The anti-inflammatory potential of diet and nonalcoholic fatty liver disease: the ATTICA study

Stefanos Tyrovolas, Demosthenes B. Panagiotakos, Ekvi N. Georgousopoulou, Christina Chrysohoou, John Skoumas, William Pan, Dimitrios Toussoulis and Christos Pitsavos

Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is correlated with low-grade inflammation and dietary habits. Until today, there have been limited epidemiologic data assessing the role of diet’s inflammatory potential on NAFLD. The aim was to evaluate the relationship between an anti-inflammatory diet, as reflected by the Dietary Anti-Inflammation Index (D-AII), and NAFLD among cardiovascular disease (CVD)-free adults.

Methods: ATTICA is a prospective, population-based study that recruited 3042 adults without pre-existing CVD from the Greek population (Whites; age ≥18 years; 1514 men and 1528 women). D-AII was calculated using a standard procedure. The baseline study captured various sociodemographic, lifestyle and clinical characteristics as well as hepatic markers. These were used to calculate four NAFLD assessment indices: triglyceride-glucose (TyG) index, fatty liver index (FLI), hepatic steatosis index (HSI), and NAFLD Fatty Liver Score (NAFLD-FLS). Specific cutoffs were applied to capture NAFLD.

Results: D-AII showed a significant inverse association with NAFLD, applying the four indices with NAFLD cutoffs [odds ratio (OR) with 95% confidence interval (CI); TyG (0.95, 0.93–0.98); HSI (0.89, 0.86–0.92); FLI (0.88, 0.85–0.91); NAFLD-FLS (0.89, 0.86–0.92)], after adjusting for various confounders. Participants in the highest D-AII tertile had lower odds of having NAFLD, compared with those in the lowest D-AII tertile [(OR, 95% CI); TyG (0.33, 0.24–0.47); HSI (0.13, 0.08–0.23); FLI (0.05, 0.02–0.11); NAFLD-FLS (0.13, 0.07–0.23)]. Anti-inflammatory nutrition was related to lower odds of NAFLD among daily alcohol drinkers and individuals with metabolic syndrome.

Conclusions: Anti-inflammatory diet is an important predictor of NAFLD among adults without pre-existing CVD. Adherence to a high anti-inflammatory diet seems to contribute to NAFLD prevention.

Keywords: anti-inflammatory diet, ATTICA study, Greece, hepatic health, liver, nonalcoholic fatty liver disease

Introduction

Hepatic steatosis is classified as a nonalcoholic fatty liver disease (NAFLD) and is a liver lipid storage condition.¹ NAFLD is the most frequent liver disease in the general Western populations, with a prevalence of 20–40%.²,³ NAFLD is accompanied by various comorbidities, mostly nutrition-related, that affect health status and quality of life.⁴ Among them are noted dyslipidemias, diabetes mellitus, obesity, and central obesity as well as insulin resistance and metabolic syndrome.⁵,⁶
The gold standard method for NAFLD assessment is biopsy; however, this procedure typically is undertaken only for severe liver disease, as it is time consuming, expensive, and often unavailable for outpatient clinical practice. For this reason, well-defined indices have been reported in the literature measuring NAFLD and hepatic steatosis with high sensitivity and accuracy, including the fatty liver index (FLI), hepatic steatosis index (HSI), NAFLD-Fatty Liver Score (NAFLD-FLS), and triglyceride-glucose (TyG) index.

Although it is widely accepted that healthy dietary patterns and lifestyle changes (weight loss, physical activities) are associated with hepatic health, there is still lack of evidence on the role of healthy nutrition in the prevention of NAFLD. Furthermore, it remains unclear whether diet quality and its improvement could prevent NAFLD development. Light alcohol consumption is still controversial; however, the frequent recommendation is total alcohol abstinence to help resolve NAFLD. In addition, the risk of NAFLD has reported high incidences of several disorders (i.e. coeliac disease), and specific dietary patterns appear beneficial. Recently well-designed studies have reported the effect of the Mediterranean diet as a therapeutic option for NAFLD, mostly through its beneficial role in the metabolic pathways of NAFLD development. Inflammation is highly related to NAFLD due to the liver fat presence associated with the synthesis of inflammatory markers. Recent evidence has shown that major inflammatory markers such as C-reactive protein (CRP) and interleukins (ILs) are increased in diagnosed individuals with NAFLD and nonalcoholic steatohepatitis (NASH). Although low-grade inflammation, NAFLD and the inflammatory potential of nutrition have interrelated pathways, only one study has described the beneficial effect of the inflammatory potential of dietary patterns (as an holistic approach) in liver status, specifically in overweight and obese populations. Given the complexity of NAFLD, its association with cardiometabolic health and inflammation, and the overall lack of data among European and other international populations, the primary aim of the present work was to evaluate the relationship between adherence to an anti-inflammatory diet (D-AII) and NAFLD in alcohol drinkers and in individuals with metabolic syndrome.

Methods

Study sample
ATTICA is a prospective population-based study that was conducted in the greater metropolitan area of Athens, Greece, and included a sample of 78% urban and 22% rural population. Participant enrolment took place in 2001–2002, with two follow-up waves after 5 years (in 2006) and 10 years (in 2012). Random, multistage sampling based on the age and gender distribution of the reference population, as defined by the Hellenic National Statistical Service Census Survey of 2001, was applied. Sampling procedures anticipated enrolling only one participant per household, while institutionalized individuals were excluded from study participation. Of the 4056 invited individuals at baseline, 3042 agreed to participate (75% participation rate). Participants were interviewed by trained personnel using standard questionnaires. Participants were excluded if they had cardiovascular disease (CVD) at baseline based on a detailed clinical evaluation by the physicians of the study. The examination was performed in the individuals’ homes or workplaces. The ATTICA study was approved by our Institutional Ethics Committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki, with all participants providing written informed consent.

For the purposes of this study, we analysed information from the baseline wave only (cross-sectional analysis), due to the lack of crucial data for the assessment of NAFLD in the follow-up waves.

Measurements
The baseline evaluation included information about demographic characteristics (age, sex), personal and family history of hypertension, hypercholesterolemia and diabetes, family history of CVD, dietary and other lifestyle habits (i.e. smoking status and physical activity). In the present analysis, a participant’s exposure to smoking over time was defined through pack years. The evaluation of the dietary habits was based on a validated semi-quantitative food-frequency questionnaire (FFQ), the EPIC-Greek questionnaire kindly
Activity Questionnaire (IPAQ) was used. For the ascertainment of physical activity status, the International Physical Activity Questionnaire (IPAQ) was used. \(^{25}\) Waist circumference was measured in the middle between the lowest rib and the iliac crest using an inelastic measuring tape to the nearest 0.5 cm, and the waist-to-hip ratio was calculated. Body mass index (BMI) was calculated as weight (in kilograms) divided by standing height (in meters squared). Obesity was defined as BMI greater than 29.9 kg/m\(^2\). Arterial blood pressure (\(\geq\)130/85 mm Hg) were measured with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA). Diabetes mellitus (yes: 2; no: 0) + 0.45 \times \text{diabetes mellitus} (yes; 2; no: 0) + 0.15 \times \text{insulin in } \mu\text{U/L} + 0.04 \times \text{AST in } \mu\text{U/L} - 0.94 \times \text{ALT}/\text{AST}. A NAFLD-FLS cutoff of -0.64 was used to categorize those with hepatic steatosis.

4. The TyG index has been proposed as a screening tool for insulin resistance, \(^{32}\) with recent reports of its use to screen liver steatosis. \(^{33}\) The TyG index was calculated using the following formula: \(\text{TyG} = \ln[TG (\text{mg/dl}) \times \text{fasting plasma glucose (mg/dl)}]/2\). A TyG cutoff of 8.5 was used to categorize those with hepatic steatosis.

Statistical analysis

Normally distributed continuous variables are presented as mean values \(\pm\) standard deviation (SD), and categorical variables as frequencies. Normality was tested using the Shapiro–Wilk criterion; the nonnormally distributed variables are presented as median values and interquartile range (IQR).

The calculation of the dietary inflammatory load of a participant’s diet was according to the methodology and the rationale of the Dietary Inflammation Index (DII), previously proposed by Shivappa \textit{et al}. \(^{26}\) Thus, a Dietary Anti-Inflammation Index (D-AII) was developed based on participants’ dietary habits. \(^{27}\)

Participants were classified either as having metabolic syndrome (MetS) or not, as defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III, revised 2005). MetS presence was defined if three or more of the following metabolic syndrome components were present: waist circumference of \(\geq\)102 cm for males or \(\geq\)88 cm for females; triglyceride level of \(\geq\)150 mg/dl; high-density lipoprotein (HDL) cholesterol level of \(<\)40 mg/dl for males or \(<\)50 mg/dl for females; blood pressure of \(\geq\)130/85 mmHg; fasting blood glucose \(\geq\)100 mg/dl. \(^{28}\)

Hepatic steatosis was evaluated using the following four indices and applying specific cutoffs:

1. **HSI** is an evaluating index of NAFLD and was calculated using the following formula:
   \[
   \text{HSI} = 8 \times (\text{ALT}/\text{AST ratio}) + \text{BMI} (+2, \text{if female}; +2, \text{if participant has diabetes mellitus}).
   \]
   Participants with HSI measurements of \(>36\) were categorized in the hepatic steatosis group. \(^{9}\)

2. **FLI** is an index assessing hepatic steatosis and was calculated using the following formula:
   \[
   \text{FLI} = (e^{0.953 \times \text{loge} (\text{triglycerides})} + 0.139 \times \text{BMI} + 0.718 \times \text{loge} (\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)/(1 + e^{0.953 \times \text{loge} (\text{triglycerides})} + 0.139 \times \text{BMI} + 0.718 \times \text{loge} (\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745) \times 100.
   \]
   Participants with FLI measurements of \(\geq 60\) were categorized in the hepatic steatosis group. \(^{30}\)

3. **NAFLD-FLS** is a score that assesses liver fat content in NAFLD and was calculated using the following formula:
   \[
   \text{NAFLD-FLS} = -2.89 + 1.18 \times \text{MetS} (\text{Yes: 1; No: 0}) + 0.45 \times \text{diabetes mellitus} (\text{yes: 2; no: 0}) + 0.15 \times \text{insulin in } \mu\text{U/L} + 0.04 \times \text{AST in } \mu\text{U/L} - 0.94 \times \text{ALT}/\text{AST}. \text{A NAFLD-FLS cutoff of -0.64 was used to categorize those with hepatic steatosis.}
   \]

4. **FLS** is a score that assesses liver fat content in NAFLD and was calculated using the following formula:
   \[
   \text{FLS} = 0.718 \times (\text{waist circumference} - 15.745)/(1 + e^{0.953 \times \text{loge} (\text{triglycerides})} + 0.139 \times \text{BMI} + 0.718 \times \text{loge} (\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745) \times 100.
   \]
   Participants with FLS measurements of \(\geq 60\) were categorized in the hepatic steatosis group. \(^{30}\)

The calculation of the dietary inflammatory load of a participant’s diet was according to the methodology and the rationale of the Dietary Inflammation Index (DII), previously proposed by Shivappa \textit{et al}. \(^{26}\) Thus, a Dietary Anti-Inflammation Index (D-AII) was developed based on participants’ dietary habits. \(^{27}\)
presented as median and first and third tertiles. Associations between categorical variables were tested by the chi-square test, whereas between continuous variables by the Pearson r or Spearman’s ρ coefficients for normally distributed and skewed variables, respectively. Continuous variables were tested for normality via P–P plots. For normally distributed variables, comparisons were performed by the Student’s t test, after controlling for equality of variances by the Levene’s test. For continuous variables without normal distribution, comparisons were performed by the nonparametric Mann–Whitney U test. Multiple logistic regression analysis was performed to evaluate the association between NAFLD as the dependent outcome (using the NAFLD cutoffs for participants’ classification) and participant’s adherence to D-AII, adjusted for multiple confounders. All p values are based on two-sided tests. Statistical analyses were performed with the Statistical Package for Social Sciences, version 22 (SPSS Inc., Chicago, IL, USA).

Results

The sample size was 3042 participants (men: n = 1514; women: n = 1528) with mean age of 45 ± 14 years old. Sociodemographic, lifestyle and bioclinical characteristics of the study participants, by D-AII tertiles, are presented in Table 1. Participants grouped in the highest D-AII tertile were younger, more likely to drink alcohol, educated, and had a high socioeconomic level (all p < 0.02). In addition, participants in the highest D-AII tertile group had better hepatic health presenting lower values of hepatic damage (NAFLD) as expressed by TyG, HSI, and FLI, while they had greater NAFLD-FLS (all p < 0.001).

The aforementioned comparisons were prone to residual confounding; therefore, multi-adjusted logistic regression analysis was performed between the four different indices of NAFLD and D-AII (Table 2). Initially, the association between D-AII and NAFLD (i.e. in all four indices’ applied cutoffs) was tested. Based on this analysis, it was observed that the higher the level of adherence to the D-AII the lower the odds of having a participant NAFLD consistently across the four different indices [odds ratio (OR) with 95% confidence interval (CI); TyG (0.95, 0.93–0.98) HSI (0.89, 0.86–0.92); FLI (0.88, 0.85–0.91); NAFLD-FLS (0.89, 0.86–0.92)], after various adjustments made (i.e. age, sex, education, hypertension, diabetes mellitus, hypercholesterolemia, obesity, smoking habits).

Table 3 presents the ORs of the logistic regression model assessing the D-AII tertiles on hepatic health. The applied analysis showed that the third D-AII tertile (high anti-inflammatory diet load) had a consistent and independent inverse association with NAFLD, as compared with the lowest one (first tertile, low anti-inflammatory diet), across all four indices. The second D-AII tertile (intermediate anti-inflammatory load), as compared with the first tertile, was inversely related with NAFLD. The association was not significant when the TyG index cutoff was tested as an outcome.

In order to further explore the effect of D-AII in hepatic health, even among liver-stressed conditions such as alcohol drinking and metabolic syndrome, an additional stratified analysis has been applied. The participants consuming a more anti-inflammatory diet had lower odds of having NAFLD, even if they had metabolic syndrome [i.e. FLS: OR = 0.86, 95% CI (0.82–0.90)] or were daily alcohol drinkers [i.e. HSI: OR = 0.88, 95% CI (0.82–0.85)]. This association was consistent among all the NAFLD indices (TyG, HSI, and FLI). The analysis did not apply to the NAFLD-FLS due to the inclusion of MetS in the equation (the above-mentioned data are shown only in text).

Discussion

While NAFLD is multifactorial, with healthy nutrition and lifestyle changes having a major role in its treatment, little is known about the effect of a holistic dietary pattern’s anti-inflammatory load as a preventive means of mitigating NAFLD in European and other international populations. The present study reports an inverse association between adherence to an anti-inflammatory diet (as reflected by D-AII) and NAFLD in a large community-based cohort of CVD-free adults. Notably, this relationship was independent of well-established risk factors such as hypercholesterolemia, obesity and diabetes mellitus. Further analysis by D-AII tertiles shows that the tertile of the highest D-AII had the lowest odds of NAFLD (as expressed by the TyG, FLI, HSI, and NAFLD-FLS cutoffs). In addition, it was reported that the higher a participant’s adherence to the D-AII, the
lower the participant’s odds of having NAFLD with metabolic syndrome or consuming daily alcohol drinks. To the best of the authors’ knowledge, this is the first study to evaluate the association between D-AII and various noninvasive NAFLD indices using a general, CVD-free, European population. These results underscore the importance of anti-inflammatory dietary patterns in relation to NAFLD and the public health actions that should be taken to improve hepatic health in Western populations.

NAFLD and healthy eating habits are interrelated through systemic inflammation and metabolism as well as through therapeutic dietary interventions for weight loss. Various diet schemas have been proposed in hepatic steatosis such as diets in low carbohydrates and increased protein intake that have shown a fast and high reduction of liver fat.\textsuperscript{34} In some cases, intake of meat and dairy products have been related with increased liver diseases.\textsuperscript{35,36} Despite the well-known intercorrelation between a diet’s inflammatory load and a body’s systemic inflammation, so far only one study, using as sample overweight and obese individuals, has reported the association between NAFLD and a diet’s inflammatory potential.\textsuperscript{21} The ATTICA applied analysis reported a robust

Table 1. NAFLD and key baseline characteristics of the ATTICA participants when categorized by D-AII tertiles.

<table>
<thead>
<tr>
<th>Baseline D-AII tertiles</th>
<th>First tertile ((n = 998))</th>
<th>Second tertile ((n = 996))</th>
<th>Third tertile ((n = 998))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 14</td>
<td>45 ± 14</td>
<td>44 ± 14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, male %</td>
<td>49</td>
<td>47</td>
<td>52</td>
<td>0.07</td>
</tr>
<tr>
<td>Smoking [% yes]</td>
<td>54</td>
<td>56</td>
<td>58</td>
<td>0.21</td>
</tr>
<tr>
<td>Physical activity, % physically active</td>
<td>38</td>
<td>41</td>
<td>41</td>
<td>0.20</td>
</tr>
<tr>
<td>Education (years of school)</td>
<td>11 ± 4</td>
<td>12 ± 4</td>
<td>12 ± 4.0</td>
<td>0.02</td>
</tr>
<tr>
<td>SES (low/middle versus high)</td>
<td>28</td>
<td>34</td>
<td>37</td>
<td>0.001</td>
</tr>
<tr>
<td>Obesity, % yes</td>
<td>18</td>
<td>16</td>
<td>19</td>
<td>0.10</td>
</tr>
<tr>
<td>Hypertension, % yes</td>
<td>32</td>
<td>28</td>
<td>29</td>
<td>0.09</td>
</tr>
<tr>
<td>Diabetes mellitus, % yes</td>
<td>08</td>
<td>07</td>
<td>06</td>
<td>0.08</td>
</tr>
<tr>
<td>Hypercholesterolemia, % yes</td>
<td>36</td>
<td>44</td>
<td>39</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>86 ± 11</td>
<td>86.4 ± 11</td>
<td>86.4 ± 11</td>
<td>0.69</td>
</tr>
<tr>
<td>Alcohol consumption, % yes</td>
<td>53</td>
<td>54</td>
<td>59</td>
<td>0.02</td>
</tr>
<tr>
<td>TyG index</td>
<td>8.5 ± 0.6</td>
<td>8.5 ± 0.7</td>
<td>8.1 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatic steatosis index [HSI]</td>
<td>38 ± 5.8</td>
<td>35 ± 5.2</td>
<td>32 ± 9.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fatty liver index [FLI]</td>
<td>52 ± 28</td>
<td>43 ± 28</td>
<td>18 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAFLD-FLS</td>
<td>-0.04 ± 1.0</td>
<td>-0.37 ± 1.1</td>
<td>-1.05 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continuous variables are presented as mean and standard deviation, and categorical as relative frequencies (percentages). \(p\)-values referring to the comparisons using chi-square test or t test for the categorical and continuous variables, respectively.

D-AII, Dietary Anti-Inflammation Index; FLI, fatty liver index; HSI, hepatic steatosis index; NAFLD, nonalcoholic fatty liver disease; FLS, Fatty Liver Score; SES, socioeconomic status; TyG, triglyceride-glucose.
and consistently beneficial effect of an anti-inflammatory diet to NAFLD using all four hepatic health indices (TyG, FLI, HSI, and NAFLD-FLS), in accordance with the aforementioned. This analysis is the first of its kind implementing diverge metrics to illustrate the major effect of adherence to anti-inflammatory diet in hepatic health.

To further explore the effect of anti-inflammatory nutrition on NAFLD, the D-AII grouped in tertiles (first tertile was reporting pro-inflammatory diet, and third tertile reported anti-inflammatory diet). In the applied regression analysis, it was reported that in all four hepatic health metrics, only the third tertile of D-AII was protective against NAFLD. The intermediate D-AII tertile (second) was related with lower odds of NAFLD using all indices except the TyG index, where there was no significance. This could be explained because the TyG index was created primarily to detect insulin resistance/sensitivity and diabetes risk, and only recently it has been proposed as a sensitive metric to assess NAFLD.

Table 2. Results from the applied logistic regression evaluating NAFLD, according to D-AII, for the baseline ATTICA participants.

<table>
<thead>
<tr>
<th>All participants</th>
<th>Odds ratios (95% confidence intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAFLD by TyG cutoff</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>1.004</td>
<td>(0.99–1.02)</td>
</tr>
<tr>
<td>Men versus women</td>
<td></td>
</tr>
<tr>
<td>1.06</td>
<td>(0.77–1.48)</td>
</tr>
<tr>
<td>Education (years of school)</td>
<td></td>
</tr>
<tr>
<td>1.01</td>
<td>(0.96–1.07)</td>
</tr>
<tr>
<td>SES (low/middle versus high)</td>
<td></td>
</tr>
<tr>
<td>1.09</td>
<td>(0.73–1.6)</td>
</tr>
<tr>
<td>Alcohol intake (y/n)</td>
<td></td>
</tr>
<tr>
<td>0.83</td>
<td>(0.62–1.12)</td>
</tr>
<tr>
<td>Smoking ever (y/n)</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>(0.56–0.98)</td>
</tr>
<tr>
<td>Physically active (y/n)</td>
<td></td>
</tr>
<tr>
<td>0.98</td>
<td>(0.96–1.06)</td>
</tr>
<tr>
<td>Obesity, % yes</td>
<td></td>
</tr>
<tr>
<td>1.34</td>
<td>(0.97–1.86)</td>
</tr>
<tr>
<td>Hypertension (y/n)</td>
<td></td>
</tr>
<tr>
<td>0.71</td>
<td>(0.52–0.96)</td>
</tr>
<tr>
<td>Diabetes mellitus (y/n)</td>
<td></td>
</tr>
<tr>
<td>0.95</td>
<td>(0.53–1.69)</td>
</tr>
<tr>
<td>Hypercholesterolemia (y/n)</td>
<td></td>
</tr>
<tr>
<td>1.20</td>
<td>(0.90–1.60)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td></td>
</tr>
<tr>
<td>3.04</td>
<td>(0.70–13.28)</td>
</tr>
<tr>
<td>D-AII (10–77)</td>
<td></td>
</tr>
</tbody>
</table>

D-AII, Dietary Anti-Inflammation Index; FLI, fatty liver index; HSI, hepatic steatosis index; NAFLD, nonalcoholic fatty liver disease; FLS, Fatty Liver Score; SES, socioeconomic status; TyG, triglyceride-glucose.
studies have noted that consuming certain foods, such as cereals, legumes, potatoes, and low-fat dairy, is inversely related to NAFLD. Adopting the Mediterranean diet has been inversely related with hepatic steatosis. Foods rich in monounsaturated and polyunsaturated fatty acids and antioxidants and flavonoids also are inversely related with NAFLD, possibly due to the high beneficial effect of their antioxidant and anti-inflammatory components in the hepatic inflammation pathway. Based on this understanding, a food’s antioxidant capacity, inflammatory, and metabolism interrelated pathways might explain the reported association between the highest D-AII tertile and NAFLD.

Although individuals with NAFLD have no symptoms, salient nutrition-related cardiometabolic risk factors coexist, such as insulin resistance, obesity, diabetes mellitus, and metabolic syndrome. Specifically, researchers have proposed that NAFLD could be the liver’s manifestation of metabolic syndrome, due to the interaction in both conditions of abnormal levels in insulin resistance, adipokines, cytokines, and inflammatory markers. In the study’s sample, obesity rates were low among all D-AII tertiles, compared with other European and Mediterranean populations older than age 45. This detail could aid the interpretation of this study’s results. In addition, although recent epidemiologic data report that a low-to-moderate consumption of alcohol could be beneficial for those with NAFLD, typical guidance cites full alcohol abstinence so the liver can recuperate more quickly. ATTICA’s stratified analysis emphasized among those with metabolic syndrome or daily alcohol drinkers has shown the consistent beneficial effect of an anti-inflammatory diet even among these cases.

Western populations suffering from NAFLD experience poor quality of life with increased cardiometabolic risk. In the coming years, NAFLD is expected to be the leading cause of hepatic-related morbidity and mortality. Already it is the main indicator for orthotopic liver transplantation (OLT). These known associations, coupled with an ageing Western populace witnessing an obesity epidemic with metabolic syndrome, raise the call for early nonpharmacological measures to promote hepatic health. Future studies are needed to evaluate the effect of anti-inflammatory dietary habits to NAFLD longitudinally.

**Strengths and limitations**

The present study has a major strength. It is the first study that evaluated the association between D-AII and NAFLD of a large sample of CVD-free adults, testing the aforementioned, in four noninvasive NAFLD metrics. Among limitations, it should be acknowledged that the baseline/entry study examination was conducted once and, hence, may be susceptible to a degree of measurement error. The assessment of dietary habits using the FFQ as a tool is known for certain biases. In addition, in our analysis, a number of continuous variables were transformed and used as binary items and were lost some information
variance. In addition, despite the fact other types of liver diseases or drugs (i.e. antibiotics)\textsuperscript{49} are associated with hepatic steatosis, the survey did not include a detailed assessment on these items and, for this reason, have not been used as confounders in the applied regression models. The calculation of TyG, FLI, HSI, and NAFLD-FLS were based on population equations that might underestimate or overestimate the actual prevalence of NAFLD. However, it has been reported that these formulas were validated and present good agreement with the classical methods of NAFLD measurement [magnetic resonance spectroscopy (MRS) or ultrasonography].\textsuperscript{30,33} In addition, these kinds of indices have been used in other European cohorts, such as the Brisighella Heart Study.\textsuperscript{50} In addition to these NAFLD formulas, liver ultrasound\textsuperscript{51} or magnetic resonance (MR),\textsuperscript{52} and other measures such as blood indices and/or FibroScan (Echosens, Waltham, MA, USA) are considered essential for NAFLD diagnosis. Therefore, the method of NAFLD diagnosis in this study is not completely in line with current recommendations, and some participants might not have been diagnosed with NAFLD in 2019. Finally, some participants might be misclassified as having NAFLD, although they did exhibit NASH. However, for this assessment, other liver fibrosis markers were needed that were not applied in this study.

Conclusions
The present work evaluated the role of anti-inflammatory diet on NAFLD of adults without pre-existing cardiovascular disease. It is of major interest now to study the anti-inflammatory burden of diet to better understand its role on hepatic health. Throughout this study, the consistent relationship of D-AII with all the noninvasive indices of NAFLD has been highlighted. A consistent inverse association between D-AII and NAFLD among individuals with metabolic syndrome and daily alcohol drinkers was also shown.

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Author contributions
Stefanos Tyrovolas and Demosthenes Panagiotakos were responsible for the study design, data analysis and manuscript draft. Ekavi Georgouso-poulou, Christina Chrysohou, John Skoumas, William Pan, Dimitrios Tousoulis and Christos Pitsavos revised the manuscript. All authors contributed to interpreting the data and approving the final manuscript.

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Conflict of interest statement
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