

The Waist–Hip Ratio is a Mediator Between Serum Levels of Brain-Derived Neurotrophic Factor and Its Val66Met Polymorphism in Adolescents

Ji Cheng Zhang, Jin Hua Wang, Jia Lin, Guo Ming Su, Yi Lin Shen, Qi Wei Guo, Jia Jing Cai, Jun Yi Liu, Ke Xin Jia, Ding Zhi Fang

Department of Biochemistry and Molecular Biology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, People's Republic of China

Correspondence: Ding Zhi Fang, Department of Biochemistry and Molecular Biology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, 610041, People's Republic of China, Email dzfang@scu.edu.cn

Aim: To explore anthropometric, metabolic and dietary factors associated with and their interplays with the Val66Met polymorphism at brain-derived neurotrophic factor (BDNF) gene (*Bdnf*) on serum BDNF levels in adolescents.

Methods: Serum BDNF levels were quantified using an enzyme-linked immunosorbent assay in 644 high school students (278 males/366 females). A polymerase chain reaction and restriction fragment length polymorphism assay were utilized for *Bdnf* Val66Met genotyping followed by verification using DNA sequencing. Serum levels of metabolic characteristics were assayed by routine methods. The intake of macro and micronutrients was collected by a three-day food record.

Results: Serum BDNF levels were found to be significantly different in the subjects with different genotypes of *Bdnf* Val66Met (Val/Val homozygotes, 60.05 ± 28.07 ng/mL vs Val/Met heterozygotes, 56.37 ± 29.34 ng/mL vs Met/Met homozygotes, 51.32 ± 24.54 ng/mL, $p = 0.022$). Among the 36 tested variables, waist–hip ratio (WHR) ($\beta = -0.163$, $p < 0.001$), iodine intake ($\beta = 0.132$, $p = 0.001$), heart rate ($\beta = 0.108$, $p = 0.005$), high-density lipoprotein cholesterol (HDL-C) ($\beta = 0.098$, $p = 0.011$) and dietary fiber intake ($\beta = 0.082$, $p = 0.084$) were the predictor of serum BDNF levels, while SBP ($\beta = 0.097$, $p = 0.013$) and WHR ($\beta = 0.091$, $p = 0.021$) were related with *Bdnf* Val66Met. Moreover, WHR was observed to play a partial mediating role in the relationship between *Bdnf* Val66Met and serum BDNF levels (95% CI $[-1.161, -0.087]$) and contribute 13.05% of its total effect on serum BDNF levels.

Conclusion: There are interplays between WHR and *Bdnf* Val66Met on serum BDNF levels, which may be among the explanations for the previous heterogeneous reports and provide novel insights into the regulation of serum BDNF levels.

Keywords: serum, brain-derived neurotrophic factor, Val66Met, waist–hip ratio, mediator, adolescent

Plain Language Summary

Brain-derived neurotrophic factor (BDNF) has pleiotropic effects on the physiology of whole organisms. Serum levels of BDNF have become a biomarker to reflect the status of diseases. Although there are a series of studies exploring the factors influencing serum BDNF levels, the results are inconsistent. Here, we systematically select the Val66Met polymorphism at the BDNF gene (*Bdnf*) and 36 anthropometric, metabolic and dietary factors and examine their associations with and their interplays on serum BDNF levels in healthy Chinese Han adolescents. The results demonstrate that *Bdnf* Val66Met is associated, while waist–hip ratio (WHR), iodine intake, heart rate, high-density lipoprotein cholesterol and dietary fiber intake are the predictors of serum BDNF levels. Moreover, systolic blood pressure and WHR are associated with *Bdnf* Val66Met. WHR plays a partial mediating role in the effect of *Bdnf* Val66Met on serum BDNF levels. These results suggest that there are interplays between WHR and *Bdnf* Val66Met on serum BDNF levels, which may be among the explanations for the previous heterogeneous reports, provide novel insights into the regulation of

serum BDNF levels and pave a novel way for precision medical interferences targeting serum BDNF concentrations in clinical practices, especially for adolescents with disturbed fat distribution.

Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family,¹ is mainly synthesized in neurons and glial cells in the brain and secreted into the blood through the blood–brain barrier.² Meanwhile, the BDNF gene (*Bdnf*) is also expressed in smooth muscle cells and macrophages of diseased cardiac tissue.³ BDNF plays an important role in neuroprotection by inducing survival signals and enhancing neural plasticity.⁴ In fact, BDNF has pleiotropic effects on the physiology of whole organisms, including responses to inflammatory cytokines,⁵ sympathetic and parasympathetic regulations of cardiovascular functions,⁶ and regulations of food intake and subsequently body weight⁷ via influencing cellular responses to glucose and insulin, mitochondrial functions and thermogenic tissue differentiation.⁸ In addition, decreased levels of serum BDNF were observed in patients with cognitive impairments,^{9–12} acute coronary syndromes,¹³ and obesity and diabetes complications.^{14–16} Therefore, understanding the regulation of circulating BDNF and its mechanism is important to elucidate the physiology of BDNF and its applications in clinical practices.

A series of factors have been examined for their influences on serum BDNF levels with discrepant results. Among them, obesity was more frequently reported in the last few years. A negative correlation between circulating BDNF levels and obesity was found in 449 college students aged between 18 and 20 years.¹⁷ Nevertheless, in a study of 24 children with normal weight and 66 with obesity, the level of circulating BDNF was observed to be significantly higher in subjects with obesity than that in the normal weight counterparts.¹⁵ However, obesity was not found to be associated with BDNF serum levels in 24 adults with obesity and 14 controls.¹⁸ Interestingly, in a study including 40 women with obesity, 40 with overweight, and 40 with normal weight, serum BDNF levels were significantly lower in patients with obesity than those with normal weight, but not significantly different between women with obesity and overweight, or between individuals with overweight and normal weight.¹⁶ Moreover, in 492 middle-aged and elderly subjects of the Baltimore Longitudinal Study of Aging, the increased level of plasma BDNF was associated with increased fat mass and increased body mass index (BMI) in females, but not in males.¹⁴ Although technical factors such as sample sizes and samples used (serum or plasma) need to be considered, these findings suggest that the relationship between serum BDNF and obesity is complex and might be affected by confounding factors. Actually, physical exercise,¹⁹ environmental enrichment,²⁰ caloric restriction²¹ and stage of life²² have been reported to influence BDNF levels.

The most frequently reported genetic factor affecting serum levels of BDNF is a single-nucleotide polymorphism in the functional coding region at *Bdnf*, resulting in an amino acid substitution from valine (Val) to methionine (Met) at codon 66 (*Bdnf* Val66Met). It has been documented to effectively influence *Bdnf* expression.²³ However, *Bdnf* Val66Met was not associated with BDNF levels in 187 participants with major depressive disorder and 55 non-depressed healthy controls.²⁴ Furthermore, Nascimento et al found that only elderly individuals carrying Met of *Bdnf* Val66Met exhibited significant improvements in peripheral BDNF levels after physical exercises.²⁵ The mechanism of the discrepant relationship between *Bdnf* Val66Met and serum BDNF levels has not been elucidated yet.

To explain the heterogeneous reports in the literature and further explore the factors influencing serum BDNF levels, we hypothesized that there were interplays of anthropometric, metabolic, and dietary characteristics and *Bdnf* Val66Met on the levels of serum BDNF. To test our hypothesis in the present study, *Bdnf* Val66Met and 36 anthropometric, metabolic and dietary factors were selected systematically, and their associations with and their interplays on BDNF levels were investigated in adolescents. This population was selected because factors associated with serum levels of BDNF were scantily reported before. Although the prevalence of obesity was high and ever-increasing in this population,^{26,27} much less previous studies were focused on the relationship between obesity and serum BDNF levels in adolescents. In fact, obesity can be identified by body mass index (BMI) or waist–hip ratio (WHR). Since BMI reflects body components^{28,29} and WHR reflects the distribution of body components,^{30,31} both were included in the factors to be tested.

Materials and Methods

Subjects

Chinese Han students at the ages of 15 to 17 years were recruited as volunteers by advertisements from a boarding high school. A total of 644 students (278 females/366 males) were finally included in the study because they (I) finished the training and understood the procedure of the study, (II) had no histories of chronic diseases such as cardiovascular, renal, endocrinological diseases and diabetes, and (III) had not taken medications or hormones for at least one month. They were healthy indicated by regular physical examinations and routine laboratory tests. The other volunteers were excluded because (I) their blood was not sampled because of personal reasons, (II) they had histories of chronic diseases, (III) they took medications or hormones within a month, and (IV) they did not finish all the questionnaires. All participants and their guardians agreed that their statistics could be used in the current study by signing the written informed consent. The Human Research Ethics Committee of Sichuan University approved the present study.

Anthropometric Measurements and Evaluations of Dietary Intake

Height, weight, waistline, and hipline of the adolescents were measured and used for the calculation of BMI and WHR. Heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were taken at rest for 5 min and recorded.

All recruited students completed a three-day food record to estimate their dietary intake. The standard bowls, plates, and spoons were used to help the students to quantify their food consumption. Compositions of minerals (including calcium, phosphorus, potassium, sodium, magnesium, iron, zinc, selenium, copper, manganese, and iodine), vitamins (including vitamin A, thiamine, riboflavin intake, vitamin B6, folic acid, niacin, vitamin C and vitamin E), macronutrients (protein, fat and carbohydrate), and dietary fiber were analyzed based on the Chinese Food Composition Table second edition³² and the nutrient composition table on the food packaging.

Blood Collection and Laboratory Analyses

Twelve-hour fasting venous blood was collected and serum was obtained after centrifugation for 10 minutes at 1000×g. Serum levels of glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were assayed by routine methods using commercially available kits (Mindray Bio-Medical Electronics, Shenzhen, China). Insulin concentrations were determined by electrochemical luminescence (Elabscience, E-EL-M2614c).

Genomic DNA was isolated from white blood cells using a DNA extraction kit (Tiandz, Cat No: 3671-50; Mianyang, China). The polymerase chain reaction and restriction fragment length polymorphism assay were utilized for *Bdnf* Val66Met genotyping, which was verified by DNA sequencing (Sangon Biotech, China). A 113-bp segment was amplified by PCR using 1 µL of forward primer of 5'-GAGGCTTGACATCATTGGCT-3' and 1 µL of reverse primer of 5'-CGTGTACAAGTCTGCGTCCT-3' (10 µM, Sangon Biotech, China) in a 25 µL reaction mixture. The cycling conditions were 94°C for 2 min, followed by 30 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 30s, with a final extension at 72°C for 5 min. Ten-microliter PCR products were then digested overnight with 2 U of PmlI (NEB, #R0532L). Digested fragments were separated on a 2.5% agarose gel and visualized with GoldenView. PCR products that contained the PmlI site (CAC|GTG) were cleaved into 2 fragments of 78 bp and 35 bp for the Val allele. The products that lacked the PmlI site migrated as 1 fragment of 113 bp for the Met allele.³³

Enzyme-Linked Immunosorbent Assay

Serum BDNF levels were quantified using an enzyme-linked immunosorbent assay (ABClonal, RK00074 with antibodies against BDNF) according to the manufacturer's instructions. The intra- and inter-assay coefficients of variations were 5% and 10%, respectively.

Statistical Analyses

Numerical data were shown as mean ± SD. The *t*-test was used to examine the numerical differences between the male and female adolescents. The Chi-Square Goodness-of-Fit Test was utilized to assess the agreement of the genotype

distribution of *Bdnf* Val66Met with Hardy-Weinberg equilibrium. Fisher's exact test was used to examine the differences in the allelic and the genotypic distribution between male and female adolescents. One-way analyses of variance (ANOVA) with Games-Howell's multiple comparisons were used to analyze the levels of serum BDNF in the subjects with different genotypes of *Bdnf* Val66Met polymorphism. Multiple linear regression analysis was applied to set up the model to estimate the impact factors of serum BDNF levels by using serum BDNF levels as an independent variable, while gender, age, genotypes of *Bdnf* Val66Met, BMI, WHR, heart rate, SBP, DBP, fasting glucose, TC, HDL-C, LDL-C, TG, Vitamin A intake, Vitamin B6 intake, Vitamin C intake, Vitamin E intake, thiamine intake, riboflavin intake, calcium intake, folic acid intake, niacin intake, phosphorus intake, potassium intake, sodium intake, magnesium intake, iron intake, zinc intake, selenium intake, copper intake, manganese intake, iodine intake, carbohydrate intake, fat intake, protein intake, and dietary fiber intake as dependent variables. Simple linear regression analysis was applied to set up models to estimate correlations between WHR and serum BDNF levels or *Bdnf* Val66Met. Bootstrap method was used to analyze the mediating effect of the other factors between *Bdnf* Val66Met and serum BDNF levels. Bootstrap confidence interval (CI) was set at 95%, and the number of bootstrap samples at 5000. If zero was not included in the interval of 95% CI, the mediating effect was significant. A *p*-value ≤ 0.05 was considered statistically significant.

Results

Characteristics of the Adolescent Population

The anthropometric, biochemical, and dietary characteristics of the study population are presented in Table 1. BMI, WHR, heart rate, TC, HDL-C, TG, and insulin were lower in the male adolescents than those in the female counterparts, while the male adolescents were older and had higher levels of SBP, fasting glucose, vitamin A intake, thiamine intake, riboflavin intake, folic acid intake, niacin intake, calcium intake, phosphorus intake, potassium intake, sodium intake,

Table 1 Anthropometric, Biochemical, and Dietary Characteristics of the Adolescents

Variables	Total (N = 644)	Male Adolescents (N = 278)	Female Adolescents (N = 366)	p*
Serum BDNF (ng/mL)	56.30 $\pm$ 28.13	55.17 $\pm$ 25.96	57.16 $\pm$ 29.68	0.375
Ages (years)	16.87 $\pm$ 0.59	16.96 $\pm$ 0.60	16.80 $\pm$ 0.57	<0.001
BMI (kg/m ²)	20.31 $\pm$ 2.36	19.83 $\pm$ 2.35	20.67 $\pm$ 2.30	<0.001
WHR	0.78 $\pm$ 0.05	0.77 $\pm$ 0.04	0.78 $\pm$ 0.05	0.015
Heart rate (beats/min)	82.74 $\pm$ 11.26	79.90 $\pm$ 10.88	84.90 $\pm$ 11.08	<0.001
SBP (mmHg)	118.9 $\pm$ 11.70	121.1 $\pm$ 12.30	117.2 $\pm$ 11.00	<0.001
DBP (mmHg)	72.87 $\pm$ 10.49	73.30 $\pm$ 10.35	72.55 $\pm$ 10.60	0.367
TC (mmol/L)	3.59 $\pm$ 0.58	3.43 $\pm$ 0.52	3.72 $\pm$ 0.59	<0.001
HDL-C (mmol/L)	1.41 $\pm$ 0.28	1.36 $\pm$ 0.27	1.45 $\pm$ 0.28	<0.001
TG (mmol/L)	1.13 $\pm$ 0.44	0.96 $\pm$ 0.35	1.25 $\pm$ 0.46	<0.001
LDL-C (mmol/L)	1.67 $\pm$ 0.49	1.63 $\pm$ 0.48	1.70 $\pm$ 0.51	0.066
Glucose (mmol/L)	5.06 $\pm$ 0.44	5.13 $\pm$ 0.44	5.01 $\pm$ 0.43	<0.001
Insulin (μ U/mL)	12.06 $\pm$ 5.64	9.72 $\pm$ 4.52	13.83 $\pm$ 5.77	<0.001
Vitamin A intake (μ g/day)	369.6 $\pm$ 795.9	455.4 $\pm$ 1061	304.5 $\pm$ 501.9	0.029
Thiamine intake (mg/day)	1.56 $\pm$ 1.00	1.84 $\pm$ 1.24	1.34 $\pm$ 0.71	<0.001
Riboflavin intake (mg/day)	0.70 $\pm$ 0.53	0.82 $\pm$ 0.72	0.60 $\pm$ 0.29	<0.001
Vitamin B6 intake (mg/day)	0.40 $\pm$ 0.25	0.42 $\pm$ 0.27	0.39 $\pm$ 0.23	0.276
Folic acid intake (μ g/day)	83.7 $\pm$ 63.07	90.17 $\pm$ 69.13	78.79 $\pm$ 57.67	0.027
Niacin intake (mg/day)	14.44 $\pm$ 11.07	17.63 $\pm$ 14.80	12.02 $\pm$ 60	<0.001
Vitamin C intake (mg/day)	60.1 $\pm$ 59.43	62.25 $\pm$ 77.15	58.47 $\pm$ 41.23	0.460
Vitamin E intake (mg/day)	27.73 $\pm$ 19.25	27.28 $\pm$ 23.18	28.08 $\pm$ 15.64	0.623
Calcium intake (mg/day)	372.0 $\pm$ 367.6	432.2 $\pm$ 471.1	326.3 $\pm$ 254.6	0.001
Phosphorus intake (mg/day)	990.9 $\pm$ 564.4	1149 $\pm$ 701.5	870.9 $\pm$ 392.7	<0.001

(Continued)

Table 1 (Continued).

Variables	Total (N = 644)	Male Adolescents (N = 278)	Female Adolescents (N = 366)	p*
Potassium intake (mg/day)	1838 ± 1401	2061 ± 1797	1669 ± 971	0.001
Sodium intake (mg/day)	3272 ± 2505	3594 ± 3277	3028 ± 1663	0.009
Magnesium intake (mg/day)	308.3 ± 209.8	350.8 ± 272.5	276.0 ± 136.9	<0.001
Iron intake (mg/day)	20.56 ± 16.36	22.3 ± 19.5	19.24 ± 13.37	0.025
Zinc intake (mg/day)	8.86 ± 6.10	10.34 ± 8.05	7.73 ± 3.67	<0.001
Selenium intake (µg/day)	50.71 ± 58.29	55.98 ± 62.29	46.70 ± 54.81	0.045
Copper intake (mg/day)	1.80 ± 1.44	2.04 ± 1.80	1.62 ± 1.06	0.001
Manganese intake (mg/day)	7.47 ± 20.98	9.46 ± 26.41	5.95 ± 15.53	0.049
Iodine intake (µg/day)	5.97 ± 13.32	8.17 ± 15.39	4.29 ± 11.25	<0.001
Protein intake (g/day)	81.95 ± 54.54	98.74 ± 70.29	69.2 ± 33.36	<0.001
Fat intake (g/day)	98.58 ± 67.84	119.3 ± 85.40	82.86 ± 44.64	<0.001
Carbohydrate intake (g/day)	373.1 ± 182.7	406.8 ± 214.3	347.4 ± 149.8	<0.001
Dietary fiber intake (g/day)	18.86 ± 16.93	19.97 ± 19.86	18.02 ± 14.29	0.167

Note: *Male adolescents vs female adolescents by t-tests.

magnesium intake, iron intake, zinc intake, selenium intake, copper intake, manganese intake, iodine intake, protein intake, fat intake, and carbohydrate intake than the female adolescents. No significant differences were observed in DBP, serum BDNF, LDL-C, vitamin B6 intake, vitamin C intake, vitamin E intake, and dietary fiber intake between the male and the female adolescents.

Serum BDNF Levels in the Subjects with Different Genotypes of *Bdnf* Val66Met

The genotype and the allele frequencies of *Bdnf* Val66Met in the study population are summarized in Table 2. The distribution of *Bdnf* Val66Met genotypes was in Hardy-Weinberg equilibrium in this adolescent population. No significant differences in the genotype or the allele frequency of *Bdnf* Val66Met were observed between the male and the female adolescents.

As presented in Figure 1, serum BDNF levels were found to be significantly different among the subjects with different genotypes of *Bdnf* Val66Met ($p = 0.022$). Games-Howell's multiple comparisons revealed that the Val/Val homozygotes had a tendency of higher serum BDNF levels than the individuals with the Val/Met genotype (60.05 ± 28.07 ng/mL vs 56.37 ± 29.34 ng/mL, $p = 0.393$) and significantly higher serum BDNF levels than the Met/Met homozygotes (60.05 ± 28.07 ng/mL vs 51.32 ± 24.54 ng/mL, $p = 0.009$), while the subjects with the Val/Met genotype had a tendency of higher serum BDNF levels than the Met/Met homozygotes (56.37 ± 29.34 ng/mL vs 51.32 ± 24.54 ng/mL, $p = 0.133$) (Figure 1).

Factors Associated with Serum BDNF Levels in the Study Population

The level of serum BDNF was utilized as the dependent variable, and gender and all the other variables in Table 1 were used as independent variables to explore the factors associated with serum BDNF levels in the study population

Table 2 Allele and Genotype Frequencies of *Bdnf* Val66Met in the Study Population

		Total (N = 644)	Hardy-Weinberg p	Male Adolescents (N = 278)	Female Adolescents (N = 366)	p*
Genotypes	Val/Val	175 (27.3)	0.954	79 (28.7)	96 (26.2)	0.832
	Val/Met	327 (50.7)		139 (49.8)	188 (51.4)	
	Met/Met	142 (22.0)		60 (21.5)	82 (22.4)	
Alleles	Val	677 (52.6)	–	297 (53.6)	380 (51.9)	0.612
	Met	611 (47.4)		259 (46.4)	352 (48.1)	

Notes: Data are expressed as n (%). *Male adolescents vs female adolescents by Fisher's exact tests.

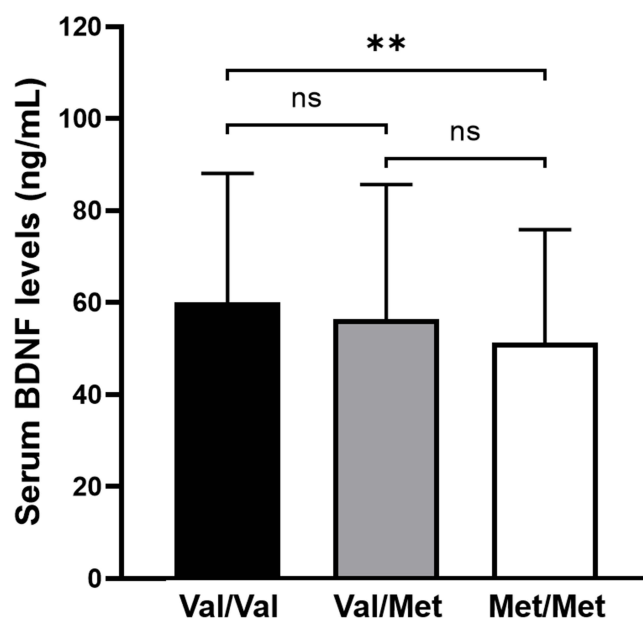


Figure 1 Serum BDNF levels in the subjects with different genotypes of *Bdnf* Val66Met. ** $p < 0.01$; ns, $p > 0.05$.

(Table 3). The results displayed that iodine intake, heart rate, HDL-C and dietary fiber intake were positive, while WHR was negative, predictors of serum BDNF levels. These findings suggested that WHR, but not BMI, might interact with iodine intake, heart rate, HDL-C and dietary fiber intake on serum BDNF levels.

Factors Associated with *Bdnf* Val66Met in the Study Population

To explore the factors associated with *Bdnf* Val66Met in the study population, the genotypes of *Bdnf* Val66Met were utilized as the dependent variable and gender and all the other variables in Table 1 except serum BDNF levels were used as independent variables (Table 4). The results displayed that SBP and WHR are positively related to the allele of *Bdnf* 66Met.

Mediation Analyses of the Factors Between *Bdnf* Val66Met and Serum BDNF Levels

Since the results indicated that WHR was correlated with both the level of serum BDNF and the *Bdnf* Val66Met, it might play a role as a mediator between *Bdnf* Val66Met and serum BDNF levels. Therefore, a model was built to examine the moderating and mediating effects of WHR in the relation between *Bdnf* Val66Met and serum BDNF levels. The results displayed that WHR significantly mediated the effect of *Bdnf* Val66Met on serum BDNF levels because zero was not included in the interval of 95% CI (-1.161 , -0.087), contributing 13.05% of its total effect on serum BDNF levels (Table 5). In addition, the direct effects between *Bdnf* Val66Met, WHR and serum BDNF levels were analyzed using simple linear regression, and the indirect effect between *Bdnf* Val66Met and serum BDNF levels through WHR was

Table 3 Factors Associated with Serum BDNF Levels in the Adolescent Population (N = 644)

Variables	Adjusted $R^2 = 0.069$		
	β	Partial Correlations	p
WHR	-0.163	-0.165	<0.001
Iodine intake	0.132	0.136	0.001
Heart rate	0.108	0.111	0.005
HDL-C	0.098	0.101	0.011
Dietary fiber intake	0.082	0.084	0.033

Abbreviation: β , standardized regression coefficient.

Table 4 Factors Associated with *Bdnf* Val66Met in the Adolescent Population (N = 644)

Variables	Adjusted R ² = 0.015		
	β	Partial Correlations	p
SBP	0.097	0.098	0.013
WHR	0.091	0.091	0.021

Note: 1 = Val/Val, 2 = Val/Met and 3 = Met/Met genotype of *Bdnf* Val66Met.

Abbreviation: β , standardized regression coefficient.

Table 5 Hypothesized Mediation Model of WHR Between *Bdnf* Val66Met and Serum BDNF Levels

	Effect	SE	Boot LLCI	Boot ULCI	Proportion
Total	-4.413	1.576	-7.507	-1.319	100%
Direct	-3.837	1.563	-6.907	-0.767	86.95%
Indirect	-0.576	0.278	-1.161	-0.087	13.05%

Abbreviations: Effect, regression coefficient; SE, standard error; Boot LLCI, bootstrapping lower limit confidence interval; Boot ULCI, bootstrapping upper limit confidence interval; Direct, direct effect of *Bdnf* Val66Met on serum BDNF levels; Indirect, indirect effect of *Bdnf* Val66Met on serum BDNF levels through WHR; Total, the sum of direct and indirect effect.

analyzed using mediation analysis. As the regression and moderated mediation model displayed in Figure 2, the direct effect of *Bdnf* Val66Met on serum BDNF levels in the regression model ($c = -0.096$) was less than the indirect effect through WHR in the simple mediation model ($c' = -0.110$) (*Bdnf* Val66Met – WHR, $a = 0.090$; WHR-serum BDNF levels, $b = -0.167$), suggesting that the Met allele increased WHR and subsequently reduced the level of serum BDNF. These results revealed that WHR plays a partial mediating role in the relationship between *Bdnf* Val66Met and serum BDNF levels.

Discussion

We hypothesized that there were interplays of anthropometric, metabolic, and dietary characteristics, and *Bdnf* Val66Met on the levels of serum BDNF in the current study. As evidenced, serum BDNF levels were observed to be significantly different in the adolescents with different genotypes of *Bdnf* Val66Met (Figure 1). Among the 36 anthropometric, metabolic and dietary factors systematically selected and listed in Table 1, iodine intake, heart rate, HDL-C, dietary fiber intake and WHR were found to be associated with serum BDNF levels (Table 3), while SBP and WHR were related to *Bdnf* Val66Met (Table 4). Subsequent mediation analyses indicated that WHR mediated the effect of *Bdnf* Val66Met on serum BDNF levels and contributed 13.05% of the total effect (Table 5). The Met allele of *Bdnf* Val66Met elevated WHR and subsequently decreased the level of serum BDNF (Figure 2). All these results demonstrated that WHR interacts with

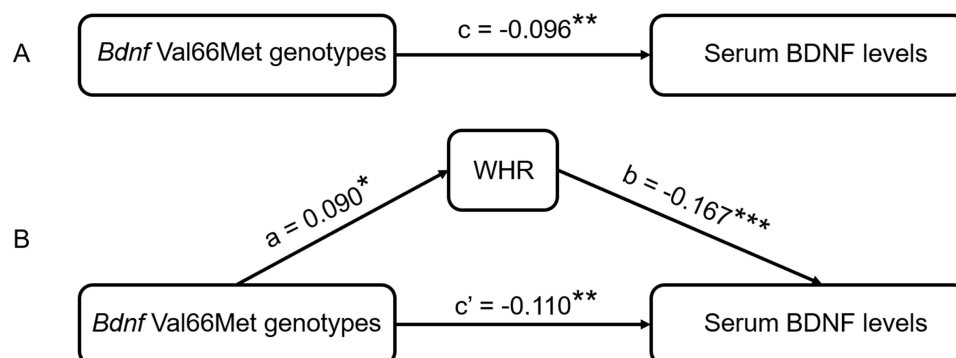


Figure 2 Mediation model for serum BDNF levels, *Bdnf* Val66Met and WHR. (A) Path diagram for the regression model. (B) The simple mediation model with WHR as a mediator of the effect of *Bdnf* Val66Met on serum BDNF levels.

Notes: Path coefficients were shown in standardized regression coefficient. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Bdnf Val66Met on the levels of serum BDNF in the current study population. To the best of our knowledge, these findings have not been reported before.

The relationship between circulating BDNF and obesity was heterogeneously reported with unknown mechanisms.^{18,34,35} Although both WHR and BMI are utilized to categorize obesity, WHR is a surrogate measure of fat distribution, while BMI is a surrogate measure of total body fat and has different cut-off points for determining overweight or obesity in different countries.³⁶ WHR is significantly affected by genetic factors and shows significant heritability of up to ~60%, even after adjusting for BMI.³⁷ As a marker of absolute and relative accumulation of abdominal fat accumulation, WHR is associated with an increased risk of myocardial infarction, heart failure and total mortality in patients with cerebrovascular disease (CVD),³⁸ underlining the importance of abdominal obesity as an independent factor of all-cause mortality in patients with CVD.³⁹ On the other hand, obesity assessed by BMI presents some limitations in the prediction of cardiovascular mortality.³⁹ In the present study, *Bdnf* Val66Met was demonstrated to significantly affect serum BDNF levels (Figure 1). Meanwhile, WHR, but not BMI, was observed to be the predictor of serum BDNF levels, together with the others in the whole study population (Table 3). Nevertheless, only WHR of these predictors plays a partial mediating role in the relationship between *Bdnf* Val66Met and serum BDNF levels (Table 5 and Figure 2). These results suggest that fat distribution, but not total fat, may interact with *Bdnf* Val66Met on the levels of serum BDNF and provide an explanation for the heterogeneous relationships between circulating BDNF and obesity reported before.

The negative correlation observed in the present study between WHR and serum BDNF levels has been reported before by others.⁴⁰ It is possible that this correlation is mediated by decreased functioning of the prefrontal cortex caused by obesity,⁴¹ or the negative effect of obesity on the integrity of neural structures, including the gray and white matter of the brain,^{42,43} because serum BDNF is mainly synthesized in the brain and secreted into the blood through the blood–brain barrier.² However, reduction of BDNF in the hypothalamus affects food intake and promotes an anorectic signal.⁴⁴ Absence of BDNF^{45,46} is associated with hyperphagia, weight gain and obesity both in mouse models and human. Both BDNF gene transfer and exogenous BDNF administration in mouse models of obesity restore normal food intake and induce weight loss.^{47,48} These findings support the concept that lower serum BDNF levels reflecting BDNF deficit in the brain can lead to obesity.^{49,50} In the current study, interplays were observed between WHR and *Bdnf* Val66Met on serum BDNF levels. More studies are needed to explore the molecular mechanism of the interplay.

Interestingly, heart rate was found to be positively associated with serum BDNF levels in the current study (Table 3). To the best of our knowledge, this finding has not been reported before. It is worth noting that BDNF is also expressed in endothelial cells with regulated releases by stimuli such as laminar shear stress and changes in intracellular calcium,^{51,52} and is recognized as a growth factor with cardiovascular functions.⁵¹ Decreased BDNF levels result in reduced endothelial cell survival and cardiac contractility, whereas activation of tyrosine kinase receptor B by BDNF is associated with angiogenesis.⁵¹ Additionally, SBP was related to *Bdnf* Val66Met genotypes (Table 4). These observations suggest specific roles of BDNF to maintain cardiac functions and pathophysiological roles of perturbed BDNF in the development of obesity-associated heart diseases.

Gender was reported to be a determining factor of serum BDNF levels in patients of some studies.^{53,54} However, no significant differences were observed in serum BDNF levels between the healthy male and the healthy female adolescents in the current study, which was in accordance with those demonstrated in other studies.^{55,56} This discrepancy may be due to the influences of disturbed healthy status of the subjects. For example, serum BDNF levels were higher in male patients than female patients with anxiety disorder, while there were no differences between the healthy male controls and the healthy female controls.⁵⁷

In summary, *Bdnf* Val66Met plays an important role in regulating serum BDNF levels, and only WHR is the mediator of the regulation among the 36 factors investigated in adolescents. These findings suggest interplays between *Bdnf* Val66Met and WHR on serum BDNF levels, which may be among the explanations for the inconsistent relationship of serum BDNF concentrations with other characteristics including *Bdnf* Val66Met and obesity, provide novel insights into the regulation of serum BDNF levels, and pave a novel way for precision medical interferences targeting serum BDNF concentrations in clinical practices, especially for adolescents with disturbed fat distribution.

Ethics Approval

This study was approved by the Human Ethics Committee of Sichuan University. All procedures performed in studies involving human participants were complying with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Acknowledgments

We thank the support of the Major Project of Sichuan for Science and Technology.

Funding

The present study was supported by the Major Project of Sichuan for Science and Technology (Grant No. 2022YFH0025). Professor Ding Zhi Fang is the recipient of the grant.

Disclosure

The authors declare no competing interests.

References

1. Sasi M, Vignoli B, Canossa M, et al. Neurobiology of local and intercellular BDNF signaling. *Pflugers Arch*. 2017;469(5–6):593–610. doi:10.1007/s00424-017-1964-4
2. Lessmann V, Brigadski T. Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neurosci Res*. 2009;65(1):11–22. doi:10.1016/j.neures.2009.06.004
3. Ejiri J, Inoue N, Kobayashi S, et al. Possible role of brain-derived neurotrophic factor in the pathogenesis of coronary artery disease. *Circulation*. 2005;112(14):2114–2120. doi:10.1161/circulationaha.104.476903
4. Marosi K, Mattson MP. BDNF mediates adaptive brain and body responses to energetic challenges. *Trends Endocrinol Metab*. 2014;25(2):89–98. doi:10.1016/j.tem.2013.10.006
5. Kozlov EM, Grechko AV, Chegodaev YS, et al. Contribution of neurotrophins to the immune system regulation and possible connection to alcohol addiction. *Biology*. 2020;9(4):63. doi:10.3390/biology9040063
6. Fujitani M, Otani Y, Miyajima H. Do neurotrophins connect neurological disorders and heart diseases? *Biomolecules*. 2021;11(11):1730. doi:10.3390/biom11111730
7. Vanevski F, Xu B. Molecular and neural bases underlying roles of BDNF in the control of body weight. *Front Neurosci*. 2013;7:37. doi:10.3389/fnins.2013.00037
8. Di Rosa MC, Zimbone S, Saab MW, et al. The pleiotropic potential of BDNF beyond neurons: implication for a healthy mind in a healthy body. *Life*. 2021;11(11):1256. doi:10.3390/life11111256
9. Hock C, Heese K, Hulette C, et al. Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch Neurol*. 2000;57(6):846–851. doi:10.1001/archneur.57.6.846
10. Zuccato C, Marullo M, Conforti P, et al. Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. *Brain Pathol*. 2008;18(2):225–238. doi:10.1111/j.1750-3639.2007.00111.x
11. Narisawa-Saito M, Wakabayashi K, Tsuji S, et al. Regional specificity of alterations in NGF, BDNF and NT-3 levels in Alzheimer's disease. *Neuroreport*. 1996;7(18):2925–2928. doi:10.1097/00001756-199611250-00024
12. Hoxha E, Lippicello P, Zurlo F, et al. The emerging role of altered cerebellar synaptic processing in Alzheimer's disease. *Front Aging Neurosci*. 2018;10:396. doi:10.3389/fnagi.2018.00396
13. Manni L, Nikolova V, Vyagova D, et al. Reduced plasma levels of NGF and BDNF in patients with acute coronary syndromes. *Int J Cardiol*. 2005;102(1):169–171. doi:10.1016/j.ijcard.2004.10.041
14. Li B, Lang N, Cheng ZF. Serum levels of brain-derived neurotrophic factor are associated with diabetes risk, complications, and obesity: a cohort study from Chinese patients with type 2 diabetes. *Mol Neurobiol*. 2016;53(8):5492–5499. doi:10.1007/s12035-015-9461-2
15. Roth CL, Elfers C, Gebhardt U, et al. Brain-derived neurotrophic factor and its relation to leptin in obese children before and after weight loss. *Metabolism*. 2013;62(2):226–234. doi:10.1016/j.metabol.2012.08.001
16. Celik Guzel E, Bakkal E, Guzel S, et al. Can low brain-derived neurotrophic factor levels be a marker of the presence of depression in obese women? *Neuropsychiatr Dis Treat*. 2014;10:2079–2086. doi:10.2147/ndt.S72087
17. Alomari MA, Khabour OF, Alawneh K, et al. The importance of physical fitness for the relationship of BDNF with obesity measures in young normal-weight adults. *Heliyon*. 2020;6(3):e03490. doi:10.1016/j.heliyon.2020.e03490
18. Hinderberger P, Rullmann M, Drabe M, et al. The effect of serum BDNF levels on central serotonin transporter availability in obese versus non-obese adults: a [(11)C]DASB positron emission tomography study. *Neuropharmacology*. 2016;110(Pt A):530–536. doi:10.1016/j.neuropharm.2016.04.030
19. Cotman CW, Berchtold NC. Physical activity and the maintenance of cognition: learning from animal models. *Alzheimers Dement*. 2007;3(2 Suppl):S30–S37. doi:10.1016/j.jalz.2007.01.013
20. van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. *Nat Rev Neurosci*. 2000;1(3):191–198. doi:10.1038/35044558
21. Mattson MP, Duan W, Guo Z. Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. *J Neurochem*. 2003;84(3):417–431. doi:10.1046/j.1471-4159.2003.01586.x

22. Erickson KI, Prakash RS, Voss MW, et al. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. *J Neurosci*. 2010;30(15):5368–5375. doi:10.1523/jneurosci.6251-09.2010
23. Ieraci A, Barbieri SS, Macchi C, et al. BDNF Val66Met polymorphism alters food intake and hypothalamic BDNF expression in mice. *J Cell Physiol*. 2020;235(12):9667–9675. doi:10.1002/jcp.29778
24. Froud A, Murphy J, Cribb L, et al. The relationship between dietary quality, serum brain-derived neurotrophic factor (BDNF) level, and the Val66met polymorphism in predicting depression. *Nutr Neurosci*. 2019;22(7):513–521. doi:10.1080/1028415x.2017.1415281
25. Nascimento CM, Pereira JR, Pires de Andrade L, et al. Physical exercise improves peripheral BDNF levels and cognitive functions in mild cognitive impairment elderly with different bdnf Val66Met genotypes. *J Alzheimers Dis*. 2015;43(1):81–91. doi:10.3233/jad-140576
26. Finucane MM, Stevens GA, Cowan MJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet*. 2011;377(9765):557–567. doi:10.1016/s0140-6736(10)62037-5
27. de Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *Am J Clin Nutr*. 2010;92(5):1257–1264. doi:10.3945/ajcn.2010.29786
28. Dosaev T, Prakash J, Livshits G. Contribution of body composition components and soft-tissue biochemical factors to genetic variation of body mass index (BMI) in an ethnically homogeneous population. *Am J Hum Biol*. 2014;26(6):760–767. doi:10.1002/ajhb.22583
29. Lebidowska A, Hartman-Petrycka M, Błońska-Fajfrowska B. How reliable is BMI? Bioimpedance analysis of body composition in underweight, normal weight, overweight, and obese women. *Ir J Med Sci*. 2021;190(3):993–998. doi:10.1007/s11845-020-02403-3
30. Chiu CH, Ko MC, Wu LS, et al. Benefits of different intensity of aerobic exercise in modulating body composition among obese young adults: a pilot randomized controlled trial. *Health Qual Life Outcomes*. 2017;15(1):168. doi:10.1186/s12955-017-0743-4
31. Carbone S, Elagizi A, Lavie CJ. Obesity and mortality risk in heart failure: when adipose tissue distribution matters. *Eur J Heart Fail*. 2018;20(9):1278–1280. doi:10.1002/ehf.1279
32. Wang GY. *Chinese Food Composition*. 2nd ed. Beijing: The Medical Press of Peking University; 2009.
33. Neves-Pereira M, Mundo E, Muglia P, et al. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet*. 2002;71(3):651–655. doi:10.1086/342288
34. Golden E, Emiliano A, Maudsley S, et al. Circulating brain-derived neurotrophic factor and indices of metabolic and cardiovascular health: data from the Baltimore Longitudinal Study of Aging. *PLoS One*. 2010;5(4):e10099. doi:10.1371/journal.pone.0010099
35. Slusher AL, Whitehurst M, Zoeller RF, et al. Brain-derived neurotrophic factor and substrate utilization following acute aerobic exercise in obese individuals. *J Neuroendocrinol*. 2015;27(5):370–376. doi:10.1111/jne.12275
36. Tan KC. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;363(9403):157–163. doi:10.1016/s0140-6736(03)15268-3
37. Schleinitz D, Böttcher Y, Blüher M, et al. The genetics of fat distribution. *Diabetologia*. 2014;57(7):1276–1286. doi:10.1007/s00125-014-3214-z
38. Dagenais GR, Yi Q, Mann JF, et al. Prognostic impact of body weight and abdominal obesity in women and men with cardiovascular disease. *Am Heart J*. 2005;149(1):54–60. doi:10.1016/j.ahj.2004.07.009
39. Kragelund C, Hassager C, Hildebrandt P, et al. Impact of obesity on long-term prognosis following acute myocardial infarction. *Int J Cardiol*. 2005;98(1):123–131. doi:10.1016/j.ijcard.2004.03.042
40. Kaur S, Gonzales MM, Tarumi T, et al. Serum brain-derived neurotrophic factor mediates the relationship between abdominal adiposity and executive function in middle age. *J Int Neuropsychol Soc*. 2016;22(5):493–500. doi:10.1017/s1355617716000230
41. Geha P, Cecchi G, Todd Constable R, et al. Reorganization of brain connectivity in obesity. *Hum Brain Mapp*. 2017;38(3):1403–1420. doi:10.1002/hbm.23462
42. Bischof GN, Park DC. Obesity and aging: consequences for cognition, brain structure, and brain function. *Psychosom Med*. 2015;77(6):697–709. doi:10.1097/psy.0000000000000212
43. Caunca MR, Gardener H, Simonetto M, et al. Measures of obesity are associated with MRI markers of brain aging: the Northern Manhattan Study. *Neurology*. 2019;93(8):e791–e803. doi:10.1212/wnl.0000000000007966
44. Lebrun B, Bariohay B, Moyse E, et al. Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. *Auton Neurosci*. 2006;126–127:30–38. doi:10.1016/j.autneu.2006.02.027
45. Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. *EMBO j*. 2000;19(6):1290–1300. doi:10.1093/emboj/19.6.1290
46. Lyons WE, Mamounas LA, Ricaurte GA, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci U S A*. 1999;96(26):15239–15244. doi:10.1073/pnas.96.26.15239
47. Cao L, Lin EJ, Cahill MC, et al. Molecular therapy of obesity and diabetes by a physiological autoregulatory approach. *Nat Med*. 2009;15(4):447–454. doi:10.1038/nm.1933
48. Bariohay B, Lebrun B, Moyse E, et al. Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. *Endocrinology*. 2005;146(12):5612–5620. doi:10.1210/en.2005-0419
49. Motamedi S, Karimi I, Jafari F. The interrelationship of metabolic syndrome and neurodegenerative diseases with focus on brain-derived neurotrophic factor (BDNF): kill two birds with one stone. *Metab Brain Dis*. 2017;32(3):651–665. doi:10.1007/s11011-017-9997-0
50. Das UN. Obesity: genes, brain, gut, and environment. *Nutrition*. 2010;26(5):459–473. doi:10.1016/j.nut.2009.09.020
51. Donovan MJ, Lin MI, Wiegand P, et al. Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. *Development*. 2000;127(21):4531–4540. doi:10.1242/dev.127.21.4531
52. Nakahashi T, Fujimura H, Altar CA, et al. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett*. 2000;470(2):113–117. doi:10.1016/s0014-5793(00)01302-8
53. Ozan E, Okur H, Eker C, et al. The effect of depression, BDNF gene val66met polymorphism and gender on serum BDNF levels. *Brain Res Bull*. 2010;81(1):61–65. doi:10.1016/j.brainresbull.2009.06.022
54. Piancatelli D, Aureli A, Sebastiani P, et al. Gene- and gender-related decrease in serum BDNF levels in Alzheimer's disease. *Int J Mol Sci*. 2022;23(23):14599. doi:10.3390/ijms232314599
55. Meng WD, Sun SJ, Yang J, et al. Elevated serum Brain-Derived Neurotrophic Factor (BDNF) but not BDNF gene Val66Met polymorphism is associated with autism spectrum disorders. *Mol Neurobiol*. 2017;54(2):1167–1172. doi:10.1007/s12035-016-9721-9

56. Kronenberg G, Gertz K, Schöner J, et al. BDNF serum concentrations in 2053 participants of the berlin aging study II. *Neurobiol Aging*. 2021;101:221–223. doi:10.1016/j.neurobiolaging.2021.01.020
57. Molendijk ML, Bus BA, Spinhoven P, et al. Gender specific associations of serum levels of brain-derived neurotrophic factor in anxiety. *World J Biol Psychiatry*. 2012;13(7):535–543. doi:10.3109/15622975.2011.587892

Diabetes, Metabolic Syndrome and Obesity

Dovepress

Publish your work in this journal

Diabetes, Metabolic Syndrome and Obesity is an international, peer-reviewed open-access journal committed to the rapid publication of the latest laboratory and clinical findings in the fields of diabetes, metabolic syndrome and obesity research. Original research, review, case reports, hypothesis formation, expert opinion and commentaries are all considered for publication. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/diabetes-metabolic-syndrome-and-obesity-journal>