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The Effect of Intermittent Fasting and Water Deprivation on Brain Growth Factors

Seyed-Hosein Abtahi-Eivary[1], Seyed-Mohammad Abtahi-Eivary[2*], Farideh Akbari[3], Elham Hassan Nezhad[4]

Abstract

Objectives: Intermittent fasting has beneficial effects on health through changing the level of growth factors. Regarding the controversy in the effect of fasting on growth factors and the possible role of age and gender in this regard, we aimed to investigate the association between fasting (with or without water deprivation) and growth factors such as brain-derived neurotrophic factor (BDNF) and its precursor (proBDNF), as well as glial cell line-derived neurotrophic factor (GDNF).

Material and Methods: In this experimental study, 84 Wistar rats (21 male immature, 21 males mature, 21 female immature, and 21 females mature) were divided into 12 equal groups (n=7 in each) based on the treatment method (control/fasting with access to water/fasting without access to water). While control groups had free access to food and water, fasting groups were deprived from food or food and water for 12 hours a day. After three weeks of treatment, serum levels of BDNF, proBDNF, and GDNF were determined using the enzyme-linked immunosorbent assay (ELISA) method and analyzed by SPSS Software version 13.

Results: Fasting was associated with significantly higher levels of BDNF, proBDNF, and GDNF in male rats. Also, there was no significant difference when water deprivation was added to fasting.

Conclusion: The effect of fasting on the levels of BDNF, proBDNF, and GDNF was gender-dependent and could be explained by the structural differences of brain tissues between male and female rats.

Keywords: Intermittent fasting, Water deprivation, Growth factors, BDNF, ProBDNF, GDNF, Rat

Introduction

Dietary interventions, such as calorie restriction and intermittent fasting have been shown to have beneficial effects on our health and longevity (1-3). Many studies have been conducted on how calorie restriction and intermittent fasting are associated with increased levels of known growth factors, especially brain-derived neurotrophic factor (BDNF) (4,5). BDNF is a member of neurotrophins which is expressed in various tissues such as central (specifically hypothalamus and hippocampus) and peripheral nervous system (6,7), skeletal muscle (8), cardiac muscle (9), endothelial cells (10), liver (6), adipose tissue (11), and platelets (12). BDNF is also detectable in circulating blood and is positively correlated with the level of BDNF in brain tissues, especially hippocampus (13).

Recently, the role of BDNF as an energy regulator has been noticed. The ventromedial and dorsomedial nuclei of hypothalamus are responsible for controlling appetite (14). Studies have shown that BDNF and its receptor, tyrosine kinase B (TrkB), are highly concentrated in these areas (14). Leptin, which is a protein secreted from adipose tissue in satiety, and is widely known as an anorexigenic factor, was shown to control appetite through increasing BDNF in hypothalamus (15). Moreover, intracerebroventricular and peripheral injection of BDNF have resulted in hypophagia and hypoglycemia; so, it has been hypothesized that BDNF normally suppresses appetite (16,17).

There are also other possible BDNF-involved mechanisms responsible for energy regulation in tissues other than brain, including pancreas, liver, skeletal muscle, and adipose tissue. BDNF increases insulin and decreases glucagon concentration in pancreatic cells (18). BDNF also is involved in insulin signaling pathways and is associated with higher insulin sensitivity of liver, skeletal muscles, and brown adipose tissue (19). Interestingly, under the condition of metabolic stress such as exercise (high energy demand) and fasting (low energy intake), the expression of BDNF is increased possibly because of an evolutionary advantage. But acute energy restriction and chronic energy excess result in lower BDNF levels (20).

Currently, there is controversy regarding the association between fasting and BDNF levels in different genders and ages. Kishi and Sunagawa reported that calorie restriction

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[1]Department of Biochemistry and Nutrition, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran. [2]Student Research Committee, Tehran University of Medical Sciences, Tehran, Iran. [3]Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. [4]Clinical Research Development Unit of Tabriz Valiasr Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.
*Corresponding Author: Seyed-Mohammad Abtahi-Eivary, Tel: +989108349319; Email: a.abtahi_51@yahoo.com

Key Message

- The fasting effect on brain growth factor is related to gender.

upregulates BDNF in hippocampus when combined with exercise but alone, it does not increase BDNF (21). Khabour et al compared the effect of exercise and fasting on hippocampal BDNF expression and revealed that despite forced swimming exercise, every-other-day fasting did not enhance memory function or hippocampal BDNF expression (22). In another study, hypothalamic BDNF expression was reduced under fasting condition in both male and female rats on postnatal days 10 and 30 (15). Varendi et al reported gender differences of BDNF level in response to fasting. In their study, unlike males, in estrous female rats, there was no significant change in ventromedial hypothalamus (VMH) BDNF expression under the condition of fasting or high fat diet (23).

The precursor of BDNF (proBDNF) yields in mature BDNF by intra- and extra-cellular cleaving enzymes (24). BDNF and proBDNF are considered as vital factors in normal and physiologic development of nervous system in first ages of life, as well as hippocampal neurogenesis in adults. Any imbalance between these factors leads to pathological mental conditions such as Alzheimer's disease, schizophrenia, bipolar disorder, and autism (25). So, as experimental studies suggest, BDNF and proBDNF are tightly intertwined with fundamental brain functions, including memory and learning (26). Despite many studies on the relationship between fasting and BDNF, our knowledge on the effect of fasting on proBDNF is very limited.

Alike BDNF, glial cell line-derived neurotrophic factor (GDNF) is a neurotrophin, which is synthesized and then secreted by glial cells in the central nervous system (CNS) and peripheral nervous system. GDNF promotes neural growth and differentiation, especially dopaminergic and motor neurons (27). The association between this growth factor and fasting is not clearly understood due to the lack of enough data.

Ramadan fasting is a special type of intermittent fasting along with water deprivation. According to Islamic regulations, every year in Ramadan month, all Muslims are obligated to abstain from food and water from sunrise to sunset for one month. Similar to fasting, dehydration and high blood osmolarity have been shown to increase BDNF in brain, especially hypothalamus (28). Some studies explored the effect of Ramadan fasting on BDNF (29,30) and reported increased BDNF level (30). However, our knowledge is limited in case of other growth factors.

This experimental study aimed to evaluate the effect of fasting on the level of some important brain growth factors, including BDNF, proBDNF, and GDNF. Also, we simulated the condition of Ramadan fasting and explored any possible difference with regular intermittent fasting. The effect of gender and age on the association between fasting and growth factors was also investigated.

Material and Methods

Study Design

In this experimental study, 84 Wistar rats (21 mature male, 21 mature female, 21 immature males, and 21 immature female) were divided into 12 equal groups (n=7 in each) as follows: four control groups, four groups of fasting with water access, and four groups of fasting without water access. According to Sengupta, we considered the rats with the weight <150 g as immature (31). Each group was treated for 3 weeks under specific circumstances as follows: four groups had free access to food and water all the time (control), four groups were fasted for 12 hours a day but had free access to water (fasting with water access), and four groups were deprived from food and water for 12 hours a day (fasting without water access). At the end of the third week, all rats were fasted daily for 12 hours, though they had free access to water. Blood samples were taken from left ventricle after anesthetizing by intraperitoneal (IP) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Serum samples were kept at -70°C until assaying. Wistar rats (age: eight weeks) with a weight of 230 ± 10 g were stored at a constant temperature of 25 ± 2°C and 30-70% humidity with 12-h light/12-h dark cycle in the animal room with free access to food and water.

Biochemical Parameters

Serum levels of the BDNF were determined using the Eastbiopharm-BDNF-Rat enzyme-linked immunosorbent assay (ELISA) kit (Eastbiopharm, China), and serum levels of proBDNF were determined by Eastbiopharm-BDNF Rat ELISA Kit, and Eastbiopharm-GDNF-Rat ELISA Kit measured GDNF levels.

Statistical Analyses

The results were analyzed using SPSS Software version 13. To evaluate the association between types of treatment and response variables, one-way analysis of variances (ANOVA) was performed. Moreover, two-way ANOVA was considered for investigating any possible interaction between age and gender.

Results

The mean and standard deviation (SD) of response variables in each of 12 groups are shown in Table 1. Regarding the serum level of BDNF, only mature male rats fasted without access to water had significantly higher levels than controls (Figure 1A). Compared to control groups, the proBDNF level was significantly increased in male immature treatment groups, male mature rats fasted without access to water, and female mature rats fasted without access to water. In female mature groups, fasting without access to water was associated with significantly

Table 1. Mean, Standard Deviation (SD), and P Value of BDNF, proBDNF, the Ratio of proBDNF/BDNF, and GDNF in Experimental and Control Groups

	BDNF (ng/mL)		ProBDNF (ng/L)		proBDNF/BDNF (x1000)		GDNF (ng/mL)	
	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]
Male immature control	0.30 ± 0.04	1	34.55 ± 3.19	1	114.75 ± 10.78	1	0.42 ± 0.05	1
Male immature fasting + water	0.76 ± 0.20	0.145	105.47 ± 22.26	0.024	139.95 ± 10.04	0.674	1.26 ± 0.34	0.016
Male immature fasting - water	0.78 ± 0.21	0.105	122.72 ± 24.15	0.002	161.05 ± 24.31	0.022	1.34 ± 0.30	0.005
Male mature control	0.32 ± 0.04	1	41.05 ± 2.46	1	129.47 ± 15.90	1	0.53 ± 0.14	1
Male mature fasting + water	0.65 ± 0.22	0.818	116.98 ± 20.15	0.073	185.89 ± 30.13	0.02	1.52 ± 0.34	0.018
Male mature fasting - water	0.94 ± 0.26	0.017	160.50 ± 32.96	0	176.84 ± 35.13	0.029	1.79 ± 0.35	0
Female immature control	0.96 ± 0.34	1	152.77 ± 67.39	1	120.91 ± 13.40	1	1.61 ± 0.27	1
Female immature fasting + water	0.76 ± 0.30	0.994	109.02 ± 40.20	0.766	146.54 ± 12.06	0.934	2.08 ± 0.36	1
Female immature fasting - water	0.84 ± 0.42	1	179.43 ± 57.17	0.991	175.22 ± 25.88	0.091	1.27 ± 0.38	0.978
Female mature control	1.05 ± 0.20	1	152.10 ± 36.57	1	143.71 ± 13.41	1	1.96 ± 0.55	1
Female mature fasting + water	1.16 ± 0.37	1	144.91 ± 41.68	1	127.59 ± 17.70	0.987	1.96 ± 0.54	1
Female mature fasting - water	1.48 ± 0.41	0.308	229.79 ± 30.69	0.023	151.29 ± 31.68	1	2.13 ± 0.69	1

[a] ANOVA test.

higher proBDNF level than fasting with access to water ($P=0.005$) (Figure 1B). Compared to control counterparts, the ratio of proBDNF/BDNF was significantly higher in male immature rats fasted without access to water, as well as male mature treatment groups (Figure 1C). The GDNF level in all male groups significantly increased compared to controls. However, this effect was not seen in any of the female groups (Figure 1D).

According to our results, age had no effect on the association between treatment and response variables. Nonetheless, gender had an effect on the association between treatment and BDNF level in immature rats ($P=0.014$). Considering proBDNF, gender in immature ($P=0.006$) and mature rats ($P=0.024$) showed a significant effect. Moreover, gender had a significant effect on the association between fasting and proBDNF/BDNF ratio in mature rats ($P=0.017$). Regarding GDNF, gender in both immature ($P<0.001$) and mature rats ($P=0.009$) had a significant effect (Figure 2).

Discussion

This study explored the association between serum levels of BDNF, proBDNF, and GDNF and fasting with/without water deprivation. We also showed the possible differences between male/female and immature/mature rats in response to fasting. The major findings of this study include: (i) fasting did not increase serum BDNF levels significantly except in mature male rats fasted without water access (Table 1); (ii) fasting significantly increased serum proBDNF levels except in immature female rats (Table 1); (iii) fasting significantly increased GDNF levels in mature and immature male rats, but this effect was not seen in females (Table 1).

In this study, serum BDNF level increased in almost all treatment groups, though it was significant only in mature male rats fasted without access to water. Lee et al reported that a 3-week dietary restriction increased BDNF levels and improved adult hippocampal neurogenesis in wild-type mice (BDNF+/+). However, this effect was lower in heterozygous BDNF knock-out mice (BDNF+/-) (32). Kishi et al reported that calorie restriction could protect against cognitive decline via up-regulation of BDNF/TrkB through its antioxidant effect in the hippocampus of dietary-induced obesity rats (33). Dasgupta et al found that BDNF and PRKCZ genes (which are neuronal metaplasticity genes) significantly upregulated in the mice fasted for 16 hours or more (and not less) every day for six months (34). These results (only mature male rats) are consistent with previous studies.

From an evolutionary standpoint, trying to find food (exercise) in the situation of food scarcity (fasting) needed better memory and brain function and this advantage has been preserved through evolution (1). Fasting and exercise have been demonstrated to have beneficial health effects, such as improved cognition and memory in humans (35). Fasting and exercise, which are two important metabolic stresses, stimulate neuronal activity in different regions of the brain (36) and enhance brain function through an activity-dependent process (37). The dominant neurotransmitter involved in active synapses is glutamate which causes Na^+ and Ca^{2+} influx into the neurons. The level of N-methyl-D-aspartate receptor, a Ca^{2+} channel indicating higher synaptic plasticity, is shown to be increased following energy restriction in middle-aged and old rats (38). However, higher Ca^{2+} concentration within neurons is associated with much more superoxide and nitric oxide production (oxidative stress). Some mechanisms have been discovered to counteract these unfavored effects. The complex of Ca^{2+}-calmodulin activates two important enzymes: Ca^{2+}-calmodulin dependent protein kinase 2 and mitogen-activated protein kinase. These enzymes trigger multiple pathways responsible for adaptive synapse response, antioxidant protection, and gene expressions including

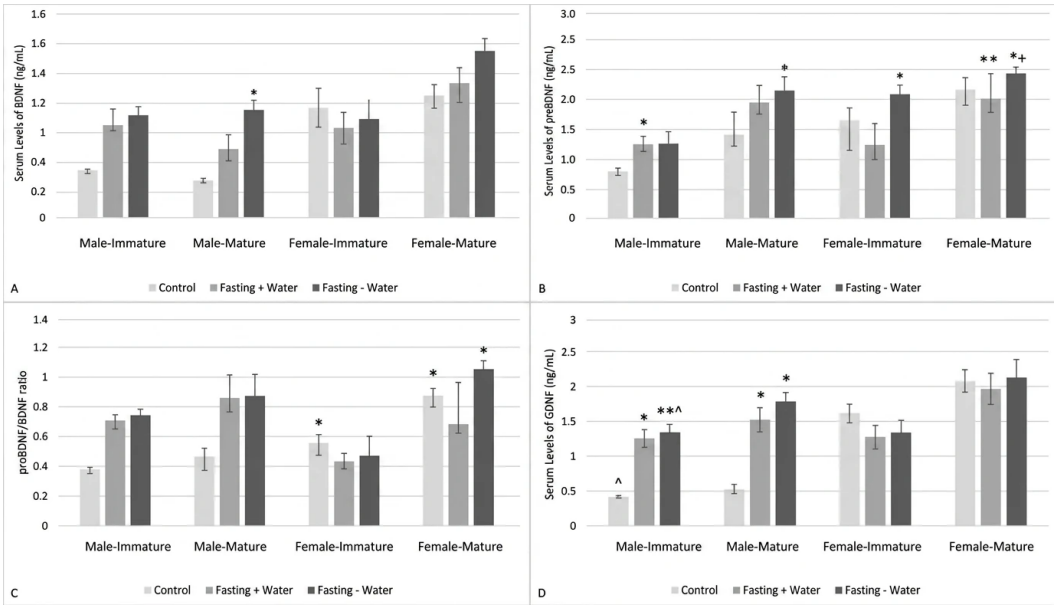

Figure 1. The amount of BDNF (A), proBDNF (B), the ratio of proBDNF/BDNF (C), and GDNF (D) compared between control, fasting + water, and fasting-water groups. The asterisk (*) and cross (†) signs show the significant difference compared to control and fasting + water groups, respectively.

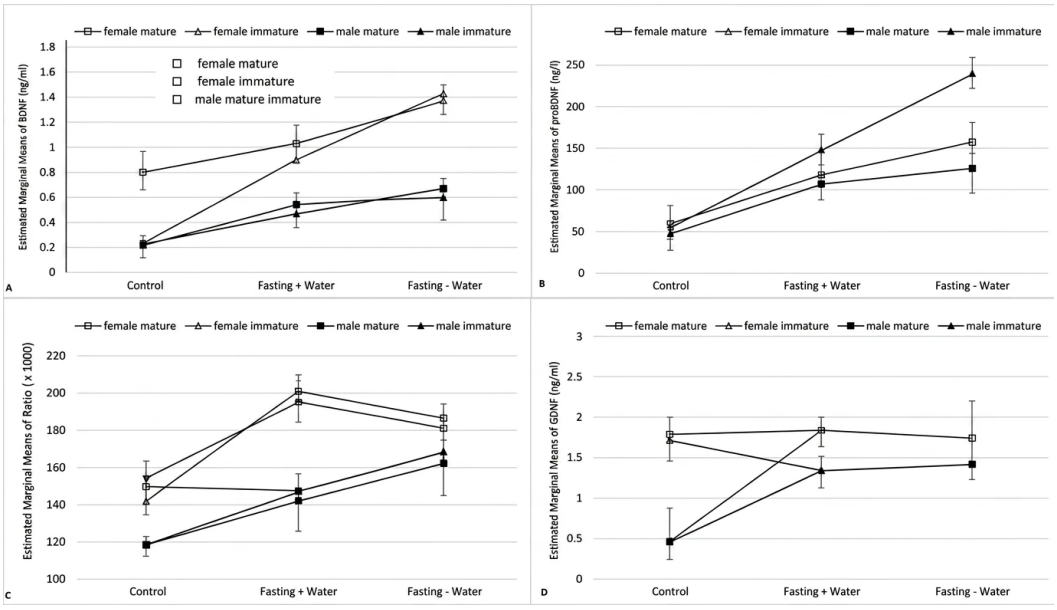

Figure 2. The Effect of Age and Gender on the Association of Treatment and BDNF, proBDNF, proBDNF/BDNF Ratio, and GDNF.

BDNF in active neurons (1,20). Overall, fasting results in oxidative stress, which is then neutralized by antioxidant pathway involving BDNF.

ProBDNF turns into mature BDNF form after intracellular enzymatic cleavage and is released from neurons. ProBDNF is also released directly into the extracellular space, where it changes to mature form by plasmin and matrix metalloproteinase (MMP) enzymes (39). While mature BDNF is an anti-apoptotic neurotrophic factor needed for cell survival and plasticity, proBDNF induces cell apoptosis by acting on p75NTR receptor (40). These anti- and pro-apoptotic activities are achieved respectively by inhibition and activation of caspase-3, an enzyme which is involved in apoptosis process (41).

Although few studies investigated the association between environmental stressors and proBDNF level, none of them involved chronic intermittent fasting. Bai et al showed that using unpredictable chronic mild stress paradigm (which randomly assigns 12 different environmental stressors including 24-hour water and food deprivation) in rats causes significant increase of proBDNF/p75 and decrease of mature BDNF/TrkB in neocortex and hippocampus of rats (42). In rat models

of depression and anxiety, proBDNF was significantly increased in hippocampus. Intrahippocampal injection of anti proBDNF monoclonal antibody showed protective effects against depression- and anxiety-like behaviors in these animal models (43).

According to the results of our study, chronic intermittent fasting was somehow associated with increased proBDNF level in male and female (not immature female) rats.

As mentioned earlier, proBDNF/BDNF ratio is a key determiner of cellular growth and differentiation or death. So, proBDNF/BDNF ratio is needed to better understand the effect of fasting on BDNF. The proBDNF/BDNF ratio was significantly higher in male rats following treatment, but not in female rats. These results suggest that while fasting causes significant higher BDNF levels in mature male rats, it also increases proBDNF level even with higher intensity; so, the proBDNF/BDNF ratio increases significantly. Therefore, despite the neuroprotective effect of fasting, there are other mechanisms counteracting favorable effects through increasing proBDNF levels following treatment.

In this study we added water deprivation to fasting as a second environmental stressor to see if water restriction alongside calorie restriction causes significant BDNF or proBDNF changes. There was no significant difference in BDNF, proBDNF, and proBDNF/BDNF ratio in the fasting groups with and without access to water, but proBDNF was significantly higher in mature female groups ($P=0.005$) (Figure 1B).

The mechanism by which dehydration and high blood osmolarity regulates BDNF expression has been investigated. Supraoptic nucleus (SON), paraventricular nucleus (PVN), and subfornical organ (SFO) are three distinct neural centers in hypothalamus which form one unique neural circuit responsible for sensing and regulating blood osmolarity via production of arginine vasopressin (AVP) (28). Studies showed the existence and expression of BDNF in these centers (44), as well as the inducing role of dehydration and osmotic stress. The level of BDNF expression in SFO following water deprivation for 24 hours, two days, and four days significantly increased (28). Aliaga et al reported that infusion of hypertonic saline significantly increased BDNF levels in SON and PVN (45). Hindmarch et al performed a microarray analysis of rat SFO following water deprivation for three days and reported that the expression of BDNF gene increased as much as 5x (46). In a study on ovariectomized rats, the animals were treated under stressful condition, including water deprivation in a specific pattern for four weeks; BDNF mRNA level significantly increased in hippocampus following treatment (47). The role of BDNF in osmotic stress is not fully understood, but it seems that BDNF increases AVP production via phosphorylation of TrkB receptor in SON (48).

Based on above-mentioned studies, the inducing effect of dehydration on BDNF expression is seen when dehydrated for at least 24 hours. So, due to the short duration of dehydration in our study (12 hours), there was not a significant difference between the two types of treatment.

The results of this study suggest that the effect of fasting on BDNF, proBDNF, and proBDNF/BDNF ratio was gender-dependent, with greater increases in immature and mature male rats compared to female counterparts (Table 1). Moreover, the effect of gender was more prominent in treatment groups (fasting with/without access to water) compared to controls.

Previous studies showed that under non-stressful circumstances, the amount of BDNF expression in cortex, hippocampus, VMH, and amygdala was significantly higher in female rats compared to males (23,49,50). In human studies, despite similar hippocampal BDNF expression in both genders, there was a higher BDNF expression in women's prefrontal cortex (51). However, there was no significant difference in circulating BDNF between men and women (52).

Interestingly, in immature rats, BDNF expression in response to stressful events is sex-related. Decreased BDNF expression after maternal deprivation (53) and isolation (54) was seen in male rats, but these stressful conditions increased BDNF in female rats (55).

Emerging evidence suggest that BDNF expression is differently regulated in male and female rats in response to environmental stressors such as fasting. Liu et al measured the amount of BDNF expression in VMH of male and female rats maintained under high-fat diet (HFD) as an energy excess condition and low-fat diet (LFD) as an energy scarcity status. Male HFD rats had lower levels of BDNF mRNA in VMH compared to LFD ones, but this association was not seen in females. Similarly, 24-hour fasting decreased BDNF mRNA levels only in male rats (23).

Sex steroids, mainly estrogen, progesterone, and testosterone, are known to have positive effect on BDNF expression (52). Among these hormones, the effect of estrogen on BDNF expression is widely investigated and multiple mechanisms have been identified. Estrogen causes hypomethylation of BDNF promoter (56), increasing Ca^{2+} influx, activating CREB pathway (57,58), and inhibiting the activity of non-ER-β-bearing GABAergic interneurons (59).

Accordingly, the regulation of BDNF in response to environmental stressors is different in male and female rats. In our study, we interestingly found that the increase of BDNF level in response to fasting was sex-dependent in immature rats which normally have low levels of sex steroids while in mature rats, the BDNF response was not significantly different. This might be partially due to the structural differences of various brain tissues between male and female rats. In mature rats, it seems that the presence of sex hormones modulates the effect of fasting on BDNF, especially estrogen which is the female sex

hormone and causes significantly lower BDNF changes in response to fasting in mature females compared to males.

Besides BDNF, we also explored the effect of gender on the association of fasting and proBDNF. The results showed that proBDNF levels increased more significantly in male rats following treatment. The proBDNF/BDNF ratio significantly increased in male rats following treatment; however, the effect of gender on this association was significant only in mature rats (Table 1). It seems that fasting increases proBDNF/BDNF ratio, significantly more in males than females. Therefore, we assume that although fasting increases BDNF, it also increases proBDNF and the proBDNF/BDNF ratio, especially in mature males. Considering the role of proBDNF-BDNF pathway in cell survival and apoptosis, we hypothesize that fasting may cause a trend toward cell apoptosis in male rats. More studies are needed to investigate the exact effect of fasting on cell survival and apoptosis.

According to our results, the effect of age on the association between fasting and BDNF, proBDNF, and proBDNF/BDNF ratio was not significant. Few studies have investigated the effect of age on the level of brain growth factors such as BDNF and proBDNF. Silhol et al determined BDNF level and its receptor (TrkB) in the hippocampus and hypothalamus of male rats from postnatal to old age. The BDNF level in hippocampus was not significantly different between postnatal and older rats, but hypothalamic BDNF was significantly higher in second to fourth weeks of age compared to older ages (60). Similarly, the level of BDNF in pituitary was reported to be significantly higher in postnatal days 7-14 (61). The amount of BDNF in spinal cord was also significantly higher in postnatal days 3-10 compared to adult rats. BDNF levels were also higher in postnatal compared to adult rats after spinal cord injury (62). So, there might be a physiologic difference in BDNF levels in CNS between immature and mature rats under non-stressful conditions; but this difference is prominent only in first days of life and fades after that.

The impact of age on the BDNF response to environmental stressors has been previously investigated. Iwasa et al showed that hypothalamic BDNF mRNA expression decreased during development from neonatal to prepubertal age in both genders. This study also revealed that after 24 hours of fasting (food deprivation), hypothalamic BDNF mRNA expression decreased in postnatal day 10 (neonatal rats) in males and females, but it did not change in postnatal days 20-30 (prepubertal age) (15).

According to the study results, it is assumed that the effect of age on the level of foregone factors under non-stressful and stressful conditions is limited to early ages (i.e., postnatal days). The difference between the results of our study and previous ones might be due to the distinct setting of study, including type of treatment and age of rats. It is worthy to note that the *P*-value of the effect of age on the association between fasting and proBDNF/BDNF ratio in females was near-significant (P=0.076). Comparing female immature and mature rats, mature control group had higher ratio level than immature controls but in treatment groups, it was the opposite. So, in females, maturity brings on a higher baseline ratio level but a slighter response to fasting, which is due to the presence of female sex hormones especially estrogen in mature rats and their regulatory roles in BDNF expression (52) and response to stressful conditions such as fasting.

In this study we also investigated the effect of fasting on the serum levels of GDNF. As Figure 1D shows, there was a significant increase in serum GDNF levels in male immature and mature treatment groups compared to controls. The results also showed that both types of treatment (fasting with or without access to water during fasting) could significantly increase the GDNF levels.

The GDNF level in response to fasting was also gender-dependent. While the level of GDNF in male control groups was significantly lower than female counterparts, fasting caused a significant higher level of GDNF only in male immature ($P<0.001$) and mature ($P=0.009$) treatment groups. This difference is partially due to the structural difference and distinct mechanisms of GDNF production and regulation between male and female tissues, especially brain.

Despite the lack of enough knowledge on the association between fasting and GDNF, according to the results of this study, there was a huge difference between the level of baseline GDNF and its response to fasting between the two genders. Some studies investigated the effect of sex hormones on GDNF expression, and reported that estrogen increased GDNF expression (63,64). However, Enterría-Morales et al reported that estrogen could not induce striatal GDNF expression (65).

Based on our results, age was not a determining factor for the effect of fasting on GDNF, as there was no significant difference between immature and mature rats in response to fasting. This may not be explained considering the effect of sex hormones on GDNF expression.

Conclusions

It seems that fasting can augment the levels of growth factors, including BDNF and GDNF. Gender was an effective factor on this association, as it was shown that fasting significantly increased BDNF only in male mature rats, and GDNF in immature and mature male rats. Fasting also increased proBDNF in immature male rats and mature male and female rats. In this study, the proBDNF/BDNF ratio, which may indicate the balance between cell apoptosis and survival, increased in fasting male rats. There was no significant difference between fasting alone and fasting plus water deprivation regarding BDNF or GDNF. Based on our results, fasting as an environmental stressor increased nervous system growth factors, but it did not have a direct relationship and was

adjusted by other factors such as age and gender. The effect of gender is not limited to post-maturity period. So, we speculate that this is because there are structural differences between genders in various tissues, especially brain, that result in different responses to fasting even in premature rats.

Authors' Contribution

Conceptualization: Seyed-Hosein Abtahi-Eivary.
Data curation: Seyed-Mohammad Abtahi-Eivary, Farideh Akbari.
Formal analysis: Elham Hassan Nezhad.
Funding acquisition: Seyed-Hosein Abtahi-Eivary.
Investigation: Seyed-Mohammad Abtahi-Eivary, Seyed-Hosein Abtahi-Eivary.
Methodology: Seyed-Hosein Abtahi-Eivary.
Project administration: Seyed-Hosein Abtahi-Eivary.
Resources: Seyed-Hosein Abtahi-Eivary.
Software: Elham Hassan Nezhad.
Supervision: Hosein Abtahi-Eivary.
Project administration: Seyed-Hosein Abtahi-Eivary.
Validation: Farideh Akbari- Seyed-Hosein Abtahi-Eivary.
Visualization: Seyed-Hosein Abtahi-Eivary.
Writing–original draft: Seyed-Hosein Abtahi-Eivary.
Writing–review & editing: Seyed-Hosein Abtahi-Eivary, Seyed-Mohammad Abtahi-Eivary.

Conflict of Interests

Authors have no conflict of interest.

Ethical Issues

This study was approved by the Ethical Committee of Gonabad University of Medical Sciences, Iran (code: IR.GMU.REC.1396.73).

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