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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, β= -1.47 (95%CI -2.73 to -0.20); T3, β= -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 β= -0.70 (95%CI: -1.92 to 0.53), high adherence β= -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/m^2 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-

131 restricted MedDiet, physical activity promotion, and behavioural support) or into a
132 control group (an energy-unrestricted MedDiet). (http://medpreventiva.es/QufSWn).
133 This clinical trial was registered (ISRCTN89898870) and conducted in accordance
134 with the Declaration of Helsinki ethical disclosure and further guidelines. All
135 participants signed an informed consent to participate at the beginning of the
136 intervention trial. The present investigation is a cross-sectional sub-study with
137 baseline data of participants from the Navarra-Nutrition centre. The sample size was
138 calculated to find a correlation coefficient with an 80% statistical power between
139 adherence to MedDiet and hepatic steatosis (r=0.20) considering a type I error of
140 5% and type II error of 10%. A total of 422 participants were registered in the pre-
141 inclusion period. Of these, we excluded 2 individuals who did not meet inclusion
142 criteria and 89 who declined participation or for other reasons. Three hundred thirty-
143 one individuals were included, but 328 had valid data for the non-invasive liver score
144 calculation, which is a number that has been shown suitable in comparable studies
145 [18,19].
146
147 **Study measurements**
148 ***Dietary assessment***
149 At baseline, trained dietitians administered face-to-face a 143-item food frequency
150 questionnaire to estimate dietary intake over last year, which was previously
151 validated in Spanish population [20]. In order to evaluate the adherence to the
152 MedDiet, a score based on nine dietary components was applied, as described

153 elsewhere [21,22]. For beneficial components (vegetables, fruits and mixed nuts,
154 legumes, cereals, fish and seafood), participants were assigned a value of 0 for the
155 consumption below the component sex-specific median and above the median were
156 assigned a value of 1 as well as for fat intake considering the ratio [MUFA/SFA].
157 Meanwhile, for components presumed to be detrimental (meat and dairy products),
158 individuals were assigned a value of 1 for the consumption below the component
159 sex-specific median and above the median were assigned a value of 0. For the
160 alcohol component, a value of 1 was assigned to men consuming 10 to <50 g/d and
161 women consuming 5 to <25 g/d and 0, otherwise. Thus, the total MedDiet
162 punctuation ranged from 0 (minimum adherence) to 9 (maximum adherence).
163 MedDiet adherence was categorized into low (0-3 points), moderate (4-5 points) or
164 high (6-9 points) adherence for analytical purposes [22].
165
166 ***Physical activity assessment***
167 Physical activity was assessed using the short REGICOR (Registre Gironi del Cor),
168 which was validated in Spanish population [23]. As described previously [24], this
169 questionnaire evaluated the total energy expenditure in leisure time physical activity
170 (Metabolic Equivalent (MET)-minute/week) considering light (<4 MET), moderate (4-
171 5.5 MET) and vigorous (≥6 MET) physical activity. Also, the number of weekly hours
172 of sedentary behavior [25]. For this study, physical activity was expressed as MET-
173 hour-week and categorized by tertiles.
174

175 ***Sociodemographic, lifestyle, and clinical variables***

176 At baseline, sociodemographic, lifestyle, history of illnesses and medication data
177 were collected during the personal interview with standardized questionnaires.
178 Smoking status was categorized as never, former, or current smoker. Trained
179 dietitians measured weight and height using calibrated equipment following the
180 PREDIMED PLUS standardized protocol [16]. The body mass index (BMI) was
181 calculated as the body weight divided by the squared height (kg/m^2). Determinations
182 of fat mass (total, trunk, android, gynoid and visceral) were performed using dual-
183 energy X-ray absorptiometry (Lunar iDXA ™, Madison, WI, USA connected with
184 enCore™ software, version 6.0) by trained personnel following the instructions of the
185 equipment as described elsewhere [26]. Overnight fasting blood was collected.
186 Serum and plasma samples were immediately frozen at -80˚C. Biochemical
187 variables, including alanine aminotransferase (ALT), aspartate aminotransferase
188 (AST), total cholesterol (CT), high-density lipoprotein cholesterol (HDL-c),
189 triglyceride (TG), glucose, and hemoglobin A1c (HbA1c), were determined with
190 specific kits according to manufacturer´s protocols, as previously described, [26,27]
191 while low-density lipoprotein cholesterol (LDL-c) and very-low-density lipoprotein
192 cholesterol (VLDL-c) were calculated using the Friedewald formula and
193 triglycerides/5, respectively [28]. The triglyceride-glucose index (TyG index) was
194 estimated as the logarithm of fasting triglyceride (mg/dL) x fasting glucose (mg/dL)/2
195 [29]. The MetS status was defined when at least 3 or more of the components were
196 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* ALT/AST + BMI + (+ 2, if type 2 diabetes, 0 otherwise) + (+ 2, if female, 0 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

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

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

355 The association between light-moderate alcohol consumption and the
356 severity and pathogenesis of NAFLD is still controversial [45]. Nevertheless,
357 moderate alcohol consumption might improve insulin sensitivity and CDV mortality
358 [45]. Ajmera and colleges suggested that subjects diagnosed with NAFLD without
359 NASH, the cardiovascular benefits of moderate alcohol consumption could have
360 outweighed by injurious effects on liver status [45]. Moreover, modest wine
361 consumption could reduce prevalence of suspected NAFLD (higher levels of ALT) in
362 patients at high risk of coronary heart disease [46]. However, further quality clinical
363 studies are crucial to better clarify the effects of moderate alcohol consumption on
364 liver health, NASH histology and NAFLD severity. When we re-calculated the
365 MedDiet adherence score without considering alcohol consumption, the inverse
366 association between MedDiet and the HSI did not change. On other hand, we noted
367 that legume consumption decreases across tertiles of HSI. In fact, when our
368 participants were stratified according to legume consumption tertiles, an apparent
369 inverse association was found with the highest HSI values. Furthermore, we also
370 observed that higher consumption of legumes was associated with 52% lower odds
371 to be in the top HSI tertile, even after controlling for potential dietary and non-dietary
372 confounders. These results are consistent with those of previous clinical studies that

373 evaluated the influence of legume intake on obesity and metabolic disorders [47,48].
374 In the PREDIMED study, it was prospectively found that greater legume intake (28
375 g/d) was associated with a lower risk of type 2 diabetes in subjects at high CVD risk
376 [48]. Several authors have claimed that the beneficial effects of legume intake are
377 attributed to the presence of vegetable protein, fibre, antioxidants, phytochemicals,
378 and other bioactive compounds [47]. Legumes are particularly rich in fibre (soluble
379 fibre and resistant starch) that might exert effects on digestibility and lowering
380 absorption rates of carbohydrates, thereby improving glycemic control [47].
381 Moreover, a hypolipidemic effect of legumes has been observed promoting a
382 reduction of intestinal fat absorption and bile acid uptake thus inducing a reduction
383 of free fatty acids and cholesterol in the liver [49]. In this regard, those with greater
384 legume intake presented a significantly lower risk of higher HSI values. This
385 suggests that legume consumption could ameliorate metabolic disorders related to
386 NAFLD in patients with MetS.

387 Only a few clinical studies have investigated the relationship between meat
388 consumption and NAFLD risk [12][50]. The link between meat intake and risk of
389 developing NAFLD and co-morbidities may rely on harmful meat components such
390 as saturated fatty acid (SFA) and heme-iron [51]. However, our findings showed no
391 differences in SFA intake among HSI tertiles. Indeed, when a multivariable analysis
392 was fully adjusted, the relationship between total meat intake and HSI values was
393 not statistically significant. This outcome may be attributed to differences in meat
394 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.

References

[1] Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018;15:11–20. doi:10.1038/nrgastro.2017.109.

[2] Rinella ME. Nonalcoholic Fatty Liver Disease. JAMA 2015;313:2263. doi:10.1001/jama.2015.5370.

[3] Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. J Hepatol 2016;65:425–43. doi:10.1016/j.jhep.2016.04.005.

[4] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–57. doi:10.1002/hep.29367.

[5] Gan L, Chitturi S, Farrell GC. Mechanisms and implications of age-related changes in the liver: nonalcoholic Fatty liver disease in the elderly. Curr Gerontol Geriatr Res 2011;2011:831536. doi:10.1155/2011/831536.

[6] Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2014;40:1209–22.

460 doi:10.1111/apt.12963.
461 [7] Bugianesi E, Rosso C, Cortez-Pinto H. How to diagnose NAFLD in 2016. J
462 Hepatol 2016;65:643–4. doi:10.1016/j.jhep.2016.05.038.
463 [8] Lee J-H, Kim D, Kim HJ, Lee C-H, Yang JI, Kim W, et al. Hepatic steatosis
464 index: A simple screening tool reflecting nonalcoholic fatty liver disease. Dig
465 Liver Dis 2010;42:503–8. doi:10.1016/j.dld.2009.08.002.
466 [9] Ros E, Martínez-González MA, Estruch R, Salas-Salvadó J, Fitó M, Martínez
467 JA, et al. Mediterranean Diet and Cardiovascular Health: Teachings of the
468 PREDIMED Study. Adv Nutr 2014;5:330S-336S. doi:10.3945/an.113.005389.
469 [10] Estruch R. Anti-inflammatory effects of the Mediterranean diet: the experience
470 of the PREDIMED study. Proc Nutr Soc 2010;69:333–40.
471 doi:10.1017/S0029665110001539.
472 [11] Godos J, Federico A, Dallio M, Scazzina F. Mediterranean diet and
473 nonalcoholic fatty liver disease: molecular mechanisms of protection. Int J
474 Food Sci Nutr 2017;68:18–27. doi:10.1080/09637486.2016.1214239.
475 [12] Eslamparast T, Tandon P, Raman M. Dietary composition independent of
476 weight loss in the management of non-alcoholic fatty liver disease. Nutrients
477 2017;9(8):800. doi:10.3390/nu9080800.
478 [13] Pintó X, Fanlo-Maresma M, Corbella E, Corbella X, Mitjavila MT, Moreno JJ,
479 et al. A Mediterranean Diet Rich in Extra-Virgin Olive Oil Is Associated with a
480 Reduced Prevalence of Nonalcoholic Fatty Liver Disease in Older Individuals
481 at High Cardiovascular Risk. J Nutr 2019:1–10. doi:10.1093/jn/nxz147.

[14] Zelber-Sagi S, Salomone F, Mlynarsky L. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. Liver Int 2017;37:936–49. doi:10.1111/liv.13435.

[15] Whitsett M, VanWagner LB. Physical activity as a treatment of non-alcoholic fatty liver disease: A systematic review. World J Hepatol 2015;7:2041–52. doi:10.4254/wjh.v7.i16.2041.

[16] Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort Profile: Design and methods of the PREDIMED-Plus randomized trial. Int J Epidemiol 2019;48:387-388o. doi:10.1093/ije/dyy225.

[17] Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the Metabolic Syndrome. Circulation 2009;120:1640–5. doi:10.1161/CIRCULATIONAHA.109.192644.

[18] Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. J Hepatol 2013;59:138–43.

[19] Kontogianni MD, Tileli N, Margariti A, Georgoulis M, Deutsch M, Tiniakos D, et al. Adherence to the Mediterranean diet is associated with the severity of non-alcoholic fatty liver disease. Clin Nutr 2014;33:678–83. doi:10.1016/j.clnu.2013.08.014.

[20] Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 2010;103:1808–16.

504 doi:10.1017/S0007114509993837.
505 [21] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a
506 Mediterranean diet and survival in a Greek population. N Engl J Med
507 2003;348:2599–608. doi:10.1056/NEJMoa025039.
508 [22] Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocké MC, Peeters
509 PH, et al. Modified Mediterranean diet and survival: EPIC-elderly prospective
510 cohort study. BMJ 2005;330:991. doi:10.1136/bmj.38415.644155.8F.
511 [23] Molina L, Sarmiento M, Peñafiel J, Donaire D, Garcia-Aymerich J, Gomez M,
512 et al. Validation of the Regicor Short Physical Activity Questionnaire for the
513 Adult Population. PLoS One 2017;12:e0168148.
514 doi:10.1371/journal.pone.0168148.
515 [24] Rosique-Esteban N, Díaz-López A, Martínez-González MA, Corella D, Goday
516 A, Martínez JA, et al. Leisure-time physical activity, sedentary behaviors,
517 sleep, and cardiometabolic risk factors at baseline in the PREDIMED-PLUS
518 intervention trial: A cross-sectional analysis. PLoS One 2017;12:e0172253.
519 doi:10.1371/journal.pone.0172253.
520 [25] Martínez-González MA, López-Fontana C, Varo JJ, Sánchez-Villegas A,
521 Martinez JA. Validation of the Spanish version of the physical activity
522 questionnaire used in the Nurses' Health Study and the Health Professionals'
523 Follow-up Study. Public Health Nutr 2005;8:920–7. doi:10.1079/phn2005745.
524 [26] Konieczna J, Abete I, Galmés AM, Babio N, Colom A, Zulet MA, et al. Body
525 adiposity indicators and cardiometabolic risk: Cross-sectional analysis in

participants from the PREDIMED-Plus trial. Clin Nutr 2018:1–9. doi:10.1016/j.clnu.2018.07.005.

[27] Salas-Salvadó J, Díaz-López A, Ruiz-Canela M, Basora J, Fitó M, Corella D, et al. Effect of a Lifestyle Intervention Program With Energy-Restricted Mediterranean Diet and Exercise on Weight Loss and Cardiovascular Risk Factors: One-Year Results of the PREDIMED-Plus Trial. Diabetes Care 2018:dc180836. doi:10.2337/dc18-0836.

[28] Bairaktari ET, Seferiadis KI, Elisaf MS. Evaluation of Methods for the Measurement of Low-Density Lipoprotein Cholesterol. J Cardiovasc Pharmacol Ther 2005;10:45–54. doi:10.1177/107424840501000106.

[29] Navarro-González D, Sánchez-Íñigo L, Pastrana-Delgado J, Fernández-Montero A, Martinez JA. Triglyceride–glucose index (TyG index) in comparison with fasting plasma glucose improved diabetes prediction in patients with normal fasting glucose: The Vascular-Metabolic CUN cohort. Prev Med (Baltim) 2016;86:99–105.

[30] Atroshenko SA, Korolyov IA, Didenko N. Standards of Medical Care in Diabetes--2014. Diabetes Care 2014;37:S14–80. doi:10.2337/dc14-S014.

[31] Kim D, Touros A, Kim WR. Nonalcoholic Fatty Liver Disease and Metabolic Syndrome. Clin Liver Dis 2018;22:133–40. doi:10.1016/j.cld.2017.08.010.

[32] Bullón-Vela MV, Abete I, Martínez JA, Zulet MA. Chapter 6 - Obesity and Nonalcoholic Fatty Liver Disease: Role of Oxidative Stress. In obesity: Oxidative Stress and Dietary Antioxidants; Marti del Moral A, García CMA,

548 Eds.; Elsevier: London, UK, 2018; pp. 111-133.
549 doi:https://doi.org/10.1016/B978-0-12-812504-5.00006-4.
550 [33] Cantero I, Abete I, Babio N, Arós F, Corella D, Estruch R, et al. Dietary
551 Inflammatory Index and liver status in subjects with different adiposity levels
552 within the PREDIMED trial. Clin Nutr 2018;37:1736–43.
553 doi:10.1016/j.clnu.2017.06.027.
554 [34] Zheng R, Du Z, Wang M, Mao Y, Mao W. A longitudinal epidemiological study
555 on the triglyceride and glucose index and the incident nonalcoholic fatty liver
556 disease. Lipids Health Dis 2018;17:1–9. doi:10.1186/s12944-018-0913-3.
557 [35] Mendez-Sanchez N, Cruz-Ramon VC, Ramirez-Perez OL, Hwang JP,
558 Barranco-Fragoso B, Cordova-Gallardo J. New aspects of lipotoxicity in
559 nonalcoholic steatohepatitis. Int J Mol Sci 2018;19.
560 doi:10.3390/ijms19072034.
561 [36] Yu AH, Duan-Mu YY, Zhang Y, Wang L, Guo Z, Yu YQ, et al. Correlation
562 between non-alcoholic fatty liver disease and visceral adipose tissue in non-
563 obese chinese adults: A CT evaluation. Korean J Radiol 2018;19:923–9.
564 doi:10.3348/kjr.2018.19.5.923.
565 [37] Jang S, Lee CH, Choi KM, Lee J, Choi JW, Kim KA, et al. Correlation of fatty
566 liver and abdominal fat distribution using a simple fat computed tomography
567 protocol. World J Gastroenterol 2011;17:3335–41.
568 doi:10.3748/wjg.v17.i28.3335.
569 [38] Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity:

570 the evidence. CMAJ 2006;174:801–9. doi:10.1503/cmaj.051351.
571 [39] Kwak M, Kim D, Chung GE, Kim W, Kim YJ, Yoon J. Role of physical activity
572 in nonalcoholic fatty liver disease in terms of visceral obesity and insulin
573 resistance. Liver Int 2015;35:944–52. doi:10.1111/liv.12552.
574 [40] Gonçalves IO, Oliveira PJ, Ascensão A, Magalhães J. Exercise as a
575 therapeutic tool to prevent mitochondrial degeneration in nonalcoholic
576 steatohepatitis. Eur J Clin Invest 2013;43:1184–94. doi:10.1111/eci.12146.
577 [41] Kalafati I-P, Borsa D, Dimitriou M, Revenas K, Kokkinos A, Dedoussis GV.
578 Dietary patterns and non-alcoholic fatty liver disease in a Greek case–control
579 study. Nutrition 2019;61:105–10. doi:10.1016/j.nut.2018.10.032.
580 [42] Cantero I, Abete I, Monreal JI, Martinez JA, Zulet MA. Fruit fiber consumption
581 specifically improves liver health status in obese subjects under energy
582 restriction. Nutrients 2017;9. doi:10.3390/nu9070667.
583 [43] Tzima N, Pitsavos C, Panagiotakos DB, Chrysohoou C, Polychronopoulos E,
584 Skoumas J, et al. Adherence to the Mediterranean diet moderates the
585 association of aminotransferases with the prevalence of the metabolic
586 syndrome; the ATTICA study. Nutr Metab (Lond) 2009;6:30.
587 doi:10.1186/1743-7075-6-30.
588 [44] Bacchetti T, Turco I, Urbano A, Morresi C, Ferretti G. Relationship of fruit and
589 vegetable intake to dietary antioxidant capacity and markers of oxidative
590 stress: A sex-related study. Nutrition 2019;61:164–72.
591 doi:10.1016/j.nut.2018.10.034.

592 [45] Ajmera VH, Terrault NA, Harrison SA. Is moderate alcohol use in nonalcoholic
593 fatty liver disease good or bad? A critical review. Hepatology 2017;65:2090–
594 9. doi:10.1002/hep.29055.
595 [46] Dunn W, Xu R, Schwimmer JB. Modest wine drinking and decreased
596 prevalence of suspected nonalcoholic fatty liver disease. Hepatology
597 2008;47:1947–54. doi:10.1002/hep.22292.
598 [47] Rebello CJ, Greenway FL, Finley JW. A review of the nutritional value of
599 legumes and their effects on obesity and its related co-morbidities. Obes Rev
600 2014;15:392–407. doi:10.1111/obr.12144.
601 [48] Papandreou C, Becerra-Tomás N, Bulló M, Martínez-González MÁ, Corella D,
602 Estruch R, et al. Legume consumption and risk of all-cause, cardiovascular,
603 and cancer mortality in the PREDIMED study. Clin Nutr 2018 Jun 9 [Epub
604 ahead of print]. doi:10.1016/j.clnu.2017.12.019.
605 [49] Rideout TC, Harding S V, Jones PJH, Fan MZ. Guar gum and similar soluble
606 fi bers in the regulation of cholesterol metabolism : Current understandings
607 and future research priorities 2008;4:1023–33.
608 [50] Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss Isakov N, Webb M, Orenstein D,
609 Shibolet O, et al. High red and processed meat consumption is associated with
610 non-alcoholic fatty liver disease and insulin resistance. J Hepatol
611 2018;68:1239–46. doi:10.1016/j.jhep.2018.01.015.
612 [51] Zelber-Sagi S, Salomone F, Mlynarsky L. The Mediterranean dietary pattern
613 as the diet of choice for non-alcoholic fatty liver disease: Evidence and

plausible mechanisms. Liver Int 2017;37:936–49. doi:10.1111/liv.13435.

[52] Kim Y, Je Y. Meat Consumption and Risk of Metabolic Syndrome: Results from the Korean Population and a Meta-Analysis of Observational Studies. Nutrients 2018;10:E390. doi:10.3390/nu10040390.

[53] Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss Isakov N, Webb M, Orenstein D, Shibolet O, et al. High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. J Hepatol 2018;68:1239–46. doi:10.1016/j.jhep.2018.01.015.

[54] Kongkachuichai R, Napatthalung P, Charoensiri R. Heme and nonheme iron content of animal products commonly consumed in Thailand. J Food Compos Anal 2002;15:389–98. doi:10.1006/jfca.2002.1080.

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

	Total	**T1**	**T2**	**T3**	**P value**
	(n=328)	**(n=110)**	**(n=109)**	**(n=109)**	
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
Gynoide 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

		Hepatic Steatosis Index				
		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.