Diet, Metabolomics, and Parkinson's Disease

Diet, Metabolomics, and Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects motor control and is characterized by several hallmark symptoms, including resting tremor, bradykinesia, rigidity, and postural instability. Existing treatments are unable to stop or slow the progression of PD. The lack of effective treatment is likely due in part to the fact that PD develops insidiously over an extended period such that there is already extensive and irreversible neurodegeneration by the time of clinical diagnosis. In order to deliver treatment before neurodegeneration is too extensive, it is therefore vital to better understand and reliably recognize the prodromal phase of PD.

In **Chapter 1**, we used blood samples from 349 matched case-control pairs in the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) to examine whether metabolites known to be associated with insulin resistance and diabetes were also associated with the development of PD. In these analyses, there was no evidence that these markers of insulin resistance and diabetes were associated with PD, corroborating previous research in these cohorts that failed to identify a relationship between diabetes and PD.

In **Chapter 2**, we used blood samples from 817 matched case-control pairs in the NHS, the HPFS, and the Cancer Prevention Study II Nutrition Survey Cohort (CPS-IIN) to investigate whether pre-diagnostic plasma metabolite levels could act as risk factors for or biomarkers of PD. Several metabolites were nominally associated with PD but, after adjustment for multiple testing, none remained significant. Further, we were unable to reliably distinguish cases from controls based on their metabolomic profiles. These results contradict several retrospective

metabolomics investigations of PD and emphasize the need for careful study design in investigations of potential biomarkers.

In **Chapter 3**, we assessed whether adherence to a Mediterranean-style diet was associated with non-motor features of prodromal PD. Here, we found that increased adherence to a Mediterranean-style diet was associated with a lower combined number of prodromal Parkinson's features as well as with three specific features. These findings add further weight to the evidence that adherence to a Mediterranean-style diet could reduce the occurrence of specific features of prodromal Parkinson's disease.

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Chapter 1

Plasma metabolic markers of insulin resistance and diabetes and rate of incident Parkinson disease

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Abstract

Background: Although there is evidence of shared dysregulated pathways between diabetes and Parkinson disease, epidemiologic research on an association between the two diseases has produced inconsistent results. We aimed to assess whether known metabolic markers of insulin resistance and diabetes contribute to predict risk of Parkinson disease.

Methods: Nested case-control study among Nurses' Health Study and Health Professionals Follow-up Study participants who had provided blood samples up to twenty years prior to Parkinson diagnosis. Cases were matched to risk-set sampled controls by age, sex, fasting status, and time of blood collection. All participants provided covariate information via regularly collected cohort questionnaires. We used conditional logistic regression models to assess whether plasma levels of branched chain amino acids, acylcarnitines, glutamate, or glutamine were associated with incident development of Parkinson disease.

Results: A total of 349 case-control pairs were included in this analysis. In the primary analyses, none of the metabolites of interest were associated with Parkinson disease development. In investigations of the association between each metabolite and Parkinson disease at different time intervals prior to diagnosis, some metabolites showed marginally significant association but, after correction for multiple testing, only C18:2 acylcarnitine was significantly associated with Parkinson disease among subjects for whom blood was collected less than 60 months prior to case diagnosis.

Conclusions: These results do not support the hypothesis that markers of diabetes or insulin resistance are associated with Parkinson disease, corroborating previous research in these cohorts that failed to identify a relationship between diabetes and Parkinson disease.

Introduction

A link between Parkinson disease (PD) and diabetes was originally suggested by the observation that individuals with PD frequently have impaired glucose tolerance[1], and was later supported by the discovery that dysregulated pathways related to mitochondrial biogenesis and respiration may play a role in the pathophysiology of both diseases[2,3]. The results of epidemiological studies, however, have been inconsistent. A significant increase in PD risk among individuals with type 2 diabetes has been reported in a cohort in Finland[4], in registry-based studies in Denmark[5] and Taiwan[6] and in two cohorts in the U.S.[7,8], but not in other large longitudinal studies.[9,10] To better understand the relation between diabetes and PD, we conducted a prospective investigation to determine whether plasma biomarkers that have been associated with an increased risk of diabetes, including increased plasma levels of branched chain amino acids (BCAAs), some species of acylcarnitines, and glutamate as well as decreased levels of plasma glutamine [11-15] , contribute to predict future PD risk.

As each of these metabolites may also directly or indirectly influence nervous system functioning[16-25], it is plausible that alterations in these metabolites may explain the observed association between diabetes and PD.

Methods

Study population

This investigation uses data and blood samples from participants in the Nurse's Health Study (NHS) and the Health Professional's Follow-up Study (HPFS). Briefly, the NHS cohort consists of 121,700 female registered nurses who were aged 30-55 and residing in one of eleven states at the time of enrollment in 1976. The HPFS cohort consists of 51,529 male health professionals who were aged 40-75 and completed a baseline questionnaire in 1986. Participants in both the NHS and HPFS complete biennial follow-up questionnaires, which

include questions regarding lifestyle practices, diet, and medical history. Between May 1989 and September 1990, blood samples were collected using heparin tubes and processed for 32,825 NHS members[26]. Blood samples were similarly collected from 18,018 HPFS participants between April 1993 and August 1995 using liquid EDTA tubes[27]. The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

Case ascertainment and Control Selection

Incident PD cases are identified through the biennial self-report questionnaires in each of the cohorts. Following a participant's first report of PD, we request permission to contact their neurologist and/or obtain copies of their medical records. Prior to 2003, cases were confirmed if the treating medical professional considered the diagnosis definite or probable, or the medical record indicated at least two of the cardinal signs of PD (resting tremor, rigidity, bradykinesia). Since 2003, the case ascertainment procedure has been modified such that medical records are requested from all cases and these records are reviewed by a movement disorders specialist. The analyses described here include 349 incident cases of confirmed PD with PD diagnosis occurring after (n=328 cases) or shortly before (n=21 cases) blood collection.

For each case, we selected one control matched by cohort, age (within one year), and month and time of blood collection (within one month), fasting status at blood collection (fasted: 8≤ hours since last meal), and race (white versus non-white). Each control was randomly selected among cohort members at risk of developing PD at the time of diagnosis of the index case.

Metabolite profiling

NHS and HPFS participants' blood samples were collected as described previously.[28] Upon sample collection, samples were stored in the vapor phase of liquid nitrogen freezers at

less than -130ºC. Following selection into this study, samples were randomly ordered and shipped on dry ice to the Broad Institute for metabolomics analysis. Plasma samples from each matched case-control pair were handled identically and assayed in the same batch. Within matched pair, the samples were randomly ordered to ensure that all assays were conducted without knowledge of case-controls status.

The metabolites were profiled at the Broad Institute (Cambridge, MA) as has been previously described.[29] In short, all the metabolites of interest in this study were profiled using hydrophilic interaction liquid chromatography in the positive ionization mode (HILIC-pos). On the HILIC-pos platform, the analyses were conducted using an LC-MS consisting of a Shimazdu Nexera X2 U-HPLC (Shimazdu Corp.) coupled to a Q Extractive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific). Plasma samples (10 µL) were extracted using nine volumes of 74.9:24.9:0.2 (v/v/v) acetonitrile/methanol/formic acid containing stable isotope-labeled internal standards (0.2 ng/µL valine-d8, Isotec; and 0.2 ng/µL phenylalanine-d8, Cambridge Isotope Laboratories). The samples were centrifuged (10 min, 9,000g, 4ºC) and the supernatants (10 µL) were injected directly onto a 150 x 2.1 mm Atlantis HILIC column (Waters). The column was eluted isocratically at a flow rate of 250µL/min with 5% mobile phase A (10 mM ammonium formate and 0.1% formic acid in water) for 0.5 minute followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 minutes. Mass spectroscopic (MS) analyses were performed using electrospray ionization in the positive ion mode using full scan analysis over 70 to 800 *m/z* at 70,000 resolution and 3 Hz data acquisition rate. The ion spray voltage was 3.5 kV, the capillary temperature was 350ºC, and the heater temperature was 300ºC.

Covariate Assessment

Information on lifestyle practices and medical history is collected biennially by self-report questionnaires in both the NHS and HPFS. Similarly, dietary data is collected every four years

by self-administered semiquantitative food frequency questionnaires (FFQ) in both cohorts, which captures average intake pattern of food and beverage during the 12 months preceding FFQ completion. For these analyses, we used covariate information from the last questionnaire cycle prior to beginning of blood collection for each cohort. In the NHS, this means that we used lifestyle practice and medical history data from the 1988 questionnaire cycle and diet information from the 1986 FFQ. In the HPFS, lifestyle practice and medical history data was used from the 1992 questionnaire and dietary data from the 1990 FFQ. In the event of a participant missing data on a given covariate, the value observed from the preceding questionnaire cycle was carried forward. Missing value indicators were used if the value from the preceding questionnaire cycle was also missing and could not be carried forward.

Statistical analyses

For samples with a missing value for a given metabolite, the missing value was replaced with half of the minimum of non-missing values observed within cohort. The coefficient of variation was calculated based on QC samples to assess inter-assay reproducibility of each metabolite. Metabolite values were log-transformed and, within cohort, standardized to the values of the control distribution. Paired t tests were used to compare mean log-transformed and standardized metabolite values between cases and controls.

For each individual metabolite of interest, we conducted conditional logistic regressions stratified on matched pair to estimate the rate ratio (RR) and corresponding 95% confidence interval. Each log-transformed and standardized metabolite value was modelled both continuously, to obtain the RR per standard deviation (SD) increase in metabolite value, as well as categorically using quartiles defined by the within-cohort control distribution, to obtain the RR comparing each of the higher quartiles to the lowest quartile. In the quartile-based analyses, we conducted additional conditional logistic regression models using the median value of each quartile to test for linear trend.

For both the continuous and quartile-based approaches, a series of models were estimated with increasing control for potential confounders, including fasting status (fasted: 4+ hours since last meal prior to sample collection), pack year category (never smoker, 0-9.9 pack years, 10-19.9 pack years, 20-29.9 pack years, 30+ pack years), cumulative average caffeine intake (mg/day) quartile (defined by within-cohort distribution), BMI category (<23, 23-24.9, 25-26.9, 27-29.9, 30+ kg/m^2), baseline history of diabetes, and log-transformed and standardized plasma uric acid level. To test whether the association between each metabolite and PD varied according to time between blood collection and diagnosis, we introduced an interaction term between metabolite level and duration of time between blood collection and date of PD diagnosis (<60, 60-179, or 180+ months). For each set of models explored, multiple testing was corrected for by calculating the false discovery rate (FDR) adjusted p-value according to the Benjamini-Hochberg approach.

To investigate the association between groups of similar metabolites and incident PD, we calculated summary scores for the following metabolite groupings, as defined in previous studies[30,31]: BCAAs (leucine, isoleucine, and valine; 3 species), short-chain acylcarnitines (C2carnitine-C7carnitine; 9 species), medium-chain acylcarnitines (C8carnitine-C14:2carnitine; 9 species), and long-chain acylcarnitines (C16carnitine-C26carnitine; 6 species). Metabolite group specific summary scores were calculated as the sum of the log-transformed and standardized metabolite values within each group of metabolites ('Summary Score 1'), which weighted each metabolite's contribution to its group equally. An alternative form of the summary score ('Summary Score 2') was also explored by summing raw metabolite values within group prior to log-transformation and standardization, which weighted each metabolite's contribution to the summary score by LC-MS peak size.

We further conducted a series of sensitivity analyses to assess how robust our results were to modeling decisions. In these analyses, we conducted analyses excluding matched pairs

for whom the case's PD diagnosis occurred prior to blood collection, restricted to participants in specific interval length categories, excluded cases and controls with a history of diabetes, and additionally adjusted for factors such as use of diabetes medications (restricted to NHS participants; data unavailable for HPFS participants at baseline), total caloric intake, and physical activity, and more minimally adjusted for only fasting status and the interaction between metabolite level and interval length category. Additionally, we assessed the possibility of a non-linear relationship between metabolite level and PD using restricted cubic splines models for both k=3 and k=4 internal knots.

All statistical analyses were performed with the use of SAS software (v9.4; SAS Institute).

Results

Baseline characteristics of the 698 (349 cases, 349 controls) participants included in these analyses are presented in Table 1.1. As expected due to matching, the age, sex, and time interval length distributions are comparable between cases and controls. Additionally, the distributions of BMI and history of diabetes were comparable between cases and controls but cases had fewer smoking pack-years and lower cumulative average caffeine intake than controls. For all metabolites of interest, the CV was less than 20% except for C7 carnitine (mean CV=47.98%) and C3-DC-CH3 carnitine (mean CV=26.80%). In general, cases tended to have lower values of most metabolites as compared to controls. Based on the results of paired t-tests, the mean levels of C9-carnitine (mean difference=-0.18, 95% CI: -0.32, -0.03; p=0.02), C12-carnitine (mean difference=-0.14, 95% CI: -0.28, -0.01; p=0.04), and C14-carnitine (mean difference=-0.17, 95% CI: -0.32, -0.02; p=0.02) were significantly different between cases and controls (Supplementary Table 1.1), but these differences did not remain significant after adjustment for multiple testing. The distribution of the summary scores for each metabolite group followed a similar pattern; cases had lower mean values of the summary scores for each

Table 1.1: Study population characteristics

	Cases	Controls
N	349	349
Age (yr)[a]	62.5 (7.7)	62.5 (7.7)
Sex (male)[b]	51.0 (178)	51.0 (178)
Fasted (≥4 hrs) [b]	73.6 (257)	75.1 (262)
Time interval (years) [a]	9.1 (5.7)	9.1 (5.7)
Time interval category [b]		
<5 years prior to diagnosis	25.5 (89)	25.2 (88)
5-15 years prior to diagnosis	57.3 (200)	57.6 (201)
15+ year prior to diagnosis	17.2 (60)	17.2 (60)
Smoking pack-years [a]	8.1 (15.1)	13.1 (17.9)
Smoking pack-year categories [b]		
Never Smoker	56.5 (197)	41.8 (146)
0-9.9 pack-years	14.0 (49)	12.9 (45)
10-19.9 pack-years	10.9 (38)	14.9 (52)
20-20.0 pack-years	6.9 (24)	10.9 (38)
30+ pack-years	9.2 (32)	16.3 (57)
Caffeine (cumulative avg. mg/day) [a]	222.4 (198.7)	253.7 (208.1)
Caffeine (cumulative avg. mg/day) quartile [b]		
Quartile 1	27.2 (95)	21.2 (74)
Quartile 2	27.2 (95)	22.6 (79)
Quartile 3	21.2 (74)	27.8 (97)
Quartile 4	22.9 (80)	26.4 (92)
History of diabetes [b]	4.3 (15)	5.2 (18)
BMI [a]	25.3 (3.4)	25.4 (3.6)

[a]Continuous variable: mean (sd)

[b]Categorical variable: % (n)

metabolite group except for the long-chain acylcarnitine scores, but these differences were not significant. To explore whether metabolite values differed between cases and controls based on length of time between blood collection and case diagnosis, Figure 1.1 shows the mean difference within case-control pair of log-transformed and standardized metabolite values for the BCAAs and the acylcarnitine groups; although some of these differences are different from 0, none are significant after adjustment for multiple testing. Glutamate and glutamine, which are not included in any of the BCAA or acylcarnitine groupings, were also not different between cases and controls at any time point.

In the single metabolite regression analyses, none of the metabolites was significantly associated with PD in either the minimally adjusted model (Supplementary Table 1.2), adjusting for pack-year category, cumulative average caffeine intake quartile, and fasting status, or the more fully adjusted model (Supplementary Table 1.3), which additionally adjusted for diabetes history, BMI category, and plasma uric acid level. The results of analyses of summary scores for each category of metabolite type were similar. As shown in Table 1.2, there was no evidence of an association between any of the metabolite groups and PD. This finding was consistent across both forms of the summary score that were assessed.

The incorporation of an interaction between metabolite level and time interval category indicates that, for some metabolites, there is a different association between the metabolite and PD among individuals at different time points prior to PD diagnosis. The results of these models are summarized according to metabolite group in Supplementary Tables 1.4a-1.7b. The results of the models for glutamate and glutamine are included in the Supplementary Table 1.4 with the BCAAs. For the single metabolite analyses, the interaction between metabolite level and time interval category contributed significantly to the median quartile-based model for the following acylcarnitines: C3-DC-CH3 ($p_{interaction}$=0.007), C12:1 ($p_{interaction}$=0.03), C14:2 ($p_{interaction}$=0.03), C16 ($p_{interaction}$=0.04), C18:1 ($p_{interaction}$=0.01), and C18:2 ($p_{interaction}$=0.003). In the continuous models,

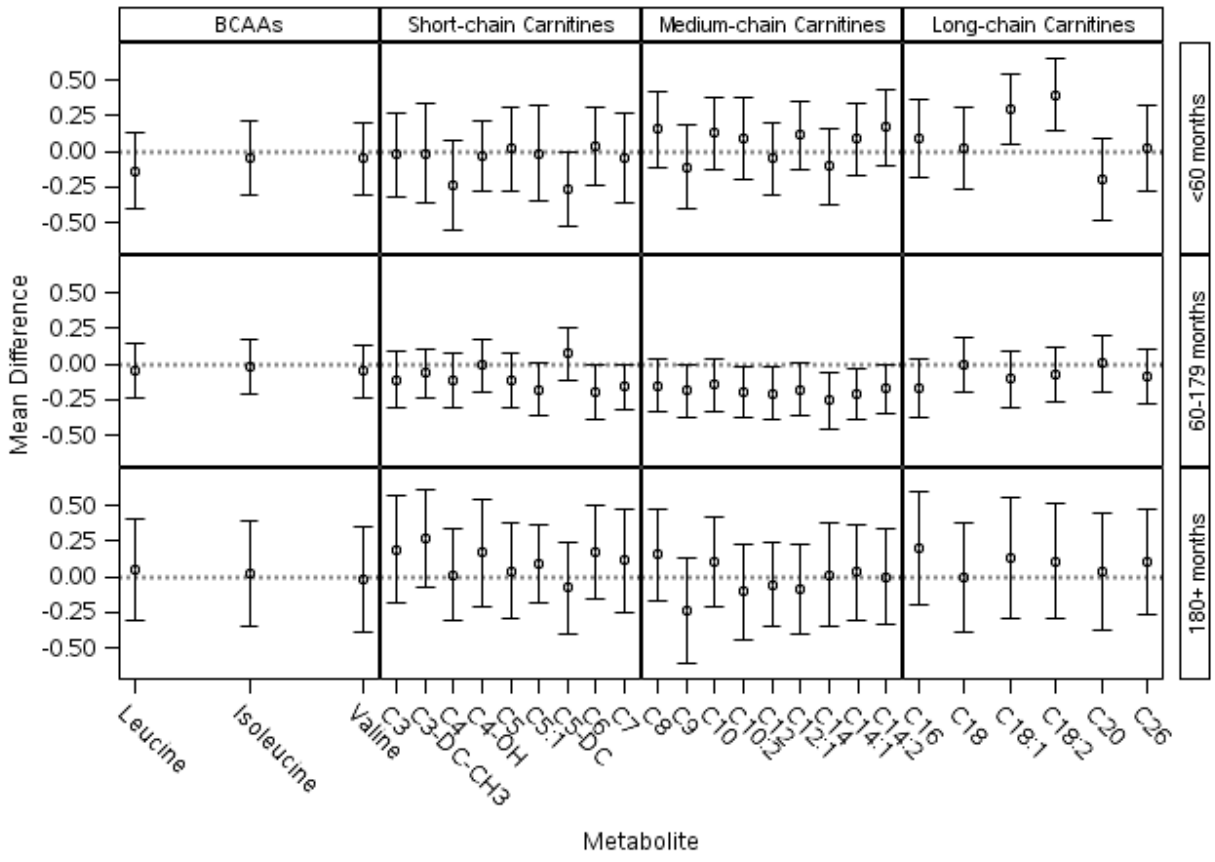

Figure 1.1: Mean difference within case-control pair of log-transformed and standardized metabolite value by metabolite group and interval category. No differences were statistically significant after adjustment for multiple testing.

Table 1.2: Association between metabolite group summary scores and incident PD for summary score 1 and summary score 2

	Summary Score 1 RR (95% CI)[a]		Summary Score 2 RR (95% CI)[b]	
Metabolite Group	Min. Adjusted	Fully Adjusted	Min. Adjusted	Fully Adjusted
BCAAs				
Per SD	0.98 (0.93, 1.04)	0.98 (0.92, 1.04)	0.95 (0.80, 1.13)	0.94 (0.78, 1.13)
Across Quartiles				
Q1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Q2	0.81 (0.52, 1.26)	0.82 (0.52, 1.29)	0.77 (0.50, 1.19)	0.75 (0.48, 1.18)
Q3	0.86 (0.55, 1.33)	0.80 (0.50, 1.26)	0.86 (0.56, 1.34)	0.80 (0.51, 1.26)
Q4	0.77 (0.49, 1.22)	0.78 (0.48, 1.26)	0.76 (0.48, 1.20)	0.74 (0.46, 1.21)
p trend	0.32	0.31	0.33	0.29
Short-Chain Carnitines				
Per SD	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.95 (0.82, 1.12)	0.95 (0.81, 1.13)
Across Quartiles				
Q1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Q2	0.74 (0.48, 1.15)	0.71 (0.45, 1.11)	0.90 (0.58, 1.39)	0.89 (0.57, 1.40)
Q3	0.72 (0.45, 1.16)	0.61 (0.37, 1.02)	0.64 (0.40, 1.02)	0.63 (0.39, 1.02)
Q4	0.80 (0.50, 1.27)	0.77 (0.47, 1.26)	0.97 (0.62, 1.52)	0.96 (0.60, 1.53)
p trend	0.32	0.24	0.69	0.67
Medium-Chain Carnitines				
Per SD	0.99 (0.97, 1.01)	0.99 (0.96, 1.01)	0.93 (0.78, 1.10)	0.91 (0.76, 1.09)
Across Quartiles				
Q1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Q2	0.86 (0.54, 1.36)	0.83 (0.52, 1.32)	0.76 (0.48, 1.20)	0.74 (0.46, 1.18)
Q3	1.02 (0.64, 1.63)	0.94 (0.58, 1.52)	1.08 (0.67, 1.73)	1.04 (0.64, 1.68)
Q4	1.01 (0.62, 1.64)	0.97 (0.59, 1.61)	0.96 (0.59, 1.55)	0.91 (0.55, 1.49)
p trend	0.82	0.95	0.84	0.99
Long-Chain Carnitines				
Per SD	1.00 (0.97, 1.03)	0.99 (0.96, 1.03)	1.02 (0.87, 1.21)	1.00 (0.84, 1.18)
Across Quartiles				
Q1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Q2	0.85 (0.54, 1.34)	0.80 (0.50, 1.27)	0.80 (0.49, 1.29)	0.76 (0.47, 1.23)
Q3	0.78 (0.48, 1.28)	0.71 (0.43, 1.18)	1.21 (0.78, 1.89)	1.13 (0.71, 1.79)
Q4	0.98 (0.63, 1.51)	0.89 (0.57, 1.40)	1.02 (0.64, 1.62)	0.96 (0.60, 1.55)
p trend	0.81	0.52	0.50	0.70

Minimally adjusted model adjusted for pack-year categories, cumulative average caffeine intake quartile and fasting status (≥4 hours); fully adjusted model additionally adjusted for history of diabetes at baseline, BMI category, and plasma uric acid level. Per SD results from model using continuous summary score value, quartile-specific results from models using summary score quartiles, *p* trend from model of summary score median quartile values.

[a]Summary Score 1 calculated by log-transforming and standardizing each individual metabolite LC-MS peak value and summing these values within metabolite group

[b]Summary Score 2 calculated by summing raw LC-MS peak intensities within metabolite group and then log-transforming and standardizing these sums

the interaction was significant for C8 ($p_{interaction}$=0.04), C12:1 ($p_{interaction}$=0.047), C14:1 ($p_{interaction}$=0.03), C14:2 ($p_{interaction}$=0.01), C18:1 ($p_{interaction}$=0.01), and C18:2 ($p_{interaction}$=0.01) acylcarnitines. In general, the significance of these interactions reflects a stronger association between these metabolites and PD in a specific time category. For example, the association between C18:2 acylcarnitine and PD is stronger among those less than 60 months prior to diagnosis (continuous model: RR=1.79 (1.20, 2.67)) as compared to among those further from diagnosis at the time of blood collection (60-179 months: RR=0.85 (0.68, 1.06); 180+ months: RR=1.04 (0.73, 1.47)). After correction for multiple testing using the FDR, only the association from the median quartile-based model between C18:2 acylcarnitine and PD among those in the shortest interval category, when cases are likely to be in the prodromal period, remained significant (FDR p_{trend}=0.054), suggesting that increased level of C18:2 acylcarnitine is associated with increased rate of developing PD. The results obtained from models restricted to individuals in a given interval category were comparable to those obtained by incorporating the interaction between interval category and metabolite level.

In models for the association between each metabolite group summary score and PD that included an interaction term between metabolite group summary score and interval category, the interaction was significant for models of both versions of the medium-chain acylcarnitines scores in the continuous model (Summary Score 1 $p_{interaction}$=0.04; Summary Score 2 $p_{interaction}$=0.04) and long-chain acylcarnitine Summary Score 2 in the continuous model ($p_{interaction}$=0.03). The results of these models are also summarized in Figure 1.2-1.3 and Supplementary Tables 1.4a-1.7b by metabolite group, and, consistent with the results of the single metabolite analyses, none of the associations between metabolite summary score and PD within interval category are significant after correction for multiple testing except for the association between the long-chain acylcarnitine Summary Score 2 and PD among individuals

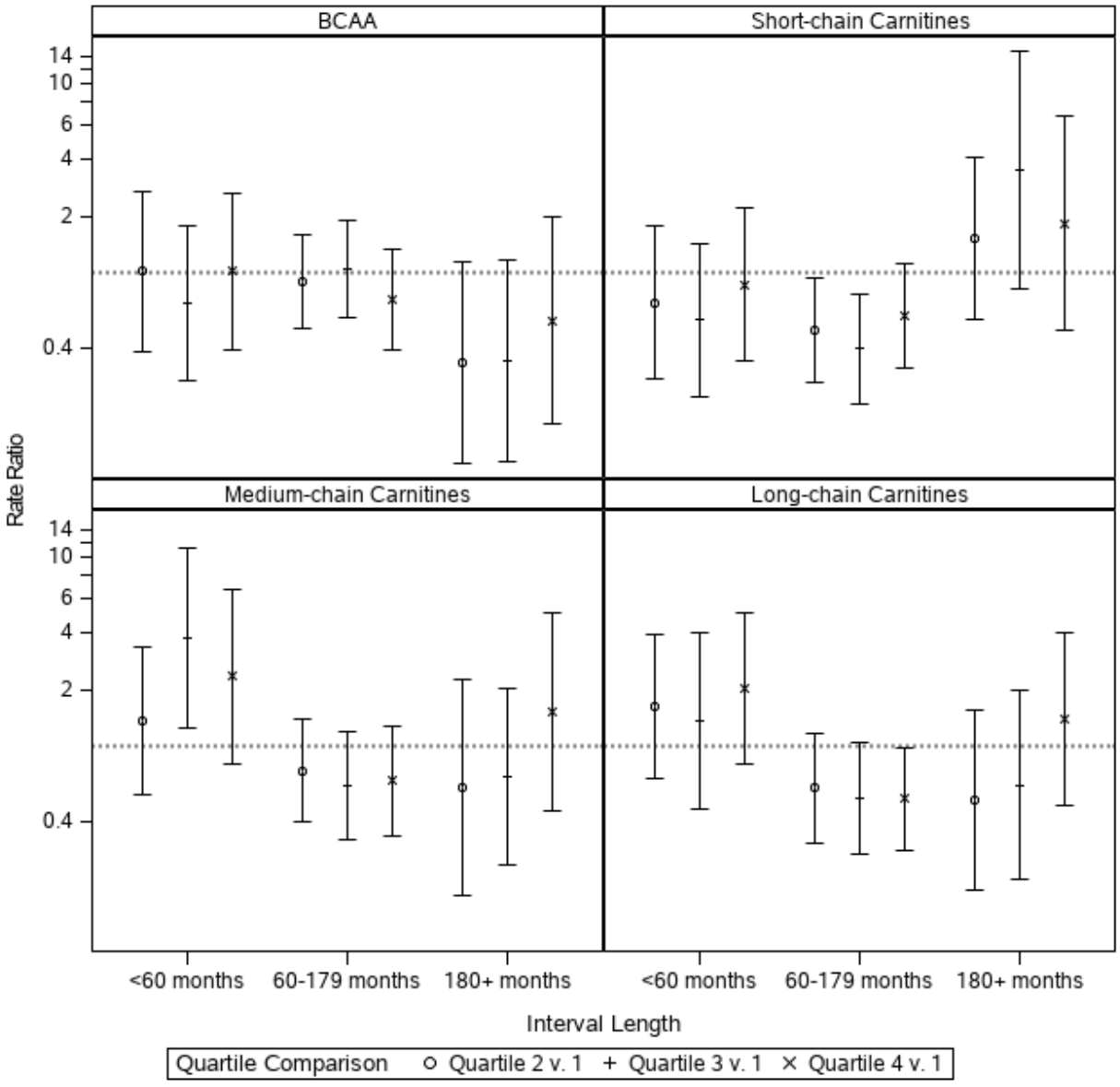

Figure 1.2: Quartile-specific associations between each metabolite group and PD by interval length for Summary Score 1 estimated using conditional logistic regression models stratified on matched pair and adjusted for pack-year category, cumulative average caffeine intake quartile, fasting status, diabetes status at baseline, BMI category, and plasma uric acid level. Y-axis is scaled using the natural logarithm.

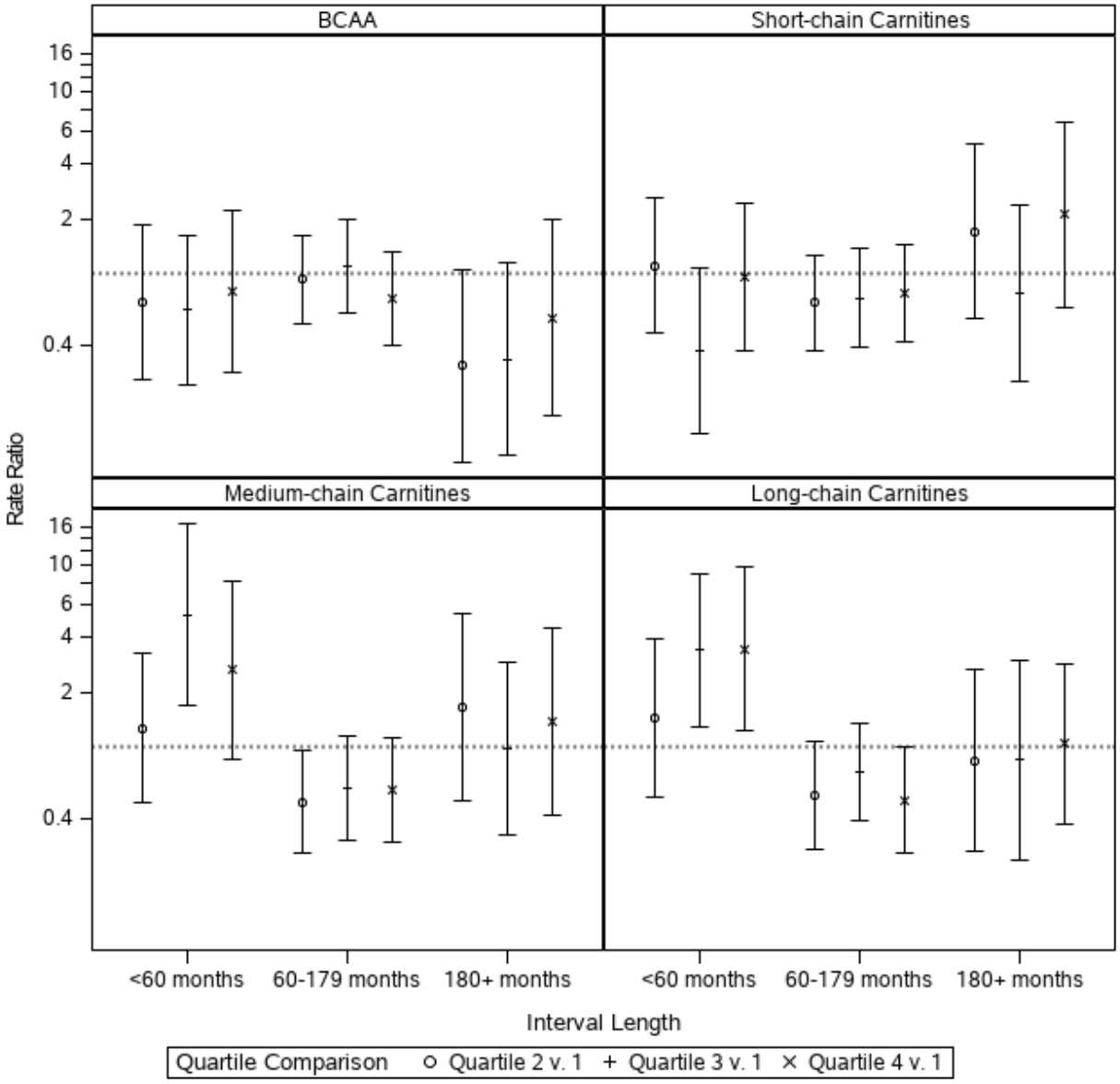

Figure 1.3: Quartile-specific associations between each metabolite group and PD by interval length for Summary Score 2 estimated using conditional logistic regression models stratified on matched pair and adjusted for pack-year category, cumulative average caffeine intake quartile, fasting status, diabetes status at baseline, BMI category, and plasma uric acid level. Y-axis is scaled using the natural logarithm.

in the shortest interval category of the quartile-based model (FDR p_{trend}=0.048), likely reflecting the association between C18:2 acylcarnitine and PD among individuals in this interval category.

The results of the main analyses described above did not change substantially when interval length was modelled continuously, when further adjustment was made for use of diabetes medications, total caloric intake, or physical activity, or when models were more minimally adjusted for only fasting status. Exclusion of matched pairs for whom the case's PD diagnosis occurred shortly before blood collection yielded similar results. Similarly, exclusion of subjects with diabetes at baseline did not substantially alter any findings. In exploring two sets of restricted cubic splines models with knots placed at the 10th 50th and 90th percentiles of the data (k=3 internal knots) as well as models with knots placed at the 5th, 35th, 65th, and 95th percentiles of the data (k=4 internal knots), the results were similar to those obtained in the quartile-based analyses described above.

Discussion

In this prospective nested case-control study, we found no evidence that branched chain amino acids, glutamate, glutamine, or acylcarnitine metabolites, all markers of insulin resistance or diabetes,[11-15] act as risk factors of PD development either individually or by metabolite class. Investigation into whether the relationship between each of these metabolites and PD differs depending on time between blood sample collection and PD diagnosis revealed that some metabolite levels may be altered at different times prior to PD diagnosis, but that no metabolite was consistently altered across each time interval assessed. After correction for multiple testing, none of the metabolites were significantly associated with PD in any of our analyses except for the association between C18:2 acylcarnitine and PD and corresponding association between the long-chain acylcarnitine Summary Score 2 and PD among those less than 60 months from diagnosis.

Recently, there have been several case-control investigations of metabolomics in relation to Parkinson disease,[32-35] including some studies[36,37] that have suggested decreased levels of long-chain acylcarnitines might act as a marker of PD. Although the findings presented here contradict these reports to some extent, it is important to note several differences between our study and other studies that may account for the inconsistencies. First, previous studies have included only samples from patients after disease onset whereas our study includes primarily pre-diagnostic samples collected prior to disease onset. As the metabolome may be influenced by changes related to disease processes or behavioral changes that might occur after diagnosis, it is difficult to directly compare the findings of this study to those of previous studies. Second, our study was nested within two large cohort studies whereas other studies have recruited participants from hospitals, ongoing clinical trials, and other source populations that may represent different underlying populations. Further, our study utilized plasma samples whereas other studies have investigated metabolomics in a variety of plasma, serum, and cerebrospinal fluid samples; it is possible that the relationship between a given metabolite and PD may differ based on the sampled biofluid.

This investigation has several key strengths. First, as a prospective nested case-control study, there are several strengths due to the study design. As a prospective study of incident PD, we were able to assess potential risk factors for the development of PD with little risk of reverse causation explaining our findings. Further, as the study is nested within two large cohorts, we minimized the risk of selection bias when selecting controls and ensured that there were no systematic differences between cases and their matched controls with respect to blood collection and processing. Another key strength of this study was the ability to combine metabolomics measurements with prospectively collected data on a variety of covariates, which allowed for careful control for potential confounders. Lastly, we conducted thorough sensitivity analyses to assess the robustness of our findings.

There are limitations of this study. It is possible that some of the less stable metabolites in our blood samples may have degraded during sample shipment or processing and that there may have been measurement error in the metabolite measurement procedures. Given the prospective nature of the study, the careful blinding of lab staff to the case status of each sample, the simultaneous analyses of cases with their matched controls, and previous quality control analyses using samples from the same cohorts and the Broad Metabolomics Platform, the effects of such measurement error or sample degradation for most metabolites are likely to be modest and nondifferential, thus resulting in a possible bias towards the null.[28] An additional limitation of the study is that we have only a single measurement for each participant, which may not be representative of long-term levels of these metabolites. However, the metabolomics validation study conducted in these cohorts also assessed reproducibility; among participants who donated two blood samples 0.8-2.3 years apart, the Spearman correlation between measurements was greater than 0.4 for more than 90% of metabolites, suggesting that a single measurement may reasonably reflect longer-term levels of metabolites.[28] Another limitation of this study is that, despite the large sample size compared to previous PD metabolomics investigations, we may be underpowered to detect effect modification, as evidenced by the wide confidence intervals in some of the interval length specific analyses.

In conclusion, our results do not support the hypotheses that metabolic markers of insulin resistance and type 2 diabetes also act as markers of pre-diagnostic Parkinson disease. These results generally support previous research conducted in the NHS and HPFS suggesting no association between diabetes and incident development of PD.[10] Given the lack of previous association between diabetes and PD in these cohorts and the low number of subjects with history of diabetes at baseline in this specific investigation, additional prospective investigation of the relationship between diabetes markers and PD in other populations is needed in order to establish the robustness of these findings.

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Chapter 2

Pre-diagnostic plasma metabolomics and risk of Parkinson's disease

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Abstract

Objective: To identify plasma metabolomic biomarkers of Parkinson disease (PD) in blood samples collected prior to diagnosis.

Methods: A nested, risk-set sampled case-control study was conducted in the Nurses' Health Study, Health Professionals Follow-up Study, and Cancer Prevention Study II Nutrition Survey Cohort. Each of the 817 incident PD cases who provided blood samples before diagnosis was matched to one control based on age, cohort membership, sex, fasting status, race, and time of blood collection. Smoking behavior, caffeine consumption, physical activity, body mass index (BMI) and other covariates were obtained from validated questionnaires. Conditional logistic regression was used to determine each metabolite's association with PD and prediction modeling techniques were used to assess whether individuals' metabolomic profiles could distinguish cases from controls.

Results: Of 345 known metabolites, 16 were associated with PD after multivariable-adjustment for confounders but none remained significant after multiple testing correction with the Benjamini-Hochberg false discovery rate approach. Across all metabolites, results were skewed such that most metabolites were decreased among cases. Different metabolites were associated with PD for subjects with different time intervals between blood collection and PD onset, but none remained significant following correction for multiple comparisons. Based on these data, we were unable to distinguish cases from controls using L_1-penalized regression, elastic net regression, or support vector machines (SVM).

Conclusions: Although no single metabolite was associated with PD and plasma metabolomic profiles could not be used to distinguish cases from controls, these data suggest that some broad disruption in metabolism may precede PD diagnosis.

Introduction

The progressive neurodegenerative process that leads to PD begins 10 years or more before the onset of any of the hallmark motor symptoms[38]. Among persons in the prodromal phase of PD, there is an increased occurrence of non-specific motor symptoms, including constipation, hyposmia, and rapid eye movement (REM) sleep behavior disorder (RBD),[39-44] but most individuals appear healthy during this phase. This appearance of health is misleading; by the time of clinical diagnosis of PD, there is already extensive and irreversible pathologic damage[45,46].

The ability to reliably recognize PD earlier in the disease process would enable the initiation of neuroprotective treatments before damage due to PD neuropathogenesis is too great for such treatments to be effective. The systemic nature of PD, the occurrence of non-motor symptoms in the years before diagnosis, and the associations between PD and metabolic-related factors such as mitochondrial dysfunction[47], adiposity[48-50], diabetes[2,4], and plasma urate[51] suggest that metabolomics could be used to identify preclinical or premotor PD. Prior investigations[32-37,52-61] indicate there may be a metabolic profile indicative of PD, however these studies were small and conducted with existing PD cases and therefore do not eliminate the possibility of reverse causation. To our knowledge, there have been no prospective metabolomics investigations of preclinical idiopathic PD. In this study, we investigated the relationship between plasma metabolomic profiles and incident PD using blood samples collected prior to PD diagnosis.

Methods

Study population

Data and blood samples collected from participants in three prospective cohorts, the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), and the Cancer

Prevention Study II Nutrition Survey Cohort (CPS-IIN), were used in these analyses. In brief, the NHS cohort consists of 121,700 female registered nurses aged 30-55 and residing in one of eleven states at the time of enrollment in 1976. The HPFS cohort consists of 51,529 male health professionals who were aged 40-75 and completed a baseline questionnaire in 1986. The CPS-IIN cohort is composed of a subset of the American Cancer Society's (ACS) Cancer Prevention Study II cohort and consists of 77,048 men and 85,360 women aged 50-75 at time of enrollment into the Nutrition Survey Cohort in 1992. All three cohorts have been followed over time using mailed questionnaires; NHS and HPFS participants have completed biennially administered questionnaires since baseline regarding lifestyle practices, diet, and medical and occupational history while CPS-IIN participants completed a similar follow-up questionnaire in 1997 and then biennially thereafter. In addition to these questionnaires, participants in each cohort were invited to provide a blood sample. Blood samples were collected from 32,825 NHS participants in 1989-90[26], from 18,018 HPFS participants in 1993-95[27], and from 39,371 CPS-IIN participants in 1998-2001[62]. NHS samples were collected in heparin blood tubes while HPFS and CPS-IIN samples were collected using liquid EDTA blood tubes.

The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

Case ascertainment and control selection

In each cohort, incident PD cases are initially identified via self-report on the regular follow-up questionnaires. Following a participant's initial PD report, we re-contact that individual to confirm their self-report and request permission to contact their neurologist and obtain copies of their medical records. Until 2003, cases were confirmed if the treating medical professional considered the diagnosis to be definite or probable or there was evidence of at least two of the three cardinal signs of PD (resting tremor, rigidity, bradykinesia) in that individual's medical record. Since 2003, medical records have been requested from all cases and reviewed by a

movement disorders specialist, who classifies each case as definite, probable, uncertain, or not PD.

Main analyses include 817 incident cases of PD with blood samples collected prior to PD diagnosis; secondary analyses were conducted excluding 112 of these cases who reported a PD diagnosis but whose medical records are either unavailable or otherwise insufficient to confirm their PD diagnosis with reasonable certainty. Further, we obtained blood samples from an additional 113 PD cases who provided a blood sample within 7.5 years after PD diagnosis. These cases were included in an additional set of secondary analyses to explore metabolomic signals that may become apparent only in clinically manifest PD. For each case that was identified, a matched control was risk-set sampled. Within each cohort, cases were matched to controls based on age (within one year), sex, month and time of blood collection (within one month), fasting status at blood collection (fasted: 8≤ hours since last meal), and race. Among the controls, 5 developed PD at some point after the diagnosis date of their matched case.

Metabolite profiling

Blood sample collection procedures in these cohorts have been previously described[62-64]. Briefly, in the NHS and the HPFS, participants donating blood arranged to have their blood drawn and shipped via overnight courier in a foam container with icepacks to the lab. Upon arrival, samples were centrifuged and aliquoted and have since been stored in liquid nitrogen vapor phase at less than -130ºC. In the NHS, 97% of samples were received within 26 hours of blood draw; in the HPFS, 95% of samples were received within 24 hours of blood draw. The blood collection procedure in CPS-IIN was similar except that the ACS coordinated with hospitals to arrange participants' blood collection, therefore only participants living in urban or suburban areas were invited to provide a sample. Among CPS-IIN participants, 94% of samples arrived at the ACS's central repository for processing within 24 hours of blood collection.

Following selection into this study, samples were randomly ordered and shipped on dry ice to the Broad Institute for metabolomics analysis. Each matched pair's plasma samples were handled identically, assayed in the same batch, and randomly ordered to ensure that assays were conducted without knowledge of case-control status. The Metabolomic Profiling Platform at the Broad Institute used three liquid-chromatography tandem mass spectrometry (LC-MS) methods, which allowed for both targeted and nontargeted analyses. Reference standards of each metabolite were used to determine chromatographic retention times and MS multiple reaction monitoring transitions, declustering potentials and collision energies for the polar metabolite profiling methods. Briefly, the three different LC-MS methods respectively measure water-soluble metabolites in the positive ionization mode (HILIC-pos), water soluble metabolites in the negative ionization mode (HILIC-neg), and polar and non-polar lipids in positive ion mode (C8-pos). The technical details of these LC-MS methods have been described[65] and will not be reiterated.

Metabolomic profiling of the samples in these analyses was conducted at three different points in time. The first group of samples, consisting of 170 NHS pairs and 184 HPFS pairs, was profiled between June-November, 2016. The second set of samples, consisting of all 313 pairs from CPS-IIN, was profiled between December, 2017 through April, 2018. Lastly, the remaining 123 NHS and 140 HPFS pairs were profiled between July-October, 2018. To enhance our ability to simultaneously analyze data from each of these time points, calibration samples were included in each run and used to align the LC-MS results across the three sets of samples.

Covariate Assessment

Lifestyle practice and medical history information is regularly collected by self-report questionnaires in each cohort. The NHS and the HPFS cohorts collect dietary data every four years via self-administered semiquantitative food frequency questionnaires (FFQ), which capture average intake pattern of food and beverage during the 12 months preceding FFQ

completion. In the CPS-IIN cohort, dietary data was collected via FFQ in 1992, 1999, and 2003[62]. For these analyses, covariate information was taken from the last questionnaire cycle prior to beginning of blood collection for each cohort. As such, we used lifestyle and medical history data from the 1988 questionnaire cycle and diet information from the 1986 FFQ for NHS subjects. Lifestyle and medical history data was used from the 1992 questionnaire and dietary data from the 1990 FFQ for HPFS participants. For participants in the CPS-IIN cohort, we used covariate and dietary data from the 1999 questionnaire. In the event of a participant missing data for a specific covariate, the value of that covariate observed from the preceding questionnaire cycle was carried forward. Missing value indicators were used if the value from the preceding questionnaire cycle was also missing and could not be carried forward.

Statistical Analyses

For these analyses, we selected known metabolites with low missingness across samples (missing in all cohorts and profiling time point ≤20% and missing in any specific cohort and profiling time point ≤50%) and a low coefficient of variation in our quality control samples (CV≤25%). When metabolites measured on multiple platforms met these inclusion criteria, the measurement with the lower CV was retained for analysis. Missing metabolite values were replaced with half the minimum non-missing values observed within sex, cohort, and samples profiled at the same time. Within these same groups, metabolite values were log-transformed and standardized to the control subjects' distribution. Subjects were additionally assigned to metabolite quartiles based on sex-, cohort-, and time of metabolomic profiling. Four metabolites (cotinine, hydroxycotinine, acetaminophen glucuronide, and acetaminophen) that met CV criteria but had biologically plausible high levels of missingness were included in these analyses. Data for these metabolites were similarly prepared except that, in place of true quartiles, individuals were categorized into four groups such that those with a missing value

were considered the lowest level and the highest three categories were determined by dividing those with non-missing values into tertiles.

To assess the association between individual metabolites and PD, we used conditional logistic regression stratified on matched pair to estimate the rate ratio (RR) and corresponding 95% confidence interval (CI). Each log-transformed and standardized metabolite value was modelled continuously to obtain the RR per standard deviation (SD) increase in metabolite value. The matching factors were accounted for by stratifying on matched pair and models were additionally adjusted for fasting status (fasted: ≥4 hours since last meal prior to sample collection), pack-year category (never smoker, 0-9.9 pack-years, 10-19.9 pack-years, 20-29.9 pack-years, ≥30 pack years), sex- and cohort-specific quartiles of cumulative average caffeine intake (mg/day) and physical activity (met-hours/week), and BMI category (<23, 23-24.9, 25-26.9, 27-29.9, 30+ kg/m^2). Secondary analyses of categorical metabolite quartiles were conducted to explore the possibility of non-linear associations. Further, to assess the extent to which time might modify the association between each metabolite and PD, we estimated models with interaction terms between metabolite value and categorical time intervals of 0-59, 60-179, and ≥180 months between blood collection and PD diagnosis. Multiple testing was corrected for by calculating the false discovery rate (FDR) adjusted p-values according to the Benjamini-Hochberg approach. The distribution of coefficients across metabolites was explored by creating volcano plots and using bootstrapping (n=1000 resamples) to characterize the range of distributions consistent with the observed data.

To evaluate the extent to which metabolomic profiles measured in pre-diagnostic blood samples could distinguish future PD cases from controls, we conducted L_1-penalized logistic regression, elastic net regression, and SVM analyses. Prediction models were fit among all matched pairs where the case's blood sample was collected prior to diagnosis and medical record review had been completed. These analyses were repeated restricting to those pairs

where the case occurred, respectively, within 180 months and 120 months of diagnosis to determine whether predictive performance improved among cases closer to diagnosis at the time of blood collection. In conducting these analyses, we separated the data into three groups; the data from the CPS-IIN subjects was set aside for use as a validation set while the data from the NHS and HPFS cohorts was split into training (80%) and test (20%) sets based on match pair ID. For these analyses, metabolites and covariates were modelled continuously; missing covariates were replaced with the sex- and cohort-specific median value. To improve model performance, only metabolites that were not highly correlated with one another ($r \leq 0.9$) but which were correlated with PD status ($r_{pb} \geq 0.07$ for full data, $r_{pb} \geq 0.1$ in time-restricted analyses) in the training data were considered in the model. In each respective analysis, 10-fold cross-validation was used to select the best tuning parameter value(s) and estimate the model. Model performance was assessed on both the held-out test and validation sets using the area under the curve (AUC).

All analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, N.C.) and R 3.5.0 (https://cran.r-project.org). P-values were considered significant at values <0.05.

Results

The characteristics of the selected matched pairs at baseline are provided in Table 2.1. Due to the matched design, the distribution of age, sex, cohort membership, and race are similar among cases and controls. On average, cases consumed less caffeine, smoked fewer pack-years, and reported lower levels of physical activity than controls.

In analyses of the association between each metabolite and PD, we found that 16 out of 345 metabolites assessed were significantly different ($p<0.05$) among cases and their matched controls in models of continuous metabolite value (Table 2.2; Figure 2.1A). Of these

Table 2.1: Study population characteristics by case status among matched pairs with prospective blood collection

	Cases (n=817)	Controls (n=817)
Age, years at baseline	63.25 (8.4)	63.25 (8.4)
Female	385 (47.1)	385 (47.1)
Cohort membership		
NHS	293 (35.9)	293 (35.9)
HPFS	284 (34.8)	284 (34.8)
CPS-IIN	240 (29.4)	240 (29.4)
Ethnicity, white	753 (92.2)	756 (92.7)
Pack-years smoked	7.99 (14.6)	11.39 (16.7)
Caffeine intake, mg/day	232.82 (197.6)	263.81 (215.9)
BMI, kg/m^2	25.42 (3.6)	25.65 (3.8)
Physical activity, met-h/week	21.48 (24.1)	23.24 (24.6)
Fasted, ≥4 hours prior to blood collection	441 (54.0)	447 (54.7)

Values are means (SD) for continuous variables and number (percentage) for categorical variables.

Table 2.2: Metabolites with nominally significant RR per SD increase among 817 matched pairs with pre-diagnostic blood sample

HMDB ID	Metabolite	RR (95%CI)	Raw *p*	FDR *p*
HMDB05066	C14 carnitine	0.86 (0.77, 0.95)	0.0048	0.7141
HMDB02250	C12 carnitine	0.86 (0.77, 0.96)	0.0076	0.7141
HMDB00201	C2 carnitine	0.88 (0.79, 0.97)	0.0129	0.7141
HMDB13331	C14:2 carnitine	0.87 (0.78, 0.97)	0.0154	0.7141
HMDB02014	C14:1 carnitine	0.87 (0.78, 0.97)	0.0154	0.7141
HMDB01046	Cotinine	0.86 (0.76, 0.97)	0.0160	0.7141
HMDB06344	Phenylacetylglutamine	1.14 (1.03, 1.28)	0.0162	0.7141
HMDB13326	C12:1 carnitine	0.87 (0.78, 0.98)	0.0185	0.7141
HMDB00670	Homoarginine	1.14 (1.02, 1.27)	0.0186	0.7141
HMDB00222	C16 carnitine	0.89 (0.8, 0.99)	0.0292	0.9468
HMDB00684	Kynurenine	0.88 (0.78, 0.99)	0.0383	0.9468
HMDB02366	C5:1 carnitine	0.9 (0.81, 1)	0.0412	0.9468
HMDB12101	C18:1 SM	0.9 (0.81, 1)	0.0450	0.9468
HMDB00705	C6 carnitine	0.89 (0.8, 1)	0.0460	0.9468
HMDB00631	Glycodeoxycholate/ glycochenodeoxycholate	1.12 (1, 1.25)	0.0474	0.9468
HMDB13130	C5-DC carnitine	0.9 (0.8, 1)	0.0474	0.9468

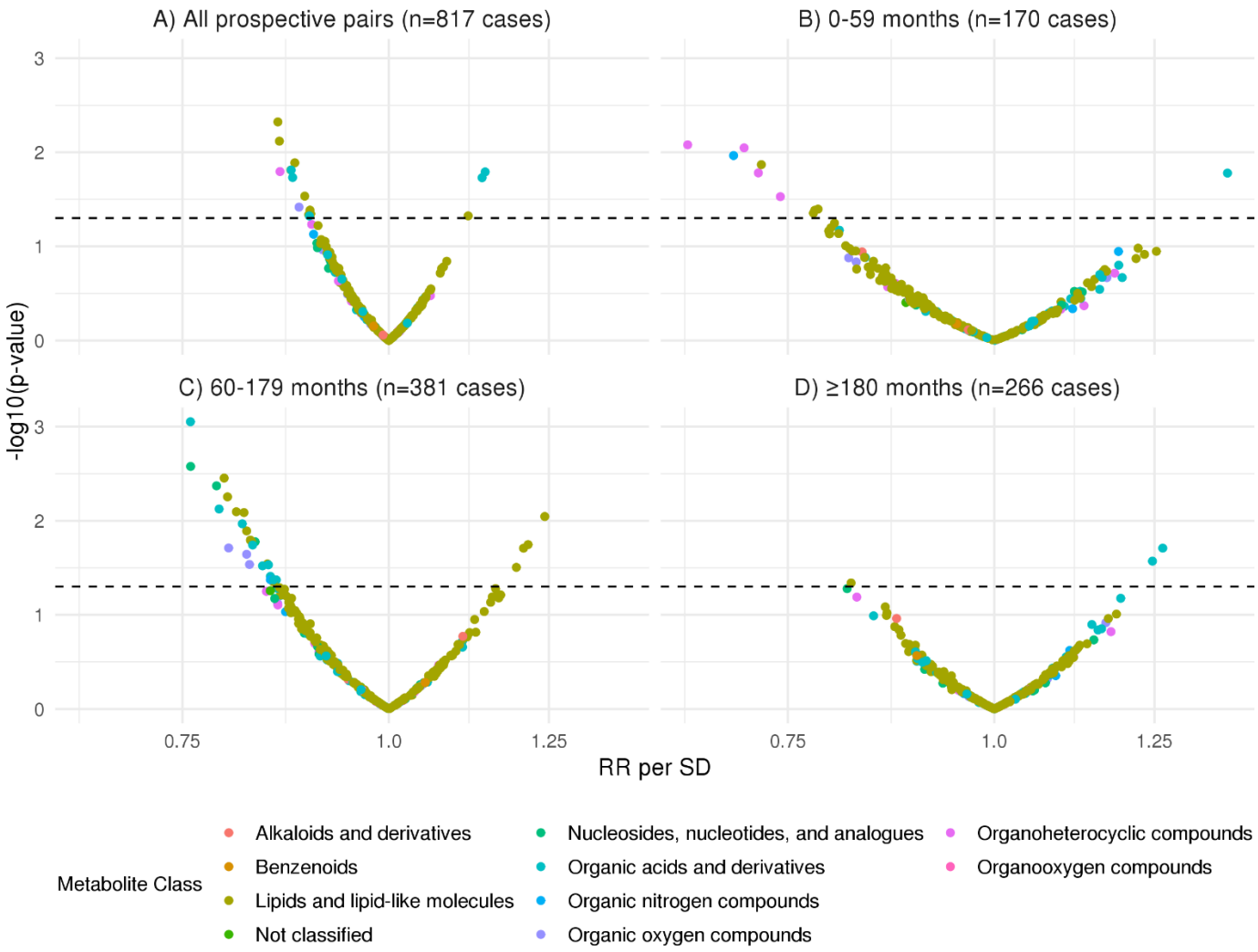

Figure 2.1: Volcano plots of overall and interval category specific RR per SD and raw p-value for matched pairs with pre-diagnostic blood samples

metabolites, 13 were inversely associated with PD (RR per SD <1.0). Following correction for multiple testing, none of these metabolites remained significantly associated with PD. The RR per SD for each individual metabolite's association with PD is provided in Supplementary Table 2.1a. Quartile-based secondary analyses produced similar results; metabolites with nominally significant joint tests of the quartile terms are provided in Supplementary Table 2.2.

To evaluate whether these results were sensitive to our inclusion criteria, we repeated the single-metabolite analyses including matched pairs where the case's blood was collected after diagnosis. In these analyses, 18 metabolites were nominally significantly associated with PD, but none remained significant after multiple testing correction. Comparing these metabolites to the metabolites that were nominally associated with PD in the 817 pairs with pre-diagnostic samples, homoarginine, C18:1 SM, and C5-DC carnitine were no longer significant but significant associations were observed for NH_4-C38:5 DAG or TAG fragment, NH_4-C56:5 TAG, NH_4-C38:5 DAG, C20:4 LPC, and lactate.

In a more restricted sensitivity analysis, we included only pairs where the case's medical record review had been completed and the case was considered either probable or definite PD (n=672 matched pairs). Here, 12 metabolites were nominally significantly associated with PD. Compared to the findings in all 817 pairs with pre-diagnostic blood collection, phenylacetylgutamine, C16, C5:1, C6, and C5-DC acylcarnitines, kynurenine, and C18:1 SM were no longer associated with PD but hydroxycotinine, cytosine, C56:5 TAG, and C20:4 LPC were. As in the main analyses, none of these results remained significant after FDR adjustment.

In analyses stratified by time, we identified 170, 381, and 266 cases with time intervals of 0-59, 60-179, and ≥180 months between blood collection and case diagnosis respectively. The interval-specific associations between each metabolite and PD are presented in Figure 2.1B-D and Supplemental Tables 2.1a-b. Among individuals with blood collection between 0-59 months before diagnosis, 11 metabolites were significantly associated with PD, including

metabolites related to smoking behavior, caffeine consumption, and several lysophosholipids. In plasma samples collected 60-179 months prior to PD onset, 32 metabolites were significantly associated with PD; this set of metabolites included several glycerophosphoethanolamines, acylcarnitines, and N1-acetylspermidine. For individuals with blood collection at least 180 months prior to diagnosis, only 3 metabolites, homocitrulline, alanine, and C5:1 acylcarnitine, were significantly associated with PD. We then repeated these time-stratified analyses including a fourth time category for pairs with case diagnosis prior to blood collection (<0 month time interval); metabolites that were nominally significant in at least one time interval in this analysis are presented in Table 2.3. Among those with blood collection after diagnosis, metabolites that were associated with PD included, among others, urate, sucrose, caffeine, and N1-acetylspermidine. As in the unstratified analyses, none of the interval-specific associations in either the analysis restricted to prospective pairs or in the analysis including all pairs remained statistically significant after adjustment for multiple testing.

In analyses using L_1-penalized logistic regression to predict PD status among prospective pairs with completed medical record review, 23 variables, including 19 metabolites and 4 covariates, were retained in the model (Table 2.4). Assessing model performance using a receiver operating characteristic (ROC) curve, the AUC was 0.600 in the NHS and HPFS training data, 0.539 in the NHS and HPFS test data, and 0.552 for the CPS-IIN validation data. Results modestly improved in the test and training sets but worsened in the validation data

Table 2.3: Interval-specific RR per SD (95% CI) for metabolites that were nominally significant in at least one time interval category among all 930 observed pairs

HMDB ID	Metabolite	Time interval between blood collection and diagnosis				*p* Interaction
		<0 months (n=223)	0-59 months (n=340)	60-179 months (n=766)	≥180 months (n=531)	
HMDB00123	Glycine	1.4 (1.04, 1.9)	0.8 (0.63, 1.01)	0.97 (0.83, 1.13)	0.99 (0.82, 1.19)	0.0367
HMDB00148	Glutamate	0.82 (0.62, 1.08)	0.9 (0.72, 1.13)	0.86 (0.74, 1)	1.05 (0.86, 1.27)	0.3617
HMDB00159	Phenylalanine	0.98 (0.72, 1.34)	0.97 (0.78, 1.2)	0.85 (0.73, 1)	1.08 (0.9, 1.3)	0.2883
HMDB00161	Alanine	1.16 (0.82, 1.64)	0.89 (0.7, 1.13)	0.92 (0.78, 1.07)	1.23 (1.02, 1.5)	0.0644
HMDB00186	Lactose	1.14 (0.84, 1.53)	0.88 (0.68, 1.14)	0.83 (0.7, 0.99)	1.17 (0.96, 1.43)	0.0403
HMDB00190	Lactate	0.87 (0.63, 1.19)	0.93 (0.73, 1.19)	0.84 (0.72, 0.99)	0.98 (0.81, 1.2)	0.6484
HMDB00206	N6-acetyllysine	1.08 (0.83, 1.41)	1.04 (0.81, 1.33)	0.79 (0.66, 0.94)	1.04 (0.86, 1.24)	0.0871
HMDB00222	C16 carnitine	0.8 (0.59, 1.09)	0.97 (0.78, 1.21)	0.85 (0.73, 0.99)	0.92 (0.76, 1.11)	0.6793
HMDB00258	Sucrose	1.43 (1.03, 1.98)	0.81 (0.63, 1.06)	0.83 (0.7, 0.99)	1.1 (0.91, 1.34)	0.0069
HMDB00289	Urate	0.73 (0.53, 0.99)	1.09 (0.86, 1.37)	0.92 (0.78, 1.08)	0.95 (0.77, 1.17)	0.2287
HMDB00670	Homoarginine	0.61 (0.44, 0.86)	1.14 (0.88, 1.47)	1.11 (0.95, 1.29)	1.18 (0.98, 1.43)	0.0068
HMDB00679	Homocitrulline	1.03 (0.8, 1.33)	1.08 (0.86, 1.35)	0.85 (0.73, 1)	1.26 (1.03, 1.54)	0.0215
HMDB00767	Pseudouridine	1.11 (0.82, 1.49)	1.05 (0.82, 1.33)	0.79 (0.67, 0.93)	1.06 (0.85, 1.34)	0.0653
HMDB00853	Acetyl-galactosamine	1.07 (0.76, 1.51)	1.17 (0.92, 1.5)	0.8 (0.66, 0.96)	1.07 (0.88, 1.32)	0.0514
HMDB00904	Citrulline	0.68 (0.48, 0.97)	0.96 (0.74, 1.24)	0.96 (0.81, 1.14)	1.03 (0.86, 1.23)	0.2437
HMDB00982	5-methylcytidine	1.39 (1, 1.92)	1.14 (0.92, 1.4)	0.89 (0.76, 1.05)	0.81 (0.66, 1)	0.0136
HMDB01046	Cotinine	0.87 (0.61, 1.26)	0.66 (0.48, 0.9)	0.86 (0.72, 1.02)	1.05 (0.86, 1.29)	0.0950
HMDB01276	N1-acetylspermidine	1.76 (1.23, 2.52)	1.17 (0.92, 1.48)	0.85 (0.73, 0.99)	0.97 (0.79, 1.19)	0.0015
HMDB01390	Hydroxycotinine	0.88 (0.59, 1.32)	0.65 (0.47, 0.92)	0.94 (0.8, 1.11)	1.02 (0.83, 1.25)	0.1713
HMDB01563	1-methylguanosine	1.16 (0.85, 1.59)	1.13 (0.89, 1.43)	0.83 (0.71, 0.97)	1.08 (0.86, 1.35)	0.0536
HMDB01847	Caffeine	0.73 (0.54, 0.99)	0.71 (0.54, 0.93)	1.05 (0.9, 1.22)	1.11 (0.92, 1.36)	0.0076
HMDB01886	3-methylxanthine	1.06 (0.83, 1.36)	0.76 (0.59, 0.99)	1.05 (0.9, 1.21)	1.07 (0.88, 1.3)	0.1563
HMDB02014	C14:1 carnitine	0.97 (0.7, 1.34)	0.96 (0.75, 1.22)	0.83 (0.71, 0.97)	0.92 (0.75, 1.12)	0.6686
HMDB02250	C12 carnitine	0.97 (0.72, 1.32)	0.93 (0.72, 1.19)	0.83 (0.71, 0.97)	0.89 (0.73, 1.09)	0.7557
HMDB03334	SDMA	1.04 (0.78, 1.4)	1.19 (0.94, 1.51)	0.84 (0.71, 0.98)	0.95 (0.78, 1.17)	0.0991
HMDB04400	5-acetylamino-6-amino-3-methyluracil	1 (0.79, 1.25)	0.72 (0.55, 0.95)	1.04 (0.9, 1.21)	1.1 (0.9, 1.33)	0.0749
HMDB04824	N2,N2-dimethylguanosine	1.11 (0.8, 1.55)	1.11 (0.88, 1.4)	0.76 (0.64, 0.91)	1.07 (0.85, 1.34)	0.0224
HMDB05066	C14 carnitine	0.89 (0.65, 1.21)	0.95 (0.75, 1.21)	0.79 (0.68, 0.93)	0.91 (0.75, 1.11)	0.5448
HMDB05406	C56:5 TAG	0.85 (0.63, 1.15)	0.93 (0.74, 1.18)	0.82 (0.7, 0.96)	1.05 (0.87, 1.27)	0.2359
HMDB06344	Phenylacetylglutamine	1.17 (0.86, 1.59)	1.43 (1.1, 1.87)	1.11 (0.94, 1.3)	1.08 (0.91, 1.29)	0.3419
HMDB07170	C38:4 DAG	0.66 (0.45, 0.96)	0.91 (0.71, 1.16)	0.92 (0.8, 1.06)	1.03 (0.85, 1.24)	0.2133
HMDB09012	C40:6 PE	0.88 (0.64, 1.21)	1 (0.81, 1.25)	0.8 (0.68, 0.94)	1.12 (0.93, 1.35)	0.0542

Table 2.3 (Continued)

HMDB09102	C38:6 PE	0.88 (0.64, 1.2)	1.02 (0.82, 1.27)	0.81 (0.69, 0.95)	1.1 (0.91, 1.32)	0.0774
HMDB10316	Acetaminophen glucuronide	0.66 (0.47, 0.93)	0.94 (0.72, 1.23)	0.86 (0.73, 1.01)	1.07 (0.9, 1.27)	0.0627
HMDB10386	C18:2 LPC	1.24 (0.91, 1.69)	0.78 (0.61, 1)	1.07 (0.91, 1.25)	0.89 (0.74, 1.07)	0.0468
HMDB10393	C18:3 LPC	0.92 (0.69, 1.23)	0.77 (0.61, 0.97)	0.96 (0.83, 1.12)	0.96 (0.78, 1.17)	0.4304
HMDB10411	C46:0 TAG	0.76 (0.56, 1.04)	0.84 (0.68, 1.04)	1.17 (1, 1.36)	0.98 (0.81, 1.2)	0.0234
HMDB11103	1,7-dimethyluric acid	0.92 (0.73, 1.16)	0.71 (0.55, 0.93)	0.99 (0.85, 1.15)	1.06 (0.88, 1.29)	0.0886
HMDB11343	C34:3 PE plasmalogen	1 (0.76, 1.32)	0.9 (0.72, 1.13)	1.23 (1.05, 1.45)	0.86 (0.72, 1.03)	0.0202
HMDB11410	C36:5 PE plasmalogen	0.86 (0.65, 1.14)	0.96 (0.76, 1.21)	1.2 (1.02, 1.4)	0.88 (0.73, 1.05)	0.0408
HMDB11441	C36:3 PE plasmalogen	1.05 (0.8, 1.38)	0.88 (0.71, 1.1)	1.21 (1.03, 1.42)	0.86 (0.72, 1.03)	0.0251
HMDB11442	C36:4 PE plasmalogen	1.01 (0.75, 1.37)	0.9 (0.72, 1.12)	1.18 (1.01, 1.39)	0.86 (0.72, 1.02)	0.0431
HMDB11506	C18:1 LPE	1.17 (0.85, 1.63)	0.7 (0.54, 0.91)	1.08 (0.92, 1.26)	0.92 (0.77, 1.11)	0.0280
HMDB11517	C20:4 LPE	1.05 (0.75, 1.47)	0.78 (0.61, 1)	0.96 (0.83, 1.11)	0.95 (0.79, 1.14)	0.4447
HMDB13130	C5-DC carnitine	1.18 (0.93, 1.5)	1.07 (0.86, 1.32)	0.76 (0.64, 0.89)	1.02 (0.83, 1.27)	0.0079
HMDB13288	C9 carnitine	1 (0.74, 1.35)	1.16 (0.93, 1.46)	0.83 (0.71, 0.97)	0.97 (0.8, 1.17)	0.1096
HMDB13331	C14:2 carnitine	1.06 (0.78, 1.44)	0.99 (0.78, 1.26)	0.82 (0.7, 0.95)	0.92 (0.75, 1.12)	0.3399
HMDB42466	C55:3 TAG	0.89 (0.63, 1.27)	1.15 (0.91, 1.46)	0.81 (0.7, 0.95)	1.05 (0.87, 1.28)	0.0503
N/A	3-(N-acetyl-L-cystein-S-yl) acetaminophen	0.71 (0.52, 0.96)	0.97 (0.75, 1.25)	0.82 (0.7, 0.97)	1.1 (0.92, 1.32)	0.0352
N/A	NH4_C14:0 CE	0.71 (0.52, 0.95)	0.89 (0.73, 1.1)	1.02 (0.88, 1.17)	0.91 (0.75, 1.09)	0.1853
N/A	NH4_C18:3 CE	0.67 (0.46, 0.98)	0.94 (0.72, 1.23)	1.02 (0.87, 1.19)	0.97 (0.8, 1.18)	0.2625
N/A	NH4_C20:5 CE	0.71 (0.51, 0.99)	0.97 (0.76, 1.24)	0.91 (0.79, 1.06)	1.08 (0.89, 1.31)	0.1720

RR per SD and corresponding 95% confidence interval for each metabolite that was nominally significant in at least one interval category. Metabolites are shaded blue if the interval-specific association was nominally significant and inverse or shaded red if the association was nominally significant and positive. Results that are unshaded within a given interval were not significant for that time category.

Table 2.4: Metabolites and covariates selected in L_1-penalized logistic regression model to predict PD

Participants	N	Increased in PD cases	Decreased in PD cases	AUC Training	AUC Test	AUC Validation
All matched pairs with case diagnosis after blood collection	Train: 816 Test: 204 Validation: 478	Age	Carnitine, Cotinine, Hydroxycotinine, C14 acylcarntine, C14:0 CE, C32:0 PC, C34:0 PC, C20:4 LPC, C20:5 LPC, C16:0 LPE, C20:4 LPE, pack-years, BMI, physical activity, interval	0.623	0.553	0.522
Matched pairs with case diagnosis 0-180 months after blood collection	Train: 498 Test: 126 Validation: 478	C30:0 PC, age	Trigonelline, C14 acylcarnitine, C14:0 CE, C30:0 PC, C40:6 PE, C20:4 LPC, 1,7-dimethyluric acid, C18:0 LPE, C22:6 LPE, NH_4-C20:5 CE, caffeine intake, pack-years, BMI, physical activity	0.655	0.621	0.510
Matched pairs with case diagnosis 0-120 months after blood collection	Train: 288 Test: 72 Validation: 356	Inosine, age	C40:6 PE, C18:1 SM, C20:0 SM, C22:0 SM, NH_4-C20:5 CE, caffeine intake, pack-years, BMI, physical activity, time interval, fasting status	0.678	0.631	0.510

when we restricted to matched pairs with case diagnosis within 180 months of blood collection (training AUC: 0.655; test AUC: 0.621; validation AUC: 0.510) or 120 months of blood collection (training AUC: 0.678, test AUC: 0.631, validation AUC: 0.510) respectively. Elastic net regression results were comparable (Table 2.5) with the exception that the model more drastically overfit the training data when restricting to pairs with less than 120 months between blood collection and PD diagnosis (training AUC: 0.763; test AUC: 0.552; validation AUC: 0.484); in this data, selecting the cross-validated lambda corresponding to the most regularized model where the AUC was within one standard error of the maximum cross-validated AUC produced a more parsimonious model (21 terms: 18 metabolites, 3 covariates) that performed similarly to the other L_1-penalized and elastic net regression model results (training AUC: 0.715; test AUC: 0.603; validation AUC: 0.496).

In addition to the elastic net and L_1-penalized regression models, we also used a SVM approach to attempt to distinguish cases and controls. In each of the three subpopulations explored, a polynomial kernel gave the best performance in the training data. Including all pairs with prospective blood collection and complete medical record review, the SVM's AUC was: 0.75 in the training data, 0.52 in the test data, and 0.53 in the validation data. As in the other prediction models, model performance improved modestly in the test data but not the validation data when restricting to pairs with cases closer to diagnosis; among those within 180 months, the AUC was 0.80 in the training data, 0.61 in the test data, and 0.50 in the validation data. Similarly, among those within 120 months of diagnosis, the AUC was 0.73 in the training data, 0.62 in the test data, and 0.49 in the validation data.

As our results suggested a majority of metabolites were decreased among future PD cases relative to their matched controls, we explored whether this proportion was statistically different from 0.5 by repeating the main and time-stratified single metabolite analyses in 1000 bootstrap resamples of the matched pairs. Replicating the main analysis, we found that the

Table 2.5: Metabolites and covariates selected in elastic net logistic regression model to predict PD

Participants	N	Alpha	Increased in future PD cases	Decreased in future PD cases	AUC Training	AUC Test	AUC Validation
All matched pairs with case diagnosis after blood collection	Train: 816 Test: 204 Validation: 478	0.1	Age	Carnitine, C16 acylcarnitine, Cotinine, Hydroxycotinine, C14 acylcarnitine, C14:0 CE, C32:0 PC, C34:0 PC, C36:0 PE, C20:4 LPC, C20:5 LPC, C16:0 LPE, C20:4 LPE, pack-years, BMI, physical activity, interval	0.622	0.543	0.525
Matched pairs with case diagnosis 0-180 months after blood collection	Train: 288 Test: 72 Validation: 356	0.7	Age	Trigonelline, C14 acylcarnitine, C14:0 CE, C40:6 PE, C20:4 LPC, 1,7-dimetyluric acid, C18:0 LPE, 22:6 LPE, C18:1 SM, NH4-C20:5 CE, pack-years, BMI, physical activity	0.651	0.614	0.505
Matched pairs with case diagnosis 0-120 months after blood collection	Train: 498 Test: 126 Validation: 478	0.8	Female, Cholesterol, Hypoxanthine, Inosine, Cytosine, C18:0 SM, 2-aminoisobutyric acid, SDMA, Phenylacetylglutamine, C36:1 PC, C14:0 LPC, NH_4-C56:5 TAG	Glutamate, Histidine, Trigonelline, C4 acylcarnitine, DMGV, C20:5 CE, C36:1 PE, C38:6 PE, C22:6 LPC, C24:0 SM, C18:1 SM, C20:0 SM, C55:2 TAG, NH_4-C14:0 CE, caffeine intake, pack-years, BMI, physical activity, age, fasting hours	0.763	0.552	0.484

mean proportion of metabolites inversely associated with PD was 0.64 (95% CI: 0.41, 0.81), which was comparable to the observed proportion of 0.7. Bootstrap distributions for each time interval are provided in Figure 2.2. Like the overall distribution, each time category's 95% confidence interval for the proportion of inverse associations contained 0.5, indicating these data are consistent an equal proportion of positive and negative associations. Comparing the distributions across time categories, there is a qualitative shift between those with ≥180 months between blood collection and diagnosis and those in each subsequently shorter time category such that those in the shorter categories have, on average, a higher proportion of metabolites inversely associated with PD.

Discussion

In this large, prospective, matched case-control study, we assessed whether plasma metabolomics could predict future PD over an interval of 15 years or more. Although we identified 16 metabolites that were nominally associated with incident PD, none remained significantly associated with PD following adjustment for multiple testing. Similarly, in analyses stratified on the length of time between blood collection and case diagnosis, different metabolites were nominally associated with PD in different interval categories, but these metabolites were neither consistently identified across time intervals nor remained significantly associated with later development of PD after multiple testing correction. Our results were not markedly changed by either including matched pairs with post-diagnosis blood collection or by excluding those pairs in which PD diagnosis was considered less certain by medical record review. In addition to these single-metabolite results, we were unable to reliably distinguish cases from controls based on plasma metabolomic profiles using a variety of prediction models. Across all our analyses, we observed a consistently high number of metabolites that had an inverse direction of association with PD. The bootstrapped distributions for the proportion of inverse associations were not consistent with more than 50% of metabolites being decreased

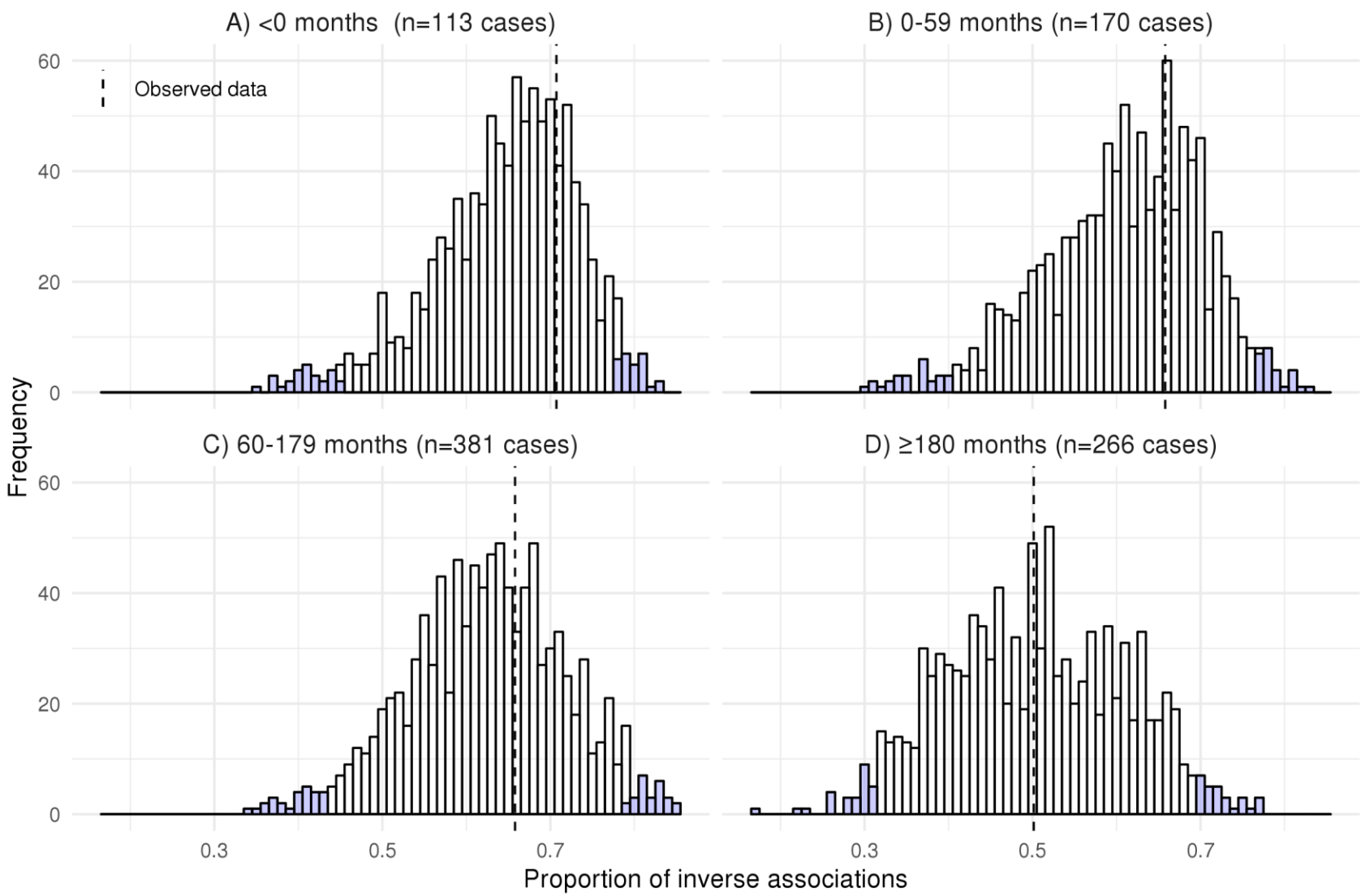

Figure 2.2: Distribution of the proportion of metabolites inversely associated with PD across 1000 bootstrap resamples of matched pairs in the analyses stratified on time interval category. The dashed line represents the proportion observed in the true data; values shaded in blue represent values above or below the 2.5 and 97.5 percentiles of the bootstrap distribution respectively.

among PD cases but, qualitatively, the distribution shifted to the right comparing those who were already diagnosed with PD or likely in the prodromal phase to those for whom disease processes were unlikely to have started; this may reflect some broad but subtle disruption in metabolism occurring as PD disease processes onset.

Recently, numerous case-control investigations of metabolomics in relation to PD[32-37,52-61] have explored whether metabolomic profiles, measured in plasma, cerebrospinal fluid, or frontal cortex samples, can be used to distinguish between previously diagnosed PD cases and controls as well as to determine whether metabolomics might be associated with the rate of disease progression or medication side effects. To our knowledge, however, the investigation reported here is not only the largest study of metabolomics and PD to date, but also the first to utilize blood samples collected prior to disease diagnosis. The results across these studies have been heterogenous, likely reflecting differences in study design, study population, and statistical analysis, and therefore any comparisons should be made with caution. Our results are largely distinct from the rest of the literature but may be most similar to those from a report suggesting a decrease in long-chain acylcarnitines was associated with PD[37]. Importantly, though, in both the analyses described here as well as in an earlier analysis on a subset of these data (S. Molsberry, K. Bjornevik, K. Hughes, Z. Zhang, S. Jeanfavre, C. Clish, et al., unpublished observations, 2019), none of these acylcarnitines remained significantly associated with PD after multiple testing correction.

Here, we were unable to reliably distinguish between future PD cases and their matched controls, even when restricting to cases with blood collected within 10 years of diagnosis, when many were likely in the prodromal phase of PD. This failure to distinguish cases from controls is in direct contrast to most of the other PD metabolomics investigations. A critical distinction between our investigation and previous investigations is that, in our study, cases and controls were directly sampled from the same source population and their blood samples were collected

and processed in an identical manner within each cohort. These conditions in conjunction with careful matching of cases and controls and standardization of metabolite levels within each analytical platform virtually eliminate the possibility of any spurious difference in metabolite levels between cases and controls. The prospective nature of this study also reduces the probability that the observed results can be attributed to reverse causation or other factors related to disease diagnosis, such as behavioral changes or medication initiation. Additionally, given the large sample size and extensive covariate information available in these cohorts, we were able to carefully control for potentially confounding factors, which most previous studies were unable to do and is important for distinguishing novel disease biomarkers from markers of established risk factors.

Although no single metabolite was significant after adjustment for multiple testing, we did observe that the overall distribution of metabolite-PD associations was skewed such that metabolites tended to be decreased among PD cases compared to controls. Bootstrapped analyses did not indicate that the proportion of metabolites inversely associated with PD was different from 0.5, but the distributions did shift away from 0.5 with increasing proximity to diagnosis. An increased number of inverse metabolite-disease associations has also been reported in studies on ALS[65], suicidal ideation (S. Mitro, B. Gelaye, S. Molsberry, M. Williams, unpublished observations, 2019), and mild cognitive impairment and Alzheimer's disease[66], suggesting that neuropsychiatric disease pathogenesis may involve subtle alterations in metabolism. Notably, beyond the differences in study design and population, such a broad yet non-specific disruption in metabolism may also explain, in part, the heterogeneity of results in the existing PD metabolomics literature.

Our investigation has some limitations. First, because we focused on plasma samples collected prior to diagnosis, it is possible that the metabolomic changes related to PD pathogenesis, if there exist any true metabolomic changes, may have been too subtle to be

useful in distinguishing future PD cases from controls at any time point prior to diagnosis. Second, only a single blood sample was collected from the individuals in this investigation. It is possible, therefore, that the plasma metabolite levels in these samples might not reflect subjects' long-term metabolomic profiles. Additionally, some metabolites are sensitive and may have been degraded during shipment. A validation study in the NHS and HPFS using the Broad's Metabolomics Platform was conducted to address these concerns[28]. In this study, the results comparing blood samples collected 1-2 years apart indicated that, for most metabolites, a single measurement reflected longer-term metabolite levels well for most metabolites. Further, this same study assessed reproducibility between samples that were processed immediately or after a 24-hour delay. Here, the results indicated that over 75% of metabolites had excellent reproducibility. It should be noted that, in both sets of reproducibility results, there was variation across metabolite groups; in general, it appeared that the results for carbohydrates and purines/pyrimidines were less reproducible than the results for other metabolite groups. An additional limitation of our study is that, although by far the largest study of PD metabolomics to date, the sample size, particularly for stratified analyses, may still have been too small to detect small effects in the nearly 350 metabolites analyzed. Of course, as in all observational research, it is also possible that there may be sources of bias that we were unable to account for, including residual and unmeasured confounding.

In conclusion, we found that, nominally, several plasma metabolites were associated with PD in pre-diagnostic blood samples. None of the metabolites remained significantly associated with PD after adjustment for multiple testing and, in analyses stratified by time, no metabolite was consistently altered across all interval categories. Further, we were unable to reliably distinguish between future PD cases and controls with metabolomic profile data using a variety of machine learning techniques. Our results suggest the possibility of a broad but non-

specific alteration in metabolism in the years preceding PD diagnosis, but further prospective research is needed to substantiate this observation.

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Chapter 3

Mediterranean diet adherence and prodromal features of Parkinson's disease

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Key points

Questions Is adherence to a Mediterranean-style diet related to risk of prodromal Parkinson's disease or specific features of prodromal Parkinson's disease?

Findings In this investigation, increased adherence to a Mediterranean-style diet was associated with a lower number of prodromal Parkinson's features as well as with 3 specific features: constipation, excessive daytime sleepiness, and depressive symptoms.

Meaning These findings add further weight to the evidence that increased adherence to a Mediterranean-style diet could reduce the occurrence of specific features of prodromal Parkinson's disease.

Abstract

Importance The prodromal phase of Parkinson's disease (PD) remains poorly understood. Identification of risk factors for common prodromal PD features could contribute to disease prevention and better understanding of disease pathogenesis.

Objective To investigate the association between Mediterranean diet adherence and non-motor features that frequently precede the clinical diagnosis of PD.

Design, Setting and Participants These analyses include 47,677 participants from two cohort studies, the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). Since 1986, both cohorts have collected dietary information every four years. In 2012, cohort participants were asked questions related to constipation and probable rapid eye movement (REM) sleep behavior disorder. For a subset of 17,400 participants who responded to the 2012 questionnaire, five additional prodromal features of PD were assessed in 2014-2015.

Exposures Using food-frequency questionnaire responses, a 9-point alternative Mediterranean diet score was assigned based on intake of fruits, vegetables, whole grains, nuts, legumes, fish, fats, red and processed meats, and alcohol. Participants were classified into quintiles based on baseline (1986) and cumulative average (1986-2006) Mediterranean diet score.

Main Outcomes and Measures Primary analyses investigate the number of prodromal Parkinson's features, categorized as 0, 1, 2, or ≥3 features. Secondary analyses investigate this same outcome excluding constipation as a feature, the association between Alternative Healthy Eating Index (AHEI) score and prodromal PD, and the relationship between diet and individual prodromal features.

Results Increased Mediterranean diet adherence was associated with lower odds of prodromal Parkinson's features; comparing extreme quintiles, the odds ratio for ≥3 versus 0 prodromal

features was 0.82 (95% CI: 0.68, 1.00; p_{trend}=0.012) at baseline and 0.67 (95% CI: 0.54, 0.83; p_{trend} <0.0001) for long-term diet; results were equally strong for the association between AHEI score and prodromal PD. Increased Mediterranean diet adherence was also inversely associated with constipation, excessive daytime sleepiness, and depression.

Conclusions and Relevance The inverse association between Mediterranean diet adherence and prodromal features of PD is consistent with previously reported findings and suggests that adherence to a Mediterranean-style diet may reduce the occurrence of non-motor symptoms that often precede the clinical diagnosis of PD.

Introduction

Several foods and nutrients[67-70] as well as adherence to specific dietary patterns[71-73] have been associated with the risk of developing Parkinson's disease (PD), but there are no longitudinal studies on the relation between diet or dietary factors and features of prodromal PD. In this study, we investigated whether long-term adherence to a Mediterranean style diet as well as to another dietary pattern, the Alternative Healthy Eating Index, was associated with prodromal features of PD.

Methods

Study population

This investigation uses data from the Nurse's Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) cohorts. The NHS cohort is composed of 121,700 female registered nurses who resided in one of eleven states and were between the ages of 30-55 at the time of enrollment in 1976. The HPFS cohort is composed of 51,529 male health professionals who responded to the baseline questionnaire and were between the ages of 40-75 at the time of enrollment in 1986. In both NHS and HPFS cohorts, the participants complete biennially administered follow-up questionnaires regarding lifestyle practices, occupational and other exposures, and medical history. Cohort participants under 85 years of age and without diagnosed PD who responded to questions assessing probable REM sleep behavior disorder (pRBD) and constipation on the 2012 questionnaire as well as the baseline (1986) FFQ are included in these analyses (NHS: n=29,899; HPFS: n=17,768). As the pRBD question is asked to the sleep partner of the participant, 13,188 NHS and 857 HPFS otherwise eligible participants who did not have a sleep partner were excluded. Due to cost constraints, secondary screening consisting of olfactory testing and an additional premotor PD questionnaire was administered to a subset of eligible participants. This subset consisted of all participants who screened positive

for either pRBD or constipation on the 2012 questionnaire but only 23% of those with neither of these features, who were randomly selected. In total, 17,400 participants (NHS: n=11,493; HPFS: n=5,907) included in these analyses completed all secondary screening and an additional 1,129 participants (NHS: n=781; HPFS: n=348) participants completed some but not all secondary screening.

The study protocol was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

Outcome assessment

Seven prodromal features are included in these analyses: constipation, probable REM sleep behavior disorder (pRBD), hyposmia, excessive daytime sleepiness, impaired color vision, depressive symptoms, and body pain. The assessment of these features in the NHS (K.C. Hughes, S.D., unpublished data, March 2019) and the HPFS[74] has previously been described. For the purposes of these analyses, constipation was assessed based on responses to the 2012 questionnaire and defined as either having a bowel movement frequency of every other day or less or using laxative twice a week or more. The 2012 questionnaire was also used to assess pRBD, which was defined as having a positive response to a screening question from the Mayo Sleep Questionnaire ("Has your spouse [or sleep partner] told you that you appear to "act out your dreams" while sleeping [punched or flailed arms in the air, shouted, or screamed], which has occurred at least three times?").[75] Hyposmia was assessed with the Brief Smell Identification Test (B-SIT), a standardized test where participants were asked to identify 12 different odorants. The Epworth Sleepiness Scale[76] was used to measure excessive daytime sleepiness, defined as a score of 10 or more. An mailed version of the Roth color discrimination test, itself an abridged version of the Farnsworth-Munsell Test,[77] was used to assess color discrimination. Body pain presence and severity were assessed using questions from the Short-form Health Survey (SF36). Depressive symptoms were measured using the Mental Health

Inventory (MHI),[78] a 5-question subscale of the SF36. We defined hyposmia, impaired color vision, body pain, and depressive symptoms as having a score in the bottom 10% of the cohort-specific distribution of participants without pRBD or constipation who completed the respective assessments. For the main analyses, we took the sum of each participant's prodromal features and categorized participants as having 0, 1, 2, or ≥3 features.

Assessment of diet and other covariates

Diet is measured every four years in both the NHS and HPFS using semi-quantitative food frequency questionnaires (FFQs), which have been validated for use in these cohorts.[79,80] Using participants' FFQ responses, a score for alternate Mediterranean diet (aMED) adherence was calculated as the sum of 9 component scores: vegetables (excluding potatoes), fruits, nuts, whole grains, legumes, fish, the ratio of monounsaturated to saturated fat, red and processed meats, and alcohol, as previously reported.[81,82] For the first seven of these components, a score of 1 was given if the participant has an intake above the cohort- and questionnaire-cycle specific median. A score of 1 was given for red and processed meat consumption if the participant reports below median intake. For alcohol intake, a score of 1 was given for moderate consumption (between 5-15 g/day for women, 10-25 g/day for men). If a participant did not meet criteria to receive a score of 1 for a given component, they receive a score of 0. As the HPFS cohort began in 1986, we used 1986 as the baseline diet assessment. For NHS participants with missing 1986 dietary information but who were otherwise eligible for this study, we carried forward information, including FFQ responses, from the 1984 questionnaire cycle.

In a set of secondary analyses, we also explored the relationship between a second dietary pattern, the Alternative Healthy Eating Index (AHEI), and prodromal PD features. Briefly, the AHEI diet is defined by 11 components: vegetables, fruits, whole grains, sugar-sweetened beverages and fruit juice, nuts and legumes, red and processed meat, trans fat, long-chain (*n*-3) fats, polyunsaturated fatty acids, sodium, and alcohol. Scores for each component, which have

been previously described,[83] are assigned on a continuous basis, ranging from 0 to 10, and are summed to create a total diet score that ranges from 0 to 110.

Information on other covariates of interest, including caffeine consumption, energy intake, body mass index (BMI), smoking pack-years, and physical activity, was collected at baseline and on subsequent questionnaires in both cohorts.

Statistical analysis

In the primary analyses, we used baseline quintiles of aMED diet score as a measure of Mediterranean diet adherence. We also conducted analyses of cumulative average aMED diet score using all available dietary information collected between 1986-2006 in order to examine the relationship between long-term Mediterranean diet adherence and prodromal features. To minimize the possibility of reverse causation, we did not consider dietary information collected after the 2006 questionnaire cycle, which allowed at least six years between the last diet assessment and earliest prodromal feature assessment. For baseline analyses in the NHS and analyses of long-term diet in both cohorts, values were carried forward one questionnaire cycle for participants missing information on either exposure or covariates. Missing value indicators were used if the value from the preceding questionnaire cycle was also missing and could not be carried forward. As baseline physical activity in the HPFS and cumulative average physical activity in both cohorts were very uncommonly missing (baseline HPFS: n=11 (0.19%); cumulative average HPFS: n=0 (0.0%), NHS: n=4 (0.03%)), participants with missing information were assigned to the median quintile to ensure that participants with each level of activity would be sampled in each bootstrap resample.

Within each cohort, multinomial logistic regression was used to estimate the odds ratio (OR) between Mediterranean diet adherence and prodromal feature combinations (1,2, or ≥ 3 vs. 0). Age-adjusted models of baseline diet were adjusted for age (years); multi-variable

models were further adjusted for caffeine intake (quintiles), caloric intake (quintiles), smoking pack-years (<5, 5 to <10, 10 to <15, 15 to <20, ≥20), BMI (<25, 25 to < 30, ≥30), and physical activity (MET hours/week; quintiles). Models of long-term diet intake were adjusted for age, cumulative average caffeine intake, caloric intake, and physical activity, as well as updated versions of smoking pack-year categories and BMI category. For each exposure of interest, the quintile median values were modeled continuously to assess linear trend across quintiles. To account for the oversampling of participants with pRBD and/or constipation on the 2012 questionnaire, all models were weighted using inverse probability weights and bootstrapping (500 resamples) was used to obtain valid standard errors. Pooled measures of association and tests of heterogeneity were obtained using random-effects meta-analysis.

A series of secondary and sensitivity analyses were also conducted. First, we completed a complementary set of analyses using quintiles of AHEI score rather than aMED score. Given that the association between alcohol consumption and PD is not specific to moderate alcohol intake,[84] we refit the aMED models excluding the alcohol component from the aMED score and instead adjusted for quintile of alcohol consumption. To further evaluate the temporal relationship between diet adherence and prodromal features of PD, we examined the association between diet and prodromal feature category at each year diet was assessed between 1986 and 2006 as well as the mean of the first two (1986, 1990) and last two (2002, 2006) diet assessments. Using multivariable-adjusted logistic regression, we additionally assessed the relationship between each specific prodromal feature of PD and diet pattern adherence among individuals who completed screening on that feature; as constipation and pRBD were measured in the entire study population, inverse probability weighting and bootstrapping were not used for these specific outcomes. To determine whether specific aMED components were driving the observed associations, we used multivariable-adjusted multinomial logistic regression to assess the relationship between the baseline and cumulative

average scores of the individual aMED components and prodromal feature category. Lastly, to investigate the extent to which excluding individuals who completed some but not all of the secondary premotor screening might have biased our results, we repeated the main analyses by first assuming that these individuals had none of the features for which they were missing data and then again assuming that they had all of the features for which they were missing data.

All analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, N.C.) and R 3.5.0 (https://cran.r-project.org). P-values were considered significant at values <0.05.

Results

The characteristics of the study population are described in Table 3.1. In both the NHS and the HPFS cohorts, individuals with higher adherence to the aMED diet had a lower BMI, were older, less likely to be current smokers, more physically active, and consumed both less caffeine and more total energy than individuals with lower aMED adherence. At baseline the quintiles corresponded, respectively, to aMED scores of 0-2, 3, 4, 5-6, and 7-9 in the HPFS and 0-2, 3, 4, 5, and 6-9 in the NHS.

Both baseline and long-term adherence to a Mediterranean-style diet were inversely associated with combinations of prodromal features (Table 3.2). In pooled analyses, the multivariable-adjusted OR for having ≥3 versus 0 prodromal features comparing those in the highest versus lowest aMED quintile was 0.82 (95% CI: 0.68,1.00; p_{trend} = 0.01) at baseline and 0.67 (95% CI: 0.54, 0.83; p_{trend} <0.001) for long-term diet. Results for the AHEI diet followed a similar pattern but were stronger in magnitude (Table 3.3); the multivariable-adjusted OR for ≥3 versus 0 prodromal features comparing extreme AHEI quintiles was 0.72 (95% CI: 0.59,0.87; p_{trend} <0.001) at baseline and 0.66 (95% CI: 0.53, 0.81; p_{trend} <0.001) for long-term diet. These associations were attenuated when constipation was excluded as a prodromal feature, but, for

Table 3.1: Age-adjusted study population characteristics at baseline by quintile of aMED adherence

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
HPFS	n=3530	n=2977	n=3191	n=5586	n=2484
aMED score	1.5 (0.6)	3.0 (0.0)	4.0 (0.0)	5.5 (0.5)	7.4 (0.6)
AHEI score	42.1 (8.7)	48.0 (9.0)	51.2 (8.8)	56.4 (9.2)	63.9 (9.1)
Age, years[b]	46.7 (5.5)	47.3 (5.6)	47.7 (6.0)	48.1 (5.7)	48.7 (5.7)
Body mass index, kg/m	25.6 (3.1)	25.4 (3.1)	25.3 (3.1)	25.1 (2.9)	24.7 (2.9)
Current smoker, %	10.7	8.6	7.7	5.6	3.3
Past smoker, %	36.9	38.9	38.3	39.4	40.5
Caucasian, %	96.4	96.2	95.7	96.5	97.1
Physical activity, met-h/week[a]	15.0 (23.0)	18.0 (25.8)	18.2 (25.8)	22.5 (26.7)	28.1 (33.9)
Caffeine, mg/day	272.8 (243.1)	255.6 (240.9)	237.0 (232.2)	223.2 (224.5)	196.9 (208.0)
Energy intake, kcal/day	1778.5 (540.9)	1874.9 (580.8)	1980.1 (599.9)	2134.0 (623.1)	2275.1 (593.6)
Probable RBD, %	11.8	11.6	13.2	12.6	11.7
Constipation, %	22.2	21.8	21.5	20.9	19.3
Hyposmia[c], %	17.5	14.3	14.4	15.2	14.0
Impaired color vision[c], %	11.9	7.7	12.2	9.3	10.0
Excessive daytime sleepiness[c], %	22.0	22.4	20.8	21.0	20.5
Body pain[c], %	14.3	15.8	15.0	15.0	17.2
Depressive symptoms[c], %	13.7	12.5	12.9	11.9	12.8
NHS	n=7255	n=5169	n=5630	n=5286	n=6559
aMED score	1.5 (0.7)	3.0 (0.0)	4.0 (0.0)	5.0 (0.0)	6.5 (0.7)
AHEI score	42.5 (8.6)	47.3 (8.9)	50.6 (9.2)	54.0 (9.4)	59.5 (9.8)
Age, years[b]	47.1 (4.9)	47.8 (5.0)	48.2 (5.1)	48.8 (5.2)	49.3 (5.2)
Body mass index, kg/m	25.0 (4.6)	24.8 (4.5)	24.8 (4.4)	24.7 (4.3)	24.3 (4.2)
Current smoker, %	20.7	17.6	14.9	12.7	11.4
Past smoker, %	30.7	33.2	36.9	38.5	40.0
Caucasian, %	91.7	91.4	92.6	92.3	92.2
Physical activity, met-h/week[a]	10.5 (17.2)	13.0 (17.8)	14.4 (18.9)	16.7 (22.5)	19.7 (25.9)
Caffeine, mg/day	310.7 (234.2)	299.9 (229.2)	297.3 (226.1)	295.2 (223.6)	273.8 (217.2)
Energy intake, kcal/day	1549.5 (459.8)	1688.7 (489.1)	1792.9 (502.3)	1910.7 (525.5)	2054.8 (528.4)
Probable RBD, %	7.1	7.5	6.9	7.0	7.1
Constipation, %	37.2	36.0	35.5	34.4	32.4
Hyposmia[c], %	20.5	19.6	18.0	18.6	18.9
Impaired color vision[c], %	14.5	15.9	15.4	14.5	14.9
Excessive daytime sleepiness[c], %	8.7	8.8	9.5	9.9	8.8
Body pain[c], %	15.4	16.2	13.9	14.3	12.9
Depressive symptom[c]s, %	16.8	17.2	14.8	15.7	14.4

Values are means (SD) for continuous variables; percentages categorical variables, and are standardized to the age distribution of the study population.

[a] Metabolic equivalents from recreational and leisure-time activities

[b] Value is not age adjusted

[c] Percentages based on 5,907 HPFS and 11,493 NHS respectively who completed all secondary screening

Table 3.2: Odds ratios for association between ≥3 vs. 0 prodromal features for each quintile of adherence to the aMED diet pattern

Cohort	Adjustment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p* Trend	*p* Heterogeneity
Including constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.82 (0.58, 1.11)	0.89 (0.64, 1.25)	0.76 (0.59, 0.99)	0.75 (0.53, 1.07)	0.07	
	Multivariable	1.0 (Ref.)	0.82 (0.58, 1.12)	0.87 (0.62, 1.22)	0.73 (0.54, 0.97)	0.74 (0.51, 1.06)	0.06	
NHS	Age	1.0 (Ref.)	1 (0.81, 1.23)	0.87 (0.7, 1.07)	0.82 (0.66, 1.03)	0.77 (0.61, 0.94)	0.005	
	Multivariable	1.0 (Ref.)	1.02 (0.83, 1.26)	0.93 (0.73, 1.15)	0.87 (0.69, 1.11)	0.86 (0.68, 1.08)	0.09	
Pooled	Age	1.0 (Ref.)	0.93 (0.78, 1.12)	0.88 (0.74, 1.04)	0.80 (0.67, 0.95)	0.76 (0.64, 0.92)	<0.001	0.70
	Multivariable	1.0 (Ref.)	0.95 (0.77, 1.17)	0.91 (0.76, 1.09)	0.81 (0.68, 0.97)	0.82 (0.68, 1.00)	0.01	0.66
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.84 (0.61, 1.18)	0.56 (0.42, 0.75)	0.63 (0.48, 0.85)	0.7 (0.5, 0.91)	0.005	
	Multivariable	1.0 (Ref.)	0.83 (0.59, 1.16)	0.54 (0.4, 0.73)	0.62 (0.46, 0.85)	0.64 (0.45, 0.86)	0.003	
NHS	Age	1.0 (Ref.)	1.09 (0.86, 1.36)	0.76 (0.6, 0.97)	0.68 (0.53, 0.86)	0.58 (0.45, 0.73)	<0.001	
	Multivariable	1.0 (Ref.)	1.12 (0.88, 1.41)	0.83 (0.64, 1.05)	0.79 (0.6, 1)	0.69 (0.52, 0.9)	<0.001	
Pooled	Age	1.0 (Ref.)	0.98 (0.77, 1.26)	0.66 (0.49, 0.90)	0.66 (0.55, 0.80)	0.62 (0.51, 0.75)	<0.001	0.19
	Multivariable	1.0 (Ref.)	0.99 (0.74, 1.32)	0.68 (0.45, 1.02)	0.71 (0.56, 0.90)	0.67 (0.54, 0.83)	<0.001	0.93
Excluding constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.77 (0.49, 1.13)	1.06 (0.7, 1.6)	0.8 (0.57, 1.11)	0.76 (0.48, 1.18)	0.2	
	Multivariable	1.0 (Ref.)	0.76 (0.48, 1.12)	1.02 (0.66, 1.52)	0.72 (0.49, 1.04)	0.69 (0.42, 1.07)	0.09	
NHS	Age	1.0 (Ref.)	1.05 (0.78, 1.37)	0.99 (0.74, 1.31)	0.8 (0.59, 1.07)	0.97 (0.74, 1.26)	0.42	
	Multivariable	1.0 (Ref.)	1.07 (0.8, 1.39)	1.02 (0.75, 1.35)	0.82 (0.61, 1.11)	1.03 (0.77, 1.36)	0.68	
Pooled	Age	1.0 (Ref.)	0.94 (0.69, 1.26)	1.01 (0.80, 1.28)	0.80 (0.64, 1.00)	0.91 (0.72, 1.15)	0.17	0.69
	Multivariable	1.0 (Ref.)	0.93 (0.68, 1.29)	1.02 (0.80, 1.30)	0.77 (0.61, 0.98)	0.89 (0.61, 1.29)	0.18	0.30
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.89 (0.58, 1.33)	0.52 (0.36, 0.76)	0.64 (0.45, 0.94)	0.76 (0.5, 1.07)	0.06	
	Multivariable	1.0 (Ref.)	0.87 (0.57, 1.3)	0.49 (0.33, 0.72)	0.59 (0.4, 0.88)	0.64 (0.4, 0.93)	0.01	
NHS	Age	1.0 (Ref.)	1.18 (0.87, 1.58)	0.81 (0.61, 1.08)	0.85 (0.61, 1.14)	0.71 (0.51, 0.97)	0.003	
	Multivariable	1.0 (Ref.)	1.19 (0.87, 1.61)	0.84 (0.62, 1.14)	0.93 (0.67, 1.26)	0.78 (0.53, 1.08)	0.07	
Pooled	Age	1.0 (Ref.)	1.05 (0.81, 1.37)	0.66 (0.43, 1.01)	0.75 (0.57, 0.99)	0.73 (0.57, 0.93)	<0.001	0.73
	Multivariable	1.0 (Ref.)	1.04 (0.77, 1.41)	0.65 (0.39, 1.09)	0.76 (0.49, 1.17)	0.72 (0.55, 0.94)	0.003	0.48

Age-adjusted models adjusted for age in years at baseline; multivariable adjusted models additionally adjusted for cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled use random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.

Table 3.3: Odds ratios for association between ≥3 vs. 0 prodromal features for each quintile of adherence to the AHEI diet pattern

Cohort	Adjustment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p* Trend	*p* Heterogeneity
Including constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.80 (0.60, 1.07)	0.55 (0.40, 0.74)	0.54 (0.40, 0.71)	0.56 (0.40, 0.74)	<0.001	
	Multivariable	1.0 (Ref.)	0.86 (0.64, 1.12)	0.60 (0.43, 0.81)	0.62 (0.45, 0.81)	0.71 (0.49, 0.96)	0.006	
NHS	Age	1.0 (Ref.)	0.84 (0.67, 1.05)	0.79 (0.64, 1.00)	0.75 (0.60, 0.94)	0.62 (0.49, 0.75)	<0.001	
	Multivariable	1.0 (Ref.)	0.86 (0.68, 1.06)	0.86 (0.68, 1.07)	0.85 (0.67, 1.07)	0.73 (0.57, 0.89)	0.01	
Pooled	Age	1.0 (Ref.)	0.83 (0.69, 0.98)	0.67 (0.47, 0.96)	0.64 (0.46, 0.89)	0.60 (0.50, 0.72)	<0.001	0.31
	Multivariable	1.0 (Ref.)	0.86 (0.72, 1.03)	0.73 (0.51, 1.04)	0.73 (0.54, 1.00)	0.72 (0.59, 0.87)	<0.001	0.41
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.64 (0.47, 0.85)	0.59 (0.43, 0.78)	0.47 (0.34, 0.62)	0.44 (0.32, 0.60)	<0.001	
	Multivariable	1.0 (Ref.)	0.71 (0.51, 0.97)	0.70 (0.51, 0.93)	0.59 (0.43, 0.79)	0.61 (0.43, 0.84)	0.002	
NHS	Age	1.0 (Ref.)	0.85 (0.67, 1.06)	0.72 (0.56, 0.90)	0.64 (0.51, 0.78)	0.50 (0.38, 0.62)	<0.001	
	Multivariable	1.0 (Ref.)	0.92 (0.73, 1.15)	0.85 (0.65, 1.09)	0.80 (0.63, 1.00)	0.69 (0.51, 0.86)	0.002	
Pooled	Age	1.0 (Ref.)	0.75 (0.56, 1.00)	0.66 (0.55, 0.80)	0.56 (0.41, 0.75)	0.48 (0.39, 0.58)	<0.001	0.78
	Multivariable	1.0 (Ref.)	0.82 (0.64, 1.07)	0.79 (0.65, 0.95)	0.70 (0.52, 0.94)	0.66 (0.53, 0.81)	<0.001	0.66
Excluding constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.78 (0.55, 1.12)	0.57 (0.37, 0.83)	0.51 (0.35, 0.76)	0.60 (0.40, 0.86)	0.003	
	Multivariable	1.0 (Ref.)	0.83 (0.57, 1.19)	0.61 (0.40, 0.88)	0.58 (0.40, 0.85)	0.76 (0.50, 1.08)	0.06	
NHS	Age	1.0 (Ref.)	0.84 (0.62, 1.11)	0.95 (0.71, 1.26)	0.85 (0.64, 1.15)	0.73 (0.55, 0.97)	0.05	
	Multivariable	1.0 (Ref.)	0.87 (0.64, 1.15)	1.06 (0.79, 1.39)	0.98 (0.73, 1.35)	0.87 (0.63, 1.14)	0.53	
Pooled	Age	1.0 (Ref.)	0.82 (0.65, 1.02)	0.75 (0.45, 1.23)	0.67 (0.41, 1.09)	0.69 (0.54, 0.86)	0.01	0.18
	Multivariable	1.0 (Ref.)	0.85 (0.68, 1.07)	0.82 (0.48, 1.40)	0.77 (0.46, 1.28)	0.83 (0.65, 1.05)	0.17	0.23
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.62 (0.41, 0.88)	0.54 (0.37, 0.75)	0.48 (0.32, 0.69)	0.40 (0.25, 0.61)	<0.001	
	Multivariable	1.0 (Ref.)	0.68 (0.46, 0.98)	0.65 (0.44, 0.90)	0.62 (0.41, 0.90)	0.56 (0.34, 0.87)	0.01	
NHS	Age	1.0 (Ref.)	0.84 (0.61, 1.13)	0.62 (0.45, 0.82)	0.70 (0.52, 0.90)	0.53 (0.39, 0.72)	<0.001	
	Multivariable	1.0 (Ref.)	0.92 (0.66, 1.25)	0.76 (0.55, 1.03)	0.93 (0.67, 1.23)	0.77 (0.53, 1.07)	0.16	
Pooled	Age	1.0 (Ref.)	0.73 (0.55, 0.98)	0.58 (0.46, 0.73)	0.59 (0.41, 0.86)	0.48 (0.36, 0.63)	<0.001	0.34
	Multivariable	1.0 (Ref.)	0.81 (0.61, 1.08)	0.71 (0.56, 0.90)	0.77 (0.52, 1.14)	0.68 (0.50, 0.92)	0.01	0.27

Age-adjusted models adjusted for age in years at baseline; multivariable adjusted models additionally adjusted for cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled using random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.

long-term diet, remained significant (aMED: p_{trend}=0.003; AHEI p_{trend}=0.01). Exclusion of moderate alcohol intake as a component of the aMED score similarly weakened but did not completely attenuate the association (baseline: p_{trend}=0.04; long-term: p_{trend}<0.001). Figure 3.1 indicates that the observed association at each subsequent dietary assessment between 1986-2006 remained inverse and became increasingly stronger. Results for 2 v. 0 and 1 v. 0 prodromal features are provided in Supplementary Tables 3.1-3.4.

The cohort-specific and pooled associations between each prodromal feature and diet pattern, comparing extreme quintiles of diet, are presented in Figure 3.2 and Supplementary Figure 3.1. In pooled multivariable-adjusted analyses, increased aMED adherence was inversely associated with constipation (baseline: p_{trend}=0.003; long-term: p_{trend}=0.004), excessive daytime sleepiness (baseline: p_{trend}=0.04; long-term: p_{trend}=0.02), and depressive symptoms (baseline p_{trend}=0.03; long-term: p_{trend}<0.001) but was not associated at baseline or long-term with pRBD, hyposmia, body pain, or impaired color vision. Results were similar for AHEI analyses with the exception that the association with depressive symptoms was only marginally significant (baseline: p_{trend}=0.06; long-term: p_{trend}=0.06). The results between cohorts were not significantly heterogeneous except for the association between aMED adherence and body pain at baseline (extreme quintiles: $p_{heterogeneity}$=0.01); there was also borderline significant heterogeneity for the association between impaired color vision and baseline aMED diet ($p_{heterogeneity}$=0.08) and between body pain and baseline AHEI diet ($p_{heterogeneity}$=0.07).

Results of analyses of the association between individual aMED components and prodromal feature combination are provided in Supplemental Table 3.5. With constipation included as a prodromal feature, baseline and long-term consumption of fruits, nuts, and vegetables as well long-term but not baseline legume consumption and moderate alcohol intake were inversely associated with having ≥ 3 versus 0 prodromal features. Excluding constipation as a prodromal feature, the association between baseline consumption of vegetables and nuts

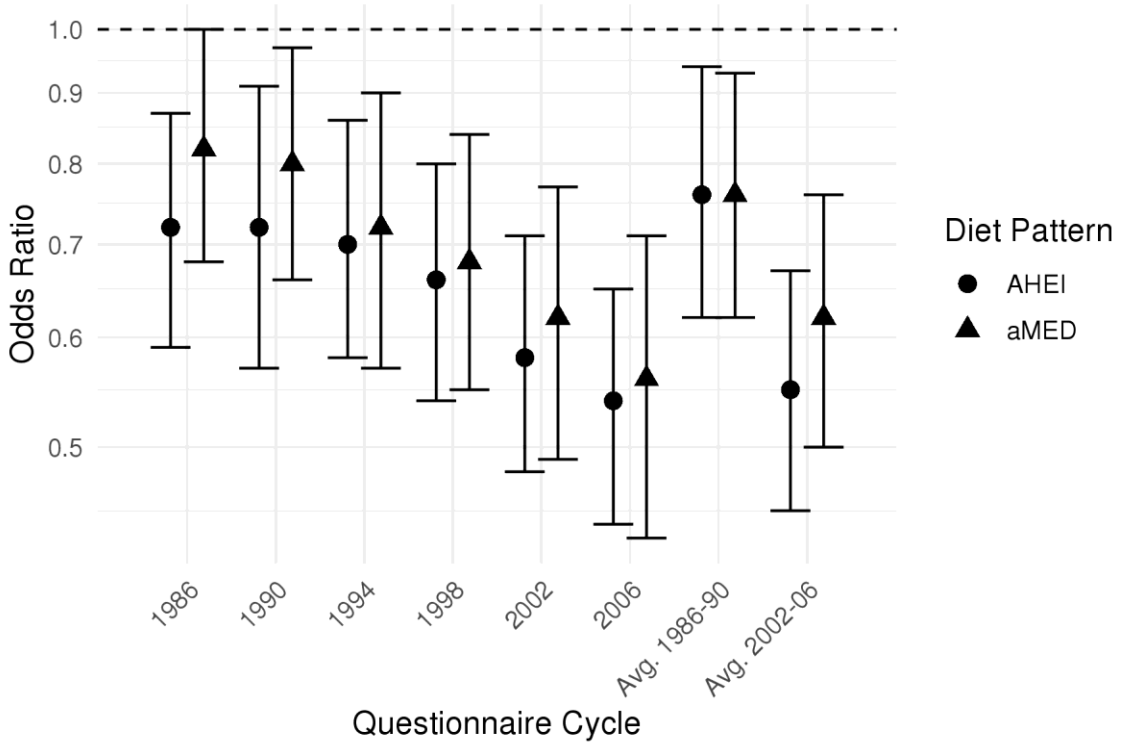

Figure 3.1: Association between ≥3 vs 0 prodromal features for highest versus lowest quintile of diet pattern adherence for each FFQ cycle between 1986 and 2006. Multivariable-adjusted pooled ORs for ≥3 versus 0 prodromal features at each time of diet assessment between 1986-2006 as well as mean diet score for first two assessments (1986,1990) and last two assessments (2002, 2006) for both aMED and AHEI dietary patterns. Models are adjusted for age (years), and cohort- and questionnaire-cycle specific quintiles of caffeine intake, energy intake, and physical activity as well as smoking pack-year and BMI categories.

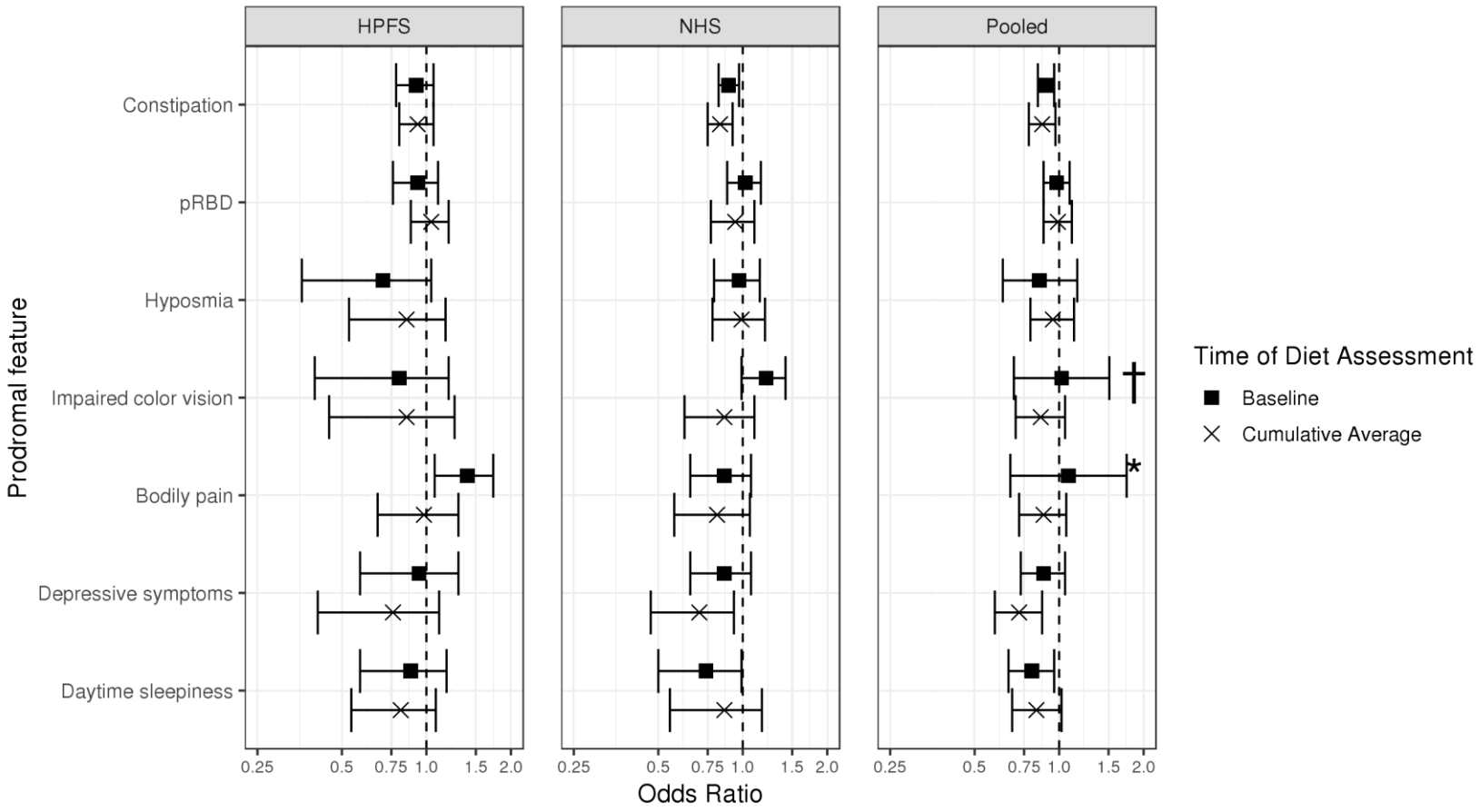

Figure 3.2: Multivariable-adjusted association for each prodromal feature comparing highest versus lowest quintile of aMED adherence. Cohort-specific and pooled multivariable-adjusted ORs for each of the 7 prodromal features comparing each the extreme quintiles of aMED adherence at baseline and for cumulative average diet between 1986-2006. Models are adjusted for age in years at baseline, cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories.
*Statistically significant heterogeneity across cohorts
†Marginally statistically significant heterogeneity across cohort

as well as long-term consumption of vegetables, nuts, and moderate alcohol intake remained significant.

Sensitivity analyses suggest that the main findings are robust to exclusion of participants completing only a portion of secondary screening. Assuming that the individuals who completed only a portion of secondary screening did not have any features for which they were missing data, the OR for ≥3 versus 0 prodromal features comparing extreme aMED quintiles was 0.83 (95% CI: 0.69, 1.01; p_{trend} = 0.01) at baseline and 0.70 (95%CI: 0.57, 0.87; p_{trend} <0.001) for long-term diet. Repeating this analysis assuming that the individuals instead had all features for which they were missing data, the OR for ≥3 versus 0 prodromal features comparing those in the highest versus lowest aMED quintile was 0.82 (95% CI: 0.67, 0.98; p_{trend} = 0.006) at baseline and 0.71 (95% CI: 0.58, 0.87; p_{trend} <0.001) for long-term adherence.

Discussion

In this pooled analysis of two large cohort studies with prospectively collected dietary information, we found that increased aMED and AHEI diet pattern scores were inversely associated with the odds of three or more prodromal PD features as well as specifically with constipation, excessive daytime sleepiness, and depressive symptoms. Analyses of individual aMED components indicate that increased consumption of vegetables, nuts, and moderate alcohol intake are each inversely associated with the odds of three or more prodromal features.

Although there have not been many investigations on the relationship between dietary pattern and either PD or prodromal PD features, these results are consistent with previous findings in the NHS and HPFS cohorts on the relationship between aMED and AHEI score and the risk of PD,[71] a small case-control study indicating that Mediterranean diet adherence is associated with both PD and PD age at onset,[73] and a recent report from the HELIAD cohort on the relationship between Mediterranean diet adherence and probability of prodromal PD.[85] Based on the results presented here, the association between diet pattern and prodromal

features of PD cannot be attributed solely to the effect of diet on constipation or the previously established association between PD and alcohol intake. Additionally, although this study and the studies described above each found an inverse association with increased adherence to a Mediterranean-style diet, the results of both studies conducted in the NHS and the HPFS are similar for the AHEI diet, suggesting that adherence to a healthy diet pattern rather than specifically to a Mediterranean-style diet may reduce risk of PD and its prodromal features. Although the mechanism by which diet pattern might influence risk of PD or its prodromal features remains unclear, growing evidence indicates the gut and enteric nervous system are involved in PD pathogenesis.[86-90] Adherence to a healthy diet pattern may therefore influence PD or prodromal PD features by protecting against alpha-synuclein aggregation in the gut or by otherwise promoting gut health[91,92] in a manner that protects against degeneration in the enteric or central nervous systems. Alternatively, because adherence to these dietary patterns is associated with consumption of foods high in antioxidant and anti-inflammatory compounds, diet pattern may instead reduce risk of PD or prodromal PD features by preventing oxidative stress[93] and neuroinflammation.[94]

There are limitations to this investigation. Most notably, prodromal features of PD were not assessed at baseline and therefore, for some participants, these features may have been present at baseline and influenced their diet. As such, it remains possible that some of the observed association is due to reverse causation, particularly for depressive symptoms and body pain, which onset early. Due to the observational nature of the investigation, it is also possible that unmeasured or residual confounding or measurement error may be biasing our results. To mitigate these biases as much as possible, we took care to adjust for several known confounders and used well-validated instruments.

This investigation also has several important strengths. The large sample size and availability of information on a range of prodromal features allowed us to investigate the

relationship between diet and combinations of prodromal features. As no individual prodromal feature considered in isolation is specific to PD but rather the co-occurrence of multiple features has been associated with PD,[74] considering these features in conjunction with one another is critical. Second, the availability of 20 years' worth of validated dietary information allowed us to assess diet at baseline, minimizing reverse causation as much as possible, and in the long-term, which may be more relevant for disease development, for multiple dietary patterns. Moreover, because dietary and covariate information is prospectively collected in these cohorts, these findings are unlikely to be significantly affected by recall or selection bias.

Conclusions

The results of this investigation suggest that increased adherence to an aMED or AHEI dietary pattern is inversely associated with a combination of prodromal PD features and specifically with constipation, excessive daytime sleepiness, and depressive symptoms. Additional prospective research is needed to determine whether increased adherence to the aMED or AHEI dietary patterns can prevent or delay conversion to PD among individuals with prodromal features.

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Appendix 1: Supplementary Tables for Chapter 1

Supplementary Table 1.1: Mean difference in metabolites within matched case-control pair

Metabolite	Mean difference (95% CI)	t	*p*	FDR *p*
Glutamate	-0.08 (-0.23, 0.06)	-1.12	0.26	0.76
Glutamine	-0.01 (-0.16, 0.14)	-0.11	0.92	0.97
BCAAs				
Leucine	-0.05 (-0.20, 0.09)	-0.73	0.47	0.85
Isoleucine	-0.02 (-0.17, 0.12)	-0.29	0.77	0.90
Valine	-0.05 (-0.19, 0.09)	-0.67	0.51	0.85
Short-Chain Acylcarnitines				
C3	-0.04 (-0.19, 0.12)	-0.46	0.65	0.85
C3-DC-CH3	0.01 (-0.14, 0.15)	0.08	0.94	0.97
C4	-0.13 (-0.27, 0.02)	-1.68	0.09	0.68
C4-OH	0.02 (-0.12, 0.15)	0.22	0.83	0.92
C5	-0.06 (-0.20, 0.09)	-0.75	0.45	0.85
C5:1	-0.04 (-0.18, 0.10)	-0.54	0.59	0.85
C5-DC	-0.09 (-0.23, 0.06)	-1.21	0.23	0.73
C6	-0.07 (-0.21, 0.07)	-1.01	0.31	0.76
C7	-0.08 (-0.22, 0.05)	-1.22	0.22	0.73
Medium-Chain Acylcarnitines				
C8	-0.02 (-0.16, 0.12)	-0.29	0.77	0.90
C9	-0.18 (-0.32, -0.03)	-2.39	0.02	0.32
C10	-0.03 (-0.17, 0.10)	-0.49	0.63	0.85
C10:2	-0.11 (-0.25, 0.03)	-1.51	0.13	0.73
C12	-0.14 (-0.28, -0.01)	-2.05	0.04	0.40
C12:1	-0.09 (-0.22, 0.05)	-1.30	0.20	0.73
C14	-0.17 (-0.32, -0.02)	-2.30	0.02	0.32
C14:1	-0.09 (-0.23, 0.04)	-1.36	0.18	0.73
C14:2	-0.06 (-0.19, 0.08)	-0.82	0.41	0.85
Long-Chain Acylcarnitines				
C16	-0.04 (-0.20, 0.11)	-0.54	0.59	0.85
C18	0.00 (-0.15, 0.15)	0.04	0.97	0.97
C18:1	0.04 (-0.11, 0.19)	0.48	0.63	0.85
C18:2	0.08 (-0.07, 0.22)	1.01	0.31	0.76
C20	-0.04 (-0.19, 0.11)	-0.52	0.60	0.85
C26	-0.03 (-0.18, 0.13)	-0.33	0.74	0.90

Mean difference and 95% confidence interval of difference (case-control) in log-transformed and standardized metabolite values. Results of paired t tests (t, *p*) and FDR adjusted *p*-values.

Supplemental Table 1.2: Minimally adjusted model results for association between individual metabolites and PD

	Quartile Model					Continuous Model		
Metabolite	Q2 RR (95% CI)	Q3 RR (95% CI)	Q4 RR (95% CI)	*p* trend	FDR *p* trend	RR per SD (95% CI)	*p*	FDR *p*
Glutamate	1.06 (0.70, 1.60)	0.78 (0.49, 1.24)	0.71 (0.44, 1.14)	0.11	0.99	0.93 (0.79, 1.09)	0.36	0.92
Glutamine	0.96 (0.61, 1.51)	0.98 (0.62, 1.54)	0.85 (0.53, 1.35)	0.51	0.99	0.97 (0.83, 1.14)	0.73	0.99
BCAAs								
Leucine	0.93 (0.61, 1.43)	0.80 (0.51, 1.25)	0.85 (0.54, 1.34)	0.41	0.99	0.95 (0.80, 1.11)	0.51	0.92
Isoleucine	0.97 (0.63, 1.50)	0.94 (0.61, 1.46)	0.89 (0.56, 1.42)	0.62	0.99	0.96 (0.81, 1.13)	0.60	0.92
Valine	0.92 (0.58, 1.46)	0.81 (0.51, 1.29)	0.88 (0.55, 1.40)	0.47	0.99	0.96 (0.81, 1.14)	0.64	0.92
Short-Chain Acylcarnitines								
C3	1.18 (0.76, 1.84)	0.95 (0.59, 1.52)	0.88 (0.55, 1.41)	0.48	0.99	0.99 (0.85, 1.16)	0.92	0.99
C3-DC-CH3	0.89 (0.57, 1.38)	0.75 (0.48, 1.19)	1.08 (0.68, 1.69)	0.99	0.99	1.07 (0.91, 1.26)	0.41	0.92
C4	0.84 (0.54, 1.29)	0.65 (0.41, 1.03)	0.82 (0.52, 1.29)	0.22	0.99	0.92 (0.79, 1.08)	0.33	0.92
C4-OH	0.84 (0.53, 1.32)	0.87 (0.55, 1.37)	1.06 (0.67, 1.67)	0.80	0.99	1.01 (0.85, 1.20)	0.89	0.99
C5	1.28 (0.82, 1.98)	0.97 (0.61, 1.52)	1.12 (0.71, 1.79)	0.85	0.99	0.98 (0.84, 1.15)	0.84	0.99
C5:1	1.03 (0.68, 1.58)	0.91 (0.59, 1.43)	0.89 (0.56, 1.42)	0.55	0.99	0.95 (0.80, 1.12)	0.52	0.92
C5-DC	1.31 (0.82, 2.10)	0.76 (0.47, 1.22)	1.14 (0.70, 1.85)	0.76	0.99	0.95 (0.81, 1.12)	0.53	0.92
C6	0.60 (0.38, 0.95)	0.95 (0.61, 1.49)	0.81 (0.51, 1.29)	0.71	0.99	0.95 (0.81, 1.12)	0.55	0.92
C7	0.91 (0.57, 1.46)	1.03 (0.64, 1.63)	0.99 (0.61, 1.61)	0.96	0.99	0.95 (0.80, 1.13)	0.57	0.92
Medium-Chain Acylcarnitines								
C8	0.62 (0.38, 1.00)	1.15 (0.73, 1.79)	1.04 (0.65, 1.69)	0.57	0.99	1.00 (0.85, 1.19)	0.96	0.99
C9	0.65 (0.41, 1.01)	0.91 (0.60, 1.40)	0.60 (0.38, 0.95)	0.09	0.99	0.85 (0.73, 1.00)	0.051	0.74
C10	0.65 (0.41, 1.03)	1.02 (0.64, 1.62)	1.09 (0.67, 1.76)	0.41	0.99	0.99 (0.83, 1.17)	0.87	0.99
C10:2	0.81 (0.51, 1.28)	1.14 (0.74, 1.75)	0.77 (0.47, 1.26)	0.54	0.99	0.88 (0.75, 1.05)	0.15	0.92
C12	0.56 (0.36, 0.88)	0.91 (0.59, 1.42)	0.75 (0.46, 1.22)	0.55	0.99	0.87 (0.73, 1.03)	0.11	0.92
C12:1	0.84 (0.54, 1.30)	0.98 (0.61, 1.56)	0.87 (0.54, 1.42)	0.65	0.99	0.91 (0.76, 1.08)	0.29	0.92
C14	0.70 (0.44, 1.12)	0.83 (0.53, 1.29)	0.82 (0.52, 1.29)	0.55	0.99	0.85 (0.73, 1.00)	0.04	0.74
C14:1	0.76 (0.48, 1.19)	1.00 (0.63, 1.57)	0.86 (0.53, 1.39)	0.80	0.99	0.90 (0.76, 1.07)	0.23	0.92
C14:2	0.75 (0.47, 1.19)	0.90 (0.56, 1.45)	0.96 (0.60, 1.54)	0.98	0.99	0.94 (0.79, 1.12)	0.50	0.92
Long-Chain Acylcarnitines								
C16	1.08 (0.69, 1.69)	1.31 (0.84, 2.04)	0.95 (0.60, 1.50)	0.94	0.99	0.96 (0.82, 1.12)	0.59	0.92
C18	0.90 (0.58, 1.39)	0.89 (0.57, 1.37)	0.99 (0.64, 1.54)	0.91	0.99	1.00 (0.85, 1.17)	0.99	0.99
C18:1	0.98 (0.62, 1.53)	1.12 (0.73, 1.74)	0.94 (0.60, 1.48)	0.90	0.99	1.02 (0.87, 1.19)	0.79	0.99
C18:2	1.16 (0.73, 1.85)	1.21 (0.77, 1.90)	1.28 (0.82, 2.01)	0.34	0.99	1.07 (0.91, 1.25)	0.43	0.92
C20	0.51 (0.33, 0.81)	0.93 (0.60, 1.43)	0.58 (0.36, 0.92)	0.07	0.99	0.93 (0.80, 1.09)	0.38	0.92
C26	1.11 (0.72, 1.70)	0.97 (0.63, 1.49)	1.04 (0.67, 1.62)	0.98	0.99	1.01 (0.87, 1.19)	0.86	0.99

Minimally adjusted model adjusted for pack-year categories, cumulative average caffeine intake quartile and fasting status. Quartile-specific results from models using metabolite quartiles, *p* trend from model of metabolite median quartile values, per SD results from model using continuous metabolite value.

Supplemental Table 1.3: Fully adjusted model results for association between individual metabolites and PD

	Quartile Model					Continuous Model		
Metabolite	Q2 RR (95% CI)	Q3 RR (95% CI)	Q4 RR (95% CI)	*p* trend	FDR *p* trend	RR per SD (95% CI)	*p*	FDR *p*
Glutamate	1.09 (0.71, 1.66)	0.78 (0.48, 1.27)	0.70 (0.42, 1.18)	0.13	0.96	0.93 (0.78, 1.11)	0.43	0.85
Glutamine	0.96 (0.60, 1.53)	0.88 (0.54, 1.42)	0.81 (0.49, 1.34)	0.37	0.96	0.95 (0.80, 1.13)	0.56	0.90
BCAAs								
Leucine	0.94 (0.60, 1.46)	0.74 (0.46, 1.18)	0.82 (0.51, 1.33)	0.33	0.96	0.94 (0.79, 1.11)	0.46	0.85
Isoleucine	0.97 (0.62, 1.52)	0.91 (0.58, 1.44)	0.90 (0.55, 1.48)	0.66	0.96	0.96 (0.80, 1.14)	0.61	0.93
Valine	0.92 (0.57, 1.48)	0.80 (0.49, 1.29)	0.86 (0.52, 1.41)	0.43	0.96	0.96 (0.80, 1.15)	0.65	0.93
Short-Chain Acylcarnitines								
C3	1.16 (0.74, 1.83)	0.93 (0.57, 1.51)	0.88 (0.53, 1.45)	0.49	0.96	0.99 (0.84, 1.17)	0.88	0.98
C3-DC-CH3	0.88 (0.56, 1.37)	0.70 (0.44, 1.13)	1.10 (0.69, 1.75)	0.99	0.99	1.06 (0.90, 1.26)	0.46	0.85
C4	0.80 (0.51, 1.24)	0.63 (0.39, 1.02)	0.84 (0.52, 1.34)	0.28	0.96	0.93 (0.79, 1.09)	0.37	0.85
C4-OH	0.87 (0.54, 1.38)	0.88 (0.55, 1.40)	1.09 (0.68, 1.74)	0.72	0.96	1.02 (0.85, 1.22)	0.85	0.98
C5	1.27 (0.81, 1.99)	0.95 (0.59, 1.52)	1.13 (0.69, 1.82)	0.85	0.96	0.99 (0.83, 1.17)	0.88	0.98
C5:1	1.09 (0.71, 1.68)	0.88 (0.56, 1.38)	0.89 (0.55, 1.43)	0.49	0.96	0.94 (0.79, 1.12)	0.47	0.85
C5-DC	1.25 (0.76, 2.06)	0.69 (0.42, 1.13)	1.06 (0.63, 1.78)	0.53	0.96	0.93 (0.78, 1.10)	0.40	0.85
C6	0.56 (0.35, 0.91)	0.94 (0.59, 1.51)	0.80 (0.49, 1.31)	0.69	0.96	0.95 (0.80, 1.13)	0.56	0.90
C7	0.85 (0.52, 1.38)	1.02 (0.63, 1.65)	0.95 (0.57, 1.57)	0.86	0.96	0.93 (0.78, 1.11)	0.42	0.85
Medium-Chain Acylcarnitines								
C8	0.59 (0.36, 0.96)	1.12 (0.71, 1.77)	1.03 (0.62, 1.69)	0.63	0.96	0.99 (0.83, 1.18)	0.92	0.98
C9	0.61 (0.38, 0.97)	0.86 (0.56, 1.33)	0.57 (0.35, 0.91)	0.06	0.83	0.82 (0.70, 0.97)	0.02	0.43
C10	0.57 (0.35, 0.93)	1.02 (0.63, 1.63)	1.06 (0.64, 1.74)	0.44	0.96	0.97 (0.81, 1.16)	0.71	0.94
C10:2	0.79 (0.50, 1.27)	1.02 (0.65, 1.60)	0.75 (0.45, 1.25)	0.42	0.96	0.87 (0.73, 1.04)	0.12	0.85
C12	0.56 (0.35, 0.89)	0.87 (0.56, 1.37)	0.73 (0.44, 1.21)	0.45	0.96	0.85 (0.71, 1.01)	0.07	0.68
C12:1	0.81 (0.52, 1.27)	0.94 (0.58, 1.51)	0.84 (0.51, 1.39)	0.57	0.96	0.89 (0.74, 1.07)	0.23	0.85
C14	0.66 (0.41, 1.06)	0.79 (0.50, 1.24)	0.74 (0.46, 1.19)	0.34	0.96	0.84 (0.71, 0.98)	0.03	0.43
C14:1	0.73 (0.46, 1.17)	0.99 (0.62, 1.58)	0.84 (0.51, 1.38)	0.77	0.96	0.89 (0.74, 1.06)	0.19	0.85
C14:2	0.71 (0.44, 1.14)	0.82 (0.50, 1.35)	0.90 (0.55, 1.45)	0.79	0.96	0.92 (0.77, 1.10)	0.38	0.85
Long-Chain Acylcarnitines								
C16	1.05 (0.66, 1.66)	1.28 (0.81, 2.02)	0.93 (0.58, 1.51)	0.98	0.99	0.94 (0.80, 1.10)	0.45	0.85
C18	0.85 (0.55, 1.33)	0.83 (0.53, 1.30)	0.91 (0.58, 1.43)	0.63	0.96	0.98 (0.84, 1.16)	0.83	0.98
C18:1	1.02 (0.64, 1.61)	1.05 (0.67, 1.65)	0.93 (0.58, 1.48)	0.75	0.96	1.00 (0.85, 1.18)	0.98	0.98
C18:2	1.06 (0.66, 1.71)	1.09 (0.68, 1.73)	1.18 (0.74, 1.87)	0.60	0.96	1.04 (0.88, 1.22)	0.67	0.93
C20	0.48 (0.30, 0.76)	0.90 (0.57, 1.41)	0.55 (0.34, 0.89)	0.047	0.83	0.90 (0.76, 1.06)	0.21	0.85
C26	1.09 (0.70, 1.68)	0.95 (0.61, 1.49)	1.02 (0.64, 1.62)	0.91	0.98	0.99 (0.84, 1.17)	0.95	0.98

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate. Quartile-specific results from metabolite quartile models, *p* trend from metabolite median quartile model, per SD results from continuous metabolite value model.

Supplementary Table 1.4a: Quartile model interval-specific results for associations between glutamate, glutamine, and BCAAs and PD

Metabolite	Interval (mon.)	Q2 RR (95% CI)	Q3 RR (95% CI)	Q4 RR (95% CI)	*p* trend	FDR *p* trend	*p* Interaction
	<60	0.49 (0.19, 1.26)	0.54 (0.21, 1.38)	0.72 (0.27, 1.91)	0.38	0.63	
Glutamate	60-179	1.27 (0.73, 2.19)	0.67 (0.35, 1.25)	0.62 (0.33, 1.17)	0.08	0.26	0.29
	180+	1.75 (0.60, 5.10)	3.33 (0.80, 13.93)	1.58 (0.31, 8.09)	0.35	0.96	
	<60	1.04 (0.45, 2.40)	1.03 (0.44, 2.44)	0.61 (0.21, 1.78)	0.41	0.63	
Glutamine	60-179	0.99 (0.53, 1.85)	0.87 (0.45, 1.69)	0.97 (0.52, 1.79)	0.89	0.92	0.55
	180+	0.70 (0.21, 2.39)	0.60 (0.20, 1.79)	0.50 (0.15, 1.73)	0.25	0.96	
BCAAs							
	<60	0.67 (0.24, 1.91)	0.54 (0.19, 1.51)	0.82 (0.30, 2.24)	0.41	0.63	
Leucine	60-179	1.14 (0.66, 1.96)	0.95 (0.52, 1.71)	0.76 (0.42, 1.38)	0.52	0.69	0.92
	180+	0.68 (0.23, 2.02)	0.48 (0.16, 1.48)	0.88 (0.26, 2.95)	0.79	0.96	
	<60	0.89 (0.32, 2.48)	0.85 (0.34, 2.16)	1.07 (0.37, 3.10)	0.83	0.93	
Isoleucine	60-179	1.16 (0.66, 2.04)	1.08 (0.61, 1.92)	0.83 (0.44, 1.54)	0.72	0.80	0.99
	180+	0.52 (0.17, 1.56)	0.50 (0.13, 1.89)	0.82 (0.25, 2.69)	0.86	0.96	
	<60	1.27 (0.45, 3.60)	0.97 (0.37, 2.53)	1.28 (0.48, 3.43)	0.91	0.95	
Valine	60-179	0.98 (0.53, 1.83)	0.99 (0.54, 1.84)	0.90 (0.48, 1.69)	0.81	0.87	0.29
	180+	0.47 (0.15, 1.45)	0.27 (0.08, 0.91)	0.38 (0.10, 1.39)	0.08	0.96	
BCAA	<60	1.02 (0.39, 2.68)	0.69 (0.27, 1.79)	1.02 (0.40, 2.64)	0.69	0.69	
Summary	60-179	0.90 (0.51, 1.60)	1.06 (0.59, 1.90)	0.73 (0.39, 1.34)	0.54	0.59	0.84
Score 1	180+	0.34 (0.10, 1.14)	0.35 (0.10, 1.18)	0.56 (0.16, 1.96)	0.34	0.81	
BCAA	<60	0.70 (0.27, 1.84)	0.63 (0.24, 1.63)	0.80 (0.29, 2.23)	0.45	0.69	
Summary	60-179	0.93 (0.53, 1.63)	1.09 (0.61, 1.96)	0.73 (0.40, 1.32)	0.59	0.59	0.83
Score 2	180+	0.31 (0.09, 1.06)	0.34 (0.10, 1.15)	0.57 (0.17, 1.98)	0.40	0.84	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate. Quartile-specific results from metabolite quartile models, *p* trend from metabolite median quartile model.

Supplementary Table 1.4b: Continuous model interval-specific results for associations between glutamate, glutamine, and BCAAs and PD

Metabolite	Interval (mon.)	RR per SD (95% CI)	*p*	FDR *p*	*p* interaction
	<60	0.88 (0.62, 1.25)	0.47	0.92	
Glutamate	60-179	0.88 (0.70, 1.10)	0.25	0.46	0.26
	180+	1.34 (0.83, 2.18)	0.23	0.92	
	<60	0.94 (0.65, 1.34)	0.72	0.96	
Glutamine	60-179	1.01 (0.82, 1.25)	0.92	0.92	0.52
	180+	0.78 (0.53, 1.16)	0.22	0.92	
BCAAs					
	<60	0.93 (0.64, 1.35)	0.69	0.96	
Leucine	60-179	0.93 (0.75, 1.15)	0.50	0.76	0.97
	180+	0.98 (0.65, 1.47)	0.92	0.92	
	<60	0.99 (0.68, 1.44)	0.96	0.96	
Isoleucine	60-179	0.95 (0.76, 1.19)	0.66	0.77	0.97
	180+	0.93 (0.62, 1.38)	0.71	0.92	
	<60	0.98 (0.67, 1.44)	0.93	0.96	
Valine	60-179	0.97 (0.77, 1.22)	0.78	0.84	0.95
	180+	0.91 (0.61, 1.34)	0.62	0.92	
BCAA	<60	0.99 (0.86, 1.13)	0.85	0.85	
Summary	60-179	0.98 (0.91, 1.06)	0.63	0.63	0.99
Score 1	180+	0.98 (0.85, 1.12)	0.74	0.96	
BCAA	<60	0.94 (0.63, 1.41)	0.77	0.85	
Summary	60-179	0.94 (0.74, 1.19)	0.60	0.63	0.99
Score 2	180+	0.94 (0.62, 1.44)	0.78	0.96	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate.

Supplementary Table 1.5a: Quartile model interval-specific results for associations between short-chain acylcarnitines and PD

Metabolite	Interval (mon.)	Q2 RR (95% CI)	Q3 RR (95% CI)	Q4 RR (95% CI)	*p* Trend	FDR *p* trend	*p* Interaction
C3	<60	1.26 (0.50, 3.17)	0.56 (0.19, 1.63)	0.89 (0.31, 2.59)	0.44	0.63	0.51
	60-179	1.08 (0.59, 1.95)	1.21 (0.66, 2.21)	0.65 (0.35, 1.23)	0.40	0.66	
	180+	1.44 (0.47, 4.36)	0.71 (0.20, 2.54)	1.90 (0.56, 6.42)	0.47	0.96	
C3-DC-CH3	<60	1.31 (0.44, 3.90)	0.74 (0.29, 1.86)	1.61 (0.64, 4.01)	0.45	0.63	0.007
	60-179	0.79 (0.44, 1.41)	0.46 (0.24, 0.86)	0.66 (0.35, 1.26)	0.06	0.26	
	180+	1.04 (0.35, 3.08)	5.57 (1.28, 24.30)	3.53 (1.04, 11.96)	0.02	0.49	
C4	<60	0.52 (0.22, 1.23)	0.41 (0.16, 1.06)	0.57 (0.23, 1.39)	0.17	0.60	0.46
	60-179	0.69 (0.37, 1.27)	0.56 (0.29, 1.07)	0.85 (0.45, 1.62)	0.48	0.67	
	180+	1.97 (0.74, 5.23)	1.61 (0.53, 4.85)	1.43 (0.43, 4.75)	0.58	0.96	
C4-OH	<60	1.23 (0.46, 3.26)	1.13 (0.43, 2.96)	1.49 (0.55, 4.01)	0.58	0.75	0.45
	60-179	0.80 (0.44, 1.47)	0.69 (0.38, 1.26)	0.86 (0.46, 1.60)	0.60	0.72	
	180+	0.74 (0.25, 2.20)	1.75 (0.50, 6.18)	1.63 (0.56, 4.70)	0.30	0.96	
C5	<60	1.19 (0.47, 3.05)	0.74 (0.27, 2.06)	1.60 (0.60, 4.25)	0.45	0.63	0.73
	60-179	1.24 (0.69, 2.21)	0.99 (0.53, 1.85)	1.02 (0.55, 1.89)	0.96	0.96	
	180+	1.55 (0.52, 4.60)	1.21 (0.43, 3.40)	0.86 (0.27, 2.81)	0.78	0.96	
C5:1	<60	0.97 (0.42, 2.23)	0.94 (0.36, 2.46)	0.34 (0.12, 0.94)	0.04	0.26	0.12
	60-179	1.11 (0.63, 1.96)	0.98 (0.55, 1.75)	1.13 (0.61, 2.10)	0.68	0.79	
	180+	1.39 (0.43, 4.48)	0.63 (0.20, 2.03)	1.47 (0.44, 4.90)	0.84	0.96	
C5-DC	<60	1.57 (0.62, 3.97)	1.26 (0.41, 3.81)	1.89 (0.74, 4.77)	0.25	0.61	0.07
	60-179	1.06 (0.55, 2.05)	0.41 (0.22, 0.78)	0.72 (0.36, 1.42)	0.048	0.26	
	180+	2.00 (0.49, 8.17)	2.23 (0.64, 7.80)	1.94 (0.41, 9.21)	0.45	0.96	
C6	<60	1.11 (0.38, 3.25)	3.31 (0.98, 11.20)	1.41 (0.48, 4.14)	0.60	0.75	0.34
	60-179	0.45 (0.24, 0.84)	0.65 (0.36, 1.17)	0.62 (0.33, 1.16)	0.23	0.45	
	180+	0.70 (0.24, 2.07)	1.13 (0.40, 3.18)	1.49 (0.44, 5.11)	0.47	0.96	
C7	<60	0.75 (0.29, 1.97)	0.75 (0.31, 1.79)	1.06 (0.45, 2.48)	0.88	0.95	0.53
	60-179	0.93 (0.49, 1.77)	0.92 (0.48, 1.76)	0.83 (0.41, 1.68)	0.48	0.67	
	180+	0.73 (0.22, 2.48)	2.70 (0.77, 9.51)	1.02 (0.29, 3.58)	0.38	0.96	
Summary Score 1	<60	0.70 (0.28, 1.77)	0.57 (0.22, 1.44)	0.87 (0.34, 2.20)	0.58	0.69	0.21
	60-179	0.50 (0.27, 0.93)	0.40 (0.20, 0.78)	0.59 (0.32, 1.12)	0.09	0.33	
	180+	1.53 (0.57, 4.09)	3.51 (0.83, 14.80)	1.83 (0.50, 6.75)	0.26	0.81	
Summary Score 2	<60	1.11 (0.47, 2.60)	0.38 (0.13, 1.07)	0.96 (0.38, 2.43)	0.60	0.69	0.49
	60-179	0.69 (0.38, 1.26)	0.74 (0.39, 1.38)	0.78 (0.42, 1.43)	0.46	0.59	
	180+	1.70 (0.57, 5.11)	0.78 (0.26, 2.40)	2.10 (0.65, 6.76)	0.37	0.81	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate. Quartile-specific results from metabolite quartile models, *p* trend from metabolite median quartile model.

Supplementary Table 1.5b: Continuous model interval-specific results for associations between short-chain acylcarnitines and PD

Metabolite	Interval (mon.)	RR per SD (95% CI)	*p*	FDR *p*	*p* interaction
C3	<60	0.97 (0.69, 1.34)	0.83	0.96	0.52
	60-179	0.94 (0.76, 1.16)	0.56	0.76	
	180+	1.20 (0.82, 1.75)	0.35	0.92	
C3-DC-CH3	<60	1.06 (0.81, 1.38)	0.67	0.96	0.23
	60-179	0.96 (0.75, 1.22)	0.73	0.81	
	180+	1.49 (0.95, 2.32)	0.08	0.92	
C4	<60	0.86 (0.63, 1.18)	0.34	0.83	0.77
	60-179	0.93 (0.75, 1.16)	0.53	0.76	
	180+	1.05 (0.67, 1.63)	0.84	0.92	
C4-OH	<60	0.98 (0.67, 1.42)	0.90	0.96	0.75
	60-179	0.99 (0.78, 1.24)	0.91	0.92	
	180+	1.16 (0.79, 1.70)	0.45	0.92	
C5	<60	1.05 (0.76, 1.47)	0.76	0.96	0.78
	60-179	0.94 (0.76, 1.17)	0.58	0.76	
	180+	1.07 (0.71, 1.61)	0.75	0.92	
C5:1	<60	0.70 (0.48, 1.01)	0.06	0.40	0.17
	60-179	1.05 (0.84, 1.32)	0.65	0.77	
	180+	0.93 (0.60, 1.43)	0.73	0.92	
C5-DC	<60	1.06 (0.80, 1.40)	0.69	0.96	0.24
	60-179	0.81 (0.64, 1.03)	0.08	0.21	
	180+	1.17 (0.69, 1.98)	0.56	0.92	
C6	<60	1.20 (0.85, 1.71)	0.30	0.80	0.08
	60-179	0.80 (0.64, 1.01)	0.07	0.21	
	180+	1.21 (0.79, 1.87)	0.38	0.92	
C7	<60	1.01 (0.75, 1.36)	0.94	0.96	0.24
	60-179	0.79 (0.60, 1.03)	0.08	0.21	
	180+	1.15 (0.78, 1.69)	0.49	0.92	
Summary Score 1	<60	0.99 (0.93, 1.06)	0.83	0.85	0.26
	60-179	0.97 (0.93, 1.01)	0.19	0.31	
	180+	1.05 (0.97, 1.14)	0.26	0.96	
Summary Score 2	<60	0.95 (0.68, 1.31)	0.74	0.85	0.64
	60-179	0.91 (0.73, 1.13)	0.39	0.52	
	180+	1.13 (0.76, 1.68)	0.55	0.96	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate.

Supplementary Table 1.6a: Quartile model interval-specific results for associations between medium-chain acylcarnitines and PD

Metabolite	Interval (mon.)	Q2 RR (95% CI)	Q3 RR (95% CI)	Q4 RR (95% CI)	*p* trend	FDR *p* trend	*p* Interaction
C8	<60	1.29 (0.44, 3.74)	6.93 (2.13, 22.57)	2.38 (0.85, 6.65)	0.10	0.42	0.18
	60-179	0.41 (0.21, 0.78)	0.54 (0.29. 0.99)	0.73 (0.37, 1.41)	0.44	0.66	
	180+	0.60 (0.19, 1.89)	1.88 (0.64, 5.58)	1.11 (0.32, 3.81)	0.55	0.96	
C9	<60	0.82 (0.33, 2.08)	0.70 (0.30, 1.65)	0.77 (0.31, 1.92)	0.46	0.63	0.95
	60-179	0.53 (0.29, 0.99)	0.96 (0.53, 1.73)	0.46 (0.24, 0.88)	0.10	0.27	
	180+	0.52 (0.17, 1.65)	0.90 (0.34, 2.43)	0.58 (0.20, 1.69)	0.48	0.96	
C10	<60	1.69 (0.62, 4.58)	5.10 (1.63, 15.94)	2.94 (0.94, 9.19)	0.07	0.32	0.08
	60-179	0.32 (0.17, 0.63)	0.53 (0.28, 0.99)	0.63 (0.33, 1.22)	0.42	0.66	
	180+	0.94 (0.28, 3.12)	1.31 (0.43, 4.01)	2.14 (0.65, 7.09)	0.20	0.96	
C10:2	<60	0.65 (0.23, 1.82)	1.82 (0.73, 4.51)	1.50 (0.57, 3.95)	0.22	0.61	0.13
	60-179	0.99 (0.52, 1.87)	0.81 (0.45, 1.47)	0.53 (0.26, 1.06)	0.07	0.26	
	180+	0.54 (0.21, 1.42)	0.96 (0.31, 2.91)	0.74 (0.22, 2.43)	0.74	0.96	
C12	<60	0.77 (0.29, 2.03)	2.17 (0.80, 5.91)	1.46 (0.50, 4.25)	0.35	0.63	0.15
	60-179	0.43 (0.23, 0.78)	0.57 (0.31, 1.03)	0.48 (0.25, 0.93)	0.07	0.26	
	180+	0.82 (0.27, 2.52)	1.15 (0.40, 3.27)	1.38 (0.41, 4.68)	0.61	0.96	
C12:1	<60	1.67 (0.65, 4.28)	5.72 (1.79, 18.29)	3.11 (1.00, 9.69)	0.04	0.26	0.03
	60-179	0.74 (0.41, 1.34)	0.61 (0.32, 1.18)	0.55 (0.29, 1.06)	0.08	0.26	
	180+	0.52 (0.16, 1.68)	0.58 (0.20, 1.63)	1.04 (0.27, 3.95)	0.67	0.96	
C14	<60	0.87 (0.31, 2.45)	1.36 (0.55, 3.39)	0.83 (0.30, 2.31)	0.97	0.97	0.22
	60-179	0.67 (0.36, 1.26)	0.52 (0.28, 0.97)	0.60 (0.33, 1.09)	0.09	0.26	
	180+	0.47 (0.16, 1.41)	1.12 (0.37, 3.40)	1.48 (0.45, 4.79)	0.32	0.96	
C14:1	<60	0.97 (0.38, 2.50)	3.53 (1.29, 9.65)	1.33 (0.44, 4.03)	0.20	0.61	0.13
	60-179	0.64 (0.34, 1.18)	0.49 (0.25, 0.93)	0.59 (0.31, 1.14)	0.14	0.31	
	180+	0.67 (0.21, 2.16)	1.32 (0.45, 3.88)	1.31 (0.41, 4.18)	0.56	0.96	
C14:2	<60	1.32 (0.51, 3.40)	2.28 (0.77, 6.75)	2.93 (1.03, 8.32)	0.045	0.26	0.03
	60-179	0.56 (0.30, 1.05)	0.61 (0.31, 1.18)	0.53 (0.28, 1.01)	0.09	0.26	
	180+	0.62 (0.19, 1.99)	0.68 (0.24, 1.98)	1.25 (0.40, 3.90)	0.89	0.96	
Summary Score 1	<60	1.36 (0.56, 3.33)	3.71 (1.25, 11.02)	2.35 (0.82, 6.77)	0.11	0.30	0.11
	60-179	0.75 (0.40, 1.41)	0.62 (0.32, 1.19)	0.66 (0.34, 1.29)	0.22	0.35	
	180+	0.61 (0.16, 2.26)	0.70 (0.24, 2.03)	1.52 (0.46, 5.02)	0.57	0.85	
Summary Score 2	<60	1.26 (0.49, 3.23)	5.31 (1.69, 16.75)	2.64 (0.85, 8.13)	0.08	0.30	0.09
	60-179	0.50 (0.26, 0.95)	0.59 (0.31, 1.14)	0.58 (0.30, 1.13)	0.21	0.35	
	180+	1.64 (0.50, 5.35)	0.97 (0.33, 2.90)	1.37 (0.42, 4.48)	0.74	0.85	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate. Quartile-specific results from metabolite quartile models, *p* trend from metabolite median quartile model

Supplementary Table 1.6b: Continuous model interval-specific results for associations between medium-chain acylcarnitines and PD

Metabolite	Interval (mon.)	RR per SD (95% CI)	*p*	FDR *p*	*p* Interaction
C8	<60	1.39 (0.97, 2.00)	0.08	0.44	0.04
	60-179	0.82 (0.65, 1.04)	0.10	0.21	
	180+	1.19 (0.76, 1.85)	0.45	0.92	
C9	<60	0.88 (0.63, 1.22)	0.44	0.91	0.89
	60-179	0.80 (0.64, 1.00)	0.05	0.21	
	180+	0.82 (0.57, 1.19)	0.30	0.92	
C10	<60	1.38 (0.94, 2.02)	0.10	0.47	0.06
	60-179	0.81 (0.64, 1.03)	0.09	0.21	
	180+	1.12 (0.71, 1.76)	0.62	0.92	
C10:2	<60	1.19 (0.86, 1.65)	0.29	0.80	0.08
	60-179	0.75 (0.59, 0.95)	0.02	0.12	
	180+	0.83 (0.54, 1.28)	0.41	0.92	
C12	<60	1.07 (0.73, 1.55)	0.73	0.96	0.28
	60-179	0.76 (0.60, 0.96)	0.02	0.12	
	180+	0.95 (0.60, 1.50)	0.82	0.92	
C12:1	<60	1.36 (0.93, 2.00)	0.12	0.47	0.047
	60-179	0.77 (0.60, 0.98)	0.03	0.15	
	180+	0.87 (0.55, 1.36)	0.54	0.92	
C14	<60	0.96 (0.67, 1.37)	0.81	0.96	0.27
	60-179	0.75 (0.61, 0.93)	0.01	0.12	
	180+	1.03 (0.70, 1.51)	0.90	0.92	
C14:1	<60	1.29 (0.90, 1.86)	0.17	0.55	0.03
	60-179	0.72 (0.57, 0.92)	0.01	0.12	
	180+	1.03 (0.68, 1.57)	0.88	0.92	
C14:2	<60	1.43 (1.00, 2.04)	0.05	0.40	0.01
	60-179	0.73 (0.57, 0.94)	0.01	0.12	
	180+	0.96 (0.64, 1.45)	0.86	0.92	
Summary Score 1	<60	1.03 (0.98, 1.08)	0.22	0.54	0.04
	60-179	0.96 (0.93, 0.99)	0.01	0.09	
	180+	1.00 (0.95, 1.06)	0.96	0.96	
Summary Score 2	<60	1.32 (0.91, 1.90)	0.14	0.54	0.04
	60-179	0.75 (0.59, 0.96)	0.02	0.09	
	180+	1.03 (0.65, 1.62)	0.91	0.96	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate.

Supplementary Table 1.7a: Quartile model interval-specific results for associations between long-chain acylcarnitines and PD

Metabolite	Interval (mon.)	Q2 RR (95% CI)	Q3 RR (95% CI)	Q4 RR (95% CI)	*p* trend	FDR *p* trend	*p* Interaction
C16	<60	1.52 (0.56, 4.10)	1.80 (0.70, 4.61)	1.85 (0.70, 4.91)	0.24	0.61	0.04
	60-179	1.08 (0.59, 1.98)	1.18 (0.65, 2.14)	0.53 (0.28, 1.01)	0.12	0.28	
	180+	0.66 (0.22, 1.97)	1.02 (0.31, 3.29)	2.49 (0.76, 8.22)	0.12	0.96	
C18	<60	1.10 (0.48, 2.55)	0.97 (0.38, 2.50)	1.19 (0.51, 2.73)	0.83	0.93	0.73
	60-179	0.69 (0.37, 1.28)	0.80 (0.45, 1.45)	0.70 (0.38, 1.30)	0.38	0.66	
	180+	1.04 (0.37, 2.95)	0.53 (0.17, 1.69)	1.37 (0.45, 4.15)	0.89	0.96	
C18:1	<60	2.49 (0.95, 6.57)	3.79 (1.41, 10.13)	3.26 (1.13, 9.38)	0.02	0.26	0.01
	60-179	0.66 (0.35, 1.24)	0.61 (0.33, 1.11)	0.52 (0.27, 0.98)	0.05	0.26	
	180+	1.10 (0.36, 3.31)	1.00 (0.31, 3.18)	1.07 (0.40, 2.91)	0.96	0.97	
C18:2	<60	1.43 (0.51, 4.02)	2.68 (0.98, 7.33)	4.44 (1.55, 12.71)	0.002	0.054	0.003
	60-179	1.04 (0.55, 1.95)	0.76 (0.42, 1.39)	0.73 (0.39, 1.37)	0.17	0.35	
	180+	1.00 (0.31, 3.27)	1.43 (0.37, 5.54)	0.99 (0.35, 2.86)	0.97	0.97	
C20	<60	0.55 (0.23, 1.34)	1.10 (0.45, 2.69)	0.59 (0.22, 1.56)	0.41	0.63	0.94
	60-179	0.46 (0.25, 0.85)	0.81 (0.44, 1.46)	0.52 (0.28, 0.95)	0.07	0.26	
	180+	0.45 (0.14, 1.41)	0.95 (0.31, 2.88)	0.60 (0.18, 1.99)	0.55	0.96	
C26	<60	1.05 (0.42, 2.65)	1.40 (0.60, 3.30)	1.11 (0.43, 2.83)	0.69	0.83	0.75
	60-179	1.14 (0.66, 1.96)	0.90 (0.50, 1.64)	0.87 (0.48, 1.58)	0.56	0.71	
	180+	0.78 (0.24, 2.59)	0.56 (0.18, 1.76)	1.31 (0.44, 3.92)	0.77	0.96	
Summary Score 1	<60	1.62 (0.68, 3.88)	1.36 (0.47, 3.95)	2.03 (0.81, 5.09)	0.17	0.34	0.06
	60-179	0.60 (0.31, 1.17)	0.54 (0.27, 1.05)	0.53 (0.28, 0.99)	0.049	0.33	
	180+	0.53 (0.18, 1.57)	0.63 (0.20, 1.97)	1.41 (0.49, 3.99)	0.71	0.85	
Summary Score 2	<60	1.44 (0.53, 3.90)	3.39 (1.30, 8.86)	3.45 (1.24, 9.61)	0.01	0.048	0.007
	60-179	0.55 (0.28, 1.08)	0.73 (0.39, 1.34)	0.51 (0.26, 0.99)	0.12	0.33	
	180+	0.84 (0.27, 2.65)	0.85 (0.24, 2.97)	1.04 (0.38, 2.85)	0.87	0.87	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate. Quartile-specific results from metabolite quartile models, *p* trend from metabolite median quartile model.

Supplementary Table 1.7b: Continuous model interval-specific results for associations between long-chain acylcarnitines and PD

Metabolite	Interval (mon.)	RR per SD (95% CI)	*p*	FDR *p*	*p* Interaction
C16	<60	1.15 (0.81, 1.62)	0.44	0.91	0.11
	60-179	0.82 (0.66, 1.01)	0.06	0.21	
	180+	1.18 (0.81, 1.72)	0.38	0.92	
C18	<60	1.03 (0.74, 1.42)	0.88	0.96	0.87
	60-179	0.95 (0.76, 1.17)	0.62	0.77	
	180+	1.04 (0.71, 1.51)	0.84	0.92	
C18:1	<60	1.64 (1.09 2.47)	0.02	0.26	0.01
	60-179	0.83 (0.67, 1.03)	0.10	0.21	
	180+	1.09 (0.78, 1.53)	0.61	0.92	
C18:2	<60	1.79 (1.20, 2.67)	0.005	0.13	0.01
	60-179	0.85 (0.68, 1.06)	0.16	0.30	
	180+	1.04 (0.73, 1.47)	0.85	0.92	
C20	<60	0.77 (0.54, 1.08)	0.13	0.47	0.51
	60-179	0.92 (0.74, 1.13)	0.42	0.72	
	180+	1.02 (0.72, 1.44)	0.92	0.92	
C26	<60	1.06 (0.77, 1.44)	0.73	0.96	0.58
	60-179	0.92 (0.74, 1.15)	0.48	0.76	
	180+	1.14 (0.77, 1.68)	0.51	0.92	
Summary Score 1	<60	1.05 (0.97, 1.13)	0.27	0.54	0.17
	60-179	0.97 (0.92, 1.01)	0.14	0.28	
	180+	1.02 (0.95, 1.10)	0.58	0.96	
Summary Score 2	<60	1.55 (1.02, 2.35)	0.04	0.31	0.03
	60-179	0.83 (0.66, 1.04)	0.11	0.28	
	180+	1.13 (0.76, 1.67)	0.54	0.96	

Models adjusted for pack-year categories, cumulative average caffeine intake quartile, fasting status, baseline diabetes, BMI category, and plasma urate.

Appendix 2: Supplementary Tables for Chapter 2

Supplementary Table 2.1a: Overall and 0-59 month interval category specific RR per SD and unadjusted p-value for each metabolite

		All subjects (n=1643)		0-59 months (n=337)	
HMDB ID	Metabolite	RR (95% CI)	p	RR (95% CI)	p
HMDB00008	Alpha-hydroxybutyrate	0.98 (0.88, 1.09)	0.7101	1.02 (0.8, 1.3)	0.8910
HMDB00011	Beta-hydroxybutyrate	0.99 (0.89, 1.11)	0.8763	0.97 (0.77, 1.23)	0.8087
HMDB00026	N-carbamoyl-beta-alanine	0.98 (0.88, 1.09)	0.7184	1.06 (0.84, 1.33)	0.6494
HMDB00062	Carnitine	0.9 (0.8, 1.01)	0.0743	0.9 (0.71, 1.13)	0.3580
HMDB00063	Cortisol	1.03 (0.93, 1.14)	0.5523	1.17 (0.93, 1.46)	0.1762
HMDB00064	Creatine	1.03 (0.93, 1.15)	0.5705	1.07 (0.84, 1.36)	0.6063
HMDB00067	Cholesterol	0.98 (0.88, 1.09)	0.6940	1 (0.77, 1.31)	0.9707
HMDB00086	Alpha-glycerophosphocholine	0.94 (0.84, 1.04)	0.1999	0.88 (0.7, 1.12)	0.3004
HMDB00092	Dimethylglycine	0.96 (0.86, 1.07)	0.4333	1.05 (0.85, 1.3)	0.6263
HMDB00112	GABA	1 (0.9, 1.11)	0.9488	1 (0.78, 1.28)	0.9954
HMDB00122	Fructose/glucose/galactose	0.95 (0.86, 1.06)	0.3485	0.88 (0.67, 1.17)	0.3946
HMDB00123	Glycine	0.94 (0.85, 1.05)	0.2579	0.81 (0.64, 1.02)	0.0673
HMDB00138	Glycocholate	1.08 (0.97, 1.2)	0.1655	1.14 (0.92, 1.42)	0.2443
HMDB00148	Glutamate	0.92 (0.83, 1.03)	0.1364	0.92 (0.73, 1.16)	0.4936
HMDB00157	Hypoxanthine	1.02 (0.9, 1.14)	0.7974	1.13 (0.83, 1.54)	0.4267
HMDB00158	Tyrosine	0.95 (0.85, 1.06)	0.3188	0.94 (0.75, 1.18)	0.6065
HMDB00159	Phenylalanine	0.95 (0.85, 1.06)	0.3453	0.96 (0.77, 1.2)	0.7284
HMDB00161	Alanine	1.01 (0.9, 1.12)	0.9045	0.9 (0.71, 1.14)	0.3957
HMDB00167	Threonine	1.05 (0.95, 1.16)	0.3695	1 (0.8, 1.24)	0.9868
HMDB00168	Asparagine	1.01 (0.91, 1.13)	0.8303	0.94 (0.75, 1.19)	0.6262
HMDB00172	Isoleucine	0.96 (0.86, 1.07)	0.4304	1 (0.79, 1.27)	0.9818
HMDB00177	Histidine	1 (0.9, 1.12)	0.9438	0.94 (0.74, 1.19)	0.5841
HMDB00182	Lysine	1.05 (0.95, 1.17)	0.3483	1.06 (0.84, 1.33)	0.6436
HMDB00186	Lactose	0.94 (0.84, 1.06)	0.3212	0.89 (0.69, 1.15)	0.3610
HMDB00187	Serine	1 (0.9, 1.11)	0.9695	0.9 (0.73, 1.12)	0.3567
HMDB00190	Lactate	0.91 (0.82, 1.02)	0.0954	0.96 (0.76, 1.23)	0.7648
HMDB00195	Inosine	1.02 (0.92, 1.13)	0.7689	1.07 (0.84, 1.36)	0.5893
HMDB00201	C2 carnitine	0.88 (0.79, 0.97)	0.0129	0.89 (0.71, 1.1)	0.2839
HMDB00206	N6-acetyllysine	0.93 (0.83, 1.04)	0.1776	1.05 (0.82, 1.34)	0.7123
HMDB00210	Pantothenate	0.95 (0.85, 1.06)	0.3322	0.86 (0.68, 1.08)	0.1998

Supplementary Table 2.1a (Continued)

HMDB00214	Ornithine	1.02 (0.91, 1.13)	0.7805	1.08 (0.86, 1.35)	0.5113
HMDB00222	C16 carnitine	0.89 (0.8, 0.99)	0.0292	0.96 (0.77, 1.19)	0.6965
HMDB00235	Thiamine	0.97 (0.86, 1.08)	0.5314	0.86 (0.66, 1.12)	0.2690
HMDB00247	Sorbitol	0.96 (0.86, 1.08)	0.4940	0.88 (0.7, 1.1)	0.2575
HMDB00251	Taurine	1.02 (0.92, 1.14)	0.6829	1.2 (0.9, 1.58)	0.2145
HMDB00258	Sucrose	0.91 (0.81, 1.02)	0.1086	0.82 (0.63, 1.06)	0.1322
HMDB00269	Sphinganine	0.96 (0.86, 1.08)	0.4826	1.12 (0.84, 1.49)	0.4591
HMDB00289	Urate	0.96 (0.86, 1.08)	0.5104	1.1 (0.87, 1.38)	0.4314
HMDB00292	Xanthine	1 (0.89, 1.11)	0.9646	1.08 (0.85, 1.37)	0.5093
HMDB00296	Uridine	0.91 (0.8, 1.02)	0.1032	0.9 (0.69, 1.17)	0.4201
HMDB00517	Arginine	1.04 (0.94, 1.15)	0.4479	0.91 (0.73, 1.14)	0.4263
HMDB00610	C18:2 CE	1.06 (0.95, 1.18)	0.3031	1.1 (0.89, 1.36)	0.3892
HMDB00630	Cytosine	0.9 (0.8, 1)	0.0583	1 (0.79, 1.28)	0.9731
HMDB00631	Glycodeoxycholate/ glycochenodeoxycholate	1.12 (1, 1.25)	0.0474	1.16 (0.93, 1.46)	0.1855
HMDB00641	Glutamine	1 (0.89, 1.11)	0.9630	1.11 (0.89, 1.4)	0.3605
HMDB00651	C10 carnitine	0.91 (0.81, 1.02)	0.0903	1.05 (0.82, 1.34)	0.6891
HMDB00658	C16:1 CE	1.03 (0.93, 1.14)	0.5880	1.07 (0.85, 1.34)	0.5845
HMDB00670	Homoarginine	1.14 (1.02, 1.27)	0.0186	1.13 (0.87, 1.46)	0.3573
HMDB00679	Homocitrulline	1.02 (0.92, 1.13)	0.7397	1.08 (0.86, 1.36)	0.4864
HMDB00682	Indoxylsulfate	1 (0.9, 1.11)	0.9835	0.84 (0.66, 1.06)	0.1311
HMDB00684	Kynurenine	0.88 (0.78, 0.99)	0.0383	0.82 (0.64, 1.07)	0.1464
HMDB00687	Leucine	0.92 (0.83, 1.03)	0.1405	0.92 (0.73, 1.15)	0.4521
HMDB00688	C5 carnitine	1.03 (0.93, 1.15)	0.5554	1.12 (0.89, 1.42)	0.3295
HMDB00696	Methionine	1.03 (0.93, 1.15)	0.5771	0.96 (0.77, 1.2)	0.7504
HMDB00699	1-methylnicotinamide	1.01 (0.92, 1.12)	0.7898	0.87 (0.69, 1.1)	0.2469
HMDB00705	C6 carnitine	0.89 (0.8, 1)	0.0460	0.92 (0.73, 1.16)	0.4583
HMDB00716	Pipecolic acid	1.01 (0.91, 1.12)	0.8405	1.01 (0.81, 1.27)	0.9075
HMDB00725	Hydroxyproline	0.98 (0.89, 1.09)	0.7152	1.13 (0.9, 1.41)	0.3002
HMDB00767	Pseudouridine	0.9 (0.8, 1.02)	0.0924	1.04 (0.82, 1.32)	0.7513
HMDB00791	C8 carnitine	0.91 (0.81, 1.02)	0.0931	1.05 (0.83, 1.32)	0.7017
HMDB00824	C3 carnitine	0.95 (0.85, 1.06)	0.3313	1.03 (0.82, 1.29)	0.7801
HMDB00848	C18 carnitine	0.92 (0.83, 1.02)	0.1150	0.91 (0.73, 1.12)	0.3514
HMDB00853	Acetyl-galactosamine	0.97 (0.87, 1.09)	0.6683	1.17 (0.91, 1.5)	0.2149
HMDB00875	Trigonelline	0.96 (0.86, 1.08)	0.4836	0.83 (0.66, 1.05)	0.1145

Supplementary Table 2.1a (Continued)

HMDB00883	Valine	0.93 (0.83, 1.04)	0.1886	0.92 (0.72, 1.18)	0.5220
HMDB00884	Ribothymidine	1.01 (0.9, 1.13)	0.9236	1.06 (0.82, 1.37)	0.6393
HMDB00885	C16:0 CE	1 (0.9, 1.12)	0.9660	1.15 (0.92, 1.44)	0.2240
HMDB00896	Taurodeoxycholate/ taurochenodeoxycholate	0.96 (0.86, 1.07)	0.4509	1.22 (0.96, 1.56)	0.1046
HMDB00897	7-methylguanine	0.95 (0.84, 1.07)	0.3829	1.18 (0.92, 1.52)	0.1926
HMDB00904	Citrulline	0.99 (0.89, 1.11)	0.9041	0.98 (0.76, 1.26)	0.8601
HMDB00918	C18:1 CE	1.05 (0.94, 1.17)	0.3890	1.1 (0.88, 1.37)	0.4048
HMDB00925	Trimethylamine-N-oxide	1.01 (0.91, 1.11)	0.8894	1.19 (0.96, 1.47)	0.1132
HMDB00929	Tryptophan	1.03 (0.93, 1.14)	0.5690	0.91 (0.72, 1.16)	0.4579
HMDB00982	5-methylcytidine	0.92 (0.83, 1.03)	0.1443	1.12 (0.91, 1.38)	0.3005
HMDB01008	Biliverdin	1 (0.9, 1.11)	0.9880	0.95 (0.74, 1.2)	0.6527
HMDB01046	Cotinine	0.86 (0.76, 0.97)	0.0160	0.65 (0.47, 0.9)	0.0083
HMDB01276	N1-acetylspermidine	0.94 (0.84, 1.04)	0.2447	1.16 (0.92, 1.47)	0.2131
HMDB01325	N6,N6,N6-trimethyllysine	1.03 (0.93, 1.14)	0.6043	1.09 (0.85, 1.4)	0.5003
HMDB01348	C18:0 SM	0.92 (0.82, 1.02)	0.1178	0.88 (0.68, 1.13)	0.3005
HMDB01390	Hydroxycotinine	0.89 (0.79, 1.01)	0.0685	0.64 (0.46, 0.9)	0.0112
HMDB01539	ADMA	0.93 (0.84, 1.04)	0.2261	1.1 (0.86, 1.41)	0.4326
HMDB01548	Pentose monophosphate	1.01 (0.91, 1.13)	0.8525	0.98 (0.76, 1.26)	0.8854
HMDB01563	1-methylguanosine	0.95 (0.85, 1.05)	0.3080	1.13 (0.89, 1.43)	0.3055
HMDB01847	Caffeine	0.99 (0.88, 1.12)	0.9119	0.72 (0.55, 0.94)	0.0166
HMDB01886	3-methylxanthine	0.98 (0.88, 1.09)	0.6901	0.74 (0.57, 0.97)	0.0296
HMDB01906	2-aminoisobutyric acid	0.97 (0.86, 1.09)	0.6015	1.16 (0.88, 1.52)	0.2860
HMDB02005	Methionine sulfoxide	0.96 (0.85, 1.08)	0.4740	0.91 (0.69, 1.19)	0.4905
HMDB02013	C4 carnitine	0.95 (0.86, 1.06)	0.3684	0.98 (0.78, 1.24)	0.8959
HMDB02014	C14:1 carnitine	0.87 (0.78, 0.97)	0.0154	0.94 (0.74, 1.19)	0.5989
HMDB02250	C12 carnitine	0.86 (0.77, 0.96)	0.0076	0.91 (0.7, 1.17)	0.4467
HMDB02366	C5:1 carnitine	0.9 (0.81, 1)	0.0412	0.83 (0.66, 1.06)	0.1310
HMDB0240212	DMGV	0.94 (0.84, 1.05)	0.2503	0.94 (0.76, 1.17)	0.5692
HMDB02815	C18:1 LPC	0.93 (0.84, 1.03)	0.1706	0.8 (0.63, 1.02)	0.0730
HMDB03282	1-methylguanine	1.06 (0.94, 1.19)	0.3337	1.1 (0.86, 1.41)	0.4649
HMDB03331	1-methyladenosine	0.96 (0.85, 1.07)	0.4468	1.04 (0.8, 1.35)	0.7707
HMDB03334	SDMA	0.94 (0.84, 1.05)	0.2637	1.19 (0.93, 1.51)	0.1582
HMDB03357	N-acetylornithine	1.01 (0.91, 1.13)	0.8070	1.07 (0.86, 1.35)	0.5373
HMDB03681	4-acetamidobutanoate	0.96 (0.86, 1.07)	0.4809	1.01 (0.8, 1.27)	0.9306

Supplementary Table 2.1a (Continued)

HMDB04400	5-acetylamino-6-amino-3-methyluracil	0.97 (0.86, 1.08)	0.5598	0.7 (0.53, 0.92)	0.0108
HMDB04824	N2,N2-dimethylguanosine	0.92 (0.82, 1.04)	0.1709	1.1 (0.87, 1.38)	0.4205
HMDB04827	Proline-betaine	0.98 (0.88, 1.09)	0.7499	0.96 (0.75, 1.24)	0.7514
HMDB04949	C16:0 Ceramide (d18:1)	0.93 (0.84, 1.03)	0.1808	1.06 (0.81, 1.37)	0.6768
HMDB04952	C22:0 Ceramide (d18:1)	0.95 (0.86, 1.06)	0.3624	0.81 (0.64, 1.04)	0.0985
HMDB04953	C24:1 Ceramide (d18:1)	0.93 (0.83, 1.03)	0.1578	0.99 (0.77, 1.26)	0.9053
HMDB04956	C24:0 Ceramide (d18:1)	0.96 (0.86, 1.06)	0.4194	0.82 (0.64, 1.04)	0.1054
HMDB05065	C18:1 carnitine	0.94 (0.85, 1.04)	0.2600	1.08 (0.85, 1.36)	0.5439
HMDB05066	C14 carnitine	0.86 (0.77, 0.95)	0.0048	0.94 (0.74, 1.19)	0.6061
HMDB05356	C48:0 TAG	1.04 (0.93, 1.16)	0.4845	0.91 (0.73, 1.13)	0.3903
HMDB05357	C50:0 TAG	1.01 (0.9, 1.12)	0.9287	0.88 (0.7, 1.1)	0.2531
HMDB05359	C48:1 TAG	1.02 (0.92, 1.14)	0.7093	0.89 (0.71, 1.11)	0.2977
HMDB05360	C50:1 TAG	1 (0.89, 1.12)	0.9937	0.88 (0.7, 1.1)	0.2605
HMDB05362	C51:2 TAG	1.07 (0.96, 1.2)	0.1925	1.07 (0.86, 1.34)	0.5399
HMDB05363	C52:4 TAG	0.99 (0.88, 1.1)	0.8083	0.99 (0.76, 1.28)	0.9112
HMDB05367	C52:1 TAG	0.98 (0.88, 1.09)	0.7011	0.87 (0.69, 1.09)	0.2164
HMDB05369	C52:2 TAG	0.96 (0.86, 1.08)	0.5102	0.9 (0.71, 1.14)	0.3932
HMDB05370	C54:4 TAG	0.98 (0.88, 1.09)	0.6570	0.99 (0.77, 1.27)	0.9440
HMDB05376	C48:2 TAG	1.02 (0.92, 1.14)	0.6715	0.89 (0.7, 1.12)	0.3185
HMDB05377	C50:2 TAG	0.98 (0.87, 1.09)	0.6941	0.91 (0.72, 1.14)	0.3942
HMDB05384	C52:3 TAG	0.98 (0.88, 1.1)	0.7884	0.98 (0.76, 1.27)	0.8854
HMDB05385	C54:5 TAG	0.92 (0.83, 1.02)	0.1005	0.82 (0.65, 1.05)	0.1118
HMDB05391	C54:6 TAG	0.95 (0.85, 1.05)	0.3123	0.97 (0.76, 1.23)	0.7768
HMDB05392	C56:8 TAG	0.99 (0.89, 1.1)	0.8315	1.07 (0.84, 1.36)	0.5882
HMDB05395	C54:1 TAG	0.98 (0.88, 1.09)	0.7028	0.9 (0.71, 1.13)	0.3578
HMDB05403	C54:2 TAG	0.96 (0.86, 1.07)	0.4576	0.91 (0.72, 1.16)	0.4384
HMDB05404	C56:2 TAG	0.96 (0.86, 1.07)	0.4834	0.91 (0.72, 1.14)	0.4076
HMDB05405	C54:3 TAG	0.96 (0.86, 1.07)	0.4898	0.92 (0.71, 1.19)	0.5194
HMDB05406	C56:5 TAG	0.91 (0.82, 1.01)	0.0868	0.93 (0.74, 1.18)	0.5666
HMDB05410	C56:3 TAG	0.95 (0.85, 1.06)	0.3393	0.93 (0.74, 1.18)	0.5665
HMDB05432	C48:3 TAG	1 (0.89, 1.11)	0.9438	0.9 (0.7, 1.15)	0.3944
HMDB05433	C50:3 TAG	1 (0.89, 1.12)	0.9981	0.93 (0.73, 1.18)	0.5571
HMDB05436	C52:6 TAG	0.95 (0.85, 1.05)	0.2935	0.98 (0.77, 1.24)	0.8458
HMDB05447	C54:7 TAG	0.93 (0.84, 1.04)	0.1968	0.95 (0.75, 1.22)	0.6995
HMDB05448	C56:9 TAG	0.98 (0.88, 1.09)	0.7335	1.05 (0.82, 1.34)	0.6895

Supplementary Table 2.1a (Continued)

HMDB05456	C56:6 TAG	0.95 (0.86, 1.06)	0.3591	0.96 (0.76, 1.2)	0.7046
HMDB05458	C58:6 TAG	0.95 (0.85, 1.05)	0.3146	1.01 (0.8, 1.28)	0.9439
HMDB05462	C56:7 TAG	0.97 (0.87, 1.08)	0.6006	1 (0.79, 1.25)	0.9815
HMDB05463	C58:9 TAG	1.02 (0.92, 1.14)	0.6992	1.09 (0.86, 1.39)	0.4739
HMDB05471	C58:7 TAG	0.99 (0.89, 1.11)	0.9025	1.06 (0.84, 1.32)	0.6310
HMDB05476	C58:10 TAG	1 (0.9, 1.12)	0.9944	1.07 (0.83, 1.36)	0.6140
HMDB05478	C60:12 TAG	1.03 (0.93, 1.15)	0.5451	1.12 (0.87, 1.43)	0.3723
HMDB05923	N4-acetylcytidine	0.99 (0.89, 1.11)	0.9191	1.01 (0.81, 1.26)	0.9110
HMDB06344	Phenylacetylglutamine	1.14 (1.03, 1.28)	0.0162	1.38 (1.06, 1.81)	0.0166
HMDB06347	C26 carnitine	0.99 (0.89, 1.1)	0.8971	1.01 (0.8, 1.28)	0.9402
HMDB06469	C18:2 carnitine	0.95 (0.85, 1.05)	0.3057	1.12 (0.9, 1.41)	0.3160
HMDB06725	C14:0 CE	1.02 (0.92, 1.13)	0.7501	0.96 (0.77, 1.2)	0.7268
HMDB06726	C20:4 CE	1.02 (0.91, 1.13)	0.7533	1.05 (0.84, 1.31)	0.6942
HMDB06731	C20:5 CE	0.99 (0.89, 1.1)	0.8589	0.98 (0.79, 1.23)	0.8912
HMDB06733	C22:6 CE	1.06 (0.95, 1.18)	0.2914	1.17 (0.93, 1.47)	0.1838
HMDB06736	C20:3 CE	1.04 (0.94, 1.16)	0.4195	1.06 (0.84, 1.34)	0.6151
HMDB06831	Butyrobetaine	0.98 (0.89, 1.07)	0.6076	1 (0.85, 1.18)	0.9731
HMDB07099	C32:1 DAG	0.96 (0.86, 1.07)	0.4663	0.87 (0.68, 1.1)	0.2460
HMDB07102	C34:1 DAG	0.93 (0.83, 1.05)	0.2421	0.87 (0.68, 1.1)	0.2421
HMDB07103	C34:2 DAG	0.95 (0.85, 1.06)	0.3567	0.94 (0.74, 1.19)	0.5961
HMDB07132	C34:3 DAG	0.98 (0.87, 1.1)	0.7274	0.96 (0.74, 1.23)	0.7286
HMDB07170	C38:4 DAG	0.95 (0.86, 1.05)	0.3294	0.92 (0.72, 1.17)	0.5015
HMDB07199	C38:5 DAG	0.92 (0.83, 1.03)	0.1322	0.79 (0.62, 1.02)	0.0737
HMDB07218	C36:2 DAG	0.94 (0.84, 1.06)	0.3153	0.89 (0.7, 1.12)	0.3196
HMDB07219	C36:3 DAG	0.94 (0.85, 1.06)	0.3143	0.92 (0.72, 1.18)	0.5292
HMDB07248	C36:4 DAG	0.94 (0.84, 1.05)	0.2699	0.94 (0.74, 1.2)	0.6175
HMDB07448	C38:3 DAG	0.95 (0.85, 1.06)	0.3387	0.93 (0.75, 1.17)	0.5481
HMDB07869	C30:0 PC	0.99 (0.89, 1.09)	0.7941	0.95 (0.74, 1.2)	0.6428
HMDB07870	C30:1 PC	1.03 (0.93, 1.14)	0.5815	1.01 (0.8, 1.28)	0.9109
HMDB07871	C32:0 PC	0.97 (0.87, 1.07)	0.5218	1.07 (0.83, 1.37)	0.5985
HMDB07873	C32:1 PC	1.01 (0.91, 1.12)	0.8200	1.01 (0.81, 1.25)	0.9355
HMDB07874	C32:2 PC	1.02 (0.91, 1.13)	0.7631	0.95 (0.74, 1.22)	0.6915
HMDB07883	C34:4 PC	0.96 (0.87, 1.07)	0.4967	0.91 (0.7, 1.17)	0.4586
HMDB07970	C34:0 PC	0.91 (0.82, 1.01)	0.0847	0.93 (0.72, 1.2)	0.5604
HMDB07972	C34:1 PC	1.01 (0.91, 1.12)	0.8726	0.95 (0.75, 1.19)	0.6364

Supplementary Table 2.1a (Continued)

HMDB07973	C34:2 PC	1.03 (0.93, 1.14)	0.6227	0.95 (0.74, 1.22)	0.7060
HMDB07983	C36:4 PC-A	0.99 (0.89, 1.1)	0.8976	0.87 (0.68, 1.12)	0.2911
HMDB07991	C38:6 PC	1.03 (0.92, 1.14)	0.6455	1.08 (0.85, 1.39)	0.5168
HMDB08006	C34:3 PC	1.01 (0.91, 1.13)	0.7990	1.05 (0.82, 1.34)	0.6954
HMDB08038	C36:1 PC	1 (0.9, 1.11)	0.9813	0.89 (0.7, 1.12)	0.3209
HMDB08039	C36:2 PC	1 (0.9, 1.11)	0.9696	0.85 (0.66, 1.11)	0.2301
HMDB08047	C38:3 PC	0.98 (0.88, 1.09)	0.7381	0.92 (0.73, 1.16)	0.4857
HMDB08048	C38:4 PC	0.93 (0.84, 1.04)	0.1906	0.86 (0.67, 1.1)	0.2357
HMDB08105	C36:3 PC	1 (0.89, 1.11)	0.9296	0.95 (0.73, 1.23)	0.6784
HMDB08138	C36:4 PC-B	0.96 (0.86, 1.06)	0.4367	0.89 (0.69, 1.16)	0.4060
HMDB08270	C38:2 PC	1.04 (0.94, 1.16)	0.4570	1.01 (0.8, 1.27)	0.9358
HMDB08511	C40:10 PC	1.01 (0.9, 1.12)	0.9099	1.04 (0.81, 1.33)	0.7526
HMDB08731	C40:9 PC	1 (0.9, 1.12)	0.9392	1.06 (0.81, 1.38)	0.6774
HMDB08925	C34:0 PE	1.01 (0.91, 1.12)	0.8238	1.03 (0.82, 1.29)	0.8268
HMDB08928	C34:2 PE	0.95 (0.86, 1.06)	0.3744	0.95 (0.76, 1.18)	0.6473
HMDB08937	C36:4 PE	0.94 (0.85, 1.04)	0.2293	0.97 (0.78, 1.22)	0.8038
HMDB08942	C38:2 PE	1.08 (0.97, 1.2)	0.1723	1.02 (0.79, 1.31)	0.8926
HMDB08952	C34:2 PE plasmalogen	1.01 (0.91, 1.12)	0.9069	0.86 (0.68, 1.09)	0.2245
HMDB08991	C36:0 PE	1 (0.9, 1.11)	0.9878	1.02 (0.81, 1.29)	0.8515
HMDB08993	C36:1 PE	0.97 (0.87, 1.08)	0.6193	0.85 (0.67, 1.07)	0.1712
HMDB08994	C36:2 PE	0.98 (0.88, 1.09)	0.6885	0.92 (0.73, 1.15)	0.4408
HMDB09003	C38:4 PE	0.94 (0.85, 1.05)	0.2863	1.02 (0.81, 1.29)	0.8455
HMDB09012	C40:6 PE	0.94 (0.85, 1.05)	0.2649	1.03 (0.82, 1.28)	0.8258
HMDB09060	C36:3 PE	0.97 (0.87, 1.08)	0.5811	0.92 (0.73, 1.17)	0.5184
HMDB09069	C38:5 PE	0.92 (0.83, 1.02)	0.1297	0.97 (0.77, 1.22)	0.7787
HMDB09082	C36:2 PE plasmalogen	1.02 (0.92, 1.13)	0.7132	0.84 (0.67, 1.07)	0.1648
HMDB09102	C38:6 PE	0.95 (0.85, 1.05)	0.2909	1.04 (0.84, 1.3)	0.7183
HMDB10169	C16:0 SM	0.96 (0.86, 1.07)	0.4373	1.02 (0.79, 1.32)	0.8530
HMDB10316	Acetaminophen glucuronide	0.95 (0.86, 1.06)	0.3820	0.94 (0.72, 1.23)	0.6604
HMDB10368	C18:0 CE	1.03 (0.93, 1.14)	0.6070	1.08 (0.86, 1.36)	0.5006
HMDB10370	C18:3 CE	1.03 (0.92, 1.15)	0.6369	0.97 (0.76, 1.24)	0.8166
HMDB10379	C14:0 LPC	0.96 (0.86, 1.07)	0.4696	0.84 (0.65, 1.09)	0.1988
HMDB10382	C16:0 LPC	0.93 (0.84, 1.03)	0.1635	0.82 (0.64, 1.05)	0.1118
HMDB10383	C16:1 LPC	0.96 (0.87, 1.06)	0.4591	0.9 (0.71, 1.13)	0.3629
HMDB10384	C18:0 LPC	0.92 (0.83, 1.02)	0.1298	0.8 (0.63, 1.01)	0.0634

Supplementary Table 2.1a (Continued)

HMDB10386	C18:2 LPC	0.95 (0.85, 1.05)	0.3072	0.78 (0.61, 0.99)	0.0413
HMDB10393	C18:3 LPC	0.93 (0.83, 1.03)	0.1663	0.78 (0.62, 0.99)	0.0402
HMDB10395	C20:4 LPC	0.91 (0.82, 1)	0.0601	0.8 (0.64, 1.01)	0.0567
HMDB10397	C20:5 LPC	0.94 (0.85, 1.04)	0.2600	0.86 (0.68, 1.08)	0.1907
HMDB10404	C22:6 LPC	0.97 (0.88, 1.08)	0.6149	0.95 (0.76, 1.2)	0.6943
HMDB10407	C16:1 LPC plasmalogen	0.94 (0.85, 1.05)	0.2657	0.86 (0.68, 1.08)	0.1962
HMDB10411	C46:0 TAG	1.03 (0.93, 1.15)	0.5394	0.86 (0.69, 1.07)	0.1694
HMDB10412	C46:1 TAG	1.02 (0.91, 1.13)	0.7464	0.86 (0.68, 1.09)	0.2014
HMDB10419	C46:2 TAG	0.99 (0.89, 1.11)	0.9041	0.86 (0.67, 1.09)	0.2071
HMDB10471	C50:5 TAG	0.95 (0.85, 1.06)	0.3678	0.93 (0.73, 1.18)	0.5595
HMDB10497	C50:6 TAG	0.94 (0.85, 1.05)	0.2666	0.88 (0.69, 1.12)	0.3081
HMDB10513	C56:10 TAG	0.97 (0.88, 1.08)	0.5776	0.96 (0.78, 1.19)	0.7291
HMDB10517	C52:7 TAG	0.95 (0.86, 1.06)	0.3522	0.97 (0.76, 1.23)	0.7800
HMDB10518	C54:8 TAG	0.98 (0.88, 1.08)	0.6528	1.06 (0.83, 1.34)	0.6496
HMDB10531	C58:11 TAG	0.99 (0.89, 1.1)	0.8022	1.02 (0.8, 1.3)	0.8873
HMDB11103	1,7-dimethyluric acid	0.93 (0.83, 1.05)	0.2331	0.71 (0.54, 0.92)	0.0090
HMDB11130	C18:0 LPE	0.91 (0.83, 1.01)	0.0885	0.84 (0.67, 1.06)	0.1438
HMDB11208	C34:1 PC plasmalogen-A	1.05 (0.94, 1.17)	0.3539	1.22 (0.94, 1.58)	0.1347
HMDB11210	C34:2 PC plasmalogen	1.03 (0.92, 1.15)	0.5790	0.97 (0.75, 1.25)	0.8005
HMDB11211	C34:3 PC plasmalogen	1.05 (0.95, 1.17)	0.3395	1.02 (0.83, 1.27)	0.8299
HMDB11220	C36:5 PC plasmalogen-B	1.01 (0.91, 1.12)	0.8929	1.03 (0.8, 1.33)	0.8272
HMDB11221	C36:5 PC plasmalogen-A	1 (0.9, 1.11)	0.9928	1.06 (0.83, 1.35)	0.6468
HMDB11229	C38:7 PC plasmalogen	1.04 (0.94, 1.16)	0.4487	1.09 (0.84, 1.41)	0.5091
HMDB11241	C36:1 PC plasmalogen	1 (0.9, 1.11)	0.9828	1.06 (0.83, 1.35)	0.6549
HMDB11243	C36:2 PC plasmalogen	1.02 (0.92, 1.14)	0.6526	1.03 (0.82, 1.3)	0.8046
HMDB11244	C36:3 PC plasmalogen-A	1.04 (0.94, 1.16)	0.4173	1.09 (0.86, 1.38)	0.4772
HMDB11252	C38:4 PC plasmalogen	1 (0.91, 1.11)	0.9604	1.06 (0.83, 1.34)	0.6549
HMDB11294	C40:7 PC plasmalogen	1.02 (0.91, 1.14)	0.7406	1.25 (0.95, 1.66)	0.1128
HMDB11310	C36:4 PC plasmalogen	1 (0.9, 1.11)	0.9832	1.23 (0.95, 1.61)	0.1216
HMDB11319	C38:6 PC plasmalogen	1.04 (0.94, 1.16)	0.4626	1.2 (0.92, 1.56)	0.1701
HMDB11343	C34:3 PE plasmalogen	1.02 (0.92, 1.13)	0.7317	0.91 (0.73, 1.14)	0.4113
HMDB11386	C38:5 PE plasmalogen	1.01 (0.91, 1.11)	0.8790	0.94 (0.75, 1.17)	0.5742
HMDB11387	C38:6 PE plasmalogen	1 (0.9, 1.1)	0.9521	0.99 (0.79, 1.24)	0.9146
HMDB11394	C40:7 PE plasmalogen	1.04 (0.94, 1.15)	0.4803	1.07 (0.86, 1.33)	0.5665
HMDB11410	C36:5 PE plasmalogen	1.03 (0.93, 1.15)	0.5443	0.97 (0.76, 1.22)	0.7709

Supplementary Table 2.1a (Continued)

HMDB11420	C38:7 PE plasmalogen	1.05 (0.95, 1.16)	0.3733	1.07 (0.84, 1.36)	0.5779
HMDB11441	C36:3 PE plasmalogen	1.01 (0.91, 1.11)	0.9219	0.89 (0.71, 1.11)	0.2888
HMDB11442	C36:4 PE plasmalogen	1 (0.9, 1.11)	0.9771	0.91 (0.73, 1.14)	0.4115
HMDB11503	C16:0 LPE	0.92 (0.83, 1.03)	0.1444	0.84 (0.65, 1.08)	0.1657
HMDB11506	C18:1 LPE	0.95 (0.86, 1.06)	0.3738	0.72 (0.56, 0.94)	0.0135
HMDB11507	C18:2 LPE	0.97 (0.87, 1.08)	0.5879	0.79 (0.62, 1.02)	0.0687
HMDB11511	C20:0 LPE	0.99 (0.89, 1.1)	0.8184	0.91 (0.73, 1.13)	0.3797
HMDB11517	C20:4 LPE	0.92 (0.83, 1.02)	0.1184	0.78 (0.61, 0.99)	0.0443
HMDB11526	C22:6 LPE	0.96 (0.86, 1.07)	0.4173	0.97 (0.77, 1.22)	0.7799
HMDB11697	C24:0 SM	1.01 (0.91, 1.12)	0.8771	0.9 (0.7, 1.15)	0.3940
HMDB11701	C51:3 TAG	1.04 (0.93, 1.16)	0.4846	1.09 (0.85, 1.39)	0.5149
HMDB11706	C49:2 TAG	1.08 (0.97, 1.21)	0.1440	1.01 (0.81, 1.26)	0.9244
HMDB12097	C14:0 SM	0.96 (0.87, 1.07)	0.4807	0.87 (0.67, 1.12)	0.2799
HMDB12101	C18:1 SM	0.9 (0.81, 1)	0.0450	0.89 (0.69, 1.15)	0.3632
HMDB12102	C20:0 SM	0.99 (0.89, 1.11)	0.9096	0.83 (0.63, 1.09)	0.1743
HMDB12103	C22:0 SM	1.01 (0.91, 1.12)	0.8330	0.93 (0.72, 1.21)	0.6012
HMDB12104	C22:1 SM	0.98 (0.88, 1.09)	0.6709	0.89 (0.68, 1.16)	0.3860
HMDB12107	C24:1 SM	1 (0.89, 1.11)	0.9397	1.07 (0.83, 1.37)	0.6173
HMDB12356	C34:0 PS	0.96 (0.86, 1.07)	0.4478	0.93 (0.72, 1.2)	0.5939
HMDB13122	C18:1 LPC plasmalogen A	0.95 (0.85, 1.05)	0.2910	1.01 (0.8, 1.27)	0.9187
HMDB13127	C4-OH carnitine	0.92 (0.82, 1.02)	0.1228	0.96 (0.75, 1.24)	0.7765
HMDB13130	C5-DC carnitine	0.9 (0.8, 1)	0.0474	1.05 (0.85, 1.31)	0.6318
HMDB13287	N6,N6-dimethyllysine	0.96 (0.87, 1.07)	0.4931	1.06 (0.85, 1.32)	0.6251
HMDB13288	C9 carnitine	0.94 (0.84, 1.04)	0.2230	1.16 (0.93, 1.45)	0.1993
HMDB13326	C12:1 carnitine	0.87 (0.78, 0.98)	0.0185	0.99 (0.77, 1.27)	0.9336
HMDB13331	C14:2 carnitine	0.87 (0.78, 0.97)	0.0154	0.97 (0.77, 1.23)	0.8060
HMDB13713	N-acetyltryptophan	1.03 (0.92, 1.15)	0.6534	1.05 (0.82, 1.35)	0.6995
HMDB13733	Trimethylbenzene	0.98 (0.88, 1.09)	0.7020	0.95 (0.75, 1.2)	0.6651
HMDB29377	Piperine	0.99 (0.9, 1.1)	0.8760	0.96 (0.75, 1.23)	0.7726
HMDB31106	C51:0 TAG	1.01 (0.9, 1.13)	0.8328	1.01 (0.8, 1.26)	0.9628
HMDB42076	C47:2 TAG	1.04 (0.93, 1.17)	0.4414	0.94 (0.75, 1.19)	0.6135
HMDB42100	C47:1 TAG	1.06 (0.95, 1.18)	0.2829	0.9 (0.72, 1.12)	0.3352
HMDB42103	C49:3 TAG	1.05 (0.94, 1.17)	0.4240	1.02 (0.8, 1.31)	0.8597
HMDB42104	C51:1 TAG	1.01 (0.9, 1.13)	0.8275	0.97 (0.78, 1.21)	0.7805
HMDB42226	C55:2 TAG	0.95 (0.85, 1.06)	0.3903	0.92 (0.73, 1.18)	0.5211

Supplementary Table 2.1a (Continued)

HMDB42466	C55:3 TAG	0.94 (0.85, 1.05)	0.2769	1.14 (0.9, 1.45)	0.2674
HMDB43058	C53:3 TAG	1 (0.9, 1.11)	0.9475	1.13 (0.88, 1.45)	0.3541
N/A	Valine-d8	0.94 (0.82, 1.08)	0.3928	0.87 (0.63, 1.19)	0.3777
N/A	N-methylproline	0.97 (0.88, 1.08)	0.6295	0.93 (0.71, 1.21)	0.5785
N/A	Ectoine	0.94 (0.85, 1.04)	0.2425	0.98 (0.79, 1.23)	0.8745
N/A	Phenylalanine-d8	0.94 (0.83, 1.05)	0.2686	0.85 (0.67, 1.08)	0.1884
N/A	3-(N-acetyl-L-cystein-S-yl) acetaminophen	0.94 (0.85, 1.05)	0.2872	0.96 (0.75, 1.24)	0.7597
N/A	NH4_C32:1 DAG	0.96 (0.86, 1.08)	0.5194	0.91 (0.72, 1.15)	0.4073
N/A	NH4_C34:3 DAG or TAG fragment	0.94 (0.84, 1.05)	0.2531	1.02 (0.8, 1.29)	0.9018
N/A	NH4_C34:3 DAG	0.95 (0.85, 1.06)	0.3802	0.96 (0.76, 1.22)	0.7589
N/A	NH4_C34:2 DAG or TAG fragment	0.93 (0.83, 1.04)	0.1764	0.96 (0.75, 1.22)	0.7166
N/A	NH4_C34:2 DAG	0.94 (0.84, 1.06)	0.3050	0.93 (0.73, 1.18)	0.5386
N/A	NH4_C34:1 DAG	0.94 (0.84, 1.05)	0.2792	0.88 (0.69, 1.11)	0.2698
N/A	NH4_C14:0 CE	0.96 (0.87, 1.06)	0.4178	0.91 (0.74, 1.11)	0.3562
N/A	NH4_C36:4 DAG or TAG fragment	0.93 (0.83, 1.04)	0.1963	1.02 (0.8, 1.31)	0.8716
N/A	NH4_C36:4 DAG	0.95 (0.85, 1.06)	0.3209	0.95 (0.74, 1.22)	0.6750
N/A	NH4_C36:3 DAG or TAG fragment	0.93 (0.84, 1.04)	0.2292	1 (0.78, 1.28)	0.9872
N/A	NH4_C36:3 DAG	0.94 (0.84, 1.05)	0.2513	0.92 (0.72, 1.18)	0.5243
N/A	NH4_C36:2 DAG or TAG fragment	0.94 (0.84, 1.05)	0.2514	0.93 (0.73, 1.17)	0.5216
N/A	NH4_C36:2 DAG	0.94 (0.84, 1.05)	0.2876	0.89 (0.7, 1.12)	0.3220
N/A	NH4_C16:1 CE	0.99 (0.89, 1.1)	0.8233	1.04 (0.83, 1.3)	0.7150
N/A	NH4_C16:0 CE	0.99 (0.88, 1.1)	0.8018	1.21 (0.92, 1.6)	0.1746
N/A	NH4_C38:5 DAG or TAG fragment	0.91 (0.82, 1)	0.0607	0.89 (0.7, 1.14)	0.3666
N/A	NH4_C38:5 DAG	0.92 (0.82, 1.02)	0.1066	0.8 (0.62, 1.03)	0.0851
N/A	NH4_C18:3 CE	1 (0.9, 1.12)	0.9926	0.98 (0.74, 1.28)	0.8610
N/A	NH4_C18:2 CE	1.04 (0.93, 1.15)	0.4928	1.1 (0.88, 1.39)	0.4004
N/A	NH4_C18:1 CE	1.03 (0.92, 1.14)	0.6480	1.03 (0.8, 1.32)	0.8075
N/A	NH4_C18:0 CE	0.99 (0.89, 1.1)	0.8987	1 (0.79, 1.27)	0.9765

Supplementary Table 2.1a (Continued)

N/A	NH4_C20:5 CE	0.98 (0.88, 1.09)	0.6600	0.99 (0.78, 1.26)	0.9493
N/A	NH4_C20:4 CE	0.98 (0.88, 1.09)	0.6637	1.01 (0.79, 1.3)	0.9219
N/A	NH4_C20:3 CE	1.02 (0.91, 1.13)	0.7518	1.05 (0.81, 1.37)	0.7006
N/A	C16:1 SM	0.91 (0.82, 1.02)	0.1048	0.88 (0.68, 1.15)	0.3459
N/A	NH4_C22:6 CE	1.05 (0.94, 1.17)	0.3544	1.25 (0.96, 1.61)	0.0949
N/A	NH4_C22:5 CE	0.97 (0.86, 1.08)	0.5322	0.97 (0.75, 1.24)	0.7826
N/A	NH4_C22:4 CE	0.98 (0.87, 1.09)	0.6607	1.13 (0.89, 1.44)	0.3161
N/A	NH4_C44:2 TAG or TAG fragment	0.99 (0.89, 1.11)	0.9251	0.83 (0.64, 1.06)	0.1316
N/A	NH4_C44:1 TAG or TAG fragment	1.02 (0.91, 1.13)	0.7474	0.84 (0.66, 1.07)	0.1515
N/A	C36:3 PS plasmalogen	1.05 (0.94, 1.17)	0.3602	1.13 (0.89, 1.43)	0.3292
N/A	C36:2 PS plasmalogen	1 (0.89, 1.11)	0.9557	1.09 (0.87, 1.37)	0.4483
N/A	C36:2 PS plasmalogen	0.99 (0.89, 1.1)	0.8516	1.09 (0.85, 1.39)	0.4934
N/A	C36:1 PS plasmalogen	1.02 (0.91, 1.14)	0.7517	0.99 (0.76, 1.29)	0.9331
N/A	NH4_C46:3 TAG or TAG fragment	0.98 (0.87, 1.09)	0.6567	0.83 (0.65, 1.07)	0.1424
N/A	NH4_C46:2 TAG or TAG fragment	1 (0.89, 1.11)	0.9404	0.85 (0.67, 1.08)	0.1856
N/A	C46:3 TAG	0.98 (0.87, 1.09)	0.6741	0.85 (0.66, 1.08)	0.1861
N/A	NH4_C47:0 TAG	1.01 (0.9, 1.12)	0.8938	0.88 (0.71, 1.1)	0.2546
N/A	C48:5 TAG	0.93 (0.84, 1.04)	0.2243	0.85 (0.67, 1.08)	0.1824
N/A	C44:13 PE plasmalogen	0.99 (0.89, 1.1)	0.8920	0.92 (0.72, 1.17)	0.4842
N/A	C48:4 TAG	0.96 (0.86, 1.07)	0.4616	0.88 (0.69, 1.13)	0.3249
N/A	NH4_C48:1 TAG	0.99 (0.89, 1.11)	0.9133	0.88 (0.7, 1.1)	0.2680
N/A	NH4_C48:0 TAG	1 (0.9, 1.12)	0.9329	0.89 (0.71, 1.11)	0.2937
N/A	NH4_C49:2 TAG	1.01 (0.9, 1.13)	0.8777	0.96 (0.76, 1.21)	0.7245
N/A	NH4_C49:1 TAG	1.02 (0.91, 1.14)	0.7725	0.93 (0.74, 1.17)	0.5197
N/A	NH4_C49:0 TAG	1 (0.9, 1.12)	0.9646	0.9 (0.72, 1.11)	0.3211
N/A	NH4_C50:3 TAG	0.97 (0.87, 1.09)	0.6313	0.95 (0.75, 1.21)	0.6806
N/A	NH4_C50:2 TAG	0.98 (0.87, 1.09)	0.6693	0.91 (0.73, 1.15)	0.4420
N/A	NH4_C50:1 TAG	0.98 (0.88, 1.1)	0.7742	0.88 (0.7, 1.1)	0.2639
N/A	NH4_C50:0 TAG	0.99 (0.88, 1.1)	0.8322	0.86 (0.69, 1.08)	0.1923
N/A	NH4_C51:3 TAG	1 (0.89, 1.11)	0.9374	0.99 (0.77, 1.26)	0.9066
N/A	NH4_C51:1 TAG	1 (0.89, 1.12)	0.9806	0.92 (0.73, 1.15)	0.4617

Supplementary Table 2.1a (Continued)

N/A	NH4_C52:4 TAG	0.95 (0.85, 1.06)	0.3715	0.97 (0.76, 1.24)	0.8090
N/A	NH4_C52:3 TAG	0.96 (0.86, 1.07)	0.4706	0.94 (0.74, 1.2)	0.6181
N/A	NH4_C52:2 TAG	0.96 (0.86, 1.07)	0.4767	0.89 (0.7, 1.12)	0.3213
N/A	NH4_C53:3 TAG	0.99 (0.89, 1.11)	0.9153	0.99 (0.78, 1.27)	0.9617
N/A	NH4_C53:2 TAG	0.98 (0.88, 1.1)	0.7446	0.93 (0.73, 1.18)	0.5556
N/A	NH4_C54:4 TAG	0.95 (0.85, 1.06)	0.3571	0.93 (0.73, 1.19)	0.5722
N/A	NH4_C54:3 TAG	0.95 (0.85, 1.06)	0.3749	0.89 (0.7, 1.13)	0.3506
N/A	NH4_C54:2 TAG	0.95 (0.85, 1.06)	0.3599	0.89 (0.7, 1.12)	0.3079
N/A	NH4_C56:8 TAG	0.96 (0.86, 1.07)	0.4260	0.99 (0.77, 1.28)	0.9540
N/A	NH4_C56:7 TAG	0.95 (0.85, 1.05)	0.3065	0.94 (0.74, 1.2)	0.6271
N/A	NH4_C56:5 TAG	0.91 (0.81, 1.01)	0.0697	0.84 (0.65, 1.07)	0.1500
N/A	NH4_C58:9 TAG	0.98 (0.88, 1.09)	0.6838	1.03 (0.8, 1.33)	0.8091
N/A	NH4_C58:6 TAG	0.93 (0.84, 1.03)	0.1665	0.91 (0.72, 1.15)	0.4251

Supplementary Table 2.1b: 60-179 and ≥180 month interval category specific RR per SD and unadjusted p-value for each metabolite

		60-179 months		≥180 months	
HMDB ID	Metabolite	RR (95% CI)	p	RR (95% CI)	p
HMDB00008	Alpha-hydroxybutyrate	0.95 (0.81, 1.11)	0.5007	1 (0.83, 1.21)	0.9654
HMDB00011	Beta-hydroxybutyrate	1.06 (0.9, 1.24)	0.5196	0.92 (0.75, 1.12)	0.3852
HMDB00026	N-carbamoyl-beta-alanine	0.99 (0.86, 1.15)	0.9384	0.9 (0.73, 1.11)	0.3113
HMDB00062	Carnitine	0.87 (0.73, 1.02)	0.0927	0.96 (0.78, 1.18)	0.6880
HMDB00063	Cortisol	1.03 (0.87, 1.23)	0.7132	0.96 (0.82, 1.13)	0.6406
HMDB00064	Creatine	1.03 (0.88, 1.21)	0.6955	1.01 (0.84, 1.22)	0.9012
HMDB00067	Cholesterol	0.96 (0.83, 1.12)	0.5997	0.99 (0.82, 1.21)	0.9602
HMDB00086	Alpha-glycerophosphocholine	0.91 (0.79, 1.05)	0.2145	1.01 (0.84, 1.21)	0.9405
HMDB00092	Dimethylglycine	0.92 (0.78, 1.08)	0.3011	0.94 (0.77, 1.14)	0.5319
HMDB00112	GABA	1.02 (0.88, 1.19)	0.7748	0.95 (0.79, 1.16)	0.6348
HMDB00122	Fructose/glucose/galactose	0.96 (0.84, 1.11)	0.5852	0.96 (0.8, 1.16)	0.6907
HMDB00123	Glycine	0.97 (0.83, 1.13)	0.6929	0.99 (0.82, 1.19)	0.9268
HMDB00138	Glycocholate	1.07 (0.91, 1.25)	0.3968	1.05 (0.86, 1.27)	0.6507
HMDB00148	Glutamate	0.85 (0.73, 0.99)	0.0425	1.04 (0.86, 1.26)	0.6831
HMDB00157	Hypoxanthine	1.02 (0.87, 1.2)	0.7916	0.96 (0.79, 1.17)	0.7022
HMDB00158	Tyrosine	0.92 (0.78, 1.07)	0.2693	1 (0.82, 1.22)	0.9985
HMDB00159	Phenylalanine	0.86 (0.73, 1.01)	0.0623	1.08 (0.9, 1.3)	0.4252
HMDB00161	Alanine	0.91 (0.78, 1.07)	0.2738	1.25 (1.03, 1.52)	0.0269
HMDB00167	Threonine	1.05 (0.9, 1.22)	0.5258	1.08 (0.9, 1.3)	0.3890
HMDB00168	Asparagine	1 (0.85, 1.17)	0.9873	1.08 (0.89, 1.29)	0.4344
HMDB00172	Isoleucine	0.96 (0.83, 1.12)	0.6271	0.92 (0.76, 1.12)	0.3929
HMDB00177	Histidine	1.01 (0.87, 1.17)	0.8889	1.04 (0.85, 1.29)	0.6833
HMDB00182	Lysine	1.01 (0.87, 1.18)	0.8719	1.11 (0.92, 1.33)	0.2795
HMDB00186	Lactose	0.82 (0.69, 0.98)	0.0291	1.17 (0.96, 1.42)	0.1210
HMDB00187	Serine	1.07 (0.92, 1.24)	0.3876	0.97 (0.81, 1.16)	0.7297
HMDB00190	Lactate	0.85 (0.72, 0.99)	0.0390	0.98 (0.81, 1.2)	0.8687
HMDB00195	Inosine	1.04 (0.9, 1.21)	0.5526	0.94 (0.78, 1.14)	0.5245
HMDB00201	C2 carnitine	0.88 (0.75, 1.02)	0.0898	0.87 (0.73, 1.04)	0.1334
HMDB00206	N6-acetyllysine	0.79 (0.66, 0.94)	0.0075	1.04 (0.87, 1.25)	0.6758
HMDB00210	Pantothenate	1.02 (0.87, 1.19)	0.8023	0.91 (0.75, 1.1)	0.3160
HMDB00214	Ornithine	1 (0.86, 1.17)	0.9713	0.99 (0.81, 1.21)	0.9164

Supplementary Table 2.1b (Continued)

HMDB00222	C16 carnitine	0.84 (0.73, 0.98)	0.0288	0.92 (0.76, 1.11)	0.3672
HMDB00235	Thiamine	0.98 (0.83, 1.14)	0.7679	1.01 (0.83, 1.22)	0.9401
HMDB00247	Sorbitol	0.99 (0.84, 1.17)	0.8852	0.99 (0.82, 1.2)	0.9341
HMDB00251	Taurine	0.93 (0.81, 1.07)	0.3278	1.12 (0.93, 1.35)	0.2362
HMDB00258	Sucrose	0.82 (0.69, 0.97)	0.0227	1.11 (0.91, 1.34)	0.3033
HMDB00269	Sphinganine	0.86 (0.74, 1.01)	0.0613	1.09 (0.88, 1.36)	0.4428
HMDB00289	Urate	0.91 (0.77, 1.07)	0.2466	0.95 (0.78, 1.17)	0.6301
HMDB00292	Xanthine	0.93 (0.8, 1.09)	0.3848	1.05 (0.86, 1.28)	0.6429
HMDB00296	Uridine	0.91 (0.77, 1.08)	0.2621	0.91 (0.73, 1.13)	0.3794
HMDB00517	Arginine	1.04 (0.9, 1.2)	0.6045	1.16 (0.95, 1.4)	0.1449
HMDB00610	C18:2 CE	1.09 (0.94, 1.27)	0.2693	0.97 (0.8, 1.19)	0.7783
HMDB00630	Cytosine	0.9 (0.77, 1.06)	0.2006	0.83 (0.67, 1.01)	0.0647
HMDB00631	Glycodeoxycholate/ glycochenodeoxycholate	1.17 (0.99, 1.37)	0.0661	1.02 (0.85, 1.24)	0.7962
HMDB00641	Glutamine	0.99 (0.85, 1.15)	0.8638	0.94 (0.77, 1.14)	0.5076
HMDB00651	C10 carnitine	0.86 (0.73, 1.01)	0.0616	0.9 (0.73, 1.1)	0.3052
HMDB00658	C16:1 CE	1.06 (0.91, 1.23)	0.4646	0.97 (0.8, 1.16)	0.7106
HMDB00670	Homoarginine	1.11 (0.95, 1.29)	0.1907	1.19 (0.99, 1.44)	0.0667
HMDB00679	Homocitrulline	0.86 (0.73, 1)	0.0522	1.26 (1.04, 1.54)	0.0195
HMDB00682	Indoxylsulfate	1.02 (0.87, 1.2)	0.7914	1.09 (0.9, 1.31)	0.3753
HMDB00684	Kynurenine	0.86 (0.72, 1.02)	0.0758	0.97 (0.78, 1.19)	0.7380
HMDB00687	Leucine	0.93 (0.8, 1.09)	0.3848	0.9 (0.75, 1.1)	0.3131
HMDB00688	C5 carnitine	0.97 (0.84, 1.13)	0.7238	1.07 (0.89, 1.3)	0.4713
HMDB00696	Methionine	1.05 (0.9, 1.22)	0.5436	1.05 (0.87, 1.28)	0.5965
HMDB00699	1-methylnicotinamide	1.07 (0.93, 1.24)	0.3424	1.02 (0.85, 1.21)	0.8593
HMDB00705	C6 carnitine	0.86 (0.74, 1)	0.0515	0.94 (0.77, 1.16)	0.5789
HMDB00716	Pipecolic acid	0.98 (0.84, 1.15)	0.8415	1.05 (0.87, 1.26)	0.6202
HMDB00725	Hydroxyproline	0.92 (0.79, 1.06)	0.2488	1 (0.83, 1.2)	0.9835
HMDB00767	Pseudouridine	0.79 (0.67, 0.93)	0.0043	1.06 (0.84, 1.32)	0.6431
HMDB00791	C8 carnitine	0.85 (0.73, 1)	0.0445	0.92 (0.74, 1.13)	0.4017
HMDB00824	C3 carnitine	0.88 (0.76, 1.03)	0.1111	1 (0.82, 1.21)	0.9934
HMDB00848	C18 carnitine	0.93 (0.8, 1.08)	0.3384	0.92 (0.76, 1.11)	0.3846
HMDB00853	Acetyl-galactosamine	0.8 (0.66, 0.96)	0.0195	1.08 (0.88, 1.32)	0.4777
HMDB00875	Trigonelline	0.94 (0.81, 1.11)	0.4827	1.11 (0.91, 1.35)	0.2981
HMDB00883	Valine	0.93 (0.8, 1.09)	0.3940	0.92 (0.76, 1.12)	0.4082

Supplementary Table 2.1b (Continued)

HMDB00884	Ribothymidine	1.02 (0.87, 1.2)	0.7807	0.93 (0.74, 1.17)	0.5328
HMDB00885	C16:0 CE	0.97 (0.83, 1.13)	0.6996	0.94 (0.76, 1.17)	0.5895
HMDB00896	Taurodeoxycholate/ taurochenodeoxycholate	0.87 (0.74, 1.02)	0.0950	0.94 (0.74, 1.19)	0.6232
HMDB00897	7-methylguanine	0.86 (0.72, 1.02)	0.0785	0.95 (0.78, 1.17)	0.6471
HMDB00904	Citrulline	0.97 (0.81, 1.15)	0.7047	1.03 (0.86, 1.23)	0.7443
HMDB00918	C18:1 CE	1.05 (0.9, 1.23)	0.5242	1.01 (0.83, 1.23)	0.9501
HMDB00925	Trimethylamine-N-oxide	0.85 (0.72, 0.99)	0.0423	1.11 (0.93, 1.32)	0.2390
HMDB00929	Tryptophan	1.07 (0.92, 1.24)	0.3922	1.05 (0.87, 1.26)	0.6028
HMDB00982	5-methylcytidine	0.89 (0.76, 1.05)	0.1564	0.81 (0.66, 1)	0.0527
HMDB01008	Biliverdin	1.02 (0.87, 1.2)	0.8182	1.01 (0.84, 1.21)	0.9456
HMDB01046	Cotinine	0.84 (0.71, 1)	0.0563	1.01 (0.83, 1.24)	0.8966
HMDB01276	N1-acetylspermidine	0.85 (0.73, 0.98)	0.0294	0.97 (0.79, 1.19)	0.7533
HMDB01325	N6,N6,N6-trimethyllysine	0.91 (0.78, 1.07)	0.2727	1.15 (0.96, 1.36)	0.1267
HMDB01348	C18:0 SM	0.93 (0.79, 1.08)	0.3193	0.93 (0.77, 1.12)	0.4434
HMDB01390	Hydroxycotinine	0.92 (0.78, 1.09)	0.3513	0.98 (0.8, 1.21)	0.8645
HMDB01539	ADMA	0.89 (0.76, 1.05)	0.1588	0.9 (0.74, 1.1)	0.2915
HMDB01548	Pentose monophosphate	1.05 (0.9, 1.22)	0.5704	0.98 (0.81, 1.18)	0.7978
HMDB01563	1-methylguanosine	0.83 (0.71, 0.97)	0.0167	1.07 (0.86, 1.34)	0.5278
HMDB01847	Caffeine	1.04 (0.89, 1.21)	0.6556	1.12 (0.92, 1.36)	0.2750
HMDB01886	3-methylxanthine	1.02 (0.88, 1.18)	0.7639	1.06 (0.88, 1.28)	0.5498
HMDB01906	2-aminoisobutyric acid	0.91 (0.77, 1.08)	0.2740	0.96 (0.79, 1.16)	0.6849
HMDB02005	Methionine sulfoxide	0.96 (0.81, 1.14)	0.6524	0.98 (0.78, 1.23)	0.8517
HMDB02013	C4 carnitine	0.96 (0.82, 1.11)	0.5445	0.93 (0.78, 1.12)	0.4575
HMDB02014	C14:1 carnitine	0.83 (0.71, 0.97)	0.0166	0.91 (0.75, 1.11)	0.3467
HMDB02250	C12 carnitine	0.82 (0.7, 0.96)	0.0161	0.89 (0.73, 1.09)	0.2456
HMDB02366	C5:1 carnitine	0.98 (0.84, 1.13)	0.7426	0.82 (0.67, 1)	0.0458
HMDB0240212	DMGV	0.88 (0.75, 1.04)	0.1236	1.02 (0.84, 1.24)	0.8412
HMDB02815	C18:1 LPC	0.97 (0.85, 1.12)	0.7230	0.94 (0.79, 1.12)	0.5220
HMDB03282	1-methylguanine	0.98 (0.83, 1.16)	0.8347	1.18 (0.94, 1.47)	0.1510
HMDB03331	1-methyladenosine	0.85 (0.72, 1.01)	0.0671	1.06 (0.88, 1.28)	0.5440
HMDB03334	SDMA	0.84 (0.72, 0.98)	0.0301	0.96 (0.78, 1.17)	0.6583
HMDB03357	N-acetylornithine	0.97 (0.83, 1.15)	0.7503	1.03 (0.85, 1.23)	0.7833
HMDB03681	4-acetamidobutanoate	0.85 (0.72, 1)	0.0554	1.11 (0.9, 1.35)	0.3252
HMDB04400	5-acetylamino-6-amino-3-methyluracil	1.02 (0.87, 1.18)	0.8405	1.08 (0.89, 1.31)	0.4365

Supplementary Table 2.1b (Continued)

HMDB04824	N2,N2-dimethylguanosine	0.76 (0.63, 0.91)	0.0026	1.06 (0.84, 1.33)	0.6283
HMDB04827	Proline-betaine	0.95 (0.82, 1.11)	0.5200	1.04 (0.87, 1.25)	0.6548
HMDB04949	C16:0 Ceramide (d18:1)	0.91 (0.79, 1.06)	0.2462	0.9 (0.75, 1.07)	0.2338
HMDB04952	C22:0 Ceramide (d18:1)	0.96 (0.83, 1.11)	0.5827	1.03 (0.86, 1.24)	0.7614
HMDB04953	C24:1 Ceramide (d18:1)	0.89 (0.76, 1.03)	0.1278	0.95 (0.8, 1.14)	0.5985
HMDB04956	C24:0 Ceramide (d18:1)	0.97 (0.84, 1.12)	0.6760	1.03 (0.86, 1.24)	0.7280
HMDB05065	C18:1 carnitine	0.91 (0.78, 1.05)	0.2093	0.92 (0.76, 1.1)	0.3503
HMDB05066	C14 carnitine	0.79 (0.68, 0.93)	0.0035	0.91 (0.75, 1.1)	0.3266
HMDB05356	C48:0 TAG	1.14 (0.98, 1.33)	0.0923	0.99 (0.82, 1.2)	0.9328
HMDB05357	C50:0 TAG	1.11 (0.94, 1.3)	0.2133	0.97 (0.8, 1.17)	0.7290
HMDB05359	C48:1 TAG	1.09 (0.93, 1.28)	0.2655	1.02 (0.84, 1.24)	0.8369
HMDB05360	C50:1 TAG	1.07 (0.9, 1.26)	0.4501	1.01 (0.83, 1.22)	0.9293
HMDB05362	C51:2 TAG	1.04 (0.89, 1.21)	0.6016	1.13 (0.94, 1.36)	0.2101
HMDB05363	C52:4 TAG	0.93 (0.8, 1.09)	0.3745	1.09 (0.89, 1.32)	0.4137
HMDB05367	C52:1 TAG	1.03 (0.88, 1.21)	0.7157	0.99 (0.82, 1.2)	0.9184
HMDB05369	C52:2 TAG	0.93 (0.8, 1.1)	0.4061	1.05 (0.87, 1.26)	0.6364
HMDB05370	C54:4 TAG	0.95 (0.82, 1.11)	0.5335	1 (0.83, 1.22)	0.9607
HMDB05376	C48:2 TAG	1.08 (0.93, 1.26)	0.3243	1.03 (0.85, 1.25)	0.7288
HMDB05377	C50:2 TAG	0.98 (0.84, 1.15)	0.8206	1.03 (0.85, 1.24)	0.7841
HMDB05384	C52:3 TAG	0.94 (0.8, 1.1)	0.4513	1.05 (0.87, 1.27)	0.5927
HMDB05385	C54:5 TAG	0.92 (0.8, 1.06)	0.2578	0.98 (0.8, 1.18)	0.8090
HMDB05391	C54:6 TAG	0.9 (0.78, 1.05)	0.1806	1.01 (0.84, 1.23)	0.9070
HMDB05392	C56:8 TAG	0.88 (0.75, 1.03)	0.1237	1.11 (0.92, 1.34)	0.2920
HMDB05395	C54:1 TAG	1.04 (0.89, 1.21)	0.6145	0.95 (0.78, 1.14)	0.5592
HMDB05403	C54:2 TAG	0.97 (0.83, 1.13)	0.6779	0.98 (0.81, 1.18)	0.8098
HMDB05404	C56:2 TAG	0.99 (0.85, 1.16)	0.9153	0.95 (0.79, 1.15)	0.6250
HMDB05405	C54:3 TAG	0.96 (0.82, 1.12)	0.5898	0.99 (0.82, 1.19)	0.9228
HMDB05406	C56:5 TAG	0.82 (0.7, 0.96)	0.0128	1.05 (0.87, 1.26)	0.6219
HMDB05410	C56:3 TAG	0.93 (0.79, 1.08)	0.3243	0.99 (0.82, 1.2)	0.9567
HMDB05432	C48:3 TAG	1.02 (0.87, 1.2)	0.8030	1.02 (0.85, 1.23)	0.8214
HMDB05433	C50:3 TAG	0.99 (0.84, 1.15)	0.8604	1.07 (0.88, 1.3)	0.4917
HMDB05436	C52:6 TAG	0.86 (0.75, 1)	0.0535	1.07 (0.89, 1.29)	0.4675
HMDB05447	C54:7 TAG	0.87 (0.76, 1.01)	0.0666	1.03 (0.86, 1.24)	0.7393
HMDB05448	C56:9 TAG	0.9 (0.77, 1.04)	0.1602	1.08 (0.9, 1.3)	0.4111
HMDB05456	C56:6 TAG	0.88 (0.75, 1.03)	0.1233	1.05 (0.88, 1.27)	0.5873

Supplementary Table 2.1b (Continued)

HMDB05458	C58:6 TAG	0.87 (0.75, 1.02)	0.0851	1.02 (0.85, 1.23)	0.7943
HMDB05462	C56:7 TAG	0.87 (0.74, 1.02)	0.0848	1.12 (0.93, 1.35)	0.2427
HMDB05463	C58:9 TAG	0.94 (0.8, 1.1)	0.4154	1.11 (0.92, 1.34)	0.2858
HMDB05471	C58:7 TAG	0.89 (0.75, 1.04)	0.1411	1.11 (0.92, 1.34)	0.2715
HMDB05476	C58:10 TAG	0.93 (0.79, 1.08)	0.3375	1.08 (0.89, 1.31)	0.4215
HMDB05478	C60:12 TAG	0.97 (0.83, 1.15)	0.7449	1.07 (0.89, 1.29)	0.4680
HMDB05923	N4-acetylcytidine	0.91 (0.77, 1.06)	0.2164	1.15 (0.94, 1.41)	0.1843
HMDB06344	Phenylacetylglutamine	1.11 (0.94, 1.31)	0.2201	1.08 (0.91, 1.29)	0.3689
HMDB06347	C26 carnitine	0.97 (0.83, 1.14)	0.7355	1.01 (0.85, 1.21)	0.9147
HMDB06469	C18:2 carnitine	0.87 (0.75, 1.02)	0.0804	0.95 (0.79, 1.14)	0.5970
HMDB06725	C14:0 CE	1.07 (0.93, 1.24)	0.3424	0.97 (0.8, 1.16)	0.7242
HMDB06726	C20:4 CE	1 (0.86, 1.16)	0.9687	1.02 (0.83, 1.26)	0.8406
HMDB06731	C20:5 CE	0.97 (0.83, 1.12)	0.6511	1.04 (0.85, 1.27)	0.6985
HMDB06733	C22:6 CE	1 (0.86, 1.17)	0.9906	1.08 (0.89, 1.31)	0.4204
HMDB06736	C20:3 CE	1 (0.86, 1.16)	0.9912	1.12 (0.91, 1.37)	0.2830
HMDB06831	Butyrobetaine	0.99 (0.84, 1.17)	0.9178	0.94 (0.81, 1.1)	0.4444
HMDB07099	C32:1 DAG	0.98 (0.83, 1.15)	0.7823	0.99 (0.82, 1.21)	0.9533
HMDB07102	C34:1 DAG	0.94 (0.8, 1.1)	0.4339	0.98 (0.8, 1.19)	0.8193
HMDB07103	C34:2 DAG	0.92 (0.79, 1.08)	0.3097	1 (0.82, 1.21)	0.9926
HMDB07132	C34:3 DAG	0.92 (0.78, 1.08)	0.2970	1.11 (0.9, 1.36)	0.3295
HMDB07170	C38:4 DAG	0.92 (0.8, 1.06)	0.2409	1.03 (0.85, 1.24)	0.7806
HMDB07199	C38:5 DAG	0.93 (0.8, 1.08)	0.3266	0.99 (0.82, 1.2)	0.9516
HMDB07218	C36:2 DAG	0.91 (0.78, 1.06)	0.2330	1.05 (0.86, 1.27)	0.6496
HMDB07219	C36:3 DAG	0.89 (0.76, 1.04)	0.1471	1.05 (0.86, 1.27)	0.6269
HMDB07248	C36:4 DAG	0.89 (0.76, 1.03)	0.1242	1.04 (0.85, 1.27)	0.6941
HMDB07448	C38:3 DAG	0.88 (0.76, 1.02)	0.0964	1.09 (0.9, 1.31)	0.3948
HMDB07869	C30:0 PC	1.02 (0.88, 1.19)	0.7619	0.96 (0.8, 1.15)	0.6224
HMDB07870	C30:1 PC	1.07 (0.92, 1.24)	0.3833	0.98 (0.82, 1.18)	0.8614
HMDB07871	C32:0 PC	0.96 (0.83, 1.11)	0.5511	0.93 (0.77, 1.12)	0.4356
HMDB07873	C32:1 PC	1.04 (0.9, 1.21)	0.5898	0.97 (0.8, 1.17)	0.7372
HMDB07874	C32:2 PC	1.04 (0.89, 1.21)	0.6142	1.02 (0.85, 1.21)	0.8358
HMDB07883	C34:4 PC	0.94 (0.81, 1.09)	0.4246	1.03 (0.86, 1.23)	0.7541
HMDB07970	C34:0 PC	0.88 (0.76, 1.03)	0.1091	0.94 (0.78, 1.14)	0.5495
HMDB07972	C34:1 PC	1.02 (0.88, 1.19)	0.7850	1.03 (0.85, 1.25)	0.7361
HMDB07973	C34:2 PC	1.1 (0.95, 1.28)	0.2063	0.96 (0.8, 1.15)	0.6554

Supplementary Table 2.1b (Continued)

HMDB07983	C36:4 PC-A	1.09 (0.93, 1.28)	0.2686	0.94 (0.79, 1.12)	0.4734
HMDB07991	C38:6 PC	0.94 (0.81, 1.1)	0.4541	1.12 (0.93, 1.36)	0.2212
HMDB08006	C34:3 PC	1.02 (0.88, 1.19)	0.7803	0.98 (0.81, 1.18)	0.8389
HMDB08038	C36:1 PC	1.04 (0.89, 1.21)	0.6251	1.02 (0.84, 1.23)	0.8319
HMDB08039	C36:2 PC	1.07 (0.92, 1.24)	0.4036	0.98 (0.82, 1.17)	0.8210
HMDB08047	C38:3 PC	0.93 (0.81, 1.08)	0.3408	1.14 (0.93, 1.39)	0.2031
HMDB08048	C38:4 PC	0.92 (0.8, 1.06)	0.2692	1 (0.83, 1.2)	0.9650
HMDB08105	C36:3 PC	1.01 (0.87, 1.17)	0.9196	1 (0.83, 1.22)	0.9815
HMDB08138	C36:4 PC-B	0.96 (0.84, 1.11)	0.6136	0.99 (0.82, 1.19)	0.8802
HMDB08270	C38:2 PC	1.04 (0.9, 1.21)	0.5980	1.06 (0.88, 1.28)	0.5356
HMDB08511	C40:10 PC	0.95 (0.82, 1.11)	0.5431	1.07 (0.89, 1.3)	0.4689
HMDB08731	C40:9 PC	0.92 (0.79, 1.07)	0.2568	1.12 (0.93, 1.35)	0.2278
HMDB08925	C34:0 PE	1.02 (0.88, 1.18)	0.7873	0.99 (0.83, 1.19)	0.9151
HMDB08928	C34:2 PE	0.95 (0.82, 1.1)	0.5218	0.96 (0.8, 1.15)	0.6695
HMDB08937	C36:4 PE	0.9 (0.77, 1.05)	0.1691	0.97 (0.81, 1.16)	0.7722
HMDB08942	C38:2 PE	1.08 (0.93, 1.26)	0.3023	1.1 (0.92, 1.32)	0.3046
HMDB08952	C34:2 PE plasmalogen	1.17 (0.99, 1.38)	0.0614	0.93 (0.79, 1.1)	0.4011
HMDB08991	C36:0 PE	0.98 (0.85, 1.13)	0.7862	1.02 (0.85, 1.22)	0.8722
HMDB08993	C36:1 PE	0.97 (0.83, 1.13)	0.6647	1.07 (0.89, 1.29)	0.4498
HMDB08994	C36:2 PE	1 (0.86, 1.16)	0.9551	1 (0.83, 1.2)	0.9894
HMDB09003	C38:4 PE	0.87 (0.75, 1.01)	0.0736	1.01 (0.84, 1.22)	0.8862
HMDB09012	C40:6 PE	0.8 (0.68, 0.94)	0.0056	1.12 (0.93, 1.35)	0.2384
HMDB09060	C36:3 PE	1 (0.86, 1.16)	0.9676	0.96 (0.8, 1.15)	0.6760
HMDB09069	C38:5 PE	0.87 (0.75, 1.01)	0.0628	0.98 (0.82, 1.18)	0.8517
HMDB09082	C36:2 PE plasmalogen	1.16 (0.99, 1.36)	0.0601	0.96 (0.8, 1.14)	0.6207
HMDB09102	C38:6 PE	0.81 (0.69, 0.95)	0.0080	1.1 (0.92, 1.32)	0.3092
HMDB10169	C16:0 SM	0.98 (0.85, 1.14)	0.8386	0.88 (0.73, 1.07)	0.2019
HMDB10316	Acetaminophen glucuronide	0.86 (0.73, 1.02)	0.0762	1.07 (0.9, 1.27)	0.4395
HMDB10368	C18:0 CE	1.01 (0.88, 1.17)	0.8671	1.02 (0.84, 1.23)	0.8668
HMDB10370	C18:3 CE	1.1 (0.94, 1.28)	0.2431	0.96 (0.78, 1.17)	0.6593
HMDB10379	C14:0 LPC	0.98 (0.85, 1.14)	0.8155	0.99 (0.82, 1.2)	0.9499
HMDB10382	C16:0 LPC	0.94 (0.81, 1.08)	0.3876	0.99 (0.81, 1.2)	0.8911
HMDB10383	C16:1 LPC	0.97 (0.84, 1.11)	0.6265	1 (0.83, 1.2)	0.9955
HMDB10384	C18:0 LPC	0.93 (0.8, 1.08)	0.3411	0.99 (0.83, 1.19)	0.9472
HMDB10386	C18:2 LPC	1.08 (0.92, 1.26)	0.3571	0.89 (0.74, 1.07)	0.2057

Supplementary Table 2.1b (Continued)

HMDB10393	C18:3 LPC	0.97 (0.84, 1.13)	0.7175	0.97 (0.79, 1.18)	0.7557
HMDB10395	C20:4 LPC	0.94 (0.82, 1.09)	0.4265	0.92 (0.76, 1.12)	0.4168
HMDB10397	C20:5 LPC	1 (0.86, 1.16)	0.9851	0.92 (0.76, 1.1)	0.3557
HMDB10404	C22:6 LPC	0.96 (0.83, 1.11)	0.5570	1.02 (0.84, 1.23)	0.8754
HMDB10407	C16:1 LPC plasmalogen	0.96 (0.83, 1.12)	0.6061	0.97 (0.81, 1.17)	0.7630
HMDB10411	C46:0 TAG	1.16 (1, 1.35)	0.0526	0.99 (0.81, 1.2)	0.9015
HMDB10412	C46:1 TAG	1.12 (0.96, 1.31)	0.1544	0.99 (0.82, 1.19)	0.8835
HMDB10419	C46:2 TAG	1.07 (0.91, 1.25)	0.4207	0.98 (0.82, 1.18)	0.8596
HMDB10471	C50:5 TAG	0.9 (0.77, 1.05)	0.1874	1.04 (0.87, 1.26)	0.6448
HMDB10497	C50:6 TAG	0.9 (0.77, 1.05)	0.1939	1.04 (0.86, 1.24)	0.7011
HMDB10513	C56:10 TAG	0.9 (0.78, 1.05)	0.1823	1.09 (0.91, 1.31)	0.3494
HMDB10517	C52:7 TAG	0.87 (0.75, 1.01)	0.0706	1.08 (0.9, 1.3)	0.4113
HMDB10518	C54:8 TAG	0.88 (0.76, 1.03)	0.1044	1.08 (0.9, 1.3)	0.3846
HMDB10531	C58:11 TAG	0.93 (0.8, 1.08)	0.3512	1.06 (0.88, 1.27)	0.5567
HMDB11103	1,7-dimethyluric acid	0.96 (0.83, 1.12)	0.6329	1.05 (0.86, 1.28)	0.6211
HMDB11130	C18:0 LPE	0.9 (0.78, 1.05)	0.1758	0.99 (0.82, 1.19)	0.8754
HMDB11208	C34:1 PC plasmalogen-A	1.04 (0.9, 1.22)	0.5800	0.98 (0.81, 1.19)	0.8507
HMDB11210	C34:2 PC plasmalogen	1.07 (0.92, 1.26)	0.3685	1.01 (0.83, 1.22)	0.9573
HMDB11211	C34:3 PC plasmalogen	1.15 (0.99, 1.35)	0.0735	0.94 (0.77, 1.14)	0.5094
HMDB11220	C36:5 PC plasmalogen-B	1.06 (0.92, 1.24)	0.4148	0.91 (0.76, 1.1)	0.3364
HMDB11221	C36:5 PC plasmalogen-A	1.02 (0.88, 1.18)	0.7724	0.93 (0.77, 1.12)	0.4617
HMDB11229	C38:7 PC plasmalogen	1.06 (0.92, 1.23)	0.4034	0.98 (0.81, 1.18)	0.8216
HMDB11241	C36:1 PC plasmalogen	1.03 (0.89, 1.2)	0.6613	0.93 (0.77, 1.11)	0.4056
HMDB11243	C36:2 PC plasmalogen	1.1 (0.95, 1.29)	0.2070	0.92 (0.77, 1.1)	0.3511
HMDB11244	C36:3 PC plasmalogen-A	1.12 (0.95, 1.3)	0.1717	0.93 (0.78, 1.12)	0.4399
HMDB11252	C38:4 PC plasmalogen	1.06 (0.92, 1.22)	0.4440	0.89 (0.75, 1.07)	0.2244
HMDB11294	C40:7 PC plasmalogen	1.01 (0.87, 1.17)	0.9212	0.93 (0.77, 1.13)	0.4884
HMDB11310	C36:4 PC plasmalogen	1.02 (0.88, 1.18)	0.8255	0.88 (0.73, 1.05)	0.1645
HMDB11319	C38:6 PC plasmalogen	1.02 (0.88, 1.18)	0.7848	0.99 (0.82, 1.2)	0.9580
HMDB11343	C34:3 PE plasmalogen	1.24 (1.06, 1.46)	0.0090	0.86 (0.72, 1.03)	0.0954
HMDB11386	C38:5 PE plasmalogen	1.13 (0.97, 1.31)	0.1120	0.89 (0.75, 1.07)	0.2222
HMDB11387	C38:6 PE plasmalogen	1.09 (0.93, 1.27)	0.2693	0.9 (0.75, 1.06)	0.2101
HMDB11394	C40:7 PE plasmalogen	1.08 (0.93, 1.25)	0.3164	0.96 (0.79, 1.15)	0.6425
HMDB11410	C36:5 PE plasmalogen	1.21 (1.03, 1.41)	0.0196	0.88 (0.73, 1.05)	0.1440
HMDB11420	C38:7 PE plasmalogen	1.06 (0.92, 1.23)	0.4122	1.01 (0.84, 1.22)	0.8976

Supplementary Table 2.1b (Continued)

HMDB11441	C36:3 PE plasmalogen	1.21 (1.03, 1.43)	0.0179	0.86 (0.72, 1.03)	0.1014
HMDB11442	C36:4 PE plasmalogen	1.19 (1.02, 1.4)	0.0313	0.86 (0.72, 1.02)	0.0822
HMDB11503	C16:0 LPE	0.93 (0.8, 1.07)	0.3139	0.97 (0.81, 1.17)	0.7712
HMDB11506	C18:1 LPE	1.08 (0.92, 1.26)	0.3415	0.93 (0.78, 1.11)	0.4250
HMDB11507	C18:2 LPE	1.13 (0.96, 1.33)	0.1529	0.9 (0.76, 1.08)	0.2680
HMDB11511	C20:0 LPE	0.99 (0.85, 1.14)	0.8452	1.06 (0.88, 1.28)	0.5538
HMDB11517	C20:4 LPE	0.96 (0.83, 1.11)	0.5884	0.95 (0.79, 1.14)	0.5839
HMDB11526	C22:6 LPE	0.89 (0.77, 1.04)	0.1512	1.06 (0.87, 1.3)	0.5482
HMDB11697	C24:0 SM	1 (0.86, 1.16)	0.9798	1.08 (0.91, 1.3)	0.3769
HMDB11701	C51:3 TAG	0.95 (0.82, 1.11)	0.5262	1.19 (0.97, 1.45)	0.0981
HMDB11706	C49:2 TAG	1.11 (0.95, 1.29)	0.1754	1.1 (0.9, 1.34)	0.3568
HMDB12097	C14:0 SM	1.01 (0.88, 1.17)	0.8527	0.93 (0.78, 1.12)	0.4596
HMDB12101	C18:1 SM	0.89 (0.76, 1.03)	0.1219	0.92 (0.76, 1.1)	0.3362
HMDB12102	C20:0 SM	0.99 (0.86, 1.15)	0.8981	1.08 (0.9, 1.3)	0.3824
HMDB12103	C22:0 SM	1 (0.86, 1.15)	0.9688	1.07 (0.9, 1.28)	0.4325
HMDB12104	C22:1 SM	0.99 (0.86, 1.14)	0.8721	1.01 (0.84, 1.22)	0.9352
HMDB12107	C24:1 SM	1.01 (0.87, 1.17)	0.9122	0.93 (0.76, 1.14)	0.4879
HMDB12356	C34:0 PS	0.97 (0.84, 1.12)	0.6744	0.96 (0.79, 1.16)	0.6725
HMDB13122	C18:1 LPC plasmalogen A	0.9 (0.77, 1.04)	0.1549	0.98 (0.82, 1.17)	0.8113
HMDB13127	C4-OH carnitine	0.92 (0.78, 1.07)	0.2732	0.9 (0.74, 1.08)	0.2477
HMDB13130	C5-DC carnitine	0.76 (0.64, 0.89)	0.0009	1.03 (0.83, 1.27)	0.7873
HMDB13287	N6,N6-dimethyllysine	0.96 (0.82, 1.12)	0.6230	0.91 (0.76, 1.09)	0.3071
HMDB13288	C9 carnitine	0.83 (0.71, 0.97)	0.0181	0.96 (0.8, 1.16)	0.6927
HMDB13326	C12:1 carnitine	0.85 (0.73, 0.99)	0.0424	0.85 (0.69, 1.03)	0.1023
HMDB13331	C14:2 carnitine	0.82 (0.7, 0.95)	0.0108	0.9 (0.74, 1.1)	0.3189
HMDB13713	N-acetyltryptophan	0.93 (0.79, 1.1)	0.4006	1.16 (0.95, 1.42)	0.1405
HMDB13733	Trimethylbenzene	1.05 (0.9, 1.23)	0.5246	0.9 (0.74, 1.09)	0.2710
HMDB29377	Piperine	1.11 (0.96, 1.28)	0.1691	0.87 (0.74, 1.03)	0.1092
HMDB31106	C51:0 TAG	1.01 (0.86, 1.19)	0.9128	1.02 (0.84, 1.24)	0.8284
HMDB42076	C47:2 TAG	1.09 (0.93, 1.28)	0.2730	1.05 (0.86, 1.28)	0.6473
HMDB42100	C47:1 TAG	1.16 (0.99, 1.35)	0.0645	1.05 (0.86, 1.29)	0.6208
HMDB42103	C49:3 TAG	1.02 (0.88, 1.19)	0.7828	1.11 (0.91, 1.36)	0.3112
HMDB42104	C51:1 TAG	1.01 (0.86, 1.19)	0.8800	1.05 (0.86, 1.28)	0.6322
HMDB42226	C55:2 TAG	0.93 (0.8, 1.09)	0.3816	1 (0.83, 1.22)	0.9723
HMDB42466	C55:3 TAG	0.82 (0.7, 0.95)	0.0082	1.06 (0.88, 1.28)	0.5433

Supplementary Table 2.1b (Continued)

HMDB43058	C53:3 TAG	0.9 (0.78, 1.03)	0.1245	1.17 (0.96, 1.42)	0.1094
N/A	Valine-d8	0.95 (0.78, 1.14)	0.5740	0.99 (0.75, 1.29)	0.9140
N/A	N-methylproline	0.96 (0.83, 1.12)	0.6360	1.01 (0.84, 1.21)	0.9047
N/A	Ectoine	0.92 (0.79, 1.07)	0.2838	0.94 (0.78, 1.13)	0.5149
N/A	Phenylalanine-d8	0.89 (0.75, 1.07)	0.2263	1.07 (0.87, 1.33)	0.5165
N/A	3-(N-acetyl-L-cystein-S-yl) acetaminophen	0.83 (0.71, 0.97)	0.0199	1.11 (0.92, 1.33)	0.2683
N/A	NH4_C32:1 DAG	0.96 (0.82, 1.12)	0.5842	1.02 (0.84, 1.24)	0.8554
N/A	NH4_C34:3 DAG or TAG fragment	0.88 (0.75, 1.03)	0.1027	0.98 (0.82, 1.18)	0.8283
N/A	NH4_C34:3 DAG	0.9 (0.77, 1.06)	0.2009	1.02 (0.84, 1.24)	0.8273
N/A	NH4_C34:2 DAG or TAG fragment	0.89 (0.76, 1.04)	0.1530	0.96 (0.8, 1.15)	0.6500
N/A	NH4_C34:2 DAG	0.91 (0.78, 1.06)	0.2384	1.01 (0.83, 1.22)	0.9356
N/A	NH4_C34:1 DAG	0.94 (0.81, 1.1)	0.4718	0.98 (0.81, 1.19)	0.8420
N/A	NH4_C14:0 CE	1.02 (0.88, 1.17)	0.8301	0.91 (0.76, 1.1)	0.3357
N/A	NH4_C36:4 DAG or TAG fragment	0.85 (0.73, 0.99)	0.0425	1.01 (0.83, 1.22)	0.9259
N/A	NH4_C36:4 DAG	0.89 (0.76, 1.03)	0.1247	1.05 (0.86, 1.29)	0.6011
N/A	NH4_C36:3 DAG or TAG fragment	0.86 (0.74, 1.01)	0.0588	1.02 (0.84, 1.23)	0.8590
N/A	NH4_C36:3 DAG	0.88 (0.75, 1.03)	0.1006	1.05 (0.86, 1.27)	0.6256
N/A	NH4_C36:2 DAG or TAG fragment	0.9 (0.77, 1.05)	0.1915	1 (0.83, 1.2)	0.9943
N/A	NH4_C36:2 DAG	0.91 (0.77, 1.06)	0.2144	1.04 (0.86, 1.26)	0.6852
N/A	NH4_C16:1 CE	1.01 (0.87, 1.17)	0.9164	0.92 (0.76, 1.11)	0.3802
N/A	NH4_C16:0 CE	0.93 (0.8, 1.08)	0.3442	0.98 (0.78, 1.22)	0.8397
N/A	NH4_C38:5 DAG or TAG fragment	0.88 (0.76, 1.03)	0.1130	0.94 (0.8, 1.1)	0.4320
N/A	NH4_C38:5 DAG	0.91 (0.79, 1.06)	0.2258	1 (0.82, 1.2)	0.9627
N/A	NH4_C18:3 CE	1.03 (0.88, 1.2)	0.7438	0.97 (0.8, 1.18)	0.7604
N/A	NH4_C18:2 CE	1.02 (0.88, 1.19)	0.7487	1.01 (0.84, 1.22)	0.8905
N/A	NH4_C18:1 CE	1.02 (0.87, 1.18)	0.8428	1.04 (0.85, 1.26)	0.7035
N/A	NH4_C18:0 CE	0.99 (0.86, 1.15)	0.9105	0.99 (0.82, 1.19)	0.9106
N/A	NH4_C20:5 CE	0.92 (0.79, 1.06)	0.2530	1.08 (0.89, 1.31)	0.4447
N/A	NH4_C20:4 CE	0.92 (0.79, 1.07)	0.2713	1.07 (0.87, 1.31)	0.5312

Supplementary Table 2.1b (Continued)

N/A	NH4_C20:3 CE	0.93 (0.8, 1.07)	0.3159	1.2 (0.98, 1.48)	0.0798
N/A	C16:1 SM	0.93 (0.81, 1.08)	0.3740	0.9 (0.75, 1.09)	0.2740
N/A	NH4_C22:6 CE	0.95 (0.81, 1.1)	0.4845	1.12 (0.93, 1.36)	0.2243
N/A	NH4_C22:5 CE	0.92 (0.79, 1.07)	0.2680	1.06 (0.86, 1.31)	0.5656
N/A	NH4_C22:4 CE	0.91 (0.78, 1.06)	0.2086	1 (0.81, 1.23)	0.9951
N/A	NH4_C44:2 TAG or TAG fragment	1.12 (0.95, 1.32)	0.1650	0.95 (0.79, 1.13)	0.5578
N/A	NH4_C44:1 TAG or TAG fragment	1.15 (0.98, 1.35)	0.0888	0.97 (0.81, 1.16)	0.7400
N/A	C36:3 PS plasmalogen	0.99 (0.84, 1.16)	0.8891	1.09 (0.91, 1.3)	0.3364
N/A	C36:2 PS plasmalogen	0.96 (0.82, 1.13)	0.6323	0.98 (0.81, 1.2)	0.8624
N/A	C36:2 PS plasmalogen	0.97 (0.84, 1.12)	0.6871	0.97 (0.8, 1.17)	0.7314
N/A	C36:1 PS plasmalogen	1.03 (0.88, 1.2)	0.7257	1.02 (0.83, 1.25)	0.8636
N/A	NH4_C46:3 TAG or TAG fragment	1.07 (0.91, 1.26)	0.4194	0.95 (0.8, 1.13)	0.5710
N/A	NH4_C46:2 TAG or TAG fragment	1.09 (0.93, 1.28)	0.2794	0.97 (0.8, 1.16)	0.7022
N/A	C46:3 TAG	1.04 (0.88, 1.22)	0.6517	0.98 (0.81, 1.18)	0.8126
N/A	NH4_C47:0 TAG	1.09 (0.94, 1.28)	0.2591	0.99 (0.81, 1.2)	0.8852
N/A	C48:5 TAG	0.94 (0.8, 1.1)	0.4341	0.98 (0.82, 1.18)	0.8522
N/A	C44:13 PE plasmalogen	1.1 (0.94, 1.3)	0.2461	0.92 (0.78, 1.09)	0.3485
N/A	C48:4 TAG	0.96 (0.82, 1.13)	0.6479	1 (0.83, 1.2)	0.9903
N/A	NH4_C48:1 TAG	1.04 (0.89, 1.22)	0.6065	1.01 (0.83, 1.23)	0.9131
N/A	NH4_C48:0 TAG	1.08 (0.93, 1.27)	0.3155	0.98 (0.81, 1.19)	0.8646
N/A	NH4_C49:2 TAG	1 (0.86, 1.17)	0.9951	1.06 (0.87, 1.29)	0.5588
N/A	NH4_C49:1 TAG	1.05 (0.89, 1.23)	0.5635	1.04 (0.85, 1.26)	0.7117
N/A	NH4_C49:0 TAG	1.07 (0.91, 1.25)	0.4256	1 (0.82, 1.21)	0.9977
N/A	NH4_C50:3 TAG	0.93 (0.8, 1.09)	0.3745	1.06 (0.87, 1.29)	0.5599
N/A	NH4_C50:2 TAG	0.97 (0.83, 1.14)	0.7271	1.03 (0.85, 1.25)	0.7677
N/A	NH4_C50:1 TAG	1.02 (0.87, 1.2)	0.7701	1.01 (0.83, 1.22)	0.9427
N/A	NH4_C50:0 TAG	1.07 (0.92, 1.26)	0.3880	0.97 (0.8, 1.17)	0.7369
N/A	NH4_C51:3 TAG	0.94 (0.8, 1.09)	0.3955	1.11 (0.91, 1.36)	0.2896
N/A	NH4_C51:1 TAG	1.01 (0.86, 1.18)	0.8914	1.04 (0.86, 1.27)	0.6725
N/A	NH4_C52:4 TAG	0.89 (0.76, 1.04)	0.1300	1.05 (0.87, 1.28)	0.6032
N/A	NH4_C52:3 TAG	0.92 (0.78, 1.07)	0.2652	1.05 (0.86, 1.27)	0.6313

Supplementary Table 2.1b (Continued)

N/A	NH4_C52:2 TAG	0.94 (0.81, 1.1)	0.4643	1.04 (0.86, 1.26)	0.6919
N/A	NH4_C53:3 TAG	0.92 (0.79, 1.07)	0.2661	1.13 (0.93, 1.37)	0.2023
N/A	NH4_C53:2 TAG	0.94 (0.81, 1.1)	0.4410	1.09 (0.9, 1.32)	0.3946
N/A	NH4_C54:4 TAG	0.92 (0.79, 1.07)	0.3027	1.01 (0.83, 1.22)	0.9347
N/A	NH4_C54:3 TAG	0.95 (0.81, 1.1)	0.4879	1 (0.83, 1.2)	0.9821
N/A	NH4_C54:2 TAG	0.97 (0.83, 1.13)	0.6707	0.97 (0.8, 1.17)	0.7295
N/A	NH4_C56:8 TAG	0.87 (0.75, 1.01)	0.0752	1.09 (0.9, 1.32)	0.3688
N/A	NH4_C56:7 TAG	0.87 (0.75, 1.01)	0.0738	1.08 (0.89, 1.3)	0.4260
N/A	NH4_C56:5 TAG	0.86 (0.74, 1)	0.0495	1.03 (0.86, 1.24)	0.7552
N/A	NH4_C58:9 TAG	0.88 (0.76, 1.03)	0.1220	1.1 (0.91, 1.32)	0.3259
N/A	NH4_C58:6 TAG	0.89 (0.77, 1.03)	0.1206	1.01 (0.84, 1.21)	0.9314

Supplementary Table 2.2: Metabolites with nominally significant joint tests of categorical quartile terms

HMDB ID	Metabolite	Q1 v Q0 RR (95% CI)	Q2 v Q0 RR (95% CI)	Q3 v Q0 RR (95% CI)	Raw *p*	FDR *p*
HMDB00201	C2 carnitine	0.73 (0.54, 0.98)	0.73 (0.54, 0.98)	0.67 (0.5, 0.91)	0.0445	0.7518
HMDB00258	Sucrose	1.19 (0.89, 1.59)	1.06 (0.78, 1.44)	0.77 (0.56, 1.05)	0.0373	0.7518
HMDB00658	C16:1 CE	1.61 (1.2, 2.16)	1.27 (0.95, 1.71)	1.16 (0.86, 1.57)	0.0143	0.7518
HMDB00848	C18 carnitine	0.64 (0.47, 0.86)	0.82 (0.62, 1.08)	0.74 (0.55, 0.99)	0.0300	0.7518
HMDB01046	Cotinine	0.69 (0.48, 0.99)	0.74 (0.51, 1.06)	0.58 (0.4, 0.84)	0.0394	0.7518
HMDB02014	C14:1 carnitine	0.78 (0.58, 1.05)	0.64 (0.47, 0.87)	0.8 (0.58, 1.08)	0.0399	0.7518
HMDB02815	C18:1 LPC	0.64 (0.48, 0.87)	0.73 (0.55, 0.98)	0.74 (0.55, 0.99)	0.0255	0.7518
HMDB04952	C22:0 Ceramide (d18:1)	1.04 (0.77, 1.39)	0.68 (0.51, 0.92)	0.85 (0.64, 1.14)	0.0248	0.7518
HMDB05370	C54:4 TAG	0.76 (0.56, 1.03)	1.15 (0.86, 1.54)	0.78 (0.57, 1.06)	0.0114	0.7518
HMDB06831	Butyrobetaine	1.28 (0.95, 1.71)	1.32 (0.98, 1.78)	0.93 (0.68, 1.26)	0.0499	0.7518
HMDB07218	C36:2 DAG	1.22 (0.92, 1.62)	0.89 (0.66, 1.19)	0.82 (0.6, 1.11)	0.0381	0.7518
HMDB08937	C36:4 PE	0.89 (0.67, 1.18)	0.61 (0.45, 0.83)	0.84 (0.63, 1.14)	0.0143	0.7518
HMDB10393	C18:3 LPC	0.98 (0.74, 1.29)	0.66 (0.48, 0.9)	0.93 (0.69, 1.26)	0.0302	0.7518
HMDB10395	C20:4 LPC	0.59 (0.44, 0.78)	0.64 (0.48, 0.86)	0.68 (0.51, 0.91)	0.0009	0.3068
HMDB10397	C20:5 LPC	0.72 (0.54, 0.96)	0.64 (0.48, 0.87)	0.82 (0.62, 1.1)	0.0212	0.7518
HMDB11503	C16:0 LPE	0.8 (0.6, 1.07)	0.66 (0.49, 0.9)	0.95 (0.71, 1.28)	0.0285	0.7518
HMDB13326	C12:1 carnitine	0.78 (0.59, 1.03)	0.61 (0.45, 0.83)	0.76 (0.56, 1.03)	0.0196	0.7518
HMDB42103	C49:3 TAG	1.38 (1.04, 1.83)	1.19 (0.88, 1.59)	0.93 (0.68, 1.28)	0.0442	0.7518
N/A	NH4_C22:4 CE	1.09 (0.82, 1.46)	1.04 (0.78, 1.39)	0.72 (0.53, 0.99)	0.0385	0.7518
N/A	NH4_C51:3 TAG	1.48 (1.1, 1.98)	1.18 (0.87, 1.59)	1.01 (0.73, 1.4)	0.0263	0.7518

Appendix 3: Supplementary Tables and Figures for Chapter 3

Supplementary Table 3.1: Odds ratios for association between 2 vs. 0 prodromal features for each quintile of adherence to the aMED diet pattern

Cohort	Adjustment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p* Trend	*p* Heterogeneity
Including constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.77 (0.58, 0.99)	0.78 (0.61, 1.03)	0.69 (0.54, 0.9)	0.83 (0.61, 1.11)	0.10	
	Multivariable	1.0 (Ref.)	0.8 (0.6, 1.02)	0.81 (0.62, 1.07)	0.73 (0.58, 0.95)	0.92 (0.64, 1.3)	0.39	
NHS	Age	1.0 (Ref.)	0.94 (0.77, 1.12)	0.9 (0.74, 1.09)	0.85 (0.71, 1.04)	0.76 (0.63, 0.92)	0.005	
	Multivariable	1.0 (Ref.)	0.97 (0.79, 1.16)	0.94 (0.78, 1.15)	0.9 (0.72, 1.11)	0.84 (0.68, 1.03)	0.10	
Pooled	Age	1.0 (Ref.)	0.87 (0.73, 1.05)	0.86 (0.73, 1.01)	0.78 (0.64, 0.95)	0.78 (0.67, 0.92)	0.001	0.66
	Multivariable	1.0 (Ref.)	0.90 (0.75, 1.08)	0.90 (0.76, 1.06)	0.82 (0.67, 1.01)	0.86 (0.72, 1.03)	0.07	0.74
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.82 (0.62, 1.07)	0.72 (0.55, 0.96)	0.72 (0.55, 0.98)	0.77 (0.58, 1)	0.05	
	Multivariable	1.0 (Ref.)	0.85 (0.63, 1.13)	0.75 (0.57, 1)	0.77 (0.57, 1.05)	0.82 (0.59, 1.08)	0.18	
NHS	Age	1.0 (Ref.)	0.96 (0.78, 1.2)	0.75 (0.61, 0.93)	0.72 (0.58, 0.89)	0.64 (0.52, 0.78)	<0.001	
	Multivariable	1.0 (Ref.)	0.98 (0.8, 1.23)	0.79 (0.63, 0.98)	0.77 (0.61, 0.96)	0.68 (0.54, 0.86)	0.001	
Pooled	Age	1.0 (Ref.)	0.91 (0.77, 1.07)	0.74 (0.63, 0.88)	0.72 (0.61, 0.85)	0.69 (0.57, 0.82)	0.001	0.15
	Multivariable	1.0 (Ref.)	0.93 (0.78, 1.11)	0.77 (0.65, 0.92)	0.77 (0.64, 0.92)	0.73 (0.61, 0.89)	0.002	0.26
Excluding constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.8 (0.59, 1.05)	0.69 (0.51, 0.92)	0.66 (0.51, 0.87)	0.78 (0.56, 1.06)	0.03	
	Multivariable	1.0 (Ref.)	0.82 (0.6, 1.09)	0.7 (0.51, 0.94)	0.68 (0.52, 0.9)	0.84 (0.58, 1.17)	0.12	
NHS	Age	1.0 (Ref.)	0.96 (0.79, 1.17)	0.86 (0.69, 1.06)	0.93 (0.74, 1.16)	0.78 (0.62, 0.96)	0.03	
	Multivariable	1.0 (Ref.)	0.96 (0.78, 1.18)	0.86 (0.7, 1.07)	0.93 (0.73, 1.16)	0.78 (0.62, 0.96)	0.04	
Pooled	Age	1.0 (Ref.)	0.90 (0.75, 1.08)	0.79 (0.64, 0.98)	0.79 (0.56, 1.12)	0.78 (0.65, 0.92)	0.002	0.79
	Multivariable	1.0 (Ref.)	0.91 (0.77, 1.08)	0.80 (0.66, 0.98)	0.80 (0.59, 1.09)	0.80 (0.66, 0.96)	0.01	0.97
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.79 (0.57, 1.05)	0.68 (0.51, 0.89)	0.72 (0.52, 0.96)	0.74 (0.57, 0.96)	0.03	
	Multivariable	1.0 (Ref.)	0.8 (0.58, 1.08)	0.69 (0.51, 0.92)	0.72 (0.52, 1.02)	0.74 (0.56, 1)	0.07	
NHS	Age	1.0 (Ref.)	1.02 (0.82, 1.26)	0.82 (0.66, 1.02)	0.78 (0.63, 0.97)	0.65 (0.52, 0.82)	<0.001	
	Multivariable	1.0 (Ref.)	1.02 (0.82, 1.27)	0.82 (0.66, 1.05)	0.79 (0.62, 1)	0.63 (0.5, 0.82)	<0.001	
Pooled	Age	1.0 (Ref.)	0.92 (0.72, 1.16)	0.76 (0.64, 0.91)	0.76 (0.64, 0.91)	0.68 (0.57, 0.81)	<0.001	0.30
	Multivariable	1.0 (Ref.)	0.93 (0.74, 1.16)	0.77 (0.64, 0.92)	0.77 (0.63, 0.93)	0.68 (0.55, 0.83)	<0.001	0.33

Age-adjusted models adjusted for age in years at baseline; multivariable adjusted models additionally adjusted for cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled using random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.

Supplementary Table 3.2: Odds ratios for association between 1 vs. 0 prodromal features for each quintile of adherence to the aMED diet pattern

Cohort	Adjustment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p* Trend	*p* Heterogeneity
Including constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.87 (0.66, 1.11)	0.89 (0.68, 1.16)	0.96 (0.77, 1.19)	1.04 (0.77, 1.36)	0.60	
	Multivariable	1.0 (Ref.)	0.87 (0.67, 1.11)	0.88 (0.67, 1.14)	0.94 (0.75, 1.17)	1.03 (0.76, 1.38)	0.72	
NHS	Age	1.0 (Ref.)	1 (0.83, 1.21)	0.98 (0.82, 1.19)	0.88 (0.73, 1.07)	0.85 (0.71, 1)	0.03	
	Multivariable	1.0 (Ref.)	1.03 (0.85, 1.24)	1.03 (0.85, 1.26)	0.94 (0.77, 1.17)	0.94 (0.76, 1.13)	0.39	
Pooled	Age	1.0 (Ref.)	0.95 (0.82, 1.11)	0.95 (0.82, 1.11)	0.91 (0.79, 1.05)	0.91 (0.76, 1.09)	0.55	0.08
	Multivariable	1.0 (Ref.)	0.97 (0.83, 1.13)	0.98 (0.84, 1.15)	0.94 (0.80, 1.09)	0.97 (0.82, 1.14)	0.67	0.41
Cumulative Average								
HPFS	Age	1.0 (Ref.)	1.02 (0.79, 1.32)	0.92 (0.72, 1.18)	0.86 (0.69, 1.09)	0.96 (0.74, 1.24)	0.39	
	Multivariable	1.0 (Ref.)	1.03 (0.79, 1.32)	0.91 (0.71, 1.18)	0.87 (0.67, 1.12)	0.95 (0.71, 1.29)	0.43	
NHS	Age	1.0 (Ref.)	1.05 (0.86, 1.29)	0.82 (0.66, 0.98)	0.76 (0.63, 0.91)	0.79 (0.65, 0.95)	<0.001	
	Multivariable	1.0 (Ref.)	1.07 (0.87, 1.32)	0.87 (0.69, 1.06)	0.84 (0.67, 1.03)	0.9 (0.7, 1.1)	0.10	
Pooled	Age	1.0 (Ref.)	1.04 (0.89, 1.22)	0.85 (0.73, 0.99)	0.79 (0.68, 0.92)	0.85 (0.71, 1.02)	0.05	0.13
	Multivariable	1.0 (Ref.)	1.06 (0.90, 1.24)	0.88 (0.75, 1.04)	0.85 (0.72, 1.00)	0.92 (0.77, 1.09)	0.08	0.63
Excluding constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.91 (0.71, 1.13)	0.97 (0.75, 1.2)	0.97 (0.79, 1.19)	1.1 (0.86, 1.41)	0.39	
	Multivariable	1.0 (Ref.)	0.91 (0.71, 1.14)	0.96 (0.73, 1.2)	0.94 (0.76, 1.16)	1.08 (0.82, 1.4)	0.61	
NHS	Age	1.0 (Ref.)	1.05 (0.89, 1.23)	0.98 (0.83, 1.17)	0.9 (0.77, 1.07)	0.93 (0.79, 1.09)	0.14	
	Multivariable	1.0 (Ref.)	1.06 (0.9, 1.24)	1 (0.85, 1.21)	0.92 (0.79, 1.09)	0.98 (0.82, 1.16)	0.40	
Pooled	Age	1.0 (Ref.)	1.00 (0.87, 1.15)	0.97 (0.85, 1.12)	0.92 (0.81, 1.05)	0.98 (0.85, 1.14)	0.81	0.11
	Multivariable	1.0 (Ref.)	1.01 (0.88, 1.16)	0.99 (0.86, 1.14)	0.93 (0.81, 1.07)	1.00 (0.87, 1.16)	0.73	0.36
Cumulative Average								
HPFS	Age	1.0 (Ref.)	1.04 (0.82, 1.3)	0.96 (0.76, 1.24)	0.9 (0.74, 1.16)	0.98 (0.77, 1.24)	0.48	
	Multivariable	1.0 (Ref.)	1.04 (0.82, 1.31)	0.94 (0.74, 1.19)	0.89 (0.71, 1.15)	0.94 (0.72, 1.19)	0.36	
NHS	Age	1.0 (Ref.)	1.01 (0.85, 1.23)	0.77 (0.66, 0.92)	0.79 (0.67, 0.92)	0.84 (0.71, 0.99)	0.003	
	Multivariable	1.0 (Ref.)	1.03 (0.86, 1.25)	0.8 (0.68, 0.97)	0.84 (0.7, 1)	0.89 (0.73, 1.07)	0.09	
Pooled	Age	1.0 (Ref.)	1.02 (0.89, 1.18)	0.85 (0.68, 1.05)	0.82 (0.72, 0.94)	0.88 (0.77, 1.01)	0.03	0.23
	Multivariable	1.0 (Ref.)	1.03 (0.89, 1.19)	0.85 (0.73, 0.99)	0.86 (0.74, 0.99)	0.91 (0.78, 1.06)	0.06	0.70

Age-adjusted models adjusted for age in years at baseline; multivariable adjusted models additionally adjusted for cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled using random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.

Supplementary Table 3.3: Odds ratios for association between 2 vs. 0 prodromal features for each quintile of adherence to the AHEI diet pattern

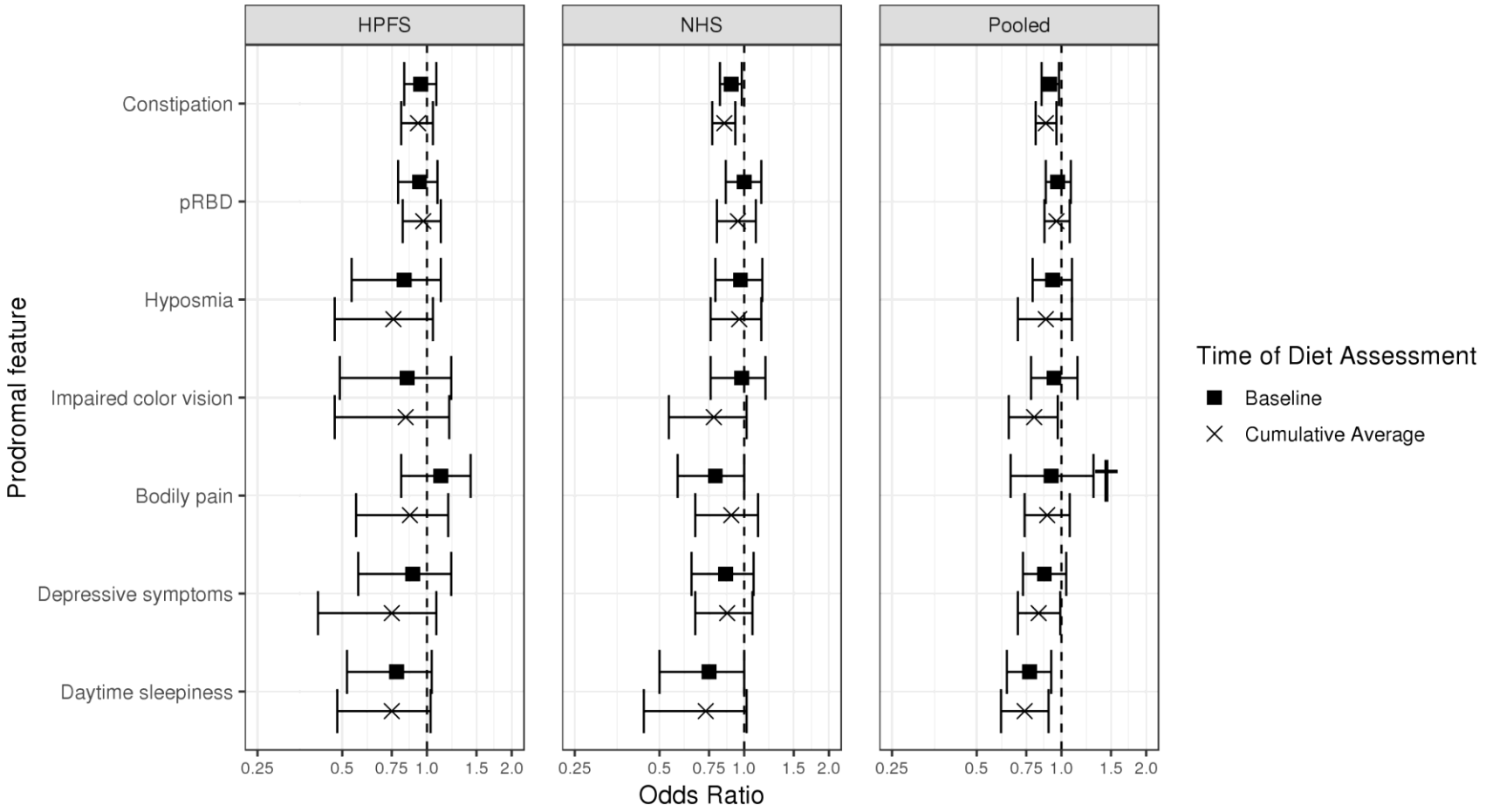
Cohort	Adjustment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p* Trend	*p* Heterogeneity
Including constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.98 (0.76, 1.27)	0.66 (0.5, 0.88)	0.76 (0.58, 1)	0.75 (0.56, 1)	0.01	
	Multivariable	1.0 (Ref.)	1.01 (0.79, 1.31)	0.69 (0.52, 0.91)	0.81 (0.62, 1.07)	0.83 (0.62, 1.14)	0.09	
NHS	Age	1.0 (Ref.)	0.97 (0.78, 1.18)	0.75 (0.59, 0.91)	0.76 (0.6, 0.95)	0.7 (0.56, 0.86)	<0.001	
	Multivariable	1.0 (Ref.)	0.99 (0.81, 1.21)	0.78 (0.62, 0.95)	0.83 (0.66, 1.03)	0.8 (0.63, 0.98)	0.02	
Pooled	Age	1.0 (Ref.)	0.97 (0.83, 1.15)	0.72 (0.60, 0.85)	0.76 (0.64, 0.90)	0.72 (0.60, 0.86)	<0.001	0.76
	Multivariable	1.0 (Ref.)	1.00 (0.84, 1.18)	0.75 (0.63, 0.89)	0.82 (0.69, 0.98)	0.81 (0.68, 0.98)	0.005	0.94
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.64 (0.48, 0.82)	0.61 (0.45, 0.81)	0.51 (0.37, 0.69)	0.57 (0.43, 0.76)	<0.001	
	Multivariable	1.0 (Ref.)	0.67 (0.5, 0.87)	0.66 (0.5, 0.9)	0.58 (0.43, 0.79)	0.69 (0.52, 0.93)	0.01	
NHS	Age	1.0 (Ref.)	0.9 (0.71, 1.09)	0.78 (0.62, 0.95)	0.68 (0.55, 0.83)	0.61 (0.49, 0.75)	<0.001	
	Multivariable	1.0 (Ref.)	0.93 (0.74, 1.12)	0.84 (0.67, 1.04)	0.77 (0.62, 0.94)	0.72 (0.58, 0.89)	<0.001	
Pooled	Age	1.0 (Ref.)	0.77 (0.55, 1.07)	0.70 (0.56, 0.89)	0.60 (0.45, 0.80)	0.60 (0.50, 0.71)	<0.001	0.93
	Multivariable	1.0 (Ref.)	0.80 (0.58, 1.10)	0.76 (0.61, 0.96)	0.68 (0.52, 0.89)	0.71 (0.59, 0.85)	<0.001	0.94
Excluding constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	0.97 (0.74, 1.3)	0.59 (0.44, 0.8)	0.77 (0.58, 1.03)	0.69 (0.51, 0.96)	0.004	
	Multivariable	1.0 (Ref.)	1 (0.78, 1.36)	0.61 (0.46, 0.83)	0.82 (0.62, 1.13)	0.78 (0.56, 1.09)	0.05	
NHS	Age	1.0 (Ref.)	0.89 (0.71, 1.11)	0.69 (0.56, 0.84)	0.72 (0.57, 0.9)	0.64 (0.51, 0.8)	<0.001	
	Multivariable	1.0 (Ref.)	0.91 (0.72, 1.1)	0.73 (0.59, 0.89)	0.8 (0.63, 1)	0.75 (0.6, 0.94)	0.01	
Pooled	Age	1.0 (Ref.)	0.92 (0.77, 1.09)	0.66 (0.55, 0.78)	0.74 (0.62, 0.88)	0.66 (0.55, 0.79)	<0.001	0.73
	Multivariable	1.0 (Ref.)	0.94 (0.79, 1.12)	0.69 (0.58, 0.82)	0.81 (0.68, 0.97)	0.76 (0.63, 0.92)	0.002	0.98
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.65 (0.5, 0.85)	0.62 (0.45, 0.83)	0.51 (0.37, 0.67)	0.59 (0.45, 0.78)	<0.001	
	Multivariable	1.0 (Ref.)	0.68 (0.52, 0.89)	0.67 (0.49, 0.91)	0.58 (0.41, 0.77)	0.71 (0.54, 0.96)	0.02	
NHS	Age	1.0 (Ref.)	0.82 (0.66, 1.01)	0.73 (0.6, 0.9)	0.59 (0.47, 0.73)	0.56 (0.44, 0.7)	<0.001	
	Multivariable	1.0 (Ref.)	0.85 (0.68, 1.06)	0.82 (0.67, 1.03)	0.68 (0.55, 0.85)	0.69 (0.54, 0.86)	<0.001	
Pooled	Age	1.0 (Ref.)	0.74 (0.60, 0.92)	0.69 (0.58, 0.83)	0.56 (0.47, 0.67)	0.57 (0.48, 0.68)	<0.001	0.50
	Multivariable	1.0 (Ref.)	0.78 (0.63, 0.97)	0.77 (0.64, 0.92)	0.64 (0.54, 0.77)	0.70 (0.58, 0.85)	<0.001	0.70

Age-adjusted models adjusted for age in years at baseline; multivariable adjusted models additionally adjusted for cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled using random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.

Supplementary Table 3.4: Odds ratios for association between 1 vs. 0 prodromal features for each quintile of adherence to the AHEI diet pattern

Cohort	Adjustment	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*p* Trend	*p* Heterogeneity
Including constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	1.02 (0.82, 1.3)	0.87 (0.67, 1.15)	0.72 (0.55, 0.93)	0.74 (0.58, 0.97)	0.002	
	Multivariable	1.0 (Ref.)	1.06 (0.85, 1.35)	0.91 (0.7, 1.21)	0.78 (0.59, 1)	0.83 (0.63, 1.08)	0.03	
NHS	Age	1.0 (Ref.)	0.96 (0.8, 1.16)	0.85 (0.71, 1.02)	0.8 (0.66, 0.95)	0.81 (0.65, 0.97)	0.009	
	Multivariable	1.0 (Ref.)	0.98 (0.81, 1.18)	0.88 (0.72, 1.07)	0.85 (0.7, 1.01)	0.87 (0.7, 1.04)	0.07	
Pooled	Age	1.0 (Ref.)	0.98 (0.85, 1.14)	0.86 (0.74, 1.00)	0.78 (0.67, 0.91)	0.79 (0.67, 0.92)	<0.001	0.38
	Multivariable	1.0 (Ref.)	1.01 (0.87, 1.17)	0.89 (0.77, 1.04)	0.82 (0.70, 0.96)	0.85 (0.73, 1.00)	0.006	0.53
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.68 (0.52, 0.89)	0.81 (0.63, 1.05)	0.63 (0.48, 0.81)	0.6 (0.46, 0.79)	<0.001	
	Multivariable	1.0 (Ref.)	0.71 (0.54, 0.95)	0.89 (0.68, 1.15)	0.71 (0.53, 0.92)	0.7 (0.52, 0.95)	0.03	
NHS	Age	1.0 (Ref.)	0.93 (0.76, 1.13)	0.86 (0.69, 1.06)	0.88 (0.73, 1.06)	0.78 (0.64, 0.95)	0.01	
	Multivariable	1.0 (Ref.)	0.94 (0.78, 1.14)	0.89 (0.71, 1.09)	0.93 (0.76, 1.12)	0.85 (0.69, 1.04)	0.15	
Pooled	Age	1.0 (Ref.)	0.80 (0.59, 1.10)	0.84 (0.71, 0.98)	0.75 (0.54, 1.04)	0.69 (0.54, 0.90)	<0.001	0.20
	Multivariable	1.0 (Ref.)	0.83 (0.64, 1.09)	0.89 (0.76, 1.05)	0.82 (0.63, 1.08)	0.80 (0.66, 0.95)	0.01	0.41
Excluding constipation								
Baseline								
HPFS	Age	1.0 (Ref.)	1.01 (0.84, 1.26)	0.87 (0.69, 1.12)	0.75 (0.59, 0.96)	0.76 (0.6, 0.97)	0.002	
	Multivariable	1.0 (Ref.)	1.05 (0.86, 1.31)	0.9 (0.71, 1.17)	0.8 (0.62, 1.05)	0.84 (0.66, 1.09)	0.04	
NHS	Age	1.0 (Ref.)	0.88 (0.76, 1.03)	0.83 (0.71, 0.97)	0.83 (0.7, 0.97)	0.78 (0.66, 0.9)	0.004	
	Multivariable	1.0 (Ref.)	0.9 (0.77, 1.05)	0.86 (0.73, 1.02)	0.89 (0.74, 1.05)	0.84 (0.7, 0.98)	0.07	
Pooled	Age	1.0 (Ref.)	0.93 (0.81, 1.06)	0.84 (0.73, 0.96)	0.80 (0.70, 0.93)	0.77 (0.67, 0.88)	<0.001	0.43
	Multivariable	1.0 (Ref.)	0.96 (0.82, 1.11)	0.87 (0.76, 1.00)	0.86 (0.74, 0.99)	0.84 (0.73, 0.97)	0.007	0.51
Cumulative Average								
HPFS	Age	1.0 (Ref.)	0.65 (0.52, 0.82)	0.83 (0.65, 1.07)	0.63 (0.5, 0.8)	0.61 (0.48, 0.8)	<0.001	
	Multivariable	1.0 (Ref.)	0.69 (0.54, 0.87)	0.92 (0.72, 1.18)	0.72 (0.56, 0.92)	0.72 (0.57, 0.96)	0.04	
NHS	Age	1.0 (Ref.)	0.89 (0.74, 1.04)	0.85 (0.7, 1.01)	0.86 (0.72, 1.01)	0.75 (0.62, 0.89)	0.002	
	Multivariable	1.0 (Ref.)	0.91 (0.77, 1.07)	0.9 (0.75, 1.08)	0.93 (0.78, 1.1)	0.84 (0.69, 1)	0.12	
Pooled	Age	1.0 (Ref.)	0.77 (0.57, 1.03)	0.84 (0.73, 0.97)	0.75 (0.55, 1.01)	0.69 (0.56, 0.85)	<0.001	0.29
	Multivariable	1.0 (Ref.)	0.80 (0.61, 1.05)	0.91 (0.78, 1.06)	0.83 (0.65, 1.07)	0.80 (0.69, 0.93)	0.01	0.50

Age-adjusted models adjusted for age in years at baseline; multivariable adjusted models additionally adjusted for cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled using random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.



Supplemental Figure 3.1: Multivariable-adjusted association for each prodromal feature comparing highest versus lowest quintile of AHEI adherence. Cohort-specific and pooled multivariable-adjusted ORs for each of the 7 prodromal features comparing the extreme quintiles of AHEI adherence at baseline and for cumulative average diet between 1986-2006. Models are adjusted for age in years at baseline, cohort-specific quintile of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories.
[†]Marginally statistically significant heterogeneity across cohorts

Supplementary Table 3.5: Association between ≥3 vs. 0 prodromal features for each component of the aMED diet pattern

Component	Time	Including constipation			Excluding constipation		
		OR (95% CI)	*p*	*p* Heterogeneity	OR (95% CI)	*p*	*p* Heterogeneity
Fruits	Baseline	0.86 (0.76, 0.98)	0.02	0.94	0.85 (0.72, 1.00)	0.05	0.98
	Cumulative Avg.	0.81 (0.67, 0.98)	0.03	0.95	0.84 (0.66, 1.06)	0.14	0.67
Vegetables	Baseline	0.83 (0.73, 0.94)	0.004	0.95	0.84 (0.71, 0.99)	0.04	0.79
	Cumulative Avg.	0.69 (0.57, 0.83)	<0.001	0.64	0.70 (0.55, 0.90)	0.005	0.73
Legumes	Baseline	0.95 (0.84, 1.07)	0.38	0.36	0.98 (0.83, 1.16)	0.81	0.68
	Cumulative Avg.	0.77 (0.64, 0.92)	0.004	0.51	0.83 (0.65, 1.06)	0.14	0.75
Nuts	Baseline	0.87 (0.77, 0.98)	0.02	0.45	0.89 (0.77, 1.04)	0.13	0.32
	Cumulative Avg.	0.63 (0.52, 0.77)	<0.001	0.97	0.66 (0.52, 0.85)	0.001	0.54
Fish	Baseline	1.00 (0.89, 1.13)	0.96	0.99	1.05 (0.89, 1.23)	0.57	0.62
	Cumulative Avg.	0.84 (0.69, 1.03)	0.10	0.28	0.91 (0.72, 1.17)	0.47	0.92
Whole grains	Baseline	0.98 (0.86, 1.10)	0.68	0.33	0.98 (0.84, 1.15)	0.80	0.34
	Cumulative Avg.	0.93 (0.66, 1.31)	0.68	0.08	0.90 (0.59, 1.38)	0.63	0.07
Ratio of monounsaturated to saturated fats	Baseline	1.06 (0.89, 1.27)	0.50	0.17	1.03 (0.78, 1.37)	0.83	0.09
	Cumulative Avg.	0.96 (0.79, 1.18)	0.72	0.80	1.01 (0.77, 1.32)	0.93	0.32
Low red and processed meat consumption	Baseline	1.05 (0.93, 1.20)	0.42	0.93	1.14 (0.96, 1.36)	0.13	0.52
	Cumulative Avg.	0.98 (0.66, 1.45)	0.90	0.05	0.93 (0.70, 1.23)	0.60	0.28
Moderate alcohol	Baseline	0.98 (0.84, 1.13)	0.75	0.42	1.00 (0.83, 1.21)	0.99	0.36
	Cumulative Avg.	0.69 (0.56, 0.86)	<0.001	0.53	0.68 (0.51, 0.90)	0.007	0.75

Baseline estimates represent association for a score of 1 versus 0 for that component in the 1986 aMED score; cumulative average estimates represent association for an average component score of 1 (i.e. always receive score of 1) versus 0 (i.e. always receive score of 0) between 1986-2006. Models are adjusted for age (years), cohort-specific quintiles of caffeine intake, caloric intake, and physical activity as well as smoking pack-year categories and BMI categories. Results from cohort-specific multinomial logistic regression models were pooled using random-effects meta-analysis. Results are provided both including and excluding constipation as a prodromal feature.