The influence of lifestyle factors and staple foods from the Mediterranean Diet on non-alcoholic fatty liver disease among elder with metabolic syndrome features

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Abstract

Objective: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver morbidity. This condition is often accompanied by obesity, diabetes and metabolic syndrome (MetS). The aim was to evaluate associations between lifestyle factors and NAFLD in subjects with MetS.

Methods: A cross-sectional study on 328 participants (55-75 years) diagnosed with MetS participating in the PREDIMED-Plus trial was conducted. NAFLD status was evaluated using the non-invasive hepatic steatosis index (HSI). Sociodemographic, clinical, and dietary data were collected. Adherence to the Mediterranean Diet (mainly assessed by the consumption of olive oil, nuts, legumes, whole grain foods, fish, vegetables, fruits and red wine) and physical activity were assessed using validated questionnaires.

Results: Linear regression analyses revealed that HSI values tended to be lower with increasing physical activity tertiles [T2, $\beta = -1.47$ (95%CI -2.73 to -0.20); T3, $\beta = -1.93$ (95%CI -3.22 to -0.65) vs T1, p-trend= 0.001] and MedDiet adherence was inversely associated with HSI values: [moderate adherence $\beta = -0.70$ (95%CI: -1.92 to 0.53), high adherence $\beta = -1.57$ (95%CI: -3.01 to -0.13) vs lower, p-trend= 0.041]. Higher tertiles of legume consumption were inversely associated with the highest tertile of HSI [T2, RRR= 0.45 (95%IC 0.22 to 0.92), p= 0.028; T3, RRR=0.48 (95%CI 0.24 to 0.97), p=0.041 vs T1].

Conclusion: Physical activity, MedDiet adherence, and legume consumption were inversely associated with a non-invasive marker of NAFLD in subjects with MetS. This data can be useful in implementing precision strategies aimed at the prevention, monitoring, and management of NAFLD. ISRCTN89898870.

Keywords: NAFLD, Inflammation, Nutrition, Obesity.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become a prevalent chronic liver disease being the principal cause of liver-related morbidity and mortality [1]. The increasing rates of NAFLD are probably accompanying the rise in obesity, type 2 diabetes, metabolic syndrome (MetS), and cardiovascular disease (CVD) incidence, [2,3] especially in Western countries [1]. Indeed, NAFLD is a multifactorial chronic condition, whose pathogenesis results from a complex interaction among genes, gut microbiota, and lifestyle factors [4]. Furthermore, the ageing process is associated with an increased risk of developing cardiometabolic abnormalities and NAFLD progression [5]. NAFLD encompasses a spectrum of liver damage features being characterized at initial stage by an excessive accumulation of intrahepatic triglycerides, which can progress to non-alcoholic steatohepatitis (NASH), and eventually lead to cirrhosis and/or hepatocellular carcinoma (HCC) if not early detected and treated [4]. Liver biopsy is the gold standard for NAFLD diagnoses [4]. However, it is an expensive and invasive procedure that may result in clinical

87 complications [4]. Thus, several alternative non-invasive liver scores have been
88 devised and developed [2–4]. The hepatic steatosis index (HSI) has demonstrated
89 good performance in several population studies and used for large scale NAFLD
90 primary screening [6–8]. The management of all stages of NAFLD has been focused
91 on improving the metabolic profile by encouraging a healthy lifestyle, such as
92 adherence to certain dietary patterns and increased physical activity [2–4]. The
93 Mediterranean Diet (MedDiet) is a healthy dietary pattern, which includes a high
94 consumption of plant-derived foods (fruits, vegetables and legumes), whole grain
95 foods, fish, olive oil, nuts, and low-moderate intake of red wine, meat and dairy
96 products [9]. Besides, MedDiet has demonstrated beneficial effects on the lipid
97 profile, glycemic control, and blood pressure [10]. The presence of these clinical
98 conditions are associated with higher risk of NAFLD and more advanced disease
99 stages [2]. Epidemiological and clinical studies have suggested that staple
100 components of the MedDiet provide specific health bioactive compounds with
101 healthy antioxidant and anti-inflammatory properties [10–13]. In fact, the effects of
102 MedDiet on liver status could be attributed to specific compounds such as
103 polyphenols, fiber, carotenoids, n-3 PUFA and oleic acid, among others [12,14]
104 Physical activity has been shown to potentially reduce hepatic steatosis, and to
105 improve insulin resistance, some MetS features, and cardiovascular events [15].
106 Current available recommendations suggest weight loss for NAFLD treatment (-5%
107 or -10% of initial body weight) as the key intervention based on energy restriction.
108 However, not only the loss of body weight is important but also the characteristics of

the nutrient composition as well as the advices for a healthy lifestyle adherence should be strongly considered in the treatment of this disease [4]. To our knowledge, there are few available data regarding lifestyle factors of elderly patients with MetS. Against this background, we hypothesized that lifestyle factors, especially adherence to MedDiet and nutritional/food characteristics as well as physical activity, would be associated with a decreased risk of NAFLD in elderly population diagnosed with MetS at high cardiovascular risk.

Methods

Study population and design

The PREDIMED-Plus study is a multi-centre randomized trial designed to investigate the effect on cardiovascular diseases (CVD) morbidity and mortality reduction. A detailed protocol of the study methods and population characteristics has been published [16]. In brief, this study recruited 6874 subjects in 23 centres located in Spain. Participants enrolled had to fulfill the following inclusion criteria: men aged 55–75 years and women aged 60–75 years with a BMI ≥ 27 and < 40 kg/m² fulfilling at least three criteria for the MetS [17]. We excluded those individuals who self-declared the following: therapy with immunosuppressive drugs, cytotoxic agents or systemic corticosteroids, liver injury at the time of recruitment (cirrhosis or liver failure), history of inflammatory bowel disease, alcohol abuse or addiction, among others. Participants were randomly assigned 1:1 into two equally sized groups, intervention group (an intensive program of weight loss based on an energy-

restricted MedDiet, physical activity promotion, and behavioural support) or into a control group (an energy-unrestricted MedDiet). (<http://medpreventiva.es/QufSWn>). This clinical trial was registered (ISRCTN89898870) and conducted in accordance with the Declaration of Helsinki ethical disclosure and further guidelines. All participants signed an informed consent to participate at the beginning of the intervention trial. The present investigation is a cross-sectional sub-study with baseline data of participants from the Navarra-Nutrition centre. The sample size was calculated to find a correlation coefficient with an 80% statistical power between adherence to MedDiet and hepatic steatosis ($r=0.20$) considering a type I error of 5% and type II error of 10%. A total of 422 participants were registered in the pre-inclusion period. Of these, we excluded 2 individuals who did not meet inclusion criteria and 89 who declined participation or for other reasons. Three hundred thirty-one individuals were included, but 328 had valid data for the non-invasive liver score calculation, which is a number that has been shown suitable in comparable studies [18,19].

Study measurements

Dietary assessment

At baseline, trained dietitians administered face-to-face a 143-item food frequency questionnaire to estimate dietary intake over last year, which was previously validated in Spanish population [20]. In order to evaluate the adherence to the MedDiet, a score based on nine dietary components was applied, as described

elsewhere [21,22]. For beneficial components (vegetables, fruits and mixed nuts, legumes, cereals, fish and seafood), participants were assigned a value of 0 for the consumption below the component sex-specific median and above the median were assigned a value of 1 as well as for fat intake considering the ratio [MUFA/SFA]. Meanwhile, for components presumed to be detrimental (meat and dairy products), individuals were assigned a value of 1 for the consumption below the component sex-specific median and above the median were assigned a value of 0. For the alcohol component, a value of 1 was assigned to men consuming 10 to <50 g/d and women consuming 5 to <25 g/d and 0, otherwise. Thus, the total MedDiet punctuation ranged from 0 (minimum adherence) to 9 (maximum adherence). MedDiet adherence was categorized into low (0-3 points), moderate (4-5 points) or high (6-9 points) adherence for analytical purposes [22].

Physical activity assessment

Physical activity was assessed using the short REGICOR (Registre Gironi del Cor), which was validated in Spanish population [23]. As described previously [24], this questionnaire evaluated the total energy expenditure in leisure time physical activity (Metabolic Equivalent (MET)-minute/week) considering light (<4 MET), moderate (4-5.5 MET) and vigorous (≥ 6 MET) physical activity. Also, the number of weekly hours of sedentary behavior [25]. For this study, physical activity was expressed as MET-hour-week and categorized by tertiles.

Sociodemographic, lifestyle, and clinical variables

At baseline, sociodemographic, lifestyle, history of illnesses and medication data were collected during the personal interview with standardized questionnaires. Smoking status was categorized as never, former, or current smoker. Trained dietitians measured weight and height using calibrated equipment following the PREDIMED PLUS standardized protocol [16]. The body mass index (BMI) was calculated as the body weight divided by the squared height (kg/m^2). Determinations of fat mass (total, trunk, android, gynoid and visceral) were performed using dual-energy X-ray absorptiometry (Lunar iDXA™, Madison, WI, USA connected with enCore™ software, version 6.0) by trained personnel following the instructions of the equipment as described elsewhere [26]. Overnight fasting blood was collected. Serum and plasma samples were immediately frozen at -80°C . Biochemical variables, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (CT), high-density lipoprotein cholesterol (HDL-c), triglyceride (TG), glucose, and hemoglobin A1c (HbA1c), were determined with specific kits according to manufacturer's protocols, as previously described, [26,27] while low-density lipoprotein cholesterol (LDL-c) and very-low-density lipoprotein cholesterol (VLDL-c) were calculated using the Friedewald formula and triglycerides/5, respectively [28]. The triglyceride-glucose index (TyG index) was estimated as the logarithm of fasting triglyceride (mg/dL) x fasting glucose (mg/dL)/2 [29]. The MetS status was defined when at least 3 or more of the components were clinically ascertained [17]. Waist circumference in Caucasian people ≥ 102 cm for

men and ≥ 88 cm for women, elevated triglycerides levels ≥ 150 mg/dL or drug treatment for hyperlipidemia; reduced HDL-c < 40 mg/dL in men and < 50 mg/dL in women or drug treatment; elevated blood pressure systolic ≥ 130 and/or diastolic ≥ 85 mmHg or current use of antihypertensive medication; elevated fasting glucose ≥ 100 mg/dL or drug treatment, according to guidelines from the International Diabetes Federation/National Heart, Lung and Blood Institute/American Heart Association (2009) [17]. Diabetes was diagnosed as described in the recommendations of the American Diabetes Association (ADA) guidelines [30].

Non-invasive liver score assessment

The non-invasive hepatic steatosis index (HSI) has been reported as a useful screening tool with valuable accuracy predictions of NAFLD [6,7] validated in a large group of subjects [8], which considers the AST/ALT ratio, body mass index (BMI), presence of diabetes mellitus, and sex (female), as follows: $HSI = 8 \times ALT/AST + BMI + (+ 2, \text{ if type 2 diabetes, } 0 \text{ otherwise}) + (+ 2, \text{ if female, } 0 \text{ otherwise})$ [8]. The lack of primary or secondary causes of hepatic fat accumulation were considered as described by American Association for the Study of Liver Diseases (AASLD) [4].

Statistical analyses

Continuous variables are presented as mean (m) and (95%IC), while categorical variables as counts (n) and frequencies (%). Categorical data were analyzed by the Chi-square test. The cohort study was stratified into HSI tertiles based on sex; HSI

219 men: T1 (≤ 40.0), T2 (>40.0 to <43.7), T3 (≥ 43.7 to ≤ 54.8); HSI women: T1 (≤ 41.0),
220 T2 (>41.0 to <46.0), T3 (≥ 46.0 to ≤ 57.4). Baseline characteristics differences among
221 groups were analyzed by ANOVA. The associations between HSI and other
222 variables were fitted by ANCOVA after adjusting for age, total energy intake and
223 alcohol intake as continuous variables with Bonferroni correction for multiple
224 comparisons. To examine the association between HSI and lifestyle variables
225 (physical activity across tertiles and MedDiet adherence), we applied linear
226 regression analyses, both performed after adjustment in model 1 for age. Further
227 adjustments for energy intake, alcohol consumption, smoking status, hypertension
228 or antihypertensive medication were accordingly applied in model 2. Model 3a was
229 further adjusted for MedDiet adherence and model 3b for MedDiet and physical
230 activity in tertiles (MET/hours/week): T1 (0 to 22.5) as reference; T2 (>22.5 to ≤ 61.4);
231 T3 (>61.4 to ≤ 321.7). MedDiet score was stratified according to adherence: low (0 to
232 3 points) as reference; moderate (4 to 5 points); high (6-9 points). To calculate p-
233 values for trend, the physical activity and MedDiet adherence were treated as
234 continuous variables. A linear regression analysis was carried out to evaluate the
235 relationship between legume consumption and HSI. Furthermore, a multinomial
236 logistic regression analysis was performed to investigate the association of legume
237 consumption categorized in tertiles T1 (≤ 16.1 g/d), T2 (>16.1 g/d to ≤ 20.8 g/d), T3
238 (>20.8 g/d) with the HSI as dependent variable categorized in tertiles, after adjusting
239 for potential confounders. The analysis in model 1 was adjusted for age, smoking
240 status, energy intake, and alcohol consumption. Model 2 was further adjusted for

physical activity and triglycerides. The effect was estimated using the relative risk ratio (RRR) with 95% confidence interval (CI). Analyses were carried out with Stata 12.0 software (StataCorp LP, College Station, TX). P-values are two tailed; $p < 0.05$ was considered statistically significant.

Results

Participant's characteristics

The unadjusted mean variables related to sociodemographic, lifestyle, anthropometric, and clinical characteristics are reported according to HSI tertiles (Table 1). The mean age was similar in all groups (65 years). Participants in the T3 group were more likely to have diabetes (52.3%). They also presented a more adverse fewer clinical status related to glycemic control such as glucose [mean 130.1 mg/dL (95% CI, 124.1-136.2)], HbA1c [mean 6.3% (95% CI, 6.1-6.5)] and disrupted insulin homeostasis represented by the TyG index [mean 9.2 (95% CI, 9.1-9.3)], as well as MetS components including waist circumference [113.8 cm (95% CI, 112.4-115.2)] and BMI measurements. No statistical differences were found in smoking status ($p = 0.134$) or high blood pressure ($p = 0.145$) among the tertiles groups.

Hepatic steatosis index, lipid profile, and body composition

Lipid profile and body composition variables and are described (Table 2). T3 group exhibited higher levels of triglycerides [mean 161.9 mg/dL (95% CI, 149.9-173.8)],

VLDL-c [mean 32.4 mg/dL (95% CI, 30.0-34.8)] serum levels, and the TG/HDL-c ratio [mean 3.9 (95% CI, 3.5-4.3)] compared to T1 (all $p < 0.05$). Nevertheless, individuals in the T2 exhibited higher total cholesterol levels compared to T1. Also, LDL-c and HDL-c levels did not differ significantly among tertiles (Table 2). Indeed, individuals from the T3 HSI had a higher total, trunk, android, gynoid, and visceral fat mass than those from the T2 and T1 ($p < 0.05$).

Relationship between hepatic steatosis index and lifestyle variables

Lifestyle variables such as physical activity and MedDiet adherence tended to decrease with increasing HSI tertiles with significant differences between T1 and T3 (Table 2). The association between HSI, physical activity, and MedDiet adherence (Table 3) revealed that participants in T2 of physical activity had a significant 1.47 lower units of HSI (95%CI -2.73 to -0.20) whereas, higher levels of physical activity (T3) were associated with 1.93 lower units of HSI (95%CI -3.22 to -0.65); p for trend = 0.001. Moreover, the change of the HSI according to MedDiet adherence was -0.70 units (95%CI -1.92 to 0.53) for moderate adherence, and -1.57 units (95%CI: -3.01 to -0.13) for high adherence; p for trend = 0.041. The daily consumption of each component of the MedDiet was also assessed according to HSI tertiles (Table 4) and no statistical differences were noted among most food groups. Interestingly, the lowest tertile group of the HSI reported a significant higher legume consumption [mean 21.6 g/d (95% CI, 20.0-23.2)] and a lower total meat intake [mean 144.0 g/d (95% CI, 134.8-153.1)] compared to highest tertile group (Table 4). Some statistical

associations were found concerning meat consumption, which were not confirmed in adjusted analyses. A linear regression analysis demonstrated a negative relationship (Figure 1) between HSI and legume consumption (R^2 -adjusted=0.027; $p=0.002$). Reinforcing this notion, a statistically significant inverse association between legume intake (g/d) and the highest tertile of HSI was observed. The RR for the HSI (T3) according to tertiles of legume consumption for the final fully adjusted model (Table 5) was as follows: 1.00 (reference); T2, 0.45 (95%IC 0.22-0.92); T3, 0.48 (95%IC 0.24-0.97).

Discussion

Emerging clinical data have established a close relationship between NAFLD and MetS [31]. In this cross-sectional cohort study, key components related to cardiometabolic risk factors disclosed a direct association with higher HSI values. To the best of our knowledge, this is the first study that evaluated the relationship between lifestyle factors, and the specific role of typical Mediterranean foods, with NAFLD characteristics in an aged population diagnosed with MetS. In particular, the HSI has been proposed as a predictor of liver steatosis [7]. The accuracy of HSI was validated in a large cohort study using ultrasonography as diagnose to fatty liver [8]. Cutoff values for the diagnosis of NAFLD were established that values >36 confirming the diagnosis of steatosis [8]. In fact, the use of non-invasive liver scores might be useful for the diagnosis and prediction of NAFLD [7,32,33]. In our study, participants at the highest HSI tertile disclosed a pro-atherogenic lipid profile. In

addition, they had higher blood glucose levels and disrupted insulin homeostasis as assessed by TyG index as an insulin resistance [29] and it could predict risk of NAFLD [34]. These findings may be explained because glucose and insulin are involved in the activation of several pathways related to lipogenesis [32]. Our results are consistent with the fact that muscle and liver insulin resistance promote the accumulation of several lipid metabolites and impairs VLDL assembly and secretion. The overproduction of VLDL particles leads to an increased free fatty acid (FFA) flux into plasma, which augments the risk of liver steatosis [32,35]. Additionally, there are several clinical studies confirming that visceral adipose tissue induces insulin resistance, inflammation and liver damage [35–37]. In our research, visceral adiposity increased across tertiles of the HSI, concurring with the observation of a strong association between visceral adipose tissue and fatty liver infiltration [36,37].

Some investigations have demonstrated the effectiveness of physical activity in the prevention and management of chronic diseases [38]. In our study, the highest tertile of physical activity (>61.4-321.7 MET/hours/week) showed a lower HSI. In agreement with our results, some studies indicated that physical activity could attenuate and/or delay NAFLD progression [15,39,40]. A recent analysis of PREDIMED-PLUS data indicated that moderate-vigorous physical activity was inversely associated with cardiometabolic risk factors such as abdominal obesity and low HDL-c as independent components of the MetS [24]. Furthermore, higher physical activity was inversely related to NAFLD and participants who had a physical activity ≥ 500 MET/min/week showed a 34% decreased risk of NAFLD compared to

sedentary individuals [39]. In fact, physical inactivity and lower aerobic fitness could have a key role in mechanisms related to fat regulation and mitochondrial dysfunction [40]. It is important to highlight that physical activity is a modifiable risk factor, which might have a protective effect on liver status. Several mechanisms for the effects of physical activity on NAFLD have been proposed, but duration or the influence of the type of exercise treatment remains unclear [15].

Few intervention studies have explored the associations between MedDiet and NAFLD [13,18,19,41]. However, specific components consumed in the context of MedDiet have shown enough scientific evidence based on epidemiological, clinical trials and animal studies on CVD and MetS features [11,14]. This healthy dietary pattern provides nutrients and bioactive compounds with antioxidant capacity and anti-inflammatory effects [10,11,33,42]. The MedDiet pattern is characterized by a high consumption of fruit, vegetables, non-refined cereals, legumes, unsaturated fatty acids (olive oil and nuts); moderate intake of fish, seafood, fermented dairy products, poultry, and eggs; low-to-moderate amounts of wine, and low consumption of red meat, processed meat and sweets [9]. The consumption of most of the healthful components of the MedDiet is associated with an improvement of the serum lipid profile, insulin resistance, liver enzymes, and other factors linked to NAFLD [13,18,43,44]. According to our data, high adherence to the MedDiet was inversely and significantly associated with the HSI after adjusting for potential confounders. Such findings are consistent with a previous study showing that MedDiet ameliorated hepatic steatosis and improved insulin sensitivity [13,18]. In

contrast, Kontogianni et al. did not find differences in the adherence to MedDiet between individuals diagnosed with NAFLD and healthy subjects [19]. In fact, authors suggested that non-dietary factors have a strong impact on pathogenesis and development of this disease [19].

The association between light-moderate alcohol consumption and the severity and pathogenesis of NAFLD is still controversial [45]. Nevertheless, moderate alcohol consumption might improve insulin sensitivity and CDV mortality [45]. Ajmera and colleagues suggested that subjects diagnosed with NAFLD without NASH, the cardiovascular benefits of moderate alcohol consumption could have outweighed by injurious effects on liver status [45]. Moreover, modest wine consumption could reduce prevalence of suspected NAFLD (higher levels of ALT) in patients at high risk of coronary heart disease [46]. However, further quality clinical studies are crucial to better clarify the effects of moderate alcohol consumption on liver health, NASH histology and NAFLD severity. When we re-calculated the MedDiet adherence score without considering alcohol consumption, the inverse association between MedDiet and the HSI did not change. On the other hand, we noted that legume consumption decreases across tertiles of HSI. In fact, when our participants were stratified according to legume consumption tertiles, an apparent inverse association was found with the highest HSI values. Furthermore, we also observed that higher consumption of legumes was associated with 52% lower odds to be in the top HSI tertile, even after controlling for potential dietary and non-dietary confounders. These results are consistent with those of previous clinical studies that

evaluated the influence of legume intake on obesity and metabolic disorders [47,48]. In the PREDIMED study, it was prospectively found that greater legume intake (28 g/d) was associated with a lower risk of type 2 diabetes in subjects at high CVD risk [48]. Several authors have claimed that the beneficial effects of legume intake are attributed to the presence of vegetable protein, fibre, antioxidants, phytochemicals, and other bioactive compounds [47]. Legumes are particularly rich in fibre (soluble fibre and resistant starch) that might exert effects on digestibility and lowering absorption rates of carbohydrates, thereby improving glycemic control [47]. Moreover, a hypolipidemic effect of legumes has been observed promoting a reduction of intestinal fat absorption and bile acid uptake thus inducing a reduction of free fatty acids and cholesterol in the liver [49]. In this regard, those with greater legume intake presented a significantly lower risk of higher HSI values. This suggests that legume consumption could ameliorate metabolic disorders related to NAFLD in patients with MetS.

Only a few clinical studies have investigated the relationship between meat consumption and NAFLD risk [12][50]. The link between meat intake and risk of developing NAFLD and co-morbidities may rely on harmful meat components such as saturated fatty acid (SFA) and heme-iron [51]. However, our findings showed no differences in SFA intake among HSI tertiles. Indeed, when a multivariable analysis was fully adjusted, the relationship between total meat intake and HSI values was not statistically significant. This outcome may be attributed to differences in meat subtypes [52]. Thus, Zelber-Sagi et al. indicated that meat consumption, especially

red and processed meat, was independently associated with the increased risk of developing NAFLD and insulin resistance [53]. In contrast, a recent meta-analysis of observational studies reported an inverse association between white meat intake and MetS [52]. It is also important to highlight that red meat, beef internal organs, and processed meat contain more heme-iron than white meat [54]. More studies will be warranted in order to evaluate the role of specific meat subtypes in NAFLD.

The strengths of this analysis include the fact that it is the first study that uses a representative and relatively large sample of elders diagnosed with MetS within the PREDIMED-Plus cohort. Additionally, the study explored the potential association between modifiable lifestyle factors and NAFLD assessed by a non-invasive liver score used for larger-scale screening studies [6,7]. However, our research has some limitations. First, the cross-sectional and non-prospective design. Second, liver fat content was not directly measured. However, we used a validated non-invasive liver marker suitable for use in clinical practice as an alternative to imaging methods or liver biopsy. Third, our study sample was made up of aged Caucasians diagnosed with MetS. This status limits the extrapolation of our results to other populations, although it concerns patients at increased cardiometabolic risk that abound in all western countries.

Conclusions

This study suggests that lifestyle modifications focused on physical activity and fostering adherence to the Mediterranean Diet in senior adults diagnosed with MetS might exert beneficial effects on liver status. Moreover, some foods such as

legumes may play a beneficial role in the improvement of hepatic steatosis reducing the risk of NAFLD. Our findings support the recommendation of lifestyle changes (nutrition and physical activity) as a cornerstone for the prevention and precise management of NAFLD in patients with MetS.

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Authorship

Author Contributions: VBV; IA; MAZ; JAM; were involved in conceptualization; design; acquisition of data; analysis and interpretation of data; writing-original draft preparation; critical revision of the manuscript for important intellectual content. JAT; XP; EC; M.AM-G; ET; DC; MM; FT; MF; RE; ER; JS-S; LD; were involved in study design, acquisition of data, interpretation data; critical revision of the manuscript for important intellectual content.

All authors approved the final version of the manuscript.

Conflicts of Interest

None of the authors reported a conflict of interest.

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625

Table 1. Main characteristics of subjects diagnosed with metabolic syndrome according to Hepatic Steatosis Index tertiles (HSI)

	Total (n=328)	T1 (n=110)	T2 (n=109)	T3 (n=109)	P value
Men, n(%)	180 (54.9)	60 (54.6)	60 (55.1)	60 (55.1)	0.996
Age (years)	65.8 (65.2-66.4)	66.2 (65.2-67.1)	66.3 (65.4-67.3)	64.9 (64.0-65.9)	0.082
BMI (kg/m ²)	32.2 (31.8-32.5)	29.5 (29.1-29.9)	31.7 (31.3-32.1)	35.3 (34.8-35.7)	<0.001
Weight (kg)	86.1 (84.8-87.4)	79.6 (77.6-81.5)	84.4 (82.4-86.3)	94.3 (92.4-96.3)	<0.001
Waist circumference (cm)	107.1 (106.1-108.1)	101.4 (100.0-102.8)	106.1 (104.7-107.5)	113.8 (112.4-115.2)	<0.001
Glucose (mg/dL)	119.3 (115.7-123.0)	108.5 (102.4-114.5)	119.6 (113.6-125.6)	130.1 (124.1-136.2)	<0.001
HbA1c (%)	6.1 (6.0-6.2)	5.9 (5.7-6.0)	6.2 (6.0-6.4)	6.3 (6.1-6.5)	0.001
TyG index	9.0 (8.9-9.0)	8.8 (8.7-8.8)	9.0 (8.9-9.1)	9.2 (9.1-9.3)	<0.001
SBP (mmHg)	142.0 (140.2-143.6)	141.2 (138.3-144.2)	143.6 (140.6-146.6)	140.9 (137.9-143.9)	0.390
DBP (mmHg)	86.2 (85.3-87.2)	84.9 (83.2-86.5)	86.3 (84.7-88.0)	87.5 (85.5-89.1)	0.093
Diabetes, n (%)	125 (38.1)	22 (20.0)	46 (42.2)	57 (52.3)	<0.001
High blood pressure or hypertensive medication, n (%)	318 (97.0)	104 (94.6)	106 (97.3)	108 (99.1)	0.145
Smoking status, n (%)					
Never smoker	133 (40.6)	52 (47.3)	43 (39.5)	38 (34.9)	0.134
Former smoker	154 (47.0)	44 (40.0)	49 (45.0)	61 (56.0)	
Current smoker	41 (12.5)	14 (12.7)	17 (15.6)	10 (9.2)	
Alcohol intake (g/d)	12.0 (10.1-13.8)	9.7 (6.6-12.9)	12.6 (9.5-15.8)	13.5 (10.3-16.7)	0.221
HSI (arbitrary units)	43.1 (42.5-43.6)	38.1 (37.6-38.5)	42.6 (42.2-43.1)	48.5 (48.1-49.0)	<0.001

Data are presented as means (95%CI) and frequencies (%), p<0.05 is considered as statistically significant.

Abbreviations: HSI, Hepatic Steatosis Index; BMI, body mass index; HbA1C, hemoglobin A1c; TyG index, triglycerides and glucose index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Lipid profile, DXA estimation and lifestyle information according to Hepatic Steatosis Index tertiles (HSI) in subjects with metabolic syndrome

	T1 (n=110)	T2 (n=109)	T3 (n=109)	P value
Lipid profile				
Total cholesterol (mg/dL)	194.7 (187.8-201.5) ^{a,b}	207.0 (200.1-213.8)	202.4 (195.5-209.3)	0.045
LDL-c (mg/dL)	123.2 (117.0-129.5)	131.1 (124.8-137.5)	125.9 (119.6-132.2)	0.212
HDL-c (mg/dL)	46.4 (44.5-48.4)	47.4 (45.4-49.3)	45.2 (43.2-47.1)	0.291
Triglycerides (mg/dL)	128.5 (116.6-140.4) ^{a,c}	149.0 (137.0-160.9)	161.9 (149.9-173.8)	<0.001
TG/HDL cholesterol ratio	3.0 (2.6-3.3) ^{a,c}	3.5 (3.1-3.9)	3.9 (3.5-4.3)	0.004
VLDL-c (mg/dL)	25.7 (23.3-28.1) ^{a,c}	29.8 (27.4-32.2)	32.4 (30.0-34.8)	<0.001
DXA estimation				
Total fat (kg)	28.7 (27.5-29.9) ^{a,b/a,c}	32.9 (31.7-34.1) ^{b,c}	40.1 (38.8-41.3)	<0.001
Trunk fat (kg)	17.3 (16.6-18.1) ^{a,b/a,c}	20.1 (19.4-20.8) ^{b,c}	24.4 (23.7-25.1)	<0.001
Android fat (kg)	3.1 (2.9-3.2) ^{a,b/a,c}	3.6 (3.5-3.8) ^{b,c}	4.4 (4.3-4.6)	<0.001
Gynoid fat (kg)	4.1 (3.9-4.4) ^{a,b/a,c}	4.7 (4.4-4.9) ^{b,c}	5.9 (5.6-6.1)	<0.001
Visceral fat (Kg)	2.0 (1.8-2.1) ^{a,b/a,c}	2.4 (2.2-2.5) ^{b,c}	2.8 (2.6-3.0)	<0.001
Lifestyle variables				
Physical activity (MET/hours/week)	58.5 (50.4-66.6) ^{a,c}	51.4 (43.3-59.6)	41.1 (32.9-49.3)	0.014
MedDiet Score (0-9)	4.7 (4.4-5.0) ^{a,c}	4.4 (4.1-4.7)	4.1 (3.8-4.4)	0.015

p<0.05 is considered statistically significant. Data are expressed as mean (95% CI). Values were adjusted for age, total energy intake and alcohol intake as continuous covariates.

a,b significant differences between T1 vs T2.

a,c significant differences between T1 vs T3.

b,c significant differences between T2 vs T3.

DXA measurements available in 268 patients (T1=85), (T2=92), (T3=89), visceral fat available in 252 patients (T1=81), (T2=88), (T3=83).

Abbreviations: HSI, Hepatic Steatosis Index; LDL-c, Low density lipoprotein cholesterol; HDL-c, High density lipoprotein cholesterol; TG/HDL cholesterol ratio, triglycerides/ High density lipoprotein cholesterol ratio; VLDL-c, Very-low-density lipoprotein cholesterol; MET, Metabolic Equivalent; MedDiet, Mediterranean Diet.

Table 3. Linear regression analyses model, exploring the association between physical activity and Mediterranean Diet adherence (as independent factors with the hepatic steatosis index (HSI) (as dependent factor) in subjects with metabolic syndrome

Physical activity (MET/hours/week)							
T1 (0-22.5)		T2 (>22.5-≤61.4)		T3 (>61.4-321.7)		R ² Adjusted	p for trend
		Regression coefficient	95% CI	Regression coefficient	95% CI		
Crude	0 Ref.	-1.51	(-2.79 -0.23)	-2.24	(-3.52 -0.96)	0.031	<0.001
Model 1	0 Ref.	-1.46	(-2.74 -0.19)	-2.11	(-3.39 -0.83)	0.037	<0.001
Model 2	0 Ref.	-1.54	(-2.81 -0.27)	-2.11	(-3.39 -0.83)	0.047	<0.001
Model 3a	0 Ref.	-1.47	(-2.73 -0.20)	-1.93	(-3.22 -0.65)	0.057	0.001
Mediterranean diet adherence (0-9 points)							
Low (0-3)		Moderate (4-5)		High (6-9)		R ² Adjusted	p for trend
		Regression coefficient	95% CI	Regression coefficient	95% CI		
Crude	0 Ref.	-1.08	(-2.31 0.15)	-1.89	(-3.32 -0.45)	0.015	0.010
Model 1	0 Ref.	-0.92	(-2.16 0.31)	-1.83	(-3.27 -0.40)	0.024	0.014
Model 2	0 Ref.	-0.72	(-1.97 0.52)	-1.88	(-3.33 -0.43)	0.033	0.016
Model 3b	0 Ref.	-0.70	(-1.92 0.53)	-1.57	(-3.01 -0.13)	0.061	0.041

Model 1: Adjusted for age, as continuous covariate.

Model 2: Adjusted for age, energy intake and alcohol consumption as continuous covariates and smoking status and high blood pressure or taking treatment as categorical covariates.

Model 3a: model 2 + MedDiet adherence as continuous covariate.

Model 3b: model 2 + physical activity as continuous covariate.

Abbreviations: HSI, Hepatic Steatosis Index; MET, Metabolic Equivalent.

Table 4. Food group and dietary intake according to Hepatic Steatosis Index tertiles (HSI) in subjects with metabolic syndrome

	T1 (n=110)	T2 (n=109)	T3 (n=109)	P value
Energy, macronutrients and fiber intake				
Total energy (Kcal/d)	2606 (2511-2702)	2559 (2463-2655)	2610 (2514-2706)	0.717
Carbohydrate (g/d)	281.1 (273.9-288.2)	284.4 (277.3-291.6)	277.9 (270.8-285.1)	0.456
Protein (g/d)	100.8 (98.2-103.4)	102.2 (99.7-104.8)	103.9 (101.3-106.5)	0.255
Lipid (g/d)	109.0 (105.8-112.2)	106.8 (103.6-110.1)	109.0 (105.8-112.3)	0.570
Monounsaturated lipids	55.5 (53.4-57.5)	54.0 (52.0-56.1)	56.0 (53.9-58.0)	0.399
Saturated lipids	26.7 (25.7-27.7)	26.4 (25.3-27.4)	27.9 (26.8-28.9)	0.103
Monounsaturated/ saturated ratio	2.2 (2.1-2.2)	2.1 (2.0-2.2)	2.1 (2.0-2.2)	0.483
Total fiber (g/d)	30.2 (28.7-31.7)	30.2 (28.6-31.7)	29.3 (27.7-30.8)	0.662
Foods and nutrient intake				
Dairy products (g/d)	361.1 (321.4-400.8)	418.0 (378.2-457.9)	383.4 (343.5-423.4)	0.137
Legumes (g/d)	21.6 (20.0-23.2) ^{a,c}	19.8 (18.2-21.5)	18.6 (16.9-20.2)	0.035
Meat (g/d)	144.0 (134.8-153.1) ^{a,c}	144.5 (135.4-153.7) ^{b,c}	161.4 (152.2-170.6)	0.013
Fruits (g/d)	447.2 (404.4- 490.0)	449.0 (406.0-492.0)	394.1 (350.9-437.2)	0.137
Vegetables (g/d)	333.2 (310.6-355.8)	328.0 (305.3-350.7)	332.5 (309.7-355.3)	0.942
Cereals (g/d)	201.1 (187.1-215.1)	202.5 (188.5-216.6)	198.2 (184.1-212.3)	0.909
Fish and seafoods (g/d)	102.1 (94.2-110.1)	95.6 (87.7-103.6)	98.0 (90.0-106.0)	0.516

p<0.05 is considered statistically significant. Data are expressed as mean (95% CI). Values were adjusted for total age, energy intake except for energy intake and alcohol intake as continuous covariates.

a,c significant differences between T1 vs T3.

b,c significant differences between T2 vs T3.

Table 5. Multivariate analysis concerning the associations between legume consumption and NAFLD according to Hepatic Steatosis Index (HSI) in subjects featured with metabolic syndrome

<i>Hepatic Steatosis Index</i>						
		T1	T2		T3	
	N		RRR (95% CI)	p value	RRR (95% CI)	p value
Legume (g/d)						
Crude						
Tertile 1 (≤ 16.1)	139	1 Ref.	1 Ref.		1 Ref.	
Tertile 2 (>16.1 - ≤ 20.8)	93	1 Ref.	0.89(0.47 to 1.68)	0.713	0.51(0.27 to 0.99)	0.046
Tertile 3 (>20.8)	96	1 Ref.	0.74(0.39 to 1.41)	0.364	0.53(0.28 to 0.99)	0.049
Model 1						
Tertile 1 (≤ 16.1)	139	1 Ref.	1 Ref.		1 Ref.	
Tertile 2 (>16.1 - ≤ 20.8)	93	1 Ref.	0.94(0.49 to 1.80)	0.846	0.50(0.26 to 0.99)	0.048
Tertile 3 (>20.8)	96	1 Ref.	0.81(0.42 to 1.59)	0.548	0.54(0.27 to 1.06)	0.072
Model 2						
Tertile 1 (≤ 16.1)	139	1 Ref.	1 Ref.		1 Ref.	
Tertile 2 (>16.1 - ≤ 20.8)	93	1 Ref.	0.85(0.44 to 1.66)	0.638	0.45(0.22 to 0.92)	0.028
Tertile 3 (>20.8)	96	1 Ref.	0.74(0.37 to 1.46)	0.382	0.48(0.24 to 0.97)	0.041

Model 1: Adjusted for age, energy intake, and alcohol consumption as continuous covariates, and smoking status as categorical covariate.

Model 2: model 1 + triglycerides and physical activity as continuous covariates.

Abbreviations: HSI, Hepatic Steatosis Index; RRR, relative risk ratio.