ADHERENCE TO MEDITERRANEAN NUTRITION PATTERN IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE: RELATIONSHIP WITH METABOLIC RISK FACTORS AND –UCP2 -866G/A GENE POLYMORPHISMS

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ABSTRACT: The current study evaluated the association between major components of Mediterranean dietary regimen with metabolic biomarkers and uncoupling protein-2 (UCP2) -866G/A gene polymorphism in patients with non-alcoholic fatty liver disease (NAFLD). In this study 75 patients with NAFLD and 76 healthy individuals were enrolled. Dietary intakes were assessed using a semi- quantitative food-frequency questionnaire (FFQ) and Mediterranean dietary quality index (Med-DQI) was calculated. Anthropometric assessments were performed; body mass index (BMI) and waist to hip ratio (WHR) were calculated. Biochemical assays including FSG (fasting serum glucose), liver enzymes and lipid profiles were measured. Polymorphism of -866G/A UCP2 gene was determined using polymerase chain reaction-restriction fragment length polymorphism method. Serum high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) concentrations were significantly lower and serum aspartate amino transferase (AST), alanine amino transferase (ALT) and triglyceride (TG) concentrations were significantly higher in NAFLD patients compared with control group (P < 0.05). NAFLD patients with higher intakes of "saturated fatty acids" and "cholesterol" had significantly higher serum TG, while patients with higher intakes of "olive" had lower serum TG and AST concentrations. According to -866A/G of UCP2 gene polymorphism between study groups, only the score of "meat" subgroup in NAFLD patients with GG genotype was higher compared with patients in other genotypes of UCP2 gene (P < 0.05). We observed a significant relationship between Mediterranean dietary quality index and metabolic risk factors. We also demonstrated a higher meat intake in GG genotype among these patients.

KEY WORDS: –866G/A polymorphism, Med-DQI, Nonalcoholic fatty liver disease, Uncoupling protein 2 gene

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) characterized by accumulation of large droplets of triglycerides within hepatocytes ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) (Farhangi et al., 2014). NAFLD as a hepatic manifestation of metabolic syndrome is strongly associated with obesity, type 2 diabetes mellitus (T2DM) (Vanni et al., 2010). It is accompanied with remarkably higher prevalence of coronary, cerebrovascular and peripheral vascular disease (Targher et al., 2007). The prevalence of the NAFLD in developed countries is up to 30% in the general population, 50% in patients with T2DM, 76% in obese individuals and nearly 100% in patients with morbid obesity (Browning et al., 2004). In Asia nearly 10% of general population is affected by NAFLD (Chitturi et al., 2011).

Numerous reports suggested the role of dietary caloric restrictions, increased physical activity (Ueno et al., 1997) and higher intakes of healthy food choices in prevention and treatment of NAFLD (Caporaso et al., 2011 and Conlon et al., 2013). Previous studies confirmed the protective role of Mediterranean diet as one of the healthiest dietary models in lower rates of chronic disease morbidity, higher life expectancy

(Mariscal-Arcas et al., 2009); Additionally, prevention of cardiovascular events (Estruch et al., 2013), T2DM (Fraser et al., 2008) and metabolic syndrome (Esposito et al., 2010). This pattern is characterized by a high intake of vegetables, legumes, fruits and nuts, cereals (that in the past were largely unrefined), a high intake of olive oil but a low intake of saturated lipids, a moderately high intake of fish, a low-to-moderate intake of dairy products, a low intake of meat and poultry and a regular but moderate intake of alcohol (Tur et al., 2005). This effective role of them in health outcomes has been related to the synergistic effect of their nutrient and non-nutrient compounds, namely dietary fiber, minerals, vitamins and phytochemicals (Georgoulis et al., 2015). Almost all of these studies focused on evaluating the relations between Mediterranean dietary pattern and risk of disease; Therefore, Mediterranean dietary quality index (Med-DQI) first developed by Gerber et al (Gerber, 2001) is a useful tool to evaluate dietary quality highlighting two different sources of fat (saturated and olive oil) and two different sources of protein (meat and fish) with the opposite scores (Table 1).

TABLE 1. Construction of the scores for the MediterraneanDietary Quality Index

Scoring	0	1	2
Saturated fatty acids (% energy)	<10	10-13	>13
Cholesterol (milligram)	<300	300-400	>400
Meats (gram)	<25	25-125	>125
Olive oil (milliliter)	>15	15-5	<5
Fish (gram)	>60	60–30	<30
Cereals (gram)	>300	300-100	<100
Vegetables+ fruits (gram)	>700	700–400	<400

The relationship of -866G>A gene polymorphism of uncoupling protein 2 (UCP2) (rs659366) with obesity and T2DM has been reported previously (Krempler et al., 2002). However, considering the lack of knowledge about the relationship between Mediterranean dietary quality index and UCP2 gene polymorphism in patients with NAFLD, in the current study we aimed to investigate Mediterranean dietary quality index in patients with non-alcoholic fatty liver disease according to different genotype of –UCP2 -866G/A gene polymorphisms.

MATERIALS AND METHODS

The present case control study was conducted among 75 patients with NAFLD and 76 age and sex-matched healthy individuals. Patients were recruited from the out patients gastroenterology clinics of Tabriz University of Medical Sciences. Disease diagnosis was confirmed by the findings of ultrasonography (US). Written informed consent was obtained from all of subjects before participation in the study. The protocol of the study has been approved by the ethics committee of Tabriz University of Medical Science (Registration Code: 11013). Patients were excluded from the study if they had any history of acute or chronic liver diseases, viral hepatitis, hemochromatosis, Wilson's disease, autoimmune or endocrine disorders, pregnancy or lactation, alcohol consumption, using hepatotoxic medications and being on weight loss diets for at least 3 months prior participation in study.

All procedures performed in the current study was in accordance with the ethical standards of the research committee of Tabriz University of Medical Science (Registration Code: 11013) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all of participants.

Anthropometric assessments

Weight was measured with a calibrated scale (SECA, Hamburg, Germany) to the nearest 0.1 kg with the minimal clothing without shoes and height using a non-stretchable measurement tape with the precision of 0.1 cm. The body mass index (BMI) was calculated as weight (Kg) divided by height squared (m²). Waist circumference (WC) was measured in standing position at the level of the umbilicus and hip circumference (HC) was measured at the maximum point between the hip and the buttock with a non-elastic tape.

Biochemical assessments

After an overnight fasting, all of patients underwent a laboratory examination. Venous blood samples were taken from individuals and approximately 2 cc of the blood was transferred into tubes containing ethylene diamine tetra acetic acid (EDTA) for genetic assays. Sera were also extracted from remaining blood samples for biochemical assays including fasting serum glucose (FSG), ALT, AST, total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C). Laboratory assessments were performed by Abbott ALCYON [™] 300 auto analyzer using commercial ELISA kits (Pars-Azmoon, Tehran, Iran). All of the biochemical assays were performed by a trained lab assistant who was blinded to group assignments. Serum LDL-C was calculated by Friedewald formula (Friedewald W et al., 1972).

Dietary intake

Dietary intakes were assessed using a semi-quantitative foodfrequency questionnaire (FFQ) adapted to the Iranian society (Mirmiran et al., 2009). The FFQ included 168 food items with specified serving sizes commonly consumed by Iranians. Participants reported their average frequency intake of each food item in terms of the number of specified serving sizes consumed per day/ week/ month/ year, or never. The reported frequency of consumed foods and portion sizes for each food item were converted to a daily intake. Then, we calculated the diet score on the basis of Mediterranean diet quality index (Med-DQI) (Table 1). The index assigns a score of 0, 1 or 2 according to the daily intake of each of the seven components and then final score was reported as a summation of all nutrient scores ranged between 0 and 14. A lower score on this index indicates a better nutrition quality (Gerber, 2001).

DNA Extraction and Genotyping

Genomic DNA was extracted from the blood cells by salting out method (Miller et al., 1988). This technique is a simple deproteinization cell procedure by dehydration and precipitation with saturated NACL solution. Single nucleotide gene polymorphism (SNP) was detected by polymerase chain reactionrestriction fragment length polymorphisms (PCR-RFLP) method. The details of this technique has been explained before (Mohseni et al., 2015). Briefly DNA fragment analogous to -866 A/G polymorphism (rs659366) in UCP2 gene was amplified by 5'- CACGCTGCTTCTGCCAGGAC -3' as forward primer and 5'- AGGCGTCAGGAGATGGACCG -3' and a reverse primer. 1µ Genomic DNA in addition to 0.2µ Taq DNA polymerase and 1µ of each primer were added to 22µ of 1x PCR master-mix. PCR conditions were: a primary denaturation at 95°C for 5 min followed by 38 cycles of denaturation at 94°C for 1 min; annealing at 59°C for1 min; extension at 72°C for 46 seconds and a final extension at 72°C for 5 minutes. The PCR products were run for visualization on 1% agarose gel and stained by ethidium bromide. 10µ of PCR products were digested by 0.3µ MLUI restriction enzyme, incubated at 37°C for 1.5 hours and ultimately separated on 2% agarose gel electrophoresis. The (-866) AA genotype was indicated by a single 369 bias-pair (bp) fragment as result of loss of MluI site, whereas, the wild-type (-866) GG genotype was digested into 297 bp and 72 bp fragments (Figure 1).

FIGURE 1. PCR –RFLP analysis for UCP2 -866G/A Polymorphism. Lane 1. GG genotype, Lane 2. AG genotype, Lane 3. AA genotype, Lane 4. Undigested PCR product, Lane 5. DNA size marker



Statistical analysis

Data analysis was performed by SPSS statistical software package version 16 (SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was performed for normality of the distributions of variables. The comparison of discrete and continuous variables between groups was performed by Chi- square test and independents sample t test or one-way ANOVA tests respectively. P values less than 0.05 considered as significance level.

RESULTS

Table 2 presents the demographic, anthropometric and biochemical variables of study population; WC and WHR in NAFLD patients were significantly higher compared with healthy group (P < 0.05). Among biochemical variables, serum HDL-C and LDL-C concentrations were significantly lower and serum AST, ALT and TG concentrations were significantly higher in NAFLD patients compared with control group (P < 0.05). The comparisons of laboratory parameters according to components of Mediterranean dietary quality index in patients with NAFLD and in healthy control group are presented in Tables 3 and 4 respectively. NAFLD patients with upper scores of "saturated fatty acids" and "cholesterol" subgroups had significantly higher serum TG concentrations while patients with upper scores of "olive" subgroup, had lower serum TG and AST concentrations. These findings were somewhat similar for healthy group; higher serum TG concentrations and lower serum AST concentrations were observed in upper scores of "cholesterol" and "olive" respectively (Table 4). In comparison of components of Mediterranean dietary quality index according to -866A/G of UCP2 gene polymorphism between study groups, only the score of "meat" subgroup in

TABLE 2. General characteristics of study subjects. BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio;vFSG, fasting serum glucose; TC, total cholesterol; TG, triglyceride; HDL, high density cholesterol; LDL, low density cholesterol; ALT, alanine amino transferase; AST, aspartate amino transferase, *p-value for sex, physical activity based on Chi-Square Tests; P value for ALT and TG based on mann-withney; otherwise based on independent T-test using equal variable. TG and ALT are presented based on median (Percentile 25th–Percentile 75th) and other variables data are presented based on mean (SD).

Variable	NAFLD (N =75)	Healthy Controls(N =76)	P *
Male [n (%)] Female [n (%])	36 (48%) 39 (52%)	29 (38.2%) 47 (61.8%)	0.25
Age (years)	40.65(8.41)	38.87(8.2)	0.18
BMI (kg/m ²)	31.78 (4.17)	31.38(4.04)	0.54
WHR	0.92(0.06)	0.89(0.06)	0.03
FSG (mg/dl)	90.59 (11.24)	89.59 (9.93)	0.71
TC (mg/dl)	183.44(36.91)	187.96(28.89)	0.40
HDL (mg/dl)	43.24(11.4)	48.29(11.6)	0.008
LDL (mg/dl)	104.11(34.62)	111.52(26.43)	0.03
AST(IU/l)	32.99(14.86)	23.08(6.12)	<0.001
ALT (IU/I)	47.00(29.00- 67.00)	25.50(18.00- 35.75)	<0.001
TG (mg/dl)	152.00(114.00- 225.00)	118.50(79.50- 198.00)	0.004

TABLE 3. Comparison of laboratory parameters according to components of Mediterranean dietary quality index in patients with NAFLD.

Characteristics		NAFLD (N=75)					
	FSG(mg/dl)	TG(mg/dl)	TC(mg/dl)	ALT(IU/l)	AST(IU/l)	HDL(mg/dl)	LDL(mg/dl)
Saturated fatty acid (n)							
0 (47)	89.94±12.25	172.24±85.06	180.48±34.66	47.76±25.38	30.82±14.89	43.54±11.709	103.12±32.32
1 (22)	91.95±9.78	217.21±42.76	191.84±45.36	58.68±24.74	39.00±14.75	40.47±10.330	107.93±43.62
2 (7)	91.67±6.37	120.67±63.30	181.50±24.94	40.67±31.81	32.00±11.24	49.50±11.023	107.87±24.08
р	0.78	0.049	0.52	0.19	0.12	0.23	0.85
Cholesterol (n)							
0 (50)	90.94±11.35	173.42±86.60	186.02±39.61	47.87±24.285	32.04±14.195	43.00±10.92	108.92±35.93
1 (19)	89.53±10.86	153.13±59.55	174.07±30.24	52.07±28.429	33.00±12.689	46.33±12.78	97.11±26.66
2 (6)	90.00±12.99	300.50±221.25	183.67±25.13	63.50±34.309	41.50±24.222	37.67±11.74	85.90±35.74
р	0.91	0.008	0.54	0.35	0.34	0.28	0.19
Meats (n)							
0 (8)	91.25± 14.37	189.63 ± 87.89	179.38±31.64	37.63± 21.99	25.25± 9.21	46.50 ±12.72	94.95±26.84
1 (66)	90.36± 10.96	178.65 ±106.75	184.59±37.56	51.21± 26.28	33.83± 15.29	43.05± 11.25	106.31±35.52
2 (1)	100.00	162.00	140.00	66.00	39.00	30.00	77.60
Р	0.69	0.47	0.94	0.36	0.51	0.31	0.28
Olive oil (n)		-					
0 (0)	-	-	-	-	-	-	-
1 (10)	89.50±15.16	171.52±80.24	183.80±37.85	56.40±26.82	31.82 ±11.69	39.60±9.72	97.92±41.49
2 (65)	90.75±10.65	231.40±196.62	183.38±37.06	48.97±25.89	40.60 ±27.74	43.80±11.61	105.76±33.70
р	0.74	0.05	0.97	0.40	0.049	0.28	0.51
Fish (n)		•					
0 (0)	-	-	-	-	-	-	-
1 (1)	77.00	86.00	145.00	45.00	22.00	48.00	79.80
2 (74)	90.77±11.204	180.89±103.80	183.96±36.88	50.03± 26.12	33.14 ±14.91	43.18 ±11.47	105.05±34.74
р	0.22	0.36	0.29	0.84	0.46	0.67	0.47
Cereal (n)							
0(74)	90.45 ±11.25	178.16±103.64	182.91± 6.87	50.04± 26.12	32.99± 14.96	43.34±11.45	104.37±34.73
1(1)	101.00	285.00	223.00	44.00	33.00	36.00	130.00
2 (0)	-	-	-	-	-	-	-
р	0.35	0.28	0.31	0.52	0.46	0.82	0.99
Fruits and vegetables (n)							
0(4)	83.25 ±14.38	151.50± 46.51	146.25±28.82	33.50± 33.13	25.2 ±14.08	42.00±13.63	73.95 ±26.09
1(20)	88.55 ±11.68	180.00± 75.21	188.90±35.07	50.60± 21.52	31.50 ± 7.76	41.60±10.45	111.30±29.86
2(51)	91.96± 10.71	181.70±116.69	184.22±37.09	51.00±27.06	34.18± 16.88	43.98±11.75	104.55±36.07
р	0.21	0.10	0.85	0.71	0.14	0.43	0.45

TABLE 4. Comparison of laboratory parameters according to components of Mediterranean dietary quality index in healthy control group

Characteristics			Healthy controls (N = 76)				
	FSG(mg/dl)	TG(mg/dl)	TC(mg/dl)	ALT(IU/l)	AST(IU/l)	HDL(mg/dl)	LDL(mg/dl)
Saturated fatty acids (n)							
0(47)	89.49 ±9.60	128.00±72.69	186.66±28.12	28.28±9.69	23.66±5.96	48.66±11.19	112.09±25.70
1(22)	89.23±10.51	149.73±76.62	189.91±32.43	25.32±10.55	22.77±6.45	50.05±11.46	109.92±28.38
2(7)	95.43±10.09	187.71±74.85	190.57±25.59	22.00±6.48	20.14±6.15	40.29±13.31	112.74±28.94
Р	0.31	0.11	0.88	0.2	0.36	0.14	0.94
Cholesterol (n)							
0 (54)	89.74±9.94	128.09±73.81	189.11±27.17	25.52±8.99	22.83±5.92	49.19±10.80	114.04±25.27
1 (16)	90.81±9.34	163.50±74.99	184.13±32.02	29.75±10.90	24.38±6.52	45.44±11.93	105.99±29.28
2(6)	89.67±12.98	181.83±70.21	187.83±39.50	31.00±12.77	21.83±7.46	47.83±17.91	103.63±29.96
Р	0.93	0.05	0.83	0.17	0.59	0.52	0.42
Meats (n)							
0(15)	92.33±8.797	140.27±84.079	186.80±23.523	30.93±10.964	24.00±6.803	45.07±11.756	113.13±21.779
1(60)	89.77±9.799	140.88±73.692	188.83±30.138	25.58±9.201	22.68±5.887	49.15±11.617	111.40±27.753
2 (1)	66.00	67.00	153.00	41.00	33.00	45.00	94.60
Р	0.03	0.46	0.62	0.46	0.79	0.067	0.21
Olive oil (n)							
0 (1)	85.00±16.103	211.00	205.00	22.00	27.00	33.00	129.80
1 (5)	91.40±9.583	140.80±95.57	180.60±32.853	32.80±10.18	22.61 ±5.99	47.20±14.30	104.00± 27.28
2(70)	89.93±9.939	138.70±74.51	188.24±28.927	26.49±9.78	29.80± 4.86	48.59±11.44	111.80± 26.59
Р	0.84	0.64	0.71	0.34	0.037	0.41	0.64
Fish (n)							
0(1)	98.00	108.00	137.00	35.00	32.00	43.00	72.40
1(1)	78.00	96.00	192.00	23.00	26.00	60.00	112.80
2 (74)	90.01±9.93	140.81±76.01	188.59±28.66	26.78±9.892	22.9 ±6.108	48.20±11.67	112.04±26.395
Р	0.35	0.77	0.21	0.66	0.31	0.77	0.54
Cereal (n)							
0(74)	90.00±10.05	138.8 ±75.81	187.51± 29.15	26.81± 9.89	23.04± 6.19	48.6 ± 11.58	110.95 ±26.55
1(2)	88.50± 4.95	176.50±48.79	204.50± 0.71	28.00± 8.48	24.50± 3.53	36.50 ± 4.95	132.70± 4.10
2(0)	-	-	-	-	-	-	-
Р	0.74	0.41	0.48	0.14	0.25	0.86	0.74
Fruits and vegetab	les (n)						
0(3)	84.33± 15.94	90.00±45.13	187.67 ±30.03	31.00 ±11.79	25.67± 8.08	50.33±13.79	119.33± 21.77
1(13)	89.15± 12.15	151.3±183.59	179.15 ±24.79	27.38± 10.09	25.31±7.57	46.46±11.17	101.95± 18.72
2(60)	90.42± 9.21	139.78±74.52	189.88 ±29.74	26.52± 9.79	22.47 ±5.65	48.58±11.76	113.21± 27.83
Р	0.56	0.48	0.45	0.81	0.33	0.73	0.24

Characteristics	NAFLD (N=75)			р	Healthy con	р		
Genotype	AA	AG	GG		AA	AG	GG	
Saturated fatty acids	-	0.45±0.11	0.54±0.11	0.56	0.4±0.4	0.35±0.08	0.51±0.13	0.29
Cholesterol	-	0.31±0.08	045±0.12	0.25	0.8±0.37	0.35±0.09	0.29±0.11	0.30
Meats	0.75±0.25	0.77±0.07	0.86±0.06	0.03	0.6±0.24	0.88±0.05	1.00 ± 0.00	0.61
Olive	1.75±0.25	1.97±0.028	1.86 ±0.086	0.85	1.80±0.20	1.86±0.05	1.88±0.06	0.25
Fish	2.00±0.00	1.91±0.063	2.00±0.00	0.42	2.00±0.00	2.00±0.00	1.96±0.03	0.34
Cereals	-	0.028±0.03	0.027 ± 0.027	0.69	-	0.023±0.02	-	0.94
Fruits and vegetables	1.75±0.25	1.82±0.076	1.67±0.095	0.48	1.80±0.2	1.55±0.1	1.62±0.06	0.46

TABLE 5. Comparison of components of Mediterranean dietary quality index according to -866A/G of UCP2 gene polymorphism between study groups

NAFLD patients with GG genotype was higher compared with patients in other genotypes of UCP2 gene. No significant difference for other components of Mediterranean dietary quality index between different genotypes of -866A/G of UCP2 gene was observed (Table 5).

DISCUSSION

In the current study according to the components of Mediterranean dietary quality index, patients with higher scores of dietary saturated fatty acids and cholesterol had higher serum TG concentrations; also we observed significantly lower AST concentrations in subjects with higher olive consumption.

Data regarding the direct effect of Mediterranean dietary pattern on NAFLD are very scarce. However numerous evidences suggested the protective role of Mediterranean dietary pattern against known risk factors of NAFLD including obesity (Romaguera et al., 2009), insulin resistance, diabetes (Martínez-González et al., 2008) and cardiovascular disease (Trichopoulou et al., 2003). In fact, over the past decades, numerous dietary models have been proposed to protect against NAFLD-related metabolic abnormalities and among them, only the Mediterranean diet demonstrated a beneficial effect (Sofi et al., 2014).

In a sub-analysis of the EPIC study analyzed a cohort of 497308 people showed that a higher adherence to the Mediterranean diet was associated with a significantly lower body mass index and waist circumference within 3 years (Romaguera et al., 2009). Likewise, the beneficial role of Mediterranean dietary pattern in protecting against CVD risk factors has been supported by much of available data. A two-point increase of Mediterranean dietary score was associated with 33% reduced risk of mortality from cardiovascular causes (RR = 0.67, 95%, CI: 0.47-0.94). In one study, higher adherence to Mediterranean dietary pattern was observed to be associated with a lesser degree of insulin resistance and severity of NAFLD (Kontogianni et al., 2014).

The Mediterranean dietary quality index (Med-DQI) is a useful tool for predicting dietary quality and has been

validated previously using nutritional biomarkers (Gerber, 2001). This index was based on the recommendations regarding the diet and health of the National Research Council and American Heart Association (Gerber et al., 2000). These recommendations are based on consumption of 30% or less of the day's total energy from fat, 10% or less of the total energy from saturated fat, 30 mg/d or less from cholesterol, 55% of energy from complex carbohydrates and 5 servings or more from fruits and vegetables.

According to the scores of Med-DQI, we demonstrated higher serum triglyceride concentrations in patients with higher scores of "saturated fatty acids" or "cholesterol" intakes (P< 0.05). Serum triglyceride response to a fatty meal strongly is dose-dependent and is proportional to the dietary fat content (Cohen et al., 1988). It has also been suggested that baseline serum triglyceride is a potent determinant of its response to high fat diet; in fact a low-fat diet is recommended to lower serum triglyceride concentrations in patients with severe hyper-triglyceridemia (Jacobs et al., 2004)

We also observed beneficial effects of olive oil in reducing serum triglyceride and AST concentrations in patients with NAFLD. The potential benefits of olive oil in dyslipidemia, T2DM (Rodríguez-Villar et al., 2004) and myocardial infarction (Fernández-Jarne et al., 2002) has been suggested in the past. For example, that olive oil decreases the triglyceride accumulation in liver of rats (Hussein et al., 2007) and protects against the development of fibrosis (Szende et al., 1994). It has recently been shown that oleuropein, an olive oil's main phenolic compound, attenuates hepatic steatosis in mice fed a high fat diet (Park et al., 2011).

In the current study we also demonstrated higher scores of "meat" intake in GG genotype of UCP2 gene polymorphisms compared with patients in other genotypes in NAFLD patients. Previous reports demonstrated that enhanced UCP2 expression is able to respond against oxidative stress by controlling production of mitochondrial superoxide (Baffy, 2005) and suggested that it may be a therapeutic target for management of oxidative damage and metabolic imbalance in NAFLD (Donadelli et al., 2014). Genetic variants in –UCP2

-866G/A gene are potent predictors of energy expenditure and dietary intakes (Deram et al., 2009). Previous studies reported that dietary protein is a determinant of UCP-2 gene expression and high protein diet up-regulates the UCP-2 gene expression in liver and skeletal muscle of rats (Souffrant et al., 2003). Further interventional studies are needed to clarify the effects of Mediterranean dietary pattern on UCP-2 gene expression in NAFLD.

In conclusion, the present study reported a significant relationship between components of Mediterranean dietary pattern and metabolic risk factors of NAFLD. We also demonstrated a gene-nutrient interaction between GG genotype of -UCP2 -866G/A gene polymorphisms and dietary meat intakes. It is clear that dietary modification is the easiest and even the most efficient way to reduce chronic disease risk factors (Loktionov, 2003). This is the first study demonstrating the effect of Mediterranean dietary pattern on metabolic biomarkers of NAFLD; however several limitations of the current study should also be addressed; first, the case control design of the study has not potential to address causeeffect relationship between variables. Second, the current study has been applied on the relatively low sample size. Further studies with interventional designs and with large sample sizes are needed to better determine the effect of components of Mediterranean dietary pattern on metabolic risk factors and UCP2 gene expression in nonalcoholic fatty liver disease.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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