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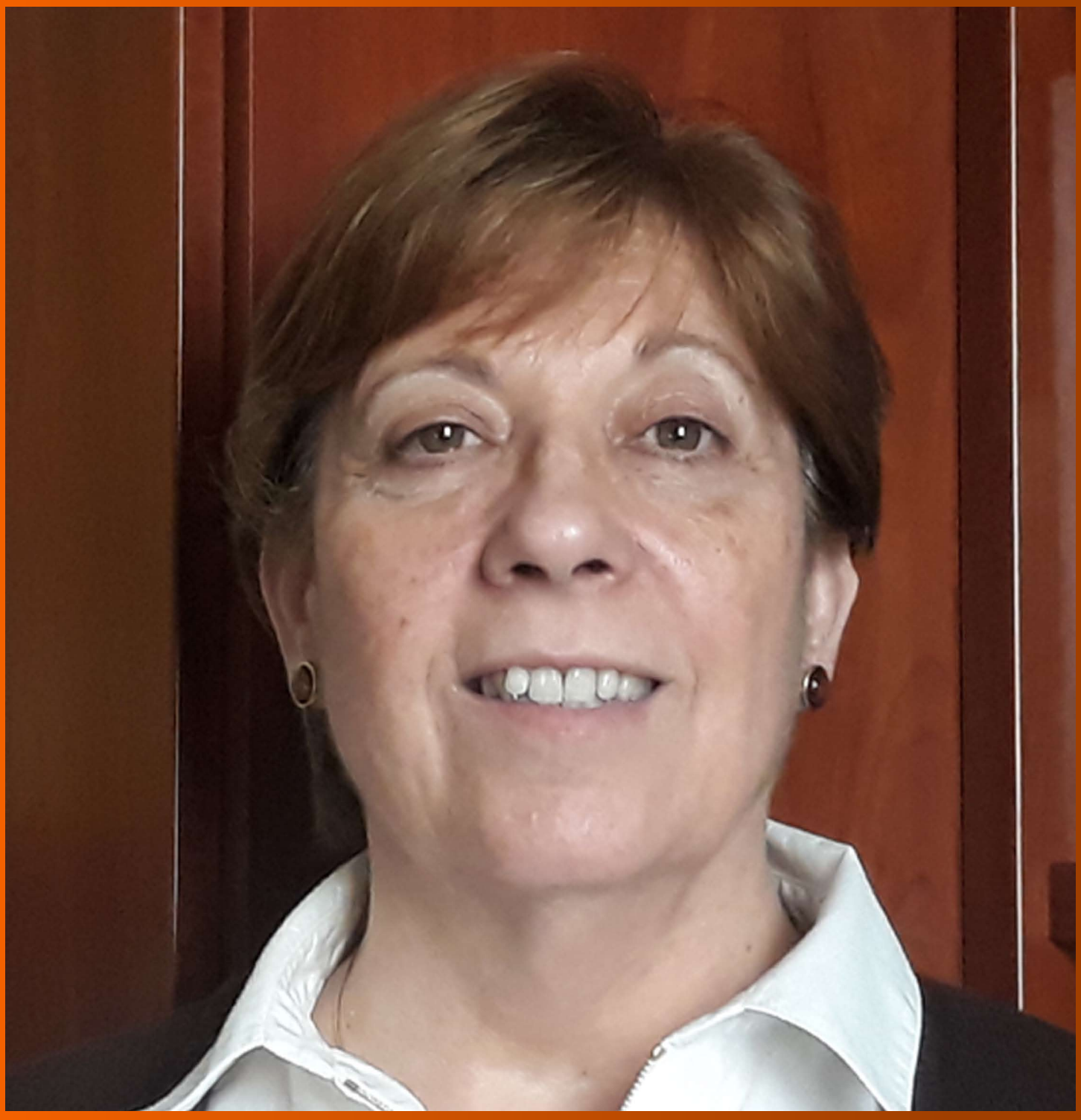

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MINIREVIEWS

Mediterranean diet and nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is emerging as the most common chronic liver disease, and is characterized by a wide spectrum of fat-liver disorders that can result in severe liver disease and cirrhosis. Inflammation and oxidative stress are the major risk factors involved in the pathogenesis of NAFLD. Currently, there is no consensus concerning the pharmacological treatment of NAFLD. However, lifestyle interventions based on exercise and a balanced diet for quality and quantity, are considered the cornerstone of NAFLD management. Mediterranean diet (MD), rich in polyunsaturated fats, polyphenols, vitamins and carotenoids, with their anti-inflammatory and anti-oxidant effects, has been suggested to be effective in preventing cardiovascular risk factors. In adults, MD has also been demonstrated to be efficacious in reducing the risk of metabolic syndrome. However, few studies are available on the effects of the MD in both adult and pediatric subjects with NAFLD. Thus, the aims of the present narrative review are to analyze the current clinical evidence on the impact of MD in patients with NAFLD, and to summarize the main mechanisms of action of MD components on this condition.

Key words: Mediterranean diet; Children; Nonalcoholic fatty liver disease; Adults

Core tip: Lifestyle interventions based on exercise and a balanced diet, are considered the cornerstone of nonalcoholic fatty liver disease (NAFLD) management. The Mediterranean diet (MD), low in saturated fats and animal protein, high in antioxidants and fibers, and with an adequate omega-3 to omega-6 fatty balance, has been suggested to be effective in NAFLD. Although the results from the available studies are encouraging, there is still need of trials with larger sample size, along with the standardization of the criteria to evaluate

adherence to the diet, before including the MD as a therapeutic dietary pattern in NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease[1,2]. It represents a wide range in liver damage that may lead to severe liver disease such as cirrhosis and hepatocellular carcinoma[3]. Adults as well as children with fatty liver display abnormal glucose and lipid metabolism. Therefore, NAFLD is now considered an important component of the metabolic syndrome (MetS)[4]. The mechanism of liver injury in NAFLD is considered to be a "multiple-hit process". The first "hit" leads to an increase in liver fat, while the next multiple factors lead to inflammation[5]. Indeed, the early manifestation of NAFLD is triglyceride accumulation in the liver associated with insulin resistance, which is considerably affected by factors such as hyperenergetic diets, sedentary lifestyle, and genetic susceptibility. Fat accumulation in the liver is associated with lipotoxic hepatocellular injury due to elevated free fatty acids, free cholesterol and other lipid metabolites. Thus, mitochondrial dysfunction with oxidative stress and endoplasmic reticulum stress-associated mechanisms are activated[6].

Obesity is considered a key player in the development of NAFLD, and the majority of patients with NAFLD are either obese or overweight. However, NAFLD has been reported also in lean subjects. "Lean" NAFLD represents subpopulation of patients with fatty liver and normal BMI. These patients are usually insulin resistant and have low HDL-C and higher triglyceride concentrations when compared to lean healthy controls[7]. Visceral obesity (as opposed to general obesity), insulin resistance, high fructose and high cholesterol intake are the most prevalent risk factors for lean NAFLD, although genetic factors (*e.g.,* Palatin-like phospholipase domain -containing 3 and Transmembrane 6 superfamily member 2 gene variants) may have an important role.

NAFLD diagnosis requires proof of steatosis, which relies on imaging techniques in clinical practice. Liver biopsy remains the gold standard to address such diagnosis and is the only valid method for differentiating NASH from simple steatosis, however it is neither feasible nor ethical to perform liver biopsy as a tool in all putative patients. Noninvasive imaging techniques, such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and proton magnetic resonance spectroscopy (MRS), can also identify fatty infiltration of the liver[8-10]. US is perhaps the most practical way to assess hepatic steatosis, due to its relatively low cost, availability, and safety. A major limitation of this operator-dependent technique is its limited sensitivitiy and specificity for diagnosing and quantifying hepatic steatosis. MRS is considered the non-invasive reference standard in the assessment of liver steatosis, because it is able to measure the real concentration of triglycerides within the hepatocytes. However, MRS is too time consuming for routine clinical practice, and requires a skilled operator to correctly perform the examination, process the data, and interpret the results. MRI has shown greater promise for the quantitative assessment of hepatic steatosis in adults and children. Until recently, the most widely used method was based on the modified Dixon technique[8]. This imaging method is reliable in the absence of magnetic field non-homogeneity and iron deposition. Recent improvement in MRI have provided measurement of the proton density fat-fraction [(PDFF): The fraction of the liver proton density attributable to liver fat],which is a inherent property of tissue and a direct measure of liver fat content. MRI-PDFF is accurate, precise, and reliable for quantifying liver steatosis having been validated against liver biopsy in both adults and children[9,10].

Currently, there is no agreement with respect to the pharmacological treatment of NAFLD. However, lifestyle interventions based on exercise and a balanced diet for quality and quantity, are considered the cornerstone of NAFLD management[11]. Mediterranean diet (MD), which is characterized by a significant amount of fibers, polyunsaturated fats and antioxidants, has been suggested to decrease the risk of cardiovascular diseases (CVD). In adults, MD has also been demonstrated to be efficacious in reducing the risk of MetS[12-15]. However, few studies are available on the effects of MD in both adults and children with NAFLD. Thus, the present narrative review aims to present an analysis of the available literature on the effects of the MD in patients with NAFLD, and to summarize the main mechanisms of action of MD components on this condition. To identify relevant studies, a systematic literature search on MEDLINE and EMBASE databases was conducted using the following keywords: "Mediterranean diet", "nonalcoholic fatty liver disease", "hepatic steatosis", "steatohepatitis". All searches were limited to studies published in English language

DIET IN NAFLD TREATMENT

Results of studies regarding pharmacological options for treatment of NAFLD are inconclusive[11]. At the moment the best treatment to manage NAFLD is lifestyle intervention to achieve weight loss[11]. A 7% to 10% body weight reduction after energy restriction and/or regular physical activity is associated with histological improvement, resolution of liver fat, necroinflammation and fibrosis[16,17]. Though weight loss is considered the

Table 1 Traditional Mediterranean diet components

Components	Consumption	Rich in
Fresh fruits	Daily, 3 servings	Vitamin C, polyphenols, carotenoids, fibers
Vegetables	Daily, 6 servings	Vitamin C, polyphenols, ω-3-PUFA, carotenoids, fibers
Olive oil	Daily[1]	MUFA, polyphenols
Unrefined cereals	Daily, 8 servings	Polyphenols, fibers
Nuts	Weekly	Polyphenols, ω-3-PUFA, fibers
Legumes	Weekly, ⩾ 3 servings	Polyphenols, fibers
Fish	Weekly, 5-6 servings	ω-3-PUFA
Red wine	Weekly, ⩾ 7 glasses	Polyphenols

[1]As the main added lipid.

most effective treatment in NAFLD, some diets that involve excessive and/or rapid weight loss (*e.g*, very low carbohydrate, high fat diets) may actually cause or exacerbate the disease, inducing insulin resistance[18,19]. As weight reduction is a consequence of physical activity and a 'healthy diet', dietary habits rather than weight loss *per se* may improve NAFLD[18]. Dietary treatment to achieve weight loss must have not only quantitative but also qualitative characteristics. Most studies conclude that energy restriction alone is not enough to treat NAFLD[20], and that the composition of the diet, with modulation of both macro and micronutrients, is crucial[21]. Therefore, a balanced nutrition and a moderate weight loss can now be considered as the best therapeutic approach in NAFLD. According to international guidelines, the first step for treating NAFLD is to limit the intake of calories, of fats (saturated fatty acids, trans fatty acids), and of fructose and, conversely, to increase the intake of lean protein, fibers, and n-3 polyunsaturated fatty acid (PUFA)[15]. Indeed, MD appears as a useful dietary option to produce weight loss followed by concomitant metabolic benefit for NAFLD.

MEDITERRANEAN DIET

MD is a nutritional model which has its origins in the States surrounding the Mediterranean Sea. It was therefore traditionally used by the populations living in these regions. Although MD pattern may vary among countries and regions owing to cultural, ethnic, religious and agricultural differences, the common MD pattern consists of eating primarily unrefined cereals, vegetables and fresh fruit, olive oil, and nuts; eating fish, white meat and legumes in moderation; limiting red meat, processed meats and sweets; and drinking red wine in moderation (Table 1). Therefore, the main characteristics of MD are beneficial fatty acid profile consisting of a low consumption of saturated fat and cholesterol, and, conversely, of a high consumption of monounsaturated fatty acid (MUFA) with a balanced PUFA omega-6 to omega-3 ratio, along with a high content of complex carbohydrates and fibers. Ancel Keys, who conducted large multinational studies in the 1950s-1980s[22-24], first reported a lower mortality rate from CVD and cancer among people living in Greece - as well as in certain parts of Italy and the former Yugoslavia - in comparison to other populations. Afterwards, other studies have confirmed these findings, recognizing MD as a healthy and useful diet for reducing the risk of CVD and cancer[25-28] as well as of obesity and type 2 diabetes[29]. Yet, MD has been proposed as a longevity determinant in these populations[30]. Many studies suggest that the protective effects of MD may be due mostly to the anti-inflammatory and anti-oxidant properties of its components. In particular, the capacity of MD to reduce the risk of development and progression of NAFLD has been attributed to the nutraceutical effect of bioactive compounds and phytochemicals with antioxidant and anti-inflammatory capacity such as fibers, monounsaturated and omega-3 fatty acids and phytosterols[31,32]. NAFLD is associated with visceral obesity, insulin resistance, dyslipidemia, and chronic inflammation all of which are features of Mets. MD may improve NAFLD by modulating the presence of these conditions. In particular, the antioxidant and anti-inflammatory effects as well as the lipid-lowering effects and gut-microbiota-mediated production of metabolites are the principal mechanisms by which MD can influence metabolic health as well as NAFLD.

CLINICAL STUDIES ON MEDITERRANEAN DIET IN NAFLD PATIENTS

Cross sectional studies

Recently, researchers have focused on the possible association between MD and NAFLD. Data from cross sectional studies suggest that MD components have a beneficial effect on NAFLD[33]. As such, the EASL-EASD-EASO clinical Practice Guidelines have recently encouraged MD as a lifestyle choice for treating the disease[16]. The available studies are presented in Table 1[34-40]. Kontogianni *et al*[34] were the first to explore the potential impact of MD on NAFLD and its severity in 73 overweight/obese adult patients, of whom 34 had liver biopsies. They found that the MD score was inversely associated to serum alanine aminotransferase (ALT) and insulin concentrations as well as to histological characteristics of severe steatosis. A higher adherence to MD (as determined by MedDietScore) was not followed by a lower likelihood of having NAFLD, even after adjustment for abdominal fat level. However, it was associated with a less severe liver disease[34]. Indeed, patients with nonalcoholic steatohepatitis (NASH) were less likely to adhere to MD (P = 0.004) versus patients without NASH. Limitations of the study are the cross-sectional design which enables to establish a casual relation; the small sample size; and patients' selection

criteria (elevated ALT, and ultrasound diagnosis of fatty liver and its severity). Similarly, in a study including 82 adult subjects with biopsy-proven NAFLD, Aller *et al*[35] demonstrated that patients with greater adherence to MD (as determined by the 14-item MD assessment tool) were less likely to present histological features of severe steatosis and NASH, as well as to have severe insulin resistance. In a population-based study involving 797 apparently healthy Chinese adults, Chan *et al*[36] evaluated the relationship between two diet-quality scores [Diet Quality Index-International (DQI-I) and MD score] in subjects with (n = 220) and without (n = 577) NAFLD [as established by proton-magnetic resonance spectroscopy (^{1}H-MRS)]. DQI-I, but not the MD score, was significantly related to the NAFLD prevalence, and this association was stronger in overweight/obese versus normal weight subjects. Lack of an association between MD and NAFLD prevalence can be explained by the fact that the intake of certain foods such as milk and milk products, olive oil, wine and nuts was lower in this study cohort than in the traditional MD[36]. Although the study by Chan *et al*[36] included a relatively large sample size and the diagnosis of NAFLD was achieved by ^{1}H-MRS, its major limitation is represented by lack of adjustment in the analysis of lifestyle factors such as physical activity. Recently, Trovato *et al*[37] in a study involving 1199 overweight/obese adult patients [with (n = 532) and without (n = 667) ultrasound-diagnosed hepatic steatosis] found that NAFLD patients were less likely to be adherent to MD. Notably, poor MD adherence strongly predicted the occurrence of NAFLD, independently of body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR), and physical activity score. Very recently, Baratta *et al*[38] showed that MD adherence was inversely related to NAFLD prevalence (as assessed by ultrasound) in a large cohort of overweight/obese adults with cardio-metabolic risk. Subjects with intermediate to high adherence to MD were less likely to have NAFLD and more likely to improve cardio-metabolic features[38]. Again, limitations of the last two studies include their cross-sectional study design; lack of a normal weight control group; and use of ultrasound for diagnosing NAFLD.

In children (Table 2), there are only two studies on the association between NAFLD and MD[39,40]. Cakir *et al*[39] first analyzed in obese youths the association between MD adherence [as assessed by the Mediterranean Diet Quality Index (KIDMED)] and NAFLD (as diagnosed by ultrasound and/or elevated ALT levels, as well as by exclusion of other causes of fatty liver disease). The authors evaluated overweight/obese children with (n = 106) and without (n = 21) NAFLD, as well as children (n = 54) with normal BMI and without known chronic disease. Subjects with a low MD adherence were more likely to present with a higher BMI, though no correlation was found with other parameters including steatosis severity. Limitations of the study are the cross-sectional design; the small sample size; assessment of fatty liver severity by ultrasound; and failure to include physical activity level[39]. Very recently, Della Corte *et al*[40] analyzed the adherence to MD (as assessed by the KIDMED score) in 243 overweight/obese youths with and without NAFLD. Of these, 100 underwent liver biopsy. Poor adherence to MD was related to severity of liver damage as well as to higher levels of C-reactive protein (CRP), insulin and HOMA-IR values, homeostatic model assessment of β cell function and blood pressure levels, thus suggesting increased inflammatory potential of unhealthy diets[40]. Lack of a normal weight control group as well as failure to adjust for confounding variables are major limitations of this study.

CLINICAL STUDIES ON MEDITERRANEAN DIET IN NAFLD PATIENTS

Longitudinal studies

Longitudinal studies are available, to our knowledge, only in adult patients (Table 3)[33,41-46]. Fraser *et al*[41] in a quasi-randomized trial evaluated the effect of three different dietary interventions [the 2003 recommended American Diabetes Association diet; a low glycemic index (LGI) diet; and a modified MD] on ALT concentrations in 259 individuals with obesity and type 2 diabetes. Food-energy intake was similar across all three diets,but diet profiles differed in fat and carbohydrate components. The lowest ALT level at 6 and 12 mo of follow-up was achieved after MD intervention, independently of weight loss, HOMA-IR or triacylglycerol values[41]. In a very small, randomized, cross-over intervention trial involving 12 non-diabetic patients with biopsy-diagnosed NAFLD, Rayan *et al*[42] compared MD to an isoenergetic standard low fat-high carbohydrate diet. After 6 wk of treatment, patients experienced after MD intervention a 38% reduction in liver steatosis (as assessed by ^{1}H-MRS) and improvement of insulin sensitivity compared to patients on low-fat, high-carbohydrate diet, independently of weight loss or waist circumference changes[42]. In a randomized,controlled study involving adult subjects wth type 2 diabetes, Bozzetto *et al*[43] evaluated the effects of an isoenergetic MUFA diet versus a diet higher in carbohydrate and fiber. They found that the hepatic fat content (as measured by ^{1}H-MRS before and after 8 wk of intervention) significantly decreased with MUFA diet, independently of exercise. Subsequently, in a single arm trial including 90 overweight NAFLD patients, Trovato *et al*[44] evaluated the Bright Liver Score at baseline and 1, 3, and 6 mo after MD intervention. Over the 6-mo period, adherence to MD resulted in a significant reduction of liver fat content, independently of other lifestyle changes[44]. In a 6-mo randomized controlled study, Abenavoli *et al*[45] compared three groups of overweight patients with ultrasound-diagnosed NAFLD who received either MD alone (n = 10), or MD supplemented with the Reasil complex including silybin (an extract of *Silybum marianum* commonly known as

Table 2 Cross sectional studies on the association between Mediterranean diet and non-alcoholic fatty liver disease

Authors, year, country[ref.]	Patient population	NAFLD Diagnosis	Adherence to MD	Comment
Kontogianni, 2014, Greece[34]	73 overweight/obese adult patients with NAFLD *vs* 58 age-, gender-, and BMI-matched controls with normal liver ultrasound/ liver chemistry	Patients who met all the following criteria: abnormal ALT and/or GGT; ultrasound evidence of hepatic steatosis and/or compatible liver histology; and no other cause of liver steatosis	Adherence to MD (as estimated by MedDietScore) did not differ significantly between patients and controls	Higher adherence to MD was not associated with lower likelihood of having NAFLD (even after adjustment with abdominal fat level). However, it was associated with lesser degree of insulin resistance and less severe liver disease among patients with NAFLD
Aller, 2015, Spain[35]	82 adult patients with NAFLD (of whom 56 had NASH, and 26 non-NASH; 35 had steatosis grade 1, and 47 steatosis grades 2 and 3)	Liver biopsy in all 82 patients	Higher adherence to MD (as estimated by the 14-item MD assessment tool) was higher in patients with low grade of steatosis than in those with high grade, in patients without NASH than in those with NASH, and in patients without liver fibrosis than in those with liver fibrosis	In the logistic regression analysis,one unit of the 14-item MD assessment tool was associated with a lower likelihood of having NASH (OR = 0.43) and steatosis (OR = 0.42)
Chan, 2015, Hong Kong[36]	797 apparently healthy Chinese adults (332 male, 465 female) of whom 220 (27.6%) had diagnosis of fatty liver	^{1}H MRS was performed to measure IHTG. Fatty liver was defined as IHTG $\geqslant$ 5%	Subjects with fatty liver showed lower gender-adjusted MD score than those without fatty liver	Multivariate adjusted regression analyses showed an inverse association between MD score and prevalence of fatty liver, which approached the level of significance
Trovato, 2016, Italy[37]	1199 overweight/ obese adult patients with (n = 532) and without (n = 667) hepatic steatosis	Hepatic steatosis and its severity were assessed by ultrasound	Greater prevalence of overweight/ obesity (as assessed by BMI) and insulin resistance (as assessed by HOMA-IR), sedentary life habits, increased TG and HDL-C, greater use of Western diet food, as well as poor adherence to MD (as assessed by 1-wk recall questionnaire) were found in patients with hepatic steatosis *vs* those without it	Multiple regression analysis, weighted by years of age, displayed BMI, HOMA-IR and adherence to MD as the most powerful predictors of hepatic steatosis severity
Baratta, 2017, Italy[38]	584 overweight/obese adult patients with $\geqslant$ 1 CVD risk factor	Ultrasound evaluation	57 (9.8%) patients had low MD adherence (as estimated by Med-Diet questionnaire), while 436 (74.6%) and 91(15.6%) had, respectively, intermediate and high MD adherence. NAFLD prevalence significantly decreased from subjects with low to high adherence to MD (from 96.5% to 71.4%, P < 0.001)	In a multiple logistic regression analysis, MD adherence (intermediate *vs* low OR = 0.115; P = 0.041; high *vs* low OR: 0.093; P = 0.030) were independently associated with NAFLD
Cakir, 2016, Turkey[39]	Overweight/obese children with (n = 106, Group 1) and without (n = 21, Group 2) hepatic steatosis; and children with normal BMI and without known chronic disease (n = 54, Group 3)	Assessment of hepatic steatosis and its severity by ultrasound	Prevalence of a low level of MD adherence (as established by KIDMED index score) was significantly higher in Group 1 children compared to those belonging to Groups 2 or 3	The level of adherence to MD was negatively correlated with BMI, but no significant correlation was found with ALT, total body fat, TG, and HOMA-IR. No significant difference in the level of MD adherence was found between patients with hepatic steatosis grade1 and those with grades 2 and 3
Della Corte, 2017, Italy[40]	4 subgroups of overweight/obese children: with and without fatty liver; with and without NASH.	Among the 243 study children, ultrasound identified and excluded fatty liver in 66 and 77, respectively. The remaining 100 underwent liver biopsy identifying and excluding NASH in 53 and 47, respectively	Prevalence of a low level of adherence to MD (as estimated by KIDMED score) was significantly higher in patients with NASH compared to those without NASH as well as to those with and without fatty liver (100% *vs* 28.8% *vs* 37.9% *vs* 9.1%; P = 0.01)	Poor adherence to MD was associated to severe liver damage, with a negative correlation with NAFLD activity score and fibrotic stage

ALT: Alanine aminotransferase; BMI: Body mass index; CVD: Cardiovascular disease; GGT: Gamma-glutamyl transferase; ^{1}H MRS: Proton magnetic resonance spectroscopy; HOMA-IR: Homeostasis model assessment of insulin resistance; IHTG: Intrahepatic triglyceride content; MD: Mediterranean diet; NAFLD: Non-Alcoholic Fatty Liver; NASH: Non-Alcoholic Steatohepatitis; OR: Odds ratio; TG: Triglycerides.

milk thistle), phosphatidylcholine and vitamin E (n = 10), or no pharmacological and nutritional treatment (n = 30) . After 6 mo of follow-up, MD either alone or in association with the Realsil complex resulted in significant improvement in fat accumulation as well as in BMI, waist circumference, total cholesterol,

Table 3 Longitudinal studies on the effects of Mediterranean diet on non-alcoholic fatty liver disease in adult patients

Authors, year, country[ref.]	Study design	Patient population	Intervention (duration, type, number of patients)	Liver outcome	Other outcomes
Fraser, 2008, Israel[41]	An open label, parallel design, quasi-randomized (allocation by alternation) controlled trial	Overweight /obese patients with T2DM	3 groups at 6/12 mo: 1. ADA diet, *n* = 64/54; 2. Low GI diet, *n* = 73/64; 3. Modified MD, *n* = 64/61. Energy contents similar in all three diets	ALT levels significantly decreased at 6 and 12 mo in modified MD *vs* low GI or ADA diets, independently of waist to hip ratio, BMI, HOMA and triacylglycerol values	
Bozzetto, 2012, Italy[43]	Randomized, controlled, parallel-group design	36 overweight /obese patients with T2DM	8 wk, 4 groups: 1. High-CHO/ high-fiber/ low GI diet, *n* = 9; 2. MUFA diet, *n* = 8; 3. High-CHO/ high-fiber/ low GI diet + exercise, *n* = 10; 4. MUFA diet + exercise, *n* = 9.	Liver fat (as measured by ^{1}H MRS) decreased more in groups 2 (-25%) and 4 (-29%) than in groups 1 (-4%) or 3 (-6%). Two-way repeated-measures ANOVA showed a significant effect on liver fat content for MUFA diet, independently of exercise. There were no significant ALT and AST changes in all groups.	At the end of intervention, there were no significant changes in body weight,WC, as well as in glucose, total cholesterol, LDL-C, HDL-C, TG, and HOMA-IR values from baseline in all groups
Ryan, 2013, Australia[42]	A randomized, controlled, cross-over study	12 non-diabetic patients with a biopsy-proven NAFLD at baseline	A cross-over 6-wk dietary intervention study comparing traditional MD *vs* low fat/high-CHO	MD group demonstrated a significant decrease in liver fat (as measured by ^{1}H MRS) compared to the low fat/ high-CHO group (39% *vs* 7%). ALT and GGT did not significantly decrease with either diet	At the end of intervention, no significant changes in body weight, WC, as well as in TG, and HDL-C in both groups. Peripheral insulin sensitivity improved only in the MD group. Systolic BP declined significantly in both groups, though to a lesser degree in the low fat/ high-CHO group
Trovato, 2015, Italy[44]	Single arm	Non-diabetic overweight/obese patients with ultrasound evaluation of liver fat changes from baseline	90 patients following intervention with MD alone for 1, 3, and 6 mo	Liver fat significantly decreased only after 6 mo of intervention. By a multiple linear regression model, changes in adherence to the MD and BMI were found to independently explain the variance of decrease of liver fat (R^2 = 0.519; P < 0.0001). No significant ALT changes were observed throughout the follow-up	Significant decrease of BMI followed by parallel increases of the MD adherence as well as of physical activity were observed from the first month of intervention. Significant decrease of HOMA-IR was observed only after 3 and 6 mo
Abenavoli, 2015, Italy[45]	Controlled randomized study	Overweight/obese patients with ultrasound evaluation of liver fat changes from baseline	6 mo, 3 groups: 1. Hypocaloric MD, *n* = 10; 2. Hypocaloric MD plus Realsil complex, *n* = 10; 3. No treatment, *n* = 10.	Compared to the group that did not undergo any treatment, MD either alone or associated with the Realsil complex led to significant improvement in liver steatosis	Compared to the group that did not undergo any treatment, those following the MD either alone or associated with the Realsil complex had improvement in BMI, WC, hip circumference, as well as in total cholesterol, and TG. Improvement in insulin sensitivity occurred only in patients receiving MD plus the Reasil complex
Misciagna, 2016, Italy[46]	Randomized, controlled, parallel-group design	A population almost composed of non-diabetic overweight/ obese patients (18 to 79 years old, without overt CVD) with ultrasound evaluation of liver fat at baseline and follow-up	3 and 6 mo, 2 groups: 1. MD with low GI, *n* = 44; 2. Control diet(based on INRAN guidelines), *n* = 46	MD with low GI was associated until 55 yr of age, in both men and women, with a more intense reduction in liver fat than a control diet, at both the 3rd and 6th month	Six months after intervention, in both groups, the number of obese patients decreased while the number of overweight subjects increased. Lower TG and glucosemia were found at 6 mo in both groups

Gelli, 2017, Italy[33]	Single arm	46 (11 normal weight; 35 overweight/obese) subjects (42 with ⩾ 1 MetS component; 4 with T2DM) with ultrasound evaluation of liver fat at baseline and follow-up	All patients followed intervention with MD alone for 6 mo	At end-intervention, the percentage of patients with hepatic steatosis grade ⩾ 2 was reduced from 93% to 48%; mean AST, ALT, GGT decreased significantly	At end of intervention, of the 35 overweight/obese patients, 12 showed ⩾ 7% weight reduction while 7 achieved normal weight; mean serum total cholesterol, HDL-C, AST, TG, glucose concentrations, and HOMA-IR values significantly improved

ADA: American Diabetes association; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; BP: Blood pressure; CHO: Carbohydrates; GGT: Gamma-glutamyl transferase; GI: Glycemic index; ^{1}H MRS: Proton magnetic resonance spectroscopy; HDL-C: High density lipoprotein-cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance; INRAN: Italian National Research Institute for Foods and Nutrition; LDL-C: Low density lipoprotein-cholesterol; MD: Mediterranean diet; MetS: Metabolic syndrome; MUFA: Monounsaturated fatty acid; T2DM: Type 2 diabetes mellitus; TG: Triglycerides; WC: Waist circumference.

triglyceride and insulin resistance values[45]. In a randomized controlled study, Misciagna *et al*[46] compared two groups of non-diabetic overweight-obese patients with moderate/severe ultrasound-diagnosed NAFLD who followed, respectively, a control diet (based on the Italian National Research Institute guidelines) and a low glycemic Index Mediterranean Diet (LGIMD). Compared to the control diet, LGIMD resulted in a major reduction of liver fat at both 3th and 6th month[46]. Finally, very recently, in a single arm, observational study, Gelli *et al*[33] treated with MD 46 normal weight (n = 11) or overweight/obese (n = 35) patients with NAFLD. They determined liver enzymes, metabolic parameters, CVD risk indexes, and ultrasound-based NAFLD severity. At the end of treatment, the proportion of patients with liver steatosis grade ⩾ 2 was reduced from 93% to 48%. Also, metabolic parameters and liver enzymes decreased significantly[33].

Several points need be considered when interpreting the results of the aforementioned studies. First, they were based on high-risk populations, therefore not representative of the general population. Second, most of them were based on a small sample size. Notably, none of the studies provided information on how sample size was calculated and how participants were randomly assigned to the intervention groups. As matter of fact, there may be synergistic and antagonistic interactions among food components of MD that may be difficult to detect unless very large samples are used. Third, MD includes a variety of eating patterns and, therefore, a wide range in assessment score items. As such, using a score for assessment of adherence to a dietary pattern is limited by subjectivity, leading therefore to a great variability in interpretation of study results. Fourth, the majority of studies utilized ultrasonography that is known to be highly operator-dependent, and to have limited repeatability and reproducibility. In addition, ultrasonography has shown low accuracy in assessing severity of liver disease including presence and extent of fibrosis[47]. Fifth, most studies failed to take into account total energy intake. Finally, most studies failed to adjust for potential confounders including physical activity, and socioeconomic and cultural levels, which might have influenced lifestyle habits of the population studied.

BIOLOGICAL MACHANISMS OF MEDITERRANEAN DIET

Anti-inflammatory and antioxidant effects of MD components

MD is based on compounds, such as polyphenols, vitamins and other biomolecules that have anti-inflammatory and antioxidant effects. This seems to be relevant, since inflammation and oxidative stress play a central role in the pathogenesis of NAFLD/NASH.

Polyphenols are present in whole-grain cereals, vegetables and fresh fruits, olive oil, nuts and red wine. They are a heterogenic group of bioactive compounds, including several hydro-soluble antioxidants, characterized by a phenolic structure[48]. Based on their chemical structure, there are two categories of polyphenols: flavonoid polyphenols, and the non-flavonoid polyphenols[49].

Flavonoids are polyphenolic compounds that are ubiquitously found[50] and provide much of the flavor and color to fruits and vegetables. They have hepato-protective effects in view of their antioxidant and anti-inflammatory potential[49,51-53]. Among non-flavonoids, resveratrol, a stilbene polyphenol content in red wine, has been shown to exert hepato-protective activity by affecting the three interacting components of homeostasis such as the vessel, the blood platelets and the clotting and the fibrinolytic system of plasma[54,55]. Vitamins, which are significant components of MD, can also be considered dietary antioxidants. They reduce cellular stress and, in this way, they have a pivotal role in preventing NAFLD progression. Vitamin E has been shown to improve histological features of NASH[56-59]. Vitamin D has immunomodulatory, anti-inflammatory and anti-fibrotic properties while vitamin D supplementation has been demonstrated to ameliorate NAFLD histopathology[60,61]. When incubated with isolated rat liver, vitamin C has been shown to decrease levels of mitochondrial reactive oxygen species generation, and to increase the levels of antioxidant enzymes and the activity of the electron transport chain[62].

Carotenoids are also part of MD; they comprise a class of natural fat-soluble pigments acting as antioxidants,

which are found in several fruits and vegetables[63]. Among them, lycopene has been investigated as a potential protective agent in NAFLD in view of its potent antioxidant effects[64]. Studies in lycopene-fed rats have shown that lycopene has a preventive effect on experimental NASH by reducing steatosis and inflammation as well as oxidative stress[65].

Lipid-lowering effect of MD components

The beneficial effects of MD on the hepatic lipid metabolism and, consequently, on NAFLD prevention, is influenced primarily by its fatty acid composition which is characterized by high MUFA content with a balanced PUFA omega-6 to omega-3 ratio due to the abundance of vegetables, legumes, nuts, olive oil and fish (instead of red meats)[66]. It has been proved that MUFA intake may prevent the development of NAFLD by improving plasma lipid levels, reducing body fat accumulation and decreasing postprandial adiponectin expression[67,68]. PUFA regulate three major transcriptional factors controlling multiple pathways involved in hepatic carbohydrate and lipid metabolism. PUFA activation of hepatic peroxisome proliferator-activated alpha (PPARα) enhances fatty acid oxidation, while PUFA suppression of sterol regulatory element binding protein-1 (SREBP-1) and of carbohydrate regulatory element binding protein (ChREBP)/Max-like factor X (MLX) results in the inhibition of glycolysis and of *de-novo* lipogenesis. As such, PUFA promote a shift in metabolism toward fatty acid oxidation and away from fatty acid synthesis and storage, and may positively affect NAFLD[69,70]. In addition to improvement in steatosis, PUFA may induce an independent, anti-inflammatory effect *via* suppression of tumor necrosis factor and interleukin-6, responsible for the inflammation occurring in NASH[71]. Opposite health effects have been found regarding the role of n-6 PUFA on NAFLD. N-6 PUFA, such as linoleic acid may have a pro-inflammatory role due to their direct relation with the production of arachidonic acid (AA). AA is metabolized to give rise to the eicosanoid family of inflammatory mediators (*e.g*. prostaglandins, leukotrienes and related metabolites), and through these to regulate the production of inflammatory cytokines[72]. Excessive amounts of omega-6 PUFA and a very high omega-6 to omega-3 ratio have been involved in the pathogenesis of many diseases, including CVD, cancer, and inflammatory and autoimmune diseases[73]. A proportionally high intake of n-6 PUFA is considered pro-inflammatory and possibly associated with an increased risk of MetS. Therefore, not only PUFA intake but also the n-6 PUFA to n-3 PUFA ratio is relevant.

Several studies have shown that a reduced intake of saturated fat is associated with a reduction of plasma concentrations of total cholesterol, very low density lipoprotein (LDL)-cholesterol and triglycerides[74].

MD can also contribute to lowering plasma cholesterol by high consumption of water-soluble fibers which are found in large concentration in some MD components, mainly beans, vegetables and fruits and whole-grain cereals. Water-soluble fibers have been shown to increase the rate of bile excretion therefore reducing serum total and LDL cholesterol[75].

GUT MICROBIOTA AND MD COMPONENTS

The liver is closely connected to the gut as it receives about 70% of its blood supply directly from the intestine *via* the portal vein. Therefore, it is one of the organs mostly exposed to gut-derived toxic products, such as bacteria and bacterial derivates. This cross-talking between the intestine and the liver is known as the "gut-liver axis" and has been linked to liver pathologies, including NAFLD. The relationship between NAFLD and altered microbiota is mainly supported by studies on animal models[76,77].There are limited data in humans[78,79]. Gut microbiota plays a crucial role in the complex pathogenesis of NAFLD through a variety of mechanisms such as predisposition to obesity, induction of insulin resistance as well as of liver inflammation, and alteration of choline metabolism[80]. Other mechanisms include increased microbiome-modulated metabolites such as bile acids, short chain fatty acids, lipopolysaccharides as well as dysbiosis-induced intestinal barrier dysfunction[81]. Many different factors may influence microbiota composition, including age, comorbid conditions, host genotype and exposure to antibiotics, and dietary habits[82]. Diet largely influences gut microbiota and its products[83]. Specific dietary factors, such as macronutrient composition (*e.g.* increased protein intake), food type (*e.g.* glycemic index or load) or the presence of specific bioactive compounds (omega-3 fatty acids, fibers or polyphenols) have been shown to influence the diversity and functionality of the gut microbiota[84]. Also protein, insoluble fibers and fat content have important effects on gut microbiota structure, function, and its secretion of metabolites that modulate immune function and multiple metabolic and inflammatory pathways[85-87]. Therefore, MD may have a significant impact on the composition and diversity of the microbiota. As MD is characterized by a high dietary fiber intake, it promotes beneficial modification of the gut microbiota with decreased *Firmicutes* and increased *Bacteroides*, which have been shown to ameliorate obesity, inflammation and related metabolic alterations. Polyphenols contained in MD induce an increase in *Bifidobacteria*, associated with various metabolic benefits such as plasma cholesterol reduction and a decrease of C-reactive protein (CRP)[88]. Gut microbial production of trimethylamine N-oxide from dietary choline and l-carnitine enhances the risk of developing CVD in both animals and humans, independently of CVD risk factors[89]. MD benefits on the gut microbiota could also be the consequence of a low content of choline and l-carnitine in MD diet.

CONCLUSION

MD, low in saturated fats and animal protein, high in antioxidants, fiber and MUFA, and with an adequate omega-3 to omega-6 fatty balance, represents an healthy dietary pattern, which has been shown to decrease CVD, MetS, and type 2 diabetes. Although MD seems particularly attractive for its potential to improve liver status, literature concerning the efficacy of this dietary pattern in patients with NAFLD is still limited to few cross-sectional as well as to few longitudinal studies with certain limitations. In particular, longitudinal studies have included small sample size, short-term follow-up, different designs, different time points of data collection, and above all poor methodology for reporting the trial or diagnosing the liver outcome and its associated comorbidities, anyone of which or any combination of which may limit the generalizability of study results. There is room for adequate randomized dietary intervention trials comparing MD with a control diet in a large sample of the general population, along with a validation of the MD indexes in the heterogeneous patient population with NAFLD.

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