Metabolic Signatures of Healthy Lifestyle Patterns and Colorectal Cancer Risk in a European Cohort

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BACKGROUND & AIMS: **Colorectal cancer risk can be lowered by adherence to the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) guidelines. We derived metabolic signatures of adherence to these guidelines and tested their associations with colorectal cancer risk in the European Prospective Investigation into Cancer cohort.**

METHODS: **Scores reflecting adherence to the WCRF/AICR recommendations (scale, 1–5) were calculated from participant data on weight maintenance, physical activity, diet, and alcohol among a**

Abbreviations used in this paper: BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition; OCFA, odd chain fatty acid; OR, odds ratio; PC, phosphatidylcholine; PLSR, partial least-squares regression; pLV1, _____; SFA, saturated fatty acid; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.

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discovery set of 5738 cancer-free European Prospective Investigation into Cancer participants with metabolomics data. Partial least-squares regression was used to derive fatty acid and endogenous metabolite signatures of the WCRF/AICR score in this group. In an independent set of 1608 colorectal cancer cases and matched controls, odds ratios (ORs) and 95% CIs were calculated for colorectal cancer risk per unit increase in WCRF/AICR score and per the corresponding change in metabolic signatures using multivariable conditional logistic regression.

RESULTS: **Higher WCRF/AICR scores were characterized by metabolic signatures of increased odd-chain fatty acids, serine, glycine, and specific phosphatidylcholines. Signatures were inversely associated more strongly with colorectal cancer risk (fatty acids: OR, 0.51 per unit increase; 95% CI, 0.29–0.90; endogenous metabolites: OR, 0.62 per unit change; 95% CI, 0.50–0.78) than the WCRF/AICR score (OR, 0.93 per unit change; 95% CI, 0.86–1.00) overall. Signature associations were stronger in male compared with female participants.**

CONCLUSIONS: **Metabolite profiles reflecting adherence to WCRF/AICR guidelines and additional lifestyle or biological risk factors were associated with colorectal cancer. Measuring a specific panel of metabolites representative of a healthy or unhealthy lifestyle may identify strata of the population at higher risk of colorectal cancer.**

Keywords: Colorectal Neoplasm; Risk Factors; World Cancer Research Fund/American Institute for Cancer Research Recommendations; Targeted Metabolomics.

Colorectal cancer is one of the most common neoplasms, with approximately 1.8 million new cases and 860,000 deaths reported worldwide in 2018.[1] Established risk factors for colorectal cancer include adiposity, smoking, adult attained height, and high intake of alcohol and red and processed meat, whereas physical activity and high intakes of whole grains, fish, and dairy products may protect against the disease.[2] Therefore, individuals may be able to minimize their risk of colorectal cancer by following a healthy lifestyle and many thousands of cases per year could be avoided.

The World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) issues continuously updated recommendations on diet, physical activity, and weight management for the prevention of cancer, based on all available evidence.[3] At their core are healthy behaviors in relation to weight maintenance, physical activity, and intakes of red and processed meat, fruit and vegetables, fiber, and alcohol. A summary score has been developed to measure individual adherence to recommendations.[4] Higher scores have since been found to be associated with colorectal cancer risk[4–8] and cancer-specific and overall mortality.[6]

Unhealthy lifestyle behaviors and low WCRF/AICR scores may increase the risk of colorectal cancer through adverse effects upon systemic metabolism. Although tumorigenesis is promoted by adiposity, hyperinsulinemia, and chronic inflammation,[9] the systemic metabolic changes that precede or precipitate these physiological states remain unclear. To identify specific metabolite patterns associated with lifestyle factors and then to investigate whether they may play a role in colorectal cancer development, we used an extensive set of participants for whom targeted metabolomics and fatty acid data had been acquired within the European Prospective Investigation into Cancer and Nutrition cohort (EPIC). The objective of this analysis was first to characterize metabolic signatures of the WCRF/AICR score in a large group of cancer-free controls and to identify which compounds contributed to these signatures, and, second, to determine whether these metabolic signatures in prediagnostic blood samples were associated with subsequent colorectal cancer development.

Materials and Methods

The European Prospective Investigation Into Cancer Cohort and Collection of Data and Samples

EPIC is a multicenter prospective cohort that was established to investigate risk factors for cancer and other chronic diseases. More than 520,000 healthy subjects were enrolled between 1992 and 2000 from 23 EPIC administrative centers in 10 European countries. The collection of participant data and biospecimens has been described previously.[10] WCRF/AICR scores were calculated for all participants from recommendations on weight maintenance, physical activity, intake of food and drinks that promote weight gain, intake of plant-based foods, intake of animal-based foods, alcohol intake, and breastfeeding (Supplementary Table 1). Although the recommendations were updated in 2018,[11] we retained the scores previously calculated in EPIC.[4] These ranged from 0 to 6 for men and from 0 to 7 for women and were grouped into quintiles for statistical modeling. The data and samples used were from all EPIC countries except Greece. Approval for the study was obtained from the International Agency for Research on Cancer and the ethical review boards of the participating institutes. All participants provided written informed consent.

Metabolomics Study Design

This analysis used a discovery set of 5738 cancer-free control participants, originating from several non-colorectal case–control studies nested within the EPIC cohort, to derive metabolic signatures of the WCRF/AICR score (ie, the linear combination of metabolites optimally related to the score). Fasted plasma and serum samples from the discovery set of controls were analyzed for either 34 fatty acids extracted from phospholipid fractions (n = 4239) or 155 endogenous metabolites assayed by the Biocrates Absolute*IDQ* P150/P180 Kit (n = 1741; Biocrates Life Sciences AG, Innsbruck, Austria). These 2 analyses are referred to as *fatty acids* and *endogenous metabolites* throughout this article. Metabolic signatures were determined separately for the 2 analyses by multivariate partial least-square regression (PLSR) models. Metabolite-predicted scores then were determined for each participant in the nested colorectal case–control study (n = 1608 cases and 1608 matched controls) for whom fatty acid or endogenous data were available, and these were regarded as the magnitude of the metabolic signature. All case–control participants had been analyzed for endogenous metabolites, while a subset of 438 cases and 438 matched controls additionally were analyzed for fatty acids. Associations between colorectal cancer risk and fatty acid signature, endogenous metabolic signature, and WCRF/AICR score then were tested separately in multivariable-adjusted models. The study design is illustrated in Figure 1.

Follow-Up Evaluation for Colorectal Cancer Incidence

Incident cases of colorectal cancer were identified from health insurance records, contact with cancer and pathology registries, and the active follow-up evaluation of participants. Cases were defined using the International Classification of Diseases, 10th revision, and the International Classification of Diseases for Oncology, 2nd revision. Cases were incidence-density matched to cancer-free controls by age and year of sampling, sex, study center, follow-up time since blood collection, fasting status, and, when relevant, menopausal status and phase of menstrual cycle at blood collection.

Acquisition of Metabolomics Data

Saturated fatty acids (SFAs), monounsaturated fatty acids, polyunsaturated fatty acids, industrial trans fatty acids, and natural trans fatty acids were extracted from plasma phospholipid fractions and quantified by gas chromatography.[12] For endogenous metabolites, the Biocrates Absolute*IDQ* p150 or p180 Kits were used to measure concentrations of amino acids, biogenic amines, hexose sugars, acylcarnitines, sphingolipids (sphingomyelins), phosphatidylcholines (PC), and lysophosphatidylcholines in serum or plasma, following the recommended procedure.[13,14] See the Supplementary Methods section for further details of analytical methodology.

What You Need to Know

Background

The World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) score is a composite of diet and lifestyle variables and has been found to be associated inversely with colorectal cancer risk in previous studies.

Findings

Blood fatty acid and endogenous metabolite signatures of the WCRF/AICR score derived from a discovery set 5738 of cancer-free participants were associated more strongly with colorectal cancer risk than the WCRF/AICR score as calculated from baseline participant data in a study of 1608 colorectal cancer cases and 1608 matched controls.

Implications for patient care

Metabolic signatures of the WCRF/AICR score may capture etiologic risk factors for colorectal cancer beyond the score itself and provide insight into metabolic changes that precede cancer development. If replicated, measurement of these metabolite signatures could help identify strata of the population at higher risk of colorectal cancer.

Statistical Analysis

Determination of metabolic signatures. Discovery set metabolite data were $\log_2$ transformed, scaled, and missing values were imputed with minimum values. The resulting matrices were transformed to the residuals of a linear model on sex, batch, center (fixed effects), and study (random effects). Metabolic signatures were derived as the loadings (coefficients) on the first latent variable of a PLSR model, denoted p_{LV1}, with metabolites as predictors and WCRF/AICR score as the response. The validated PLSR models then were used to predict WCRF/AICR scores in the case–control study on a continuous scale of 1 to 5. Pearson correlations between metabolite concentrations also were calculated in a subset of participants. See the Supplementary Methods section for further details.

Association of metabolic signatures of World Cancer Research Fund/American Institute for Cancer Research score with adherence to recommendations and colorectal cancer risk. Partial Pearson correlations were calculated between metabolic signatures and adherence to the 6 individual components of the WCRF/AICR score (as given earlier, each on a scale of 0, 0.5, or 1), adjusting for height, highest education level attained, and smoking

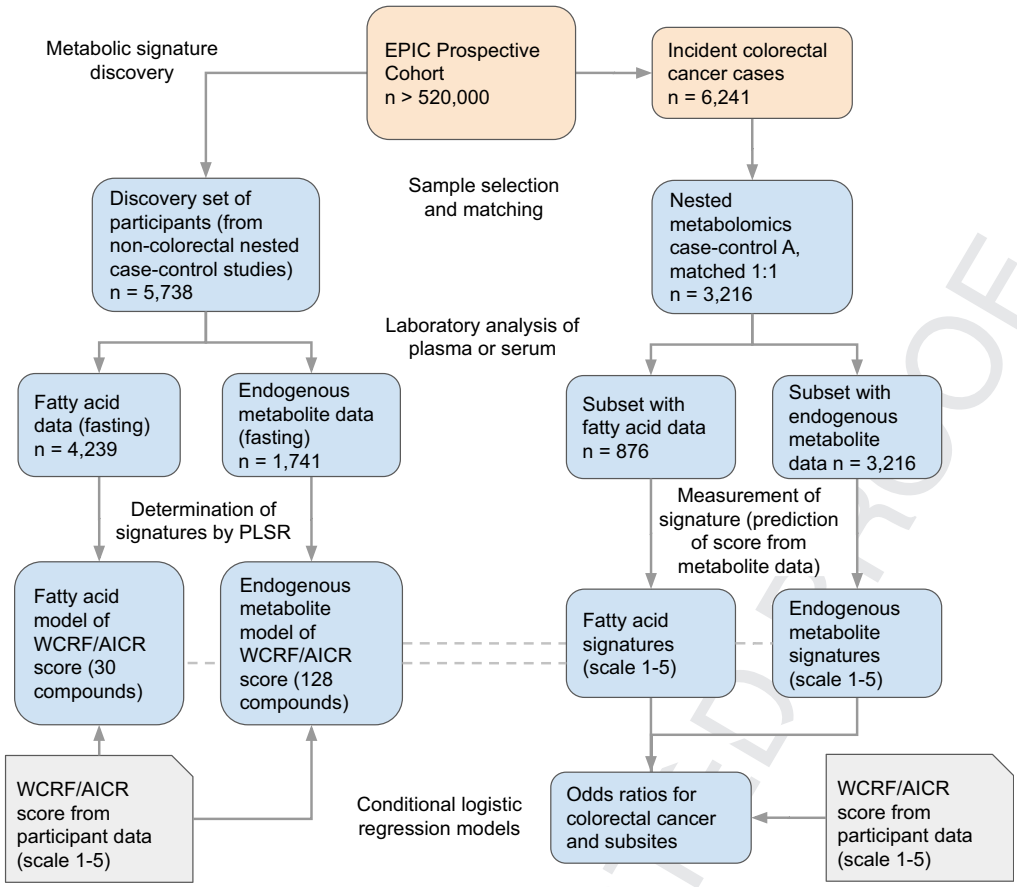

Figure 1. Overview of the study design. An independent set of healthy controls (*left*) was used to derive metabolic signatures of the WCRF/AICR score, which then were used to predict score categories in the nested case–control study (*right*). EPIC, European Prospective Investigation into Nutrition and Cancer; PLSR, partial least-squares regression; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.

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status and intensity. Odds ratios and 95% CIs were calculated for risk of colorectal cancer and subsites with a metabolic signature or WCRF/AICR score as the main explanatory variable in multivariable conditional logistic regression models. Additional models were fit for individual WCRF/AICR components. Sensitivity analyses also were performed, additionally adjusting for smoking duration, intake of dairy products, or, in signature models only, WCRF/AICR score. Extra analyses were performed for strata of follow-up time and, for signature only, body mass index (BMI) and WCRF/AICR score. All analyses were performed using R statistical software, version 3.6.2.

Results

Characteristics of Nested Case–Control Study Participants

Participant characteristics for the nested case–control study are shown in Table 1. Cases were followed up for an average of 7.7 years before a colorectal cancer diagnosis. Cases had a higher BMI and larger waist circumference than controls at baseline, were taller, and attained lower WCRF/AICR scores. Participant characteristics for the discovery set are shown in Supplementary Table 2.

Metabolomics Data and Metabolic Signatures of World Cancer Research Fund/American Institute for Cancer Research Score

A total of 155 endogenous metabolites and 34 fatty acids were measured in both discovery and case–control data sets (Supplementary Table 3). Many high correlations ($r > 0.9$) were noted within metabolite classes (Supplementary Figure 1), but fewer were noted between compounds from fatty acid and endogenous metabolite platforms, with r greater than 0.6 for only 25 of 4964 possible correlations (Figure 2*A* and Supplementary Table 4). In the discovery set, the case–control study of origin contributed most variability to endogenous metabolite profiles ($R_{partial}^2 = 20.3\%$) (Supplementary Figure 2), while the study center explained most variability in fatty acid profiles ($R_{partial}^2 = 3.0\%$).

After exclusion of compounds with insufficient detection rates or high coefficient of variations, 128 endogenous compounds and 30 fatty acids remained for the derivation of metabolic signatures. Of these, SFAs 17:0 and SFAs 15:0 ($p_{LV1} = 0.149$ and 0.076, respectively) were increased most markedly in the fatty acid signature of high WCRF/AICR scores (Table 2 and Figure 2*B*), while monounsaturated fatty acids 16:1n-7/n-9 and SFAs 16:0 were most diminished ($p_{LV1} = -0.058$ and -0.043, respectively). The endogenous metabolic

Table 1. Characteristics of the Colorectal Cancer Cases and Matched Controls in EPIC

	Controls	Cases	*P* value[a]
N	1608	1608	
Sex			
Male	730 (45.4)	730 (45.4)	–
Female	878 (54.6)	878 (54.6)	
Age at blood collection, *y*	56.8 ± 7.5	56.9 ± 7.5	.74
Time to diagnosis, *y*	–	7.7 ± 4.4	–
Country			
France	52 (3.2)	52 (3.2)	–
Italy	387 (24.1)	387 (24.1)	
Spain	317 (19.7)	317 (19.7)	
United Kingdom	243 (15.1)	243 (15.1)	
The Netherlands	139 (8.6)	139 (8.6)	
Germany	163 (10.1)	163 (10.1)	
Denmark	307 (19.1)	307 (19.1)	
Tumor site			
Proximal colon	–	599 (37.7)	–
Distal colon	–	657 (41.3)	
Rectum	–	233 (14.7)	
Other	–	100 (6.3)	
Unknown	–	19 (1.2)	
Confirmed histologic verification			
Yes	–	1387 (86.3)	–
No	–	221 (13.7)	
Smoking status			.06
Nonsmoker	759 (47.2)	683 (42.5)	
Never smoker	480 (29.9)	519 (32.3)	
Smoker	353 (22.0)	390 (24.3)	
Height, *cm*	165.6 ± 9.3	166.1 ± 9.3	.008
BMI, kg/m^2	26.4 ± 3.9	27.0 ± 4.4	<.001
Waist circumference, *cm*	88.0 ± 12.2	90.4 ± 13.2	<.001
Total energy intake, *kcal*	2177 ± 643	2160 ± 702	.41
Physical activity, *MET*	87.7 ± 52.7	84.3 ± 52.6	.66
Alcohol intake, *g/d*	15.0 ± 18.9	16.7 ± 21.5	.09
WCRF/AICR score	2.54 ± 1.02	2.46 ± 1.02	.03
Fatty acid metabolic signature	2.64 ± 0.41	2.59 ± 0.42	<.001
Endogenous metabolic signature	2.51 ± 0.27	2.47 ± 0.30	.015

NOTE. Means and SD or frequency and percentage are shown unless stated otherwise.
BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition cohort; MET, metabolic equivalent of task; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.
[a]*P* value for paired *t* test, Wilcoxon signed-rank test, or chi-squared test. Matching factors were age, sex, study center, follow-up time since blood collection, fasting status, menopausal status, and phase of menstrual cycle at blood collection.

signature of the WCRF/AICR score was dominated by phosphatidylcholines (PCs). Lysophosphatidylcholines a 17:0, PC ae 40:6 and PC ae C36:2 were most increased for high scores (p_{LV1} = 0.035, 0.032, and 0.032, respectively), while PC aa C32:1 and PC aa C38:4 were most diminished (p_{LV1} = -0.037 and -0.034, respectively).

Association Between Metabolic Signatures, World Cancer Research Fund/American Institute for Cancer Research Score Components and Colorectal Cancer Risk

Both metabolic signatures were correlated significantly with adherence to the weight maintenance and alcohol avoidance recommendations (Figure 2*C*). Fatty acid signatures captured the alcohol guideline to the greatest extent (r = 0.43) and endogenous metabolite weight maintenance (r = 0.33). A 1-unit increase in the fatty acid signature was associated with a 49% lower risk of colorectal cancer (odds ratio [OR], 0.51 per unit increase; 95% CI, 0.29–0.90), while a 1-unit increment in the endogenous metabolic signature (scale, 1–5) was associated with a 38% lower risk of colorectal cancer (OR, 0.62 per unit; 95% CI, 0.50–0.78). In comparison, a 1-unit increase in the WCRF/AICR score was associated with a 7% lower risk in the whole case–control study (OR, 0.93 per unit; 95% CI, 0.86–1.00) (Table 3). For comparison, associations between adherence to individual WCRF/AICR components and colorectal cancer risk are shown in Supplementary Table 5. By anatomic subsite, a 1-unit increment in the metabolic signature of endogenous metabolites was associated with a 35% lower risk of colon cancer (OR, 0.65 per unit; 95% CI, 0.50–0.84) and a 56% lower risk of rectal cancer (OR, 0.44 per unit; 95% CI, 0.25–0.79). As an additional analysis, when signature models additionally were adjusted for the WCRF/AICR score, the association between colorectal cancer risk and the fatty acid signature lost statistical significance (OR, 0.59 per unit; 95% CI, 0.33–1.07), whereas the association for the endogenous metabolic signature was not changed appreciably (OR, 0.62 per unit; 95% CI, 0.49–0.79). Sensitivity analyses are presented in Supplementary Table 6.

Discussion

In this analysis, we have derived fatty acid and endogenous metabolite signatures associated with the WCRF/AICR score from a large group of cancer-free control participants. Signatures were characterized by specific profiles of odd chain fatty acids (OCFAs), PCs, and amino acids, and principally captured the weight management and alcohol avoidance aspects of the WCRF/AICR guidelines. Both signatures were associated more strongly with colorectal cancer risk than the traditional WCRF/AICR score in the same participants. Measuring these signatures could provide a more sensitive assessment of colorectal cancer risk than questionnaire data and physical measurements alone because they may encompass a greater range of lifestyle

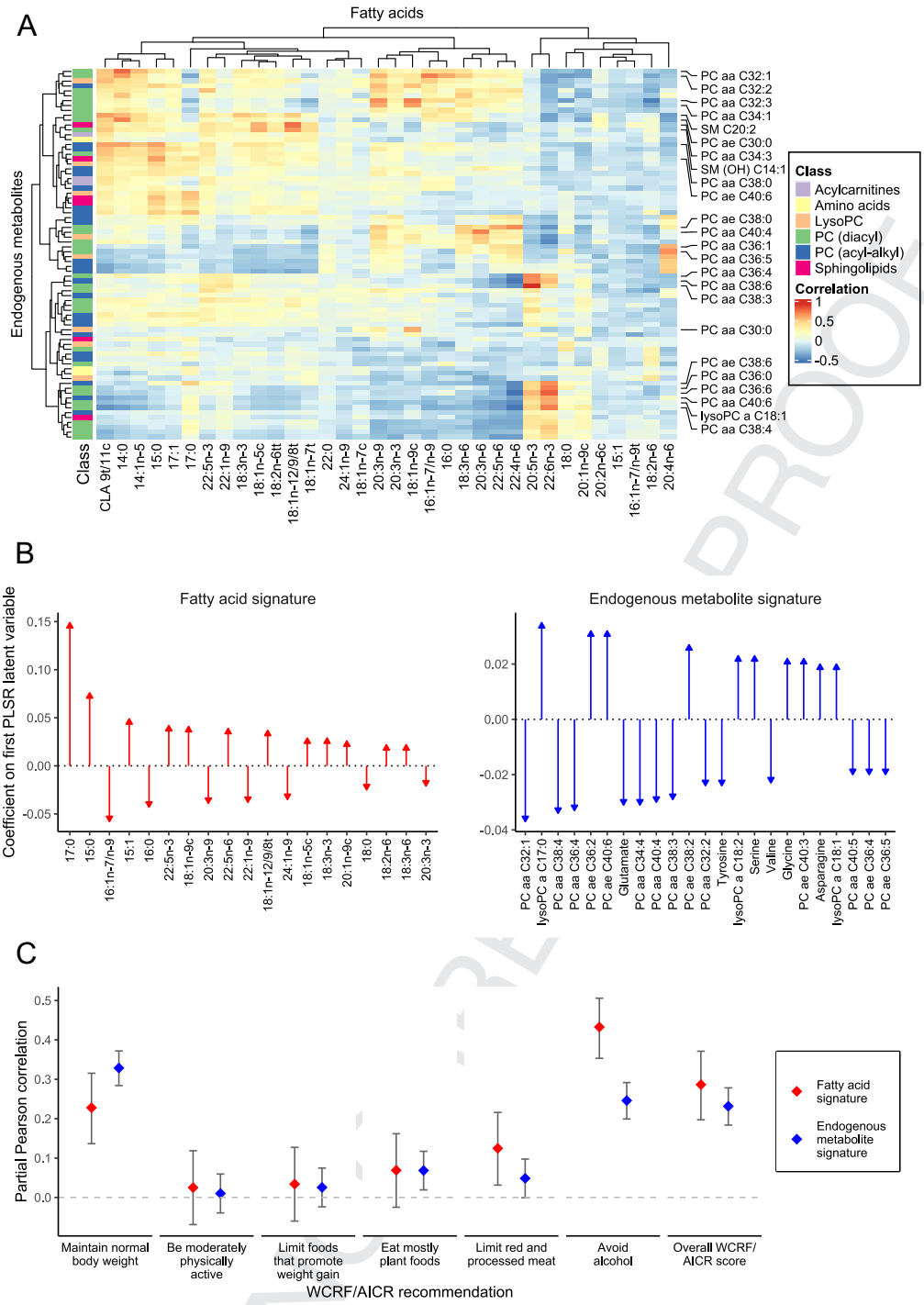

Figure 2. (*A*) Pearson correlations between fatty acids and endogenous metabolites in 439 control participants. Endogenous metabolites with no correlations greater than 0.25 with fatty acids have been omitted. (*B*) Strongest components of fatty acid and endogenous metabolite signatures of high WCRF/AICR scores in order of coefficient magnitude in PLSR models. (*C*) Partial correlations between individual WCRF/AICR recommendation scores and metabolic signatures in control participants. Partial correlations were adjusted for height, energy intake, highest educational level attained, smoking status, and smoking intensity. lyso PC, lysophosphatidylcholine; PC, phosphatidylcholine; PLSR, partial least-squares regression; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.

behaviors and characteristics than captured by the WCRF/AICR recommendations.

Adherence to the WCRF/AICR guidelines has been associated with a reduced risk of colorectal cancer in EPIC and other cohorts. Previous studies have used custom weightings for score components; for example, to best capture colorectal cancer–specific risk factors.[7] We weighted score components evenly to characterize the metabolic profiles that accompany general cancer-preventing or cancer-promoting lifestyles. In terms of individual compounds, OCFA 17:0 and 15:0 were strikingly influential in the fatty acid signature. OCFAs originate from dairy fat and significant correlations between total OCFAs and dairy product intakes have been reported previously.[15,16] However, adjustment for total dairy product intake in our analysis changed risk estimates minimally. Other factors also may affect circulating OCFAs, such as alcohol[16] and fiber intake via de novo formation from propionate.[17] OCFAs also have been associated positively with a lower incidence of type 2

Table 2. Compounds Contributing Most to Metabolic Signatures of WCRF/AICR Score by Coefficient in the First PLSR Latent Variable

Components of metabolic signature	Metabolite subclass or description	Coefficient from first LV of PLSR model, p_{LV1}[a]	OR (95% CI) for association with colorectal cancer[b]
Fatty acids[c]			
Increased for higher WCRF/AICR scores			
17:0	Saturated FA (odd chain)	0.149	**0.81 (0.71–0.99)**
15:0	Saturated FA (odd chain)	0.076	**0.78 (0.65–0.93)**
15:1	Monounsaturated FA	0.049	0.99 (0.85–1.16)
22:5n-6	Polyunsaturated FA	0.042	0.95 (0.80–1.13)
18:1n-9c	Monounsaturated FA	0.041	1.07 (0.92–1.26)
PC ae C40:3	Phosphatidylcholines, acyl-alkyl	0.022	0.84 (0.65–1.08)
Diminished for higher WCRF/AICR scores			
16:1n-7/n-9	Monounsaturated FA	-0.058	0.96 (0.80–1.14)
16:0	Saturated FA	-0.043	0.92 (0.78–1.09)
20:3n-9	Polyunsaturated FA	-0.039	0.99 (0.84–1.17)
22:1n-9	Monounsaturated FA	-0.038	1.10 (0.91–1.32)
Endogenous metabolites[d]			
Increased for higher WCRF/AICR scores			
lysoPC a C17:0	Lysophosphatidylcholine	0.035	0.80 (0.62–1.02)
PC ae C40:6	Phosphatidylcholine, acyl-alkyl	0.032	0.90 (0.72–1.14)
PC ae C36:2	Phosphatidylcholine, acyl-alkyl	0.032	**0.72 (0.54–0.97)**
PC ae C38:2	Phosphatidylcholine, acyl-alkyl	0.027	0.90 (0.70–1.15)
Serine	Amino acid	0.023	0.87 (0.63–1.20)
lysoPC a C18:2	Lysophosphatidylcholine	0.023	0.85 (0.66–1.10)
Glycine	Amino acid	0.022	0.83 (0.62–1.13)
Diminished for higher WCRF/AICR scores			
PC aa C32:1	Phosphatidylcholine, diacyl	-0.037	0.94 (0.72–1.23)
PC aa C38:4	Phosphatidylcholine, diacyl	-0.034	1.13 (0.89–1.42)
PC aa C36:4	Phosphatidylcholine, diacyl	-0.033	1.08 (0.83–1.39)
Glutamate	Amino acid	-0.031	1.12 (0.64–1.97)
PC aa C34:4	Phosphatidylcholine, diacyl	-0.031	0.83 (0.66–1.06)
PC aa C40:4	Phosphatidylcholine, diacyl	-0.030	1.04 (0.83–1.30)
PC ae C38:3	Phosphatidylcholine, acyl-alkyl	-0.029	0.79 (0.61–1.02)

FA, fatty acid; LV, latent variable; lysoPC, lysophosphatidylcholine; OR, odds ratio; PC, phosphatidylcholine; PLSR, partial least-squares regression; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.

[a]After adjustment for center, batch, and study using the residuals method. Coefficients for all compounds are shown in Supplementary Table 3.

[b]Fatty acids, odds ratio per SD increase in concentration; endogenous metabolites, odds ratio for fourth vs first quartile of compound concentration. Adjusted for body mass index, alcohol intake, red and processed meat intake, height, energy intake, highest educational level attained, smoking status, and smoking intensity.

[c]Compounds with coefficients in the top or bottom quintiles for the first PLSR LV.

[d]Compounds with coefficients in the top or bottom 5 percentiles for the first PLSR LV.

diabetes[18] and an anti-inflammatory profile of adipokines.[19] Fatty acid intake is known to modulate biomarkers of inflammation.[20]

Fatty acids obtained from the diet also are incorporated into PCs, which are components of biological membranes but also signaling molecules that govern processes such as gene regulation and homeostatic control of serum glucose.[21] PCs that are influential in the endogenous metabolite signature have been linked to individual lifestyle behaviors in previous studies. LysoPC a C17:0 and PC ae C36:2, increased in the signature of a high WCRF/AICR score, were associated inversely with alcohol intake in 3 separate prospective studies.[22,23] PC aa C32:1, conversely, was associated positively with alcohol intake in the same studies, and associated independently with high total meat intake, smoking, and risk of type 2 diabetes.[24–27] Because PCs are perturbed easily by diet and lifestyle factors and fine differences in structure impart distinct bioactivities, dedicated studies are needed to elucidate their relationship to tumorigenesis. Glycine, increased in the endogenous signature of a high WCRF/AICR score, has been reported to be associated inversely with total red meat intake[28] and type 2 diabetes risk,[26] but associated positively with total weekly physical activity.[29] Glutamate, conversely, appeared in metabolic profiles of a high BMI[30] and was associated with insulin resistance.[31] Our observations regarding amino acids thus largely were consistent with previous studies.

Both signatures captured weight management and alcohol avoidance more strongly than other components of the WCRF/AICR score, despite the orthogonality of the 2 platforms. Alcohol avoidance was captured strikingly by the fatty acid signature. OCFAs in particular have been reported to be associated inversely with alcohol intake,[16,32] although ethanol exposure may attenuate fatty acid absorption and incorporation into phospholipids by diverse mechanisms such as inhibition of

Table 3. ORs and 95% CI for Colorectal Cancer Risk and Metabolic Signatures or WCRF/AICR Score by Sex and Anatomic Subsite

	Colorectal OR (95% CI)	Colon OR (95% CI)	Proximal colon OR (95% CI)	Distal colon OR (95% CI)	Rectal OR (95% CI)
	N = 3216	N = 2504	N = 1190	N = 1314	N = 468
Fatty acids					
N, women	876 (530)	792 (486)	358 (226)	434 (260)	
WCRF/AICR score[a]					
All	**0.77 (0.66–0.91)**	**0.75 (0.63–0.89)**	0.83 (0.63–1.10)	**0.70 (0.55–0.90)**	
Women	**0.78 (0.63–0.98)**	**0.77 (0.61–0.97)**	0.87 (0.58–1.29)	0.73 (0.53–1.01)	
Men	**0.75 (0.58–0.96)**	**0.69 (0.52–0.92)**	0.74 (0.48–1.15)	0.64 (0.42–0.97)	
P het	.36	.28	.44	.49	
Metabolic signature[a,b]					
All	**0.51 (0.29–0.90)**	**0.53 (0.29–0.97)**	0.78 (0.31–1.97)	**0.40 (0.18–0.91)**	
Women	0.73 (0.34–1.57)	0.77 (0.34–1.71)	0.67 (0.18–2.44)	0.70 (0.24–2.00)	
Men	**0.31 (0.13–0.75)**	**0.33 (0.13–0.83)**	0.84 (0.18–4.00)	**0.23 (0.06–0.83)**	
P het	.072	.11	.43	.18	
Metabolic signature adjusted for WCRF/AICR score					
All	0.59 (0.33–1.07)	0.61 (0.33–1.14)	0.79 (0.30–2.02)	0.52 (0.22–1.21)	
Endogenous metabolites					
N, women	3216 (1752)	2504 (1418)	1190 (712)	1314 (706)	468 (258)
WCRF/AICR score[a]					
All	**0.93 (0.86–1.00)**	0.93 (0.85–1.02)	1.00 (0.87–1.14)	0.89 (0.79–1.01)	0.89 (0.72–1.08)
Women	1.01 (0.91–1.12)	1.05 (0.93–1.18)	1.07 (0.90–1.29)	1.04 (0.87–1.23)	0.96 (0.70–1.31)
Men	**0.85 (0.76–0.95)**	**0.80 (0.70–0.92)**	0.90 (0.73–1.12)	**0.72 (0.59–0.87)**	0.83 (0.62–1.11)
P het	.022	.002	.12	.005	.83
Metabolic signature[a,b]					
All	**0.62 (0.50–0.78)**	**0.65 (0.50–0.84)**	0.78 (0.53–1.14)	**0.57 (0.40–0.82)**	**0.44 (0.25–0.79)**
Women	0.82 (0.59–1.12)	0.89 (0.62–1.26)	0.92 (0.55–1.54)	0.87 (0.52–1.43)	0.60 (0.25–1.46)
Men	**0.44 (0.32–0.61)**	**0.44 (0.25–0.79)**	0.59 (0.33–1.06)	**0.36 (0.21–0.62)**	**0.41 (0.19–0.86)**
P het	.029	.03	.21	.12	.46
Metabolic signature adjusted for WCRF/AICR score					
All	**0.62 (0.49–0.79)**	**0.63 (0.48–0.83)**	**0.61 (0.42–0.90)**	**0.67 (0.45–1.00)**	**0.52 (0.29–0.94)**

OR, odds ratio; P het, P heterogeneity; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.
[a]On a scale of 1 to 5, and after adjustment for height, energy intake, highest educational level attained, and smoking status or intensity.
[b]Magnitude of the metabolic signature is defined as the metabolite-predicted WCRF/AICR score derived from partial least-squares regression models trained on endogenous metabolite and fatty acid data from the discovery set.

enzyme catalysts, disruption of gut microbiota, or physiological changes to hepatocytes.[23,33] Weight management was captured most strongly by the endogenous signature, whose amino acid components are implicated in adiposity and insulin resistance. In sensitivity analysis, the endogenous signature remained associated strongly with colorectal cancer risk after additional adjustment for the WCRF/AICR score, showing a capability to capture intrinsic or longer-term abnormalities in metabolism related to the disease. The fact that associations for metabolic signatures were stronger than those of WCRF/AICR scores suggests that signatures, rather than acting as biomarker surrogates of score, reflect aspects of metabolic health that are not measured directly by conventional approaches.[34]

The association of the metabolic signatures with colorectal cancer was more apparent in men and the associations were weaker and nonsignificant in women. This may reflect sex-specific differences in the association of the composite risk factors within the score such as BMI and alcohol consumption, which are stronger risk factors for colorectal cancer in men than in women.[35] In addition to this heterogeneity, it is known that colorectal cancer risk factors and associations by sex may differ by anatomic subsite,[36] and in our study associations for colon cancer were driven disproportionately by distal tumors. Interestingly, rectal cancer, however, was associated strongly with endogenous metabolic signatures of the WCRF/AICR score, despite the influence of biologic, lifestyle, and dietary factors upon risk being less clear than for colon cancer.[37] Overall, these differences require follow-up evaluation in other cohorts, but if reproduced may point toward specific biological pathways that deserve mechanistic investigation.

Our study is unique in deriving metabolic signatures from a large fasting discovery group on 2 complementary platforms and measuring their magnitude prospectively

in a nested case–control study of substantial size. One limitation is that we have been unable to test these signatures in external cohorts to date. Participants nonetheless were from different combinations of EPIC centers and samples were analyzed in different laboratories. Because endogenous metabolite and fatty acid data were not always available for the same participants, an overall signature derived from both platforms could not be determined, and the fatty acid signature was derived from a data set of mostly female participants and therefore may have been less applicable to males. Another drawback was the unavailability of data on colorectal cancer screening and family history and use of nonsteroidal anti-inflammatory drugs in some EPIC centers, meaning we were unable to adjust for these potential confounders.

In conclusion, the stronger associations of signatures with colorectal cancer compared with the WCRF/AICR scores suggest that metabolite profiles reflect a broader spectrum of behavioral and biological characteristics than are included in the recommendations and can be used to better assess colorectal cancer risk or gain insight into metabolic risk factors. Further studies of healthy lifestyle patterns and their relationship with metabolism and cancer are merited.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.11.045.

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Conflicts of interest

The authors disclose no conflicts.

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Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

Supplementary Methods

Laboratory Methods

Serum and plasma samples were stored at the International Agency for Research on Cancer (Lyon, France) at -196°C in liquid nitrogen, apart from those of Sweden (-80°C in freezers) and Denmark (-150°C in nitrogen vapor). Data and the biospecimens used were from all EPIC countries except Greece.

Fatty acid profiling was performed at the International Agency for Research on Cancer for both discovery and case–control samples. SFAs, monounsaturated fatty acids, polyunsaturated fatty acids, industrial trans fatty acids, and natural trans fatty acids were extracted from plasma phospholipid fractions and quantified using an Agilent 7890 gas chromatograph instrument (Agilent Technologies, Santa Clara, CA). Concentrations were expressed as the percentage of total fatty acids. For endogenous metabolites, analyses were performed at the International Agency for Research on Cancer (all discovery and approximately one third of case–control samples), and the Helmholtz Zentrum, München, Germany (all other case–control samples). The Absolute*IDQ* p150 or p180 Kits were used to measure concentrations of amino acids, biogenic amines, hexose sugars, acylcarnitines, sphingolipids, PCs, and lysoPCs in serum or plasma, following the recommended procedure. The International Agency for Research on Cancer method used a 1290 Series liquid chromatography instrument with a Q-Trap 5500 mass spectrometer (Agilent Technologies, Les Ulis, France). The Helmholtz method was based on a 1200 series liquid chromatography instrument (Agilent, Böblingen, Germany) with an API 4000 triple–quadrupole mass spectrometer (AB Sciex, Darmstadt, Germany). Case–control pairs were analyzed in the same batch, and coefficients of variation were calculated for each metabolite. The full details of the laboratory procedures have been published.[1–3]

Statistical Analysis

Determination of metabolic signatures. This analysis used a discovery set of 5738 cancer-free control participants, originating from several noncolorectal case–control studies nested within the EPIC cohort,[1,4–6] to derive metabolic signatures of the WCRF/AICR score. Discovery set metabolite matrices were prepared for derivation of metabolic signatures, separately for fatty acids and endogenous metabolites. Compounds not measured in both discovery and case–control sets were excluded, as well as those that were missing (outside the limits of quantification) for more than 40% of participants. For the remainder, missing concentrations were replaced with half the minimum in the whole data set. The discovery metabolite matrices then were $\log_2$ transformed, centered, and unit variance–scaled. Second, unwanted variability was removed from the data. The principal component partial R-squared technique was used to identify covariates that contributed the most toward variability in metabolomics data. The principal component partial R-squared technique combines principal component analysis and multivariable regression to estimate the relative effects of metadata variables upon a matrix of omics measurements.[7] Each metabolite concentration then was transformed by the residuals method[8] using models on sex, batch, center (fixed effects), and study (random effects). Pearson correlations between concentrations also were calculated in a subset of participants.

PLSR was used to determine metabolic signatures of the WCRF/AICR score[6] (ie, the linear combination of metabolite concentrations most correlated with the score). Models were selected that balanced simplicity and low root mean square error of cross-validation. Loadings (coefficients) on the first latent variable of the PLSR model fit, denoted p_{LV1}, were calculated for each compound as a measure of contribution to each signature. ORs and 95% CIs were calculated for colorectal cancer risk for baseline concentrations of compounds that contributed the most to these signatures, adjusting for BMI, height, energy intake, highest educational level attained, red and processed meat intake, alcohol intake, smoking status, and smoking intensity in conditional logistic regression models.

The case–control metabolite matrix was prepared similarly to that of the discovery set. The validated PLSR models then were used to predict WCRF/AICR scores, applying coefficients to metabolites, on a continuous scale of 1 to 5 for each subject in the case–control study. These predicted scores were regarded as the magnitude of the metabolic signature with distributions comparable with those of WCRF/AICR scores.

Association of metabolic signatures of the World Cancer Research Fund/American Institute for Cancer Research score with adherence to recommendations and colorectal cancer risk. Partial Pearson correlations were calculated between metabolic signatures and adherence to the 6 individual components of the WCRF/AICR score (as described earlier, each on a scale of 0, 0.5, or 1), adjusting for height, highest education level attained, smoking status, and intensity. Odds ratios and 95% CIs were calculated for risk of colorectal cancer and subsites, with the metabolic signature or the WCRF/AICR score as the main explanatory variable in multivariable conditional logistic regression models. Heterogeneity by sex was determined by likelihood ratio test, comparing unpaired logistic regression models with and without interaction terms between sex and the WCRF/AICR score or metabolic signature. Matching factors additionally were included in these models. Additional models were fit for individual WCRF/AICR components. Sensitivity analyses also were performed, additionally adjusting for smoking duration, intake of dairy products, or, in signature models only, WCRF/AICR score. Subgroup analyses

were performed for strata of follow-up time and, for signature only, BMI and WCRF/AICR score. All analyses were performed using R statistical software, version 3.6.2.

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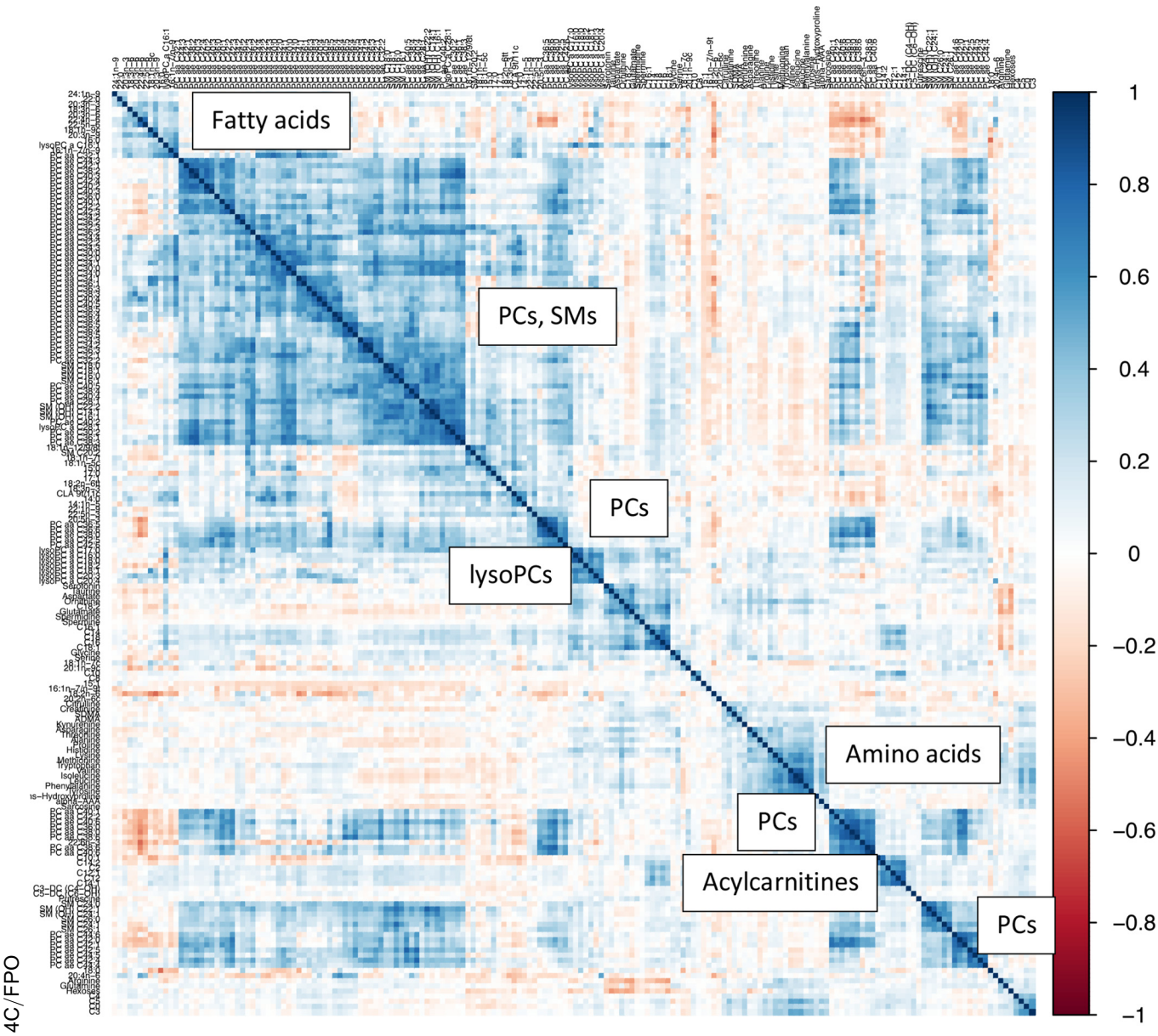
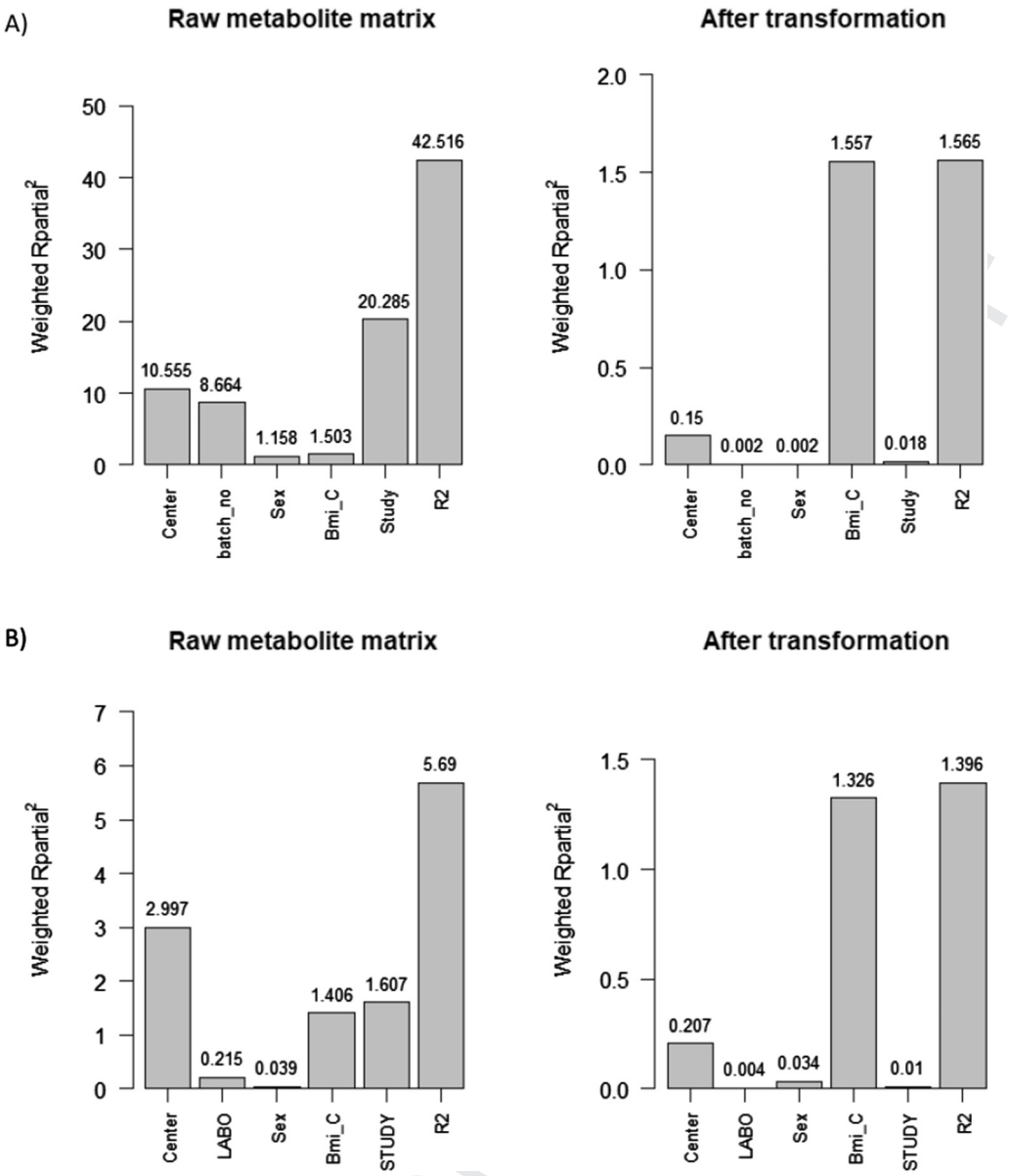


PC, phosphatidylcholines; SM, sphingomyelins.Concentrations were $\log_2$ transformed.

Supplementary Figure 1. Pearson correlations between 159 endogenous metabolites and 31 fatty acids measured in a subset of 439 colorectal study control participants. Concentrations were $\log_2$ transformed. lysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelins.



Supplementary Figure 2. Variability in discovery metabolomics data explained by different metadata variables as determined by the principal component partial R-square technique. For calculation of metabolic signatures, each column of the metabolite matrix was transformed to the residuals of a mixed-effects model whose explanatory variables were technical confounders: (*A*) 155 endogenous metabolites (n = 1741), and (*B*) 34 fatty acids (n = 4239). Bmi_C, Q26 _____; LABO, ____.

Supplementary Table 1. Summary of WCRF/AICR Recommendations and Scoring System Used in the Present Study

Characteristic	Criteria (operationalization)	Score attributed
Maintain a healthy body weight	BMI, 18.5–24.9	1
	BMI, 25–29.9	0.5
	Other BMI	0
Be moderately physically active, equivalent to brisk walking, for ≥30 min every day	Manual/heavy manual job, or >2 h/wk of vigorous PA, or >30 min/d of cycling/sports	1
	15–30 min/d of cycling or sport	0.5
	<15 min/d of cycling or sport	0
Avoid food and drinks that promote weight gain	Energy dense foods: <125 kcal/100 g/d	1
	125–175 kcal/100 g/d	0.5
	>175 kcal/100 g/d	0
	or sugary drink intake: 0 g/d	1
	0–250 g/d	0.5
	>250 g/d	0
Intake of plant foods	Intake of fruits and vegetables: >400 g/d	1
	200–400 g/d	0.5
	<200 g/d	0
	or dietary fiber intake: >25 g/d	1
	12.5–25 g/d	0.5
	<12.5 g/d	0
Limit intake of animal foods	Intake of red and processed meat or processed meat: <500 g/wk and 3 g/d	1
	<500 g/wk and 3–50 g/d	0.5
	>500 g/wk and >50 g/d	0
Avoid alcohol	Ethanol intake: <20 g/d for men or <10 g/d for women	1
	20–30 g/d for men or 10–20 g/d for women	0.5
	>30 g/d for men or >20 g/d for women	0
Breastfeeding	Cumulative breastfeeding >6 mo	1
	0–6 mo	0.5

BMI, body mass index; PA, physical activity; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.

Supplementary Table 2. Baseline Characteristics for the Discovery Set of EPIC Controls Used to Determine Metabolic Signatures of WCRF/AICR Score

	Participants with endogenous metabolite data	Participants with fatty acid data
N	1741	4239
Study of origin		
Breast	562 (32.3)	2876 (67.8)
Kidney	213 (12.2)	0 (0.0)
Ovary	0 (0.0)	1060 (25.0)
Pancreas	0 (0.0)	303 (7.1)
Prostate	891 (51.2)	0 (0.0)
Liver	75 (4.3)	0 (0.0)
Sex		
Male	1046 (60.1)	118 (2.8)
Female	695 (39.9)	4121 (97.2)
Age at recruitment, *y*	54.50 ± 7.2	53.5 ± 8.1
Height, *cm*	165.6 ± 8.4	161.5 ± 6.8
BMI, kg/m^2	26.8 ± 3.9	25.3 ± 4.2
Total energy intake, *kcal*	2328 ± 670	1964 ± 550
Country		
France	53 (3.0)	638 (15.1)
Italy	903 (51.9)	868 (20.5)
Spain	558 (32.1)	425 (10.0)
United Kingdom	36 (2.1)	825 (19.5)
The Netherlands	11 (0.6)	727 (17.2)
Germany	143 (8.2)	601 (14.2)
Sweden	37 (2.1)	0 (0)
Norway	0 (0)	155 (3.7)
Physical activity, *MET*	81.0 ± 53.9	102.7 ± 53.0
Alcohol intake, *g/d*	18.0 ± 21.5	8.8 ± 12.5
Smoking status		
Nonsmoker	740 (42.5)	2383 (56.2)
Never smoker	564 (32.4)	1046 (24.7)
Smoker	426 (24.5)	729 (17.2)
WCRF/AICR score	2.61 ± 1.01	2.49 ± 1.03
Adherence to individual WCRF/AICR score components (full adherence = 1)		
Weight maintenance	0.56	0.68
Physical activity	0.42	0.40
Intake of foods that promote weight gain	0.59	0.55
Intake of plant foods	0.72	0.60
Intake of animal foods	0.23	0.34
Alcohol intake	0.66	0.79

NOTE. Means and SD or frequency and percentage are shown unless stated otherwise.

BMI, body mass index; EPIC, European Prospective Investigation into Cancer and Nutrition cohort; MET, metabolic equivalent of task; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.

Supplementary Table 3. Details of 155 Endogenous Metabolites and 34 Fatty Acids Measured in Both the Discovery Set and the Colorectal Nested Case–Control Studies

Platform and compound class	Compound name	Included in signatures or reason for exclusion	Coefficient PLS model (importance in signature)	CV 1[a]	CV 2[a]
Endogenous metabolites					
Acylcarnitines					
1	C0	Included	-0.017	NA	6.1
2	C10	Excluded missings		NA	9.2
3	C10:1	Excluded missings		NA	8.3
4	C12	Excluded missings		7.4	10.9
5	C12:1	Excluded missings		7.6	11.8
6	C14	Excluded missings		8.4	16.5
7	C14:1	Included	0.007	7.2	12.3
8	C14:2	Excluded missings		10.4	14.1
9	C16	Included	-0.001	8.4	11
10	C16:1	Excluded missings		12.3	9.4
11	C18	Included	0.006	6.8	15.6
12	C18:1	Included	0.003	7	8.9
13	C18:2	Included	0.001	9.5	10.4
14	C2	Included	-0.011	4.7	6.8
15	C3	Included	0.001	6.1	8.7
16	C4	Included	-0.003	5.5	9.2
17	C5	Included	-0.007	6.8	12
18	C8	Excluded missings		5	10.5
19	C3-DC (C4-OH)	Excluded missings		9.3	12.7
20	C4:1	Excluded missings		10.8	16.5
21	C5-DC (C6-OH)	Excluded missings		8.8	21
22	C5-M-DC	Excluded missings		8.6	17.9
23	C7-DC	Excluded missings		13.2	16.5
24	C9	Excluded missings		12.8	19.6
25	C5:1-DC	Excluded missings		12.3	24.1
Amino acids					
26	Alanine	Included	-0.016	6.3	NA
27	Arginine	Included	0.003	5.2	8.1
28	Asparagine	Included	0.02	6.4	NA
29	Aspartate	Included	-0.001	11.5	NA
30	Citrulline	Included	0.013	7.2	NA
31	Glutamine	Included	0.019	7.6	8
32	Glutamate	Included	-0.031	5.7	NA
33	Glycine	Included	0.022	6.9	7.3
34	Histidine	Included	0.005	4.5	7.5
35	Isoleucine	Included	-0.017	7.1	NA
36	Leucine	Included	-0.018	6.9	NA
37	Lysine	Included	-0.008	9.4	NA
38	Methionine	Included	-0.002	11.4	9.5
39	Ornithine	Included	-0.003	11.6	7.2
40	Phenylalanine	Included	-0.011	6.2	8
41	Proline	Included	-0.009	5	6.8
42	Serine	Included	0.023	5	7.3
43	Threonine	Included	0.002	6.1	7.3
44	Tryptophan	Included	0.001	8	7.1
45	Tyrosine	Included	-0.024	6.5	8.3
46	Valine	Included	-0.023	9.1	6.9
Biogenic amines					
47	α-AAA	Excluded missings		121.2	NA
48	Creatinine	Included	0	3.7	NA
49	Kynurenine	Included	-0.011	7	NA
50	Putrescine	Excluded missings		35.9	NA
51	Sarcosine	Included	-0.011	8.6	NA
52	Serotonin	Excluded missings		5.9	NA
53	Spermidine	Excluded missings		15.5	NA
54	Spermine	Excluded missings		8.8	NA
55	Transhydroxyproline	Included	-0.019	4.7	NA
56	Taurine	Included	0.001	2.9	NA

Supplementary Table 3. Continued

Platform and compound class	Compound name	Included in signatures or reason for exclusion	Coefficient PLS model (importance in signature)	CV 1[a]	CV 2[a]
57	ADMA	Included	0.002	9.3	NA
58	SDMA	Included	0.006	12	NA
LysoPCs					
59	LysoPC a C16:0	Included	-0.003	7.1	6.6
60	LysoPC a C16:1	Included	-0.017	6.7	7.7
61	LysoPC a C17:0	Included	0.035	9	8.3
62	LysoPC a C18:0	Included	0.007	7.5	6.6
63	LysoPC a C18:1	Included	0.02	9.4	6.5
64	LysoPC a C18:2	Included	0.023	8.7	7
65	LysoPC a C20:3	Included	-0.002	7.9	9
66	LysoPC a C20:4	Included	-0.006	9.2	7
67	LysoPC a C28:1	Excluded missings		12.6	31.7
68	LysoPC a C24:0	Excluded missings		13.9	14.4
69	LysoPC a C14:0	Excluded missings		4.7	4.5
70	LysoPC a C28:0	Excluded missings		20	31.7
Monosaccharides					
71	Hexoses	Included	-0.018	4.9	5.5
PCs, diacyl					
72	PC aa C28:1	Included	-0.004	6.4	8.8
73	PC aa C30:0	Included	-0.015	6.1	9.6
74	PC aa C32:0	Included	-0.01	5.2	7.4
75	PC aa C32:1	Included	-0.037	5.7	10
76	PC aa C32:2	Included	-0.024	8.4	11.5
77	PC aa C32:3	Included	0.003	6.8	9.9
78	PC aa C34:1	Included	-0.019	5.3	7.7
79	PC aa C34:2	Included	-0.009	5.9	6.6
80	PC aa C34:3	Included	-0.016	4.9	7.1
81	PC aa C34:4	Included	-0.031	7.2	7.9
82	PC aa C36:0	Included	0	9.9	11.4
83	PC aa C36:1	Included	-0.015	5.7	7.4
84	PC aa C36:2	Included	-0.008	5.3	6.5
85	PC aa C36:3	Included	-0.012	5.2	6.1
86	PC aa C36:4	Included	-0.033	4.4	5.9
87	PC aa C36:5	Included	-0.011	5.3	9.2
88	PC aa C36:6	Included	-0.005	8.3	13.5
89	PC aa C38:0	Included	0.018	5.1	8.5
90	PC aa C38:3	Included	-0.029	5.1	6.1
91	PC aa C38:4	Included	-0.034	4.9	5.9
92	PC aa C38:5	Included	-0.013	5.4	6.6
93	PC aa C38:6	Included	-0.002	5	8.1
94	PC aa C40:1	Excluded missings		4.8	13.1
95	PC aa C40:2	Included	0.007	6.7	13.4
96	PC aa C40:3	Included	0.007	11.7	11.3
97	PC aa C40:4	Included	-0.03	4.5	6.4
98	PC aa C40:5	Included	-0.02	6.7	6.5
99	PC aa C40:6	Included	-0.004	8.3	8.2
100	PC aa C42:0	Included	0.011	6.2	9.4
101	PC aa C42:1	Included	0.009	10.5	12.1
102	PC aa C42:2	Included	0.015	6.3	12
103	PC aa C42:4	Included	0.003	7.8	12.3
104	PC aa C42:5	Included	0.004	6.1	11
105	PC aa C42:6	Included	0.004	8	13.8
106	PC aa C24:0	Excluded missings		36.1	40.3 Q27
PCs, acyl-alkyl					
107	PC ae C30:0	Included	0.011	6.1	17.3
108	PC ae C30:2	Included	0.004	13.2	10.2
109	PC ae C32:1	Included	0.005	7.1	9.2
110	PC ae C32:2	Included	0.001	4.6	11.5
111	PC ae C34:0	Included	0.005	7.6	11.2
112	PC ae C34:1	Included	0.015	4.7	7.5
113	PC ae C34:2	Included	0.019	5.2	6.6
114	PC ae C34:3	Included	0.009	4.5	6.7

Supplementary Table 3. Continued

Platform and compound class	Compound name	Included in signatures or reason for exclusion	Coefficient PLS model (importance in signature)	CV 1[a]	CV 2[a]
115	PC ae C36:0	Included	-0.009	16.6	13.9
116	PC ae C36:1	Included	0.016	5.8	6.5
117	PC ae C36:2	Included	0.032	5.3	6.6
118	PC ae C36:3	Included	0.015	5.9	6.5
119	PC ae C36:4	Included	-0.02	6	5.9
120	PC ae C36:5	Included	-0.02	4.7	5.7
121	PC ae C38:0	Included	0.007	7	9
122	PC ae C38:2	Included	0.027	10	8.2
123	PC ae C38:3	Included	0.014	7.3	6.8
124	PC ae C38:4	Included	-0.003	5.9	5.8
125	PC ae C38:5	Included	-0.005	6.8	5.8
126	PC ae C38:6	Included	0.001	6.1	6.8
127	PC ae C40:1	Included	0.003	7	12
128	PC ae C40:2	Included	0.01	5.2	8
129	PC ae C40:3	Included	0.022	6.6	7.4
130	PC ae C40:4	Included	0.009	5.5	6.9
131	PC ae C40:5	Included	0.017	5.8	6.2
132	PC ae C40:6	Included	0.032	3.9	7.4
133	PC ae C42:1	Included	0.005	7.4	13.8
134	PC ae C42:2	Included	0.007	6.1	11.6
135	PC ae C42:3	Included	0.014	5.4	10.8
136	PC ae C42:4	Included	0.016	7.7	8.8
137	PC ae C42:5	Included	0.018	6.8	5.6
138	PC ae C44:3	Included	0	13.4	15.7
139	PC ae C44:4	Included	0.015	12	11.3
140	PC ae C44:5	Included	0.015	5.7	7.5
141	PC ae C44:6	Included	0.012	4.5	7.2
Sphingolipids					
142	SM (OH) C14:1	Included	0.014	5.1	7.5
143	SM (OH) C16:1	Included	0.012	8.2	7.1
144	SM (OH) C22:1	Included	0.004	9.9	7.3
145	SM (OH) C22:2	Included	0.015	7.1	7.9
146	SM (OH) C24:1	Included	0.006	12.7	12.5
147	SM C16:0	Included	0.005	8.1	6.5
148	SM C16:1	Included	-0.007	5.2	6.7
149	SM C18:0	Included	-0.016	6.2	6.8
150	SM C18:1	Included	-0.01	5.7	6.5
151	SM C20:2	Included	0.003	23.1	14.7
152	SM C24:0	Included	-0.012	5.9	7
153	SM C24:1	Included	0.001	11.5	7.6
154	SM C26:1	Excluded high CV		13.6	25
155	SM C26:0	Excluded high CV		17.1	50.6
Fatty acids					
Industrial trans					
1	18:1n-12/9/8t	Included	0.037	13.2	
2	18:2n-6tt	Excluded high CV		22.6	
Monounsaturated					
3	14:1n-5	Excluded high CV		31.9	
4	15:1	Included	0.049	13.7	
5	16:1n-7/n-9t	Included	0.009	NA	
6	16:1n-7/n-9	Included	-0.058	NA	
7	17:1	Included	0.005	7.3	
8	18:1n-9c	Included	0.041	2.5	
9	18:1n-7c	Included	-0.004	2.1	
10	18:1n-5c	Included	0.029	6.6	
11	20:1n-9c	Included	0.026	2.3	
12	22:1n-9	Included	-0.038	15.6	
13	24:1n-9	Included	-0.035	12.1	
Natural trans					
14	18:1n-7t	Excluded high CV		32.7	
15	CLA 9t/11c	Included	-0.016	NA	

Supplementary Table 3. Continued

Platform and compound class	Compound name	Included in signatures or reason for exclusion	Coefficient PLS model (importance in signature)	CV 1[a]	CV 2[a]
Polyunsaturated					
16	18:2n-6	Included	0.022	0.7	
17	18:3n-6	Included	0.022	8	
18	20:2n-6c	Included	0.001	1.3	
19	20:3n-9	Included	-0.039	4.1	
20	20:3n-6	Included	0.006	1.4	
21	20:4n-6	Included	-0.011	1.3	
22	22:4n-6	Included	0.014	2.1	
23	22:5n-6	Included	0.039	2.9	
24	18:3n-3	Included	0.029	7.4	
25	20:3n-3	Included	-0.021	8	
26	20:5n-3	Included	-0.02	7	
27	22:5n-3	Included	0.042	1.7	
28	22:6n-3	Included	-0.002	2.7	
Saturated					
29	14:0	Included	0.011	8.6	
30	15:0	Included	0.076	2.7	
31	16:0	Included	-0.043	1.3	
32	17:0	Included	0.149	1.2	
33	18:0	Included	-0.025	1.4	
34	22:0	Excluded high CV		28.2	

ADMA, _____; CLA, _____; CV, coefficient of variation; lysoPC, lysophosphatidylcholine; NA, not available; PC, phosphatidylcholine; PLS, partial least-square; QC, quality control; SDMA, _____; SM, sphingomyelin.

[a]Laboratory 1: International Agency for Research on Cancer; 13 plates of serum samples with 2 QCs per plate for endogenous compounds, 56 batches of plasma samples, 2 QCs per batch for fatty acids. Laboratory 2: Helmholtz Zentrum; 29 plates of serum samples with 5 aliquots of a reference serum as a QC.

Supplementary Table 4. Highest Pearson Correlations Between 159 Endogenous Metabolites and 31 Fatty Acids in 439 Colorectal Study Control Participants

Fatty acid	Endogenous metabolite	Pearson correlation r, $\log_2$ transformed concentrations
PUFA 20:5n-3	PC aa C36:5	0.892
PUFA 22:6n-3	PC aa C38:6	0.767
SFA 14:0	PC aa C30:0	0.746
ITFA 18:1n-12/9/8t	SM C20:2	0.728
PUFA 22:6n-3	PC aa C38:0	0.696
PUFA 22:6n-3	PC aa C40:6	0.694
MUFA 18:1n-9c	PC aa C34:1	0.690
MUFA 16:1n-7/n-9	PC aa C32:1	0.689
PUFA 20:3n-6	PC aa C38:3	0.685
SFA 14:0	PC aa C32:2	0.683
PUFA 20:5n-3	PC aa C36:6	0.669
PUFA 22:4n-6	PC aa C40:4	0.661
PUFA 20:3n-9	PC aa C34:1	0.657
PUFA 20:5n-3	PC ae C38:0	0.653
PUFA 22:6n-3	PC ae C40:6	0.651
PUFA 20:4n-6	PC aa C38:4	0.649
ITFA 18:1n-12/9/8t	PC aa C32:3	0.631
SFA 14:0	PC aa C32:1	0.618
MUFA 18:1n-9c	PC aa C36:1	0.611
SFA 0.625	PC ae C30:0	0.604

ITFA, industrial trans fatty acid; MUFA, monounsaturated fatty acid; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SM, sphingomyelin.

Supplementary Table 5. Odds Ratios and 95% CI for Individual WCRF/AICR Score Components in the Colorectal Cancer Nested Case–Control Study

Cancer subsite	WCRF/AICR recommendation	OR (95% CI)[b]
Colorectal		
$N = 3216$	Maintain normal body weight	**0.68 (0.67–0.93)**
	Be physically active	**0.87 (0.63–0.99)**
	Limit foods that promote weight gain	**1.10 (0.59–0.99)**
	Eat mostly plant foods	0.93 (0.69–1.26)
	Limit red and processed meat	1.50 (1.13–1.98)
	Avoid alcohol	0.92 (1.77–1.11)
	Overall WCRF score	**0.92 (0.86–1.00)**
Colon		
$N = 2504$	Maintain normal body weight	**0.66 (0.51–0.84)**
	Be physically active	0.85 (0.70–1.04)
	Limit foods that promote weight gain	1.17 (0.77–1.77)
	Eat mostly plant foods	0.91 (0.64–1.28)
	Limit red and processed meat	1.59 (1.17–2.17)
	Avoid alcohol	0.92 (1.74–1.15)
	Overall WCRF score	0.92 (0.84–1.01)
Rectal		
$N = 468$	Maintain normal body weight	0.79 (0.45–1.37)
	Be physically active	0.91 (0.57–1.46)
	Limit foods that promote weight gain	0.65 (0.22–1.89)
	Eat mostly plant foods	0.93 (0.42–2.06)
	Limit red and processed meat	1.10 (1.43–2.83)
	Avoid alcohol	0.78 (0.49–1.22)
	Overall WCRF score	0.89 (0.73–1.09)

OR, odds ratio; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.
[a]Scored on a scale of 0, 0.5, or 1 according to criteria for individual components.
[b]Adjusted for height, energy intake, highest educational level attained, smoking status, and smoking intensity.

Supplementary Table 6. Additional Sensitivity and Subgroup Analyses in the Nested Case–Control Study

Metabolite platform and anatomic subsite	N	Model[a]	Odds ratio (95% CI) for association per unit increase in the WCRF/AICR score or change in metabolic signature[ab]	
			WCRF/AICR score[a]	Metabolic signature[ab]
Fatty acids				
Colorectal	876	Base co-variates only	**0.78 (0.66–0.91)**	**0.48 (0.28–0.83)**
	876	Base + smoking intensity	**0.77 (0.66–0.91)**	**0.51 (0.29–0.90)**
	876	Base + smoking duration	**0.78 (0.66–0.91)**	**0.49 (0.28–0.85)**
	876	Base + dairy product intake	**0.78 (0.67–0.92)**	**0.50 (0.29–0.88)**
	130	Base + smoking intensity, normal BMI only	–	2.64 (0.25–27.43)
	406	Base + smoking intensity, overweight or obese BMI only	–	0.40 (0.17–0.95)
	210	Base + smoking intensity, WCRF/AICR scores 1 or 2	–	0.38 (0.11–1.33)
	246	Base + smoking intensity, WCRF/AICR scores 3, 4 or 5	–	0.82 (0.23–2.93)
	768	Base model, cases diagnosed after 2 years of follow-up only	**0.84 (0.71–0.99)**	**0.54 (0.30–0.97)**
Endogenous				
Colorectal	3210	Base co-variates only	0.93 (0.85–1.02)	**0.61 (0.49–0.77)**
	3210	Base + smoking intensity	0.93 (0.85–1.02)	**0.62 (0.50–0.78)**
	3210	Base + smoking duration	0.93 (0.85–1.02)	**0.62 (0.49–0.77)**
	3210	Base + dairy product intake	0.94 (0.86–1.03)	**0.62 (0.49–0.77)**
	478	Base + smoking intensity, normal BMI only	–	1.22 (0.63–2.36)
	1352	Base + smoking intensity, overweight or obese BMI only	–	**0.50 (0.35–0.71)**
	722	Base + smoking intensity, WCRF/AICR scores 1 or 2	–	**0.56 (0.35–0.90)**
	848	Base + smoking intensity, WCRF/AICR scores 3, 4 or 5	–	0.69 (0.43–1.11)
	2860	Base model, cases diagnosed after 2 years of follow-up only	0.94 (0.86–1.03)	**0.63 (0.50–0.80)**
Colon	2504	Base co-variates only	0.92 (0.84–1.01)	**0.63 (0.49–0.81)**
	2504	Base + smoking intensity	0.93 (0.85–1.02)	**0.65 (0.50–0.84)**
	2504	Base + smoking duration	0.93 (0.85–1.01)	**0.63 (0.49–0.82)**
	2504	Base + dairy product intake	0.93 (0.85–1.01)	**0.63 (0.49–0.81)**
	2274	Base model, cases diagnosed after 2 years of follow-up only	0.93 (0.85–1.02)	**0.64 (0.49–0.84)**
Rectal	468	Base co-variates only	0.94 (0.78–1.14)	**0.53 (0.31–0.91)**
	468	Base + smoking intensity	0.89 (0.72–1.08)	**0.44 (0.25–0.79)**
	468	Base + smoking duration	0.95 (0.79–1.14)	**0.54 (0.32–0.93)**
	468	Base + dairy product intake	0.97 (0.80–1.17)	**0.55 (0.32–0.95)**
	366	Base model, cases diagnosed after 2 years of follow-up only	0.91 (0.74–1.12)	**0.48 (0.26–0.89)**

BMI, body mass index; WCRF/AICR, World Cancer Research Fund/American Institute for Cancer Research.
[a]Base models were adjusted for height, energy intake, highest educational level attained, and smoking status.
[b]Measurement of metabolic signature is defined as the metabolite predicted WCRF/AICR score derived from partial least-square regression models fit with endogenous metabolite and fatty acid data in the discovery set.