# α-lipoic acid attenuates obesity-associated hippocampal neuroinflammation and increases the levels of brain-derived neurotrophic factor in ovariectomized rats fed a high-fat diet

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**Abstract.** Overweight or obesity in post-menopausal women is known to induce metabolic disorders associated with neuro-inflammation. α-lipoic acid (α-LA), which has been clinically used to treat diabetic peripheral neuropathy, has been found to exert anti-inflammatory and neuroprotective effects in patients with Alzheimer's disease (AD). In this study, to investigate the effects of α-LA on neuroinflammatory factors and the levels of hippocampal brain-derived neurotrophic factor (BDNF) in ovariectomized rats fed a high-fat diet (HFD), Wistar ovariecto-mized rats were fed HFD alone or HFD and treated with α-LA for 12 weeks. Metabolic parameters in serum were detected, real-time polymerase chain reaction (RT-PCR), western blot analysis and ELISA were used to evaluate the levels of inflammatory factors and BDNF in the hippocampus. α-LA markedly reduced body weight, the levels of serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), as well as the levels of leptin and insulin resistance in ovariectomized rats fed HFD. It also significantly increased the levels of serum adiponectin and high-density lipoprotein cholesterol (HDL-C). In the hippocampal tissues of ovariectomized rats fed HFD, the protein and mRNA expression of BDNF was upregulated following the administration of α-LA. Conversely, the levels of interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) were decreased following treatment with α-LA. These findings demonstrate that α-LA significantly increases the expression of BDNF in the hippocampus of obese ovariectomized rats fed HFD, possibly by improving lipid metabolism, enhancing insulin sensitivity and reversing central inflammation.

## Introduction

Overweight and obesity not only increase the risk of developing insulin resistance and cardiovascular disease, but also contribute to cognitive impairment and accelerate the progression of Alzheimer's disease (AD) (1). Obesity is typically characterized by the secretion of adipokines and inflammatory cytokines, including leptin, adiponectin, interleukin (IL)-6 and tumour necrosis factor-α (TNF-α) (2,3), which collectively induce insulin resistance (4). Insulin resistance in peripheral tissues has been shown to reduce central insulin levels (5), suggestive of the potential role of peripheral insulin resistance in the pathogenesis of AD. A previous study suggested that the inflammatory system plays a critical role linking obesity-induced peripheral insulin resistance with neurodegenerative diseases, including AD (6).

Menopause, owing to estrogen deficiency, is associated with increased visceral adiposity and is closely associated with metabolic disorders, including dyslipidemia, insulin resistance, as well as cognitive deficits (7,8). Epidemiological data have demonstrated that post-menopausal women have up to a 3-fold higher risk of developing AD than men (9). In women, the dysregulation of inflammatory factors and cytokines is induced during menopause (10), which may predispose women to brain-associated inflammatory disorders and neurodegeneration. Furthermore, studies have shown that ovariectomy (OVX) or natural estrogen deficiency selectively induces the production of inflammatory factors, including IL-6, TNF-α and IL-1β. These factors may act on the hippocampus, a crucial brain region for recognition, causing cognitive deficits (11,12).

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors, which plays an important role in the survival and differentiation of neurons. BDNF is highly expressed in the hippocampus, whereby it regulates hippocampal synaptic plasticity associated with the

process of cognition and memory (13). A recent study indicated that pro-inflammatory cytokines negatively regulate hippocampal BDNF mRNA levels and synaptic transmission (14). Other studies have shown that higher levels of IL-6 and TNF-α in the mammalian brain, induced by either a high-fat diet (HFD) or OVX, are closely linked to the reduction of BDNF levels and cognitive dysfunction (15,16).

α-lipoic acid (α-LA) is a compound with strong antioxidant properties. It has been demonstrated that α-LA improves dyslipidemia and insulin resistance in obese rats (17,18). Moreover, α-LA has been shown to exert neuroprotective effects in patients with AD (19).

Thus far, to our knowledge, the effects of α-LA on cerebral inflammation under estrogen-deficient and hyperlipidemic conditions have not been documented. In the present study, we address this issue using a rat model of OVX- and HFD-induced obesity. In addition, the effects and mechanism of action of α-LA on hippocampal BDNF expression are also investigated.

## Materials and methods

*Animals.* Forty female Wistar rats, aged 3 months, were provided by the Shandong University Laboratory Animal Center, Jinan, China. The rats were housed in separate cages under diurnal lighting conditions and allowed free access to food and water. After 1 week of acclimatization, the rats were randomly divided into 4 groups (n=10 per group): the sham-operated group fed a normal diet (ND) (SHAM + ND); the sham-operated group fed HFD (SHAM + HFD); the OVX group fed HFD (OVX + HFD); and the OVX group fed HFD and treated with α-LA (OVX + HFD + α-LA). The rats, which underwent either sham operation or bilateral OVX, were anaesthetized with pentobarbital sodium [50 mg/kg body weight, intraperitoneally (ip)]. After the establishment of models, the rats were fed either ND or HFD (60%). The rats were administered 200 mg/kg of α-LA (Sigma, St. Louis, MO, USA) solution as previously described (20) or an equal volume of sodium carboxymethyl cellulose solution by gavage daily for 12 weeks. Body weight was measured once a week. All procedures were conducted in accordance with the National Institutes of Health and Nutrition Guidelines for the care and use of laboratory animals.

*Preparation of blood and hippocampus samples.* After an overnight fast, the rats were anaesthetized with pentobarbital sodium (50 mg/kg body weight, ip). Blood samples were obtained from the femoral artery and were then centrifuged at 2,500 rpm at 4°C for 15 min; serum was separated and stored in a refrigerator at -80°C. The rats were then sacrificed by decapitation. The bilateral hippocampi were collected and frozen in liquid nitrogen and then stored at -80°C until use.

*Biochemical assays.* The serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were determined by enzymatic colorimetric methods. Serum 17β-estradiol (E2) levels were determined using the Iodine[$^{125}$I] Estradiol Radioimmunoassay Kit (JD Bio. Co., Tianjin, China) according to the manufacturer's instructions; serum leptin, adiponectin and insulin levels were measured by individual enzyme-linked immunosorbent assay kits (R&D Systems,

Minneapolis, MN, USA) according to the manufacturer's instructions.

*Protein measurements.* Hippocampal tissues were homogenized with protease inhibior cocktail (Amresco, Solon, OH, USA). The homogenates were then centrifuged at 12,000 rpm at 4°C for 20 min, the supernatants were collected and the total protein concentration was determined using the BCA Protein Assay kit (Beyotime Institute of Biotechnology, Shanghai, China). BDNF, TNF-α and IL-6 protein levels in the hippocampal homogenates were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Promega, Madison, WI, USA) according to the manufacturer's instructions.

*Western blot analysis.* Protein from hippocampal homogenates was separated by electrophoresis on a 10% SDS-PAGE gel and transferred onto a Hybond-P PVDF membrane. The membrane was blocked with 4% skim milk in Tris-buffered saline with Tween-20 (TBST; 10 mM Tris-HCl, pH 7.5, 200 mM NaCl, 0.05% Tween-20) for 2 h at room temperature and then incubated with BDNF (Abcam, Cambridge, MA, USA) or β-actin (ZSGB-BIO, Beijing, China) antibodies (in WB antibody diluent) overnight at 4°C. The membranes were washed 3 times with TBS-T and incubated with secondary antibody (peroxidase-conjugated goat anti-rabbit IgG) for 2 h at room temperature. After washing with TBS, the bound primary antibody was visualized by enhanced chemiluminescence (ECL; Pierce Biotechnology, Inc., Rockford, IL, USA) and exposed to films.

*Real-time polymerase chain reaction (RT-PCR).* Total RNA was extracted from the right hippocampal tissues using TRIzol reagent (TakaraBio., Inc., Shiga Japan) and reverse-transcribed into cDNA in 20 $\mu$l of reaction mixture using a reverse transcription kit (Takara) according to the manufacturer's instructions. RT-PCR was performed using SYBR-Green technology. The forward and reverse primers used in the current study were as follows: BDNF-F, 5'-TGTGACAGT ATTAGCGAGTGGGT-3' and BDNF-R, 5'-CGATTGGTA GTTCGGCATT-3'; IL-6-F, 5'-AGAAAAGAGTTGTGC AATGGCA-3' and IL-6-R, 5'-GGCAAATTTCCTGGTT ATATCC-3'; TNF-α-F, 5'-CCAACAAGGAGGAGAA GTTCC-3' and TNF-α-R, 5'-TCTGCTTGGTGGTTTGC TAC-3'; β-actin-F, 5'-GACAGGATGCAGAAGGAGA TTACT-3' and β-actin-R, 5'-TGATCCACATCTGCTGG AAGGT-3'. The annealing temperatures were 56°C (BDNF), 53°C (IL-6) and 56°C (TNF-α). The results are expressed as the ratio to β-actin. To confirm the amplification specificity, the PCR product of each gene was subjected to melting curve and subsequent sequence analysis.

*Statistical analysis.* All data are expressed as the means ± SEM. Statistical analyses were performed using the Student's t-test or two-way analysis of variance (ANOVA) with post hoc least significant difference (LSD) tests for comparisons between two groups or multiple groups, respectively. Statistical software used was the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA; version 18.0 J for Windows). A value of P<0.05 was considered to indicate a statistically significant difference.

Table I. Changes in body weight of rats.

| Groups | Initial weight (g) | Terminal weight (g) |
|---|---|---|
| SHAM + ND | 201±10 | 365±26 |
| SHAM + HFD | 201±8 | 407±24[a] |
| OVX + HFD | 200±13 | 501±27[b,c] |
| OVX + HFD+ α-LA | 201±11 | 433±31[d] |

Data are the means ± SEM (n=8-10 per group). [a]P<0.05 vs. sham-operated group fed normal diet (ND); [b]P<0.01 vs. sham-operated group fed ND (SHAM + ND); [c]P<0.01 vs. sham-operated group fed high-fat diet (HFD) (SHAM + HFD); [d]P<0.01 vs. ovariectomized rats fed HFD (OVX + HFD).
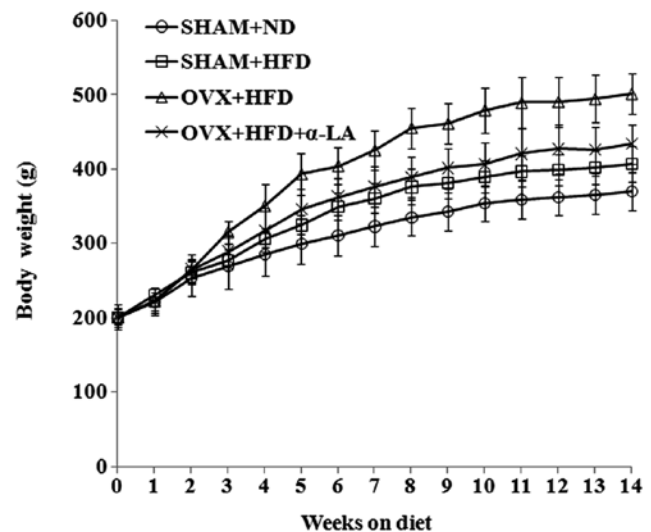


Figure 1. Evolution of the body weight of rats (n=8-10 per group). Rats in the ovariectomy fed high-fat diet (OVX + HFD) group showed a significant increase in weight as compared with the sham-operated group fed a normal diet (SHAM + ND) and the sham-operated group fed a high-fat diet (SHAM + HFD). Body weights of the rats in the ovariectomy fed high-fat diet group treated with α-lipoic acid (α-LA) (OVX + HFD + α-LA) had a significantly reduced body weight as compared with the rats in the ovariectomy fed fed high-fat diet (OVX + HFD) group. Data are the means ± SEM.
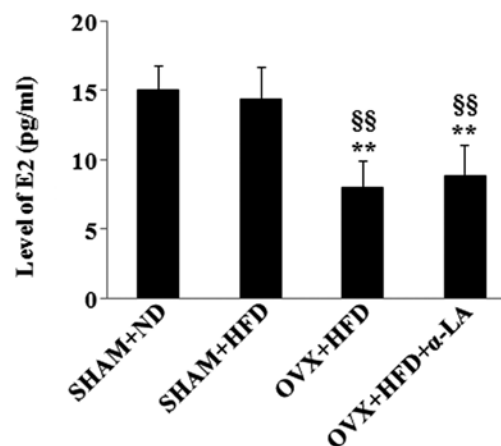
## Results

*Changes in body weight of rats.* The curves in Fig. 1 indicate the mean body weights of the rats in the 4 groups. Before the sham operation or OVX, the initial body weights of the rats were similar among the 4 groups (SHAM + ND, 201±10 g; SHAM + HFD, 201±8 g; OVX + HFD, 200±13 g; OVX + HFD + α-LA, 201±11 g; p>0.05; Table I). After 12 weeks of HFD or HFD plus OVX, in the SHAM groups, the rats fed HFD gained approximately 164±16 g and reached a terminal weight of 407±24 g, which was higher than the weight of the rats in the SHAM + ND group (P<0.05). Among the HFD groups, the final body weight of the rats in the OVX + HFD group increased significantly (301±14 g) compared with the SHAM + HFD group (501±27 vs. 407±24 g; P<0.01). Nevertheless, the rats administered α-LA weighed less during the experimental period and α-LA significantly neutralized the increase in body weight in the ovariectomized rats fed HFD (433±31 vs. 501±27 g; P<0.01).

*Serum levels of E2 in ovariectomized rats.* As illustrated in Fig. 2, 12 weeks after OVX, the concentration of serum E2 was markedly decreased in the OVX groups (OVX + HFD, 8.0±1.89 pg/ml; OVX + HFD + α-LA, 8.9±2.17 pg/ml) as compared with the SHAM groups (SHAM + ND, 15.04±1.74 pg/ml; SHAM + HFD, 14.36±2.31 pg/ml; P<0.01), which indicated that the procedure was successful.

*Effects of α-LA on serum levels of TG, TC, HDL-C, LDL-C and fasting blood glucose (FBG) in ovariectomized rats fed HFD.* The serum TG, TC, HDL-C, LDL-C and FBG levels were measured in the 4 groups to observe whether the ovariectomized hyperlipidemic and hyperglycemic rat model was successfully established (Table II). The concentration of TG in the SHAM + ND, SHAM + HFD and OVX + HFD groups was similar (p>0.05). Of note, in the OVX + HFD+ α-LA group, the rats had lower levels of serum TG than those in the OVX + HFD group (P<0.05). In the SHAM groups, the rats fed HFD showed a significant increase in serum TC and LDL-C levels as compared with the rats fed ND (P<0.05). However, the ovariectomized rats fed HFD had more severe hypercholesterolemia than the rats in the SHAM + HFD group (P<0.05).



Figure 2. Serum levels of E2 (n=8-10 per group). Data represent the means ± SEM. [**]P<0.01 vs. sham-operated group fed a normal diet (SHAM + ND); [§§]P<0.01 vs. sham-operated group fed a high-fat diet (SHAM + HFD). α-LA, α-lipoic acid.

Conversely, compared with the OVX + HFD group, 12 weeks of α-LA administration markedly decreased the serum levels of TC and LDL-C (P<0.05). In addition, OVX plus HFD did not reduce the levels of serum HDL-C, but increased HDL-C levels as compared with the rats in the SHAM groups (OVX + HFD vs. SHAM + ND; OVX + HFD vs. SHAM + HFD; P<0.01) and the α-LA-treated rats had higher levels of serum HDL-C than the rats in the OVX + HFD group (P<0.05). Rats in the SHAM + HFD group had higher levels of FBG than the rats in the SHAM + ND group (P<0.01). In addition, as compared with the rats in the SHAM + HFD group, the ovariectomized rats fed HFD showed a marked increase in FBG levels (P<0.01). However, after 12 weeks of α-LA administration, the levels of FBG in the rats in the OVX + HFD + α-LA group significantly decreased (P<0.05).

Table II. Effects of α-LA on serum levels of TG, TC, HDL-C, LDL-C and FBG in ovariectomized rats fed a high-fat diet.

| | Animal groups | | | |
|---|---|---|---|---|
| | SHAM + ND | SHAM + HFD | OVX + HFD | OVX + HFD + α-LA |
| TG (mmol/l) | 1.54±0.43 | 1.56±0.36 | 1.63±0.35 | 1.23±0.27[e] |
| TC (mmol/l) | 1.62±0.28 | 2.25±0.49[a] | 2.93±0.56[a,c] | 2.29±0.36[a,e] |
| LDL-C (mmol/l) | 0.17±0.05 | 0.25±0.05[a] | 0.27±0.03[a] | 0.21±0.06[e] |
| HDL-C (mmol/l) | 0.63±0.30 | 0.59±0.16 | 1.25±0.23[b,d] | 1.53±0.20[e] |
| FBG (mmol/l) | 5.34±0.66 | 6.87±0.68[b] | 8.54±0.49[b,d] | 5.97±0.69[e] |

Data are the means ± SEM (n=8-10 per group). [a]P<0.05, [b]P<0.01 vs. sham-operated group fed normal diet (ND) (SHAM + ND); [c]P<0.05, [d]P<0.01 vs. sham-operated group fed high-fat diet (HFD) (SHAM + HFD); [e]P<0.05 vs. ovariectomized rats fed HFD (OVX + HFD). TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose.
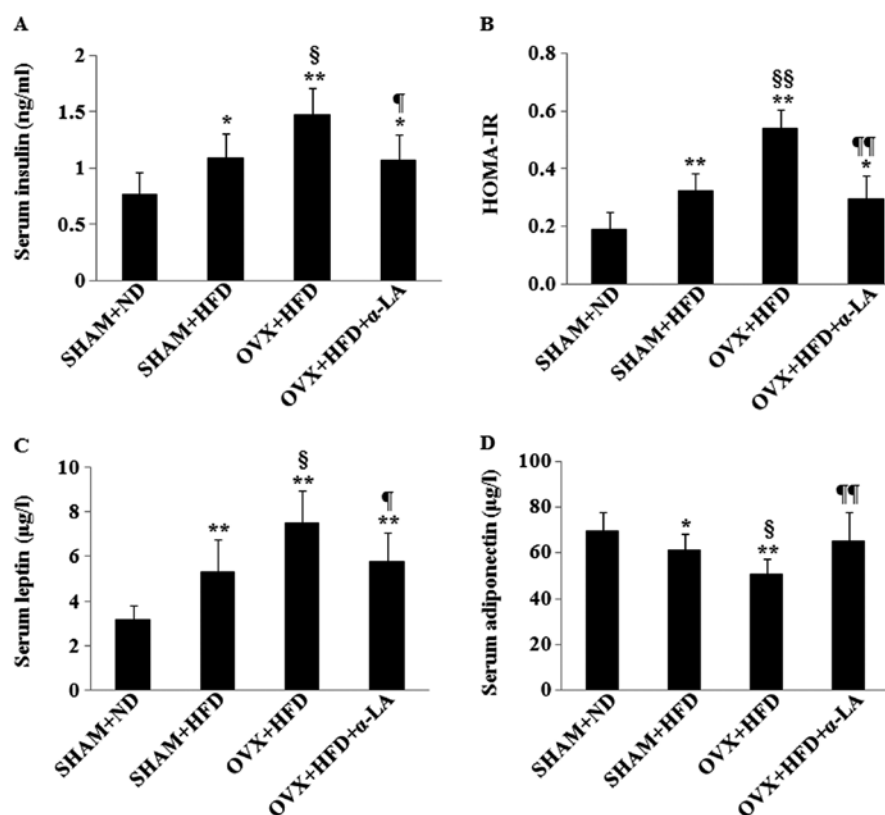


Figure 3. Effect of α-lipoic acid (α-LA) on metabolic parameters in ovariectomized rats fed a high-fat diet (n=8-10 per group). Data represent the means ± SEM. (A) Serum insulin levels were measured using the ELISA kit. (B) Homeostasis model assessment of insulin resistance (HOMA-IR) values were calculated using the formula: FBG x insulin/22.5. (C) Serum leptin levels were measured using the ELISA kit. (D) Serum adiponectin levels were measured using the ELISA kit. *P<0.05, **P<0.01 vs. sham-operated group fed a normal diet (SHAM + ND); §P<0.05, §§P<0.01 vs. sham-operated group fed a high-fat diet (SHAM + HFD). ¶P<0.05, ¶¶P<0.01 vs. ovariectomized rats fed a high-fat diet (OVX + HFD).

*α-LA improves insulin sensitivity in ovariectomized rats fed HFD.* To determine the effects of α-LA on the metabolic parameters in the OVX + HFD rats, we observed the content of serum insulin by ELISA. In addition, we calculated the homeostasis model assessment of insulin resistance (HOMA-IR) values to evaluate insulin resistance in obese rats. As shown in Fig. 3A and B, compared with the SHAM + ND group, HFD alone markedly increased the serum levels of insulin (0.77±0.20 vs. 1.09±0.21 ng/ml; P<0.05), as well as HOMA-IR values (0.19±0.06 vs. 0.32±0.06 ng/ml; P<0.01),

whereas OVX rats fed HFD developed hyperinsulinemia (P<0.05) and had higher HOMA-IR values (P<0.01) than the rats in the OVX + HFD group. Of note, α-LA markedly counteracted the increase in serum insulin levels (1.07±0.23 vs. 1.47±0.24 ng/ml; P<0.05) and HOMA-IR values (0.30±0.08 vs. 0.54±0.06 ng/ml; P<0.01) induced by OVX plus HFD.

*Effects of α-LA on serum levels of adiponectin and leptin in ovariectomized rats fed HFD.* Fig. 3C demonstrates that the concentration of leptin in the SHAM + HFD and OVX + HFD
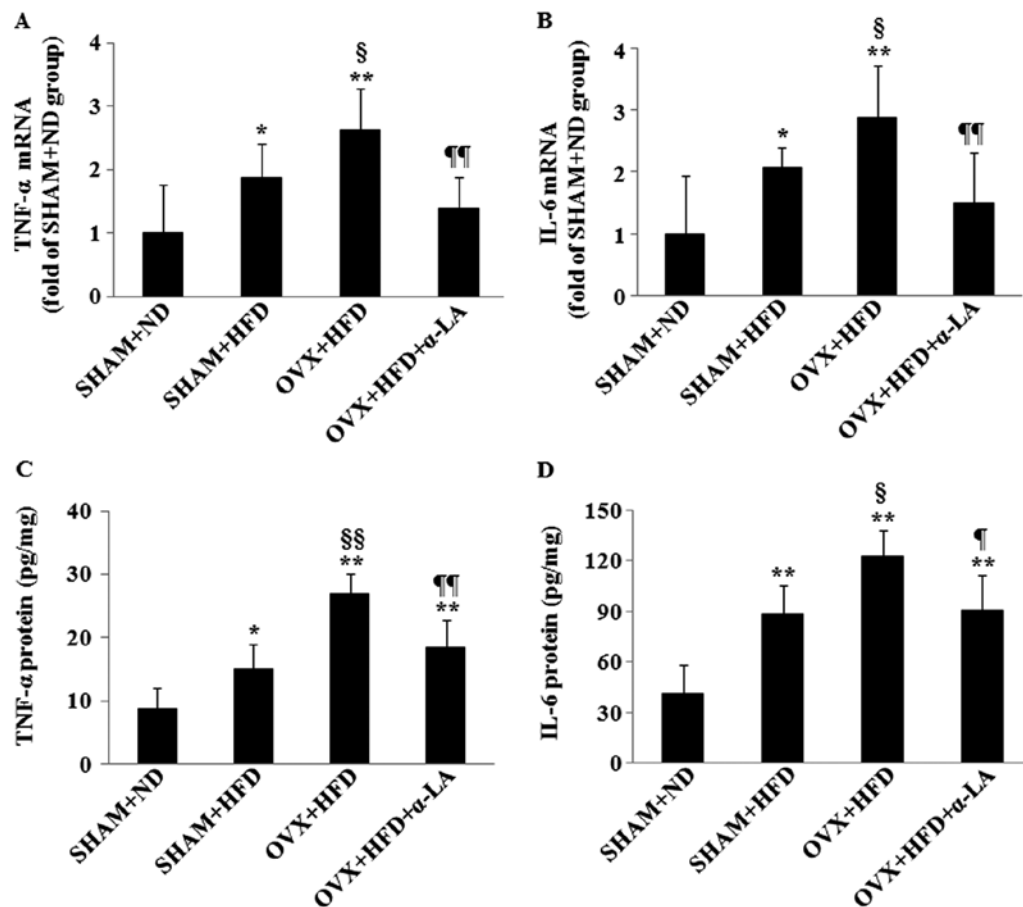
Figure 4. mRNA and protein expression of TNF-α and IL-6 in the hippocampus (n=8-10 per group). Data represent the means ± SEM. (A) The mRNA expression of TNF-α was measured by RT-PCR. (B) The mRNA expression of IL-6 was measured by RT-PCR. (C) The protein expression of TNF-α in the hippocampus was measured by ELISA. (D) The protein expression of IL-6 in the hippocampus was measured by ELISA. $^*P<0.05$, $^{**}P<0.01$ vs. sham-operated group fed a normal diet (SHAM + ND); $^{§§}P<0.01$ vs. sham-operated group fed a high-fat diet (SHAM + HFD). $^{¶}P<0.05$, $^{¶¶}P<0.01$ vs. ovariectomized rats fed a high-fat diet (OVX + HFD). α-LA, α-lipoic acid.

groups was significantly increased as compared with that in the SHAM + ND group (P<0.01). However, the ovariectomized rats fed HFD had severe hyperleptinemia compared with the rats fed HFD alone (P<0.05). Following treatment with α-LA for 12 weeks, the rats in the OVX + HFD + α-LA group had lower leptin levels as compared with the rats in the OVX + HFD group (P<0.05). As shown in Fig. 3C, in the SHAM groups, serum adiponectin levels were lower in the rats fed HFD alone than the others (P<0.05), Nevertheless, the content of adiponectin was markedly reduced in the OVX + HFD group as compared with the SHAM + HFD group (P<0.05); α-LA administration significantly reversed hypoadiponectinemia induced by OVX plus HFD (P<0.01 vs. OVX + HFD).

*Effects of α-LA on hippocampal cytokine levels.* To explore neruoinflammation in the hippocampus, we evaluated the mRNA expression of TNF-α and IL-6 by RT-PCR and the protein levels of TNF-α and IL-6 by ELISA. Fig. 4A and B illustrates that obesity induced by HFD alone significantly increased the mRNA expression of TNF-α and IL-6 in the hippocampus (P<0.05) compared with SHAM + ND. Additionally, as compared with SHAM + HFD, OVX in conjunction with HFD induced a marked increase in TNF-α and IL-6 mRNA expression in the hippocampus (P<0.01).

However, after 12 weeks of α-LA administration (OVX + HFD + α-LA), TNF-α and IL-6 mRNA levels were markedly downregulated (P<0.01 vs. OVX + HFD).

Similarly, as shown in Fig. 4C and D, the protein expression of TNF-α (P<0.05) and IL-6 (P<0.01) in the hippocampus was significantly increased in the SHAM + HFD group as compared with the SHAM + ND group. Furthermore, OVX plus HFD aggravated the increase in TNF-α (P<0.01) and IL-6 (P<0.05) protein levels compared with the rats fed HFD alone. In accordance with the significant effect on TNF-α and IL-6 mRNA expression, the protein levels of TNF-α and IL-6 in the hippocampus were markedly decreased following treatment with α-LA (TNF-α, P<0.01 vs. OVX + HFD; IL-6, P<0.05 vs. OVX + HFD).

*Effects of α-LA on BDNF mRNA levels in the hippocampus.* Fig. 5 illustrates the changes in BDNF mRNA expression in the 4 groups. Rats in the SHAM + HFD group had lower BDNF mRNA levels than the rats in the SHAM + ND group (P<0.05). Furthermore, compared with the SHAM + ND group, OVX in conjunction with HFD significantly downregulated the mRNA expression of BDNF (P<0.01). Of note, the administration of α-LA significantly reversed the decrease in BDNF mRNA levels in the hippocampus induced by OVX and HFD (P<0.01).
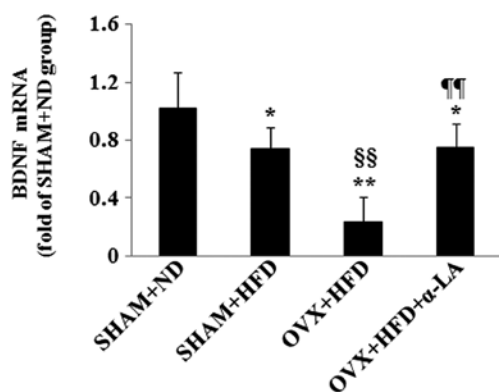
Figure 5. Effects of α-lipoic acid (α-LA) on hippocampal brain-derived neurotrophic factor (BDNF) mRNA expression (n=8-10 per group). Data represent the means ± SEM. The mRNA expression of BDNF was measured by RT-PCR. *P<0.05, **P<0.01 vs. sham-operated group fed a normal diet (SHAM + ND); §§P<0.01 vs. sham-operated group fed a high-fat diet (SHAM + HFD). ¶¶P<0.01 vs. ovariectomized rats fed a high-fat diet (OVX + HFD).



Figure 6. Effects of α-lipoic acid (α-LA) on hippocampal brain-derived neurotrophic factor (BDNF) protein expression (n=8-10 per group). Data represent the means ± SEM. The protein level of BDNF in the hippocampus was measured by western blot analysis and ELISA. *P<0.05, **P<0.01 vs. sham-operated group fed a normal diet (SHAM + ND); §P<0.05 vs. sham-operated group fed a high-fat diet (SHAM + HFD). ¶P<0.05 vs. ovariectomized rats fed a high-fat diet (OVX + HFD).
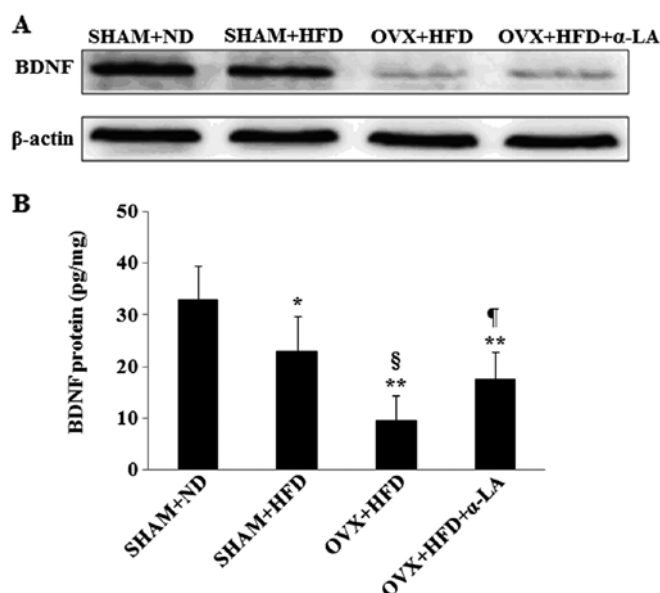
*Effects of α-LA on BDNF protein levels in the hippocampus.* We evaluated the effects of α-LA on BDNF protein levels in the hippocampus in ovariectimized rats fed HFD by western blot analysis and ELISA. As illustrated in Fig. 6, the changes in the BDNF protein levels in the 4 groups were similar to those observed for BDNF mRNA expression in the hippocampus. The levels of BDNF protein in the sham-operated group fed HFD were lower than those in the SHAM + ND group (P<0.05). In addition, OVX in conjunction with HFD significantly decreased the BDNF concentration in comparison with SHAM + HFD (P<0.05). Following treatment with α-LA, the levels of BDNF protein in the hippocampus were markedly upregulated as compared with OVX + HFD (P<0.05).

## Discussion

A recent study investigated the positive association between obesity-induced chronic inflammation and cognitive defects (21). Severely obese exhibit abnormal cytokines in the brain due to macrophage infiltration into excessive adipose tissue (22) and this aggravated neuroinflammation can adversely influence the process of neural plasticity and neurotransmitter metabolism, which participate in the regulation of cognition (23). Additionally, obesity can induce neuronal insulin resissitance owing to the activation of inflammatory pathways, ultimately impairing neural signaling pathways and leading to cognitive function deficits (24,25). Furthermore, menopause contributes to a disordered production of inflammatory factors (10) and obesity in post-menopausal women amplifies the primary state of inflammation (26). The changes described above may be related to the incidence of metabolic disorders, such as insulin resissitance, dyslipidemia, as well as cognitive impairment (7,27).

It had been reported that α-LA exerts neuroprotective effects on AD and attenuates the spatial learning impairment of mice induced by HFD (19,28). However, to our knowledge, the effects of α-LA on neuroinflammation and BDNF expression in ovariectomized rats fed HFD have not been explored to date. In the present study, an animal model to mimick the hyperlipidemic state post-menopause was induced by OVX and HFD and the effects of α-LA on central inflammation and neuroprotection, as well as on peripheral metabolic parameters were examined. Our data demonstrated that α-LA not only improved insulin sensitivity and metabolic parameters, but also increased hippocampal BDNF expression and the underlying mechanisms may be associated with inhibiting neuroinflammatory responses. These results provide further support for the neuroprotective role of α-LA in rats with obesity induced by OVX and HFD.

It has been suggested that OVX or post-menopause lead to a higher risk of obesity, responsible for the lack of hormonal protection and increase the susceptibility to abnormal serum lipid levels (29). In the present study, although there were no differences observed in the levels of TG in the 4 groups, serum TC, LDL-C levels and the body weights of the rats in OVX + HFD group were significantly increased. In addition, the ovariectomized rats fed HFD exhibited hyperinsulinemia and insulin resistance. Moreover, our results demonstrated that OVX did not increase the level of LDL-C in rats fed HFD. It has been confirmed that α-LA not only plays a role in inhibiting fat accumulation in diabetic rats (30), but also significantly decreases appetite and fat accumulation through the regulation of central and peripheral adenosine monophosphate-activated protein kinase (AMPK) activities in rats with obesity induced by OVX (20). In the current study, our data illustrated that α-LA significantly ameliorated the increase in body weight and improved the dysregulation of metabolism, including lipid and insulin in ovariectomized rats fed HFD. The alleviation of peripheral hyperlipidemia and hyperinsulinemia may be conductive to reduce lipid levels in the hippocampus, as well as nueral insulin resistance, which restores cognitive ability.

Apart from abnormal serum lipid and insulin levels, ovariectomized rats fed HFD presented higher levels of leptin and lower adiponectin levels in the peripheral circulation. A

previous study published by Trujillo *et al* (31), as well as another study, demonstrated that adiponectin not only increased insulin sensitivity, but also exhibited potent anti-inflammatory effects in vascular tissue and in the hippocampus by affecting the AMPK/ACC signaling pathway (31,32). Leptin, participating in the regulation of energy homeostasis, increases appetite and body weight and is associated with inflammatory response (33). Recent evidence supports that α-LA downregulates serum leptin levels and upregulates circulating adiponectin levels in ovariectimized rats (20). In accordance with the results mentioned above, in our study, the rats treated with α-LA had lower levels of leptin and higher levels of adiponectin and these alterations may be beneficial for ameliorating brain inflammation.

It is well established that obesity-related cognitive impairment is closely connected with brain inflammation. In this study, in order to evaluate the lipid profile in the hippocampus, we observed the changes in cytokines secreted by macrophages infiltrating adipocytes, such as IL-6 and TNF-α which are known to affect cognitive behavior (15,16). The mRNA and protein expression of IL-6 and TNF-α was markedly increased in the hippocampus of ovariectomized rats fed HFD, suggesting that the inflammatory pathway in the hippocampus was activated in the obese ovariectomized rats fed HFD. Mounting evidence demonstrates that brain inflammation can adversely influence cognition, learning and memory by disturbing the process of long-term potentiation and synaptic plasticity (34-36). As observed in previous studies, α-LA exerts neuroprotective effects partly by attenuating inflammation through the cAMP/protein kinase A (PKA) signaling cascade (19,37). In accordance with these studies, our results demonstrated that following the administration of α-LA for 12 weeks, rats in the OVX + HFD + α-LA group had lower levels of IL-6 and TNF-α in the hippocampus, which indicated that α-LA administration attenuated obesity-associated hippocampal neuroinflammation induced by OVX and HFD and improved cognitive deficits in the rats.

To ascertain the alterations in rat cognitive ability in each group, the mRNA and protein expression of BDNF was detected in the hippocampal tissue. BDNF, one of the neurotrophic factors, is a pivotal regulator in the process of synaptic plasticity and memory formation (38). A recent study demonstrated that BDNF is a biomarker of neurodegenerative diseases, including AD (39) and it has been reported that cognitive impairment induced by HFD or OVX correlates with the downregulation of BDNF expression in the hippocampus (40,41). In agreement with the above data, our data demonstrated that OVX and HFD significantly aggravated the decrease in BDNF mRNA and protein levels in the rat hippocampus, which indicated that HFD accentuates cognitive impairment in estrogen-deficient rats. Of note, the alteration in BDNF levels negatively correlated with the changes in IL-6 and TNF-α levels in the hippocampus; these results further corroborate the evidence that the expression of BDNF in the hippocampus is negatively regulated by cytokines, as described in previous studies (42,43). Furthermore, the decreased mRNA and protein levels of BDNF in the hippocampus were reversed by treatment with α-LA; conversely, the levels of IL-6 and TNF-α were reduced following treatment with α-LA. These results suggest that the increased levels of cytokines observed in the hippocampus of obese rats may ascribe to reducing the expression of BDNF and the administration of α-LA increases the expression of hippocampal BDNF in ovariectomized rats fed HFD, exerting neuroprotective effects by attenuating neuroinflammation.

In conclusion, the present study demonstrates that α-LA markedly increases the expression of BDNF in the hippocampus of rats with obesity induced by OVX and HFD, possibly by improving lipid metabolism, enhancing insulin sensitivity and regulating neuroinflammation. Therefore, this study provides a theoretical basis that α-LA may prove useful for the prevention of AD in obese post-menopausal women.

## Acknowledgements

## References

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