Basic and Clinical
NEUROSCIENCE
January & February 2026, Vol 17, No. 1

Research Paper

Vitamin D Improves the Maternal Hypothyroidism-induced Cognitive Decline in the Rat Offspring

Hanieh Hajipour[1], **Katayoun Sedaghat**[2*]

1. Student Research Committee, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.
2. Department of Physiology, Research Center of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

Use your device to scan and read the article online

Citation Hajipour, H., & Sedaghat, K. (2026). Vitamin D Improves the Maternal Hypothyroidism-induced Cognitive Decline in the Rat Offspring. *Basic and Clinical Neuroscience, 17*(1), 143-158. http://dx.doi.org/10.32598/bcn.2026.1792.2

http://dx.doi.org/10.32598/bcn.2026.1792.2

Article info:
Received: 18 Nov 2025
First Revision: 29 Nov 2025
Accepted: 02 Des 2025
Available Online: 01 Jan 2026

Keywords:
Maternal hypothyroidism, Vitamin D, Cognition, Oxidation, Pro-inflammation, Brain-derived neurotrophic factor (BDNF)

ABSTRACT

Introduction: Thyroid hormones (THs) are vital for fetal and neonatal nervous system development. Mild maternal hypothyroidism might render cognitive impairment in offspring during adulthood, by producing oxidative stress, inflammation, and lowering brain-derived neurotrophic factor (BDNF). Vitamin D has anti-oxidant and anti-inflammatory actions. Therefore, we investigated whether vitamin D administration during gestation in hypothyroid dams or after birth would improve cognitive function in offspring.

Methods: For this study, we used propylthiouracil (PTU) to induce hypothyroidism in pregnant rats from the sixth day of gestation until delivery. A group of pregnant rats received vitamin D (5 or 10 mg/kg) along with PTU, while another group received that after delivery until the weaning of offspring and continued for the offspring until they were sacrificed. At the 60th postnatal day, a novel object recognition (NOR) test was performed. Protein assays for lipid peroxidation, superoxide dismutase (SOD), interleukin-6 (1L-6:), tumor necrosis factor alpha (TNF-α), and BDNF were performed in the hippocampus and prefrontal cortex.

Results: Results indicated that maternal hypothyroidism reduced cognitive functions in the offspring, and that vitamin D administered during gestation improved memory decline and recognition of the novel object. Vitamin D, either during or after birth, markedly altered oxidative stress, inflammation, and BDNF levels in the brain.

Conclusion: This study indicates that maternal hypothyroidism-induced oxidation, inflammation, and decreased BDNF are passed and remain with the offspring into their adulthood, as possible causes of cognitive decline. Vitamin D administration, especially during pregnancy, may improve cognitive function by modulating the underlying mechanisms.

*** Corresponding Author:**
Katayoun Sedaghat, Associate Professor.
Address: *Department of Physiology, Research Center of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.*
Tel: *+98 (910) 9088967*
E-mail: *katsedaghat@gmail.com*

Highlights

- Maternal hypothyroidism reduced cognitive function in the adult offspring, which is possibly related to the oxidative stress, increase in pro-inflammatory cytokines and decrease in the brain derived nerve growth factor.

- Vitamin D administration to dams during gestation markedly restored cognitive ability in the NOR test.

- Vitamin D administration either pre- or postnatally, markedly adjusted oxidative stress, inflammatory cytokines, and BDNF levels in the offspring hippocampus and prefrontal cortex.

Plain Language Summary

Hypothyroid mothers usually give birth to children with different levels of nervous system deficiency. This study is based on a model of mild maternal hypothyroidism (MH)-induced cognitive deficiency in male rat offspring. We measured cognitive ability using the novel object recognition (NOR) test, a standard test that evaluates a type of memory used to distinguish between old and new objects in a set, over a 24-hour interval. Our study showed that adult offspring with MH display lower cognitive ability in compare with healthy offspring. In addition, our molecular part of the study showed that in two brain parts that are highly related to learning and memory, the hippocampus and pre-frontal cortex, higher levels of harmful chemicals called oxidants and inflammatory factors and lower brain growth factors were found compared to healthy offspring's brain. Vitamin D is an internal hormone that we use as a food supplement, and it has a strong antioxidant and anti-inflammatory capability. In this study, we injected vitamin D twice a week to mothers during pregnancy (group 1, G1) or after giving birth for 2 weeks (during breastfeeding), following injecting twice to offspring from day 15 to 60 after birth (group 2, G2). G1 offspring responded much closer to normal offspring in NOR in comparison to G2, while vitamin D in both groups lowered the levels of oxidative and inflammatory and improved the level of growth factor in the offspring's brains. This study showed for the first time that mild maternal hypothyroidism can induce cognitive disabilities in offspring even during adulthood and that administering vitamin D (best along with thyroid hormone treatment) during or after birth can help restore cognitive ability.

1. Introduction

Thyroid hormones (THs) are critical for normal central nervous system development during the fetal or neonatal periods. Offspring from a mother with even mild TH insufficiency during pregnancy show cognitive impairments. TH regulates neuronal myelination, dendrite proliferation, neurogenesis, and synapse formation during early developmental periods. Based on multiple studies, maternal hypothyroidism during the fetal period usually causes serious neurological impairments in the offspring, such as intellectual disability and cerebral spastic diplegia (Gilbert et al., 2016), autism spectrum disorders, anxiety, schizophrenia, increased susceptibility to seizures, and epilepsy (Alcaide Martin et al., 2023). These disorders may be along with normal serum TH levels or without any hypothyroid symptoms. Since the availability of T4 to the fetal brain and body tissues depends on maternal free T4 levels, maternal hypothyroidism will severely impair this supply. However, cognitive impairment in neonatal thyroid insufficiency is less severe, although there may be deficits in memory, understanding, and learning (Gilbert et al., 2016; Sedaghat et al., 2015; Williams, 2008).

TH acts through two subtypes of nuclear receptors: TRα and TRβ. The thyroid receptor (TR), while not bound to TH, binds to DNA sequences, known as thyroid receptor response elements (TREs). In this form, the receptor acts as a transcription repressor. Upon binding the TH to the TR, the receptor acts as a transcription activator. For this reason, the absence of T3 produces more damaging effects than the absence of the TR, as seen in various TR knockout models (Gilbert et al., 2013).

The role of THs in brain development is multifactorial and complex. TH may act through different pathways mediated by various factors. The role of neurotrophins as mediators for TH action in brain development is crucial. Neurotrophins are highly implicated in neuronal plasticity during development. Based on previous studies, although hypothyroxinemia during development mildly affects the neurotrophin gene expression, it impairs hippocampal-based learning and LTP-induced neuro-

trophin expression. Some studies have reported reduced BDNF levels in the limbic system of rat offspring born to a hypothyroid dam, along with deficits in cognitive abilities (Chakraborty et al., 2012; Lasley et al., 2011; Madhusudhan et al., 2022). Brain-derived neurotrophic factor (BDNF) stabilizes hippocampal glutamate-dependent excitatory synapses (Gilbert et al., 2016) and, therefore, is essential for normal hippocampal cognitive performance. Thus, it appears that TH is necessary for the physiological actions of neurotrophins.

TH is also essential for the regulation of oxygen, O_2 metabolites, and anti-oxidant balance in the body. TH deficiency affects the electron transport chain in the mitochondria; therefore, it renders reactive oxygen species (ROS) production, in addition to the reduction in the levels of anti-oxidant activity and lipid metabolism (dos Anjos Cordeiro et al., 2022; Sahoo et al., 2008). Previously, it has been demonstrated that maternal hypothyroidism could cause oxidative stress (OS), an increase in levels of hypoxia inducible factor-1alpha and hypoxia in offspring, and disruption of anti-oxidant enzymes expression and function (dos Anjos Cordeiro et al., 2022). OS and subsequent neural death are relevant to cognitive dysfunction and decline in learning and memory (Ramos-González et al., 2024). The other side of OS in hypothyroidism is that protein oxidation and lipid peroxidation in the brain can lead to mitochondrial and neural cell damage, activating pro-inflammatory cytokines and promoting neuroinflammation, neurodegeneration, and cognitive dysfunction (Hussain et al., 2016). The rise in pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)α is reported in human hypothyroid cases (Lasa et al., 2022). Recent studies have shown that prenatal exposure to proinflammatory cytokines like IL–6, IL–8, and TNFα may put the nervous system at risk of neurodevelopmental diseases like autism or schizophrenia by prompting neuromorphological and neurochemical changes in the fetal brain (Popa-Wagner et al., 2013). Elevated levels of these cytokines have a negative effect on the hypothalamic-pituitary-thyroid (HPT) axis, and, for this reason, some studies have shown that administering levothyroxine decreases pro-inflammatory cytokine levels. Furthermore, THs regulate the activity of the immune system, including neutrophils, macrophages, natural killer cells, dendritic cells (innate immune system), and B- and T-lymphocytes (adaptive immune system). Therefore, hypothyroidism leads to a less activated immune system (Lasa et al., 2022). Altogether, hypothyroidism is accompanied by activated pro-inflammatory factors along with reduced immune defense, which may lead to cognitive deficiencies and impairments (Chamas et al., 2022).

Obviously, the best treatment for hypothyroidism and accompanying dilemmas is the THs themselves. However, a combination of THs and another agent, such as an internal hormone working toward the same goals and through the same targets, would be undoubtedly far more beneficial and prevent the nervous system from further damage and perhaps accelerate the repair. One of the best candidates to fit the position is vitamin D. It is well documented that vitamin D has anti-oxidative and anti-inflammatory effects, and therefore, as our previous studies have shown, is one of the best for the prevention of psychiatric deficiencies such as depression, followed by chronic mild stress in rodents (Sedaghat et al., 2021; Sedaghat et al., 2019), and in humans (Lally et al., 2019). The relation between vitamin D and its anti-oxidative/anti-inflammatory actions is well documented. It improves mitochondrial respiratory function, prevents OS, and, by doing so, protects the body from metabolic diseases such as autoimmunity, infection, cancer, insulin resistance, hypertension, and, among others, memory disorders (Wimalawansa, 2019). Vitamin D improves production of glutathione and superoxide dismutase (SOD) (Sedaghat et al., 2021; Wimalawansa, 2019). It reduces the inflammatory reactions by reducing the C-reactive protein, IL-6, and TNF-α (Menéndez et al., 2024; Sedaghat et al., 2021). It inhibits nuclear factor-κB (NF-κB) dependent pathways, which altogether prevents or reduces production of the inflammatory cytokines and free radicals and therefore, fades OS and inflammation away (Barbalho et al., 2024). On the other hand, vitamin D as a neurosteroid improves the functions of the nerve growth factors, such as nerve growth factor (NGF), and its role in the cholinergic action in the brain, as well as glial cell line-derived (GDNF) and BDNF, and their role in development and protection, specifically for, dopamine neurons (Cui et al., 2022; Dewi et al., 2025).

In this study, we employed a moderate gestational hypothyroidism model in male offspring that has previously shown impairments of hippocampus-associated cognitive functions in adult offspring (Amano et al., 2018). We subjected a group of male offspring to propylthiouracil (PTU) during the gestational period; a subset received vitamin D via maternal injection during the fetal period, while another group did not. After the birth, those born to hypothyroid dams without receiving vitamin D were again divided into two groups: those that received vitamin D from birth up to the 65th day postnatal and those who did not. On the 60th postnatal day, their cognitive abilities were tested using passive-avoidance and novel-object recognition tests. Later, they were sacrificed, and the hippocampus and prefrontal cortex were extracted and examined for OS, pro-inflammatory cytokines, and

BDNF. The aim of these groupings in this study was to evaluate and compare the effects of vitamin D on cognitive abilities in the brain when administered during fetal life versus postnatally.

Materials and Methods

Study animals

For this study, 30 adult female Wistar rats weighing 180–200 g were used. Rats were house-bred at the Research Center of Physiology, Semnan University of Medical Sciences (SEMUMS) in Semnan, Iran. They were housed in plastic cages measuring 45×25×15 cm, with three rats in each cage. They were kept on a 12/12 light/dark cycle at 22±2 ˚C and had access to food and water ad libitum. Rats were allowed to acclimate to the environment for 7 days before mating. For the mating, an adult male rat was added to each cage for 2 nights. The pregnancy was confirmed by observing the vaginal plug. Female rats were weighed before mating and then weekly throughout the gestational period. The day after delivery, blood samples were collected from dams to measure T4 levels. Male offspring were weaned at 1 month and kept in groups of 3 per cage until 60 days after birth, when the behavioral experiments began. In this study, the National Research Council's guide for the care and use of laboratory animals was followed, and the study was performed in accordance with the approved proposal submitted to the Ethics Committee of Semnan University of Medical Sciences.

Study drugs

PTU was obtained from Sigma-Aldrich (USA). A fresh PTU solution was made by adding 100 mg PTU to 1 L of water. Vitamin D (obtained from Osveh Pharmaceutical Co., Tehran, Iran) was prepared as 1 µg/µL dissolved in 100% ethanol (stock) and then diluted in 5% ethanol in distilled water for intraperitoneal (IP) injections at 2 concentrations: 5 and 10 µg/kg, twice weekly (Sedaghat et al., 2021; Sedaghat et al., 2019).

Experimental groups

Our model was based on subjecting the fetus to maternal PTU only during the gestational period. After the dams gave birth to their offspring, PTU was cut off from their drinking water, and they received only pure tap water. We used only male offspring in this study to avoid potential hormonal cycles in females that might affect hypothyroidism or vitamin D administration at the behavioral and cellular levels. Since the offspring were the subjects of this study, the grouping was based on whether their mothers received vitamin D during gestation or after delivery. Group 1 (G1) consisted of offspring whose mothers received PTU and vitamin D IP, 5 and 10 µg/kg, twice weekly) only during gestation. After delivery, they did not receive vitamin D in any form. Group 2 (G2) consisted of offspring whose mother received PTU during gestation and started receiving vitamin D injections (IP, 5 and 10 µg/kg, twice weekly) right after delivery and during breastfeeding. After weaning, offspring continued to receive vitamin D (with the same protocol as their mother) until day 60 after birth. For groups 1 and 2, PTU was discontinued for the mothers immediately after giving birth.

Therefore, the offspring experimental groups in this study were as follows: 1- Hypothyroid: (mother received PTU 100 mg/L during pregnancy), 2- G1: hypothyroid received vitamin D (5 or 10 µg/kg) during gestation, 3- G2: hypothyroid received vitamin D (5 or 10 µg/kg) after birth until sacrificed, and 4- euthyroid (mother did not receive PTU during pregnancy) (Figure 1). In this study to control the litter effect, we randomly took 2 male pups from each littermate. In other words, we randomly allocated 5 dams to each group, and from each dam, 2 male offspring were taken for the experiment. The number of rats per group ranged from 6 to 8.

Behavioral experiment

The method for the novel object recognition (NOR) experiment was adapted with minor modifications from Bevins and Besheer (2006). In this study, we used a black color box with (100×100×50 cm) dimensions. One day before the experiment, the rats were habituated to the experiment box by placing them in the box, facing the wall, and letting them explore the environment for 5 minutes. On the day of experiment, the rat was first allowed to explore the box for 5 minutes before being removed. Then, 2 similar objects were placed on one side of the box, parallel to each other. The rat was again placed into the box facing the wall opposite the objects and allowed to explore the objects for 5 minutes, and then it was returned to its cage. Twenty-four hours later, one of the objects was replaced with a new one.

The rat was placed into the box facing the wall and allowed to explore for 5 minutes. A charge-coupled device camera (Panasonic Inc., Japan) was installed above the apparatus, and the time rats spent around familiar and new objects was measured and analyzed using EthoVision software, version XT7 (Noldus Information Technology, Wageningen, Netherlands). The chamber was

wiped with a disposable tissue and ethanol 70% after each animal. The preference index (PI) was calculated as TN/(TN+TF) ×100, where TN stands for (exploration) time for the new object, and TF, time for the familiar object. The PI above 50% indicated a preference for the new object.

Tissue preparation

The day after the NOR test, rat blood samples were obtained from the heart, and then the rats were decapitated, and the brain was extracted, and the hippocampus and prefrontal cortices from both hemispheres were dissected. Samples were frozen and kept in a (-70˚C) freezer for later processing.

ELISA studies

Plasma T4 was measured using a T4 enzyme linked immunosorbent assay (ELISA) kit (Pishtazteb, Tehran, Iran). To perform ELISA on the brain samples, we first measured the protein levels using the Bradford protein assay kit (Cat No: DB0017, DNA BioTech). SOD enzyme activity was assessed using Nasdox superoxide dismutase activity assay kit (Navand Lab kit, Tehran, Iran) and lipid peroxidation by malondialdehyde (MDA) assay kit (Navand Lab kit, Tehran, Iran). Pro-inflammatory cytokines: Interleukin-6 (1L-6: Cat No: CSB-E04640r, Cusabio, USA) and Tumor Necrosis Factor Alpha (TNF-α: Cat No: RTA00, Bio-techne, USA), and brain neurotrophic factor, BDNF (BDNF: Cat No: DBNT00, R&D, USA), were measured using named rat ELISA kits. All the assays were performed according to the protocols provided with the kits.

Statistical analysis

GraphPad Prism software, version 8.0.2 (GraphPad Software Inc., 2019) was used for statistical analysis and graph creation. Data were evaluated for homogeneity of variances using the Brown-Forsythe test. We used a one-way analyses of variance (ANOVA) followed by multiple Tukey test for paired comparisons. Statistical significance was defined as $P<0.05$.

Results

Serum T4 measurement

One-way ANOVA for dams' T4 levels indicated significantly lower T4 (about 60% decline) in dams that received PTU (hypothyroid), compared to the euthyroid group ($F_{3, 8}=47.15$, $P<0.0001$). Vitamin D (5 and 10 mg/kg) did not affect the T4 levels of hypothyroid dams in G1, which means hypothyroid dams who took vitamin D still had lower T4 levels (57%-65%, for vitamin D 10 mg/kg and 5 mg/kg, respectively) compared to euthyroid group.

One-way ANOVA revealed no statistical significance in T4 levels among all offspring groups. Although the level of serum T4 was lower in offspring from hypothyroid mothers relative to euthyroid offspring, it was not significant ($P>0.05$). Vitamin D did not affect T4 levels in all groups.

Behavioral study

This study showed that maternal hypothyroidism caused offspring to show impaired ability to recognize a novel object. One-way ANOVA revealed a significant difference between groups ($F_{5, 31}=7.156$, $P=0.0001$). Tukey's multiple comparison test showed a marked reduction (42.02%) in the NOR in the hypothyroid group relative to the euthyroid group ($P<0.0001$). However, administering vitamin D (5 and 10 mg/kg) in the G1 group improved the ability to recognize the novel object (28.86 and 33.56%, respectively) by increasing the difference in means with the hypothyroid group ($P<0.05$ and $P<0.01$, respectively), whereas vitamin D in the G2 group did not affect their cognitive ability (Figure 2).

Lipid peroxidation marker (MDA)

Regarding the hippocampus, one-way ANOVA revealed a significant difference in MDA expression among groups ($F_{5, 16}=20.34$, $P<0.0001$). Tukey's multiple comparison test showed a marked increase in MDA levels (88.27%) in the hypothyroid hippocampus relative to the euthyroid group ($P<0.0001$). On the other hand, both vitamin D doses (5 and 10 mg/kg) reduced MDA levels in the G1 group (90% and 85%, respectively) and the G2 group (69% and 70%, respectively) relative to the hypothyroid ($P<0.001$ and $P<0.0001$, respective to vit D-5 and -10, in both G1 and G2 groups) (Figure 3A).

Regarding the prefrontal cortex (PFC), same as hippocampus, there was a considerable difference in MDA expression among groups in this area ($F_{5, 16}=13.14$, $P<0.0001$). Tukey's multiple comparison test revealed MDA levels in the hypothyroid PFC were markedly increased (79%) relative to the euthyroid ($P<0.0001$). However, vitD-5 and 10 reduced MDA levels dose-dependently in the G1 group (56% and 78%, $P<0.01$ and $P<0.0001$, respectively). In the G2 group, only vitD-10

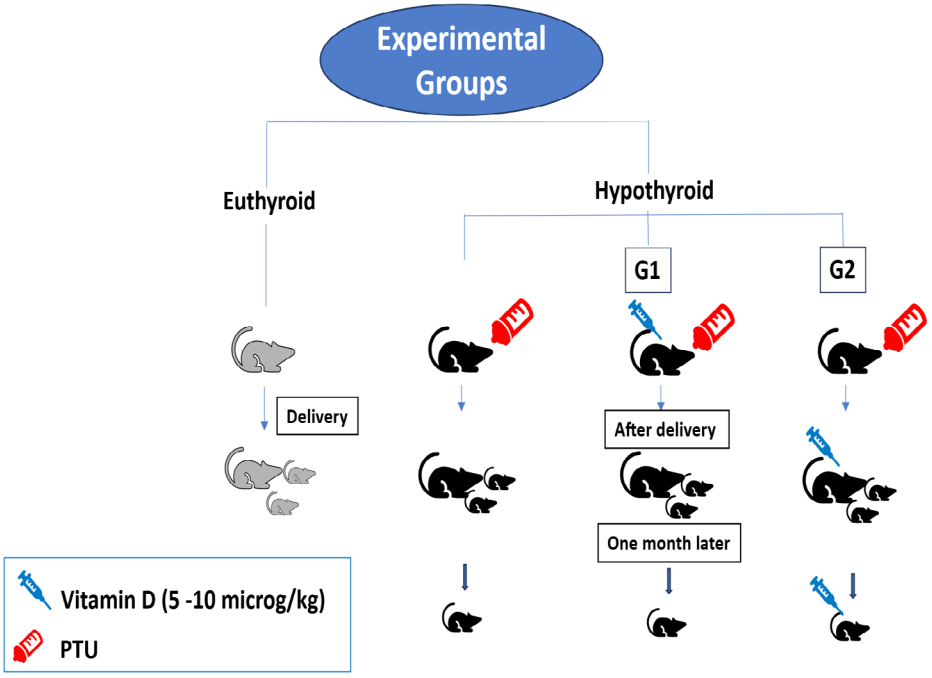


Figure 1. The study's experimental groups: G1, G2: groups 1 and 2

NEUROSCIENCE

reduced MDA levels significantly (48%) relative to the hypothyroid group (P<0.01, Figure 3B).

Anti-oxidative enzyme (SOD)

Regarding the hippocampus, A significant difference in SOD expression was detected among groups ($F_{5, 16}$=29.11, P<0.0001). Tukey's multiple comparison test revealed a marked reduction (59%) in SOD levels in the hypothyroid group relative to the euthyroid group (P<0.0001). Whilst vitD-5 and 10 increased the SOD levels markedly (100%) relative to the hypothyroid in the G1 group (P<0.0001). In addition, only vitamin D-10 increased SOD levels in the G1 (39%) relative to G2 (P<0.01). In the G2 group, only vitD-10 increased SOD

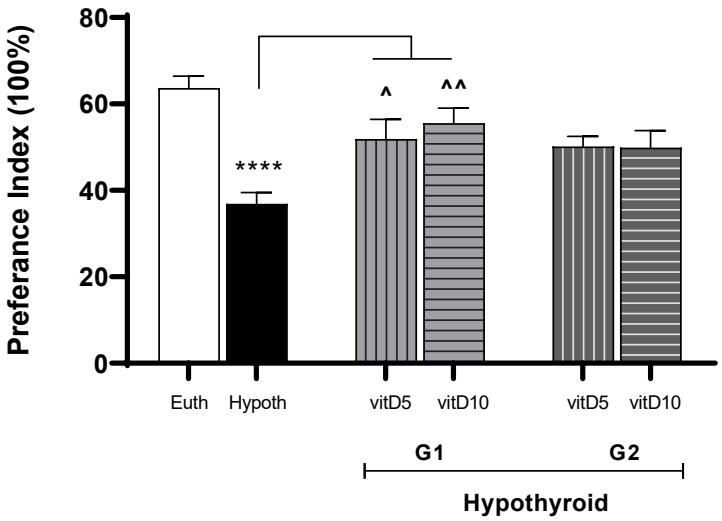
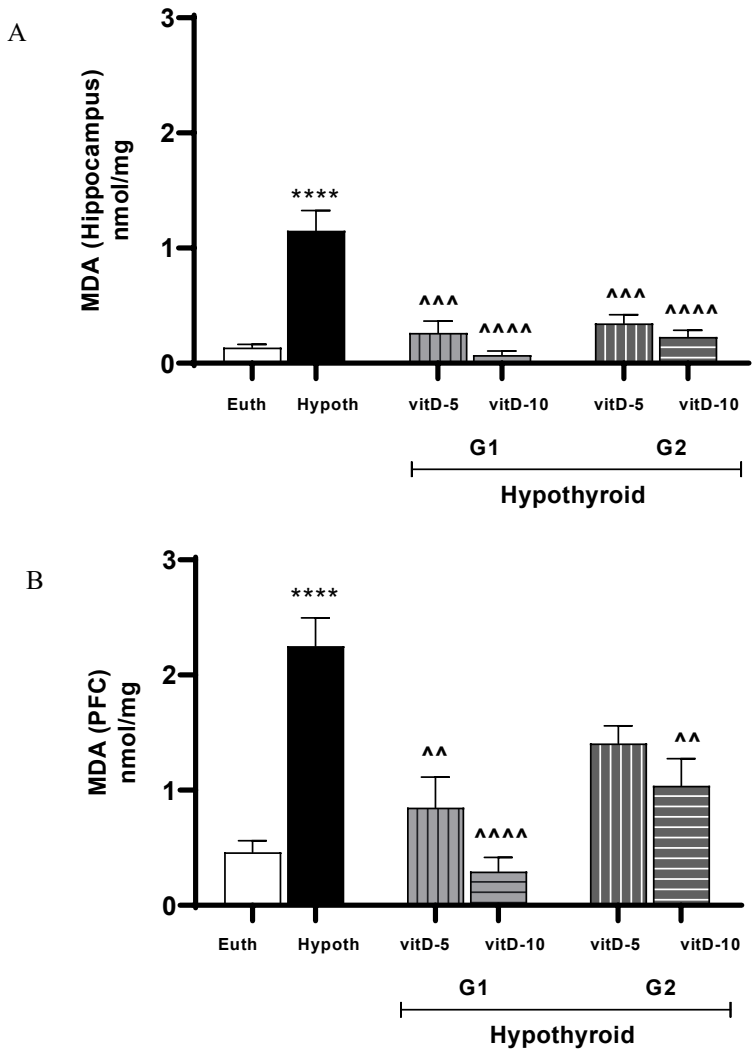


Figure 2. The PI in the NOR test

NEUROSCIENCE

Abbreviations: Euth: Euthyroid; Hypoth: Hypothyroid; Vitd: Vitamin D (5-10 µg/kg); G1: Group 1; G2: Group 2.

Note: ****P<0.0001, ^P<0.05, ^^P<0.01, representing the difference compared to the euthyroid group.



NEUROSCIENCE

Figure 3. Marker of lipid peroxidation, MDA (nmol/mg) levels in A) Hippocampus, B) PFC, in the experimental groups

Abbreviations: Euth: Euthyroid; Hypoth: Hypothyroid; Vitd: Vitamin D (5-10 µg/kg); G1: Group 1; G2: Group 2.

Note: ****P<0.0001, representing the difference compared to the euthyroid group. ^^P<0.01, ^^^P<0.001, ^^^^P<0.0001, representing the difference compared to the hypothyroid group.

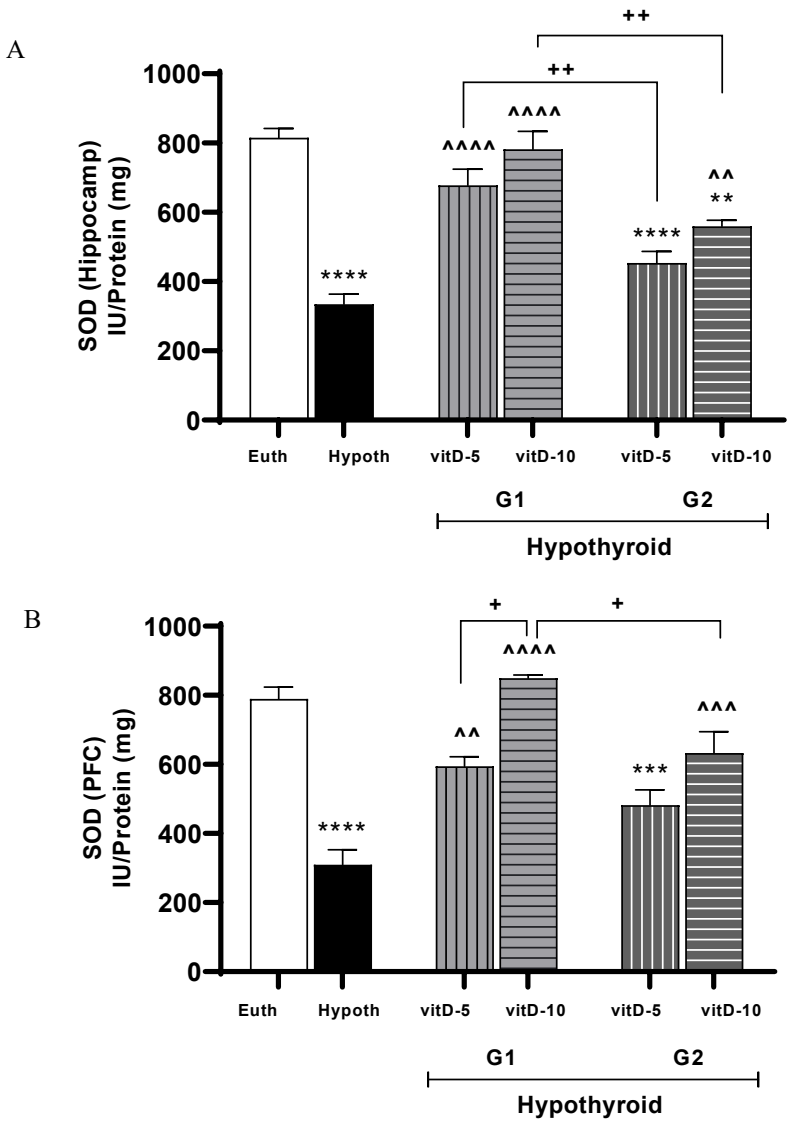
levels compared with the hypothyroid (67%), although it remained markedly lower than the euthyroid group (P<0.01) (Figure 4A).

PFC

Regarding the PFC, the difference in SOD levels was noteworthy among groups ($F_{5, 16}$=21.26, P<0.0001). Tukey's multiple comparison test detected a marked decrease in SOD levels (60%) in the hypothyroid compared to the euthyroid group (P<0.0001). Almost similar to hippocampus, vitamin D increased the SOD levels in the G1 group with both 5 and 10 concentrations (92% and 100%, P<0.01 and P<0.0001, respectively), while in the G2 group, only vitD-10 raised the SOD levels (100%) compared to the hypothyroid group (P<0.001). Likewise, vitD-10 increased SOD (43%) higher than vitD-5 (P=0.017) in the G1 group and vitD-10 in the G2 group (34%, P=0.034) (Figure 4B).

BDNF

Regarding the hippocampus, a marked difference in BDNF levels was detected among groups ($F_{5, 16}$=65.95, P<0.0001). Paired-comparison also revealed a significant decline (69%) in hippocampus BDNF levels for the hypothyroid group relative to the euthyroid group (P<0.0001). Both vitamin D-5 and 10 increased BDNF



NEUROSCIENCE

Figure 4. SOD (IU/protein mg) levels in A) Hippocampus, B) PFC

Abbreviations: Euth: Euthyroid; Hypoth: Hypothyroid; Vitd: Vitamin D (5-10 mg/kg); G1: Group 1; G2: Group 2.

Note: ***P<0.001, ****P<0.0001, representing the difference compared to the euthyroid group. ^^P<0.01, ^^^P<0.001, ^^^^P<0.0001, representing the difference in compare to the hypothyroid group. +P<0.05, representing the difference between G1 and G2-vitD-10 and G1-vitD-5 and 10. ++P<0.01 representing the difference between G1 and G2-vitD-5 and vitD-10.

levels in the G1 and G2 groups (about 2-fold or more, P<0.0001). In addition, relative to the euthyroid group, only G1 with vitD-10 showed a higher level (19%) of BDNF (P=0.0197). In the G1 and G2 groups, vitD-10 increased BDNF higher than vitD-5 (P=0.0016 and P=0.0365, respectively). In addition, vitD-10 raised BDNF higher (20%) in the G1 group relative to the G2 group (P=0.024, Figure 5A).

Regarding the PFC, like the hippocampus, there was a significant difference in BDNF levels between groups ($F_{5, 16}$=35.57, P<0.0001). Tukey's multiple comparison test showed that PFC BDNF levels decreased by 62% in the hypothyroid group compared to the euthyroid group (P<0.0001). In the G1 group, vitamin D increased BDNF levels by 1 to 2 times higher than in the hypothyroid group (P=0.0003, P<0.0001 for vitD-5 and 10, respectively). In the G2 group, vitamin D also increased BDNF levels compared with the hypothyroid group (P=0.0082, P<0.0001 for vitD-5 and vitD-10, respectively). Additionally, BDNF levels were 20% higher in the G1 group with vitD-10 compared to the G2 group with vitD-10

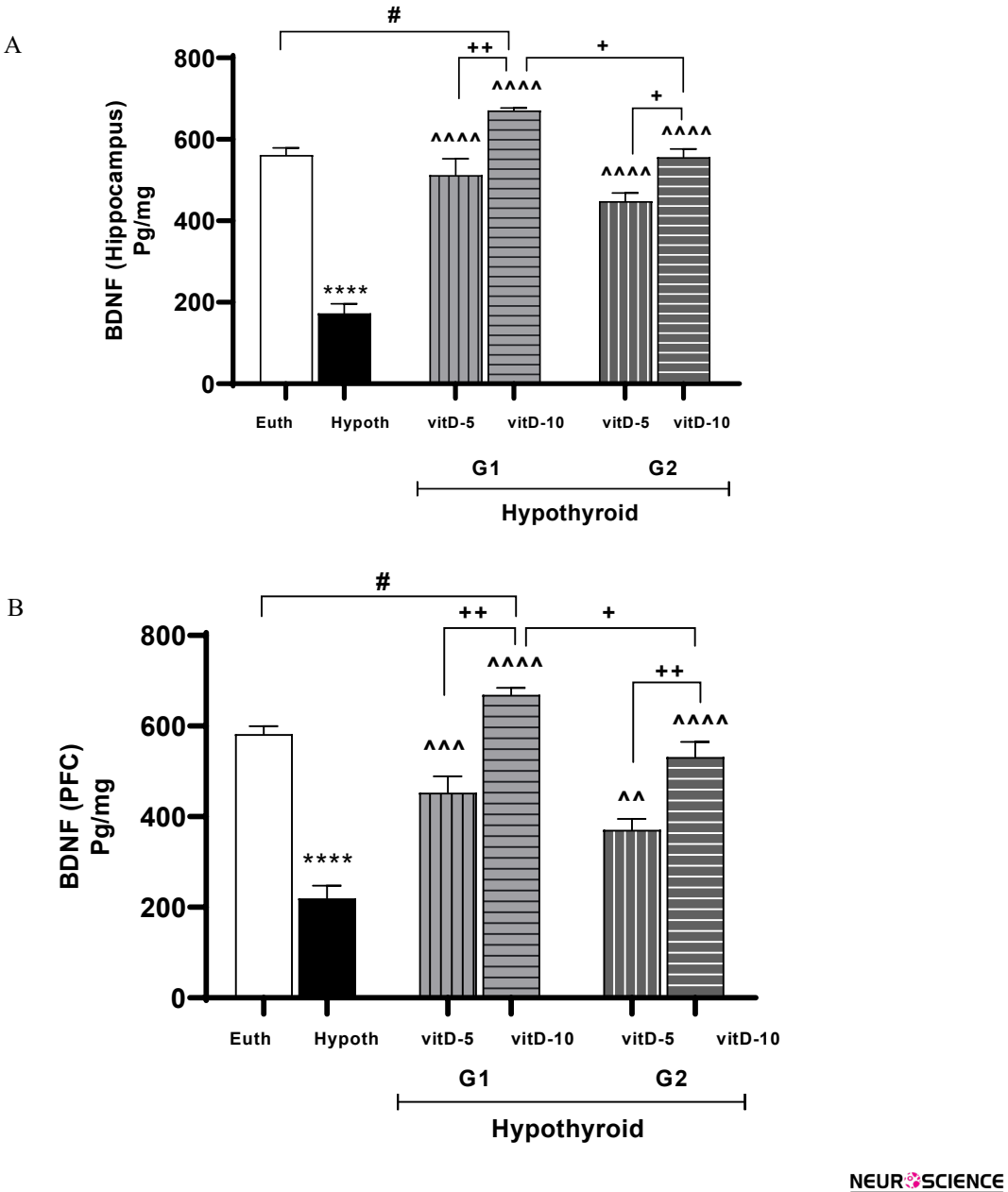


Figure 5. BDNF (pg/mg) levels in A) Hippocampus, B) PFC

Abbreviations: Euth: Euthyroid; Hypoth: Hypothyroid; Vitd: Vitamin D (5-10 µg/kg); G1: Group 1; G2: Group 2.

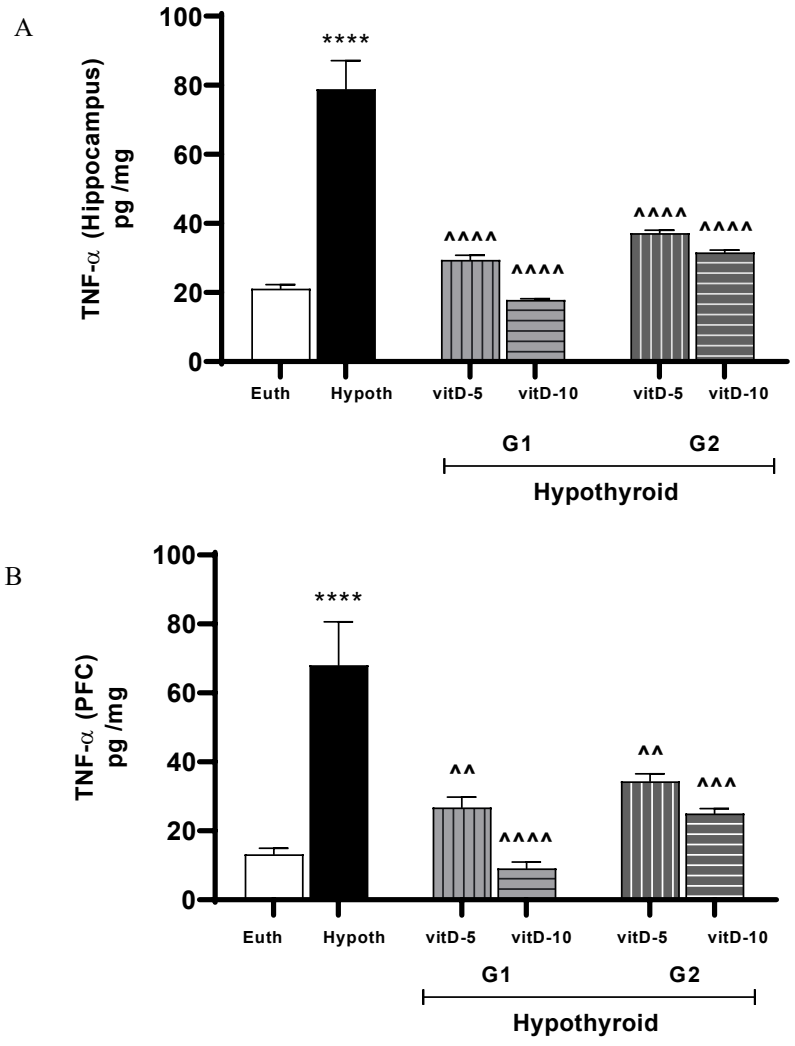
Note: ****P<0.0001, representing the difference compared to the euthyroid group. $^{^^}$P<0.01, $^{^^^}$P<0.001, $^{^^^^}$P<0.0001, respectively, representing the difference compared to the hypothyroid group. $^{\#}$P<0.05, representing the difference between the G1-vitD-10 and euthyroid groups. $^{+}$P<0.05, $^{++}$P<0.01, respectively representing the difference between G2-vitD-5 and 10

(P=0.0305). Rats in the G1 and G2 groups that received vitD-10 showed higher BDNF levels than those that received vitD-5 in each group (47% and 43%, respectively; P=0.0012 and 0.0049, respectively; Figure 5B).

Pro-inflammatory cytokines

TNFa

Regarding the hippocampus, the differences in TNFα levels among the groups was significant ($F_{5, 16}$=15.70, P<0.0001). Tukey's multiple comparison test showed over a 2-fold increase in TNFα in the hypothyroid compared to



NEUROSCIENCE

Figure 6. TNF-α (pg/mg) protein levels in the A) Hippocampus, B) PFC, in the experimental groups

Abbreviations: Euth: Euthyroid; Hypoth: Hypothyroid; Vitd: Vitamin D (5-10 µg/kg); G1: Group 1; G2: Group 2.

Note: ****P<0.0001, representing the difference from the euthyroid group. ^^P<0.01, ^^^P<0.001, ^^^^P<0.0001, represent the difference from the hypothyroid group.

the euthyroid group (P<0.0001). In the G1 group, vitD-5 and vitD-10 decreased TNFα levels by 62% (P=0.0003) and 77% (P<0.0001), respectively. The same was observed for the G2 group, with decreases of 52% (P=0.0005) and 59% (P=0.0009) for vitD-5 and vitD-10, respectively (Figure 6A).

Regarding the PFC, like the hippocampus, there was a significant difference among groups for TNF-α in the PFC ($F_{5, 16}$=12.91, P<0.0001). Tukey's multiple comparison test showed a four-fold increase in TNF-α levels in the hypothyroid group compared to the euthyroid group (P<0.0001). Vitamin D lowered TNF-α levels in the G1 group (60%, P=0.0026 and 86%, P<0.0001 for vitD-5 and 10, respectively) and the G2 group (49%, P=0.0078 and 63%, P=0.0008 for vitD-5 and 10, respectively) relative to the hypothyroid group (Figure 6B).

Interleukine-6 (IL-6)

Regarding the hippocampus, a marked difference in IL-6 levels was found among the groups ($F_{5, 15}$=21.15, P<0.0001). Tukey's multiple comparison test also revealed that IL-6 was significantly increased (64%) in the hypothyroid group compared with the euthyroid group (P=0.0004). Furthermore, vitamin D decreased IL-6 levels in both G1 (54% and 60% for vitD-5 and 10, respectively) and G2 groups (48% and 59% for vitD-5 and 10, respectively) for both vitD-5 and 10 (P<0.0001) (Figure 7A).

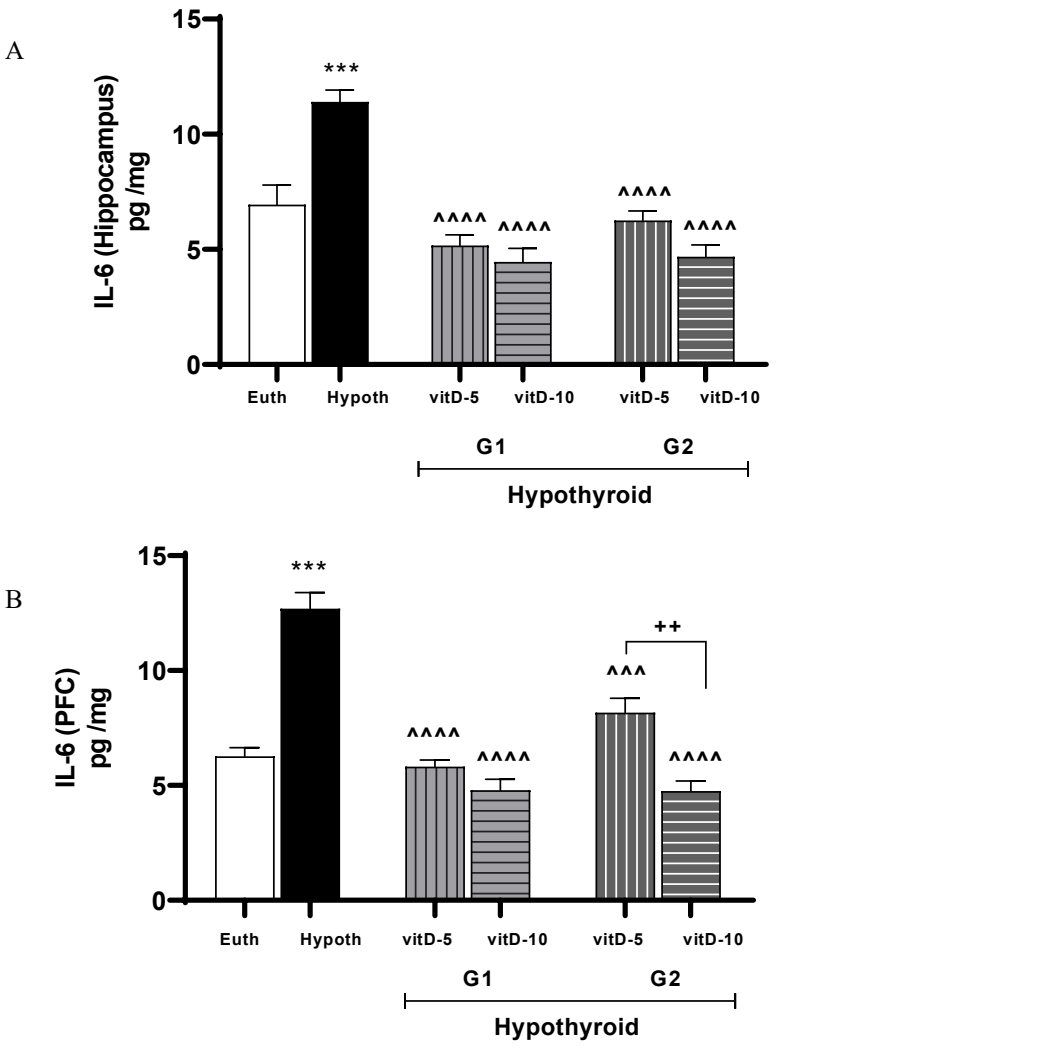


Figure 7. IL-6 (pg/mg) protein levels in A) Hippocampus, B) PFC, in the experimental groups

Abbreviations: Euth: Euthyroid; Hypoth: Hypothyroid; Vitd: Vitamin D (5-10 µg/kg); G1: Group 1; G2: Group 2.

Note: ***P<0.001, representing the difference from the euthyroid group. ^^^P<0.001, ^^^^P<0.0001, representing the difference from the hypothyroid group. ++P<0.01, representing the difference between G2-vitD-5 and 10.

PFC

Regarding the PFC, similar to the hippocampus, the difference in IL-6 levels between groups was significant ($F_{5, 14}$=41.26, P<0.0001). Tukey's multiple comparison test revealed a 2-fold increase in IL-6 levels in the hypothyroid group relative to the euthyroid group (P<0.001). Vitamin D decreased IL-6 levels in the G1 group (P<0.0001) (54% and 62%, for vitD-5 and 10, respectively) and the G2 group (35%, P=0.0002, and 62%, P<0.0001, for vitD-5 and 10, respectively) (Figure 7B).

Discussion

THs are critical for normal fetal brain development and neural construction during gestation. Maternal hypothyroidism causes changes in the neural morphology and dysfunction leading to cognitive impairment; decreased intellectual ability, memory, language, and non-verbal cognition, in addition to decreased hippocampal and cortical volume in the offspring (Kampouri et al., 2021). In the present study, to evaluate the effect of maternal hypothyroidism on the cognitive ability of the offspring, we examined the offspring's ability to recognize a new object in an otherwise familiar environment (NOR test).

The hippocampus and prefrontal cortex are the two brain areas engaged in the cognitive processes during the NOR test, suggesting that the hippocampus is more involved in recognizing object place and recency. In contrast, the prefrontal cortex is involved in the preference for the novel object (Cordner et al., 2015). Severe hypothyroid models of animals using PTU or methimazole with high doses have revealed a crucial influence of THs on hippocampal development. These models have demonstrated organizational and practical alterations in the hippocampal formation due to disrupted hippocampal formation, including learning deficits, delayed migration of hippocampal neurons, and abnormal synaptic function and plasticity (Amano et al., 2018). Previous studies indicate that postnatal PTU-induced hypothyroidism in rats (Bakalov et al., 2023) or methimazole in mice (Rutigliano et al., 2023) displayed lower behavioral functioning in the NOR (reduced short or long-term memory function) relative to the euthyroid animals. Fewer studies have performed experiments with gestational or prenatal hypothyroidism models. A couple of studies, using methimazole to induce gestational hypothyroidism, revealed that offspring displayed longer freezing behavior in the fear-conditioned test (Hipólito et al., 2023; Sanaiee et al., 2024). In our study, hypothyroid offspring showed impaired recognition of the new object compared to euthyroid offspring. On the other hand, vitamin D improved recognition of a new object in offspring when delivered only during gestation.

THs are among the main factors controlling OS in various tissues of the body. In hypothyroidism, the decline in antioxidant levels and alterations in lipid metabolism are most likely the causes of OS in the brain and other organs. Previous studies showed low total anti-oxidant capacity in hypothyroid patients. TH controls lipid metabolism, and therefore the nervous system is more sensitive to OS in hypothyroidism. Human hypothyroid patients exhibit elevated plasma MDA, a marker of lipid peroxidation, with no change in SOD levels compared with controls (Mancini et al., 2016). In our study, we demonstrated higher MDA levels and reduced SOD activity in the hippocampus and PFC of hypothyroid offspring. In line with our results, previous studies showed that inducing maternal hypothyroidism markedly reduced offspring SOD, catalase (CAT), and glutathione and increased lipid peroxidation (MDA) in the cortex and cerebellum of 3-week-old postnatal rats (Ahmed et al., 2012). Likewise, maternal hypothyroidism increased hypoxia-inducible factor 1-alpha, HIF-1α, MDA, and ROS in the placental and decidual tissue on the 14th and 18th day of gestation, which demonstrated that in hypothyroid females, the fetal-placental interface is associated with hypoxia and dysregulation of anti-oxidant enzymes and increased OS (Dos Anjos Cordeiro et al., 2022).

In the next part, our results showed that maternal hypothyroidism altered the expression levels of proinflammatory cytokines in offspring. We demonstrated a considerable rise in TNFα and IL-6 levels in both the hippocampus and PFC, on day 60th postnatal. Previous studies also reported increases in pro-inflammatory cytokines IL-1β, IL-6, IL-17A, and TNFα in the blood of both offspring sexes, and an elevation of IL-17 in the prefrontal and hippocampus of male offspring of hypothyroidism mouse dams (González-Madrid et al., 2024). In another study which hypothyroidism was induced in the rat with PTU, an increase in the oxidative factors, such as MDA, decrease in the anti-oxidative enzymes like SOD1, CAT, and glutathione peroxidase (GPx), in addition to the elevation in the inflammatory factors (TNFα, IL-6, IL-1β,and NOD-, LRR- and pyrin domain-containing protein 3[NLRP3]) was demonstrated in the placenta and decidua on the 18th day of gestation (Dos Anjos Cordeiro et al., 2024).

Several studies point to the impact of maternal hypothyroidism on defects in fetal and offspring brain development and cognitive functions. Previous studies clearly show that the cognitive decline or impairments seen in the offspring of hypothyroid humans or animals, such as rats, are most likely related to impairments in the growth factors during gestation. One of the most important growth factors for brain cognitive function is BDNF (Asadian et al., 2022). Regarding the subject, previous studies reported that pregnant female rats treated for hypothyroidism during gestation gave birth to offspring with lower hippocampal BDNF levels (Chakraborty et al., 2012) or hypermethylation of the BDNF promoter in offspring of hypothyroxinemia dams (Kawahori et al., 2018). Furthermore, the role of microRNA-206 (miRNA-206) as a post-transcriptional inhibitor of the BDNF gene in hypothyroid pregnant rats has been studied (Xing et al., 2018). Also, maternal hypothyroidism is accompanied by cognitive impairments, such as a decline in learning and memory, along with suppression of BDNF (Shafiee et al., 2016). Similarly, in a model of subclinical hypothyroidism, poor spatial memory performance in the Morris Water Maze was observed, along with reduced BDNF levels in the hippocampus and a lower amplitude of EPSP on days 10 and 13 postnatal (Wang et al., 2012).

In this study, in addition to PTU treatment, we administered vitamin D at 5 and 10 µg/kg during gestation (G1) or postnatally (G2). We found that both doses of vitamin D altered lipid peroxidation, pro-inflammatory activation, and BDNF levels in the hippocampus and PFC of offspring in both G1 and G2 groups.

In line with our study results, previous studies indicate that vitamin D is an effective anti-oxidant and anti-inflammatory factor by significantly increasing anti-oxidative enzymes GPx, SOD, and reducing IL-6 and TNFα in the PFC and hippocampus of the rat brain exposed to chronic stress (Sedaghat et al., 2021). Also, in a rat hippocampus injected with lipopolysaccharide (LPS), vitamin D decreased IL-6 and MDA and increased SOD, CAT, and total thiol (Mokhtari-Zaer et al., 2020). Likewise, another study reported that vitamin D suppressed the immune activation of microglia previously activated by Interferon γ and LPS, and reduced the release of pro-inflammatory factors IL-6, IL-12, and TNFα, and increased the release of IL-10 as an anti-inflammatory cytokine (Boontanrart et al., 2016).

Previous studies have emphasized the role of vitamin D in brain development, specifically in the cortex and hippocampus, by regulating the proliferation and differentiation of neurons in cooperation with nerve growth factors (Lardner, 2015). In a mouse model of post-stroke depression, vitamin D increased vitamin D receptor (VDR) and elevated BDNF levels and improved cardinal depression symptoms, including anhedonia and helplessness (Xu et al., 2021). Another study indicated that levels of BDNF, T4, and vitamin D are important for the prognosis of cognitive impairment in patients with hypothyroidism (Kamyshna et al., 2022). Furthermore, vitamin D's positive role in elevating BDNF levels has been demonstrated in a couple of previous studies, such as the alleviation of depressive-like behaviors (Sedaghat et al., 2019; Yang et al., 2024) or improving the symptoms and glucose levels in diabetic models (Alqudah et al., 2022), which are indicators of the relation between this vitamin and BDNF in neural development, integrity, and function.

Conclusion

This study points to an interesting finding that offspring from a hypothyroid mother show higher levels of the pro-inflammatory and oxidative and lower levels of anti-oxidative and neurotrophic factors. Changes in the blood chemistry may be well related to poor cognitive abilities. Administering vitamin D might help maintain the cognitive function-related abilities of brain regions that were harmed during gestation by low levels of maternal THs, pre- or postnatally, and dose-dependently.

Study limitations

In this study, we used male rat offspring, whereas female offspring may present congenital hypothyroidism defects in different ways, or their response to vitamin D during gestation or after may be altered at various time points in the female hormonal cycle. In addition, studying neuronal changes in size, dendrite arborization and number would be a valuable addition to present structural defects in brain cells under the lack of gestational THs, and how they might be able to survive or reconstruct under the neuroprotective effect of vitamin D. Furthermore, it would be worthwhile to measure vitamin D levels in different groups of offspring and hypothyroid dam to study the impact of hypothyroidism on serum vitamin D levels also, assessing wider range of vitamin D as a dose-response study to gain better understanding of intervention of vitamin D with THs deficit during gestation.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Semnan University of Medical Sciences, Semnan, Iran (Code: IR.SEMUMS.REC.1400.015).

Data availability

The data used to support the findings of this study is available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

"Grammarly" was used for checking and correction of the grammatical errors in the text.

Funding

This work was supported by the Semnan University of Medical Sciences, Semnan, Iran (Grant No.: 1882).

Authors' contributions

Conceptualization, investigation, resources, writing, supervision, and funding acquisition: Katayoun Sedaghat; Methodology, software, validation, formal analysis, data curation, visualization, and project administration: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors thank the Sorena Laboratory for assisting with the ELISA studies.

References

Ahmed, O. M., Ahmed, R. G., El-Gareib, A. W., El-Bakry, A. M., & Abd El-Tawab, S. M. (2012). Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: II-the developmental pattern of neurons in relation to oxidative stress and antioxidant defense system. *International Journal of Developmental Neuroscience: the Official Journal of the International Society for Developmental Neuroscience, 30*(6), 517–537. [DOI:10.1016/j.ijdevneu.2012.04.005] [PMID]

Alcaide Martin, A., & Mayerl, S. (2023). Local thyroid hormone action in brain development. *International Journal of Molecular Sciences, 24*(15), 12352. [DOI:10.3390/ijms241512352] [PMID]

Alqudah, M., Khanfar, M., Alfaqih, M. A., Al-Shboul, O., Ghazi Al-U'Datt, D., & Al-Dwairi, A., et al. (2022). Correlation between vitamin D and serum brain derived neurotropic factor levels in type 2 diabetes mellitus patients. *Biomedical Reports, 16*(6), 54. [DOI:10.3892/br.2022.1537] [PMID]

Amano, I., Takatsuru, Y., Khairinisa, M. A., Kokubo, M., Haijima, A., & Koibuchi, N. (2018). Effects of mild perinatal hypothyroidism on cognitive function of adult male offspring. *Endocrinology, 159*(4), 1910–1921. [DOI:10.1210/en.2017-03125] [PMID]

Asadian, N., Parsaie, H., Vafaei, A. A., Dadkhah, M., Omoumi, S., & Sedaghat, K. (2022). Chronic light deprivation induces different effects on spatial and fear memory and hippocampal BDNF/TRKB expression during light and dark phases of rat diurnal rhythm. *Behavioural Brain Research, 418,* 113638. [DOI:10.1016/j.bbr.2021.113638] [PMID]

Bakalov, D., Iliev, P., Sabit, Z., Tafradjiiska-Hadjiolova, R., & Bocheva, G. (2023). "Attenuation of hypothyroidism-induced cognitive impairment by modulating serotonin mediation." *Veterinary Sciences, 10*(2), 122. [DOI:10.3390/vetsci10020122] [PMID]

Barbalho, S. M., Sloan, L. A., Araujo, A. C., Laurindo, L. F., & Sloan, K. P. (2024). Vitamin D and its role on inflammation, oxidative stress and cardiovascular disease. In P. S. Tappia, A. K. Shah & N. S. Dhalla (Ed.), *Lipophilic Vitamins in Health and Disease. Advances in Biochemistry in Health and Disease, vol 28.* Cham: Springer. [DOI:10.1007/978-3-031-55489-6_15]

Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: A one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nature Protocols, 1*(3), 1306-1311. [DOI:10.1038/nprot.2006.205] [PMID]

Boontanrart, M., Hall, S. D., Spanier, J. A., Hayes, C. E., & Olson, J. K. (2016). Vitamin D3 alters microglia immune activation by an IL-10 dependent SOCS3 mechanism. *Journal of Neuroimmunology, 292,* 126–136. [DOI:10.1016/j.jneuroim.2016.01.015] [PMID]

Chakraborty, G., Magagna-Poveda, A., Parratt, C., Umans, J. G., MacLusky, N. J., & Scharfman, H. E. (2012). Reduced hippocampal brain-derived neurotrophic factor (BDNF) in neonatal rats after prenatal exposure to propylthiouracil (PTU). *Endocrinology, 153*(3), 1311–1316. [DOI:10.1210/en.2011-1437] [PMID]

Chamas, L., Seugnet, I., Poirier, R., Clerget-Froidevaux, M. S., & Enderlin, V. (2022). A Fine Regulation of the Hippocampal Thyroid Signalling Protects Hypothyroid Mice against Glial Cell Activation. *International Journal of Molecular Sciences, 23*(19), 11938. [DOI:10.3390/ijms231911938] [PMID]

Cordner, Z. A., & Tamashiro, K. L. (2015). Effects of high-fat diet exposure on learning & memory. *Physiology & Behavior, 152*(Pt B), 363–371. [DOI:10.1016/j.physbeh.2015.06.008] [PMID]

Cui, X., & Eyles, D. W. (2022). Vitamin D and the Central Nervous System: Causative and Preventative Mechanisms in Brain Disorders. *Nutrients, 14*(20), 4353. [DOI:10.3390/nu14204353] [PMID]

Dewi, M. M., Imron, A., Risan, N. A., Mediana, G., Judistiani, R. T. D., & Setiabudiawan, B. (2025). The Association of Vitamin D, Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), and Glial Cell-Derived Neurotrophic Factor (GDNF) with development in children. *Children (Basel, Switzerland), 12*(1), 60. [DOI:10.3390/children12010060] [PMID]

Dos Anjos Cordeiro, J. M., Santos, L. C., de Oliveira, L. S., Santos, B. R., Santos, E. O., & Barbosa, E. M., et al. (2022). Maternal hypothyroidism causes oxidative stress and endoplasmic reticulum stress in the maternal-fetal interface of rats. *Free Radical Biology & Medicine, 191,* 24–39. [DOI:10.1016/j.freeradbiomed.2022.08.033] [PMID]

Dos Anjos Cordeiro, J. M., Santos, L. C., Santos, B. R., de Jesus Nascimento, A. E., Santos, E. O., & Barbosa, E. M., et al. (2024). Manganese porphyrin-based treatment improves fetal-placental development and protects against oxidative damage and NLRP3 inflammasome activation in a rat maternal hypothyroidism model. *Redox Biology, 74,* 103238.[DOI:10.1016/j.redox.2024.103238] [PMID]

Gilbert, M. E., & Lasley, S. M. (2013). Developmental thyroid hormone insufficiency and brain development: A role for brain-derived neurotrophic factor (BDNF)?. *Neuroscience, 239,* 253–270. [DOI:10.1016/j.neuroscience.2012.11.022] [PMID]

Gilbert, M. E., Sanchez-Huerta, K., & Wood, C. (2016). Mild thyroid hormone insufficiency during development compromises activity-dependent neuroplasticity in the hippocampus of adult male rats. *Endocrinology, 157*(2), 774–787. [DOI:10.1210/en.2015-1643] [PMID]

González-Madrid, E., Rangel-Ramírez, M. A., Opazo, M. C., Méndez, L., Bohmwald, K., & Bueno, S. M., et al. (2024). Gestational hypothyroxinemia induces ASD-like phenotypes in behavior, proinflammatory markers, and glutamatergic protein expression in mouse offspring of both sexes. *Frontiers in Endocrinology, 15,* 1381180. [DOI:10.3389/fendo.2024.1381180] [PMID]

Hipólito, L. T. M., Batista, T. H., Dos Anjos-Garcia, T., Giusti-Paiva, A., & Vilela, F. C. (2023). Methimazole-induced gestational hypothyroidism affects the offspring development and differently impairs the conditioned fear in male and female adulthood rodents. *International Journal of Developmental Neuroscience: The Official Journal of The International Society for Developmental Neuroscience, 83*(1), 108–120. [DOI:10.1002/jdn.10243] [PMID]

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative stress and inflammation: what polyphenols can do for us? *Oxidative Medicine and Cellular Longevity, 2016,* 7432797. [DOI:10.1155/2016/7432797] [PMID]

Kampouri, M., Margetaki, K., Koutra, K., Kyriklaki, A., Karakosta, P., & Anousaki, D., et al. (2021). Maternal mild thyroid dysfunction and offspring cognitive and motor development from infancy to childhood: The Rhea mother-child cohort study in Crete, Greece. *Journal of Epidemiology and Community Health, 75*(1), 29–35. [DOI:10.1136/jech-2019-213309] [PMID]

Kamyshna, I. I., Pavlovych, L. B., & Kamyshnyi, A. M. (2022). Prediction of the cognitive impairment development in patients with autoimmune thyroiditis and hypothyroidism. *Endocrine Regulations, 56*(3), 178–189. [DOI:10.2478/enr-2022-0019] [PMID]

Kawahori, K., Hashimoto, K., Yuan, X., Tsujimoto, K., Hanzawa, N., & Hamaguchi, M., et al. (2018). Mild maternal hypothyroxinemia during pregnancy induces persistent DNA hypermethylation in the hippocampal brain-derived neurotrophic factor gene in mouse offspring. *Thyroid: Official Journal of the American Thyroid Association, 28*(3), 395–406. [DOI:10.1089/thy.2017.0331] [PMID]

Lally, J., & Gaughran, F. (2019). Vitamin D in schizophrenia and depression: A clinical review. *BJPsych Advances, 25*(4), 240-248. [DOI:10.1192/bja.2019.10]

Lardner, A. L. (2015). Vitamin D and hippocampal development-the story so far. *Frontiers in Molecular Neuroscience, 8,* 58. [DOI:10.3389/fnmol.2015.00058] [PMID]

Lasa, M., & Contreras-Jurado, C. (2022). Thyroid hormones act as modulators of inflammation through their nuclear receptors. *Frontiers in Endocrinology, 13,* 937099. [DOI:10.3389/fendo.2022.937099] [PMID]

Lasley, S. M., & Gilbert, M. E. (2011). Developmental thyroid hormone insufficiency reduces expression of brain-derived neurotrophic factor (BDNF) in adults but not in neonates. *Neurotoxicology and Teratology, 33*(4), 464–472. [DOI:10.1016/j.ntt.2011.04.001] [PMID]

Madhusudhan, U., Singaravelu, M, K., V., Ganji, V., John, N., & Gaur, A. (2022). Brain-derived neurotrophic factor-mediated cognitive impairment in hypothyroidism. *Cureus, 14*(4), e23722. [DOI:10.7759/cureus.23722]

Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., & Meucci, E., et al. (2016). Thyroid hormones, oxidative stress, and inflammation. *Mediators of Inflammation, 2016,* 6757154. [DOI:10.1155/2016/6757154] [PMID]

Menéndez, S. G., & Manucha, W. (2024). Vitamin D as a Modulator of Neuroinflammation: Implications for Brain Health. *Current Pharmaceutical Design, 30*(5), 323–332. [DOI:10.2174/0113816128281314231219113942] [PMID]

Mokhtari-Zaer, A., Hosseini, M., Salmani, H., Arab, Z., & Zareian, P. (2020). Vitamin D3 attenuates lipopolysaccharide-induced cognitive impairment in rats by inhibiting inflammation and oxidative stress. *Life Sciences, 253,* 117703. [DOI:10.1016/j.lfs.2020.117703] [PMID]

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., & Buga, A. M. (2013). ROS and brain diseases: The good, the bad, and the ugly. *Oxidative Medicine and Cellular Longevity, 2013,* 963520. [DOI:10.1155/2013/963520] [PMID]

Ramos-González, E. J., Bitzer-Quintero, O. K., Ortiz, G., Hernández-Cruz, J. J., & Ramírez-Jirano, L. J. (2024). Relationship between inflammation and oxidative stress and its effect on multiple sclerosis. *Neurologia, 39*(3), 292–301.[DOI:10.1016/j.nrleng.2021.10.010] [PMID]

Rutigliano, G., Bertolini, A., Grittani, N., Frascarelli, S., Carnicelli, V., & Ippolito, C., et al. (2023). Effect of combined levothyroxine (L-T4) and 3-Iodothyronamine (T1AM) supplementation on memory and adult hippocampal neurogenesis in a mouse model of hypothyroidism. *International Journal of Molecular Sciences, 24*(18), 13845. [DOI:10.3390/ijms241813845] [PMID]

Sahoo, D. K., Roy, A., Bhanja, S., & Chainy, G. B. (2008). Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. *General and Comparative Endocrinology, 156*(1), 63–70. [DOI:10.1016/j.ygcen.2007.11.007] [PMID]

Sanaiee, A., Hassanpour, S., & Vazir, B. (2024). Protective role of the ginsenoside Rg1 against methimazole-induced gestational hypothyroidism on reflexive behaviors, conditioned fear and cortical antioxidant levels in mice offspring. *IBRO neuroscience Reports, 16,* 485–496. [DOI:10.1016/j.ibneur.2024.03.010] [PMID]

Sedaghat, K., Naderian, R., Pakdel, R., Bandegi, A. R., & Ghods, Z. (2021). Regulatory effect of vitamin D on pro-inflammatory cytokines and anti-oxidative enzymes dysregulations due to chronic mild stress in the rat hippocampus and prefrontal cortical area. *Molecular Biology Reports, 48*(12), 7865–7873. [DOI:10.1007/s11033-021-06810-2] [PMID]

Sedaghat, K., Yousefian, Z., Vafaei, A. A., Rashidy-Pour, A., Parsaei, H., Khaleghian, A., & Choobdar, S. (2019). Mesolimbic dopamine system and its modulation by vitamin D in a chronic mild stress model of depression in the rat. *Behavioural Brain Research, 356,* 156–169. [DOI:10.1016/j.bbr.2018.08.020] [PMID]

Sedaghat, K., Zahediasl, S., & Ghasemi, A. (2015). Intrauterine programming. *Iranian Journal of Basic Medical Sciences, 18*(3), 212–220. [PMID]

Shafiee, S. M., Vafaei, A. A., & Rashidy-Pour, A. (2016). Effects of maternal hypothyroidism during pregnancy on learning, memory and hippocampal BDNF in rat pups: Beneficial effects of exercise. *Neuroscience, 329,* 151–161. [DOI:10.1016/j.neuroscience.2016.04.048] [PMID]

Wang, S., Teng, W., Gao, Y., Fan, C., Zhang, H., & Shan, Z. (2012). Early levothyroxine treatment on maternal subclinical hypothyroidism improves spatial learning of offspring in rats. *Journal of Neuroendocrinology, 24*(5), 841–848. [DOI:10.1111/j.1365-2826.2011.02275.x] [PMID]

Williams G. R. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone. *Journal of Neuroendocrinology, 20*(6), 784–794. [DOI:10.1111/j.1365-2826.2008.01733.x] [PMID]

Wimalawansa S. J. (2019). Vitamin D Deficiency: Effects on Oxidative Stress, Epigenetics, Gene Regulation, and Aging. *Biology, 8*(2), 30. [DOI:10.3390/biology8020030] [PMID]

Xing, Q., Shan, Z., Gao, Y., Mao, J., Liu, X., & Yu, J., et al. (2018). Differential expression of MicroRNAs and miR-206-mediated downregulation of BDNF expression in the rat fetal brain following maternal hypothyroidism. *Hormone and Metabolic Research = Hormon- und Stoffwechselforschung = Hormones et Metabolisme, 50*(9), 696–703. [DOI:10.1055/a-0658-2095] [PMID]

Xu, Y., & Liang, L. (2021). Vitamin D3/vitamin D receptor signaling mitigates symptoms of post-stroke depression in mice by upregulating hippocampal BDNF expression. *Neuroscience Research, 170,* 306–313. [DOI:10.1016/j.neures.2020.08.002] [PMID]

Yang, X., Miao, J., Huang, Y., Li, L., & Zhuang, G. (2024). Preventive and therapeutic effect of vitamin D on depression-like behavior in a mouse adolescent depression model and its association with BDNF protein expression. F*rontiers in Psychiatry, 15,* 1425681. [DOI:10.3389/fpsyt.2024.1425681] [PMID]