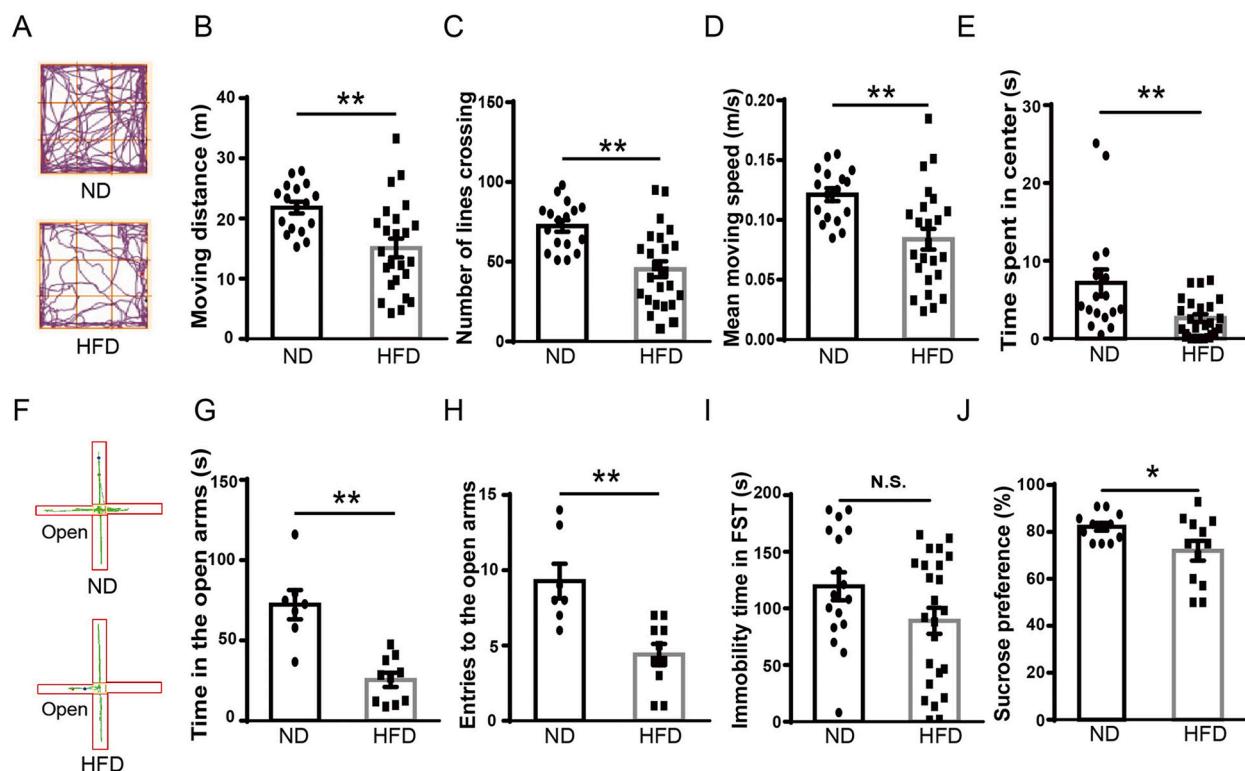


Fig. 2. The effect of high-fat diet on depression and anxiety-like behavior. Representative traces of ND and HFD mice of OFT and EPM (A and F, respectively). In the OFT, compared with the ND group, the total distance, the number of threading, the average speed, and the central area time were reduced significantly in the HFD group (ND, $n = 17$ mice; HFD, $n = 24$ mice) (B-E). In the EPM, compared with the ND group, the mice in the HFD group spent less time in the open arms (G) and entries to the open arms (H) were significantly reduced (ND, $n = 7$ mice; HFD, $n = 9$ mice). In the FST, no significant difference in the immobility time was observed between the two groups (ND, $n = 17$ mice; HFD, $n = 24$ mice) (I). In the SPT, compared with the ND group, the HFD group showed a lower sugar water preference rate (ND, $n = 12$ mice; HFD, $n = 12$ mice) (J). Data are represented as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; N.S., not significant.

investigated whether a high-fat diet would cause neuroinflammation, we detected some inflammasomes and pro-inflammatory cytokine protein levels in the hippocampus of mice after a high-fat diet by western blot and found that levels of NLRP3, ASC, IL-1 β , and TNF- α were upregulated leaving NLRP1 unchanged, suggesting that a high-fat diet increases neuroinflammation in the hippocampus of mice ($t_{10} = 0.9556$; $p = 0.3618$; $t_{10} = 2.396$; $p = 0.0376$; $t_{10} = 2.354$; $p = 0.0403$; $t_4 = 3.116$; $p = 0.0356$; $t_{10} = 4.018$; $p = 0.0024$; Fig. 3A, B and D). BDNF was found to be downregulated, suggesting that a high-fat diet decreased BDNF level in the hippocampus ($t_{10} = 2.695$; $p = 0.0225$; Fig. 3C and D). In addition,

we also found an activated microglia and astrocyte in HFD hippocampus by immunofluorescent staining ($t_8 = 5.393$; $p = 0.0003$; $t_{10} = 4.531$; $p = 0.0011$; $t_6 = 5.081$; $p = 0.0023$; Fig. 3E and F). The pro-inflammatory cytokines TNF- α and IL-1 β were also found to be increased in mice after a high-fat diet ($t_6 = 3.673$; $p = 0.0104$; $t_6 = 2.540$; $p = 0.0401$; Fig. 3G), suggesting that a high-fat diet also increases inflammation in peripheral blood. Together, these data suggested that a high-fat diet can activate microglia and astrocytes, which leading to neuroinflammation in hippocampus.

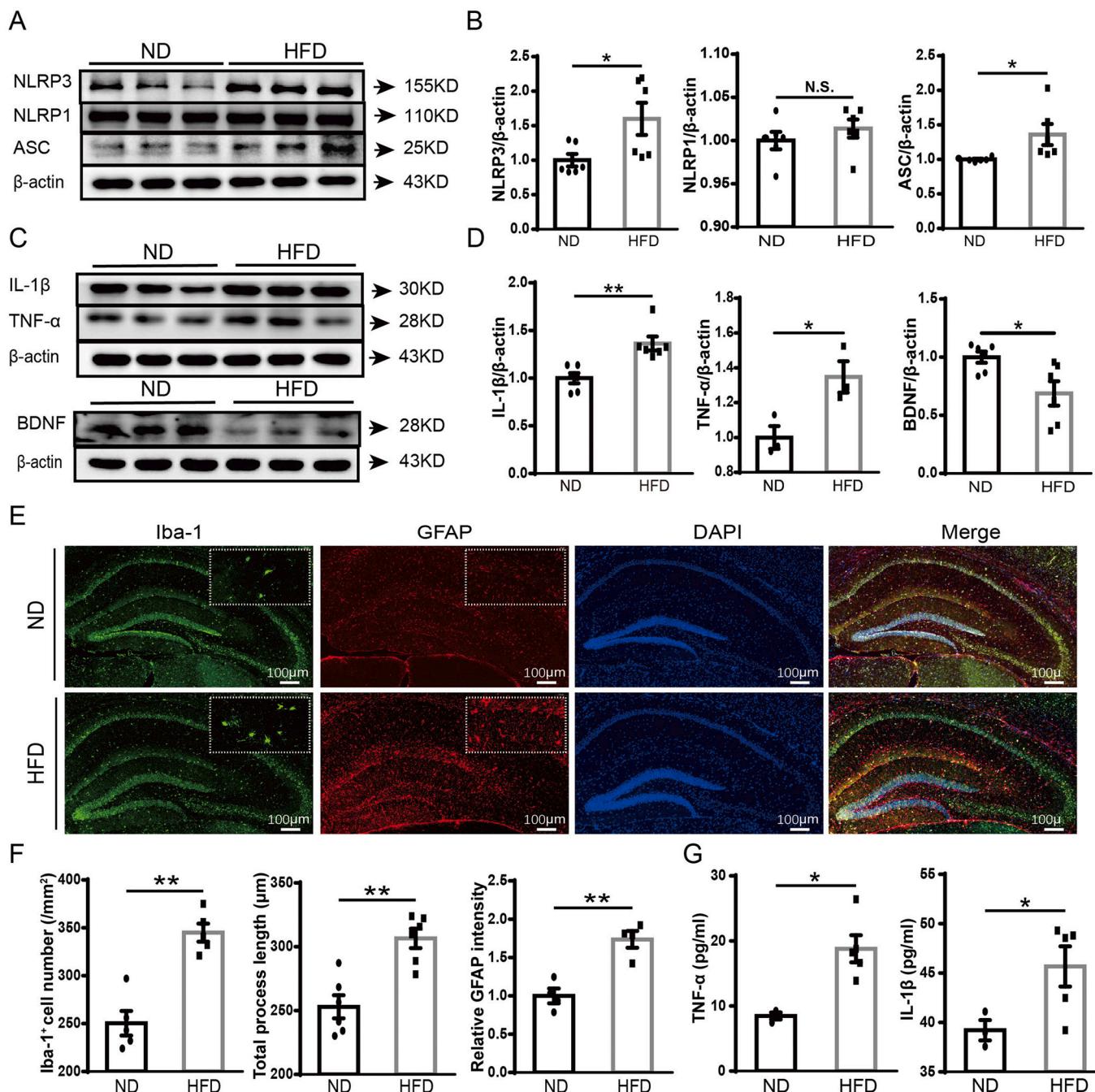


Fig. 3. Effect of high-fat diet on inflammasome, pro-inflammatory cytokines, and BDNF levels. Representative western blotting bands of NLRP1, NLRP3, ASC, IL-1 β , TNF- α , and BDNF in the hippocampus of ND and HFD groups (A and C, respectively). Statistical results showed that high-fat diet increased NLRP3, ASC, IL-1 β , and TNF- α levels and decreased BDNF level in the hippocampus while leaving NLRP1 level unchanged (ND, $n = 6$ mice; HFD, $n = 6$ mice) (B and D). Representative immunofluorescent staining images of Iba-1+ and GFAP+ cells in the hippocampus (E). Statistical results showed that Iba-1+ cell number and total process length, and GFAP+ cells were increased after high-fat feeding (ND, $n = 4$ –6 mice; HFD, $n = 4$ –6 mice) (F). TNF- α and IL-1 β levels in serum were increased in the HFD groups (ND, $n = 4$ mice; HFD, $n = 5$ mice) (G). Data are represented as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; N.S., not significant.

3.4. High-fat diet activates mTOR phosphorylation and downregulates autophagy level in mice

Autophagy is involved in the developmental process of several neurological diseases, such as Parkinson's disease, Alzheimer's disease, and Huntington's disease (Deng et al., 2017). To investigate whether a

high-fat diet affects autophagy, we examined the levels of autophagy-related proteins in the hippocampus of mice after a high-fat diet by western blot and immunofluorescent staining and found that the levels of Atg7, Beclin1, and LC3 were down-regulated ($t_{10} = 4.688; p = 0.0009$; $t_{10} = 7.644; p = 0.0001$; $t_{10} = 2.502; p = 0.0313$; $t_6 = 8.997; p = 0.0001$; $t_6 = 5.032; p = 0.0024$; Fig. 4B, E, and F), while P62 level was up-

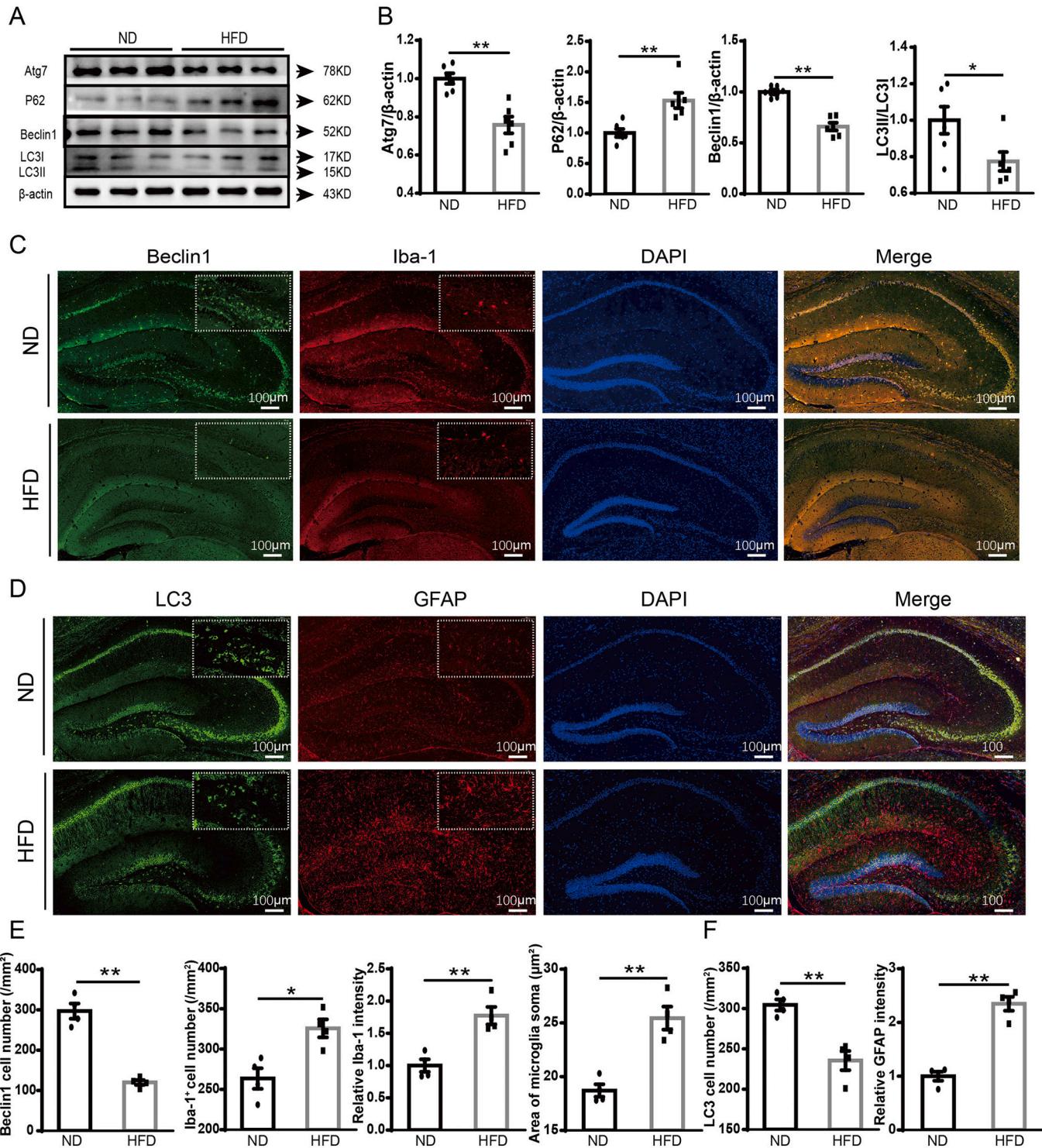


Fig. 4. Effect of high-fat diet on autophagy-related protein levels. Representative western blotting bands of Atg7, P62, Beclin1, and LC3 in the hippocampus of ND and HFD groups (A). Statistical results showed that high-fat diet decreased Atg7, Beclin1, and LC3 levels and increased P62 level in the hippocampus (ND, $n = 6$ mice; HFD, $n = 6$ mice) (B). Representative immunofluorescence staining images of Beclin1, Iba-1, and LC3, GFAP in the hippocampus (C and D, respectively). Statistical results showed that Beclin1 and LC3 cell numbers were decreased, and Iba-1⁺ cell number and GFAP⁺ cells were increased after high-fat feeding (ND, $n = 5$ mice; HFD, $n = 5$ mice) (E and F). Data are represented as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$.

regulated ($t_{10} = 3.734; p = 0.0039$; Fig. 4B). AMPK promotes autophagy initiation, while mTOR is a negative regulator of the autophagic pathway. AMPK activates cellular autophagy through both direct phosphorylation and inhibition of mTOR (Winden et al., 2018). To investigate whether a high-fat diet affects autophagy through the AMPK/mTOR pathway, we examined the levels of AMPK/mTOR pathway-related proteins in the hippocampus of mice after a high-fat diet by western blot and immunofluorescence staining, and found that the level of p-AMPK was down-regulated ($t_{10} = 4.775; p = 0.0008$; $t_6 = 7.724; p = 0.0002$; Fig. 5D and H), while the level of p-mTOR was up-regulated ($t_{10} = 4.594; p = 0.001$; $t_6 = 10.69; p = 0.0001$; Fig. 5B and F). These data suggested that a high-fat diet can inhibit autophagy by reducing AMPK phosphorylation and promoting mTOR shift to phosphorylation.

3.5. Rapamycin ameliorated excessive obesity and disorders of lipid metabolism in mice caused by high-fat diet

As shown in Fig. 6A, after one month of a high-fat diet, rapamycin was injected intraperitoneally (2 mg/kg per day) and saline was injected as control, and high-fat feeding was continued during the administration period. The mice in the HFD-rapamycin group showed a decreasing trend in body weight ($t_{15} = 7.858; p = 0.0001$; Fig. 6B), which suggested that rapamycin could ameliorate obesity caused by a high-fat diet. In addition, rapamycin can also reduce TG ($t_{10} = 2.349; p = 0.0407$; Fig. 6C), TC ($t_{10} = 3.712; p = 0.004$; Fig. 6D), and LDL-C ($t_{10} = 6.132; p = 0.0001$; Fig. 6E) levels, leaving HDL-C unchanged ($t_{10} = 0.4441; p = 0.6664$; Fig. 6F). These results suggested that rapamycin can ameliorate excessive obesity and hyperlipidemia in mice caused by high-fat diet.

3.6. Rapamycin ameliorates depressive and anxiety-like behaviors caused by a high-fat diet

To investigate the effect of rapamycin on the behavioral phenotype of mice after a high-fat diet, a series of behavioral tests were performed. Firstly, mice were examined for spontaneous movements in the open field. Compared to HFD-Saline group, HFD-Rapamycin group mice showed significantly increased total distance ($t_{27} = 4.396; p = 0.0002$; Fig. 7B), threading times ($t_{27} = 3.099; p = 0.0045$; Fig. 7C), and time in the central area ($t_{27} = 2.362; p = 0.0256$; Fig. 7D). In the EPM, compared to HFD-Saline group, HFD-Rapamycin group mice showed significantly increased number of trips to the open arm ($t_{27} = 3.155; p = 0.0039$; Fig. 7H) and more dwell time ($t_{27} = 3.036; p = 0.0053$; Fig. 7G). In SPT, after treating with rapamycin, the mice showed significantly increased rate of preference for sugar water ($t_{27} = 9.309; p = 0.0001$; Fig. 7J) compared to saline control. Next, we performed TST and FST, and found the immobility time was significantly reduced in HFD-Rapamycin group mice ($t_{27} = 4.149; p = 0.0003$; $t_{27} = 4.806; p = 0.0001$; Fig. 7E and I). While rapamycin showed no significant effect on depressive and anxiety-like behaviors in normal diet mice (Suppl-Fig. 1). Together, these results indicated that rapamycin improves depressive and anxiety-like behaviors in HFD mice.

3.7. Rapamycin alleviates microglial and astrocytes activation, reduces the neuroinflammation, and upregulates BDNF level in HFD mice

To investigate whether rapamycin has an effect on neuroinflammation after a high-fat diet, we examined some inflammasome and pro-inflammatory cytokine protein levels in the hippocampus of mice after a high-fat diet by western blot, and found that levels of NLRP3, ASC, IL-1 β , and TNF α were down-regulated ($t_4 = 3.264; p = 0.0310$; $t_4 = 7.551; p = 0.0016$; $t_4 = 3.516; p = 0.0254$; $t_4 = 4.276; p = 0.0129$; Fig. 8B and D), which suggested that rapamycin can reduce the increased neuroinflammation level in the hippocampus of HFD mice. Besides, BDNF was found to be up-regulated after rapamycin administration ($t_4 = 3.287; p = 0.0303$; Fig. 8D), which suggested that

rapamycin could improve the neurotrophic damage caused by high-fat diet to some extent. In addition, we found a reduced number of activated microglia and astrocytes in the HFD-Rapamycin hippocampus by immunofluorescent staining ($t_{16} = 2.696; p = 0.0159$; $t_{14} = 0.1125; p = 0.9120$; $t_{14} = 5.128; p = 0.002$; Fig. 8G). The pro-inflammatory cytokines TNF- α and IL-1 β were also found to be decreased in mice after rapamycin administration ($t_{10} = 3.023; p = 0.0128$; $t_{10} = 2.939; p = 0.0148$; Fig. 8H). Together, these data suggest that rapamycin can inhibit microglia and astrocyte activation, and reduce neuroinflammation in the hippocampus of HFD mice.

3.8. Rapamycin alleviates decreased autophagy level in HFD mice

To investigate the effect of rapamycin on autophagy after high-fat diet, we examined the levels of autophagy-related proteins in the hippocampus of mice after high-fat diet by western blot and immunofluorescent staining, and found that the levels of Atg7, Beclin1, and LC3 were up-regulated ($t_4 = 3.077; p = 0.0370$; $t_4 = 7.224; p = 0.0091$; $t_4 = 2.847; p = 0.0465$; $t_6 = 8.013; p = 0.0002$; Fig. 9B and D), while P62 level was down-regulated ($t_4 = 3.022; p = 0.0391$; Fig. 9B). These data suggested that rapamycin can ameliorate the reduced autophagy level caused by a high-fat diet.

4. Discussion

This study aimed to investigate the effects of high fat diet-induced obesity on depressive and anxiety-like behaviors in mice and its possible mechanisms of action. In this study, we found that 8 weeks of high-fat feeding effectively induced metabolic disorders, including obesity and hyperlipidemia in mice. Interestingly, the mice also showed depressive and anxiety-like behaviors. In addition, high-fat diet-induced obesity activated microglia and astrocyte, increased neuroinflammation, decreased BDNF level, inhibited autophagy by decreasing p-AMPK/AMPK ratio and increasing p-mTOR/mTOR ratio in the hippocampus. By treating with the mTOR signaling pathway inhibitor, rapamycin, level of autophagy was elevated in the hippocampus, neuroinflammation and the number of glial cells were reduced. Meanwhile the mice showed reduced lipid level and obesity, and the symptoms of depression and anxiety were also improved to moderate extent. Collectively, these results suggest that a high fat diet-induced obesity may inhibit AMPK phosphorylation and promote mTOR shift to phosphorylation to inhibit autophagy, which leads to depressive and anxiety-like behaviors in mice finally.

Obesity is associated with a high risk of depression, and clinical studies have found a link between the two (Milaneschi et al., 2017). It has been found that chronic consumption of high-fat food-induced obesity can cause disturbances in lipid metabolism in the body further contributing to depression and anxiety-like behaviors (Takase et al., 2016; Sharma and Fulton, 2013). In addition, high-fat diet-induced obesity can inhibit hypothalamic PKA signaling, which resulting in depressive-like behavior in mice (Vagena et al., 2019). Obesity is not only a weight problem, but behind obesity lies a series of problems such as metabolic disorders, diabetes, and cardiovascular disease (Saltiel and Olefsky, 2017), which can lead to an exponential increase in the probability of developing depression (Schmitz et al., 2016; Hazuda et al., 2019). Although a short-term high-calorie diet can reduce bad mood, a long-term high-calorie diet can lead to obesity and aggravate depression, forming a vicious circle of “depression-more food-obesity-depression” (Sharma and Fulton, 2013). The most direct effect of obesity will lead to changes in lipid metabolism (Li et al., 2017), and lipids, as an important part of the brain, act as signaling molecules starting from structural development, nerve impulse conduction, neurogenesis, synapse formation, myelin formation, etc., and play a variety of physiological activities in the brain (Peng et al., 2019; Oliveira et al., 2016; Xu et al., 2019), at the same time, the lipid bilayer also maintains the structural integrity of the physiological functions of proteins, therefore,

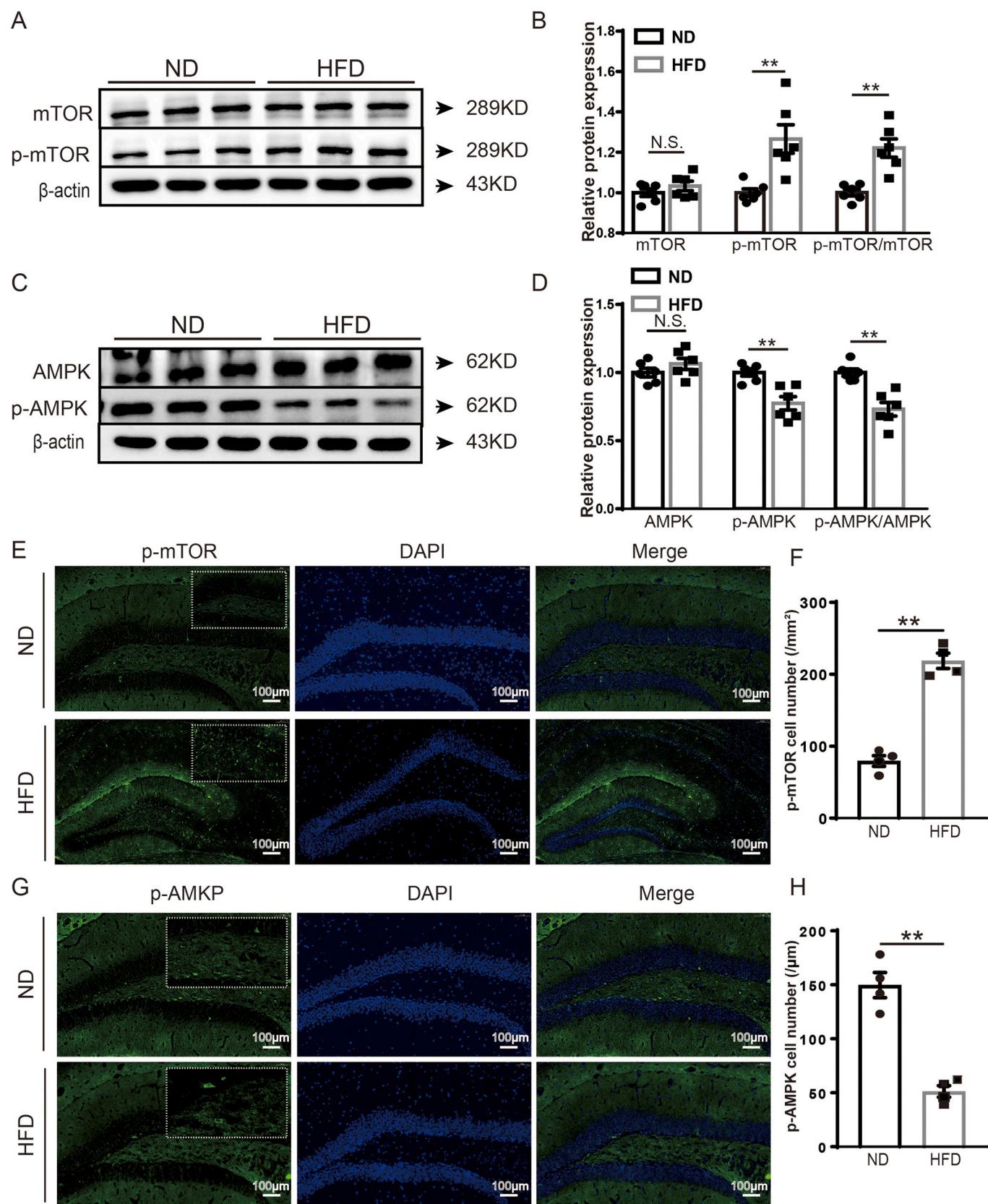


Fig. 5. Effect of high-fat diet on AMPK/mTOR pathway proteins levels. Representative western blotting bands of mTOR, p-mTOR, AMPK, and p-AMPK in the hippocampus of ND and HFD groups (A and C respectively). Statistical results showed that high-fat diet increased p-mTOR level and p-mTOR/mTOR ratio, and decreased p-AMPK level and p-AMPK/AMPK ratio in the hippocampus, while leaving mTOR and AMPK levels unchanged (ND, $n = 6$ mice; HFD, $n = 6$ mice) (B and D). Representative immunofluorescence staining images of p-mTOR and p-AMPK in the hippocampus (E and G respectively). Statistical results showed that p-mTOR cell number was increased, while p-AMPK cell number was decreased after high-fat feeding (ND, $n = 4$ mice; HFD, $n = 4$ mice) (F and H). Data are represented as mean \pm SEM. ** $p < 0.01$, N.S., not significant.

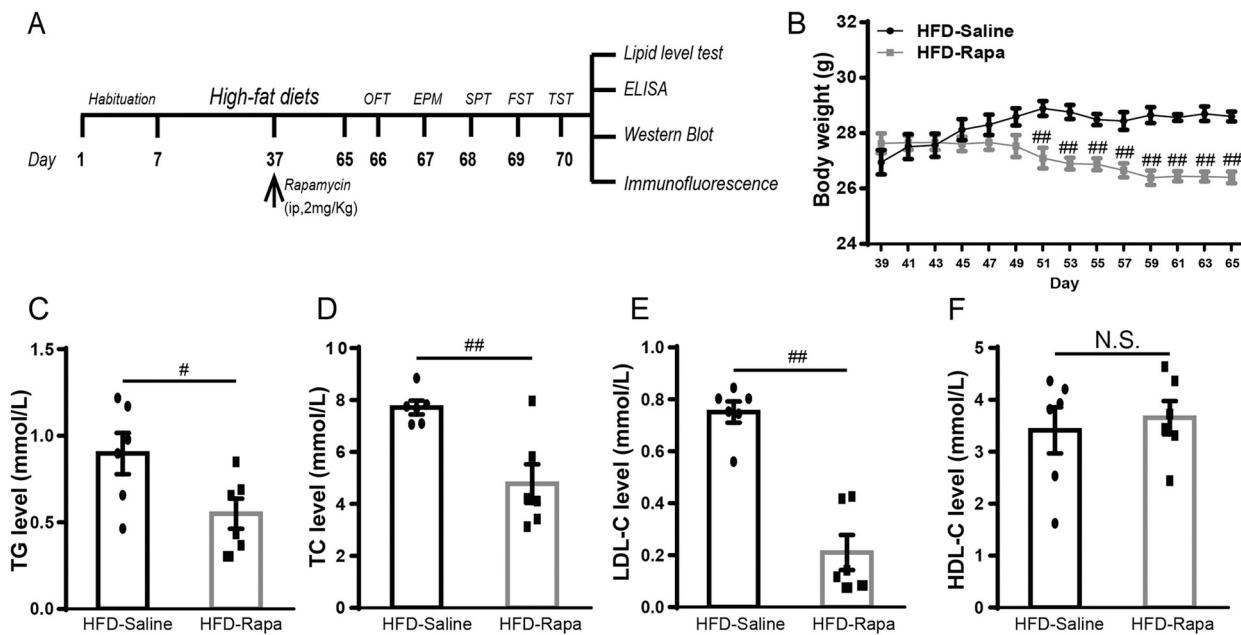


Fig. 6. Timeline diagram of the study design. In the experiments, the mice were allowed to acclimate to their environment for 7 days, then after one month of high-fat feeding, rapamycin (2 mg/kg per day) was injected intraperitoneally and saline was injected as a control, and high-fat feeding continued during the administration, and behavioral tests for OFT, EPM, SPT, and FST were performed sequentially after 28 days. After the behavioral tests were completed, tissues and sera were extracted for tests such as western blot, lipid level test, immunofluorescent staining, and ELISA (A). Changes in body weight (measured every two days) (HFD-Saline, $n = 8$ mice; HFD-Rapamycin, $n = 9$ mice) (B). Levels of TG, TC, LDL-C, and HDL-C in serum (HFD-Saline, $n = 6$ mice; HFD-Rapamycin, $n = 6$ mice) (C-F). Data are represented as mean \pm SEM. $^{\#}p < 0.05$, $^{##}p < 0.01$, N.S., not significant.

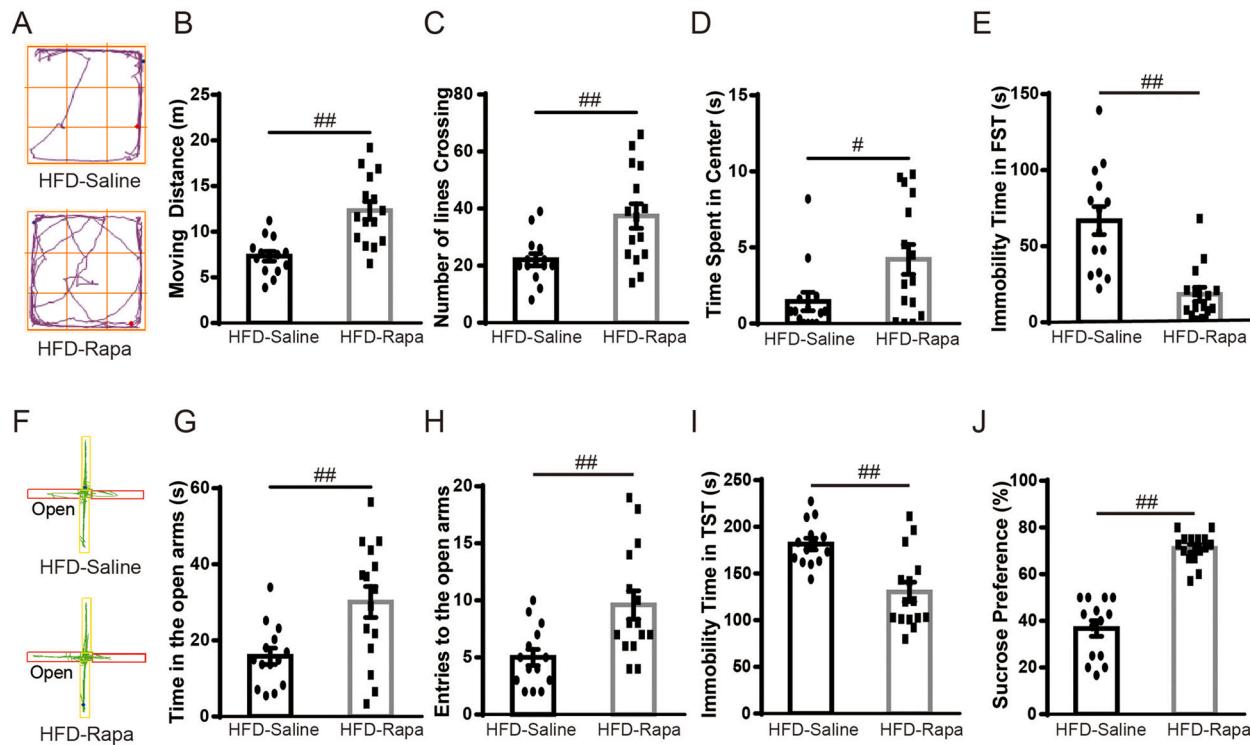


Fig. 7. Effects of rapamycin on depression and anxiety-like behaviors resulting from a high-fat diet. Representative traces of HFD-Saline and HFD-Rapamycin mice of OFT and EPM (A and F, respectively). In the OFT, compared with the HFD-Saline group, the total distance, the number of threading, and the central area time were increased significantly in the HFD-Rapamycin group (B-D). In the FST, compared with the HFD-Saline group, the immobility time of HFD-Rapamycin group mice was significantly reduced (E). In the EPM, compared with the HFD-Saline group, the mice in the HFD-Rapamycin group spent more time in the open arms (G) and entries to the open arms (H) were significantly increased. In the TST, compared with the HFD-Saline group, the mice in the HFD-Rapamycin group immobility time were significantly reduced (I). In the SPT, compared with the HFD-Saline group, the HFD-Rapamycin group showed a higher sugar water preference rate (J). Data are expressed as mean \pm SEM. $^{\#}p < 0.05$, $^{##}p < 0.01$, (HFD-Saline, $n = 14$ mice; HFD-Rapamycin, $n = 15$ mice).

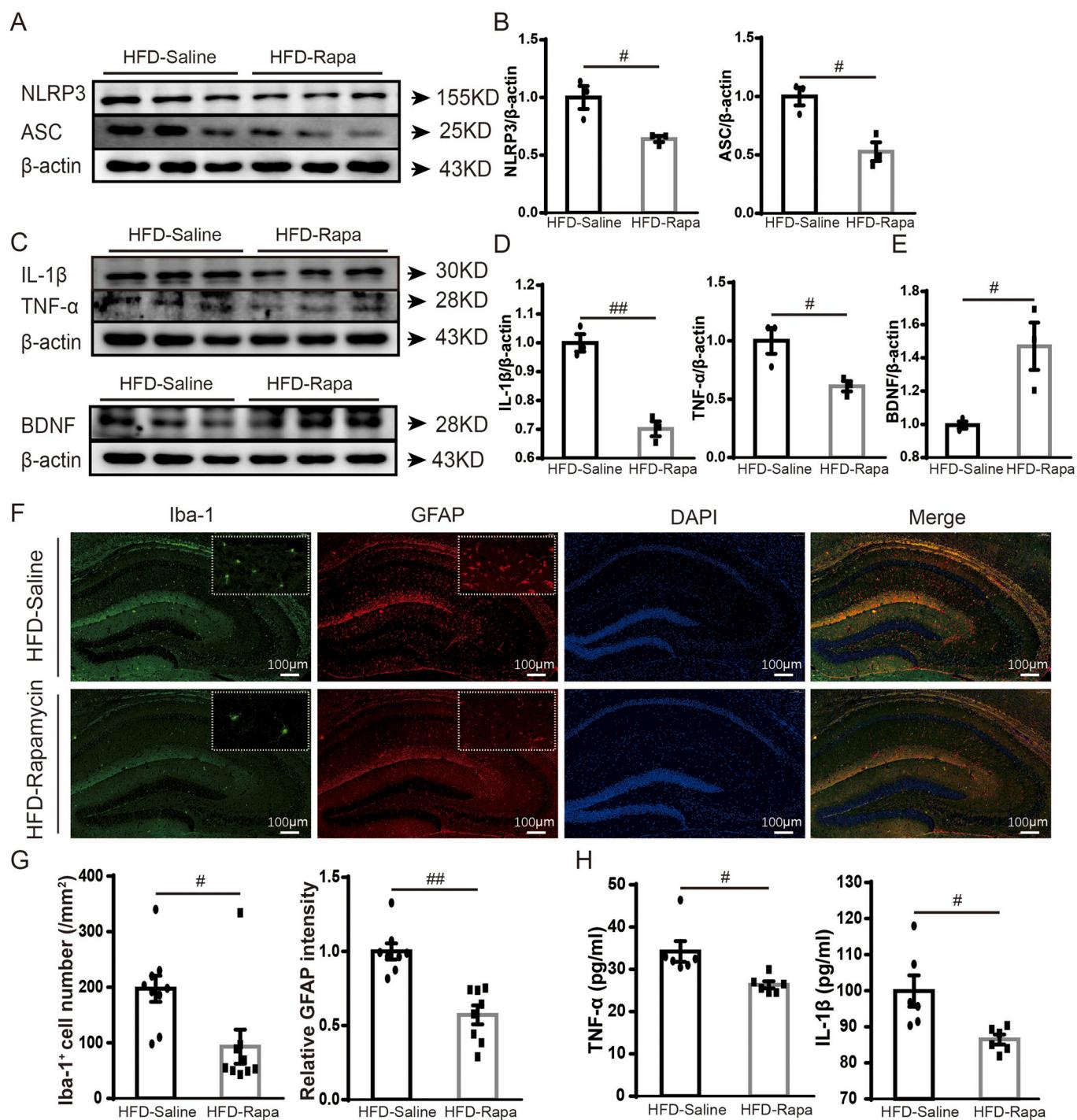


Fig. 8. Effect of rapamycin on inflammasome, pro-inflammatory cytokines, and BDNF levels after high-fat diet. Representative western blotting bands of NLRP3, ASC, IL-1 β , TNF- α , and BDNF in the hippocampus of HFD-Saline and HFD-Rapamycin group (A and C, respectively). Statistical results showed that rapamycin decreased NLRP3, ASC, TNF- α , and IL-1 β levels and increased BDNF level in the hippocampus after high-fat diet (HFD-Saline, $n = 3$ mice; HFD-Rapamycin, $n = 3$ mice) (B and D). Representative immunofluorescent staining images of Iba-1+ and GFAP+ cells in the hippocampus (F). Statistical results showed that the number of Iba-1+ cells and GFAP+ cells decreased after rapamycin treatment (HFD-Saline, $n = 3$ mice; HFD-Rapamycin, $n = 3$ mice) (G). TNF- α and IL-1 β levels in serum were decreased in the HFD-Rapamycin group (HFD-Saline, $n = 6$ mice; HFD-Rapamycin, $n = 6$ mice) (H). Data are expressed as mean \pm SEM. # $p < 0.05$, ## $p < 0.01$; N.S., not significant.

impaired metabolism of lipids and their intermediates or alterations may be associated with the development of various brain disorders including neurodegenerative diseases, neurological and neuropsychiatric disorders (Bornstein et al., 2020; Wong et al., 2017; Yu et al., 2020). Most studies have shown that patients with major depression have lower high-density lipoprotein (HDL) levels and higher triglyceride TG levels, while

lower HDL levels remain independently associated with depressive features (Lasserre et al., 2017; Ma et al., 2017), with HDL acting as the body's "lipid scavenger" (Bianconi et al., 2018; Riwanto and Landmesser, 2013), the only member of the lipoprotein family that transports exogenous cholesterol to the liver, and changes in these indicators are biological evidence supporting a disruption of lipid metabolism in

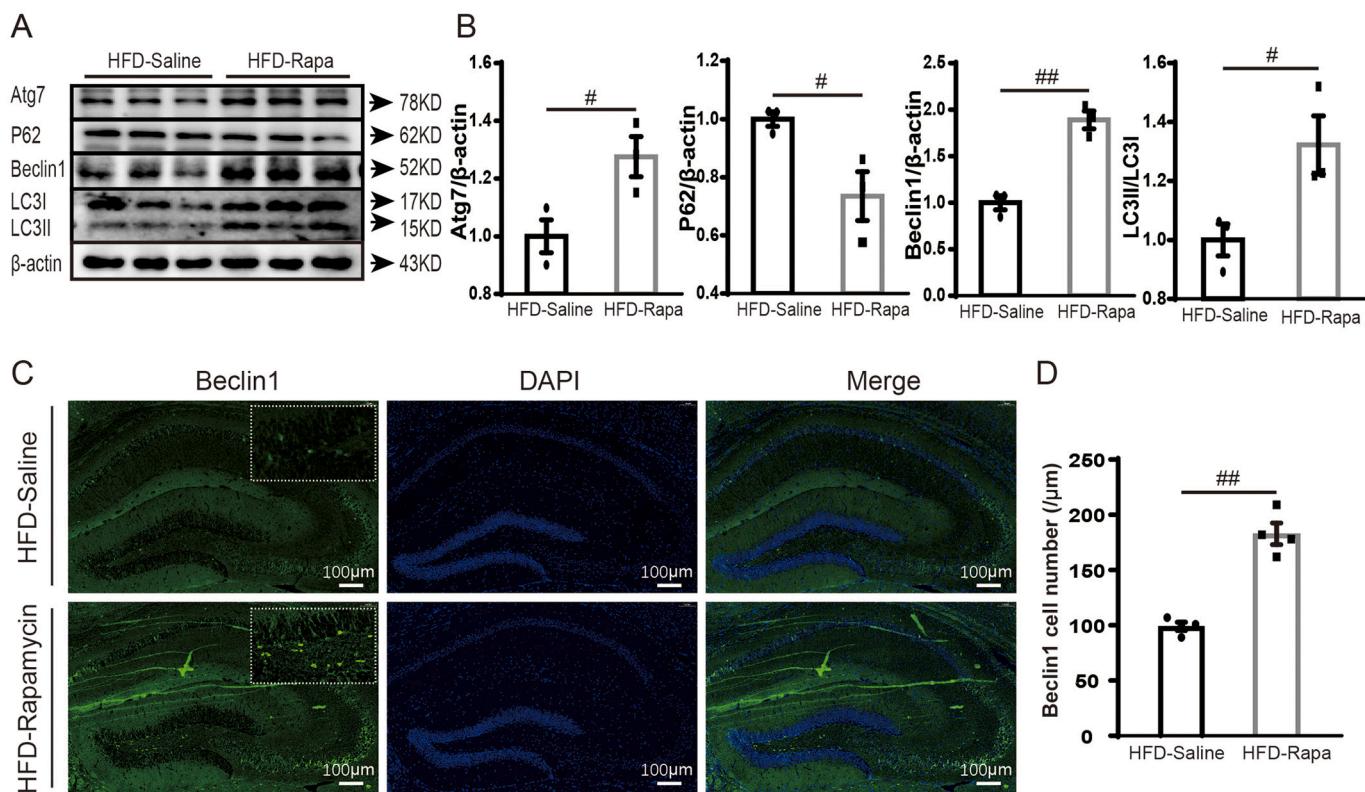


Fig. 9. Effect of rapamycin on autophagy-related protein levels after high-fat diet. Representative western blotting bands of Atg7, P62, Beclin1, and LC3 in the hippocampus of HFD-Saline and HFD-Rapamycin groups (A). Statistical results showed that rapamycin increased the Atg7, Beclin1, and LC3 levels and decreased P62 levels in the hippocampus after high-fat diet (B). Representative immunofluorescent staining images of Beclin1 in the hippocampus of two groups (C). Statistical results showed that rapamycin increased Beclin1 cell number in HFD group mice (D). Data are expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$; $n = 3$ mice per group.

depressed patients (Kloiber et al., 2007; Quimet et al., 2019). To verify the relationship between obesity and depression, we fed mice with high-fat diets for 8 weeks, which showing a significant increase in body weight to meet the criteria for obesity, and we found the HFD mice showed depressive and anxiety-like behaviors compared to the ND mice. Then the serum lipid levels were measured, and the result indicated the levels of TC, TG, and LDL-C were significantly elevated, leaving HDL-C unchanged. Although HDL-C, which plays as a “lipid scavenger” (Bianconi et al., 2018; Riwanto and Landmesser, 2013) in the body did not change here, the excessive increase of other lipid levels that could not be cleared by HDL in time could be a major cause of lipid metabolism disorder. This may be one of the reasons why obesity causes disorders of lipid metabolism, which lead to depressive and anxiety-like behaviors in mice finally.

A large body of evidence confirms the role of immune-inflammatory dysregulation in depression (Miller and Raison, 2016; Wittenberg et al., 2020). Neuroinflammation is an innate immune response to tissue damage, characterized by microglial activation, which may be accompanied by astrocyte activation (Colonna and Butovsky, 2017), increased levels of inflammatory factors and chemokines (Mathur et al., 2017; Morganti-Kossmann et al., 2019), and plays an important role in many central nervous system disorders, including psychiatric disorders (Schwartz and Deczkowska, 2016). While inflammatory vesicles are a key component of the innate immune response and have been reported to be involved in the mechanisms of inflammation-related neurological disorders (Heneka et al., 2018). NLRP3 as an inflammatory vesicle consists of NLRP3 protein, apoptosis-associated spot-like protein (ASC), and caspase1, which can be activated by a variety of endogenous stimuli, such as ATP, serum amyloid β , uric acid crystalline salts, saturated fatty acids (He et al., 2016). Activation of NLRP3 inflammatory vesicles mediates the release of pro-inflammatory cytokines IL-1 β , IL-6, IL-18,

etc., and promotes the development of tissue inflammation (Swanson et al., 2019). According to clinical studies, the levels of inflammatory response factors such as IL-1 β , IL-6, and TNF- α are significantly increased in depressed patients. It has also been found that levels of IL-1 β and IL-6 are positively correlated with the risk of depression (Wong et al., 2016), and that chronic low-grade inflammation is also a marker of obesity (Tilg et al., 2020), where white adipocytes infiltrated by macrophages and other immune cells produce more pro-inflammatory cytokines, such as TNF- α and IL-6 than normal BMI individuals (Saitel and Olefsky, 2017; Winer et al., 2017). Obesity also revealed elevated NLRP3 and ASC protein expression in the hippocampus and significantly increased levels of IL-1 β and TNF- α in the periphery and hippocampus, which may be attributed to the disruption of lipid metabolism in mice by a high-fat diet.

Autophagy is a process of degradation and reuse of cytoplasmic components such as senescent and abnormal functioning organelles, damaged proteins, and lipids through the intracellular lysosomal pathway, and is an important mechanism for cellular self-protection (Sciarretta et al., 2018; Doherty and Baehrecke, 2018). Autophagy is extremely sensitive to changes in the nutritional status of the body, especially to high-fat or high-calorie dietary intake (Toledo et al., 2018). Changes in autophagy may trigger metabolic dysregulation by interfering with endocrine regulatory mechanisms (Oh et al., 2016). Autophagy is regulated by a variety of signaling molecules. AMPK and mTOR are two of the most important ones (Yan et al., 2019). AMPK is a highly conserved energy receptor that is sensitive to changes in the adenosine monophosphate (AMP) or adenosine diphosphate (ADP)/adenosine triphosphate (ATP) ratio, and in states of metabolic stress such as energy deprivation Elevated AMP or ADP/ATP ratios lead to AMPK activation, which promotes the initiation of autophagy (Garcia and Shaw, 2017; Herzig and Shaw, 2018). mTOR is a nutrient sensor and negative

regulator of autophagy, forming two structurally and functionally distinct complexes, mTORC1 and mTORC2, due to binding to different proteins. mTOR activation increases the level of phosphorylation, which in turn allows the complexes mTORC1 and mTORC2 to exert their respective roles (Winden et al., 2018; Ni et al., 2019). mTORC1 is mainly involved in cell growth, proliferation and apoptosis processes, and its activity can be regulated by integrating many signals, and it can inhibit autophagy by directly phosphocreatine ULK1 and Atg13. Therefore, it has been considered as a negative regulator of cellular autophagy and is extremely sensitive to rapamycin (Odle et al., 2020). mTORC2 has also been shown to be a key regulator of lipid metabolism, regulating adipogenesis, lipolysis, and the formation of adiposity (Jia and Bonifacino, 2019). It has been found that cAMP/protein kinase A can modulate the mTOR signaling pathway and thus regulate autophagy (Kim et al., 2010; Shillingford et al., 2006; Torres and Harris, 2014; Ye et al., 2017; Zhu et al., 2017). It is reported that some drugs or foods, which have direct antidepressant effect may target on mTOR signaling (Nowak et al., 2019). In addition, inhibition of mTORC2 can regulate lipid formation, ameliorate metabolic syndrome and have potential to treat obesity (Micheli et al., 2019; Thompson et al., 2012). Our results showed that HFD resulted in decreased p-AMPK level and increased p-mTOR level in the hippocampus, which could be due to eutrophication caused by high-fat diet (Huang et al., 2018; Muranen et al., 2017). Meanwhile, activated mTOR may impair autophagy by reducing levels of beclin1, LC3, and Atg7. Interestingly, after treated with rapamycin, autophagy level was increased. This may be due to the inhibition of the mTOR signaling, which protects neuronal cells by regulating lipolysis, inhibiting lipid accumulation, preventing apoptosis, and limiting inflammation and oxidative stress, thereby improving depressive and anxiety-like behaviors in mice.

BDNF is an important growth factor in the central and peripheral nervous system (Sikandar et al., 2018), particularly in the hippocampus and cortical areas, and it plays different roles in inflammation, metabolism, and cardiovascular aspects (Gray et al., 2018; Wood et al., 2018; McGregor et al., 2017). In a recently published study, obesity was found to be associated with reduced BDNF concentrations in the hippocampus, but the exact cause-and-effect relationship was not clearly described (Xie et al., 2019). BDNF plays a key role in adult neurogenesis and learning and memory processes (Arango-Lievano et al., 2019), and serum BDNF levels have been found to be associated with depressive symptoms or impaired memory function (Hashikawa et al., 2017). Many preclinical and clinical studies provide direct evidence that regulation of BDNF expression may be involved in depression-related behaviors and that BDNF may mediate the therapeutic effects of antidepressants (Jiang et al., 2018). Some antidepressant therapies, such as selective serotonin reuptake inhibitors and electroconvulsive therapy, increase BDNF expression in the hippocampus of depressed patients to achieve anti-depressant effects, meaning that depressed patients have reduced BDNF expression compared to normal subjects (Duman et al., 2016; Zhu et al., 2017). Interestingly, we also found that BDNF expression was reduced in the hippocampus of HFD mice. Several studies indicate that BDNF plays a crucial role in food intake, and the lack of BDNF can cause overeating and cause severe obesity. mTORC1, a key component of the nutrient sensing network that controls cellular metabolism, is also regulated by BDNF (Takei et al., 2014). In our study, we also found that the mTOR signaling was activated through high-fat diet induce obesity. After treated with rapamycin, the level of BDNF was up-regulated. This indicates that reduced BDNF level may activate the mTOR signaling, which was involved in depression and anxiety disorders.

In summary, we found that a high-fat diet-induced obesity inhibits AMPK phosphorylation and promoting mTOR shift to phosphorylation to reduce autophagy level in the hippocampus, leading to oxidative stress and neuroinflammatory responses, which results in depressive and anxiety-like behaviors. Rapamycin can activate autophagy by inhibiting mTOR phosphorylation, and ameliorate depressive and anxiety-like behaviors induced by a high-fat diet. Our study may provide clues for

future clinical studies on obesity depression.

5. Conclusion

In this study, we showed that 8 weeks of high-fat diet evoked alterations in depressive and anxiety-like behaviors, disruption of lipid metabolism, increased neuroinflammation and reduced autophagy level, which was partially reversed by rapamycin. Our study provides new insights into the molecular mechanisms of obesity depression. Therefore, improving lipid metabolism or enhancing autophagy through the AMPK/mTOR pathway could be potential targets for the treatment of obesity depression.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.exneuro.2021.113949>.

Author's contribution

Ming Chen, Wenning Wu and Jinyu Mei conceived, designed, and directed the research project and provided financial support. Yong Li, Yujie Cheng, Yuan Zhou, Hongmei Du, Cui Zhang, Zhentao Zhao, and Yuenan Chen completed the research project. Yong Li, Yujie Cheng, and Yuan Zhou analyzed the experimental data, interpreted the results, and wrote the manuscript. Ming Chen, Wenning Wu, and Jinyu Mei reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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