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Interactions of dietary insulin index and dietary insulin load with brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in relation to cardiometabolic markers in Iranian diabetic patients: a cross-sectional study

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Abstract

The progression of cardiometabolic diseases is determined by both genetic and environmental factors. Gene–diet interactions may therefore be important in modulating the risks of developing metabolic diseases. The objectives were to investigate the effect of the interaction between brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms and dietary insulin index (DII) and dietary insulin load (DIL) on cardiometabolic markers among diabetic patients. In this cross-sectional study, blood samples were collected from 667 patients. DIL and DII were defined using a validated FFQ. Genotyping the BDNF Val66Met polymorphism was conducted by the PCR-Restriction fragment length polymorphism (RFLP) method. Interactions between dietary indices and gene variants were evaluated using a generalised linear model. PGF2a concentrations were significantly higher among Val homozygotes than Met-allele carrier. This study revealed that, compared with individuals with the Val/Val genotype, those with the Met/Val or Met/Met genotype had lower BMI ($P_{interaction} = 0{\cdot}04$), TAG ($P_{interaction} = 0{\cdot}04$), leptin ($P_{interaction} = 0{\cdot}01$), LDL ($P_{interaction} = 0{\cdot}04$) and total cholesterol ($P_{interaction} = 0{\cdot}01$) when they consumed diets higher on the DIL index. Moreover, the highest quartile of the DIL, compared with the lowest, showed increase in waist circumference ($P_{interaction} = 0{\cdot}02$) and LDL/HDL ($P_{interaction} = 0{\cdot}04$) for Val/Val homozygotes compared with Met-allele carriers. BDNF Val66Met variants may interact with DIL and DII, thus be involved in the development of cardiometabolic risk factors. If diabetic patients with Met alleles regulate dietary intakes, they have a protective opportunity to regulate their cardiometabolic markers.

Key words: **BDNF Val66Met polymorphism: Type 2 diabetes mellitus: Nutrigenetic: Dietary insulin index**

Type 2 diabetes mellitus (T2DM) is a metabolic disorder and is a known health risk associated with various body organs[1]. T2DM has also been considered as a risk factor for CVD[2]. Several studies suggest that T2DM and CVD are induced by environmental conditions, hormonal factors, lifestyle behaviours and genetic variations[3–5].

Findings have shown that one of the key genetic targets is brain-derived neurotrophic factor (BDNF), which plays a critical role in cardiovascular functions[6]. Numerous genome-wide association studies have revealed that the BDNF gene is associated with T2DM and CVD through various mechanisms[7]. The BDNF genes are polymorphic in humans, of which BDNF Val66Met variants have been more commonly assessed in terms of BDNF polymorphisms[8]. Previous research has shown an association between BDNF Val66Met variants and CVD risk factors such as inflammation, hypertension, insulin resistance (IR) and dyslipidaemia, but other findings have reported no associations[9].

Additionally, dietary pattern is a major factor relating to T2DM and CVD[10]. Diet has been shown to affect postprandial insulin secretion and control metabolic disorders, including IR, dyslipidaemia, obesity and T2DM[11]. Many studies have estimated the insulinogenic effect of diet through the glycaemic index and glycaemic load, in terms of food able to enhance blood glucose levels (postprandial hyperglycaemia) *v.* reference foods (either glucose or white bread)[12]. However, while glycaemic index

Abbreviations: BDNF, brain-derived neurotrophic factor; DII, dietary insulin index; DIL, dietary insulin load; IR, insulin resistance; TC, total cholesterol; T2DM, type 2 diabetes mellitus; WC, waist circumference.

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and glycaemic load demonstrate the effect of carbohydrates on plasma glucose, these indices are not able to estimate accurate insulin responses[13]. Insulin indices, including dietary insulin index (DII) and dietary insulin load (DIL), can thus provide more valuable and accurate estimates of insulin response for different dietary food items with various carbohydrate values[14]. Numerous recent studies have indicated that insulin indices are more suitable in terms of examining correlations between insulin levels and the occurrence of metabolic disorder, compared with glycaemic indices[15].

There is currently limited research reporting the correlations between DIL, DII and CVD risk factors, and more nutrition studies are required in the field[16]. However, the existing contradictory results may be due to genetic variations[17,18]. Therefore, nutrigenetic approaches, which specifically relate genetics and nutrition, are an extremely promising avenue of research. There are several studies examining associations between several nutrients and Val66Met polymorphism, which have indicated that BDNF polymorphisms interact with dietary carbohydrate consumption in chronic diseases, including hypertension, obesity, IR, dyslipidaemia and T2DM[19].

To the authors' knowledge, there is no present research in the literature assessing the interaction between DII, DIL and BDNF polymorphism towards CVD risk factors. Therefore, the present study proposes to investigate interactions among BDNF Val66Met variants and insulin indices on cardiometabolic markers in T2DM patients (Iranian diabetic adults).

Method

Sample

For this cross-sectional study, 667 T2DM patients (260 men, 407 women) aged 35–65 years were recruited from diabetes referral centres. All the inclusion and exclusion criteria were taken based on the authors' previous, larger investigation[20] Diabetic patients who had fasting blood sugar > 126 mg/dl or were using glucose-lowering medications without history of inflammatory diseases, CVD, stroke and cancers were included in the study. Besides, patients who were pregnant, addict, taking anti-inflammatory medications and using insulin, lactating patients and also their total energy intake was not in range between 800 and 4200 were excluded. All subjects signed a written informed consent form, which was approved by the Ethics Committee of the Tehran University of Medical Sciences (protocol no:4128).

Biochemical assessments

Laboratory assessments were collected after 12–14 h fasting. Baseline circulating plasma lipid (total cholesterol (TC), TAG, LDL and HDL) was profiled by a standard enzymatic technique with a commercial kit (Pars Azmoon Co.). Serum inflammatory markers including pentraxin-3, IL-18 and high-sensitivity C-reactive protein were measured by Crystal Day Biotech (Shanghai Crystal Day Biotech Co. Ltd.) and DBC (Diagnostics Biochem Canada Inc.), respectively. Total antioxidant capacity and superoxide dismutase enzymatic activity were assessed using spectrophotometry and colorimetry methods. ELISA method (Bioassay Technology Co) was used to assess serum levels of ghrelin and leptin.

General, anthropometric and physical activity assessments

Information about age, sex, medical history, smoking status, duration of diabetes and its complications, use of supplements or medication either lipid- or glucose lowering was obtained through interviews by an expert dietitian. BMI, waist circumference (WC) and physical activity level were measured based on the previous study's results[21].

Genotyping

A salting-out method was used for DNA extraction[22]. Val66Met polymorphism was genotyped by the PCR-RFLP method. PCR amplification of rs6265 was performed by the following primers: forward, 5'-CACTAGCCCAGAGAGAGGAGTG-3', reverse, 50-TGAGCCCAGCCGCACACTAAC-. The final volume of PCR product contained 25 µl including 75 ng genomic DNA, 0·6 mM of each primer and 2X Taq DNA Polymerase Master Mix (Amplicon). PCR cycles were designed with denaturation at 95°C for the 30 s (40 cycles), annealing at 8°C for 30 s and 40 s of extension at 72°C, with a final extension at 65°C for 30 min. Finally, electrophoresis of the products was performed on 2 % agarose gel.

Dietary assessments

Self-reported macronutrient intake data were obtained via FFQ[23]. All portion sizes of consumed foods were reported in household measures, and then converted to g/d using a validated Iranian food composition table[24]. The daily dietary intake of nutrients and energy were analysed using the US Department of Agriculture Food database, as adjusted for Iranian foods. The modified USDA nutrient database contains the compositions of some Iranian foods that were not available in the original USDA database. The compositions of these foods were assessed previously[25]. DII was computed from a previous study by Brand-Miller[26]. Briefly, DII measures the incremental insulin AUC over 2 h in response to the consumption of a 1000-kJ portion of the test food, divided by the AUC after ingestion of a 1000-kJ portion of the reference food[15,26,27]. Food items in the present study were matched to the Brand-Miller items, based on the correlations between their content of energy, fibre, carbohydrate, protein and fat. The insulin index of each food was multiplied its energy content to calculate the insulin of food as follows:

Insulin load of food = Insulin index of food × energy content of food (kcal/d)

DIL was obtained by summing the overall insulin loads of the foods.

Statistical analysis

Adherence to Hardy–Weinberg equilibrium was determined using the χ^2 test. Biochemical variables were assessed for normality of distribution by Kolmogorov Smirnov test and were normalised by log10 as appropriate. One-way ANOVA and χ^2 test were used to calculate unadjusted means of continuous and categories variables, respectively, by quartile of each dietary index

(DIL and DII). Independent *t* test was performed for the comparison of cardiometabolic variables between genotype groups. Interactions between genotypes and (DIL and DII) on quantitative variables were analysed using a generalised linear regression model. We included all confounders including age, marital status, education, physical status, family history of diabetes, dietary supplement use and dietary variables into the models and then in the final analysis, which was presented in the manuscript, we retained those variables that had a to the outcome. Data were analysed using Statistical Package for Social Sciences (SPSS Inc., version 25) and *P*-value < 0·05 was considered as significant.

Results

Associations between cardiometabolic markers and brain-derived neurotrophic factor Val66Met polymorphism

Six hundred and sixty-seven patients with T2DM were investigated in this cross-sectional study. The overall prevalence of rs6265 genotypes was 55·6, 35·2 and 9·1 % for Val/Val, Met/Val and Met/Met, respectively. The genotype distributions were within Hardy–Weinberg equilibrium (*P*-value > 0·05). Details of the biochemical variables between rs6250 genotypes are presented in Table 1. PGF2a concentrations were significantly higher among Val homozygotes than Met-allele carrier. Additionally, there were no significant differences for other variables, according to BDNF genotypes.

Association between cardiometabolic markers and dietary insulin index and dietary insulin load

The basic information of diabetic patients between the DIL and DII groups is presented in Table 2. All patients were divided into four groups, based on their DIL and DII scores. Patients in the fourth quartile of DII were more likely to be male ($P < 0·001$) and younger ($P = 0·01$) compared with those in the first group of DIL. Patients in the last quartile of DIL had higher alcohol consumption ($P = 0·001$) and energy intake ($P < 0·001$), with higher WC ($P = 0·04$), although greater adherence to DIL reduced HDL ($P = 0·03$). Regarding DII, patients in the last quartile were more likely to be male ($P = 0·008$) and they consumed higher energy intake ($P < 0·001$), with higher LDL plasma levels ($P = 0·04$). There was a significant difference in leptin and ghrelin serum across the DII quartiles. Patients in the last quartile of DII had more leptin and less ghrelin, compared with the first quartile of DII. There were no significant associations found regarding other basic characteristics and biochemical parameters between the DIL and DII groups.

Table 1. The association between metabolic markers and the genotypes of BDNF Val66Met polymorphism in T2DM patients (Mean values and standard deviations)

	BDNF polymorphism				
	Met/Met, Met/Ala		Ala/Ala		
	Mean	SD	Mean	SD	*P*
Age (year)	53·69	6·71	54·36	6·3	0·19
BMI (kg/m^2)	29·40	4·64	29·18	4·64	0·53
HDL-cholesterol (mg/dl)	52·21	12·3	53·68	11·78	0·07
LDL-cholesterol (mg/dl)	106·27	34·69	107·26	32·74	0·5
CH (mg/dl)	192·21	59·28	196·36	63·45	0·48
LDL/HDL	2·08	0·67	3·06	13·34	0·75
TAG (mg/dl)	173·10	90·98	178·88	93·77	0·47
Leptin (ng/ml)	26·32	16·18	24·52	13·19	0·83
Ghrelin (ng/ml)	2·27	1·14	2·54	1·5	0·34
CRP (mg/l)	2·06	1·54	2·41	1·46	0·18
PTX3 (ng/ml)	2·61	0·51	2·65	0·49	0·57
IL18 (pg/ml)	250·19	29·61	246·55	27·23	0·48
TAC (g/dl)	2·55	0·58	2·46	0·57	0·32
SOD (U/ml)	0·14	0·04	0·14	0·04	0·93
PGF2α (pg/ml)	71·23	5·74	73·48	6·67	0·04

CH, cholesterol; CRP, C-reactive protein; PTX3, pentraxin3; TAC, total antioxidant capacity; SOD, superoxide dismutase.

Interaction between dietary insulin index and dietary insulin load with brain-derived neurotrophic factor Val66Met variants on cardiometabolic markers

The interaction between BDNF Val66Met polymorphism and quartiles of DIL and DII scores on cardiometabolic marker is shown in Figs 1 and 2.

Significant interactions were observed between DIL score and rs6265 SNP in terms of BMI, WC, TC, TAG, LDL, LDL/HDL and leptin. This study revealed that, compared with individuals with the Val/Val genotype, those with the Met/Val or Met/Met genotype had lower BMI ($P_{interaction} = 0·04$), TAG ($P_{interaction} = 0·04$), leptin ($P_{interaction} = 0·01$), LDL ($P_{interaction} = 0·04$) and TC ($P_{interaction} = 0·01$) when they consumed diets higher on the DIL index, although these scores increased for those with Val/Val genotypes.

Moreover, when comparing Val/Val homozygotes to Met/Met Met/Val homozygotes, the highest quartile of the DIL showed an increase in WC ($P_{interaction} = 0·02$). Also, higher DIL scores were associated with higher levels of LDL/HDL ($P_{interaction} = 0·04$) in individuals with the Val homozygotes, compared with Met-allele carriers (Fig. 1).

Besides, the highest quartile of the DII, compared with the lowest, showed increases in BMI among Val/Val genotypes ($P_{interaction} = 0·03$) (Fig. 2).

Discussion

This cross-sectional study explored the interactions of the BDNF Val66Met polymorphism and DIL and DII on cardiometabolic markers. The results showed that higher DIL scores were more likely to be seen among males and younger participants. Patients in the lowest group of DIL consumed more alcohol and had higher energy intake, with higher WC and lower HDL. A higher DII score, however, was associated with higher energy intake, LDL plasma levels, leptin and lower serum ghrelin, compared with the first quartile of DII. There have been limited studies on the associations between DIL, DII and metabolic markers including lipid profiles and inflammatory markers; these previous results have been inconsistent. A similar conclusion was reached by Katharina Nimptsch *et al.*, revealing that DIL and DII were significantly associated with lower HDL concentrations, although they did not find a significant relationship with

Table 2. The association between baseline characteristic and metabolic markers with dietary insulin load (DIL) and dietary insulin index (DII) in T2DM patients (Numbers and percentages)

	Quartiles of DIL									Quartiles of DII								
	Q1		Q2		Q3		Q4		*P*	Q1		Q2		Q3		Q4		*P*
	n	%	*n*	%	*n*	%	*n*	%		*n*	%	*n*	%	*n*	%	*n*	%	
n	159		158		158		159			159		158		158		159		
Sex																		
Male	41/118		53/105		81/77		77/82		< 0·001	49	19·4	58	23	67	26·6	78	31	0·008
Female										109	28·5	101	26·4	91	23·8	81	21·2	
Cigarette smoking																		
Yes	25	22·1	23	20.	36	31·9	29	25·7	0·57	26	23	26	23	27	23·9	34	30·1	0·89
No	132	25·8	132	25·8	119	23·3	128	25		130	25·4	130	25·4	128	25	123	24·1	
Alcohol consumption																		
Yes	3	15·8	1	5·3	3	15·8	12	63·2	0·001	7	36·8	4	21·1	7	36·8	1	5·3	0·14
No	156	25·4	157	25·5	146	23·7	156	25·0		151	24·6	155	25·2	151	24·6	158	25·7	
Fat medications																		
Yes	94	59·11	90	56·96	89	55·94	83	55·97	0·68	104	65·82	87	54·71	86	54·43	78	49·05	0·41
No	65	40·88	68	24·4	70	44·02	76	27·2		54	34·17	72	45·28	72	45·56	81	50·94	
Familial history of diabetes																		
Yes	129	79·24	133	84·17	127	80·37	129	81·13	0·36	132	83·54	124	77·98	123	77·84	136	85·53	0·5
No	33	27·7	25	21	31	26·1	30	25·2		26	16·45	35	22·01	35	22·15	23	14·46	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (year)	55·36	6·3	54·16	6·53	53·79	6·38	52·94	6·57	0·01	54·96	5·93	53·97	6·93	53·68	6·79	53·65	6·22	0·23
BMI (kg/m^2)	29·06	4·66	29·15	4·52	29·39	4·53	29·51	4·86	0·78	29·15	4·74	29·66	4·55	28·99	4·7	29·29	4·57	0·55
WC (cm)	90·71	10·41	91·62	11·34	93·63	10·19	92·91	9·87	0·04	90·77	10·65	93·22	10·53	92·32	10·77	92·56	9·98	0·16
PA (Met.week)	37·36	4·85	38·53	5·6	37·73	6·35	38·01	5·04	0·28	38·15	5·64	37·7	6·32	37·82	5·01	37·96	4·92	0·9
Energy intake (kcal/d)	1755·78	359·53	2249·21	393	2647·69	372·36	2990·85	764·26	< 0·001	2334·27	605·72	2366·32	592·17	2327·88	654·90	2613·57	812·19	< 00·1
HDL-cholesterol (mg/dl)	55·89	12·05	54·36	11·96	50·91	12·38	53·96	11·52	0·03	53·8	12·23	51·91	13·11	52·93	11·07	53·49	11·63	0·33
LDL-cholesterol (mg/dl)	109·47	35·61	106·53	35·81	103·09	31·9	108·18	30·72	0·34	108·89	36·32	102·04	33·57	105·55	33·15	110·82	30·75	0·04
CH (mg/dl)	201·84	67·08	197·07	64·04	187·48	58·83	191·69	55·54	0·22	197·08	64·36	197·2	68·26	195·48	61·84	188·38	50·89	0·79
LDL/HDL	2·13	0·72	3·09	13·61	2·08	0·64	3·21	14·6	0·84	3·16	13·61	2·01	0·64	2·03	0·65	3·3	14·59	0·19
TAG (mg/dl)	174·89	93·66	181·16	94·49	176·23	91·21	173·05	91·35	0·86	173·54	88·46	177·37	84·01	188	105·29	166·44	90·54	0·24
Leptin (ng/ml)	27·15	12·57	26·57	13·14	22·79	16·91	24·38	14·92	0·1	21·59	12·88	23·35	14·46	26·14	14·34	29·72	14·98	0·01
Ghrelin (ng/ml)	2·62	1·34	2·6	1·59	2·33	1·47	2·22	1·18	0·32	2·26	1·17	2·84	1·67	2·57	1·44	2·1	1·1	0·03
hs-CRP (mg/l)	2·11	1·46	2·37	1·43	1·92	1·33	2·66	1·69	0·43	2·32	1·35	2·19	1·62	2·1	1·34	2·48	1·73	0·79
PTX3 (ng/ml)	2·72	0·4	2·53	0·53	2·63	0·48	2·62	0·59	0·42	2·57	0·48	2·6	0·41	2·69	0·5	2·68	0·6	0·79
IL18 (pg/ml)	249·12	29·31	247·2	29·49	245·37	27·68	249·56	26·85	0·93	252·37	28·74	251·41	30·92	243·26	26·4	244·42	26·28	0·44
TAC (g/dl)	2·41	0·53	2·71	0·65	2·47	0·53	2·43	0·55	0·14	2·54	0·57	2·42	0·57	2·48	0·62	2·56	0·54	0·65
SOD (U/ml)	0·14	0·03	0·14	0·04	0·15	0·04	0·14	0·05	0·63	0·14	0·04	0·13	0·03	0·15	0·04	0·14	0·05	0·41
PGF2α (pg/ml)	72·65	6·4	71·38	5·74	72·65	6·85	73·45	6·66	0·65	73·18	5·6	73·22	6·35	70·5	6·38	73·3	7·1	0·2

WC, waist circumference; CH, cholesterol; hs-CRP, high-sensitivity C-reactive protein; PTX3, pentraxin-3; TAC, total antioxidant capacity; SOD, superoxide dismutase.

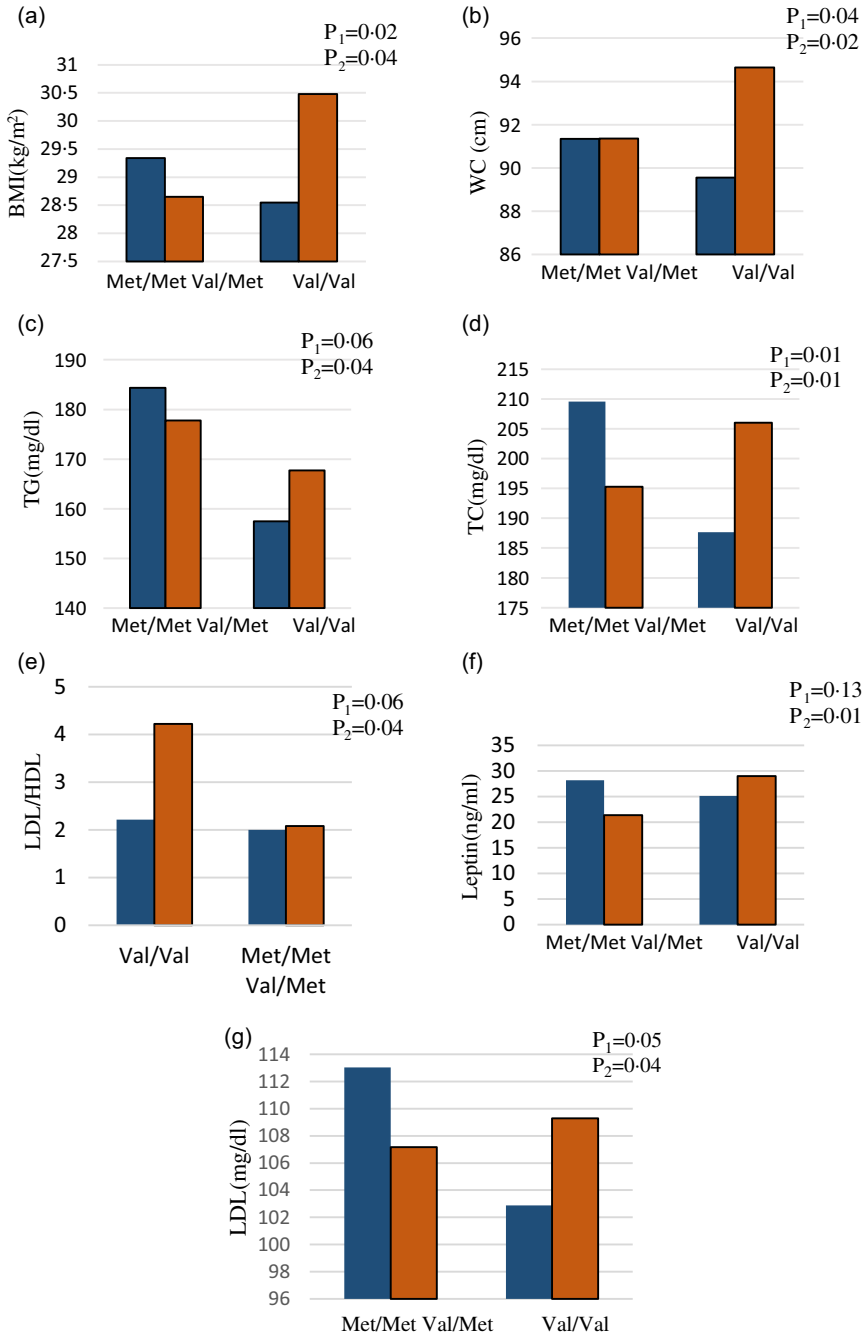

Fig. 1. The interaction between BDNF and DIL on (a) BMI, (b) WC, (C) TAG, (d) TC, (e) LDL/HDL, (f) leptin, (g) LDL. The interaction between BDNF Val66Met and DIL was obtained with generalised linear model (GLM). P_1= *P* value with unadjusted (crude) model. P_2= *P* value with adjustments for potential confounding factors including (age, sex, smoking, alcohol consumption, PA and energy intake). The bars indicate mean values and standard deviations. (a) Mean values of BMI across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. (b) Mean values of WC across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. (c) Mean values of TG across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. (d) Mean values of TC across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. (e) Mean values of LDL/HDL across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. (f) Mean values of leptin across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. (g) Mean values of LDL across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DIL intake. BDNF, brain-derived neurotrophic factor; DIL, dietary insulin load; WC, waist circumference; TC, total cholesterol. ■, Q1; ■, Q4

LDL and inflammatory markers[15]. Recent studies in this regard have shown significant associations between DIL scores and weight and WC[28]. Joslowski *et al.* revealed a positive correlation between DII/DIL scores and body fat in younger subjects[29]. Chaput *et al.* showed that postprandial hyperinsulinaemia may be able to predict changes in anthropometric data such as weight and WC[30]. One of the proposed mechanisms involved in the effect of DIL and DII on metabolic markers is a persistent demand for β cells, which results from the long-term intake of foods that can reduce insulin sensitivity. These foods also

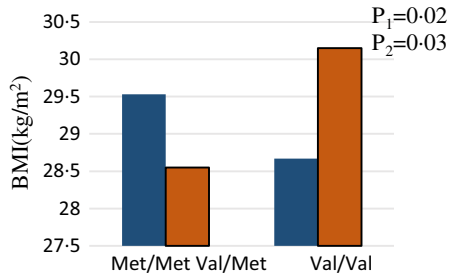

Fig. 2. The interaction between BDNF Val66Met and DII on BMI. The interaction between BDNF Val66Met and DII was obtained with generalised linear model (GLM). P_1= *P* value with unadjusted (crude) model. P_2= *P* value with adjustments for potential confounding factors including (age, sex, smoking, alcohol consumption, PA and energy intake). The bars indicate mean values and standard deviations. (a) Mean values of BMI across (Met/Met +Met/Val) and Val/Val genotypes based on low and high DII intake. BDNF, brain-derived neurotrophic factor; DII, dietary insulin index. ■, Q1; ■, Q4.

increase the risks of developing obesity factors, such as higher WC and lower HDL. Other results were broadly in line with Gargari *et al.*, demonstrating a negative relationship between dietary fat intake and serum leptin. This study also showed a significant inverse relationship between carbohydrate intake and serum ghrelin among polycystic ovarian incidence, although did not find a significant relationship between leptin and ghrelin with macronutrients in control subjects[31]. Several studies have shown the role of insulin in the regulation of ghrelin concentrations. Ghrelin alterations were conversely associated with insulin; ghrelin serum was down-regulated with increases in insulin concentration after meal consumption[32–37]. In rodents, high insulin concentrations decrease ghrelin production by the stomach *in vitro*, and *in vivo*, high insulin induced via clamp decreases ghrelin[38,39]. Some authors have shown hyperinsulinaemia to be conversely associated with ghrelin concentration[40,41]. It is commonly acknowledged that carbohydrates are the most effective component for ghrelin suppression, due to their insulin-secretin and rapid absorption effects.

To the authors' knowledge, previous studies in this field have primarily focused on investigating the associations between DIL and DII scores with obesity and cancer. However, the interaction between diet and genetics has rarely been studied directly; thus, the present study investigated the interplay between dietary (IL and II) and BDNF Val66Met polymorphism in relation to cardiometabolic markers in diabetic patients. A gene–dietary interaction was found in diabetic patients. The novelty of the present study is that there was a significant interaction between DIL, DII score and BDNF Val66Met polymorphism on BMI, WC, TC, TAG, LDL/HDL, LDL and leptin. A further novel finding is that diabetic patients who are carriers of the Met allele were less likely to increase their cardiometabolic risks with high adherence to DIL and DII. However, Val/Val participants with high DIL or DII intake were found to be more likely to increase their cardiometabolic risks. Met/Val and Met/Met genotypes had lower BMI, TAG, leptin and TC when they consumed diets higher in DIL; these values were increased for those carrying Val/Val. Xian-Yong Ma *et al.* assessed the interactions of BDNF Val66Met polymorphism with dietary intake for obesity traits and revealed a significant interaction between BDNF Val66Met and PUFA in terms of obesity risks[42]. A further study demonstrated that the relationship between T2DM and BDNF Val66Met polymorphisms may be diet dependent. BDNF Val/Met lowered the risk of T2DM with high carbohydrate intake, low energy and protein intake groups in comparison with Val/Val. BDNF Val/Met did not compensate for T2DM development with high-energy intake, having higher homeostatic model assessment (HOMA-B) levels. However, Val/Val participants (even in low-protein and energy intake groups) were found to be more likely to develop diabetes, and participants with Val/Met allele were found to have higher HOMA-IR levels than others[43].

Prior studies have revealed significant correlations between BDNF Val66Met and food intake regulation[44,45]. Moreover, there are already studies indicating that high carbohydrate intake reduces BDNF mRNA and protein content, whereas dietary restriction and carbohydrate restrictions increase brain BDNF expression[46]. Additionally, previous studies have emphasised a significant association between glucose and insulin metabolism, adiposity regulation and serum levels of BDNF[47]. Some authors have also suggested that hyperphagia, hyperinsulinaemia and higher levels of serum leptin and body weight following a decrease in BDNF levels, among BDNF-knockout mice[48]. However, the relation of Val66Met polymorphism and DNA expression rate and serum levels of BDNF remains unclear. Despite findings being inconsistent, it seems that Met carriers tend to display increased serum BDNF[49–52]. The present study revealed the negative effect of high glycaemic index foods among Val/Val carriers on cardiometabolic factors, which may be a cause of a decrease in BDNF levels. This is an especially interesting protective effect among Met carriers, as Daily *et al.* showed in high-energy, high-carbohydrate diets[43]. It seems that Met carriers who consume high-energy, high-carbohydrate diets can be compared with Val/Val patients in terms of increased insulin secretion (HOMA-B) to eliminate the effects of IR. It is suggested that dietary insulin indices might adjust the relationship between BDNF genotype and metabolic markers by changes in BDNF expression; these findings remain to be further investigated in future studies.

Limitations of the present study including the cross-sectional design, so any causality cannot be argued; the use of FFQ for dietary assessing, which may have resulted in memory bias. Furthermore, our participants were from the Iranian country which may not be generalised due to racial and regional difference. Despite the limitations mentioned above, this is the first effort to study the interaction between BDNF Val66Met polymorphism and dietary insulin indices on cardiometabolic risk factors. Recognition of these gene–diet interactions could be determining in prescribe personalised nutritional recommendations for the improvement and management of CVD risk in T2DM patients. Personalised nutrition recommendations, based on the knowledge of an individual's genetic background, will improve the outcomes of specific dietary intervention and will represent a promising dietary approach to improve health, reducing obesity and its metabolic consequences between diabetic patients.

Conclusion

These results suggest that subjects with T2DM and Val/Val allele are more likely to be at risk of cardiovascular risk factors than patients carrying Met alleles, even in low DIL or DII. In the present study, Met-allele carriers had higher energy intakes. Interestingly, the risk of CVD was reduced in spite of higher DIL and DII in patients with this allele. If diabetic patients with Met alleles regulate dietary intakes, they have a protective opportunity to regulate their cardiometabolic markers. BDNF Val66Met polymorphism may be related to DII and DIL in terms of the development of cardiometabolic risk factors.

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