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

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Funding: The PREDIMED trial was supported by the Instituto de Salud Carlos III (ISCIII): RETIC G03/140 (to R. E.), CIBERobn and RETIC RD 06/0045 (to M. A. M.-G.). ICG holds a grant from the Centre for Nutrition Research of the University of Navarra.

Disclosure: The authors declare no conflict of interest

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

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

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

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

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

INTRODUCTION

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

NAFLD has been related with obesity, insulin resistance, hypertension and dyslipidemia, and it is regarded as a key liver manifestation of the metabolic syndrome (3). In fact, excessive body mass index (BMI kg/m^2) and visceral fat (%) are known to be important risk factors in NAFLD onset and at least two-thirds of population with obesity and diabetes show hepatic steatosis (4). In addition, inflammation has been hypothesized as an underlying mechanism in the link between obesity and NAFLD. The pathogenesis of NAFLD is multifactorial and denoted by environmental factors such as unbalanced diets and overnutrition as well as by lack of physical activity in the context of a genetic predisposition (5, 6). Currently, the treatment of NAFLD is founded on diet and lifestyle modifications (7). Weight lowering, exercise and healthy eating habits are the main tools to fight NAFLD (8). Nevertheless, there is no a specifically characterized dietary pattern and further studies are needed (9). Moreover, because not all overweight individuals develop NAFLD, it is unknown if specific dietary

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

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

METHODS

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

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

Dietary Inflammatory Index

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

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

Fatty Liver Index

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

FLI = [e 0.953*log[e] [triglycerides] + 0.139*BMI + 0.718*log[e] (GGT) + 0.053*waist circumference - 15.745] / [1 + e 0.953*log[e] [triglycerides] + 0.139*BMI + 0.718*log[e] [GGT] + 0.053*waist circumference - 15.745] * 100

The FLI ranges from 0 to 100. Thus. FLI scores of <30 and FLI ≥ 60 indicated the absence or presence, respectively, in fatty liver with a good diagnostic accuracy (22).

Statistical analysis

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

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

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

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

RESULTS

At baseline, the average age of participants was 67 years old, of which 55% were women and only 12% were current smokers. Also, the main characteristics of the participants according to phenotype, quality of the diet and dietary consumption are reported (Table 1). The analysis of the dietary pattern according to main groups evidenced that polyunsaturated fat, Ω3 fatty acids (long-chain n-3 polyunsaturated fatty acids), Ω6, vitamin E and D, dairy and total energy intake were higher ($p<0.05$) in subjects with BMI ≤ 26.9 kg/m^2, while red meat consumption was higher in obese participants (Table 1). Differences in the adherence to the Mediterranean diet ($p<0.001$) were observed, and individuals with obesity showed lower adherence. Interestingly, a higher DII ($p<0.001$) was also found in obese participants (Figure

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

The consumption of carbohydrate, fiber, PUFA, fruits, vegetables, dairy, animal protein, fish, nuts, omega-3, omega-6, linoleic acid, Vit. B1, Vit. B6, Vit. B2, Vit. C, Vit. D, Vit. B9, Na, k, Mg, P, Ca, betacarotens, phytoesterols were higher in patients with the lowest FLI scores. In addition, the anti-inflammatory pattern measured by DII was lower in these individuals (≤ p50 FLI). In order to further evaluate these results several correlations analyses were carried out. Fiber, protein (%E), vegetables, fruits, dairy, semi-skimmed milk, fresh cheese, fatty fish, nuts, Ω 3 marine, Vit.B6, Vit.B2, Vit.C, Vit. D, Vit. B9, K, Mg, P, Ca, betacarotens were negatively correlated with FLI (Figure 2). However, red meat and sugar showed a positive, significant relationship. Interestingly, the DII score was positively associated (p<0.001) with non-invasive liver markers (ALT, AST, GGT and FLI) (Figure 3). A linear regression analysis was carried out to assess the influence that some factors might have on FLI. Variables were independently studied by univariable linear regression. Thus, variables associated with the FLI were: DII (β= 2.02, R= 0.019, p<0.001), age (β= -0.54, R= 0.020, p<0.001), Total energy intake (β= 0.0004, R= 0.619, p<0.001), Gender (β= -4.67, R=0.003, p= 0.010), ALT (U/L) (β= 0.32, R=0.038, p<0.001), METs (β= -0.46, R= 0.006, p= 0.011). When these variables were jointly considered, the predictors of the model explained up to 9.2% (Table 4) of the variation of the FLI (adjusted R^2 = 0.099, P_{model} <0.001).

DISCUSSION

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

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

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

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

LIMITATIONS

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

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

CONCLUSION

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

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Acknowledgements

1. The authors are very grateful to all the participants of the study; the PREDIMED personnel for their assistance; and the personnel of primary care centres.
2. Author**s acknowledge Cassondra Saande (native English speaker, Iowa State University) for reviewing the final version of the manuscript.**
3. Conflict of Interest: None declared. The PREDIMED trial was supported by the Instituto de Salud Carlos III (ISCIII): RTIC G03/140 (to R. E.), CIBERobn and RETIC RD 06/0045 (to M. A. M.-G.). ICG holds a grant from the Centre for Nutrition Research of the University of Navarra.

Table 1. Description of the phenotypic characteristics, quality of the diet, nutrient and food consumption in PREDIMED Trial.

	≤ 26.9 kg/m^2	**≥ 27 - <29.9 kg/m^2**	**≥ 30 kg/m^2**	p
n=794	n= 182	n= 235	n=377	
Phenotypic characteristics				
Waist Circumference (cm)	93 (6)	99 (6)#	106 (7)*†	<0.001
Diabetes (%)	58	54	51	0.228
Hypercholesterolemia (%)	63	68	70	0.267
Hypertension (%)	78	84	87*	0.015
Quality of the diet				
Dietary Inflammatory Index	-0.8 (1)	-0.6 (1)	-0.1 (1)*	0.003
High Adherence to MedDiet (n)	52	53	38*†	<0.001
Energy and Macronutrients				
Total energy intake (Kcal/d)	2384 (575)	2349 (611)	2279 (582)	0.109
Carbohydrate (%E)	39 (6)	39 (6)	40 (6)	0.086
Total Protein (%E)	16 (2)	16 (2)	16 (2)	0.055
Animal (%E)	67 (6)	67 (7)	68 (7)	0.089
Vegetable (%E)	32 (6)	32 (7)	31 (7)	0.263
Lipid(%E)	41 (6)	41 (6)	40 (6)	0.516
Saturated Fat (g/d)	28 (9)	27 (9)	27 (9)	0.386
Monounsaturated fat (g/d)	54 (15)	53 (17)	51 (15)	0.210
Polyunsaturated fat (g/d)	17 (6)	17 (6)	16 (5)*	0.007
Marine Ω3 (g/d)	0.8 (0.5)	0.7 (0.4)	0.7 (0.4)*	0.024
No marine Ω3 (g/d)	1.6 (0.6)	1.5 (0.6)	1.4 (0.5)*	0.002
Ω 6 (g/d)	14 (5)	14 (5)	13 (5)*	0.016
Micronutrients				
Fibre (g/d)	24 (8)	23 (7)	23(7)	0.223
Vitamin E (µg /d)	10 (3)	10 (3)	9 (3)	0.046
Vitamin D (µg /d)	6 (3)	5 (3)	5 (3)	0.019
Vitamin A (µg /d)	1324 (854)	1303 (1019)	1292 (942)	0.931
Vitamin C (µg /d)	184 (79)	178 (74)	179 (75)	0.672
Alcohol intake (g/d)	8 (11)	9 (15)	7 (12)	0.208
Food groups				
Vegetables (g/d)	322 (136)	312 (133)	297 (131)	0.100
Fruits (g/d)	310 (168)	315 (178)	310 (172)	0.947
Legumes (g/d)	17 (8)	17 (8)	18 (8)	0.820
Cereals (without potato) (g/d)	150 (87)	148 (8)	148 (78)	0.940
Potato (g/d)	107 (48)	101 (45)	101 (45)	0.298
Dairy products (g/d)	401 (232)	376 (217)	352 (194)*	0.035
Whole milk (g/d)	36 (126)	41 (114)	33 (95)	0.700
Semi-skimmed milk (g/d)	138 (205)	125 (184)	119 (165)	0.499
Skimmed milk (g/d)	96 (170)	90 (167)	76 (138)	0.291
Meat (g/d)	145 (55)	138 (57)	149 (60)	0.089
Red meat (g/d)	85 (43)	80 (44)	89 (48)	0.087
White meat (g/d)	60 (32)	57 (33)	60 (32)	0.664
Fish (g/d)	107 (47)	99 (42)	101 (43)	0.223
Virgin Olive oil (g/d)	41 (17)	41 (18)	41 (17)	0.958

Continuous variables are shown as means (SDs), and categorical variables are shown as percentages.
MedDiet, Mediterranean Diet; PREDIMED, PREvención con DIeta MEDiterránea.
p<0.05 was considered statistically significance.
*p was significant between participants with BMI ≤ 26.9 Kg/m^2 and BMI ≥ 30 kg/m^2
† p was significant between participants with BMI ≥ 27 - <29.9 kg/m^2 and BMI ≥ 30 kg/m^2
# p was significant between participants with BMI ≤ 26.9 kg/m^2 and ≥ 27 - <29.9 kg/m^2

Table 2 Liver markers and Fatty Liver Index in the PREDIMED trial (n= 794).

	≤ 26.9 kg/m^2 n=182	**≥ 27.0 - <29.9 kg/m^2 n=235**	**≥ 30 kg/m^2 n= 377**	p
TG (mg/dl)	119 (70)	130 (67)	143 (72)*	<0.001
ALT(UI/L)	22 (15)	23 (16)	23 (10)	0.405
AST (UI/L)	21 (7)	20 (6)	21 (12)	0.574
AST/ALT	1 (0.3)	1 (0.6)	0.9 (0.5)	0.167
GGT (UI/L)	32 (559	29 (26)	29 (23)	0.562
FLI	37 (18)	56 (17)	76 (14)*	<0.001

All variables are shown as means ± SD. AST, Aspartate Aminotransferase. ALT, Alanine Aminotransferase. GGT, Gamma-Glutamyltransferase. FLI, Fatty Liver Index. p<0.05 was considered statistically significance.

*p was significant between participants with BMI ≤ 26.9 Kg/m^2 and BMI ≥ 30.0 kg/m^2

Table 3 Nutrients and food consumption (mean and SD) according to FLI in PREDIMED trial (n = 794).

	Fatty Liver Index				
n=794	≤FLI P_{50}	>FLI P_{50}	P_{anova1}	P_{anova2}	Correlation with FLI (p)
Macronutrients					
Carbohydrate (%E)	40 (3)	39 (0.3)	0.056	<0.001	-0.01 (0.651)
Dietary fiber (g)	24 (8)	22 (7)	0.003	<0.001	-0.10 (0.004)
Protein (%E)	16 (0.1)	16 (0.1)	<0.001	<0.001	-0.11 (0.001)
Animal protein (g)	65 (17)	64 (17)	0.003	<0.001	-0.05 (0.111)
Vegetal protein (g)	30 (9)	30 (9)	<0.001	<0.001	-0.03 (0.297)
Lipids (%E)	40 (0.3)	41 (0.2)	0.222	<0.001	0.08 (0.807)
PUFA (g)	17 (6)	16 (5)	<0.001	<0.001	-0.02 (0.570)
MUFA (g)	52 (16)	53 (16)	<0.001	<0.001	0.03 (0.270)
SFA (g)	27 (9)	27 (9)	<0.001	<0.001	0.02 (0.406)
Food groups					
Vegetables (g/d)	324 (6)	291 (6)	<0.001	<0.001	-0.11 (0.001)
Fruits (g/d)	324 (8.9)	299 (8.3)	0.197	<0.001	-0.06 (0.071)
Cereals (without potato) (g/d)	144 (4)	153 (4)	<0.001	<0.001	0.04 (0.206)
Potato (g/d)	103 (46)	102 (46)	0.040	<0.001	0.01 (0.695)
Legumes (g/d)	17 (0.4)	18 (0.4)	0.270	<0.001	0.04 (0.906)
Dairy (g/d)	393 (10)	346 (10)	<0.001	<0.001	-0.15 (<0.001)
Whole milk (g/d)	37 (117)	35 (101)	0.222	<0.001	-0.01 (0.705)
Semi-skimmed milk (g/d)	142 (201)	112 (161)	0.009	<0.001	-0.08 (0.022)
Skimmed milk (g/d)	93 (160)	78 (149)	0.137	0.017	-0.05 (0.110)
Cheeses (g/d)	16 (169)	17 (17)	0.028	<0.001	0.03 (0.388)
Fresh cheese (g/d)	12 (19)	8 (13)	<0.001	<0.001	-0.11 (0.001)
Meat (g/d)	142 (2.8)	148 (3)	<0.001	<0.001	0.06 (0.055)
Red meat (g/d)	82 (49)	88 (49)	<0.001	<0.001	0.10 (0.003)
White meat (g/d)	59 (32)	59 (32)	0.989	<0.001	-0.02 (0.502)
Fish (g/d)	103 (2)	101 (8)	0.054	<0.001	-0.05 (0.147)
White fish (g/d)	43 (27)	43 (26)	0.520	<0.001	-0.02 (0.440)
Fatty fish (g/d)	24 (20)	20 (18)	0.012	<0.001	-0.08 (0.018)
Nuts (g/d)	8 (9)	6 (8)	<0.001	<0.001	-0.12 (<0.001)
Olive oil (g/d)	40 (0.9)	42 (0.8)	0.079	<0.001	0.05 (0.139)
Jams (g/d)	0.8 (2)	1 (3)	0.549	<0.001	0.01 (0.583)
Sugar (g/d)	4.5 (9)	5.8 (9.6)	0.021	<0.001	0.07 (0.031)
Carbonated drinks (g/d)	12 (52)	17 (57)	0.073	<0.001	0.06 (0.076)
Diet carbonated drinks (g/d)	19 (79)	26 (104)	0.200	0.441	0.02 (0.469)
Micronutrients					
Marine Ω3 (g)	0.8 (0.02)	0.7 (0.02)	0.007	<0.001	-0.08 (0.015)
No marine Ω3 (g)	1.5 (0.03)	1.4 (0.02)	<0.001	<0.001	-0.06 (0.075)
Ω 6 (g)	13.9 (0.2)	13.6 (0.6)	<0.001	<0.001	-0.00 (0.882)
Linoleic acid (g)	1.5 (0.6)	1.4 (0.4)	<0.001	<0.001	-0.06 (0.065)
Vit B1 (mg/d)	2.4 (0.8)	2.3 (0.8)	0.042	<0.001	-0.06 (0.062)
Vit B6 (mg/d)	2.3 (0.6)	2.2 (0.5)	<0.001	<0.001	-0.10 (0.002)
Vit B12 (mg/d)	11 (5)	11 (6)	0.016	<0.001	-0.02 (0.540)
Vit B2 (mg/d)	20 (0.5)	19 (0.5)	<0.001	<0.001	-0.12 (<0.001)
Vit C (mg/d)	189 (80)	173 (71)	0.009	<0.001	-0.11 (0.001)
Vit E (µg /d)	10 (0.17)	10 (0.17)	<0.001	<0.001	-0.05 (0.155)
Vit D (µg /d)	6.2 (0.17)	5.5 (0.16)	0.001	<0.001	-0.08 (0.016)
Folic Acid (µg /d)	391 (6)	364 (5)	<0.001	<0.001	-0.13 (<0.001)
Na (mg/d)	2608 (951)	2569 (937)	<0.001	<0.001	-0.02 (0.565)
K (mg/d)	4376 (1126)	4164 (1035)	<0.001	<0.001	-0.09 (0.006)
Fe (mg/d)	16 (4)	16 (4)	<0.001	<0.001	-0.04 (0.210)
Mg (mg/d)	380 (104)	362 (92)	<0.001	<0.001	-0.10 (0.003)
P (mg/d)	1769 (447)	1675 (409)	<0.001	<0.001	-0.12 (<0.001)
Ca (mg/d)	1080 (367)	999 (345)	<0.001	<0.001	-0.13 (<0.001)
Betacarotens (µg /d)	3438 (1923)	3076 (1994)	0.005	<0.001	-0.11 (0.001)
Phytoesterols (mg/d)	350 (5)	338 (4.9)	<0.001	<0.001	-0.053 (0.135)
Score					
Dietary Inflammatory Index	-0.7(0.07)	-0.33 (0.07)	<0.001	<0.001	0.139 (0.001)

Continuous variables are shown as means ± SDs, and categorical variables are shown as percentages. P<0.05 was considered statistically significance.

P_{anvoa1} was adjusted by BMI, gender and age.

P_{anova2} was adjusted by BMI, gender, age, high adherence to MedDiet, smoking habit (dichotomous), hypertension (dichotomous), diabetes (dichotomous), hypercholesterolemia (dichotomous), alcohol intake (g/d), total energy intake (Kcal/d), physical activity (METS- h/d), waist circumference, and waist/height ratio.

P_{50} FLI cut off value= 65.09

Table 4 Analysis of linear regression model of association between Dietary Inflammatory Index and Fatty Liver Index in PREDIMED trial (n= 794).

n= 794	β	**Std. Err.**	**P**	**[95% Conf. Interval]**	
DII	3.057	0.590	<0.001	1.897	4.216
Age (years)	-0.503	0.132	<0.001	-0.763	-0.243
Total energy intake	0.004	0.001	0.011	0.009	0.007
Gender	-2.699	1.627	0.012	-5.893	0.495
ALT (U/L)	0.276	0.057	<0.001	0.164	0.389
METs	-0.579	0.189	0.002	-0.951	-0.208
R^2 adjusted				0.099	
P model				<0.001	

P<0.05 was considered statistically significance