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The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition

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Abstract

A diet high in saturated fat (HF) decreases levels of brain-derived neurotrophic factor (BDNF), to the extent that compromises neuroplasticity and cognitive function, and aggravates the outcome of brain insult. By using the antioxidant power of vitamin E, we performed studies to determine the role of oxidative stress as a mediator for the effects of BDNF on synaptic plasticity and cognition caused by consumption of the HF diet. Male adult rats were maintained on a HF diet for 2 months with or without 500 IU/kg of vitamin E. Supplementation of the HF diet with vitamin E dramatically reduced oxidative damage, normalized levels of BDNF, synapsin I and cyclic AMP-response element-binding protein (CREB), caused by the consumption of the HF diet. In addition, vitamin E supplementation preserved the process of activation of synapsin I and CREB, and reversed the HF-impaired cognitive function. It is known that BDNF facilitates the synapse by modulating synapsin I and CREB, which have been implicated in synaptic plasticity associated to learning and memory. These results show that oxidative stress can interact with the BDNF system to modulate synaptic plasticity and cognitive function. Therefore, studies appear to reveal a mechanism by which events classically related to the maintenance of energy balance of the cell, such as oxidative stress, can interact with molecular events that modulate neuronal and behavioural plasticity.

Introduction

Emerging evidence indicates that lifestyle is a critical factor for determining the capacity of the brain to compensate for insults encountered in our daily life. Recent studies emphasize the importance of specific dietary components on modulating the expression and function of molecular systems involved with maintenance of neuronal health and function. In particular, a diet high in saturated fat (HF) decreases levels of brain-derived neurotrophic factor (BDNF) to the extent that compromises cognitive performance (Molteni *et al*., 2002). The HF diet can also aggravate the outcome of traumatic brain injury on neuroplasticity and cognitive function (Wu *et al*., 2003) using a mechanism associated with the action of BDNF. In addition, cognitive impairment was observed in rats fed high-fat diets (Greenwood & Winocur, 1996). It is well known that BDNF supports synaptic plasticity and neuronal excitability (Jovanovic *et al*., 2000) and seems important for normal learning and memory function (Linnarsson *et al*., 1997; Croll *et al*., 1998; Hall *et al*., 2000; Kovalchuk *et al*., 2002; Ying *et al*., 2002). Although abundant evidence maintains that a dysfunction of the BDNF system can affect normal memory function, it is not clear how HF diet consumption can translate into decreased levels of BDNF, with subsequent impairments in neuronal plasticity and function.

The mechanisms by which a HF diet can affect BDNF expression are largely unknown. Based on the effects of a HF diet on oxidative stress (OS), and the fundamental role that OS plays in neuronal function, we performed studies to evaluate a possible involvement of OS with the effects of the HF diet in our paradigm. Recent evidence indicates that a high-fat diet can lead to increase of free radicals (Beltowski *et al*., 2000), and that supplementation with antioxidant-enriched diets can reduce the adverse effects of free radicals on neuronal function and cognition (Joseph *et al*., 1999; Lim *et al*., 2001; Farr *et al*., 2003). Thus, we have evaluated the possibility that OS can interact with the mechanisms by which HF reduces BDNF levels, impairs cognition, and compromises neuroplasticity. To test this possibility, we have used the antioxidant power of vitamin E, which has been shown to be neuroprotective against many insults (Morris *et al*., 2002; Gonzalez-Polo *et al*., 2003; Mishima *et al*., 2003; Nagai *et al*., 2003). BDNF seems to affect synaptic function through modulation of molecules such as synapsin I and cyclic AMP-response element-binding protein (CREB), which have been implicated in synaptic function underlying learning and memory.

It is known that OS can result in cognitive decline (Liu *et al*., 2003; Nagai *et al*., 2003), and that BDNF can enhance synaptic plasticity associated to cognitive function (Korte *et al*., 1995; Patterson *et al*., 1996; Linnarsson *et al*., 1997). It is unknown, however, whether BDNF and OS can interrelate to affect synaptic plasticity and cognition. In this study we provide novel evidence showing possible mechanisms by which OS associated to the consumption of a HF diet can interact with the BDNF system to affect hippocampal synaptic plasticity and cognition.

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Materials and methods

Experimental design and tissue preparation

Forty male Sprague–Dawley rats (Charles River Laboratories, Inc., Wilmington, MA, USA) weighing between 200 and 240 g were housed in cages (two rats per cage) and maintained in environmentally controlled rooms (22–24 °C) with a 12-h light : 12-h dark cycle. After acclimatization for 1 week on standard rat chow, the rats were randomly assigned to HF diet or regular diet (RD) with or without 500 IU/kg vitamin E for 2 months. The diets, fed *ad libitum*, were provided in powder (Purina Mills Inc, TestDiet Inc., Richmond, IN, USA) in a large bowl and contained a standard vitamin and mineral mix with all essential nutrients, as described previously (Molteni *et al*., 2002; Wu *et al*., 2003). RD is low in saturated fat (13% of energy from fat). HF diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil, 39% energy). The rats were killed by decapitation; the fresh tissues including hippocampus were dissected, frozen in dry ice and stored at −70 °C until use for biochemical analyses. For immunohistochemistry, the rats were deeply anaesthetized (Nembutal, 75 mg/kg) and then transcardially perfused with 400 mL 4% paraformaldehyde and 100 mL 20% sucrose. The fixed brains were then removed and stored at −70 °C until use. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of oxidized proteins

The amounts of oxidized proteins containing carbonyl groups were measured by using an Oxyblot kit (Intergen, Purchase, NY, USA). Briefly, the protein sample (10 μg) was reacted with 1× dinitrophenylhydrazine (DNPH) for 15 min, followed by neutralization with a solution containing glycerol and β-mercaptoethanol. These samples were electrophoresed on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. After blocking, membranes were incubated overnight with a rabbit DNPH antibody (1 : 150) at 4 °C, followed by incubation in goat anti-rabbit (1 : 300) for 1 h at room temperature. After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to the manufacturer's instructions.

Real-time quantitative RT-PCR

Total RNA was isolated by using the RNA STAT-60 (TEL-TEST, Inc., Friendswood, TX). Briefly, after tissue homogenization (1 mL/50–100 mg tissue), 0.2 mL of chloroform per 1 mL of the RNA STAT-60 was added. Samples were centrifuged and aqueous phase was mixed with isopropanol (0.5 mL/mL). Samples were centrifuged again. Supernatant was removed and the RNA pellet was washed with 75% ethanol, centrifuged, dried and dissolved in water. The mRNAs for BDNF, synapsin I, and CREB were measured by TaqMan real-time quantitative RT-PCR using ABI PRISM 7700 Sequence detection system (Perkin Elmer, Branchburg, NJ, USA). This system directly detects the reverse transcription polymerase chain reaction (RT-PCR) product without downstream processing. This is accomplished with the monitoring of the increase in fluorescence of a dye-labelled DNA probe specific for BDNF, synapsin I, or CREB plus a probe specific for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene used as an endogenous control for the assay. Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Perkin-Elmer, Branchburg, NJ, USA). The sequences of probes, forward and reverse primers were:

BDNF:
(5′-AGTCATTTGCGCACAACTTTAAAAGTCTGCATT-3′),
forward: (5′-GGACATATCCATGACCAGAAAGAAA-3′),
reverse: (5′-GCAACAAACCACAACATTATCGAG-3′);
Synapsin I:
(5′-CATGGCACGTAATGGAGACTACCGCA-3′),
forward: (5′-CCGCCAGCTGCCTTC-3′),
reverse: (5′-TGCAGCCCAATGACCAAA-3′);
CREB:
(5′-CATGGCACGTAATGGAGACTACCGCA-3′),
forward: (5′-CCGCCAGCATGCCTTC-3′),
reverse: (5′-TGCAGCCCAATGACCAAA-3′).

An oligonucleotide probe specific for the rat GAPDH gene (5′-CCGACTCTTGCCCTTCGAAC-3′) was used as an endogenous control to standardize the amount of sample RNA. The GAPDH gene is a constitutively expressed housekeeping gene, which has been shown to be suitable to correct variations in RNA quantity and quality (Medhurst *et al*., 2000). The RT-reaction conditions were 2 min at 50 °C as initial step to activate uracil glycosylase (UNG), followed by 30 min at 60 °C as reverse transcription and completed by uracil glycosylase-deactivation at 95 °C for 5 min. The 40 cycles of two-step PCR reaction conditions were 20 s at 94 °C and 1 min at 62 °C.

ELISA

Hippocampal tissue was homogenized in a lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/mL aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged, the supernatants were collected and total protein concentration was determined according to MicroBCA procedure (Pierce, Rockford, IL, USA), using bovine serum albumin as standard. BDNF protein was quantified using an enzyme-linked immunosorbent assay (ELISA) kit (BDNF Emax ImmunoAssay System kit, Promega Inc., Madison, WI, USA) according to manufacturer's protocol.

Western blot

The total proteins from hippocampal tissue were extracted as described above. Synapsin I, phospho-synapsin I (p-synapsin I), CREB and phospho-CREB (p-CREB) were analysed by Western blot. Briefly, protein samples were separated by electrophoresis on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Non-specific binding sites were blocked in TBS-T containing 2% BSA and 0.1% Tween-20 overnight at 4 °C. Membranes were rinsed for 10 min in buffer (0.1% Tween-20 in TBS) and then incubated with antiactin, antisynapsin I or antip-synapsin I (1 : 2000; Santa Cruz Biotechnology, Santa Cruz, CA), followed by anti-goat IgG horseradish peroxidase-conjugate; anti-CREB, anti-*p*-CREB (1 : 1000; Cell Signalling Technology, Beverly, MA, USA), followed by anti-rabbit IgG horseradish peroxidase-conjugate. After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH Image software. The actin was used as internal standard for normalization.

Immunohistochemistry for BDNF and synapsin I

Serial coronal sections (25 μm) were cut on a cryostat, mounted to gelatin-coated slides and processed for immunohistochemistry, as previously described (Gomez-Pinilla *et al*., 2001). A 1 : 1000 dilution was used for the rabbit polyclonal anti-BDNF (Chemicon International Inc., Temecula, CA, USA) and anti-synapsin I (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The specificity of these antibodies has been shown by our previous studies (Molteni *et al*., 2002; Wu *et al*., 2003). Immunohistochemistry controls were performed by omission of

the primary antibody. The results of immunohistochemistry controls were negative as no staining was observed in cell structures.

Detection of oxidized nucleosides

The sections were prepared as described above and incubated with monoclonal anti-8-hydroxy-2′-deoxyguanosine/ 8-hydroxyguanosine (8OHdG/8OHG; 1 : 2000; QED Bioscience, San Diego, CA, USA) and visualized by using standard immunohistochemical methods (Gomez-Pinilla *et al*., 2001).

Cognitive testing

To evaluate the effect of vitamin E and diet on cognitive function, all rats were tested in a Morris water maze as previously described (Molteni *et al*., 2002; Wu *et al*., 2003). The swimming pool (130 cm diameter, 50 cm height) was divided into four quadrants as four zones. The quadrant where the escape platform (12 cm diameter) was located in a fixed position 2 cm under the water surface was defined as target zone; the other three quadrants were left, right and opposite zone. The water ($22 \pm 2\,^{\circ}C$) was made opaque with white nontoxic biodegradable dye to prevent the rats from seeing the platform. The rats were trained in the water maze with ten consecutive trials per day for 3 days. The animals were placed into the tank facing the wall from one of the equally spaced start locations that was randomly changed every trial. The spatial cues for reference around the pool were maintained constant throughout the duration of the experiment. Each trial lasted until the rat has found the platform or for a max of 2 min. If the rat failed to find the platform in the allocated time, it was gently placed on the platform. At the end of each trial, the animals were allowed to rest on the platform for 1 min. The latency to locate the platform was recorded. In order to assess spatial memory retention, spatial probe tests were performed at 72 h after the last try by removing the platform from the pool. The rats were allowed to swim for 1 min in the pool without the escape platform, and the percentage of time spent in each zone was calculated.

Statistical analysis

GAPDH and actin were used as internal standards for real-time RT-PCR and for Western blot, respectively. For quantification of TaqMan RT-PCR results, fluorescent signal intensities were plotted against the number of PCR cycles on a semilogarithmic scale (ABI sequence detector software version 1.6.3; Perkin Elmer, Branchburg, NJ, USA). Taqman RT-PCR values for GAPDH were subtracted from BDNF, synapsin I, or CREB values. The resulting corrected values were expressed as percentage of control according to manufacturer's instructions. For Western blot, the values were expressed as a ratio of actin value and then converted to per cent of RD group as presented in the bar figures. The data were analysed by ANOVA followed by Fisher's protected least significant difference *post hoc* test. Statistical differences were considered significant at $P < 0.05$.

Results

HF induces increased oxidative stress

Our previous studies show that exposure to a HF diet reduces levels of BDNF and impairs cognition, motivating our interest in determining the mechanisms involved with these effects. To evaluate the possible role of OS as an intermediate mechanism for the adverse effects of HF diet, we assessed two markers of OS, oxidized protein levels and nucleic acids. The levels of oxidized proteins were determined using Western blot analysis, in which carbonyl groups on oxidized proteins were derivatized with DNPH and detected using a DNPH antibody. A representative example of an Oxyblot is shown in Fig. 1A. HF significantly increased the protein carbonyl levels (160%) compared with control rats fed RD (Fig. 1B). Vitamin E treatment dramatically abolished the HF-elicited elevation of oxidized protein (67%) relative to RD animals (Fig. 1B).

It is known that free radicals can damage nucleic acids, accordingly, we investigated the possibility that increased OS subsequent to consumption of the HF diet could affect the integrity of nucleic acids. We evaluated possible treatment effects on oxidized nucleic acids by immunostaining cells with an 8OHdG/8OHG antibody that detects oxidized nucleic acids. HF produced significant increase in the levels of oxidized nucleic acids in the hippocampus (Fig. 2B) compared with RD rats (Fig. 2A). The accumulation of staining was present in the hippocampal subfield pyramidal cells as well as dentate granule cells. Treatment with vitamin E markedly eliminated staining (Fig. 2C).

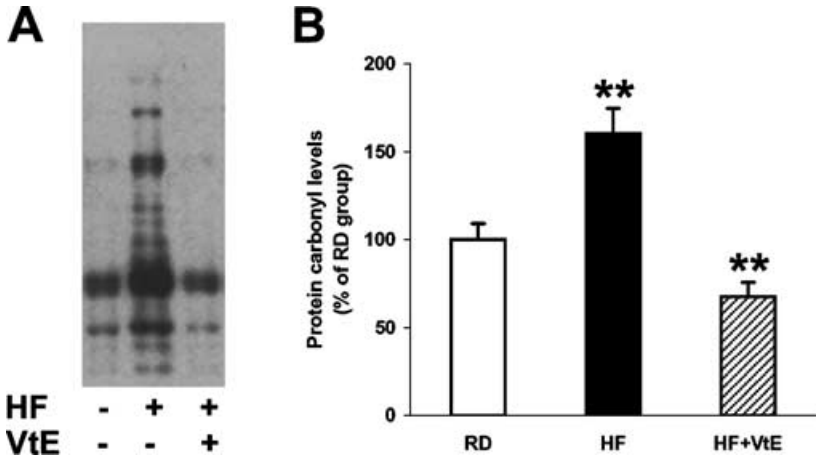

FIG. 1. Measurement of oxidized protein levels in the hippocampus. The oxidized protein levels were determined by Oxyblot kit. (A) Representative example of Oxyblot from hippocampal tissue of each group. (B) Effects of HF and vitamin E (VtE) on oxidized protein levels. ANOVA showed a significant HF effect, in which the oxidized protein levels were higher (160%) in HF-fed rats compared with RD rats. $^{**}P < 0.01$. ANOVA showed that levels of oxidized protein were lower (67%) in animals fed HF diet containing VtE relative to animals fed RD. $^{**}P < 0.01$. RD, regular diet. HF, high fat. VtE, vitamin E. Values in B represent mean ± SEM.

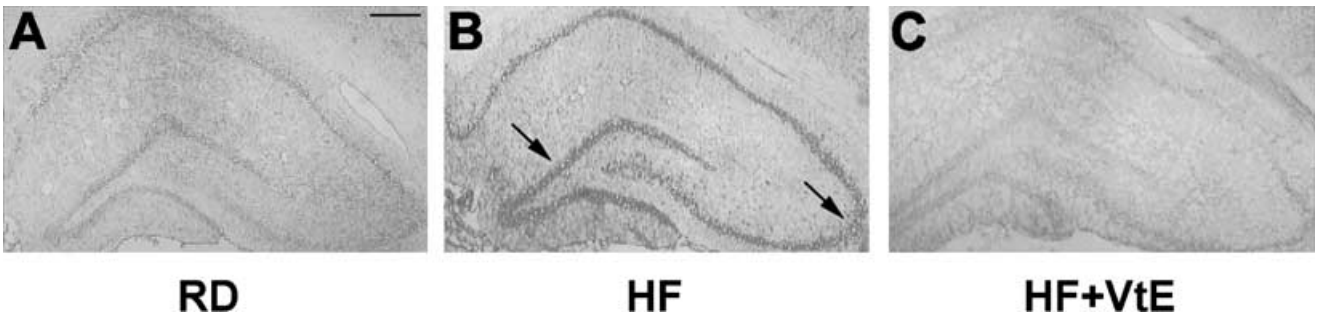

FIG. 2. Detection of oxidized nucleic acids in the hippocampus. Rats fed HF diet with or without vitamin E were perfused with 4% paraformaldehyde. Serial coronal sections (25 μm) were cut on a cryostat, mounted to gelatin-coated slides and processed for immunohistochemistry by using a monoclonal anti-8OHdG/8OHG as described in Materials and methods. Note the marked increase in 8OHdG/8OHG immunoreactivity in HF-fed rats (B) compared with RD rats (A) and a clear decrease in rats fed HF diet containing VtE (C). Arrows indicate strong immunoreactivity for 8OHdG/8OHG. Scale bar, 250 μm.

HF-induced oxidative damage reduces BDNF expression

In order to determine whether OS may be involved in HF-induced reductions of BDNF mRNA and protein levels, we supplemented the HF diet with the antioxidant vitamin E. The results showed that vitamin E alone did not affect BDNF mRNA, but completely counteracts the HF-elicited reduction in levels of BDNF mRNA (96% in rats fed HF containing vitamin E vs. 77% in HF-fed rats; Fig. 3A) and protein (103% in rats fed HF containing vitamin E vs. 68% in HF-fed rats; Fig. 3B). BDNF immunohistochemistry showed that vitamin E treatment preserved BDNF immunostaining within the CA3 and DG of hippocampus (Fig. 3C). Consistent with our previous observations, the HF diet reduced BDNF mRNA levels (Fig. 3A), and decreased BDNF protein level (Fig. 3B) relative to control rats fed RD. The pattern of BDNF immunostaining was consistent with our previous observations (Wu *et al*., 2003), showing a marked reduction in BDNF labelling in the CA3 and DG of hippocampus in HF-fed rats (Fig. 3C).

Oxidative damage-reduced BDNF is associated with decreased activation of synapsin I and CREB

To establish whether oxidative damage could play a role in the HF-induced decrease of synapsin I mRNA and protein levels, we measured synapsin I mRNA and protein in rats fed HF with or without vitamin E treatment. The results demonstrated that vitamin E significantly

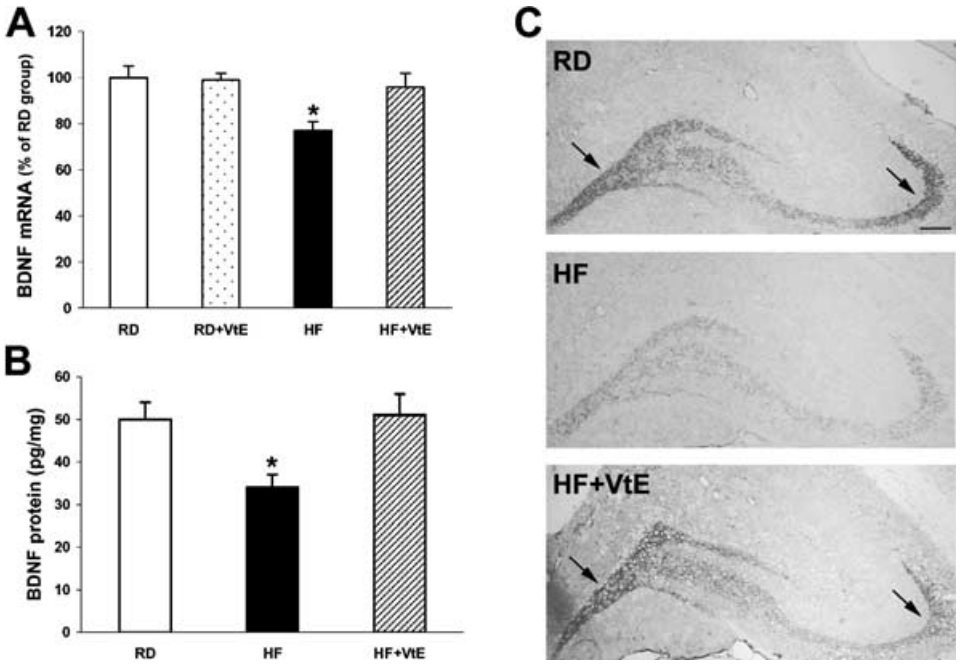

FIG. 3. Effects of HF and vitamin E on BDNF expression in the hippocampus. (A) mRNA level was measured by Taqman real-time quantitative RT-PCR. ANOVA showed that the level of BDNF mRNA was significantly decreased in animals fed HF diet, but normalized in animals fed HF diet containing VtE compared with RD animals. Vitamin E alone did not affect BDNF mRNA. $^{*}P < 0.05$. (B) Protein level was determined by ELISA. ANOVA showed that BDNF was significantly reduced in animals fed HF diet, but normalized in animals fed HF diet containing VtE relative to RD animals. $^{*}P < 0.05$. (C) Immunohistochemistry for BDNF showed that BDNF labelling was predominantly distributed along the mossy fibre system between CA3 and the dentate gyrus (DG), and in the molecular layer of the DG of the hippocampal formation. Note that vitamin-E reversed HF-reduced BDNF immunostaining in the CA3 and DG. Arrows indicate strong immunostaining for BDNF. Scale bar, 250 μm (C).

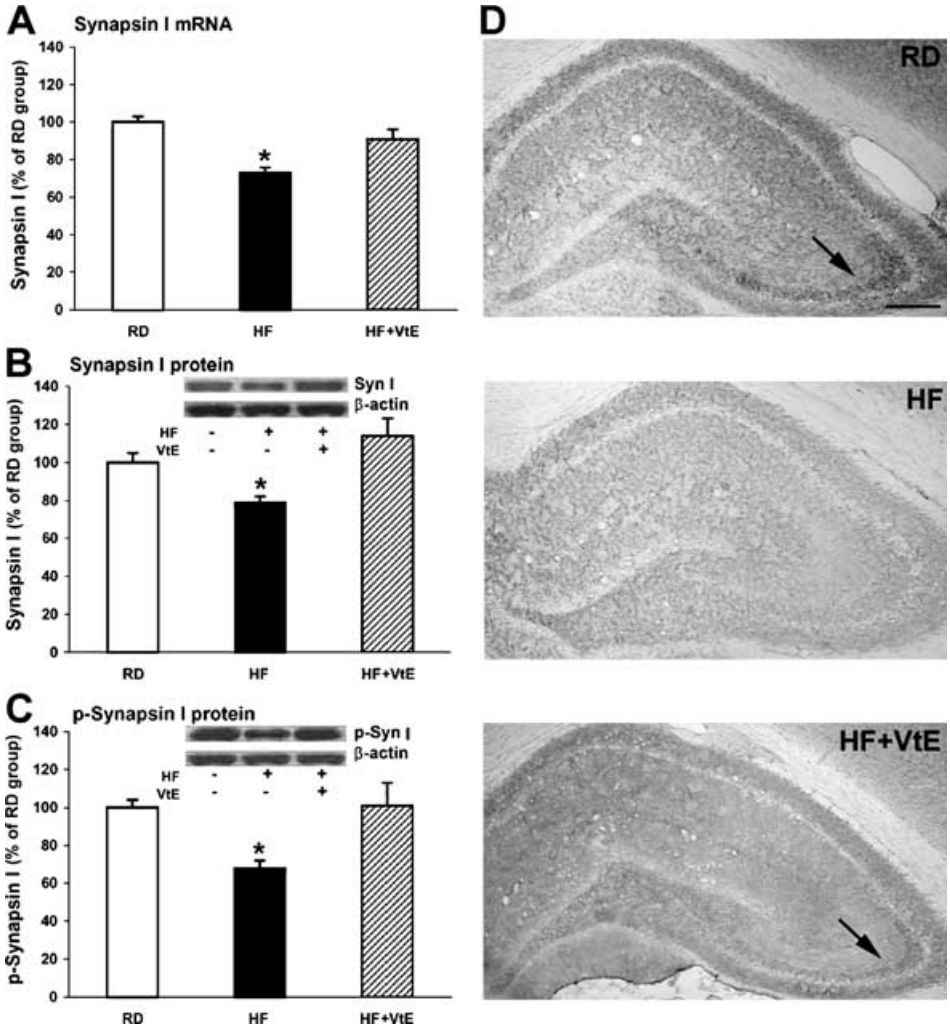

FIG. 4. Effects of HF and vitamin E on synapsin I and p-synapsin I in the hippocampus. (A) mRNA level was measured by Taqman real-time quantitative RT-PCR. ANOVA showed that level of synapsin I mRNA was significantly decreased in animals fed HF diet, but normalized in animals fed HF diet containing VtE compared with RD animals. $^{*}P < 0.05$. Protein levels of synapsin I (B) and p-synapsin I (C) were determined by Western blot. ANOVA revealed that synapsin I and p-synapsin I were significantly reduced in animals fed HF diet, but normalized in animals fed HF diet containing VtE relative to RD animals. $^{*}P < 0.05$. (D) Immunohistochemistry for synapsin I showed that vitamin E eliminated HF-induced reduction in synapsin I immunostaining in the CA3 of hippocampus. Arrows indicate strong immunostaining for synapsin I. Scale bar, 250 μm.

counteracted the HF-induced reduction of synapsin I mRNA (91% in rats fed HF containing vitamin E vs. 73% in HF-fed rats; Fig. 4A), synapsin I protein (114% in rats fed HF containing vitamin E vs. 79% in HF-fed rats; Fig. 4B), and p-synapsin I (101% in rats fed HF containing vitamin E vs. 68% in HF-fed rats; Fig. 4C) protein levels. The effects of vitamin E were also reflected on preserving normal immunoreactivity for synapsin I in the CA3 of hippocampus (Fig. 4D). It is well documented that BDNF facilitates synaptic transmission by regulating synapsin I phosphorylation (Wang *et al*., 1995; Jovanovic *et al*., 1996). To determine the role of oxidative damage in the relationship between BDNF and activation of synapsin I, we performed a correlation analysis between BDNF and p-synapsin I. This analysis revealed that the vitamin E treatment reversed the disrupted correlation between BDNF and p-synapsin I caused by the HF diet ($r = 0.96$, $P < 0.01$ in RD rats; $r = 0.58$, $P > 0.05$ in HF-fed rats; $r = 0.91$, $P < 0.05$ in rats fed HF containing vitamin E). In addition, vitamin E treatment restored the disrupted correlation between p-synapsin I and total synapsin I caused by the HF diet ($r = 0.94$, $P < 0.01$ in RD rats; $r = 0.14$, $P > 0.05$ in HF-fed rats; $r = 0.94$, $P < 0.05$ in rats fed HF containing vitamin E).

To further evaluate how HF-induced oxidative damage interacts with molecular systems associated with the action of BDNF on neural plasticity, we assessed the expression of CREB mRNA and protein levels. The results demonstrated that vitamin E significantly reversed the reduction of CREB mRNA (113% in rats fed HF containing vitamin E vs. 80% in HF-fed rats; Fig. 5A), CREB protein (112% in rats fed HF containing vitamin E vs. 74% in HF-fed rats; Fig. 5B), and p-CREB (111% in rats fed HF containing vitamin E vs. 63% in HF-fed rats; Fig. 5C) protein levels induced by the HF diet. It is well known that BDNF can modulate phosphorylation of CREB (Finkbeiner, 2000; Ying *et al*., 2002). To evaluate a possible role of oxidative damage in the activation of CREB by BDNF, we performed a correlation analysis between BDNF and p-CREB. This analysis revealed that the vitamin E treatment reversed the disrupted correlation between BDNF and p-CREB caused by the HF diet ($r = 0.90$, $P < 0.05$ in RD rats; $r = 0.29$, $P > 0.05$ in HF-fed rats; $r = 0.96$, $P < 0.01$ in rats fed HF containing

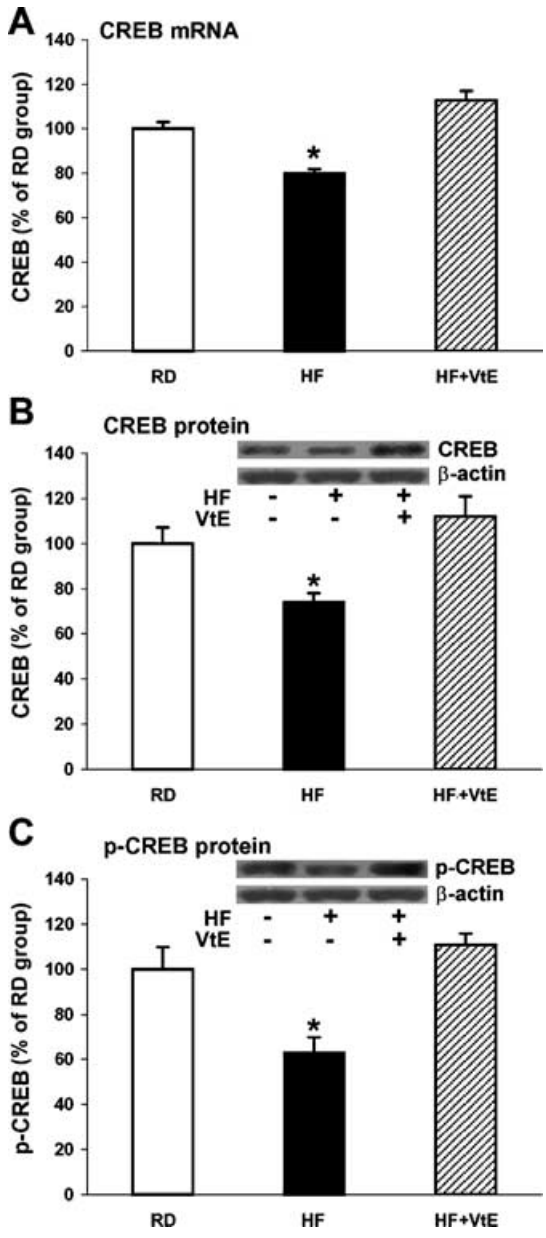

FIG. 5. Effects of HF and vitamin E on CREB and p-CREB in the hippocampus. (A) mRNA level was measured by Taqman real-time quantitative RT-PCR. ANOVA showed that level of CREB mRNA was significantly decreased in animals fed HF diet, but normalized in animals fed HF diet containing VtE compared with RD animals. $^{*}P < 0.05$. Protein levels of CREB (B) and p-CREB (C) were determined by Western blot. ANOVA revealed that CREB and p-CREB were significantly reduced in animals fed HF diet, but normalized in animals fed HF diet containing VtE relative to RD animals. $^{*}P < 0.05$.

vitamin E). In addition, vitamin E treatment restored the disrupted correlation between p-CREB and total CREB ($r = 0.95$, $P < 0.01$ in RD rats; $r = 0.59$, $P > 0.05$ in HF-fed rats; $r = 0.95$, $P < 0.05$ in rats fed HF containing vitamin E) caused by the HF diet.

HF-induced oxidative damage results in cognitive impairment

To evaluate the role of oxidative damage in HF-induced cognitive decline, we maintained rats on HF or RD with or without vitamin E for two months before performing cognitive testing in a Morris water maze. The results demonstrated that HF resulted in longer escape latencies to find the platform (Fig. 6A). The spatial probe trial test was performed by allowing rats to swim for 1 min in the same pool without escape platform, and the percentage of time spent in each zone was calculated. The animals fed HF spent less time in the target zone compared to RD rats (31% vs. 52%; Fig. 6B), showing an obvious impairment to recognize the target zone (Fig. 6C), consistent with our previous report of cognitive impairment in HF-fed rats (Molteni *et al.*, 2002). Supplementation with vitamin E improved cognitive performance by reducing latency to find the platform in HF-fed rats (Fig. 6A). Further, rats fed HF containing vitamin E spent a similar time in the target zone as those animals fed RD (50% vs. 52%; Fig. 6B) with a clear preference for the target zone in the probe trial test (Fig. 6C). In addition, in this study we found a significant and important relationship between poor performance in the water maze, scored as time spent in the target zone during probe trial test, and increased oxidized protein levels in the hippocampus of HF-fed rats (correlation coefficient, $r = -0.84$, $P < 0.05$).

Discussion

Nutrition as an integral component of our daily life routine has the potential to modulate brain health and function including cognition. There is great concern to learn about the mechanisms by which the diet can affect neuroplasticity and cognitive function. Our recent reports indicate that HF diet consumption can compromise cognitive performance (Molteni *et al.*, 2002) and aggravate the outcome of brain insult on neuroplasticity and cognition (Wu *et al.*, 2003), which may be associated with the disruption of BDNF-related molecular systems. Here we provide novel evidence that oxidative damage mediates the deleterious effects of the HF diet on synaptic function and cognition by reducing BDNF and its downstream effectors synapsin I and CREB (Fig. 7).

Our findings demonstrated that HF induces oxidative damage associated with increases in two markers of OS; but the question is: How can oxidative damage lead to cognitive impairment in HF-fed rats? Our results provide novel evidence showing that HF-induced oxidative damage is associated with reduced expression of BDNF. In particular, HF-elicited increase of OS accompanied reduction of BDNF protein and mRNA levels. Treatment with vitamin E significantly prevented this reduction, suggesting that oxidative damage was involved in the adverse effects of HF diet on the transcription and translation of BDNF. It is well accepted that BDNF has beneficial actions on learning and memory (Korte *et al.*, 1995) and is a powerful synaptic facilitator (Thoenen, 1995; Kang & Schuman, 1996; Bolton *et al.*, 2000). For example, animals lacking BDNF show deficits in long-term potentiation and learning and memory, which can be amended by exogenous BDNF (Korte *et al.*, 1995; Patterson *et al.*, 1996; Linnarsson *et al.*, 1997). Therefore, the HF feeding evoked oxidative damage and subsequent reduction of BDNF may contribute to cognitive impairment (Fig. 7).

Although the mechanisms by which oxidative damage reduces BDNF expression are complex, it can be suggested that several factors might be involved. First, the decrease of DNA-binding activities of activator protein-1 and CREB may play a role. It has been reported that OS leads to the decrease of DNA-binding activities of activator protein-1 and CREB (Iwata *et al.*, 1997), which is associated with reduction of BDNF gene expression (Vellucci *et al.*, 2001). Second, increased OS can result in energy depletion and then impair NMDA channel function (Light *et al.*, 2001; Lu *et al.*, 2001), while the impairment of NMDA channel function is related to the decrease in BDNF gene expression (Hayashi *et al.*, 2001; Roceri *et al.*, 2002).

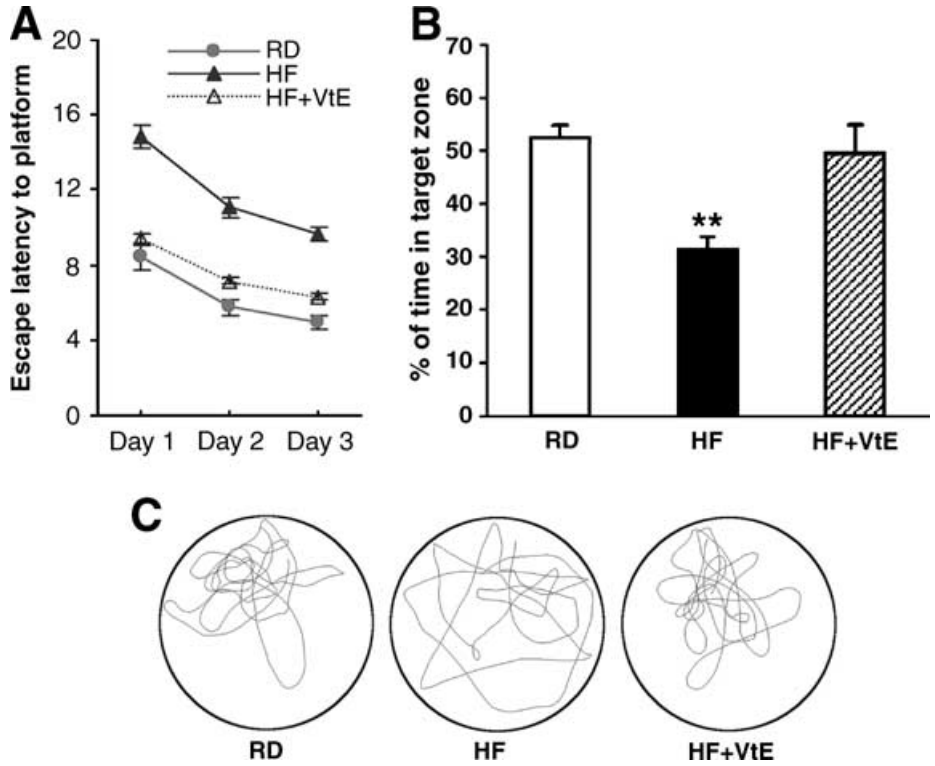

FIG. 6. Effects of HF and vitamin E on spatial learning and memory retention. At the end of two months of diet feeding, spatial learning and memory retention were tested in a Morris water maze as described in Materials and methods. (A) The learning performance was calculated as average of latencies to find the platform. The latency was significantly longer in rats fed HF diet compared with RD animals, but similar in rats fed HF diet containing vitamin E to that in rats fed RD. (B) Memory retention in probe trial test was expressed as percentage of time spent in target zone as described in Materials and methods. ANOVA indicated that rats fed HF diet spent less time in the target zone relative to animals fed RD, whereas animals fed HF containing vitamin E showed similar time spent in target zone as that in RD-fed rats. $^{**}P < 0.01$. (C) Typical swim paths from the various groups indicated that the HF-fed animals swam randomly, showing an obvious impairment to recognize the target zone compared with other groups, while vitamin E reversed this impairment.

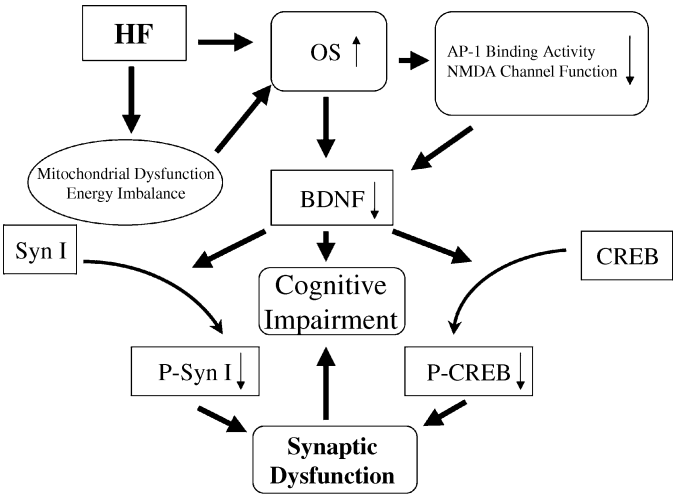

FIG. 7. Possible mechanisms underlying HF-induced impairment in cognition and neuroplasticity. HF results in accumulative OS, subsequently leading to reduction of BDNF, synapsin I, CREB, and activated state of synapsin I and CREB, which in turn contributes to synaptic dysfunction and cognitive impairment. The mechanisms underlying HF-increased oxidative stress are currently unknown, but it can be suggested that energy imbalance may be involved. Abundant evidence indicates that energy balance and OS are tightly regulated. High fat intake, a great source of fuel, may induce mitochondrial dysfunction and cause energy imbalance, resulting in accumulative OS, which in turn can lead to synaptic dysfunction.

However, which factor plays a key role or whether it is multifactorial effects needs further investigations.

Our results also show that vitamin E significantly prevented the HF-induced decrease of synapsin I and CREB, suggesting that OS is involved in the pathways by which HF reduces CREB and synapsin I, and interferes with the activation process of CREB and synapsin I. Further, HF-elicited OS disrupted the positive association of BDNF with activated state of CREB and synapsin I, suggesting that HF may impair the downstream effects of BDNF. It is well documented that BDNF facilitates synaptic transmission by regulating synapsin I phosphorylation (Wang *et al*., 1995; Jovanovic *et al*., 1996), playing important roles in modulation of transmitter release (Jovanovic *et al*., 2000), formation and maintenance of presynaptic structure (Takei *et al*., 1995), and axonal elongation (Akagi *et al*., 1996). BDNF also modulates the phosphorylation of CREB (Finkbeiner, 2000; Ying *et al*., 2002), which play important roles in gene expression (Tao *et al*., 1998; Conti *et al*., 2002) and long-term memory (Yin & Tully, 1996; Tully, 1997; Taubenfeld *et al*., 2001). Our results show that vitamin E completely reverses protein oxidation, with subsequent effects on normalizing levels of synapsin I and CREB, suggesting that OS might be involved in synaptic dysfunction and cognitive impairment related to HF diet (Fig. 7).

It is believed that an imbalance in production and removal of ROS may cause the increase of OS in pathological conditions, which in turn can damage proteins, lipids, and nucleic acids (Liu *et al*., 2001). OS-induced damage has been implicated in normal ageing and cognitive declines seen in neurodegenerative diseases (Ames *et al*., 1993;

Veinbergs *et al.*, 2000; Fukui *et al.*, 2002; Liu *et al.*, 2002; Mattson *et al.*, 2003). Our findings indicate that HF also can lead to oxidative damage to proteins and nucleic acids. In particular, we found markedly increased protein oxidation in the hippocampus, which was completely reversed by treatment with antioxidant vitamin E. This same treatment resulted in a complete reversal of cognitive decline induced by HF, suggesting that protein oxidation may be critical for decline in cognitive function in HF-fed animals. Consistent with this point, we observed a significant negative association between performance in the water maze of HF-fed rats and hippocampal levels of protein oxidation.

We further found elevated oxidized nucleic acids especially in the hippocampus critically involved in spatial learning and memory (Sugaya *et al.*, 1996; Clayton *et al.*, 2002; Steffenach *et al.*, 2002), which was dramatically decreased by vitamin E treatment. The oxidized nucleic acids may lead to errors of transcription and translation. The increased oxidized nucleic acids may indicate the HF-induced damage to DNA repair systems as the presence of 8-hydroxy-2′-deoxyguanosine in DNA (also 8-hydroxyguanosine in RNA) is a reliable marker of base modifications (Liu *et al.*, 2001). These base modifications caused by oxidative damage have been detected in various insults (Nunomura *et al.*, 1999; Won *et al.*, 1999; Liu *et al.*, 2003). Our findings support the possibility that HF may lead to DNA damage and impair DNA repair systems, which in turn may cause the reduction of mRNAs in BDNF and its downstream effectors.

In summary, in this study we provide novel evidence showing that BDNF and OS can interrelate to affect synaptic plasticity and cognitive function. Our results also suggest that vitamin E supplementation might be a potent therapeutic agent for preventing oxidative damage-mediated neurological disorders induced by unhealthy dietary factors. But perhaps the most important finding provided by these studies is a potential mechanism by which energy balance factors can modulate synaptic plasticity and cognition.

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Abbreviations

BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP-response element-binding protein; DNPH, dinitrophenylhydrazine; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HF, high fat; 8OHdG/8OHG, 8-hydroxy-2′-deoxyguanosine/8-hydroxyguanosine; OS, oxidative stress; p-CREB, phospho-CREB; p-synapsin-I, phospho-synapsin-I; RD, regular diet.

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