

# The Combined Effect of Sleep Deprivation and Western Diet on Spatial Learning and Memory: Role of BDNF and Oxidative Stress

Karem H. Alzoubi · Omar F. Khabour · Heba A. Salah · Baraa E. Abu Rashid

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**Abstract** Either sleep deprivation or Western diet can impair learning and memory via induction of oxidative stress, which results in neuronal damage and interference with the neurotransmission. In this study, we examined the combined effect of sleep deprivation and Western diet on hippocampus-dependent spatial learning and memory. In addition, possible molecular targets for sleep deprivation and Western diet-induced cognitive impairments were investigated. Sleep deprivation was induced in rats using the modified multiple platform model simultaneous with the administration of Western diet for 6 weeks. Thereafter, spatial learning and memory were tested using radial arm water maze. At the molecular level, BDNF protein and antioxidant markers including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG, and thiobarbituric acid reactive substances (TBARS) were assessed. The results of this study revealed that sleep deprivation, Western diet, or a combination of both impair short- and long-term memory ( $P<0.05$ ). The magnitude of the impairment induced by the combined treatment at the 24-h long-term memory was higher than that caused by each factor alone ( $P<0.05$ ). In addition, the combined treatment reduced the levels of hippocampal BDNF, a reduction

that was not detected with each factor alone. Moreover, the combined treatment reduced the hippocampal activities of SOD, catalase, GPx, ratio of GSH/GSSG, and elevated TBARS level ( $P<0.05$ ). In conclusion, the combination of sleep deprivation and Western diet decreases BDNF levels and increases oxidative stress in the hippocampus, thus inducing memory impairment that is greater than the impairment produced by each factor alone.

**Keywords** Sleep deprivation · Western diet · Maze · Memory · BDNF · Oxidative stress · Learning · Hippocampus

## Introduction

Sleep plays an important role in human life by buffering out body metabolites and thus maintaining general and mental health (Greene and Siegel 2004; Ohlmann and O'Sullivan 2009). Sleep is divided into two stages that alternate with each other: non-rapid eye movement and rapid eye movement (REM) (Guyton and Hall 2006; Silber et al. 2007). REM sleep is involved in memory consolidation (Harrison and Horne 2000; McDermott et al. 2006; Lopez et al. 2008; McCarley 2011). This was suggested by the fact that REM sleep duration is increased by learning (Smith and Rose 1997), whereas it was decreased in patients with Alzheimer disease (Montplaisir et al. 1998).

Numerous animal and human studies have described memory deficits following sleep deprivation (Smith and Kelly 1988; Jiang et al. 2009; Alhaider et al. 2010b; Aleisa et al. 2011a, b; Alzoubi et al. 2012; Zagaar et al. 2012). Recent studies have shown that sleep deprivation-induced memory deficits may possibly be caused by increasing oxidative stress in the hippocampus, which might be caused, at least in part, by accumulation of reactive oxygen species during the wake cycle (Reimund 1994; Ramanathan et al.

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K. H. Alzoubi (✉) · B. E. Abu Rashid  
Department of Clinical Pharmacy, Faculty of Pharmacy,  
Jordan University of Science and Technology,  
Irbid, Jordan 22110  
e-mail: khalzoubi@just.edu.jo

O. F. Khabour  
Department of Medical Laboratory Sciences, Faculty of Applied  
Medical Sciences, Jordan University of Science and Technology,  
Irbid, Jordan

H. A. Salah  
Department of Physiology, Faculty of Medicine,  
Jordan University of Science and Technology,  
Irbid, Jordan

2002; Silva et al. 2004; Alzoubi et al. 2012) and could also be manifested by changes in BDNF (Alhaider et al. 2010a).

Diet is also a major contributor to neuronal and cognitive health. Emerging data show that the consumption of Western diet in human populations is associated with cognitive decline and enhanced vulnerability to brain injury (Baran et al. 2005; Ansari et al. 2008; Srivareerat et al. 2009). Similarly, experimental studies in animal models confirm a profile of heightened brain vulnerability and decreased cognitive function (Greenwood and Winocur 1990; Alzoubi et al. 2009; White et al. 2009; Kanoski and Davidson 2010; Yu et al. 2010; Kanoski and Davidson 2011). Although the physiologic mechanisms whereby Western diet adversely affects the brain are poorly understood, both experimental and human studies have shown that obesity is associated with increased oxidative stress markers and lipid peroxidation (Mattson et al. 2003; Fachinnetto et al. 2005; Xie and Du 2005; Zhang et al. 2005; Park et al. 2010; Kanoski and Davidson 2011).

Available data from animal models indicate that Western diet can decrease neuronal plasticity via regulation of BDNF (Molteni et al. 2002). The effect of BDNF on hippocampus-dependent memory is well documented. Interfering with BDNF-related signaling pathway resulted in deficit in spatial learning (Linnarsson et al. 1997) and memory formation (Mizuno et al. 2000). In addition, the deletion of BDNF hippocampal genes impaired hippocampal tasks such as object recognition and spatial memory in Morris maze (Heldt et al. 2007). Furthermore, BDNF expression was increased in rats' hippocampus after spatial learning and memory tasks (Falkenberg et al. 1992). Finally, treatments that enhance memory formation were associated with elevation in BDNF expression (Khabour et al. 2010, 2012).

On the other hand, antioxidative stress mechanisms are important for cognitive functions. The term oxidative stress describes the situation of imbalance between reactive oxygen species and the antioxidant opposing forces (Gupta et al. 2003). Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into hydrogen peroxide, which is then neutralized by catalase (Zelko et al. 2002; Chelikani and Loewen 2004). The glutathione system is composed of the enzymatic antioxidants including GPx and non-enzymatic free radicals scavenger glutathione (GSH) (Meister and Anderson 1983). Oxidative stress in the brain is associated with neuronal damage and the subsequent impaired spatial learning and memory (Benzi et al. 1990; Fukui et al. 2002; Jhoo et al. 2004).

Prevalence of sleep deprivation and obesity are increasing in parallel all over the world (Chamorro et al. 2011; Freedman 2011). Several studies showed an association between sleep deprivation and obesity (Kripke et al. 2002; Spiegel et al. 2004; Leproult and Van Cauter 2010), but the cause and effect relationship is still controversial (Taheri et al. 2004; Vorona et al. 2005). Since sleep deprivation and

Western diet separately lead to memory impairment, it was reasonable to question the extent to which the combination of sleep deprivation and Western diet leads to learning and memory impairment. In this study, we investigated the hypothesis that a combination of sleep deprivation and Western diet exacerbates the learning and memory impairment induced by each factor alone. Both behavioral approach using the radial arm water maze (RAWM) to test learning and memory functions and molecular approach using enzymatic assays were used to test this hypothesis.

## Methods

Adult male Wistar rats weighing 180–250 g ages (7–8 weeks old) were used in this study. The animals were housed in metal cages, at four to five rats in each cage, with wood shaving as bedding, under hygiene conditions, and maintained at room temperature with free access to food and water. The rats were housed at a 12-h light/dark cycle (light at 7 am). All experimental procedures were performed during the light cycle. The procedures performed were approved by the Animal Care Use Committee of Jordan University of Science and Technology.

### Animals and Treatments

Animals were randomly assigned into five groups: control, wide platform (WPF), sleep deprivation (SD), Western diet (WD), and sleep deprivation with Western diet (SD + WD). Animals' randomization was carried out according to their weight so that the average weights in all groups were similar. Animals were allowed 2 weeks to acclimate before any experimental manipulation began. Among the five animal groups, both WD and SD + WD groups were fed only Western diet containing (g%): 25 % total fat including 11 % unsaturated fats, 44 % carbohydrates, 18 % proteins, 13 % fibers, ash, and other ingredients. The remaining groups were fed conventional diet containing (g%): 5 % total fat including 2 % unsaturated fats, 62 % carbohydrates, 20 % proteins, 13 % fibers, ash, and other ingredients (Sahil-Huran Animal Food Company, Ramtha, Jordan) (Alzoubi et al. 2009). In both diets, casein was the main source of protein, butter and soybean oil were the main source of fat, and starch was the main source of carbohydrate. Both diets contained the same amount of standard vitamins and mineral mix with all essential nutrients. Food was provided ad libitum for the duration of the experiments. All manipulations including SD, WD, and the combination were started on the same day and continued for 6 weeks. The RAWM training was carried out immediately after 6 weeks of treatment. SD and/or WD treatments continued throughout the RAWM testing days.

## Induction of Sleep Deprivation

SD and SD + WD groups were exposed to chronic sleep deprivation for 6 weeks, which was induced using a columns-in-water (modified multiple platform) model as described (McDermott et al. 2006; Alhaider et al. 2010a; Aleisa et al. 2011a, b; Alhaider et al. 2011; Alzoubi et al. 2012). Briefly, animals were placed on platforms (20 platforms; 20 cm in height and 5 cm in diameter, 7 cm apart edge-to-edge) surrounded by water ( $24 \pm 1$  °C) in an aquarium where water and food were accessible. The water level in the aquarium was about 2 cm below the edge of the platform. This method has been reported to interfere with total sleep, but it mainly eliminates REM sleep (Grahnstedt and Ursin 1985). During REM sleep, animals lose their muscle tone, fall into the water, and wake up. Furthermore, to test the possible stresses caused by the aquarium environment, wide platforms (diameter, 12 cm) were used, which allow the WPF rats group to sleep without falling into the water.

## Radial Arm Water Maze

All the five groups were tested for spatial learning and memory performance on the radial arm water maze task (Diamond et al. 1999; Park et al. 2001; Gerges et al. 2004; Alzoubi et al. 2006, 2009). The RAWM is a black, circular, water-filled tub (water temperature,  $24 \pm 1$  °C) with six V-shaped stainless steel plates arranged to form a swimming path of an open central area and six radiating arms. All the experiments were done in a dimly light room, and two different pictures were placed in fixed positions on the wall to serve as cues for the rats. The animals had to find a hidden platform (2 cm under water) at the far end of one of the swim arms (the goal arm). The goal arm was not changed for a particular rat. Rats were allowed 12 consecutive acquisition trials; the first six trials were separated by 5 min of resting time. The 12 trials were followed at 30 min later by a short-term memory test and at 5 and 24 h later by long-term memory tests. Every trial/test was started in a different starting arm (except the goal arm) in a particular day for a particular rat. In each trial, the rat was allowed to swim freely for 1 min in the maze to find the hidden platform. Once on the platform, the rat was allowed 15 s to observe cues before the next trial. When a rat was unable to find the platform within the 1-min period allowed, it was guided toward the platform for the 15-s stay. During the 1-min period, each time the rat entered an arm other than the goal arm, an error was registered. Entry is defined as the entry of the entire body of the rat including the tail into the particular arm.

## Hippocampus Dissection

The animals were killed after 6 weeks of SD and/or WD, and the hippocampus was dissected as described (Alzoubi et

al. 2005; Gerges et al. 2005). In brief, the brain was removed immediately from the skull and placed on a filter paper soaked with normal saline over a petri dish filled with crushed ice. The hippocampus was immediately collected in pre-labeled Eppendorf tubes and placed in liquid nitrogen. Finally, the samples were frozen at  $-30$  °C, until the time of tissue processing.

## Biochemical Testing

The hippocampus tissues were homogenized in phosphate buffer saline (8 g NaCl, 0.2 g KCl, 0.24 g  $\text{KH}_2\text{PO}_4$ , and 1.44 g  $\text{Na}_2\text{HPO}_4$  in 1 L distilled water) using a plastic pestle. EDTA (5 mM) was dissolved in the saline as a preservative (Alzoubi et al. 2012). The homogenized tissues were centrifuged to remove the insoluble materials ( $15,000 \times g$  for 10 min at 4 °C). Total protein concentration was estimated using BioRAD procedure (Hercules, CA, USA). Hippocampal BDNF levels were measured using enzyme-linked immunosorbent assay (Human BDNF, DuoSet ELISA Development Systems, R&D System, USA). The activities of antioxidant enzymes, SOD, and catalase were determined calorimetrically according to the kit manufacturer's instructions (SOD: Sigma-Aldrich Corp, MI, USA; catalase: Cayman Chem, Ann Arbor, MI, USA). The activity of GPx was determined kinetically using Glutathione Peroxidase Cellular Activity Assay Kit (CGP1, Sigma-Aldrich, MI, USA) and a spectrophotometer (UV-vis spectrophotometer, UV-1800, Shimadzu, Japan). To quantify glutathione hippocampal levels, samples were pre-treated with 5 % salicylic acid. The reduced GSH levels were quantified calorimetrically according to the kit manufacturer's instructions (Sigma-Aldrich Corp, MI, USA). The oxidized glutathione (GSSG) was also measured using the same procedure used to estimate GSH, but the tissue homogenates were first treated with 1 M 4-vinylpyridine. Thiobarbituric acid reactive substances (TBARS) levels were also evaluated in the homogenized tissue using TBARS assay kit (Cayman Chem, Ann Arbor, MI, USA). ELISA plates were read at the kit-specified wavelengths using an automated reader (ELx800, Biotek Instruments, Plate Reader, Highland Park, Winooski, VT, USA).

## Statistical Analysis

One-way ANOVA followed by Tukey's test was applied to assess differences among groups. All statistical tests were carried out using GraphPad Prism software (version 4.0, GraphPad software, La Jolla, CA, USA). Significance levels were considered at  $P < 0.05$ , and values are presented as mean  $\pm$  standard error of mean (SEM).

## Results

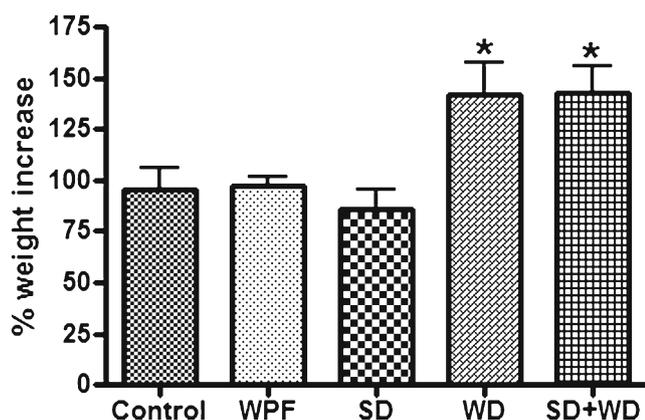
### Percentages of Body Weight Increase

The normal increase in body weight over the duration of the experiments in control, WPF, and SD groups was in the range of 85–97 %. However, WD and SD + WD groups showed a significant elevation in body weight gain (130–142 %,  $P < 0.05$ ,  $n = 14$  rats/group; Fig. 1) when compared to all other groups over the same time period.

### The Effect of Sleep Deprivation and Western Diet on Learning and Memory

RAWM learning and memory test was performed directly at the end of the treatment course. At the beginning of the learning trials, rats in all groups made a high number of errors. As the trials proceeded (the acquisition phase), the number of errors committed by the rats decreased, with no significant difference among groups in all learning trials (Fig. 2a).

In short-term memory tests, the number of errors committed by SD, WD, and SD + WD groups ( $2.14 \pm 0.34$ ,  $2.33 \pm 0.31$ , and  $2.87 \pm 0.61$ , respectively;  $P < 0.05$ ,  $n = 14$  rats/group) were significantly higher than that of control and WPF groups ( $0.4 \pm 0.16$  and  $0.41 \pm 0.23$ , respectively;  $n = 14$  rats/group; Fig. 2b). In long-term memory tests performed 5 h after the end of the acquisition phase, SD, WD, and SD + WD groups showed a significant elevation in the number of errors ( $3.64 \pm 0.37$ ,  $2.93 \pm 0.56$ , and  $4.01 \pm 0.50$ , respectively;  $P < 0.05$ ,  $n = 14$  rats/group) committed compared to the control and WPF groups ( $0.53 \pm 0.27$  and  $0.58 \pm 0.19$ , respectively;  $n = 14$  rats/group; Fig. 1). In the 24-h long-term memory testing, SD, WD, and SD + WD groups also made



**Fig. 1** Western diet is associated with increase in body weight. The percentage of increase in body weight over the experimental period was significantly more in the WD and SD + WD groups than that of control or SD groups. All values are mean  $\pm$  S.E.M. Asterisk indicates significant difference ( $P < 0.05$ ,  $n = 14$  rats/group) from the control value

a significantly higher number of errors compared to control and WPF groups ( $P < 0.05$ ;  $n = 14$ –15 rats/group; Fig. 2d). Moreover, SD + WD group made a significantly more number of errors ( $5.8 \pm 0.56$ ;  $n = 14$ –15 rats/group,  $P < 0.05$ ) compared to SD and WD groups ( $4.2 \pm 0.24$ ;  $3.47 \pm 0.35$ ,  $n = 14$ –15 rats/group; Fig. 2d). These results indicate that sleep deprivation and Western diet result in a memory impairment that is not exacerbated on a short-term level by their combination. However, the combination of sleep deprivation and Western diet exacerbates the 24-h long-term memory impairment induced by sleep deprivation and Western diet separately.

### The Effect of the Combination of Sleep Deprivation and Western Diet on Hippocampus BDNF Levels

No significant change was observed in BDNF levels in SD and WD groups ( $120 \pm 8.4$  and  $120.3 \pm 9.9$ ,  $n = 10$  rats/group, respectively) compared to control and WPF groups ( $114.3 \pm 1,047$  and  $117.6 \pm 17.6$ , respectively;  $n = 10$  rats/group; Fig. 3). However, the combination of sleep deprivation and Western diet (SD + WD group) resulted in a significant reduction in hippocampal BDNF levels ( $89.5 \pm 4.9$ ;  $P < 0.05$ ;  $n = 10$  rats/group) compared to all other animal groups.

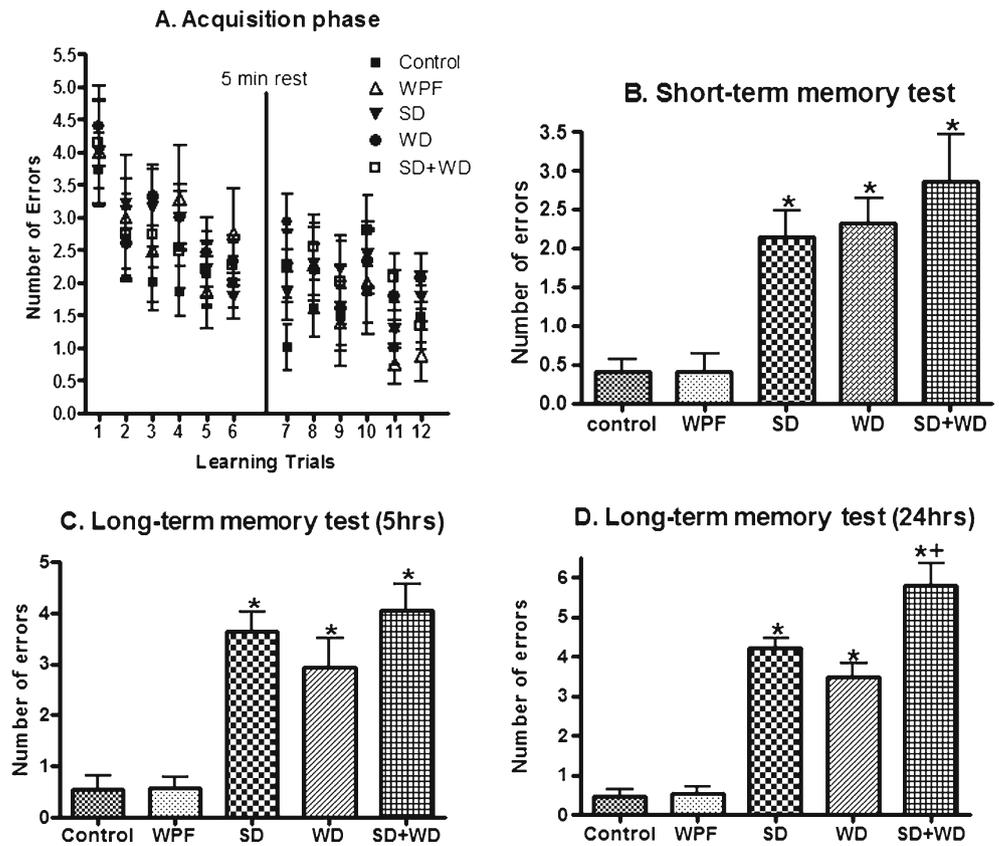
### The Combined Effect of Sleep Deprivation and Western Diet on Hippocampal Oxidative Stress Markers

**SOD Activity** SOD activity in SD and WD groups was significantly lower ( $2.97 \pm 0.28$  and  $2.67 \pm 0.17$ , respectively;  $P < 0.05$ ;  $n = 10$  rats/group) than in control and WPF groups ( $4.13 \pm 0.40$  and  $4.59 \pm 0.25$ , respectively; Fig. 3;  $n = 10$  rats/group). In addition, SOD activity in SD + WD in hippocampus ( $2.82 \pm 0.17$ ;  $P < 0.05$ ;  $n = 10$  rats/group) was lower than in control group, but not different from SD or WD alone ( $P > 0.05$ ; Fig. 4;  $n = 10$  rats/group).

**Catalase Activity** Catalase activity was significantly decreased in SD and WD groups ( $9.57 \pm 1.32$  and  $8.41 \pm 1.13$ , respectively;  $P < 0.05$ ;  $n = 10$  rats/group) compared to control and WPF groups ( $15.68 \pm 2.26$  and  $14.4 \pm 1.28$ , respectively; Fig. 5;  $n = 10$  rats/group). Reduction in catalase activity in the SD + WD group was comparable to that in the SD or WD groups ( $7.34 \pm 1.37$ ;  $P < 0.05$ ;  $n = 10$  rats/group) and significantly different from the control group (Fig. 5).

**GPx Activity** GPx activity was significantly lower in SD ( $1.334 \pm 0.40$ ;  $P < 0.05$ ;  $n = 10$  rats/group) when compared to control and WPF groups ( $1.98 \pm 0.18$  and  $1.97 \pm 0.12$ , respectively;  $n = 10$  rats/group; Fig. 6). On the other hand, no significant change was observed in GPx activity in WD group compared to control. Nevertheless, a significant reduction in GPx activity was observed in SD + WD group ( $1.35 \pm 0.09$ ;

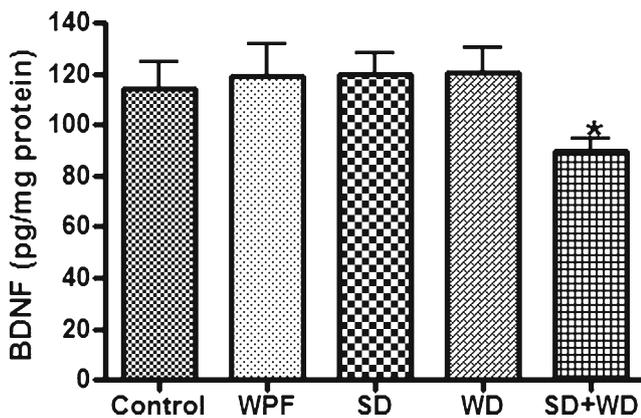
**Fig. 2** Animal groups' performance during RAWM. In the acquisition phase (a), the number of errors committed by all groups declined as learning proceeded with no significant change among different groups. Memory tests performed after 30 min, 5 h, and 24 h of the acquisition (b–d) showed a significant increase in the number of errors committed by SD, WD, and SD + WD groups compared to control. Furthermore, the 24-h long-term memory test indicated that SD + WD rats made a significantly higher number of errors compared to SD and WD groups (c). Each point is the mean ± SEM of 14–15 rats. Asterisk indicates significant difference from control group, and plus sign indicates significant difference from WD and SD + WD groups ( $P < 0.05$ )



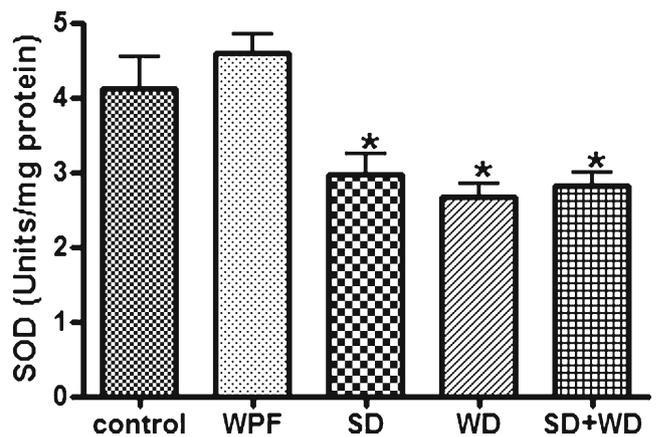
$P < 0.05$ ;  $n = 10$  rats/group) compared to the control group, but no further reduction was observed compared to the SD group.

**Levels of the GSH, GSSG and GSH/GSSG Ratio** No significant change was observed in hippocampal GSH levels among various groups (Fig. 7a). However, SD, WD, and SD + WD groups showed a significant elevation in GSSG

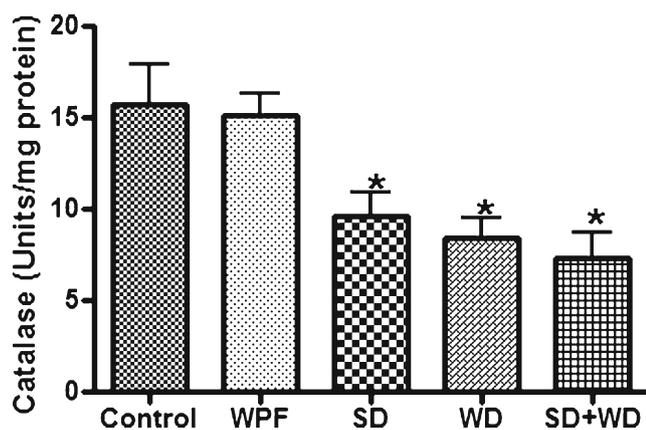
levels ( $3.11 \pm 0.28$ ,  $2.91 \pm 0.46$ , and  $2.90 \pm 0.20$ , respectively;  $P < 0.05$ ;  $n = 10$  rats/group) compared to control and WPF groups ( $1.93 \pm 0.23$  and  $1.96 \pm 0.09$ , respectively;  $n = 10$  rats/group; Fig. 7b). The GSH/GSSG ratio was significantly reduced in SD and SD + WD groups ( $1.93 \pm 0.19$  and  $1.88 \pm 0.12$ , respectively;  $P < 0.05$ ;  $n = 10$  rats/group) compared to control, WPF, and WD groups ( $2.71 \pm 0.45$ ,  $2.72 \pm 0.25$ , and  $2.22 \pm 0.24$ , respectively;  $n = 10$  rats/group; Fig. 7c).



**Fig. 3** Hippocampal BDNF levels. No change in the levels of BDNF was observed in either SD or WD groups when compared to control. However, the combination of SD + WD showed a significant reduction in BDNF levels when compared to all other groups. Each point is the mean ± SEM of 10 rats. Asterisk indicates significant difference compared to all other groups ( $P < 0.05$ )



**Fig. 4** Hippocampal SOD activity. SOD activity was significantly reduced in SD, WD, and SD + WD groups compared to control. SOD activity in SD + WD group was comparable to that in SD and WD groups. Each point is the mean ± SEM of ten rats. Asterisk indicates significant difference compared to control ( $P < 0.05$ )

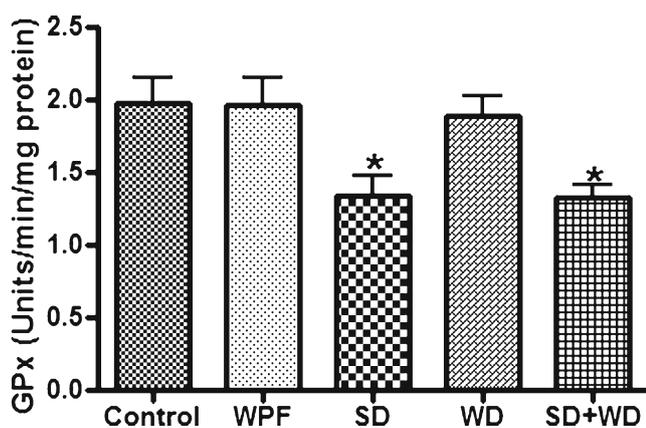


**Fig. 5** Hippocampal catalase activity. Catalase activity was significantly reduced in SD, WD, and SD + WD groups compared to control. Catalase activity in SD + WD was similar to that detected in SD and WD groups. Each point is the mean  $\pm$  SEM of ten rats. Asterisk indicates significant difference compared to control ( $P < 0.05$ )

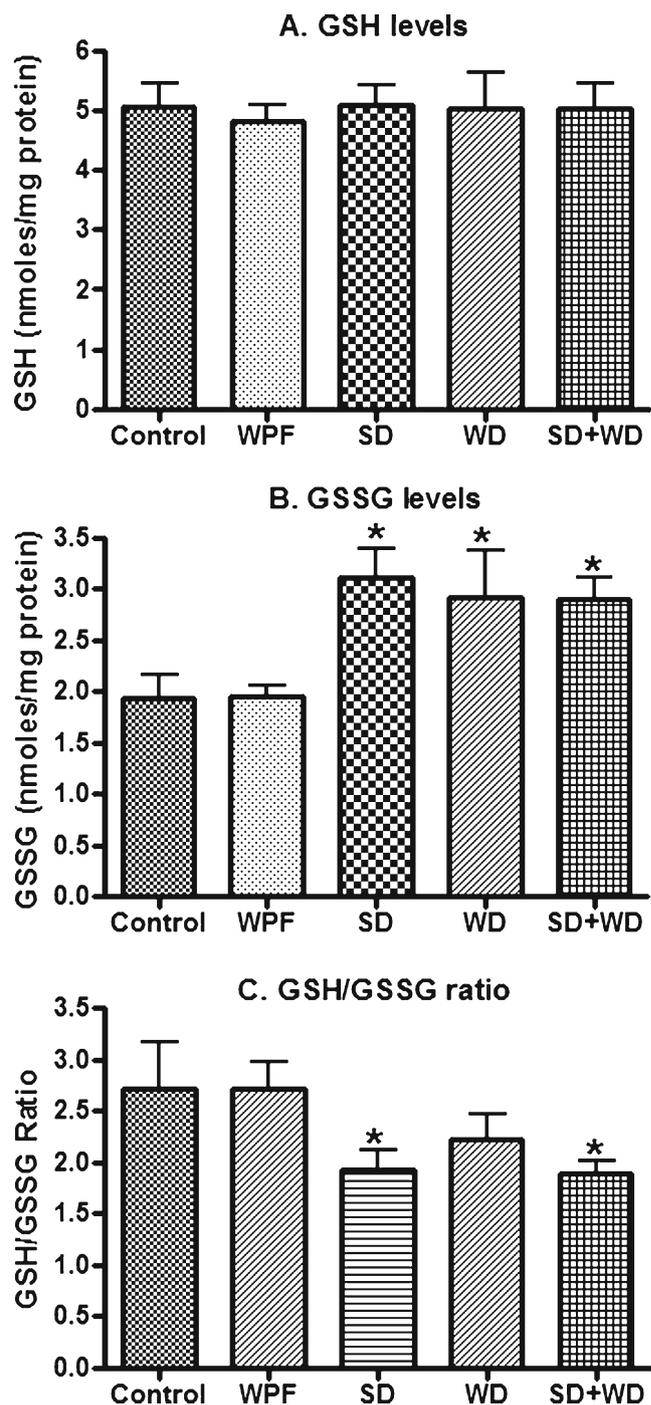
**TBARS Levels** In the SD group, no significant change was observed in hippocampal TBARS levels ( $9.26 \pm 1.09$ ) compared to control and WPF groups ( $8.74 \pm 0.94$  and  $11.25 \pm 1.21$ , respectively;  $n = 10$  rats/group; Fig. 8). However, WD and SD + WD resulted in a significant elevation in TBARS levels ( $18.64 \pm 3.22$ ;  $17.66 \pm 1.16$ ;  $P < 0.05$ ;  $n = 10$  rats/group) compared to control and other groups.

## Discussion

Sleep deprivation and Western diet are lifestyle patterns that negatively affect cognitive function. The current study shows that the combination of sleep deprivation and Western diet leads to short- and long-term memory impairment. Moreover,

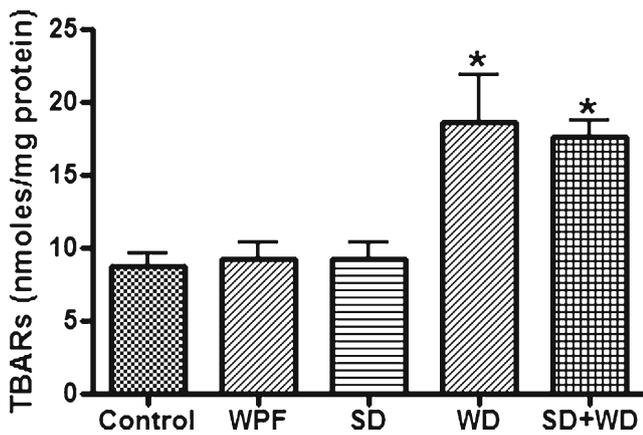


**Fig. 6** Hippocampal GPx activity. SD group showed a significant reduction in GPx activity compared to control. However, in WD-fed animals, no change was observed in GPx activity compared to control group. Nevertheless, no change in GPx activity was observed when comparing SD and SD + WD groups. Each point is the mean  $\pm$  SEM of ten rats. Asterisk indicates significant difference compared to control ( $P < 0.05$ )



**Fig. 7** Hippocampal levels of different forms of glutathione. No change was observed in the GSH levels among different groups (a). However, SD + WD elevated the GSSG levels compared to control (b) yet did not cause further elevation in the GSSG levels compared to SD and WD groups. SD + WD also reduced the GSH/GSSG ratio compared to control and WD groups (c). Each point is the mean  $\pm$  SEM of ten rats. Asterisk indicates significant difference compared to control ( $P < 0.05$ )

the long-term memory impairment induced by the combination was greater than the impairment produced by sleep deprivation or Western diet alone. The worsening in the long-term



**Fig. 8** Levels of TBARS in the hippocampus. Whereas the levels of TBARS were not altered in the SD group, in WD and SD + WD groups the levels of TBARS were significantly increased. Each point is the mean  $\pm$  SEM of ten rats. Asterisk indicates significant difference compared to control ( $P < 0.05$ )

memory was associated with a reduction in BDNF hippocampal levels but was not associated with further changes in hippocampal oxidative stress markers compared to sleep deprivation or Western diet alone.

Extensive studies have shown an association between sleep deprivation and cognitive processes in humans (Harrison and Horne 2000; Mednick et al. 2002) and animals (Smith and Kelly 1988; Jiang et al. 2009; Alhaider et al. 2010b; Aleisa et al. 2011a, b; Alzoubi et al. 2012; Zagaar et al. 2012). The current study showed that chronic REM sleep deprivation (8 h/day for 6 weeks) impaired short- and long-term spatial memory compared to control, which confirms our previous results (Alzoubi et al. 2012). The results of this study are also supported by several animal studies. For example, 48 h of REM sleep deprivation impaired the spatial relevance memory in Morris water maze (Li et al. 2009). In another study, 24 h of sleep deprivation using the multiple platform model impaired long-term memory in RAWM (Aleisa et al. 2011a). Furthermore, REM sleep deprivation for 3 h per day for a net of 7 days impaired reference memory at 7 days after the sleep deprivation (Jiang et al. 2009). Thus, both acute and chronic forms of sleep deprivation seem to interfere with cognitive functions.

Recent studies indicated that Western diet is a risk factor for cognitive decline. Our study show that Western diet consumption causes short- and long-term memory impairment. This finding is supported by several studies that demonstrated the negative effect of Western diet on learning and memory. For instance, 3-month administration of diet rich in saturated fats and carbohydrates to rats impaired their learning and memory, which was tested by the radial arm maze (Greenwood and Winocur 1990), and the RAWM (Alzoubi et al. 2009). Moreover, a recent study showed a decrease in spatial and working memory in the radial maze

following only 72 h of feeding the rats with Western diet (Kanoski and Davidson 2010, 2011).

Since sleep deprivation and Western diet separately resulted in a cognitive impairment, it was reasonable to wonder to what extent does the combination of both factors affects cognition. Our study indicated that the combination of sleep deprivation and Western diet did not exacerbate short-term and 5-h long-term memory impairment produced by each factor alone. However, the combination intensified the 24-h long-term impairment caused by sleep deprivation and Western diet separately. This effect can be explained, at least in part, by the molecular mechanisms underlying the cognitive impairment produced by sleep deprivation and Western diet. Several studies showed that the cognitive decline noticed in both factors could be attributed to a decrease in synaptic plasticity via the reduction of BDNF levels (Molteni et al. 2002; Alhaider et al. 2010a, 2011) or an increase in oxidative stress markers in the hippocampus (Studzinski et al. 2009; Alzoubi et al. 2012). Thus, in an attempt to explore the mechanisms underlying the behavioral results, several molecular tests were performed. BDNF is a neurotrophin that enhances the growth and differentiation of neurons and synapses (Acheson et al. 1995). In the CNS, BDNF is active in the hippocampus, cortex, and forebrain, the areas that have a vital role in learning and memory (Yamada and Nabeshima 2003). Our study showed that the combination of sleep deprivation and Western diet caused a significant reduction in hippocampal BDNF levels. However, chronic REM sleep deprivation alone or Western diet alone did not affect the hippocampal BDNF levels. Earlier studies on the hippocampal BDNF levels in sleep deprivation and Western diet separately were controversial. For instance, REM sleep deprivation for 8 to 48 h resulted in a decrease in BDNF mRNA levels in the rats' hippocampus (Guzman-Marin et al. 2006) and hippocampal protein levels (Alhaider et al. 2010a). In contrast, REM sleep deprivation for 6 h suppressed BDNF levels in the cerebellum and brainstem without producing significant changes in the hippocampus (Sei et al. 2000). Similar to sleep deprivation, 2 months of Western diet in rats reduced BDNF levels in the hippocampus (Molteni et al. 2002), while 6 weeks of Western diet in rats did not change the hippocampal BDNF levels (Zeeni et al. 2009), which correlates with the current results.

The mechanism by which the combination of sleep deprivation and Western diet induce a reduction in BDNF was not investigated in this study. REM sleep theta waves have been shown to enhance both BDNF gene expressions and its release from stored vesicles (Guzman-Marin et al. 2006). In contrast, Western diet has been shown to decrease the BDNF levels by inhibiting its transcription and translation (Molteni et al. 2002). Other studies showed that the oxidative stress produced by fat content in diets impairs the natural DNA repair systems, which tend to decrease BDNF

expression (Pistell et al. 2010). The decrease in BDNF levels, produced by sleep deprivation and Western diet separately, might be mild to cause a significant reduction in hippocampal BDNF levels. However, a combination of both factors resulted in a pronounced decrease in BDNF levels, possibly through a combination of the aforementioned mechanisms. Thus, a collective additive effect of the molecular mechanisms underlying the combination of sleep deprivation and Western diet is thought to induce a reduction in hippocampal BDNF.

Oxidative stress appears to play a role in diminishing cognitive processes; thus, several oxidative stress markers have been evaluated in the hippocampus. Sleep is thought to be responsible for the elimination of reactive oxygen species produced during the wake cycle. Thus, sleep deprivation is thought to increase oxidative stress in certain brain areas (Reimund 1994). Our study showed that sleep deprivation for 8 h/day for 6 weeks caused significant changes in hippocampal oxidative stress markers. The antioxidant enzyme activities, SOD, catalase, and GPx were reduced by sleep deprivation. In addition, the GSH/GSSG ratio was reduced, decreasing the scavenging effect of glutathione in the hippocampus. The reduction in the antioxidant defense mechanisms increased the oxidative stress in the hippocampus and provided a reasonable explanation for the memory deficits accompanying sleep deprivation. In support of this conclusion, several animal studies showed that sleep deprivation increased the oxidative stress markers in the hypothalamus and the thalamus (D'Almeida et al. 1998). It was also demonstrated that sleep deprivation increases the rats' hippocampal oxidative stress by decreasing the glutathione level, increasing the GSSG/GSH ratio (Silva et al. 2004), reducing superoxide dismutase activity in the hippocampus and brainstem (Ramanathan et al. 2002), and reducing catalase activity (Harikesh Kalonia 2006).

Our study showed that Western diet consumption for 6 weeks had also reduced hippocampal antioxidative capacity by reducing the activities of SOD, and catalase, and increasing the levels of the oxidized form of glutathione. Moreover, Western diet administration significantly increased hippocampal TBARS levels compared to control. These results are in accordance with the finding that diet rich in fat impairs hippocampal neurogenesis through elevation of lipid peroxidation and increase of non-protein thiol group levels (Park et al. 2010). Also, the consumption of a Western-style meal by a morbidly obese individual resulted in a significant reduction in the activity of plasma superoxide dismutase (Garcia-Fuentes et al. 2010).

The combination of sleep deprivation and Western diet exacerbated 24-h long-term memory impairment induced by either treatment alone. This could be related to the remarkable reduction in BDNF due to the combined treatment which was not observed by either treatment alone. Another

explanation is the collective effect of combining sleep deprivation and Western diet on oxidative stress markers where sleep deprivation decreases SOD, catalase, and GPx activities and GSH/GSSG ratio, while Western diet adds to these effects via an elevation in TBARS levels.

In conclusion, either sleep deprivation or Western diet induces short- and long-term memory impairments. When combining both factors, the impairment in long-term memory is exacerbated compared to each factor alone. This could be explained possibly by reduced hippocampal BDNF levels and/or changes in oxidative stress markers such as SOD, catalase, and GPx activities, GSH/GSSG ratio, and TBARS levels. .

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