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Identifying Metabolomic Profiles of Inflammatory Diets in Postmenopausal Women:

Metabolomic profiles of inflammatory diets

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CONFLICT OF INTEREST STATEMENT

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SUMMARY

Background: We previously showed that a food-based empirical dietary inflammatory pattern (EDIP) score is associated with circulating inflammatory biomarkers. Metabolomic profiling of inflammatory diets may therefore provide insights on mechanisms contributing to disease etiology and prognosis. We aimed to elucidate metabolites associated with inflammatory diets among postmenopausal women, utilizing a robust study design that incorporates independent discovery and validation datasets.

Methods: This baseline cross-sectional investigation evaluated associations between continuous EDIP scores calculated from food frequency questionnaires and 448 log-transformed plasma metabolites as outcomes in multivariable-adjusted linear regression analyses. Metabolites were measured with liquid chromatography tandem mass spectroscopy. Metabolite discovery was conducted among 1,109 Women's Health Initiative (WHI) Hormone Therapy trial participants and results were replicated in an independent dataset of 810 WHI Observational Study participants. Secondary analyses were stratified by standard body mass index (BMI, kg/m^2) categories. In discovery and replication datasets statistical significance was based on false-discovery rate adjusted P<0.05.

Results: After adjusting for energy intake, BMI, physical activity, and other confounding variables, 23 metabolites were significantly associated with EDIP score in the discovery dataset. Of these, the following ten were replicated: trigonelline, caffeine, acethylamino-6-amino-3-methyluracil, 7-methylxanthine, 1,7-dimethyluric acid, 3-methylxanthine, C18:3CE, glycine, associated with lower dietary inflammatory potential; whereas C52:3 triacylglycerol and linoleate associated with higher dietary inflammatory potential. Four of the ten were associated [glycine (inversely), caffeine, 1,7-dimethyluric acid, C52:3 triacylglycerol, (positively)], with C-reactive protein levels. In secondary analyses, associations showed differences by BMI category. Four metabolites, related to coffee/caffeine metabolism were inversely associated among normal weight women, and 83 metabolites associated with EDIP among overweight/obese women, including 40 (48%) that were also associated with C-reactive protein.

Conclusion: Metabolites associated with coffee/caffeine and lipid metabolism may reflect the inflammatory potential of diet. Potential differences by BMI and the linkage to disease outcomes, require further study.

Keywords

INTRODUCTION

Diet is known to influence development and progression of chronic diseases, such as cancer, cardiovascular disease and diabetes. However, to date the biological pathways through which diet influences chronic disease risk have not been fully elucidated. Findings in this area, show systemic inflammation as one potential pathway through which diet may influence chronic disease risk in men and women [1–4]. In place of single analyte biomarkers, metabolomics approaches allow for a holistic view of multiple pathways implicated in the pathophysiology of disease by profiling multiple metabolites in biofluids, cells, and tissues. High-throughput metabolomics assays may shed light on specific metabolites and metabolic pathways influenced by diet, which could provide insights into the underlying biological mechanisms that drive dietary pattern - disease associations; and may inform dietary intervention strategies and/or intervention monitoring. Metabolomic analyses of human samples have now extended to population-based analyses, where the aim is to identify new or novel biomarkers of disease or metabolite profiles that are associated with different human exposures such as diet and lifestyle [5, 6]. Given the influence of dietary patterns on chronic systemic inflammation [1, 2, 7], dietary patterns associated with inflammation may therefore influence chronic disease outcomes [3, 8]. Elucidating the metabolomic profiles of inflammatory diets may therefore provide insights into specific biological pathways.

We previously developed the empirical dietary inflammatory pattern (EDIP) score to identify the dietary components that explain maximal variation in inflammatory biomarkers, including c-reactive protein (CRP), interleukin-6 (IL6) and tumor necrosis factor (TNF) alpha receptor 2 [1]. The EDIP is a food-based index that characterizes the inflammatory potential of a diet based on circulating levels of inflammatory biomarkers. Its relative validity was evaluated in two independent cohorts of health professionals [1, 9]; and in the Women's Health Initiative cohort [2]. Previous studies have shown differences by BMI in the association of the EDIP and several inflammatory markers and with weight change [1, 2, 7, 10]. For example, long-term changes in the EDIP were associated with greater weight changes among overweight/obese men and women than among normal weight participants: overweight/obese participants who changed their diets towards more anti-inflammatory dietary patterns experienced significantly less weight gain compared to those who made minimal changes to their diets [10]. In other previous studies, EDIP predicted higher concentrations of inflammatory biomarkers among overweight or obese participants than among normal weight participants [1, 2, 7]. These findings suggest that the EDIP may be sensitive to dietary patterns associated with obesity and therefore may be more strongly associated with obesity-related inflammation. In this cross-sectional study in a population of postmenopausal women, we aimed to identify metabolites associated with the inflammatory potential of diet and to examine the role of body weight in these associations.

METHODS

Study population

The Women's Health Initiative (WHI) enrolled 161,808 postmenopausal women 50 to 79 years old with a predicted >3-year survival, in 40 sites in the United States between 1993

and 1998. Women of racial or ethnic minority groups represented 17.1% of the overall WHI sample [11]. Participants were enrolled into an observational study (OS) or one or more of four clinical trials, two of which were hormone therapy (HT) trials. One of the HT trials randomly assigned 16,608 women to estrogen plus progestin or placebo, whereas the other randomized 10,739 women with prior hysterectomy, to estrogen or placebo. The full WHI-OS consisted of 93,676 women not eligible or unwilling to participate in the clinical trials [11].

At the baseline clinic visit, certified staff drew blood samples and performed physical measurements including blood pressure, height and weight. For the current study, we used data from 2,306 participants from the *Metabolomics of CHD in the WHI* study [12], a matched case-control study that selected participants from the OS and HT. The cases (who developed coronary heart disease (CHD) after the baseline blood draw) were frequency matched to controls on 5-year age groups, race/ethnicity, hysterectomy status, and 2-year enrollment window. We excluded women with implausible total energy intake values (600 kcal/d or 5000 kcal/d, n=92), very low or very high body mass index (BMI) values (<15 kg/m^2 or >50 kg/m^2, n=15), or those who self-reported diabetes at baseline (n=280). After exclusions, the analytic dataset included 1,919 women: 1,109 in the WHI-HT (discovery dataset) and 810 in the WHI-OS (replication dataset) (Supplemental Figure 1). The WHI protocol was approved by the institutional review boards at the Clinical Coordinating Center at the Fred Hutchinson Cancer Research Center in Seattle, WA, and at each of the 40 Clinical Centers [11]. The current study was approved by the institutional review board at the Brigham and Women's Hospital.

Dietary assessment and calculation of the empirical dietary inflammatory pattern (EDIP) score

At baseline, all participants completed a 122-item semi-quantitative food frequency questionnaire (FFQ) by self-report, developed for the WHI to estimate average daily dietary intake over the previous 3-month period [11, 13]. The WHI FFQ has produced results reasonably comparable to those from four 24-hour dietary recall interviews and four days of food diaries recorded within the WHI [14].

The EDIP development and validation studies have been described [1, 9]. The EDIP score was developed in a sample of 5,230 women in the Nurses' Health Study (NHS) and validated in NHS-II (n=1,002) and Health Professionals Follow-up Study (HPFS: n=2632) [1]. Also, the score was previously applied in a multi-racial sample of 31,472 WHI participants with diet and biomarker data, and was found to be significantly associated with several inflammatory biomarkers [2]. The goal for the EDIP was to empirically create a score for overall inflammatory potential of whole diets defined using food groups. Thirty-nine pre-defined food groups [15] were entered into reduced rank regression models [16] followed by stepwise linear regression analyses to identify a dietary pattern most predictive of three plasma inflammatory biomarkers: CRP, IL6 and TNF receptor 2 [1]. The EDIP score is the weighted sum of 18 food groups which assesses the inflammatory potential of diet on a continuum from maximally anti-inflammatory to maximally pro-inflammatory, with lower (more negative) scores indicating anti-inflammatory diets and higher (more

positive) scores indicating pro-inflammatory diets. Food groups that were positively related to concentrations of inflammatory biomarkers are the following: processed meat, red meat, organ meat, non-dark (non-oily) fish, other vegetables (i.e., vegetables other than green leafy vegetables and dark-yellow vegetables), refined grains, high-energy beverages (cola and other carbonated beverages with sugar, fruit drinks), low-energy beverages (low-energy cola and other low-energy carbonated beverages - the WHI FFQ did not separately assess low-energy beverages), and tomatoes. The remaining food groups were inversely related to concentrations of the inflammatory biomarkers, and include: beer, wine, tea, coffee, dark-yellow vegetables, green leafy vegetables, snacks (popcorn, corn chips, potato chips, crackers), fruit juice, and pizza [1].

Metabolite assessment

Plasma samples were collected using EDTA tubes and processed immediately. Specimens were stored in a –70°C freezer within 2 hours of collect ion or stored at –20°C for up to 2 days and shipped on dry ice and stored at –70°C until proces sing. Metabolomics measurements were conducted at the Broad Institute (Boston, MA), using 4 complimentary liquid chromatography tandem mass spectroscopy (LC-MS) methods, described in detail elsewhere [12], resulting in 509 metabolites. For each method, pooled plasma reference samples were included every 20 samples, and results were standardized using the ratio of the value of the sample to the value of the nearest pooled reference multiplied by the median of all reference values for the metabolite [12]. MultiQuant 1.2 software (AB SCIEX) was used to identify and quantify the metabolites [17]. All signals were inspected to ensure quality and integration, and a signal-to-noise ratio <10 was considered unquantifiable [18–20]. For each method, metabolite identities were confirmed using authentic reference standards or reference samples. Coefficients of variation (CVs) were calculated using pooled plasma samples from the first 800 WHI-OS participants [12]. After excluding metabolites with a CV >20%, 448 known metabolites were retained for analyses. In the pilot testing of the metabolomics platform, 92% of metabolites had acceptable assay reproducibility (CV<20%) and almost 90% of metabolites were stable over 1 – 2 years (Spearman correlation or ICC 0.4) [21].

Covariates

Data on potential confounding variables were collected by self-administered questionnaires on demographics, medical history, and lifestyle factors at baseline, and has been previously described [11]. Covariates included in the models were the following: total energy intake (kcal/day); age at WHI baseline (years); body mass index [BMI=weight (kg)/(height (m) x height (m))]; racial/ethnic groups (American Indian or Alaskan Native, Asian or Pacific Islander, Hispanic/Latino, African American, European American, and other race groups); educational levels categorized into some high school or lower educational level, high school graduate or some college or associate degree, and 4 years of college; smoking status (current, former, and never); regular use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) (yes/no); where regular use of medications was defined as: 2 times in each of the two weeks preceding the interview; physical activity, calculated by summing the metabolic equivalent tasks for all reported activities for each individual (e.g., walking, aerobics, jogging, tennis, swimming, biking outdoors, exercise machine, calisthenics,

popular or folk dancing) (MET-hours/week) [22]; category and duration of estrogen use and category and duration of combined estrogen and progestin use categorized into five groups (none, ≤4.9y, 5–10.0y, 10.1–14.9y, and ≥15.0y); CHD case-control status, dietary modification trial arm (intervention, control, not randomized to the trial); hormone therapy trial arm (estrogen-alone intervention, estrogen-alone control, combined estrogen and progestin intervention, estrogen and progestin control, not randomized to trial); and calcium and vitamin D arm (intervention, control, not randomized to trial).

Statistical analysis

All metabolites were log-transformed and scaled to render with mean of zero and unit variance. Missing values below the limit of detection were assigned to half the lowest observed value. We described participants' characteristics using means (standard deviations) for continuous variables, and frequencies (%) for categorical variables across quintiles of the EDIP score. Analyses were adjusted for covariates listed above.

For the discovery analysis in the larger WHI-HT dataset (n=1109), each metabolite was evaluated individually in multivariable-adjusted linear regression models in relation to 5-SD increments in the EDIP score. All models included BMI and the other factors listed above. Statistical significance was based on a two-sided P<0.05 and a corresponding false discovery rate-adjusted P-value of 0.05. Metabolites discovered in WHI-HT were evaluated individually in the independent WHI-OS dataset (n=810) using the same models as in the discovery analysis. Metabolite associations were considered to have been replicated based on a two-sided P<0.05 and a corresponding false discovery rate-adjusted p value of 0.05.

In addition, the subset of metabolites that were significantly associated with the EDIP score in the discovery dataset were evaluated in the discovery and validation datasets for association with CRP as the main predictor of interest, adjusting for the same factors as in the EDIP models. Plasma CRP was measured using a high sensitivity immunoturbidimetric assay on the Hitachi 911 (Roche Diagnostics - Indianapolis, IN), using reagents and calibrators from DiaSorin (Stillwater, MN).

In secondary analyses, we conducted subgroup analysis in BMI categories: normal weight (15 to <25 kg/m^2), overweight/obese (25 to ≤50 kg/m^2), while adjusting for continuous BMI within BMI categories. Due to power considerations, stratified analyses were conducted in the combined WHI-OS and WHI-HT datasets. Statistical significance was based on a two-sided P<0.05 and a corresponding false discovery rate-adjusted P-value of 0.05. Additionally, and even though statistical power was limited, we conducted exploratory analyses in the BMI subgroups in separate discovery and replication datasets, similar to the primary analyses but at an FDR-adjusted P<0.10 for statistical significance.

RESULTS

Table 1 presents characteristics of study participants by EDIP quintiles. In both the discovery and replication datasets, women consuming the most anti-inflammatory diets (EDIP quintile 1) showed lower CRP concentrations, lower BMI and reported higher physical activity, compared to the women consuming the most pro-inflammatory diets

(quintile 5). The proportions of obese women, African Americans, Hispanic/Latinos, and those with lower educational levels increased across EDIP quintiles (Table 1). Table 2 shows the distribution of foods and nutrients across EDIP quintiles. In terms of average weekly food intake, women with anti-inflammatory diets consumed 1.3 servings less red meat, 1.7 servings less processed meat, 5.9 servings less sugar-sweetened beverages, 1.3 servings less refined grains than women classified with pro-inflammatory diets. Also, women with anti-inflammatory diets consumed 17 glasses more wine, 4.2 cups more tea or coffee, and about 2.2 servings more dark-yellow vegetables and green leafy vegetables than the women with pro-inflammatory dietary patterns. Servings are defined in Table 2, footnote #3.

In the discovery dataset, the EDIP score was associated with 77 metabolites at an FDR adjusted P-value <0.05 in multivariable-adjusted models and after additionally adjusting for BMI, 23 metabolites remained significantly associated (Table 3). Of the 23 metabolites, ten were replicated in the WHI-OS after adjustment for all covariates. The eight metabolites with inverse associations included: trigonelline, caffeine, 5-acethylamino-6-amino-3-methyluracil, 7-methylxanthine, 1,7-dimethyluric acid, 3-methylxanthine, C18:3 cholesterol ester, and glycine. Of the ten metabolites, four were also associated with CRP levels: Caffeine, 1,7-dimethyluric acid and C52:3 triacylglycerol were positively associated with CRP, whereas glycine was inversely associated (Table 4). Caffeine and 1,7-dimethyluric acid showed positive associations with CRP, in contrast with their inverse associations with the EDIP score.

Figure 1 is a heat map in the replication dataset showing partial Spearman correlations between the 23 discovered metabolites with FDR P-value <0.05 and EDIP score, BMI and CRP, ordered by hierarchical clustering. The carnitines were highly clustered and showed no association with EDIP, CRP and BMI. The strongest inverse correlations with the EDIP were shown by trigonelline, −0.26, 1,7-dimethyluric acid, −0.17, 5-acetylamino-6-amino-3-methyluracil, −0.13, 7-methylxanthine, −0.13, and 3-methylxanthine, −0.12 (all P<0.001); whereas the strongest positive correlations with the EDIP were shown by C52:3 TAG, 0.12, C56:5 TAG, 0.10 (all P<0.004) (Figure 1).

Secondary analyses suggested differences by BMI category. Among normal weight women (BMI: 15 to <25 kg/m^2, n=630, including 20 thin women with BMI 15 - <18.5), after adjustment for covariates, including continuous BMI; four metabolites, related to coffee/caffeine metabolism (5-acetylamino-6-amino-3-methyluracil, trigonelline, 1,7-dimethyluric acid and caffeine) were inversely associated with the EDIP score (FDR adjusted P<0.05). None of the four metabolites was associated with CRP levels (Table 5). In contrast, among normal weight women, 63 metabolites showed significant associations with CRP after multivariable adjustment (FDR adjusted P<0.05). However, none of the four metabolites that associated with EDIP were among the 63 associated with CRP concentrations (data not shown). In the exploratory analyses, 26 metabolites were associated with EDIP at the raw P<0.05, and none at FDR-adjusted P<0.10, among normal weight women in the discovery dataset (Hormone Therapy trial: n=317) (Supplemental Table 1); therefore no replication was conducted among normal weight women.

Among overweight or obese women (BMI: 25 to 50 kg/m^2, n=1289), after adjustment for covariates, including continuous BMI, 110 metabolites were significantly associated with the EDIP score after multivariable adjustment (FDR adjusted P<0.05) (data not shown); and 83 remained significant after additionally adjusting for BMI (FDR adjusted P<0.05) (Table 6). In multivariable-adjusted models, 196 metabolites were significantly associated with CRP concentrations and 178 remained significant with additional adjustment for continuous BMI (FDR adjusted P<0.05) (data not shown). Of the 83 metabolites associated with EDIP, 40 (48%) were also associated with CRP (Table 6). The 83 metabolites included alkaloids and derivatives, cholesterol esters, lysophosphatidylcholines, sphingomyelins, amino acids, purines and pyrimidines and that were significantly higher with lower dietary inflammatory potential, whereas diacyleglycerols, triacylglycerols and bile acids increased with higher dietary inflammatory potential. In the exploratory analyses among overweight and obese women, we found 45 metabolites to be significantly associated with EDIP at FDR-adjusted P<0.10 in the discovery dataset (Hormone Therapy trial, n=792) (Supplemental Table 2). In the replication dataset (Observational Study: n=497), 16 of these metabolites associated with EDIP at FDR-adjusted P<0.05 (Supplemental Table 3).

A summary of the associations of the ten replicated metabolites in the discovery and replication datasets and in BMI categories, is presented in Figure 2. In both the discovery and validation datasets, all ten metabolites were significant, both nominally at the 2-sided P<0.05 and at the FDR adjusted P<0.05. Among normal weight women, eight of the ten (except the two TAGs) were nominally significant and four of these were significant at FDR adjusted P<0.05; whereas among overweight or obese women, nine of the ten (except C56:5 TAG) were significant both nominally and at FDR adjusted P<0.05. For CRP associations, in the discovery dataset, three of the ten metabolites were nominally significant and two of these were also FDR significant, whereas in the replication dataset, four metabolites were both nominally and FDR significant. Among normal weight women, only one metabolite (glycine) was associated both nominally and at FDR adjusted P<0.05, whereas among overweight or obese women, four metabolites were associated both nominally and at FDR adjusted P<0.05. All associations (including in the subgroups) were adjusted for continuous BMI.

DISCUSSION

In the current study, using a robust methodology, we identified ten metabolites correlated with the inflammatory potential of diet, independent of energy intake, race/ethnicity, BMI, physical activity and smoking; Adding biologic plausibility, four of the ten were also significantly associated with CRP levels.

Body weight appeared to play an important role in modifying these associations. For example, among normal weight women, four metabolites related to coffee/caffeine metabolism (5-acetylamino-6-amino-3-methyluracil, trigonelline, 1,7-dimethyluric acid and caffeine) were inversely associated with the EDIP score, but none of them were associated with CRP levels. In contrast, among overweight or obese women, up to 83 metabolites including lower levels of alkaloids and derivatives (n=6), cholesterol esters (n=7), lysophosphatidylcholines (n=6), sphingomyelins (n=6), amino acids (n=10) and purines &

pyrimidines (n=4) decreased with higher dietary inflammatory potential; and higher levels of diacyleglycerols (n=13), triacylglycerols (n=18) and bile acids (n=2) were associated with higher inflammatory potential of the diet.

Six of the ten replicated metabolites were coffee/caffeine-related metabolites (trigonelline, caffeine, 7-methylxanthine, 5-acethylamino-6-amino-3-methyluracil, 3-methylxanthine, 1,7-dimethyluric acid) which were all associated with lower inflammatory potential of the diet. Two of the other four metabolites, a cholesterol ester (C18:3 CE) and a nonessential amino acid (glycine) were also inversely associated with dietary inflammatory potential whereas the remaining two replicated metabolites contributing to higher dietary inflammatory potential were a triacylglycerol (C52:3 TAG) and an omega-6 fatty acid (linoleate). These ten metabolites also reflect the food group composition of the EDIP score. For example, the six caffeine-related metabolites are indicative of coffee and tea, two inverse components of the EDIP score with a naturally high caffeine content [23], and C18:3 CE (a cholesterol ester) may be indicative of the vegetable components of the EDIP. Cholesterol esters generally contain high proportions of C18 fatty acids including alpha-linolenic acid found in plant-based oils [24]. However, excess omega-6 fatty acids (e.g., linoleate) from vegetable oils and meat-based foods may interfere with the health benefits of omega-3 fats, partly because they compete for the same rate-limiting enzymes [25]. In addition, omega-6 fatty acids are typically associated with pro-inflammatory responses [26, 27] in line with the current study findings for linoleate. The triacylglycerol is also indicative of the meat-based components which contributes to higher dietary inflammatory potential in the EDIP score [28]. Triacylglycerols are major components of very low density lipoprotein cholesterol, and play an important role in metabolism as energy sources and transporters of dietary fat [28].

In the biologic validation analysis, four of the ten replicated metabolites were also associated with CRP levels. The two caffeine-related metabolites (caffeine, and 1,7-dimethyluric acid), although inversely associated with the EDIP score, were positively associated with CRP levels. This is in line with previous studies of coffee and caffeine showing a predominant anti-inflammatory action of coffee but not of caffeine consumption [29]. A review of 15 clinical trials of coffee (8 studies) and caffeine (7 studies) found increased adiponectin levels in four of seven trials comparing filtered or caffeinated coffee with placebo; or comparing its levels at baseline and after consumption of medium or dark roasted coffee; but no change in adiponectin levels in the caffeine trials. Caffeine increased interleukin (IL)-6 levels in three of five studies and IL-10 levels in two of three trials [29]. Moreover, in a large previous study of 15,538 women and 7,393 men, we found that habitual coffee consumption (4 cups/day of total coffee compared to nondrinkers) was associated with lower concentrations of inflammatory biomarkers (CRP, IL6, TNF alpha receptor 2), and the associations were not different by caffeine content [30]. These findings suggest that caffeine may actually have a pro-inflammatory effect and that the anti-inflammatory effects of coffee may therefore be mediated through other metabolites. For example, in the current study, high trigonelline levels were associated with lower dietary inflammatory potential but not associated with systemic inflammation (CRP). Trigonelline is an alkaloid in coffee, and is degraded to an extent during coffee roasting to produce niacin (vitamin B3), which has been shown to suppress colonic inflammation [31] and colon cancer in mice [32]. In addition, a randomized crossover trial that enrolled 15 overweight men to evaluate the acute effects of decaffeinated

coffee and major coffee compounds including chlorogenic acid and trigonelline, found that these two metabolites reduced early glucose and insulin secretion [33].

Glycine was inversely associated with both EDIP and CRP whereas C52:3 TAG was positively associated with both predictors. Several amino acids including glycine have been shown to have anti-inflammatory properties [34]. In a study that treated 74 diabetes patients with 5g/day glycine or 5 g/day placebo, patients treated with glycine had a significant decrease in HbA1C, pro-inflammatory cytokines and IFN-gamma [35]. C52:3 TAG (palmitic acid, C16:0) is obtained mainly from vegetable oil and animal fats, and though endogenous synthesis contributes a significant portion of this saturated fatty acid in the circulation [36], serum levels have been found to correlate well with dietary intake [37]. Recent findings suggest that dietary fats can influence gut microbiota composition and that this can affect inflammatory status in vivo (31) but whether dietary fats substantially influence disease outcomes by affecting the inflammatory status of people via alterations in their gut microbiome remains untested.

Associations differed by body weight. The metabolites identified among normal weight women were all inversely associated with the EDIP score, indicating that these women were generally consuming an anti-inflammatory (high quality) dietary pattern. Additionally, normal weight women had lower (more anti-inflammatory) EDIP scores. An anti-inflammatory dietary pattern would be expected to be associated with lower concentrations of circulating CRP concentrations. However, none of the four metabolites was associated with CRP. It is possible that among normal weight women, the beneficial effects of a high-quality dietary pattern are mediated via pathways other than those directly involving systemic inflammation. In contrast, among overweight or obese women, 83 metabolites were significantly associated with the EDIP score, and about half were also associated with CRP. In contrast to normal weight women, the 83 metabolites identified in overweight/obese women include several metabolite categories other than alkaloid and derivatives. In addition, the alkaloid trigonelline that was inversely associated with EDIP but not with CRP among normal weight women, was significantly inversely associated with both predictors among overweight/obese women (n=1289), suggesting that the lack of associations with CRP among normal weight women (n=630) could also be due to low statistical power. Among the overweight/obese group, most of the metabolite associations with EDIP were consistent with the CRP associations. Higher CRP concentrations were associated with higher levels of most triacylglycerols and diacylglycerols and with lower levels of most lysophosphatidylcholines, cholesterol esters and fatty acids, but were generally not associated with alkaloids and derivatives or sphingomyelins.

The differences by body weight in the metabolomic profiles observed in the current study, reflect differences by body weight in the association of the EDIP score with weight change and with several inflammatory biomarkers in previous studies [1, 2, 7, 10]. In these previous studies, EDIP predicted higher concentrations of inflammatory biomarkers and weight changes among overweight or obese participants than among normal weight participants. Interestingly, higher EDIP scores were strongly associated with lower concentrations of adiponectin in these previous studies, especially among overweight or obese participants. Adiponectin is a hormone secreted by adipose tissue [38] and lower adiponectin

concentrations are associated with higher adiposity and inflammation [39]. Taken together, these findings suggest that the EDIP is sensitive to dietary patterns associated with obesity and therefore may be more strongly associated with obesity-related inflammation in line with the metabolomic profile identified among overweight/obese women.

Among overweight or obese women, results showed a positive association between higher EDIP scores and higher levels of diacylglycerols and triacylglycerols consistent with reduced lipid turnover rate and increased storage of triglycerides observed in obesity [40]. Specifically, lysophospholipids are signaling molecules involved in modulating processes such as inflammation, insulin secretion and insulin sensitivity through their interaction with G protein-coupled receptors [41, 42]. Lysophospholipids may therefore be important in obesity and related diseases such as cancer. A study assessing plasma lysophospholipids in obesity found that a multivariable-adjusted combination of 26 lysophospholipids could discriminate between normal weight and obese participants with an accuracy of 98% [43]. Among the obese participants, the authors found decreased concentrations of different lysophospholipid species including lysophosphatidylcholines, and lysophosphatidylethanolamines [43]. These results are consistent with those observed in the current study, in which nine lysophospholipids were inversely associated with higher dietary inflammatory potential among overweight/obese women, and seven of these were also inversely associated with CRP, a widely studied biomarker of inflammation.

Our study has several strengths, including a well-validated metabolomics platform, detailed covariate data, and a robust methodology. Notably, our methodology includes the integration of dietary data with metabolomics and biomarker data to characterize the metabolomics profiles of inflammatory diets – an analytic approach that to t he best of our knowledge, has not yet been conducted. Limitations of our study include known measurement error in using an FFQ for the assessment of diet, such as underreporting of energy and protein intake [44, 45]. Given that energy misreporting may depend on BMI, we adjusted all models for BMI including the adjustment of BMI subgroup analysis for continuous BMI. In addition, BMI as a measure of body fatness (adiposity) is limited in not being able to distinguish between excess fat, muscle, or bone mass, and does not measure body fat distribution. However, BMI is a reasonable measure of weight status in populations and as is a good screening tool to identify potential weight problems in individuals. Our findings may also have limited generalizability, therefore additional data are needed to identify whether these results are specific to postmenopausal women or whether there are similar profiles in younger premenopausal women or in men. In addition, we had limited sample sizes in BMI subgroup analyses and could not conduct statistical replication of the metabolites found, but we note that nine of the ten replicated metabolites were among the 83 significant metabolites in overweight/obese women at the FDR adjusted $P<0.05$. In addition, we also conducted similar subgroup analyses using CRP. Furthermore, we did not have data on other inflammatory biomarkers (e.g., IL6, TNF alpha receptor 2 etc), and CRP may not be comprehensively reflective of systemic inflammation.

In summary, in two separate samples of postmenopausal women, multiple metabolites associated with the inflammatory potential of diet were identified, statistically replicated and biologically validated using plasma CRP levels. We found that metabolites associated with

coffee/caffeine and lipid metabolism may inform on the underlying biological pathways through which inflammatory diets may influence disease outcomes. Also, metabolites identified may provide insights on the role of inflammatory dietary patterns in obesity-related inflammation. The linkage of these findings to inflammation-related disease requires further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

BMI	Body mass index
EDIP	Empirical dietary inflammatory pattern score
FDR	False Discovery Rate
FFQ	Food frequency questionnaire
HMDB	Human Metabolome Database
MET-hours	Metabolic equivalent hours
NHS	Nurses' Health Study
NSAIDs	Non-steroidal anti-inflammatory drugs
PA	Physical activity
US	United States
WHI-HT	Women's Health Initiative – Hormone Therapy
WHI-OS	Women's Health Initiative – Observational Study

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Highlights

- Diet may influence disease risk and progression through inflammation

- The specific mechanisms involved are not fully understood

- Metabolomic profiling of inflammatory diets may therefore provide insights

- We identified and replicated 10 metabolites associated with diet-related inflammation

- Metabolites associated with lipid metabolism may reflect diet-related inflammation
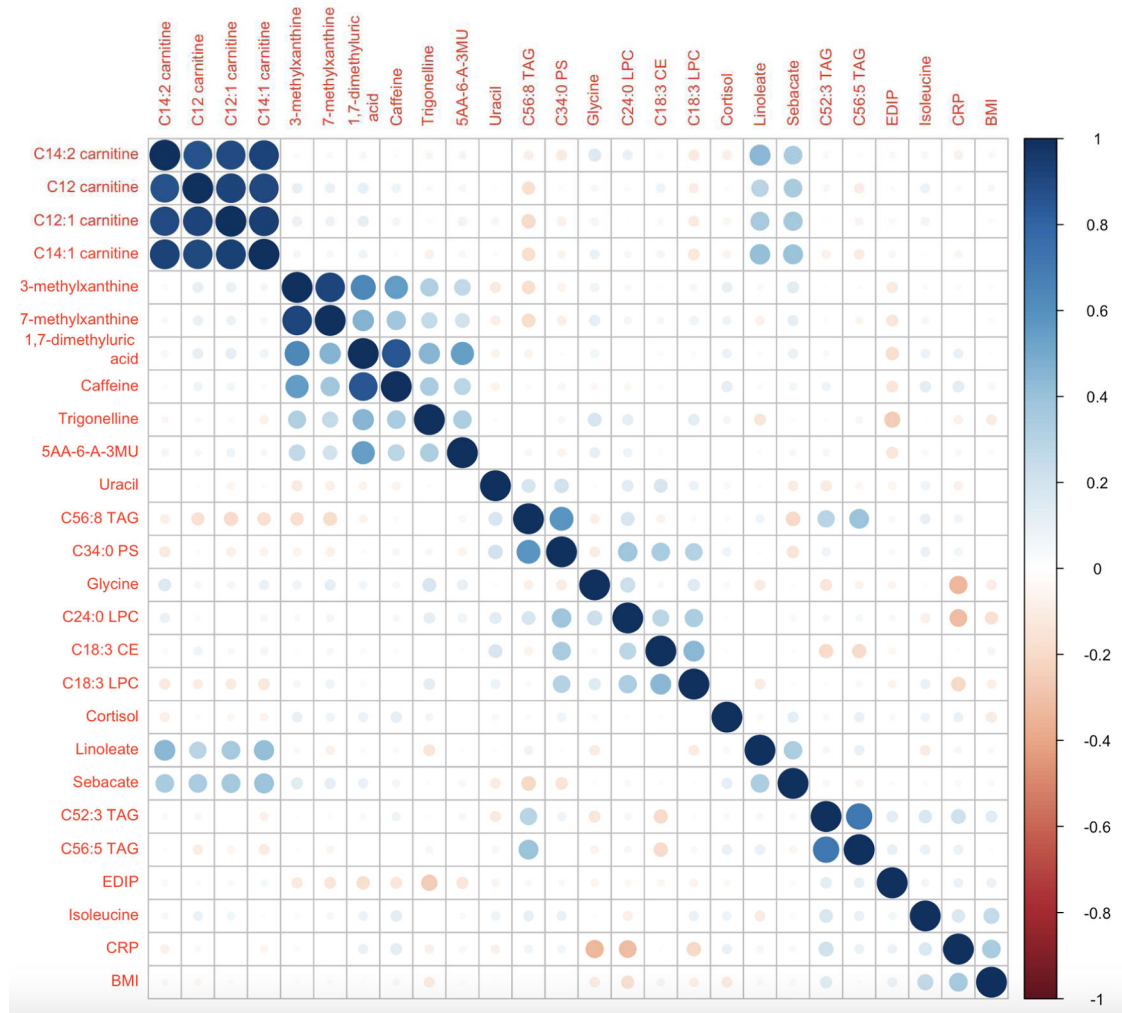
Figure 1.
Heat map in the replication dataset showing partial Spearman correlations between the 23 discovered metabolites and EDIP score, BMI and CRP. Ordering is by hierarchical clustering. Correlations were adjusted for body mass index (continuous) age, physical activity, educational level, race/ethnicity, aspirin/NSAIDs use, smoking status, WHI Hormone Therapy trial arm, CHD case-control status. 5AA-6-A-3MU, 5-acetylamino-6-amino-3-methyluracil; BMI, body mass index; CE, cholesterol ester; CRP, C-reactive protein; EDIP, empirical dietary inflammatory pattern score; LPC, lysophosphatidylcholine; TAG, triacylglycerol
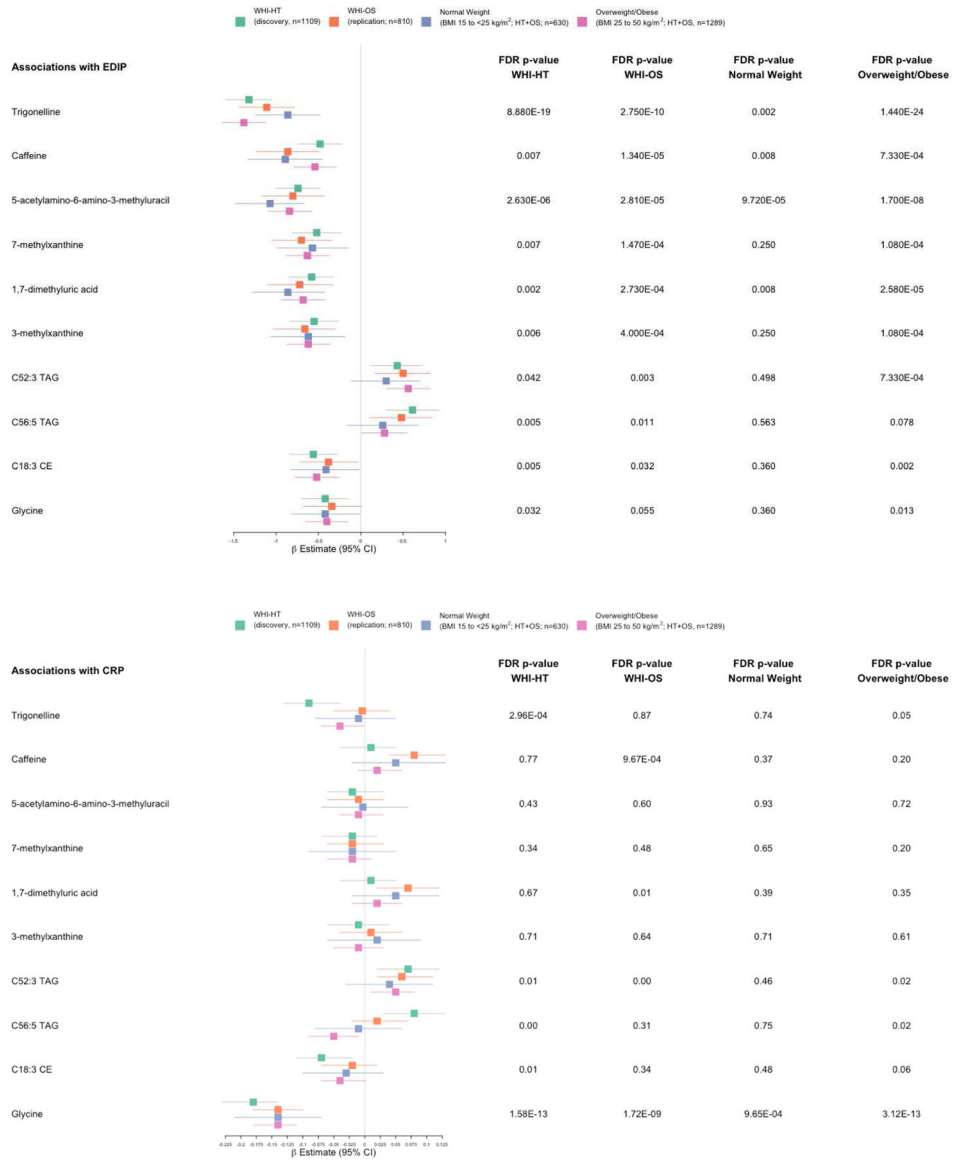
Figure 2.
Summary of EDIP and CRP associations with the 10 replicated metabolites in the discovery and replication datasets and in body mass index categories. Associations were adjusted for body mass index (continuous) age, physical activity, educational level, race/ethnicity, aspirin/NSAIDs use, smoking status, WHI Hormone Therapy trial arm, CHD case-control status.

Table 1.

Baseline characteristics of the study population across EDIP quintiles [1,2,3]

| Characteristic	WHI-HT: discovery (n=1109)					WHI-OS: replication (n=810)				
	Quintile 1 (−4.99, <−0.76) n=221	Quintile 2 (−0.76, <−0.18) n=222	Quintile 3 (−0.18, <0.28) n=222	Quintile 4 (0.28, <0.77) n=222	Quintile 5 (0.77, 5.14) n=222	Quintile 1 (−4.23, <−0.75) n=162	Quintile 2 (−0.75, <−0.21) n=162	Quintile 3 (−0.21, <0.30) n=162	Quintile 4 (0.30, 0.74) n=162	Quintile 5 (0.74, 3.63) n=162
Race/ethnicity										
Black	3.6	7.2	10.4	10.4	17.6	6.8	8.6	11.1	12.3	24.7
Hispanic	1.4	0.9	0.9	4.5	5.4	3.1	1.2	1.8	1.9	6.2
White	94.6	90.5	86.9	82.0	73.9	85.2	84.6	80.9	74.7	54.9
Other[3]	0.4	1.4	1.8	3.1	3.1	4.9	5.6	6.2	11.1	14.2
CRP, mg/L	3.9 ± 5.0	4.3 ± 6.0	4.6 ± 8.3	5.1 ± 6.1	5.4 ± 5.3	4.1 ± 7.3	4.1 ± 5.2	4.3 ± 5.4	5.3 ± 7.5	5.6 ± 10.7
Age, years	66.6 ± 7.4	67.4 ± 6.5	67.5 ± 6.8	67.1 ± 6.4	65.9 ± 7.6	67.2 ± 6.6	68.6 ± 6.1	68.2 ± 6.4	67.8 ± 6.4	66.0 ± 7.8
BMI, kg/m^2	27.5 ± 5.1	28.7 ± 5.5	28.3 ± 5.2	28.7 ± 5.5	30.3 ± 6.1	26.4 ± 5.4	26.7 ± 4.4	26.7 ± 4.9	27.2 ± 5.7	28.6 ± 5.9
Body mass index categories, %										
15 - <18.5	0.5	0.5	0.9	0.9	1.8	1.9	0.6	0	1.9	1.8
18.5 - <25	34.4	28.8	27.5	27.5	20.3	43.8	35.8	41.4	35.8	30.3
25-<30	38.0	31.5	41.9	33.3	27.5	37.0	39.5	37.0	36.4	31.5
30-50	27.1	39.2	29.7	38.3	50.4	17.3	24.1	21.6	25.9	36.4
Physical activity, MET-hours/week	8.2 ± 11.5	7.4 ± 12.6	5.7 ± 8.5	5.1 ± 7.8	4.0 ± 8.0	11.3 ± 13.7	8.5 ± 11.5	7.8 ± 11.5	7.2 ± 9.8	6.1 ± 9.8
Smoking, %										
Never	42.1	53.1	46.4	48.2	52.3	45.1	47.5	49.4	46.9	49.4
Former	40.3	32.9	40.1	41.0	32.4	46.9	46.3	45.1	44.5	39.5
Current	17.6	14.0	13.5	10.8	15.3	8.0	6.2	5.6	8.6	11.1
Aspirin/NSAIDs use, %	15.4	18.5	20.3	14.0	14.9	14.8	15.4	17.3	12.3	10.5
Educational level, %										
Less than high school	5.4	4.5	6.3	9.4	15.3	3.1	4.3	3.7	6.2	13.6
High school/GED	57.9	63.1	68.0	68.5	65.3	56.8	57.4	63.6	64.8	54.9
4 years of college	36.7	32.4	25.7	22.1	19.4	40.1	38.3	32.7	29.0	31.5

EDIP, empirical dietary inflammatory pattern score; MET-h, metabolic equivalent hours; NSAID, nonsteroidal anti-inflammatory drugs; CRP, C-reactive protein; WHI, Women's Health Initiative; HT, Hormone Therapy trial; OS, Observational Study.

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[1] Values are percentages or means ± SDs.

[2] EDIP scores were adjusted for total energy intake using the residual method. Lower EDIP scores indicate anti-inflammatory diets whereas higher scores indicate pro-inflammatory diets.

[3] Other race category included American Indian or Alaskan Native, Asian or Pacific Islander

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Table 2.

Distribution of dietary intakes across quintiles of the EDIP score (combined discovery and replication datasets)

	Quintile 1 (−4.99 to <−0.76) n=383	Quintile 2 (−0.76 to <−0.19) n=384	Quintile 3 (−0.19 to <0.29) n=384	Quintile 4 (0.20 to <0.76) n=384	Quintile 5 (0.76 to 5.14) n=384
Food/food groups, servings/week					
Red meat	3.4 ± 3.0	3.2 ± 2.7	3.2 ± 2.8	3.3 ± 2.9	4.5 ± 4.5
Processed meat	1.7 ± 2.0	1.6 ± 1.7	1.6 ± 1.7	1.9 ± 2.1	2.9 ± 3.5
Sugar-sweetened beverages	0.7 ± 2.4	0.6 ± 2.0	0.6 ± 1.7	0.9 ± 2.2	4.1 ± 8.9
Tomatoes	3.9 ± 3.5	3.6 ± 3.2	3.4 ± 3.6	3.6 ± 3.6	4.1 ± 4.5
Refined grains	21.4 ± 12.2	20.3 ± 11.4	20.0 ± 11.6	21.6 ± 12.5	27.7 ± 16.6
Wholegrains	8.7 ± 5.7	8.1 ± 5.1	7.5 ± 5.3	7.5 ± 5.1	8.1 ± 6.4
Wine	3.4 ± 5.8	1.5 ± 2.8	0.8 ± 1.6	0.4 ± 1.3	0.2 ± 0.7
Fruit juice	4.9 ± 5.0	4.5 ± 4.4	4.3 ± 4.7	3.8 ± 4.0	3.8 ± 4.0
Dark-yellow vegetables	7.5 ± 5.3	6.0 ± 3.7	4.9 ± 4.1	4.5 ± 3.3	3.6 ± 2.9
Green-leafy vegetables	8.6 ± 6.5	6.8 ± 4.7	5.7 ± 4.0	4.7 ± 3.7	3.7 ± 3.2
Coffee or tea	29.4 ± 16.6	18.1 ± 9.9	13.5 ± 8.0	9.4 ± 7.1	7.0 ± 8.4
Pizza	0.5 ± 1.2	0.4 ± 0.5	0.3 ± 0.4	0.3 ± 0.4	0.2 ± 0.3
Nutrient intakes					
Total fiber, g/d	17.6 ± 7.2	16.3 ± 6.5	15.0 ± 6.4	14.5 ± 6.2	14.3± 6.9
Total carbohydrate, g/d	213 ± 76	200 ± 71	185 ± 74	186 ± 72	211 ± 95
Total protein, g/d	72.7 ± 28.8	66.0± 25.8	61.3 ± 26.1	61.5 ± 26.5	70.0 ± 34.6
Total fat, g/d	61.9 ± 33.0	57.0 ± 26.8	53.8 ± 30.9	55.4 ± 29.4	66.6 ± 41.7
Saturated fat, g/d	20.7 ± 11.7	19.3 ± 9.9	18.2 ± 11.4	18.2 ± 10.3	22.2± 14.8
Total cholesterol, g/d	230 ± 149	209 ± 116	194 ± 110	204 ± 138	246 ± 177
Dietary calcium, mg/d	928 ± 484	828 ± 437	758 ± 428	739 ± 422	739 ± 449
Lycopene, mcg/d	5362 ± 3647	4832 ± 3070	4551 ± 3187	4564 ± 3223	4559 ± 3456

EDIP, empirical dietary inflammatory pattern score

[1] Values are means ± standard deviations.

[2] EDIP scores were adjusted for total energy intake using the residual method. Lower EDIP scores indicate anti-inflammatory diets whereas higher scores indicate pro-inflammatory diets.

[3] The EDIP component foods (servings per day) in the WHI were the following: processed meat (hot dogs, chorizo, other sausage, bacon, breakfast sausage, scrapple; lunch meat such as ham, turkey; other lunch meat such as bologna); red meat (ground meat including hamburgers, beef, pork, and lamb as a main dish or as a sandwich; stew, pot pie, and casseroles with meat; gravies made with meat drippings); organ meat (liver, including chicken liver; other organ meats); fish other than dark-meat fish (fried fish, shrimp, lobster, crab and oysters, canned tuna, tuna salad, and tuna casserole, white fish such as sole, snapper, cod); other vegetables (i.e., vegetables other than green leafy vegetables and dark yellow vegetables: red peppers and red chilies, green peppers, green chilies, jalapenos, and green chili salsa, corn, and hominy); refined grains (total grain variable minus whole grain variable, both WHI-computed food groups); high-energy beverages [all regular (not diet) soft drinks]; low-energy beverages (the WHI FFQ did not assess low-energy beverages); tomatoes (fresh tomato, tomato juice, tomato sauce, cooked tomato, salsa and salsa picante); beer (all types); wine (red wine, white wine); coffee or tea (all types); dark-yellow vegetables (carrots, including mixed dishes with carrots; summer squash, zucchini, nopales, and okra; winter squash, such as acorn, butternut, and pumpkin; sweet potatoes and yams; other potatoes, cassava, and yucca—boiled, baked, or mashed); green leafy vegetables (cooked greens such as spinach, mustard greens, turnip greens, collards; lettuce and plain lettuce salad; mixed lettuce or spinach salad with vegetables); pizza (low-fat pizza; other pizza); fruit juice (orange juice and grapefruit juice; other fruit juices such as apple and grape); snacks (snacks such as potato chips, corn chips, tortilla chips, Ritz and cheese crackers; saltines, Snackwell's, fat-free tortilla chips and fat-free potato chips; popcorn).

Table 3.

Associations of the EDIP score with metabolites in the discovery (WHI-HT) and replication (WHI-OS) datasets[1,2,3,4]

Metabolite	HMDB #	Associations in WHI-HT (discovery, n=1109)		Associations in WHI-OS (replication, n=810)	
		Beta estimate (95%CI)	FDR –adjusted P-value	Beta estimate (95%CI)	FDR-adjusted P-value
Trigonelline	HMDB0000875	−1.32 (−1.59, −1.05)	8.88 E-19	−1.11 (−1.44, −0.78)	2.75 E-10
Caffeine	HMDB0001847	−0.48 (−0.74, −0.22)	0.007	−0.86 (−1.23, −0.49)	1.34 E-05
5-acetylamino-6-amino-3-methyluracil	HMDB0004400	−0.74 (−1.00, −0.48)	2.63 E-06	−0.80 (−1.16, −0.43)	2.81 E-05
7-methylxanthine	HMDB0001991	−0.52 (−0.81, −0.23)	0.007	−0.70 (−1.05, −0.34)	1.47 E-04
1,7-dimethyluric acid	HMDB0011103	−0.58 (−0.85, −0.31)	0.002	−0.72 (−1.10, −0.33)	2.73 E-04
3-methylxanthine	HMDB0001886	−0.55 (−0.84, −0.26)	0.006	−0.66 (−1.03, −0.30)	4.00 E-04
C52:3 TAG	HMDB0005384	0.43 (0.13, 0.73)	0.042	0.50 (0.17, 0.82)	0.003
Linoleate	HMDB0000673	0.61 (0.30, 0.92)	0.005	0.48 (0.11, 0.84)	0.011
C18:3 CE	HMDB0010370	−0.56 (−0.84, −0.28)	0.005	−0.38 (−0.72, −0.03)	0.032
Glycine	HMDB0000123	−0.42 (−0.70, −0.15)	0.032	−0.34 (−0.68, 0.01)	0.055
Isoleucine	HMDB0000172	0.38 (0.12, 0.65)	0.042	0.23 (−0.11, 0.58)	0.190
Uracil	HMDB0000300	−0.41 (−0.70, −0.13)	0.042	−0.31 (−0.61, 0.02)	0.067
C18:3 LPC	HMDB0010387	−0.50 (−0.80, −0.20)	0.016	−0.26 (−0.59, 0.08)	0.131
C24:0 LPC	HMDB0008038	−0.40 (−0.68, −0.13)	0.042	−0.19 (−0.51, 0.14)	0.261
Sebacate	HMDB0000792	−0.50 (−0.81, −0.18)	0.027	0.17 (−0.17, 0.52)	0.328
C34:0 PS	HMDB0012356	−0.39 (−0.67, −0.12)	0.045	−0.16 (−0.53, 0.20)	0.374
Cortisol	HMDB0000063	−0.55 (−0.84, −0.26)	0.006	−0.20 (−0.55, 0.15)	0.265
C12 carnitine	HMDB0002250	0.44 (0.15, 0.73)	0.032	0.20 (−0.16, 0.57)	0.277
C12:1 carnitine	HMDB0013326	0.53 (0.24, 0.81)	0.007	0.16 (−0.21, 0.52)	0.402
C14:1 carnitine	HMDB0002014	0.44 (0.15, 0.72)	0.032	0.22 (−0.15, 0.58)	0.245
C14:2 carnitine	HMDB0013331	0.42 (0.14, 0.71)	0.040	0.22 (−0.15, 0.58)	0.238
C56:5 TAG	HMDB0005406	0.45 (0.13, 0.77)	0.048	−0.06 (−0.40, 0.28)	0.910
C56:8 TAG	HMDB0005392	0.50 (0.21, 0.79)	0.012	−0.02 (−0.29, 0.26)	0.721

HMDB, Human Metabolome Database

[1] All values are beta estimates obtained from multivariable-adjusted linear regression models with 5-SD increments of the EDIP score as the main predictor of interest and metabolite as the main response variable of interest.

[2] Metabolites in **bold font** are the 10 replicated metabolites

[3] Models were adjusted for body mass index (continuous) age, physical activity, educational level, race/ethnicity, aspirin/NSAIDs use, smoking status, WHI Hormone Therapy trial arm, CHD case-control status.

[4] Statistical significance was defined as false-discovery rate adjusted p<0.05.

Table 4.

Associations of CRP with metabolites in the discovery (WHI-HT) and replication (WHI-OS) datasets [1,2,3,4]

Metabolite	HMDB #	Associations in WHI-HT (discovery, n=1109)		Associations in WHI-OS (replication, n=810)	
		Beta estimate (95%CI)	FDR-adjusted P-value	Beta estimate (95%CI)	FDR –adjusted P-value
Trigonelline	HMDB0000875	−0.09 (−0.13, −0.04)	**2.96 E-04**	−0.004 (−0.05, 0.04)	0.867
Caffeine	HMDB0001847	0.01 (−0.04, 0.05)	0.767	0.08 (0.04, 0.13)	**9.67 E-04**
5-acetylamino-6-amino-3-methyluracil	HMDB0004400	−0.02 (−0.06, 0.03)	0.431	−0.01 (−0.06, 0.03)	0.603
7-methylxanthine	HMDB0001991	−0.02 (−0.07, 0.02)	0.341	−0.02 (−0.06, 0.03)	0.477
1,7-dimethyluric acid	HMDB0011103	0.01 (−0.04, 0.05)	0.673	0.07 (0.02, 0.12)	**0.007**
3-methylxanthine	HMDB0001886	−0.01 (−0.06, 0.04)	0.708	0.01 (−0.04, 0.06)	0.642
C52:3 TAG	HMDB0005384	0.07 (0.02, 0.12)	**0.012**	0.06 (0.02, 0.11)	**0.005**
Linoleate	HMDB0000673	0.08 (0.03, 0.13)	**0.004**	0.02 (−0.02, 0.07)	0.307
C18:3 CE	HMDB0010370	−0.07 (−0.11, −0.02)	**0.007**	−0.02 (−0.07, 0.02)	0.339
Glycine	HMDB0000123	−0.18 (−0.23, −0.14)	**1.58 E-13**	−0.14 (−0.18, −0.10)	**1.72 E-09**
Isoleucine	HMDB0000172	0.12 (0.08, 0.16))	**8.32 E-07**	0.04 (−0.002, 0.09)	0.062
Uracil	HMDB0000300	−0.02 (−0.07, 0.02)	0.314	−0.06 (−0.10, −0.01)	**0.011**
C18:3 LPC	HMDB0010387	−0.18 (−0.23, −0.13)	**2.65 E-11**	−0.10 (−0.14, −0.05)	**2.33 E-05**
C24:0 LPC	HMDB0008038	−0.16 (−0.20, −0.11)	**3.82 E-10**	−0.12 (−0.16, −0.08)	**5.59 E-08**
Sebacate	HMDB0000792	−0.05 (−0.10, −0.001)	0.047	0.03 (−0.01, 0.08)	0.158
C34:0 PS	HMDB0012356	−0.04 (−0.09, 0.003)	0.069	0.03 (−0.02, 0.08)	0.202
C12 carnitine	HMDB0002250	0.03 (−0.01, 0.08)	0.152	0.01 (−0.04, 0.06)	0.665
C12:1 carnitine	HMDB0013326	0.07 (0.02, 0.12)	**0.005**	0.02 (−0.02, 0.07)	0.300
C14:1 carnitine	HMDB0002014	0.06 (0.01, 0.10)	**0.024**	0.01 (−0.04, 0.06)	0.703
C14:2 carnitine	HMDB0013331	0.03 (−0.02, 0.08)	0.187	0.01 (−0.04, 0.05)	0.785
Cortisol	HMDB0000063	−0.02 (−0.07, 0.03)	0.462	0.002 (−0.04, 0.05)	0.932
C56:5 TAG	HMDB0005406	−0.05 (−0.10, 0.002)	0.058	−0.01 (−0.04, 0.05)	0.699
C56:8 TAG	HMDB0005392	0.02 (−0.03, 0.07)	0.407	−0.02 (−0.06, 0.01)	0.212

[1] All values are beta estimates obtained from multivariable-adjusted linear regression modeling 5-unit increments of CRP levels as the main predictor of interest and metabolite as the main response variable of interest.

[2] Metabolites in **bold font** are the 10 replicated metabolites

[3] Models were adjusted for body mass index (continuous) age, physical activity, educational level, race/ethnicity, aspirin/NSAIDs use, smoking status, WHI Hormone Therapy trial arm, CHD case-control status.

[4] Statistical significance was defined as false-discovery rate adjusted p<0.05.

Table 5.

Metabolite discovery among normal weight women (n=630), and an evaluation of association with C-reactive protein[1,2,3]

Metabolite	HMDB #	Association with EDIP score		Association with CRP	
		Beta estimate (95%CI)	FDR-adjusted *P*-value	Beta estimate (95%CI)	FDR-adjusted *P*-value
5-acetylamino-6-amino-3-methyluracil	HMDB0004400	−1.07 (−1.48, −0.67)	9.72 E-05	−0.003 (−0.07, 0.07)	0.932
Trigonelline	HMDB0001847	−0.86 (−1.24, −0.48)	0.002	−0.01 (−0.08, 0.05)	0.736
1,7-dimethyluric acid	HMDB0011103	−0.86 (−1.28, −0.43)	0.008	0.05 (−0.02, 0.12)	0.392
Caffeine	HMDB0001847	−0.89 (−1.33, −0.45)	0.008	0.05 (−0.02, 0.13)	0.372

[1] All values are beta estimates obtained from multivariable-adjusted linear regression modeling 5-SD increments in EDIP or 5-unit increments in CRP as the main predictor of interest and metabolite as the main response variable of interest.

[2] Models were adjusted for body mass index (continuous) age, physical activity, educational level, race/ethnicity, aspirin/NSAIDs use, smoking status, WHI Hormone Therapy trial arm, CHD case-control status.

[3] Statistical significance was defined as false-discovery rate adjusted p<0.05.

Table 6.

Metabolite discovery among overweight or obese women (n=1289), and an evaluation of the associations with C-reactive protein [1,2,3,4,5]

Metabolite	Category and metabolic pathway	HMDB #	Association with EDIP score		Association with CRP	
			Beta estimate (95%CI)	FDR adjusted *P*-value	Beta estimate (95%CI)	FDR adjusted *P*-value
Trigonelline	Alkaloids and derivatives	HMDB0000875	−1.38 (−1.63, −1.13)	1.44 E-24	**−0.04 (−0.07, −00009)**	**0.049**
1,7–dimethyluric acid	Alkaloids and derivatives	HMDB0011103	−0.68 (−0.94, −0.42)	2.59 E-05	0.02 (−0.02, 0.06)	0.346
7–methylxanthine	Alkaloids and derivatives	HMDB0001991	−0.63 (−0.88, −0.37)	1.08 E-04	−0.02 (−0.06, 0.01)	0.202
3–methylxanthine	Alkaloids and derivatives	HMDB0001886	−0.62 (−0.87, −0.36)	1.08 E-04	−0.01 (−0.05, 0.03)	0.612
Caffeine	Alkaloids and derivatives	HMDB0001847	−0.54 (−0.79, −0.29)	7.33 E-04	0.02 (−0.01, 0.06)	0.199
Piperine	Alkaloids and derivatives	HMDB0029377	0.33 (0.08, 0.58)	0.034	0.01 (−0.02, 0.05)	0.482
C18:1 CE	Cholesterol esters	HMDB0000918	−0.43 (−0.70, 0.17)	0.010	**−0.06 (−0.09, −0.02)**	**0.006**
C18:2 CE	Cholesterol esters	HMDB0000610	−0.45 (−0.75, −0.19)	0.007	**−0.05 (−0.09, −0.01)**	**0.009**
C18:3 CE	Cholesterol esters	HMDB0010370	−0.52 (−0.78, −0.25)	0.002	−0.04 (−0.07, 0.002)	0.064
C20:2 CE	Cholesterol esters	HMDB0006734	−0.39 (−0.64, −0.13)	0.015	**−0.05 (−0.09, −0.02)**	**0.006**
C20:3 CE	Cholesterol esters	HMDB0006736	−0.34 (−0.61, −0.07)	0.043	0.02 (−0.02, 0.05)	0.394
C20:4 CE	Cholesterol esters	HMDB0006726	−0.33 (−0.59, −0.06)	0.047	−0.01 (−0.04, 0.03)	0.741
C22:4 CE	Cholesterol esters	HMDB0006729	−0.40 (−0.67, −0.14)	0.016	**−0.04 (−0.08, −0.002)**	**0.040**
C15:0 LPC	Lysophosphatidylcholines	HMDB0010381	−0.33 (−0.60, −0.06)	0.047	**−0.12 (−0.15, −0.08)**	**2.16 E-08**
C18:2 LPC	Lysophosphatidylcholines	HMDB0010386	−0.40 (−0.67, −0.14)	0.014	**−0.15 (−0.19, −0.12)**	**1.31 E-14**
C18:3 LPC	Lysophosphatidylcholines	HMDB0010387	−0.44 (−0.72, −0.17)	0.011	**−0.10 (−0.14, −0.06)**	**1.75 E-06**
C20:1 LPC	Lysophosphatidylcholines	HMDB0010391	−0.51 (−0.79, −0.24)	0.003	**−0.09 (−0.13, −0.05)**	**3.89 E-05**
C20:5 LPC	Lysophosphatidylcholines	HMDB0010397	−0.40 (−0.66, −0.14)	0.015	**−0.15 (−0.19, −0.11)**	**6.29 E-14**
C24:0 LPC	Lysophosphatidylcholines	HMDB0008038	−0.44 (−0.70, −0.18)	0.007	**−0.12 (−0.16, −0.08)**	**2.72 E-09**
C36:0 PC	Phosphatidylcholines	HMDB0008036	−0.33 (−0.60, −0.06)	0.048	−0.03 (−0.07, 0.01)	0.111
C40:11 PC plasmalogen	Phosphatidylcholine plasmalogens	NA	−0.34 (−0.59, −0.08)	0.038	0.01 (−0.03, 0.05)	0.653
C18:1 LPE	Lysophosphatidylethanolamines	HMDB0011506	−0.33 (−0.60, −0.06)	0.047	**−0.07 (−0.11, −0.04)**	**5.37 E-04**
C14:0 SM	Sphingomyelins	HMDB0012097	−0.45 (−0.72, −0.19)	0.007	0.01 (−0.03, 0.05)	0.633
C20:0 SM	Sphingomyelins	HMDB0012102	−0.34 (−0.61, −0.07)	0.043	0.01 (−0.02, 0.05)	0.471
C22:0 SM	Sphingomyelins	HMDB0012103	−0.33 (−0.61, −0.06)	0.047	0.03 (−0.01, 0.07)	0.109
C22:1 SM	Sphingomyelins	HMDB0012104	−0.32 (−0.59, −0.05)	0.050	−0.01 (−0.05, 0.03)	0.634

Metabolite	Category and metabolic pathway	HMDB #	Association with EDIP score		Association with CRP	
			Beta estimate (95%CI)	FDR adjusted *P*-value	Beta estimate (95%CI)	FDR adjusted *P*-value
C24:0 SM	Sphingomyelins	HMDB0011697	−0.41 (−0.68, −0.14)	0.015	−0.01 (−0.04, 0.03)	0.764
C24:1 SM	Sphingomyelins	HMDB0012107	−0.34 (−0.60, −0.07)	0.043	**0.05 (0.01, 0.09)**	**0.016**
5-acetylamino-6-amino-3-methyluracil	Amino acids	HMDB0004400	−0.84 (−1.09, −0.58)	1.70 E-08	−0.01 (−0.04, 0.03)	0.723
Phenylalanine-d8	Internal Standard		−0.44 (−0.72, −0.17)	0.012	0.003 (−0.04, 0.04)	0.887
Glycine	Amino acids	HMDB0000123	−0.40 (−0.65, −0.15)	0.013	**−0.14 (−0.18, −0.11)**	**3.12 E-13**
Asparagine	Amino acids	HMDB0000168	−0.36 (−0.61, −0.11)	0.022	**−0.10 (−0.13, −0.06)**	**1.27 E-06**
Taurine	Amino acids	HMDB0000251	−0.36 (−0.63, −0.09)	0.032	0.005 (−0.03, 0.04)	0.817
Proline betaine	Amino acids	HMDB0004827	−0.35 (−0.60, −0.09)	0.034	−0.02 (−0.06, 0.02)	0.311
ADMA	Amino acids	HMDB0001539	−0.34 (−0.61, −0.07)	0.043	**−0.05 (−0.09, −0.01)**	**0.016**
Serine	Amino acids	HMDB0000187	−0.33 (−0.60, −0.06)	0.048	**−0.12 (−0.16, −0.08)**	**2.96 E-08**
N-methylproline	Amino acids	NA	−0.31 (−0.57, −0.05)	0.050	−0.03 (−0.06, 0.01)	0.174
Isoleucine	Amino acids	HMDB0000172	0.31 (0.07, 0.56)	0.043	**0.07 (0.03, 0.10)**	**4.45 E-04**
Uracil	Purines and pyrimedines	NA	−0.47 (−0.74, −0.21)	0.005	−0.03 (−0.07, 0.01)	0.130
Uridine	Purines and pyrimedines	NA	−0.38 (−0.64, −0.13)	0.016	−0.04 (−0.07, 0.001)	0.060
AMP	Purines and pyrimedines	NA	−0.33 (−0.60, −0.06)	0.046	0.03 (−0.01, 0.07)	0.110
GMP	Purines and pyrimedines	NA	−0.33 (−0.60, −0.06)	0.047	0.01 (−0.03, 0.05)	0.495
Indole-3-propionate	Indoles and derivatives	NA	−0.31 (−0.57, −0.06)	0.047	**−0.05 (−0.09, −0.01)**	**0.010**
Cortisol	Steroids	HMDB0000063	−0.46 (−0.73, −0.20)	0.006	−0.01 (−0.05, 0.03)	0.605
Pantothenate	Vitamins and minerals	HMDB0000210	−0.37 (−0.63, −0.11)	0.022	0.01 (−0.03, 0.05)	0.555
Hexose-monophosphate	NA	NA	−0.31 (−0.58, −0.05)	0.050	0.02 (−0.02, 0.06)	0.320
Eicosapentaenoate	Fatty acids	HMDB0001999	−0.46 (−0.71, −0.21)	0.004	**−0.09 (−0.12, −0.05)**	**1.19 E-05**
2-hydroxyhexadecanoate	Fatty acids	HMDB0031057	0.33 (0.06, 0.59)	0.047	0.02 (−0.02, 0.06)	0.302
C32:0 DAG	Diacylglycerols	HMDB0007098	0.49 (0.23, 0.75)	0.003	**0.07 (0.03, 0.11)**	**6.73 E-04**
C32:1 DAG	Diacylglycerols	HMDB0007099	0.40 (0.14, 0.66)	0.014	**0.04 (0.01, 0.08)**	**0.022**
C34:0 DAG	Diacylglycerols	HMDB0007100	0.49 (0.22, 0.76)	0.004	**0.04 (0.002, 0.08)**	**0.037**
C34:1 DAG	Diacylglycerols	HMDB0007102	0.53 (0.27, 0.79)	0.001	**0.06 (0.03, 0.10)**	**0.001**
C34:2 DAG	Diacylglycerols	HMDB0007103	0.51 (0.25, 0.77)	0.002	**0.05 (0.01, 0.09)**	**0.008**
C34:3 DAG	Diacylglycerols	HMDB0007132	0.40 (0.14, 0.66)	0.014	0.02 (−0.01, 0.06)	0.193
C36:1 DAG	Diacylglycerols	HMDB0007216	0.50 (0.24, 0.76)	0.002	**0.05 (0.01, 0.08)**	**0.018**

Metabolite	Category and metabolic pathway	HMDB #	Association with EDIP score		Association with CRP	
			Beta estimate (95%CI)	FDR adjusted *P*-value	Beta estimate (95%CI)	FDR adjusted *P*-value
C36:0 DAG	Diacylglycerols	HMDB0007158	0.36 (0.10, 0.61)	0.028	0.002 (−0.03, 0.04)	0.918
C36:2 DAG	Diacylglycerols	HMDB0007218	0.47 (0.20, 0.73)	0.005	0.03 (−0.01, 0.07)	0.108
C36:3 DAG	Diacylglycerols	HMDB0007219	0.44 (0.17, 0.71)	0.010	0.01 (−0.03, 0.05)	0.664
C36:4 DAG–A	Diacylglycerols	HMDB0007248	0.34 (0.07, 0.60)	0.042	−0.01 (−0.03, 0.04)	0.739
C36:4 DAG–B	Diacylglycerols	HMDB0007248	0.36 (0.11, 0.61)	0.022	**0.04 (0.003, 0.07)**	**0.034**
C38:3 DAG	Diacylglycerols	HMDB0007168	0.41 (0.14, 0.68)	0.014	0.02 (−0.02, 0.06)	0.291
C48:0 TAG	Triacylglycerols	HMDB0005356	0.39 (0.09, 0.61)	0.035	**0.07 (0.03, 0.11)**	**5.37 E-04**
C48:1 TAG	Triacylglycerols	HMDB0005359	0.38 (0.12, 0.64)	0.021	**0.05 (0.01, 0.09)**	**0.012**
C50:0 TAG	Triacylglycerols	HMDB0005357	0.36 (0.10, 0.62)	0.028	**0.06 (0.02, 0.09))**	**0.005**
C50:1 TAG	Triacylglycerols	HMDB0044109	0.54 (0.29, 0.80)	7.33 E-04	**0.08 (0.04, 0.12)**	**8.94 E-05**
C50:2 TAG	Triacylglycerols	HMDB0005377	0.50 (0.24, 0.75)	0.002	**0.07 (0.04, 0.11)**	**4.02 E-04**
C50:3 TAG	Triacylglycerols	HMDB0005433	0.39 (0.13, 0.65)	0.015	**0.04 (0.002, 0.08)**	**0.040**
C51:1 TAG	Triacylglycerols	HMDB0042104	0.36 (0.11, 0.62)	0.024	**0.04 (0.01, 0.08)**	**0.024**
C51:2 TAG	Triacylglycerols	HMDB0049721	0.30 (0.05, 0.56)	0.050	0.03 (−0.01, 0.07)	0.106
C51:3 TAG	Triacylglycerols	NA	0.36 (0.10, 0.62)	0.028	0 (−0.04, 0.04)	0.990
C52:0 TAG	Triacylglycerols	HMDB0005365	0.34 (0.08, 0.61)	0.039	0.03 (−0.01, 0.07)	0.103
C52:1 TAG	Triacylglycerols	HMDB0005367	0.47 (0.22, 0.73)	0.004	**0.08 (0.04, 0.12)**	**0.002**
C52:2 TAG	Triacylglycerols	HMDB0005369	0.56 (0.31, 0.82)	5.77 E-04	**0.06 (0.03, 0.10)**	**0.002**
C52:3 TAG	Triacylglycerols	HMDB0005384	0.56 (0.30, 0.82)	7.33 E-04	**0.05 (0.01, 0.08)**	**0.018**
C52:4 TAG	Triacylglycerols	HMDB0005363	0.41 (0.15, 0.68)	0.014	0.01 (−0.03, 0.05)	0.590
C54:1 TAG	Triacylglycerols	HMDB0005395	0.36 (0.10, 0.62)	0.028	0.04 (−0.001, 0.07)	0.057
C54:2 TAG	Triacylglycerols	HMDB0005403	0.47 (0.21, 0.73)	0.004	**0.04 (0.01, 0.08)**	**0.020**
C54:3 TAG	Triacylglycerols	HMDB0005405	0.43 (0.17, 0.70)	0.010	0.02 (−0.02, 0.05)	0.397
C56:8 TAG	Triacylglycerols	HMDB0005392	0.43 (0.16, 0.70)	0.013	0.03 (−0.01, 0.06)	0.197
Glycolithocholate	Bile acids	HMDB0000698	0.32 (0.08, 0.56)	0.036	−0.03 (−0.06, 0.01)	0.120
Deoxycholate isomer–G	Bile acids	NA	0.30 (0.05, 0.55)	0.050	**−0.04 (−0.07, −0.001)**	**0.044**
C6 carnitine	Acylcarnitines	HMDB0000705	0.32 (0.05, 0.59)	0.047	**0.04 (0.01, 0.08)**	**0.022**

[1] All values are beta estimates obtained from multivariable-adjusted linear regression modeling 5-SD increments in EDIP score or 5-unit increments in CRP levels as the main predictor of interest and metabolite as the main response variable of interest.

[2] Metabolites in **bold font** are 9 of the 10 replicated metabolites or metabolites (n=40 metabolites) also significantly associated with CRP.

[3] Models were adjusted for body mass index (continuous) age, physical activity, educational level, race/ethnicity, aspirin/NSAIDs use, smoking status, WHI Hormone Therapy trial arm, CHD case-control status.

[4] Statistical significance was defined as false-discovery rate adjusted p<0.05.

[5] In the multivariable models, 110 metabolites were significantly associated with the EDIP score and 83 remained significant after additionally adjusting for BMI. In multivariable-adjusted models, 196 metabolites were significantly associated with CRP and 178 remained significant with additional adjustment for continuous BMI. Of the 83 metabolites associated with EDIP, 40 (48%) were also associated with CRP.