



High Fat Diet- Induced Neurotoxicity Alters Following vitamin E and C Administration in Hippocampus of Male Rats

Iraj Salehi,¹ Massoud Saidijam,¹ Ali Asghar Vahidinia,² Maryam Sohrabi,³ and Sara Soleimani Asl^{4,*}

¹Ph.D, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

²Ph.D, Department of Biochemistry and Nutrition, School of Medical, Hamadan University of Medical Sciences, Hamadan, IR Iran

³Ph.D, Anatomy Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

⁴Ph.D, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran

*Corresponding author: Sara Soleimani Asl, Ph.D, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel/Fax: +98-8118380208, E-mail: s.soleimaniasl@umsha.ac.ir

Received 2017 July 21; Revised 2017 August 31; Accepted 2017 September 28.

Abstract

Background: Consumption of a high fat diet (HFD) leads to spatial memory impairment and hippocampal cell death.

Objectives: The present study evaluated the capacity of antioxidant supplementation to interact with the effects of high fat diet at the molecular level.

Methods: Animal groups were exposed to HFD for 9 months with free access to high fat diet and high fat diet with antioxidant supplementation (vitamin E, vitamin C, and Astaxanthin). At the end of the study, brains were removed and the level of brain-derived neurotrophic factor (BDNF), Tropomyosin receptor kinase B (Trk β), synapsin I, and the transcription factor Cyclic AMP Response Element Binding protein (CREB) and caspase-3 expression in the hippocampi were measured.

Results: Antioxidants protected against HFD-induced cell death and the up-regulation of BDNF, Trk β , Synapsin I, CREB, and Caspase-3.

Conclusions: This data suggested the possible benefit of vitamin E and C in the treatment of neurotoxicity among, those with a HFD.

Keywords: Diet, High-Fat, Apoptosis, Neurotrophic Factor, Antioxidant

1. Background

It is clear that life style and nutritional status play critical roles in general health, neuronal function, memory, and learning throughout the life span of individuals (1). It has been shown that the worldwide prevalence of obesity has been increasing and it continues to rise in developed and developing countries that can increase the risk of cardiovascular disease and neurological disorders (2, 3). The increased consumption of saturated fats in high fat diets (HFD) contributes to obesity. High fat diets influence neuronal growth of the brain, disrupts cognitive function and neuronal plasticity, and finally impairs memory and learning in adult rats (4, 5).

A number of studies have reported that high fat diets impair hippocampal long-term potentiation (LTP) in the granular cells of the dentate gyrus and alter neurogenesis in the hippocampus (6, 7). It has been indicated that high dietary fat intake can disrupt hippocampal neurogenesis, probably through an increase in serum corticosterone levels. Park et al. reported that seven weeks of HFD significantly decreased the number of newly generated cells

in the dentate gyrus and the level of brain-derived neurotrophic factor (BDNF) in the hippocampus (8).

There is evidence that BDNF density decreases following HFD consumption, which leads to a decrease in neuronal plasticity and cell death (8, 9). Furthermore, BDNF is a member of the neurotrophin family of growth factors that promotes neuronal survival and synaptic plasticity through its interaction with Tropomyosin receptor kinase B (Trk β) (10). Also, BDNF affects neuronal plasticity by molecules, such as synapsin I and Cyclic AMP- Response Element Binding protein (CREB). Synapsin I takes a role in synaptogenesis and axonogenesis, influences synaptic vesicle exocytosis, and subsequently mediates BDNF modulation of neurotransmitter release (11, 12). Cyclic AMP-response element binding protein, as the best known transcription factor in the brain, is phosphorylated under control of BDNF and participates in learning and memory (13).

Several lines of evidence suggest that oxidative stress plays a critical role in the HFD-induced neurotoxicity (14-16). Oxidative stress is an imbalance between reactive oxygen species (ROS) and internal antioxidants. Oxidative

stress leads to the formation of hydroxyl radicals, lipid peroxidation, and apoptotic cell death.

It is clear that dietary enrichment with nutritional antioxidants could improve brain damage and cognitive function (17, 18). It can scavenge free radicals and protect unsaturated fatty acids from lipid peroxidation. Vitamin C and E, as an antioxidant has been reported to prevent the production of free radicals and lipid peroxidation (19, 20). Additional studies show that vitamin C and E could improve spatial memory performance and increase BDNF expression in the hippocampus (21, 22).

The hypothesis of this study was that if vitamin C and E could scavenge free radicals, an important factor in producing brain damage induced by HFD, they might be able to improve neurotoxicity related to HFD group via reduction of oxidative stress.

2. Methods

2.1. Animals and Diets

A total of 15 adult Wistar male rats (250 to 300 g; 6 to 8 weeks) were used in this experimental study. The animals were kept in animal houses, according to standard laboratory conditions at a temperature of $22 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 5\%$, and on a 12-hour light/12-hour dark cycle with free access to food and water. Ethical approval for all experiments was provided by the ethical committee of Hamadan University of Medical Sciences.

The rats were randomly assigned to the following groups:

- 1- Intact control group received a standard laboratory rodent chow diet for nine months that had a caloric density of approximately 3.0 kcal/g.
- 2- The HFD group received HFD (D12492) for 9 months that was designed according Furnes et al. (23) and consisted of 60.9% fat, 8.3% protein, and 2.3 % carbohydrate, with a caloric density of approximately 5.24 kcal/g.
- 3- The antioxidant group received HFD plus antioxidant (0.2 g/kg vitamin E, 0.2 g/kg vitamin C, and 0.6 g/kg Astaxanthin) for 9 months.

2.2. Tissue Preparation for Molecular Studies

The animals were decapitated and their brain was removed. The hippocampi were dissected out on ice, frozen in liquid nitrogen, and maintained at -80°C until use. The left hippocampi were used for real time polymerase chain reaction (RT-PCR) and the right one for western blot.

2.3. Reverse Transcription Polymerase Chain Reaction

Total mRNA of the hippocampus was extracted using the phenol-chloroform method. The hippocampus was homogenized in RNATM (1000 μL) and centrifuged (Eppendorf, Hamburg, Germany) in chloroform at 12,000 g for 20 minutes at 4°C . Next, the RNA of the supernatant was precipitated with isopropanol and washed with 75% ethanol. A cDNA synthesis kit (Qiagen, Hilden, Germany) for reverse transcribing of the total RNA (1 μg) to complementary DNA (cDNA) was used following the protocol outlined by the manufacturer (Bioneer, Republic of Korea).

The PCR analyses were performed using QuantiFast SYBR Green PCR Kit (Bioneer, Korea) and primers (Table 1) for BDNF, Synapsin, Trk β , and CREB, and GAPDH.

Table 1. Primer sequence for Quantitative Real Time-Polymerase Chain Reaction

Genes	Primers
BDNF	Forward GATTAGGTGGCTTCATAGGAGAC
	Reverse AGAACAGAACAGAACAGAACAGG
Trk β	Forward TATGCCGTGGTGGTGATTG
	Reverse TGGAGATGTGGTGGAGAGG
Synapsin I	Forward CTCAGCAGCACAAACATACC
	Reverse TTCTGGACACGCACATCG
CREB	Forward CCAGAAGATGAAGCGAGTC
	Reverse TTGATGTTGAGGCAGAAGG
GAPDH	Forward TTCACCGCCACAGTCAAGG
	Reverse CTCAGCACCAGCATCACC

The PCR involved initial denaturation at 95°C for 3 minutes, followed by 40 cycles at 95°C for 5 seconds, 57°C , 53.2°C , 55.1°C , and 51.6°C (BDNF, Synapsin, Trk β , and CREB, respectively) for 30 seconds and 72°C for 30 seconds with a final elongation time at 72°C for 7 minutes. The reaction was terminated by an elongation period at 72°C for 7 minutes. The same annealing temperature was used for GAPDH.

Cycle threshold (Ct) values were obtained through the auto Ct function. Following efficiency correction, the mean CT value was calculated and then normalized to the reference using delta (Δ) CT.

2.4. Western Blot

Caspase 3 was quantified using a western blot method (24). The hippocampi extract were prepared in a lysis buffer that contained Ripa buffer with protease inhibitor cocktail (2:10) and centrifuged at 12,000 g for 20 minutes at a temperature of 4°C . The supernatants were collected

and protein concentration was measured using the Bradford method. The denatured protein was separated on a SDS page (sodium dodecyl sulfate polyacrylamide gel) and transferred to a Hybond-ptm nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Next, the membrane was blocked with 5% powdered milk and incubated with polyclonal anti body to caspase 3 (1:1000, Santa Cruz Biotechnology) and anti-actin (1:1000, Santa Cruz Biotechnology) for 2 hours followed by alkaline phosphatase conjugated secondary antibody (1: 10,000, Santa Cruz Biotechnology) for 1 hour. Bands were detected by chromogenic substrate 5- bromo-4-chloro-3-indolyl phosphate in the presence of nitroblue tetrazolium and analyzed using densitometric measurements by an image analysis system (UVIDoc, Houston, TX, USA).

2.5. Statistical Analysis

Statistical analyses of the results were carried out using the SPSS version 16 software. Data was presented as mean \pm SEM and analyzed using the one-way analysis of variance (ANOVA) and Tukey post-hoc comparison. Values of $P < 0.05$ were considered significant.

3. Results

Effects of HFD and antioxidants on expression of BDNF, Synapsin I, CREB, and Trk- B in the hippocampus in the real time-polymerase chain reaction

Following electrophoresis on 1.5% agarose gel at 100 volts, one single band was detected for quantification of all genes (Figure 1).

Figures 2 to 5 show the delta CT (Δ CT) values of BDNF, Trk β , Synapsin I, and CREB, respectively. Analysis of variance of data showed that HFD causes upregulation of BDNF, which was insignificant when compared to the control group (Figure 2). There was no significant difference between antioxidant and HFD groups.

Analysis of Δ CT values of Trk β showed that there was a significant difference between control and other groups ($P < 0.01$ and $P < 0.001$ for HFD and antioxidant, respectively, Figure 3) and the control group expressed less Trk β than others. Furthermore, administration of antioxidant insignificantly increased Trk β when compared to the HFD group.

As shown in Figure 4, there was a significant increase in synapsin I expression in the HFD, and antioxidant-treated groups compared with the control group ($P < 0.05$ and $P < 0.001$ for HFD and antioxidant, respectively). Administration of antioxidant increased synapsin I expression compared to HFD groups, significantly ($P < 0.05$).

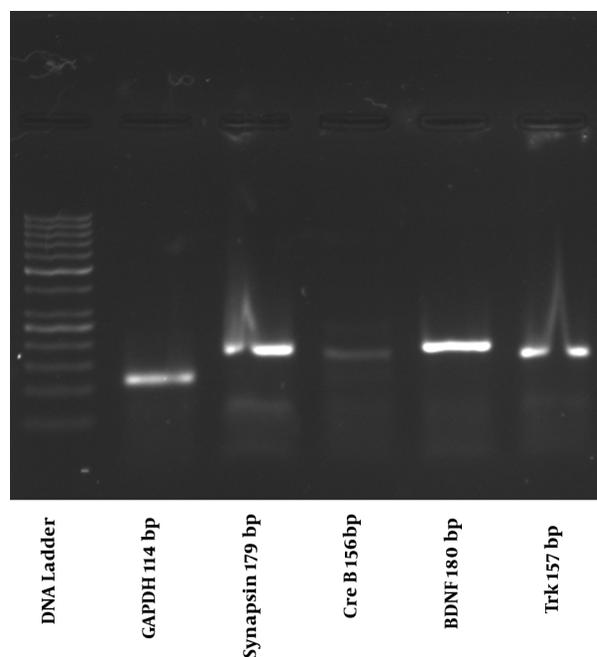
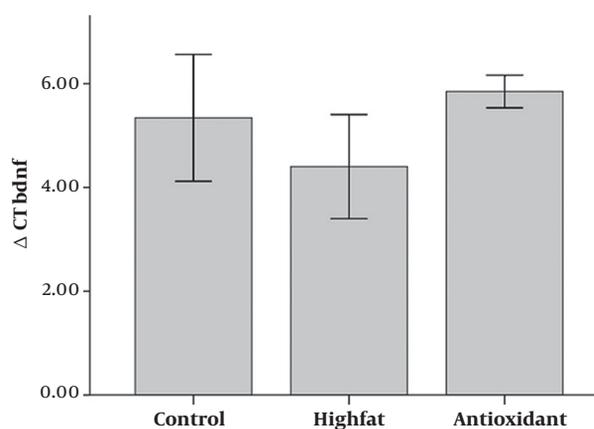


Figure 1. Electrophoresis Gel of the Synapsin (179 bp), Trk (157 bp), CreB (156 bp), GAPDH (114 bp), and BDNF (180 bp) genes

Figure 2. Mean Value (\pm S.E.M) of BDNF mRNA Concentration in Hippocampi

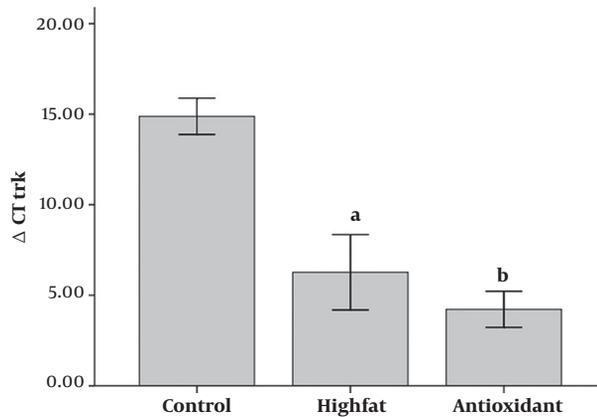


Data were analyzed with one-way ANOVA followed by Tukey's test for multiple comparisons.

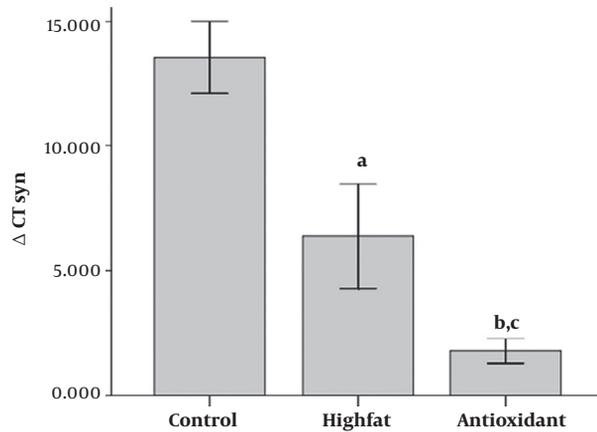
Furthermore, expression of CREB increased in HFD and antioxidant groups ($P < 0.001$ for both groups, Figure 5) in comparison to the control group.

Administration of antioxidant significantly increased CREB expression compared to the HFD group.

Effects of HFD and antioxidants on expression of caspase 3 in the western blot

Figure 3. Mean Value (\pm S.E.M) of Trk β mRNA Concentration in Hippocampi

Data were analyzed with one-way ANOVA followed by Tukey's test for multiple comparisons. (a; $P < 0.01$ vs. control group and b; $P < 0.001$ vs. HFD group).

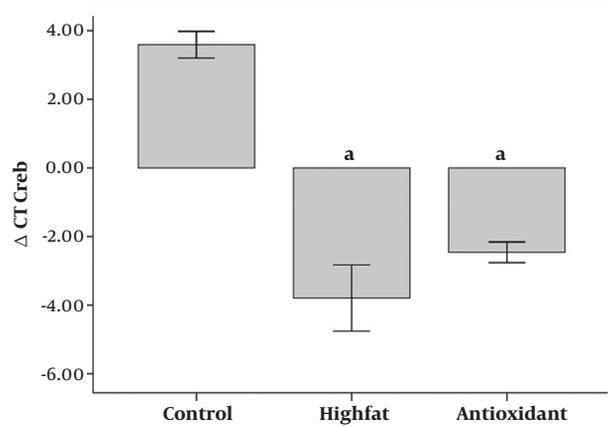
Figure 4. Mean value (\pm S.E.M) of Synapsin I mRNA Concentration in Hippocampi

Data were analyzed with one-way ANOVA followed by Tukey's test for multiple comparisons. (A; $P < 0.01$, B; $P < 0.001$ vs. control group and C; $P < 0.05$ vs. HFD group).

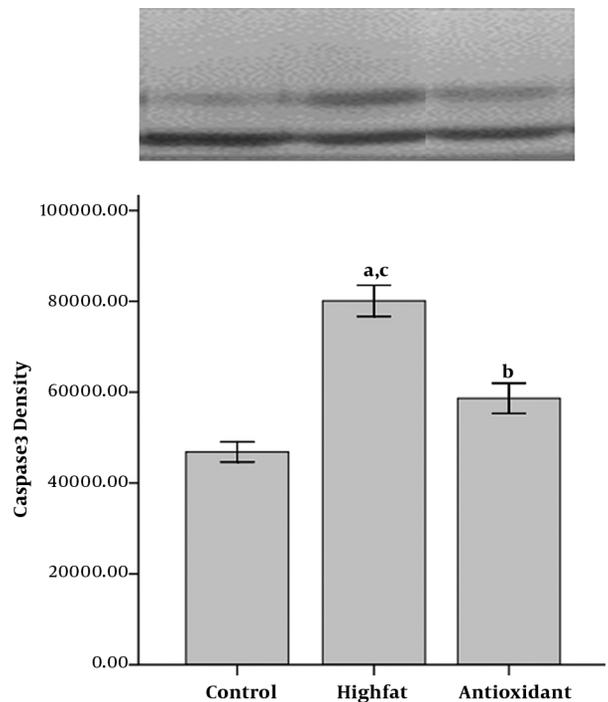
Densitometry from the membranes showed significantly less expression of caspase 3 in the control group compared to the other groups ($P < 0.001$ for HFD and $P < 0.05$ for antioxidant group, Figure 6). There was a significant difference in caspase 3 expression between the antioxidant and the HFD groups ($P < 0.001$).

4. Discussion

Several studies using rodent models have addressed the relationship between HFD and neurotrophic factors in the brain. Yu et al. Showed that BDNF and Trk β decreased following HFD in the hippocampus and ventromedial hy-

Figure 5. Mean Value (\pm S.E.M) of CEREB mRNA Concentration in Hippocampi

Data were analyzed with one-way ANOVA followed by Tukey's test for multiple comparisons. (A; $P < 0.001$ vs. control group).

Figure 6. Mean Value (\pm S.E.M) of Caspase 3 Protein Concentration in Hippocampi

Data were analyzed with one-way ANOVA followed by Tukey's test for multiple comparisons. (A; $P < 0.001$, B; $P < 0.05$ vs. control group and C; $P < 0.001$ vs. Antioxidant group).

pothalamic nucleus of male rats (25). Furthermore, consuming a high fat refined sugar diet for 2 month decreased hippocampal BDNF and performance in the Morris water

maze. They reported that the downstream effectors for the action of BDNF on synaptic plasticity, including synapsin I, CREB, and growth-associated protein 43 mRNA were reduced proportionally to BDNF levels (7).

Inconsistent to the mentioned studies, the present study showed that administration of HFD for 9 months led to an increase in BDNF, Trk β (BDNF receptor), synapsin I (important for neurotransmitter release), and CREB (required for various forms of memory and is under regulatory control of BDNF). Furthermore, the results showed that the antioxidant could exaggerate HFD-induced increase in BDNF, Trk β , CREB, and synapsin I. It has been reported that vitamin C and E could exert protective effects by enhancing BDNF expression in stressed rats (26). A study by Coskun *et al.* showed that vitamin C supplementation failed to protect the brain tissue against exercise-induced oxidative damage and behavior as a pro-oxidant (27). In another study, vitamin E ameliorated the ethanol-induced changes on secretion of BDNF and neurotrophin-3 (28).

In this study, HFD was used for 9 months, and longer than other studies. Such changes may be long-lasting changes, in which neurotrophic factors increase compensatory. Consistent with the current study, BDNF expression in the ventral tegmental area was increased by cocaine and they suggested that this may be essential for modulating cocaine-rewarding effects (29).

In another study, rats, fed a diet rich in sugar, exhibited increased hippocampal inflammation (TNF- α and IL-1 β mRNA) and oxidative stress, as indicated by an upregulation of NRF1 mRNA compared to control rats. In contrast, these markers were not significantly elevated in rats that received the cafeteria diet without added sucrose. Hippocampal BDNF mRNA was similar across all groups (30), which confirms the current results.

The other finding of this study was attenuation of apoptosis following antioxidant administration in the HFD group. It has been reported that consumption of a fat-rich diet blunts leptin and insulin orexigenic signaling by a mechanism dependent on in situ activation of inflammation that could induce apoptosis of neurons and a reduction of synaptic inputs in the arcuate nucleus and lateral hypothalamus (31, 32). Wang *et al.* showed that apoptotic hepatocytes were significantly greater in livers of rats fed HFD, and these were associated with a higher level of cleaved caspase-3 (33). Caspase-3 is one of the key executioners of apoptosis that is synthesized as inactive pro-forms, which upon receiving an apoptotic signal, is cleaved and forms the active enzyme (34). The ability of vitamin C or vitamin E to reduce cell damage elicited by various apoptotic stimuli has also been well studied (35, 36). Vitamin C is an effective scavenger of hydroxyl radicals, and vitamin E is a lipid radical chain breaker that scavenges oxygen rad-

icals and alkyl radicals. Taken together, it is possible that vitamin E and C inhibit ROS generation and might be implicated in the protection of HFD-induced neurotoxicity.

In conclusion, the current findings demonstrate that administration of vitamin E and C significantly attenuated HFD-induced neurotoxicity in the rat hippocampus. Therefore, it is likely that they may be useful as a potential treatment for the adverse effects associated with HFD.

Acknowledgments

This research was supported by a grant from Hamadan University of Medical Sciences No.p/16/35/3396.

Footnote

Conflict of Interest: None of the authors of this paper had any financial interest to report.

References

1. Parletta N, Milte CM, Meyer BJ. Nutritional modulation of cognitive function and mental health. *J Nutr Biochem.* 2013;**24**(5):725–43. doi: [10.1016/j.jnutbio.2013.01.002](https://doi.org/10.1016/j.jnutbio.2013.01.002). [PubMed: [23517914](https://pubmed.ncbi.nlm.nih.gov/23517914/)].
2. Bastien M, Poirier P, Lemieux I, Despres JP. Overview of epidemiology and contribution of obesity to cardiovascular disease. *Prog Cardiovasc Dis.* 2014;**56**(4):369–81. doi: [10.1016/j.pcad.2013.10.016](https://doi.org/10.1016/j.pcad.2013.10.016). [PubMed: [24438728](https://pubmed.ncbi.nlm.nih.gov/24438728/)].
3. Stachowiak EK, Srinivasan M, Stachowiak MK, Patel MS. Maternal obesity induced by a high fat diet causes altered cellular development in fetal brains suggestive of a predisposition of offspring to neurological disorders in later life. *Metab Brain Dis.* 2013;**28**(4):721–5. doi: [10.1007/s11011-013-9437-8](https://doi.org/10.1007/s11011-013-9437-8). [PubMed: [24043569](https://pubmed.ncbi.nlm.nih.gov/24043569/)].
4. Gomez-Pinilla F. Brain foods: the effects of nutrients on brain function. *Nat Rev Neurosci.* 2008;**9**(7):568–78. doi: [10.1038/nrn2421](https://doi.org/10.1038/nrn2421). [PubMed: [18568016](https://pubmed.ncbi.nlm.nih.gov/18568016/)].
5. Asadbegi M, Yaghmaei P, Salehi I, Komaki A, Ebrahim Habibi A. Investigation of thymol effect on learning and memory impairment induced by intrahippocampal injection of amyloid beta peptide in high fat diet fed rats. *Metab Brain Dis.* 2017;**32**(3):827–39. doi: [10.1007/s11011-017-9960-0](https://doi.org/10.1007/s11011-017-9960-0). [PubMed: [28255862](https://pubmed.ncbi.nlm.nih.gov/28255862/)].
6. Lindqvist A, Mohapel P, Bouter B, Frielingsdorf H, Pizzo D, Brundin P, *et al.* High-fat diet impairs hippocampal neurogenesis in male rats. *Eur J Neurol.* 2006;**13**(12):1385–8. doi: [10.1111/j.1468-1331.2006.01500.x](https://doi.org/10.1111/j.1468-1331.2006.01500.x). [PubMed: [17116226](https://pubmed.ncbi.nlm.nih.gov/17116226/)].
7. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience.* 2002;**112**(4):803–14. [PubMed: [12088740](https://pubmed.ncbi.nlm.nih.gov/12088740/)].
8. Park HR, Park M, Choi J, Park KY, Chung HY, Lee J. A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. *Neurosci Lett.* 2010;**482**(3):235–9. doi: [10.1016/j.neulet.2010.07.046](https://doi.org/10.1016/j.neulet.2010.07.046). [PubMed: [20670674](https://pubmed.ncbi.nlm.nih.gov/20670674/)].
9. Wu A, Molteni R, Ying Z, Gomez-Pinilla F. A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor. *Neuroscience.* 2003;**119**(2):365–75. [PubMed: [12770552](https://pubmed.ncbi.nlm.nih.gov/12770552/)].

10. Broad KD, Mimmack ML, Keverne EB, Kendrick KM. Increased BDNF and trk-B mRNA expression in cortical and limbic regions following formation of a social recognition memory. *Eur J Neurosci*. 2002;**16**(11):2166–74. [PubMed: [12473084](#)].
11. Jovanovic JN, Benfenati F, Siow YL, Sihra TS, Sanghera JS, Pelech SL, et al. Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. *Proc Natl Acad Sci U S A*. 1996;**93**(8):3679–83. [PubMed: [8622996](#)].
12. Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS. Synapsins as mediators of BDNF-enhanced neurotransmitter release. *Nat Neurosci*. 2000;**3**(4):323–9. doi: [10.1038/73888](#). [PubMed: [10725920](#)].
13. Silva AJ, Kogan JH, Frankland PW, Kida S. CREB and memory. *Annu Rev Neurosci*. 1998;**21**:127–48. doi: [10.1146/annurev.neuro.21.1.127](#). [PubMed: [9530494](#)].
14. White CL, Pistell PJ, Purpera MN, Gupta S, Fernandez-Kim SO, Hise TL, et al. Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: contributions of maternal diet. *Neurobiol Dis*. 2009;**35**(1):3–13. doi: [10.1016/j.nbd.2009.04.002](#). [PubMed: [19374947](#)].
15. Zhang X, Dong F, Ren J, Driscoll MJ, Culver B. High dietary fat induces NADPH oxidase-associated oxidative stress and inflammation in rat cerebral cortex. *Exp Neurol*. 2005;**191**(2):318–25. doi: [10.1016/j.expneurol.2004.10.011](#). [PubMed: [15649487](#)].
16. Ganji A, Salehi I, Sarihi A, Shahidi S, Komaki A. Effects of hypericum scabrum extract on anxiety and oxidative stress biomarkers in rats fed a long term high fat diet. *Metab Brain Dis*. 2017;**32**(2):503–11. doi: [10.1007/s11011-016-9940-9](#). [PubMed: [27981406](#)].
17. Azad N, Rasoolijazi H, Joghataie MT, Soleimani S. Neuroprotective effects of carnosic Acid in an experimental model of Alzheimer's disease in rats. *Cell J*. 2011;**13**(1):39–44. [PubMed: [23671826](#)].
18. Mehdizadeh M, Dabaghian F, Nejehadi A, Fallah-Huseini H, Choopani S, Shekarriz N, et al. Zingiber Officinale Alters 3,4-methylenedioxyamphetamine-Induced Neurotoxicity in Rat Brain. *Cell J*. 2012;**14**(3):177–84. [PubMed: [23508562](#)].
19. Chapple IL, Matthews JB, Wright HJ, Scott AE, Griffiths HR, Grant MM. Ascorbate and alpha-tocopherol differentially modulate reactive oxygen species generation by neutrophils in response to Fcγ₃ and TLR agonists. *Innate Immun*. 2013;**19**(2):152–9. doi: [10.1177/1753425912455207](#). [PubMed: [22914919](#)].
20. Kobori Y, Ota S, Sato R, Yagi H, Soh S, Arai G, et al. Antioxidant co-supplementation therapy with vitamin C, vitamin E, and coenzyme Q10 in patients with oligoasthenozoospermia. *Arch Ital Urol Androl*. 2014;**86**(1):1–4. doi: [10.4081/aiua.2014.1.1](#). [PubMed: [24704922](#)].
21. R Rai A. A comparison of resveratrol and vitamin C therapy on expression of BDNF in stressed rat brain homogenate. *IOSR J Pharm*. 2013;**3**(10):22–7. doi: [10.9790/3013-0310022-7](#).
22. Rendeiro C, Vauzour D, Rattray M, Waffo Tegu P, Merillon JM, Butler LT, et al. Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain derived neurotrophic factor. *PLoS One*. 2013;**8**(5):63535. doi: [10.1371/journal.pone.0063535](#). [PubMed: [23723987](#)].
23. Furnes MW, Zhao CM, Chen D. Development of obesity is associated with increased calories per meal rather than per day. A study of high-fat diet-induced obesity in young rats. *Obes Surg*. 2009;**19**(10):1430–8. doi: [10.1007/s11695-009-9863-1](#). [PubMed: [19506986](#)].
24. Gharebaghi A, Amiri I, Salehi I, Shahidi S, Komaki A, Mehdizadeh M, et al. Treadmill exercise attenuates 3,4 methylenedioxyamphetamine induced memory impairment through a decrease apoptosis in male rat hippocampus. *J Neurosci Res*. 2017;**95**(12):2448–55. doi: [10.1002/jnr.24078](#). [PubMed: [28493333](#)].
25. Yu Y, Wang Q, Huang XF. Energy-restricted pair-feeding normalizes low levels of brain-derived neurotrophic factor/tyrosine kinase B mRNA expression in the hippocampus, but not ventromedial hypothalamic nucleus, in diet-induced obese mice. *Neuroscience*. 2009;**160**(2):295–306. doi: [10.1016/j.neuroscience.2009.01.078](#). [PubMed: [19217934](#)].
26. Tagliari B, Scherer EB, Machado FR, Ferreira AG, Dalmaz C, Wyse AT. Antioxidants prevent memory deficits provoked by chronic variable stress in rats. *Neurochem Res*. 2011;**36**(12):2373–80. doi: [10.1007/s11064-011-0563-6](#). [PubMed: [21822921](#)].
27. Coskun S, Gonul B, Guzel NA, Balabanli B. The effects of vitamin C supplementation on oxidative stress and antioxidant content in the brains of chronically exercised rats. *Mol Cell Biochem*. 2005;**280**(1–2):135–8. doi: [10.1007/s11010-005-8421-y](#). [PubMed: [16311914](#)].
28. Heaton MB, Madorsky I, Paiva M, Siler-Marsiglio KI. Ethanol-induced reduction of neurotrophin secretion in neonatal rat cerebellar granule cells is mitigated by vitamin E. *Neurosci Lett*. 2004;**370**(1):51–4. doi: [10.1016/j.neulet.2004.07.064](#). [PubMed: [15489016](#)].
29. Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci*. 2007;**10**(8):1029–37. doi: [10.1038/nn1929](#). [PubMed: [17618281](#)].
30. Beilharz JE, Maniam J, Morris MJ. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. *Brain Behav Immun*. 2014;**37**:134–41. doi: [10.1016/j.bbi.2013.11.016](#). [PubMed: [24309633](#)].
31. Moraes JC, Coope A, Morari J, Cintra DE, Roman EA, Pauli JR, et al. High fat diet induces apoptosis of hypothalamic neurons. *PLoS One*. 2009;**4**(4):5045. doi: [10.1371/journal.pone.0005045](#). [PubMed: [19340313](#)].
32. Dalvi PS, Chalmers JA, Luo V, Han DY, Wellhauser L, Liu Y, et al. High fat induces acute and chronic inflammation in the hypothalamus, effect of high fat diet, palmitate and TNF alpha on appetite regulating NPY neurons. *Int J Obes (Lond)*. 2017;**41**(1):149–58. doi: [10.1038/ijo.2016.183](#). [PubMed: [27773938](#)].
33. Wang Y, Ausman LM, Russell RM, Greenberg AS, Wang XD. Increased apoptosis in high-fat diet-induced nonalcoholic steatohepatitis in rats is associated with c-Jun NH₂-terminal kinase activation and elevated proapoptotic Bax. *J Nutr*. 2008;**138**(10):1866–71. [PubMed: [18806094](#)].
34. Mirzayans R, Andrais B, Kumar P, Murray D. The growing complexity of cancer cell response to DNA damaging agents: caspase 3 mediates cell death or survival? *Int J Mol Sci*. 2016;**17**(5). doi: [10.3390/ijms17050708](#). [PubMed: [27187358](#)].
35. Ebokaiwe AP, Mathur PP, Farombi EO. Quercetin and vitamin E attenuate Bonny Light crude oil-induced alterations in testicular apoptosis, stress proteins and steroidogenic acute regulatory protein in Wistar rats. *Drug Chem Toxicol*. 2016;**39**(4):424–31. doi: [10.3109/01480545.2015.1137303](#). [PubMed: [26821606](#)].
36. Hao J, Li WW, Du H, Zhao ZF, Liu F, Lu JC, et al. Role of Vitamin C in Cardioprotection of Ischemia/Reperfusion Injury by Activation of Mitochondrial KATP Channel. *Chem Pharm Bull (Tokyo)*. 2016;**64**(6):548–57. doi: [10.1248/cpb.c15-00693](#). [PubMed: [27250789](#)].