High Dietary Inflammatory Index associates with inflammatory proteins in plasma

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Abstract

Background and aim:
Unhealthy dietary habits and highly caloric foods induce metabolic alterations and promote the development of inflammatory consequences of obesity and insulin resistance, which are epidemic conditions leading to diabetes and cardiovascular diseases. Describing an inflammatory effect of diet is difficult to pursue, owing to the lack of quali-quantitative dietary assessment standardization. The Dietary Inflammatory Index (DII) has been proposed as an estimator of the pro- or anti-inflammatory effect of nutritional components. Higher DII values, which indicate an increased intake of nutrients with pro-inflammatory effects, relates to an increased risk of metabolic and cardiovascular diseases in epidemiological studies. Whether higher DII values reflect biologically relevant variations of inflammatory proteins in plasma, has been poorly described today.

Methods:
In this cross-sectional study, seven-days dietary records from 663 subjects in primary prevention for cardiovascular diseases were analyzed to derive the intake of nutrients, foods and to calculate DII. To associate DII with the Normalized Protein eXpression (NPX), an index of abundance, of a targeted panel of 368 inflammatory biomarkers (Olink™) measured in the plasma, we divided the population by the median value of DII (1.60 (0.83–2.30)).

Results:
332 subjects with estimated DII over the median value reported a higher intake of saturated fats but lower intakes of poly-unsaturated fats, including omega-3 and omega-6 fats, versus subjects with estimated dietary DII below the median value (N = 331). The NPX of 61 proteins was increased in the plasma of subjects with DII > median vs subjects with DII < median. By contrast, in the latter group, we underscored only 3 proteins with increased NPX. Only 23, out of these 64 proteins, accurately identified subjects with DII > median (Area Under the Curve = 0.601 (0.519–0.668), p = 0.035).

Conclusion:
This large-scale proteomic study supports that higher DII reflects changes in the plasmatic abundance of inflammatory proteins. Larger studies are warranted to validate.

Introduction
Metabolic alterations induced by unhealthy dietary habits and by consumption of highly caloric foods, including obesity and insulin resistance, are epidemic conditions leading to diabetes and cardiovascular
diseases. To prevent their occurrence, current guidelines constantly advise to contain the intake of calorie-dense nutrients, upon the concept that reducing their metabolic burden will constrain the inflammatory consequences of unhealthy dietary habits.

The understanding of a pro-inflammatory effect of diet, to link the intake of specific nutritional components of foods with the activation of inflammatory mechanisms, is difficult to pursue, because of shortcomings in the standardization of qualitative assessments (e.g. Food Frequency Questionnaires “FFQs”) and quantitative analyses of dietary consumption. The Dietary Inflammatory Index (DII) is a validated score, generally calculated from the analysis of FFQs, that has been associated with the presence or the occurrence of cardio-metabolic alterations and cardiovascular diseases in epidemiological studies. DII normalizes the intake of each nutrient present in the foods consumed over the period of the dietary assessment for a correction factor (“inflammatory effect score”), which is either positive, if that nutrient is expected to exert pro-inflammatory effect (e.g. saturated fats to which the highest score is addressed), or negative, if an anti-inflammatory effect is predicted based on experimental evidence from literature (e.g. fiber, to which the lowest score is addressed).

Sparse data indicate that changes in DII reflect biologically relevant variations in the abundance of some inflammatory proteins. Indeed, some data indicate that high DII relates to increased plasma levels of C-Reactive Protein (CRP), while others do not support this relation or failed to find an association with other common markers of inflammation. Also, the association between high DII, immune cell counts, and few other targeted cytokines has been only recently evaluated in marginalized population or in comorbid patients. To elucidate the relation between higher DII and inflammatory markers, we conducted a plasma proteomic study, measuring the abundance of 368 proteins, that we previously associated with increased cardiovascular risk in independent cohorts. By harnessing Proximity Extension Assay (PEA; Olink™), a technology that combines the use of antibodies with unique oligonucleotides to run DNA amplification steps, we simultaneously measured each protein with an elevated degree of sensitivity, reaching ng-pg/ml concentration ranges, and, consequently, we provided a superior power of investigation compared to the common existing literature published for this purpose.

Materials and Methods

Study design and population

The “PLIC” (Progressione delle Lesioni Intimali Carotidi) Study was developed and followed at the Center for the Study of Atherosclerosis at E. Bassini Hospital (Cinisello Balsamo, Milan, Italy). 2,606 participants were initially included in the PLIC study from 2001 to 2003 and all the information needed for the purpose of this study was available on 663 subjects. Supplemental Fig. 1 reports the flowchart of the study. Further information about ethic statements, inclusion criteria, sample selection, sample size statistical analysis, and selection bias see Supplemental Material. This work is an cross-sectional
study and it was conducted following the standards of the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) initiative\textsuperscript{27}.

**Measurement of biochemical and clinical parameters**

Blood samples were collected from antecubital vein after 12 hours fasting on NaEDTA tubes (BD Vacuette) and then, centrifuged at 3,000 rpm for 12 minutes (Eppendorf 580r, Eppendorf, Hamburg, Germany) for biochemical parameters profiling including total cholesterol, HDL-C, triglycerides, Apolipoprotein B (ApoB), Apolipoprotein A-I (ApoA-I), glucose and C-Reactive Protein. Measurements were performed using immuno-turbidimetric and enzymatic methods through automatic analyzers (Randox, Crumlin, UK). LDL-C was derived from the Friedewald formula.

Data on pathological, pharmacological history (including lipid-lowering, glucose-lowering, anti-hypertensive and antiplatelets therapy). Clinical and anthropometrical measures (systolic and diastolic blood pressure, Body Mass Index (BMI), waist and hips circumferences, height, and weight) and lifestyle habits, were collected during the outpatient activity using a validated questionnaire approved for the PLIC Study, as described elsewhere\textsuperscript{25}.

**Dietary data analysis**

Seven-day dietary records of each subject were processed by trained dietitians to derive the daily intakes of calories and nutrients from foods consumed. This analysis was performed following data reported in publicly available datasets\textsuperscript{28}. Single food items were then classified according to the food category reported in Italian publicly available datasets\textsuperscript{29}. The intake of macro-and micro-nutrients derived for each of the seven-day was employed to calculate the DII, as reported by Shivappa N. et al\textsuperscript{13}. Further details are provided as Supplemental Material.

**Proteomics analysis**

Proteins were measured by Proximity Extension Assay (PEA) strategy and the complete list of the proteins that are included in the Cardiovascular II, Cardiovascular III, Cardiometabolic and Inflammation panels of the Olink™ platform have been previously indicated\textsuperscript{22}. Further methodological details are reported as Supplemental Material.

**Statistics:**

The statistical analyses were performed using the SPSS software (version 28.0) for Windows. Graphs were prepared using GraphPad Prism (version 8).

Linear data are presented as mean with standard deviation or as median (interquartile ranges) after verifying for normal distribution (Kolmogrov-Smirnov test). The comparison within each group was performed with simple t-test (if linear distribution) or Mann-Whitney U-test (if not-normal distribution). The variations in the expression of plasma proteins between groups of subjects were analyzed by calculating the fold changes (on log\textsubscript{2} scale).
To validate the biological relevance of the DII, we built a binary outcome prediction (DII > median cohort vs DII < median cohort) model with XGboost algorithm.

**Gradient boosting Machine Learning (ML) model**

The model included all the significantly different proteins measured among those with DII > median vs DII < median. The total sample was split randomly into train set (60% of the entire cohort) and test set (40% of the entire cohort). The XGBoost classifier model was trained in the train set with 1000 iteration rounds and < 0.001 learning rate. Hyperparameter optimization was performed by k-fold iteration internal to the training set. The most important proteins found in the optimized model were then listed by relative importance in the Random Forest classifier plot. Then we assessed the predicting performance of the algorithm in the test set by Receiver Operating Characteristic (ROC) analysis. Models were built in Python 6.4.5 with pandas, scikit-learn, NumPy, XGboost.

**Results**

**Specific food patterns and nutritional profiles from habitual diets characterize higher DII.**

By the analysis of the seven-days dietary records of the 663 subjects, we classified the food patterns and averaged their amount consumed daily (Table 1), to then quantify the total daily energy intake, the percentages of the energy deriving from the main macronutrients (%En/day) and the absolute intakes of the micro-nutrients present in the consumed food patterns (either as milligrams/day (mg/day) or micrograms/day (mcg/day)) (Table 2). With this information, we then calculated that DII was 1.60 on average (0.83–2.30) and to explore which foods and nutrients mostly reflect higher DII values, we compared the nutritional and dietary profiles of the subjects with DII > median (n = 332, DII = 2.30 (1.97–2.73)) with those of subjects with DII < median (n = 331, DII = 0.83 (0.29–1.18)). The clinical characteristics of these two groups are available in **Supplemental Table 1**.
Table 1
Intakes of food groups reported to be consumed in the seven days’ dietary reports according to lower or higher DII.

<table>
<thead>
<tr>
<th>Daily intake of foods</th>
<th>Total sample (n = 663)</th>
<th>DII &lt; median (n = 331)</th>
<th>DII &gt; median (n = 332)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/day</td>
<td>Median (25th-75th percentiles)</td>
<td>Median (25th-75th percentiles)</td>
<td>Median (25th-75th percentiles)</td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>200.42 (147.14-254.82)</td>
<td>234.57 (191.04-295.65)</td>
<td>166.55 ± 66.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Legumes</td>
<td>12.86 (0-28.57)</td>
<td>14.29 (3.17–33.33)</td>
<td>5.7 (0-21.43)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fruits</td>
<td>261.43 (167.55-350.14)</td>
<td>307.14 (238.57-411.43)</td>
<td>216.2 ± 126.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bread, crispbread, rusks</td>
<td>87.14 (54.29-119.29)</td>
<td>95.71 (62.86-127.14)</td>
<td>78.18 (47.93-108.06)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>28.57 (14.29-50)</td>
<td>35.71 (20.71-60)</td>
<td>25.36 (7.14–41.79)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Oils and vegetables fats</td>
<td>22.86 (17.14–29.21)</td>
<td>25.17 (19.06–32.33)</td>
<td>20.71 (14.58–25.96)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>71.43 (9.76-202.75)</td>
<td>105.98 (20.47-226.21)</td>
<td>50 (3.39-175.28)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Condiments and mayonnaise</td>
<td>2.68 (0.78–6.32)</td>
<td>3.05 (0.98–7.14)</td>
<td>2.14 (0.71–5.29)</td>
<td>0.002</td>
</tr>
<tr>
<td>Milk and yogurt</td>
<td>135.71 (59.9-197.04)</td>
<td>144.17 (82.5-206.48)</td>
<td>128.21 (53.57-182.37)</td>
<td>0.007</td>
</tr>
<tr>
<td>Potatoes</td>
<td>21.43 (6.77-40)</td>
<td>24.07 (7.14–44.84)</td>
<td>19.01 (5.63–35.35)</td>
<td>0.019</td>
</tr>
<tr>
<td>Cereals and cereals products (except bread)</td>
<td>74.11 (53.39–97.21)</td>
<td>77.14 (57.14–98.57)</td>
<td>70.51 (51.63–93.4)</td>
<td>0.065</td>
</tr>
<tr>
<td>Cakes</td>
<td>35.71 (20-59.67)</td>
<td>39.29 (20.29–61.43)</td>
<td>33.47 (19.88–55.71)</td>
<td>0.088</td>
</tr>
<tr>
<td>Meat and meat products</td>
<td>93.57 (67.42-124.02)</td>
<td>96 (69.71-125.64)</td>
<td>88.93 (65.46-121.18)</td>
<td>0.143</td>
</tr>
<tr>
<td>Coffee, tea and herbal drinks</td>
<td>98.57 (62.14-166.43)</td>
<td>102.86 (64.29-168.57)</td>
<td>92.26 (57.74-158.57)</td>
<td>0.166</td>
</tr>
<tr>
<td>Sugar and confectionery</td>
<td>20.36 (9.43–37.51)</td>
<td>21.43 (10.31–41.43)</td>
<td>19.29 (9.29–34.86)</td>
<td>0.186</td>
</tr>
<tr>
<td>Eggs</td>
<td>8.33 (1.81–14.46)</td>
<td>8.57 (2.14–16.43)</td>
<td>7.41 (1.53-14.29)</td>
<td>0.240</td>
</tr>
<tr>
<td>Daily intake of foods</td>
<td>Total sample (n = 663)</td>
<td>DII &lt; median (n = 331)</td>
<td>DII &gt; median (n = 332)</td>
<td>p-value</td>
</tr>
<tr>
<td>-----------------------------</td>
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</tr>
<tr>
<td>Cheeses</td>
<td>34.14 (21.43–47.81)</td>
<td>33.85 (20.71–47.81)</td>
<td>34.27 (23.03–47.5)</td>
<td>0.348</td>
</tr>
<tr>
<td>Non-alcoholic beverages</td>
<td>0 (0-17.86)</td>
<td>0 (0-10.5)</td>
<td>0 (0-25.54)</td>
<td>0.492</td>
</tr>
<tr>
<td>Bouillons</td>
<td>7.51 (0-33.75)</td>
<td>7.71 (0-31.43)</td>
<td>6.85 (0-35.71)</td>
<td>0.783</td>
</tr>
<tr>
<td>Butter and animal fats</td>
<td>2.86 (0-6.43)</td>
<td>2.86 (0-6.43)</td>
<td>2.85 (0-6.31)</td>
<td>0.888</td>
</tr>
</tbody>
</table>
Table 2
Intakes of nutrients reported to be consumed in the seven days’ dietary reports according to lower or higher DII.

<table>
<thead>
<tr>
<th>Daily intake of nutrients</th>
<th>Total sample (n = 663)</th>
<th>DII &lt; median (n = 331)</th>
<th>DII &gt; median (n = 332)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (25th -75th percentiles)</td>
<td>Median (25th -75th percentiles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (Kcal/day)</td>
<td>1711.96 (1469.94-2045.29)</td>
<td>1882.61 (1587.64-2168.17)</td>
<td>1592.03 (1344-1848.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Energy from macronutrients:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy from lipids (%En/day)</td>
<td>35.11 ± 5.61</td>
<td>34.73 ± 5.53</td>
<td>35.49 ± 5.68</td>
<td>0.083</td>
</tr>
<tr>
<td>Energy from saturated fat (%En/day)</td>
<td>11.56 ± 2.50</td>
<td>11.08 ± 2.36</td>
<td>12.05 ± 2.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Energy from monounsaturated fat (%En/day)</td>
<td>16.25 (14.3-18.25)</td>
<td>16.25 (14.31–18.33)</td>
<td>16.26 (14.22–17.93)</td>
<td>0.647</td>
</tr>
<tr>
<td>Energy from polyunsaturated fat (%En/day)</td>
<td>4.04 (3.47–4.77)</td>
<td>4.12 (3.55–4.9)</td>
<td>3.88 (3.39–4.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Energy from omega-3 polyunsaturated fat (%En/day)</td>
<td>0.6 (0.5–0.73)</td>
<td>0.64 (0.53–0.77)</td>
<td>0.57 (0.49–0.69)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Energy from omega-6 polyunsaturated fat (%En/day)</td>
<td>3.32 (2.81-4)</td>
<td>3.42 (2.88–4.12)</td>
<td>3.24 (2.73–3.88)</td>
<td>0.013</td>
</tr>
<tr>
<td>Energy from proteins (%En/day)</td>
<td>16.04 (14.67–17.62)</td>
<td>16.04 (14.65–17.59)</td>
<td>16.04 (14.73–17.66)</td>
<td>0.553</td>
</tr>
<tr>
<td>Energy from carbohydrates (%En/day)</td>
<td>50 ± 6.27</td>
<td>50 ± 6.27</td>
<td>49.48 ± 6.36</td>
<td>0.284</td>
</tr>
<tr>
<td>Energy from soluble carbohydrates (%En/day)</td>
<td>16.66 (14.07–19.52)</td>
<td>17.21 (14.76–20.08)</td>
<td>15.84 (13.29–18.96)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Daily intake of micronutrients:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/day)</td>
<td>638.21 (503.51-782.09)</td>
<td>713.53 (568.12-847.42)</td>
<td>569.42 (474.34-705.86)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Iron (mg/day)</td>
<td>9.74 (7.93–11.58)</td>
<td>11.09 (9.57–12.67)</td>
<td>8.11 (7.07–10.08)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sodium (mg/day)</td>
<td>1772.92 (1411.09-2275.05)</td>
<td>1927.06 (1519.7-2425.92)</td>
<td>1671.76 (1308.76-2075.68)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Daily intake of nutrients</td>
<td>Total sample (n = 663)</td>
<td>DII &lt; median (n = 331)</td>
<td>DII &gt; median (n = 332)</td>
<td>p</td>
</tr>
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</tr>
<tr>
<td></td>
<td>Median (25th-75th percentiles)</td>
<td>Median (25th-75th percentiles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium (mg/day)</td>
<td>2596.59 (2192.95-2990.21)</td>
<td>2931.34 (2653.53-3266.4)</td>
<td>2223.86 (1985.62-2503.77)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phosphorus (mg/day)</td>
<td>1053.82 (889.49-1234.9)</td>
<td>1154.56 (988.41-1305.85)</td>
<td>959.42 (834.21-1112.65)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Zinc (mg/day)</td>
<td>8.96 (7.71-10.53)</td>
<td>9.82 (8.57-11.4)</td>
<td>8.22 (7.13-9.34)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Magnesium (mg/day)</td>
<td>156.2 (129.01-186.14)</td>
<td>172.57 (145.16-203.69)</td>
<td>139.48 (116.32-164.95)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Selenium (mg/day)</td>
<td>29.87 (22.1-40.26)</td>
<td>33.7 (25.51-44.18)</td>
<td>26.04 (19.84-35.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin B1 (mg/day)</td>
<td>0.89 (0.75-1.08)</td>
<td>1.01 (0.86-1.15)</td>
<td>0.79 (0.68-0.94)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin B2 (mg/day)</td>
<td>1.4 (1.17-1.64)</td>
<td>1.58 (1.38-1.78)</td>
<td>1.25 (1.08-1.43)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin C (mg/day)</td>
<td>108.49 (75.8-151.17)</td>
<td>139.24 (109.92-177.62)</td>
<td>80.62 (59.38-107.27)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin B3 (mg/day)</td>
<td>16.57 (14.19-19.75)</td>
<td>18.36 (15.6-20.99)</td>
<td>15.35 (12.94-17.82)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin B6 (mg/day)</td>
<td>1.62 (1.37-1.89)</td>
<td>1.83 (1.61-2.04)</td>
<td>1.43 (1.24-1.64)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Folates (mcg/day)</td>
<td>256.19 (209.88-310.85)</td>
<td>302.4 (257.66-345.18)</td>
<td>219.93 ± 56.29</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pantothenic acid (mg/day)</td>
<td>2.48 (2.03-3.01)</td>
<td>2.79 (2.34-3.35)</td>
<td>2.23 (1.89-2.62)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Biotin (mg/day)</td>
<td>16.66 (13.54-20.43)</td>
<td>18.78 (15.05-22.51)</td>
<td>15.22 (12.51-17.86)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin B12 (mcg/day)</td>
<td>4 (2.96-6)</td>
<td>4.39 (3.22-6.85)</td>
<td>3.63 (2.78-5.05)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin A (RE/day)</td>
<td>718.38 (547.99-941.6)</td>
<td>862.68 (721.98-1095.18)</td>
<td>575.93 (476.17-713.22)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vitamin E (mcg/day)</td>
<td>9.7 (8.07-11.77)</td>
<td>11.23 (9.44-13.07)</td>
<td>8.72 ± 2.38</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Subjects with DII > median reported to consume not only less vegetables, legumes, and fruits, but also less daily amount of bread/crispbread/rusks, fish/shellfish, oils/vegetable fats, condiments/mayonnaise, milks/yogurts, and potatoes compared to subjects with DII < median; By contrast, the consumption of other food patterns, including cereal products, cakes, meats and processed meats, coffee/tea, eggs, cheeses, butter and animal fats was comparable (Table 1).

In addition to these differences in the food patterns consumed, the daily caloric intake of subjects with DII > median was lower compared to that of the subjects DII < median (1592.03 (1344.00-1848.50) Kcal/die vs 1882.61 (1587.64-2168.17) Kcal/die, p < 0.001; Table 2). In search of a possible explanation of this difference, we profiled the percentages of energy deriving from macronutrients. While the percentage of energy from carbohydrates, soluble carbohydrates, and proteins were comparable between subjects with DII > median vs subjects with DII < median (49.48 ± 6.36% vs 50.00 ± 6.27% from carbohydrates, p = 0.284; 15.84 (13.29–18.96)% vs 17.21 (14.76–20.08)% from soluble carbohydrates, p < 0.001; 16.04 (14.73–17.66)% vs 16.04 (14.65–17.59)% from proteins (Table 2)), the energy deriving from the intake of lipids was numerically, although not statistically, higher in the group of subjects with DII > median (35.49 ± 5.68% vs 34.73 ± 5.53%, p = 0.083 (Table 2)). This trend was explained by an increased energy deriving from the intake of saturated fats in subjects with DII > median (12.05 ± 2.55% vs 11.08 ± 2.36%, p < 0.001 (Table 2)), a comparable energy deriving from the intake of monounsaturated fats (MUFA) (16.26 (14.22–17.93)% vs 16.25 (14.31–18.33)% p = 0.647 (Table 2)), but reduced energy deriving from the intake of polyunsaturated fats (PUFA) versus subjects with DII < median (3.88 (3.39–4.60)% vs 4.12 (3.55–4.90)% p = 0.002 (Table 2)). Furthermore, the percentages of energy deriving from the intakes of omega-3 PUFA and omega-6 PUFA were reduced in subjects with DII > median vs subjects with DII < median (0.57 (0.49–0.69)% vs 0.64 (0.53–0.77)% for omega-3, p < 0.001; 3.24 (2.73–3.88)% vs 3.42 (2.88–4.12)% for omega-6, p = 0.013 (Table 2)). Finally, higher DII was also
associated with significantly less intake in the entire spectrum of micronutrients and vitamins (Table 2) which, although not providing energetic supply, significantly contribute to the “inflammatory effect score” used to estimate their anti-inflammatory potential.

Together these findings indicate that a distinct food and nutritional profile contributes to calculating a higher DII from the quantitative analysis of the seven days’ dietary records.

**Higher Dietary Inflammatory Index is associated with plasma markers of inflammation.**

In addition to the differences in the clinical profile, we also found that higher DII associated with higher CRP levels, which were increased in the plasma of subjects with DII > median versus the group of subjects with DII < median (0.10 (0.06–0.07) vs 0.08 (0.04–0.15) mg/L respectively, p = 0.004; Supplemental Table 1). This finding, in line with data from literature but in contrast with others, prompted us to investigate whether higher DII reflects biologically relevant variations in the plasmatic abundance of a larger spectrum of inflammatory proteins.

Compared to subjects with DII < median, the NPX of 61 proteins were significantly increased while the NPX of 3 proteins was significantly reduced in the plasma of subjects with DII > median (Fig. 1A) Supplemental Table 2 reports the mean and the standard errors of each protein in both groups, the p values and the log2fold of change of the variation of the NPX of each protein in the group of subjects with DII > median versus the median value of the NPX in the group of subjects with DII < median).

To identify which proteins, out of the ones significantly changed between subjects with DII > median vs subjects with DII < median, associated with higher DII, we employed a machine learning boosting prediction model trained on a subset of 194 subjects in the group with DII > median compared to 203 subjects in the group with DII < median (“training sets”). This model was then tested in internal test sets (138 subjects with DII > median vs 128 subjects with DII < median) (“test sets”; see methods). Of note, this model achieved significant performance in discriminating subjects with DII > median versus subjects DII < median (Area Under the Curve (AUC) of Receiver Operating Characteristic (ROC) = 0.601 (0.519–0.668), and p = 0.035) (Fig. 1B) and underscored 23 proteins that, emerging significant in the train set, were also confirmed as hit predictors of higher DII in test sets (Fig. 1C, reporting Random Forest classifier in descending order the importance of each protein in the model). Out of these proteins, 22 were increased in the plasma of subjects with DII > median, compared to subjects with DII < median and included, by descending order of predicted importance (Random Forest classifier; see methods) Galectine-9 (Gal9), Sulfotransferase 1A1 (ST1A1), Vascular Endothelial growth factor A (VEGFA), Platelet glycoprotein Ib alpha chain (GP1A1), Stem cell factor (SCF), Junctional adhesion molecule A (JAM-A), Programmed death-ligand 1 (PD1L1), Sirtuin-2 (SIRT2), Colony Stimulating Factor 1 (CSF1), Interleukine-24 (IL-24), Interleukine-6 (IL-6), Selectin-P (SELP), Caspase 3 (CASP3), Fibroblast growth factor 3 (FGF23), Chemokine-ligand 5 (CCL5), Chemokine-ligand 18 (CCL18), Spondin-1 (SPON1), Hepatocyte growth factor (HGF), tumor necrosis factor receptor superfamily member 10A (TNFRSF10A), CD8 subunit alpha (CD8A), Integrin Subunit Beta 1 Binding Protein 2 (ITGB1BP2), Serpin Family A Member 7 (SERPINA7) were
significantly increased. By contrast, only Interleukin-27 (IL-27), known to exert anti-inflammatory properties, while was significantly reduced in subjects with DII > median vs subjects with DII < median.

Together these data indicate that the predicted inflammatory potential of diet, marked by higher DII, reflects biologically relevant changes in the plasmatic abundance of a large set of inflammatory proteins.

**Discussion**

Our data support that higher DII reflects significant variations in the plasmatic abundance of inflammatory proteins that we previously associated with increased cardiovascular risk \(^{22,23}\). By using quantitative seven-days’ dietary records, alternative to the qualitative FFQs, and by a harnessing high sensitivity technique to measure a large set of plasma proteins, our findings contribute to a better understanding of the inflammatory consequences of unhealthy dietary habits, as a risk factor for the development of obesity, cardiometabolic and cardiovascular diseases. To date, significant shortcomings affect studies to properly find a plausible inflammatory effects of diet and they principally include lacks of standardizations for the dietary assessments and a poor accuracy, both of the publicly available biobanks (including the ones for the Italian population\(^{31}\)) and of the scores/indices used so far to distinguish healthy from unhealthy dietary patterns (e.g. the PREDIMED score\(^{32}\)), to provide a realistic quantitative dietary and nutritional intakes. By contrast, the seven-days’ dietary records, although being representative of the adherence to a specific dietary pattern in a short-term period, they allowed to unmask the different associations between DII, the consumed foods and the caloric/nutritional intakes that we found in this study; This information could not have been provided by the qualitative assessment of the FFQs used in large epidemiological studies\(^{3,8–11}\). From a methodological standpoint, our protocol for the dietary assessment involved the analysis of the seven days’ records by two expert and independent dieticians, hence minimizing the risk of biases in the self-reporting/underestimation. Accordingly, the total caloric intakes were in line with the current dietary surveys for the Italian population \(^{31}\).

Our study is a first-in-class biological validation of the DII, including the plasmatic quantification of an unprecedented large set of proteins, that we still associated with increased cardiovascular risk\(^{22}\), by using a high-sensitivity technology.

The predictions of the machine learning model find a match with biological relevance. Indeed, 22 proteins, which displayed increased plasma abundance in subjects with DII > median, included those relevant for systemic inflammatory responses (e.g: Gal9, PDL1, SIRT2, IL-24, IL-6, SELP, CCL5, CCL18, TNFRSF10A, CD8A). By contrast IL27, with known immunoregulatory potential\(^ {33}\), was the unique protein more abundant in plasma of subjects with DII < median and the second predictor identified by the machine learning algorithm. Anyhow, the PEA technology, employed for this proteomics analysis, although ensuring an elevated degree of sensitivity, provides an information of a relative abundance (NPX values), but not an absolute quantity, of each marker \(^ {34}\). Therefore, the future step of our study will be to confirm such data of abundance into absolute quantities by techniques of mass-spectrometry.
Furthermore, we also acknowledge other limits in our study. Multiple aspects related to diet (e.g. the geographic locations\textsuperscript{35}, the socioeconomic status\textsuperscript{36}, the processing and quality of foods\textsuperscript{37}) could significantly impact and cannot be unmasked in this single-center experience. Validation studies in independent cohorts and in subjects with more advanced cardio-metabolic impairment are warranted.

Finally, longitudinal studies still demonstrated that dietary changes towards adherence to healthier dietary patterns result into reduction of DII\textsuperscript{38,39}, and whether such changes also lead to reductions in the plasma abundance of inflammatory proteins will be a matter of analysis in our future studies.

**Conclusions**

Higher DII, calculated from the quantitative analysis of the consumption of specific food patterns and nutritional intakes, associates with significant variation of a large set of inflammatory proteins in plasma.

**Abbreviations**

DII
Dietary Inflammatory Index
CRP
C-Reactive Protein
Gal-9
Galectine-9
ST1A1
Sulfotransferase 1A1
VEGFA
Vascular Endothelial growth factor A
GP1A1
Platelet glycoprotein Ib alpha chain
SCF
Stem cell factor
JAM-A
Junctional adhesion molecule A
PDL1
Programmed death-ligand 1
SIRT2
Sirtuin-2
CSF1
Colony Stimulating Factor 1
IL-24
Interleukine-24
IL-6
Declarations

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Ethic approval and consent to participate

The PLIC study, including clinical assessments, collection of biological samples and analysis of individual data, was approved in 2001 by the Scientific Ethic Committee of the University of Milan (SEFAP/Pr.0003).

Consent for publication

Not applicable.
Availability of data and materials

The pooled data that support the findings of this study are available from the author A.L.C., upon reasonable request.

Competing interests

The authors declare no conflicts of interest relevant to the submitted work.

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Authors contributions

All the authors approved the manuscript in its contents and details and agree to be personally accountable for the author’s own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature. A.B., E.M. contributed to conceptualization, investigation, formal analyses and use of software, data curation, writing, reviewing and editing the original draft. A.L.C. contributed to conceptualization, investigation, supervision, reviewing and editing the original draft. E.P., F.A., L.R., A.N., S.T., N.S., L.G., F.P. contributed to methodology and formal analyses., A.B. and N.S. contributed to visualization and supervision. A.B., and A.L.C. contributed to resources and funding acquisition. All authors have read and agreed to the published version of the manuscript.

References


3. Denova-Gutiérrez E, Muñoz-Aguirre P, Shivappa N, Hébert JR, Tolentino-Mayo L, Batis C, Barquera S. Dietary Inflammatory Index and Type 2 Diabetes Mellitus in Adults: The Diabetes Mellitus Survey of


28. BDA | Food Composition Database for Epidemiological Studies in Italy [Internet]. [cited 2022 Apr 30];Available from: http://www.bda-ieo.it/wordpress/en/


34. What is NPX? - Olink [Internet]. [cited 2022 Sep 2];Available from: https://www.olink.com/faq/what-is-npx/


**Figures**

Figure 1
**Pro-inflammatory potential of diet marks variation of inflammatory proteins.**

(A) Volcano plot, showing how much the plasmatic expression of the 368 proteins in subjects with pro-inflammatory potential of diet (DII over the median value of the population) change as compared to the plasmatic expression of the same proteins in subjects with anti-inflammatory potential of diet (DII below the median value of the population). Data are expressed as fold of changes in log$_2$ scale (x axis). On the y axis, the p-value of the difference between the two groups is reported (as $-\log_{10}$).

(B) Receiving Operating Curve (ROC) reporting the performance of the machine learning model (as sensitivity and 1-specificity to detect subjects with pro-inflammatory potential of diet (DII over the median value of the population) including the 368 proteins measured in plasma). The Area Under the Curve (AUC), the upper and lower limits of the 95% confidence interval and the p-value are reported.

(C) Random forest classifier plot showing, in descending order, the relative importance of each protein identified as the top predictors of pro-inflammatory potential of diet (DII over the median value of the population) by the machine learning model.

**Supplementary Files**

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- [Supplemental07.06.23.docx](#)
- [FigureS1.tif](#)