Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

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

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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

Received: 17 March 2023
Accepted: 26 May 2023
Published: 30 May 2023

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 weight[7] 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 restriction[21] and stage of life[22] 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 scantly 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 edition[32] 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 ± 28.13	55.17 ± 25.96	57.16 ± 29.68	0.375
Ages (years)	16.87 ± 0.59	16.96 ± 0.60	16.80 ± 0.57	<0.001
BMI (kg/m^2)	20.31 ± 2.36	19.83 ± 2.35	20.67 ± 2.30	<0.001
WHR	0.78 ± 0.05	0.77 ± 0.04	0.78 ± 0.05	0.015
Heart rate (beats/min)	82.74 ± 11.26	79.90 ± 10.88	84.90 ± 11.08	<0.001
SBP (mmHg)	118.9 ± 11.70	121.1 ± 12.30	117.2 ± 11.00	<0.001
DBP (mmHg)	72.87 ± 10.49	73.30 ± 10.35	72.55 ± 10.60	0.367
TC (mmol/L)	3.59 ± 0.58	3.43 ± 0.52	3.72 ± 0.59	<0.001
HDL-C (mmol/L)	1.41 ± 0.28	1.36 ± 0.27	1.45 ± 0.28	<0.001
TG (mmol/L)	1.13 ± 0.44	0.96 ± 0.35	1.25 ± 0.46	<0.001
LDL-C (mmol/L)	1.67 ± 0.49	1.63 ± 0.48	1.70 ± 0.51	0.066
Glucose (mmol/L)	5.06 ± 0.44	5.13 ± 0.44	5.01 ± 0.43	<0.001
Insulin (μIU/mL)	12.06 ± 5.64	9.72 ± 4.52	13.83 ± 5.77	<0.001
Vitamin A intake (μg/day)	369.6 ± 795.9	455.4 ± 1061	304.5 ± 501.9	0.029
Thiamine intake (mg/day)	1.56 ± 1.00	1.84 ± 1.24	1.34 ± 0.71	<0.001
Riboflavin intake (mg/day)	0.70 ± 0.53	0.82 ± 0.72	0.60 ± 0.29	<0.001
Vitamin B6 intake (mg/day)	0.40 ± 0.25	0.42 ± 0.27	0.39 ± 0.23	0.276
Folic acid intake (μg/day)	83.7 ± 63.07	90.17 ± 69.13	78.79 ± 57.67	0.027
Niacin intake (mg/day)	14.44 ± 11.07	17.63 ± 14.80	12.02 ± 60	<0.001
Vitamin C intake (mg/day)	60.1 ± 59.43	62.25 ± 77.15	58.47 ± 41.23	0.460
Vitamin E intake (mg/day)	27.73 ± 19.25	27.28 ± 23.18	28.08 ± 15.64	0.623
Calcium intake (mg/day)	372.0 ± 367.6	432.2 ± 471.1	326.3 ± 254.6	0.001
Phosphorus intake (mg/day)	990.9 ± 564.4	1149 ± 701.5	870.9 ± 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 Val/Met Met/Met	175 (27.3) 327 (50.7) 142 (22.0)	0.954	79 (28.7) 139 (49.8) 60 (21.5)	96 (26.2) 188 (51.4) 82 (22.4)	0.832
Alleles	Val Met	677 (52.6) 611 (47.4)	–	297 (53.6) 259 (46.4)	380 (51.9) 352 (48.1)	0.612

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

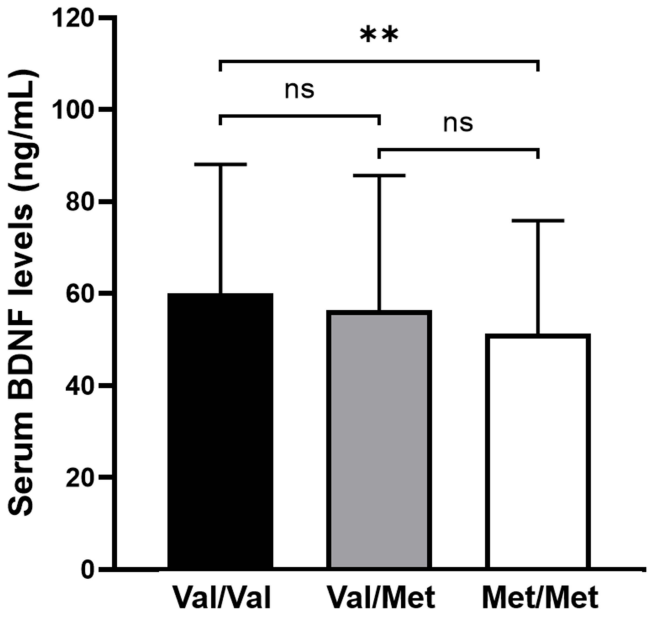

Figure 1 Serum BDNF levels in the subjects with different genotypes of *Bdnf* Val66Met. **p<0.01; ns, p>0.05.

(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

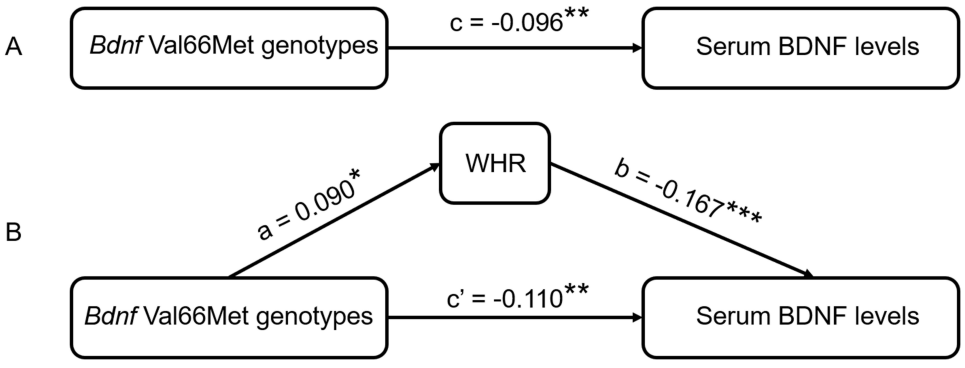

Figure 2 Mediation model for serum BDNF levels, *Bdnf* Val66Met and WHR. (**A**) Path diagram for the regression model. (**B**) The simple mediation model with WHR as a mediator of the effect of *Bdnf* Val66Met on serum BDNF levels.
Notes: Path coefficients were shown in standardized regression coefficient. *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 counties.[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.

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