



## The effect of four-week intermittent fasting from dawn to sunset on circulating brain-derived neurotrophic factor levels in subjects with metabolic syndrome and healthy subjects



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### ABSTRACT

**Background:** Brain-derived neurotrophic factor (BDNF) is a key neurotrophin that regulates food intake and energy hemostasis. BDNF also promotes neurogenesis, neuroplasticity, and neuroprotection. There are conflicting reports regarding how intermittent fasting affects circulating BDNF levels. We tested the hypothesis that 4-week intermittent fasting from dawn to sunset (4-week-IF) would decrease circulating BDNF levels in subjects with metabolic syndrome and healthy subjects.

**Methods:** We conducted pilot studies in subjects with metabolic syndrome and healthy subjects who fasted from dawn to sunset for more than 14 h for four consecutive weeks. We measured serum BDNF levels and metabolic parameters before 4-week-IF, at the end of 4th week during 4-week-IF, and one week after 4-week-IF.

**Results:** We enrolled 28 subjects, 14 with metabolic syndrome (women/men:6/8) with a mean age of 59 years and 14 healthy subjects (women/men:1/13) with a mean age of 32 years. Overall, BDNF levels decreased at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (mean paired difference =  $-98.5$  ng/ml,  $P = 0.0006$ ). When subjects with metabolic syndrome were compared with healthy subjects, subjects with metabolic syndrome had a lower mean paired reduction in BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference =  $-27.6$  ng/ml vs.  $-169.5$  ng/ml,  $P = 0.003$ ). Multivariate linear regression analysis showed a positive correlation between the change in tumor necrosis factor- $\alpha$  and change in BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF in subjects with metabolic syndrome ( $P = 0.040$ ) and healthy subjects ( $P = 0.007$ ). The change in weight and body mass index independently predicted the change in BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF in subjects with metabolic syndrome.

**Conclusion:** Four-week-IF resulted in a reduction in the BDNF levels at the end of 4th week during 4-week-IF. Higher BDNF levels and a lower reduction in BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF in subjects with metabolic syndrome than healthy subjects suggest a potential BDNF resistance similar to insulin and leptin resistance in metabolic syndrome. A positive correlation between the change in BDNF and change in tumor necrosis factor- $\alpha$  levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF suggests that BDNF is a biomarker of inflammation and endothelial dysfunction in addition to its neurotrophic and anorexigenic features.

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## 1. Introduction

The human brain derived neurotrophic factor (BDNF) gene is located on the chromosome 11 and encodes for BDNF [1], a master neurotrophin that promotes neurogenesis, neuroplasticity, and neuroprotection [2,3]. BDNF is also an anorexigenic protein that regulates food intake and energy hemostasis [3–5]. BDNF has a biased expression in the brain [1], and its highest expression and signaling occur in the hippocampus and cerebral cortex that play a key role in memory and learning processes [6]. Obese BDNF heterozygous mutant mice were shown to have adipocyte hypertrophy, increased weight, leptin, and insulin levels compared with non-obese BDNF heterozygous mutant and wild-type mice [7]. Subjects with BDNF haploinsufficiency were shown to have hyperphagia, obesity, and reduced serum BDNF levels [8].

Animal studies showed conflicting reports regarding how fasting affects BDNF levels. A study showed a significant reduction in the dorsal vagal complex BDNF levels in the rats that had a 48-h fasting compared with the dorsal vagal complex BDNF levels in the rats that had ad libitum eating [5]. The same study showed a significant increase in the dorsal vagal complex BDNF levels in the rats that had 15-h fasting followed by 1-h refeeding compared with the dorsal vagal complex BDNF levels in rats that had 16-h fasting without refeeding [5]. These changes in the BDNF levels with food deprivation and refeeding were not observed in the hypothalamus [5]. Another study conducted in rats showed that while hippocampus BDNF levels decreased in female and male rats with alternate-day fasting, plasma BDNF levels increased in female rats and had no change in male rats [9]. Altogether, these rodent studies showed that the effect of fasting on BDNF levels could vary depending on the brain region where BDNF levels were measured, type of specimen (brain tissue vs. plasma), and type of fasting (48-h fasting vs. alternate-day fasting).

Human studies also showed conflicting reports regarding how fasting affects BDNF levels. Among subjects who fasted from dawn to sunset during the month of Ramadan, whereas one study showed decreased serum BDNF levels [10], another study showed increased [11] plasma BDNF levels at the end of 4th week during 4-week intermittent fasting from dawn to sunset (4-week-IF) compared with the levels before 4-week-IF. The cause for these conflicting reports might be related to blood medium (serum vs. plasma) where the BDNF level was measured or the timing of blood collection from the last food intake [12,13]. Circulating BDNF levels were shown to be substantially different between plasma and serum and affected by several factors including age, sex, time of blood collection, fasting status, diet, and exercise [12–15].

Given these conflicting reports related to the effect of intermittent fasting on the circulating BDNF levels, we conducted a study to assess the effect of 4-week-IF on serum BDNF levels and determine the associations between BDNF and metabolic parameters in subjects with metabolic syndrome and healthy subjects.

## 2. Methods

### 2.1. Study subjects

After obtaining approval from the Institutional Review Board of the Baylor College of Medicine (Protocol number H-31612) and informed consent from all subjects, we conducted pilot studies in subjects with metabolic syndrome and healthy subjects who observed the month of Ramadan in 2019 and 2018, respectively [16,17].

We previously reported the inclusion and exclusion criteria for both studies [16,17]. Briefly, for the study conducted on subjects with metabolic syndrome, we included those who were 18 years of age or older, agreed to have a FibroScan® [18] and planned to fast during the month of Ramadan [17]. Study exclusion criteria were the inability to consent, use of alcohol or recreational substances, pregnancy or breastfeeding, active cancer and infection, seizure disorder, and a cardiovascular event that occurred during the last six months before enrollment [17].

For the study conducted on healthy subjects, we included those who were 18 years of age or older and reported to be in excellent health [16]. Study exclusion criteria were a body mass index equal to or greater than 30 kg/m<sup>2</sup>, history of acute, sub-acute, or chronic disease, use of alcohol and illicit substances, and daily medication use excluding the use of over-the-counter pain medications [16].

### 2.2. Intermittent fasting from dawn to sunset for 4 weeks

Subjects with metabolic syndrome fasted from dawn to sunset for more than 14 h daily for 29 days [17]. Healthy subjects fasted from dawn to sunset for more than 14 h daily for 30 days, excluding one subject who fasted for 26 days instead of 29 days [16]. Both study cohorts had strict dry fasting (no food, drink or water) from dawn to sunset [16,17].

### 2.3. Study procedures

We performed anthropometric measurements (weight, height, and waist circumference), measured blood pressure and collected blood samples within 4 weeks before 4-week-IF (ad libitum eating), at the end of 4th week during 4-week-IF (during fasting), and 1 week after 4-week-IF (return to ad libitum eating). For each study time point, subjects fasted for at least 8 h before the clinical parameters were measured and blood was collected [16,17].

### 2.4. Laboratory analysis

BDNF levels were measured in the serum using human BDNF ELISA Kit (Abcam, Cambridge, MA).

### 2.5. Study endpoints

The primary endpoint was a statistically significant change in the BDNF levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF compared with the levels before 4-week-IF. The secondary endpoint was a statistically significant association between the changes in the BDNF and metabolic parameter levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF compared with the levels before 4-week-IF.

### 2.6. Statistical analysis

We analyzed data using SAS (Version 9.4 TS Level 1M5 X64\_10PRO platform, SAS, Cary, NC) [19] and Minitab (Version 19 (Stage College, PA) [20] softwares. We assessed the changes in

BDNF levels by calculating the paired difference in the BDNF levels for each subject (i.e., paired difference = BDNF level at the end of 4th week during 4-week-IF - BDNF level before 4-week-IF, paired difference = BDNF level 1 week after 4-week-IF - BDNF level before 4-week-IF, and paired difference = BDNF level 1 week after 4-week-IF - BDNF level at the end of 4th week during 4-week-IF). Positive and negative paired differences indicated an increase and a decrease in the BDNF levels, respectively. We performed a paired *t*-test to determine statistically significant differences in the BDNF levels at the end of 4th week during 4-week-IF and one week after 4-week-IF in subjects with metabolic syndrome and healthy subjects. We performed an independent *t*-test to determine statistically significant differences in the BDNF levels between subjects with metabolic syndrome and healthy subjects. We performed a multivariate linear regression analysis to determine the associations between the BDNF and metabolic parameters before 4-week-IF and associations between the changes in the BDNF and metabolic parameters at the end of 4th week during 4-week-IF and 1 week after 4-week-IF.

### 3. Results

#### 3.1. Subjects

A total of fourteen subjects with metabolic syndrome and 14 healthy subjects were enrolled [16,17]. Among 14 subjects with metabolic syndrome, there were 8 men and 6 women, with a mean age of 59 years [17]. Among 14 healthy subjects, there were 13 men and 1 woman, with a mean age of 32 years [16]. Twelve out of 14 subjects with metabolic syndrome were diagnosed with nonalcoholic fatty liver disease (5 out of 14 subjects had hepatic fibrosis stage F2 or greater hepatic fibrosis) based on FibroScan® findings [17]. All 28 subjects completed the study without any complications or adverse events. There was no missing data for BDNF levels.

#### 3.2. Comparison of the changes in BDNF levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF in all subjects

Including all subjects, there was a significant reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (mean paired difference = -98.5 ng/ml, standard deviation (SD) = 135.0,  $P = 0.0006$ ). There was a significant increase in the BDNF levels 1 week after 4-week-IF with the return to ad libitum eating compared with the levels at the end of the 4th week during 4-week-IF (mean paired difference = 101.1 ng/ml, SD = 151.0,  $P = 0.002$ ). There was no significant change in the BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference = 2.6 ng/ml, SD = 135.8,  $P = 0.920$ ).

#### 3.3. Comparison of the changes in BDNF levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF in subjects with metabolic syndrome (Fig. 1)

Fig. 1 shows the mean BDNF levels before 4-week-IF and mean paired differences in the BDNF levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF in subjects with metabolic syndrome. Overall, there was a reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF; however, it did not reach statistical significance (BDNF mean paired difference = -27.6 ng/ml,  $P = 0.321$ ). While women had a significant reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference = -58.5 ng/ml, SD = 45.5,  $P = 0.025$ ), men had a mild reduction in

their BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference = -4.4 ng/ml, SD = 125.1,  $P = 0.923$ ). There was a significant increase in the BDNF levels 1 week after 4-week-IF with the return to ad libitum eating compared with the levels at the end of the 4th week during 4-week-IF (BDNF mean paired difference = 54.9 ng/ml,  $P = 0.042$ ). There was no significant change in the BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference = 27.4 ng/ml,  $P = 0.194$ ).

#### 3.4. Comparison of the changes in BDNF levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF in healthy subjects (Fig. 1)

Fig. 1 shows the mean BDNF levels before 4-week-IF and mean paired differences in the BDNF levels at the end of 4th week during 4-week-IF and 1 week after 4-week-IF in healthy subjects. There was a significant reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference = -169.5 ng/ml,  $P = 0.0003$ ). There was a significant increase in BDNF levels 1 week after 4-week-IF with the return to ad libitum eating compared with the levels at the end of the 4th week during 4-week-IF (BDNF mean paired difference = 147.3 ng/ml,  $P = 0.011$ ). There was no significant change in the BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF (BDNF mean paired difference = -22.1 ng/ml,  $P = 0.648$ ).

#### 3.5. Comparison of the changes in BDNF levels at the end of 4th week during 4-week-IF and one week after 4-week-IF between subjects with metabolic syndrome and healthy subjects (Fig. 1)

When subjects with metabolic syndrome were compared with healthy subjects, subjects with metabolic syndrome had a lower mean paired reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the BDNF levels before 4-week-IF (BDNF mean paired difference = -27.6 ng/ml vs. -169.5 ng/ml,  $P = 0.003$ ) (Fig. 1). The mean paired difference between the BDNF levels 1 week after 4-week-IF and the end of 4th week during 4-week-IF ( $P = 0.111$ ), and mean paired difference between the BDNF levels 1 week after 4-week-IF and before 4-week-IF ( $P = 0.349$ ) were not significant when two cohorts were compared with each other.

#### 3.6. Comparison of the BDNF levels before 4-week-IF, at the end of 4th week during 4-week-IF and one week after 4-week-IF between subjects with metabolic syndrome and healthy subjects (Fig. 2)

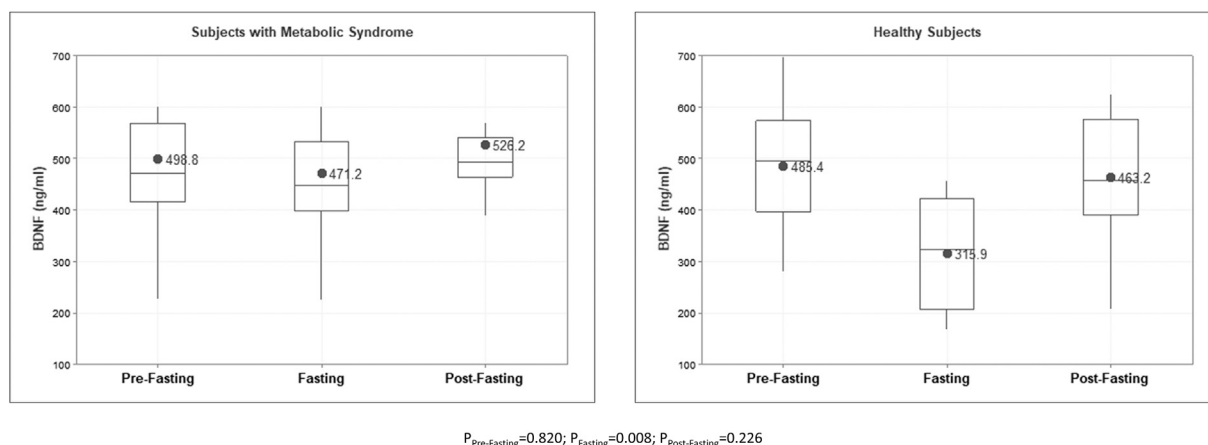
There was a significant difference in the BDNF levels at the end of 4th week during 4-week-IF between subjects with metabolic syndrome and healthy subjects (mean BDNF = 471.2 ng/ml, SD = 176.4 vs. mean BDNF = 315.9 ng/ml, SD = 102.2,  $P = 0.008$ ) (Fig. 2). The mean BDNF levels before 4-week-IF (mean BDNF = 498.8 ng/ml, SD = 184.9 vs. 485.4 ng/ml, SD = 115.7,  $P = 0.820$ ) and 1 week after 4-week-IF (mean BDNF = 526.2 ng/ml, SD = 147.0 vs. mean BDNF = 463.2 ng/ml, SD = 120.4,  $P = 0.226$ ) did not differ between subjects with metabolic syndrome and healthy subjects, respectively.

#### 3.7. Associations between BDNF and metabolic parameter levels in subjects with metabolic syndrome (Fig. 3)

In a multivariate linear regression analysis controlled for age and sex, HDL (adjusted R-square = 0.66, beta coefficient = 12.1,

Cohort	BDNF levels before 4-week-IF		BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF				BDNF levels 1 week after 4-week-IF compared with the levels at the end of 4th week during 4-week-IF				BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF			
	Mean	SD	Mean paired difference <sup>1</sup>	SD	Paired P value <sup>2</sup>	Unpaired P value <sup>3</sup>	Mean paired difference <sup>1</sup>	SD	Paired P value <sup>2</sup>	Unpaired P value <sup>3</sup>	Mean paired difference <sup>1</sup>	SD	Paired P value <sup>2</sup>	Unpaired P value <sup>3</sup>
Subjects with metabolic syndrome n=14, women/men=6/8, mean (SD) age=59 years (16)	498.8	184.9	-27.6	99.9	0.321	0.003	54.9	91.2	0.042	0.111	27.4	74.7	0.194	0.349
Healthy subjects n=14, women/men=1/13, mean (SD) age=32 years (10)	485.4	115.7	-169.5	130.6	0.0003		147.3	185.5	0.011		-22.1	177.3	0.648	

**Fig. 1.** Changes in BDNF levels at the end of 4th week during 4-week intermittent fasting from dawn to sunset (4-week-IF) and one week after 4-week-IF in subjects with metabolic syndrome and healthy subjects, and comparison of these changes between subjects with metabolic syndrome and healthy subjects. Paired difference indicates the change in BDNF level for each subject (e.g., paired difference = BDNF at the end of 4th week during 4-week-IF - BDNF before 4-week-IF). <sup>1</sup>Positive and negative paired difference indicates an increase and decrease in BDNF level, respectively. <sup>2</sup>Paired P values compare the mean paired differences within cohorts (e.g., there was a significant reduction in BDNF levels at the end of 4th week during 4-week IF compared with the levels before 4-week-IF in healthy subjects, mean paired difference = - 169.5 ng/ml, P = 0.0003). <sup>3</sup>Unpaired P values compare the mean paired differences between two cohorts (e.g., when subjects with metabolic syndrome were compared with healthy subjects, subjects with metabolic syndrome had a lower mean paired reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the BDNF levels before 4-week-IF, mean paired difference = -27.6 ng/ml vs. -169.5 ng/ml, P = 0.003). SD=Standard deviation.



**Fig. 2.** Boxplot analysis of BDNF levels before 4-week-intermittent fasting (pre-fasting/ad libitum eating), at the end of 4th week during 4-week intermittent fasting (fasting) and one week after 4-week-intermittent fasting (post-fasting/return to ad libitum eating) in subject with metabolic syndrome (N = 14) and healthy subjects (N = 14). Black circles in the boxes represent mean values of BDNF levels. P values compare the mean BDNF levels before fasting, during fasting and post-fasting between subject with metabolic syndrome and healthy subjects. There was a statistically significant difference in BDNF levels at the end of 4th week during 4-week-IF between subjects with metabolic syndrome and healthy subjects (mean BDNF = 471.2 ng/ml, SD = 176.4 vs. mean BDNF = 315.9 ng/ml, SD = 102.2, P = 0.008).

P = 0.007) and adiponectin (adjusted R-square = 0.60, beta coefficient = 3.5, P = 0.017) were independent predictors of BDNF before 4-week-IF (Fig. 3). The change in tumor necrosis factor-alpha levels independently predicted the change in BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF (adjusted R-square = 0.28, beta coefficient = 17.5, P = 0.040). The change in weight (adjusted R-square = 0.55, beta coefficient = 23.6, P = 0.042) and body mass index (adjusted R-square = 0.55, beta coefficient = 69.5, P = 0.043) independently predicted the change in BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF.

**3.8. Associations between BDNF and metabolic parameter levels in healthy subjects (Fig. 4)**

In a multivariate linear regression analysis controlled for age, HDL (adjusted R-square = 0.41, beta coefficient = -13.3, P = 0.007) was an independent predictor of BDNF (Fig. 4). The change in tumor necrosis factor-alpha (adjusted R-square = 0.41, beta coefficient = 57.4, P = 0.007) and glucose (adjusted R-square = 0.21, beta coefficient = 5.1, P = 0.041) levels independently predicted the change in BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF.

Associations between BDNF and metabolic parameter levels before 4-week-IF <sup>1</sup>				Associations between changes in BDNF and metabolic parameter levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF <sup>2</sup>				Associations between changes in BDNF and metabolic parameter levels 1 week after 4-week-IF compared with the levels before 4-week-IF <sup>2</sup>			
Metabolic Parameter	Beta Coefficient	SE	P Value	Paired difference in metabolic parameter	Beta Coefficient	SE	P Value	Paired difference in metabolic parameter	Beta Coefficient	SE	P Value
Weight (kg)	-2.3	5.1	0.657	Weight (kg)	15.9	15.7	0.336	Weight (kg)	23.6	10.1	0.042
WaistC (inch)	-25.1	13.6	0.096	WaistC (inch)	-7.3	12.8	0.583	WaistC (inch)	-3.0	6.3	0.648
BMI (kg/m <sup>2</sup> )	-11.9	14.1	0.419	BMI (kg/m <sup>2</sup> )	50.5	47.5	0.313	BMI (kg/m <sup>2</sup> )	69.5	30.0	0.043
SBP (mmHg)	3.2	3.5	0.378	SBP (mmHg)	-0.2	2.5	0.934	SBP (mmHg)	-0.4	1.3	0.786
DBP (mmHg)	-7.0	4.8	0.180	DBP (mmHg)	-2.2	4.1	0.600	DBP (mmHg)	-3.4	3.1	0.301
Insulin (μU/ml)	-11.4	6.9	0.132	Insulin (μU/ml)	-11.6	6.8	0.118	Insulin (μU/ml)	-1.2	5.7	0.843
Glucose (mg/dl)	-1.2	1.6	0.486	Glucose (mg/dl)	-3.3	1.9	0.115	Glucose (mg/dl)	-0.004	1.2	0.997
TG (mg/dl)	-0.6	0.7	0.418	TG (mg/dl)	0.04	0.4	0.925	TG (mg/dl)	-0.6	0.4	0.176
HDL (mg/dl)	12.1	3.5	0.007	HDL (mg/dl)	1.9	5.0	0.711	HDL (mg/dl)	0.7	4.0	0.863
TC (mg/dl)	-1.0	1.3	0.442	TC (mg/dl)	0.9	1.1	0.421	TC (mg/dl)	-0.3	0.6	0.589
LDL (mg/dl)	-2.0	1.5	0.197	LDL (mg/dl)	0.7	1.4	0.606	LDL (mg/dl)	-0.1	0.7	0.861
Leptin (pg/ml)	0.002	0.008	0.847	Leptin (pg/ml)	0.003	0.005	0.607	Leptin (pg/ml)	0.003	0.004	0.538
ADIPO (μg/ml)	3.5	1.2	0.017	ADIPO (μg/ml)	-0.3	1.8	0.873	ADIPO (μg/ml)	-0.5	0.9	0.582
TNF-α (pg/ml)	4.3	10.1	0.679	TNF-α (pg/ml)	17.5	7.4	0.040	TNF-α (pg/ml)	3.5	6.7	0.616
IL-1β (pg/ml)	-25.1	41.1	0.555	IL-1β (pg/ml)	-3.1	48.2	0.951	IL-1β (pg/ml)	9.8	37.8	0.801
IL-6 (pg/ml)	25.0	29.0	0.410	IL-6 (pg/ml)	-12.2	61.4	0.847	IL-6 (pg/ml)	-26.4	38.8	0.512
IL-8 (pg/ml)	-3.4	6.4	0.606	IL-8 (pg/ml)	21.4	12.8	0.124	IL-8 (pg/ml)	-0.5	2.5	0.852
HCY (μmol/l)	-17.3	10.6	0.131	HCY (μmol/l)	2.6	19.0	0.893	HCY (μmol/l)	2.0	18.1	0.916
CRP (mg/L)	-8.3	46.2	0.862	CRP (mg/L)	25.9	19.1	0.203	CRP (mg/L)	-14.0	15.0	0.371

**Fig. 3.** Associations between BDNF and metabolic parameter levels in subjects with metabolic syndrome. <sup>1</sup>Multivariate linear regression analysis controlled for age and sex: BDNF (ng/ml) = metabolic parameter + age + sex. <sup>2</sup>Multivariate linear regression analysis controlled for age and sex: Paired difference in BDNF (ng/ml) = paired difference in metabolic parameter + age + sex. Paired difference indicates the change in BDNF and metabolic parameter level for each subject (e.g., paired difference = BDNF at the end of 4th week during 4-week-IF - BDNF before 4-week-IF). SE=Standard error, BMI=Body mass index, WaistC = Waist circumference, SBP = systolic blood pressure, DBP = Diastolic blood pressure, TG = Triglyceride, HDL=High density lipoprotein, TC = Total cholesterol, LDL = Low density lipoprotein, ADIPO = Adiponectin, TNF-α = Tumor necrosis factor-alpha, IL-1β = Interleukin-1 beta, IL-6= Interleukin-6, IL-8 = Interleukin-8, HCY=Homocysteine, CRP=C-reactive protein.

Associations between BDNF and metabolic parameter levels before 4-week-IF <sup>1</sup>				Associations between changes in BDNF and metabolic parameter levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF <sup>2</sup>				Associations between changes in BDNF and metabolic parameter levels 1 week after 4-week-IF compared with the levels before 4-week-IF <sup>2</sup>			
Metabolic Parameter	Beta Coefficient	SE	P Value	Paired difference in metabolic parameter	Beta Coefficient	SE	P Value	Paired difference in metabolic parameter	Beta Coefficient	SE	P Value
Weight (kg)	-0.6	4.7	0.897	Weight (kg)	8.6	18.2	0.648	Weight (kg)	-11.0	20.2	0.598
BMI (kg/m <sup>2</sup> )	3.0	13.4	0.826	BMI (kg/m <sup>2</sup> )	24.6	54.5	0.660	BMI (kg/m <sup>2</sup> )	-36.7	58.7	0.544
SBP (mmHg)	-6.3	4.3	0.169	SBP (mmHg)	-1.2	3.2	0.718	SBP (mmHg)	-6.3	4.5	0.186
DBP (mmHg)	-0.2	4.2	0.956	DBP (mmHg)	3.1	3.3	0.375	DBP (mmHg)	-3.2	4.3	0.469
Insulin (μU/ml)	8.0	7.9	0.333	Insulin (μU/ml)	12.5	6.5	0.083	Insulin (μU/ml)	0.6	10.3	0.955
Glucose (mg/dl)	1.9	3.2	0.571	Glucose (mg/dl)	5.1	2.2	0.041	Glucose (mg/dl)	-1.4	2.5	0.590
TG (mg/dl)	-0.04	1.4	0.975	TG (mg/dl)	0.1	1.1	0.925	TG (mg/dl)	2.0	1.7	0.266
HDL (mg/dl)	-13.3	4.0	0.007	HDL (mg/dl)	1.4	6.6	0.833	HDL (mg/dl)	14.2	9.2	0.152
TC (mg/dl)	-2.0	1.0	0.073	TC (mg/dl)	1.8	1.2	0.159	TC (mg/dl)	2.1	1.6	0.222
LDL (mg/dl)	-2.1	1.3	0.137	LDL (mg/dl)	2.2	1.3	0.119	LDL (mg/dl)	1.8	1.9	0.380
Leptin (pg/ml)	-0.001	0.01	0.947	Leptin (pg/ml)	-0.003	0.03	0.926	Leptin (pg/ml)	0.02	0.02	0.316
ADIPO (μg/ml)	0.9	2.4	0.700	ADIPO (μg/ml)	1.4	2.7	0.616	ADIPO (μg/ml)	28.8	16.9	0.116
TNF-α (pg/ml)	32.4	17.6	0.093	TNF-α (pg/ml)	57.4	17.2	0.007	TNF-α (pg/ml)	53.2	28.4	0.088
IL-1β (pg/ml)	25.3	39.2	0.532	IL-1β (pg/ml)	-59.5	43.1	0.194	IL-1β (pg/ml)	32.0	68.9	0.651
IL-6 (pg/ml)	1.0	4.0	0.807	IL-6 (pg/ml)	-12.6	12.3	0.330	IL-6 (pg/ml)	-9.7	52.6	0.856
IL-8 (pg/ml)	3.5	16.4	0.835	IL-8 (pg/ml)	0.7	13.7	0.963	IL-8 (pg/ml)	5.1	7.2	0.487
HCY (μmol/l)	11.0	9.7	0.282	HCY (μmol/l)	35.6	23.2	0.153	HCY (μmol/l)	12.5	8.8	0.183
CRP (mg/L)	-0.9	11.8	0.941	CRP (mg/L)	17.2	10.3	0.123	CRP (mg/L)	-8.0	8.5	0.365

**Fig. 4.** Associations between BDNF and metabolic parameter levels in healthy subjects. <sup>1</sup>Multivariate linear regression analysis controlled for age: BDNF (ng/ml) = metabolic parameter + age. <sup>2</sup>Multivariate linear regression analysis controlled for age: Paired difference in BDNF (ng/ml) = paired difference in metabolic parameter + age. Paired difference indicates the change in BDNF and metabolic parameter level for each subject (e.g., paired difference = BDNF level at the end of 4th week during 4-week-IF - BDNF level before 4-week-IF). SE=Standard error, BMI=Body mass index, SBP = systolic blood pressure, DBP = Diastolic blood pressure, TG = Triglyceride, HDL=High density lipoprotein, TC = Total cholesterol, LDL = Low density lipoprotein, ADIPO = Adiponectin, TNF-α = Tumor necrosis factor-alpha, IL-1β = Interleukin-1 beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, HCY=Homocysteine, CRP=C-reactive protein.

### 3.9. Associations between BDNF levels and demographics (age and sex) in subjects with metabolic syndrome

Controlling for age, female sex was associated with higher BDNF levels before 4-week-IF (adjusted R-square = 0.33, beta coefficient = 222.0,  $P = 0.020$ ). In addition, sex was associated with change in BDNF levels 1 week after 4-week-IF compared with the levels before 4-week-IF (adjusted R-square = 0.38, beta coefficient = -100.2,  $P = 0.010$ ). There was no association between sex and change in BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF ( $P = 0.380$ ). There was no association between age and BDNF levels before 4-week-IF ( $P = 0.421$ ), between age and change in BDNF levels at the end of 4th week during 4-week-IF ( $P = 0.400$ ), and between age and change in BDNF levels 1 week after 4-week-IF ( $P = 0.828$ ) compared with the levels before 4-week-IF.

### 3.10. Associations between BDNF levels and age in healthy subjects

There was no association between age and BDNF levels before 4-week-IF ( $P = 0.917$ ), between age and change in BDNF levels at the end of 4th week during 4-week-IF ( $P = 0.863$ ) and between age and change in BDNF levels 1 week after 4-week-IF ( $P = 0.608$ ) compared with the levels before 4-week-IF. As there was only one woman among healthy subjects, the association between BDNF and sex was not analyzed.

## 4. Discussion

This study is the first comprehensive report of the effect of 4-week-IF on circulating BDNF levels measured in serum and associations between BDNF and key metabolic parameters in subjects with metabolic syndrome and healthy subjects. We previously reported that 4-week-IF from dawn to sunset resulted in an anti-cancer and anti-metabolic syndrome proteome [16,17]. In this study, in the same subjects, we showed that serum BDNF levels decreased at the end of 4th week during 4-week-IF and returned almost to baseline levels 1 week after 4-week-IF with the return to ad libitum eating. These findings suggest that the reduction in circulating BDNF levels during 4-week-IF could be due to increased cellular uptake and expression at the tissue level in response to nutrient deprivation. In fact, a study showed an approximately 3.5 fold increase in the BDNF mRNA expression in the human muscle tissue after 48-h fasting [21].

We found decreased BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF. Similar to our findings, a study conducted in schizophrenia patients with or without metabolic syndrome who fasted from dawn to sunset for 4 weeks during the month of Ramadan showed a reduction in the serum BDNF levels at the end of 4th week during 4-week-IF compared with the baseline BDNF levels [10]. In contrast to our findings, a study conducted in 29 healthy subjects who fasted for 4 weeks during the month of Ramadan showed an increase in the plasma BDNF levels at the end of 4th week during 4-week-IF compared with the baseline BDNF levels [11]. These different results could be related to multiple factors, including differences in the analysis medium where the BDNF was analyzed (serum vs. normal plasma vs. platelet-poor plasma) [12], age, sex, selection criteria, and timing of blood collection [12–15] between study cohorts.

We found an increase in the BDNF levels 1 week after 4-week-IF with the return to ad libitum eating compared with the levels at the end of 4th week during 4-week-IF. Similar to our findings, a study showed a significant increase in the dorsal vagal complex BDNF levels in the rats that had 15-h fasting followed by 1-h refeeding

compared with the dorsal vagal complex BDNF levels in the rats that had 16-h fasting without refeeding [5].

Metabolic syndrome is a cluster of metabolic abnormalities, including central obesity, insulin resistance, elevated blood pressure, high triglyceride, and low high-density lipoprotein levels [22], and a major risk factor for nonalcoholic fatty liver disease, coronary heart disease, chronic kidney disease, and several common cancers [22,23]. Metabolic syndrome is associated with hyperinsulinemia, hyperleptinemia and hypoadiponectinemia [24,25]. One study showed that serum BDNF levels were significantly elevated in patients with obesity and type II diabetes mellitus compared with healthy subjects [26]. Similar to the findings of this study, we observed that overall, the BDNF levels were higher in subjects with metabolic syndrome compared with healthy subjects (Fig. 2). Additionally, when the subjects with metabolic syndrome were compared with the healthy subjects, subjects with metabolic syndrome had a lower mean paired reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the BDNF levels before 4-week-IF (Fig. 1). Altogether, these findings suggest that there could be a BDNF resistance equivalent to insulin and leptin resistance or a compensatory increase in BDNF levels in subjects with metabolic syndrome.

Metabolic syndrome is also a prothrombotic and proinflammatory state, which is a risk factor for endovascular dysfunction [27,28]. We observed that the changes in BDNF levels at the end of 4th week during 4-week-IF was significantly associated with the changes in tumor necrosis factor- $\alpha$  levels (Fig. 3). These findings suggest that the reduction in the BDNF levels could be associated with a reduction in metabolic syndrome-induced inflammation and endothelial dysfunction.

We previously reported that the subjects with metabolic syndrome enrolled in this study had a significant reduction in weight and body mass index at the end of 4th week during 4-week-IF and 1 week after 4-week-IF compared with baseline [17]. In this study, we observed a positive correlation between changes in the BDNF levels and weight 1 week after 4-week-IF compared with the levels before 4-week-IF in subjects with metabolic syndrome (Fig. 3). Our findings are in line with the findings of the studies conducted to assess the effect of caloric reduction and bariatric surgery on the circulating BDNF levels [15,29]. A pilot study conducted among 18 women who underwent bariatric surgery showed that BDNF levels significantly decreased three months after surgery compared with the baseline levels, and these changes co-occurred with a significant postoperative weight loss [29]. A randomized controlled study conducted in 50 overweight or obese healthy subjects showed that circulating BDNF levels decreased in women after 12 weeks of diet (very low energy diet for 8 weeks followed by weight maintenance diet for 4 weeks) [15].

The strengths of our study are three-fold. Firstly, we evaluated serum BDNF levels in subjects with metabolic syndrome comparing with serum BDNF levels in healthy subjects. This provided us a better perspective in understanding the effect of 4-week-IF on the serum BDNF levels in subjects with metabolic syndrome. Secondly, we assessed the association of BDNF levels with adiposity, oxidative stress, and inflammatory biomarkers in a multivariate analysis controlling for known BDNF confounders, including age and sex. Thirdly, we not only assessed BDNF levels at the end of 4th week during 4-week-IF but also one week after 4-week-IF. This provided us to evaluate the carryover effect of 4-week-IF on serum BDNF levels.

As expected from pilot studies, the sample size in both cohorts was the limitation of our study. A randomized controlled study of intermittent fasting from dawn to sunset with a larger sample size is needed for external validation of our findings. The second limitation was that we only measured BDNF levels in the serum. Future

studies of intermittent fasting should measure circulating BDNF levels in both serum and plasma to determine the differences in both mediums.

In conclusion, serum BDNF levels decreased at the end of 4th week during 4-week-IF and returned almost to baseline levels 1 week after 4-week-IF with the subjects' return to ad libitum eating. Higher BDNF levels and a lower reduction in the BDNF levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF in subjects with metabolic syndrome than healthy subjects suggest a potential BDNF resistance in subjects with metabolic syndrome similar to insulin and leptin resistance. The change in weight and body mass index was positively correlated with the change in BDNF levels 1 week after 4-week-IF compared with BDNF levels before 4-week-IF in subjects with metabolic syndrome. The change in tumor necrosis factor- $\alpha$  was an independent predictor of the change in BDNF levels at the end of 4th week during 4-week-IF compared with BDNF levels before 4-week-IF in subjects with metabolic syndrome and healthy subjects. A positive correlation between the change in BDNF and change in tumor necrosis factor- $\alpha$  levels at the end of 4th week during 4-week-IF compared with the levels before 4-week-IF suggests that BDNF is a biomarker of inflammation and endothelial dysfunction in addition to its neurotrophic and anorexigenic features.

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## Declaration of competing interest

None of the authors has a conflict of interest.

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