Systematic Review

Biomarkers of dietary patterns: a systematic review of randomized controlled trials

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Context: *Most methods for assessing dietary intake have considerable measurement error. Dietary biomarkers are objective tools for dietary assessment. Dietary biomarkers of dietary patterns have not been well described, despite modern dietary guidelines endorsing dietary patterns.* ***Objective:*** *This systematic review sought to describe the dietary biomarkers commonly used to assess dietary patterns, and the novel biomarkers of dietary patterns identified by exploratory studies.* ***Data Sources:*** *MEDLINE, Embase, Cochrane Central, PreMEDLINE, and CINAHL databases were searched.* ***Data Extraction:*** *Data extraction and bias assessment were undertaken in duplicate.* ***Data Analysis:*** *A qualitative approach was applied, without statistical analysis.* ***Conclusion:*** *In controlled settings, dietary biomarkers of single nutrients or of individual foods or food groups are commonly used to assess compliance with dietary patterns. However, currently, there are no dietary biomarkers or biomarker profiles that are able to identify the specific dietary pattern that has been consumed by an individual. Future work should seek to validate novel dietary biomarkers and biomarker profiles that are indicative of specific dietary patterns and their characteristics. A dietary biomarker panel consisting of multiple biomarkers is almost certainly necessary to capture the complexity of dietary patterns.*
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INTRODUCTION

A "single-nutrient approach" that focuses on individual nutrients, foods, and food groups has traditionally been the dominant modality for nutrition research and policy. This approach, however, may not fully capture the complexities of dietary intake, given nutrient–nutrient interactions, intercorrelations, and food matrix

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characteristics.[1] An alternative approach is to view nutrition through the prism of dietary patterns, which capture the overall combination of the dietary components, including the quality, quantity, and frequency of consumption.[1] This approach acknowledges the synergistic and antagonistic effects of nutrients and foods, and, therefore, better aligns with the complexity of real-world dietary intake.[2]

The application of both approaches is impaired by the relatively low accuracy of current methods for assessing habitual dietary intake. Food frequency questionnaires are commonly used in large-scale clinical trials and cohort studies, despite the inaccuracy inherent in recall of habitual intake over an extended period of time. Multiple short-term dietary recalls using multipass methods are increasingly seen as the most accurate method of assessing usual dietary intake,[3] although this method is not exempt from random or systematic measurement error due to the subjective nature of self-report, estimation of portion size, etc. Also, a high number of recalls is required to capture infrequently consumed nutrients or foods, especially at an individual level.[4] More-objective dietary assessment would enhance nutritional research and improve the evidence base for the development of dietary guidelines.

Dietary biomarkers are an attractive alternative or additional measure for assessing dietary intake. Defined as measurable and quantifiable biological indicators of dietary intake or nutritional status,[5] dietary biomarkers have not only been used in validating dietary assessment tools,[6] but have demonstrated the ability to predict dietary intake independent of traditional dietary assessment tools.[7] Dietary biomarkers can be categorized as either direct biomarkers of dietary exposure (ie, measures of consumed nutrients) or as biomarkers of nutritional status (ie, indicators of dietary intake affected by metabolism and nutrient–nutrient interactions).[8] Both types of dietary biomarkers are commonly used for assessing consumption of specific nutrients, or of individual foods or food groups.[9–11] The widespread adoption of metabolomics has shifted the field further during the past decade.[12] Metabolomics is the study of the different molecules synthesized by an organism and can provide a broad profile of the metabolites that are present in a biospecimen,[13] including many associated with dietary intake. One strength of metabolomics, in comparison with the use of traditional nutritional biomarkers, is that it is often applied in an unbiased manner as part of exploratory research; however, this approach requires subsequent validation. A key weakness of metabolomics is that these metabolites may be associated with a variety of foods, and as indicators they may lack specificity; however, they have great potential for development and application within biomarker profiles of dietary patterns.

One previous systematic review investigated urinary biomarkers of dietary intake, including dietary patterns. That review included 191 articles on urinary biomarkers, but only 4 of the articles described their relationship to dietary patterns[14]; the review reported on the Mediterranean diet, the vegetarian diet, diets based on dietary guidelines, and dietary clusters identified by cluster analysis. However, the use of urinary biomarkers as dietary pattern indicators was not a focus and was not discussed in depth. The present comprehensive review of biomarkers for dietary patterns sought to identify the nature of those currently being used within clinical trials, and those for which there is strong evidence for their value as biomarkers of dietary patterns.

Accordingly, a systematic review of randomized controlled trials (RCTs) was undertaken to identify dietary biomarkers currently used to assess compliance with dietary patterns, as well as those biomarkers and metabolites affected by intake of a distinct dietary pattern in exploratory metabolomics studies.

METHODS

Protocol and registration

The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO, Registration no. CRD42019129839), and conducted as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[15] The PRISMA checklist is included in Appendix S1 in the Supporting Information online.

Selection criteria

The inclusion criteria were (1) RCT study design; (2) healthy adult participants, including some participants who have a chronic disease; (3) food-based intervention focusing on a dietary pattern, without nutritional supplementation; (4) having an appropriate comparator, ie, a different dietary pattern; (5) dietary biomarkers or metabolomic profiles were reported based on different dietary patterns. The exclusion criteria were (1) studies with all participants selected on the basis of a current diagnosis of metabolic syndrome, diabetes, cardiovascular diseases, cancer, or other diseases; (2) study participants selected on the basis of pregnancy or lactation; (3) the dietary intervention focused on one component of the diet such as a single nutrient, or individual foods or food groups; (4) the intervention involved supplementation or energy restriction; (5) the control group(s) differed in less than 2 components (foods/food groups) of the intervention diet; (6) article not written in English language; (7) research only published in the form of a

scientific abstract. The PICOS (participants, interventions, comparators, outcomes, and study design) criteria are shown in Table 1.

Information source

The information was sourced from databases, including MEDLINE, Embase, Cochrane Central, PreMEDLINE, and CINAHL until November 16, 2020. Reference lists from included publications and from reviews on dietary pattern analysis were also reviewed to supplement the electronic database search.

Search strategy

The search strategy was developed in MEDLINE and adapted for Embase, Cochrane Central, PreMEDLINE, and CINAHL. The search terms used in MEDLINE were "Biomarkers," "Metabolome" and "Metabolomics," which were then combined with "Diet, Carbohydrate Loading," "Diet, Atherogenic," "exp Diet, Carbohydrate-Restricted," "Diet, Fat-Restricted," "Diet, Gluten-Free," "Diet, High-Fat," "exp Diet, High-Protein," "Diet, Mediterranean," "Diet, Paleolithic," "Diet, Protein-Restricted," "Diet, Sodium-Restricted," "exp Diet, Vegetarian," "Diet, Western," "Dietary Approaches To Stop Hypertension," and "Healthy Diet." A line of key word searching was also included as "([Biomarker* or biochemical marker* or biological marker* or metabolomic* or metabolite* or metabolome*] adj5 [nutrition* or food* or diet* or DASH or eating]).tw." The search was then limited to RCTs in human adults published in the English language. The study type and animal filters used were derived from the Scottish Intercollegiate Guidelines Network (SIGN) systematic review filters.[16] The full electronic search strategy for MEDLINE is presented in Appendix S2 in the Supporting Information online.

Study selection

First-round screening was undertaken by reviewer S.L. A random sample of 12% of all abstracts was checked by reviewer F.M.O'L. and a 100% agreement was reached. The full text of each article was then assessed in duplicate by 3 reviewers (S.L., R.F.N., and C.A.T.) independently, and any discrepancies were resolved by consultation with reviewers (F.M.O'L., M.R.S., and K.S.B.-A.). The screening process was completed on reference management software EndNote (version 9, Clarivate Analytics, Philadelphia, PA, USA).

Table 1 **PICOS criteria for inclusion of studies**

Parameter	Criteria
Participants	Healthy adult participants; however, including studies in which some of the participants have a chronic disease
Interventions	Food-based intervention focusing on a dietary pattern, without nutritional supplementation
Comparators	A different dietary pattern
Outcomes	Dietary biomarkers, including metabolomic profiles
Study design	Randomized controlled trials

Data collection process

Data extraction was completed with the use of Research Electronic Data Capture (REDCap), a secure online application for data collection and management.[17,18] Data were extracted from each of the identified articles by 2 reviewers (S.L. and R.F.N.) independently, and verified by reviewers F.M.O'L. and M.R.S.

Data extracted include study title, first author, publication year, country, study design, aim, selection criteria, study time frame, population size, participant characteristics, dietary pattern details, biomarkers, biological compartment, assay technique, statistical method, conclusions, limitations, and funding sources.

Risk of bias

The Cochrane risk-of-bias tool version 2[19] was used by 2 reviewers (S.L. and R.F.N.) independently to assess the risk of bias of each publication. A total of 5 domains were assessed: (1) bias arising from the randomization process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in measurement of the outcome, and (5) bias in selection of the reported result. An overall risk of bias judgment was made as "Low risk of bias" (if judged as having "low risk of bias" for all domains), "Some concerns" (if judged as having "some concerns" for at least 1 domain and not judged as having "high risk of bias" for any domain), and "High risk of bias" (if judged as having "high risk of bias" for at least 1 domain, or "some concerns" for multiple domains in a way that substantially increased the risk of bias).

RESULTS

Study selection

The complete study selection procedure is summarized in Figure 1. Briefly, the literature search retrieved 3930 records; a total of 2610 records were screened for titles and abstracts, of which 2160 records were excluded. From the remaining 450 full texts screened, a total of 30 publications met the inclusion criteria. A number of publications provided data on different biomarkers

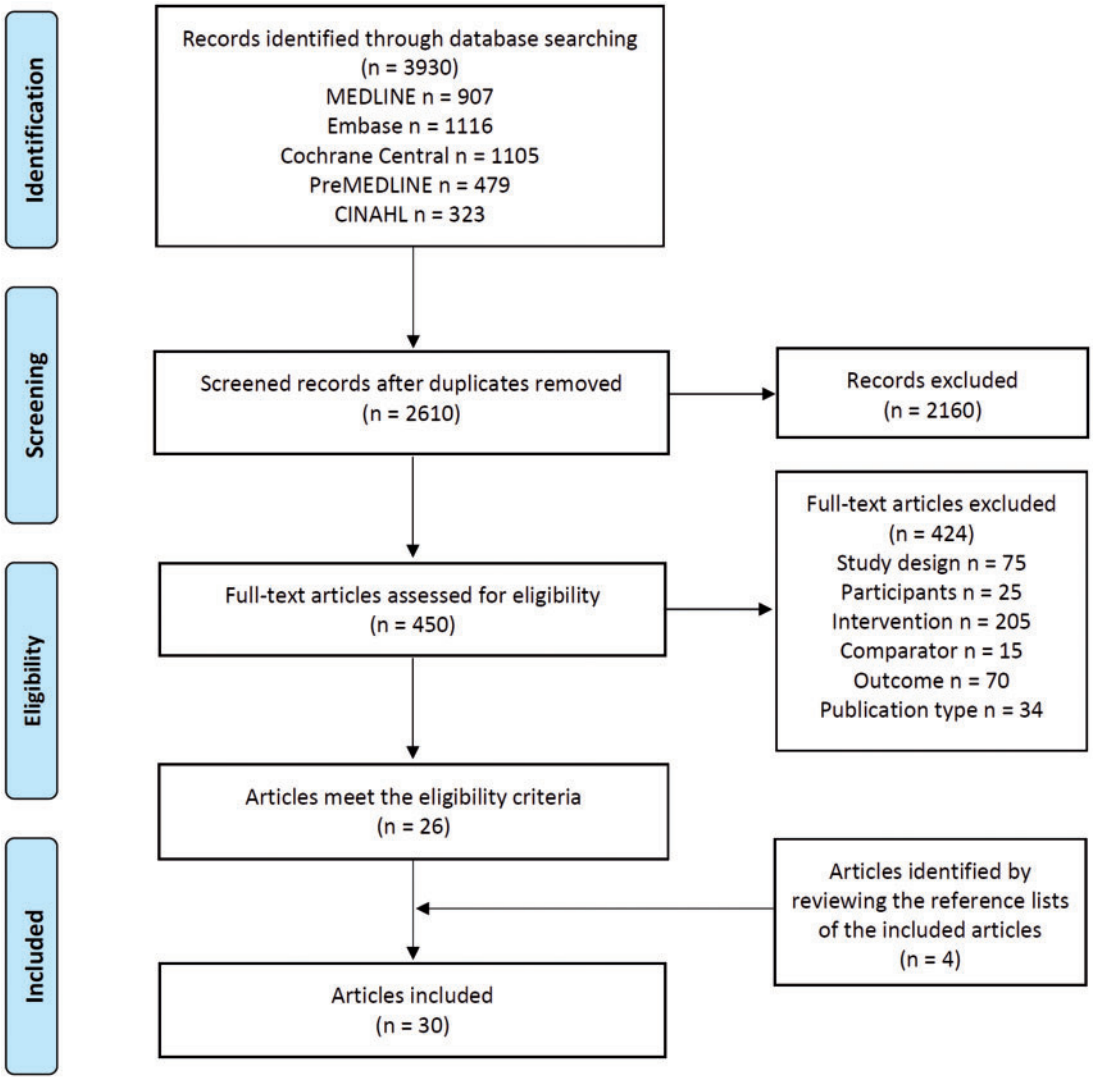

Figure 1 **PRISMA flowchart of the study selection procedure.**

from the same RCTs,[20–32] using various biospecimen types, or measurement approaches; thus, results were included from 22 RCTs.

Study characteristics

The main characteristics of the included studies are shown in Table 2.[20–49] Of the 30 publications identified, 13 utilized prospectively identified dietary biomarkers.[23,25–27,29,33–35,37–39,42,45] These dietary biomarkers have all been previously proposed as indicators of a dietary characteristic (eg, nutrient, food, or food group). The other 17 publications applied a metabolomics approach to identify novel biomarkers of dietary patterns.[20–22,24,28,30–32,36,40,41,43,44,46–49] Publications were from the United States (n = 9),[22,25–28,30,35,43,49] Denmark (n = 5),[20,21,24,29,31] and Spain (n = 3),[23,32,39] 2 each from Australia,[33,42] Germany,[34,38] and the UK,[36,45] and 1 each from Canada,[48] Italy,[40] Korea,[46] the Netherlands[41] and Sweden.[44] Two publications were based on multinational trials from European[37] and Nordic countries[47] (n = 1 each). The 30 publications reported findings from 22 trials, with 5 papers from a Danish trial,[20,21,24,29,31] 4 from a US trial,[25–27,30] and 2 each from Spanish[23,32] and US trials.[22,28] Sixteen publications reported trials with sample size of <100 participants,[22,28,32,34–36,38–44,46,48,49] 10 with a sample size of 100–200 participants,[20,21,24,26,27,29,31,33,45,47] and 4 with a sample size of >300 participants[23,25,30,37] (maximum 1127 subjects[37]). The participants' mean age ranged from 22 to 71 years, and between 31% to 100% of participants were female.

The main dietary patterns studied were the Mediterranean diet (n = 11),[23,32–35,37,39–41,43,49] the healthy Nordic diet (n = 6),[20,21,24,29,31,47] the DASH diet (n = 5),[25–27,30,42] a low-glycemic-load (GL) diet (n = 2),[22,28] a vegetarian diet (n = 2),[38,44] diets based on national and international guidelines (n = 2) (ie, UK

Table 2 **Characteristics of included randomized controlled trials**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
Mediterranean diet								
Davis et al (2017)[33]	Australia		Parallel	n = 152, 71 y, 56% F, 27.0 kg/m^2	Intervention: 6 months Mediterranean diet: based on a traditional Mediterranean diet, with small adaptations to the Australian food supply Written guide, recipe book, checklists Key foods were provided (EVOO, nuts, Greek yogurt, canned legumes, and canned tuna).	Habitual diet: Written instructions detailing the conditions of the diet	Established biomarkers[a] Serum Within-group differences, 24 wks—baseline =: no change within either group *$\log_{10}$-transformed β-cryptoxanthin* (=) ($P = 1.00$ within both groups) Lycopene* (=) ($P = 1.00$ within both groups) α-carotene*, ng/mL (46.5 ± 202.2, $P < 0.01$ within intervention group), ($P = 0.50$ within control group) β-carotene* (=) ($P = 0.10$ within intervention group; $P = 1.00$ within control group) Lutein: zeaxanthin* (=) ($P = 0.40$ within intervention group; $P = 1.00$ within control group) Erythrocyte % SFAs (decreased from $43.3\% \pm 0.1\%$ to $42.8\% \pm 0.1\%$, $P < 0.001$ within intervention group) ($P = 1.00$ within control group) % TFAs (-0.1 ± 0.1, $P < 0.001$ within intervention group) ($P = 0.34$ within control group) % MUFAs (increased from $18.4\% \pm 0.1\%$ to $19.3\% \pm 0.1\%$, $P < 0.001$ within intervention group) ($P = 1.00$ within control group)	Linear mixed-effects models (diet × visit interaction) Serum (HPLC) Erythrocyte (direct transesterification)

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							% *n*–3 FAs (=) ($P = 1.00$ within intervention group; $P = 0.30$ within control group) % *n*–6 FA (=) ($P = 0.07$ within intervention group; $P = 1.00$ within control group) % DHA (increased from 5.8% ± 0.14% to 6.1% ± 0.13%, $P = 0.01$ within intervention group) *n*–6:*n*–3 (–0.1 ± 0.4, $P = 0.05$ within intervention group) ($P = 0.15$ within control group) *n*–3 index* (0.2 ± 0.9; $P = 0.02$ within intervention group) ($P = 0.28$ within control group) 24-h urinary (=) Sodium, potassium, calcium, and magnesium	
Diekmann et al (2019)[34]	Germany		Crossover	n = 26, 70 y, 31% F, 30.3 kg/m^2	Intervention: 4.5 h Washout: 2 wks Mediterranean diet Ciabatta, smoked salmon, muesli, fruit and vegetables, canola oil Food was provided.	Western diet high-fat meal Croissants, bread rolls, jam, butter, cold cuts, boiled eggs, cream yogurt	Established biomarkers[a] Plasma α-tocopherol (+) ($P = 0.032$) Retinol (=) ($P = 0.837$) β-carotene (+) ($P = 0.002$) Vitamin C (+) ($P < 0.001$)	Linear mixed models α-tocopherol, retinol, β-carotene: Normal-phase HPLC Vitamin C: HPLC with ultraviolet detection
Djuric et al (2009)[35]	USA		Parallel	n = 69, 44 y, 100% F, 24 kg/m^2	Intervention: 24 wks Mediterranean diet n = 27 Dietary counseling was provided.	Habitual diet n = 33 Provided written materials (National Cancer Institute's Action Guide to Healthy Eating) to correct nutritional deficiencies	Established biomarkers[a] Plasma 18:1, *n*–9 (oleic acid) (+) ($P < 0.05$) 18:2, *n*–6 (linoleic acid) (–) ($P < 0.05$) 18:3, *n*–3 (α-linolenic acid) (=) NS 20:4, *n*–6 (arachidonic acid) (=) NS 20:5, *n*–3 (EPA) (=) NS 22:6, *n*–3 (DHA) (=) NS Lutein (=) NS	ANOVA GC-MS HPLC

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Zeaxanthin (+) ($P < 0.05$) β-cryptoxanthin (+) ($P < 0.05$) α-carotene (+) ($P < 0.05$) β-carotene (+) ($P < 0.05$) Lycopene (=) NS Total carotenoids (+) ($P < 0.05$) Cyclolycopene (+) NS γ-tocopherol (+) ($P < 0.05$) α-tocopherol (=) NS Plasma Phospholipid SFA (=) NS MUFA (+) NS PUFA (=) NS 18:1, *n*–9 (oleic acid) (+) NS 18:2, *n*–6 (linoleic acid) (=) NS 18:3, *n*–3 (α-linolenic acid) (=) NS 20:4, *n*–6 (arachidonic acid) (=) NS 20:5, *n*–3 (EPA) (=) NS 22:6, *n*–3 (DHA) (=) NS	
Fitó et al (2014)[23]	Spain	PREDIMED	Parallel	n = 930, 67 y, 55% F, 29.5 kg/m^2 48% of participants had type 2 diabetes.	Intervention: 1 y Mediterranean diet + VOO OR Mediterranean diet + nuts Dietary recommendation Key foods were provided.	Low-fat diet (reduce all fat and American Heart Association guidelines)	Established biomarkers[a] (n = 450) Urinary Tyrosol (concentration) (virgin olive oil) (increased in Mediterranean diet + VOO group vs baseline, and low-fat group, $P < 0.05$) Hydroxytyrosol (concentration) (VOO) (increased in Mediterranean diet + VOO group vs baseline, low-fat group, and Mediterranean diet + nuts group, $P < 0.05$) Plasma α-linolenic acid (%) (walnut) (increased in Mediterranean diet + nuts group vs baseline, $P < 0.05$) (no differences	ANOVA GC-MS GC

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							in other inter- or intragroup comparisons)	
Jennings et al (2020)[37]	Europe (France, Italy, Netherlands, Poland, UK)	New Dietary Strategies Addressing the Specific Needs of the Elderly Population for Healthy Aging in Europe (NU-AGE)	Parallel	n = 1142, 71 y, 55% F, 26.6 kg/m^2	Intervention: 1 y Mediterranean-style diet Key foods were provided.	Habitual Western diet Requested to continue with usual diet	Established biomarkers[a] Serum Selenium (=) ($P = 0.91$) (n = 1076) Ferritin (=) ($P = 0.41$) (n = 1118) Iron (=) ($P = 0.62$) (n = 1118) Soluble transferrin receptor (+) ($P = 0.06$) (n = 1127)	Linear mixed-effect models (time × treatment interaction) Selenium: Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Micro- mass), with a plasma gas (argon) nebulizer; Ferritin, iron: soluble transferrin receptor: COBAS system (Roche Diagnostics)
Marin et al (2011)[39]	Spain		Crossover	n = 20, 67.1 y, 50% F, 31.9 kg/m^2 No evidence of chronic illness (eg, hepatic, renal, thyroid, or cardiac dysfunction) Six patients had high BP, 2 had hyperlipidemia, 3 had diabetes.	Intervention: 4 wks each diet Mediterranean diet, enriched in MUFAs, with virgin olive oil, containing 15% of energy as protein, 47% as carbohydrate, and 38% as fat Food was provided.	Low-fat, high-carbohydrate diet enriched in *n*–3 PUFAs (CHO-α-linolenic acid diet), with 15% of energy as protein, 55% as carbohydrate, and 30% as fat SFA-rich diet, with 15% of energy as protein, 47% as carbohydrate, and 38% as fat	Established biomarkers[a] Plasma Mediterranean diet β-carotene, μmol/L·×·10^{-3} (+) (compared with the other 2 diets) (58.2 ± 38.6, $P = 0.003$) α-tocopherol (=) ($P = 0.360$)	One-factor ANOVA with a post hoc Bonferroni test ($P < 0.05$) Adjustment Gender Reversed-phase HPLC
Meslier et al (2020)[40]	Italy		Parallel	n = 82, 43 y, 52% F, 31.1 kg/m^2	Run-in: 2 wks on habitual diet Intervention: 8 wks Mediterranean diet (n = 43) 7-day food diaries were completed every 2 wks.	Habitual (Western) diet (n = 39)	Metabolomics[b] Targeted Plasma: TMAO (NS) Carnitine (meat) (–) ($P < 0.001$) Choline (NS) Creatinine (NS) Betaine (NS)	Unpaired Wilcoxon rank-sum test (targeted) PLS-DA (untargeted) Liquid Chromatography tandem MS (targeted)

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Urinary: TMAO (NS) Carnitine (meat) (–) ($P < 0.001$) Choline (NS) Creatinine (NS) Betaine (NS) Untargeted Fecal: Oxindole-3-acetic acid (vegetables/berries) (+) Leucine (animal-based foods) (–) Isoleucine (animal-based foods) (–) Bile acids (meat products) (–) Urinary: 3-(3,5-dihydroxyphenyl) propanoic acid-glucuronide (whole grains) (+) Benzoxazinoids (whole grains) (+) Pipecolic acid betaine (whole grains) (+) Tryptophan betaine (legumes) (+) Pyrogallol-sulfate (legumes) (+) 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) (fish) (+) Urolithins (nuts) (+) Carnitine (meat) (–) Aromatic amino acids (–) N-acetylcadaverine p-cresol sulfate (proteolysis) (–) Indoxyl-sulfate (proteolysis) (–) Phenylacetylglutamine (proteolysis) (–) Short-chain and medium-chain acylcarnitines (complex CHO and protein metabolism) (–)	UPLC-MS (untargeted)

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							TMAO (fish/meat-derived proteins) (=) Serum: TMAO (fish/meat-derived proteins) (=)	
Michielsen et al (2019)[41]	Netherlands	FoodBALL	Parallel	n = 47, 56 y, 57% F, 27.4 kg/m^2	Run-in: 2 wks on Western diet Intervention: 8 wks Mediterranean-type diet Higher in fatty fish, legumes, nuts, unrefined grain products, and red wine, and lower in dairy products and meat 90% of energy needs were provided; the remaining 10% was chosen from a list of low-fat and low-fiber products.	Western diet High in SFA MUFA diet Western-type diet in which part of the SFA was replaced by MUFA	Metabolomics[b] Targeted Serum: Most important for the separation between the 3 diets: Mediterranean-type diet–associated DHA: total FA (fish) ($P < 0.001$) *n*–3 FA: total FA (fish) ($P < 0.001$) Western Diet–associated Conjugated linoleic acid (CLA) (butter) ($P < 0.001$) CLA: FA (butter) ($P < 0.001$) MUFA Diet–associated MUFA: FA (olive oil) ($P < 0.002$)	Sparse PLS-DA ANOVA false discovery rate–corrected *P*-values ^{1}H-NMR
Park et al (2019)[43]	USA		Crossover	n = 18, 31 y, 35% F, 22.6 kg/m^2	Intervention: 4 wks Washout: 4 wks Mediterranean diet (South Beach) 3-day food records were completed at completion of each dietary phase.	High-fat diet (Atkins) Very-low-fat diet (Ornish)	Metabolomics[b] Targeted[c] Plasma (n = 14): Not significant when compared with baseline in each dietary phase or compared with Atkins diet: Choline Betaine Carnitine Butyrobetaine Crotonobetaine Iso-leucine Phenylalanine Tyrosine; Significantly higher levels in Atkins phase when compared with Ornish phase:	ANOVA LC-MS/MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
Vázquez-Fresno et al (2015)[32]	Spain	PREDIMED (nondiabetic)	Parallel	n = 98, 66.6 y, 71.3% F, 30.2 kg/m^2 Participants had at least 3 cardiovascular risk factors (current smoking, hypertension, hypercholesterolemia, BMI ≥25, family history of premature CVD).	Intervention: 1 y and 3 y Mediterranean diet + EVOO n = 41 OR Mediterranean diet + nuts n = 27 Dietary recommendation Key foods were provided.	Low-fat diet n = 30 (reduce all fat and American Heart Association guidelines)	TMAO ($P = 0.01$) Leucine ($P = 0.01$) Valine ($P = 0.005$) Metabolomics[b] Untargeted[c] Urinary: Significant at both 1 y and 3 y if not specified Mediterranean diet–associated 3-HB (+) Leucine (+) Isobutyric acid (+) 2-oxoisovaleric (+) (EVOO vs LFD at 3 y) 4-DTEA (4-deoxythreonic acid) (+) N-Ac (N-acetylglycoproteins) (+) (EVOO vs LFD) Glycine (+) (3 y) p-cresol (+) (EVOO vs LFD 1 y, Nuts vs LFD 3 y) Suberic acid (+) Oleic acid (+) Proline (+) Mediterranean diet + Nuts–associated PAGN (+) N-AGN (+) Creatine (3 y, Nuts vs EVOO, Nuts vs LFD) Mediterranean diet + EVOO–associated Creatinine (+) Citrate (+) (1 y and 3 y, EVOO vs Nuts, EVOO vs LFD; 3 y, EVOO vs LFD) Cis-aconitate (+) (y1, EVOO vs Nuts, Nuts vs LFD) Low-fat diet–associated Hippurate (+) TMAO (+) (3 y, LFD vs EVOO)	OSC-PLS-DA (orthogonal signal correction) and 2-way Hierarchical Clustering Analysis VIP score threshold: >1.5 ANCOVA ($P < 0.05$) (controlled by baseline values: age, gender, BMI, smoking status, hypertension, hypercholesterolemia) ^{1}H-NMR

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Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Anserine (+) (1 y), (–) (3 y, LFD vs EVOO) Histidine (+) (1 y) 3-methylhistidine (+) (1 y, LFD vs EVOO) 1-methylhistidine (+) (1 y, LFD vs Nuts) Carnosine (+) (1 y, LFD vs Nuts; 3 y, LFD vs EVOO) Proline betaine (+) (3 y, LFD vs EVOO, LFD vs Nuts) Xanthosine (+) (1 y, LFD vs Nuts; 3 y, LFD vs EVOO)	
Zhu et al (2020)[49]	USA		Crossover	n = 10, 22 y, 50% F, 24.4 kg/m^2	Intervention: 4 d Washout: 4 d Mediterranean diet: rich in vegetables, whole grains, olive oil, nuts, and fish Food was provided.	Fast-food diet: Burgers and fries Exact dollar amounts were provided for purchase at a specific restaurant.	Metabolomics[b] Untargeted Plasma: Increased after Mediterranean diet and decreased after Fast-food diet Indole-3-lactic acid ($P = 0.003$) Indole-3-propionic acid ($P = 0.021$) Indole-3-acetic acid ($P = 0.021$) Increased after Fast-food diet and decreased after Mediterranean diet Tryptophan ($P = 0.014$) Indole-6-carboxaldehyde ($P = 0.014$) 4-(1-piperazinyl)-1H-indole ($P = 0.030$) No change after either Mediterranean diet or Fast-food diet TMAO (=) Choline (=) Increased after Mediterranean diet but not after Fast-food diet Oleoylcarnitine ($P = 0.038$)	Differential abundance analyses using a linear mixed model. Multiple test adjustments were performed. LC-MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Acetylcarnitine ($P = 0.052$) Betaine ($P = 0.032$) Hippuric acids ($P = 0.04$)	
Dietary approaches to stop hypertension								
McClure et al (2019)[25]	USA	DASH Trial	Parallel	n = 397, 45 y, 48% F, 28.2 kg/m^2	Run-in: 3 wks on control diet Intervention: 8 wks DASH diet Food was provided.	Fruit and vegetables diet TAD	Established biomarkers[a] 24-h Urinary: Fruit and vegetables vs TAD Phosphorus (=) ($P = 0.74$) All other pairwise comparisons Phosphorus (+) ($P < 0.001$)	ANOVA followed by pairwise comparisons with Tukey's honest significant difference test only if ANOVA $P < 0.05$
Miller et al (2005)[27]	USA		Parallel	n = 103, 52 y, 56% F, 29.6 kg/m^2 14% current smokers 75% African American	Run-in: 2 wks on control diet Intervention: 3 months DASH diet: rich in fruits, vegetables, low-fat dairy products; included whole grains, poultry, fish, and nuts; and was reduced in red meat, sweets, sugar-containing beverages, saturated fat, total fat and cholesterol (n = 51) Food was provided. Alcoholic and caffeinated beverages were limited and monitored.	TAD, n = 52	Established biomarkers[a] Serum: Lutein (+) ($P < 0.001$) Cryptoxanthin (+) ($P < 0.001$) Zeaxanthin (+) ($P < 0.001$) β-carotene (+) ($P < 0.001$) Lycopene (–) ($P < 0.001$) γ-tocopherol (–) ($P < 0.001$) Retinol (=) NS α-tocopherol (=) NS α-carotene (–) ($P < 0.01$)	Regression analysis Adjustments Baseline measurement (age, gender, ethnicity, smoking status, BMI) HPLC
Miller et al (1998)[26]	USA	Ancillary study within the DASH trial	Parallel	n = 123, 48.5 y, 47% F, 27.5 kg/m^2	Run-in: 3 wks on control diet Intervention: 8 wks Combination (DASH) diet n = 41 Emphasized fruit and vegetables (10 servings per day),	TAD, n = 40 Fruit and vegetable diet, n = 42 Nine servings of fruit and vegetables per day, rich in potassium, magnesium, and fiber;	Established biomarkers[a] Serum (n = 34): Combination (DASH) diet (n = 12) AND Fruit and vegetable diet (n = 10) compared with TAD (n = 12) β-carotene (+) ($P < 0.05$) Cryptoxanthin (+) ($P < 0.05$)	*t*-test (ANCOVA) with Bonferroni adjustment HPLC

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
					low-fat dairy and other reduced-fat foods, rich in potassium, magnesium, fiber, calcium, and protein and reduced in saturated fat, total fat, and cholesterol	otherwise similar to control diet	Zeaxanthin (+) ($P < 0.05$) Combination (DASH) diet compared with TAD Lutein (+) ($P < 0.05$) Among diets Lycopene (=) NS Retinol (=) NS α-tocopherol (=) NS	
Nowson et al (2009)[42]	Australia		Parallel	n = 95, 59.2 y, 100% F, 29.6 kg/m^2 36.8% of participants were using antihypertensives.	Run-in: 3–4 wks Intervention: 14 wks Vitality diet n = 46 Low dietary acid load; based on low-sodium DASH diet rich in fruits and vegetables and including lean red meat; lower in sodium, higher in potassium and magnesium Key foods were provided (red meat, low-sodium bread, no-added-salt baked beans, salt-free margarine, and low-sodium stock powder).	Reference healthy diet, n = 49 Based on general dietary guidelines to reduce fat intake, particularly saturated fat, and increase intake of cereals and breads with a high acid load (Regular-salt margarine and baked beans)	Established biomarkers[a] 24-h Urinary: Sodium (–) ($P < 0.001$) Potassium (+) ($P = 0.08$) Calcium (–) ($P = 0.02$) Magnesium (=) ($P = 0.89$) Chloride (–) ($P = 0.001$) Phosphate (–) ($P = 0.2$) Urea (=) ($P = 0.92$) Sodium:potassium ratio (–) ($P < 0.001$)	Student's *t*-test Photometry: Randox Daytona automated clinical chemistry analyzer (Antrim, United Kingdom)
Rebholz et al (2018)[30]	USA	DASH Trial subset	Parallel	n = 329, Age category, % (n): 18–30 y, 11.9 (39); 31–55 y, 69.0 (227); ≥56 y, 19.2 (63) 47% F, 28 kg/m^2	Run-in: 3 wks on control diet Intervention: 8 wks DASH diet (n = 110) Food was provided.	Fruit and vegetable diet, n = 111 (similar to DASH, high fiber; potassium and magnesium close to 75th percentile of US consumption; more fruit and vegetables, fewer CHO-rich sweet desserts	Metabolomics[b] Untargeted Serum: The 10 metabolites most able to distinguish the DASH diet from the Fruit and vegetable diet: 2-methylserine (+) (amino acid: glycine, serine, and threonine metabolism)	PLS-DA GC-MS LC-MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
						and snacks. Otherwise, similar to control diet) TAD, n = 108 (macronutrient intake similar to average US consumption, potassium, magnesium, and calcium 25th percentile of US consumption)	S-allylcysteine (+) (Xenobiotics: Food component/plant) 4-allylphenol sulfate (+) (Xenobiotics: Food component/plant) Linoleoyl-linolenoyl-glycerol (18:2/18:3) [1] (+) (lipid: diacylglycerol) # Linoleoyl-linolenoyl-glycerol (18:2/18:3) [2] (+) (lipid: diacylglycerol) # Linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) (+) (lipid: diacylglycerol) # Heptenedioate (C7:1-DC) (–) (lipid: fatty acid, dicarboxylate) # Suberoylcarnitine (C8-DC) (–) (lipid: fatty acid metabolism [acyl carnitine]) Adipoylcarnitine (C6-DC) (–) (lipid: fatty acid metabolism [acyl carnitine]) # 3-methylglutarylcarnitine (–) (amino acid: leucine, isoleucine, and valine metabolism) The 10 metabolites most able to distinguish the DASH diet from the TAD N-methylproline (+) (amino acid: urea cycle; arginine and proline metabolism) Stachydrine (+) (xenobiotics: Food component/plant) Tryptophan betaine (+) (amino acid: tryptophan metabolism) Chiro- inositol (+) (lipid: inositol metabolism)	

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Methyl glucopyranoside ($\alpha + \beta$) (+) (Xenobiotics: Food component/plant) β-cryptoxanthin (+) (cofactors and vitamins: Vit A metabolism) Theobromine (–) (xenobiotics: xanthine metabolism) 7-methylurate (–) (xenobiotics: xanthine metabolism) 3-methylxanthine (–) (xenobiotics: xanthine metabolism) 7-methylxanthine (–) (xenobiotics: xanthine metabolism) # significant for both DASH vs Fruit and vegetable and DASH vs TAD	
Healthy Nordic diet								
Acar et al (2019)[20]	Denmark	SHOPUS	Parallel	n = 146, age range 18–65 y, 68% F, BMI not reported Centrally obese	Run-in: 1 wk Intervention: 26 wks New Nordic diet (organic diet high in fruit and vegetables, whole grains and fish; based on Nordic Nutrition Recommendations 2004 but higher in protein) Food was provided from study shop, collected ad lib.	Average Danish diet (Higher in imported and processed foods, refined grains, meat, dairy, sugary products, convenience foods, low fiber vegetables, imported fruit)	Metabolomics[b] Untargeted Plasma: New Nordic diet Prolyl hydroxyproline (fish collagen) Phosphatidylcholine (40:9) (lipid and fatty acid metabolism) Phosphatidylcholine (18:0/22:6) (Fish C [22:6]) Pipecolic acid betaine (whole grain) TMAO (fish) Hydroxydecanoic acid (unknown) Lysophosphatidylcholine (16:0) (fish) Phosphatidylcholine (P-38:4) (unknown) Average Danish diet Phosphatidylcholine (18:0/20:3) (lipid and fatty acid metabolism)	PLS-DA Ultra-performance liquid chromatography, coupled to quadruple time-of-flight mass spectrometer (UPLC-qTOF-MS)

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Theobromine (chocolate) Butyryl carnitine (lipid metabolism) Cyclo-(pro-val) (food heating) Proline betaine (citrus fruits) Phosphatidylcholine (36:3) (lipid and fatty acid metabolism) Phosphatidylcholine (O-18:0/20:4) (fish C [20:4]) Phosphatidylcholine (36:3) (unknown) Threonine or allothreonine (animal protein) Phosphatidylcholine (P-18:0/20:3) (lipid and fatty acid metabolism) 2- or 3-hydroxy-3-methylbutyrate (amino acid metabolism) Phosphatidylcholine (18:0/20:3) (lipid and fatty acid metabolism) 3-indolelactic acid (tryptophan metabolite)	
Anderson et al (2014)[21]	Denmark	SHOPUS	Parallel	n = 107, 42.9 y, 74% F, 29.7 kg/m^2 Centrally obese	Run-in: 1 wk Intervention: 26 wks New Nordic diet	Average Danish diet	Metabolomics[b] Untargeted Urinary: New Nordic diet Hydroquinone glucuronide (no info) TMAO (fish) Hippuric acid (no info) (2-oxo-2,3-dihydro-1H-indol-3-yl) acetic acid (no info) 3,4,5,6-tetrahydrohippurate (no info) Average Danish diet Pyrraline (heat treatment) Theobromine (chocolate) 7-methyluric acid (chocolate) 6-amino-5-[N-methylformylamino]-1-methyluracil (chocolate)	PLS-DA (with feature selection) Ultra-performance liquid chromatography, coupled to quadruple time-of-flight mass spectrometer (UPLC-qTOF-MS)

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							3,7-dimethyluric acid (chocolate) 7-methylcanthine (chocolate) Proline betaine (citrus cluster) Pyroglutamyl proline (no info) P-menth-1-ene-6,8,9-triol (limonene) Perillic acid-8,9-diol-glucuronide (limonene) Limonene-8,9-diol-glucuronide (limonene) Dihydroperillic acid glucuronide (limonene) Limonene-1,2-diol glucuronide (limonene) Octanoyl-glucuronide (no info) 3-indoleacetyl-glucuronide (no info)	
Khakimov et al (2016)[24]	Denmark	SHOPUS	Parallel	n = 145, age range 18–65 y, 69% F, BMI not reported Centrally obese	Run-in: 1 wk Intervention: 26 wks New Nordic diet	Average Danish diet	Metabolomics[b] Untargeted Plasma: New Nordic diet 3-hydroxybutanoic acid Erythritol (fruit and vegetables) 2-hydroxybenzoic acid (fruit and vegetables) Aspartic acid 2,3,4-trihydroxybutanoic acid Xylitol (fruit and vegetables) N-acetylaspartic acid 2,5-dimethoxyphenylpropionic acid Palmitoleic acid; Average Danish diet Lactic acid Oxalic acid Alanine Threonine (grains, mushrooms) Diethyl phthalate 2,6-diisopropylnaphthalene Citric acid Cholesterol	PLS-DA GC-MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
Poulsen et al (2014)[29]	Denmark	SHOPUS	Parallel	n = 181, 42 y, 71% F, 30.2 kg/m^2 Centrally obese	Run-in: 1 wk Intervention: 26 wks New Nordic diet	Average Danish diet	Established biomarkers[a] 24-h urinary (n = 143) Nitrogen (+) ($P = 0.12$) Sodium (=) ($P = 0.33$) Whole blood (n = 145) % SFAs (–) ($P = 0.14$) % MUFAs (=) ($P = 0.56$) % PUFAs (+) ($P = 0.79$) % *n*–6 FA (=) ($P = 0.17$) % *n*–3 FA (+) ($P < 0.001$) % EPA + DHA (+) ($P < 0.001$) % *n*–6/*n*–3 ratio (–) ($P < 0.001$) % *n*–3 HUFA in total HUFA (+) ($P < 0.001$)	Student's *t*-test
Trimigno et al (2020)[31]	Denmark	OPUS	Parallel	n = 181, 42 y, 48% F, BMI not reported Centrally obese	New Nordic diet	Average Danish diet	Metabolomics[b] Untargeted Urinary (n = 142): Acetate (+) (q = 0.203) Acetone (unbalanced diet) (–) (q = 0.808) Alanine (low-protein, high-carbohydrate diet; average Danish diet) (–) (q = 0.279) Glycine betaine (fish; cereals and fibers) (+) (q = 0.001) Carnitine (red meat; protein) (–) (q = 0.094) Creatine (meat; game meat; fish) (+) (q = 0.719) Dimethyl sulfone (onions; grains and fibers; cabbage and asparagus; meat, egg, dairy and fish) (–) ($q = 2.34 \times 10^{-8}$) Dimethylamine (fish; red meat, eggs and dairy) (+) (q = 0.167) Fumarate (+) (q = 0.268) Glucose (fruits, honey and sugar) (+) ($q = 1.34 \times 10^{-4}$) Glucose, lactose, maltose (+) ($q = 6.85 \times 10^{-4}$)	PLS-DA with false discovery rate-corrected ANOVA q values ^{1}H NMR

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Glycine (protein-rich foods; NND) (+) (q = 0.336) Guanidinoacetate (amino acids) (+) (q = 0.427) Hippurate (vegetarian diet; plant-based foods; green and black tea; dairy products) (+) (q = 0.013) Isoleucine, leucine (cheese) (–) (q = 0.314) Lysine (+) (q = 0.418) Methanol (alcohol; pectins in fruit and fruit juices) (–) (q = 0.380) Phenylalanine (protein-rich foods; fish, pulses and nuts) (+) (q = 0.026) Propylene glycol (lacto-ovo vegetarian breakfast; diet of people at risk for poverty) (–) (q = 0.004) Succinate (low-protein diet) (–) (q = 0.554) Tartrate (red wine; grapes) (–) ($q = 5.73 \times 10^{-5}$) Taurine (omnivorous diet; high-protein diet) (+) (q = 0.013) TMA (fish; red meat, eggs and dairy; pulses) (+) (q = 0.0841) TMAO (fish) (+) ($q = 8.75 \times 10^{-5}$) Tyrosine (cheese) (–) (q = 0.474)	
Tuomainen et al (2019)[47]	Nordic countries (Finland, Sweden, Denmark, Iceland)	SYSDIET	Parallel	n = 164, 55 y, 66% F, 31.7 kg/m^2	Intervention: 18 or 24 wks (depending on the study center) Healthy Nordic diet Increased whole grains, canola oil,	Control group (typical Nordic diet) Low-fiber cereal, milk fat, restricted amounts of fish and berries	Metabolomics[b] Targeted[c] Plasma: 4-aminovaleric acid betaine (*P*-value NA) (n = 5) 5-aminovaleric acid betaine (=) (*P* = 0.920) (n = 163)	ANOVA (significant at $P < 0.0036$) LC with triple quadrupole MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
					berries, and fish. Nordic nutrition recommendations used. Key foods were provided.		Acetyl L-carnitine (=) ($P = 0.651$) (n = 163) Alanine betaine (=) ($P = 0.193$) (n = 140) Choline (=) ($P = 0.420$) (n = 163) Glycine betaine (=) ($P = 0.198$) (n = 163) γ -Butyrobetaine (=) ($P = 0.097$) (n = 163) Hydroxyproline betaine (=) ($P = 0.906$) (n = 71) L-carnitine (=) ($P = 0.890$) (n = 163) Pipecolic acid betaine (coffee; citrus fruits) (+) ($P = 0.00032$) (n = 130) Phenylalanine betaine (=) ($P = 0.419$) (n = 97) Proline betaine (=) ($P = 0.234$) (n = 162) TMAO (=) ($P = 0.027$) (n = 162) Trigonelline (=) ($P = 0.505$) (n = 157) Tryptophan betaine (=) ($P = 0.261$) (n = 154)	
Diet based on dietary guidelines								
Garcia-Perez et al (2017)[36]	UK		Crossover	n = 19, 55.8 y, 47% F, 25.6 kg/m^2	Intervention: 72 h inpatient period on 4 occasions Washout: $\geq$5 d WHO healthy eating guidelines (increased fruits, vegetables, whole grains, and dietary fiber; decreased fats, sugars, and salt) Four diets with a stepwise variance in concordance	Diet 4: least concordant with WHO healthy eating guidelines.	Metabolomics[b] Untargeted Urinary: Diet 1 (higher concentration compared with diet 4) 3-aminoisobutyrate (unknown) ($P = 6.22 \times 10^{-25}$) Rhamnitol (Fruits) ($P = 6.81 \times 10^{-11}$) Lysine (unknown) ($P = 1.92 \times 10^{-3}$) Acetate (unknown) ($P = 2.97 \times 10^{-3}$) N-acetyl-S-(1Z)-propenyl-cysteine-sulfoxide (vegetables) ($P = 6.85 \times 10^{-26}$)	PLS-DA 1H-NMR spectroscopy

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
					with WHO healthy eating guidelines: Diet 1: most concordant, Diet 4: least concordant The diets had a range of energy densities. DASH scores were calculated based on 4-day dietary records. Food was provided.		Dimethylamine (fish) ($P = 5.90 \times 10^{-4}$) N-acetyl-S-methyl-cysteine-sulfoxide (cruciferous vegetables) ($P = 5.75 \times 10^{-21}$) S-methyl-cysteine-sulfoxide (cruciferous vegetables) ($P = 2.03 \times 10^{-23}$) Creatine ([Red] meats) ($P = 2.38 \times 10^{-5}$) 1-methylhistidine (lean [white] meats) ($P = 1.26 \times 10^{-27}$) 3-methylhistidine (lean [white] meats) ($P = 7.31 \times 10^{-25}$) TMAO (fish, meats) ($P = 6.64 \times 10^{-16}$) N-methyl-2-pyridine-5- carboxamide (Niacin [Vit B3]) ($P = 2.80 \times 10^{-10}$) 4-hydroxyhippurate (Fruits) ($P = 3.58 \times 10^{-6}$) Hippurate (fruits, vegetables) ($P = 2.81 \times 10^{-14}$) Tartrate (grapes) ($P = 1.62 \times 10^{-19}$) N-methylnicotinate (Niacin [Vit B3]) ($P = 1.09 \times 10^{-12}$) N-methylnicotinamide (Niacin [Vit B3]) ($P = 4.23 \times 10^{-16}$) Urea (protein) ($P = 8.87 \times 10^{-28}$); Diet 4 (higher concentration compared with diet 1) Fatty acids (C5–C10) (fats) ($P = 7{\cdot}34 \times 10^{-5}$) Alanine (unknown) ($P = 6{\cdot}95 \times 10^{-19}$) N–acetyl neuraminate (unknown) ($P = 7{\cdot}87 \times 10^{-5}$) Phenylacetylglutamine (unknown) ($P = 4.34 \times 10^{-26}$) O-acetylcarnitine ([Red] meats) ($P = 7.50 \times 10^{-16}$)	

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Carnitine ([Red] meats) ($P = 2.48 \times 10^{-14}$) Glucose (sugars) ($P = 6.38 \times 10^{-22}$) Glycine (unknown) ($P = 3.87 \times 10^{-10}$) Glycolate (unknown) ($P = 6.04 \times 10^{-13}$) Applied model developed to confirm the association between urinary metabolic and dietary profiles in two cohorts: INTERMAP UK (n = 225) ($P < 0.0001$) (24-h urinary) Danish (n = 66) ($P < 0.001$) (spot urinary) Single metabolites quantified in INTERMAP: (+): higher concentration with high DASH score compared with low DASH score, $P \leq 0{\cdot}05$) (Kruskal-Wallis test and Wilcoxon rank sum post hoc tests) Hippurate (fruits and vegetables) (+) (*no difference between high and intermediate DASH score, $P = 0.051$; *no difference between intermediate and low DASH score, $P = 0.096$) 4-hydroxyhippurate (fruits) (+) (*no difference between intermediate and low DASH score, $P = 0.15$) S-methyl-L-cysteine-sulfoxide (cruciferous vegetables) (+) (*no difference between high and intermediate DASH score, $P = 0.19$)	

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Proline betaine (citrus fruits) (+) ($P < 0.05$ in all pair-wise comparisons)	
Reidlinger et al (2015)[45]	UK	CRESSIDA	Parallel	n = 162, 52 y, 60% F, 26.2 kg/m^2 Nonsmoking healthy individuals	Intervention: 12 wks UK dietary guidelines Reduced intake of sodium, total fat, saturated fatty acids, and nonmilk extrinsic sugars Increased consumption of oily fish, and whole grains Key foods were provided (low-SFA and -TFA margarine, liquid vegetable oil).	Traditional British diet Refined cereals, meats, full-fat dairy, no restriction on salt/sugar intake, but limited confectionery and snack foods (butter-based spread and unhydrogenated vegetable oil)	Established biomarkers[a] 24-h urinary: Sodium (salt) (–) ($P < 0.01$) Potassium (fruit and vegetables) (+) ($P < 0.01$) Sucrose and fructose (added sugars) (–) ($P < 0.01$) Erythrocyte lipids: *n*–3 index (oily fish) (+) ($P < 0.01$) Plasma: Alkylresorcinol (whole grains) (+) ($P < 0.01$) Serum: 25-hydroxyvitamin D (higher intake of oily fish) (+) ($P < 0.001$) Folate (avoidance of folic acid-fortified breakfast cereals) (–) ($P = 0.001$) Ferritin (iron status index) (=) ($P = 0.43$) Homocysteine (folate status index) (=) ($P = 0.206$)	ANCOVA ($P < 0.01$) Regressed against baseline value, age group, sex, ethnicity, and BMI category
Low-glycemic-load diet								
Barton et al (2015)[22]	USA	Carbohydrates And Related Biomarkers (CARB) study	Crossover	n = 19, 31.6 y, 53% F, BMI not reported 58% overweight/obese	Intervention: 4 wks Washout: 4 wks habitual diet Low-GL diet: 125 GL/d, 55 g fiber/d Food was provided.	High-GL diet: 250 GL/d, 28 g fiber/d Identical distribution of macronutrients compared with low-GI diet	Metabolomics[b] Targeted Plasma: Kynurenate (tryptophan metabolism) (+) ($P = 0.0002$) Methyl succinate (dicarboxylic acids and derivatives) (+) (0.004) Cystamine (taurine and hypotaurine metabolism) (–) ($P = 0.004$) Proline (arginine and proline metabolism) (+) ($P = 0.010$)	PLS-DA (VIP Score > 2) *P*-value calculated using paired *t*-test Adjustments Weight change Body fat % Fat distribution LC-MS/MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Acetylcholine (glycerophospholipid metabolism) (–) ($P = 0.014$) Hydroxyproline (arginine and proline metabolism) (–) ($P = 0.016$) Creatine (glycine, serine, threonine metabolism; arginine and proline metabolism) (–) ($P = 0.016$) TMAO (gut bacterial metabolite) (+) ($P = 0.025$) Carnitine (fatty acid metabolism) (+) ($P = 0.030$) Homovanillate (tyrosine metabolism) (+) ($P = 0.036$) Lysine (biotin metabolism; carnitine synthesis) (+) ($P = 0.039$) Nitrotyrosine (product of reactive nitrogen species) (+) ($P = 0.042$) Niacinamide (vitamin B3) (–) ($P = 0.046$) Dimethylguanosine (nucleoside) (+) ($P = 0.047$)	
Navarro et al (2019)[28]	USA	Carbohydrates And Related Biomarkers (CARB) study	Crossover	n = 80, 30 y, 50% F, BMI not reported	Intervention: 4 wks Washout: 4 wks Low-GL diet Whole grains, legumes, fruits, vegetables, nuts, and seeds Food was provided.	High-GL diet High in refined grains and added sugars	Metabolomics[b] Targeted Plasma: Inositol (+) ($P = 1.46 \times 10^{-23}$)* Melatonin (–) ($P = 3.58 \times 10^{-22}$)* Hydroxyphenylpyruvate (+) ($P = 3.02 \times 10^{-12}$)* Betaine (–) ($P = 1.75 \times 10^{-10}$)* Creatine (–) ($P = 1.19 \times 10^{-7}$)* Acetylcholine (–) ($P = 8.54 \times 10^{-7}$)* Citrulline (+) ($P = 1.34 \times 10^{-4}$)* Ornithine (+) ($P = 3.83 \times 10^{-4}$)*	Linear mixed model LC-MS/MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							13-Hydroxyoctadecadienoic acid (+) ($P = 4.32 \times 10^{-4}$)* Aspartic acid (–) ($P = 7.25 \times 10^{-4}$)* Hydroxyproline (–) ($P = 7.77 \times 10^{-4}$)* Methylhistidine (–) ($P = 8.18 \times 10^{-4}$)* Tryptophan (–) ($P = 9.19 \times 10^{-4}$)* Cystamine (–) ($P = 0.002$)* Glutamine (+) ($P = 0.003$)* Carnitine (–) ($P = 0.004$)* Trimethylamine (–) ($P = 0.007$)* Oxaloacetate (+) ($P = 0.007$)* Xanthurenic acid (–) ($P = 0.03$) Shikimic acid (+) ($P = 0.06$) *Significant with Benjamini-Hochberg False Discovery Rate < 0.05	
Vegetarian diet Lederer et al (2019)[38]	Germany		Parallel	n = 53, 32 y, 62% F, 23.1 kg/m^2	Run-in: 1 wk on a balanced mixed diet Intervention: 4 wks Vegan diet Strict Extensive training on assigned diet; detailed information material; recipe book; free meals offered	Meat-rich diet >150 g of meat daily	Established biomarkers[a] Serum: Vitamin B_{12} (–) ($P < 0.001$) Holotranscobalamin (–) ($P < 0.001$) Methylmalonic acid (+) ($P = 0.090$) Homocysteine (+) ($P = 0.140$) Arachidonic acid (–) ($P = 0.046$) DHA (–) ($P = 0.298$) Eicosanoic acid (=) ($P = 0.211$) Eicosenoic acid (+) ($P = 0.906$) Linoleic acid (+) ($P = 0.532$) Linolenic acid (+) ($P = 0.705$) Oleic acid (–) ($P = 0.602$) 25-hydroxyvitamin D_2/D_3 (=) ($P = 0.903$) Urinary: Creatinine (=) ($P = 0.158$) Plasma:	ANCOVA adjusted for baseline values Serum biomarkers LC-Electrospray Ionization (ESI)-MS/MS FA profiles GC-MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Nitrite and nitrate (+) ($P = 0.003$)	
Raådjursöga et al (2018)[44]	Sweden		Crossover	n = 32, 29 y, 50% F, 22.1 kg/m^2	Intervention: 3 h Breakfast meal crossed over during 3 consecutive days Vegan diet Lacto-ovo vegetarian diet Food was provided.	Omnivore diet	Metabolomics[b] Untargeted[c] Serum: Lacto-ovo vegetarian vs Vegan Increased in concentration after lacto-ovo vegetarian diet: 3-hydroxyisobutyrate ($P < 0.0001$) Acetoacetate ($P < 0.0001$) Carnitine & acetoacetate ($P < 0.0001$) Creatinine & creatine & creatine phosphate ($P < 0.0001$) Isoleucine ($P < 0.0001$) Leucine ($P < 0.0001$) Leucine & arginine ($P < 0.0001$) Lysine ($P < 0.0001$) Methionine ($P < 0.0001$) N-acetylcysteine & proline & glutamate ($P < 0.0001$) Proline ($P < 0.0001$) Proline & glutamate & unknown ($P < 0.0001$) Propylene glycol ($P = 0.004$) Threonine ($P < 0.0001$) Tyrosine ($P < 0.0001$) Valine ($P < 0.0001$) Increased in concentration after vegan diet Lipids/FFA ($P < 0.0001$) Lacto-ovo vegetarian vs Omnivore diets Increased in concentration after lacto-ovo vegetarian diet: 3-hydroxyisobutyrate ($P = 0.004$) Alanine ($P = 0.03$) Carnitine + acetoacetate ($P = 0.007$)	Orthogonal projections to Latent Structures with Effect Projection (OPLS-EP) *P*-value calculated using Wilcoxon signed ranked test ^{1}H-NMR

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Lactate ($P = 0.06$) N-acetylcysteine & proline & glutamate ($P = 0.005$) Proline ($P = 0.003$) Proline & glutamate & unknown ($P = 0.003$) Propylene glycol ($P = 0.02$) Pyruvate ($P = 0.2$) Succinic acid ($P = 0.2$) Tyrosine ($P = 0.005$) Increased in concentration after omnivore diet: Ascorbate ($P = 0.02$) Betaine ($P = 0.02$) Choline ($P = 0.003$) Creatinine & Creatine & Creatine phosphate ($P = 0.003$) Isoleucine ($P = 0.08$) Lipids/FFA ($P = 0.003$) Lysine ($P = 0.3$) Serine & tyrosine ($P = 0.001$)	
Prudent diet Wellington et al (2019)[48]	Canada	Subset of Diet and Gene Intervention (DIGEST) pilot study	Parallel	n = 42, 47 y, 64% F, 27 kg/m^2	Intervention: 2 wks Prudent diet Based around minimally processed foods including lean protein and whole grains, and high in fresh fruits and vegetables Food was provided: allotment picked up at store, or delivered to home;	Western diet Reflecting a typical Canadian macronutrient profile with higher intake of processed foods	Metabolomics[b] Untargeted Plasma: Proline betaine (+) ($P = 0.007$) 3-methylhistidine (+) ($P = 0.001$) Proline (–) ($P = 0.02$) Carnitine (–) ($P = 0.005$) Deoxycarnitine or γ-butyrobetaine (–) ($P = 0.008$) Linoelaidic acid (–) ($P < 0.001$) Pentadecanoic acid (–) ($P < 0.001$) Alanine (–) ($P = 0.018$) Ketoleucine or 4-methyl-2-oxopentanoic (+) ($P = 0.043$) 3-hydroxybutyric (+) ($P = 0.097$) α-linoleic acid (–) ($P = 0.002$)	Paired orthogonal PLS-DA Mixed ANOVA High-resolution MS/MS

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Ketovaline or α-isovaleric acid (+) ($P = 0.125$) Myristic acid (+) ($P < 0.001$) Linoleic acid (–) ($P < 0.001$) Urinary: 3-methylhistidine (+) ($P = 0.008$) 5-hydroxypipecolic acid (+) ($P = 0.293$) Imidazole propionic acid (+) ($P = 0.002$) Proline betaine (+) ($P = 0.002$) Valinyl-valine (+) ($P = 0.06$) Enterolactone glucuronide (+) ($P = 0.01$) Dihydroxybenzoic acid (+) ($P = 0.01$) Dimethylglycine (+) ($P = 0.065$) Acesulfame K (–) ($P < 0.05$)	
Korean diet Shin et al (2019)[46]	Korea		Crossover	n = 54, 41 y, 48% F, 27.5 kg/m^2	Intervention: 4 wks Washout: 2 wks Typical Korean Diet Based on the Korean Food Guide of the Dietary Reference Intakes for Koreans Comprised of 5 food groups: grains (mix of whole and refined); meat, fish, eggs, and beans; vegetables; fruits; milk and dairy products Traditional Korean preparation techniques used Food was provided.	Recommended American diet (RAD) Sample menus from the 2010 Dietary Guidelines for Americans developed by the US Department of Agriculture TAD What We Eat in America dietary survey from NHANES 2001–2004	Metabolomics[b] Targeted (+) increased compared with baseline (–) decreased compared with baseline Serum: Significantly altered after ($P < 0.01$): Typical Korean diet: Acetate (+) Isoleucine (–) Leucine (–) Lactate (–) Proline (–) Valine (–) RAD: 2-aminobutyrate (+) 3-hydroxybutyrate (+) Acetate (+) Ascorbate (+) Mannose (+)	Univariate statistical analysis (Wilcoxon Signed-Rank test) ^{1}H-NMR

(continued)

Table 2 **Continued**

Reference	Country	Trial name (if available)	Study design	Participants (sample size, mean age, % female (F), mean BMI)	Intervention	Control	Biomarkers	Statistical method, Technique
							Myo-inositol (+) TAD: Ethanol (–) Glutamine (+) Glycine (–) Proline (–) Pyruvate (–) Tyrosine (+) Urinary: Significantly altered after: All diets: Glycolate (–) $P = 0.001$ in Typical Korean diet and RAD, $P = 0.003$ in TAD Taurine (+) in Typical Korean diet and RAD, $P < 0.001$; (–) in TAD, $P < 0.001$ RAD: 3-hydroxy-3-methylglutarate (3-HMGA) (–) ($P = 0.004$) Citrate (–) ($P < 0.001$) Dimethylamine (–) ($P = 0.007$) Hippurate (–) ($P < 0.001$) Homovanillate (–) ($P < 0.001$) TAD: 1-methylnicotinamide (+) ($P = 0.001$) Carnitine (+) ($P = 0.001$) Pyruvate (–) ($P = 0.003$)	

[a]Values are means ± SDs; +, higher in intervention group; –, lower in intervention group; =, no difference between groups, unless otherwise indicated. Use of established dietary biomarkers of specific foods or nutrients.

[b] Use of metabolomics to study biomarkers and biomarker profiles; targeted metabolomics focused on defined subsets of metabolites within specific metabolic pathways, and untargeted metabolomics quantified all metabolites globally (including known and unknown) in a biospecimen.

[c] Metabolomics type (targeted or untargeted) was ascertained from the reported methodology as this information was not specified in the article.

Abbreviations: ^{1}H-NMR, hydrogen-1 proton nuclear magnetic resonance; 3-HB, 3-hydroxybutyrate; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BMI, body mass index; BP, blood pressure; CHO, carbohydrate; CVD, cardiovascular disease; DASH, dietary approaches to stop hypertension; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EVOO, extra virgin olive oil; FAs, fatty acids; FFA, free fatty acid; GC-MS, Gas Chromatography–Mass Spectrometry; GL, glycemic load; HPLC, high-performance liquid chromatography; HUFA, highly unsaturated fatty acid; LC-MS, liquid chromatography–mass spectrometry; LFD, low-fat diet; MUFAs, monounsaturated fatty acids; *n*–3, omega 3; *n*–6, omega 6; N-AGN, n-acetylglutamine; NHANES, National Health and Nutrition Examination Survey; NS, not significant; PAGN, phenylacetylglutamine; PLS-DA, partial least-squares discriminant analysis; PUFAs, polyunsaturated fatty acids; RAD, recommended American diet; SFAs, saturated fatty acids; TAD, typical American diet; TFAs, trans fatty acids; TMAO, trimethylamine N-oxide; VOO, virgin olive oil.

and World Health Organization [WHO] Eating Guidelines),[36,45] Korean diet [n = 1],[46] and a prudent diet (n = 1).[48] The Mediterranean diet was compared with a Western habitual diet (n = 8),[33–35,37,39–41,49] a low-fat diet (n = 4),[23,32,39,43] a low-carbohydrate, high-fat, high-protein (Atkins) diet (n = 1),[43] and a monounsaturated fatty acid (MUFA)-rich diet but otherwise similar to a Western habitual diet (n = 1).[41] The healthy Nordic diet was compared with a typical Nordic[47] or Danish diet[20,21,24,29,31] (n = 6), while the DASH diet was compared with a typical American diet (n = 4),[25–27,30] a diet high in fruit and vegetables (n = 3),[25,26,30] and a healthy diet based on the Australian dietary guidelines (n = 1).[42]

Two studies detailed dietary biomarkers within the acute postprandial stage (3 and 4.5 hours)[34,44]; the intervention period of the other studies ranged from 3 days to 5 years. Most of the RCTs used a parallel design (n = 14)[20,21,23–27,29–33,35,37,38,40–42,45,47,48] and the rest used a crossover design (n = 8).[22,28,34,36,39,43,44,46,49] The majority of the trials provided intervention foods (n = 19)[20,22,23,26,30,33,34,36–39,41,42,44–49]; most provided all foods (n = 13),[20,22,26,30,34,36,38,39,41,44,46,48,49] but some trials provided only key food items (n = 6).[23,33,37,42,45,47] Meals were monitored[20,22,26,30,34,36,44,46,49] or assessed by food records or questionnaires[23,33,37–39,41,42,45,47,48] in trials that provided foods. Food was not provided in 3 trials, but food records were collected to confirm dietary compliance.[35,40,43]

Risk of bias within studies

Thirteen publications included in the review were rated as having low risk of bias, 16 publications were rated as having some concerns, and 1 publication was rated as having high risk of bias, mostly due to issues with the randomization process. The full results are shown in Table 3.[20–49]

Mediterranean diet

Eleven publications generated from 10 studies reported dietary biomarkers of the Mediterranean diet. Three were conducted in the United States, 2 in Spain, and 1 each in Australia, Germany, Italy, the Netherlands, and Europe.

A study from Australia measured serum carotenoids (β-cryptoxanthin, lycopene, α-carotene, β-carotene, and lutein:zeaxanthin ratio), erythrocyte fatty acids (saturated fatty acids, trans fatty acids [TFAs], MUFAs, omega-3 [*n*–3], omega-6 [*n*–6], docosahexenoic acid [DHA], *n*–6:*n*–3, and *n*–3 index), and 24-h urinary electrolytes (sodium, potassium, calcium and magnesium) in 152 participants to assess their compliance with a Mediterranean diet intervention in comparison with that of a habitual diet group.[33] The serum α-carotene, and erythrocyte MUFA %, DHA %, and *n*–3 index were higher, and the percentage of saturated fatty acids and TFA % were lower at 6 months compared with baseline in the Mediterranean diet group. No within- or between-group differences were identified in the other biomarkers tested.

A study conducted in the United States analyzed plasma lipids and carotenoids in 69 females following a Mediterranean diet or their habitual diet for 6 months.[35] Oleic acid, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, total carotenoids, and γ-tocopherol were higher in the Mediterranean diet group compared with the control group, whereas linoleic acid was lower.

A multinational trial from Europe assessed serum selenium, ferritin, iron, and soluble transferrin receptor in 1142 participants who followed a Mediterranean-style diet or a habitual Western diet for 1 year.[37] No differences were observed between the groups in these nutritional biomarkers.

Plasma α-tocopherol, retinol, β-carotene, and vitamin C were assessed 4.5 hours after the consumption of a meal during two study visits in a study in Germany, with the 26 study participants consuming a Mediterranean-type diet meal and a Western-diet high-fat meal in a random order.[34] Vitamin C, α-tocopherol, and β-carotene were higher, but retinol did not differ following the Mediterranean-type diet meal compared with the Western-diet high-fat meal.

Plasma β-carotene and α-tocopherol levels were also determined in a 3-arm crossover trial conducted in Spain in which 20 participants followed the Mediterranean diet, a high-saturated fat diet, and a low-fat/high-carbohydrate diet.[39] β-carotene was higher following a 4-week Mediterranean diet compared with either a high-saturated fat diet or a low-fat/high-carbohydrate diet. No variation was found in the plasma α-tocopherol level between groups.

The other study from Spain, the PREDIMED study, is one of the largest dietary RCTs with a parallel design and it compared the Mediterranean diet with virgin olive oil (VOO) or mixed nuts with a low-fat diet. That study measured urinary tyrosol and hydroxytyrosol, which are the main phenolic components in olive oil, and plasma α-linolenic acid in a random sample of 450 participants.[23] Tyrosol increased in the Mediterranean diet + VOO group when compared with the low-fat group. Hydroxytyrosol increased in the Mediterranean diet + VOO group when compared with the low-fat, and compared with the Mediterranean diet + nuts group. For α-linolenic acid, the only intragroup difference identified was an increase in participants on the Mediterranean diet + nuts group at 1 year.

Table 3 **Risk of bias for individual studies**

Reference	Randomization process	Deviations from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall
Acar et al (2019)[20]	Low	Low	Low	Low	Low	Low
Anderson et al (2014)[21]	Low	Low	Low	Low	Low	Low
Barton et al (2015)[22]	Low	Low	Low	Low	Low	Low
Davis et al (2017)[33]	Low	Low	Low	Low	Low	Low
Diekmann et al (2019)[34]	Some concerns	Low	Low	Low	Low	Some concerns
Djuric et al (2009)[35]	Some concerns	Low	Low	Low	Low	Some concerns
Fitó et al (2014)[23]	High	Low	High	Low	Some concerns	High
Garcia-Perez et al (2017)[36]	Low	Low	Low	Low	Low	Low
Jennings et al (2020)[37]	Some concerns	Low	Low	Low	Low	Some concerns
Khakimov et al (2016)[24]	Low	Low	Low	Low	Low	Low
Lederer et al (2019)[38]	Low	Low	Low	Low	Low	Low
Marin et al (2011)[39]	Some concerns	Low	Low	Low	Low	Some concerns
McClure et al (2019)[25]	Some concerns	Low	Low	Low	Low	Some concerns
Meslier et al (2020)[40]	Some concerns	Low	Low	Low	Low	Some concerns
Michielsen et al (2019)[41]	Some concerns	Low	Low	Low	Low	Some concerns
Miller et al (2005)[27]	Some concerns	Low	Low	Low	Low	Some concerns
Miller et al (1998)[26]	Some concerns	Low	Low	Low	Low	Some concerns
Navarro et al (2019)[28]	Low	Low	Low	Low	Low	Low
Nowson et al (2009)[42]	Some concerns	Low	Low	Low	Low	Some concerns
Park et al (2019)[43]	Some concerns	Low	Low	Low	Low	Some concerns
Poulsen et al (2014)[29]	Low	Low	Low	Low	Low	Low
Ra˚djursöga et al (2018)[44]	Some concerns	Low	Low	Low	Low	Some concerns
Rebholz et al (2018)[30]	Low	Low	Low	Low	Low	Low
Reidlinger et al (2015)[45]	Low	Low	Low	Low	Low	Low
Shin et al (2019)[46]	Some concerns	Low	Low	Low	Some concerns	Some concerns
Trimigno et al (2020)[31]	Low	Low	Low	Low	Low	Low
Tuomainen et al (2019)[47]	Some concerns	Low	Low	Low	Low	Some concerns
Vázquez-Fresno et al (2015)[32]	Some concerns	Low	Low	Low	Low	Some concerns
Wellington et al (2019)[48]	Low	Low	Low	Low	Low	Low
Zhu et al (2020)[49]	Some concerns	Low	Low	Low	Low	Some concerns

Urinary metabolomics was determined in a subset of 98 participants without diabetes in the PREDIMED study.[32] Statistical analysis by partial least-squares discriminant analysis and analysis of covariance identified a number of Mediterranean diet–associated metabolites, including ketone bodies, amino acids and their derived metabolites, gut microbiota cometabolites, and fatty acids. Metabolites associated with consumption of a low-fat diet were hippurate, trimethylamine N-oxide (TMAO), histidine and its derived metabolites, as well as carnosine, proline-betaine, and xanthosine.

The other metabolomics studies were from the United States (n = 2) and the Netherlands and Italy (n = 1 each). Zhu et al from the United States assigned 10 participants to a Mediterranean diet and a fast food diet, each for 4 days in a random order,[49] and found 3 indole derivatives that are related to tryptophan metabolism increased after the Mediterranean diet and decreased after the fast food diet, while 2 other indole derivatives changed in opposite direction. TMAO and choline were stable with both diets. In the other metabolomics study from the United States, Park et al assessed intra- and intergroup differences in plasma metabolites in 14 subjects after a 4-week Mediterranean diet, a high-fat (Atkins) diet, or a very-low-fat (Ornish) diet.[43] No differences were identified except for higher TMAO, leucine, and valine in the Atkins diet when compared with the Ornish diet. Michielsen et al in the Netherlands analyzed serum metabolome after an 8-week Mediterranean-type diet, a Western diet high in saturated fat, or a Western-type diet in which part of the saturated fatty acid was replaced by MUFA.[41] Five metabolites were identified by sparse partial least-squares discriminant analysis as having the most important differences between the diets; all of these 5 metabolites were associated with fatty acid metabolism, with 2 reflecting fish consumption, 2 reflecting butter consumption, and 1 reflecting olive oil consumption. In a trial in Italy, Meslier et al collected blood, urine, and fecal samples from 82 participants who followed a Mediterranean diet or a habitual Western diet for 8 weeks.[40] Metabolites that were lower in the Mediterranean diet group were generally associated with meat and animal-based foods, while those that were higher were associated with vegetables/berries, whole grains, legumes, fish, and nuts.

Taken together, the most important biomarkers for measurement of a Mediterranean-style diet are those relating to fruit and vegetables, fish, olive oil, and nut intake. These biomarkers include but are not limited to carotenoids, vitamin C, DHA, *n*–3 index, MUFA %, tyrosol, hydroxytyrosol, α-linolenic acid, and γ-tocopherol.

DASH diet

The 5 publications that examined the DASH and DASH-based diets were derived from 3 studies: 2 conducted in the United States and 1 in Australia.

A study from Baltimore in the United States compared a DASH diet, a fruit and vegetable diet, and a typical American diet, using an 8-week 3-arm parallel design. Three publications based on this study were included in this review. One analyzed established serum biomarkers in 34 participants: β-carotene, cryptoxanthin, and zeaxanthin were increased in the DASH and fruit and vegetable diet groups, when compared with the typical American diet ($P < 0.05$).[26] Lutein was increased in the DASH diet group when compared with the typical American diet ($P < 0.05$). Lycopene, retinol, and α-tocopherol levels did not differ among or between groups. McClure et al reported higher 24 h-urinary phosphorus in the DASH diet group when compared with the other 2 groups in 397 subjects.[25] The third publication (with 329 subjects) identified 44 serum metabolites that were significantly different in participants consuming the DASH diet compared with those consuming either the fruit and vegetable diet or the typical American diet.[30] The majority of the metabolites identified were lipids (n = 41); the others included the amino acid trans-4-hydroxyproline and 2 vitamin A metabolites (isomers of carotene diol). Two panels of the 10 most useful metabolites in distinguishing between consumers of the 3 diets are listed in Table 2; 1 distinguished the DASH diet from the fruit and vegetable diet; the other distinguished consumers of the DASH from consumers of the typical American diet.

The other US study, from the same group, compared a 3-month DASH diet with a typical American diet, and measured serum carotenoids, retinol, and tocopherols in 103 participants.[27] Lutein, cryptoxanthin, zeaxanthin, and β-carotene were higher in the DASH diet group ($P < 0.001$), whereas lycopene, α-carotene, and γ-tocopherol were lower ($P < 0.001$). There were no significant differences for retinol or α-tocopherol.

A study from Australia with 95 participants looked at a DASH-type diet (with a low dietary acid load) compared with a healthy diet based on dietary guidelines (with a high acid load).[42] The group measured 24-h urinary metabolites to assess compliance with the 14-week interventions. They reported lower sodium, calcium, chloride, and phosphate excretion, and higher excretion of potassium in the DASH-type diet group compared with the control group, although the results for phosphate and potassium were not significant. No differences were reported for magnesium and urea.

Overall, these results indicate that sodium, potassium, and carotenoids are the primary biomarkers in assessing compliance with a DASH diet. Emerging evidence indicates that metabolites relating to lipid and protein metabolism may also be useful.

Healthy Nordic diet

Six publications from 2 trials investigating the healthy Nordic diet were eligible for inclusion in this systematic review.

A trial from Copenhagen, Denmark, randomly allocated participants to a 26-week intervention of either a New Nordic diet or an average Danish diet. The New Nordic diet was based on the Nordic Nutrition Recommendations[50] but with a slightly higher protein content. Plasma and urinary samples underwent untargeted metabolomics determination, as described in 4 publications (2 each for plasma and urinary metabolomics). The plasma samples were analyzed by gas chromatography–mass spectrometry (n = 145)[24] and again by an ultra-performance liquid chromatography (UPLC) system coupled to quadruple time-of-flight mass spectrometry (n = 146).[20] The analysis using gas chromatography–mass spectrometry identified 9 metabolites associated with the New Nordic diet and 8 metabolites associated with the average Danish diet (Table 2). The UPLC-quadruple time-of-flight mass spectrometry analysis reported complementary information. Eight metabolites were identified as being associated with the New Nordic diet; of these, 4 have been suggested to relate to fish intake, and 1 each to whole grains and fat intake; 2 do not have known dietary associations. Thirteen metabolites were identified as being associated with the average Danish diet: 5 that relate to lipid metabolism, 3 to protein metabolism, 1 each to intake of chocolate, citrus fruits, and fish, 1 to food heating, and 1 with unknown food origin. The urinary samples were analyzed by UPLC (n = 107)[21] and hydrogen-1 proton nuclear magnetic resonance (n = 142).[31] Analysis using UPLC identified 5 metabolites associated with the New Nordic diet: 1 relating to fish intake and the others with no clear food origin. Fifteen metabolites were associated with the average Danish diet: 5 limonene metabolites relating to orange juice, wine gums, and soft drink intakes, 1 citrus metabolite relating to citrus fruit intake, 5 relating to chocolate intake, 1 metabolite relating to heat-treated food that has undergone Maillard reactions,[51] and 3 of unknown food origin. Using hydrogen-1 proton nuclear magnetic resonance and partial least-squares discriminant analysis, 15 metabolites were deemed to be higher in the New Nordic diet group and 10 lower. Suggested food origins are detailed in Table 2. The use of established biomarkers within the same trial was reported in Poulsen et al.[29] No significant differences between the 2 diets were identified in terms of 24-h urinary nitrogen or sodium levels in consumers (n = 143). Whole-blood lipids were also assessed (n = 145). The percentages of *n*–3 fatty acids, the *n*–3 index, the *n*–6/*n*–3 ratio, and the proportion of total highly unsaturated fatty acids as *n*–3 highly unsaturated fatty acids differed significantly between the groups, whereas percentages of saturated fatty acids, MUFAs, polyunsaturated fatty acids, and *n*–6 fatty acids in whole blood did not differ.

Similarly, a Nordic multinational trial[47] randomly assigned 164 participants to a healthy Nordic diet based on the Nordic Nutrition Recommendations,[50] or a typical Nordic diet for 18 or 24 weeks. Plasma metabolomics were measured, and the only difference in metabolites found between the 2 groups was that pipecolic acid betaine (homostachydrine, which is a substance found in coffee beans and citrus fruits) was higher in the healthy Nordic diet group.

These results suggest that biomarkers related to fish, fruit and vegetables, whole grains, and lipids are the top candidates for healthy Nordic diet measurement.

Diets based on dietary guidelines

Two trials from the United Kingdom investigated diets based on government healthy eating guidelines. The CRESSIDA trial[45] compared the UK dietary guidelines with a traditional British diet in 162 participants. In order to verify participants' compliance, biomarkers measured included 24-h urinary electrolytes, erythrocyte lipids, plasma alkylresorcinol, and serum vitamin D, folate, ferritin, and homocysteine. Potassium, *n*–3 index, alkylresorcinol, and vitamin D were reported to be higher in the intervention group compared with the control group, whereas sodium, folate, sucrose, and fructose were lower. No differences were reported for ferritin or homocysteine.

The other study was from London (UK) in which 19 participants consumed 4 diets that varied in their concordance with the WHO healthy eating guidelines.[36] Twenty-four-hour urinary samples were collected during each 72-h intervention period. A total of 28 metabolites had significant changes, with 19 of them being present in higher concentrations, and 9 metabolites having lower concentrations, after the consumption of the diet with the highest concordance with WHO healthy eating guidelines, compared with the diet with the lowest concordance (Table 2). Most of the metabolites had well-known dietary associations with individual foods, including 7 associated with fruit and/or vegetable intake, 7 with fish or meat intake, and 6 with single nutrients. Food and nutrient origins were

unknown for the remaining 8 metabolites. The metabolomic model developed in this study was further validated against DASH scores in 225 subjects from a UK cohort of the INTERMAP study, as well as an additional external Danish cohort of 66 subjects. Significant associations between urinary metabolic levels and DASH scores were observed. Additionally, 4 metabolites related to healthy eating were quantified individually in the INTERMAP study samples. All 4 metabolites increased in participants with higher, compared with lower, DASH scores (Table 2).

Due to the limited evidence, no definitive summary can be provided. However, possible candidates include biomarkers of fruit and vegetables, fish, whole grains, salt, and sugar intakes.

Low-glycemic-load diet

One trial from Seattle, United States, investigated the plasma metabolome response to GL. Subjects followed a low-GL or a high-GL diet for 4 weeks each in a random crossover design. Targeted metabolomics were reported in 2 articles derived from this 1 trial, with 19[22] and 80[28] participants, respectively. One article reported the levels of 14 metabolites as differing significantly between the low-GL and high-GL intervention diets.[22] Nine metabolites were found to be higher in concentration in the low-GL diet, while 5 were found to be lower. The other article reported on 20 metabolites, of which 8 were found to be higher and 12 were found to be lower in the low-GL diet when compared with the high-GL diet.[28] Five metabolites were found to differ in both of the metabolomics determinations: cystamine, acetylcholine, hydroxyproline, and creatine were consistently lower in the low-GL diet; however, carnitine was higher in the low-GL diet in 1 article but lower in the other (Table 2).

Vegetarian diet

A Swedish study from the University of Gothenburg examined the postprandial metabolic responses in 32 participants consuming a lacto-ovo vegetarian diet, compared with a vegan diet or an omnivore diet, using a crossover design.[44] Serum 3-hydroxyisobutyrate, proline, propylene glycol, and tyrosine, in addition to variables that included overlapping metabolites (carnitine & acetoacetate, N-acetylcysteine & proline & glutamate, proline & glutamate & unknown), were consistently higher post lacto-ovo vegetarian diet consumption compared with the vegan or omnivore diets. The metabolites that increased in participants consuming the vegan or omnivore diets are listed in Table 2.

Lederer et al randomly assigned 53 participants to a 4-week strict vegan diet or a meat-rich diet[38] and measured the levels of a group of nutritional biomarkers. Serum vitamin B_{12}, holotranscobalamin, and arachidonic acid were lower in consumers of the vegan diet compared with consumers of the meat-rich diet, whereas plasma nitrite and nitrate concentrations were higher. No significant differences were detected in serum methylmalonic acid, homocysteine, DHA, eicosanoic acid, eicosenoic acid, linoleic acid, linolenic acid, oleic acid, 25-OH-vitamin D_2/D_3, or urinary creatinine.

These results indicate that biomarkers related to vitamin B_{12} metabolism, as well as nitrites and nitrates are potentially important markers of this diet. However, more studies are required.

Prudent diet

A group from Hamilton, Canada, contrasted a prudent diet with a typical Western diet and reported metabolomics in 42 participants following a 2-week intervention.[48] Fourteen metabolites in plasma and 9 in urine samples were selected as the top-ranked metabolites distinguishing the contrasting diets (Table 2). The limited evidence supported the fatty acid profile as being a potential marker of a prudent diet.

Korean diet

A trial conducted at the Seoul National University compared a typical Korean diet based on the Korean food guide, with a recommended American diet and a typical American diet using a crossover design.[46] Fifty-four participants followed each intervention for 4 weeks. At the end of each dietary phase, serum and urinary metabolites that changed significantly compared with baseline were identified and are listed in Table 2. While limited, the current evidence showed inverse associations between the Korean diet and some serum essential amino acid (isoleucine, leucine, and valine) concentrations.

DISCUSSION

This is the first systematic review to describe the use of dietary biomarkers for assessing dietary patterns. A number of biomarkers used in clinical trials were identified, and information gained from metabolomic studies on the metabolites that characterize dietary pattern intakes was synthesized. These biomarkers/metabolites and their related dietary patterns are summarized in Table 4. Currently, the most commonly used biomarkers for assessing dietary patterns are those related to specific nutrients and foods characteristic of the patterns, including the *n*–3 index, 24-h urinary electrolytes, and carotenoids. The metabolites most frequently

identified in exploratory studies were those broadly associated with fish, protein, and lipid intakes.

The common dietary biomarkers in use for assessing dietary patterns relate to specific foods or nutritional aspects of a diet, but appear to lack specificity for a given dietary pattern. For example, the *n*–3 index is the combined proportion of eicosapentaenoic acid and DHA as a percentage of total fatty acids in erythrocytes or whole blood. The *n*–3 index is frequently included as a biomarker of oily fish intake to assess adherence to fish-rich dietary patterns, including the Mediterranean diet,[33] the New Nordic diet,[29] and a diet based on the UK dietary guidelines.[45] While this index may be useful under certain controlled conditions, it not only reflects fish consumption, but also a shift in fatty acid equilibrium, as the index is by definition inversely proportional to the levels of all other fatty acids combined and therefore may lack sensitivity.[52]

Twenty-four-hour urinary sodium, potassium, and nitrogen are established biomarkers for sodium, potassium, and protein intake, respectively. They are not affected by metabolism, and thus there is a direct relationship with absolute intake, making them robust dietary intake biomarkers.[53,54] Urinary sodium and potassium were included to assess compliance with the Mediterranean diet,[33] DASH diet,[42] New Nordic diet,[29] and the diet based on the UK dietary guidelines,[45] to inform on salt (sodium), and fruit and vegetables (potassium) intake. Twenty-four-hour urinary nitrogen was used in the study comparing the New Nordic diet with the average Danish diet.[29] These biomarkers only differentiate between dietary patterns where the difference in the corresponding dietary characteristics (eg, salt, fruit and vegetables, and protein) differ as individual components or characteristics of the dietary patterns tested. Therefore, the choice of such biomarkers needs to be carefully considered based on the knowledge of the specific characteristics of the dietary pattern being studied.

Serum or plasma carotenoids are established biomarkers for fruit and vegetable intake. These biomarkers were measured in 4 studies that investigated the Mediterranean diet[33,34,39] or the DASH diet.[26,27] No consistent results were seen across the studies. Although it was not the intention to assess dietary compliance but to study oxidative stress in 3 of the studies,[27,34,39] these studies were included in this review as they are about established dietary biomarkers and inform the research question. The lack of consistency seen in these results, may be explained by the broad spectrum of carotenoids that are present in various fruit and vegetables, the analysis methods used, or that the comparator dietary patterns had similar levels of carotenoid-rich fruit and vegetables. Future research may seek to identify whether a panel of specific carotenoids, or indeed a panel of dietary biomarkers, may better reflect a specific dietary pattern.

The other dietary biomarkers identified were 24-h urinary sucrose and fructose, which are biomarkers commonly used for assessing sugar intake. These biomarkers successfully differentiated between a diet based on the UK dietary guidelines and a typical American diet, where added sugar was discouraged in the former and no restriction was placed in the latter.[45] It should be noted that urinary sucrose and fructose cannot distinguish between the dietary intakes of naturally occurring or added sources and so is a marker of total sugar intake[55]; in this study, however, it was interpreted as reflecting added sugar intake.[45] Plasma alkylresorcinol, serum 25-hydroxyvitamin D, and folate were also measured in the aforementioned study to capture intakes of whole grains, oily fish, and folic-acid–fortified breakfast cereals, respectively.[45] All 3 of these biomarkers performed well in differentiating between the 2 dietary patterns. In another study using serum 25-hydroxyvitamin D as a biomarker, no difference was seen when an unsupplemented vegan diet and a meat-rich diet were compared.[38] This is not surprising, as the meat-rich diet did not emphasize oily fish intake, while other protein sources in the diet were not necessarily good sources of vitamin D.

Serum or plasma α-tocopherol concentrations differed between dietary patterns in only 1[34] of 5 included studies,[26,27,34,35,39] suggesting poor specificity. There is evidence that α-tocopherol is not a reliable biomarker of intake, given its plasma concentration is highly regulated and affected by factors other than dietary intake, such as genetic differences in absorption and metabolism.[56] Two studies assessed γ-tocopherol and both reported a significant difference between dietary patterns. One compared the Mediterranean diet and a habitual American diet,[35] the other compared the DASH diet and a typical American diet.[27] In contrast to α-tocopherol, γ-tocopherol is more closely associated with dietary intake[56] and therefore may be superior as a biomarker of vitamin E intake in dietary patterns.

Biomarkers of nutritional status that are indicators of not only dietary intake, but also nutrient metabolism[8] and that reflect longer-term intakes,[57] are possible contenders for dietary pattern biomarkers. Some examples of these include retinols, B vitamin, and iron status indicators. Serum or plasma retinol, ferritin, and homocysteine were measured in multiple trials for vitamin A, iron, and B vitamin status[26,27,34,37,38,45]; serum selenium, iron, soluble transferrin receptor,[37] methylmalonic acid, and urinary creatinine[38] were measured in only 1 trial. No differences across dietary patterns were reported for any of these biomarkers. These biomarkers reflect dietary intake but are also affected by nutrient

Table 4 **Summary of biomarkers/metabolites and their related dietary patterns**

Biomarker	Related dietary pattern(s)
25-hydroxyvitamin D	Diets based on dietary guidelines
α-linolenic acid	Mediterranean diet
γ-tocopherol	Mediterranean diet
	DASH diet[a]
n–3 index	Mediterranean diet
	Healthy Nordic diet
	Diets based on dietary guidelines
Alkylresorcinol	Diets based on dietary guidelines
Calcium	DASH diet[a]
Carotenoids	Mediterranean diet
Chloride	DASH diet[a]
Docosahexaenoic acid (DHA)	Mediterranean diet
Folate	Diets based on dietary guidelines[a]
Linoleic acid	Mediterranean diet[a]
MUFA	Mediterranean diet
	MUFA-rich Western diet[b]
Nitrites and nitrates	Vegetarian diet
Nitrogen	Healthy Nordic diet
Oleic acid	Mediterranean diet
Phosphorus	DASH diet
Potassium	DASH diet
	Diets based on dietary guidelines
Saturated fatty acids (SFAs)	Mediterranean diet[a]
Sodium	DASH diet[a]
	Diets based on dietary guidelines[a]
Sucrose, fructose	Diets based on dietary guidelines[a]
Total fatty acids	Mediterranean diet[a]
Trimethylamine N-oxide	Healthy Nordic diet
	Diets based on dietary guidelines
	Low-GL diet
	Atkins diet[a, b]
	Low-fat diet[b]
Tyrosol, hydroxytyrosol	Mediterranean diet
Vitamin B_{12}, holotranscobalamin	Vegetarian diet[a]
Vitamin C	Mediterranean diet
Essential amino acids, eg, isoleucine, leucine, valine[c]	Mediterranean diet
	Healthy Nordic diet
	Vegetarian diet
	Korean diet
	Atkins diet[b]
Animal-based foods/meat-related metabolites[c]	Mediterranean diet
	Healthy Nordic diet
	Diets based on dietary guidelines
	Vegetarian diet
	Prudent diet
	Western diet[b]
Fish intake–related metabolites[c]	Healthy Nordic diet
	Diets based on dietary guidelines
	Average Danish diet[b]
Fruit and vegetable intake–related metabolites[c]	Mediterranean diet
	Healthy Nordic diet
	Diets based on dietary guidelines
	Prudent diet
	Fast-food diet[b]
Lipid intake–related metabolites[c]	DASH diet
	Healthy Nordic diet
	Low-GL diet
	Vegetarian diet
	Prudent diet
	Western diet[b]
	Average Danish diet[b]

(continued)

Table 4 **Continued**

Biomarker	Related dietary pattern(s)
Protein intake–related metabolites[c]	DASH diet Healthy Nordic diet Low-GL diet Vegetarian diet Prudent diet Low-fat diet[b] Average Danish diet[b]
Whole grains intake–related metabolites[c]	Mediterranean diet Healthy Nordic diet

[a]Dietary biomarker was inversely associated with the dietary pattern.
[b]Dietary pattern investigated as a control group.
[c]For essential amino acids and intake-related metabolites, there are both direct and inverse associations (see Table 2 for details).
Abbreviations: DASH, Dietary Approaches to Stop Hypertension; GL, glycemic load; MUFA, monounsaturated fatty acid.

metabolism.[58] The only biomarkers of nutritional status that differed in the context of a dietary pattern were serum vitamin B_{12} and holotranscobalamin[38]; however, these biomarkers were only assessed in 1 study. The usefulness of these and other nutritional status biomarkers as a means by which to discriminate between different dietary patterns should be further investigated.

Established biomarkers of specific foods or nutrients lack specificity for a given dietary pattern, and there is considerable overlap between biomarkers used for various dietary patterns. For instance, 24-hour urinary sodium and potassium were determined in all dietary pattern studies included in this systematic review. Healthy dietary patterns share many aspects in common, including increased intake of fruit and vegetables, fish, whole grains, nuts, and healthy fats, and decreased intake of sodium and added sugar. Accordingly, these established biomarkers are currently unable to individually quantify dietary pattern intakes in observational situations. Taken together, these results suggest that a biomarker profile consisting of multiple biomarkers that reflect relevant foods, nutrients, and/or changes in metabolic status is likely required to accurately identify and discriminate between individual dietary patterns.

Exploratory studies have sought to identify dietary biomarker profiles that reflect different dietary patterns in an unbiased way. As such, inclusion of these exploratory studies within this systematic review complements the findings for established dietary biomarkers by providing unbiased information on the associations of a broad set of metabolic markers with the consumption of distinct dietary patterns. Exploratory studies have predominantly leveraged metabolomic methodologies, although these metabolomic profiles have generally not yet been validated in subsequent independent trials. Only 1 trial validated[36] their metabolomic profile model developed from a diet based on WHO healthy eating guidelines in independent cohorts. However, the association that was confirmed was between the metabolomic profiles and the DASH diet, rather than the original dietary pattern that the model was developed from (ie, the diet based on WHO healthy eating guidelines). This result reinforces the similarities shared by healthy dietary patterns.

Of the individual metabolites identified as having a dietary source, the most frequently identified were associated with fish (n = 29), proteins (n = 21), and lipids (n = 18), followed by meat (n = 17), vegetables (n = 9), fruit (n = 8), dairy (n = 6), chocolate (n = 6), vitamins (n = 6), whole grains (n = 4), legumes (n = 4), nuts (n = 2), sugar (n = 2), and wine/grape (n = 1). Most of these dietary components overlap across many dietary patterns, again suggesting a lack of specificity for a given dietary pattern if used in isolation. For example, TMAO has been shown to be a biomarker for both red meat and deep-sea fish consumption.[59] These foods can be consumed in distinct dietary patterns with distinctly different associations with mortality and heart disease.[60,61] Biomarker profile is also dependent on the nature of the sample and the error in the analytic method. Urinary samples from one study were analyzed by UPLC–quadruple time-of-flight mass spectrometry[21] and hydrogen-1 proton nuclear magnetic resonance.[31] Only 1 overlapping metabolite (TMAO) was identified.[59] In another study, plasma metabolomic profiles were assessed twice using the same analysis technique LC-MS/MS in 19[22] and 80[28] participants. Fourteen and 20 metabolites were identified, respectively, with only 5 of them being identified on both occasions. Accordingly, there remain analytic challenges with handling complex data from metabolomics, which adds to the difficulty of developing objective dietary pattern assessment tools.

Currently, the metabolic profiles for identifying particular dietary patterns are not sufficiently developed; they require external validation and determination of sensitivity and specificity. This systematic review and summary of the current evidence base provides a

clear direction for future research seeking to identify dietary biomarkers profiles. Biomarkers related to intakes of fish, proteins, and lipids, are likely to be important for objective assessment of dietary patterns. Future metabolomic studies should be hypothesis-driven, investigating a priori metabolic profiles, but should also test for study-specific exploratory associations.

One of the strengths of this review is the comprehensive search that was conducted. A total of 5 databases were searched without restricting biological specimen type, intervention duration, or publication year. To the authors' knowledge, this is the first systematic review on dietary biomarkers of dietary patterns. A limitation is that this review is based on studies conducted in generally healthy populations, and the findings may not be generalizable to those with noncommunicable diseases. Furthermore, assessing the dietary biomarkers of dietary patterns was not the primary aim of some of these studies; however, they nonetheless provided relevant data that was suitable for inclusion in this systematic review.

CONCLUSION

Using dietary biomarkers of single nutrients or of individual foods or food groups can be useful for assessing dietary compliance in controlled settings. However, identifying an individual's specific or broad dietary pattern on the basis of their biomarker profile remains an area for future research. This is particularly challenging given the large degree of variation within dietary patterns. The application of established biomarkers is limited due to their poor specificity. A framework that incorporates a panel of dietary biomarkers is likely necessary in order to accurately capture the full complexity of dietary patterns.

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Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 **PRISMA 27-item checklist**
Appendix S2 **MEDLINE search**

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