Comparing metabolite profiles of habitual diet in serum and urine[1–3]

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ABSTRACT

Background: Diet plays an important role in chronic disease etiology, but some diet-disease associations remain inconclusive because of methodologic limitations in dietary assessment. Metabolomics is a novel method for identifying objective dietary biomarkers, although it is unclear what dietary information is captured from metabolites found in serum compared with urine.

Objective: We compared metabolite profiles of habitual diet measured from serum with those measured from urine.

Design: We first estimated correlations between consumption of 56 foods, beverages, and supplements assessed by a food-frequency questionnaire, with 676 serum and 848 urine metabolites identified by untargeted liquid chromatography mass spectrometry, ultra-high performance liquid chromatography tandem mass spectrometry, and gas chromatography mass spectrometry in a colon adenoma case–control study (n = 125 cases and 128 controls) while adjusting for age, sex, smoking, fasting, case-control status, body mass index, physical activity, education, and caloric intake. We controlled for multiple comparisons with the use of a false discovery rate of $<$0.1. Next, we created serum and urine multiple-metabolite models to predict food intake with the use of 10-fold crossvalidation least absolute shrinkage and selection operator regression for 80% of the data; predicted values were created in the remaining 20%. Finally, we compared predicted values with estimates obtained from self-reported intake for metabolites measured in serum and urine.

Results: We identified metabolites associated with 46 of 56 dietary items; 417 urine and 105 serum metabolites were correlated with ≥1 food, beverage, or supplement. More metabolites in urine (n = 154) than in serum (n = 39) were associated uniquely with one food. We found previously unreported metabolite associations with leafy green vegetables, sugar-sweetened beverages, citrus, added sugar, red meat, shellfish, desserts, and wine. Prediction of dietary intake from multiple-metabolite profiles was similar between biofluids.

Conclusions: Candidate metabolite biomarkers of habitual diet are identifiable in both serum and urine. Urine samples offer a valid alternative or complement to serum for metabolite biomarkers of diet in large-scale clinical or epidemiologic studies. *Am J Clin Nutr* 2016;104:776–89.

Keywords: metabolomics, metabolite, serum, urine, diet, food, nutrition assessment, biomarker

INTRODUCTION

Chronic diseases account for two-thirds of deaths worldwide, and some risk factors, including the consumption of an unhealthy diet, are modifiable (1). However, inconsistent findings for some diet-disease associations have contributed to a shifting evidence base, curbing the strong promotion of dietary guidance in disease prevention. These inconsistent findings may partly reflect methodologic limitations in measuring diet with the use of self-reported questionnaires, which are subject to recall bias and measurement errors that may weaken diet-disease associations (2–4). Dietary biomarkers have the potential to improve the measurement of dietary exposures by validating diet questionnaires (5), by replacing inadequate dietary data (6), or for regression calibration (7). Recovery biomarkers that are excreted in proportion to food intake exist for protein and energy (8, 9). Concentration biomarkers, which are correlated with but not directly proportional to actual intake, have been identified for some foods (e.g., carotenoids for vegetables). However, valid biomarkers for other macronutrients and many foods are unestablished (10).

Metabolomics platforms measure hundreds of identifiable (and up to thousands of currently unidentified) metabolic parent compounds, substrates, and products in biospecimens with the use of mass spectrometry or proton nuclear magnetic resonance (11, 12), creating an opportunity to improve dietary assessment by establishing objective biomarkers (13–15). Metabolites reflect biologically relevant components of food and their metabolic effects, concentrations of which are determined by nutrient

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[3] Supplemental Figures 1 and 2, Supplemental Tables 1–15, and Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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availability after food preparation, nutrient interactions, baseline nutrient status, environment, genetics, and the gut microbiome are accounted for (6, 16).

Metabolite profiling has been used in several studies to identify candidate dietary biomarkers and to explore diet-disease mechanisms (13, 15–19). However, the critical question of which biospecimens typically collected in clinical and epidemiologic studies (i.e., blood or urine) provide metabolite biomarkers that better characterize habitual diet remains unanswered. Few studies have measured dietary metabolites in serum and urine concurrently. Urine is cheaper and easier to collect on a large scale, so this information has implications for widespread use.

With the use of biospecimens collected from participants in a colorectal adenoma case–control study, our objectives were to *1*) conduct agnostic analyses of the associations between usual diet (food items or groups, beverages, and dietary supplements) and serum and urine metabolites to identify candidate nutritional biomarkers of habitual diet, and *2*) compare diet–biomarker correlations between serum and urine to assess the comparative value of each biofluid for predicting specific dietary exposures.

METHODS

Study design and population

This case-control study was conducted at the National Naval Medical Center in Bethesda, Maryland, between April 1994 and September 1996, as described previously (20). Briefly, new or recurrent colorectal adenoma cases and adenoma-free controls were selected from those undergoing routine colorectal cancer screening. Eligible participants (n = 289 cases and 314 controls) were aged 18–74 y and were study area residents, with no previous diagnosis of Crohn disease, ulcerative colitis, colorectal neoplasms, or cancer, besides nonmelanoma skin cancer. Of these, 241 cases (84%) and 231 controls (74%) participated (**Supplemental Figure 1**). Reasons for nonparticipation included subject refusal (12% of cases and 21% of controls), illness (3% of cases and 4% of controls), and other reasons (1% of cases and 1% of controls). Controls were matched individually to cases by age, sex, smoking status, and date of blood draw. Both dietary data and biological samples were ascertained from 258 participants. Procedures followed were in accordance with the Institutional Review Board of the National Cancer Institute and National Naval Medical Center, Bethesda, Maryland, and approval was obtained from these committees. All participants provided written informed consent.

Metabolite assessment

Biological sample collection

Samples were included for 253 participants (n = 125 cases and 128 controls) after those with an implausible caloric intake (<500 or >3500 kcal/d; n = 5) were excluded (**Supplemental Figure 2**). All participants provided one sample each from serum and urine. Nonfasting serum samples were collected at a clinic visit scheduled separately from the screening visit (23% were collected during screening, thus were fasting). A 12-h overnight nonfasting urine sample was collected at a separate home visit.

Metabolite platform

Serum and urine parent compounds and their metabolites (<1000 Da) were measured by Metabolon in September 2011. The metabolite measurement process has been described previously (18, 19, 21, 22). Briefly, samples were stored at −80°C before processing. Aqueous methanol extracted the protein fraction. Aliquots were dried and reconstituted with the use of organic solvents that contained internal standards. After assay preparation, samples were analyzed with the use of untargeted ultrahigh-performance liquid chromatography mass spectrometry, ultra-high performance liquid chromatography tandem mass spectrometry, and gas chromatography mass spectrometry. To identify individual metabolites, data were compared with a chemical reference library of pure standards of known metabolite identity based on retention time and index, *m/z*, and chromatographic data.

Metabolites with concentrations below the limit of detection (LOD)[9] for ≥95% of participants were excluded. This value was chosen to enable analysis of metabolites related to diet items that were consumed only episodically or only by a small proportion of participants. In addition, some metabolites may have been present in concentrations higher than the LOD in one biofluid only, such as in urinary excretion products.

Dietary and covariate assessment

Covariate data were ascertained by baseline questionnaire. Usual dietary intake (frequency and portion size) over the 12 mo before colorectal screening was measured by self-administered food-frequency questionnaire (FFQ) on the basis of a modified 100-item Block Health Habits and History Questionnaire at a clinic visit (23); responses were verified at a home visit by trained interviewers. Participants were asked about dietary supplement use, dose, and frequency, including for multivitamin use and individual vitamin supplements, separately. Food serving size and frequency data were converted to grams per day and categorized into predefined groupings on the basis of the USDA My Plate classification of the Dietary Guidelines for Americans with similar nutrient profile [fruits, vegetables (dark-green, starchy, red and orange, or beans and peas), grains (whole or refined), protein foods (meat, poultry, seafood, eggs, or nuts), dairy (milk-containing), oils, and sugars] (24, 25) (**Supplemental Table 1**). Foods that did not fall into these groups or previously were shown to have unique metabolite profiles (e.g., citrus or coffee) (15, 26) were analyzed separately. The fruit category included juice. Dietary supplement use was categorized as binary (yes or no). All continuous dietary variables were ln-transformed before statistical analysis.

Statistics

Metabolite values were batch-normalized by dividing metabolite peak intensity by median nonmissing batch values to control for some day-to-day mass spectral drift, followed by

[9] Abbreviations used: CEHC, 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate; FDR, false discovery rate; FFQ, food-frequency questionnaire; LASSO, least absolute shrinkage and selection operator; LOD, limit of detection; TMAO, trimethylamine *N*-oxide; α-CEHC, α-2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman.

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ln-transformation and centering to account for nonnormal distribution. Missing values for a particular metabolite generally were from the value falling below the LOD for the metabolomics platform; therefore, missing values were imputed as the value at the minimum of nonmissing values. This is the lowest level that could be confirmed, and it tended to result in conservative, rather than exaggerated, estimates of correlations.

Demographic characteristics and dietary intake were compared by case-control status with the use of Wilcoxon's signed rank test and independent sample *t* tests for continuous variables, and chi-square tests for categorical variables. Associations between dietary variables and metabolites were estimated with the use of a partial Pearson correlation while adjusting for factors previously associated with metabolites and dietary intake (27–35), including age (years), sex, smoking (never, past, or current), fasting status at time of blood draw (fasting or nonfasting), case-control status, BMI (kg/m^2), physical activity index (weekly frequency of moderate-intensity leisure activity plus 2 × weekly frequency of vigorous-intensity leisure activity), education (≤12 y, vocational or 1–3 y college, ≥4 y college, or graduate), and daily caloric intake (kcal/d). Analyses of single vitamin and nutrient supplement use were adjusted for multivitamin use. To account for multiple comparisons while also allowing for biomarker discovery and hypothesis generation, a false discovery rate (FDR) < 0.1 was applied (Benjamini-Hochberg procedure) (36, 37). Diet–metabolite correlations first were ranked from smallest to highest *P* value. The FDR threshold for statistical significance for each metabolite–food correlation was calculated as [0.1/(total number of diet–metabolite correlations/rank value)], and *Q* values were calculated as [(*P* value × total number of diet–metabolite correlations)/rank value]. Correlations were considered to be FDR-significant if the *Q* value was < 0.1 and the *P* value was less than the FDR-threshold *P* value. Although less stringent than Bonferroni correction, an FDR < 0.1 allowed for the fact that many metabolites are part of the same metabolic pathways and thus are not independent. It was also more conservative than the FDR < 0.25 recommended for gene-set enrichment analyses and generally considered to be a reasonable standard for hypothesis-generating analyses (38).

We compared prediction of dietary intake by multiple-metabolite linear models in serum and urine with the use of 10-fold crossvalidation least absolute shrinkage and selection operator (LASSO) regression (39), which imposed a penalty on the β coefficients to reduce variance inflation, because the number of metabolites exceeded the number of observations (40). For binary dietary supplement variables, we performed logistic regression on potential confounders and used the fitted values as an offset in binary LASSO regression. Ten iterations of predictive models were generated with the use of 80% data and tested in the remaining 20% (described in detail in **Supplemental Methods**).

Finally, we created *1*) metabolite and *2*) dietary variable partial Pearson correlation matrices. Results for $r > 0.5$ (metabolites) and $r > 0.2$ (diet) are presented in **Supplemental Tables 2–4**.

Quality control

Reliability was assessed by including 30 blinded pooled serum samples from a separate, independent sample of nonsmoking adult US volunteers and 4 duplicates taken from different main study subjects. For urine, there were 29 pooled quality-control samples with 3 duplicates from separate main study subjects. Biological variability was expressed by intraclass correlation coefficients [the sum of between-individual (σ^2_B) and within-individual (σ^2_W) variation divided by total variation (σ^2_T)—the sum of between- and within-individual variability and laboratory reproducibility (σ^2_E)] (41).

Sensitivity analysis

Heterogeneity of diet–metabolite correlations by case-control and fasting status was tested with the use of Fisher's *r* to Z transformation and Z test for comparing correlation coefficients with significance determined at the Bonferroni level (42). To examine the influence of consumption frequency on the crossvalidation LASSO prediction models, we performed a median split (≤2 times/wk or >2 times/wk) and compared the prediction in serum with that in urine in each category.

Partial correlation and sensitivity analyses were performed with the use of SAS, version 9.3 (Proc CORR with PARTIAL statement); crossvalidation LASSO was conducted in R, version 3.1.2 (cv.glmnet from the glmnet package).

RESULTS

Study population

Characteristics of the study population are shown in **Table 1**. The mean ± SD age of participants was 57 ± 9 y. The majority were male (77%) with nonfasting serum (77%) and nonfasting urine (100%) samples. Most participants were not current smokers (49% never and 44% former). The physical activity index was a mean ± SD of 10 ± 11. Eighty-nine percent of participants had a college-level education. Mean ± SD BMI (in kg/m^2) was 27 ± 5, and mean ± SD self-reported daily caloric intake was 1639 ± 581 kcal/d. Demographic characteristics were similar in cases and controls (all $P > 0.05$). There were more participants who had fasted before their baseline blood draw in the control group than in the case group ($P < 0.0001$).

Habitual dietary intake based on the FFQ is presented in **Table 2**. Results did not differ by case-control status after control for multiple comparisons.

A total of 848 metabolites were detected in urine and 675 in serum. After excluding metabolites lower than the LOD in ≥95% of samples (24 in urine and 27 in serum), we detected 824 metabolites in urine, and 648 in serum (known metabolites detailed in **Supplemental Tables 5–6**). The median (IQR) intraclass correlation coefficient was 0.96 (0.90, 0.98) for urine and 0.85 (0.56, 0.95) for serum.

Diet-related metabolites

Overall, 46 of 56 dietary items were associated with ≥1 metabolite after controlling for multiple comparisons. There were 417 urine and 105 serum diet–metabolite correlations observed at an FDR < 0.1. A total of 188 urine and 39 serum metabolites were uniquely associated with one food; the remainder were associated with multiple foods.

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TABLE 1
Baseline characteristics of participants in the Navy Adenoma Study[1]

Characteristic	Total[2]	Cases (n = 125)	Controls (n = 128)	P[3]
Age, y	57 ± 9	58 ± 9	57 ± 9	0.62
Sex				0.80
M	77	38	39	
F	23	12	11	
Fasting status				<0.001
Nonfasting	77	47	30	
Fasting	23	2	20	
Smoking status				1.00
Never	49	24	25	
Former	44	22	23	
Current	7	4	4	
Education				0.08
≤12 y	10	15	5	
Vocational or 1–3 y college	21	21	21	
≥4 y college	21	19	23	
Graduate	47	45	50	
Physical activity index[4]	10 ± 11	10 ± 14	9 ± 7	0.60
BMI, kg/m^2	27 ± 5	27 ± 4	27 ± 5	0.57
Daily caloric intake, kcal/d	1639 ± 581	1637 ± 575	1647 ± 597	0.90

[1]Values are means ± SDs for continuous variables and percentages for categorical variables, n = 253.
[2]Values may not sum to 100% because of rounding.
[3]Comparison by case-control status was significant at $P < 0.05$ on the basis of a chi-square test for categorical variables and a t test for continuous variables.
[4]Calculated by adding the number of times per week engaged in moderate-intensity leisure-time physical activity plus 2 times the number of times per week engaged in vigorous-intensity leisure-time physical activity.

Food and supplements

Urine and serum metabolites that were significantly associated with food and supplements at an FDR < 0.1 with $r \geq \pm 0.25$ are presented in **Table 3** (for full results, see **Supplemental Tables 7–8**). We identified 220 significant positive or inverse food or supplement–metabolite correlations in urine (r = −0.30–0.50; $P = 9.57 \times 10^{-17}$ to 9.20×10^{-4}); approximately one-half (n = 115) were with unknown metabolites. There were 69 correlations ≥ ±0.25, including with total citrus and juice (stachydrine, r = 0.50 and 0.43, respectively), fish [3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF), r = 0.27], nuts (tryptophan betaine, r = 0.41), processed and red meat (acetylcarnitine, r = 0.26 and 0.32, respectively), shellfish (CMPF, r = 0.26), meat fat (creatine, r = 0.30), multivitamins (pantothenate, r = 0.36; pyridoxate, r = 0.30; and riboflavin, r = 0.27), and vitamin E supplements [α-2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (α-CEHC) glucuronide, r = 0.40; and α-CEHC-sulfate, r = 0.33].

In serum, 55 food or supplement–metabolite correlations were observed (r = −0.44–0.55; $P = 4.17 \times 10^{-20}$ to 2.74×10^{-4}). Approximately 40% (n = 25) of the associations were with unknown metabolites. Food and supplement–metabolite correlations ≥ ±0.25 that were unique to serum included grapefruit (deoxycarnitine, r = −0.25), juice (tryptophan betaine, r = −0.25), other fruit (mannitol, r = −0.25), cruciferous vegetables (kynurenine, r = −0.28), fish (docosahexaenoate, r = 0.36, and 2 unknown metabolites, r = 0.37 and 0.28, respectively), shellfish (unknown metabolite, r = 0.38), meat fat (3 unknown metabolites, r = 0.26, 0.25, and −0.25), salty snacks (unknown metabolite, r = 0.27), and multivitamin (α-tocopherol, r = 0.31, and 2 unknown metabolites, r = 0.27 and 0.26), and vitamin E (α-tocopherol, r = 0.37, and γ-tocopherol, r = −0.44) use. Sixteen food and supplement–metabolite correlations in serum were similar to those in urine, including biomarkers of citrus (stachydrine, scyllo-inositol, *N*-methylproline, and chiro-inositol), juice (stachydrine, *N*-methylproline, and scyllo-inositol), fish (3-carboxy-4-methyl-5-propyl-2-furanpropanoate and CMPF), nuts (tryptophan betaine), shellfish (CMPF and 2-hydroxybutyrate), and multivitamin supplement use (pantothenate, pyridoxate).

Beverages

Nonjuice beverage–metabolite correlations in urine and serum (FDR < 0.1, $r \geq \pm 0.25$) are presented in **Table 4** (for full results, see Supplemental Tables 7–8). More urine metabolites (n = 198) correlated with specific beverages than did those in serum (n = 50). We found only 2 inverse metabolite correlations with decaffeinated coffee in serum. The majority of the remainder of total coffee metabolites were associated with caffeinated coffee. Eighteen metabolites were associated with caffeinated coffee in both serum and urine. Catechol sulfate (r = 0.29) and cyclo(Leu-Pro) (r = 0.23) were associated uniquely with caffeinated coffee in serum.

Of the 3 alcohol types investigated, wine had the strongest association with 2,3-digydroxyisovalerate in urine (r = 0.31); and liquor had the strongest association with ethyl glucuronide in serum (r = 0.26). 3-Methylglutaroylcarnitine was associated with sugar-sweetened beverages in serum only (r = 0.25). Other beverage–metabolite associations were observed primarily in urine (e.g., tea and an unknown metabolite, r = 0.38).

Urine and serum comparison

There were 37 FDR-significant diet–metabolite correlations that were the same in both serum and urine (excluding total coffee); correlation magnitudes were similar between biospecimens. We observed diet–metabolite associations that, to our knowledge, have not been reported in larger-scale epidemiologic studies, including citrus (*N*-methyl glutamate, r = 0.32), added sugar [trimethylamine *N*-oxide (TMAO), r = 0.23], shellfish (ciliatine and 2- and 3-hydroxybutyrate, r = 0.25), red meat (cinnamoylglycine, r = 0.23, and methyl-α-glucopyranoside, r = 0.22), desserts (3- and 7-methylxanthine, r = 0.23 and 0.24, respectively) and wine (2,4,6-trihydroxybenzoate, r = 0.23). In serum, potentially novel associations included those with leafy green vegetables (threonate, r = 0.24) and sugar-sweetened beverages (methylglutaroylcarnitine, r = 0.25). For a list of potentially novel associations, see **Supplemental Table 9**; for a replication of diet–metabolite associations in previous studies, see **Supplemental Table 10**.

The comparison of correlations between self-reported diet and diet predicted by multiple-metabolite profile is presented in **Figure 1** (95% CIs provided in **Supplemental Table 11**). These correlation magnitudes, which broadly reflect strength of serum and urine multiple-metabolite prediction of diet based on statistical modeling (i.e., prediction based on a training data set and testing with the use of a testing data set), were generally comparable in serum and urine, with evidence that results did not

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TABLE 2
Self-reported usual dietary intake in the Navy Colon Adenoma Study[1]

USDA food group and food categories, g/d	Values
Fruits	
Apples, pears	15 (5, 39)
Total citrus (oranges, lemons, grapefruit)	99 (32, 198)
Grapefruit	2 (0, 14)
Juices	81 (21, 186)
Melons	3 (1, 8)
Oranges	8 (0, 23)
Strawberries	2 (0, 3)
Other fruit (bananas, peaches, others)	37 (17, 84)
Vegetables	
Beans	5 (0, 11)
Corn	4 (1, 8)
Cruciferous (broccoli, mustard or collard greens, turnips, cabbage, cauliflower)	12 (7, 23)
Leafy greens (green salad or raw or cooked spinach)	42 (20, 70)
Orange or yellow (carrots, winter squash)	8 (3, 16)
Peas	4 (1, 8)
Tomatoes	14 (6, 28)
Other vegetables (onions, summer squash, green beans, others)	11 (6, 21)
Total potatoes (white, sweet, fried)	36 (18, 55)
Sweet potatoes	0 (0, 3)
White potatoes	27 (11, 40)
Grains	
High-fiber (high-fiber cereal, dark bread)	14 (5, 29)
Average fiber (spaghetti, white bread, corn bread, cooked/cold/fortified cereal, biscuits, muffins)	91 (58, 141)
Protein foods	
Chicken	21 (7, 41)
Eggs	8 (2, 14)
Fish[2]	6 (3, 11)
Nuts (peanuts, other nuts, nut butters)	2 (0, 5)
Processed meat (lunch meat, sausages, hot dogs, bacon, liverwurst)	9 (3, 20)
Red meat (steak, hamburger, lamb, pork, venison)	21 (10, 36)
Shellfish	1 (0, 2)
Dairy	
Milk	112 (35, 249)
Cheese	8 (2, 16)
Yogurt	8 (0, 32)
Fats and oils	
Butter (butter, added fat, whipped butter)	0 (0, 2)
Margarine (soft, diet, stick)	4 (0, 9)
Meat fat (lard, gravy, meat fat)[2]	26 (16, 36)
Mayonnaise or salad dressing	1 (0, 9)
Oil used in cooking	1 (0, 3)
Shortening	0 (0, 0)
Sugars	
Added sugar	2 (0, 8)
Sugar-sweetened beverages (caffeinated, decaffeinated)	185 (52, 430)
Miscellaneous	
Fried potatoes (includes french fries)	7 (0, 15)
Salty snacks (potato chips, corn chips, popcorn)	4 (1, 8)
Ice cream	5 (0, 14)
Sweets (cookies, pie, candy, chocolate)	13 (4, 29)
Alcohol	
Total alcohol (beer, wine, liquor)	144 (7, 364)
Beer	144 (0, 360)
Wine	0 (0, 40)
Liquor	0 (0, 17)
Beverages	
Total coffee (caffeinated, decaffeinated)	366 (207, 732)
Caffeinated	366 (180, 366)
Decaffeinated	0 (0, 180)
Tea (all varieties)	72 (0, 180)
Dietary supplements	
Multivitamin use	
No	53
Yes	47
Vitamin C	
No	73
Yes	27
Vitamin E[3]	
No	77
Yes	23
Calcium[3]	
No	80
Yes	19

[1]Values are medians (25th, 75th percentiles) or percentages, n = 253. Measured with the use of the Block Health Habits and History Questionnaire.

[2]Self-reported intake differs for cases compared with controls (Wilcoxon's Signed Rank Test for comparison of dietary intake by case-control status; 2-sided significance, $P < 0.05$). Cases had a lower intake of fish and higher intake of meat fat.

[3]One participant each was missing information on supplemental vitamin E use and supplemental calcium use.

vary by frequency of consumption. Correlations of urine and serum metabolites with food intake differed significantly for apples, butter, and tea ($P < 0.05$). A similar number of foods (n = 26 in urine and n = 23 in serum) were predicted to some degree by both urine and serum metabolite profiles, as evidenced by positive prediction ($r > 0.1$).

Sensitivity analyses

We found no effect modification by case-control or fasting status that was statistically significant, after accounting for multiple testing (**Supplemental Tables 12–15**).

DISCUSSION

In this analysis of the associations between metabolites and habitual diet in a case-control study of 253 adults, we identified 417 urine and 105 serum diet–metabolite correlations after controlling for multiple comparisons. The strongest diet–metabolite associations were for total citrus, juice, fish, shellfish, nuts, processed and red meats, meat fat, multivitamin and vitamin E supplement use, and caffeinated coffee. Several of these have not been reported in free-living participants, to our knowledge, and thus may represent novel candidate nutritional biomarkers. Urine and serum multiple-metabolite profiles similarly predicted intake for most foods and beverages.

The emergence of candidate dietary biomarkers creates new opportunities to better evaluate mechanisms driving diet-disease associations. Ideal biomarkers are reliably measured, exposure-specific, and easily collected (43). Urine, as a biospecimen, is less

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TABLE 3
Urine and serum metabolites associated with food and dietary supplements in the Navy Colon Adenoma Study[1]

Metabolite/Metabolon, Inc. identification	Platform	RI	*m/z*	Super pathway	Metabolite identification	Urine		Serum	
						r^2	P^3	r^2	P^3
Fruit									
Apples									
X - 17351	LC-MS −	3914.4	245.1			0.25	5.97×10^{-5}		
Total citrus									
Stachydrine	LC-MS +	860.0	144.1	XEN	HMDB04827	0.50	9.57×10^{-17}	0.55	4.17×10^{-20}
X - 12111	LC-MS +	1723.0	144.1			0.41	1.45×10^{-11}		
Scyllo-inositol	GC-MS	1893.8	318.2	LIP	HMDB06088	0.37	2.00×10^{-9}	0.33	1.42×10^{-7}
N-methylproline or *N*-methyl proline	LC-MS +	833.0	130.1	AA	557	0.36	8.32×10^{-9}	0.40	1.72×10^{-10}
X - 12109	LC-MS +	1751.0	401.9			0.33	1.48×10^{-7}		
N-methylglutamate	GC-MS	1665.0	260.2	AA	439377	0.32	2.92×10^{-7}		
Chiro-inositol	GC-MS	1857.0	318.1	LIP	HMDB34220	0.31	6.17×10^{-7}	0.35	3.10×10^{-8}
X - 17350	LC-MS −	2728.5	345.3			0.31	7.98×10^{-7}		
X - 13847	LC-MS −	1426.0	383.1			0.29	3.89×10^{-6}		
X - 17145	LC-MS −	3844.7	257.2			0.29	6.24×10^{-6}	0.29	6.24×10^{-6}
Betonicine or 4-hydroxyproline betaine	LC-MS +	746.0	160.1	XEN	HMDB29412	0.28	7.72×10^{-6}		
X - 17453	LC-MS +	4000.6	591.3			−0.26	4.63×10^{-5}		
X - 13696	LC-MS +	4057.0	457.1			0.25	9.46×10^{-5}		
Grapefruit									
Deoxycarnitine	LC-MS +	759.0	146.1	LIP	HMDB01161			−0.25	7.24×10^{-5}
Juices									
Stachydrine	LC-MS +	860.0	144.1	XEN	HMDB04827	0.43	1.59×10^{-12}	0.42	1.30×10^{-11}
X - 12111	LC-MS +	1723.0	144.1			0.35	1.60×10^{-8}		
N-methylglutamate	GC-MS	1665.0	260.2	AA	439377	0.31	7.84×10^{-7}		
N-methylproline or *N*-methyl proline	LC-MS +	833.0	130.1	AA	557	0.30	1.31×10^{-6}	0.30	2.40×10^{-6}
Scyllo-inositol	GC-MS	1893.8	318.2	LIP	HMDB06088	0.29	3.58×10^{-6}	0.25	7.71×10^{-5}
X - 12109	LC-MS +	1751.0	401.9			0.28	6.63×10^{-6}		
X - 13847	LC-MS −	1426.0	383.1			0.28	9.45×10^{-6}		
X - 17350	LC-MS −	2728.5	345.3			0.26	3.02×10^{-5}		
Chiro-inositol	GC-MS	1857.0	318.1	LIP	HMDB34220			0.26	4.90×10^{-5}
Betonicine or 4-hydroxyproline betaine	LC-MS +	746.0	160.1	XEN	HMDB29412	0.25	1.00×10^{-4}		
Tryptophan betaine	LC-MS +	2464.0	247.1	AA	HMDB61115			−0.25	7.24×10^{-5}
Other fruit									
X - 10593	LC-MS +	1256.0	172.1			−0.30	2.57×10^{-6}		
X - 12128	LC-MS +	1725.0	162.1			−0.28	1.15×10^{-5}		
X - 13722	LC-MS −	893.0	160.1			−0.28	1.18×10^{-5}		
X - 12819	LC-MS −	2702.0	170.1			−0.25	9.72×10^{-5}		
Mannitol	GC-MS	1839.0	319.1	CHO	HMDB00765			−0.25	1.21×10^{-4}
Vegetables									
Corn									
X - 17346	LC-MS −	1742.8	253.2			−0.26	2.83×10^{-5}		
X - 12831	LC-MS −	3214.0	433.2			−0.26	3.71×10^{-5}		
Pimelate (heptanedioate)	GC-MS	1602.9	155.0	LIP	HMDB00857	−0.26	4.15×10^{-5}		
X - 12814	LC-MS −	2597.0	405.2			−0.26	5.01×10^{-5}		

(Continued)

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TABLE 3 (*Continued*)

Metabolite/Metabolon, Inc. identification	Platform	RI	*m/z*	Super pathway	Metabolite identification	Urine		Serum	
						r^2	P^3	r^2	P^3
X - 13452	LC-MS +	3606.0	192.2			−0.26	5.27×10^{-5}		
Mannitol	GC-MS	1839.0	319.1	CHO	HMDB00765	−0.25	5.86×10^{-5}		
Cytosine	GC-MS	1535.5	254.0	NUC	HMDB00630	−0.25	6.68×10^{-5}		
Suberate (octanedioate)	LC-MS +	3603.0	175.0	LIP	HMDB00893	−0.25	7.55×10^{-5}		
Homovanillate sulfate	LC-MS −	1050.0	261.1	AA	HMDB11719	−0.25	9.30×10^{-5}		
X - 12101	LC-MS +	1646.0	164.1			−0.25	9.74×10^{-5}		
X - 12244	LC-MS +	1147.0	269.2			−0.25	1.09×10^{-4}		
X - 11880	LC-MS −	5378.0	537.4					−0.25	1.06×10^{-4}
Cruciferous vegetables									
Kynurenine	LC-MS +	1902.0	209.1	AA	HMDB00684			−0.28	8.40×10^{-6}
X - 13553	LC-MS −	1406.0	263.0			−0.25	1.01×10^{-4}		
Orange and yellow vegetables									
X - 12707	LC-MS −	1260.0	249.1			−0.25	9.71×10^{-5}		
Grains—low-fiber grains									
X - 05492	GC-MS	1584.6	122.0			−0.26	5.30×10^{-5}		
X - 16397	LC-MS +	2200.8	248.1			−0.25	1.04×10^{-4}		
Protein									
Fish									
CMPF	LC-MS −	2815.0	239.1	LIP	HMDB61112	0.27	2.00×10^{-5}	0.37	4.00×10^{-9}
X - 02269 (X - 11469)	LC-MS +	1551.0	255.1					0.37	3.00×10^{-9}
DHA (22:6n–3)	LC-MS −	5518.0	327.3	LIP	HMDB02183			0.36	5.00×10^{-9}
X - 12644	LC-MS −	5650.0	524.3					0.28	8.96×10^{-6}
Nuts									
Tryptophan betaine	LC-MS +	2464.0	247.1	AA	HMDB61115	0.41	1.44×10^{-11}	0.36	1.20×10^{-8}
4-Vinylphenol sulfate	LC-MS −	3323.0	199.1	XEN	HMDB04072	0.31	8.97×10^{-7}		
X - 13735	LC-MS +	1514.0	238.2			0.28	7.05×10^{-6}		
X - 04500	GC-MS	1462.0	172.0			0.25	8.37×10^{-5}		
Processed meat									
X - 12855	LC-MS +	1404.0	248.2			0.29	2.79×10^{-6}		
Acetylcarnitine	LC-MS +	1203.0	204.2	LIP	HMDB00201	0.26	4.42×10^{-5}		
X - 11858	LC-MS −	4400.0	437.1					−0.25	1.06×10^{-4}
Red meat									
Acetylcarnitine	LC-MS +	1203.0	204.2	LIP	HMDB00201	0.32	3.05×10^{-7}		
X - 12855	LC-MS +	1404.0	248.2			0.31	8.63×10^{-7}		
Xylitol	GC-MS	1677.6	307.2	CHO	HMDB02917, HMDB00568	0.28	8.24×10^{-6}		
3-Dehydrocarnitine	LC-MS +	1020.0	160.2	LIP	HMDB12154	0.26	5.00×10^{-5}		
Ethyl glucuronide	LC-MS −	1574.1	240.0	XEN	152226	0.25	6.20×10^{-5}		
X - 12212	LC-MS −	3607.0	229.1					−0.27	2.66×10^{-5}
Shellfish									
CMPF	LC-MS −	2815.0	239.1	LIP	HMDB61112	0.26	2.83×10^{-5}	0.38	1.00×10^{-9}
X - 02269 (X - 11469)	LC-MS +	1551.0	255.1					0.38	8.10×10^{-10}
Lysine	GC-MS	1836.7	317.2	AA	HMDB00182	0.25	5.60×10^{-5}		
Ciliatine (2-aminoethylphosphonate)	GC-MS	1707.7	398.2	AA	HMDB11747	0.25	8.20×10^{-5}		

(*Continued*)

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TABLE 3 (*Continued*)

Metabolite/Metabolon, Inc. identification	Platform	RI	*m/z*	Super pathway	Metabolite identification	Urine		Serum	
						r^2	P[3]	r^2	P[3]
2-Hydroxybutyrate	GC-MS	1169.4	130.9	AA	HMDB00008	0.25	9.46×10^{-5}		
Fats and oils—meat fat									
Creatine	LC-MS +	758.0	132.1	AA	HMDB00064	0.30	1.76×10^{-6}		
X - 15461	LC-MS +	2125.0	160.1			0.28	1.27×10^{-5}		
N-acetyltyrosine	LC-MS +	1677.0	222.2	AA	HMDB00866	0.26	4.28×10^{-5}		
N-acetylglutamine	LC-MS −	783.0	187.1	AA	HMDB06029	0.25	7.24×10^{-5}		
X - 16135	LC-MS +	3201.2	510.9					0.26	5.96×10^{-5}
X - 16133	LC-MS +	2640.8	453.3					0.25	6.55×10^{-5}
X - 11470	LC-MS −	4151.0	525.2					−0.25	8.94×10^{-5}
Miscellaneous—salty snacks									
X - 12742	LC-MS −	2534.0	241.2			0.28	9.22×10^{-6}		
X - 11880	LC-MS −	5378.0	537.4					0.27	1.98×10^{-5}
Dietary supplement use (yes or no)									
Multivitamin use									
Pantothenate	LC-MS −	2218.0	220.1	CV	HMDB00210	0.36	1.00×10^{-8}	0.31	6.37×10^{-7}
α-Tocopherol	GC-MS	2305.4	502.5	CV	HMDB01893			0.31	1.06×10^{-6}
Pyridoxate	LC-MS − (urine) or LC-MS + (serum)	2210.0	182.1	CV	HMDB00017	0.30	1.56×10^{-6}	0.38	1.00×10^{-9}
Riboflavin (vitamin B-2)	LC-MS +	3111.0	377.2	CV	HMDB00244	0.27	2.43×10^{-5}		
X - 12095	LC-MS +	1638.0	153.1					0.27	1.73×10^{-5}
X - 12015	LC-MS −	1318.0	216.2			0.26	4.80×10^{-5}		
X - 12094	LC-MS +	1692.0	153.1					0.26	3.80×10^{-5}
Vitamin E[4]									
γ-Tocopherol	GC-MS	2264.0	488.4	CV	HMDB01492			−0.44	6.78×10^{-13}
α-CEHC glucuronide	LC-MS −	2623.1	453.2	CV	477200–36-5	0.40	1.98×10^{-10}	0.27	2.31×10^{-5}
α-Tocopherol	GC-MS	2305.4	502.5	CV	HMDB01893			0.37	3.00×10^{-9}
α-CEHC sulfate (X - 12435)	LC-MS −	3174.0	357.2	CV		0.33	1.90×10^{-7}		

[1] $n = 253$. Partial Pearson correlation analyses adjusted for age (years, continuous), sex, case-control status (case or control), fasting status (yes or no), smoking status (current, former, or never), daily caloric intake (kcal/d), BMI (kg/m^2), education (≤12 y, vocational or 1–3 y college, ≥4 y college, or graduate), and physical activity index (weekly frequency of moderate-intensity leisure activity plus 2 × weekly frequency of vigorous-intensity leisure activity). Metabolite IDs are referenced from the HMDB (http://www.hmdb.ca/metabolites/) and from PubChem (https://pubchem.ncbi.nlm.nih.gov). AA, amino acid; CHO, carbohydrate; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoate; CV, cofactor/vitamin; FDR, false discovery rate; GC-MS, gas chromatography–mass spectrometry; HMDB, Human Metabolome Database; LC-MS, liquid chromatography–mass spectrometry; LIP, lipid; NUC, nucleotide; RI, retention index; X, unknown identity; XEN, xenobiotic; α-CEHC, α-2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman.

[2] $r \geq \pm 0.25$.

[3] All results presented were significant at an FDR <0.1 [$\alpha < 0.1$/(number of metabolites × number of foods)/P value rank].

[4] Controlled for multivitamin use (yes or no).

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TABLE 4
Urine and serum metabolites associated with beverages in the Navy Colon Adenoma Study[1]

Metabolite/Metabolon, Inc. identification	Platform	RI	*m/z*	Super pathway	Metabolite identification	Urine		Serum	
						r^2	P^3	r^2	P^3
Caffeinated coffee									
X - 14473	LC-MS +	3363.0	211.2			0.49	5.53×10^{-16}	0.42	6.92×10^{-12}
X - 14062	LC-MS −	3139.0	283.1			0.48	1.17×10^{-15}		
Quinate	GC-MS	1791.5	345.1	XEN	HMDB03072	0.43	1.41×10^{-12}	0.47	2.33×10^{-14}
X - 17352	LC-MS −	3398.6	495.3			0.43	2.73×10^{-12}		
Paraxanthine	LC-MS +	2444.0	181.2	XEN	HMDB01860	0.42	7.35×10^{-12}	0.30	2.08×10^{-6}
X - 13733	LC-MS −	2186.0	317.0			0.41	1.95×10^{-11}		
Theophylline	LC-MS −	2361.0	179.1	XEN	HMDB01889	0.41	2.91×10^{-11}	0.34	5.80×10^{-8}
X - 12039	LC-MS −	1617.0	174.0			0.41	3.03×10^{-11}	0.37	4.00×10^{-9}
X - 14465	LC-MS +	3306.0	211.2			0.39	1.99×10^{-10}	0.42	6.92×10^{-12}
X - 13840	LC-MS −	1130.0	270.1			0.39	2.54×10^{-10}		
1-Methylxanthine	LC-MS +	1974.0	167.0	XEN	HMDB10738	0.39	3.21×10^{-10}	0.33	1.12×10^{-7}
Trigonelline (*N*′-methylnicotinate)	LC-MS +	757.0	138.1	CV	HMDB00875	0.38	7.04×10^{-10}	0.34	9.50×10^{-8}
X - 12738	LC-MS −	2422.0	231.1			0.38	1.00×10^{-9}		
X - 14082	LC-MS −	4487.0	511.2			0.38	1.00×10^{-9}		
X - 17300	LC-MS +	1475.2	116.2			0.38	1.00×10^{-9}		
X - 12816	LC-MS −	2676.0	495.3			0.36	7.00×10^{-9}	0.33	2.15×10^{-7}
1-Methylurate	LC-MS +	1827.0	183.1	XEN	HMDB03099	0.36	9.00×10^{-9}		
Caffeine	LC-MS +	2820.0	195.1	XEN	HMDB01847	0.36	1.10×10^{-8}	0.34	8.50×10^{-8}
X - 12837	LC-MS −	3605.0	495.2			0.35	1.30×10^{-8}		
1,3-Dimethylurate	LC-MS − (serum) and LC-MS + (urine)	2161.0	197.1	XEN	HMDB01857	0.35	1.40×10^{-8}	0.26	5.25×10^{-5}
5-Acetylamino-6-formylamino-3-methyluracil	LC-MS +	1547.0	227.1	XEN	HMDB11105	0.33	1.33×10^{-7}		
X - 04524	GC-MS	2071.3	210.1			0.33	1.67×10^{-7}		
X - 05507	GC-MS	1678.8	210.0			0.32	3.29×10^{-7}		
1,7-Dimethylurate	LC-MS −	1607.0	195.1	XEN	HMDB11103	0.32	5.01×10^{-7}	0.30	2.77×10^{-6}
X - 05426	GC-MS	1792.1	245.1			0.32	4.18×10^{-7}	0.32	4.18×10^{-7}
X - 12230	LC-MS −	3360.0	217.1			0.31	5.58×10^{-7}	0.34	7.10×10^{-8}
Nicotinate	GC-MS	1334.1	180.0	CV	HMDB01488	0.31	7.73×10^{-7}		
X - 13741	LC-MS −	2782.0	203.0			0.29	3.65×10^{-6}	0.29	3.86×10^{-6}
Catechol sulfate	LC-MS −	1928.0	188.9	XEN	HMDB59724			0.29	5.55×10^{-6}
X - 17320	LC-MS +	2715.6	184.1			0.29	4.32×10^{-6}		
X - 13703	LC-MS −	2172.0	367.1			0.28	6.46×10^{-6}		
N-(2-furoyl)glycine	LC-MS +	2076.0	170.1	XEN	HMDB00439	0.28	9.59×10^{-6}	0.26	4.75×10^{-5}
X - 11429	LC-MS −	1151.0	245.1			−0.28	1.17×10^{-5}		
X - 13844	LC-MS −	1425.0	209.1			0.28	1.22×10^{-5}		
Hippurate	LC-MS +	2802.0	180.1	XEN	HMDB00714	0.28	1.22×10^{-5}		
5-Acetylamino-6-amino-3-methyluracil	LC-MS −	1025.0	197.1	XEN	HMDB04400	0.27	1.32×10^{-5}		
X - 17303	LC-MS +	1916.8	176.1			0.27	1.34×10^{-5}		
X - 13706	LC-MS −	2588.0	367.1			0.27	1.69×10^{-5}		
X - 13462	LC-MS −	1594.0	289.1			−0.26	3.82×10^{-5}		
X - 12329	LC-MS −	1867.0	188.1			0.26	4.86×10^{-5}	0.29	4.64×10^{-6}
1,3,7-Trimethylurate	LC-MS −	1988.0	209.1	XEN	HMDB02123	0.25	6.84×10^{-5}	0.30	2.36×10^{-6}

(Continued)

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TABLE 4 (*Continued*)

Metabolite/Metabolon, Inc. identification	Platform	RI	*m/z*	Super pathway	Metabolite identification	Urine		Serum	
						r^2	P^3	r^2	P^3
Pseudouridine	LC-MS −	1104.0	243.1	NUC	HMDB00767	−0.25	9.45×10^{-5}		
Homovanillate sulfate	LC-MS −	1050.0	261.1	AA	HMDB11719	−0.25	9.47×10^{-5}		
X - 17185	LC-MS −	3069.1	215.2			0.25	1.07×10^{-4}	0.37	4.00×10^{-9}
Decaffeinated coffee									
1,7-Dimethylurate	LC-MS −	1607.0	195.1	XEN	HMDB11103			−0.26	3.90×10^{-5}
3-Methoxytyrosine	LC-MS +	1765.0	212.1	AA	HMDB01434			−0.25	1.11×10^{-4}
Tea									
X - 17345	LC-MS −	1544.0	215.2			0.38	1.00×10^{-9}		
X - 17440	LC-MS −	2431.5	287.2			−0.26	5.14×10^{-5}		
X - 16580	LC-MS +	1681.8	222.0			−0.25	8.14×10^{-5}		
X - 17324	LC-MS +	3320.5	342.3			−0.25	1.08×10^{-4}		
Sugar-sweetened beverages[4]									
Methylglutaroylcarnitine (3-methylglutarylcarnitine)	LC-MS +	1900.0	290.1	AA	HMDB00552			0.25	6.91×10^{-5}
X - 17145	LC-MS −	3844.7	257.2					−0.25	1.15×10^{-4}
X - 17351	LC-MS +	3914.4	245.1			−0.25	6.98×10^{-5}		
Beer									
X - 12556	GC-MS	1374.0	116.9			−0.25	7.27×10^{-5}		
Glycerol 3-phosphate	GC-MS	1719.7	357.1	LIP	HMDB00126	−0.25	7.83×10^{-5}		
Homovanillate sulfate	LC-MS −	1050.0	261.1	AA	HMDB11719	−0.25	9.50×10^{-5}		
Liquor									
Ethyl glucuronide	LC-MS +	1574.1	240.0	XEN	152226	0.29	2.75×10^{-6}	0.26	3.69×10^{-5}
Wine									
X - 17306	LC-MS +	2202.6	290.2			0.31	7.71×10^{-7}		
2,3-Dihydroxyisovalerate	GC-MS	1422.0	131.0	CV, XEN	HMDB12141	0.31	1.16×10^{-6}		
2-Isopropylmalate	LC-MS −	732.0	175.2	XEN, CHO	HMDB00402	0.26	3.98×10^{-5}		
Nicotine	LC-MS +	1284.8	163.2	XEN	HMDB01934	0.26	4.26×10^{-5}		
Total alcohol									
X - 11799	LC-MS +	1572.0	226.0			0.27	1.51×10^{-5}		
X - 13529	LC-MS +	3088.0	190.1			−0.26	4.08×10^{-5}		
X - 15646	LC-MS −	2888.0	343.2			−0.25	7.28×10^{-5}		

[1] $n = 253$. Partial correlation analyses adjusted for age (years, continuous), sex, case-control status (case or control), fasting status (yes or no), smoking status (current, former, or never), daily caloric intake (kcal/d), BMI (kg/m^2), education (≤12 y, vocational or 1–3 y college, ≥4 y college, or graduate), and physical activity index (weekly frequency of moderate-intensity leisure activity plus 2 × weekly frequency of vigorous-intensity leisure activity). Metabolite IDs are referenced from the HMDB (http://www.hmdb.ca/metabolites/) and from PubChem (https://pubchem.ncbi.nlm.nih.gov). AA, amino acid; CHO, carbohydrate; CV, cofactor/vitamin; FDR, false discovery rate; GC-MS, gas chromatography–mass spectrometry; HMDB, Human Metabolome Database; LC-MS, liquid chromatography–mass spectrometry; LIP, lipid; NUC, nucleotide; RI, retention index; X, unknown identity; XEN, xenobiotic.

[2] $r \geq \pm 0.25$.

[3] All results presented were significant at an FDR <0.1 [$\alpha < 0.1$/(number of metabolites × number of foods)/*P* value rank].

[4] Excludes juice.

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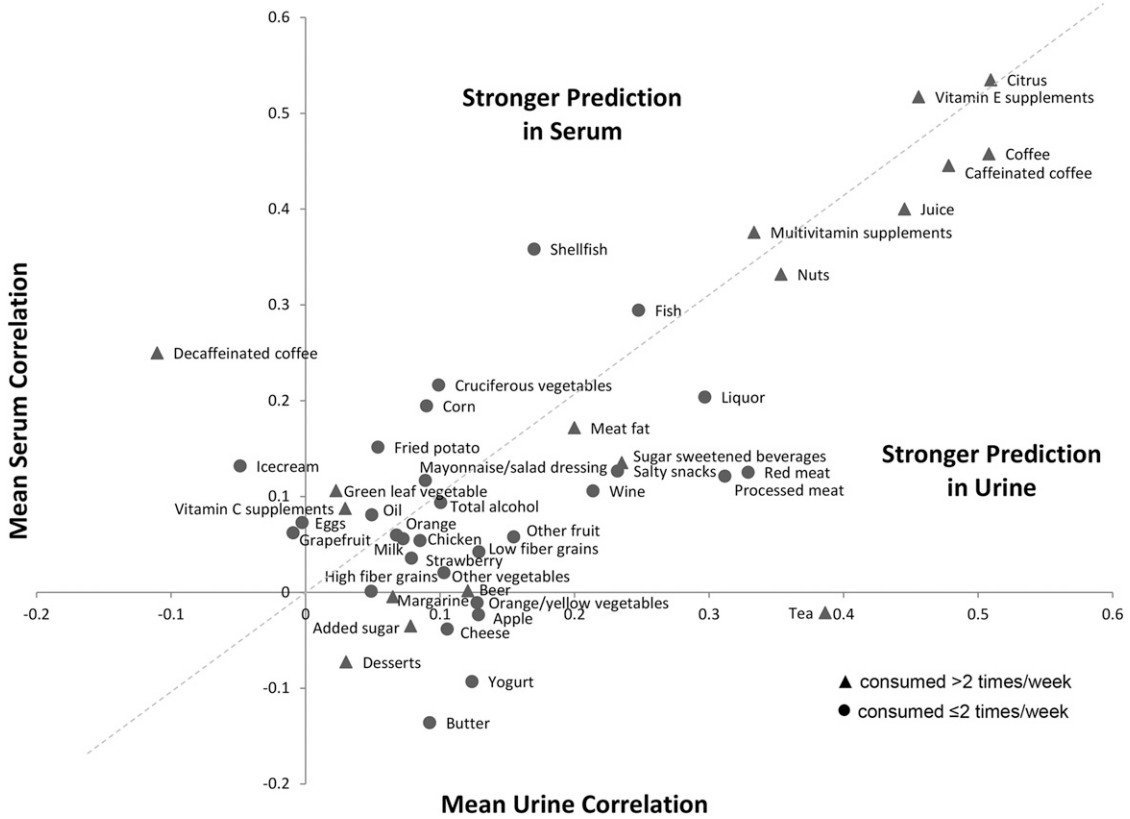

FIGURE 1 Accuracy of multiple-metabolite profiles for predicting dietary intake amounts in serum compared with urine in the Navy Adenoma Study (n = 253). Data represent the correlation between observed dietary intake and predicted intake based on 10-fold crossvalidated LASSO regression of metabolites at the dietary intake level. Metabolites were adjusted residually for age, sex, smoking history, fasting status, case-control status, BMI, education, physical activity, and daily caloric intake. Residual-adjusted metabolite values were used for the LASSO analysis. Training and validation were conducted in a random 80% of the sample; testing was conducted in the remaining 20%. This process was repeated 10 times with the use of different random data splits. Final estimates averaged correlations between observed and predicted dietary intake levels. Correlations of urine and serum metabolites with food intake differed significantly for apples, butter, and tea ($P < 0.05$). LASSO, least absolute shrinkage and selection operator.

invasively collected than blood, is less expensive to obtain, and affords greater collection volumes, making it a potentially highly useful biological resource for large clinical and population studies. However, because urine metabolite concentrations can be highly variable because of ionic strength, pH, osmolarity, and dilution, it is sometimes assumed that urine provides less valuable data on usual biomarker status, including for diet-related metabolites (44). To our knowledge, few epidemiologic studies have tested this assumption by comparing blood and urine for their usefulness in identifying stable and reliable candidate dietary biomarkers with the use of metabolomics.

Our analyses identified, to our knowledge, novel diet–metabolite associations measured from human samples (predominantly urine). These associations require replication but may suggest new hypotheses. For example, TMAO is produced from the metabolism of L-carnitine (high in red meat) to trimethylamine by intestinal microbiota, with conversion to TMAO in the liver (45). We found that TMAO was associated with added sugar intake (r = 0.23). Sucrose previously has been shown to modify the gut microbiome (46, 47); thus, our findings possibly could indicate an indirect sugar effect on TMAO via changes to the gut microbiome.

Our findings for specific food-metabolite associations replicate ≥31 associations previously reported from clinical and population studies, including for fruit and juice (13–15, 48–52), red meat (13, 53–55), fish (15, 51, 56), nuts (15, 51), liquor and wine (15, 57), coffee (15, 16, 51, 58, 59), and multivitamin supplement (15) intake. Our serum findings parallel those reported by the Atherosclerosis Risk in Communities study (n = 1977) (51) and the Prostate, Lung, Colorectal, and Ovarian Cancer screening trial (n = 502) (15) for metabolites associated with citrus (stachydrine, chiro-inositol, scyllo-inositol, and N-methyl proline), coffee (trigonelline, quinate, paraxanthine, 1-methylxanthine, and caffeine), fish (CMPF), nuts (tryptophan betaine), alcohol (ethyl glucuronide) and multivitamins (pyridoxate and pantothenate).

In addition to our findings for metabolites with known chemical identity, we also found significant diet correlations with currently unidentified metabolites, predominantly from urine. Some unknown metabolites had notably strong correlations with diet items of interest—e.g., tea, red meat, and caffeinated coffee—that warrant follow-up in future studies. Updated metabolomics platforms capable of identifying the chemical structure of more metabolites than the platforms used for the current analysis may detect a greater diversity of candidate biomarkers for exposures that are currently underrepresented, such as fruit and vegetables.

Serum and urine capture complementary information about different aspects of food metabolism; as a result, the specific metabolites correlated with each food may vary by biospecimen. We found that approximately one-half of the strongest diet correlations were observed with both serum and urine metabolites; the

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remainder were unique to one biofluid, primarily urine. However, more serum metabolites were correlated $r \geq \pm 0.25$ with fish and dietary supplement intake. Urine metabolites often reflect nutrient excretion after degradation or detoxification, whereas serum metabolite concentrations may be regulated for homeostasis (6, 60). Detectability in a biofluid also may be influenced by metabolite half-life, lipid solubility, gut absorption site, and bile secretion and reabsorption (61–63), in addition to the metabolomics platform detection limit. For example, red meat was associated with urine acetylcarnitine, but not serum acetylcarnitine. Acetylcarnitine is hydrolyzed rapidly in the blood; rising blood concentrations of precursor metabolites trigger increased reabsorption and urinary clearance, preventing substantial accumulation in the blood (64). Excretion site also influences detection. We found that caffeinated coffee was associated with serum catechol sulfate, but not urinary catechol sulfate. Catechol, a derivative of coffee processing, is conjugated to catechol sulfate to facilitate absorption (65, 66). Conjugated polyphenol metabolites generally are eliminated in feces (61).

Suitable candidate nutritional biomarkers may be indicated by strong positive diet–metabolite correlations that are unique to certain foods. A substantial fraction of the dietary metabolites we identified were phytochemicals (classified here as food or plant xenobiotic), which are food constituents and therefore are of particular interest for further follow-up. Consumption of some foods may reduce concentrations of endogenous metabolites, yielding negative diet–metabolite correlations. We found that vitamin E use was positively correlated with known vitamin E biomarkers [α-tocopherol ($r = 0.37$) and α-CEHC glucuronide ($r = 0.27$)], but had a stronger inverse correlation with γ-tocopherol ($r = -0.44$). Vitamin E is a collective term for 8 tocopherols and tocotrienols. The administration of α-tocopherol, the most common form in dietary supplements, has been shown to decrease circulating γ-tocopherol in healthy adults, because the α-tocopherol isomer is preferentially circulated to protect lipoproteins from peroxidation, whereas γ-tocopherol is metabolized in the liver and excreted in urine or feces (67).

Study strengths include identification of a large number of metabolites in both serum and urine from the same individuals with high reliability. There are several limitations. Results were based on a self-reported FFQ with a known measurement error that likely attenuated the observed correlations. The period of exposure measurement (12 mo) also may be weakly correlated with recent intake, although our primary aim was to identify candidate biomarkers of foods that would be reflected in biospecimens with regular, habitual intake. Serum and urine samples were collected on different days without control for time of day, introducing additional metabolite variability and possibly increasing the likelihood that prediction differed from chance; however, both biofluids identified many of the same diet–metabolite associations. In addition, 23% of participants provided fasting serum samples; in agreement with some previous studies (68, 69), we found limited influence of fasting status on metabolites. Our sample included people with colorectal adenoma, which could in theory limit generalizability, but we did not find any differences in diet–metabolite associations between study groups. Metabolites were based on a single sample collection, and reliability may be improved with multiple measurements. Metabolite peak intensity concentrations account for gut microbiota influences and genetic effect modification of metabolic processes. We were unable to evaluate these factors, which could influence the extent to which a metabolite can act as a universal dietary biomarker (70). Our sample was primarily non-Hispanic white, and, therefore, results may not generalize to more diverse racial groups. Finally, we cannot rule out that some findings may be from chance, but we attempted to minimize this by controlling for multiple comparisons.

In conclusion, metabolites are correlated with habitual diet in both serum and urine with similar magnitudes. Serum and urine provide complementary information about food metabolism. Urine samples may offer a valid alternative or complementary addition to serum for nutritional metabolomics analyses in large-scale clinical and epidemiologic studies.

The authors' responsibilities were as follows—MCP, JNS, RS, NR, and SCM: designed the research; AJC, RS, KAG, KAM and NR: provided essential materials; MCP and JNS: performed the statistical analysis; MLI, STM, and RS-S: advised on statistical analysis and interpretation of the findings; MCP: wrote the paper and had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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