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Original

Mediterranean diet is associated with liver histology in patients with non alcoholic fatty liver disease

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Abstract

Background: **clinical data on impact of the Mediterranean diet on the the stage of non alcoholic fatty liver disease are limited and these studies have heterogeneous designs.**

Aim: **we decide to explore any potential associations between adherence to the Mediterranean diet and histological characteristics of patients with NAFLD.**

Methods: **a sample of 82 patients was analyzed in a cross sectional study. To evaluate the level of adherence to the Mediterranean dietary pattern the 14-Item Mediterranean Diet Assessment Tool was used.**

Results: **thirty five patients (42.7%) had a low grade of steatosis (grade 1 of classification) and 47 patients (57.3%) had a high grade of steatosis (grade 2 and 3). Fifty-six patients (68.3%) had liver steatohepatitis and forty-two patients (51.2%) had liver fibrosis. In the logistic regresion analysis, one unit of the 14-Item Mediterranean Diet Assessment Tool was associated with a lower likehood of having steatohepatitis odds ratio 0.43 (CI:95%: 0.29-0.64) and steatosis 0.42 (CI:95%: 0.26-0.70). Secondly, one unit of HOMA-IR was associated with higher likehood of having steatosis odds ratio 2.01 (CI:95%: 1.08-3.71) and liver fibrosis 1.38 (CI:95%: 1.10-1.80) .**

Conclusions: **greater adherence to the Mediterranean diet was associated with lower likelihood of high grade of steatosis and presence of steatohepatitis.**

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Key words: *Insulin resistance. Mediterranean diet. Non-alcoholic fatty liver disease. Liver biopsy.*

LA DIETA MEDITERRÁNEA SE ASOCIA CON LA HISTOLOGÍA HEPÁTICA EN PACIENTES CON ENFERMEDAD DEL HÍGADO GRASO NO ALCOHÓLICO

Resumen

Antecedentes: **los datos clínicos sobre el impacto de la dieta mediterránea en el estadio de la enfermedad son limitados, y los estudios existentes tienen diseños heterogéneos.**

Objetivo: **decidimos explorar las posibles asociaciones entre la adhesión a la dieta mediterránea y las características histológicas de los pacientes.**

Métodos: **se analizó una muestra de 82 pacientes en un estudio de corte transversal. Para evaluar el nivel de adhesión al patrón de dieta mediterránea se utilizó la herramienta de evaluación de la dieta mediterránea de 14-ítem.**

Resultados: **treinta y cinco pacientes (42,7%) tenían un bajo grado de esteatosis (grado 1 de la clasificación) y 47 pacientes (57,3%) tenían un alto grado de esteatosis (grados 2 y 3). Cincuenta y seis pacientes (68,3%) tenían esteatohepatitis y cuarenta y dos pacientes (51,2%) tenían fibrosis hepática. En el análisis de regresión logística, el aumento de una unidad de la Herramienta de Evaluación de Dieta Mediterránea de 14-ítems se asoció con un menor probabilidad de desarrollar esteatohepatitis 0,43 (IC del 95%: 0,29 hasta 0,64) y esteatosis 0,42 (IC: 95%: 0,26- 0,70). En segundo lugar, una unidad de HOMA-IR se asoció con mayor probabilidad de esteatosis 2,01 (IC del 95%: 1,08 a 3,71) y la fibrosis hepática 1,38 (IC: 95%: 1,10 a 1,80).**

Conclusiones: **la mayor adhesión a la dieta mediterránea se asoció con una menor probabilidad de presentar esteatosis y esteatohepatitis.**

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Palabras clave: *Resistencia a la insulina. Dieta mediterránea. Hígado graso no alcohólico. Biopsia hepática.*

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Introduction

NAFLD is defined as the accumulation of lipids, primarily in the form of triacylglycerols in individuals who do not consume significant amounts of alcohol and in whom other known causes of steatosis, such as certain toxins and drugs, have been excluded[1]. The spectrum of NAFLD includes simple fatty liver, non-alcoholic steatohepatitis (NASH), cirrhosis post NASH, hepatocellular carcinoma and advanced liver disease, which leads to liver related death[2].

Although not all patients with NAFLD are obese, obesity is considered the most important risk factor. Insulin resistance is a significant predictor of NAFLD in most patients[3], even a percentage of patients who are not overweight[4]. NAFLD is a multifactorial disease that involves a complex relation of diet and genetics.

A cornerstone of the management strategy in such patients is the use of diet. No proven treatment for patients with NAFLD is currently available. Weight reduction with diet changes is usually recommended as the first step in treatment of patients with this condition but the results are inconsistent[5,6]. The impact of both positive energy balance and diet´s composition on the risk and the stage of NAFLD must be explored. Various treatment strategies such as thiazolidinediones, metformin, lipid-lowering agents and antioxidants have been studied[7].

Mediterranean diet is a dietary pattern that has been extensively associated with favorable helth outcomes, in relation with cardiovascualr risk factors and cancer[8]. In relation to the metabolic syndrome[9], adhering a Mediterranean dietary pattern has a beneficial effect both on the prevention and the resolution of this syndrome. Clinical data on impact of the Mediterranean diet on the stage of NAFLD are limited[10-12] and these studies have heterogeneous designs.

Considering the limited evidence that adherence to a Mediterranean dietary could be related with liver lesions in patients with NAFLD, we decided to explore any potential associations between adherence to the Mediterranean diet and histological characteristics of patients with NAFLD.

Subjects and methods

Subjects

A sample of 82 patients was enrolled. Exclusion criteria were hepatitis B, C, cytomegalovirus, Epstein Barr infections, non organ-specific autoantibodies, alcohol consumption, diabetes mellitus, impaired glucose tolerance , medication (blood-pressure lowering medication and statins) and hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency). The study was approved by the institutional Ethics Committee and all patients signed an informed consent.

Liver biopsy

The diagnosis of NAFLD was confirmed by percutaneous liver biopsy performed in all subjects with a 1.6 mm Menghini-type biopsy needle. Liver samples were routinely processed, sectioned, and stained with hematoxilin-eosin and Manson's trichome. A semi quantitative scoring system for NAFLD had been applied. Defined as the unweighted sum of scores for: Steatosis (<5%=0, 5 to 33%=1,>33 to 66%=2,>66%=3), Lobular inflammation (no foci=0,<2 foci per 200 x field=1, 2 to 4 foci per 200 x field=2,>4 foci pr 200 x field=3), Ballooning (none=0, few balloon cells=1, many cells/prominent ballooning=2) and Fibrosis was not include in the NAS score. The maximum score was 8. Definitive non alcoholic steatohepatitis (NASH) was defined as a NAS score>=5[13].

In our study presense of liver fibrosis was divided as absent (stage 0) or present (stage 1 to 4). The system also includes: stage 0: no fiboris, stage 1: zone 3 perivenular perisinusoidal/pericellular fibrosis, focal or extensive; stage 2: as above with focal or extensive periportal fibrosis; stage 3: bridging fibrosis, focal or extensive; stage 4: cirrhosis.

Steatosis was graded as follows: grade 1 (>5% and<33% of hepatocytes affected); grade 2 (33-66%); or grade 3 (>66% of hepatocytes affected). Grades 2 and 3 were combined for statistical analysis (high grade) and grade 1 (low grade).

Procedure

The following variables were specifically recorded: age, weight, waist circumference, blood pressure, body mass index (BMI). Fasting basal glucose, transaminases, insulin, HOMA-R, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, adiponectin and leptin blood levels were measured.

Dietary assessment

To evaluate the level of adherence to the Mediterranean dietary pattern the 14-Item Mediterranean Diet Assessment Tool was used. A dietitian completed a 14-item Mediterranean Diet adherence screener in a face-to-face interview with the participant. The 14-Item Mediterranean Diet Assessment Tool was developed in a Spanish case-control study of myocardial infarction[14], where the best cut-off points for discriminating between cases and controls were selected for each food or food group. The lowest adherence to the Mediterranean diet was a score under or equal to 7 and those with higher levels of adherence (above 8 or 9) were considered subjects with a

Mediterranean diet. The question of test (criteria for 1 point)were; *1) Do you use olive oil as main culinary fat? (Yes), 2) How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc.)? (> = 4 tablespoon), 3) How many vegetable servings do you consume per day? (1 serving : 200 g [consider side dishes as half a serving]) (> = 2 (> = 1 portion raw or as a salad)), 4) How many fruit units (including natural fruit juices) do you consume per day? (> = 3), 5) How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving: 100–150 g) (< 1), 6) How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g) (< 1), 7) How many sweet or carbonated beverages do you drink per day? (< 1), 8) How much wine do you drink per week? (> = 7 glasses), 9) How many servings of legumes do you consume per week? (1 serving : 150 g) (> = 3), 10) How many servings of fish or shellfish do you consume per week? (1 serving 100–150 g of fish or 4–5 units or 200 g of shellfish) (> = 3), 11) How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard? (< 3), 12) How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g) (> = 3), 13) Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage? (Yes), 14) How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?(> = 2).*

Assays

Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California).Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA-R) was calculated using these values[15].

Alanine amino transferase, aspartate aminotransferase activity, bilirrubin and gamaglutamine transferase were determined by enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Geneve, Switzerland). Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml[16]. Adiponectin was measured by ELISA (R&D systems, Inc., Mineapolis, USA) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml[17].

Anthropometric measurements

Body weight was measured to an accuracy of 0.1 Kg (BMI computed as body weight/height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too.

Statistical analysis

Sample size was calculated to detect differences over 1 points of the 14-Item Mediterranean Diet Assessment Tool with 90% power and 5% significance. A sample of 80 patients was needed. The results were expressed as mean +/-standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's-t test. Non-parametric variables were analyzed with the W-Wilcoxon test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. 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 A logistic model was used to study the dependent variables (grades of steatosis; low grade vs high grade), (grades of fibrosis; absence and presence) and (non-alcoholic steatohepatitis; ausence or presence).A p-value under 0.05 was considered statistically significant.

Results

Eighty two patients gave informed consent and were enrolled in the study. The mean age was 44.2+11.3 years, the mean BMI 32.9+8.8 and the sex distribution (26 females (31.7%) and 56 males (68.3%)). Thirty five patients (42.7%) had a low grade of steatosis (grade 1 of classification) and 47 patients (57.3%) had a high grade of steatosis (grade 2 and 3). Twenty six patients (31.7%) did not have liver steatohepatitis and fifty-six patients (68.3%) had liver steatohepatitis. Forty patients (48.8%) did not have liver fibrosis and forty-two patients (51.2%) had liver fibrosis.

Table I shows differences in clinical and biochemical parameters between grades of steatosis. BMI, weight, systolic blood pressure, diastolic pressure, insulin, HOMA-IR, AST and leptin were higher in patients with high grade of steatosis. Adiponectin and 14-Item Mediterranean Diet Assessment Tool score were higher in patients with low grade of steatosis.

Table II shows differences among levels of biochemical and clinical parameters between patients with and without steatohepatitis. BMI, weight, systolic

Table I
Clinical and epidemiological characteristics (low grade vs high grade of steatosis)

Characteristics	*Low Grade*	*High Grade*	*P*
Age(years)	44.3 ± 10.2	43.7 ± 12.5	ns
Sex(female/male)	9/26	17/30	ns
BMI(kg/m^2)	29.8 ± 5.6	35.3 ± 10.3	p = 0.001
Weight (kg)	84.7 ± 18.5	98.1 ± 27.9	p = 0.01
Waist circumference (cm)	96.7 ± 7.5	100.2 ± 12.9	ns
Waist to hip ratio	0.94 ± 0.07	0.93 ± 0.09	ns
SBP (mmHg)	122.8 ± 16.6	140.2 ± 18.3	p = 0.02
DBP (mmHg)	68.7 ± 14.7	86.6 ± 15.5	p = 0.02
Total cholesterol (mg/dl)	219.5 ± 21.3	193.3 ± 35.3	ns
LDL-cholesterol (mg/dl)	129.1 ± 31.1	118.5 ± 31.2	ns
HDL-cholesterol (mg/dl)	52.5 ± 14.5	52.8 ± 11.2	ns
Tryglicerides (mg/dl)	142.5 ± 95.3	138.5 ± 68.1	ns
HOMA-R	2.7 ± 1.4	5.4 ± 3.3	p = 0.015
Glucose (mg/dl)	101.1 ± 11.4	107.2 ± 9.3	ns
Insulin (mU/L)	11.4 ± 5.5	19.4 ± 15.3	p = 0.045
ALT (UI/L)	74.1 ± 20.6	83.3 ± 50.1	ns
AST (UI/L)	43.8 ± 20.6	49.3 ± 31.1	p = 0.039
Adiponectin (ng/ml)	28.1 ± 15.3	14.7 ± 20.9	p = 0.011
Leptin (ng/ml)	27.9 ± 26.1	44.2 ± 36.7	p = 0.032
MEDAS (points)	10.2 ± 1.1	6.4 ± 2.5	p = 0.001

HOMA-IR: Homeostatic model assessment (glucose (mmol/L*insulin mU/L)/22.5). ALT (Alanine aminotransferase activity) and AST (aspartate aminotransferase activity).

blood pressure, diastolic pressure, insulin, HOMA-R, AST and leptin were higher in patients with liver inflammation. The 14-Item Mediterranean Diet Assessment Tool score was higher in patients without liver inflammation.

Table III shows differences among levels of biochemical and clinical parameters between patients with and without liver fibrosis. BMI, weight, systolic blood pressure, insulin, HOMA-IR, AST and leptin were higher in patients with liver fibrosis. The 14-Item Mediterranean Diet Assessment Tool score was higher in patients without liver fibrosis.

The 14-Item Mediterranean Diet Assessment Tool score correlated significantly to weight (r=-0.29,p=0.007), BMI (r=-0.306,p=0.005), HOMA-IR (r=-0.36,p=0.002), logALT (r=-0.20,p=0.01), log AST (r=-0.22,p=0.01), LDL levels (r=0.23,p=0.01) and HDL levels (r=0.24,p=0.01).

In the logistic regresion analysis with a dependent dichotomous variable (grades of steatosis; low grade vs high grade), the 14-Item Mediterranean Diet Assessment Tool score and HOMA-IR remained in the model, with an odds ratio to protect from high grade of steatosis of 0.42 (CI:95%: 0.26-0.70) with each 1 point of Mediterranean diet score and 2.01 (CI:95%: 1.08-3.71) to develop high grade of steatosis with each 1 unit of HOMA-R adjusted by age, sex and BMI.

In the second logistic regression analysis with a dependent dichotomous variable (liver inflammation; present vs absent), the 14-Item Mediterranean Diet Assessment Tool score remained in the model, with an odds ratio to protect from liver inflammation of 0.43 (CI:95%: 0.29-0.64) with each 1 point of Mediterranean diet score adjusted by age, sex and BMI.

In the third logistic regression analysis with a dependent dichotomous variable (of fibrosis; present vs absent), the HOMA-IR remained in the model, with an odds ratio to develop of 1.38 (CI:95%: 1.10-1.80) with each 1 unit of HOMA-R adjusted by age, sex and BMI.

Discussion

In this study, greater adherence to Mediterranean diet, as estimated by the 14-Item Mediterranean Diet Assessment Tool score, was significantly associated with less steatosis and steatohepatitis. The second finding of our study was that insulin resistance (HOMA-IR) is rela-

Table II
Clinical and epidemiological characteristics (ausence vs presence os steatohepatitis)

Characteristics	*Absence*	*Presence*	*P*
Age(years)	44.7 ± 10.2	42.3 ± 12.7	ns
Sex(female/male)	16/40	10/16	ns
BMI(kg/m^2)	31.5 ± 7.5	35.9 ± 10.7	p = 0.001
Weight (kg)	88.6 ± 18.5	100.6 ± 33.3	p = 0.01
Waist circumference (cm)	98.6 ± 11.5	100.9 ± 12.7	ns
Waist to hip ratio	0.94 ± 0.06	0.95 ± 0.07	ns
SBP (mmHg)	128.8 ± 19.6	141.2 ± 17.3	p = 0.008
DBP (mmHg)	75.9 ± 11.7	85.8 ± 12.5	p = 0.007
Total cholesterol (mg/dl)	209.5 ± 52.3	195.3 ± 35.3	ns
LDL-cholesterol (mg/dl)	129.1 ± 31.1	118.5 ± 31.2	ns
HDL-cholesterol (mg/dl)	51.3 ± 14.5	55.1 ± 11.2	ns
Tryglicerides (mg/dl)	140.8 ± 87.3	139.5 ± 71.1	ns
HOMA-R	3.7 ± 2.4	5.4 ± 3.2	p = 0.023
Glucose (mg/dl)	106.3 ± 8.4	110.2 ± 7.3	ns
Insulin (mU/L)	14.2 ± 7.5	18.8 ± 9.1	p = 0.047
ALT (UI/L)	74.5 ± 46.6	81.3 ± 45.1	ns
AST (UI/L)	45.1 ± 20.6	51.3 ± 31.1	p = 0.039
Adiponectin (ng/ml)	21.4 ± 22.3	18.9 ± 15.9	ns
Leptin (ng/ml)	31.1 ± 27.1	50.3 ± 39.7	p = 0.020
MEDAS (points)	9.4 ± 2.1	5.2 ± 1.7	p = 0.001

HOMA-IR: Homeostatic model assessment (glucose (mmol/L*insulin mU/L)/22.5). ALT (Alanine aminotransferase activity) and AST (aspartate aminotransferase activity).

ted to liver histology and may be indicative of grade of steatosis and presence of liver fibrosis. Moreover, higher adherence to the Mediterranean diet was associated with lower degree of insulin resistance which is a major pathogenic mechanism of NAFLD.

Mediterranean diet is a pattern characterized by high consumption of foods such as vegetables, fruits, non-refined cereals, legumes, moderate consumption of poultry and fish and low consumption of red meats and full fat dairies. And olive oil is the basic fat used during food preparation and consumption. The Mediterranean diet has been extensively investigated in terms of benefits in relation to reduction of cardiovascular risk[18] and improvement in insulin sensitivity[19], however, studies specifically examining its effect or relation on NAFLD are scarce.

In a recent study, there was no significant difference in the adherence to the Mediterranean diet between NAFLD patients and healthy controls[10]. However, greater adherence to the Mediterranean diet, as estimated by MedDietScore, was significantly associated with less steatosis as estimated by liver biopsy. A limitation of this study[10] is the low number of patients with liver biopsy (only 34 subjects of 58). The second limitation, it is the lack of the analysis of liver fibrosis or the steatosis grade. Our present study show a protective effect of adherence to Mediterranean Diet with some parameters of liver biopsy. According to logistic regression analysis, one unit increase in the 14-Item Mediterranean Diet Assessment Tool score was associated with 58% lower likelihood of having higher grade of steatosis and secondly, this score was associated (one unit) with 57% lower likelihood of having steatohepatitis. Recently, Ryan *et al*.[20] 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. In other study, Trovato *et al*.[12] has shown that adherence to Mediterranean diet is a significant predictor of changes in the fat content of the liver assessed by ultrasound in overweight patients with NAFLD. To the best of our knowledge, our study is the largest epidemiological study (n = 82) evaluating the impact of adherence to Mediterranean Diet on liver biopsies of patients with NAFLD.

The relation of insulin resistance with histological changes in the liver are well-known. In our design,

Table III
Clinical and epidemiological characteristics (absence and presence of liver fibrosis)

Characteristics	*No Fibrosis*	*Fibrosis*	*P*
Age(years)	41.7±9.8	47.0±12.0	ns
Sex(female/male)	18/24	8/32	ns
BMI(kg/m^2)	30.4±5.2	35.4±10.1	p=0.001
Weight (kg)	88.6±16.7	96.1±30.9	p=0.01
Waist circumference (cm)	97.4±8.5	101.0±14.7	ns
Waist to hip ratio	0.94±0.06	0.94±0.08	ns
SBP (mmHg)	127.1±19.6	138.0±17.1	p=0.001
DBP (mmHg)	76.4±18.1	81.6±17.1	ns
Total cholesterol (mg/dl)	212.5±48.3	197.3±50.3	ns
LDL-cholesterol (mg/dl)	129.4±30.1	127.8±45.2	ns
HDL-cholesterol (mg/dl)	52.4±15.5	52.5±10.2	ns
Tryglicerides (mg/dl)	131.8±68.3	149.5±93.1	ns
HOMA-R	3.0±1.3	5.4±3.5	p=0.001
Glucose (mg/dl)	102.5±11.4	108.9±7.2	ns
Insulin (mU/L)	12.6±5.8	18.7±11.2	p=0.007
ALT (UI/L)	72.9±35.6	86.2±55.1	ns
AST (UI/L)	43.2±23.6	50.6±20.1	p=0.041
Adiponectin (ng/ml)	19.4±19.3	21.4±17.9	ns
Leptin (ng/ml)	26.9±25.1	46.3±36.7	p=0.010
MEDAS (points)	9.6±2.2	6.5±2.5	p=0.001

HOMA-IR: Homeostatic model assessment (glucose (mmol/L*insulin mU/L)/22.5). ALT (Alanine aminotransferase activity) and AST (aspartate aminotransferase activity).

insulin resistance was measured by the homeostasis model assessment method; this method correlates closely with other tests, such as the euglycemic glucose clamp[21]. Some authors[22] have demonstrated a closely correlation between insulin resistance (HOMA-R) and NAFLD. Also, other authors have detected this relation using the clamp technique[23] with results supporting our conclusions. The nature of the connection between insulin resistance and hepatic steatosis or fibrosis could be explain by the "two hit" model. In obese patients, the primary abnormality may be genetically induced insulin resistance (first hit), with a secondary increase of serum triglyceride levels due to enhance of peripheral lipolysis. The resulting hepatic supply of fatty acids and insulin may increase triglyceride deposition in the liver[24] and this fatty acid deposition increases substrates for oxidative stress and adipokines (second hit). The role of adipokines on liver histology of these morbid obese patients remains unclear. Severe fibrosis has been related to higher serum leptin[25]. Low levels of adiponectin have been also correlated with increased liver steatosis[26,27]; however, others[25] did not find any similar correlation. In our logistic model, the univariant relationship of these adipokines with liver damage disappeared. Our logistic analysis are controlled by Mediterranean Diet adherence, and this is a strength of our results. In previous studies dietary saturated fat intake correlated negatively with circulating adiponectin, whereas omega-3 fatty acids were positively associated with this adipokine[28]. Perhaps, the relationship between insulin resistance and adipokines with liver damage in patients with NAFLD, can be modulated by the different nutrients that are part of the Mediterranean Diet as suggesting our results. With a predominant role of HOMA-R in the liver fibrosis, in which the role of adherence to the Mediterranean diet seems secondary.

A limitation of our study was the cross-sectional design, our study cannot establish causal relations but may only generate hypotheses for associations. Further interventional studies are needed (randomized clinical trial) to confirm the role of Mediterranean Diet in the severity of NAFLD or other type of diets[29-31]. Another limitation is the small sample size used in the study, a possible type 2 error cannot be excluded.

In conclusion, greater adherence to the Mediterranean diet was associated with lower likelihood of high grade of steatosis and presence of steatohepatitis in NAFLD patients. Insulin resistance determined

with HOMA model is associated with high grade of steatosis and liver fibrosis with a relationship with adherence to Mediterranean Diet. Further studies in this topic area[31-34] are needed to elucidate new therapeutic approaches.

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