A High-Protein/Low-Fat Diet May Interact with Vitamin D-Binding Protein Gene Variants to Moderate the Risk of Depression in Apparently Healthy Adults

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Keywords

Dietary intake · Vitamin D-binding protein · Polymorphisms · Depression

Abstract

Background: Recent studies have shown that depression is inversely correlated with high protein and low fat intake and positively correlated with vitamin D-binding protein (VDBP). Therefore, the aim of this study was to examine the interaction between protein/fat dietary patterns and VDBP genotypes with regard to the risk of depression in apparently healthy adults who have not been diagnosed with any chronic disease. **Methods:** In this study, 265 individuals (126 males and 139 females) aged 18–55 years were recruited from the communities of central and west Tehran based on convenience sampling. Body composition was measured with a body composition analyzer and depression symptoms were categorized as normal, moderate depression, or severe depression using the Depression Anxiety Stress Scales 21 (DASS-21) questionnaire. Dietary patterns were determined by a semiquantitative food frequency question-

naire to assess typical food intake during the 12-month period. Blood samples were collected from and biochemical measurements performed on all participants. An analysis of two polymorphisms (rs7041 and rs4588) in the GC gene, which encodes VDBP, was performed by polymerase chain reaction-restriction fragment length polymorphism. **Results:** A statistically significant association was found between depression and diet ($p = 0.03$) after having categorized the participants into three groups: a high-protein/low-fat (HP/LF) group, a moderate-protein/moderate-fat (MP/MF) group, and a low-protein/high-fat (LP/HF) group. Moreover, the findings demonstrated that depression was related to both the rs7041 and the rs4588 polymorphism ($p = 0.05$ and $p = 0.02$, respectively). We next used multinomial logistic modeling to investigate the risk of depression. A significant interaction was observed between HP/LF diet and the rs7041 polymorphism in the moderate- and severe-depression groups ($\beta = -0.30$, $p = 0.05$, and $\beta = -0.48$, $p = 0.01$, respectively). **Conclusion:** This study showed that an HP/LF diet interacts with the rs7041 polymorphism, with T allele carriers having a greater prevalence of moderate and severe depression.

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Introduction

Depression is a prevalent mental disorder and the second leading cause of global disability, with a high prevalence estimated at 350 million people worldwide [1]. Depression is characterized by a range of symptoms, including a loss of interest in daily activities for more than 2 weeks [1]. The outcomes associated with depressive disorders include a negative impact on relationships, families, and work. It has been estimated that depressive disorders are a significant cause of disease burden in both men and women [1, 2]. Several epidemiological studies have reported that a low serum 25-hydroxy-vitamin D level is a risk factor for depression [3, 4].

Calcitriol ($1,25[\text{OH}]_2\text{D}_3$) helps to maintain both the number of neurons and neuronal structure via detoxification mechanisms including inhibition of inducible nitric oxide synthase synthesis and increasing glutathione levels [5] and by regulating the synthesis of neurotrophins [6, 7] – all of which are factors affecting the risk of depression. The effect of calcitriol on depression may be due to the presence of vitamin D receptor, vitamin D-binding protein (VDBP), and/or 1-alpha-hydroxylase enzyme, which converts $25(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ in the brain. These findings have encouraged efforts to describe the relationship between neuropsychiatric disorders and brain development [6].

In the present study, our goal was to examine the effects of polymorphisms in the GC gene, which encodes VDBP, and not of circulating VDBP levels. VDBP is found in human cerebrospinal fluid [8] and is a serum protein encoded by the GC gene – the main carrier protein responsible for the transfer of calcitriol to target neurons [2]; however, little is known about the role of VDBP in the central nervous system [9]. Two common functional single nucleotide polymorphisms (SNPs) are present in exon 11 of the GC gene, and they result in nucleotide substitutions in codon 416 (GAT to GAG; Asp to Glu; rs7041) and codon 420 (AAG to ACG; Thr to Lys; rs4588) [10]. The T allele in rs7041 and the A allele in rs4588 correspond to the risk alleles related to depression [11–14].

Many studies have established a relationship between diet and depression. For example, there is a relationship between high-protein/low-fat (HP/LF) diet and depression [15, 16]. A number of studies have shown the effect of protein intake on depression [16, 17]. The investigation into the effects of dietary protein intake on depression has centered on levels of serotonin and brain concentrations of tryptophan [17]. Plasma concentrations of

VDBP were found to be sensitive to dietary protein deficiency, though the mechanisms are unknown [18].

In the present study, the interaction between dietary protein/fat patterns and GC polymorphisms, as well as their effect on depression risk, were examined in a population of healthy Iranian adults free of chronic disease.

Subjects and Methods

Participants

The study subjects consisted of 265 individuals (126 males and 139 females) aged between 18 and 55 years who were recruited from all the regions of west and central Tehran, using community-based convenience sampling [19]. The information was collected between May and September 2017. Exclusion criteria were pregnancy; being diagnosed with hepatic diseases such as viral hepatitis; thyroid, renal, or cardiovascular diseases; heart failure; malignancies; diabetes mellitus; being in any acute or chronic inflammatory state that affects inflammatory markers; having any kind of infection; being a current smoker; having a history of hypertension; or a self-reported indication of alcohol or drug abuse.

Anthropometric Assessments

For all participants in the study, weight (kg), height (cm), and waist circumference and hip circumference (cm) were measured. Body weight was measured in light clothing with electronic scales, height was measured barefoot, and hip circumference was measured at the largest part of the hip over light clothing. Waist circumference was measured using an anthropometric tape by determining the distance midway between the lowest rib and the iliac crest with the subject standing [20, 21].

Complete Body Composition Analysis

The body composition of all individuals was assessed using a BC-418MA Segmental Body Composition Analyzer (Tanita, UK) [22]. Measurements included weight, BMI, fat mass, body fat percentage, abdominal fat mass, muscle mass, fat-free mass, and visceral fat mass. Total body water was obtained using bioelectrical impedance analysis. The participants had all fasted overnight (10–12 h) and were barefoot when they were assessed by bioelectrical impedance analysis [19]. Taking measurements after strenuous exercise was avoided; the clinicians waited until the individuals had rested sufficiently in order to prevent possible differences in the measured values.

Dietary Intake Assessment and HP/LF Diet Scoring

The participants consumed their usual diet. Dietary intake was evaluated with the use of a valid and reliable [23], 147-item, semi-quantitative food frequency questionnaire (FFQ) to assess the usual food intake of individuals during the previous 12 months. The consumption frequency of each food item on a daily, weekly, or monthly basis was converted into daily intakes; portion sizes were then converted to grams and milliliters using Nutritionist software (Nutritionist version 4.0; Tinuviel Software, Warrington, UK). Eventually, total energy intake was calculated with this software. Protein/fat dietary patterns were calculated according to the following method. Using data obtained from the FFQ, the amount of protein and

fat intake was determined and the percentage of protein and fat intake was calculated using the following formulae: (protein intake [g] $\times$ 4/kcal) $\times$ 100 and (fat intake [g] $\times$ 9/kcal) $\times$ 100. The rank method for tertile scoring was used. The tertile scoring of protein intake was done as follows: (1) high protein intake, (2) moderate protein intake, and (3) low protein intake; the tertile scoring was the opposite for fat intake. Then, the scores were pooled together, and the final score was tertiled into (1) an HP/LF, (2) a moderate-protein/moderate-fat (MP/MF), and (3) a low-protein/high-fat (LP/HF) pattern. The mean protein percentage in the HP/LF, MP/MF, and LP/HF dietary patterns was 16.9, 14.7, and 12.7%, respectively.

Depression Symptoms

Data were collected using the Depression Anxiety Stress Scales 21 (DASS-21) questionnaire. This questionnaire is a psychological screening instrument which is able to differentiate between symptoms of depression, stress, and anxiety. It is a reliable and validated tool with 21 items in three domains (depression, anxiety, and stress) [24]. The individuals were asked to indicate the presence of symptoms related to each dimension over the past week, scored from 0 to 3. The scores on each domain were added together and then categorized based on the DASS manual as normal, moderate depression, or severe depression [25].

Blood Pressure Measurement

Resting blood pressure was measured after 15 min seated in a chair by the same person. Blood pressure was measured using an Automatic Inflate Blood Pressure Monitor (Samsung BA507S Automatic Digital Blood Pressure Monitor; Samsung America, Inc.).

Laboratory Measurements

All of the participants were referred to Shariati Hospital's outpatient clinic. All baseline blood samples were collected between 8:00 and 10:00 in the morning, following 10–12 h of overnight fasting. After centrifugation, serum was isolated and stored at a temperature of -80°C . All measurements were performed at the Endocrinology and Metabolism Research Center Laboratory of Shariati Hospital.

Biochemical Measurements

The GPO-PAP (glycerol-3-phosphate oxidase phenol 4-aminopyridine peroxidase) method was used for the measurement of triglyceride (TG) levels, and fasting serum glucose was measured by the GOD-PAP (glucose oxidase phenol 4-aminopyridine peroxidase) method [20]. Total cholesterol (TC) levels as well as direct high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured with an enzymatic clearance assay and the enzymatic endpoint method, respectively. All measurements were performed using Randox laboratory kits (Randox Laboratories Ltd., Ardmore, UK). Serum $1,25(\text{OH})_2\text{D}_3$ was measured using a BioSource kit (BioSource Europe SA, Nivelles, Belgium) by the radioimmunoassay method. Serum high-sensitivity C-reactive protein (hs-CRP) was assessed by the immunoturbidimetric assay method (high-sensitivity assay; Hitachi 902). Quality control for all biochemical assessments was performed using control serum, as per the manufacturers' instructions.

Polymorphism Analysis of the GC Gene

Genomic DNA was extracted from 200 mL of whole blood using the GeneAll Mini Columns Type kit (GeneAll, South Korea). The extracted DNA was used to assess the rs4588 and rs7041 SNPs

located in the GC gene. Polymerase chain reaction (PCR) was performed on the rs4588 and rs7041 SNPs using forward and reverse primers: forward, 5'-CAAGTCTTATCACCATCCTG-3'; reverse, 5'-GCCAAGTTACAATAACAC-3'. PCR was carried out on a total volume of 20 μL , which included 10 pmol of each primer, 50 ng/mL of DNA, and 1 M of dimethyl sulfide.

The amplification protocol consisted of a primary denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 60°C for 1 min, annealing at 94°C for 45 s, and extension at 72°C for 1 min, and final extension at 72°C for 10 min. To auscultate the SNPs rs4588 (Asp, 416, Glu) and rs7041 (Thr, 420, Lys), *StyI* and *HaeIII* enzymes were used as follows: for *HaeIII*, PCR product 5 μL , *HaeIII* 1 μL , Buffer Y/Tango 10 $\times$ 1 μL , and distilled water (D.W.) 8 μL , and for *StyI*, PCR product 5 μL , *StyI* 1 μL , Buffer Y/Tango 10 $\times$ 1 μL , and D.W. 8 μL .

The digestion products were stained with ethidium bromide on a 2% agarose gel and imaged. Individuals homozygous for the Asp allele had a nondigested band at 809 bp, while those homozygous for the Glu allele showed two bands (577 and 232 bp) in the GC gene (rs7041). The homozygous Lys allele showed two bands (584 and 225 bp), while the Thr allele in rs4588 appeared as a single band (809 bp). Haplotypes were determined by observing the digestion products of both restriction enzymes. GC 1S has the *HaeIII* but not the *StyI* site. GC 1F has neither the *HaeIII* nor the *StyI* site. GC 2 has the *StyI* but not the *HaeIII* site. The existence of both restriction sites on a single haplotype has not yet been characterized [26].

Statistical Analysis

Data and statistical analyses were performed using the SPSS 20 statistical package (SPSS Inc., Chicago, IL, USA). According to the Peduzzi method, the sample size was estimated using a binary logistic equation [27]. Normal data distribution was determined using the Kolmogorov-Smirnov test. Individuals were categorized according to depression scores. The rank method was used for tertile scoring of the three dietary groups (HP/LF, MP/MF, and LP/HF).

A general linear model and ANOVA were used to assess differences in biochemical measurements and characteristics between depression groups, dietary patterns, and rs7041 and haplotype groups. A general linear model adjusted for age, BMI, total energy, and systolic and diastolic blood pressure as confounder effects was used. An independent-samples *t* test was used to assess differences between the two rs4588 groups in biochemical measurements and characteristics. Coding of the SNPs was performed using an additive model.

Multinomial logistic regression analysis was carried out to assess interactions between VDBP and protein/fat dietary patterns, and it was adjusted for differences in age, sex, and BMI. The study population characteristics are reported as mean $\pm$ standard deviation (SD). Statistical significance was defined as $p \leq 0.05$ for all analyses.

Results

Study Population Characteristics

The participants in the current study were apparently healthy, with a mean age, weight, height, and BMI of 35.08 ± 8.78 years, 73.51 ± 15.66 kg, 168.23 ± 9.43 cm, and 25.93 ± 4.89 , respectively.

Table 1. Baseline characteristics and biochemical measurements among participants separated according to depression status

Characteristics	Normal	Moderate depression	Severe depression	p^1	p^2
Age, years	35.44±9.04	34.84±8.94	35.42±7.95	0.88	0.76
Sex, n (%)	128 (51)	81 (32)	40 (16)	0.01*	0.22
Anthropometrics					
Weight, kg	72.93±16.31	74.95±14.62	72.25±16.67	0.58	0.386
Height, cm	167.89±9.01	169.24±9.89	166.93±10.11	0.40	0.906
Waist circumference, cm	88.35±12.91	89.87±11.88	88.84±12.84	0.70	0.04*
Hip circumference, cm	102.40±9.86	103.03±8.98	101.94±9.99	0.82	0.133
BMI	25.86±5.17	26.11±4.52	25.76±4.58	0.91	0.362
Fat percentage	25.88±9.15	25.08±9.58	25.20±9.26	0.82	0.002*
SBP, mm Hg	11.96±1.20	11.98±1.23	11.80±1.41	0.77	0.650
DBP, mm Hg	7.82±0.88	7.68±0.93	7.64±0.96	0.43	0.006*
Biochemical tests					
HDL-C, mg/dL	48.46±12.25	48.80±11.26	48.95±12.38	0.96	0.097
LDL-C, mg/dL	99.03±21.57	104.54±29.60	100.62±23.51	0.29	0.03*
TC, mg/dL	181.95±34.70	187.88±42.55	185.02±35.64	0.53	0.183
TG, mmol/L	132.38±106.30	121.61±91.10	119.95±84.44	0.66	0.006*
AST, IU/L	20.44±6.87	20.64±6.70	19.92±6.49	0.85	0.336
ALT, IU/L	16.51±12.14	18.32±10.66	16.12±8.62	0.44	0.531
FBS, mmol/L	95.79±23.23	93.90±14.98	89.82±7.46	0.22	0.008*
hs-CRP, mg/L	2.28±3.44	2.41±3.60	2.35±2.73	0.96	0.890
1,25(OH) ₂ D ₃ , nmol/L	20.74±12.91	25.65±30.94	23.09±16.27	0.40	0.34

Values are presented as mean ± SD unless specified otherwise. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; AST, aspartate transaminase; ALT, alanine transaminase; FBS, fasting blood sugar; hs-CRP, high-sensitivity C-reactive protein. * $p < 0.05$. ¹ p value of ANOVA. ² p value of ANCOVA after adjustment for age, sex, BMI, and total energy.

Association between the Clinical and Biochemical Characteristics of the Participants and Depression Status

Table 1 presents the clinical and biochemical characteristics of the 265 participants (126 males and 139 females). The subjects were categorized based on depression status and divided into three groups: normal, moderate depression, and severe depression. ANOVA revealed that there were no statistically significant differences between the three depression status groups in demographic characteristics and biochemical measurements; however, the sex ratio was significantly different ($p = 0.01$). After adjusting for age, BMI, total energy, and systolic and diastolic blood pressure using the general linear model, the relationships between depression status and waist circumference ($p = 0.04$), fat percentage ($p = 0.002$), diastolic blood pressure ($p = 0.006$), LDL ($p = 0.03$), TG ($p = 0.006$), and fasting blood sugar (FBS; $p = 0.008$) were statistically significant. However, a post hoc analysis was unable to show statistically significant differences between the groups. Nevertheless, in the severe depression group, waist circumference

and LDL tended to be more similar to the values of the normal group, while fat percentage, diastolic blood pressure, TG, and FBS in the severe depression group were less similar to the values of the normal group.

HP/LF Diet

A validated, 147-item, semiquantitative FFQ was employed to assess dietary intake [23]. In order to estimate the protein/fat dietary pattern, the participants were categorized according to their protein and fat consumption. The sources of dietary protein included red meat, processed red meat, poultry, full-fat dairy, low-fat dairy, fish, eggs, and legumes. Table 2 shows the age, weight, height, BMI, waist and hip circumference, fat percent, systolic and diastolic blood pressure, protein intake, and biochemical measurements for the HP/LF, MP/MF, and LP/HF groups.

After categorization, statistically significant differences were observed in age ($p = 0.001$), depression score ($p = 0.03$), weight ($p < 0.001$), height ($p < 0.001$), systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p = 0.006$), HDL ($p = 0.004$), LDL ($p = 0.001$), TC ($p < 0.001$), TG

Table 2. Characteristics of the participants separated into protein/fat dietary pattern groups

Characteristics	HP/LF	MP/MF	LP/HF	p^1	p^2
Age, years	35.07±8.70 ^a	34.06±8.84 ^b	36.68±8.69 ^{a,b}	0.001*	<0.001*
Protein intake	93.09±22.41	80.33±22.31	72.39±23.11	<0.0001*	<0.0001*
Depression status				0.03*	0.29
Normal	51.2%	50.3%	53.4%		
Moderate depression	41.0%	31.2%	31.0%		
Severe depression	7.8%	18.5%	15.5%		
Anthropometrics					
Weight, kg	73.46±14.38 ^c	71.90±16.67 ^d	76.63±14.22 ^{c,d}	<0.001*	0.002*
Height, cm	167.61±9.19 ^e	167.47±8.85 ^f	171.04±10.41 ^{e,f}	<0.001*	<0.001*
Waist circumference, cm	88.40±11.80	87.84±13.07 ^g	89.98±11.56 ^g	0.09	0.39
Hip circumference, cm	101.70±7.45 ^h	102.28±10.68	103.40±8.22 ^h	0.14	0.005*
BMI	26.00±3.75	25.59±5.29	26.21±4.72	0.21	0.47
Fat percentage	26.01±7.16	25.33±10.04	25.31±9.39	0.64	0.12
SBP, mm Hg	12.26±1.55 ^{i,j}	11.81±1.22 ⁱ	11.92±1.14 ^j	<0.001*	<0.001*
DBP, mm Hg	7.90±0.99 ^{k,l}	7.69±0.93 ^k	7.63±0.77 ^l	0.006*	0.001*
Biochemical tests					
HDL-C, mg/dL	46.60±8.10 ^{m,n}	49.71±12.49 ^m	48.68±12.26 ⁿ	0.004*	0.002*
LDL-C, mg/dL	103.65±26.24 ^o	98.07±23.42 ^{o,p}	104.74±31.94 ^p	0.001*	0.06
TC, mg/dL	189.08±38.38 ^q	179.39±36.06 ^{q,r}	189.83±44.51 ^r	<0.001*	0.007*
TG, mmol/L	154.17±119.74 ^{s,t}	108.86±54.86 ^{s,u}	133.87±115.34 ^{t,u}	<0.001*	<0.001*
AST, IU/L	19.84±5.38	20.42±7.46	20.41±5.80	0.52	0.81
ALT, IU/L	16.98±5.38	16.65±13.05	17.90±8.41	0.33	0.71
FBS, mmol/L	96.00±27.83 ^v	92.86±16.83 ^v	95.19±11.02	0.06	0.25
hs-CRP, mg/L	1.85±2.21 ^w	2.66±4.02 ^{w,x}	1.86±2.27 ^x	0.001*	0.001*
1,25(OH) ₂ D ₃ , nmol/L	2.11±1.35 ^y	1.71±0.94	1.41±1.25 ^y	0.001*	0.94

Values are presented as mean ± SD unless specified otherwise. HP/LF, high-protein/low-fat; MP/MF, moderate-protein/moderate-fat; LP/HF, low-protein/high-fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; AST, aspartate transaminase; ALT, alanine transaminase; FBS, fasting blood sugar; hs-CRP, high-sensitivity C-reactive protein. * $p < 0.05$. ¹ p value of ANOVA. ² p value of ANCOVA after adjustment for age, sex, BMI, and total energy.

($p < 0.001$), and hs-CRP ($p = 0.001$). After factoring in age, sex, BMI, total energy, and systolic and diastolic blood pressure, significant differences were preserved between the three groups for age ($p < 0.001$), protein intake ($p < 0.0001$), weight ($p = 0.002$), height ($p < 0.001$), hip circumference ($p = 0.005$), systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p = 0.001$), HDL ($p = 0.002$), TC ($p < 0.007$), TG ($p < 0.001$), and hs-CRP ($p = 0.001$). The percentage of severe depression among the participants in the HP/LF group (7.8%) was significantly lower than among the individuals in the LP/HF group (15.5%).

Characteristics and Biochemical Measurements among Different GC Genotypes

In the multinomial model, sex, age, and BMI were considered as dependent. When analyzing individuals based on the rs7041 and rs4588 polymorphisms, the partici-

pants were divided into three and two groups, respectively (rs7041: GG, TG, and TT; rs4588: CC and AC+AA), while in the haplotype model individuals were categorized into four groups (TC, GA, GC, and TA). The present study revealed rs7041 as being significantly associated with depression score ($p = 0.05$), weight ($p = 0.008$), height ($p = 0.001$), hip circumference ($p = 0.01$), systolic blood pressure ($p = 0.002$), diastolic blood pressure ($p = 0.01$), LDL ($p < 0.001$), TC ($p < 0.001$), TG ($p = 0.04$), AST ($p < 0.001$), ALT ($p < 0.001$), FBS ($p < 0.001$), and hs-CRP ($p = 0.04$). The mean depression score among the participants with the TG genotype was higher than in the individuals with either the TT or the GG genotype.

In the present study, rs4588 was positively associated with depression score ($p = 0.02$), weight ($p = 0.002$), height ($p = 0.003$), waist circumference ($p = 0.02$), hip circumference ($p = 0.003$), HDL ($p = 0.03$), LDL ($p = 0.005$),

Table 3. Interaction between HP/LF diet and GC genotypes

		$\beta \pm \text{SE}$	OR (95% CI)	p^1
<i>rs7041</i>				
Crude model	moderate	-0.30 ± 0.15	0.73 (0.54–1.00)	0.05*
	severe	-0.48 ± 0.20	0.61 (0.41–0.92)	0.01*
Adjusted model	moderate	-0.25 ± 0.16	0.77 (0.56–1.06)	0.11
	severe	-0.47 ± 0.21	0.62 (0.41–0.93)	0.02*
<i>rs4588</i>				
Crude model	moderate	-0.26 ± 0.21	0.77 (0.50–1.18)	0.23
	severe	-0.27 ± 0.29	0.75 (0.42–1.35)	0.35
Adjusted model	moderate	-0.30 ± 0.22	0.73 (0.47–1.13)	0.16
	severe	-0.24 ± 0.30	0.78 (0.43–1.41)	0.42
<i>Haplotype</i>				
Crude model	moderate	-0.92 ± 0.08	0.91 (0.77–1.06)	0.24
	severe	-0.17 ± 0.10	0.84 (0.68–1.03)	0.10
Adjusted model	moderate	-0.07 ± 0.08	0.92 (0.79–1.08)	0.36
	severe	-0.16 ± 0.10	0.84 (0.68–1.04)	0.11

We consider the normal as reference. Adjusted model: after adjustment for age, sex, and BMI. HP/LF, high-protein/low-fat. * $p < 0.05$. ¹ p value of multinomial logistic regression analysis.

AST ($p < 0.001$), and ALT ($p = 0.006$). After categorization, the individuals in the haplotype model had significant associations with hip circumference ($p = 0.03$) and AST ($p = 0.01$). However, there were no significant associations in the haplotype model regarding other measurements ($p > 0.05$). The depression scores of the participants with the GA and TA genotypes in the haplotype model were not significantly lower than those of the participants with GC and TC alleles.

Interaction between GC Genotype and HP/LF Diet regarding Depression Risk

Multinomial logistic regression analysis was performed to determine the interaction between HP/LF diet and the various genotypes related to the two SNPs (rs4588 and rs7041) with regard to depression (Table 3). There was no considerable heterogeneity in the interaction between HP/LF diet and rs4588 polymorphism and the haplotype model in the depression groups before and after adjustment for age, sex, and BMI ($p > 0.05$). However, a significant interaction was noted between HP/LF diet and rs7041 polymorphism in the moderate- and severe-depression groups before and after controlling for covariates ($p = 0.05$, OR = 0.73, 95% CI 0.54–1.00, and $p = 0.01$, OR = 0.61, 95% CI 0.41–0.92, respectively). For the rs7041 polymorphism, the interaction with the severe-depression group was higher than with the moderate-depression group (se-

vere-depression group: $\beta = -0.48$, 95% CI 0.41–0.91, $p = 0.01$). Also, in Figure 1, a considerable interaction between HP/LF diet and rs7041 polymorphism was seen, with the percentage of depressed participants with the risk allele T being greater (50% in the moderate-depression group and 10% in the severe-depression group).

Discussion

This cross-sectional study demonstrated that an HP/LF diet may interact with the VDBP genotype to moderate the depression risk in apparently healthy adults free of chronic disease. Three depression statuses were determined: normal, moderate depression, and severe depression. After categorization, individuals consuming an HP/LF diet demonstrated statistically significant differences in depression scores. These findings demonstrate that depression scores correlated with rs7041 and rs4588 polymorphisms, and that there was a strong interaction between HP/LF diet and being a T allele carrier for rs7041 in the moderate- and severe-depression groups. However, we did not identify an interaction between the HP/LF dietary pattern and rs4588.

Depression may be associated with lipid profiles and inflammatory cytokines [28, 29]. In this study, after categorization by depression status, a significant association was

Fig. 1. A significant interaction was noted between high-protein/low-fat diet and rs7041 polymorphism in the moderate- and severe-depression groups. The normal group was considered as the reference group. Regarding rs7041 polymorphism, the interaction in the moderate-depression group (a) was weaker than in the severe-depression group (b). The frequency of the TT genotype

among the depressed participants was higher than the frequencies of the other genotypes. The number of participants in the moderate-depression group was 81 (GG, $n = 16$; TG, $n = 25$; TT, $n = 40$), and in the severe-depression group there were 40 subjects (GG, $n = 4$; TG, $n = 16$; TT, $n = 20$).

found between depression status and waist circumference, fat percentage, diastolic blood pressure, LDL, TG, and FBS. Some previous studies have presented data supporting similar outcomes. In a Norwegian study among schizophrenic ($n = 344$) and nonschizophrenic groups ($n = 308$), depression was related to LDL and TC levels, while in another study it was shown that depression was associated with elevated levels of several inflammatory cytokines [29–31]. In contrast to our results, Shah et al. [32] reported that there was no association of HbA_{1c}, blood pressure, and LDL cholesterol with depression in adults with type 2 diabetes mellitus. The reason for the conflict in results between our study and this previous study may stem from using different questionnaires to assess depression (i.e., Patient Health Questionnaire-9 [PHQ-9] vs. DASS-21).

In the present study, after having categorized the individuals based on protein/fat dietary patterns, we observed that a high protein intake was associated with a decreased incidence of depression. Some previous studies have reported data that support these findings [17, 33]. For example, Nanri et al. [33] reported that protein intake was associated with decreased depressive symptoms. In contrast, in a Spanish study on 140 elderly participants [34] and a Japanese study on 279 elderly individuals [35], protein intake was not related to depressive symptoms. The contrasting findings in some of these former reports may be due to the inclusion of elderly participants and/or the lack of adjustment for potential confounding variables associated with depressive symptoms, such as sleeping habits and physical activity [33]. The mechanism linking depressive symptoms and protein intake is unknown. Invest-

igation into the effects of protein intake on behavior and mood has focused on levels of tryptophan and serotonin [17]. Indeed, tryptophan has an antidepressant-like effect through its conversion into serotonin [36]. Given this connection, a high protein intake may have a greater beneficial effect on mental health [33].

In the present study, we demonstrated that rs7041 polymorphism was associated with depression and weight, height, hip circumference, systolic and diastolic blood pressure, LDL, TC, TG, AST, ALT, FBS, and hs-CRP, while rs4588 polymorphism was correlated with depression and waist circumference, LDL, TC, TG, AST, and ALT. These findings are in contrast to those of previous studies. For example, some prior studies have observed no relationship between various biochemical measures and rs7041 or rs4588 [37, 38]. This contradiction in findings may be due to the imbalance between the two populations, as well as the low prevalence of the SNPs. However, Lee et al. [2] reported significant associations between GC gene expression levels, depression symptoms, and serum lipid profiles. The distribution of neurons targeted by calcitriol suggests an influence of synthesis levels of acetylcholine acetylase, testosterone, and serotonin [39, 40], which have all been linked to the pathogenesis of depression. Specifically, a reduction in neurotransmitters has been related to an enhanced risk of depression.

The current study also suggests that there is an interaction between HP/LF diet and rs7041 in moderate- and severe-depression groups, as well as showing a strong interaction between the T allele in rs7041 and a HP/LF diet. The T allele in rs7041 has been reported as a risk allele

[41]. It seems that the depression rates among the participants carrying the T allele were higher. In contrast, we found no association between HP/LF diet and rs4588 polymorphism. These findings have considerable significance in view of the high prevalence of low-protein diets and VDBP genotype changes among apparently healthy depressed adults. This could lead to practical strategies to help with the control or prevention of depression. The researchers' interpretation of the associations described in the present study will require interventional studies in order to provide causal evidence.

The main limitation of the present study is its basis on a sample size, which led to insufficient numbers of participants in some subgroups for genotype categorization. The strength of the current study is that – to the best of our knowledge – it is the first to evaluate interactions between GC gene variants and protein/fat dietary patterns in apparently healthy depressed subjects.

Conclusions

The current study suggests that an HP/LF diet may interact with the rs7041 genotype in moderate and severe depression. Also, another important finding of this study is that there was an interaction effect identified in carriers of the T allele in rs7041 with HP/LF diet.

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Statement of Ethics

All individuals gave their informed, written consent to participate in the study. The study was approved by the Ethics Committee of the Endocrinology and Metabolism Research Center of the Tehran University of Medical Sciences (Ethics No. 93-04-159-28031-144521).

Disclosure Statement

The authors declare no conflicts of interest.

Author Contributions

Study concept and design: K.M. and S.P.; data acquisition: K.M., S.P., M.H.R., M.M., L.K.-N., and Z.M.; data analysis and interpretation: K.M. and Z.M.; drafting of the manuscript: S.P., Y.N., M.H.R., M.M., and L.K.-N.

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