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Inflammation-Related Marker Profiling of Dietary Patterns and All-cause Mortality in the Melbourne Collaborative Cohort Study

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ABSTRACT

Background: Nutritional epidemiology research using self-reported dietary intake is prone to measurement error. Objective methods are being explored to overcome this limitation.

Objectives: We aimed to examine *1*) the association between plasma markers related to inflammation and derive marker scores for dietary patterns [Mediterranean dietary score (MDS), energy-adjusted Dietary Inflammatory Index (E-DII™), Alternative Healthy Eating Index 2010 (AHEI)] and *2*) the associations of these marker scores with mortality.

Methods: Weighted marker scores were derived from the cross-sectional association between 30 plasma markers and each dietary score (assessed using food-frequency questionnaires) using linear regression for 770 participants in the Melbourne Collaborative Cohort Study (aged 50–82 y). Prospective associations between marker scores and mortality ($n = 249$ deaths) were assessed using Cox regression (median follow-up: 14.4 y).

Results: The MDS, E-DII, and AHEI were associated ($P < 0.05$) with 9, 14, and 11 plasma markers, respectively. Healthier diets (higher MDS and AHEI, and lower anti-inflammatory, E-DII) were associated with lower concentrations of kynurenines, neopterin, IFN-γ, cytokines, and C-reactive protein. Five of 6 markers common to the 3 dietary scores were components of the kynurenine pathway. The 3 dietary-based marker scores were highly correlated (Spearman ρ: –0.74, –0.82, and 0.93). Inverse associations (for 1-SD increment) were observed with all-cause mortality for the MDS marker score (HR: 0.84; 95% CI: 0.72–0.98) and the AHEI marker score (HR: 0.76; 95% CI: 0.66–0.89), whereas a positive association was observed with the E-DII marker score (HR: 1.18; 95% CI: 1.01–1.39). The same magnitude of effect was not observed for the respective dietary patterns.

Conclusions: Markers involved in inflammation-related processes are associated with dietary quality, including a substantial overlap between markers associated with the MDS, the E-DII, and the AHEI, especially kynurenines. Unfavorable marker scores, reflecting poorer-quality diets, were associated with increased mortality. *J Nutr* 2021;151:2908–2916.

Keywords: inflammation, diet, dietary pattern, mortality, biomarker, kynurenine

Introduction

Diet is a key modifiable risk factor for chronic conditions that contribute to morbidity and premature mortality (1). However, a well-known limitation of self-reported dietary assessment in epidemiological research has been random and systematic error, leading to potential misclassification of dietary intake, which can influence study conclusions and lead to inconsistencies in

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findings across studies. One way to potentially overcome this is to identify objective nutritional biomarkers (2).

Examining dietary patterns rather than nutrients and food groups enables researchers to capture the complexity of diet, including potential synergism between components, and better reflects real-world behavior (3). Identifying circulating markers that may help discriminate between consumption of healthy versus unhealthy diets has been of increasing interest (4).

Animal studies and human trials have demonstrated how dietary components, including saturated fat, omega-3 fatty acids, dietary fiber, and polyphenols, affect classic markers of inflammation such as C-reactive protein (CRP) and cytokines (e.g., IL-6) (5). Several studies have explored the relations of dietary patterns, including the Mediterranean diet score (MDS), the Dietary Inflammatory Index (DII™), and the Alternative Health Eating Index 2010 (AHEI), with CRP and cytokines (6–10). This includes evidence that inflammatory biomarkers are responsive to changes in dietary scores such as the MDS (6). Kynurenines are another set of markers that are elevated during inflammation but are understudied in relation to diet. Dietary tryptophan (an essential amino acid) is the substrate and B vitamins act as enzyme cofactors within the kynurenine pathway. Kynurenines are also increasingly recognized as playing a role in chronic diseases and mortality (11, 12); therefore, understanding their relation with diet is important. However, only 1 study has examined the relation between a Mediterranean dietary intervention and kynurenines (13).

We aimed to fill this knowledge gap by exploring whether there are relations between evidence-based dietary scores [MDS (14), the energy-adjusted DII (E-DII™) (15), and the AHEI (16)] and plasma markers of inflammation, kynurenines, and B-vitamins—hereafter referred to as inflammation-related markers. We refer to these as markers because not all have been validated as biomarkers. From the individual marker–dietary score associations, we then aimed to derive marker scores to reflect adherence to these dietary scores and evaluate their associations with all-cause mortality.

Methods

Study population and design

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,513 men and women from Melbourne, Australia, aged between 27 and 76 y (99% were 40–69 y) at recruitment (17). All participants were of white European descent, including 13% born in Italy, 11% Greece, and 6% in the United Kingdom, and had attended baseline and up to 2 waves of follow-up (wave 1 and wave 2). This analysis used data from a subset of 976 MCCS participants with blood samples available at baseline (during 1990–1994) and wave 2 (during 2003–2007) (**Supplemental Figure 1**). Participants were selected as controls from pre-existing case-control studies, had a wide range of socioeconomic profiles, assessed as the Socio-Economic Indexes of Areas (SEIFA), and had plasma samples available to measure markers associated with inflammation and immune response. Our main analyses included a subgroup of participants who attended wave 2 and had a complete set of all 30 plasma inflammation-related markers and dietary information (n = 770 for MDS and n = 787 for E-DII and AHEI) (Supplemental Figure 1). MCCS participants provided informed consent and the Cancer Council Victoria Human Research Ethics Committee approved the study (17).

Dietary assessment

At wave 2, participants completed a 144-item validated self- or interviewer-administered semi-quantitative food-frequency questionnaire (FFQ) (18), including food and beverage groups (grain-based foods, dairy foods and fats, meat, fish and seafood, fruit, vegetables, miscellaneous, tea/coffee, and alcoholic beverages), with photos to help estimate portion size. Nutrient intakes per day from the FFQs were calculated using nutrition composition data from NUTTAB 2010 and AUSNUT 2007 (19, 20). Total energy intake was estimated by combining energy from foods, drinks, and alcohol. Three dietary scores—the MDS (14, 21), the E-DII (15, 22), and the AHEI (16)—were calculated according to methods previously published (**Supplemental Methods**).

Other factors

Sociodemographic and lifestyle factors were collected via an interviewer-administered questionnaire at baseline and wave 2. Residential postcodes were used to assign participants to a decile of socioeconomic status based on SEIFA (23). Physical activity data were obtained using the long-form International Physical Activity Questionnaire and then converted into domain-specific metabolic equivalent of task (MET) hours per week (24). Baseline physical activity was modelled as a score based on time spent walking, in moderate physical activity, and in vigorous activity (25). Weight was measured at baseline and wave 2 to the nearest 100 g using a digital electronic scale, while height was measured only at baseline, within 1 mm, using a stadiometer. BMI was calculated as weight (kilograms) divided by height (meters) squared.

Marker measurement and quality control

Baseline blood samples were collected in Lithium-Heparin tubes (during 1990–1994) and wave 2 samples were collected into Ethylenediaminetetraacetic acid (EDTA) tubes (during 2003–2007). The time between blood collection and plasma separation was not recorded but may have varied between baseline and wave 2. After separation, plasma was stored in liquid nitrogen. Samples were stored for several years. Plasma samples were thawed, separated into aliquots, and sent to the respective laboratories for biochemical analyses, Bevital (www.bevital.no) or the International Agency for Research on Cancer (IARC). Each individual's

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Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization. Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database.

Supplemental Figure 1, Supplemental Methods, and Supplemental Tables 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: AHEI, Alternative Healthy Eating Index 2010; CRP, C-reactive protein; E-DII, energy-adjusted Dietary Inflammatory Index; EDIP, Empirical Dietary Inflammatory Pattern; EDTA, Ethylenediaminetetraacetic acid; FFQ, food-frequency questionnaire; IARC, International Agency for Research on Cancer; IL, interleukin; IFN-γ, interferon gamma; LOD, limit of detection; MCCS, Melbourne Collaborative Cohort Study; MDS, Mediterranean dietary score; SEIFA, Socio-Economic Indexes of Areas, TNF-α, tumour necrosis factor alpha.

baseline and wave 2 samples were assayed at the same time using the same methods described below.

The following plasma markers were measured at Bevital in Bergen, Norway: thiamin and thiamin monophosphate (vitamin B-1, in nmol/L); riboflavin and flavin mononucleotide (vitamin B-2 in nmol/L); nicotinic acid, nicotinamide, N^1-methylnicotinamide (vitamin B-3, in nmol/L); pyridoxal 5'-phosphate, pyridoxal, pyridoxine, and 4-pyridoxic acid (vitamin B-6, in nmol/L). Components of the tryptophan-kynurenine pathway were also measured: 3-hydroxykynurenine (in nmol/L), kynurenic acid (in nmol/L), xanthurenic acid (in nmol/L), anthranilic acid (in nmol/L), 3-hydroxyanthranilic acid (in nmol/L), picolinic acid (in nmol/L), and quinolinic acid (in nmol/L); neopterin (in nmol/L), cystathionine (in μmol/L), and trigonelline (a marker of coffee consumption, in μmol/L); and cotinine and trans-3′-hydroxycotinine (nmol/L). All of the above biomarkers were measured by liquid chromatography–tandem mass spectrometry (LC-tandem MS) (26). Kynurenine (in μmol/L) and tryptophan (in μmol/L) were measured using gas-chromatography–tandem mass spectrometry (GC-tandem MS) (27). CRP (in μg/mL), serum amyloid A (in μg/mL), calprotectin (in μg/mL), and cystatin C (in μg/mL) were measured using immuno matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS (28). Three plasma samples were included for quality control in all batches. Separate aliquots were shipped to the laboratories of IARC in Lyon, France, for measurement of markers of inflammation, including IL-6, IL-8, IL-10, and IL-13 (in pg/mL), IFN-γ (in pg/mL), and TNF-α (in pg/mL), using the Meso Scale Discovery 6-Plex kit. From 35 markers, we excluded markers where ≥80% of participants' measurements were missing, not available, or below the limit of detection (LOD), resulting in 30 markers included in this study (nicotinic acid, pyridoxine, cotinine, and trans-3′-hydroxycotinine and IL-13 were excluded). Cotinine and 3′-hydroxycotinine are markers of tobacco smoking and very few participants smoked at wave 2; therefore, plasma concentrations were negligible. Measurements that fell below the LOD, assigned by the respective laboratories, for CRP, IL-6, and IL-10 were assigned half the value at LOD, according to laboratory procedures. Thus, markers involved in inflammation were chosen pragmatically.

Ascertainment of deaths

Vital status was ascertained through linkage of the cohort to the Victorian Registry of Births, Deaths and Marriages and the National Death Index. Death registration was assumed to be complete up to 31 October 2019 from the National Death Index.

Statistical analysis

Complete-case analysis was performed for each dietary score separately. All inflammation-related markers were log-transformed to approximate the normal distribution before winsorizing them at ±3 SDs from the mean to reduce the influence of extreme values. Each marker was then converted to a comparable *z*-score for marker score development. We calculated Spearman correlations between all markers and each dietary score. To derive marker scores that may reflect specific dietary patterns, we used multivariable linear regression to assess the association between individual markers (independent variable) and each dietary score (dependent variable). The following risk factors were considered potential confounders and adjusted for sequentially—model 1: age, sex, country of birth (Australia/New Zealand, Northern Europe, or Southern Europe) and total energy intake; model 2: model 1 plus physical activity, smoking >7 cigarettes/wk (yes/no), and SEIFA; and model 3: model 2 plus BMI and cystatin C as a marker of renal function [except in analyses involving cystatin C (included as a component of the E-DII marker score)], which may be mediators of the association. Markers found to be associated with each dietary score at model 2 adjustment ($P < 0.05$) were combined into a marker score, calculated as the weighted average of marker values with weights being the regression coefficient of their individual association with the respective dietary scores. Cox regression, using age as the underlying time variable, was performed to examine the associations of dietary and marker scores at wave 2 with all-cause mortality. The same covariates were used. Participants were followed up from wave 2 until death or 31 October 2019, whichever occurred first.

Sensitivity analyses included deriving marker scores from marker and diet score associations accounting for multiple testing using the false discovery rate (29). Additionally, the associations between marker score and diet score with mortality were conducted *1*) excluding participants with implausible total energy intake [those within the top and bottom 1% of our distribution of energy intake from the whole cohort at wave 2 (males: bottom 1%: <4200 kJ/d, top 1%: >20,100 kJ/d; females: bottom 1%: <3720 kJ/d, top 1%: >15400 kJ/d)] and *2*) excluding participants who reported symptomatic inflammatory conditions (hay fever, eczema, food allergy, asthma, and arthritis).

Replication analysis

MCCS baseline data (1990–1994) were used to replicate the linear regression analyses between individual markers and dietary scores ($n = 870$) using the same method as for wave 2 markers, because currently there are no external studies with comparable diet and marker data. All data on exposures and covariates were collected at baseline using similar methods as described for wave 2. At baseline, blood samples were stored in lithium-heparin tubes with similar sample stability as in EDTA (used at wave 2), except for folate, 3-hydroxyanthranilic acid (30), and calprotectin (31). We used the same statistical analysis method as described for wave 2.

Results

Of the 787 participants at wave 2, 69% were male, the mean age was 69 y (SD: 8 y), mean BMI (in kg/m^2) was 27 (SD: 4), and mean total energy intake was 8960 kJ/d (SD: 2300 kJ/d) (**Table 1**). Some participants suffered from chronic inflammatory conditions, including 11% with asthma and 41% with arthritis. Dietary scores ranged from 0 to 9 for the MDS (maximum of 9), –4.7 to +3.9 for the E-DII (possible range from –9 to +8), and 23 to 81 for the AHEI (maximum of 100).

Nine markers were associated ($P < 0.05$) with the MDS, 14 with the E-DII, and 11 with the AHEI, after adjustment for potential confounding in model 2 (**Figure 1**, **Supplemental Table 1**). No substantial changes in these associations were observed when models were further adjusted for BMI and kidney function, except for the associations of IL-8 and trigonelline with the MDS and of calprotectin, cystathionine, and xanthurenic acid with the E-DII. There was no strong evidence of association between these markers with dietary patterns ($P > 0.05$) and therefore were not included in any marker scores. Six markers were common to all 3 dietary scores, i.e., CRP and 5 components of the kynurenine pathway (kynurenine, kynurenine acid, anthranilic acid, 3-hydroxykynurenine, quinolinic acid). Less-healthy dietary scores (lower MDS and AHEI and higher proinflammatory E-DII) were associated with higher concentrations of these markers. After accounting for multiple testing, 7, 11, and 9 markers were associated with MDS, E-DII, and AHEI, respectively, and associations between marker scores and their respective diet scores remained similarly strong (**Supplemental Table 2**). There was an association between each dietary score and their respective marker score, which was evident in both men and women and those with a BMI </≥25 (**Table 2**). The dietary scores were weakly correlated with marker scores (Spearman ρ: 0.16 to 0.27) and marker scores were strongly correlated with each other (Spearman ρ: –0.74 for MDS and E-DII, –0.82 for AHEI and E-DII, and 0.93 for AHEI and MDS) (**Supplemental Table 3**).

TABLE 1 Characteristics of the Melbourne Collaborative Cohort Study participants at wave 2[1]

Characteristics	*n*	Values	Characteristics	Median	IQR
Sex, %			Activity MET, h/wk	17.3	(6.6, 35.9)
Male	546	69.4	Follow-up,[2] y	14.4	(12.6, 15.3)
Female	241	30.6	Plasma B vitamins		
Country of birth, %			Thiamin, nmol/L	3.86	(2.75, 5.68)
Australian/New Zealand	628	79.8	Thiamin monophosphate, nmol/L	7.95	(6.42, 9.80)
Northern Europe	67	8.5	Riboflavin, nmol/L	15.8	(10.3, 25.2)
Southern Europe	92	11.7	Flavin mononucleotide, nmol/L	13.7	(11.2, 17.4)
			Nicotinamide, nmol/L	447	(344, 567)
Age at wave 2 (mean ± SD), y	787	68.6 ± 8.1	N1-methylnicotiname, nmol/L	154	(115, 203)
SEIFA (mean ± SD) 10	787	6.2 ± 2.9	Pyridoxal 5′-phosphate, nmol/L	47.5	(34.4, 70.7)
BMI (mean ± SD), kg/m^2	787	27 ± 4.1	Pyridoxal, nmol/L	12.3	(8.9, 19.3)
Waist circumference (mean ± SD), cm			4-Pyridoxic acid, nmol/L	25.7	(19.3, 36.6)
Male	546	97.8 ± 10	Plasma tryptophan and metabolites		
Female	241	86.4 ± 12.2	Tryptophan, μmol/L	59.1	(51.4, 66.4)
Total energy intake (mean ± SD), kJ/d	787	8960 ± 2300	Kynurenine, μmol/L	1.62	(1.39, 1.94)
MDS score (mean ± SD)	770	4.7 ± 1.7	3-Hydroxykynurenine, nmol/L	38.8	(31.4, 48.8)
Range (min, max)		(0, 9)	Kynurenic acid, nmol/L	53.2	(43.0, 69.2)
DII, energy adjusted (mean ± SD)	787	−0.7 ± 1.4	Xanthurenic acid, nmol/L	15.3	(10.9, 20.4)
Range (min, max)		(−4.8, 3.9)	Anthranilic acid, nmol/L	16.6	(13.8, 20.6)
AHEI-2010 (mean ± SD)	787	52.2 ± 10.8	3-Hydroxyanthranilic acid, nmol/L	31.8	(26.0, 41.4)
Range (min, max)		(23, 81)	Picolinic acid, nmol/L	33.6	(26.2, 43.2)
			Quinolinic acid, nmol/L	445	(356, 591)
Smoking (≥7 cigarettes/wk),[3] %			Others		
No	751	95.4	Trigonelline, μmol//L	0.66	(0.37, 1.23)
Yes	36	4.6	Neopterin, nmol/L	9.98	(8.05, 12.60)
Hay fever, %			Cystathionine, μmol/L	0.24	(0.17,0.36)
No	41	5.2	Plasma peptides and protein markers		
Yes	15	1.9	CRP,[4] μg/mL	1.02	(0.45, 2.12)
Not asked	731	92.9	Cystatin C, μg/mL	0.97	(0.86, 1.14)
Eczema, %			Calprotectin, μg/mL	0.81	(0.69, 1.05)
No	49	6.2	Serum amyloid A, μg/mL	2.39	(1.54, 4.01)
Yes	7	0.9	Plasma cytokines		
Not asked	731	92.9	IL-6,[4] pg/mL	0.78	(0.56, 1.13)
Food allergy, %			IL-8, pg/mL	4.54	(3.56, 6.17)
No	51	6.5	IL-10,[4] pg/mL	0.24	(0.18, 0.34)
Yes	5	0.6	IFN-γ, pg/mL	5.53	(3.76, 8.57)
Not asked	731	92.9	TNF-α, pg/mL	1.99	(1.44,2.65)
Asthma,[5] %					
No	703	89.3			
Yes	84	10.7			
Arthritis,[5] %					
No	457	58.1			
Yes	326	41.4			

[1] AHEI, Alternative Healthy Eating Index; BMI, body mass index; CRP, C-reactive protein; E-DII, energy-adjusted Dietary Inflammatory Index; IL, interleukin; IFN-γ, interferon gamma; IQR, interquartile range; MDS, Mediterranean diet score; MET, metabolic equivalent of task; N, number; SD, standard deviation; SEIFA, Socio-Economic Indexes of Areas; TNF-α, tumour necrosis factor alpha; wk, week.
[2] Follow-up time (years) based on follow-up until death or censor date of 31 October 2019.
[3] Smoking status: 400 participants were "not asked" at wave 2. These were replaced with data from baseline about smoking status (all reported to be never smokers at baseline so categorized as "no" at wave 2). This assumes participants who were not smoking at baseline did not initiate smoking between baseline and wave 2.
[4] Plasma markers represent raw values (pre-transformation and winsorization). Markers that were assigned a value of half the limit of detection (LOD) because they were <LOD: CRP, was 0.05 μg/mL ($n = 45$); IL-6, which was 0.09 pg/mL ($n = 1$); and IL-10, which was 0.045 pg/mL ($n = 35$).
[5] Asthma and arthritis status: participants classified as "not asked" at wave 2 were replaced with condition status reported at baseline.

We examined the consistency of plasma marker and dietary pattern associations at baseline compared with wave 2 in 845 baseline participants. Although there were generally weaker associations or no strong evidence for associations at baseline compared with wave 2, the directions of associations were consistent with those observed at wave 2 for all but 3 markers (3-hydroxyanthranilic acid, nicotinamide with MDS, and flavin mononucleotide with E-DII) (**Supplemental Table 4**).

In 770 participants with complete wave 2 data on all markers and dietary scores, 249 deaths were observed during a median follow-up of 14.4 y. With model 2 adjustment, associations with all-cause mortality per SD of marker scores were as follows: for the MDS, HR = 0.84 (95% CI: 0.72, 0.98); for the

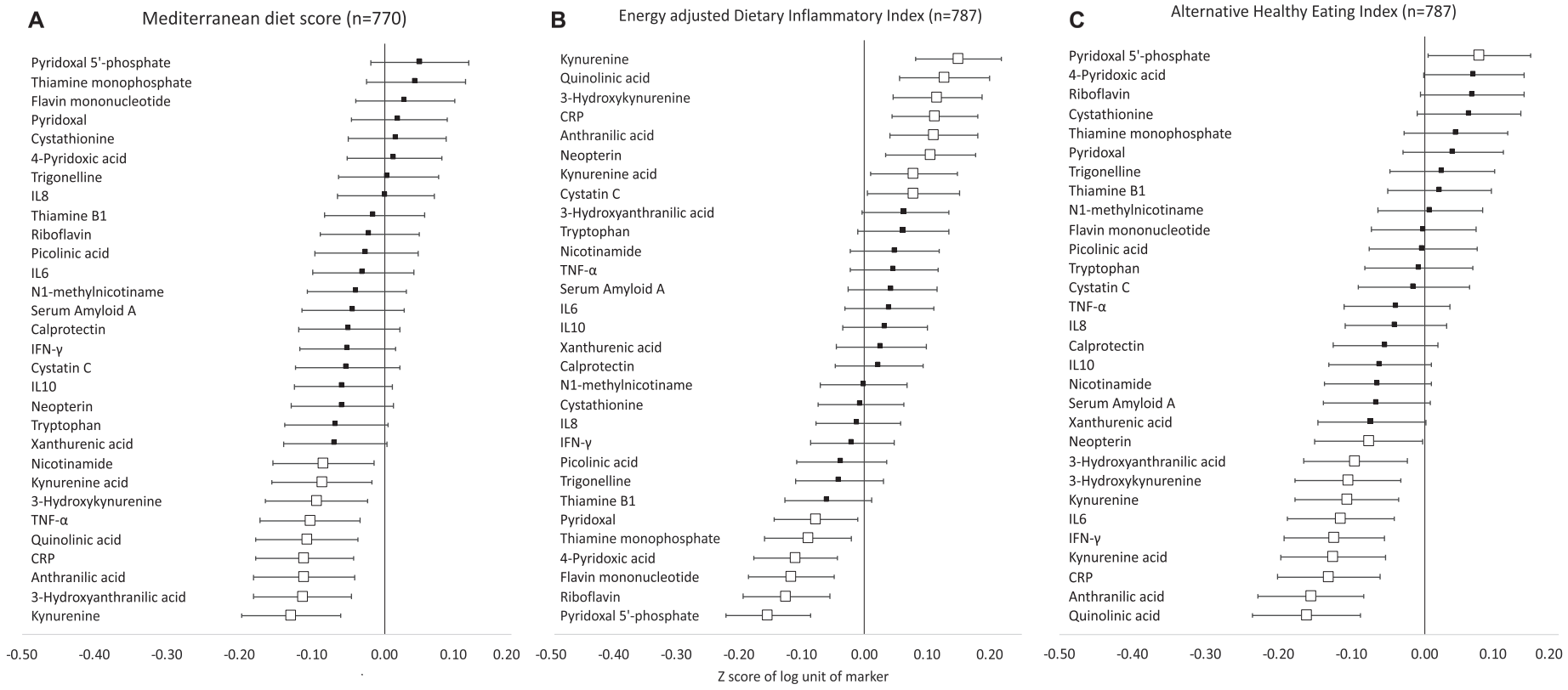

FIGURE 1 Association between 30 inflammation-related markers and 3 dietary scores in the Melbourne Collaborative Cohort Study. (A) Mediterranean diet score; (B) energy-adjusted Dietary Inflammatory Index; (C) Alternative Healthy Eating Index. The *x* axis represents the *z* score of log unit in each marker and the association with their respective dietary score. Associations are based on model 2 (Supplemental Table 1). White markers indicate those with *P* values <0.05. Multivariable linear regression adjusted for age, sex, country of birth, total energy intake (food, drink, and alcohol), physical activity (activity METs), smoking, and SEIFA. CRP, C-reactive protein; MET, metabolic equivalent of task; SEIFA, Socio-Economic Indexes of Areas.

TABLE 2 Association between marker scores and their respective dietary scores in the Melbourne Collaborative Cohort Study[1]

Marker scores (per SD)	*n*	B	SE	*P*
MDS	770	0.17	0.04	8×10^{-7}
E-DII	787	0.28	0.03	6×10^{-16}
AHEI	787	0.19	0.04	2×10^{-7}
Stratified by sex				
MDS				
Male	532	0.16	0.04	0.0001
Female	238	0.20	0.06	0.002
E-DII				
Male	546	0.25	0.04	1×10^{-8}
Female	241	0.30	0.06	2×10^{-7}
AHEI				
Male	546	0.15	0.04	0.0007
Female	241	0.25	0.06	4×10^{-5}
Stratified by BMI				
MDS				
<25 kg/m^2	259	0.22	0.06	0.0002
≥ 25 kg/m^2	511	0.13	0.05	0.005
E-DII				
<25 kg/m^2	263	0.25	0.06	2×10^{-5}
≥ 25 kg/m^2	524	0.28	0.04	4×10^{-10}
AHEI				
<25 kg/m^2	263	0.20	0.06	0.001
≥ 25 kg/m^2	524	0.15	0.04	0.001

[1] Linear regression of the association between per SD increase in marker score and their respective dietary score. Analyses performed across the whole population and by sex and BMI categories. Analyses were unadjusted because the marker scores were derived from adjusted models (see Supplemental Table 1). Marker scores were independent variables and dietary scores were dependent variables. AHEI, Alternative Healthy Eating Index; β: beta coefficient; BMI, body mass index; E-DII, energy adjusted Dietary Inflammatory Index; MDS, Mediterranean diet score; P, *P*-Value; SD, standard deviation; SE, standard error.

E-DII, HR = 1.18 (95% CI: 1.01, 1.39); and for the AHEI, HR = 0.76 (95% CI: 0.66, 0.89) (**Table 3**). The associations of dietary scores with mortality were (per SD) as follows: MDS, HR = 0.98 (95% CI: 0.85, 1.13); E-DII, HR = 0.93 (95% CI: 0.81, 1.06); and AHEI, HR = 0.88 (95% CI: 0.77, 1.00) (Table 3). After excluding individuals with implausible energy intakes and inflammatory conditions, associations of marker scores with mortality were all attenuated (Table 3). In the sensitivity analysis that additionally considered multiple testing to select included markers, the associations with mortality were similar for the MDS and AHEI marker scores (**Supplemental Table 5**).

Discussion

Our study of a community-based sample of Australian adults is the first to examine the association of well-established dietary scores with a set of 30 markers reflecting inflammation, immune activation, and nutrition, including B vitamins and compounds in the kynurenine pathway. Several markers were associated with 3 dietary scores (MDS, E-DII and AHEI), and 5 markers within the kynurenine pathway were consistently associated with each of them. In combination as marker scores reflecting adherence to these dietary scores, unfavorable marker scores were associated with mortality. Our findings indicate the value of these markers in health and their potential as biomarkers of dietary patterns, but this needs to be further validated.

Consistency with the literature

The E-DII was associated with 14 of 30 markers. Another inflammation-based diet score, the Empirical Dietary Inflammatory Pattern (EDIP), was associated with 10 of 448 markers related to lipid metabolism and immune function in a cross-sectional study of 1109 postmenopausal women (32). The study found an association with trigonelline (product of niacin metabolism and found in coffee), which we also examined but was not associated with the MDS, E-DII, or the AHEI in our study. The discrepant results may reflect the different populations studied and that the EDIP included coffee intake, unlike the dietary scores that we derived (E-DII includes caffeine, a compound within coffee). Published studies have not examined EDIP with other markers measured in our study. We confirmed the expected inverse association between the E-DII and B vitamins, including pyridoxal 5′-phosphate (i.e., in the opposite direction to associations with proinflammatory markers including CRP and kynurenine) (33). The MDS was inversely associated with 9 markers and AHEI with 11 markers. Previous studies that examined the association of the MDS or AHEI with CRP, IL-6, or TNF-α found associations directionally consistent with ours (6–9). Only 1 other study has examined the association between 2 types of Mediterranean diet interventions and kynurenines over 1 y, with no consistency in changes to kynurenines across the interventions (13).

Shared metabolites across 3 dietary scores

The consistency between markers associated with the 3 diet scores, and their very strong correlations (**Figure 2**, Supplemental Table 3), suggests that the kynurenine pathway, along with

TABLE 3 Association of marker and diet scores with all-cause mortality in the Melbourne Collaborative Cohort Study[1]

Marker score (per SD)	HR	95% CI	*P*	Diet score	HR	95% CI	*P*
MDS				MDS			
Model 1[2]	0.80	(0.70, 0.92)	0.002	Model 1	0.98	(0.85, 1.13)	0.75
Model 2[3]	0.84	(0.72, 0.98)	0.02	Model 2	0.98	(0.85, 1.13)	0.79
Sensitivity[4]	0.80	(0.64, 1.00)	0.05				
E-DII				E-DII			
Model 1[2]	1.19	(1.02, 1.38)	0.03	Model 1	0.93	(0.82, 1.07)	0.33
Model 2[3]	1.18	(1.01, 1.39)	0.04	Model 2	0.93	(0.81, 1.06)	0.29
Sensitivity[4]	1.13	(0.91, 1.41)	0.27				
AHEI				AHEI			
Model 1[2]	0.75	(0.65, 0.86)	4×10^{-5}	Model 1	0.87	(0.77, 1.00)	0.05
Model 2[3]	0.76	(0.66, 0.89)	0.0006	Model 2	0.88	(0.77, 1.00)	0.06
Sensitivity[4]	0.79	(0.64, 0.99)	0.04				

[1]Multivariable Cox regression of the association between per SD increase in diet or markers scores (independent variable) and all-cause mortality (dependent variable) with the following covariates. $n = 770$, 249 deaths. AHEI, Alternative Healthy Eating Index; CI, confidence interval; E-DII, energy-adjusted Dietary Inflammatory Index; HR, hazard ratio; MDS, Mediterranean diet score; MET, metabolic equivalent of task; P, *P*-Value; SD, standard deviation; SEIFA, Socio-Economic Indexes of Areas.
[2]Model 1: adjustment for age (underlying time variable), sex, country of birth, total energy intake (food, drink and alcohol), physical activity (activity METs), smoking, and SEIFA.
[3]Model 2: model 1, with additional adjustment for BMI, cystatin C (marker of kidney function) except for E-DII marker score
[4]Sensitivity: sensitivity analyses for model 2, excluding implausible energy intake and those with an inflammatory condition (hay fever, eczema, food allergy, asthma, arthritis). $n = 393$.

CRP, are important in relation to healthy diets. We observed that healthy diets (high MDS, high AHEI, and low, anti-inflammatory E-DII) were associated with lower concentrations of kynurenines (kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and quinolinic acid), nicotinamide, and CRP. These markers may be considered potential biomarkers reflective of a generally healthy diet.

Several reviews have identified a reduction in inflammatory markers with consumption of high-quality diets, particularly plant-based diets (6, 7, 34–36). A systematic review of 25 randomized controlled trials among participants with obesity or a metabolic condition found adherence to diets including the Mediterranean diet, Nordic diet, Dietary Approaches to Stop Hypertension diet, and vegetarian diets led to lower concentrations of CRP (mean difference of –0.55; 95% CI: –0.78, –0.32) and IL-6 (mean difference of –0.25; 95% CI: –0.56, 0.06) (7). Our findings provide support for an association between high-quality diets and inflammatory status, including with components of the kynurenine pathway. The consistency between markers associated with the dietary scores may not be surprising given that all score diets high in fruit, vegetables, whole grains, nut and legumes and with limited intake of meat and saturated fat as "healthy." Yet, these 3 dietary scores were only weakly or moderately correlated with each other in our study.

Marker scores and mortality

In our study, marker scores were associated with all-cause mortality in the expected direction. Kynurenines may contribute to this, given that they have been linked to a wide range of conditions, which together may accelerate aging and increase the risk of death (11, 37–39). A study of 7015 Norwegian participants followed for 14 y reported a nonlinear positive association between kynurenines (kynurenine: tryptophan ratio, anthranilic acid, and 3-hydroxykynurenine) and neopterin with all-cause mortality, even when participants with baseline cancer, cardiovascular disease, and diabetes mellitus were excluded (12). A mediation analysis in a cohort of 25,994 females in the United States found that markers of inflammation (CRP, fibrinogen, soluble intercellular adhesion molecule 1 [sICAM-1], glycoprotein acetylation) accounted for 29% of the protective effect of MDS on cardiovascular events (40). Formal mediation analysis could be used to quantify the extent to which kynurenines would explain the effect of diet on mortality, but this is beyond the scope of the current study.

The stronger association with mortality for the marker scores than for their respective dietary scores in our study may be because markers reflect both the metabolic effect of diet (i.e., post-digestion and absorption) and the health/inflammation status of the individual: for example, inflammation (e.g., due to acute illness or chronic disease with a low-grade inflammatory profile) may increase kynurenines (39) independently of diet and may explain the attenuation observed after excluding participants with chronic inflammatory conditions at baseline (Table 3). In addition, the dietary scores based on FFQs may be more prone to measurement error than circulating metabolites. Supplemental Table 4 demonstrates the reproducibility of the direction of effect for marker and dietary score associations over time (baseline and at wave 2) despite differences in FFQs. Participants were, on average, 11 y younger at baseline, an age at which inflammation-related processes might play a less prominent role. Future mechanistic studies and feeding trials are warranted to understand if and to what extent these markers reflect food constituents, de novo synthesis, physiological changes, or the health/inflammation status of participants and, hence, can be considered as nutritional biomarkers.

Limitations of this study include our sample consisting of a higher proportion of males than the full wave 2 MCCS sample and the cross-sectional nature of the marker and dietary score data collection. Findings may need to be interpreted with caution given the relatively small sample size. Data on possible confounders such as B-vitamin supplements and anti-inflammatory medications were not available at wave 2 of the MCCS. It is also unknown whether these markers reflected recent or long-term dietary status. Individual markers are present in various marker scores; therefore, these overlapping markers may explain the high correlation between marker scores. Possible residual confounding and measurement errors of self-reported intake also may have contributed to weaker associations between all-cause mortality and dietary scores than with markers, which highlights the potential objectivity of the derived marker scores in this context. Despite consistency in the associations for marker and dietary scores at baseline and wave 2 measurements, there was moderate correlation

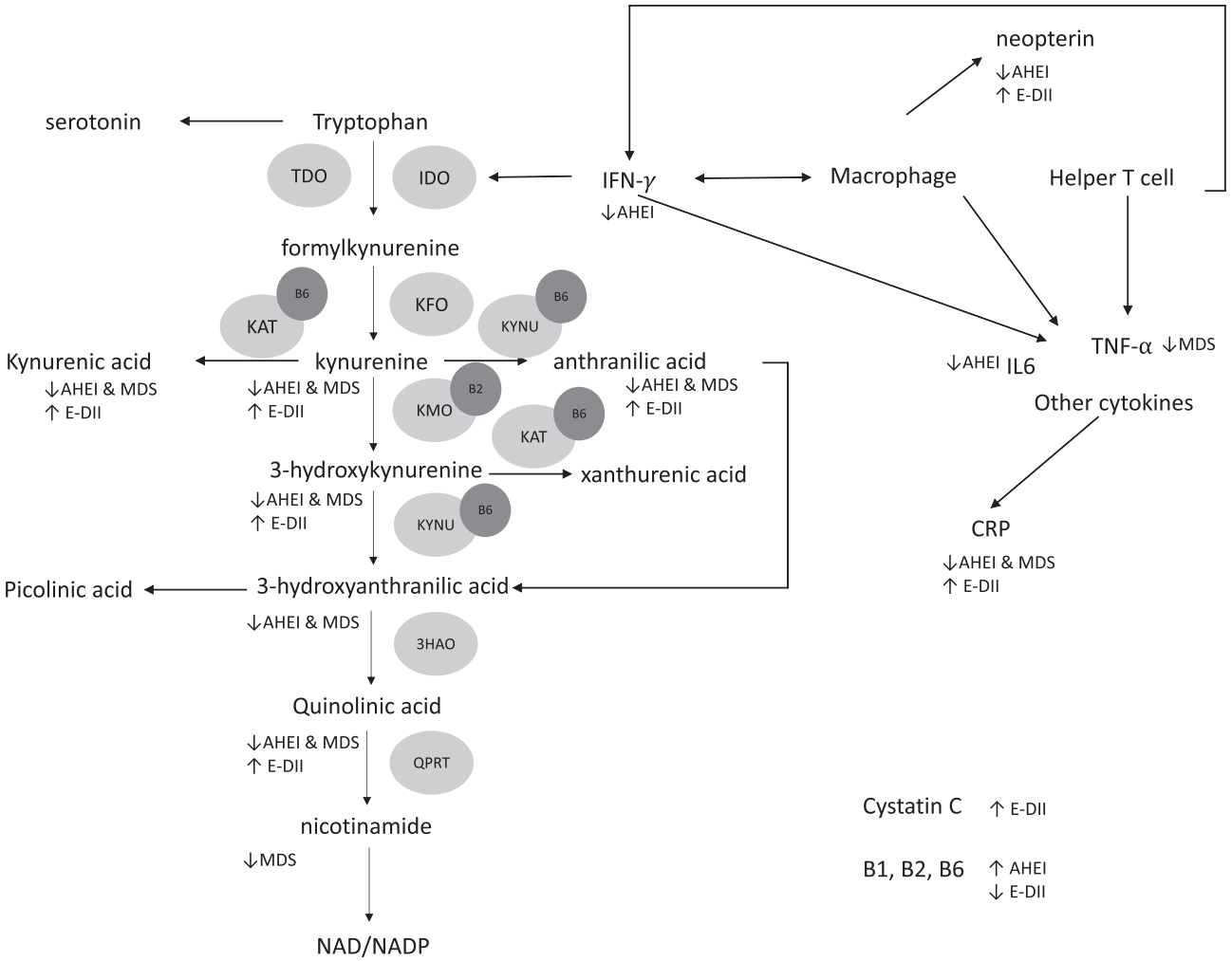

FIGURE 2 Tryptophan-kynurenine pathway and directions of association observed with the MDS, AHEI, and/or E-DII (11, 33, 41, 42). Direction of associations is based on model 2 (Supplemental Table 1). The figure is adapted from the KEGG pathway (43). AHEI, Alternative Healthy Eating Index; B1, thiamin or derivate; B2, riboflavin or derivate; B3, niacin or derivate; B6, pyridoxine or derivate; CRP, C-reactive protein; E-DII, energy-adjusted Dietary Inflammatory Index; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine transaminase; KFO, kynurenine formylase; KMO, kynurenine monooxygenase; KYNU, kynureninase; MDS, Mediterranean diet score; QPRT, quinolinate phosphoribosyl transferase; TDO, tryptophan 2,3-dioxygenase; 3HAO, 3-hydroxyanthranilate dioxygenase.

for dietary scores and markers between these time points (e.g., due to measurement error, change in dietary habits, or change in other aspects of lifestyle). Further research is therefore warranted to confirm our findings and strengthen the evidence of the role played by diet in inflammation, particularly for the kynurenine pathway. We derived marker scores that represent the inflammatory potential of 3 diets; the association patterns observed in our study also require further validation in additional cohort or controlled dietary intervention studies.

In conclusion, our findings provide evidence that markers involved in inflammation-related processes, including those within the kynurenine pathway, are associated with dietary quality assessed by well-established dietary scores, with substantial overlap between markers associated with the MDS, the E-DII, and the AHEI. Favorable marker scores, reflecting higher-quality diets, were associated with reduced mortality. These results contribute to the evidence on the role of inflammation with diet and health outcomes. Our marker scores may be useful for future biomarker discovery related to dietary patterns.

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Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

References

1. Benziger CP, Roth GA, Moran AE. The Global Burden of Disease Study and the preventable burden of NCD. Glob Heart 2016;11:393–7.

2. Kuhnle GGC. Nutritional biomarkers for objective dietary assessment. J Sci Food Agric 2012;92(6):1145–9.
3. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002;13(1):3–9.
4. Brennan L, Hu FB. Metabolomics-based dietary biomarkers in nutritional epidemiology—current status and future opportunities. Mol Nutr Food Res 2019;63(1):1–5.
5. Christ A, Lauterbach M, Latz E. Western diet and the immune system: an inflammatory connection. Immunity 2019;51(5):794–811.
6. Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. Nutr Metab Cardiovasc Dis 2014;24(9):929–39.
7. Eichelmann F, Schwingshackl L, Fedirko V, Aleksandrova K. Effect of plant-based diets on obesity-related inflammatory profiles: a systematic review and meta- analysis of intervention trials. Obes Rev 2016;17(11):1067–79.
8. Piccand E, Vollenweider P, Guessous I, Marques-Vidal P. Association between dietary intake and inflammatory markers: results from the CoLaus study. Public Health Nutr 2019;22(3):498–505.
9. Guillermo C, Boushey CJ, Franke AA, Monroe KR, Lim U, Wilkens LR, Marchand LLe, Maskarinec G. Diet quality and biomarker profiles related to chronic disease prevention: the Multiethnic Cohort Study. J Am Coll Nutr 2020;39(3):216–23.
10. Shivappa N, Hebert JR, Marcos A, Diaz L-E, Gomez S, Nova E, Michels N, Arouca A. Association between dietary inflammatory index and inflammatory markers in the HELENA study. Mol Nutr Food Res 2017;61:1–10.
11. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. Science 2017;357(6349):1–8.
12. Zuo H, Ueland PM, Ulvik A, Eussen S, Vollset SE, Nygård O, Midttun Ø, Theofylaktopoulou D, Meyer K, Tell GS. Plasma biomarkers of inflammation, the kynurenine pathway, and risks of all-cause, cancer, and cardiovascular disease mortality: the Hordaland Health Study. Am J Epidemiol 2016;183(4):249–58.
13. Yu E, Ruiz-Canela M, Guasch-ferr M, Zheng Y, Toledo E, Clish CB, Salas-salvad J, Liang L, Wang DD, Corella D, et al. Increases in plasma tryptophan are inversely associated with incident cardiovascular disease in the Prevencion con Dieta Mediterranea (PREDIMED) study. J Nutr 2017;147:314–22.
14. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348(26):2599–608.
15. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr 2014;17(8):1689–96.
16. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142(6):1009–18.
17. Milne RL, Fletcher AS, MacInnis RJ, Hodge AM, Hopkins AH, Bassett JK, Bruinsma FJ, Lynch BM, Dugué PA, Jayasekara H, et al. Cohort profile: the Melbourne Collaborative Cohort Study (Health 2020). Int J Epidemiol 2017;46(6):1757–1757i.
18. Bassett JK, English DR, Fahey MT, Forbes AB, Gurrin LC, Simpson JA, Brinkman MT, Giles GG, Hodge AM. Validity and calibration of the FFQ used in the Melbourne Collaborative Cohort Study. Public Health Nutr 2016;19(13):2357–68.
19. Food Standards Australia New Zealand. AUSNUT 2007 [Internet]. 2019[cited 2020 May 10]. Available from: https://www.foodstandards.gov.au/science/monitoringnutrients/ausnut/Pages/ausnut2007.aspx.
20. Food Standards Australia New Zealand. Australian Food Composition Database (NUTTAB 2019)[Internet]. 2019 [cited 2020 May 10]. Available from: https://www.foodstandards.gov.au/science/monitoringnutrients/afcd/pages/default.aspx.
21. Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocké M, Peeters P. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ 2005;330(7498):991.
22. Hébert JR, Shivappa N, Wirth MD, Hussey JR, Hurley TG. Perspective: the Dietary Inflammatory Index (DII)—lessons learned, improvements made, and future directions. Adv Nutr 2019;10(2):185–95.
23. Australian Bureau of Statistics. 2033.0.55.001—Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA), Australia, 2016[Internet]. 2018 [cited 2020 May 10]. Available from: https://www.abs.gov.au/ausstats/abs@.nsf/mf/2033.0.55.001.
24. International Physical Activity Questionnaire (IPAQ). Guidelines for data processing and analysis of the international physical activity questionnaire (IPAQ)-short and long forms. 2005 [Internet]. 2021 [cited 2020 May 10]. Available from: https://sites.google.com/site/theipaq/questionnaire_links.
25. MacInnis RJ, English DR, Hopper JL, Gertig DM, Haydon AM, Giles GG. Body size and composition and colon cancer risk in women. Int J Cancer 2006;118(6):1496–500.
26. Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23(9):1371–9.
27. Midttun Ø, McCann A, Aarseth O, Krokeide M, Kvalheim G, Meyer K, Ueland PM. Combined measurement of 6 fat-soluble vitamins and 26 water-soluble functional vitamin markers and amino acids in 50 μl of serum or plasma by high-throughput mass spectrometry. Anal Chem 2016;88(21):10427–36.
28. Meyer K, Ueland PM. Targeted quantification of C-reactive protein and cystatin C and its variants by immuno-MALDI-MS. Anal Chem 2014;86(12):5807–14.
29. Hochberg Y, Benjaminit Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 1995;57:289–300.
30. Hustad S, Eussen S, Midttun Ø, Ulvik A, Van De Kant PM, Mørkrid L, Gislefoss R, Ueland PM. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. Clin Chem 2012;58(2):402–10.
31. Pedersen L, Birkemose E, Gils C, Safi S, Nybo M. Sample type and storage conditions affect calprotectin measurements in blood. J Appl Lab Med 2018;2(6):851–6.
32. Tabung FK, Liang L, Huang T, Balasubramanian R, Zhao Y, Chandler PD, Manson JAE, Cespedes Feliciano EM, Hayden KM, Van Horn L, et al. Identifying metabolomic profiles of inflammatory diets in postmenopausal women. Clin Nutr 2020;39(5):1478–90.
33. Ueland PM, McCann A, Midttun Ø, Ulvik A. Inflammation, vitamin B6 and related pathways. Mol Aspects Med 2017;53:10–27.
34. Hosseini B, Berthon BS, Saedisomeolia A, Starkey MR, Collison A, Wark PAB, Wood LG. Effects of fruit and vegetable consumption on inflammatory biomarkers and immune cell populations: a systematic literature review and meta-analysis. Am J Clin Nutr 2018;108(1):136–55.
35. Navarro SL, Tarkhan A, Shojaie A, Randolph TW, Gu H, Djukovic D, Osterbauer KJ, Hullar MA, Kratz M, Neuhouser ML, et al. Plasma metabolomics profiles suggest beneficial effects of a low-glycemic load dietary pattern on inflammation and energy metabolism. Am J Clin Nutr 2019;110(4):984–92.
36. Pickworth CK, Deichert DA, Corroon J, Bradley RD. Randomized controlled trials investigating the relationship between dietary pattern and high-sensitivity C-reactive protein: a systematic review. Nutr Rev 2019;77(6):363–75.
37. Dadvar S, Ferreira DMS, Cervenka I, Ruas JL. The weight of nutrients: kynurenine metabolites in obesity and exercise. J Intern Med 2018;284(5):519–33.
38. Liu JJ, Movassat J, Portha B. Emerging role for kynurenines in metabolic pathologies. Curr Opin Clin Nutr Metab Care 2019;22(1):82–90.
39. Savitz J. The kynurenine pathway: a finger in every pie. Mol Psychiatry 2020;25(1):131–47.
40. Ahmad S, Moorthy MV, Demler OV, Hu FB, Ridker PM, Chasman DI, Mora S. Assessment of risk factors and biomarkers associated with risk of cardiovascular disease among women consuming a Mediterranean diet. JAMA Network Open 2018;1(8):e185708.
41. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol 2018;9:1–11.
42. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 2014;6(10):a016295.
43. KEGG: Kyoto Encyclopedia of Genes and Genomes. Tryptophan metabolism—reference pathway. [Internet]. 2021[cited 2020 May 11]. Available from: https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map00380&keyword=kynurenine.