RESEARCH PAPER

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Sensory-motor performance and neurochemical effects in the cerebral cortex of brain-derived neurotrophic factor heterozygous mice fed a high-cholesterol diet

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Reports suggest that a high-cholesterol diet may induce neuroinflammation, oxidative stress, and neurodegeneration in brain tissue. Brain-derived neurotrophic factor (BDNF) might play a role in protecting against changes induced by high cholesterol. We aimed to assess behavioral correlates and biochemical alterations in the motor and sensory cortices following a high-cholesterol diet under normal and reduced BDNF concentrations. C57Bl/6 strain, wild-type (WT) and BDNF heterozygous (+/-) mice were used to reveal the effects of endogenous BDNF concentrations. We compared diet and genotype effects using four experimental groups: WT and BDNF heterozygous (+/-) groups of mice were each fed a normal or high-cholesterol diet for 16 weeks. The cylinder test and wire hanging test were performed to evaluate neuromuscular deficits and cortical sensory-motor functions, respectively. In addition, neuroinflammation was assessed by tumor necrosis factor alpha and interleukin 6 levels measured in the somatosensory and motor areas. Additionally, MDA levels and SOD and CAT activity were evaluated as oxidative stress parameters. Results showed that a high-cholesterol diet significantly impaired behavioral performance in the BDNF (+/-) group. Diet did not change the levels of neuroinflammatory markers in any of the groups. However, MDA levels, an indicator of lipid peroxidation, were significantly higher in the high-cholesterol-fed BDNF (+/-) mice. The results suggest that BDNF levels might be a critical factor in determining the extent of neuronal damage induced in the neocortex by a high-cholesterol diet.

Key words: high cholesterol diet, BDNF, cortex, neuroinflammation, oxidative stress, mouse

INTRODUCTION

Cholesterol is an essential component of the human brain, the most cholesterol-rich organ in the body. Cholesterol is tightly regulated between the major brain cells (neurons, glia, astrocytes, microglia, and oligodendrocytes) and is essential for synapse and dendrite formation (Goritz et al., 2005, Fester et al., 2009) and axonal guidance (Posse de Chaves et al., 1997) during normal brain development. Synaptic vesicles are composed primarily of cholesterol and their formation, shape, and release properties are controlled by cholesterol content (Segatto et al., 2014). Defects in cholesterol metabolism lead to structural and functional central nervous system diseases (Orth and Bellosta, 2012). Cholesterol reduction leads to synaptic and dendritic spine degeneration, failure in neurotransmission and decreased synaptic plasticity (Koudinov and Koudinova, 2005).

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Cholesterol itself cannot pass through the blood-brain barrier and most of the brain cholesterol is re-synthesized from its precursors within the brain. However, dietary cholesterol still affects central nervous system metabolism. Side chains, which are metabolites of cholesterol, like 24-hydroxycholesterol (24-OHC) and 27-hydroxycholesterol (27-OHC), can cross the blood-brain barrier and play a role in neuroinflammation (Gamba et al., 2015; Testa et al., 2016). At high concentrations, cholesterol has been shown to increase neuroinflammation and exert detrimental effects on neuronal function. High 27-OHC levels in the brain, due to dietary cholesterol, caused an increase in neurodegeneration in the hippocampus (Brooks et al., 2017). Increasing plasma cholesterol levels with diet caused disruption in the blood-brain barrier in the hippocampus and increased its permeability (Chen et al., 2018; de Oliveira et al., 2020); additionally, it was shown to cause hippocampal neuroinflammation, reduced cholinergic activities and cognitive deficits (Rui et al., 2017).

Brain-derived neurotrophic factor (BDNF) is a regulatory neuropeptide that has neurotrophic roles in physiological processes such as neuronal development, differentiation, and morphological changes in the central nervous system (Gottmann et al., 2009). BDNF also facilitates protective and regenerative effects after neuronal damage (Zhai et al., 2019). In a previous study conducted in our laboratory, endogenous BDNF was found to have a protective effect against the high-fat-diet-induced loss of synaptic proteins in the cortex (Abidin et al., 2018). High-fat diets have been shown to reduce the expression of BDNF, reduce neurogenesis and increase lipid peroxidation (Park et al., 2010). In particular, inflammation and oxidative stress may play a role in modulating BDNF levels (Molteni et al., 2004). It seems that diet-induced neuronal damage is associated with reduced BDNF levels, in turn, reduced levels of BDNF – as in the case of BDNF heterozygous mice, make the brain more susceptible to diet-induced changes (Abidin et al., 2018).

A high-fat diet is also closely associated with chronic metabolic disorders like obesity, diabetes, and increased blood pressure, as well as elevated cholesterol levels. In the present study, independent of the systemic effects of a high-fat diet, we aimed to differentially identify the effects of a high-cholesterol diet in the brain. This study aimed to investigate the role of endogenous BDNF in the cholesterol-mediated changes. For this purpose, we have employed BDNF heterozygous mice, which are characterized by decreased BDNF expression and decreased BDNF concentrations in the brain. In the literature, most studies have reported the effects of a high-cholesterol diet on the hippocampus; our focus was its effect on cortical changes. Cortical functions were evaluated with two different sensory-motor tests. Additionally, inflammatory markers and oxidative stress parameters from somatosensory and motor cortex were analyzed.

METHODS

Animals

In the study, 20-25 g male C57BL/6, wild-type – WT, and BDNF heterozygous – BDNF (+/-), mice were used. Animals were housed under standard conditions of 12:12 light-dark cycle, 20–21°C room temperature and 55–65% relative humidity. The study was approved by Karadeniz Technical University Animal Care and Ethics Committee. WT female and BDNF (+/-) male mice were used for breeding and the offspring were genotyped. Genotyping was performed for BDNF by polymerase chain reaction (PCR) using samples taken from the tail tips (Abidin et al., 2011). WT and BDNF (+/-) mice were placed in IVC (ventilated mouse cage) cages and the following groups were created: Group I: 9 WT mice fed with a standard diet; CD: WT; Group II: 8 WT mice fed with a high cholesterol diet; HCD: WT; Group III: 6 BDNF (+/-) mice fed with a standard diet; CD: HT; Group IV: 7 BDNF (+/-) mice fed with a high cholesterol diet; HCD: HT.

All groups were fed with standard mouse food for 15 days for standardization. The weights of the mice were recorded and weighing continued at 15 day intervals. Group II and IV mice were administered a high-cholesterol diet (HCD) (Research Diets, D12109C, USA) for 16 weeks. The diet was high fat rodent diet with regular casein and 1.25% added cholesterol, and consisted of 20% kcal protein, 40% kcal fat, 40% kcal carbohydrate, briefly. Similarly, a standard mouse diet (CD) was given to the control groups. At the end of the 4-month diet protocol, two separate behavioral experiments were performed to test sensory-motor nerve functions. These experiments were carried out in the animals' own habitats and the same researcher interacted with the animals from the beginning of the experiment.

Genotyping

Genotyping was performed for BDNF and neomycin by PCR using samples taken from the tail tips as described previously (Abidin et al., 2011). The presence of only the BDNF band indicated that that animal only had the BDNF gene, that is, it was the WT genotype; the presence of a neomycin band (+/-) was the marker for heterozygosity.

Behavioral tests

Cylinder test: The cylinder test measures spontaneous forefoot use; this test has been used to assess sensory-motor function in rodents in a range of injury models that cause forefoot use asymmetry (Schallert et al., 2000). The test is easy to administer and was able to identify motor disturbances that other behavioral tests failed to detect (Lundblad et al., 2002). The mouse was placed in a 12 cm glass cylinder beaker and the number of times that both paws were used simultaneously within 120 s was scored. The experiment was repeated twice with an interval of 30 min (Roome and Vanderluit, 2015; Au et al., 2016).

Wire hanging test: The wire hanging test was chosen to assess neuromuscular deficits and motor function (Carter and Shieh, 2015). The mouse was left on a suspended 0.5 cm-thick wire, for a maximum of 120 s. The behaviors of maintaining balance on the wire and holding on to the wire were scored. A pillow was placed 35 cm below. The trial was repeated twice with an interval of 1 hour (Li et al., 2004; Abe et al., 2009).

Behavioral experiments were recorded with a camera. Scoring and analysis were later confirmed from images. At the end of the behavioral experiments, the animals were sacrificed by decapitation method. The brain was rapidly removed and cortical tissues were dissected for use in biochemical experiments; right and left cortical tissues were frozen separately and stored at -80°C until use.

Biochemical studies

Homogenization: For the biochemical analyses, the sensory and motor cortices were carefully dissected. Frontal areas and visual cortices were discarded. The harvested left cortical tissues were used for determining the levels of the inflammatory markers tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) *via* enzyme-linked immunosorbent assay (ELISA) tests; the right cortical tissues were used to measure oxidative stress parameters. The left cortical tissues were weighed and 1 mg tissue was mixed with a 10 µl volume of the homogenization solution (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4-monobasic, 7.7 mM Na_2HPO_4-dibasic, 1% Triton X-100, 5µg/ml aprotinin, 5µg/ml leupeptin, pH 7.4) and then homogenized by an ultrasonic cell disrupter (Sonics Vibracell, Newtown, CT, USA). The homogenate was centrifuged at 20,000 g for 20 min and its supernatant was separated for ELISA tests. The right cortical tissues were homogenized in a solution of 1.15% KCl solution containing 2 ml ice cold Triton X-100 (0.5 ml/L), 2 ml ice cold Tris-HCl (50 mmol/L, pH 7.4) for the determination of superoxide dismutase (SOD) and catalase (CAT) activities.

Determination of protein concentrations: Supernatants from the prepared cortical tissue homogenates were diluted in 150 µl amounts with their own homogenization buffer at 5×, 10×, 20×, 40× and vortexed. 2 mg/ml bovine serum albumin (BSA) was used as a standard and loaded into the standard well in a volume of 25 µl. Then, the solution obtained by mixing bicoquinonic acid (BCA) solution and $CuSO_4$ was added to each well in a volume of 200 µl. It was mixed with shaking for 30 s and incubated at 37°C for 30 min. Measurements were taken at 562 nm with a spectrophotometer. The protein in the alkaline solution reduces Cu^{+2} to Cu^{+1} by a biuret reaction. BCA chelates with reduced Cu, turning from green to purple. Protein determination was performed by measuring the reduced Cu concentration as purple chelate at 562 nm. Protein concentrations (µg/ml) were calculated according to the linear standard plots.

ELISA measurements: Quantities of tumor necrosis factor alpha and interleukin 6 were determined according to the procedures of Elabscience mouse TNF-α (Catalog No: E-EL-M0049, Lot No: 33JUTNM2VP) and Elabscience mouse IL-6 (Catalog No: E-EL-M0044, Lot No: IAPTLLKG3D) ELISA kits, respectively.

Oxidative stress parameters measurements: The end product of peroxidation, malondialdehyde (MDA), forms a complex with thiobarbituric acid. Thus, its levels in tissue protein can be determined by measuring the absorbance of this complex at 532 nm (Benzie, 1996). The activity of SOD in the cortex was measured using Sun et al.'s (1988) method, in which the reduction of nitroblue tetrazolium results in the formation of purple-colored formazan, which can be measured by taking the absorbance at 560 nm. The activity of CAT was measured according to the method developed by Goth et al. (1991), where the amount of catalase in the sample leading to a decrease in the absorbance of hydrogen peroxide (H_2O_2) was measured at a 240 nm wavelength over time *via* a UV/Vis spectrophotometer. The activity of CAT was measured in each sample by taking three measurements at 30 s intervals.

Statistical analysis

In the study, there are two variables of diet and genotype. When the data were normally distributed, statistical comparisons of the data were made by the two-way ANOVA test using GraphPad Prism software. Dunnett's *post hoc* test was used to compare the study groups with the control WT group. Data were presented as means ± standard error of mean (SEM). A p value of $p<0.05$ was considered statistically significant.

RESULTS

Body weights

There was no difference in body weight between groups before the diet protocol was applied. The body weights were measured after 16 weeks of dietary protocol and the results were recorded as 24.4±0.3 g for the CD: WT group and 27.5±0.3 g for the CD: HT group. On the other hand, the body weights of the HCD: WT and HCD: HT groups, which were fed with a high-cholesterol diet, were recorded as 25.15±0.57 g and 27.9±1.19 g, respectively. Genotype affected body weight significantly ($F_{(1,26)}$=16,32; P<0.0005). Additionally, the CD: HT group weighed more than the CD: WT group (P<0.05) and the body weight of the HCD: HT group was significantly higher than the control group, CD: WT (P<0.001) (Fig. 1).

Behavioral tests

Sensory-motor performance was evaluated with two different behavioral tests. For the cylinder test, the number of times that both paws were used simultaneously was scored. The number of these contacts decreased in heterozygous mice for both diet types. Two-way ANOVA revealed a significant effect of genotype ($F_{(1,26)}$=9.169; P<0.05) but no effect of diet on the number of contacts. Dunnett's *post hoc* test showed that the number of contacts changed significantly between the CD: WT (5.48±1.06), CD: HT (2.83±0.75) (P<0.05) and HCD: HT (1.95±0.54) (P<0.005) groups (Fig. 2A).

In the wire hanging test, wire holding time was scored. There was a significant effect of diet on holding time ($F_{(1,26)}$=4.713; P<0.05) but no effect of genotype. The holding time of the CD: HT group (67.8±12 s) was observed to be higher than that of the HCD: HT (30.3±5.43 s) group, however, the difference was statistically insignificant. When compared to the WT control group (63.47±11.7 s), wire holding time was significantly lower in the HCD: HT group (30.3±5.43 s) (P<0.05) (Fig. 2B). The wire holding time in the HCD: WT group was recorded as 54.3±10.9 s.

Oxidative stress parameters

We analyzed MDA levels, as well as SOD and CAT activity, in the neocortex of mouse brain to explore whether a high-cholesterol diet and BDNF heterozygosity affected oxidative stress. Two-way ANOVA showed a significant effect of diet ($F_{(1,26)}$=8.765, P<0.05) but not genotype on MDA levels. Dunnett's *post hoc* test revealed that MDA levels were significantly increased in the HCD: HT group (679.88±50.47 nmol/g) compared to the CD: WT group (533.42±21.7 nmol/g) (P<0.005) (Fig. 3A). The MDA levels were 581.2±59.7 nmol/g and 601.13±18.4 nmol/g in the CD: HT group and HCD: WT group, respectively.

SOD activity was also significantly affected by diet ($F_{(1,26)}$=8.765, P<0.05) but not genotype. As shown in Fig. 3B, SOD activity levels were found to be higher in the CD: WT group (491.84±74.05 U/g) than in the HCD: WT group (230.04±54.55 U/g) (P<0.05). SOD activity in the CD: HT group (516.58±82.4 U/g) was also higher than in the HCD: WT group (P<0.05). However, the SOD activity levels of the HCD: HT group (377.9±50.4 U/g) were not significantly different from the other groups.

CAT activity levels were not significantly different between groups (Fig. 3C). The values for CAT activity were 0.11±0.006 U/g, 0.114±0.007 U/g, 0.13±0.014 U/g and 0.1±0.011 U/g in CD: WT, CD: HT, HCD: WT and HCD: HT groups, respectively.

Neuroinflammation

We evaluated levels of IL-6β and TNF-α to investigate neuroinflammation. Two-way ANOVA revealed a significant effect of diet on IL-6 ($F_{(1,26)}$=9.823, P<0.005). Dunnett's test revealed no significant differences in IL-6 levels for any groups compared to control. The IL-6 levels were 74.3±4.8, 67.21±4.94, 84.39±3.41 and 83.76±3.17 pg/mg in the CD: WT, CD: HT, HCD: WT and HCD: HT groups, respectively (Fig. 4A). The nonparametric Friedman test showed no significant differences in TNF-α levels between groups (Fig. 4B). The TNF-α levels for CD: WT, CD: HT, HCD: WT and HCD: HT groups were 113.8±9.4 pg/mg, 103.6±8.62 pg/mg, 123.8±6.04 pg/mg and 123.5±4.33 pg/mg, respectively.

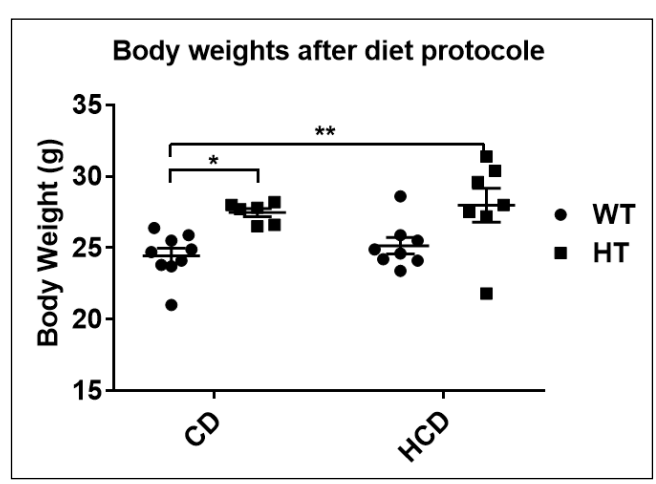

Fig. 1. The bar charts show the effects of a high-cholesterol diet and BDNF heterozygosity on body weight in the experimental groups. CD: control diet, HCD: high-cholesterol diet, WT: wild-type, HT: BDNF heterozygous. The level of significance is denoted as *p<0.05, **p<0.001.

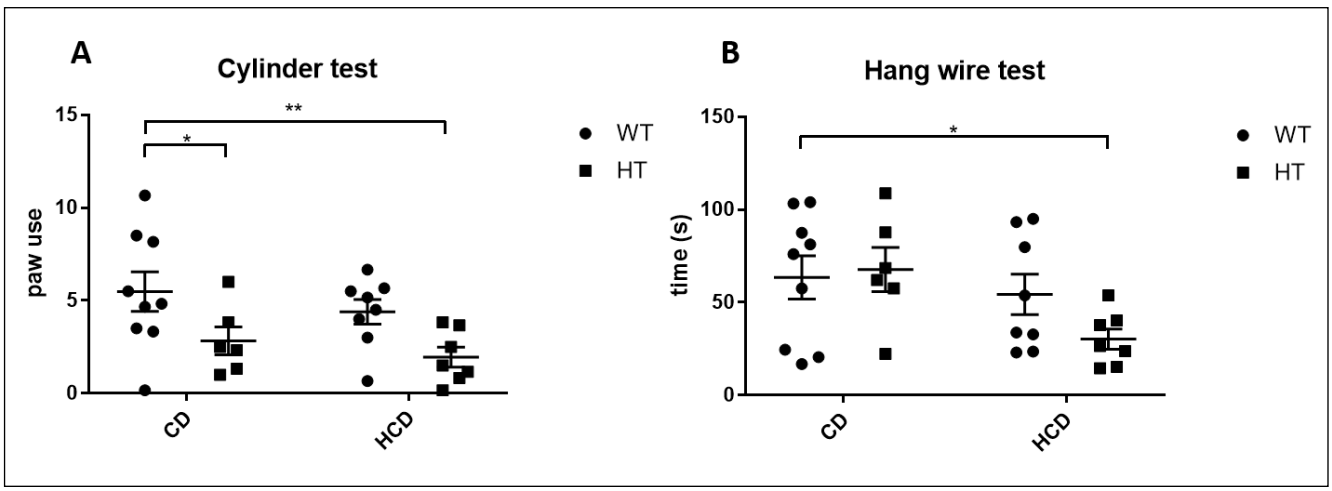

Fig. 2. The bar charts show the effects of the high-cholesterol diet and BDNF heterozygosity on behavioral performance. (A) the number of times both paws were used simultaneously in the cylinder test and (B) holding time in the wire hanging test. CD: control diet, HCD: high-cholesterol diet, WT: wild-type, HT: BDNF heterozygous. The level of significance is denoted as *p<0.05, **p<0.001.

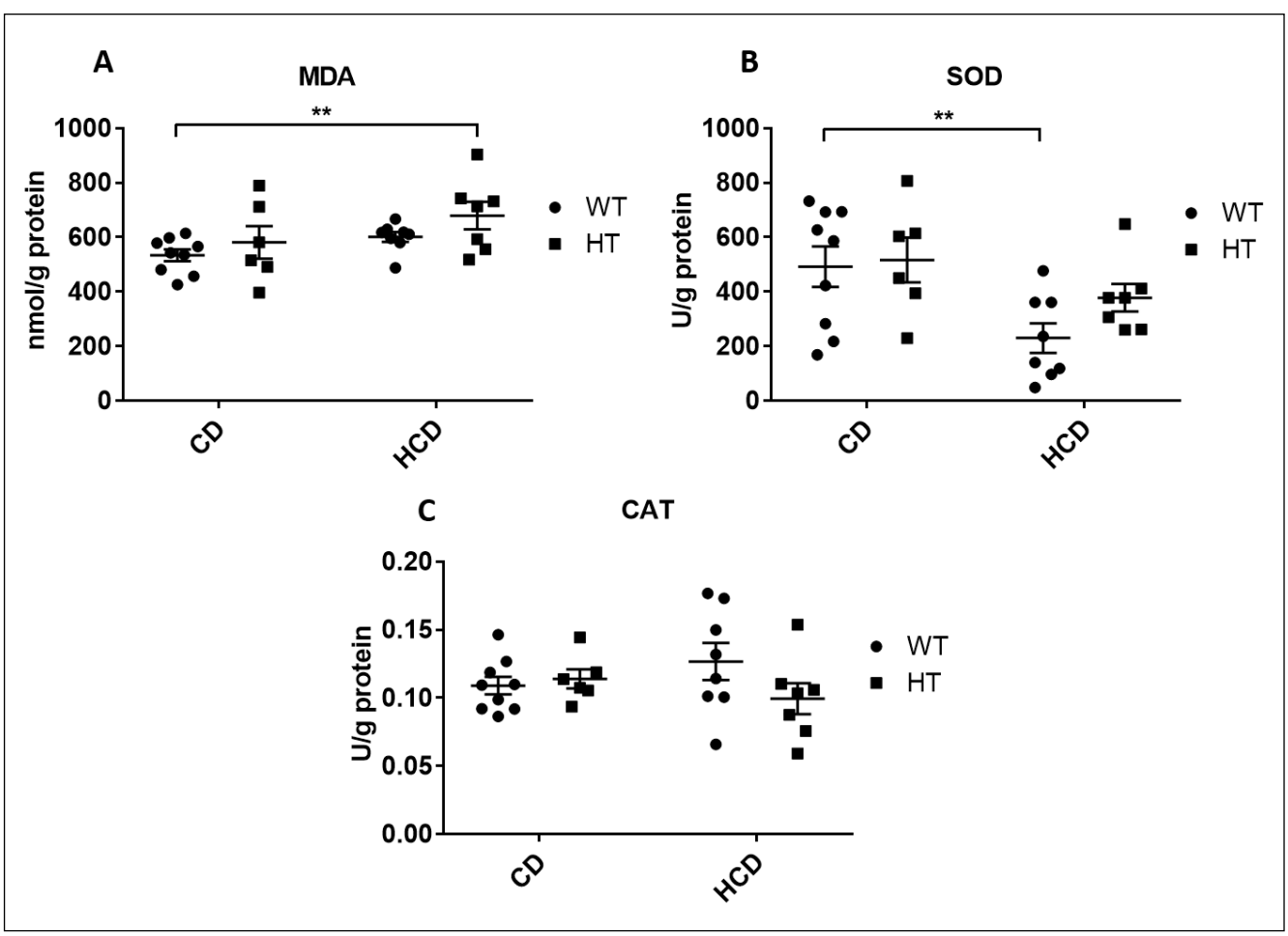

Fig. 3. The bar charts show the effects of high cholesterol and BDNF heterozygosity on oxidative stress parameters. (A) alterations in the MDA levels, (B) alterations in SOD activity, and (C) alterations in CAT activity between the groups. CD: control diet, HCD: high-cholesterol diet, WT: wild-type, HT: BDNF heterozygous. The level of significance is denoted as *p<0.05, **p<0.001.

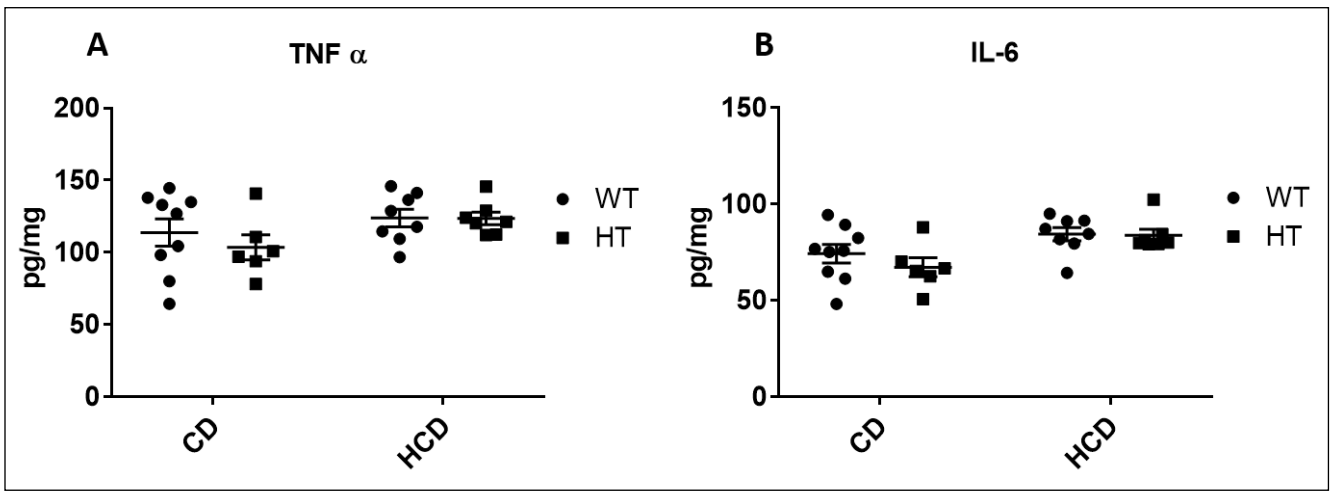

Fig. 4. The bar charts show the effects of a high-cholesterol diet and BDNF heterozygosity on neuroinflammation. (A) IL-6 levels and (B) TNFα levels in the cortices of each group. CD: control diet, HCD: high-cholesterol diet, WT: wild-type, HT: BDNF heterozygous. Group comparisons showed no significant differences.

DISCUSSION

The cerebral cortex has six layers which contain excitatory and inhibitory neurons. It is responsible for cognitive, sensory and motor functions, and locates to the exterior regions of the cerebral hemispheres. The tasks performed by the cerebral cortex depend on many molecular mechanisms and the development of synaptic connections (Kwan et al., 2012; Greig et al., 2013). Since oxidative stress and neuroinflammation cause a decrease in the mitochondrial activity, they damage cellular structures and disrupt these processes (Mecocci et al., 2018).

Although cholesterol is one of the main components of the cell membrane and necessary for neuronal and synaptic homeostasis, it has been shown that a high-cholesterol diet causes inflammation and loss of neuronal function. BDNF is an endogenous factor with neuroprotective effects against neuroinflammation and oxidative stress. This study investigated the effects of a high-cholesterol diet in the neocortex in mice with normal and reduced concentrations of BDNF. Our findings suggested a protective role for endogenous BDNF, as a high-cholesterol diet administered for four months did not induce any significant changes in WT mice, however, it significantly increased lipid peroxidation and reduced sensory-motor performance selectively in the high-cholesterol diet heterozygous group.

A heterozygous BDNF phenotype has been associated with obesity (Fox et al., 2013). In the present study, similar to our previous studies that used the same mouse model, we observed that the body weights of BDNF heterozygous groups were significantly higher than WT controls (Bodur et al., 2019). However, in the present study, high-cholesterol diet did not lead to higher body weights. This finding is in line with previous reports (Tanaka et al., 2013).

A high-cholesterol diet was reported to result in different kinds of behavioral changes. Hypercholesteremic mice exhibited motor abnormalities in psychomotor behaviors (akinesia, swimming ability, gait pattern) as well as changes in dopamine and serotonin metabolism in corticostriatal regions (Paul et al., 2017), where the observed alterations were similar to those present in Parkinson's disease. In another study, depression- and anxiety-like changes, as well as impulsivity, were reported for mice fed a high-cholesterol diet and associated with systemic and central upregulation of toll-like receptor 4 mRNA and protein (Strekalova et al., 2015). A high-cholesterol diet has also been shown to reduce learning and other types of cognitive performance. For instance, a high-fat, cholesterol-enriched diet impaired spatial memory and object recognition performance, which were rescued by an enriched environment (de Souza et al., 2018). Additionally, a high-cholesterol diet was shown to decrease short-term spatial memory without effecting long-term recognition memory (Mayagoitia et al., 2020). Object location, forced swimming and elevated plus-maze tests were used to determine that hypercholesterolemia increased acetylcholinesterase levels in the prefrontal cortex and hippocampus and led to short-term memory impairment (Moreira et al., 2014). In the present study, we investigated how a high-cholesterol diet changes sensory-motor performance associated with neocortical regions. We also tested whether this effect was related to BDNF

concentration. The results of the cylinder test revealed that a missing allele of BDNF led to impaired sensory-motor performance when the mice were fed a control diet. In addition, the results of the cylinder test demonstrated that BDNF heterozygous mice fed a high-cholesterol diet exhibited the lowest levels of simultaneous use of both paws among all the experimental groups. Similarly, in the wire hanging test, BDNF heterozygous mice fed with a high-cholesterol diet spent the shortest time hanging on the wire among all the experimental groups. Therefore, deletion of one allele of BDNF led to impaired performance in both tests, when the mice were fed with a high-cholesterol diet. The results from the present study reveal for the first time the effects of both BDNF deficiency and a high-cholesterol diet on sensory-motor performances. In addition, the observed neuroinflammatory marker and MDA levels also support the cylinder test results.

Neuroinflammation is a factor that mechanistically links high cholesterol and impaired neuronal function. It has been demonstrated that neuroinflammation and blood-brain barrier dysfunction play a role in cognitive disorders caused by hypercholesterolemia (de Oliveira et al., 2020). A high-cholesterol diet was reported to lead to a decrease in the number of pyramidal cells and an increase in neuroinflammation in the hippocampus in aged rats (Rui et al., 2017). Also, when the rats were fed with a high-salt and high-cholesterol diet, the activity of the nuclear factor kappa B signaling pathway was observed to increase in brain, together with an increase in brain nitric oxide levels and an increase in TNF-α and interleukin-10 (IL-10) levels in blood (Husain et al., 2017). Increased mRNA levels of IL-1β, IL-6, TNF-α, IL-4 and IL-10 were observed in the hippocampus of aged mice fed with a high-cholesterol diet (Chen et al., 2018). In this study, we observed that a high-cholesterol diet slightly increased the levels of inflammatory cytokines in the neocortex, especially IL-6 levels. The increase in inflammatory cytokine levels was more pronounced in BDNF heterozygous mice when fed a high-cholesterol diet. Therefore, the results implied that a high-cholesterol diet triggers neuroinflammation in the presence of BDNF heterozygosity, which is noteworthy, as neuroinflammation and mitochondrial dysfunction were previously reported to play a role in many neurodegenerative diseases such as Alzheimer's disease (Giordano et al., 2013).

Oxidative stress is an important factor involved in the mechanism of high-cholesterol-diet-induced neuronal damage. The synaptic sites require high amounts of energy, which inevitably is associated with reactive oxidative stress. When synaptic mitochondria are inadequate, reactive oxidative species are produced, which leads to altered synaptic function and synaptic loss (Crispino et al., 2020). Dietary supplementation with 2% cholesterol for 4 weeks has been found to increase oxidative stress in the rabbit hippocampus and cortex (Aytan et al., 2008). A high-fat/high-cholesterol diet was observed to increase inflammation and oxidative stress in the mouse hippocampus (Thirumangalakudi et al., 2008).

We investigated the changes in a set of oxidative stress parameters caused by a high-cholesterol diet, as well as a potential antioxidant role for BDNF in the neocortex. The interaction between oxidative stress and the BDNF system modulated the diet-induced changes in synaptic plasticity and cognitive function. Neuronal and behavioral plasticity can be modulated by molecular events that are affected by oxidative stress (Wu et al., 2004). In another study from our laboratory, reduced levels of BDNF in the cortex resulted in increased MDA levels and loss of synaptic proteins (Abidin et al., 2018). Previous studies have also shown that elevated MDA levels and oxidative stress parameters were correlated with impaired cortical function (Yaras et al., 2003; Yargiçoğlu et al., 2004). Similarly, in our study, it was observed that a high-cholesterol diet increased oxidative stress and neuroinflammation in mouse neocortex tissue. The present findings indicated that MDA, which is the product of lipid peroxidation, levels were the highest in the HCD: HT group. Additionally, the WT groups of both diet types had lower MDA levels in comparison to the HT groups. The activity of the antioxidant defense enzyme SOD was also observed to be higher in the HT groups. Based on the observed levels of oxidative stress parameters, we can suggest that BDNF provides an antioxidant effect in cortical tissues and plays a protective role in the presence of a high-cholesterol diet. In this way, BDNF may be a genetic factor that determines the effects of dietary cholesterol in brain tissue. Moreover, the BDNF concentration and cholesterol relationship points to a signaling pathway waiting to be elucidated by detailed molecular studies.

There are some limitations of this study. Since the main focus was on the effects of the BDNF concentration and cholesterol relationship on sensory-motor functions, influences of the applied diet protocol on general metabolic changes were not evaluated, such as changes in plasma cholesterol levels or weekly variations in body weight of the animals. In the current literature, there are a number of studies that verified serum cholesterol elevations following diet changes, as well as a number of studies that did not made such a measurement. Ultimately, in the present study, diet was the independent variable of interest; hence, the differences can be attributed to the high-cholesterol diet.

CONCLUSION

Our results revealed that a high-cholesterol diet increased inflammation and increased MDA levels in the neocortex. Among these factors, BDNF heterozygous mice were more vulnerable to oxidative damage. The expression of BDNF might be an endogenous factor that contributing to modulation of high-cholesterol-induced damage in the central nervous system.

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