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RESEARCH ARTICLE

The impact of the Mediterranean diet on liver steatosis and fibrosis in patients with type 2 diabetes and metabolic dysfunction-associated steatotic liver disease

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Objective: The study investigated the association between markers of liver steatosis and fibrosis and the adherence to the Mediterranean dietary pattern, evaluated by a diet-quality score, in patients with type 2 diabetes (T2DM) and metabolic dysfunction-associated steatotic liver disease (MASLD).

Methods: Patients with T2DM and MASLD underwent a comprehensive medical evaluation, which included lifestyle, clinical, laboratory, and liver ultrasound assessment. The natural consumption of foods specific to the Mediterranean Diet (MedDiet) was investigated by a previously validated 14-item questionnaire (MedDiet Score). For the estimation of liver steatosis, the Index of NASH (Non-alcoholic steatohepatitis) (ION) was calculated by sex-specific formulas, while liver fibrosis was estimated by the Fibrosis-4 (FIB-4) score.

Results: Data from 271 patients were analyzed. The mean MedDiet Score was 4.55±1.59 points, and most patients scored 3 points (19.93%), 4 points (28.78%), and 5 points (21.40%). Patients with a MedDiet Score ≥5 points had lower fasting blood glucose, ferritin, C-peptide and HOMA-IR, and lower ION values (19.96 ±14.63 vs. 23.50±14.77; p=0.025). No significant differences were noted for FIB-4 values. MedDiet Score was negatively correlated with fasting blood glucose, ferritin, C-peptide, HOMA-IR, and ION values (r=-0.14 [-0.25; -0.01]; p=0.026), and positively with LDL cholesterol levels. Drinking less than one portion of sweet or carbonated beverages daily and eating at least 3 portions of nuts weekly was associated with lower ION values.

Conclusions: Low MedDiet Score was associated with markers of hepatic steatosis (but not fibrosis), worse insulin resistance, higher fasting hyperglycemia, and serum ferritin levels in patients with T2DM and MASLD.

Keywords: Mediterranean diet, metabolic dysfunction-associated steatotic liver disease, type 2 diabetes

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a chronic liver disease with an increasing prevalence worldwide, which is forecasted to exceed half of adult population by 2040 [1]. MASLD is characterized by the accumulation of excessive fat in the liver (not induced by secondary causes, including excessive alcohol intake) in association with at least one cardio-metabolic risk factor, and comprises a spectrum of liver conditions (from simple steatosis to steatohepatitis without/with various degrees of fibrosis, and hepatocellular carcinoma) [2, 3].

Early identification and intervention to halt disease progression (or even reverse it) would be of utmost importance for liver and overall health [4]. In fact, lifestyle interventions (i.e. healthy dietary patterns and physical activity) that promote weight reduction have been recommended as the cornerstone of MASLD management to improve hepatic health, mainly in the context of cardio-metabolic risk factors [3-6]. A body weight loss of at least 5% (obtained through lifestyle interventions) was shown to be associated with significant reduction of liver steatosis, while ≥ 10% was associated with improvement of liver fibrosis [7].

Currently, the European guidelines recommend the Mediterranean diet (MedDiet) as the preferred dietary intervention for subjects with MASLD [7]. Several studies have shown that MedDiet reduces the hepatic fat content or markers of steatosis, and improves insulin sensitivity [8, 9]. There is less evidence for the impact of MedDiet on hepatic fibrosis [4]. Still, a few observational studies reported that adherence to MedDiet was associated with lower risk of liver fibrosis and even improvement of fibrosis markers (mainly in association with physical exercise) [10-13].

The MedDiet generally describes a dietary pattern that characterizes traditional eating habits of individuals from the Mediterranean area, i.e. South Europe, North Africa and some Middle Eastern countries [14]. The key elements of the MedDiet include high intake of (extra virgin) olive oil, vegetables and legumes, fruits, cereals (mostly who-

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legrain), nuts and seeds, moderate intake of fish, seafood and poultry, dairy products, and red wine, and low intake of eggs, red meat and sweets [14, 15]. Several components of the MedDiet have been shown to have lipid lowering, anti-steatotic, anti-oxidative and anti-inflammatory effects (extensively reviewed by Sualeheen A and colleagues) [16]. The beneficial effects of the MedDiet come mainly from the monounsaturated fatty acids (MUFAs) (found in olive oil in large amounts), which inhibit lipogenesis and increase lipid oxidation, thus decreasing the accumulation of lipids (triglycerides) in the liver and contributing to the reduction of hepatic steatosis [17]. Additionally, polyphenolic compounds (predominantly found in fruits, vegetables, and legumes, but also in olive oil and wholegrains), modulate lipid metabolism and have anti-oxidative and anti-inflammatory effects [18, 19]. Furthermore, the omega-3 polyunsaturated fatty acids (PUFAs) from fish/fish oil suppress de novo lipogenesis, promote triglyceride metabolism and attenuate inflammation [20].

Several studies have demonstrated that MedDiet significantly improves insulin resistance and liver fat content, independent of other factors [21-24]. However, most studies were performed in Mediterranean (mostly European) countries, although data from non-Mediterranean, Western regions are emerging [16, 25].

The aim of this work was to investigate the association between the natural consumption of foods specific to the Mediterranean dietary pattern and markers of liver steatosis and fibrosis in patients with T2DM and MASLD from a non-Mediterranean country. Secondarily, we have explored which dietary components had a significant influence on liver health.

Methods

Study population

Patients with T2DM and MASLD were enrolled in this study from July 2022 until July 2023. The study was approved by the Ethics Committees of the Emergency County Clinical Hospital of Târgu Mureș (nr. 8120/05.04.2022), County Clinical Hospital of Târgu Mureș (nr. 4873/24.05.2022), and George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș (nr. 1806/22.06.2022). All patients signed a written informed consent at study entry.

Details regarding the inclusion and exclusion criteria, as well as other aspects of the materials and methods used have been published before [25]. Briefly, the inclusion criteria were: adult (30 years or older) patients with T2DM and NAFLD (based on medical history and liver ultrasound). At study entry (in July 2022) the Non-alcoholic fatty liver disease (NAFLD) definition (hepatic steatosis/steatohepatitis in the absence of other secondary causes of liver disease) was used as an inclusion criterion. However, in June 2023, there was a change in terminology and definition of steatotic liver disease [26]. MASLD was the new widely adopted term that we have used thereafter to characterize this study population, since all patients fulfilled the MASLD definition (liver steatosis associated with at least one additional cardio-metabolic risk factor, i.e. T2DM). Exclusion criteria were other types of diabetes, other chronic liver diseases, malignant diseases in the last 5 years, severe autoimmune diseases, severe valvulopathy, and significant pericardial collections [25].

Clinical assessment

Demographic and medical data were collected, which included medical history, vital signs, anthropometric parameters, as well as information about patients' lifestyle (obtained through several questionnaires). Blood pressure measurement and the anthropometric evaluation (which included height, weight, waist and hip circumferences) were done by standard methods. The body mass index (BMI) was calculated with the formula: BMI=weight/height^2 (kg/m^2). Additional anthropometric parameters were collected by using an InnerScan BC-545N segmental body composition monitor (Tanita, Japan).

Liver steatosis was assessed by abdominal ultrasonography (Hitachi Arietta v70 system; Hitachi Ldt., Japan), through the subjective assessment of several ultrasonographic (US) parameters: the brightness of the liver (liver-kidney contrast), the appearance of liver parenchyma, intrahepatic vessels, and the diaphragm [27].

Dietary assessment

To evaluate the food and alcohol intake, several questionnaires were used. Here we report data resulting from the evaluation of the natural consumption of foods specific to the MedDiet assessed by a previously validated diet-quality score (MedDiet Score) [28]. The survey is based on 14 questions, which evaluate several dietary characteristics of a traditional Mediterranean diet. Two questions (Q1 and Q13) are qualitative (yes/no) and are related to food intake habits, while the other 12 items are quantitative and assess food consumption frequency [28]. Each item was scored 0 or 1 point, as described previously [28]. A maximum of 14 points could be obtained finally, a higher score indicating a better adherence to the Mediterranean dietary pattern. The patients filled out the questionnaire and a member of the team verified that all questions had a response.

Laboratory assessment

Blood samples were collected in fasting conditions on the first visit, and serum aliquots were stored at –80°C for further analysis of several parameters: fasting blood glucose, glycated hemoglobin (HbA1c), lipid panel (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides), C-peptide, uric acid, liver panel (albumin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), direct bilirubin, gamma-glutamyl transpeptidase (GGT)), ferritin, haptoglobin, creatinine, sex hormone binding globulin (SHBG). A Cobas Integra 400plus system (Roche

Diagnostic; Mannheim, Germany) was used for the analysis of the biochemical parameters, which was performed by an immunoturbidimetric method (for albumin, HbA1c and haptoglobin) and a spectrophotometric method (for the rest of the parameters, except for C-peptide, SHBG and ferritin). The latter three parameters were analyzed by a solid phase, two-site chemiluminescent immunometric assay (Immulite 2000 XPI system; Siemens Healthcare Diagnostics, Erlangen, Germany). In addition, blood for the complete blood count (CBC) was drawn and analyzed shortly afterwards on an automated hematology equipment (Mindray BC6200, India).

The Homeostatic Model Assessment (HOMA) for Beta-Cell Function (HOMA-B) and for Insulin Resistance (HOMA-IR) were calculated by the HOMA calculator version 2.2.3 [29]. The Neutrophil-to-Lymphocyte Ratio (NLR), Systemic Immune-Inflammation Index (SIII) (platelet count x neutrophil count/lymphocyte count) were calculated as markers of inflammation [30].

Estimation of liver steatosis and fibrosis

For the estimation of liver steatosis, the Index of NASH (Non-alcoholic steatohepatitis) (ION) was calculated by sex-specific formulas: ION = 0.02 x triglycerides (mg/dl) + 0.24 x ALAT (U/l) + 9.61 x HOMA-IR – 13.99 (for women) and ION = 1.33 x waist-to-hip ratio + 0.03 x triglycerides (mg/dl) + 0.18 x ALAT (U/l) + 8.53 x HOMA-IR – 13.93 (for men) (a score >22 is indicative of steatosis, and >50 of NASH) [31]. For the estimation of liver fibrosis, the Fibrosis-4 (FIB-4) score was calculated by using the formula: FIB-4 = age (years) x ASAT (U/l) / [platelet (10^9/l) x ALAT1/2 (U/l)] (a score >2.67 rules in advanced fibrosis (F≥2), a score <1.3 (<2 in subjects ≥65 years old) rules out advanced fibrosis, while in-between values are indeterminate) [32].

Statistical analysis

Data are presented as mean ± standard deviation (for variables with Gaussian distribution) or median (min-max) (for non-parametric variables). Normality of data was verified with the Kolmogorov-Smirnov test. The Fisher's exact test was used to analyze the categorical variables, while the comparisons of continuous variables were performed by using the unpaired t test (for normally distributed data) or Mann-Whitney unpaired test (for non-parametric data). The correlation between two variables of interest was analyzed with Pearson's test (if both sets of variables were normally distributed) or Spearman's test (if one or both sets of variables were not normally distributed). GraphPad InStat3 software was used for statistical analysis, and the statistical significance was set at $p<0.05$.

Results

A total of 278 T2DM patients with MASLD were enrolled in this study, of which seven met the exclusion criteria. Thus, data from 271 patients were analyzed, with a mean age of 65.0±8.4 years and a mean BMI of 34.1±5.3 kg/m^2.

Adherence to the Mediterranean dietary pattern

The mean MedDiet Score was 4.55±1.59 [4.0 (1.0-10.0)] points, which overall indicates a reduced intake of foods specific to the MedDiet. Furthermore, most patients scored 3 points (19.93%), 4 points (28.78%), and 5 points (21.40%), which indicate a low adherence to the MedDiet, and only one patient reached a score ≥ 10 points, which indicates a high adherence to the Mediterranean dietary pattern (high intake of foods specific to the MedDiet) (Figure 1).

Patients scored higher for questions 7, 11 and 13 (referring to the intake of sweet or carbonated beverages, sweets and pastries, and preferential use of white over red meat, hamburger or sausage, respectively), and lowest for questions 2, 8 and 10 (referring to the use of olive oil, wine and fish/shellfish, respectively) (Figure 2).

Given the scores obtained in our study population, we have divided it into two groups, delimited by the median MedDiet Score (4.0 points) (median-split). We have considered values ≥ 5 points as indicating moderate-high ad-

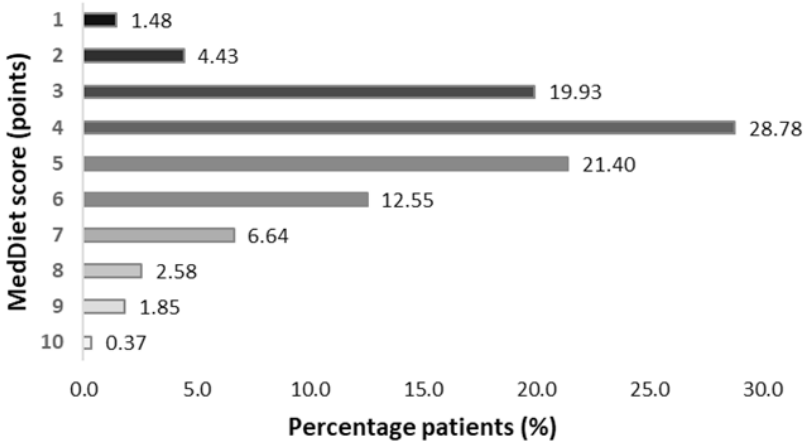


Fig. 1. Frequency of MedDiet Scores obtained in the study population (each horizontal bar represents a specific MedDiet Score, ranging from 1 to 10 points, and indicates the percentage of patients achieving that score)

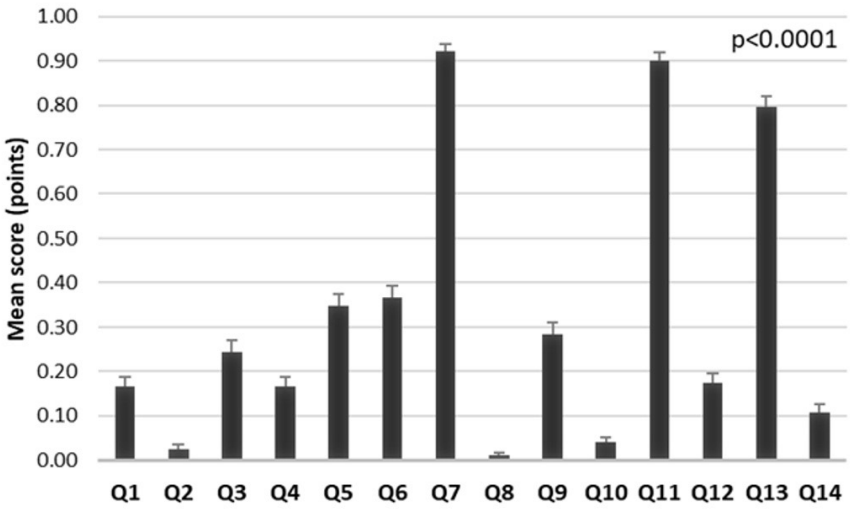


Fig. 2. Average scores obtained for each individual question (data are means ± standard error of means (SEM))

herence, and values ≤ 4 points as indicating low adherence to the Mediterranean dietary pattern. There were no significant differences between the two groups with regards to age (65.98±7.48 [67.0 (45.0; 82.0) years vs. 64.13±8.96 [65.0 (36.0; 82.0)] years; p=0.1312), diabetes duration (9.70±4.47 [10.0 (0.0; 23.0)] years vs. 9.67±5.33 [9.5 (0.5; 33.0)] years; p=0.5232) or sex (56.9% females/43.1% males vs. 53.4% females/46.6% males; p=0.6241). Patients in the moderate-high MedDiet Score group had lower fasting blood glucose, ferritin, C-peptide and HOMA-IR values (Table 1). No other significant differences were noted for the rest of the laboratory parameters analyzed, inflammatory indexes, blood pressure values or anthropometric measurements (Table 1).

Patients in the moderate-high MedDiet Score group scored higher in questions 1 and 14 (indicating higher olive oil intake), 3 and 9 (indicating higher intake of vegetables and legumes), 5 and 13 (indicating lower intake of red meat and meat products), 6 (indicating lower intake of butter, margarine or cream) and 12 (indicating higher intake of nuts) compared with low MedDiet Score group (Figure 3).

The mean ION values (which indicate hepatic steatosis/steatohepatitis) were significantly lower in the moderate-high MedDiet Score group (19.96 ±14.63 [16.140 (-5.09; 69.08)] vs. 23.50±14.77 [21.05 (-5.29; 75.59)]; p=0.025) (Figure 4). In turn, there were no significant differences between mean FIB-4 values of the two groups (1.69±1.03 [1.365 (0.58; 8.79)] vs. 1.67±1.56 [1.34 (0.40; 17.30)]; p=0.3759).

Association between MedDiet Score and clinical and laboratory parameters

The MedDiet Score was further analyzed in correlation with clinical and laboratory parameters, ION and FIB-4. MedDiet Score was negatively correlated with fasting blood glucose, ferritin, C-peptide, HOMA-IR and ION values, and positively with LDL cholesterol levels (Table 2). In turn, no significant correlation was found for FIB-4 (r=-0.019 [95%CI: -0.14; 0.10]; p=0.7538) or for the rest of the laboratory, clinical or anthropometric parameters.

To evaluate which of the MedDiet components was associated with liver steatosis, the ION was analyzed in correlation with each component (question) of the MedDiet Score, both as individual question scores and raw data (quantities). Drinking more sweet or carbonated beverages per day was associated with higher ION values (drinking less than one portion per day was associated with lower ION values). In addition, eating at least 3 portions (≥ 90 grams) of nuts per week was associated with lower ION values (Table 3).

Discussions

The dietary pattern analysis in this study demonstrated an inverse association between higher consumption of foods specific to the MedDiet and markers of liver steatosis and insulin resistance (but not with liver fibrosis) in a group of T2DM subjects with MASLD from a non-Mediterranean country.

The overall adherence to the Mediterranean dietary pattern in our study population was low, with a mean score of 4.55 out of maximum 14 points, and more than three quarters of subjects scoring ≤ 5 points. This was not an unexpected result, given the different traditional dietary pattern followed by the inhabitants of this geographical area, which is characterized by high intake of meat (mainly pork, poultry and beef), meat-based products, and fats [33-35]. Indeed, a lower intake of olive oil and fish/shellfish was noted in the study population. By using different scoring systems to evaluate the adherence to the MedDiet, other studies from Central/Northern European countries have reported relatively similar results [36, 37]. The mean pyramid-based MedDiet score was 9.07 and 8.45, respectively, out of maximum 15 points in two independent population-based cohorts of middle-aged healthy adults from East England, UK and Lausanne, Switzerland [36]. The

Table 1. Clinical and laboratory characteristics of study groups, according to the MedDiet Score

	Moderate-High MedDiet Score (n=123)	Low MedDiet Score (n=148)	p
MedDiet Score (points)	5.94±1.16 6.0 (5.0-10.0)	3.39±0.75 4.0 (1.0-4.0)	<0.0001
Clinical parameters			
BMI (kg/m^2)	33.73 (24.25; 55.17)	33.99±4.82	0.9206
Waist circumference (cm)			
Females	108.84±12.49	109.38±11.04	0.7766
Males	114.97±10.84	114.66±10.99	0.8764
Hip circumference (cm)			
Females	110.47±11.37	109.93±9.63	0.7567
Males	107.5 (95.2; 153.0)	106.2 (88.9; 134.6)	0.8242
%Body fat			
Females	41.59±5.58	41.44±4.77	0.8613
Males	31.04±4.75	30.58±5.09	0.6080
Systolic blood pressure (mm Hg)	137.5 (95.0; 190.0)	134.59±14.937	0.7579
Diastolic blood pressure (mmHg)	80.0 (51.0; 102.5)	81.5 (57.5; 107.5)	0.1216
Laboratory parameters and indexes			
Fasting blood glucose (mg/dl)	133.35 (91.85; 320.11)	142.30 (87.34; 326.20)	0.0083
HbA1c (%)	6.7 (4.6; 10.0)	6.9 (5.6; 10.2)	0.0565
Total cholesterol (mg/dl)	155.41 (90.04; 376.17)	152.97 (96.75; 301.66)	0.2274
HDL cholesterol (mg/dl)	44.34 (23.12; 79.18)	43.58 (22.48; 75.75)	0.2581
LDL cholesterol (mg/dl)	86.10 (36.57; 270.60)	78.23 (31.20; 221.68)	0.1025
Triglycerides (mg/dl)	140.86 (62.37; 573.36)	159.12 (65.36; 609.08)	0.0517
Uric acid (mg/dl)	5.92±1.45	5.86±1.47	0.7070
C-peptide (ng/ml)	2.91 (0.28; 8.36)	3.29 (0.37; 10.50)	0.0325
HOMA-IR	2.39 (0.45; 7.58)	2.78 (0.52; 8.40)	0.0214
HOMA-B	82.96±34.19	78.45 (16.00; 240.20)	0.5542
Albumin (g/dl)	4.63±0.21	4.62 (4.17; 5.31)	0.4107
ALAT (U/l)	17.01 (4.18; 80.94)	18.82 (2.32; 92.79)	0.2755
ASAT (U/l)	21.09 (9.75; 130.85)	19.78 (10.03; 78.41)	0.9237
GGT (U/l)	28.820 (4.97; 313.66)	29.005 (4.02; 338.18)	0.4592
Ferritin (ng/ml)	80.40 (8.79; 561.00)	112.00 (6.41; 811.00)	0.0396
Direct bilirubin (mg/dl)	0.19 (0.07; 0.54)	0.20 (0.08; 0.90)	0.3263
Creatinine (mg/dl)	0.84 (0.46; 1.98)	0.82 (1.70; 0.54)	0.7174
Haptoglobin (g/l)	1.67±0.63	1.66 (0.50; 3.47)	0.9944
SHBG (nmol/l)	35.70 (11.20; 118.00)	32.00 (7.62; 100.00)	0.1460
Neutrophil-to-Lymphocyte Ratio	1.96 (1.04; 8.68)	1.94 (0.65; 6.49)	0.2927
SIII	458.45 (4.56; 1996.5)	445.59 (27.04; 2128.7)	0.4232

No=number; BMI=body mass index, HbA1c=glycated hemoglobin; HDL=high-density lipoprotein; LDL=low-density lipoprotein; HOMA-IR=Homeostatic Model Assessment for Insulin Resistance; HOMA-B=Homeostatic Model Assessment for Beta-Cell Function; ASAT=aspartate aminotransferase; ALAT=alanine aminotransferase; GGT=gamma glutamyl transpeptidase; SHBG=sex hormone-binding globulin; SIII=Systemic Immune-Inflammation Index.

study reported an inverse association between the MedDiet scores and prevalence of US-based hepatic steatosis [36]. Similarly, the mean MedDiet score was 8.9 out of 18 points in a group of individuals without diabetes from Germany, with a slightly lower value (8.4) in subjects with NAFLD [37]. There was a negative correlation between the

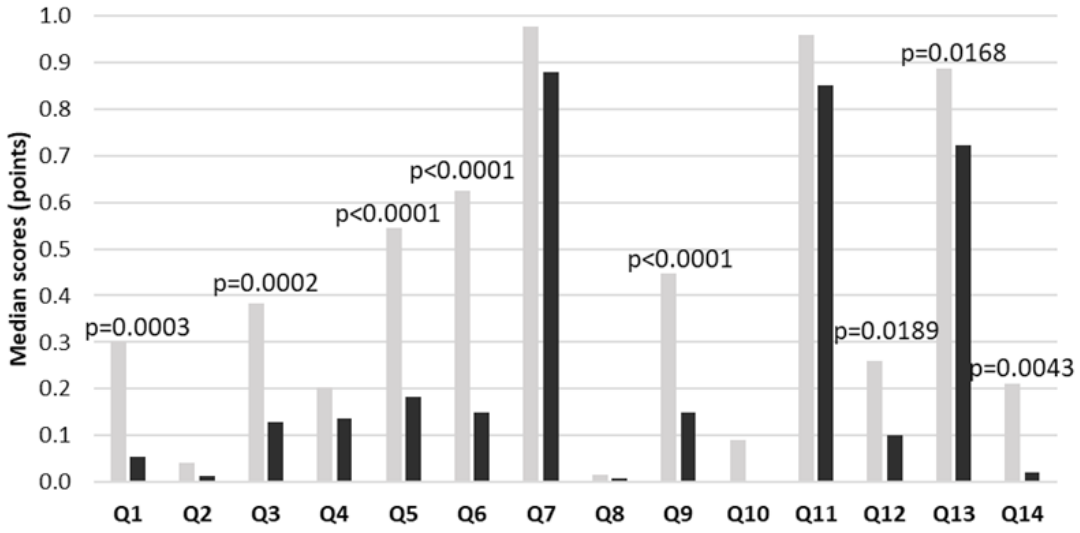


Fig. 3. Average scores for each individual question in the two median-split MedDiet Score subgroups (dark bars represent the low MedDiet Score group, gray bars represent the moderate-high MedDiet Score group)

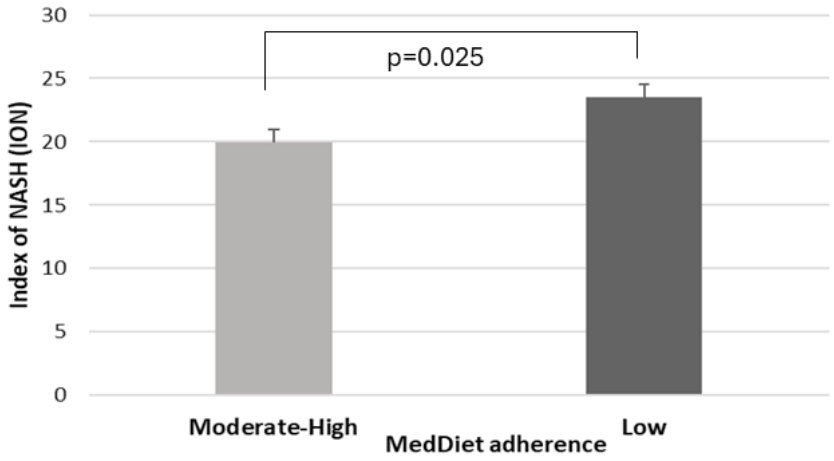


Fig. 4. The Index of NASH (ION) scores in the two median-split MedDiet Score study subgroups

Table 2. Parameters and indexes significantly associated with the MedDiet Score

	r [95%CI]	p value
Fasting blood glucose	-0.15 [-0.26; -0.02]	0.0155
LDL cholesterol	0.13 [0.003; 0.24]	0.0393
Ferritin	-0.13 [-0.24; -0.003]	0.0391
C-peptide	-0.13 [-0.25; -0.01]	0.0334
HOMA-IR	-0.14 [-0.26; -0.02]	0.0202
ION	-0.14 [-0.25; -0.01]	0.0260

Legend. CI: confidence interval; ION: Index of NASH.

Table 3. Correlation between ION values and individual question score or quantity

Question	Individual scores r [95%CI]	Quantity r [95%CI]
Q1	-0.05 [-0.17; 0.07]	N/A
Q2	-0.07 [-0.19; 0.06]	0.001 [-0.12; 0.12]
Q3	0.002 [-0.12; 0.13]	-0.06 [-0.18; 0.06]
Q4	-0.04 [-0.16; 0.08]	-0.09 (-0.21; 0.04]
Q5	-0.05 [-0.17; 0.07]	0.01 [-0.11; 0.13]
Q6	-0.12 [-0.23; 0.01]	0.11 [-0.02; 0.23]
Q7	-0.15 [-0.27; -0.03]*	0.18 [0.05; 0.29]**
Q8	-0.01 [-0.13; 0.11]	-0.01 [-0.13; 0.12]
Q9	0.04 [-0.08; 0.17]	0.07 [-0.06; 0.19]
Q10	-0.04 [-0.17; 0.08]	-0.001 [-0.12; 0.12]
Q11	0.03 [-0.09; 0.15]	-0.01 [-0.22; 0.03]
Q12	-0.14 [-0.26; -0.02]*	-0.10 [-0.22; 0.02]
Q13	-0.11 [-0.23; 0.01]	N/A
Q14	0.11 [-0.02; 0.23]	0.06 [-0.07; 0.18]

*p value<0.05; **p value<0.01

MedDiet score and magnetic resonance-assessed % liver fat content (β coefficient = –0.195, p=0.012) [37]. George ES and colleagues reporting results from the 10-year follow-up of the ATTICA Study, carried out in the greater metropolitan area of Athens, Greece, showed that subjects with non-invasive markers of hepatic steatosis had lower mean MedDiet scores (21.8 out of maximum 55 points) [38].

In line with these and other results, our data showed that patients with a higher consumption of foods specific to the MedDiet, even in the context of a different traditional dietary pattern, had lower ION values, indicating a potentially favorable effect of MedDiet on hepatic steatosis in this high-risk group. Furthermore, consistent with a growing body of research highlighting the benefits of the MedDiet on metabolic markers, the results of our study demonstrated that better adherence to MedDiet was associated with lower fasting blood glucose, C-peptide and HOMA-IR values, indicating higher insulin sensitivity (even if body adiposity was similar to the low-adherence to MedDiet group), as well as lower serum ferritin levels. The 6-week dietary intervention study by Ryan MC et al. has demonstrated in a small group of subjects with NAFLD without diabetes that MedDiet significantly improved the hepatic steatosis evaluated by localized magnetic resonance 1H spectroscopy (39±4% versus 7±3% in the control diet, in the context of a similar weight loss) [38]. In addition, this study showed a good positive correlation between intrahepatic lipid content and baseline HOMA-IR (r=0.6, p=0.006) [39]. In fact, the decrease in lipid fat content was accompanied by a significant improvement in insulin sensitivity (HOMA-IR) and a reduction in insulin levels [39]. These results are congruent with our data suggesting better insulin sensitivity in subjects that have a better adherence to MedDiet. The metabolic and hepatic benefits are attributed to the MedDiet composition, which is rich in unsaturated fatty acids (mainly from olive oil, nuts and seeds) that indirectly may improve insulin resistance via their anti-inflammatory effects, and in bioactive compounds such as plant-based polyphenols (e.g. oleuropein and its derivative secoiridoids), that regulate lipid metabolism and ameliorate insulin resistance [40].

An interesting finding of our study was that subjects with moderate-high intake of foods specific to the MedDiet had lower ferritin levels. This might indicate a milder inflammatory status, although other inflammatory indexes were similar between the two study groups. In fact, Beaton MD et al. has previously shown that serum ferritin was related to liver iron storage and not inflammation in steatotic liver disease [41]. Furthermore, it has been suggested that MedDiet is a low-iron-available diet, with higher proportion of iron absorption inhibitors (such as polyphenols, phytates, and dairy products) versus enhancers, and may be associated with lower body iron stores on long-term

in individuals that follow this dietary pattern [42]. Other possible mechanisms might be involved, which warrant further investigation.

While the association of MedDiet with improved liver steatosis is strongly supported by the literature, data related to its effect on liver fibrosis is not as consistent so far [10, 39, 43, 44]. The meta-analysis by Haigh L et al. reported that MedDiet interventions (n=5 studies; 271 subjects) decreased liver stiffness measurement (LSM), although with high heterogeneity (standardized mean difference=-0.75; I2=87%, p=0.05) [43]. Perez-Diaz-Del-Campo N et al. has indicated that lower adherence to the MedDiet was independently associated with higher risk of significant liver fibrosis (LSM ≥ 7.1 kPa) [10]. In contrast, we did not find an association between the adherence to the MedDiet and FIB-4. Similarly, the study by Baratta F et al. did not indicate a significant difference in another liver fibrosis biomarker (Aspartate aminotransferase-to-Platelet Ratio Index) between various MedDiet adherence categories (p=0.900) [44]. These differences might be due to variations in methodology used to assess liver outcomes (and dietary adherence), but also possibly in population characteristics.

Further investigation of dietary components in our study revealed that a higher intake of sweetened and carbonated beverages was associated with higher ION values, while the opposite was seen with the consumption of at least three portions of nuts per week. Indeed, sugar-sweetened beverages (SSB) have been shown to increase liver fat and the risk of incident MASLD [45]. In fact, SSB appear to be key drivers of MASLD, through enhanced de novo lipogenesis (which worsens hepatic lipid accumulation), inflammation, oxidative stress, etc. [6, 46]. Therefore, healthcare providers might consider dietary counseling that emphasizes the benefits of reducing sweetened beverages intake and incorporating at least three portions of nuts per week. Of course, while the findings of this study underscore the potential benefits of incorporating MedDiet principles into the dietary recommendations for patients with T2DM and MASLD, future research is needed to validate the associations observed in this study, as well as to clarify the underlying mechanistic links.

Although many investigations report consistent improvements in metabolic and liver health with greater adherence to the MedDiet, the magnitude of these benefits might be influenced by heterogeneity in patient demographics and their clinical profiles. Nevertheless, even in our high-risk population from a non-Mediterranean country with different traditional dietary habits, incorporating elements of the MedDiet had proven significant benefits.

Our study has several limitations. The cross-sectional design of the study precluded a better understanding of the causal relationships between higher consumption of foods specific to the Mediterranean dietary pattern and the observed metabolic and hepatic outcomes. Additionally, the observational nature of the study did not allow us to address all confounding factors that may influence the outcomes. Moreover, we could not employ the gold-standard method to define hepatic steatosis or fibrosis (i.e. liver biopsy), but instead we have used well accepted non-invasive indexes. Finally, as the data obtained in the MedDiet questionnaire were self-reported, there is a reasonable possibility of less accurate responses to some degree, therefore interpretation of findings should be done with care.

Conclusion

Low intake of foods specific to the Mediterranean dietary pattern was associated with markers of hepatic steatosis (but not fibrosis), worse insulin resistance, higher fasting hyperglycemia, and serum ferritin levels in T2DM patients with MASLD. A higher intake of sweet and carbonated beverages correlated positively with ION values.

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Authors' contributions

SC (Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing – original draft, review & editing)
DO (Investigation; Validation; Writing – review & editing)
ALR (Investigation; Validation; Writing – original draft, review & editing)

Conflict of interest

The authors declare no conflicts of interest related to this paper.

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