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ORIGINAL PAPER

Cardiotrophin-1 (CT-1) Improves High Fat Diet-Induced Cognitive Deficits in Mice

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Abstract Previous studies demonstrated that a high fat diet (HFD) results in a loss of working memory in mice correlated with neuroinflammatory changes as well as synaptodendritic abnormalities and brain insulin resistance. Cardiotrophin-1 (CT-1), a member of the gp130 cytokine family, has been shown to potentially play a critical role in obesity and the metabolic syndrome. Our recent studies have demonstrated that CT-1 attenuates cognitive impairment and glucose-uptake defects induced by amyloid-β in mouse brain through inhibiting GSK-3β activity. In this study, we evaluated the effect of CT-1 on cognitive impairment induced by brain insulin resistance in mice fed a HFD, and explored its potential mechanism. CT-1 (1 μg/day, intracerebroventricular injection) was given for 14 days to mice that were fed with either a HFD or normal diet for 18 weeks. After 20 weeks of treatment, our results showed that in the HFD group, CT-1 significantly improved learning and memory deficits and alleviated neuroinflammation demonstrated by decreasing brain levels of proinflammatory cytokine tumour necrosis factor-α and interleukin-1β, and increasing brain levels of anti-inflammatory cytokine IL-10. CT-1 significantly reduced body weight gain, restored normal levels of blood glucose, fatty acids and cholesterol. Furthermore, CT-1 significantly enhanced insulin/IGF signaling pathway as indicated by increasing the expression levels of insulin receptor substrate 1 (IRS-1) and the phosphorylation of Akt/GSK-3β, and reducing the phosphorylation of IRS-1 in the hippocampus compared to control. Moreover, CT-1 increased the level of the post-synaptic protein, PSD95, and drebrin, a dendritic spine-specific protein in the hippocampus. These results indicate a previously unrecognized potential of CT-1 in alleviating high-fat diet induced cognitive impairment.

Keywords CT-1 · High fat diet · Cognitive deficits · Insulin/IGF signaling · Neuroinflammation

Abbreviations

CT-1	Cardiotrophin-1
GSK-3β	Glycogen synthase kinase-3β
HFD	High fat diet
IL-10	Interleukin-10
IL-1β	Interleukin-1β
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
IIS	Insulin/IGF signaling
PSD95	Postsynaptic density protein 95
SYP	The action of synapsin I
TNF-α	Tumor necrosis factor-α

Introduction

Obesity is defined as abnormal or excessive fat accumulation and has become one of the most serious health

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problems with increased risk of several diseases such as type-2 diabetes mellitus, insulin resistance, and metabolic syndrome [1–4]. There is increasing evidence that a high-fat diet can induce neuroinflammation and synaptic plasticity impairment, ultimately resulting in neurodegeneration and cognitive deficits [5–7]. Recent findings suggest that neuronal insulin resistance induced by high-dietary cholesterol is a contributing factor [8]. There is a substantial amount of experimental and clinical evidence that brain insulin resistance and impaired insulin/IGF signaling (IIS) are implicated in the pathogenesis of cognitive impairment and neurodegeneration [9–14].

Cardiotrophin-1 (CT-1), a member of the gp130 cytokine family, expressed at high levels in the embryonic limb bud and secreted by differentiated myotubes [15, 16], has been shown to exhibit impressive neuroprotective effects and delay the procession of motor neuron degenerative disorder in mouse models of amytrophic lateral sclerosis (ALS) [17], progressive motor neuropathy (PMN) [18, 19] and spinal muscular atrophy (SMA) [20] and in adult rats with spinal cord injuries [21]. CT-1 is not only expressed in peripheral tissues but also in the postnatal and adult central nervous system [16, 22], including the hippocampus, an important area for learning and memory. It has been shown that CT-1 is a key regulator of glucose and lipid metabolism in cell lines [23, 24], obesity-associated animal models [25] and clinical obesity patients [26]. Recently, one study reported that CT-1 attenuated inflammation, improved insulin signaling, and eliminated hepatic steatosis in high fat diet (HFD) induced obese mice [27]. Another member of the gp130 cytokine family, ciliary neurotrophic factor (CNTF), has been demonstrated to be effective in the reduction of body weight, together with an improvement of insulin sensitivity in diabetes animal models [28, 29]. Our previous studies have demonstrated that CT-1 might improve learning and memory and glucose uptake in Aβ-induced Alzheimer's disease mouse model [30]. However, no reports are available which are aimed to investigate whether CT-1 has a protective effect against cognitive impairment induced by exposure to HFD and its medical comorbidities in mice fed on a HFD. The purpose of our present study is designed to investigate the potential mechanism of protective effects of CT-1 on cognitive impairment induced by exposure to HFD.

Materials and Methods

Animals

C57BL/6 mice were housed individually in plastic rodent cages and maintained on a 12 h light/dark cycle with ad libitum access to conventional standard rodent chow and water, with the constant temperature (23 ± 1 °C) and relative humidity (65 %). Protocols were conducted according to the University Policies on the Use and Care of Animals and were approved by the Institutional Animal Experiment Committee of Henan University of Science and Technology, China.

Group and Treatment

Forty 4-week-old male mice were randomized into four groups: control diet (CD); CD + CT-1 (CDC); HFD; HFD + CT-1 (HFDC), and assigned to either HFD or CD chow (Research Diets, Inc., New Brunswick, NJ) for 20 weeks (n = 10 each). CD consisted of the following (in Kcal): protein (19 %), carbohydrate (68 %), and fat (13 %), and HFD consisted of the following (in Kcal): protein (15 %), carbohydrate (43 %), and fat (42 %). 18 weeks later, CDC and HFDC groups were received intracerebroventricular (*i.c.v.*) injection of recombinant mouse CT-1 (1 μg/day) for 14 consecutive days. In parallel, CD and HFD mice were administered saline via *i.c.v.* injection in similar manner. The dose of CT-1 was selected based on other experimental studies [25, 31]. The experimental procedures are shown in Fig. 1. After the behavioural testing, mice were sacrificed and brain tissue was collected immediately for experiments or stored at −70 °C.

Surgery and *i.c.v.* Microinjections

Mice anesthetized with anesthetic ether, were shaved on the dorsal skull surface and cleaned with 70 % isopropyl alcohol followed by 10 % betadine iodine solution, then were transferred to a stereotactic apparatus (Stoelting Company, Wood Dale, IL, USA). A 26-gauge stainless-steel guide cannula (Plastics One, Roanoke, VA, USA) was directed toward the midhypothalamus in the third ventricle using flat-skull coordinates from bregma (AP 0 mm, ML 0 mm, DV −5.1 mm). The guide cannula was secured with cyanoacrylate gel (Plastics One) and acrylic dental cement (Jet Denture Repair, Lang Dental Manufacturing Co., Wheeling, IL, USA); the incision was closed with 4-0 silk suture (Syneture; Tyco Healthcare Group, Mansfield, MA, USA). Mice were received 1 μl of 1 mg/ml recombinant

Fig. 1 Timeline of experimental procedure

mouse CT-1 in PBS, or PBS alone, slowly infused through a 30-gauge internal cannula (Plastics One) with a 2-µl Hamilton syringe (Fisher Scientific; Nepean, ON, Canada). Microinjections were administered over 14 consecutive days. Correct placement of the cannula was confirmed by injection of angiotensin II (50 ng). Animals not displaying a prompt and sustained drinking response were excluded from the study.

Behavioural Tests

Novel Object Recognition Test

The test procedure consisted of three sessions: habituation, training, and retention. Each mouse was habituated to the box (30 × 30 × 35 cm), with 10 min of exploration in the absence of objects for 3 days (habituation session). During the training session, two objects were placed at the back corner of the box. A mouse was then placed in the box and the total time spent exploring the two objects (blue wooden cubes of size 3 cm) was recorded for 10 min. During the retention session, the mice were placed back in the same box 24 h after the training session, in which one of the familiar objects used during the training was replaced with a novel object (a yellow wooden cylinder of diameter 3 cm and height 3 cm). The animals were then allowed to explore freely for 5 min, the exploration time for the familiar (TF) or the new object (TN) during the test phase was recorded. The exploration time for the familiar (T_F) or the new object (T_N) during the test phase was videotaped and analyzed using the Noldus Ethovision XT software (Noldus Information Technology, Wageningen, The Netherlands). Memory was defined by the recognition index (RI) for the novel object as the following formula: $RI = T_N/(T_N + T_F)$. To control for odor cues, the OF arena and the objects were thoroughly cleaned with 10 % odorless soap, dried, and ventilated for a few minutes between mice [32, 33].

Morris Water Maze

Spatial learning and memory was tested using the Morris water maze, performed after the end of novel object recognition test. The protocol for the Morris water maze test was modified from previously reported methods [34, 35]. Briefly, the apparatus included a pool with a diameter of 100 cm that was filled with opaque water at approximately 22 ± 1 °C. An escape platform (15 cm in diameter) was placed 0.5 cm below the water surface. Geometric objects with contrasting colours were set at the remote ends of the water tank as references. Room temperature was constant, and the lighting was even throughout the room. Spatial memory is assessed by recording the latency time for the animal to escape from the water onto a submerged escape platform during the learning phase. The mice were subjected to four trials per day for 5 consecutive days. The mice were allowed to stay on the platform for 15 s before and after each trial. The time that it took for an animal to reach the platform (latency period) was recorded. Twenty-four hours after the learning phase, the mice swam freely in the water tank without the platform for 60 s, and the time spent in the region, and number of passes through the region and the quadrant of the original platform were recorded. Monitoring was performed with a video tracking system (Noldus Ltd, Ethovision XT, Holland).

Body Weight and Biochemical Analysis

Body weight was measured every week. After 20 weeks on a HFD, for glucose/insulin measurements, blood samples were taken by tail venipuncture using heparin-coated capillary tube [36]. For insulin measurements, whole blood was centrifuged at 16,000*g* for 7 min to pellet blood cells. The plasma was transferred to a fresh tube and placed on dry ice, after which it was stored at −70 °C. Serum insulin levels were measured with the appropriate enzyme-linked immunosorbent assay kits (ALPCO Diagnostics, Windham, NH, USA). Plasma levels of non-esterified fatty acids were measured using a non-esterified fatty acids assay C kit (Wako Chemicals, Richmond, VA, USA) according to the manufacturer's instructions. Total cholesterol was measured directly in whole blood using a Cardiochek meter (PTS, Indianapolis, IL, USA).

Elisa

Mouse hippocampus (n = 5 each) was sampled and 100 mg of tissue per animal was homogenized in 1.0 mL of 0.9 % NaCl solution containing 0.1 % PMSF (Sigma, MO, USA). After centrifugation at 14,000*g* for 15 min at 4 °C, the resulting supernatants were sampled in triplicate to detect the levels of TNF-α, IL-1β, and IL-10 by an ELISA kit (R&D Systems and Invitrogen) according to the provided instructions. Standard curves were generated using purified recombinant protein supplied by the manufacturers. Quantitation was performed on an ELISA microplate reader (Molecular Devices Corp, San Diego, CA). All samples were run in quadruplicate, with results expressed as mean f standard error of the mean.

Western Blot Analysis

Following behavioral assessment, animals were deeply anesthetized with isoflurane and sacrificed by decapitation. The hippocampus (n = 5 each) was directly homogenised in RIPA buffer containing 0.1 % PMSF and 0.1 % protease

inhibitor cocktail (Sigma, MO, USA). The lysates were centrifuged at 14,000*g* for 30 min at 4 °C and the supernatant was used for protein analyses. The protein concentration in supernatants was determined using the BCA method. Equal amounts of soluble protein were separated by SDS-PAGE and transferred onto a nitrocellulose membrane (Immobilon NC; Millipore, Molsheim, France). Immunoblotting was performed with antibodies specific for phospho-IRS-1-Ser612 (3203), IRS-1 (3407), p-AKT-Ser473 (4060), AKT (4691), p-GSK3β-Ser9 (9323), GSK3β (9315) (Cell Signaling Technology), SYP (ab8049), PSD-95 (ab18258), drebrin (ab12350) (Abcam). Primary antibodies were visualised using anti-rabbit HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, Inc.) and a chemiluminescent detection system (Western blotting Luminal Reagent; Santa Cruz Biotechnology, Inc.). Variations in sample loading were normalised relative to GAPDH.

Statistical Analysis

All data were expressed as the mean ± SEM. For the Morris water maze tests, escape latency in the hidden platform trial were analysed with two-way ANOVA of repeated measures, while one-way ANOVA was conducted on the data obtained from the probe trial. The other data were analysed by one-way ANOVA, followed by LSD. All analyses were performed with SPSS statistical package (version 13.0 for Windows, SPSS Inc., USA). Differences were considered significant at a p value <0.05.

Results

Behavioural Test

CT-1 Ameliorates Recognition Memory of HFD-Induced Obese Mice in Novel Object Recognition

To evaluate cognitive function, a novel object recognition test was carried out in CD mice, CDC mice, HFD mice and HFDC mice. In the test section, there was a significant overall group difference in the RI [$F(3, 36) = 18.48$, $p < 0.01$] among the four groups. Compared with CD mice, the RI ($p < 0.01$) was significantly reduced in HFD mice. CT-1 markedly increased the RI by 45.4 % ($t_{36} = 3.99$, $p = 0.000$) in the HFDC versus HFD group (Fig. 2a). There was no significant difference in RI between CD mice and CDC mice ($p > 0.05$). In addition, there was no significant difference in the RI (Fig. 2b) in training session between the four groups of mice ($p > 0.05$).

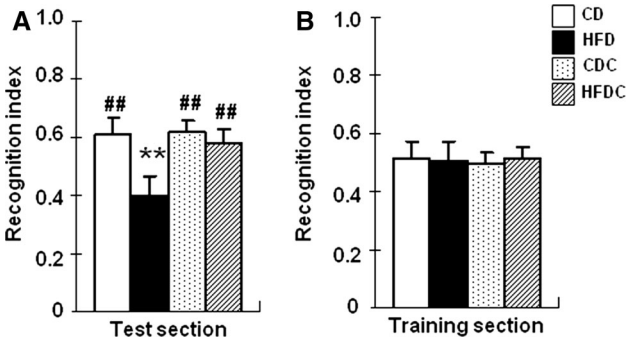

Fig. 2 Effect of CT-1 on the recognition memory in HFD-induced obese mice detected by a novel object recognition test. The RI in the test section (**a**) and training section (**b**) of mice on a CD and treated with CT-1 per se (CDC), HFD, high-fat diet and treated with CT-1 (HFDC) were measured. Values are presented as mean ± SEM. The analysis was performed using one-way ANOVA with a LSD post hoc test between groups (n = 10, $^{**}p < 0.01$ vs. CD mice; $^{\#\#}p < 0.01$ vs. HFD mice)

CT-1 Improves the Learning and Memory of HFD-Induced Obese Mice in the Morris Water Maze

To assess spatial reference learning and memory function, all mice underwent testing in the Morris water maze after 20 weeks administration. Spatial learning was assessed in the hidden platform task in all mice. As shown in Fig. 3a, there was a significant overall group difference in escape latency among the four groups [group effect: $F(3, 36) = 22.32$, $p < 0.01$; training day effect: $F(4, 144) = 72.78$, $p < 0.01$; group × training day interaction: $F(12, 144) = 0.49$, $p > 0.05$]. In the hidden platform test, the HFD group showed significantly increased escape latencies from day 3 compared to CD controls ($p < 0.01$ for day 3–4; $p < 0.05$ for day 5). When compared with the HFD group, escape latencies were significantly decreased in HFDC group from day 3 ($t_{36} = -3.49$, $p = 0.001$ for day 3; $t_{36} = -4.43$, $p = 0.000$ for day 4; $t_{36} = -2.47$, $p = 0.018$ for day 5). Note that all the mice had the same level of performance at the start of the experiment (no significant individual effects were observed in the first five trials on day 1).

In the probe test, the time spent in target quadrant and the crossing-target numbers were measured for 60 s on the 6th day after the last acquisition test. As shown in Fig. 3b, there was a significant overall group difference in the time spent in target quadrant [$F(3, 36) = 25.46$, $p < 0.01$] and crossing-target number [$F(3, 36) = 15.33$, $p < 0.01$] amongst the four groups. The HFD mice showed an obvious 48.8 % decrease in the time spent in target quadrant and a 51.7 % decrease in crossing-target number compared to the CD controls. The time in target quadrant and the crossing-target number of the HFDC mice were significantly increased by 54.5 % ($t_{36} = 3.99$, $p = 0.000$, vs.

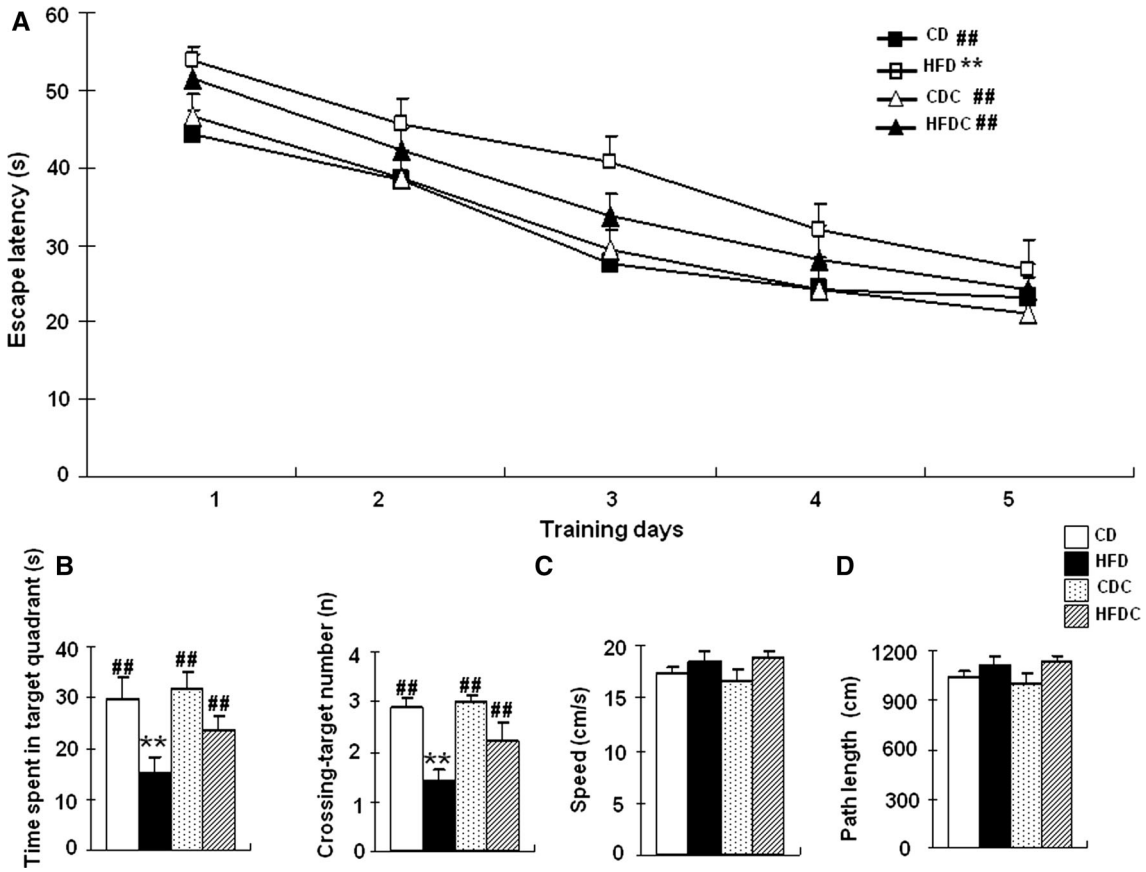

Fig. 3 Effect of CT-1 on learning and memory in HFD-induced obese mice using the Morris water maze. Escape latency during 5 days of hidden platform tests (**a**), the time spent in target quadrant and the crossing-target number in the probe test (**b**), swimming speed (**c**), and path length (**d**) in the probe test were tabulated. All data are presented as mean ± SEM (n = 10, $^{**}p < 0.01$ vs. CD mice; $^{\#\#}p < 0.01$ vs. HFD mice)

HFD group) and 57.1 % ($t_{36} = 2.99$, $p = 0.005$, vs. HFD group), respectively. Moreover, the results suggested that CT-1 treatment improved but not completely ameliorated deficits in the probe test through comparing HFDC mice with CDC mice. There was no significant difference in the time in target quadrant and the crossing-target number between CD mice and CDC mice ($p > 0.05$). In addition, there was no significant difference in swimming speed (Fig. 3c) and path length (Fig. 3d) in the probe test between the four groups of mice ($p > 0.05$).

CT-1 Reduces Body Weight Gain, Restores Abnormal Glucose, Fatty Acid and Cholesterol Metabolism to Near-Normal Levels in HFD-Induced Obese Mice

A substantial amount of evidence has demonstrated that obesity can induce excess lipid accumulation, abnormalities in intracellular energy fluxes and nutrient availability, might be a chronic stimulus for insulin resistance and inflammation [4]. On the basis of these observations, we further examined body weight, the levels of blood insulin, glucose, free fatty acids and cholesterol in each group. Our data showed that after 20 weeks of a high fat diet, mice exhibited significant differences in body weight [$F(3, 36) = 22.51$, $p < 0.01$] (Fig. 4a). Moreover, feeding a high fat diet to mice for 20 weeks caused significantly increased levels of blood insulin [$F(3, 36) = 48.00$, $p < 0.01$] (Fig. 4b), glucose [$F(3, 36) = 29.07$, $p < 0.01$] (Fig. 4c), free fatty acids [$F(3, 36) = 18.17$, $p < 0.01$] (Fig. 4d), and cholesterol [$F(3, 36) = 34.94$, $p < 0.01$] (Fig. 4e). However, CT-1 treated mice fed a HFD for 20 weeks markedly reversed the metabolic changes ($p < 0.01$ vs. HFD group). As shown Fig. S1, during the CT-1 treatment there was no difference in food intake among the CD mice and HFD mice. The food intake was also decreased in CDC group but no statistically significant versus the CD group. Moreover, CT-1 treatment suppressed the food intake in the HFDC group versus the HFD group ($p < 0.05$).

CT-1 Alleviates Neuroinflammation in the Brain of HFD-Induced Obese Mice

Neuroinflammatory molecules are important factors contributing to the pathogenesis and development of cognitive

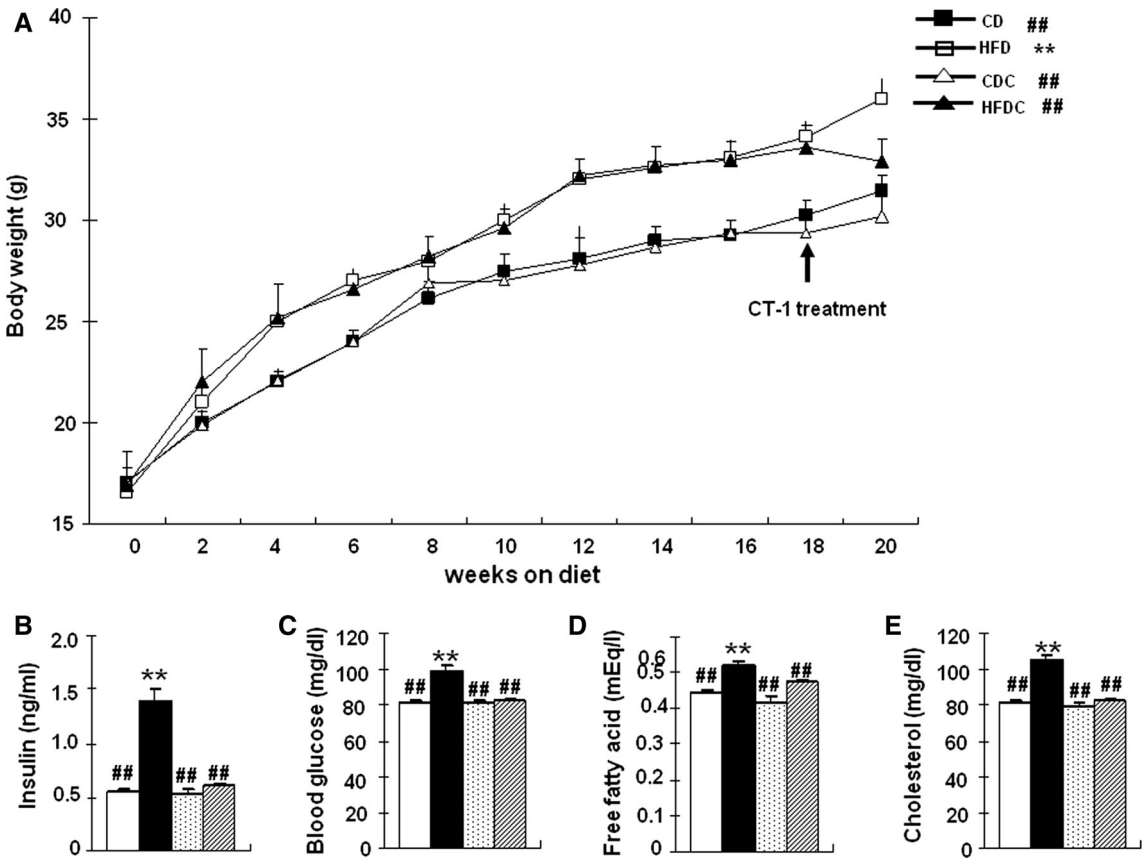

Fig. 4 CT-1 reduces body weight gain and restores abnormal glucose, fatty acid and cholesterol metabolism in HFD-induced obese mice. Total body weight (**a**), blood insulin (**b**), glucose (**c**), free fatty acids (**d**), and cholesterol levels (**e**) were performed. All data are presented as mean ± SEM (n = 10, $^{**}p < 0.01$ vs. CD mice; $^{\#\#}p < 0.01$ vs. HFD mice)

impairment, and increased levels of inflammatory cytokines can disrupt hippocampal synaptic plasticity. Thereafter, the effect of CT-1 on the levels of neuroinflammatory molecules, such as IL-1β, TNF-α, and IL-10 in the brain were determined by ELISA. As shown in Fig. 5, there was a significant overall group difference in the levels of TNF-α [$F(3, 16) = 31.52$, $p < 0.01$] (Fig. 5a), IL-10 [$F(3, 16) = 29.72$, $p < 0.01$] (Fig. 5b), and IL-1β [$F(3, 16) = 21.38$, $p < 0.01$] (Fig. 5c) amongst the four groups. We observed a significant elevation in the levels of TNF-α ($p < 0.01$) and IL-1β ($p < 0.01$), and a remarkable decrease in the level of IL-10 ($p < 0.01$) in the brain of HFD mice compared to the CD controls. CT-1 markedly decreased the levels of TNF-α by 41.4 % ($t_{16} = -6.99$, $p = 0.000$) and IL-1β by 31.7 % ($t_{16} = -5.94$, $p = 0.000$), and increased the level of IL-10 by 47.7 % ($t_{16} = 4.76$, $p = 0.000$) in the HFDC versus HFD group.

CT-1 Attenuates Impaired Insulin Signalling in HFD-Induced Obese Mice

In order to explore the effect of CT-1 on brain insulin signaling, the expression levels of IRS-1 and the phosphorylation of IRS-1, Akt/GSK-3β were investigated in hippocampus. The levels of the IRS-1 ($p < 0.01$), p-Akt ($p < 0.01$), and p-GSK-3β ($p < 0.01$) were significantly decreased and the level of the p-IRS-1 ($p < 0.01$) was significantly increased in HFD mice compared to the CD group. CT-1 significantly attenuated insulin signalling impairments as evidenced by a 33.3 % increase in the expression levels of IRS-1 ($t_{16} = 4.23$, $p = 0.001$), a 46.1 % decrease in the p-IRS-1 ($t_{16} = -6.25$, $p = 0.000$), a 1.10-fold increase in the p-Akt ($t_{16} = 8.85$, $p = 0.000$) and a 71.1 % increase ($t_{16} = 6.68$, $p = 0.000$) in the p-GSK-3β in the hippocampus of the HFDC versus HFD group (Fig. 6). In addition, there was no significant difference in the levels of total Akt ($p > 0.05$) and total GSK-3β ($p > 0.05$) between the four groups of mice (data not shown). When p-IRS-1 was compared, there was no significant difference in the levels of total IRS-1 ($p > 0.05$) between the four groups of mice (data not shown).

CT-1 Increases the Expression of Synaptic Proteins in HFD-Induced Obese Mice

To assess the variations of the expression of synapticproteins, we tested the determined the expressions of synapsin I

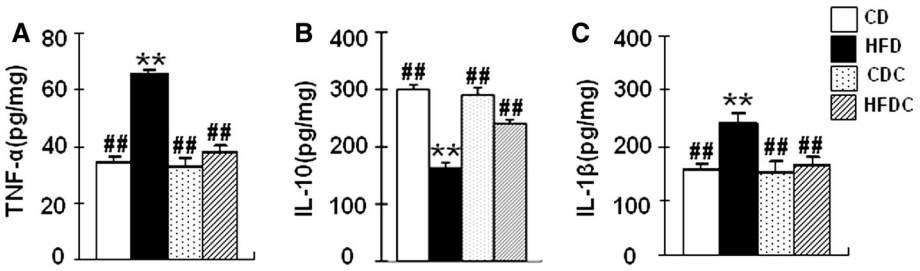

Fig. 5 CT-1 alleviates neuroinflammation in the brain of HFD-induced obese mice. After 20 weeks of administration, brain tissue from CD mice, HFD mice, CDC mice and HFDC mice were sampled and total lysates were isolated. The levels of TNF-α (**a**), IL-10 (**b**), and IL-1β (**c**) were detected by ELISA kits. All data are presented as mean ± SEM (n = 5, $^{**}p < 0.01$ vs. CD mice; $^{\#\#}p < 0.01$ vs. HFD mice)

(SYP), the post-synaptic protein (PSD95), and drebrin in hippocampus. The levels of PSD95 ($p < 0.01$) and drebrin ($p < 0.01$) were significantly decreased in HFD mice compared to the CD controls. CT-1 increased the levels of PSD95 by 44.4 % ($t_{16} = 5.23, p = 0.000$) and the levels of drebrin by 48.9 % ($t_{16} = 4.10, p = 0.001$) in the HFDC versus HFD group (Fig. 7). However, there was no significant difference in the SYP expression between the four groups.

Discussion

In the present study, we found CT-1 improved HFD induced cognitive deficits as indicated by enhancing learning and memory (i.e., increasing the RI by 45.4 % in the novel object recognition test and inducing a 57.1 % increase in the crossing-target number in the probe test) in mice. The results demonstrated that CT-1 reduced metabolic disorder, alleviated neuroinflammation, enhanced IIS pathway, and increased the post-synaptic protein expression in HFD induced mice.

A substantial amount of evidence indicates that obesity can contribute to the development of type-2 diabetes and ultimately result in cognitive impairment that might be caused by neuronal insulin resistance, brain inflammation and defective neural signalling pathways [13, 37, 38]. Recent findings indicate that a HFD also induces insulin resistance and IIS dysfunction [8, 39, 40], which is considered to be an underlying mechanism of neurotoxicity [41]. Insulin promotes cell survival through the inhibition of apoptosis-inducing peptides, facilitates neuronal growth and differentiation by enhancing neurite outgrowth and synapse formation, and regulates expression and localization of GABA [42], NMDA [43, 44], and AMPA [45, 46] receptors, and plays a critical role in synaptic plasticity [47, 48] and cognitive function [49–52]. Previous studies indicated that disruption of insulin action in the brain leads to deleterious neurobehavioral outcome and cognitive deficits in HFD-induced animal model [13, 39, 53]. Consistent with this, our results also demonstrate defective IIS, as indicating by the decreased the expression levels of insulin receptor substrate 1 (IRS-1) and the phosphorylation of

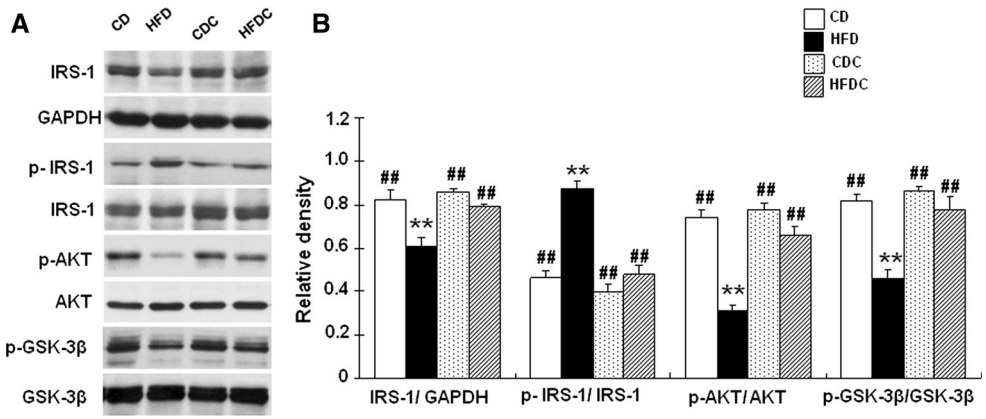

Fig. 6 CT-1 improves insulin signalling dysfunctions by increasing the expression levels of IRS-1 and the phosphorylation of Akt/GSK-3β, and reducing the phosphorylation of IRS-1 in the hippocampus of HFD-induced obese mice. The relative levels of IRS-1, p-IRS-1, p-AKT and p-GSK-3β were detected by Western blotting from hippocampus tissues of CD mice, HFD mice, CDC mice and HFDC mice, and a representative experiment was shown (**a**). The quantitative analysis of IRS-1, p-IRS-1 p-AKT, and p-GSK-3β using GAPDH, IRS-1, AKT, and GSK-3β as normalization, respectively (**b**). All data are presented as mean ± SEM (n = 5, $^{**}p < 0.01$ vs. CD mice;, $^{\#\#}p < 0.01$ vs. HFD mice)

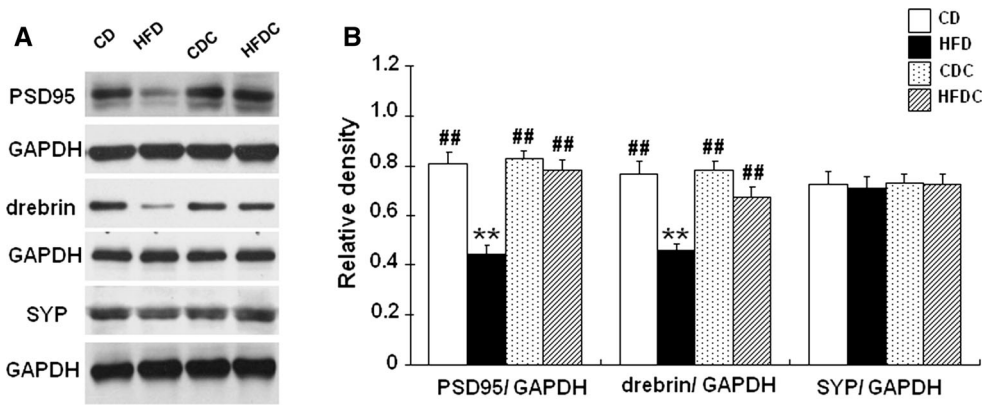

Fig. 7 CT-1 restores low levels of synaptic protein in the hippocampus of HFD-induced obese mice. The relative level of SYP, PSD95, and drebrin was detected by Western blotting from hippocampus tissues of CD mice, HFD mice, CDC mice and HFDC mice, and a representative experiment was shown (**a**). The quantitative analysis of SYP, PSD95, and drebrin using GAPDH as normalization (**b**). All data are presented as mean ± SEM (n = 5, $^{**}p < 0.01$ vs. CD mice; $^{\#\#}p < 0.01$ vs. HFD mice)

IRS-1, Akt/GSK-3β, and impaired learning and memory in HFD-induced obese mice. CT-1 has been shown to modulate insulin sensitivity and IIS through regulating the expression of the SOCS3 [24] and the peroxisome proliferator-activated receptor γ (PPARγ) and insulin receptor substrate-1 protein [23], and the level of insulin-stimulated Akt phosphorylation [25]. Furthermore, it has been demonstrated that CT-1 can phosphorylates Akt and prolongs cell survival in cardiac myocytes [54]. The amelioration of insulin resistance and impaired IIS in CNS is an effective way of preventing or reversing the cognitive deficits and attenuating the atrophy that is observed in obesity [55–58]. In this present study, CT-1 can attenuate insulin signaling dysfunction that is observed in HFD-induced obese mice to that which is observed in CD mice (Fig. 5), suggesting that CT-1 may exert positive effect on cognition.

Compelling evidence indicates that chronic low-grade inflammation in brain is involved in the type-2 diabetes and obesity-induced cognitive deficits. Neuroinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and IL-1β are activated and interleukin-10 (IL-10) are down-regulated in obesity-associated animal models and obese patients. CT-1 possesses anti-inflammatory effects, as have been reported in other gp130-dependent cytokines. Previous studies indicated that CT-1 decreases the levels of TNF-α and IL-1β, and increases the level of IL-10 in cell lines [59] and animal models [59, 60]. Consistent with this, our results show that CT-1 can repress the levels of TNF-α and IL-1β, and up-regulate anti-inflammatory cytokine IL-10 in brain. It has been demonstrated that IL-10 suppresses NF-κB activation and TNF-α production. Furthermore, CT-1 can inhibit TNF-a production in vivo and in vitro [59]. CT-1's ability to anti-inflammation may be caused by an improvement of insulin sensibility or/and an inhibition of NF-κB signal pathway. However, this hypothesis needs to be investigated further. Neuroinflammatory cytokines have an inhibitory effect on the tyrosine kinase activity of the IR and IGF-1R [61, 62], thus reduced the IIS cascade and associated down-stream mechanisms, and exacerbated insulin resistance, compounding the impact of obesity on the learning and memory loss.

Our results also indicated that CT-1 increased the level of the post-synaptic protein, PSD95, and drebrin, a dendritic spine-specific protein in the hippocampus compared with the control mice (Fig. 6). An inadequate IIS might have led to the reduction in the levels of PSD95 since its expression is known to be regulated by insulin signaling via PI3K-Akt-mTOR pathway [63]. The actin-binding protein, drebrin, is involved in shaping the dendritic spine morphology which is closely associated with spine function in learning and memory [64]. The receptors which access this pathway include those that recognize growth factors, hormones, antigens and inflammatory stimuli, and the cellular events known to be regulated include cell growth, survival, proliferation and movement [65]. It is an obvious connection between IIS and drebrin expression based on the observation that enhancement of IIS positively regulates the expression of drebrin via PI3K pathway [66]. Meanwhile, this present study observed unaltered levels of presynaptic protein synaptophysin in HFD-induced obese mice, suggesting that altered IIS was perhaps responsible for the observed postsynaptic changes. However, this hypothesis needs to be investigated further.

The hypothalamus plays an important role in regulating food intake and energy expenditure. Recent studies have shown that AMPK and its down-stream signaling pathways comprise a crucial regulatory system for food intake and body weight [67–69]. Activation of AMPK in the hypothalamus increases feeding and body weight gain,

whereas inhibition of hypothalamic AMPK activity promotes a reduction of food intake and weight lost [67, 70, 71]. Furthermore, previous studies have demonstrated that regulation of AMPK activity is involved in the improvement of CT-1 on metabolic disorders [27]. Interestingly, our results show that CT-1 treatment can significantly reduce food intake (Fig. 4a) and inhibit hypothalamic AMPK activity (data not shown). Based on these, we speculate that hypothalamic AMPK may be participated in the effects of CT-1 on food intake and body weight. However, this hypothesis needs to be investigated further. In this present study, the illumination of CT-1 on food intake is limited and the further studies are required to delineate the underlying mechanism.

In conclusion, CT-1 prevents obesity and reverses HFD-induced cognitive deficits. The enhancement of insulin/IGF-1 signaling and the inhibition of neuroinflammation in the mouse brain may be involved in the mechanisms by which CT-1 affects HFD-induced cognitive damage. CT-1 could be recommended as a possible candidate for the prevention and therapy of cognitive deficits induced by HFD.

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Conflict of interest The authors declare that they have no conflict of interest.

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