

1 **Dietary Inflammatory Index and liver status in subjects with different adiposity levels**
2 **within the PREDIMED trial**

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43 **Background & Aims:** To assess the possible association between a validated Dietary
44 Inflammatory Index (DII) and specific dietary components with suitable non-invasive markers
45 of liver status in overweight and obese subjects within the PREDIMED study.

46 **Methods:** A cross-sectional study encompassing 794 randomized overweight and obese
47 participants (mean \pm SD age: 67.0 \pm 5.0 y, 55% females) from the PREDIMED (PREvención
48 con DIeta MEDiterránea) trial was conducted. DII is a validated tool evaluating the effect of
49 diet on six inflammatory biomarkers (IL-1b, IL-4, IL-6, IL-10, TNF- α and C-reactive
50 protein). Furthermore, a validated 137-item food-frequency-questionnaire was used to obtain
51 the information about the food intake. In addition, anthropometric measurements and several
52 non-invasive markers of liver status were assessed and the Fatty Liver Index (FLI) score was
53 calculated.

54 **Results:** A higher DII and lower adherence to Mediterranean diet (MeDiet) were associated
55 with a higher degree of liver damage (FLI>60) in obese as compared to overweight
56 participants. Furthermore, the DII score was positively associated with relevant non-invasive
57 liver markers (ALT, AST, GGT and FLI) and directly affected FLI values. Interestingly, a
58 positive correlation was observed between liver damage (>50th percentile FLI) and nutrients
59 and foods linked to a pro-inflammatory dietary pattern.

60 **Conclusions:** This study reinforced the concept that obesity is associated with liver damage
61 and revealed that the consumption of a pro-inflammatory dietary pattern might contribute to
62 obesity and fatty liver disease features. These data suggest that a well-designed precision diet
63 including putative anti-inflammatory components could specifically prevent and ameliorate
64 non-alcoholic fatty liver manifestations in addition to obesity.

65

66 **Keywords:** liver, diet, inflammation, NAFLD, obesity.

67 **INTRODUCTION**

68 Non-alcoholic fatty liver disease (NAFLD) is a condition of excessive hepatic lipid accumulation
69 in subjects that consume less than 20g ethanol per day, without other known causes such as
70 drugs prescription or exposure to toxins (1). In developed countries, NAFLD affects
71 approximately 20-30% in the general adult population. The term NAFLD encompasses a range
72 of conditions, from simple steatosis to non-alcoholic steatohepatitis (NASH), which
73 eventually can lead to cirrhosis and, in some cases, hepatocellular carcinoma (1). Liver biopsy
74 is considered the “gold standard” of steatosis, fibrosis and cirrhosis. However, it is rarely
75 performed because it is an invasive procedure with a significant degree of sampling error;
76 therefore, investigators and clinicians are focusing on the design and application of non-
77 invasive liver damage markers and scores for diagnosis (2).

78 NAFLD has been related with obesity, insulin resistance, hypertension and dyslipidemia, and
79 it is regarded as a key liver manifestation of the metabolic syndrome (3). In fact, excessive
80 body mass index (BMI kg/m²) and visceral fat (%) are known to be important risk factors in
81 NAFLD onset and at least two-thirds of population with obesity and diabetes show hepatic
82 steatosis (4). In addition, inflammation has been hypothesized as an underlying mechanism in
83 the link between obesity and NAFLD. The pathogenesis of NAFLD is multifactorial and
84 denoted by environmental factors such as unbalanced diets and overnutrition as well as by
85 lack of physical activity in the context of a genetic predisposition (5, 6). Currently, the
86 treatment of NAFLD is founded on diet and lifestyle modifications (7). Weight lowering,
87 exercise and healthy eating habits are the main tools to fight NAFLD (8). Nevertheless, there is
88 no a specifically characterized dietary pattern and further studies are needed (9). Moreover,
89 because not all overweight individuals develop NAFLD, it is unknown if specific dietary

90 patterns may prevent or protect against the development of NAFLD overweight or obese
91 subjects. For this reason, more information about the interplay between diet and fatty liver,
92 taking into account inflammation as an implicated mechanism, is required to design effective
93 strategies in the prevention and treatment of obesity-associated NAFLD.

94 In this context, the current study investigated associations of a validated DII, recognized as a
95 tool for assessing the inflammatory capacity of the diet according to six inflammatory
96 biomarkers (IL-1b, IL-4, IL-6, IL-10, TNF- α and C-reactive protein (CRP)), with non-
97 invasive liver markers in overweight and obese subjects within the PREDIMED study.

98

99 **METHODS**

100 The ‘PREDIMED’ study was a parallel-group, multi-centre, clinical trial targeting the
101 primary prevention of Cardiovascular Disease (CVD) via the MeDiet
102 (<http://www.predimed.com>). A description of the study design has been previously published
103 (10, 11). This research was registered as an International Standard Randomized Controlled
104 Trial, number ISRCTN355739639. Men aged 55–80 years and women aged 60–80 years were
105 enrolled by eleven centres in Spain between October 2003 and December 2010. Eligible
106 participants had no previous CVD event, but were at high CVD risk at baseline due to the
107 diagnosis of type 2 diabetes mellitus or the presence of at least three of the following major
108 cardiovascular risk factors: smoking (more than one cigarette per d during the last month);
109 elevated blood pressure (systolic blood pressure \geq 140 mmHg or diastolic blood pressure \geq 90
110 mmHg or antihypertensive medication); high LDL-cholesterol levels (\geq 160 mg/dl); low HDL-
111 cholesterol levels ($<$ 40 mg/dl in men or $<$ 50 mg/dl in women, independently of lipid-lowering
112 therapy); BMI \geq 25.0 kg/m²; or family history of premature Chronic Heart Disease (CHD).
113 Fasting blood samples were collected and trained dietitians assisted participants in completing

114 a validated Food Frequency Questionnaire including 137 food-items (FFQ). As previously
115 described (12), an ad hoc computer program was created using the information available from
116 validated standard Spanish food composition tables (13) to translate food consumption
117 obtained from FFQ into nutrients (macronutrients and micronutrients) and energy.
118 Furthermore, participants completed the Minnesota physical activity questionnaire validated
119 for Spanish-language (14) and adherence to the MeDiet was assessed using a fourteen-item
120 dietary screen (≥ 9 points from the Mediterranean test) as described elsewhere (15). A total of
121 794 randomized subjects from the PREDIMED trial were included in the present study to
122 evaluate the DII with respect to liver status at baseline. For some analyses, the subjects were
123 categorized depending on adiposity level and according with the Spanish Association for the
124 Study of Obesity (16) as ($\text{BMI} \leq 26.9 \text{ kg/m}^2$; $\text{BMI} > 27.0 - \leq 29.9 \text{ kg/m}^2$; $\text{BMI} \geq 30.0 \text{ kg/m}^2$).

125 **Dietary Inflammatory Index**

126 The DII is a scoring algorithm that evaluates the effect of diet on six inflammatory
127 biomarkers; IL-1 β , IL-4, IL-6, IL-10, TNF- α , and CRP. In the present study, habitual dietary
128 intake of each participant was derived from a 137-item FFQ, which has been validated in
129 Spain (12), and has been extensively used in Mediterranean studies of nutritional
130 epidemiology. Dietary intake data achieved by this FFQ was then used in the estimation of DII
131 at baseline (17, 18). The DII was calculated as described previously (19, 20). Briefly, dietary
132 parameters were scored according to their effect (pro-inflammatory, anti-inflammatory, or no
133 effect) on six well-established inflammatory biomarkers (IL-1 β , IL-4, IL-6, IL-10, TNF- α and
134 CRP). An inflammatory effect score was computed for every food and standardized to a
135 composite database representative of food consumption patterns of a diverse population.
136 Inflammatory effect scores for each food were converted to food parameter specific DII
137 scores, which were then summed to obtain an overall DII score for each participant. Positive

138 values represent a pro-inflammatory diet, whereas negative values represent an anti-
139 inflammatory diet. DII scores range from 7.98 (maximally pro-inflammatory) to -8.87
140 (maximally anti-inflammatory), as reported elsewhere (21).

141

142 **Fatty Liver Index**

143 The FLI score was designed after a bootstrapped stepwise logistic regression analysis (22).
144 Out of thirteen variables (including gender, age, ethanol intake, ALT, AST, GGT, BMI, Waist
145 Circumference (WC), sum of four skinfolds, glucose, insulin, Triglycerides (TG) and
146 cholesterol) four predictors remained within the equation:

$$147 \text{ FLI} = \left[\frac{e^{0.953 \cdot \log[e] [\text{triglycerides}] + 0.139 \cdot \text{BMI} + 0.718 \cdot \log[e] (\text{GGT}) + 0.053 \cdot \text{waist}}}{1 + e^{0.953 \cdot \log[e] [\text{triglycerides}] + 0.139 \cdot \text{BMI} + 0.718 \cdot \log[e] (\text{GGT}) + 0.053 \cdot \text{waist}}} \right] \cdot 100$$

150 The FLI ranges from 0 to 100. Thus, FLI scores of <30 and $\text{FLI} \geq 60$ indicated the absence or
151 presence, respectively, in fatty liver with a good diagnostic accuracy (22).

152

153 **Statistical analysis**

154 Normality distributions of the analyzed variables were assessed according to the Shapiro–
155 Wilk test. Statistical analyses were stratified by BMI in order to consider overweight and
156 obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$) categories. Continuous variables (total energy intake, Metabolic
157 Equivalent of Task (METs), DII, FLI, alcohol intake, nutrient and food consumption) were
158 compared between groups by the Student's t-test or the Mann–Whitney U test for parametric
159 or non-parametric variables, respectively. Intakes of carbohydrate, protein and fat (also fat
160 subtypes) were expressed as percentage of total energy intake. Categorical variables were
161 compared by the chi-squared test and are defined as High Adherence to the MedDiet (High

162 adherence) ≥ 9 , smoking habit (never: no smoking habit; former: >5 years gave up smoking;
163 current smoker: at least 1 cigarette/day).

164 Two new groups were defined considering the median of the FLI score as the cut off ($< 50^{\text{th}}$
165 percentile FLI vs $\geq 50^{\text{th}}$ percentile FLI) and a new analysis considering these two groups was
166 carried out using the tests described before.

167 The relationship between anthropometric measurements, dietary factors and NAFLD
168 biomarkers was assessed by means of Pearson and Spearman's correlation tests for parametric
169 and non-parametric variables, respectively. Variables associated with NAFLD biomarkers
170 were selected to be included in a linear regression model. Thus, a linear regression model
171 was set up to find out the influence of independent variables such as DII, and anthropometric
172 and biochemical parameters on the variability of the FLI score. Analyses were performed
173 using STATA version 12.0 (Stata Corp). All p-values are two-tailed, and differences were
174 considered statistically significant at $p < 0.05$.

175

176 **RESULTS**

177 At baseline, the average age of participants was 67 years old, of which 55% were women and
178 only 12% were current smokers. Also, the main characteristics of the participants according to
179 phenotype, quality of the diet and dietary consumption are reported (Table 1). The analysis of
180 the dietary pattern according to main groups evidenced that polyunsaturated fat, $\Omega 3$ fatty
181 acids (long-chain n-3 polyunsaturated fatty acids), $\Omega 6$, vitamin E and D, dairy and total
182 energy intake were higher ($p < 0.05$) in subjects with $\text{BMI} \leq 26.9 \text{ kg/m}^2$, while red meat
183 consumption was higher in obese participants (Table 1). Differences in the adherence to the
184 Mediterranean diet ($p < 0.001$) were observed, and individuals with obesity showed lower
185 adherence. Interestingly, a higher DII ($p < 0.001$) was also found in obese participants (Figure

186 1). In addition, DII was negatively correlated with MeDiet adherence ($r = -0.263$; $p < 0.001$).
187 Liver-related markers (Table 2) particularly FLI and TG, were higher in the obese group
188 ($p < 0.0011$). Furthermore, individuals were classified according to the median value of FLI
189 and food consumption was explored (Table 3). All nutrients showed significant differences
190 with model 2 of Ancova Test except for diet carbonated drinks.

191 The consumption of carbohydrate, fiber, PUFA, fruits, vegetables, dairy, animal protein, fish,
192 nuts, omega-3, omega-6, linoleic acid, Vit. B1, Vit. B6, Vit. B2, Vit. C, Vit. D, Vit. B9, Na, k,
193 Mg, P, Ca, betacarotens, phytoesterols were higher in patients with the lowest FLI scores. In
194 addition, the anti-inflammatory pattern measured by DII was lower in these individuals ($\leq p50$
195 FLI). In order to further evaluate these results several correlations analyses were carried out.

196 Fiber, protein (%E), vegetables, fruits, dairy, semi-skimmed milk, fresh cheese, fatty fish,
197 nuts, $\Omega 3$ marine, Vit.B6, Vit.B2, Vit.C, Vit. D, Vit. B9, K, Mg, P, Ca, betacarotens were
198 negatively correlated with FLI (Figure 2). However, red meat and sugar showed a positive,
199 significant relationship. Interestingly, the DII score was positively associated ($p < 0.001$) with
200 non-invasive liver markers (ALT, AST, GGT and FLI) (Figure 3). A linear regression
201 analysis was carried out to assess the influence that some factors might have on FLI.

202 Variables were independently studied by univariable linear regression. Thus, variables
203 associated with the FLI were: DII ($\beta = 2.02$, $R = 0.019$, $p < 0.001$), age ($\beta = -0.54$, $R = 0.020$,
204 $p < 0.001$), Total energy intake ($\beta = 0.0004$, $R = 0.619$, $p < 0.001$), Gender ($\beta = -4.67$, $R = 0.003$,
205 $p = 0.010$), ALT (U/L) ($\beta = 0.32$, $R = 0.038$, $p < 0.001$), METs ($\beta = -0.46$, $R = 0.006$, $p = 0.011$).

206 When these variables were jointly considered, the predictors of the model explained up to
207 9.2% (Table 4) of the variation of the FLI (adjusted $R^2 = 0.099$, $P_{\text{model}} < 0.001$).

208

209

210 **DISCUSSION**

211 The DII is a relatively novel tool for evaluating the inflammatory potential of a diet that
212 reflects both a robust scientific framework and standardization of individual intakes to global
213 referent values (23). Results based on this index indicate that it reliably predicts
214 concentrations of inflammatory markers, such as C-reactive protein, IL-6 (21, 23-25). In the
215 present study, a higher pro-inflammatory diet was observed in participants with higher BMI,
216 suggesting that diet-induced inflammation may increase or maintain obesity, particularly
217 central obesity, in an overweight or obese population with high WC values. Indeed,
218 inflammation is induced by adiposity (26, 27), but this relationship can be bidirectional. Thus,
219 a pro-inflammatory diet can increase obesity and accompanying comorbidities in liver,
220 triggering a continuous cycle. These findings support the idea that overall dietary patterns
221 play an essential role in the metabolism of inflammation process (28). Remarkably, a previous
222 ancillary study carried out within of the PREDIMED trial showed that the DII was inversely
223 associated with the adherence to a MeDiet and healthy foods consumption (25). Other trials
224 have also evidenced correlations of specific nutrients such as total dietary fiber intake (29),
225 vitamin E and C intake (30) and antioxidants with lower levels of inflammatory markers (31,
226 32). Furthermore, one analysis reported the anti-inflammatory capacity of the MeDiet (33-35).
227 Moreover, a number of trials have reported the association between specific dietary anti-
228 inflammatory components and better liver status, suggesting that the prevalence or incidence
229 of NAFLD might be mediated through different inflammatory pathways where food play a
230 key role, but not as a whole.

231 The liver is the principal location of amino acid synthesis, protein degradation, carbohydrate
232 metabolism, cholesterol synthesis, the production of TG and the bulk of lipoprotein synthesis,
233 as well as several other regulatory and growth factors (36). Thus, fatty liver is not only

234 considered hepatic manifestation of metabolic syndrome, but it could also promote the
235 emerging metabolic-related extra-hepatic complications (37-39). Currently, there are no
236 effective therapies available for the treatment of NAFLD. Recent investigations have focused
237 on identifying biomarkers to predict NASH or NAFLD. However, these strategies have rarely
238 benefited clinical practice in terms of NAFLD diagnosis or discrimination of the pathological
239 evolution of NAFLD. Therefore, the development of current non-invasive evaluations of
240 NAFLD could be useful for the diagnosis of fatty liver (40). In this context, Bedogni et al.
241 developed a simple scoring system called FLI which considers TG, GGT, BMI, and WC and
242 is easily calculated (41). FLI was developed for the prediction of fatty liver disease evidenced
243 a good area under the curve of 0.84. Thus, FLI accuracy has been validated in comparison
244 with liver ultrasonography (22). Also, WC and TG have been used to predict the presence of
245 liver fibrosis in a clinical study in children (42). In addition, BMI and GGT have been
246 reported as independent predictors of fatty liver in other research studies (43, 44). Regarding
247 the relationship between diet and the FLI, significant differences were observed when
248 stratified based on the median value of the FLI. However, there is no a well characterized
249 dietary pattern for fatty liver disease patients and further randomized controlled trials are
250 needed (45). In the present study, different dietary patterns were found. First, patients with
251 less fatty liver consumed more animal protein from white meat and fish. In contrast, the
252 consumption of red meat was higher in patients with high FLI. These results are reinforced by
253 a study of Freedman et al, (2010) where the authors found that red meat may be associated
254 with increased chronic liver disease, whereas white meat may be associated with reduced risk
255 (46). Another controversial finding is emphasized in the dairy group. All dairy food showed a
256 negative relationship with fatty liver, with the exception of cheeses high in fat, which
257 increased fatty liver. One study from the EPIC-cohort suggests that elevated consumption of

258 dairy products, specifically milk and cheese, can be associated with increased liver diseases
259 (47). In contrast, another study reported that three low-fat-dairy servings per day ameliorated
260 both inflammation and liver function in metabolic syndrome subjects (48). In addition, the
261 consumption of cereals and potatoes showed a positive correlation with FLI, which contrasts
262 with the study of Shi L et al., (2012), where authors found that people with NAFLD
263 consumed a reduced quantity of potatoes and cereals compared to the control group (49).
264 Another research disclosed the consumption of cereals in general increased the likelihood of
265 having NAFLD (50) . Surprisingly, a positive correlation with the consumption of legumes
266 was noted, but not statistically significant. Legumes are known as a healthy food and most
267 food studies classify legumes as a pattern of a healthy and anti-inflammatory diet (51, 52). In
268 contrast, in a multicenter study from India, the high-legume consumption did not reveal
269 association with obesity and metabolic-related comorbidities (53). Taking into account these
270 results, a higher consumption of all antioxidants, flavonoids, healthy fatty acids were
271 inversely related with FLI. These results are consistent with a recent review, which found that
272 flavonoids and an antioxidant components had beneficial effects in patients with NAFLD
273 (54).

274

275 **LIMITATIONS**

276 Our study is a transversal design, which identifies associations; however, these studies are
277 remarkably similar to those from Randomized Control trials on the same topic (55). A large
278 cross-sectional study such as this one contributes to the design of new dietary patterns in
279 NAFLD pathology and to establishing new hypotheses for large prospective studies and
280 clinical trials. One limitation of this study is that liver fat was not directly measured, i.e
281 magnetic resonance spectroscopy (MRS) or magnetic resonance imaging (MRI). Another

282 limitation is the lack of a control group comprised of exclusively normoweight subjects due
283 to the inclusion criteria requirements for PREDIMED study participants. Lastly, the FFQ are
284 known to contain a certain degree of measurement error, which might affect results that
285 depend on such evaluation. However, validity of the FFQ used in our cohort has been
286 evaluated, showing good association with nutrient intake according to repeated food records
287 (14). In addition, the participants were from a Mediterranean area and had a high risk of
288 cardiovascular disease, which could limit the generalizability of our findings to different
289 settings.

290

291 **CONCLUSION**

292 To our knowledge, this is the first study evaluating the association between DII and liver
293 status in overweight and obese subjects. Participants with higher liver damage (FLI) and
294 adiposity ($BMI \geq 30\text{kg/m}^2$) showed a pro-inflammatory dietary pattern (DII) and worse
295 adherence to the MeDiet. In addition, the DII score was positively associated with non-
296 invasive liver damage markers. These findings suggest that the consumption of an anti-
297 inflammatory dietary pattern might contribute the reduction of obesity and related co-
298 morbidities, especially NAFLD following precision nutrition guidelines.

299

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