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Plasma metabolomic profiles of plant-based dietary indices reveal potential pathways for metabolic syndrome associations

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ABSTRACT

Background and aims: Plant-based dietary patterns have been associated with improved health outcomes. This study aims to describe the metabolomic fingerprints of plant-based diet indices (PDI) and examine their association with metabolic syndrome (MetS) and its components in a Danish population.
Methods: The MAX study comprised 676 participants (55% women, aged 18-67 y) from Copenhagen. Sociodemographic and dietary data were collected using questionnaires and three 24-h dietary recalls over one year (at baseline, and at 6 and 12 months). Mean dietary intakes were computed, as well as overall PDI, healthful (hPDI) and unhealthful (uPDI) scores, according to food groups for each plant-based index. Clinical variables were also collected at the same time points in a health examination that included complete blood tests. MetS was defined according to the International Diabetes Federation criteria. Plasma metabolites were measured using a targeted metabolomics approach. Metabolites associated with PDI were selected using random forest models and their relationships with PDIs and MetS were analyzed using generalized linear mixed models.
Results: The mean prevalence of MetS was 10.8%. High, compared to low, hPDI and uPDI scores were associated with a lower and higher odd of MetS, respectively [odds ratio (95%CI); hPDI: 0.56 (0.43–0.74); uPDI: 1.61 (1.26–2.05)]. Out of 411 quantified plasma metabolites, machine-learning metabolomics fingerprinting revealed 13 metabolites, including food and food-related microbial metabolites, like hypaphorine, indolepropionic acid and lignan-derived enterolactones. These metabolites were associated with all PDIs and were inversely correlated with MetS components ($p < 0.05$). Furthermore, they had an explainable contribution of 12% and 14% for the association between hPDI or uPDI, respectively, and MetS only among participants with overweight/obesity.
Conclusions: Metabolites associated with PDIs were inversely associated with MetS and its components, and may partially explain the effects of plant-based diets on cardiometabolic risk factors.

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1. Introduction

Metabolic and cardiovascular diseases (CVDs) are major global causes of morbidity and mortality [1]. Many of the identified cardiometabolic risk factors have been directly associated with unhealthy lifestyles, which are still highly prevalent and poorly tackled [2]. In parallel, metabolic alterations, clustered within metabolic syndrome (MetS), make an important contribution to the attributable burden of CVD [1]. The prevalence of MetS has increased over time globally, with nearly 20–25% of the adult population currently having MetS [3,4].

Following a healthy diet is one of the most important modifiable lifestyle factors in preventing MetS and cardiometabolic diseases [5]. In recent years, there has been a growing interest in plant-based and vegetarian diets and their relationships with environmental sustainability, animal welfare, and its concomitant health benefits [6,7]. Vegetarian diets are a subset of plant-based diets that include both traditional foods based on vegetables and fruits, but more recently also plant-based protein isolates and concentrates from traditional and new sources used as meat and dairy analogues. Overall, these diets are characterized by a low/null consumption of animal foods [8]. From their first approach by Martínez-González et al. [9] in 2014 to the present, different plant-based dietary patterns have been captured by proposed *a priori* defined diet indices (PDIs). Originally, PDIs scored all plant-based foods positively without any particular organization. Now, there is a high acceptance of the classification of PDIs scoring animal food groups, and healthy and less healthy plant foods, although classifications as "healthy" or "unhealthy" are highly context related [10–12]. The healthy plant-based diet index (hPDI) emphasizes a higher intake of healthy plant foods (e.g., fruits and vegetables, whole grains, nuts and seeds, legumes, tea and coffee), and the unhealthy plant-based diet index (uPDI) is based on less healthy plant foods (e.g., refined grains, sweets and desserts, sugar-sweetened beverages, potatoes, fruit and vegetable juices), while all PDIs reverse score animal foods (e.g., meats, dairy products, fish, eggs, animal fat, and miscellaneous) [13]. Although one may seem the inverse of the other, these two indices exhibit differences in their association with health outcomes [8,10,13]. A recent meta-analysis of prospective cohort studies showed that a higher overall PDI and hPDI adherence was associated with a lower risk of CVDs [8]. Moreover, a South Korean prospective cohort found that a higher uPDI was associated with an elevated risk of MetS [13]. A few studies have analyzed the relationships between differentiated PDI and MetS or cardiovascular risk factors [10,12,13], but further research is needed to confirm such potential relationships, especially in different populations.

As a complementary strategy, the use of large-scale targeted metabolomics platforms, which offer high-sensitivity, reproducibility, and compound coverage, has emerged in recent years for use in quantitative metabolomics analysis, with great applicability in nutrimetabolomics and exposome research [14]. This allows new opportunities to investigate the molecular mechanisms linking diet with health and to improve prediction and stratification for precision nutrition. Identifying metabolite signatures associated with plant-based diets may, for example, provide new insights into the underlying biological processes, including host and gut microbiota metabolic processes, behind these associations with cardiometabolic diseases [15]. Indeed, a recent study found that the plasma metabolites related to plant-based diets explained up to 37% of the type 2 diabetes risk reduction associated with plant-based dietary patterns [16].

Dietary patterns in Nordic populations are distinguishable from Asian, Western, or even other European populations [17]. Only a few studies have yet identified and investigated metabolite patterns shared across several healthy dietary patterns and their association with disease outcomes as recently reviewed [18]. To the best of our knowledge, no previous study in Nordic populations has described the associations between plant-based diets and cardiometabolic risk factors, using a nutrimetabolomics approach. Thus, we aimed to investigate the relationships of different self-reported plant-based diet indices (PDI, hPDI, and uPDI), and their associated metabolomic fingerprints, with MetS in the Danish Diet, Cancer and Health – Next Generations (DCH-NG) MAX study, a subcohort of the main DCH-NG.

2. Patients and methods

2.1. Study population/design

The current analysis is based on the DCH-NG MAX study, a validation subsample within the DCH-NG cohort. This large population-based family study, established in Denmark between August 2015 and April 2019, is an extension of the Diet, Cancer and Health (DCH) cohort [19]. The DCH-NG cohort includes 39,554 adult participants with complete data collection and incorporates biological children, their spouses, and the grandchildren of the participants in the DCH cohort [20]. From August 2017 to January 2018, 720 participants of the DCH-NG MAX study, aged 18 or older, were enrolled and both questionnaire data and biological samples were collected at baseline, and at 6 and 12 months (Supplementary Fig. 1). At each time point, participants completed two main questionnaires concerning lifestyle and dietary habits, and participated in a health examination that included collection of blood, spot urine, saliva and fecal samples, as well as anthropometry and blood pressure measurements. Not all participants had available data at the three time points (Supplementary Fig. 1).

The DCH-NG research project was approved by the Danish Data Protection Agency (journal number 2013–41- 2043/2014–231-0094). The Committee on Health Research Ethics for the Capital Region of Denmark approved the full DCH-NG project March 2015 and the DCH-NG MAX sub-study July 2017 (journal number H-15001257). The participants provided their written informed consent to participate in the study.

2.2. Dietary assessment

Participants (n = 676) of the DCH-NG MAX study completed one 24-h dietary recall (24-HDR) at least once. For dietary assessment, the Danish version of the web-based tool myfood24 (www.myfood24.org/) from Leeds University [21] was used. This application has been linked with the Danish national food composition database and other food databases [22], which includes approximately 1600 Danish food items, and also features a recipe maker. Portion sizes were based on reports from the Danish Food Institute.

2.3. Plant-based diet indices

We calculated the overall PDI, the hPDI and the uPDI, following the procedures described in previous publications [11,13]. Eighteen food groups were classified into larger categories of healthy and less healthy plant foods, and animal foods. Healthy plant food groups included whole grains, fruits, vegetables, nuts, legumes, vegetable oils, and tea/coffee. Less healthy plant food groups included fruit juices, sugar-sweetened beverages, refined grains, potatoes, and sweets/desserts. Animal food groups included animal fats, dairy, eggs, fish/seafood, meat, and miscellaneous animal-based foods. Supplementary Table 1 details examples of food items constituting each food group. Intake data from all the available time points of each participant were averaged to calculate the PDIs. Afterwards, we adjusted the mean consumption (g/d) of each food group for the mean of total energy intake using the residual method [9]. The energy-adjusted estimates were ranked in quintiles. Each quintile was assigned a score between 1 and 5. The scoring system (positive *vs.* reverse) is presented in Supplementary Table 2.

For the PDI, all plant foods were positively scored. For example, individuals in the highest quintile of fruit consumption received a score of 5, and those in the lowest quintile received a score of 1. Another example, for hPDI the less healthy plant-based foods were reversely scored, so individuals in the lowest quintile of e.g. refined grains intake

received a score of 5. In all PDIs, animal foods were reversely scored, so individuals in the highest quintile of e.g. fish intake received a score of 1. The 18 food group scores for an individual were summed to obtain the indices, with a theoretical range of 18 (lowest possible score) to 90 (highest possible) for all PDIs.

Alcoholic beverages were not included in the indices. The associations between alcoholic beverages, at the low-modest intake levels reported in the present study and health outcomes are controversial and not clear [11]. Nonetheless, statistical analyses were controlled for alcohol consumption.

2.4. Biological, anthropometrical, and clinical data

The anthropometric measures for height and weight were performed using a stadiometer (Seca 264, Germany) and a medical Body Composition Analyzer (Seca 515/514, Germany), respectively. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. Waist circumference (WC) was measured midway between the lowest rib margin and the iliac crest and to the nearest 0.1 cm, using a measuring tape. Blood pressure was measured on the left arm, three times after at least 5 min of rest, using an automatic manometer (Omron M − 10 IT and Omron HB-1300, Germany). Blood samples were collected in a nonfasting status (from 1 to 9 h since last meal, mean fasting time 5 h). Measurements of high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and high-sensitivity *C*-reactive protein (hs-CRP) were carried out in lithium heparin plasma, and hemoglobin A1c (HbA1c) in blood tubes with K_2EDTA using standardized clinical laboratory methods. All analyses were performed on a Cobas® 6000 analyzer by Roche Diagnostics. A complete description of the physical examination and measurements in biological samples is presented elsewhere [20].

2.5. Metabolic syndrome and cardiometabolic risk factors

MetS was defined as the presence of three or more of its five components according to the International Diabetes Federation (IDF) definition [23], including: WC (>88 cm in women and >102 cm in men); high serum TG concentration ≥1.7 mmol/L; reduced serum HDL-C (<1.3 mmol/L in women and <1.0 mmol/L in men); high blood pressure, high systolic blood pressure (SBP) (>130 mmHg) and/or high diastolic blood pressure (DBP) (>85 mmHg); and high HbA1c (>42 mmol/mol or 6.0%) as a biomarker for long-term glycemic control, replacing fasting plasma glucose [24,25]. Furthermore, we included high hs-CRP as a cardiovascular risk factor (≥2.0 mg/L) [26,27]. Most of the cutoffs of lower cardiometabolic risk were in line with the recommendations of the European Guidelines on CVD Prevention in Clinical Practice [2]. Cardiometabolic risk factors were used as categorical variables following the metabolic risk classification.

2.6. Targeted metabolomics analysis and quality control assessment

A large-scale targeted metabolomics approach was available for plasma samples of 625 participants at baseline, 380 at 6 months and 348 at 12 months (Supplementary Fig. 1), which encompasses simultaneous detection and quantification of (poly)phenolic and other food derived compounds, and its phase I/II metabolites, gut microbiota-transformed derivatives, and endogenous metabolites. Plasma samples were first subjected to protein precipitation in a Sirocco Plate (Waters, Milford, MA, USA) as previously described [14]. Supernatants were transferred to 96-well injection plates after the addition of a set of 14 labeled internal standards. Analyses were carried out by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), using the operating conditions described elsewhere [14]. Calibration curves were prepared at 10 concentration levels in the range 0.01–1000 μg/L.

Metabolomics data preprocessing was performed using the POMA R/Bioconductor package (https://github.com/nutrimetabolomics/POMA) [28]. Data preprocessing included the removal of metabolites with more than 40% missing values, and those with a coefficient of variation (CV) > 30% in an internal quality control. The imputation of the remaining missing values was carried out using the KNN algorithm, correction of batch effects using the ComBat function ('sva' R package) [29], and data normalization using auto-scaling. Afterwards, distances to the group centroid were computed based on Euclidean distances to remove outliers from the data matrix (±1.5 × interquartile range [IQR]). Thus, the metabolites were preprocessed, and outliers were removed, imputed, batch effect corrected, standardized, and normalized. The working metabolomics data set comprised 411 metabolites out of the 1353 putative metabolites from the exposome-based metabolomics method developed at the Nutrimetabolomics laboratory of the University of Barcelona [14].

2.7. Statistical analysis

Baseline characteristics were presented as median (p25 – p75) or percentages for dichotomous variables according to tertiles of PDIs. To include all participants in the baseline descriptive analysis, including 28 participants who only had available data at 6 or 12 months, their first available evaluation was considered as baseline. PDI scores were replicated at the different time points for each participant, reflecting their adherence to the dietary pattern during the year of the study. All clinical and metabolomics data available were included for all the participant at each time point (total observations, k = 1353), and generalized linear mixed models (GLMMs) were used for inferential statistics.

Associations between PDIs (as continuous per standard deviation (SD) or categorical variables per tertile) and the prevalence of MetS and cardiometabolic risk factors (as categorical variables) were assessed using GLMMs in a random intercepts model. The p-value for trend was calculated by modeling PDI tertiles as a continuous variable.

GLMM models for studying the association between PDIs and MetS were constructed based on previous knowledge as shown in the directed acyclic graph (Supplementary Fig. 2). Results are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Model 1 was unadjusted; Model 2 was adjusted for age (y), sex (male *vs.* female), total energy intake (kcal/d), physical activity (regular *vs.* no regular exercise), smoking (never, former, and current smoker), and alcohol intake (g/d).

The associations between dietary patterns and targeted metabolomics data were evaluated with random forest models using the R package 'ranger' in baseline data with a split at 70% and 30% of the sample for training and testing, respectively. Furthermore, model fit was evaluated with data sets at 6 and 12 months and accepted using a minimum R^2 threshold of 0.15. Variable importance was attributed according to the permutation criteria, which emphasizes the effects of the metabolites based on the mean squared error reduction. For each PDI, we selected the top 20 metabolites (Venn diagram) with the highest permutation importance. The association between these individual metabolites and cardiometabolic risk factors was analyzed using the Spearman's rank correlation coefficient. In addition, we presented the top 10 metabolites' highest permutation importance for all PDIs (Supplementary Fig. 3). The associations between PDIs, the selected metabolites, and MetS were analyzed using GLMM.

The percentual contribution of the metabolites to the association between the PDIs and MetS was calculated by including the metabolites separately in Model 2 (OR base model – OR adjusted model)/(OR base model − 1) × 100%. These last analyses were performed in participants categorized by normal weight (BMI <25 kg/m^2) or overweight/obesity (≥25 kg/m^2) due to the mediation effect of BMI (Supplementary Fig. 2). Additionally, we conducted an analysis including BMI in the models since BMI and waist circumference are variables highly intercorrelated (Supplementary Fig. 4).

p-values <0.05 were considered statistically significant in two-tailed tests. IBM SPSS 27.0 and R version 4.1.3 (R foundation, Austria) were

used for statistical analysis.

3. Results

The baseline characteristics according to tertiles of PDIs are shown in Table 1. Participants with higher scores in overall PDI and hPDI were more likely to be women, have a lower BMI, be more physically active, and were less likely to smoke than participants with lower PDI and hPDI scores. In contrast, participants in the highest *vs.* those in the lowest uPDI tertile were more likely to be men, to be overweight or have a high waist circumference, and to be more physically inactive and smoke. With regards to cardiometabolic risk factors, participants with the highest PDI and hPDI scores were more likely to have lower blood pressure, HbA1c, LDL-C, and hs-CRP, and higher HDL-C.

The mean food group intakes according to tertiles of PDIs are presented in Supplementary Table 3. Participants with higher scores in overall PDI and hPDI (as well as those with lower uPDI scores) showed higher intakes of wholegrains, vegetables, tea, and coffee and lower intakes of sugar-sweetened beverages, sweets, and animal food groups (i.e., dairy and miscellaneous animal-based foods) than participants with lower overall PDI and hPDI scores (higher uPDI scores).

The mean prevalence of MetS was 10.8% (155 cases out of 676 participants), with minor differences over the three time points ($p > 0.05$): baseline 11.6% (n = 648), 6 months 8.6% (n = 406), and 12 months 11.8% (n = 382). In adjusted models, a 1 SD higher PDI and hPDI score was significantly associated with a 30% and 44% lower odds of MetS (OR: 0.70; 95%CI: 0.55–0.90]) and (OR: 0.56; 95%CI: 0.43–0.74]), respectively (Table 2). Conversely, 1 SD higher uPDI score was significantly associated with 61% higher odds of MetS (OR: 1.61; 95%CI: 1.26–2.05]). Furthermore, we observed a linear trend across the tertiles in all associations between PDIs and MetS ($p < 0.05$). The inclusion of BMI as a covariate attenuated the observed associations of PDI (OR: 0.94; 95%CI: 0.77–1.44]), hPDI (OR: 0.84; 95%CI: 0.69–1.03]) and uPDI (OR: 1.18; 95%CI: 0.98–1.43]) with MetS (Supplementary Fig. 4, panel b). Moreover, in analyses stratified by BMI categories, significant associations between hPDI or uPDI and MetS odds were only observed among participants with overweight/obesity (Table 3).

Regarding MetS components, associations between all PDIs and high WC were consistently significant in all models (Supplementary Table 4 and Supplementary Fig. 5). Moreover, we found an inverse association between a higher hPDI and the prevalence of low HDL-C and elevated hs-CRP. Similar associations, but in the opposite direction, were observed for uPDI (Supplementary Fig. 5).

From a total of 411 metabolites, using a machine-learning algorithm we selected the top 20 metabolites associated with each PDI. A common set of 13 metabolites was associated with all three PDIs (Fig. 1B). These metabolites were tridecanoyl-carnitine, hydroxyvaleryl-carnitine, linoleoyl-carnitine, 2,6-dihydroxybenzoic acid (2,6-DHBA), 3,4-dihydroxybenzoic acid (3,4-DHBA), trigonelline, catechol-sulfate, 2-hydroxyphenylacetic acid, urolithin A-glucuronide, enterolactone-glucuronide, enterolactone-sulfate, indolepropionic acid, and hypaphorine. In correlation analyses, most of these metabolites (enterolactone sulfate/glucuronide, 2,6-DHBA and 3,4-DHBA, indolepropionic acid, and hypaphorine) were inversely correlated with several cardiometabolic risk factors (Fig. 1A). Among the specific metabolites selected for PDIs, most of them showed a negative correlation with HDL-C and a positive correlation with TG concentrations (Fig. 1). Metabolites specifically associated with hPDI included testosterone, 1-methylhistidine, valeryl-carnitine, and 2-methylbutyryl-carnitine.

Next, we divided the metabolomic fingerprint into two sets of metabolites, those common to all three PDIs, and those specific to each PDI (Fig. 1, named common and specific metabolites, respectively). To

Table 1
Baseline characteristics of MAX study according to tertiles of plant-based diet indices (n = 676)[a].

Plant-based diet indices	Overall PDI		hPDI		uPDI		
	Tertile 1	Tertile 3	Tertile 1	Tertile 3	Tertile 1	Tertile 3	All
Sample size, n[b]	220	230	230	216	221	223	676
Median score, range[b]	43 (29–47)	56 (53–74)	46 (33–50)	62 (58–79)	49 (35–53)	64 (59–81)	50 (29–74)
Sex, female (%)	45.5	64.8	47.8	70.8	63.3	51.6	54.9
Age, years	48.0 (30.0–54.0)	46.5 (30.7–52.0)	47.0 (32.7–53.0)	48.0 (30.0–52.7)	49.0 (34.0–54.0)	46.0 (30.0–53.0)	48.0 (31.0–53.0)
BMI (kg/m2)	25.3 (22.6–28.0)	23.7 (21.5–26.0)	25.5 (23.1–28.6)	23.4 (21.4–25.7)	23.6 (21.8–26.0)	25.1 (22.8–28.6)	24.5 (22.2–27.2)
Waist circumference (cm)	90.0 (80–98)	82.9 (76.2–91.7)	90.0 (81.0–98.3)	81.8 (75.2–89.8)	82.5 (76.0–92.0)	88.5 (79.2–98.0)	86.1 (78.5–95.3)
Alcohol intake (g/d)	0.0 (0.0–14.0)	0.0 (0.0–12.7)	0.0 (0.0–9.0)	0.0 (0.0–11.7)	0.0 (0.0–14.2)	0.0 (0.0–1.4)	0.0 (0.0–12.4)
Total energy intake (kcal/d)	1869 (1452–2382)	2001 (1615–2593)	2167 (1637–2602)	1836 (1446–2283)	1929 (1563–2404)	1919 (1436–2489)	1976 (1528–2473)
Physical activity (%)							
Regular	80.9	87.0	79.1	86.6	90.0	74.4	83.1
Not regular	19.1	13.0	20.9	13.4	10.0	25.6	16.9
Smoking status (%)							
Never	49.1	57.9	53.5	53.7	51.6	51.6	52.2
Former	24.5	28.3	24.3	27.8	31.2	22.4	27.5
Current	26.4	13.9	22.2	18.5	17.2	26.0	20.3
Risk factors							
SBP (mmHg)	119 (107–131)	113 (102–124)	119 (107–128)	112 (102–122)	113 (104–124)	118 (106–129)	116 (106–126)
DBP (mmHg)	81 (75–89)	79 (71–85)	81 (74–89)	77 (71–84)	79 (71–86)	80 (73–89)	80 (72–87)
HbA1c (mmol/mol)	34 (32–37)	33 (31–36)	35 (32–37)	34 (31–36)	34 (32–36)	34 (32–36)	34 (32–36)
TG (mmol/L)	1.2 (0.8–2.0)	1.0 (0.7–1.5)	1.1 (0.8–1.9)	1.0 (0.7–1.4)	1.0 (0.7–1.4)	1.2 (0.8–1.8)	1.12 (0.80–1.67)
HDL-C (mmol/L)	1.4 (1.2–1.7)	1.5 (1.2–1.8)	1.3 (1.1–1.7)	1.6 (1.3–1.9)	1.6 (1.3–1.9)	1.4 (1.1–1.6)	1.49 (1.23–1.79)
LDL-C (mmol/L)	3.0 (2.4–3.7)	2.8 (2.3–3.4)	3.0 (2.4–3.6)	2.8 (2.3–3.4)	2.9 (2.3–3.4)	3.0 (2.4–3.6)	2.99 (2.40–3.55)
hsCRP (mg/L)	0.8 (0.3–1.6)	0.4 (0.2–1.1)	0.9 (0.3–2.0)	0.4 (0.2–0.9)	0.5 (0.2–1.2)	0.8 (0.3–2.0)	0.71 (0.31–1.49)
Healthy plant food (score/d)[c]	13 (11–16)	22 (18–25)	14 (11–16)	22 (18–25)	20 (17–23)	29 (26–31)	17 (14–21)
Less-healthy plant foods (score/d)[c]	11 (9–13)	15 (12–17)	14 (12–17)	20 (17–22)	11 (9–13)	15 (13–17)	13 (10–16)
Animal food (score/d)[3]	17 (15–21)	20 (18–23)	17 (15–19)	22 (19–24)	17 (15–20)	21 (19–23)	19 (17–22)

BMI, body mass index. WC, waist circumference. SBP, systolic blood pressure. DBP, diastolic blood pressure. HbA1c, glycosilated hemoglobin. TG, triglycerides. HDL, high-density lipoprotein. HsCRP, high-sensitivity *C*-reactive protein.

[a] Values are median (p25-p75) for continuous variables or percentages for dichotomous variables, unless otherwise indicated.

[b] The tertiles do not have equal sample size because many participants received the same scores. For participants that did not have a baseline (n = 28) considered the starting time point (i.e. six or twelve months) as baseline.

[c] Energy-residual adjusted.

Table 2
Associations between plant-based diet indices and prevalent MetS (n = 676).

	Tertile 1	Tertile 2	Tertile 3	*p*-trend	Per SD
Overall plant-based diet					
Number of cases	72	53	30		155
K-measures	474	467	495		1436
Model 1	Ref.	0. 73 (0.45–1.18)	0.37 (0.21–0.64)	<0.001	0.68 (0.54–0.85)
Model 2	Ref.	0.80 (0.48–1.34)	0.43 (0.24–0.79)	0.007	0.70 (0.55–0.90)
Healthful plant-based diet					
Number of cases	85	43	27		155
K-measures	490	474	472		1436
Model 1	Ref.	0.46 (0.28–0.75)	0.27 (0.15–0.48)	<0.001	0.60 (0.47–0.75)
Model 2	Ref.	0.38 (0.22–0.65)	0.26 (0.14–0.49)	<0.001	0.56 (0.43–0.74)
Unhealthful plant-based diet					
Number of cases	35	49	71		155
K-measures	481	490	465		1436
Model 1	Ref.	1.50 (0.86–2.60)	2.48 (1.45–4.24)	<0.001	1.50 (1.21–1.86)
Model 2	Ref.	1.49 (0.82–2.72)	2.70 (1.50–4.85)	<0.001	1.61 (1.26–2.05)

Total mean plant-based scores were used as a continuous variable, after SD transformation. The data represent the OR (odds ratios) and confidence interval (CI). n, subjects. k, measures. Model 1 was an unadjusted model. Model 2 was adjusted for age, sex, time point, total energy intake, physical activity, smoking and alcohol intake.

Table 3
Associations between plant-based diet indices and prevalent MetS according to the BMI status (n = 676).

	Tertile 1	Tertile 2	Tertile 3	*p*-trend	Per SD
Overall plant-based diet					
Number of cases	5	5	2		12
K-measures	225	245	327		797
Model (N)	Ref.	1.01 (0.40–2.56)	0.84 (0.33–2.14)	0.696	0.93 (0.63–1.36)
Number of cases	67	48	28		143
K-measures	249	222	168		639
Model (OV/OB)	Ref.	0.79 (0.44–1.40)	0.57 (0.29–1.12)	0.103	0.78 (0.58–1.04)
Healthful plant-based diet					
Number of cases	4	6	2		12
K-measures	218	248	331		797
Model (N)	Ref.	0.97 (0.38–2.50)	0.79 (0.30–2.08)	0.626	0.89 (0.60–1.32)
Number of cases	81	37	25		143
K-measures	272	226	141		639
Model (OV/OB)	Ref.	0.41 (0.23–0.74)	0.42 (0.21–0.86)	0.004	0.69 (0.51–0.92)
Unhealthful plant-based diet					
Number of cases	6	2	4		12
K-measures	314	254	229		797
Model (N)	Ref.	0.81 (0.32–2.02)	0.96 (0.38–2.40)	0.922	1.01 (0.68–1.48)
Number of cases	29	47	67		143
K-measures	167	236	236		639
Model (OV/OB)	Ref.	1.37 (0.68–2.74)	2.49 (1.25–4.97)	0.006	1.57 (1.18–2.08)

Total mean plant-based scores were used as a continuous variable, after SD transformation. The data represent the OR (odds ratios) and confidence interval (CI). n, subjects. k, measures. The models were stratified for normal (N: <25 kg/m^2) and (OV/OB: ≥25 kg/m^2) by nutritional status according to BMI index. All models were adjusted for age, sex, time point, total energy intake, physical activity, smoking and alcohol intake.

evaluate whether these sets could explain the association between PDIs and MetS, we sequentially included them by sets in the regression models and calculated their percentual contribution to the PDIs' protective effects. Fig. 2 shows the calculated contribution of the metabolites in Model 2 (fully adjusted model) among participants according to BMI categories (normal weight *vs.* overweight/obesity). In individuals with a normal BMI, the specific metabolites of overall PDI and hPDI made a relevant contribution (27 and 71%) to the association between the respective PDI score and odds of MetS (Fig. 2C). In participants with overweight/obesity, common metabolites of hPDI and uPDI made a relevant contribution (12 and 14%) to the association between the respective PDI score and odds of MetS (Fig. 2D).

Additionally, further analyses were performed comparatively in Model 2 and in Model 2 including further adjustment for BMI (Supplementary Fig. 3A: without BMI, and Panel b: with BMI). Without BMI, the set of metabolites reflecting all PDIs explained between 15% and 30% of the association between PDIs and MetS risk (Supplementary Fig. 3A). However, in the Model that includes BMI, only the set of metabolites specific to hPDI showed an explainable contribution of 25% (Supplementary Fig. 3B). Conversely, for the other indices, no sets of metabolites made important contributions when BMI was included as covariate (Supplementary Fig. 3B).

4. Discussion

In this study of middle-aged Danish adults, metabolomics fingerprinting revealed a set of 13 food- and food-related microbial metabolites common to all three PDIs, as well as some minor sets of metabolites specific for each individual index. This common set of metabolites explained almost 12–14% of the association between hPDI or uPDI, respectively, and MetS risk among participants with overweight/obesity. Therefore, these food and gut microbiota related metabolites may be more relevant and sensitive to guide precision nutrition interventions in individuals at high-risk for cardiometabolic diseases. The graphical abstract summarizes our study findings (Fig. 3).

Most of the common metabolites included indole propionic and phenolic acids, and enterolactone metabolites, that are the result of the interaction between diets and microbe-host environment (e.g. gut permeability and grade of inflammation) [30,31]. Interestingly, beneficial associations have been described between these metabolites and

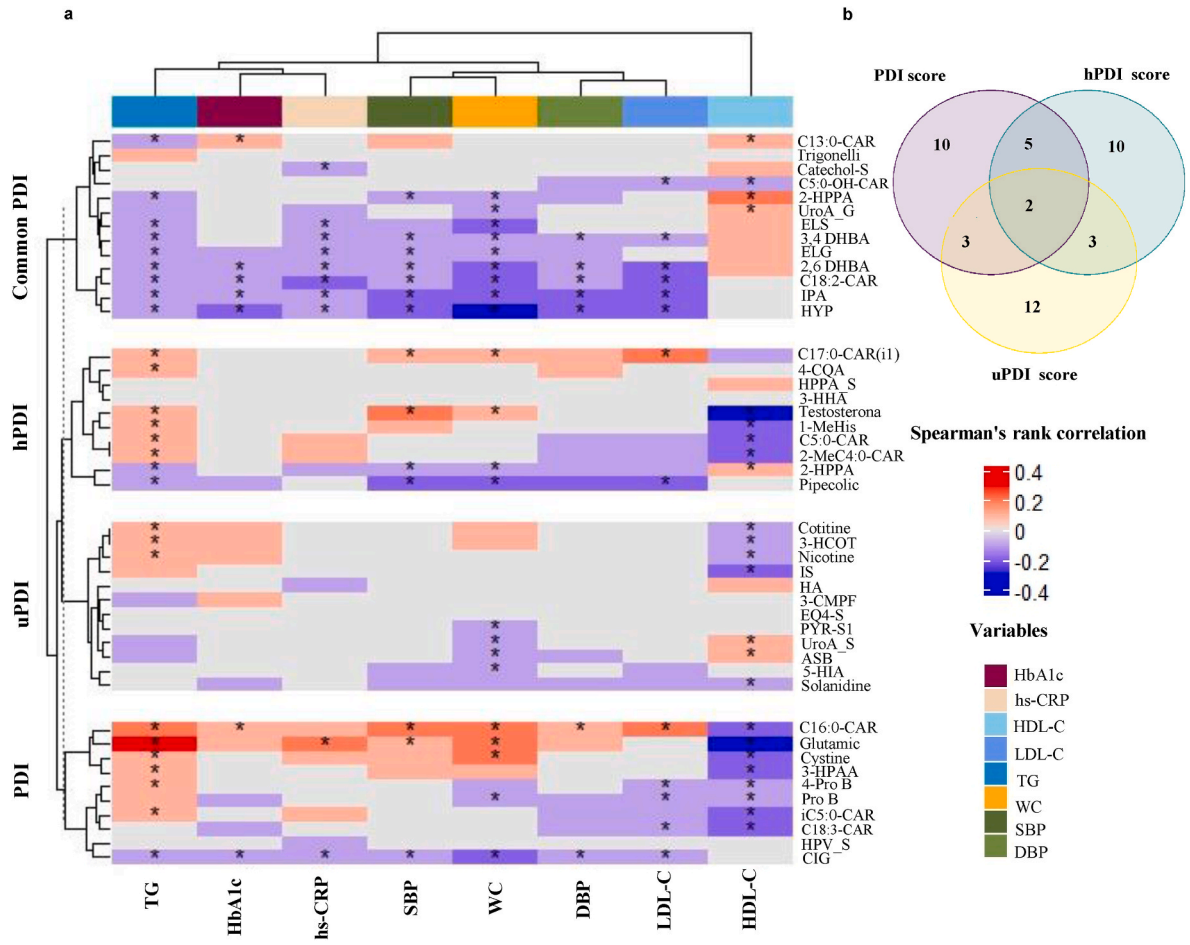

Fig. 1. Correlations between the specific and common metabolites selected from plant-bases diet indices by metabolomics profiling and cardiometabolic risk factors (n: 625, k = 1353).

(A) PDI: overall plant-based diet index. uPDI, unhealthful plant-based diet index. hPDI: healthful plant-based diet index. C13:0-CAR, tridecanoyl-carnitine. Catechol-S, catechol sulfate. C5:0-OH-CAR, hydroxyvaleryl-carnitine. 2-HPAA, 2-hydroxyphenylacetic acid. UroA_G, urolithin A glucuronide. ELS, enterolactone sulfate. 3,4-DHBA, 3,4-dihydroxybenzoic acid. ELG, enterolactone glucuronide. 2,6-DHBA, 2,6-dihydroxybenzoic acid. C18:2-CAR, linoleoyl-carnitine. IPA, indolepropionic. HYP, hypaphorine. C17:0-CAR(i1), heptadecanoyl-carnitine (isomer 1). 4-CQA, 4-caffeoylquinic acid. HPPA_S, hydroxyphenylpropionic acid sulfate. 3-HHA, 3-hydroxyhippuric acid. 1-MeHis: 1-methylhistidine. C5:0-CAR: valeryl-carnitine. 2-MeC4:0-CAR: 2-methylbutyryl-carnitine. 2-HPPA: 3-(2-hydroxyphenyl)propionic acid. 3-HCOT: 3-hydroxycotinine. IS, indoxyl sulfate. HA, hippuric acid. 3-CMPF: 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid. EQ4-S, equol 4′-sulfate. PYR-S1, pyrogallol sulfate 1. UroA_S, urolithin A sulfate. ASB, arsenobetaine. 5-HIA, 5-hydroxyindoleacetic. C16:0-CAR: palmitoyl-carnitine. 3-HPAA: 3-hydroxyphenylacetic acid. 4-Pro B, 4-hydroxyproline betaine. Pro B: proline betaine. iC5:0-CAR, isovaleryl-carnitine. C18:3-CAR, linolenoyl-carnitine. 3-HPV_S: 5-(3′-hydroxyphenyl)-γ-valerolactone 3′-sulfate. CIG, cinnamoylglycine. HbA1c, glycosilated hemoglobin. HDL-C, high-density lipoprotein cholesterol. hs-CRP: high-sensitivity C-reactive protein. WC, waist circumference. SBP, systolic blood pressure. DBP, diastolic blood pressure. TG, triglyceride; colors denote the association directions (red, positive; blue, inverse). Asterisk in the colored cell represent association significance ($*p < 0.05$). (B) Venn diagram of specific and common metabolites for the metabolite profiling of PDI, hPDI, and uPDI. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cardiometabolic risk factors [32–34]. Among the common metabolites, indolepropionic acid and enterolactones have been increasingly highlighted for its positive cardiometabolic effects [31,35,36]. Indolepropionic acid is a microbial metabolite of dietary tryptophan and it has been associated with the improvement of chronic low-grade inflammation and higher dietary fiber intake [31]. Moreover, some of the effects of increased dietary fiber on weight control and gut bacteria composition could be linked to the higher production of short-chain fatty acids and indolepropionic acid [37]. Enterolactones are metabolites produced by gut microbiota from plant lignans, and some studies suggest that these enterolignans provide health benefits associated with chronic and cardiometabolic diseases [15,38].

Among the hPDI-specific metabolites, some metabolites could be related to male sex and the consumption of dairy or meat (testosterone, 1-methylhistidine, valeryl-carnitine, and 2-methylbutyryl-carnitine) [39,40]. Among the 12 metabolites specific to the metabolomics fingerprint of uPDI, some were related to smoking and the uremic toxin indoxyl-sulfate. In the case of overall PDI, some of the specific metabolites could be related to the intake of saturated fatty acids (e.g., palmitoyl-carnitine) and meat (e.g., isovaleryl-carnitine). These highlight the notion that, although the metabolomic fingerprints were built based on PDIs, they may provide information related to lifestyle characteristics in general, when using an exposome-based metabolomics method.

Metabolomics fingerprinting allowed us to analyze the differences between the PDIs signatures beyond dietary intake and to study their associations with MetS risk. Remarkably, in the associations between dietary indices and MetS risk in models adjusted for BMI only the set of specific metabolites of hPDI showed a significant contribution (Supplementary Fig. 4). This finding has two complementary interpretations: i) hPDI may provide a more comprehensive estimate of the relationship between diet and cardiometabolic health than the other PDIs, which

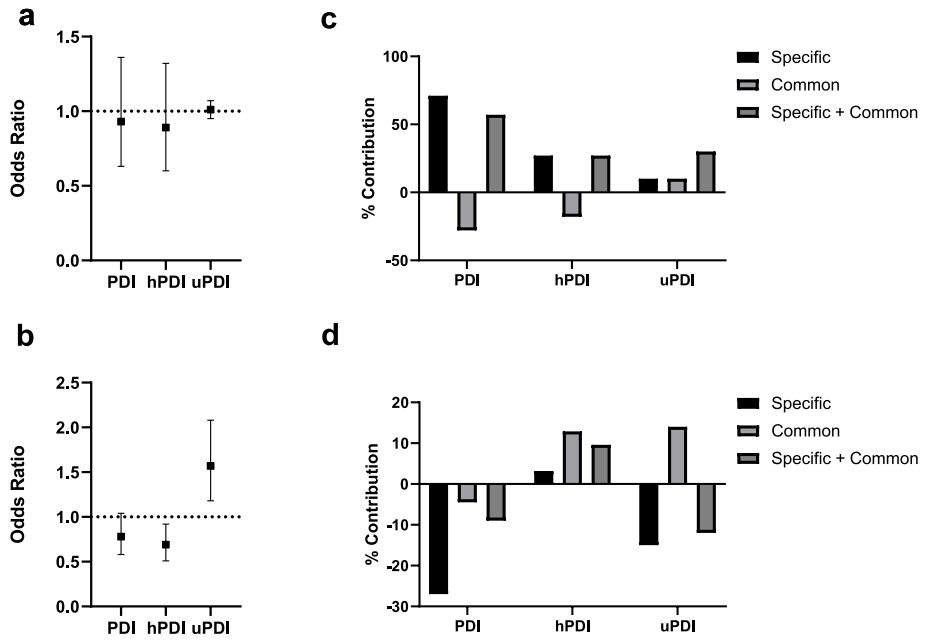

Fig. 2. Odds ratio for metabolic syndrome of each plant-based diet indices according to participants with normal BMI (n = 377, k = 816) (A) and overweight and obesity (n = 248, k = 537) (B).
GLMM models were adjusted for age, sex, time point, total energy intake, physical activity, smoking and alcohol intake. The percentage of change in total effect of the plant-based diet indices after controlling for selected metabolites (C and D). The percentage of change was calculate using the formula (OR base model – OR adjusted model)/(OR base model − 1) × 100%). Model (a) was additionally adjusted for: specific, common, and specific and common metabolites (as appropriate, for calculations of % of change in total effect).

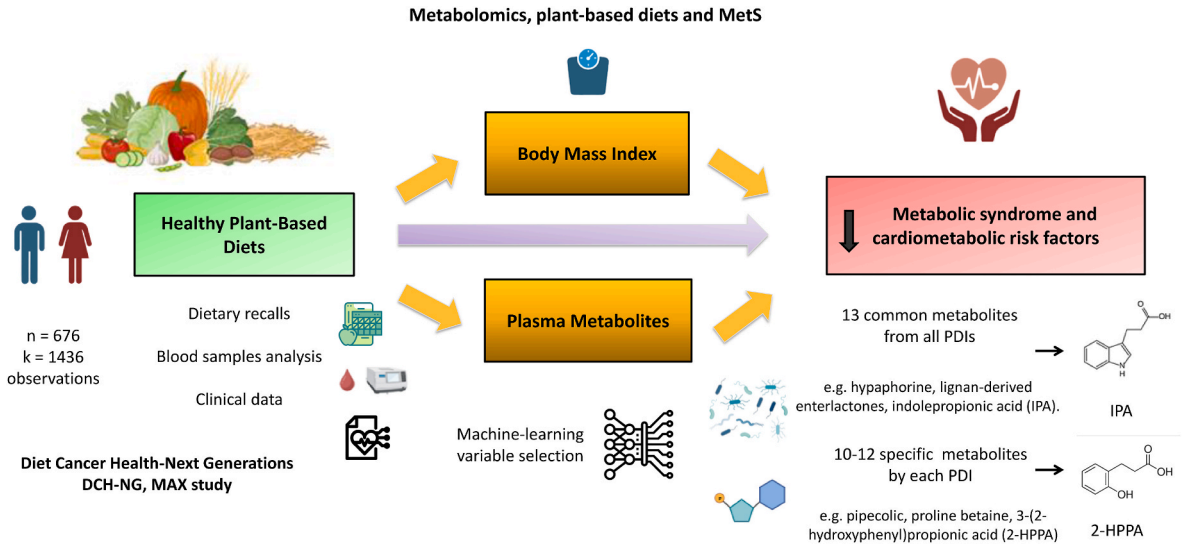

Fig. 3. Using a targeted metabolomics method of food and microbiota-derived metabolites, as well lifestyle, contaminants, exposome, and clinical metabolites, a common signature of 13 metabolites was associated with three different plant-based dietary indices (PDIs).
High, compared to low, healthful PDI and unhealthful PDI scores were associated with a lower and higher odds of metabolic syndrome. Metabolomic fingerprinting of PDIs support the notion that not all PDIs are equal in terms of their impact on cardiometabolic health.

mainly reflected the association mediated by BMI; and ii) the metabolomic fingerprint of hPDI may provide a 'broader' outlook of the diet and lifestyle-related metabolites involved in cardiometabolic risk determinants when compared to the other PDIs.

As we previously mentioned, the plant-based diet has been linked with beneficial or even detrimental cardiovascular health effects according to the type of the diet, particularly in vegan diets [41]. However, to the best of our knowledge, only one study has reported a metabolomic fingerprint for PDIs [16]. Wang et al. using elastic net regression, identified a metabolomic signature of 55 metabolites for overall PDI, 93 metabolites for hPDI, and 75 metabolites for uPDI. Remarkably, the hPDI contributed to 51% of the effects of higher adherence to hPDI on a lower risk of incident type 2 diabetes. Differences in the metabolomic methods, which in our study include gut microbial metabolites and dietary biomarkers, and statistical methodologies make comparisons between studies difficult. There are different methodologies for reducing high-dimensional data for metabolic fingerprinting, such as random forests, principal component analysis, partial least-squares regression, or even elastic net regression [16,42,43]. In particular, random forests do not need normally distributed data and have shown better

performance when compared to other machine-learning algorithms [44]. On the other hand, elastic net regression may be used to obtain metabolic fingerprints 'adjusted' for confounding or mediation variables (e.g., sex, BMI, and other lifestyles) [16,45]. Including covariates in metabolite selection methods could be misleading as assumptions of linear regression may not be satisfied for all the metabolites, leading to inappropriate results. Thus, we must be aware of the implications of each methodological process, data analysis, results, and their interpretation.

In general, in participants with a higher adherence to plant-based dietary patterns, there is a high consumption of foods that are rich in dietary fiber and phytochemicals (e.g. phenolic compounds), which have been identified as key factors for positive health effects, such as glycemic control [46]. Although (poly)phenols are consumed in less quantity than fiber (mg/day *vs.* g/day, respectively), increasing scientific literature has demonstrated its impact as a prebiotic acting via direct microbe-host interactions and therefore on the gut bacterial community, and indirectly through the production of gut microbial metabolites, that may display beneficial health effects. In addition, PDIs, and specially overall PDI or uPDI, may be affected by high intakes of plant-based ultra-processed foods of poor nutritional value (e.g. few minerals/vitamins and a lot of energy, sugar and additives) such as sugar-sweetened beverages or refined grains [11,47]. Moreover, the length and accessibility of the food supply chain may contribute to the development of cardiovascular risk among individuals who adhere to a specific PDI [48]. Another layer of complexity to the study of the diet-health relationship has been recently highlighted due to the importance of differentiating and integrating short and long-term PDI data in order to identify and establish associations with gut microbial diversity, taxonomic composition, and their protective role on cardiometabolic health [49].

The present study has three main strengths. First, we included a large-scale targeted metabolomics approach and biochemical data from individuals evaluated three times over a one-year period. Second, we applied a comprehensive multi-metabolite targeted metabolomics method and a nonparametric, machine-learning, random forest algorithm, which, compared to other machine-learning methods, is independent of the conformity with the assumptions of linear regression. Also, to the best of our knowledge, no previous studies have analyzed the PDIs, combining diet assessment data and a targeted metabolomics approach, in relation to MetS and cardiometabolic risk factors. Lastly, our analytical method focused on plant-based foods and their derivatives. However, another approach could capture additional metabolites from endogenous pathways.

Our study also has limitations, including weaknesses related to the limitations of the analytical procedures in nutrimetabolomics studies, such as difficulties in detecting specific compounds, and the unknown bias and intra-individual variation in metabolomics measurements (only 287 participants had metabolomics measurements at baseline, and 6 and 12 months). In addition, this is a one-year study and we do not know whether the associations found, or even the metabolomics fingerprint, are stable over longer time periods. With regard to dietary assessment, one of the limitations is that the repeated 24-HDR during three times over the year to estimate the PDIs may be less representative of the habitual diet than a food frequency questionnaire. It is worth noting that participants may have also engaged in social desirability bias, including underreporting the intake of energy-dense foods perceived as unhealthy. This, in turn, has the potential to attenuate the observed association between diet and disease [50]. However, in this case, we worked with the mean of the three timepoints to improve our estimations of dietary indices. On the other hand, the 24-HDR usually provides more food items and is more accurate in terms of dietary components than other dietary assessment methods. As all self-reported diet intake, 24-HDR are prone to random and systematic errors [51]. The studied population of the MAX study has a relatively low prevalence of obesity (10.2%) and of MetS (11.6%) compared to some other studies and even the general population [4,52,53]; thus, the generalizability of our results might be limited. Finally, the observational design of our study does not allow to establish cause effect relationships, and further studies should confirm if these metabolites are directly involved in metabolic health benefits associated with PDIs or are biomarkers of a more diverse and more healthy gut bacteria ecosystem.

In conclusion healthy and unhealthy PDIs were associated with lower and higher MetS risk, respectively. Additionally, we identified specific and common metabolites associated with PDIs and MetS risk, which may be relevant to explain the effects of diet on cardiometabolic risk factors independently of BMI. Other metabolites in the metabolic fingerprint may reflect other exposures such as clinical or lifestyle characteristics (e.g. sex, meat consumption, or smoking, among others). Future studies need to evaluate if these metabolites are useful targets for novel precision nutrition interventions for cardiometabolic disease prevention.

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CRediT authorship contribution statement

Fabian Lanuza: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, contributed the conceptualization and methodology of the current analysis. performed the formal analysis and wrote the first draft of the manuscript. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Tomas Meroño:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, contributed the conceptualization and methodology of the current analysis. supervised the first draft of the manuscript. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Raul Zamora-Ros:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, contributed the conceptualization and methodology of the current analysis. supervised the first draft of the manuscript. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Nicola P. Bondonno:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, contributed the conceptualization and methodology of the current analysis. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Agnetha Linn Rostgaard-Hansen:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, collected the data from the DCH-NG cohort and the MAX study.

computed the plant-based diet indices. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Alex Sánchez-Pla:** Supervision, Formal analysis, supervised the statistical analysis. **Berta Miro:** Supervision, supervised the statistical analysis. **Francesc Carmona-Pontaque:** Supervision, supervised the statistical analysis, All authors have read and agreed to the published version of the manuscript. **Gabriele Riccardi:** Writing – review & editing, reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Anne Tjønneland:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, contributed the conceptualization and methodology of the current analysis. conceived and conceptualized the MAX study. collected the data from the DCH-NG cohort and the MAX study. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Rikard Landberg:** Conceptualization, Writing – review & editing, conceived and conceptualized the MAX study. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Jytte Halkjær:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, contributed the conceptualization and methodology of the current analysis. conceived and conceptualized the MAX study. collected the data from the DCH-NG cohort and the MAX study. reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices. **Cristina Andres-Lacueva:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, contributed the conceptualization and methodology of the current analysis. supervised the first draft of the manuscript, reviewed, edited, and contributed to the final version of the manuscript. computed the plant-based diet indices.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2023.117285.

Abbreviations

BMI	body mass index
DCH-NG	Diet, Cancer and Health – Next Generations
HbA1c	glycosylated hemoglobin
HDL-C	high-density lipoprotein cholesterol
hPDI	healthful plant-based index
hs-CRP	high-sensitivity *C*-reactive protein
MetS	metabolic syndrome
PDI	overall plant-based diet index
TG	triglyceride
uPDI	unhealthful plant-based diet index
WC	waist circumference
24-HDRs	24-h dietary recalls

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