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Original article

Brain-derived neurotrophic factor in obese women and its relationship with leptin and ghrelin

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Summary. *Objectives:* We aimed to assess the brain-derived neurotrophic factor (BDNF) level and its relation with leptin and ghrelin levels in obese women. *Methods:* In this case-control cross-sectional study, forty obese women and 40 healthy normal weight women aged (20- 50 y) were participated. Anthropometric indices were measured and serum levels of BDNF, leptin and ghrelin were assessed by the ELISA method. *Results:* Obese women had significantly higher leptin, but lower ghrelin levels compared with normal weight women. BDNF level was comparable between the two groups of women. There was a strong positive correlation between leptin and anthropometric parameters. A significant negative association was found between BDNF and leptin levels in normal weight women (r= -0.41, p= 0.02). *Conclusion:* Our results in women do not support a relationship between BDNF and obesity. Anthropometric indices may be important predictors of the leptin level. Further studies are required to elucidate the exact link between BDNF and leptin.

Key words: obesity, BDNF, leptin, ghrelin

Introduction

Obesity is defined as an extreme increase proportion of in body fat to total lean body mass that imposes adverse health consequences of poor health (1). Today, Obesity is one of the most common health problems in the world and its prevalence is increasing in both developed and developing nations with changes in dietary habits and activity levels. In 2014, the World Health Organization estimated that more than 1.9 billion adults were overweight and of these over 600 million were obese (2). The obesity prevalence had increased to 35.7% among U.S. adults by 2009 - 2010 (3). The overall prevalence of overweight and obesity in Iran was 34.1% and 15.4%, respectively (4).

Several factors are involved in the pathogenesis of obesity, involving genetic factors, lifestyle, lack of exercise, intake of medication such as corticosteroids, diseases such as hypothyroidism, Cushing's syndrome, neurological and psychiatric disorders, hormonal disorders, etc (5). Human and economic burden of obesity and its high prevalence suggest an urgent need to better treatments and better understanding of involved factors in the regulation of energy balance (6). According to various separate studies, there is association between the hormones leptin, ghrelin, brain-derived neurotrophic factor (BDNF) with obesity.

Leptin is a hormone that is secreted from adipocites and serum leptin concentration is associated with fat mass and percentage of body fat (7, 8). The primary role of leptin is informing the central nervous system (CNS) about the energy storage to regulate energy balance (9). Several studies have suggested that leptin reduces appetite and human leptin deficiency in both children and adults causes severe obesity. In addition they found that leptin concentration was higher in the blood of obese people and also said that these people are resistant to leptin (10, 11).

On the other hand, ghrelin is a hormone produced by the stomach and it has stimulating effect on the hypothalamic hunger-signaling pathway. When the stomach is empty, this factor level goes up and it decreases after meals. This hormone increases appetite and desire to take high-calorie foods (12, 13). Recent studies showed that the levels of ghrelin in obese individuals are reduced (10).

Another interesting factor is BDNF which is a member of the family of growth factors, neurotrophins, capable of signaling to cells for survival, differentiation or growth (14). BDNF has also a major role in energy metabolism, eating behavior (15, 16). Numerous animal studies have suggested the contribution of BDNF in developing of obesity phenotype. Deletion of BDNF gene from excitatory neurons in the brain of mice leads to obesity in these animals (16, 17). Moreover, there are substantial evidences that both central and peripheral administration of BDNF causes loss of appetite and weight loss in mice (18). However, data on BDNF level in adult obese individual is insufficient. Therefore, we aimed to assess the BDNF level in obese women. On the other hand, since BDNF and leptin peptides have similar effect on food intake and energy expenditure, we hypothesized that these two peptides may act synergistically in energy metabolism and obesity. Hence, the second aim of the study was to examine the relationship between BDNF, leptin and ghrelin in obese adults.

Material

Participants

In this case-control cross-sectional study, forty obese women with BMI≥ 30, aged (20- 50 y) and 40 healthy normal weight women with 18.5≤BMI<25 were recruited from medical weight loss center, prior to treatment. Volunteers with a mental illness such as depression, chronic diseases, certain physiological conditions such as pregnancy, lactation, menopause, any use of complementary medicine and regular physical activity in the last 6 months were excluded.

Personal data (including age, education, occupation, marital status, smoking, following a special diet, and the risk of other diseases, drugs) and physical activity of all subjects were collected by using appropriate questionnaires. The stress level of participants was assessed by means of the Holmes and Rahe stress scale. Anthropometric indices including height, weight, waist circumference (WC) and hip circumference were measured by an expert individual. Body weight was measured using a digital scale, with the examinee wearing a light gown. Height was measured barefoot using a wall-mounted stadiometer to the nearest 0.5 cm. BMI was calculated as body weight (kg) divided by the square of height (m^2). According to WHO, 18.5≤ BMI <25 kg/m2 was defined as normal weight and a BMI greater than or equal to 30 was considered as obesity.WC was measured by a tape measure at the midpoint between the lower costal margin and iliac crest to the nearest 0.5 cm. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. Waist-to-hip ratio (WHR) was calculated as waist measurement divided by hip measurement.

A written informed consent was obtained from all the participants. The study was approved by Ethical committee at Tabriz University of Medical Sciences, Tabriz, Iran.

Biochemical assays

Fasting blood sample were collected and sera were immediately collected after centrifugation at 5000 RPM for 3 minutes. Then serum samples were stored at -70 ^oC until analysis. Serum levels of BDNF, leptin and ghrelin were measured by the ELISA method according to the protocol provided by the manufactures.

Statistical analysis

All analyses were performed using SPSS version 16.0. Data are expressed as mean± SD or median for quantitative variables and as percentages for categorical variables. Student independent t-test and Mann-Whitney U-test was used for normally and non-normally distributed continuous variables, respectively, and chi-square test for categorical variables. Bivariate Spearman correlation coefficient was used to assess the significance association between variables. Statistical significance was set at $P<0.05$.

Results

A total of 80 women participated in the study and the data of 77 subjects including 37 obese and 40 normal- weight women were analyzed. Three of obese subjects were excluded from the study due to their extremely hemolyzed or lipolyzed samples.

Demographic and anthropometric parameters

The demographic and anthropometric parameters of the studied subjects are shown in (Table 1). As expected, the mean BMI, waist circumference, waist to hip ratio (WHR) were significantly greater in obese subjects compared to normal weight subjects (p< 0.0001). Significant difference was observed in education level between the two groups (P= 0.004). More than half of obese subjects had education at diploma or under diploma level, whereas 80% of normal weight subject had education at academic level. No significant difference was observed for age, marital status, physical activity level, stress level between the two groups.

Serum levels of BDNF, ghrelin and leptin

Table 2 presents the comparison of biochemical variables between the two groups. In obese women serum leptin concentrations were significantly higher (p< 0.0001) and serum ghrelin concentrations were significantly lower (p= 0.03) compared with control group. Median of BDNF level of obese subjects was 21 pg/ml compared with 23 pg/ml in healthy controls with no statistically significant difference.

Correlation of biochemical variables with anthropometric parameters

As shown in Table 3, there was a strong positive relationship between leptin and weight (r= 0.59, p<

Table 1. Characteristics of obese and normal weight women

	obese	Normal weight	P value
Age (years)	37.2± 7.48	35.2± 7.9	0.27
Marital status			0.07
married	27 (73.0)	21(52.5)	
divorce	1(2.7)	-	
single	9 (24.3)	19 (47.5)	
Education levela			0.004
under Diploma, diploma	20 (54.1)	8 (20.0)	
master	14 (37.8)	20 (50.0)	
post graduate	3 (8.1)	12 (30)	
BMI (kg/m2)	31.2 (30.1-32.8)	23.2 (21.5-24.7)	<0.0001
Waist circumstance (cm)	97.2± 8.2	77.3± 7.9	<0.0001
Waist to hip ratio	0.84± 0.6	0.76± 0.6	<0.0001
Physical activity			0.31
low	33 (89.2)	33 (82.5)	
moderate	4 (10.8)	7 (17.5)	
high			
Stress			0.16
low	24 (64.9)	23 (57.5)	
moderate	9 (24.3)	16 (40.0)	
severe	4 (10.8)	1 (2.5)	

Data presented as mean± SD or median (IQ 25-75) for continuous variables and number for categorically distributed variables.
T-test and Mann-Whitney U-test was used for normally and non-normally distributed continuous variables, respectively, and chi-square test for categorical variabl.
[a] were presented as frequency (percent).

Table 2. Comparison of biochemical variables between obese and normal weight women

	Obese	Normal weight	P value
Leptin (ng/ml)	8.2± 3.8	3.8± 3.1	<0.0001
Gherelin (ng/ml)	3.8 (2.7, 7.2)	6.3 (3.4, 11.1)	0.03
BDNF (pg/ml)	21(10.6, 49.5)	23 (10.3, 49.2)	0.99

Data expressed as mean± SD or median (IQ 25, 75) for continuous variables.
T-test and Mann-Whitney U-test was used for normally and non-normally distributed continuous variables, respectively.

0.0001), BMI (r= 0.63, P< 0.0001), WC (r= 0.58, P< 0.0001) and WHR (r= 0.37, P= 0.001). Considerable, but not statistically significant, negative correlation was observed between ghrelin and body weight of subjects (r= -0.18, p= 0.05) and also between BDNF with WHR (r= -0.18, p= 0.06).

Correlation of serum BDNF with leptin and ghrelin

As shown in Table 4, in total, there was notable, but not statistically significant, inverse association between serum levels of BDNF with leptin (r= -0.16, p= 0.08). Also a significant negative correlation was found between BDNF and leptin levels in normal weight women (r= -0.41, p= 0.02). We also observed that the plasma ghrelin levels tended to correlate negatively with leptin, but the correlation was not statistically significant(r= -0.19, p= 0.05)

Discussion

In the current study, we found that obese woman had higher concentrations of leptin and lower ghrelin compared to normal weight women. These results are in consistent with previous findings that reported leptin levels were significantly higher in obese subjects (19, 20). There are also evidences that plasma ghrelin concentrations in patients with obesity are lower than those of healthy subjects with normal body weight (21, 22). This downregulation might be a consequence of raised leptin, because serum ghrelin levels inversely associated with serum leptin levels. It is speculated that the reduced ghrelin levels observed in obesity represent a physiological adaptation to the positive energy balance associated with obesity.

Additionally, current studies show that BDNF serum concentrations in obese women were comparable with those of non-obese women. There are controversial findings from previous studies in this area. In agreement to our finding, Lee et al. have reported no difference in serum BDNF levels between men with or without metabolic syndrome (23, 24). In obese human, some investigators have reported lower levels of serum or plasma BDNF compared to normal weight subjects (25, 26), while , Roth et al., found higher BDNF serum concentrations in obese vs. lean children and suggested a relationship between BDNF and fat mass (27). Since, anti-obesity effect of BDNF has

Table 3. Correlation of biochemical variables with anthropometric indices

	Weight	BMI	WC	WHR
BDNF	r= -0.04	r= -0.12	r= -0.08	r= -0.18
	p= 0.38	p= 0.14	p= 0.24	p= 0.06
Leptin	r= 0.60	r= 0.69	r= 0.58	r= 0.37
	p< 0.0001	p< 0.0001	p< 0.0001	p= 0.001
Ghrelin	r= -0.18	r= -0.14	r= -0.13	r= 0.02
	p= 0.05	p= 0.11	p= 0.13	p= 0.44

r: correlation coefficient

Table 4. Correlation between biochemical variables

	Leptin		Ghrelin	
	r	p	r	p
BDNF (total)	-0.16	0.08	-0.14	0.12
obese	-0.16	0.13	-0.05	0.36
normal weight	-0.41	0.02	-0.19	0.17
Leptin (total)	-	-	-0.19	0.05
obese	-	-	-0.16	0.13
normal weight	-	-	-0.02	0.46

r: correlation coefficient

been documented and in an animal model study has been reported that chronic administration of BDNF in the hypothalamic paraventricular nucleus reverses obesity induced by high-fat diet (19, 29). Moreover, humans with mutations in the BDNF gene exhibit severe obesity (29). An obese phenotype is also observed in BDNF-conditional knockout mice (16). Taken all together, reduction of BDNF in obese people seems be more reaseonable. Inconsistent finding in this area across the different studies might be due to differnce in obesity grade of studied people. For instance, the studies that demonstrated lower levels of BDNF, were on children and extremly obese subjects (25, 26), whereas in Roth et al. study that showed higher levels of BDNF in obese subjects, in fact, children in early stage of obesity (overweight) have been compared with partialy lean children (BMI was 28.9 vs. 18.9 kg/m^2). Recently, a neurotrophic hypothesis suggests that neurotrophins have a different role in the early or late stage of metabolic diseases. It has been suggested that neurotrophin levels are high in early stage of metabolic diseases in order to compensate and attenuate emerging inflammatory events, but when metabolic diseases criteria are developed, concentration of neurotrophins begin to reduce gradually because of proinflammatory cytokines effects on the neurotrophins. Therefore, a hyponeurotrophinemia appears during the developed stage of the diseases (30, 31).

We observed that serum levels of leptin positively correlated with anthropometric indices, in the study subjects. This result is in line with previous findings which reported fasting serum leptin significantly correlated with BMI, weight, waist circumstance and WHR (32,33). The results indicate the importance of anthropometric parameters as predictors of the hormone level.

In the present study, a notable inverse association between serum ghrelin and leptin levels was found. The present result confirm previous observations by different studies regarding the negative association between ghrelin and leptin levels (34-36). It is well known that ghrelin and leptin are two hormones which have an important role in the energy balance and homeostasis. Both the hormones acts through hypothalamus pathway to regulate appetite, food intake and body weight, oppositely. In the brain, the receptor for leptin, is found on the same cells in the hypothalamic cells as the receptor for ghrelin. Leptin, satiety hormone, operates to inhibit food intake and increase energy expenditure, whereas, ghrelin, hunger hormone, acts to increase hunger, and to increase gastric acid secretion and gastrointestinal motility to prepare the body for food intake. Therefore, it is rational that a negative interaction between the two hormones exists, as demonstrated in the present study.

We also reported a significant inverse association between concentrations of serum BDNF and leptin in healthy subjects but no association in obese patients. In the present work, for two reasons we expected to observe a positive relation between BDNF and leptin. First, as it pointed out in the introduction section, BDNF and leptin have similar function on feeding behavior and energy expenditure. Second, the findings of earlier animal interventional investigations supported our hypothesis. Komori et al. demonstrated that administration of leptin causes an increase in expression of BDNF mRNA and protein in the ventromedial hypothalamic (37). Yamada et al. reported that in lean mice subcutaneous leptin injections augmented hippocampal BDNF expression (38). Therefore, the hypothesis that the two peptides may act synergistically in energy metabolism and obesity might be true in the CNS and target organs, where the two peptides act their role directly. But in the circulating, evidence about the relation between BDNF and leptin is remarkably scarce. Nakagawa et al. in a study on rodents revealed that repetitive administration of BDNF significantly lowers serum leptin concentrations compared with vehicle-treated groups (18). They didn't describe about its mechanism. Possibly, BDNF has a role in leptin entrance to the brain or enhances its utilization by cells. If this be true, the observed inverse association between leptin and BDNF in serum could be explained. In healthy individuals, increased secretion of BDNF might enhances leptin entry to the brain or cells and lowers its serum concentration and vice versa. However, further investigations are needed to clarify the issue.

In conclusion, serum BDNF concentration in obese women was comparable with those of non-obese women. It appears that there is a link between BDNF and leptin, but further research is necessary to clarify exact direction of the link with molecular pathways.

The direct relation of anthropometric indices with leptin levels indicates the importance of anthropometric parameters as predictors of the hormone level.

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