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Interaction of Vitamin D Receptor Gene FokI Variants and Omega-3 Fatty Acids on Perceived Stress Score and Serum Cortisol Levels in Nurses: A Cross-Sectional Study

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ABSTRACT

Background: There is a high prevalence of perceived stress among nurses. Recent studies have shown that omega-3 fatty acids and genetic variants contribute to perceived stress. This study aimed to examine interactions between vitamin D receptor (VDR) gene FokI polymorphism and omega-3 fatty acids on perceived stress score and serum cortisol levels in nurses. **Methods:** A total of 268 Iranian nurses (248 women; 20 men) participated in this cross-sectional study. Omega-3 fatty acids and perceived stress score of participants were evaluated using a 3-day food record and the Perceived Stress Scale (PSS)-10, respectively. Serum cortisol concentrations were evaluated by ELISA. VDR FokI polymorphism was genotyped using the restriction fragment length polymorphism method. **Results:** No significant relationship was found between omega-3 fatty acids with perceived stress score or cortisol level in FF genotype carriers, but lower intake of PUFA was related to higher cortisol level (P=0.04) in Ff carriers. Significant interactions were observed between VDR FOKI polymorphism and intakes of eicosapentaenoic acid (EPA) (P Interaction=0.06), linoleic acid (P Interaction=0.06), and docosahexaenoic acid (DHA) (P Interaction=0.06) on serum cortisol, so that lower intake of EPA, linoleic acid, and DHA was associated with an increase in cortisol levels in individuals with ff genotype. Moreover, in carriers of ff genotype, lower intake of EPA was related to the elevated perceived stress score (P Interaction=0.06). **Conclusion:** FokI polymorphism interacts with omega-3 fatty acids (EPA, linoleic acid, and DHA) to increase cortisol level and with EPA to increase perceived stress score in nurses.

Keywords: *Fatty acids, Omega-3; Eicosapentaenoic acid; Docosahexaenoic acids; Linoleic acid; Receptors calcitriol; Gene-environment interaction*

Introduction

Stress, with the overall prevalence of 69% among nurses (Gheshlagh *et al.*, 2017), is a growing worldwide issue (Maddahi *et al.*, 2020), which potentially affects mental health, the quality of patient care, and job performance of nurses (Bernburg *et al.*, 2019).

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Shift work in nursing typically comprises long hours, high pressure, heavy patient loads, and complicated interdisciplinary coordination (Higbee *et al.*, 2020), which expose them at risk of stress. Accumulating evidence has revealed that chronic stress is a significant initiator of poor eating behaviors and may be related to the increased food intake (Junne *et al.*, 2017). Nevertheless, evidence regarding the association between omega-3 fatty acids and stress is somewhat inconclusive. For the majority of people, stress increases food intake, especially energy dense, fatty and sweet foods although in some individuals, omega-3 fatty acids is reduced in response to stress (El Ansari and Berg-Beckhoff, 2015). The so-called stress-eaters are susceptible to obesity and have elevated levels in cortisol, insulin, and cholesterol following a stressful condition (Wingenfeld *et al.*, 2017). The Hypothalamic-Pituitary-Adrenocortical (HPA) axis is a leading pathway in stress response, and might contribute to the association between omega-3 fatty acids and stress (George *et al.*, 2010). Activation of HPA axis leads to the secretion of cortisol, as the most important biological indicator of stress, that increases appetite and stimulates the motivation for the consumption of highly palatable foods (Rodriguez *et al.*, 2015).

To date, no persuasive explanation has been identified for the stress-related under- or overeating behaviors in different individuals (El Ansari and Berg-Beckhoff, 2015). Religious or cultural beliefs might be potential explanations (El Ansari and Berg-Beckhoff, 2015). Moreover, the diversity of the associations between omega-3 fatty acids and stress in different people is probably because of differences in genetic background and gene-diet interactions (Harbron *et al.*, 2014). Genetic variance in the vitamin D receptor (VDR) gene is a potential genetic candidate affecting the susceptibility to mood disorders (Kuningas *et al.*, 2009). Beyond its well-defined role in osteoporosis (Alizadeh *et al.*, 2018), vitamin D has potential neuroactive impacts on brain function through the VDR (Yalamanchili and Gallagher, 2012). A decreasing effect of vitamin D on stress has been reported, which is reported to be mediated through the suppression of HPA-axis activity (Rolf *et al.*, 2018) and thus a reduction in cortisol concentration (Fitzgerald *et al.*, 2018). VDR is widely expressed in various parts of brain, including hippocampus, prefrontal cortex, hypothalamus, cingulate gyrus, thalamus, and substantia nigra (Glocke *et al.*, 2013), indicating the essential role of VDR in the brain health. In this sense, variants in the rs2228570 (FokI) near the VDR has been strongly related to abnormality in mental health (Glocke *et al.*, 2013), and significantly modified the relation of omega-3 fatty acids to some diseases (Kim *et al.*, 2001). It remains, however, to assess the impact of FokI single nucleotide polymorphism (SNP) on the association of omega-3 fatty acids with perceived stress. Given the high prevalence of perceived stress among nurses, the current study was carried out to explore the interaction of FokI SNP and PUFA omega-3 on perceived stress score and serum cortisol levels in nurses.

Materials and Methods

Study design and population: This cross-sectional study was performed during 2015-2016 on a total of 268 nurses (248 women; 20 men) with mean age 35.5±6.31 years working in all the hospitals (14 hospitals) covered by Tehran University of Medical Sciences, Tehran, Iran. The list of nursing staffs working in all the hospitals were taken from the nursing office of each hospital, and then subjects were randomly selected. The selected individuals were invited to participate in the study after explaining the goals and benefits of the study by telephone. The inclusion criteria were having a bachelor's degree or higher in nursing, being premenopausal for women and age <45 years for men, and willingness to collaborate in the interview until the end of the study. Individuals with cardiovascular disease, heart failure, liver disease, kidney disease, thyroid disease, cancer, diabetes, and acute or chronic infections were excluded from the study because of the likely

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disease-related changes in their dietary patterns. We also excluded people with specific clinically confirmed neurological and psychiatric disorders, such as mood disorders and depression as well as those using drugs that affect the expression and function of VDR, such as corticosteroids (Bhatia *et al.*, 2010).

$$n = \frac{z^2_{1-\alpha/2}\, p(1-p)}{d^2} = \frac{(1.96)^2 \times 0.87 \times (1-0.87)}{(0.05)^2} = 173$$

n=173*1.55=268

General and anthropometric measurements: General information of individuals including age, sex, marital status, education level, and shift-working status was recorded using a self-administered questionnaire. The weight and height of participants were assessed in the morning with the least amount of clothing, no shoes, in standing position, and with an accuracy of 100 grams and 0.5 cm, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference (WC) was measured using a non-elastic meter in the middle of the distance between the lower edge of the last rib of the thoracic region and the iliac crest with an accuracy of 0.5 cm. Hip circumference (HC) in the largest part of the hip was measured with an accuracy of 0.5 cm. All measurements were performed by a trained nurse.

Clinical parameters assay: Blood samples were taken following a 10-12 hour overnight fasting. Half of the whole blood was immediately centrifuged and the other half was not centrifuged and stored at -80 °C. Serum cortisol concentrations were evaluated by ELISA. Systolic (SBP) and diastolic (DBP) blood pressure of subjects was assessed in sitting and lying down position after five minutes of rest with the use of a mercury sphygmomanometer on the right arm.

Assessment of omega-3 fatty acids and perceived stress score: Omega-3 fatty acids were evaluated using a 3-day food record. The daily macro- and micro-nutrient intakes as well as energy intake of individuals were determined using nutritionist IV software (First Databank, Inc., Hearst Corporation). Perceived stress score of participants was assessed using the validated perceived stress scale (PSS)-10 (Maroufizadeh *et al.*, 2014), which includes 10 questions and is the most widely tool used for evaluating perceived stress. Notably, higher PSS score has been related to higher level of biological markers of stress, such as cortisol (Walvekar *et al.*, 2015). This scale evaluated the perception of stressful experiences by asking the participants to rate the frequency of their thoughts and feelings associated with situations and events that a person has had over the past month. PSS scores were calculated by reversing responses to the four positively stated questions, including items 4, 5, 7, and 8 (scoring: 0 = 4, 1 = 3, 2 = 2, 3 = 1, and 4 = 0) and then summing across all scale items. The total score ranged from 0 to 40 and higher scores represented higher perceived stress.

DNA extraction and gene sequencing: DNA was extracted from blood samples with the use of a DNA extraction kit (QIAGEN GmbH, Germany) based on the manufacturer's protocol. NanoDrop spectrophotometer (Thermo Scientific Company, USA) was used to obtain the concentration and purity of the extracted DNA. The extracted DNA was then stored at 4 °C until the genotype was detected. The VDR fokI (rs10735810) polymorphism (major allele: F; minor allele: f) was genotyped by polymerase chain reaction-restricted length polymorphism (PCR–RFLP) technique using forward (5'-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3') and reverse (5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC -3') primers. PCR reactions were carried out in a final volume of 25.5 µl containing 1 µl extracted DNA, 12.5 µl Master Mix, 11 µl distilled water, 0.5 µl primers F, and 0.5 µl primers, and under the following laboratory situations in a DNA thermocycler: primary denaturation at 94 °C for 3 min; 36 cycles of denaturation at 95 °C for 3 min, annealing at 58°C for 40 seconds, extension at 72 °C for 1 min; with a final extension at 72 °C for 3 min. Amplified DNA (10 µl) was digested with 0.5 µl of FokI FasDigen restriction enzyme at 37 °C overnight. Electrophoresis in agarose gel was applied to visualize all products. Then, fragments including three likely genotypes were recognized including FF, Ff, and ff.

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Ethical consideration: A written informed consent was obtained from all included participants. The study protocol was approved by the ethics committee of Tehran University of Medical Sciences (TUMS) (Ethics number: IR.TUMS.REC.1394.1688).

Data analysis: Data distribution was assessed based on the Kolmogorov–Smirnov test. Deviation from Hardy- Weinberg equilibrium was assessed with the use of the χ^2 test. The χ^2 and ANOVA tests were used to compare the qualitative and quantitative variables among different quartiles of perceived stress score and cortisol levels followed by LSD post-hoc test. General linear model (GLM) was used to investigate the interaction between genotypes of VDR and omega-3 fatty acids on serum cortisol levels and perceived stress score. In GLM model, VDR genotypes and omega-3 fatty acids were considered as covariate, and cortisol level and perceived stress score as dependent variables and P=0.05 was considered statistically significant interaction. Intake of all dietary components was adjusted for total energy intake using linear regression. All analyses were carried out by a statistical Package for Social Science (Version 20.0; SPSS Inc., Chicago IL, USA).

Results

A total of 268 participants completed the study. The mean age of participants was 35.50±6.31 years. Moreover, the mean serum cortisol level and perceived stress score of subjects was 13.40±8.75 pmol/l and 15.55±5.04, respectively, with no significant difference between men and women (P>0.05). **Table 1** presents general demography, anthropometry, and omega-3 fatty acids of participants across quartiles of perceived stress score. In the crude analysis, there was a significant decreasing trajectory in weight (P=0.04), BMI (P=0.03), waist circumferences (P=0.03), hip circumferences (P=0.02), and systolic blood pressure (P=0.04) across quartiles of perceived stress score (from Q1 to Q4). However, after adjustment for potential covariates including age, weight, sex, and energy intake, these associations were disappeared. In the adjusted model, higher perceived stress score were significantly associated with lower intake of polyunsaturated fatty acids (P=0.05) and higher carbohydrate intake (P=0.04) (**Table 1**). No significant difference in omega-3 fatty acids was observed across quartiles of serum cortisol level (**Table 2**).

Genotype frequency of VDR FOKI SNP among 268 nurses participated in this study is reported in **Table 3**. The frequency of the FF, Ff, and ff genotypes were 179, 74, and 15, respectively. There was no significant difference in terms of demography, anthropometry, blood pressure, marital status, shiftwork status, education level, as well as serum cortisol and perceived stress score among genotypes of FOKI SNP. Omega-3 fatty acids of participants according to the categories of perceived stress score and serum cortisol level stratified by VDR FOKI genotypes is reported in **Table 4**. No significant relationship was found between omega-3 fatty acids with perceived stress score or cortisol level in FF genotype carriers; lower intake of PUFA was related to higher cortisol level (P=0.04) in Ff carriers, and in individuals with ff genotype, lower omega-3 fatty acids and dietary fat were marginally associated with higher cortisol levels (P=0.05).

After adjustment for energy intake, significant interactions were observed between VDR FOKI polymorphism and omega-3 of eicosapentaenoic acid (EPA) (P Interaction = 0.06), linoleic acid (P Interaction = 0.06), and docosahexaenoic acid (DHA) (P Interaction = 0.06) on serum cortisol, so that lower intake of EPA, linoleic acid, and DHA, compared to higher intake, was associated with an increase in cortisol levels in individuals with ff genotype. Moreover, in carriers of ff genotype, lower intake of EPA was related to the elevated perceived stress score (P Interaction = 0.06) (**Figure 1**). No significant interaction was found between FOKI polymorphism and intakes of carbohydrate, protein, total fat, saturated fat, PUFA, oleic acid, linolenic acid, Vitamin D, Vitamin E, and Vitamin A on serum cortisol and score of perceived stress.

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Table 1. Dietary intakes and characteristics of participants across quartiles of perceived stress score.

Variables	Quartiles of perceived stress score				P-value	P-value[a]
	Q1	Q2	Q3	Q4		
Sex					0.34	
Male	7 (35.0)[b]	7(35.0)	3(15.0)	3(15.0)		
Female	44(20.6)	62(29.1)	57(26.7)	50(23.4)		
Marital status					0.64	
Single	16(24.2)	16(24.2)	17(25.7)	17(25.7)		
Married	35(21.0)	52(31.3)	43(25.9)	36(21.6)		
Other	0(0.0)	1(100)	0(0.0)	0(0.0)		
Education levels					0.99	
Bachelor	47(21.4)	67(30.5)	56(25.5)	49(22.3)		
Higher than bachelor	3(21.4)	4(28.5)	4(28.5)	3(21.4)		
Shiftwork status					0.94	
Morning shift	19(22.3)	28(32.9)	21(24.7)	17(20.0)		
Evening and night shifts	12(18.4)	20(30.7)	19(29.2)	14(21.5)		
Rotating morning shift	21(25.3)	23(27.7)	20(24.0)	19(22.8)		
Age (y)	35.9±7.4[c]	35.5±6.31	34.86±6.42	33.87±5.74	0.37	0.12
Height (cm)	161.90±9.14	161.51±7.72	161.93±7.47	160.74±7.62	0.84	0.38
weight (kg)	69.37±13.48	65.38±12.87	66.03±10.74	62.90±9.71	0.04	0.07
BMI (kg/m^2)	26.41±4.59	24.97±3.93	25.17±3.63	24.26±2.79	0.03	0.29
Waist circumferences (cm)	80.12±11.70	75.82±10.35	75.87±9.81	74.77±6.86	0.03	0.69
Hip circumferences (cm)	97.15±12.62	93.97±8.57	94.28±8.59	91.47±7.79	0.02	0.10
Systolic blood pressure (mm Hg)	112.78±11.78	106.91±13.21	109.59±11.36	106.62±14.40	0.04	0.57
Diastolic blood pressure (mm Hg)	70.56±9.08	68.78±9.98	71.14±10.13	69.46±11.28	0.57	0.38
Serum cortisol (Pmol/l)	12.01±4.61	14.11±6.79	12.16±4.27	13.60±7.33	0.13	0.71
Protein (g/day)	65.95±20.40	62.46±17.73	63.88±21.47	63.65±17.50	0.91	0.53
Fat (g/day)	74.76±1.88	71.19±1.90	74.40±1.76	67.99±1.76	0.17	0.07
Carbohydrate (g/day)	251.29±43.68	254.70±39.78	260.16±42.02	267.13±41.13	0.22	0.04
SFA (g/day)	18.19±7.12	16.70±4.34	19.11±7.16	18.82±7.55	0.15	0.66
PUFA (g/day)	19.86±8.13	18.24±7.77	18.66±9.29	16.29±6.59	0.15	0.05
Linoleic acid (g/day)	31.17±4.07	31.85±5.65	29.56±4.8	29.35±4.68	0.98	0.86
Linolenic acid (g/day)	25.80±9.15	38.01±10.43	27.60±8.73	31.87±9.92	0.99	0.85
Oleic acid (g/day)	27.66±8.95	25.98±9.36	25.99±8.98	24.71±8.22	0.38	0.06
Vitamin D (µg/day)	15.01±5.59	13.10±5.01	7.42±4.60	5.19±3.81	0.86	0.33

SFA: Saturated fatty acids; PUFA: Polyunsaturated fatty acids; BMI: body mass index; [a]: P-value from ANCOVA analysis after adjustment for age, weight, sex, and energy intake; [b]: n (%); [c]: Mean±SD.

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Table 2. Dietary intake of participants according to the categories of perceived stress score and serum cortisol level stratified by VDR FOKI .

Dietary intake (per day)	Participants with wild-type genotype (FF)									
	Quartiles of perceived stress score					Quartiles of serum cortisol levels				
	Q1	Q2	Q3	Q4	P-value	Q1	Q2	Q3	Q4	P-value[a]
Protein (g)	58.61±16.12[b]	58.65±16.69	61.01±18.50	61.94±13.81	0.77	58.56±15.98	57.52±14.48	62.36±17.80	61.97±17.25	0.48
Fat (g)	73.57±20.02	70.43±18.37	74.36±19.54	65.84±16.14	0.21	73.25±19.46	67.80±19.12	70.47±19.39	74.21±16.48	0.45
Carbohydrate (g)	256.46±50.56	263.29±41.26	252.04±42.71	270.87±39.74	0.28	257.14±42.30	272.80±47.55	258.45±44.42	250.18±35.18	0.14
SFA (g)	18.60±7.14	16.54±3.35	19.59±6.92	17.90±5.57	0.15	18.21±5.05	18.36±6.99	17.98±5.69	18.07±5.87	0.99
PUFA (g)	19.14±8.65	17.72±7.44	17.97±9.94	15.57±6.05	0.39	19.43±9.06	17.52±6.01	17.03±8.47	18.83±7.02	0.26
EPA (g)	0.03±0.01	0.02±0.00	0.05±0.01	0.63±0.01	0.53	0.04±0.01	0.04±0.01	0.03±0.00	0.06±0.01	0.80
DHA (g)	0.11±0.03	0.07±0.01	0.16±0.05	0.19±0.05	0.52	0.14±0.04	0.13±0.02	0.11±0.02	0.18±0.05	0.79
Oleic acid (g)	27.38±9.34	26.12±9.37	25.80±9.71	24.24±8.11	0.57	26.79±9.11	24.47±9.47	24.90±8.05	24.00±9.39	0.32
Linoleic acid (g)	36.63±27.01	21.94±20.61	47.56±32.12	53.80±31.05	0.62	27.19±9.59	26.71±6.45	21.01±5.44	36.04±10.93	0.82
Linolenic acid (g)	22.89±6.41	14.51±3.05	31.81±10.90	36.99±11.28	0.53	39.07±31.92	39.76±23.99	34.33±23.55	52.62±33.30	0.65
Vitamin D (µg)	18.84±6.73	3.33±1.58	8.90±5.17	3.09±0.57	0.41	3.06±0.60	3.54±3.83	8.73±4.70	18.87±6.65	0.52

SFA: Saturated fatty acids; PUFA: Polyunsaturated fatty acids; EPA: Ecosapantaenoeic acid; DHA: Docosahexaenoic acid; [a]: P-value from ANCOVA analysis after adjustment for age, weight, sex, and energy intake; [b]: Mean±SD.

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Table 3. Dietary intake of participants according to the categories of perceived stress score and serum cortisol level stratified by VDR FOKI in

Dietary intake (per day)	Participants with heterozygote genotype (Ff)									
	Quartiles of perceived stress score					Quartiles of serum cortisol levels				
	Q1	Q2	Q3	Q4	P-value	Q1	Q2	Q3	Q4	P-value[a]
Protein (g)	67.98±17.04[b]	63.93±32.10	55.20±18.00	56.86±16.04	0.07	62.93±15.51	63.62±18.82	63.20±14.55	56.84±15.97	0.61
Fat (g)	74.02±16.10	72.37±20.12	75.26±13.54	75.25±19.04	0.94	70.57±19.88	68.21±16.80	81.41±14.13	74.38±16.27	0.10
Carbohydrate (g)	245.87±24.87	254.12±46.27	258.80±29.98	259.93±47.73	0.52	258.82±35.03	258.82±39.03	250.34±37.01	258.82±39.03	0.18
SFA (g)	17.53±7.65	16.67±5.60	17.35±5. 52	20.89±11.00	0.34	15.81±5.04	16.67±5.84	18.52±5.08	11.16±2.17	0.09
PUFA (g)	20.12±7.35	19.16±8.11	20.47±8.06	17.63±8.11	0.74	18.33±8.90	17.62±6.55	23.86±7.30	17.54±6.90	0.04
EPA (g)	0.05±0.02	0.10±0.03	0.00±0.00	0.03±0.01	0.27	0.07±0.00	0.05±0.01	0.09±0.00	0.00±0.00	0.50
DHA (g)	0.14±0.05	0.29±0.12	0.00±0.00	0.11±0.04	0.26	0.22±0.08	0.14±0.04	0.28±0.09	0.00±0.00	0.47
Oleic acid (g)	27.40±8.80	26.17±9.50	26.06±7.15	25.51±9.02	0.94	24.54±10.84	24.24±7.68	29.51±7.17	27.41±7.40	0.19
Linoleic acid (g)	43.80±35.33	85.67±51.25	16.66±8.21	34.39±28.47	0.32	44.14±16.78	27.25±9.80	54.71±20.19	11.00±1.19	0.50
Linolenic acid (g)	28.02±11.50	57.89±23.08	1.09±0.99	8.76±2.02	0.30	66.10±41.28	41.09±30.23	80.27±51.51	16.91±6.57	0.34
Vitamin D (µg)	3.60±1.67	7.47±2.12	3.72±3.29	9.43±5.39	0.77	23.78±8.78	31.21±0.64	3.33±1.16	9.75±5.94	0.51

SFA: Saturated fatty acids; PUFA: Polyunsaturated fatty acids; EPA: Ecosapantaenoeic acid; DHA: Docosahexaenoic acid; [a]: P-value from ANCOVA analysis after adjustment for age, weight, sex, and energy intake; [b]: Mean±SD.

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Table 4. Dietary intake of participants according to the categories of perceived stress score and serum cortisol level stratified by VDR FOKI in

Dietary intake (per day)	Participants with Homozygote genotype (ff)									
	Quartiles of perceived stress score					Quartiles of serum cortisol levels				
	Q1	Q2	Q3	Q4	P-value	Q1	Q2	Q3	Q4	P-value[a]
Protein (g)	52.48±8.96[b]	44.14±12.53	53.54±18.30	61.01±13.79	0.87	50.14±12.81	58.45±13.23	67.19±84.20	50.53±12.80	0.26
Fat (g)	91.56±8.01	70.88±24.31	72.17±18.23	69.62±8.36	0.41	95.11±12.47	73.37±10.05	56.66±15.45	75.19±16.94	0.05
Carbohydrate (g)	221.14±21.48	267.98±44.11	266.3±23.76	263.00±13.07	0.42	216.56±21.87	255.16±16.83	285.83±42.12	264.14±44.19	0.18
SFA (g)	16.96±5.73	18.02±4.36	21.97±14.45	18.27±3.66	0.86	45.34±19.92	22.36±10.62	32.52±17.41	17.44±10.03	0.84
PUFA (g)	26.57±0.72	17.46±9.70	17.74±8.46	15.35±3.38	0.30	27.59±2.26	18.28±8.88	18.58±5.88	18.61±6.62	0.07
EPA (g)	0.09±0.00	0.07±0.00	0.02±0.00	0.00±0.00	0.32	0.09±0.06	0.09±0.06	0. 01±0.00	0. 13±0.00	0.18
DHA (g)	0.23±0.13	0.00±0.00	0.20±0.10	0.00±0.00	0.50	0.23±0.13	0.23±0.13	0.00±0.00	0. 00±0.00	0.39
Oleic acid (g)	33.68±3.02	23.98±10.24	27.54±10.69	25.72±4.74	0.50	35.51±4.72	26.95±5.51	18.53±6.48	27.55±8.80	0.09
Linoleic acid (g)	45.77±27.53	0.57±0.50	39.68±20.58	0.65±0.25	0.50	46.07±27.17	45.60±27.41	0.32±0.09	0.97±0.53	0.38
Linolenic acid (g)	67.15±65.23	16.44±9.33	52.52±46.73	14.84±3.90	0.32	66.29±66.23	59.25±56.70	11.05±5.67	17.90±6.60	0.21
Vitamin D (µg)	3.00±0.35	4.97±4.13	2.79±0.02	3.42±0.67	0.57	2.89±0.18	2.78±0.21	3.50±0.29	4.83±3.72	0.57

SFA: Saturated fatty acids; PUFA: Polyunsaturated fatty acids; EPA: Ecosapantaenoeic acid; DHA: Docosahexaenoic acid; [a]: P-value from ANCOVA analysis after adjustment for age, weight, sex, and energy intake; [b]: Mean±SD.

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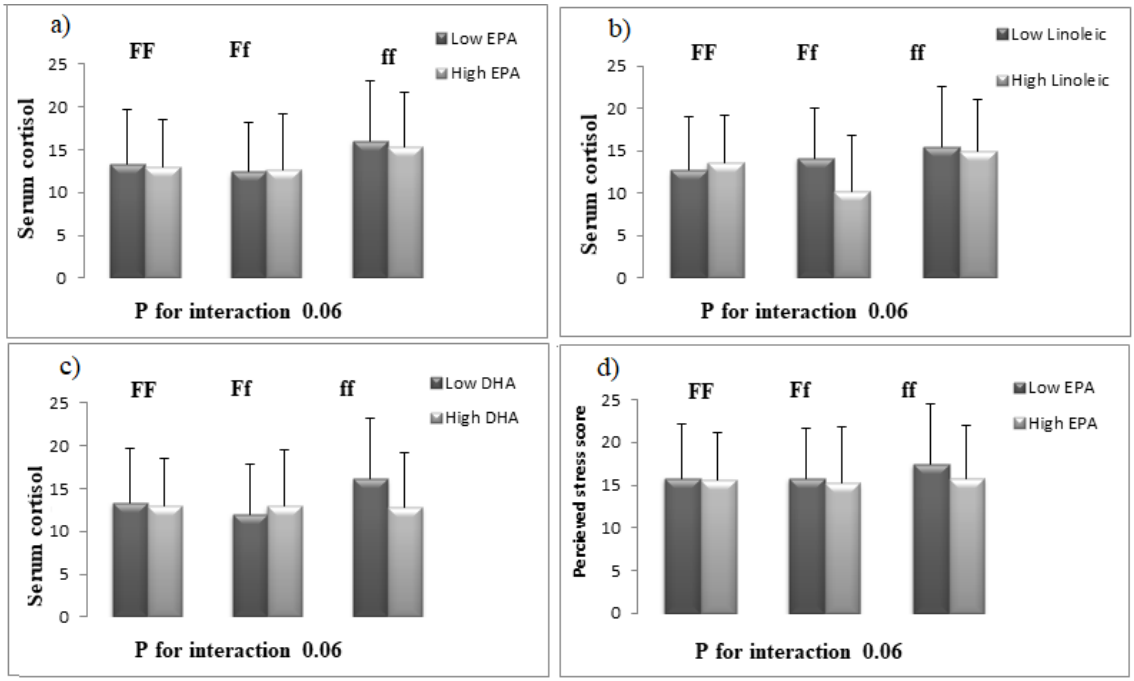

Figure 1. Interaction of fatty acids intake of EPA, linoleic acid, DHA, and VDR FOKI polymorphism on serum cortisol (a) (b) (c), and interaction of fatty acids intake of EPA and VDR FOKI polymorphism on perceived stress score (d).

Discussion

Omega-3 fatty acids and genetic variants potentially affect brain function and mental health. In an attempt to identify factors involved in predisposition to perceived stress, this study aimed to investigate the interaction between omega-3 fatty acids and VDR FOKI polymorphism on serum cortisol and perceived stress score in nurses, as a population with high prevalence of stress. It was revealed that higher perceived stress score was significantly associated with lower intake of PUFA. The main finding of the study is that VDR FOKI SNP can modify the association between omega-3 fatty acids of EPA, DHA, and linoleic acid and serum cortisol concentration. Nurses with lower intake of EPA, linoleic acid, and DHA and the homozygous risk genotype (ff genotype) of VDR FOKI polymorphism had a higher cortisol level. Moreover, lower intake of EPA was associated with an increase in perceived stress score in individuals with the ff genotype. No significant relationship was found between omega-3 fatty acids with perceived stress score or cortisol level in the carriers of wild type homozygous genotype, but lower intake of PUFA was related to higher cortisol level in Ff carriers, showing the interaction between VDR and omega-3 fatty acids on perceived stress and circulating levels of cortisol.

In line with the present study findings, higher omega-3 fatty acids has been found to be associated with the decreased risk of developing symptoms of stress, as well as other mood disorders including depression and anxiety (Taylor and Holscher, 2020). Intake of DHA has been related to a lower level of cortisol and perceived stress and a slighter increase in cortisol in response to the stressor (Keenan *et al.*, 2014). Similarly, supplementation with omega-3 fatty acids in fish oil decreased cortisol concentrations and perceived stress (Barbadoro *et al.*, 2013). Omega-3 fatty acids play roles in a broad range of brain functions like neuroinflammation, neurotransmission, and neurogenesis. EPA and DHA have been associated with mental health maintenance, and their deficiency have been shown to be involved in the pathogenesis of mental complications (Lange, 2020). Mechanistically, the stress-protective role

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of omega-3 PUFA (Hamazaki *et al.*, 2000) is possibly associated with their inhibitory impacts on HPA activity (Miller *et al.*, 2009). Omega-3, acting via the γ-aminobutyric acid type A (GABAA) receptor-mediated mechanism, can potentiate GABAergic inhibitory drive on corticotropin releasing factor (CRF)-secreting hypothalamic neurons (Takeuchi *et al.*, 2003). Also, accumulating evidence has identified that omega-3 could decrease the production of proinflammatory cytokines by microglia and block the signaling pathway of interleukin-1 (IL-1) (Layé, 2010), hence blunting a central trigger of CRF secretion and HPA activation (Miller *et al.*, 2009). Although clinical trial studies examining the therapeutic impacts of PUFA on mental health have reported inconsistent findings, their use in psychiatric practice is limited (Lange, 2020). Heterogeneity in the evidence might be due to the differences in the dietary assessment methods used, sample size, participants' characteristics, study design, and lack of controlling for confounders. The present study revealed that the relation of PUFA to serum cortisol level or perceived stress depends on VDR FokI polymorphism. Modulation of the association of PUFA with serum cortisol level or perceived stress by VDR SNP may partly justify the inconclusive findings of previous studies exploring the relation of PUFA intake with mental health. It has been identified that VDR might contribute to the pathogenesis of stress in animal studies (Ji *et al.*, 2014), thereby providing an alternative candidate gene for optimal therapeutic approaches for stress. Moreover, the absence of VDR might cause anxiety (Kalueff *et al.*, 2004) and genetic variants in VDR gene may affect the cognition function and depression (Kuningas *et al.*, 2009), showing the vital role of VDR in the regulation of behavior and brain function. A relatively high expression of VDR was detected in the limbic system (Eyles *et al.*, 2005), the brain region that regulates emotional behaviors such as stress. Hypersensitivity of the HPA axis and elevated cortisol level is typical of individuals suffering from stress (Guilliams and Edwards, 2010) and vitamin D suppresses HPA-axis activity (Rolf *et al.*, 2018) and thus reduces cortisol concentration (Fitzgerald *et al.*, 2018) through VDR. It suggests that VDR may be involved in down-regulation of stress response. The potential clinical significance of diet-gene interaction seen in the current investigation is to improve personalized dietary recommendations for the prevention of stress and its related health consequences based on the genetic background of individuals.

To the best of the authors' knowledge, this is the first study to identify the interaction between FokI SNP in VDR and omega-3 fatty acids on cortisol levels as well as perceived stress. Nevertheless, several limitations are necessary to be noted. First, given that the study is cross-sectional, causality cannot be inferred; therefore, additional studies, in particular with prospective cohort or clinical trial design, are needed to confirm these findings. Second, this study recruited a relatively small number of participants and the majority of participants were women, which makes it difficult to generalize the findings to men. Thus, we suggest performing similar surveys on both sexes and with larger sample sizes. Third, only one of the most common SNP in VDR gene was examined in the present study, while, more than 30 SNP of the VDR gene have been recognized so far (Alizadeh *et al.*, 2017); hence, other polymorphisms in this gene are proposed to be explored by future investigations.

Conclusion

The study provided preliminary data that FokI polymorphism interacts with omega-3 fatty acids of EPA, linoleic acid, and DHA to increase cortisol level and with EPA to increase perceived stress score. This evidence emphasizes that people with ff genotype of FokI in VDR following a diet low in PUFA fatty acids are more susceptible to have an increased level of cortisol and stress. Further investigations, particularly clinical trials, are required to clarify the biology of VDR FokI SNP and its effect on the perceived stress.

Authors' contributions

All co-authors agreed with the contents of the manuscript. Veysi Z conceived and designed the

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experiments. Sanjari M performed the experiments. Mirzae K analyzed the data. Dehghani A contributed reagents/materials/ analysis tools. Veysi Z wrote the paper.

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Conflict of interest

The authors declare no conflict of interest.

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