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Adherence to the Mediterranean diet is associated with the severity of non-alcoholic fatty liver disease☆

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SUMMARY

Background & aims: Nutrition has been proposed as a potential environmental factor affecting the risk of non-alcoholic fatty liver disease (NAFLD). In the present study, the impact of adherence to the Mediterranean diet (MD) on the presence and severity of NAFLD was explored.
Methods: Seventy-three consecutive adult patients with recent NAFLD diagnosis were included. Adherence to the MD was estimated with MedDietScore. Demographic and anthropometric data, body composition analysis and several biochemical and inflammatory markers were estimated. Liver stiffness measurements by transient elastography were available in 58 patients and liver biopsies in 34 patients. Fifty-eight patients were matched with 58 healthy controls in terms of age, sex and body mass index.
Results: MedDietScore was negatively correlated to patients' serum alanine aminotransferase ($p = 0.03$) and insulin levels ($p = 0.001$), insulin resistance index ($p = 0.005$) and severity of steatosis ($p = 0.006$) and positively to serum adiponectin levels ($p = 0.04$). Patients with non-alcoholic steatohepatitis (NASH) exhibited lower adherence to MD (29.3 ± 3.2 vs. 34.1 ± 4.4, $p = 0.004$) compared to those with simple fatty liver. Logistic regression analysis revealed that one unit increase in the MedDietScore was associated with 36% lower likelihood of having NASH (odds ratio: 0.64, 95% confidence interval: 0.45–0.92), after adjusting for sex and abdominal fat level. No difference in the MedDietScore was observed between patients and controls.
Conclusions: Higher adherence to the Mediterranean diet is not associated with lower likelihood of having NAFLD, but it is associated with less degree of insulin resistance and less severe liver disease among patients with NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease in Western countries.[1] NAFLD is the result of fat accumulation in the liver (liver fat >5–10% of liver weight) which is not due to excess alcohol consumption or other causes of steatosis, while its severity ranges from simple hepatic steatosis or fatty liver to non-alcoholic steatohepatitis (NASH).[1] NAFLD is considered as the hepatic manifestation of the metabolic syndrome with insulin resistance being the most prevailing pathogenetic mechanism.[2] Dysregulations of proinflammatory cytokines and adipokines are almost universally detected in NAFLD patients, while oxidative stress and apoptosis appear to contribute to the development of NASH.

Both genetic and environmental factors have been proposed to be implicated in the etiology of NAFLD. Thus, nutrition is reasonably

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BIA, bioelectrical impedance analysis; FFQ, food frequency questionnaire; GGT, gamma-glutamyl transpeptidase; HDL, high density lipoprotein; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IL-8, interleukin-8; LDL, low density lipoprotein; LSM, liver stiffness measurements; MedDietScore, Mediterranean Diet Score; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; TGF-β1, transforming growth factor beta-1; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial factor.

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considered to be a potential environmental factor affecting the risk for this disease.[3] The impact of both positive energy balance and diet's composition on the risk and the stage of NAFLD have been explored, mainly through retrospective observational studies. Although there is consistent evidence that overweight due to energy overconsumption increases the risk for and the prevalence of NAFLD,[4] the role of diet's composition, in terms of macro- or micronutrients, in the pathogenesis of the disease remains controversial.[5]

Current trends in clinical nutrition and nutritional epidemiology propose that pattern analysis, examining the effects of the overall diet, is the most realistic approach to examine associations between diet and disease instead of looking at individual nutrients or foods and food groups.[6] Mediterranean diet is a dietary pattern that has been extensively associated with favorable health outcomes, mainly in relation to mortality, cardiovascular disease risk factors and cancer.[7] In relation to the metabolic syndrome, according to a recent systematic review and meta-analysis,[8] adhering or adopting a Mediterranean dietary pattern has a beneficial effect both on the prevention and the resolution of the metabolic syndrome, as well as on individual metabolic parameters. To our knowledge, there are no epidemiological or clinical data on the impact of the Mediterranean diet on the risk for and stage of NAFLD.

The aim of this study was to explore any potential associations between adherence to the Mediterranean diet and the clinical and histological characteristics of patients with NAFLD. A control group was also enrolled to explore any potential involvement of the adherence to the Mediterranean diet in the presence of NAFLD.

2. Materials and methods

The study sample consisted of 73 consecutive adult (18–65 years old) patients with recent (within the last 6 months) diagnosis of NAFLD, who visited the outpatient liver clinics of the 2nd Academic Department of Internal Medicine at Hippokration General Hospital of Athens between May 2009 and December 2010. Participants included were free of diabetes mellitus and liver neoplasm. NAFLD was diagnosed in patients who met all the following criteria: abnormal alanine aminotransferase (ALT) and/or gamma-glutamyl transpeptidase (GGT), ultrasonographic evidence of hepatic steatosis and/or compatible liver histology, and no other cause of liver injury and steatosis. In particular, all patients should have had negative serological markers for hepatitis B (HBsAg), hepatitis C (anti-HCV) and human immunodeficiency virus (anti-HIV), weekly alcohol consumption less than 210 g for men or 140 g for women, no use of potentially hepatotoxic agents, no evidence of metabolic or autoimmune liver disease, and absence of any known systemic disease with potential liver involvement. In addition, patients who had changed their habitual eating habits since NAFLD diagnosis and those following a weight loss diet, as well as patients with diabetes mellitus and/or any diagnosed malignancy were excluded from the study. Medical records were thoroughly reviewed and laboratory data were recorded, namely complete blood count, prothrombin time, uric acid, urea, creatinine, liver enzymes [ALT, aspartate aminotransferase (AST), alkaline phosphatase, GGT, total protein, albumin], serum copper, ceruloplasmin, iron and ferritin, as well as detection of HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV, liver autoantibodies (anti-nuclear, anti-smooth muscles, anti-microsomial, anti-mitochondrial). Participants' habits such as smoking, alcohol consumption and reception of any medication were also recorded. The history of alcohol use was taken from the patients and was confirmed by the patients' relatives or friends.

Moreover, 58 healthy controls matched for age, sex and BMI with 58 patients were evaluated. Controls were either subjects who visited the outpatient clinics of the same department during the study period for routine examinations or people working at the same Department and the universities involved in the study. Controls had stable dietary and exercise habits during the last year and normal liver enzymes. Moreover to minimize the selection bias they underwent ultrasonography to confirm the absence of hepatic steatosis. For all the controls, dietary, physical activity, body composition and biochemical assessments were conducted. The study was approved by the Ethics Committee of the Hippokration General Hospital of Athens and by the Ethics Committee of Harokopio University and was carried out in accordance with the Declaration of Helsinki.[9] All participants were informed about the aims and procedures of the study and gave their written consent.

2.1. Dietary & physical activity assessment

Participants' habitual dietary intake over the last 12 months was assessed through a 60 items semi-quantitative food frequency questionnaire (FFQ), enriched with foods and beverages commonly consumed in Greece.[10] Based on the FFQ data, dietary intake was expressed in terms of food groups (e.g. dairy foods, fruits, vegetables) and individual foods and beverages (e.g. nuts, potatoes, beverages). To evaluate the level of adherence to the Mediterranean dietary pattern the Mediterranean Diet Score (MedDietScore) was used.[11] In particular, for the score calculation, the consumption of food items from 9 food groups (non-refined starchy food, potatoes, fruit, vegetables, legumes, fish, meat and meat products, poultry, and full fat dairy products), as well as olive oil, and alcoholic beverages, were taken into account. For the consumption of food items that are close to this dietary pattern, a score 0 was assigned for no consumption, and scores 1 to 5 for rare to daily consumption, respectively. On the other hand, for the consumption of foods that are away from this pattern, the opposite scores were assigned (i.e. 0 for almost daily consumption to 5 for rare or no consumption). Consumption of various alcoholic beverages was calculated in wine glasses of 100 mL and 12-g ethanol concentrations. For alcohol, score 5 was assigned for consumption of less than 300 ml/day, score 0 for consumption of more than 700 ml/day or 0 ml/day and scores 1 to 4 for consumption of 600–700, 500–600, 400–500 and 300–400 ml per day, respectively. The range of the MedDietScore is 0–55. Higher values of the dietary score indicate greater adherence to the Mediterranean diet.

Physical activity level of the participants was assessed trough a questionnaire that has been previously used in adults.[12,13] Briefly, the questionnaire recalls previous day's physical activities by recording the duration, the type and the intensity of each activity, as well as time spent daily in television viewing or computer use. Based on this information, daily time spent in moderate and vigorous activities (structured or not) and in sedentary activities were calculated. Participants were also given a pedometer (Digi-Walker, Yamax, SW-200, Japan) for a 7-day period (including weekend days) and each participant was asked to record his/her daily steps according to the measurements appearing on the pedometer screen.

2.2. Anthropometric & body composition assessment

Body weight of participants was measured with digital scale (Seca robusta 813, Hamburg, Germany), to the nearest 100 g and height to the nearest 0.5 cm. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference was tape measured to the nearest 0.1 cm.

Abdominal fat compartments, namely trunk fat % and abdominal fat level were estimated by abdominal bioelectrical impedance analysis (Tanita Viscan AB140, Japan).[14]

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2.3. Biochemical and inflammatory markers

Liver enzymes [ALT, AST, alkaline phosphatase and GGT] were recorded from the medical records. Fasting blood samples were collected following a 12 h fast. The obtained serum and plasma samples were immediately frozen at −80 °C. Serum glucose, total cholesterol and HDL-cholesterol were measured using enzymatic colorimetric method (analyzer Cobas 8000, Roche) and LDL cholesterol was calculated using the Friedewald formula.[15] Serum triglycerides were measured with chromatometric enzymatic method (analyzer Cobas 8000, Roche) and insulin with chemiluminescence (Centaur analyzer, Siemens). The insulin resistance index HOMA-IR (Homeostasis Model of Assessment-Insulin Resistance) was calculated using the formula by Matthews et al.[16] High sensitivity CRP (hsCRP) was measured using a nephelometric assay (BN II® nephelometer, Siemens). Tumor necrosis factor-α (TNF-α), (IL-6), interleukin-8 (IL-8), vascular endothelial factor (VEGF), transforming growth factor beta-1 (TGF-β1) and adiponectin, were measured by sensitivity enzyme-linked immunosorbent assay (ELISA, Quantikine/immunoassay kit, R&D Systems, Minneapolis, MN, USA). The intra-assay coefficient of variation was <7% for TNF-α, IL-8 and VEGF and <5% for IL-6, TGF-β1 and adiponectin. The inter-assay coefficient of variation was <8% for TNF-α, IL-6 and adiponectin and <10% for IL-8, TGF-β1 and VEGF.

2.4. Transient elastography

Reliable liver stiffness measurements (LSM) (in kPa) by transient elastography (FibroScan®, Echosens, France) were available in 58 of the 73 patients. The examination was considered to be reliable, if 10 successful measurements were obtained, with a success rate >60% and a ratio of interquartile range (IQR) to mean stiffness <30%. For patients who underwent both transient elastography and liver biopsy, LSM was performed a few hours before liver biopsy in most or within 4 weeks before or after liver biopsy in some patients. In any case, LSM was performed within the first 4 weeks from the diagnosis of NAFLD.

2.5. Liver histology

Adequate liver biopsies were available in 34 of the 73 patients. They were evaluated by a single hepatopathologist (D.T.), who was blinded to the clinical data. A liver biopsy was considered to be adequate, if at least 6 portal tracts were identified and the specimen length was ≥1.5 cm. The diagnosis of NASH was based on the overall pattern of injury and the criteria of Brunt et al.[17] modified by Kleiner et al.[18] Global grade of necroinflammatory activity and stage of fibrosis were assessed according to Brunt et al.[17] Severity of steatosis and NAFLD activity score (NAS) were evaluated according to Kleiner et al.[18]

2.6. Statistical analysis

SPSS 18.0 software (SPSS Inc. 2009, Chicago, Illinois, USA) was used for all the statistical calculations. The normality of the data was assessed graphically using histograms. Skewed variables were log-transformed to become normal. Continuous variables are presented as mean values ± standard deviation, while categorical variables are presented as frequencies. Correlations between MedDietScore and anthropometric, biochemical and histological parameters were tested using the Spearman or the Pearson's correlation coefficients. For multiple comparisons the Bonferroni correction was applied. Contingency tables and the calculation of chi-squared test, two samples *t*-test or Mann–Whitney *U* test were used were appropriate to test differences between patients and controls. Mann–Whitney *U* test was used to test differences among patients with NASH and simple fatty liver. Multiple linear regression model was applied to test the association between the MedDietScore and insulin resistance, after controlling for several potential confounders. Standardized residuals were used to test model's goodness of fit. The odds ratios of having NAFLD and their corresponding 95% confidence intervals were calculated through logistic regression analysis. The same was also performed for estimating the odds of having NASH, according to liver biopsies. All reported *p*-values were based on two-sided tests and compared to a significance level of 5%.

3. Results

3.1. Patients' characteristics

Patients anthropometric, lifestyle, biochemical and histological characteristics are presented in Table 1. Their mean age was 45 years, whereas the majority (69%) of the patients was males. The mean liver stiffness in 58 patients who underwent transient elastography was 6.5 ± 2.0 kPa. In the patients with available liver biopsies, the mean grade of necroinflammation (according to Brunt) was 1.1 ± 0.9 and the mean stage of fibrosis 1.4 ± 1.2. Of these 34 patients, 11 (32%) cases were diagnosed to have simple fatty liver and 23 (68%) cases to have NASH.

3.2. Correlations of MedDietScore with laboratory parameters in participants with NAFLD

MedDietScore correlated significantly to age ($r = 0.28$, $p = 0.016$) and tended to correlate to number of steps per day ($r = 0.23$, $p = 0.06$) waist circumference ($r = -0.22$, $p = 0.06$) and

Table 1
Main characteristics of 73 patients with non-alcoholic fatty liver disease (NAFLD).

Gender – males, *n* (%)	50 (68.5)
Age, years	45.4 ± 11.3
Body mass index, kg/m^2	29.7 ± 4.2
Waist circumference, cm	
Males	103 ± 9
Females	102 ± 12
Increased waist circumference,[a] *n* (%)	45 (61.6)
Abdominal fat level (1–59)	16.4 ± 5.1
Smoking habits – current smokers, *n* (%)	20 (27.4)
Mediterranean Diet Score (0–55)	32.5 ± 5.0
Number of steps per day	6957 ± 3409
Sedentary activities (h/day)	3.07 ± 1.70
Biochemical characteristics	
HOMA-IR	3.6 ± 2.46
hsCRP mg/L	1.62 ± 1.71
Adiponectin μg/mL	6.27 ± 4.30
TNF-α pg/mL	5.19 ± 4.14
Interleukin-6 pg/mL	2.68 ± 4.22
Interleukin-8 pg/mL	29.2 ± 46.8
TGF-β-1 μg/mL	40.1 ± 8.3
VEGF pg/mL	315 ± 167
Liver stiffness measurements, kPa (in 58 patients)	7.6 ± 4.9
Histological characteristics in 34 patients	
Grade[b] (necroinflammatory activity)	1.1 ± 0.9
NAFLD activity score[c]	4.0 ± 2.1
Stage (fibrosis severity)	1.4 ± 1.2
Severity of steatosis[c]	2.2 ± 0.9
Non-alcoholic steatohepatitis, *n* (%)	23 (68)

Data are presented as mean ± SD or frequencies.
HOMA-IR: Homeostasis Model of Assessment of insulin resistance; hsCRP: high sensitivity CRP; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial factor; TGF-β-1: transforming growth factor beta-1.
[a] >102 cm for males and >88 cm for females.
[b] According to Brunt et al.[25]
[c] According to Kleiner et al.[26]

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abdominal fat level ($r = -0.18$, $p = 0.10$). Regarding biochemical variables, MedDietScore significantly correlated to serum loginsulin ($r = -0.38$, $p = 0.001$), logHOMA-IR ($r = -0.33$, $p = 0.005$), total cholesterol ($r = 0.257$, $p = 0.03$) and logALT ($r = -0.25$, $p = 0.034$), while it tended to correlate to logAST ($r = -0.21$, $p = 0.08$), HDL ($r = 0.20$, $p = 0.09$) and LDL levels ($r = 0.22$, $p = 0.06$). Among the inflammatory markers assessed in the present study, MedDietScore correlated only to logadiponectin ($r = 0.25$, $p = 0.039$).

3.3. Correlations of MedDietScore with patients' histological characteristics

Table 2 shows the correlations between MedDietScore and liver stiffness or histological parameters in the patients with available transient elastography and/or liver biopsy. MedDietScore was negatively correlated to liver stiffness measurements, stage of fibrosis and severity of steatosis. However, when the Bonferroni correction rule was applied, MedDietScore was significantly associated only with the severity of steatosis (rho $= -0.52$, $p = 0.006$).

When the 34 patients with available liver biopsies were classified as having simple fatty liver or NASH, those with NASH had higher BMI (30.4 ± 4.1 vs. 27.0 ± 2.7, $p = 0.028$), higher abdominal fat level (18.1 ± 6.1 vs. 12.7 ± 4.1, $p = 0.012$) and exhibited lower adherence to the Mediterranean diet (29.3 ± 3.2 vs. 34.1 ± 4.4, $p = 0.004$) (Fig. 1), whereas no differences in physical activity indices were recorded. Moreover, patients with NASH had higher HOMA-IR (4.90 ± 3.11 vs. 2.19 ± 1.25, $p < 0.001$) and IL-6 levels (2.97 ± 4.2 vs. 1.26 ± 0.63, $p = 0.013$) and lower HDL levels (22.8 ± 12.5 vs. 32.3 ± 10.6, $p = 0.001$) compared to those with simple fatty liver.

According to logistic regression analysis, one unit increase in the MedDietScore was associated with 36% lower likelihood of having NASH (odds ratio: 0.64, 95% confidence interval: 0.45–0.92, $p = 0.02$), after adjusting for sex and abdominal fat level. Given that insulin resistance plays a major role in NAFLD pathogenesis, the association between adherence to the Mediterranean diet and HOMA-IR was explored using multiple regression analysis. MedDietScore was negatively associated with logHOMA-IR (standardized beta coefficient:-0.303, $p = 0.005$) after adjustment for age, sex, smoking, abdominal fat level and serum adiponectin levels (Table 3). The above association of MedDietScore and logHOMA-IR remained unchanged when the number of steps was included in the independent variables.

3.4. Comparisons between participants with NAFLD and matched controls

Comparisons between the 58 patients and their 58 matched healthy controls are presented in Table 4. Although the two groups were matched for BMI, controls exhibited lower abdominal fat level ($p = 0.008$), lower levels of HOMA-IR, hsCRP, and TNF-α and higher adiponectin levels (all $p < 0.05$). Interestingly, neither adherence to the Mediterranean Diet Score nor physical activity indices (i.e. daily number of steps and hours of sedentary activities) differed significantly between the two groups. Table 5 shows the odds ratios of having NAFLD based on the adherence to the Mediterranean diet and after adjusting for age, sex, abdominal fat level and adiponectin levels. Obviously, adherence to the Mediterranean diet was not associated with the likelihood of having NAFLD.

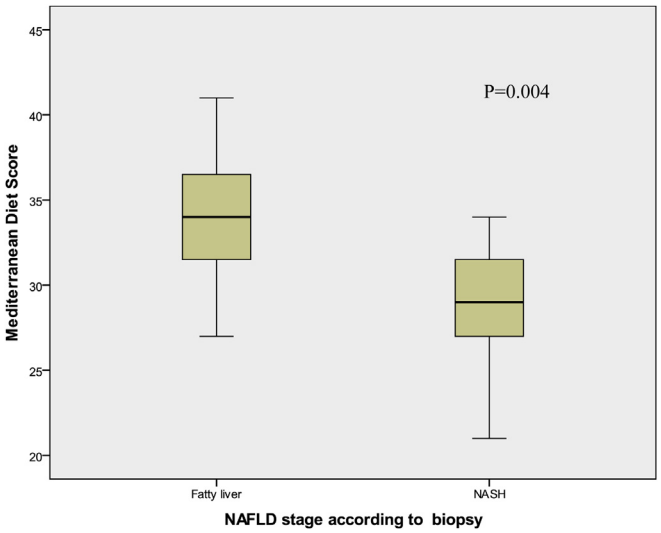

Fig. 1. Mediterranean Diet Score in patients with simple fatty liver and non-alcoholic steatohepatitis (NASH). NAFLD: non-alcoholic fatty liver disease.

4. Discussion

In the present study, any potential associations between adherence to the Mediterranean diet and clinical characteristics of patients with NAFLD were explored. According to our results, greater adherence to the Mediterranean diet, as estimated by MedDietScore, was significantly associated with less liver steatosis as estimated by liver biopsy. Patients with NASH exhibited lower adherence to the Mediterranean diet compared to patients with simple fatty liver. Moreover, higher adherence to the Mediterranean diet was associated with lower degree of insulin resistance, which is thought to be a major pathogenic mechanism in NAFLD, after adjustment for other factors known to affect insulin resistance.

Table 2
Correlations of adherence to the Mediterranean diet with histological parameters and liver stiffness measurements.

	N	MedDietScore	
		Rho	*p*
Grade of necroinflammatory activity	34	−0.28	0.11
NAFLD activity score	34	−0.19	0.28
Stage of fibrosis	34	−0.37	0.03
Severity of steatosis	34	−0.30	0.08
Steatosis, %	34	−0.52	0.006*
Liver stiffness, kPa	59	−0.28	0.03

Rho: Spearman correlations coefficient; MedDietScore: Mediterranean Diet Score. The Bonferroni correction rule was applied to adjust for multiple comparisons; *<0.008.

Table 3
Multiple regression analyses model, exploring the association between adherence to the Mediterranean diet and insulin resistance as assessed by the homeostasis model ($N = 73$).

Variables	LogHOMA-IR	
	Standardized beta coefficient	*p*
Age (y)	0.018	0.87
Sex (male vs. female)	−0.456	0.002
Smoking (current vs. ex/never smokers)	0.088	0.37
Abdominal fat level (1–59 units)	0.623	<0.001
Adiponectin (μg/mL)	−0.257	0.03
MedDietScore (0–55 units)	−0.303	0.005
R^2 *adjusted*	0.410	

MedDietScore: Mediterranean Diet Score; HOMA-IR: Homeostasis Model of Assessment of insulin resistance.

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Table 4
Comparisons between patients with non-alcoholic fatty liver disease (NAFLD) and matched controls.

	NAFLD ($N = 58$)	Controls ($N = 58$)	p^*
Gender – males, *n* (%)	36 (62.1)	36 (62.1)	>0.99
Age, years	44.5 ± 11.6	44.6 ± 12.3	0.96
Body mass index, kg/m^2	28.7 ± 3.9	27.7 ± 3.6	0.15
Waist circumference, cm			
Males	99 ± 7	97 ± 8	0.15
Females	101 ± 7	95 ± 7	0.07
Abdominal fat level (1–59)	15.0 ± 4.4	12.5 ± 5.1	0.008
Smoking habits – current smokers, *n* (%)	14 (24)	26 (45)	0.03
Number of steps per day	7076 ± 3178	7371 ± 3401	0.67
Sedentary activities (h/day)	2.8 ± 1.6	2.7 ± 1.4	0.66
Mediterranean Diet Score (0–55)	33.1 ± 5.0	33.0 ± 4.5	0.75
Biochemical characteristics			
HOMA-IR	3.1 ± 2.2	1.9 ± 1.5	<0.001
hsCRP mg/L	1.81 ± 2.0	1.18 ± 1.1	0.03
Adiponectin μg/mL	6.6 ± 4.7	10.0 ± 7.7	0.007
TNF-α pg/mL	4.8 ± 3.7	2.5 ± 3.6	<0.001
Interleukin-6 pg/mL	2.3 ± 3.9	3.7 ± 9.3	0.19
Interleukin-8 pg/mL	32.4 ± 51.3	33.8 ± 84.8	0.10

Data are presented as mean ± SD or frequencies.
*Probability values as derived by chi-squared test, two samples *t*-test or Mann–Whitney *U* test.
HOMA-IR: Homeostasis Model of Assessment of insulin resistance; hsCRP: high sensitivity CRP; TNF-α: tumor necrosis factor-α.

Mediterranean diet, as described by the index used in the present study,[11] is a pattern characterized by high consumption of foods such as fruits, vegetables, non-refined cereals, legumes and potatoes, moderate consumption of fish and poultry and low consumption of full fat dairies, red meat and its products. Moreover, olive oil is the basic fat used during food preparation and consumption, and meals are often accompanied by low-to-moderate amounts of wine. To the best of our knowledge, this is the first epidemiological study exploring the potential impact of a whole dietary pattern (i.e. Mediterranean diet) on NAFLD characteristics and severity. Prior studies have focused either on macro- and micronutrients, whereas there is a limited number of studies' exploring the role of certain foods in NAFLD risk and severity. In specific, a cross-sectional study comparing nutritional habits of subjects having NAFLD to those not having the disease revealed that subjects with NAFLD consumed more meat and less fish, as well as more soft drinks.[19] The detrimental effect of soft drinks consumption on NAFLD has been also supported by two case-control studies.[20,21] Moreover, a recent study aimed to evaluate the association between modest alcohol drinking and NASH, among subjects with NAFLD and according to its findings alcohol consumption up to two drinks per day was associated with half the odds of steatohepatitis, and with a lesser severity of fibrosis and ballooning hepatocellular injury.[22] In the present sample mean daily alcohol intake of both patients and controls was around one drink (8.5 and 13.3 g of ethanol, respectively) reflecting a low alcohol consumption that has been proposed to exhibit health benefits. Furthermore, in vivo studies in rats with experimental NAFLD have shown that olive oil decreases the accumulation of triglycerides in the liver of rats,[23] can protect against the development of fibrosis compared to polyunsaturated fats,[24] whereas oleuropein, an olive oil's main phenolic compound, has been recently shown to attenuate hepatic steatosis in mice fed with high-fat diet.[25]

Table 5
Logistic regression analysis models, exploring the association between adherence to the Mediterranean diet and the likelihood of the presence of NAFLD ($N = 58$ cases and 58 controls).

	Wald	*p*	OR	95% CI
Age (y)	0.10	0.75	0.99	0.94–1.04
Sex, male	3.02	0.08	3.58	0.85–15.08
Abdominal fat level (1–59)	2.86	0.09	1.10	0.99–1.24
Mediterranean Diet Score (0–55)	0.33	0.57	1.03	0.93–1.15
HOMA-IR	5.02	0.03	1.42	1.05–1.94
Adiponectin (μg/mL)	7.49	0.006	0.86	0.77–0.96

OR: odds ratio, CI: confidence interval, HOMA-IR: Homeostasis Model of Assessment of insulin resistance.

Although there are no data linking Mediterranean diet to NAFLD, there is strong evidence both from epidemiological and clinical studies supporting the protective role of the adherence to the Mediterranean diet against metabolic syndrome, as well as its components, namely hypertension, hypertriglyceridemia, and impaired glucose metabolism.[8,26] In an effort to explore which mechanisms could be possibly affected by the adherence to the Mediterranean diet in patients with NAFLD, potential predictors of insulin resistance were tested in a multivariate model and adherence to the Mediterranean diet was inversely associated with insulin resistance, independent from other confounders. Quite recently Ryan et al.[27] in a randomized, cross-over intervention trial showed that Mediterranean diet, compared to a low fat high carbohydrate diet, improved insulin sensitivity and hepatic steatosis in patients with biopsy proven NAFLD in the absence of weight loss.

According to our findings, there was no significant difference in the adherence to the Mediterranean diet between NAFLD patients and healthy controls matched for age, sex and BMI. Despite the BMI matching, however, the two groups differed in several parameters that have been shown to affect the probability of NAFLD development, such as levels of abdominal fat and of HOMA-IR, hsCRP, TNF-α and adiponectin. These findings suggest that positive energy balance has different effects on different subjects and therefore non-dietary, most probably genetic, factors are more important in the pathogenesis and development of NAFLD.[28] This hypothesis is also supported by findings showing that the likelihood of NAFLD differs according to the race[29] or that there may be a familial clustering of NAFLD with an overall heritability of around 40%.[30] Of course, positive energy balance and increased BMI substantially increase the risk of NAFLD, perhaps in genetically predisposed individuals,[4,28,30] as NAFLD is not always present in obese subjects or may develop in individuals with normal BMI as well.[31] The strong involvement of non-dietary factors in the development of NAFLD may be responsible for the controversial findings on the role of diet's composition, in terms of macro- or micronutrients, in the pathogenesis of this disease.[4,5] Prospective epidemiological, perhaps case-control, studies in normal BMI individuals should be conducted aiming to shed light on this issue.

The "two hit" model proposed for the pathogenesis of NAFLD involves excessive hepatocyte triglyceride accumulation, resulting from insulin resistance (first hit) as well as oxidative stress, NF-κB-dependent inflammatory cytokine expression and adipocytokines (second hits).[32] Based on our findings, one could speculate that although genetic predisposition and positive energy balance may be far more crucial for the "first hit" leading to the development of NAFLD, diet composition may affect the severity of the disease by influencing mainly the second hit and the subsequent inflammation and oxidative stress. Mediterranean diet is a pattern with clear anti-inflammatory and anti-oxidant effects[33,34] and these anti-inflammatory effects could also partly explain the lower likelihood of having NASH, as the adherence to this pattern increased in patients of our study.

Among the strengths of the study, it should be mentioned that this is the first one exploring potential associations between adherence to a dietary pattern, namely the Mediterranean diet and

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clinical characteristics of NAFLD, also including data from biopsies for a subgroup of patients. However, given the cross-sectional design, our study cannot establish causal relations but may only generate hypotheses for associations. These hypotheses should be tested in the context of randomized clinical trials to confirm the role of the Mediterranean diet in the severity and treatment of NAFLD and also in prospective epidemiological studies regarding this pattern's involvement in the development of the disease. Another limitation is the relatively small sample size used in the case-control analysis ($N = 116$) leading to a small statistical power (<50%) for evaluating odds ratios equal to 0.70 (±0.10) at 5% significance level. Thus, a possible type 2 error cannot be excluded. Given the design of the present study, it is also vulnerable to recall bias and findings apply only to patients with NALFD and elevated liver enzymes and not in patients with ultrasonographic evidence of liver steatosis and normal liver enzymes activity.

In conclusion, although greater adherence to the Mediterranean diet was not associated with lower likelihood of having NAFLD, higher adherence to this pattern seems to have a beneficial effect on the severity of disease (i.e. lower likelihood of NASH, less severe liver steatosis and lower degree of insulin resistance) in patients with NAFLD.

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Statement of authorship

MDK, YM, GP designed the study; NT, MG, AM conducted the study; MD, DT, EF, RZ, conducted assessments and assays, MDK analyzed data; MDK and NT drafted the manuscript; YM and GP interpreted the data and critically revised the manuscript. All authors read and approved the final manuscript.

Conflict of interest

All authors declare no conflicts of interest.

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References

1. Argo CK, Caldwell SH. Epidemiology and natural history of non-alcoholic steatohepatitis. *Clin Liver Dis* 2009;**13**:511–31.
2. Levene AP, Goldin RD. The epidemiology, pathogenesis and histopathology of fatty liver disease. *Histopathology* 2012;**61**:141–52.
3. Erickson SK. Nonalcoholic fatty liver disease. *J Lipid Res* 2009;**50**(Suppl.):S412–6.
4. Utzschneider KM, Kahn SE. Review: the role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2006;**91**:4753–61.
5. Mouzaki M, Allard JP. The role of nutrients in the development, progression, and treatment of nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2012;**46**:457–67.
6. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002;**13**:3–9.
7. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* 2010;**92**:1189–96.
8. Kastorini CM, Milionis HJ, Esposito K, Giugliano D, Goudevenos JA, Panagiotakos DB. The effect of mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. *J Am Coll Cardiol* 2011;**57**:1299–313.
9. World Medical Association declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *J Am Med Assoc* 1997;**277**:925–6.
10. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S. Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. *Nutr J* 2004;**3**:13.
11. Panagiotakos DB, Pitsavos C, Arvaniti F, Stefanadis C. Adherence to the mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. *Prev Med* 2007;**44**:335–40.
12. Manios Y, Moschonis G, Koutsikas K, Papoutsou S, Petraki I, Bellou E, et al. Changes in body composition following a dietary and lifestyle intervention trial: the postmenopausal health study. *Maturitas* 2009;**62**:58–65.
13. Petrogianni M, Grammatikaki E, Pitsavos C, Moschonis G, Alexopoulos EC, Manios Y. Changes in CVD risk factors after combined dietary counselling and supplementation with lipid-lowering milk product: the effect of compliance. *e-SPEN J* 2012;**1**–**6** [Ahead of print].
14. Browning LM, Mugridge O, Chatfield MD, Dixon AK, Aitken SW, Joubert I, et al. Validity of a new abdominal bioelectrical impedance device to measure abdominal and visceral fat: comparison with MRI. *Obesity (Silver Spring)* 2010;**18**:2385–91.
15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499–502.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–9.
17. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;**94**:2467–74.
18. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;**41**:1313–21.
19. Zelber-Sagi S, Nitzan-Kaluski D, Goldsmith R, Webb M, Blendis L, Halpern Z, et al. Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *J Hepatol* 2007;**47**:711–7.
20. Assy N, Nasser G, Kamayse I, Nseir W, Beniashvili Z, Djibre A, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol* 2008;**22**:811–6.
21. Abid A, Taha O, Nseir W, Farah R, Grosovski M, Assy N. Soft drink consumption is associated with fatty liver disease independent of metabolic syndrome. *J Hepatol* 2009;**51**:918–24.
22. Dunn W, Sanyal AJ, Brunt EM, Unalp-Arida A, Donohue M, McCullough AJ, et al. Modest alcohol consumption is associated with decreased prevalence of steatohepatitis in patients with non-alcoholic fatty liver disease (NAFLD). *J Hepatol* 2012;**57**:384–91.
23. Hussein O, Grosovski M, Lasri E, Svalb S, Ravid U, Assy N. Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. *World J Gastroenterol* 2007;**13**:361–8.
24. Szende B, Timar F, Hargitai B. Olive oil decreases liver damage in rats caused by carbon tetrachloride (CCl4). *Exp Toxicol Pathol* 1994;**46**:355–9.
25. Park S, Choi Y, Um SJ, Yoon SK, Park T. Oleuropein attenuates hepatic steatosis induced by high-fat diet in mice. *J Hepatol* 2011;**54**:984–93.
26. Martinez-Gonzalez MA, de la Fuente-Arrillaga C, Nunez-Cordoba JM, Basterra-Gortari FJ, Beunza JJ, Vazquez Z, et al. Adherence to mediterranean diet and risk of developing diabetes: prospective cohort study. *BMJ* 2008;**336**:1348–51.
27. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J Hepatol* 2013;**59**:138–43.
28. Puppala J, Siddapuram SP, Akka J, Munshi A. Genetics of nonalcoholic fatty liver disease: an overview. *J Genet Genomics* 2013;**40**:15–22.
29. Mohanty SR, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J Hepatol* 2009;**50**:797–804.
30. Tilg H, Moschen A. Update on nonalcoholic fatty liver disease: genes involved in nonalcoholic fatty liver disease and associated inflammation. *Curr Opin Clin Nutr Metab Care* 2010;**13**:391–6.
31. Margariti A, Deutsch M, Manolakopoulos S, Tiniakos D, Papatheodoridis GV. The severity of histologic liver lesions is independent of body mass index in patients with nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2013;**47**:280–6.
32. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010;**51**:679–89.
33. Estruch R. Anti-inflammatory effects of the mediterranean diet: the experience of the PREDIMED study. *Proc Nutr Soc* 2010;**69**:333–40.
34. Bullo M, Lamuela-Raventos R, Salas-Salvado J. Mediterranean diet and oxidation: nuts and olive oil as important sources of fat and antioxidants. *Curr Top Med Chem* 2011;**11**:1797–810.

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