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# EXERCISE REVERSES THE HARMFUL EFFECTS OF CONSUMPTION OF A HIGH-FAT DIET ON SYNAPTIC AND BEHAVIORAL PLASTICITY ASSOCIATED TO THE ACTION OF BRAIN-DERIVED NEUROTROPHIC FACTOR

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**Abstract—A diet high in total fat (HF) reduces hippocampal levels of brain-derived neurotrophic factor (BDNF), a crucial modulator of synaptic plasticity, and a predictor of learning efficacy. We have evaluated the capacity of voluntary exercise to interact with the effects of diet at the molecular level. Animal groups were exposed to the HF diet for 2 months with and without access to voluntary wheel running. Exercise reversed the decrease in BDNF and its downstream effectors on plasticity such as synapsin I, a molecule with a key role in the modulation of neurotransmitter release by BDNF, and the transcription factor cyclic AMP response element binding protein (CREB), important for learning and memory. Furthermore, we found that exercise influenced the activational state of synapsin as well as of CREB, by increasing the phosphorylation of these molecules. In addition, exercise prevented the deficit in spatial learning induced by the diet, tested in the Morris water maze. Furthermore, levels of reactive oxygen species increased by the effects of the diet were decreased by exercise. Results indicate that exercise interacts with the same molecular systems disrupted by the HF diet, reversing their effects on neural function. Reactive oxygen species, and BDNF in conjunction with its downstream effectors on synaptic and neuronal plasticity, are common molecular targets for the action of the diet and exercise. Results unveil a possible molecular mechanism by which lifestyle factors can interact at a molecular level, and provide information for potential therapeutic applications to decrease the risk imposed by certain lifestyles.** 

**Key words: synapsin I, CREB, neuronal plasticity, cognitive function, hippocampus, water maze.**

Dietary factors are important predictors of the general health of individuals; however, the actual impact of diet on brain structure and function remains poorly understood. As the brain can neither synthesize nor store energy reserves, daily diet provides the immediate source of energy to the brain, thereby a means to influence brain function. A diet rich in saturated fat and refined sugar (HF), similar in composition to the average popular diet of most industrialized Western societies, can threaten neuronal plasticity and compromise the capacity of the rodent brain for learning (Greenwood and Winocur, 1996; Winocur and Greenwood, 1999; Molteni et al., 2002). On the other hand, epidemiological studies indicate that exercise can decrease cognitive decay associated to aging (Kramer et al., 1999) and is inherently beneficial for reducing the risk of various diseases (Friedland et al., 2001; Laurin et al., 2001). Experimental studies show that exercise can improve cognitive function in both young and aged animals (Radak et al., 2001; Churchill et al., 2002). Although the potential of exercise to protect against neurological damage is well recognized, the capacity of exercise to interact with specific molecular systems impacted by insult has not been experimentally scrutinized. Information on the mechanisms by which exercise repairs the brain at the molecular levels is critical for the development of therapeutic interventions based on exercise.

A HF diet reduces brain-derived neurotrophic factor (BDNF) in the hippocampus and this decrease is associated with reduced learning performance (Molteni et al., 2002). BDNF holds a well-established protective role in the adult brain such that genetic deletion of the BDNF gene in mice increases the incidence of apoptosis (Linnarsson et al., 1997). The expression of BDNF in the hippocampus is elevated by exercise (Neeper et al., 1995), in line with recent studies showing a clear involvement of BDNF with regulation of neuronal excitability (Kafitz et al., 1999; Bolton et al., 2000). BDNF is synthesized predominantly by neurons located in the hippocampus, a brain region intimately associated with the processing of cognitive function (Wetmore et al., 1991). BDNF facilitates synaptic transmission (Kang and Schuman, 1995; Levine et al., 1998; Sherwood and Lo, 1999; Tyler and Pozzo-Miller, 2001), and hippocampal BDNF seems necessary for the induction of long-term potentiation (Patterson et al., 1996; Linnarsson et al., 1997), a physiological correlate of learning. Synapsin I is a nerve terminal phospho-protein involved in neurotransmitter release, axonal elongation and maintenance

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*Abbreviations:* ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP response element binding protein; DNPH, dinitrophenylhydrazine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HF, high-fat, refined sugar diet; LTP, long-term potentiation; MWM, Morris water maze; RD, regular diet; ROS, reactive oxygen species; RT-PCR, real-time quantitative reverse transcription polymerase chain reaction.

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of synaptic contacts (Wang et al., 1995; Brock and O'Callaghan, 1987) whose synthesis (Wang et al., 1995) as well as phosphorylation (Jovanovic et al., 2000) are affected by BDNF. Cyclic AMP response element binding protein (CREB), a transcription factor involved in learning and memory, is an important modulator of gene expression induced by BDNF (Finkbeiner, 2000).

We have focused this study on the possibility that the harmful effects of diet and the protection provided by exercise can share common molecular mechanisms, involving oxidative stress, BDNF, and their interaction with molecular systems critical for synaptic plasticity. We have included oxidative stress, as this is a fundamental cellular mechanism through which stimuli such as exercise and nutrients can affect neural function.

## EXPERIMENTAL PROCEDURES

### Subjects and experimental paradigm

Female Fisher 344 rats (Harlan Sprague Dawley Inc., San Diego, CA, USA), 2 months old, were maintained in a 12-h light/dark cycle at 22–24 °C. After acclimatization of the animals for 1 week on standard rat chow, the rats were assigned to one of four groups ($n=6$ each group): regular diet (RD)/Sedentary; HF/Sedentary; RD/Exercise, HF/Exercise and housed individually in standard polyethylene cages. Animals engaged in voluntary physical activity had free access to a running wheel (diameter=31.8 cm, width=10 cm; Nalgene Nunc International, Rochester, NY, USA) that rotated against a resistance of 100 g. Wheel revolutions were recorded automatically by computer using VitalViewer Data Acquisition System software (Mini Mitter Company, Inc., Sunriver, OR, USA) and were positively counted irrespective of the direction of wheel rotation.

### Diet

Diets containing a standard vitamin and mineral mix with all essential nutrients (Roberts et al., 2000) were provided in powder form *ad libitum* (Purina Mills Inc., Test Diets Inc., Richmond, IN, USA) in large bowls. The HF diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil, approximately 39% energy) and high in refined sugar (sucrose, approximately 40% energy). The RD, is low in saturated fat (approximately 13% of energy from fat) and contains complex carbohydrate (starch, 59% energy). Under our experimental conditions, female rats do not develop hypertension (Roberts et al., 2000), and do not show atherosclerosis (Barnard et al., 1993). The animals were killed by decapitation after 2 months in the morning immediately following the last period of exercise, the hippocampi were rapidly dissected, frozen on dry ice and stored at −70 °C for biochemical analysis. All efforts were made to minimize animal suffering and to reduce the number of animals employed in the study. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of California at Los Angeles, Animals Research Committee.

### Cognitive performance

The effects of 1 and 2 months of diet and exercise on cognitive function were assessed using the Morris water maze (MWM). In order to have experimentally homogenous groups, we performed a water maze test before starting the diet/exercise period. According to these results, animals with comparable performance were distributed equally in the four experimental groups. The swimming pool (130 cm diameter, 50 cm height), with the escape platform (12 cm diameter) placed 1 cm beneath the water surface and 32 cm from the wall of the pool, is divided into four quadrants; i.e. platform (P), platform left (L), platform right (R) and opposite (O). The water (24 °C) was made opaque with white nontoxic biodegradable dye to prevent the rats from seeing the platform. The rats were trained on the water maze using 10 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 were 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 had found the platform or for a max of 2 min. If the rat failed to find a platform, it was placed gently on the platform. At the end of each trial, the animals were allowed to rest on the platform for 1 min. Time to locate the platform was recorded and an average latency was calculated from the values of 10 trials at each day. To assess spatial memory retention, spatial probe tests were performed 3 days after the last day of behavioral test by removing the platform from the pool. The rats were allowed to swim for 1 min in the pool without the escape platform. The percentage of swim distance in each quadrant was calculated against the total distance.

### Isolation of total RNA and real-time quantitative reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated using RNA STAT-60 kit (TEL-TEST, Inc., Friendswood, TX, USA) as per manufacturer's protocol. The mRNAs for BDNF, synapsin I, and CREB were measured by real-time quantitative RT-PCR using PE Applied Biosystems prism model 7700 sequence detection system (Perkin-Elmer, Branchburg, NJ, USA). Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Perkin-Elmer). The sequences of probes, forward and reverse primers, designed by Integrated DNA Technologies (Coralville, IA, USA), 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′. The mRNA levels for BDNF, Synapsin I, and CREB were normalized for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

### Protein measurements

Hippocampal extracts were prepared in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate). Homogenates were centrifuged to remove insoluble material (12,000 r.p.m. for 20 min at 4 °C) and total protein concentration was determined according to the MicroBCA procedure (Pierce, Rockford, IL, USA). BDNF protein was quantified using an enzyme linked immunosorbent assay (ELISA; BDNF Emax ImmunoAssay system Kit; Promega Inc., Madison, WI, USA) as per manufacturer's protocol. Synapsin I, phosphosynapsin I, total-CREB, and phospho-CREB proteins were analyzed by Western blot as previously described (Gómez-Pinilla et al., 2001), quantified by densitometric scanning of the film under linear exposure conditions and normalized for actin levels. Membranes were incubated with the following primary antibodies: anti-synapsin I (1:2000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), anti-phospho-synapsin I (1:2000; Santa Cruz Biotechnology), anti-total CREB (1:1000; Cell Signaling Technology, Inc., Beverly, MA, USA), anti-phospho-CREB (1:1000; Cell Signaling Technology, Inc.), anti-actin (1:2000; Santa Cruz Biotechnology) followed by anti-goat IgG horseradish peroxidase conjugate for

synapsin, phospho-synapsin and actin or anti-rabbit IgG horseradish peroxidase conjugate for total CREB and phospho-CREB (Santa Cruz Biotechnology). 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.

### Measurement of oxidized proteins

The amounts of oxidized proteins containing carbonyl groups were measured using an Oxyblot kit (Intergen, Purchase, NY, USA). Briefly, the protein sample (10 μg) was reacted with 1× dinitrophenylhydrazine (DNPH) for 15–30 min, followed by neutralization with a solution containing glycerol and â-mercaptoethanol. These samples were electrophoresed on an 8% polyacrilamide gel and electrotransferred to a nitrocellulose membrane. After blocking, membranes were incubated overnight with a rabbit anti-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.) according to the manufacturer's instructions and then quantified.

### Statistical analyses

GAPDH and actin were employed as internal standards for real-time RT-PCR and for Western blot respectively, as diet or exercise did not alter their expressions. An analysis of variance (ANOVA) with repeated measures was conducted for analyzing data of the water maze. We analyzed the biochemical data using ANOVA, with diet and exercise as independent factors and BDNF, synapsin I, and CREB levels as dependent variables. When appropriate, further differences were analyzed by Scheffe post hoc test. Statistical differences were considered significant when $P<0.05$. The results were expressed as mean percent of control (RD/Sedentary) values for graphic clarity and represent the mean±S.E.M. of five to six independent determinations.

## RESULTS

### BDNF (Fig. 1)

BDNF mRNA levels increased to 135% ($P<0.01$) of the controls levels in animals fed RD who had access to voluntary wheel running for 2 months (Fig. 1A). Conversely, in sedentary rats exposed to the HF diet, BDNF mRNA levels decreased to 76% ($P<0.05$; Fig. 1A). In turn, exposure to exercise throughout the period of consumption of the HF diet was able to reduce the decrease in BDNF mRNA from 76% to 91% ($P<0.05$; Fig. 1A). We performed an ELISA to determine whether the changes produced by diet and exercise on BDNF mRNA levels translated into protein. We found that the modulation of BDNF protein levels by diet and exercise followed the same profile observed for the mRNA, but with more pronounced effects. In fact, voluntary wheel running induced a dramatic increase in BDNF in the RD group (from 100% to 185%; $P<0.01$), and increased BDNF beyond control levels in the HF group (from 61% to 141%; $P<0.01$; Fig. 1B).

### Synapsin I (Figs. 2 and 3)

To gain insight into the mechanisms by which exercise compensates for the effects of the HF diet, we assessed the expression of synapsin I, a molecule with a key role in the modulation of neurotransmitter release by BDNF (Jovanovic et al., 2000). Although exercise did not have an affect on synapsin I mRNA in the animals fed RD, exercise completely reverted the decrease in synapsin I ($P<0.01$) as a result of the HF diet, increasing synapsin I mRNA from 76% to 105% ($P<0.01$; Fig. 2A). Western blot analysis of total synapsin I protein showed a similar tendency to the one observed at the mRNA level (Fig. 2B, D).

Since phosphorylation regulates the function of synapsin I (Hosaka et al., 1999), we examined the effect of exercise on the phosphorylated form of synapsin I (phospho-synapsin I) by Western blot. Exercise elevated the levels of phospho-synapsin I in the RD group up to 123%, ($P<0.05$) of sedentary values. In turn, exercise completely reversed the decrease ($P<0.01$) in phospho-synapsin I

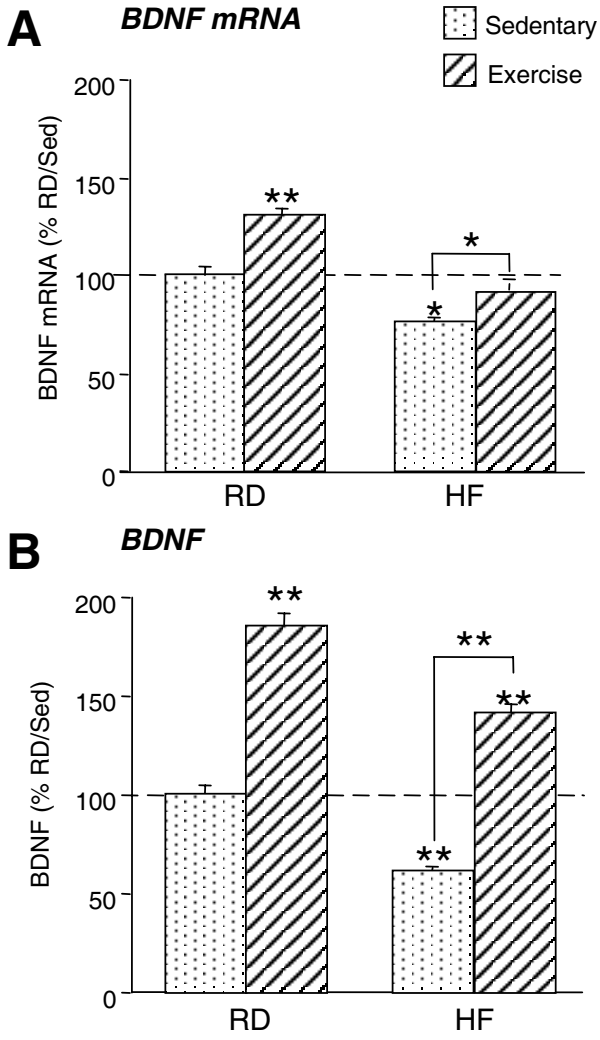


**Fig. 1.** Differential effects of a HF diet, exercise, or both combined on levels of BDNF mRNA and protein in the hippocampus. Exercise increased levels of BDNF mRNA (A) and protein (B) in control rats. The HF diet decreased levels of BDNF mRNA (A) and protein (B) in sedentary rats while exercise elevated levels of BDNF. Experimental treatment extended for 2 months. Each value represents the mean±S.E.M ($n=6$ for each experimental group; * $P<0.05$ and ** $P<0.01$).

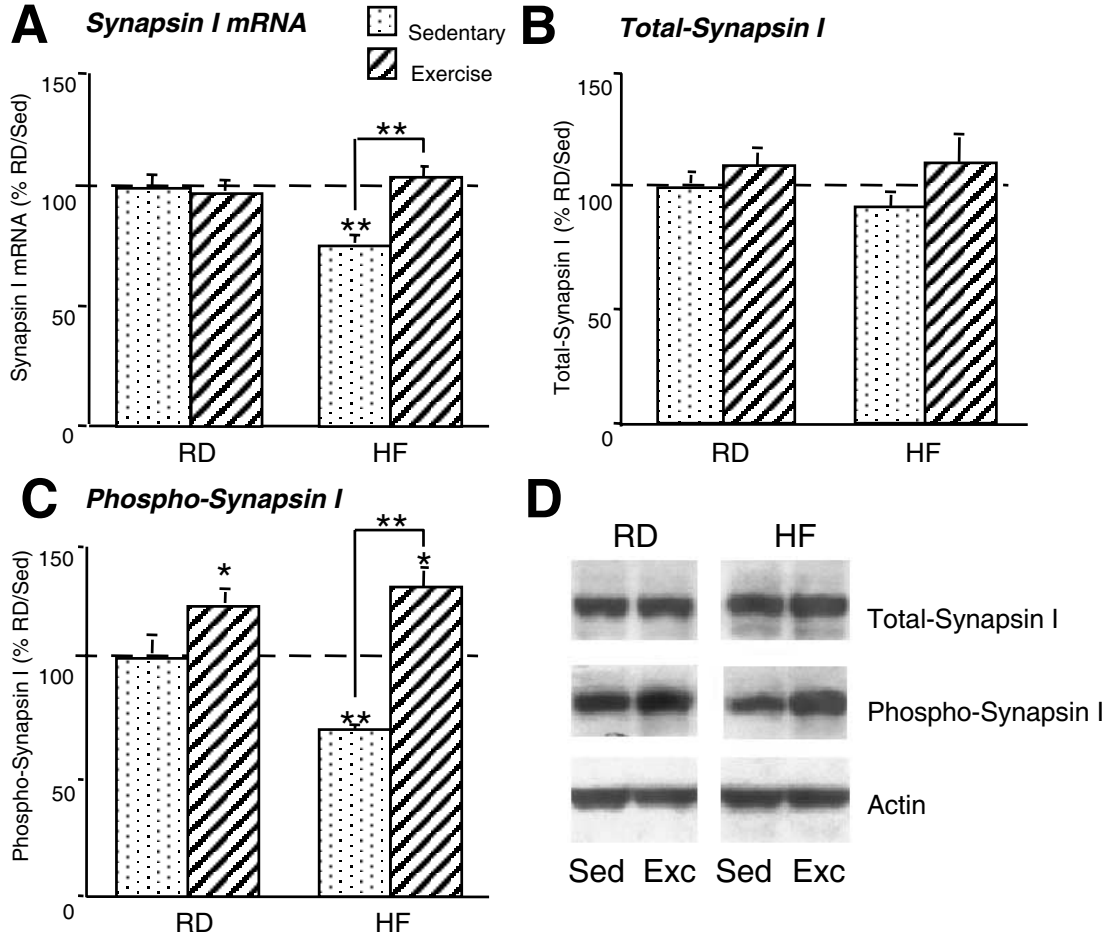


**Fig. 2.** Changes in synapsin I mRNA and protein in the hippocampus after 2 months of HF diet, exercise, or both conditions. (A) The HF diet decreased synapsin I mRNA in sedentary rats relative to the RD. (B) Levels of total synapsin I protein showed a decreasing tendency in the HF rats. (C) The HF diet reduced phospho-synapsin I in sedentary rats ($P<0.01$), while exercise raised phospho-synapsin I levels in both RD ($P<0.05$) and HF ($P<0.05$) groups. (D) Representative Western blot gels for group data shown in B and C. Each value represents the mean±S.E.M. ($n=6$ for each experimental group; * $P<0.05$ and ** $P<0.01$).

caused by the HF diet from 70% to 131% ($P<0.01$; Fig. 2C, D). In addition, to evaluate the possibility that exercise and diet could effect the activation of synapsin I, we performed a correlation analysis between levels of total and phosphorylated synapsin I. There was a significant and positive correlation between levels of phospho-synapsin I and total-synapsin I in sedentary ($r=0.85$, $P<0.05$; Fig. 3A) and exercised ($r=0.93$, $P<0.05$; Fig. 3B) animals fed RD. Interestingly, exposure to the HF diet disrupted such correlation in sedentary rats ($r=0.15$; Fig. 3C), but exercise provided along the HF diet period preserved the correlation ($r=0.84$, $P<0.05$; Fig. 3D).

### CREB (Figs. 4 and 5)

To evaluate possible effects of diet and exercise on the molecular machinery involved with synaptic plasticity underlying learning and memory, we assessed the transcription activator CREB. CREB involvement with neuronal and behavioral plasticity is associated to the action of BDNF (Ying et al., 2002). Contrary to the case of synapsin I, exercise diminished CREB mRNA in the RD group from 100% to 76% ($P<0.05$), but did not change levels of CREB mRNA in the HF group (Fig. 4A). Exercise decreased levels of total-CREB from 100% to 70% ($P<0.01$) in RD rats, and restored levels of total-CREB that had been decreased ($P<0.05$) because of the HF diet (Fig. 4B, D).

Phosphorylation of CREB is a crucial step for its action on activity- and neurotrophin-mediated gene expression (Bito et al., 1996; Finkbeiner et al., 1997; Silva et al., 1998). We assessed levels of phospho-CREB by Western blot analysis using an antibody specific for CREB phosphorylated (phospho-CREB) at Ser-133. Exercise had no effects on levels of phospho-CREB in RD rats. The HF diet reduced levels of phospho-CREB to 78% ($P<0.05$) of controls, but exercise was able to elevate these levels higher than controls from 78% to 164% ($P<0.01$; Fig. 4C, D).

We performed a correlation analysis to evaluate possible effects of diet and exercise on the activation of CREB. We found a significant positive correlation between the levels of total-CREB and phospho-CREB in the sedentary animals fed RD ($r=0.87$, $P<0.05$; Fig. 5A). Interestingly, exercise maintained levels of total and phosphorylated

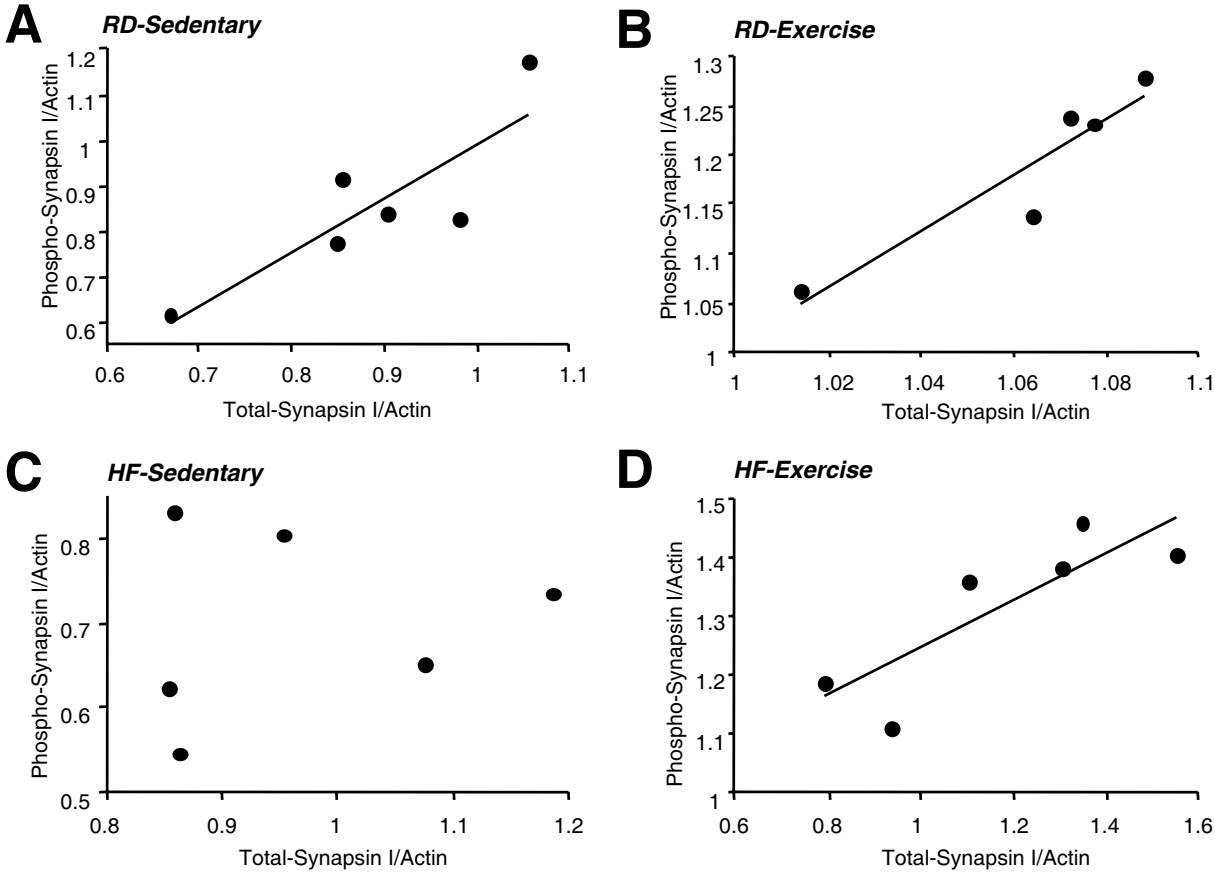


**Fig. 3.** Differential effects of diet and exercise on the relationship between total-synapsin I and phospho-synapsin I levels in the hippocampus. Levels of phospho-synapsin changed according to levels of total-synapsin I in rats fed RD and maintained under sedentary (A, $n=6$; $r=0.85$; $P<0.05$) or exercise (B, $n=5$; $r=0.93$; $P<0.05$) conditions. Consumption of the HF diet disrupted the correlation between total- and phospho-synapsin (C, $n=6$; $r=0.15$), and exercise restored this correlation (D, $n=6$; $r=0.84$; $P<0.05$).

CREB correlated for the RD rats, but inverting the slope resulting in a negative correlation ($r=0.92$, $P<0.01$; Fig. 5B). Consumption of the HF diet disrupted the correlation between total and phospho-CREB ($r=0.52$; Fig. 5C). Interestingly again, exposure to exercise throughout the diet period restored the correlation but inverted its phase ($r=0.97$, $P<0.01$; Fig. 5D).

### Spatial learning (Fig. 6)

Exposure to the HF diet for 2 months reduced spatial learning performance in the MWM as evidenced by an increase in the latency to find the hidden platform (Fig. 6A). We evaluated the effects of exercise on spatial learning efficacy, as a compensatory strategy to revert the learning deficit as a result of the HF diet. We found that exercise provided simultaneous with the diet decreased the latency to find the platform in RD and HF groups. That is, HF rats engaged in voluntary wheel running required the same time as control rats (RD/SED) to find the platform after 1 and 2 months of diet and exercise (Fig. 6A). To evaluate memory retention, the same animals were re-tested 3 days after the last trial of the second testing period in the same maze but without platform. Control rats showed preference for the quadrant where the platform was previously located (P quadrant; 45% in P quadrant), while HF animals maintained under sedentary conditions swam randomly in all quadrants (25% in P quadrant, $P<0.05$ vs. Controls). RD rats with access to exercise showed increased preference for the P quadrant (68% in P quadrant, $P<0.05$ vs. Controls). Animals fed HF diet that were exposed to exercise performed similar to controls expending 43% of their time in the P quadrant (Fig. 6B).

### Spatial learning vs. CREB and BDNF (Fig. 7)

Phosphorylation of CREB has been found to be a good predictor of learning performance, therefore, we have evaluated a possible relationship between levels of phospho-CREB and spatial learning performance in animals exposed to the effects of HF diet and exercise. Results showed that animal with more phospho-CREB took shorter to find the platform, as shown by a significant negative correlation between the mean escape latency to find the platform and phospho-CREB (Fig. 7A). Interestingly, levels of phospho-CREB in these animals were directly related to levels of BDNF, as early predicted based on the known interactions between BDNF and CREB (Fig. 7B).

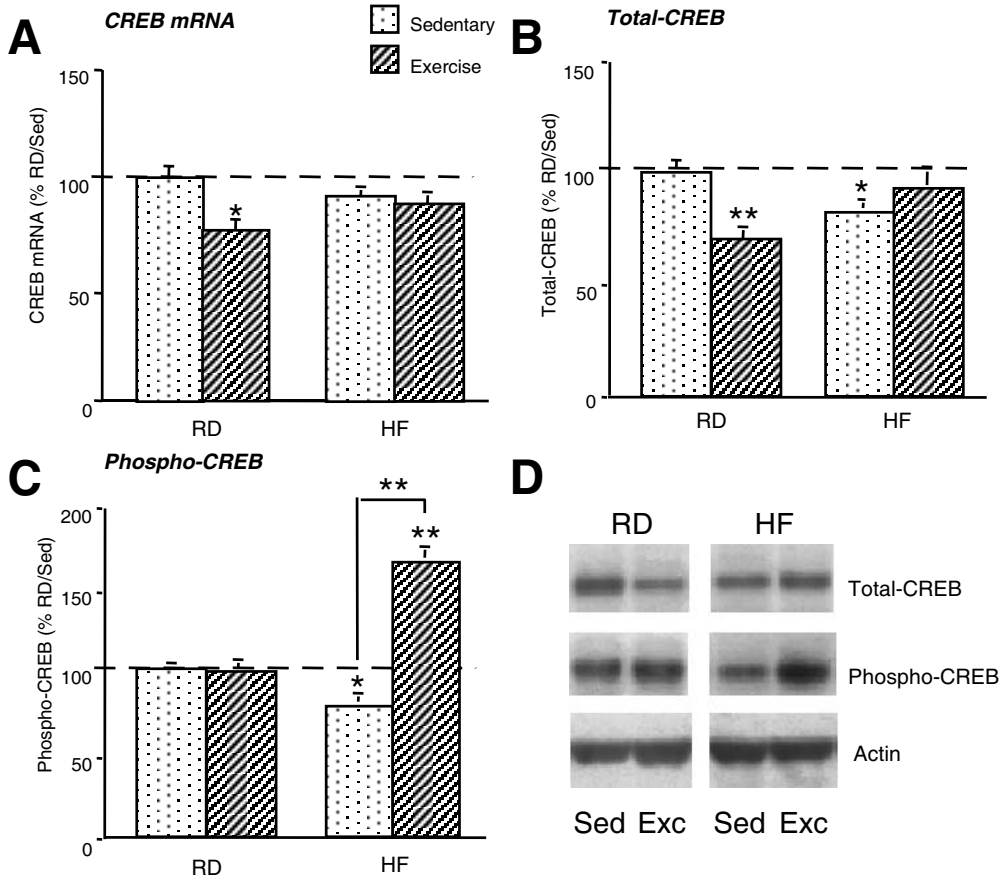


**Fig. 4.** The HF diet, exercise, or both combined differentially affected levels of CREB mRNA and protein in the hippocampus. (A) Exercise decreased CREB mRNA in RD rats ($P<0.05$) relative to sedentary rats. (B) Exercise decreased levels of total-CREB in RD rats ($P<0.01$), and restored levels that had been reduced by the effects of the HF diet ($P<0.05$). (C) The HF diet reduced levels of phospho-CREB ($P<0.05$), and exercise abundantly increased phospho-CREB ($P<0.01$). (D) Representative Western blot gels for group data shown in B and C. Each value represents the mean±S.E.M. ($n=6$ for each experimental group; * $P<0.05$ and ** $P<0.01$).

### Reactive oxygen species (ROS; Fig. 8)

The possibility that oxidative damage can play a role on the effects of the HF diet and exercise was assessed. We used a convenient Western blot analysis, in which carbonyl groups on oxidized proteins were derivatized with DNPH and detected using an anti-DNP antibody. Results indicate that animals exposed to the HF diet had a significantly higher level of oxidized proteins (142%; $P<0.05$) compared with RD sedentary rats. In turn, animals exposed to the HF diet that received exercise throughout the diet period showed reduced levels of oxidized proteins (78%; $P<0.05$) compared with RD sedentary rats. Interestingly, exercise per se had an effect reducing oxidized protein levels in RD rats to 83% ($P<0.05$) of RD sedentary values.

## DISCUSSION

Our results indicate that the detrimental effects of a HF diet and the salutary effects of exercise interact on a common molecular machinery (Fig. 9), with opposite effects on synaptic plasticity on a molecular level and learning and memory on a behavioral level. The effects of both diet and exercise target the hippocampus, a brain region important for learning and memory. Oxidative stress and the BDNF system seem to play a central role in the cascade of events activated by diet and exercise. Diet and exercise are able not only to influence the quantitative state of synaptic plasticity molecules, but also more significantly, affect their activation. Overall results indicate that physical activity compensates for the deleterious effects of a HF diet.

### Exercise and diet affect oxidative stress and BDNF

Exercise and diet affected levels of DNPH-derived carbonyls, an indicator of protein oxidation. Exercise was able to lower elevated levels of protein oxidation resulting from consumption of the HF diet. Although our data are not conclusive to ascertain the role that free radical formation plays in the sequence of molecular events resulting in decreased plasticity, likely oxidative stress is one of the earliest events subsequent to consumption of the HF diet. Free radical formation takes place at the mitochondria, where dietary fuels are transformed into energy metabolism. It is possible that ROS formation can play a role in the reduced BDNF expression resulting from the HF diet. Imbalance between the normal cellular production of free radicals and the ability of cells to buffer them is referred to

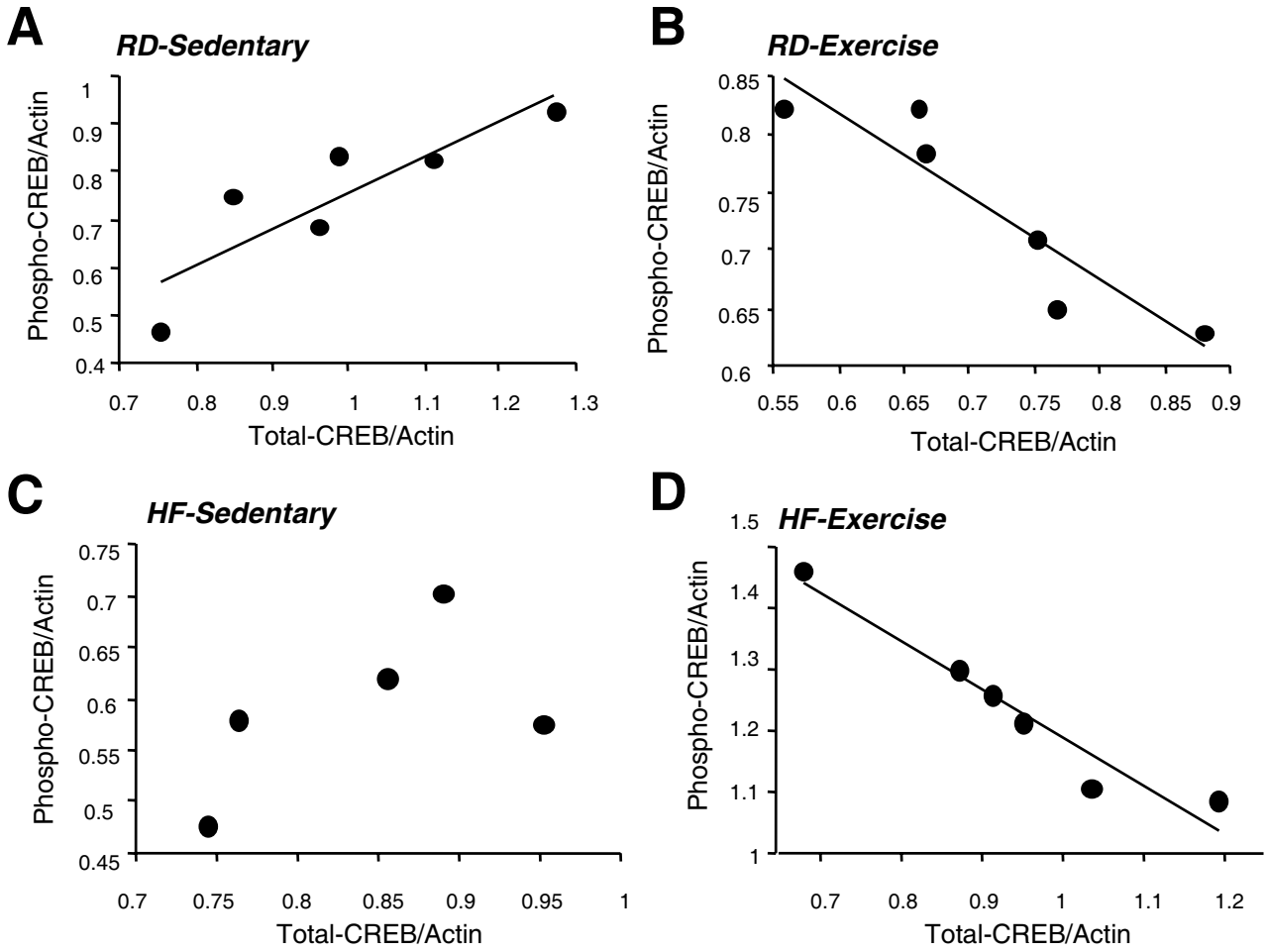


**Fig. 5.** The type of diet and exercise differentially affected the relationship between total-CREB and phospho-CREB levels. Phospho-CREB levels changed proportionally to total-CREB levels in sedentary RD animals (A, $n=6$; $r=0.87$; $P<0.05$). Exercise reversed this relationship such that phospho-CREB decreased proportionally to total-CREB values (B, $n=6$; $r=0.92$; $P<0.01$). The HF diet disrupted the relationship between phospho-CREB and total-CREB (C, $n=5$; $r=0.52$). Exercise restored the relationship between phospho-CREB and total-CREB but inverting its dependency such that the correlation was negative (D, $n=6$; $r=0.97$; $P<0.01$).

as oxidative stress. Oxidative stress is associated with a paucity in the capacity of neurons to remain optimally functional, and elevated susceptibility to damage. It is known that BDNF protects neurons against oxidative stress (Cheng and Mattson, 1994); therefore, a reduction of BDNF resulting from the HF diet can expose neurons to further damage. In this context, our results showing that high levels of ROS are accompanied by low BDNF in HF rats emphasize the heavy toll for brain plasticity imposed by the HF diet. Indeed, our results showing that the bad diet can affect synaptic plasticity and cognition may be a demonstration. Further studies remain to be performed to clarify the relationship between oxidative stress and BDNF production.

### The role of BDNF on the restorative effects of exercise counteracting the deleterious decline imposed by a HF diet

In a previous study, we showed that a HF diet reduces BDNF mRNA levels in the hippocampus, but not in the cerebral cortex. These changes were associated with a decrease in hippocampal-dependent learning performance (Molteni et al., 2002). Here we show that exercise completely counteracted the reduction in hippocampal BDNF protein levels produced by a HF diet (Fig. 1B). The restorative effects of exercise on a HF diet are most prominently presented in the MWM performance of the HF animals, who show a memory capability comparable to the RD/Sed animals (Fig. 6A, B).

The importance of modulating hippocampal BDNF levels may be explicated by BDNF involvement in activity-dependent events regulating neuronal function and behavior, distinctively targeting those involved in learning and memory processes. BDNF is synthesized predominantly by neurons located in brain areas associated with the processing of cognitive functions, i.e. the hippocampus (Wetmore et al., 1991). Moreover, increases in hippocampal BDNF expression appear to be constitutive for the induction of long-term potentiation (LTP), a physiological correlate of learning (Patterson et al., 1992). A failure to

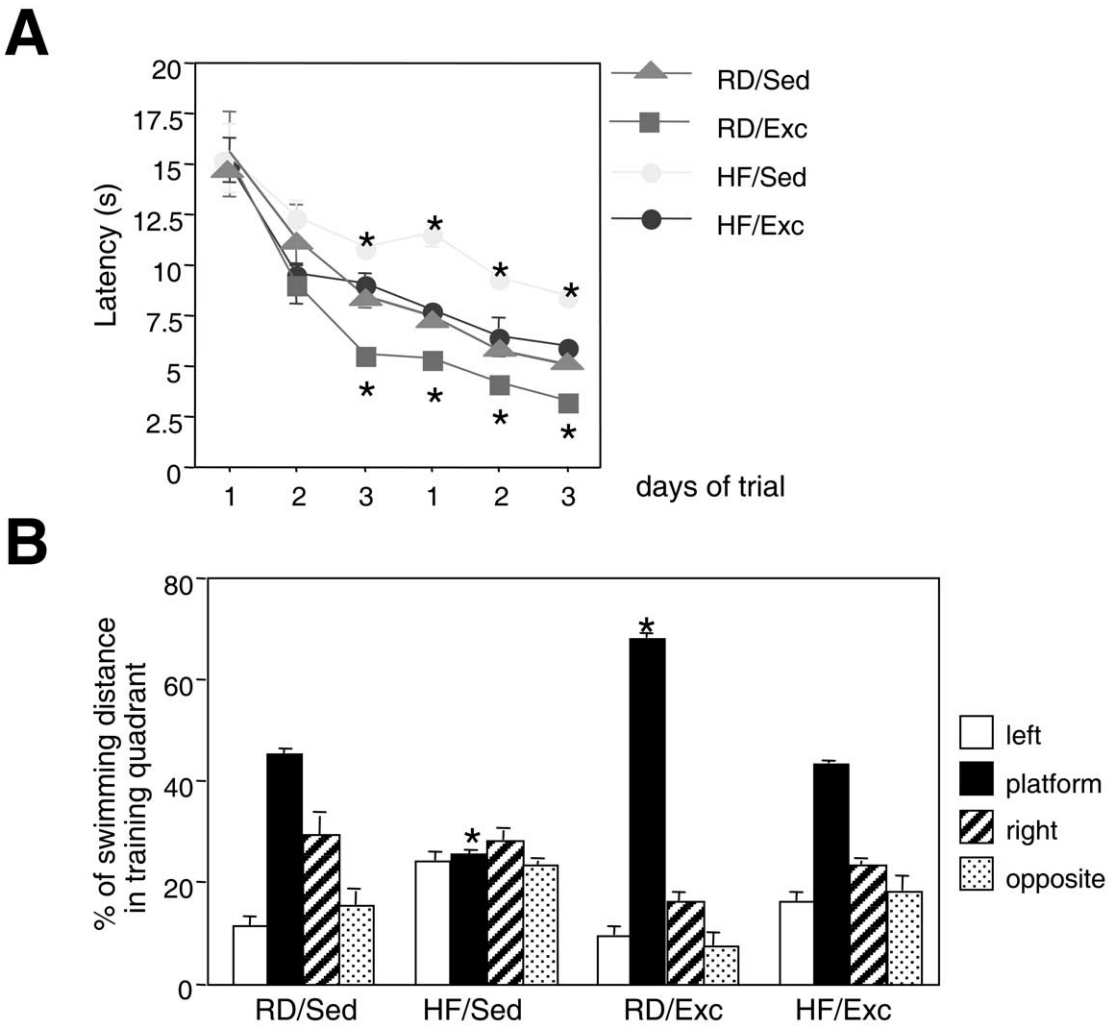


**Fig. 6.** Effects of diet and exercise on spatial learning performance using the water maze. (A) The HF diet increased the latency to find the platform in sedentary rats shown for testing days 1, 2, and 3 for the first and second months of diet. Exercise decreased the latency in the animals fed RD. Exercise normalized the latency in animals fed HF performing similar to controls (RD/Sed). (B) To evaluate memory retention, the same animals were re-tested 3 days after the last trial using the same maze but without the platform. Distribution of the average percent of swimming distances for the four quadrants is displayed, showing clear differences between the four groups (each value represents the mean±S.E.M.; * $P<0.05$ for the P quadrant is shown relative to control).

exhibit LTP occurs in transgenic animals with diminished BDNF expression (Linnarsson et al., 1997) but can be reinstated with exogenous hippocampal BDNF (Patterson et al., 1996).

In addition, BDNF holds a well-established role in protecting neurons against insults. For example, BDNF gene deletion in mice increases the incidence of apoptosis (Linnarsson et al., 1997) and addition of BDNF in cultured rat hippocampal neurons suppresses accumulation of peroxides and protects neurons against excitotoxicity (Mattson et al., 1995). BDNF may partake in a protective mechanism that exercise employs to protect the brain from insults or risk factors imposed by a HF diet, possibly by influencing downstream effectors such as CREB and synapsin I. We have previously shown that exercise can increase levels of BDNF (Gómez-Pinilla et al., 2002) and synapsin I (Vaynman et al., 2003) in a dose-dependent manner. It is possible, therefore, that the restorative effects of exercise can involve downstream BDNF effectors which are differentially modulated by doses of exercise.

### Exercise alters the phospho-CREB to total-CREB relationship; implications for synaptic plasticity and memory formation

In the hippocampus, the phosphorylation of CREB is required for the institution of its transcriptional activity. Consequently, it is interesting that under normal conditions phospho-CREB production was correlated with total-CREB (Fig. 5A). The HF diet decreased phospho-CREB (Fig. 4D) and disrupted the correlation between phospho-CREB and total-CREB (Fig. 5C). Exercise was able to alter the activation of CREB, intensely increasing the levels of phospho-CREB in HF animals (Fig. 4C). Furthermore, the correlation analysis revealed an effect of exercise on the

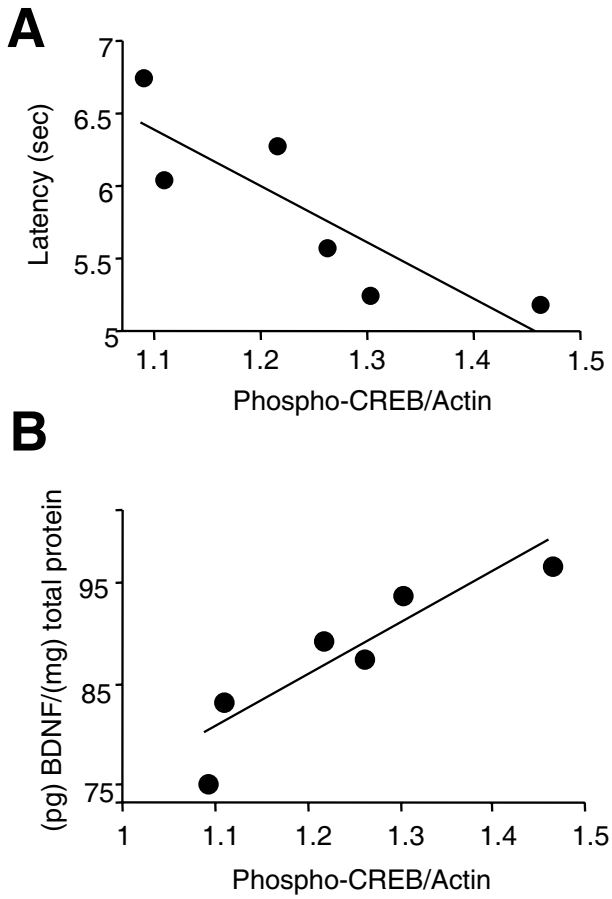


**Fig. 7.** Relationship between spatial learning performance and levels of phospho-CREB and BDNF in animals fed HF diet exposed to exercise. (A) Animals that performed better in the water maze showed proportionally higher levels of phospho-CREB (A, $n=6$; $r=0.86$; $P<0.05$), and levels of phospho-CREB varied according to levels of BDNF (B, $n=6$; $r=0.91$; $P<0.05$).

activational state of CREB in both RD and HF animals: i) in RD animals, exercise inverted the relationship phospho-CREB vs. total-CREB, apparently depleting levels of total CREB (Figs. 4 C and 5B); ii) in HF rats, exercise restored and inverted the relationship phospho-CREB vs. total-CREB, apparently increasing phospho-CREB at expense of total-CREB (Fig. 5D). Thus, changes in the phosphorylation of CREB may represent a crucial functional aspect of the effect of exercise on neuronal plasticity.

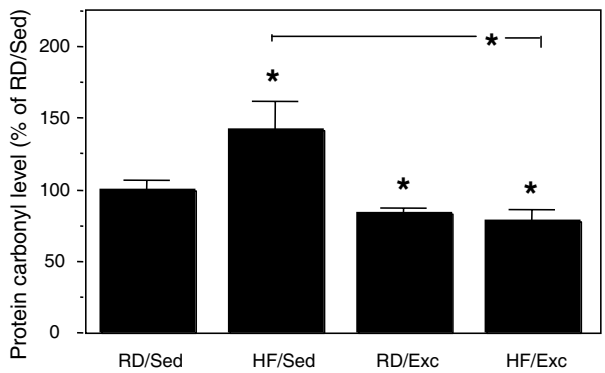


**Fig. 8.** Relative levels of oxidized protein in the hippocampus determined by a quantifiable Western blot analysis of DNPH-derivatized carbonyls. Levels of protein oxidation were increased in animal exposed to the HF diet. Access to voluntary exercise concurrently to the diet reduced levels of oxidized proteins. Values represent mean±S.E.M. (* $P<0.05$; $n=6$/group).

As phospho-CREB is a potent transcriptional activator, a consequence of heightened phospho-CREB formation may be to promote synaptic plasticity. Active CREB can alter neuronal responses to future stimuli, making them more sensitive to weaker stimuli and more receptive to diverse stimuli (Mermelstein et al., 2001). In fact, studies have shown that hippocampal neurons sustain an increase in phospho-CREB during memory formation (Impey et al., 1998) and that "amnesiac" rats, severely impaired in retaining memory of a task, fail to exhibit an increase in hippocampal CREB phosphorylation (Taubenfeld et al., 1999). In this context, it is significant that rats fed a HF diet and exposed to exercise performed equally as well as control rats (RD/Sed). Exercise only augmented phospho-CREB levels in HF animals. Inferentially, the increase in phospho-CREB may contribute to the overall ability of exercise to counteract the impairment in neuronal and behavioral plasticity associated with the consumption of a HF diet. This possibility is strongly supported by the findings that increased levels of phospho-CREB in HF rats that performed exercise was significantly correlated with the latency to find the platform.

This situation contrasts sharply with HF/Sed rats that showed the poorest learning, commensurable to a decrease in phospho-CREB levels and a disruption in the phospho-CREB vs. total-CREB relationship. Indeed, it has been reported that inhibiting the phosphorylation of CREB increases the degree of neuronal injury following insult (Mabuchi et al., 2001). Conformably, RD rats that underwent exercise training performed superlatively on the MWM task, probably by a dynamic utilization of phospho-CREB based on the depletion of total-CREB and inverted correlation between the two.

The HF diet decreases CREB in concert with BDNF (Molteni et al., 2002). Moreover, the current data indicate that the restorative effect of exercise on learning and memory was associated with increased levels of phospho-CREB (Fig. 7A), and this in turn was associated with increased levels of BDNF (Fig. 7B). This relationship between CREB and BDNF may incorporate the propensity of CREB to be disposed to phosphorylation by BDNF (Tully, 1997) and in turn the ability of phospho-CREB to govern BDNF transcription (Tao et al., 1998; Shieh and Ghosh, 1999). Thus, BDNF and CREB may partake in a protective mechanism, activated when neuronal function is compromised, such as in the presence of a HF diet.

### Exercise alters the synapsin I and phospho-synapsin I relationship; implications for synaptic plasticity

Our results indicate that exercise elevated phospho-synapsin I protein levels in both RD and HF animals. Synapsin I is a well-characterized member of the synapsin family of

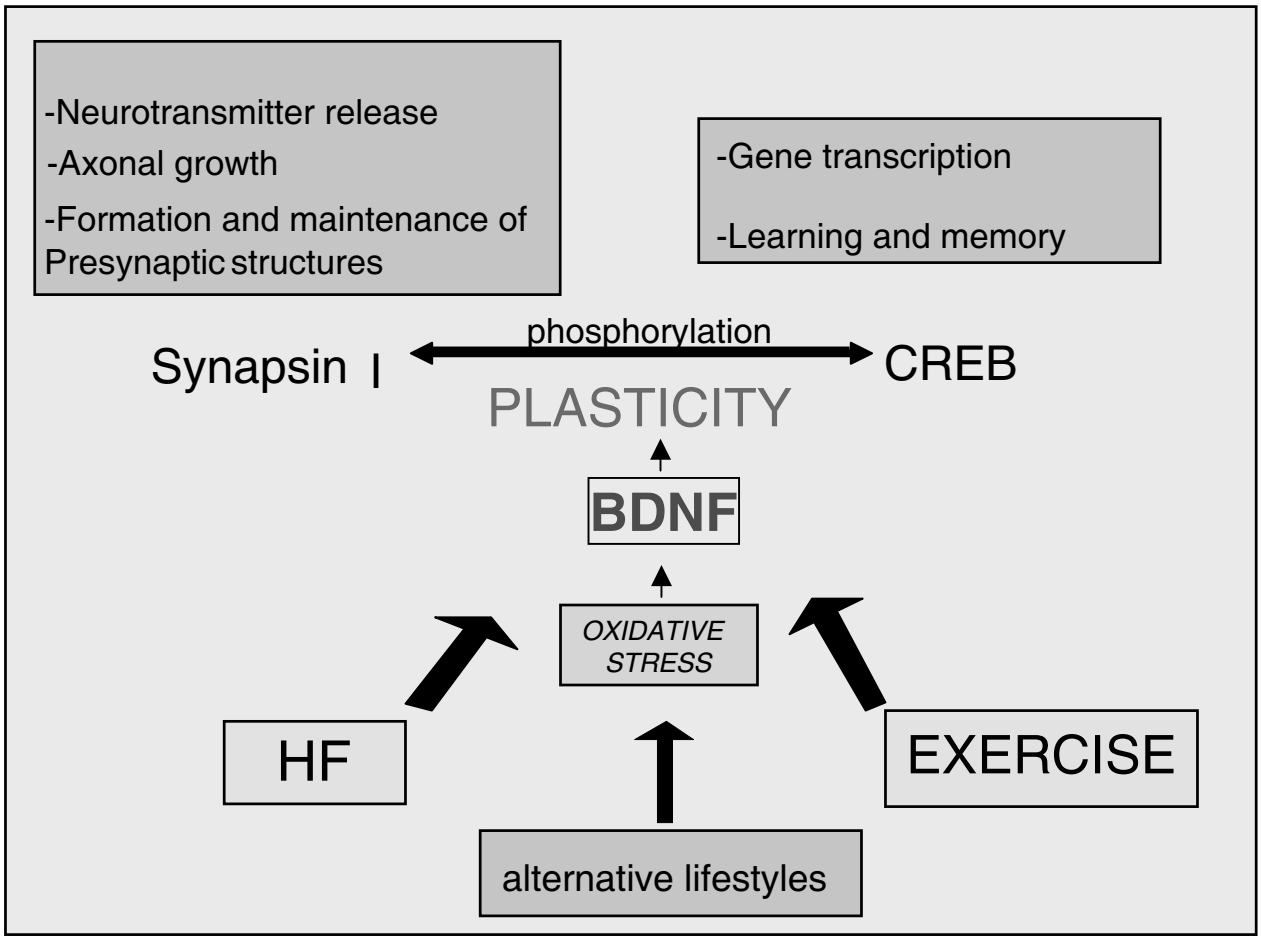


**Fig. 9.** Proposed mechanisms by which exercise and diet can modulate neuronal plasticity. Physical activity, nutritional factors, and others lifestyles affect BDNF expression and function. A HF diet decreases hippocampal BDNF levels while exercise compensates for these effects. Oxidative stress may be involved with the molecular events by which a HF diet and exercise can affect BDNF and neuroplasticity. Changes in BDNF levels can subsequently affect the phosphorylation and synthesis of molecules involved with regulation of synaptic transmission and function, such as synapsin I and CREB.

neuro-specific proteins, whose ability to cluster synaptic vesicles (Greengard et al., 1993) enables it to modulate neurotransmitter release and synaptic plasticity (Hilfiker et al., 1999). BDNF grasps a tight regulation over synapsin I, promoting the phosphorylation of synapsin I and subsequent neurotransmitter release (Jovanovic et al., 2000). In fact, BDNF gene deletion results in fewer docked vesicles and impaired neurotransmitter release (Pozzo-Miller et al., 1999). Thus, the exercise-induced increase in phospho-synapsin I may signify enhanced synaptic efficacy and responsiveness for release.

HF animals, although exhibiting normal synapsin I protein levels, show decreased synapsin I mRNA (Fig. 2A) and phospho-synapsin I levels (Fig. 2C), and fail to hold a relationship between phospho-synapsin I and synapsin I (Fig. 3C). A disruption of the positive correlation between phospho-synapsin I and total-synapsin I levels, despite normal levels of both, suggests a profound effect of the HF diet on the state of neurotransmitter reserve and release pools. Exercise increased synapsin I mRNA (Fig. 2A), and phospho-synapsin (Fig. 2C), and restored the phospho-/total-synapsin relationship (Fig. 3D). Thus, it appears that exercise may trigger a cascade of events whose molecular consequences become evident when the system is compromised by the deleterious effects of a HF diet.

## CONCLUSIONS

Several lines of evidence illustrate the beneficial action of physical activity in maintaining and improving neural function in humans and animals. Exercise has been shown to reduce the cognitive decline associated with aging (Friedland et al., 2001; Laurin et al., 2001), help recover functional loss after CNS damage (Mattson, 2000), and promote neurogenesis in the adult hippocampus (van Praag et al., 1999). Despite these strong examples of the beneficial role of exercise, underlying mechanisms have remained elusive. Our results show that exercise restore the function of molecular systems affected by select insults such a “bad diet,” involving oxidative stress, BDNF and its effects on the quantitative and activational states of molecules implicated in the establishment of memory pro-

cesses. Oxidative stress and a lack of trophic factor support are involved with the pathology of various degenerative diseases including Alzheimer and Parkinson. Therefore, the present results emphasize the influence of lifestyle on mechanisms of degeneration as well as healing, and the possibility for their manipulation.

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