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# Differences in Metabolomic Profiles Between Black and White Women and Risk of Coronary Heart Disease: an Observational Study of Women From Four US Cohorts

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**BACKGROUND:** Racial differences in metabolomic profiles may reflect underlying differences in social determinants of health by self-reported race and may be related to racial disparities in coronary heart disease (CHD) among women in the United States. However, the magnitude of differences in metabolomic profiles between Black and White women in the United States has not been well-described. It also remains unknown whether such differences are related to differences in CHD risk.

**METHODS:** Plasma metabolomic profiles were analyzed using liquid chromatography-tandem mass spectrometry in the WHI-OS (Women's Health Initiative-Observational Study; 138 Black and 696 White women), WHI-HT trials (WHI-Hormone Therapy; 156 Black and 1138 White women), MESA (Multi-Ethnic Study of Atherosclerosis; 114 Black and 219 White women), JHS (Jackson Heart Study; 1465 Black women with 107 incident CHD cases), and NHS (Nurses' Health Study; 2506 White women with 136 incident CHD cases). First, linear regression models were used to estimate associations between self-reported race and 472 metabolites in WHI-OS (discovery); findings were replicated in WHI-HT and validated in MESA. Second, we used elastic net regression to construct a racial difference metabolomic pattern (RDMP) representing differences in the metabolomic patterns between Black and White women in the WHI-OS; the RDMP was validated in the WHI-HT and MESA. Third, using conditional logistic regressions in the WHI (717 CHD cases and 719 matched controls), we examined associations of metabolites with large differences in levels by race and the RDMP with risk of CHD, and the results were replicated in Black women from the JHS and White women from the NHS.

**RESULTS:** Of the 472 tested metabolites, levels of 259 (54.9%) metabolites, mostly lipid metabolites and amino acids, significantly differed between Black and White women in both WHI-OS and WHI-HT after adjusting for baseline characteristics, socioeconomic status, lifestyle factors, baseline health conditions, and medication use (false discovery rate <0.05); similar trends were observed in MESA. The RDMP, composed of 152 metabolites, was identified in the WHI-OS and showed significantly different distributions between Black and White women in the WHI-HT and MESA. Higher RDMP quartiles were associated with an increased risk of incident CHD (odds ratio=1.51 [0.97–2.37] for the highest quartile comparing to the lowest;  $P_{\text{trend}}=0.02$ ), independent of self-reported race and known CHD risk factors. In race-stratified analyses, the RDMP-CHD associations were more pronounced in White women. Similar patterns were observed in Black women from the JHS and White women from the NHS.

**CONCLUSIONS:** Metabolomic profiles significantly and substantially differ between Black and White women and may be associated with CHD risk and racial disparities in US women.

**GRAPHIC ABSTRACT:** A [graphic abstract](#) is available for this article.

**Key Words:** heart diseases ■ health status disparities ■ metabolomics ■ plasma ■ race ■ women

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**In this Issue, see p 559 | Meet the First Author, see p 560 | Editorial, see p 616**

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## Novelty and Significance

### What Is Known?

- In the United States, Black women have higher prevalence of coronary heart disease (CHD) than White women, and such differences cannot be fully explained by racial disparities in education, income, and known CHD risk factors.
- Metabolomic profiles are influenced by many external factors (eg, diet, lifestyle, environmental pollution, and social exposures) and have been associated with risk of CHD.
- Limited data have shown some differences in metabolomic profiles between Black and White individuals in the United States, but the magnitude of such differences has not been well-described.

### What New Information Does This Article Contribute?

- Levels of several metabolites, especially lipid metabolites and amino acids, significantly differed between Black and White women.
- We identified a racial difference metabolomic pattern composed of 152 metabolites that had significantly different distributions between Black and White women.
- The racial difference metabolomic pattern and a few lipid metabolites with large differences ( $>1$  SD) by race were significantly associated with incident CHD, independent of self-reported race and known risk factors for CHD.

Differences in CHD risk between Black and White women in the United States have been well-recognized and cannot be fully explained by racial differences in socioeconomic status and known CHD risk factors. Metabolomic profiles reflect the status of human metabolism, which is influenced by many external factors including social exposures. Alterations in metabolomic profiles have been associated with CHD risk in women. However, it remains unknown the magnitude of differences in metabolomic profiles between Black and White women and their roles in racial differences in CHD risk. In this study, we observed substantial and significant differences in metabolomic profiles between Black and White women. We identified and validated a racial difference metabolomic pattern that characterizes differences in the metabolomic patterns between Black and White women. We also found that a few metabolites with large differences ( $>1$  SD) by race and the racial difference metabolomic pattern were significantly associated with risk of incident CHD, independent of self-reported race and known cardiovascular risk factors. Our findings indicate that metabolomics may act as a tool that sums the impact of cumulative exposures to differences in the lived and social experiences between Black and White women on racial disparities in cardiovascular diseases.

### Nonstandard Abbreviations and Acronyms

<b>BMI</b>	body mass index
<b>CHD</b>	coronary heart disease
<b>CVD</b>	cardiovascular disease
<b>FDR</b>	false discovery rate
<b>HDL</b>	high-density lipoprotein
<b>JHS</b>	Jackson Heart Study
<b>MESA</b>	Multi-Ethnic Study of Atherosclerosis
<b>NHS</b>	Nurses' Health Study
<b>OR</b>	odds ratio
<b>RDMP</b>	racial difference metabolomic pattern
<b>WHI-HT</b>	WHI-Hormone Therapy
<b>WHI-OS</b>	Women's Health Initiative-Observational Study

The prevalence and mortality rates of coronary heart disease (CHD) in the United States has been declining over the past several decades; however, racial disparities in CHD have remained.<sup>1,2</sup> Compared with non-Hispanic White women, Black/

African American women have higher CHD morbidity and mortality as well as significantly and consistently poorer cardiovascular health.<sup>2,3</sup> Classifications of race are social and cultural constructs,<sup>4</sup> which both create and reflect racial differences in lifetime exposure to environmental (eg, physical environment), social (eg, socioeconomic status, cultural factors, and structural racism), and individual (eg, lifestyle and behavioral factors) factors contributing to health.<sup>5,6</sup> However, racial disparities in CHD between Black and White women cannot be fully explained by racial differences in socioeconomic status and other known risk factors for CHD, which are mostly individual level factors.<sup>7-9</sup> Racial disparities in health are also caused by adverse social factor exposures at the neighborhood level, which are usually hard to quantify.<sup>6,9</sup> Given the difficulties in measuring the impact of social exposures on health outcomes, there is a critical need to identify novel measures of the cumulative impact of the social experience of race on cardiovascular health disparities.<sup>6</sup>

Recent advances in metabolomic profiling enable the assessment of a wide array of small-molecule metabolites that reflect human metabolic status. Human metabolism

is partially determined by the genome<sup>10</sup> but is influenced by a large variety of exogenous factors, including dietary and lifestyle factors, environmental exposures, and social exposures.<sup>11–14</sup> Recently, metabolomic alterations have been associated with incident CHD.<sup>15–17</sup> Limited data on differences in the metabolomic profiles between Black and White individuals in the United States are available, with small sample sizes in populations with high prevalence of specific conditions, such as overweight/obesity (n=500),<sup>18</sup> high blood pressure (n=52),<sup>19</sup> or bladder cancer (n=73).<sup>20</sup> Thus, the magnitude of differences in the metabolomic profiles by race in the United States has not been well-described. It remains unknown whether differences in metabolomic profiles between racial groups might contribute to racial disparities in CHD.

In this study, we determined whether the metabolomic profiles of Black women differ from those of White women, and then investigated whether differences in the metabolomic patterns between Black and White women might be associated with CHD risk, based on data from the Women's Health Initiative (WHI).<sup>15</sup> We performed discovery analyses to (1) estimate differences in metabolite levels between Black and White women and (2) established the racial difference metabolomic pattern (RDMP) representing differences in the metabolomic pattern by race in the WHI-OS (WHI-Observational Study), and these findings were internally validated in the WHI-HT (WHI-Hormone Therapy) trials and externally replicated in the MESA (Multi-Ethnic Study of Atherosclerosis) to assess the generalizability. To estimate the impact of racial differences in metabolomic patterns on racial disparities in CHD risk, we estimated associations of metabolites showing large race differences and the RDMP with incident CHD risk in the WHI and estimated race-specific associations. Findings were then replicated in Black women from the JHS (Jackson Heart Study) and White women from the NHS (Nurses' Health Study).

## METHODS

### Data Availability

WHI and NHS metabolomics data used in this study are available from the corresponding author upon reasonable request and compliance with the data request processes associated with each cohort. Data access for the MESA was approved by the TOPMed Publications and Presentations Steering Committees with data access provided by an approved project (#10106). The JHS metabolomics data used in this study have been submitted to the JHS Data Coordinating Center and to dbGaP; until posted in dbGaP, all JHS data are available from the JHS Data Coordinating Center on request.

### Study Population

In the WHI, MESA, and NHS, participants were asked to self-report their race and ethnicity. Black indicates self-reported

Black or African American (not of Hispanic origin), and White indicates self-reported White (not of Hispanic origin). We sought to examine differences in metabolomic profiles between Black and White women, male participants (from MESA and JHS) and participants who reported other races and ethnicities were excluded. The JHS only recruited participants who self-identified as Black. All participants included in this study were free of known clinical cardiovascular disease (CVD) at baseline. The study protocols were approved by the Institutional Review Board of Mass General Brigham/Brigham and Women's Hospital, the MESA Metabolomics Working Group, and the Beth Israel Deaconess Medical Center. A detailed description of the study cohorts is included in the [Supplemental Methods](#). A flow chart of the analysis approach and the role of each of the cohorts is included in [Figure S1](#).

### Women's Health Initiative

The WHI-OS enrolled 93 676 postmenopausal women across the United States, between 1994 and 1998, who were ineligible or unwilling to participate in the WHI hormone or dietary trials. In the WHI-HT, one of the trials randomly assigned 16 608 postmenopausal women with an intact uterus to estrogen-plus-progestin or placebo, whereas in the other trial 10 739 women with prior hysterectomy were randomly assigned to estrogen or placebo. All participants provided written informed consent. Women included in this study were drawn from a prior nested case-control study of the metabolomics of CHD and were free of CVDs at the study baseline.<sup>15</sup> CHD was defined as myocardial infarction or death attributable to CHD. Each CHD case was matched to a control on baseline age (5-year range), self-reported race, hysterectomy status, and enrollment groups (2-year range).<sup>15</sup>

All WHI participants included in this study had baseline metabolomics data and had no missing covariate data. In analyses examining racial differences in metabolomic profiles, the discovery/training set included 834 women (Black/White: 138/696) from the WHI-OS, and the validation/testing set included 1294 women (Black/White: 156/1138) from the WHI-HT.

In analyses examining associations of metabolites with large differences by race and the RDMP with CHD risk, the discovery set combined women from both WHI-OS and WHI-HT placebo arms to maximize the statistical power. We excluded women from the WHI-HT intervention arms from the CHD analysis because (1) active hormone therapy (HT) use was found to substantially change metabolomic profiles from baseline<sup>21</sup> and (2) estrogen-plus-progestin use was associated with increased risk of CHD in the WHI while estrogen-alone use was not.<sup>22,23</sup> After exclusions, 717 incident CHD cases (Black/White: 109/608), with a median time to event of 4.8 years, and 719 matched controls (Black/White: 108/611) were included in the analysis.

### Multi-Ethnic Study of Atherosclerosis

As a replication set in analyses examining racial differences in metabolomic profiles, 333 women (Black/White: 114/219) from the MESA who had available baseline metabolomics data (blood samples were collected between 2000 and 2002) were included. All participants in the MESA cohort provided written informed consent for participation. Women included in this study were drawn from a multi-omics pilot study in which participants were randomly selected.

### Jackson Heart Study

As a replication set in analyses examining associations of metabolites with large differences by race and the RDMP with CHD risk in Black women, we included 1465 Black women from a previous study of the metabolomics of CHD in the JHS, who were free of CVD at baseline and had available baseline metabolomics data (blood samples were collected between 2000 and 2004).<sup>16</sup> CHD was defined as definite fatal CHD, definite or probable myocardial infarction, silent myocardial infarction between examinations (as determined by electrocardiography), or coronary revascularization. During a median follow-up of 11.7 years, 107 incident CHD cases were documented.<sup>16</sup>

### Nurses' Health Study

As a replication set in analyses examining associations of metabolites with large differences by race and the RDMP with CHD risk in White women, this study included 2506 White women from the NHS, who were free of CVD and cancers at the time of blood collection (between 1989 and 1990) and had both metabolomic profiles and blood lipid data measured previously.<sup>24</sup> Metabolomic data were available from 10 prior substudies (nested case-control studies) in the NHS that were originally designed for different outcomes<sup>25</sup> (see details in the [Supplemental Methods](#)). CHD was defined as fatal or nonfatal myocardial infarction or coronary death. During a median follow-up of 24.3 years, 136 incident CHD cases were documented.

### Metabolomics Profiling

Metabolomic profiling in baseline plasma samples of participants from the WHI was performed using liquid chromatography-tandem mass spectrometry at the Broad Institute,<sup>15</sup> and the same methods were used for MESA, JHS, and NHS samples. A detailed description of metabolomics profiling methods for each cohort is included in the [Supplemental Methods](#). Metabolites with >20% missing values were excluded from the analysis. After quality control, 472 named metabolites were used in the WHI analyses. Of these 472 metabolites, 322 were available in the MESA, 101 were available in the JHS, and 169 were available in the NHS. Differences in the number of available metabolites between cohorts were mainly due to differences in liquid chromatography-tandem mass spectrometry panels used and the number of metabolites annotated. For the metabolites included in the analyses, missing values were imputed to one-half the minimum observed value in the WHI, MESA, and JHS. In the NHS, the imputation was performed within each substudy (see details in the [Supplemental Methods](#)).

### Covariates

Information on age, lifestyle factors (smoking, alcohol consumption, and physical activity), body mass index (BMI), education, family income, female-specific variables (hysterectomy, menopausal status, and HT use), baseline health conditions (diabetes, hypertension, and depression), medication use (aspirin, lipid-lowering, antihyperglycemic, antihypertensive, and antidepressants), dietary factors (macronutrients intake, total calorie intake, and Healthy Eating Index-2005<sup>26</sup>), and psychological indicators (emotional well-being, hostility, general health, optimism, social support, social functioning, social

strain, and sleep disturbance) was collected at study baseline in the WHI, MESA, and JHS. In the NHS, the above information was collected from biennial questionnaires preceding blood collections and a separate questionnaire completed at the time of blood draw. Total and HDL (high-density lipoprotein) cholesterol levels were measured in plasma samples for all cohorts included in this study. Fasting glucose levels were available in a subset of participants in the WHI-OS (n=217) and the WHI-HT (n=1289).

### Statistical Analyses

Metabolite levels were converted to standard normal distributions using inverse normal transformation. Statistical analyses were carried out using R. The statistical analysis approaches are illustrated in [Figure S1](#).

### Metabolome-Wide Association Analysis of Race

#### Discovery

In WHI-OS, we used linear regression to estimate associations between levels of each metabolite (continuous; dependent variables) and self-reported race (binary; Black versus White), adjusting for CHD case-control status, matching factors (age, hysterectomy, and enrollment window), HT use status (never/past/current users), BMI, smoking status, alcohol consumption, education, family income, physical activity, baseline health conditions (diabetes, hypertension, and depression), and medication use (aspirin, lipid-lowering, antihyperglycemic, antihypertensive, and antidepressants). The false discovery rate (FDR) was calculated using the Benjamini-Hochberg procedure to account for multiple comparison, and an FDR<0.05 was considered significant. Sensitivity analyses were performed with additional adjustment for total and HDL-cholesterol, dietary factors (Healthy Eating Index-2005 and intake of proteins, total carbohydrates, total fat, and total calories), fasting glucose levels (in a subset of participants), or psychological characteristics (emotional well-being, hostility, general health, optimism, social support, social functioning, social strain, and sleep disturbance).

#### Validation

Metabolites with an FDR<0.05 in the WHI-OS were then tested in the WHI-HT using linear regression models adjusting for all above covariates plus HT trial type (estrogen-plus-progestin or estrogen-alone trial) and randomized treatment arms (intervention or placebo). Metabolites with an FDR<0.05 in both WHI-OS and WHI-HT were considered to be validated and, therefore, significantly differ between Black and White women.

#### Replication

All validated metabolites were tested as available in the MESA; linear regression models were adjusted for age, BMI, HT use, smoking, alcohol consumption, education, family income, physical activity, baseline health conditions (diabetes, hypertension, and depression), and medication use (aspirin, lipid-lowering, antihyperglycemic, antihypertensive, and antidepressants). Sensitivity analyses were performed with additional adjustment for total and HDL-cholesterol levels.

We calculated Pearson correlation coefficients to estimate the concordance of association coefficients estimated in the WHI-OS and those in the WHI-HT and MESA.

## Estimation and Validation of the RDMP

### Training

Elastic net regression was used in the WHI-OS to select a sparse metabolite set associated with self-reported race and to estimate a corresponding metabolite score of racial differences (the RDMP score). The elastic net regression is a regularized regression that shrinks regression coefficients without indiscriminate elimination of correlated predictors.<sup>27</sup> The R package *glmnet* was used to fit the elastic net regression model with adjustment for age.<sup>28</sup> The optimal model was chosen using 10-fold cross-validation. We included all 472 metabolites in the elastic net model. Regression coefficients of each selected metabolite and age in the optimal model were used to calculate the RDMP.

### Testing

For internal testing, we calculated the RDMP and compared its distribution between Black and White women in the WHI-HT. For external testing, the RDMP was calculated in the MESA, and the distribution between races was compared. To avoid overfitting, the RDMP was calculated in the training set (WHI-OS) using leave-one-out cross-validation. This approach has been used previously to estimate a metabolic signature that robustly reflects the adherence and metabolic response to a Mediterranean diet.<sup>24</sup> We also calculated the RDMP in the JHS and NHS. Additionally, since not all the metabolites used to calculate the RDMP were available in MESA, JHS, and NHS, 3 restricted RDMP scores (rRDMP) were calculated using overlapping metabolites in the WHI-HT (ie, rRDMP1 for overlapping metabolites with the MESA, rRDMP2 for overlapping metabolites with the JHS, and rRDMP3 for overlapping metabolites with the NHS). Finally, to estimate the performance of the RDMP, we calculated the area under the receiver operating characteristic curves for self-reported race in both WHI and MESA.

### Associations of Metabolites That Differed by Race and the RDMP With CHD Risk

Because CHD cases were matched to controls in the WHI (combining women from the WHI-OS and placebo arms of the WHI-HT), we applied conditional logistic regression models to estimate associations of selected metabolites (whose levels significantly [FDR<0.05] differed by >1-SD and 0.5-1 SD between Black and White women; continuous) and the RDMP (Z score and in quartiles) with incident CHD, adjusting for substudies (OS/estrogen-alone trial/estrogen-plus-progestin trial), BMI, HT use status, smoking status, diabetes, antihyperglycemic medication use, systolic blood pressure, antihypertensive medication use, aspirin use, lipid-lowering medication use, and total and HDL-cholesterol. We selected 0.5-SD and 1-SD as the cut points because such differences were considered large in magnitude that might potentially be clinically significant. Finally, sensitivity analyses were performed with additional adjustment for education, family income, diet quality, and psychosocial characteristics.

In stratified analyses, we repeated the above analyses separately in Black and White women in the WHI. Because almost all the Black women were distributed in the highest quartile of the RDMP (Figure S2), we calculated race-specific RDMP quartiles and estimated their associations with CHD risk in Black and White women separately, adjusting for the same set of covariates. As a replication, we examined the

associations of metabolites with large differences by race (differed >0.5-SD in WHI-HT) and the RDMP with incident CHD risk in Black women from the JHS and White women from the NHS. Cox proportional-hazards models were used to estimate associations of metabolites (continuous) and RDMP (Z score and in quartiles) with incident CHD, adjusting for batch (JHS only), age, BMI, menopausal status, HT use status, smoking status, diabetes, antihyperglycemic medication use, systolic blood pressure, antihypertensive medication use, aspirin use, lipid-lowering medication use, and total and HDL-cholesterol. Similarly, sensitivity analyses were performed with additional adjustment for education (WHI and JHS), family income (WHI and JHS), diet quality (WHI and NHS), and psychosocial characteristics (WHI only).

## RESULTS

In the WHI, Black women were relatively younger, had a higher BMI and HDL-cholesterol, and had lower physical activity, alcohol intake, dietary quality, education, family income, and total cholesterol, compared with White women. Black women were more likely to be current smokers and had higher prevalence of diabetes and hypertension (Table 1). Baseline characteristics of MESA and WHI participants were mostly similar but women in MESA were younger than the WHI (Table S1). MESA participants had higher total physical activity because both leisure and nonleisure physical activity were assessed, whereas only leisure physical activity was assessed in the WHI. Women from the JHS and NHS were also younger than those from the WHI, but their characteristics were mostly similar to Black and White women in the WHI, respectively (Table S2).

### Differences in Individual Metabolites Between Black and White Individuals

Of 472 tested metabolites, 259 (54.9%) significantly differed between Black and White women (FDR<0.05) in both WHI-OS and WHI-HT in the fully adjusted model (Figure 1 and Table S3). A majority of these metabolites were lipids (n=181; including 75 glycerolipids, 80 glycerophospholipids, 15 sterol lipids, and 11 sphingolipids), followed by amino acids (n=21), purines and pyrimidines (n=14), fatty acids (n=10), and other metabolites across different categories (n=33).

Figure 2 shows the direction and magnitude of the association between self-reported race and each metabolite. In the fully adjusted model (model 1), most of the lipid metabolites had lower abundance in Black than White women, except for several long-chain polyunsaturated triacylglycerols (C56-C60 with 8-12 double-bonds), phosphatidylcholine plasmalogens, phosphatidylethanolamine plasmalogens, cholesteryl esters, and sphingomyelins. Additional adjustment for total and HDL-cholesterol slightly reduced the number of lipid metabolites and amino acids that differed by race but not metabolites from other metabolite classes. Results from

**Table 1. Baseline Characteristics of WHI Participants by Race**

Mean ± SD or n (%)	Women from the WHI-OS			Women from the WHI-HT		
	Black (n = 138)	White (n = 696)	P	Black (n = 156)	White (n=1138)	P
Case/control status, CHD cases%	50.0	50.0		50.0	50.0	
Age, years	65.7 ± 7.9	67.9 ± 6.3	0.0003	64.2 ± 6.9	67.2 ± 6.8	3.0×10 <sup>-7</sup>
Body mass index, kg/m <sup>2</sup>	30.6 ± 6.3	27.5 ± 6.1	1.2×10 <sup>-7</sup>	31.7 ± 6.2	29.0 ± 5.8	1.2×10 <sup>-7</sup>
Alcohol intake, g/day	2.5 ± 10.8	5.8 ± 11.0	0.001	2.1 ± 7.2	4.6 ± 11.0	0.01
Physical activity, MET-hour/week	8.4 ± 11.6	13.4 ± 13.3	0.0001	8.7 ± 15.3	9.6 ± 11.1	0.39
Total cholesterol, mg/dL	218.2 ± 44.7	234.6 ± 46.9	0.0002	235.5 ± 48.4	237.3 ± 40.0	0.59
HDL-cholesterol, mg/dL	54.5 ± 16.3	54.0 ± 17.0	0.76	52.3 ± 13.2	48.9 ± 12.4	0.001
Hysterectomy	72 (52.2%)	280 (40.2%)	0.01	117 (75%)	579 (50.9%)	2.4×10 <sup>-8</sup>
Baseline diabetes	28 (20.3%)	61 (8.8%)	0.0001	35 (22.4%)	154 (13.5%)	0.005
Baseline hypertension	89 (64.5%)	367 (52.7%)	0.01	123 (78.8%)	617 (54.2%)	9.3×10 <sup>-9</sup>
Depressive disorders	52 (37.7%)	291 (41.8%)	0.42	73 (46.8%)	465 (40.9%)	0.19
Healthy Eating Index-2005	65.3 ± 12.8	69.2 ± 10.7	0.0002	61.7 ± 11.1	66.8 ± 10.6	3.6×10 <sup>-8</sup>
Dietary proteins, g/day	59.4 ± 31.0	67.5 ± 28.8	0.004	64.3 ± 32.8	69.0 ± 29.4	0.08
Dietary total carbohydrate, g/day	188.0 ± 83.0	201.9 ± 74.8	0.06	201.8 ± 99.8	199.0 ± 77.3	0.70
Dietary total fat, g/day	54.5 ± 35.6	55.2 ± 29.7	0.81	70.5 ± 42.1	63.1 ± 34.4	0.02
Dietary Energy, kcal /day	1464 ± 677	1583 ± 600	0.04	1687 ± 832	1642 ± 643	0.46
Smoking status			0.02			0.41
Never smokers	62 (44.9%)	337 (48.4%)		65 (41.7%)	543 (47.7%)	
Past smokers	57 (41.3%)	313 (45.0%)		61 (39.1%)	416 (36.6%)	
Current smokers	19 (13.8%)	46 (6.6%)		25 (16.0%)	157 (13.8%)	
Unknown	0 (0%)	0 (0%)		5 (3.2%)	22 (1.9%)	
Hormone therapy status			5.2×10 <sup>-6</sup>			0.02
Non-users	70 (50.7%)	229 (32.9%)		92 (59.0%)	537 (47.2%)	
Past users	37 (26.8%)	146 (21.0%)		54 (34.6%)	475 (41.7%)	
Current users	30 (21.7%)	311 (44.7%)		8 (5.1%)	69 (6.1%)	
Unknown	1 (0.7%)	10 (1.4%)		2 (1.3%)	57 (5.0%)	
Medication use						
Aspirin	21 (15.2%)	180 (25.9%)	0.01	26 (16.7%)	360 (31.6%)	0.0002
Lipid-lowering	9 (6.5%)	75 (10.8%)	0.17	24 (15.4%)	178 (15.6%)	1.00
Antihypertensive medications	56 (40.6%)	161 (23.1%)	3.2×10 <sup>-5</sup>	73 (46.8%)	314 (27.6%)	1.4×10 <sup>-6</sup>
Antihyperglycemic medications	16 (11.6%)	36 (5.2%)	0.01	23 (14.7%)	103 (9.1%)	0.04
Antidepressants	5 (3.6%)	47 (6.8%)	0.23	7 (4.5%)	79 (6.9%)	0.33
Education			2.9×10 <sup>-6</sup>			8.3×10 <sup>-12</sup>
Below high school	22 (15.9%)	26 (3.7%)		34 (21.8%)	72 (6.3%)	
High school diploma or GED	17 (12.3%)	137 (19.7%)		40 (25.6%)	276 (24.3%)	
Vocational or training school	15 (10.9%)	85 (12.2%)		16 (10.3%)	171 (15.0%)	
Some college or associate degree	39 (28.3%)	209 (30.0%)		41 (26.3%)	294 (25.8%)	
Baccalaureate degree and above	44 (31.9%)	233 (33.5%)		21 (13.5%)	319 (28.0%)	
Unknown	1 (0.7%)	6 (0.9%)		4 (2.6%)	6 (0.5%)	
Family income			3.6×10 <sup>-6</sup>			1.3×10 <sup>-6</sup>
Less than \$19,999	48 (34.8%)	115 (16.5%)		80 (51.3%)	331 (29.1%)	
\$20,000 to \$34,999	28 (20.3%)	200 (28.7%)		29 (18.6%)	327 (28.7%)	
\$35,000 to \$49,999	13 (9.4%)	141 (20.3%)		26 (16.7%)	208 (18.3%)	
\$50,000 to \$74,999	24 (17.4%)	110 (15.8%)		8 (5.1%)	132 (11.6%)	
\$75,000 or more	12 (8.7%)	86 (12.4%)		5 (3.2%)	83 (7.3%)	
Unknown	13 (9.4%)	44 (6.3%)		8 (5.1%)	57 (5.0%)	

(Continued)

**Table 1. Continued**

Mean $\pm$ SD or n (%)	Women from the WHI-OS			Women from the WHI-HT		
	Black (n = 138)	White (n = 696)	P	Black (n = 156)	White (n=1138)	P
Psychosocial characteristics						
Emotional well-being	78.2 $\pm$ 14.1	79.1 $\pm$ 14.3	0.53	74.4 $\pm$ 15.7	78.8 $\pm$ 14.1	0.0004
Hostility	5.4 $\pm$ 3.0	3.7 $\pm$ 2.7	1.4 $\times$ 10 <sup>-11</sup>	5.8 $\pm$ 3.1	3.9 $\pm$ 2.7	2.4 $\times$ 10 <sup>-16</sup>
General health	66.7 $\pm$ 19.0	73.2 $\pm$ 17.9	0.0001	62.3 $\pm$ 19.3	72.1 $\pm$ 17.6	1.6 $\times$ 10 <sup>-10</sup>
Optimism	23.1 $\pm$ 3.3	23.1 $\pm$ 3.6	0.87	22.6 $\pm$ 3.0	22.9 $\pm$ 3.4	0.28
Social functioning	86.0 $\pm$ 20.0	90.1 $\pm$ 17.7	0.01	81.1 $\pm$ 23.4	89.2 $\pm$ 18.1	6.1 $\times$ 10 <sup>-7</sup>
Social strain	7.2 $\pm$ 3.1	6.4 $\pm$ 2.4	0.001	8.0 $\pm$ 3.2	6.4 $\pm$ 2.5	1.8 $\times$ 10 <sup>-12</sup>
Social support	33.8 $\pm$ 9.1	35.9 $\pm$ 8.0	0.01	34.8 $\pm$ 7.5	35.3 $\pm$ 7.9	0.42
Sleep disturbance	6.1 $\pm$ 4.8	7.0 $\pm$ 4.4	0.03	7.7 $\pm$ 4.9	7.2 $\pm$ 4.5	0.14

CHD indicates coronary heart disease; GED, General Educational Development; HDL, high-density lipoprotein; MET, metabolic equivalent; WHI-HT, Women's Health Initiative-Hormone Therapy Trials; and WHI-OS, Women's Health Initiative-Observational Study.

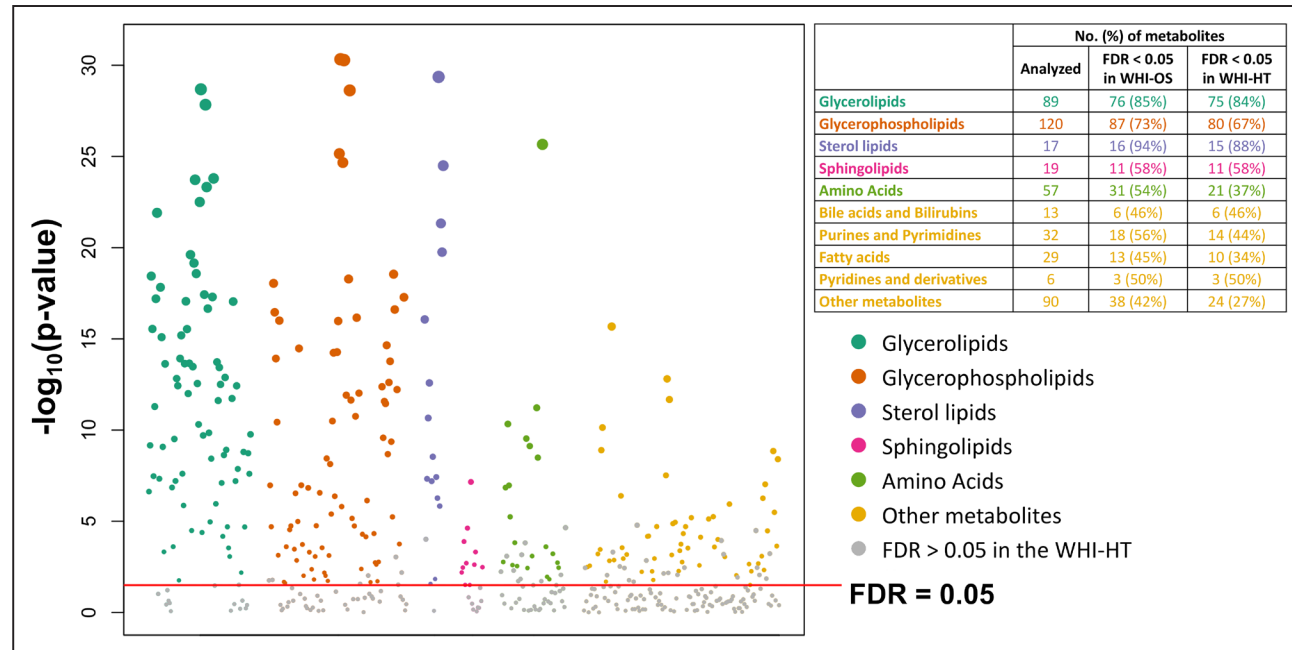
sensitivity analyses with additional adjustment for dietary factors, fasting glucose levels, or psychological characteristics were consistent with the main findings (Figure 2 and Table S3).

The regression coefficients for each metabolite were highly correlated in the WHI-OS and WHI-HT ( $r=0.98$ ), and regression coefficients from the MESA were concordant with those from the WHI-OS ( $r=0.52$ ; Figure S3). Although regression coefficients observed in MESA were largely nonsignificant, possibly because of the small sample size, 5 metabolites (C36:3 phosphatidylethanolamine,

C20:5 cholesteryl ester, C18 carnitine, proline, and hypoxanthine) significantly differed by race (raw  $P<0.05$ ), with the regression coefficients in the same direction as observed in the WHI-HT (Table S4).

### Associations Between Metabolites With Large Differences by Race and CHD Risk

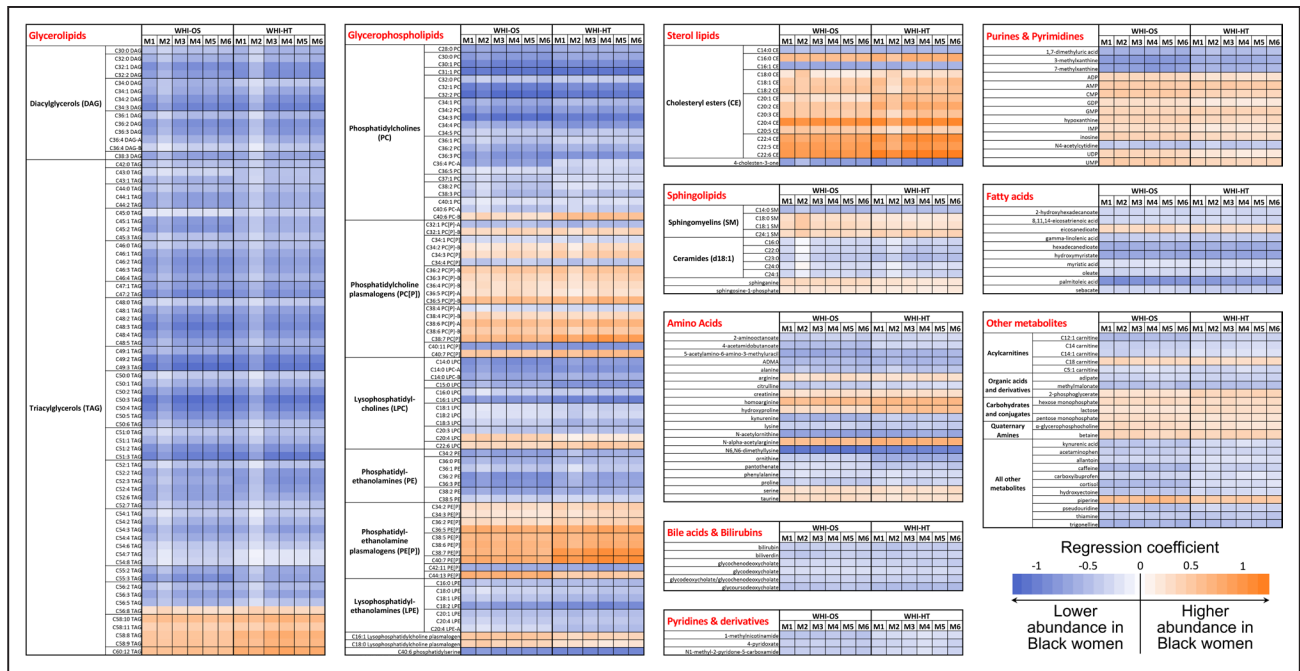
The direction and magnitude of differences in metabolite levels (SD units) in the WHI-HT is displayed in Figure 3A. Ten lipid metabolites differed in abundance by  $>1$ -SD between Black and White women and were tested



**Figure 1. Metabolites that significantly differed between Black and White women from the Women's Health Initiative (WHI).**

The Manhattan plot shows the distributions of  $P$  values for regression coefficients of each metabolite in the discovery set (WHI-OS [WHI-Observational Study]). Linear regression models were adjusted for coronary heart disease (CHD) case-control status, matching factors (age, hysterectomy status, and enrollment window), hormone therapy use status, body mass index, smoking status, alcohol consumption, education, family income, physical activity, baseline health conditions (diabetes, hypertension, and depression), and medication use (aspirin, lipid-lowering, antihyperglycemic, antihypertensive, and antidepressants). The table shows the numbers of analyzed metabolites in each category, as well as the numbers and percentages of metabolites that significantly differed by race in each category. False discovery rate (FDR) was estimated for 472 comparisons in the WHI-OS (ie, the number of all metabolites included in the analysis) and 299 comparisons in the WHI-HT (WHI-Hormone Therapy; ie, the number of metabolites with an  $FDR<0.05$  in the WHI-OS).





**Figure 2. Differences in metabolite levels between Black and White women in the Women's Health Initiative (WHI).**

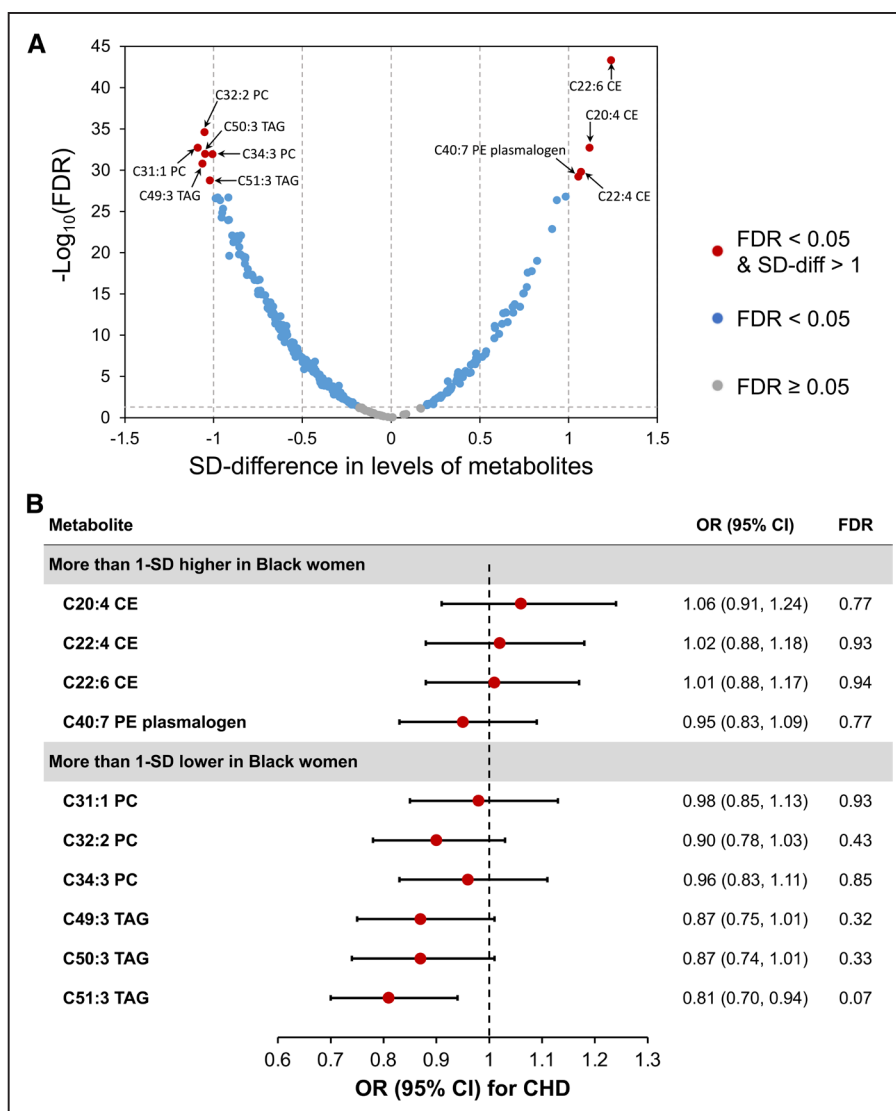
The heatmap shows regression coefficients for each metabolite in each category. Model 1 (M1; fully adjusted) is adjusted for coronary heart disease (CHD) case-control status, matching factors (age, hysterectomy, and enrollment window), hormone therapy use status, body mass index, smoking status, alcohol consumption, education, family income, physical activity, baseline health conditions (diabetes, hypertension, and depression), and medication use (aspirin, lipid-lowering, antihyperglycemic, antihypertensive, and antidepressants). Model 2 (M2; lipid-adjusted): M1 plus total and high-density lipoprotein cholesterol. Model 3 (M3; diet quality-adjusted): M1 plus Healthy Eating Index-2005. Model 4 (M4; macronutrient-adjusted): M1 plus dietary intake of proteins, total carbohydrates, and total fat. Model 5 (M5; calorie-adjusted): M1 plus total calorie intake. Model 6 (psychological factors-adjusted): M1 plus psychological indicators (emotional well-being, hostility, general health, optimism, social support, social functioning, social strain, and sleep disturbance). WHI-HT indicates WHI-Hormone Therapy; and WHI-OS, WHI-Observational Study.

for their associations with CHD in the WHI. For the 4 metabolites that were  $>1$ -SD higher in Black women, we did not observe significant associations with CHD risk ( $FDR > 0.5$ ); however, for the remaining 6 metabolites that were  $>1$ -SD lower in Black women, higher levels of C51:3 triacylglycerol were marginally significantly associated with a decreased risk of CHD (odds ratio [OR]=0.81 [95% CI, 0.70–0.94]) after accounting for multiple comparisons ( $FDR < 0.1$  for 472 comparisons; Figure 3B). In stratified analysis by race, similar results were observed in White women, but the associations in Black women were nonsignificant (Figure S4 and Table S5). Additionally, for the 25 metabolites that were 0.5–1 SD higher in Black women, 3 lipid metabolites were marginally significantly associated with higher CHD risk ( $FDR < 0.1$ ), with one metabolite (C36:2 phosphatidylcholine plasmalogen) showing a significant association (OR=1.18 [95% CI, 1.09–1.49];  $FDR = 0.03$ ). For the 89 metabolites that were 0.5–1 SD lower in Black women, 8 lipid metabolites (5 triacylglycerols and 3 diacylglycerols) were marginally significantly associated with decreased risk of CHD ( $FDR < 0.1$ ), with 2 metabolites (C52:6 triacylglycerol and C36:4 diacylglycerol) showing significant associations ( $FDR = 0.03$ ). When stratified by self-reported race, similar results were observed in Black

and White women in the WHI. In replication analyses in Black women from the JHS and White women from the NHS, findings were similar to race-specific results from the WHI for metabolites showing marginally significant associations with CHD risk (Table S5).

### Estimation and Validation of the RDMP

To create a composite score that reflects the cumulative differences in metabolomic profiles between Black and White women, we established the RDMP using elastic net regression. The RDMP consists of 152 metabolites, including 48 lipid metabolites, 32 amino acids, 18 purines and pyrimidines, 11 fatty acids, 11 acylcarnitines, and 32 metabolites from other classes (Figure 4A and Table S6). As expected, the RDMP had high performance in both WHI (area under the receiver operating characteristic curves  $> 0.97$ ) and MESA (area under the receiver operating characteristic curves = 0.95; Figure S5). Significant differences in distributions of the RDMP between Black and White women were observed in WHI-OS ( $P = 1.6 \times 10^{-70}$ ), WHI-HT ( $P = 2.6 \times 10^{-83}$ ), and MESA ( $P = 1.8 \times 10^{-40}$ ; Figure 4B). Notably, 210 (96.8%) of all 217 Black women included in this analysis from the WHI were distributed in the highest RDMP quartile (Figure S2). Because not all studies measured the exact same



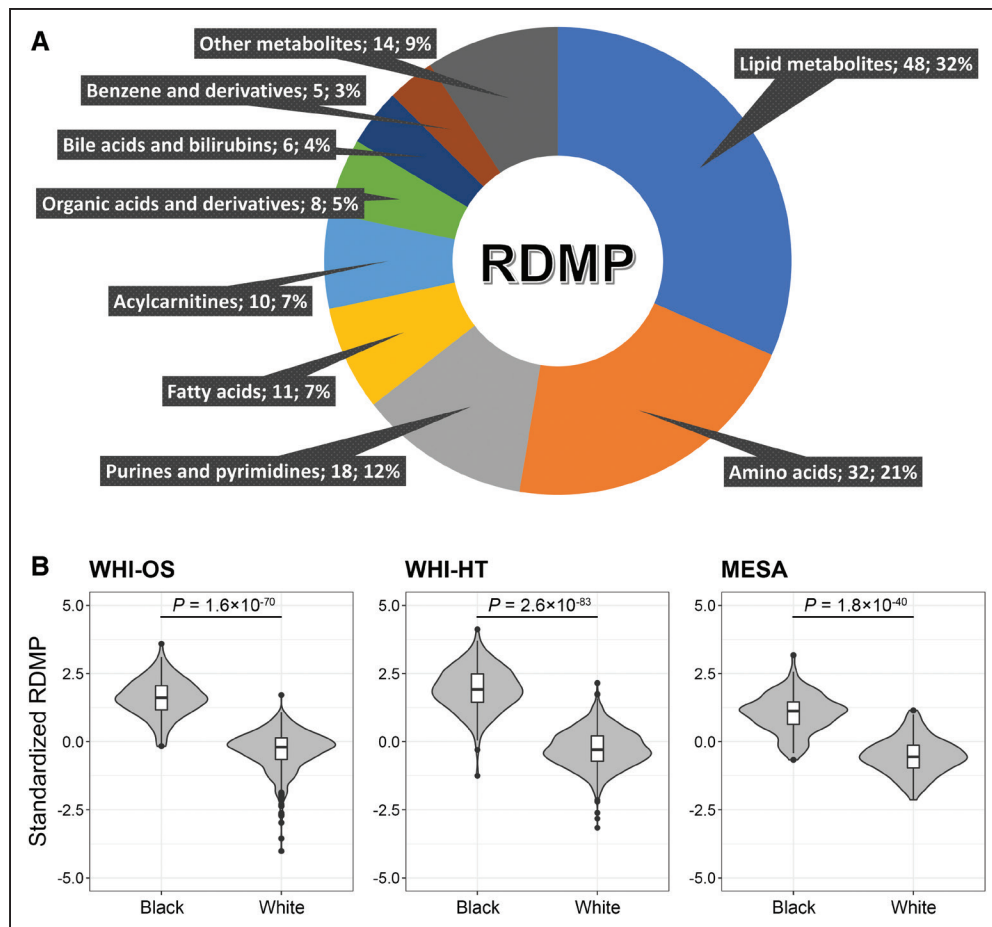
**Figure 3. Magnitude of difference in metabolites between Black and White women, and their associations with coronary heart disease (CHD) risk in the Women's Health Initiative (WHI).**

**A**, The volcano plot highlights 12 metabolites whose levels differed by  $>1$ -SD between Black and White women in the WHI-HT (WHI-Hormone Therapy). The x-axis is SD-difference in levels of each metabolite; the y-axis is  $-\log_{10}$ (false discovery rate [FDR]) for each metabolite. Metabolites with SD-difference  $\leq 1$  were highlighted using gray (FDR $\geq 0.05$ ) or blue (FDR $< 0.05$ ) dots. Red dots highlight metabolites with SD-difference  $> 1$  and FDR $< 0.05$ . Models were adjusted for age, CHD case-control status, hysterectomy, hormone therapy use, enrollment window, body mass index, smoking, alcohol consumption, education, family income, physical activity, baseline health conditions, and medication use. **B**, Associations between metabolites with  $>1$ -SD difference and CHD risk in the combined dataset of WHI-OS (WHI-Observational Study) and placebo arms of WHI-HT. CHD cases were matched to controls on age, self-reported race, hysterectomy, and enrollment window. Conditional logistic regression models were adjusted for substudies (OS/estrogen-alone trial/estrogen-plus-progestin trial), body mass index, hormone therapy use status, smoking, diabetes, antihyperglycemic medication use, systolic blood pressure, antihypertensive medication use, aspirin use, lipid-lowering medication use, and total and high-density lipoprotein cholesterol. FDR for associations between metabolites and CHD risk were estimated for 472 comparisons to test the statistical significance in the whole metabolomics profile in the WHI. CE indicates cholesteryl ester; OR, odds ratio; PC, phosphatidylcholine; PE, phosphatidylethanolamine; and TAG, triacylglycerol.

set of metabolites as the WHI, to further test the generalizability of the identified RDMP, we calculated rRDMP1 (using the 93 metabolites available in the MESA), rRDMP2 (using the 79 metabolites available in the JHS), and rRDMP3 (using the 98 metabolites available in the NHS) in the WHI-HT and found significant differences in distributions between Black and White women from the WHI-HT for all 3 rRDMP scores (Figure S6).

#### Associations Between the RDMP and CHD Risk

To determine whether metabolite differences by race, as summarized by the RDMP, are associated with risk of incident CHD, we combined women from the WHI-OS and the placebo arms of the WHI-HT to increase the statistical power, where 717 CHD cases were matched to 719 controls on self-reported race and other factors. After adjusting for baseline characteristics and known



**Figure 4. Constituent metabolites of the racial difference metabolomic pattern (RDMP), and the median RDMP in Black and White individuals from Women's Health Initiative (WHI) and MESA (Multi-Ethnic Study of Atherosclerosis).**

**A**, Constituent metabolites of the RDMP in each category. For a full list of metabolites, please see Table S5. **B**, Violin plots were used to compare the distributions of RDMP for Black and White individuals from the WHI and MESA, and *P* values were calculated using Wilcoxon signed-rank test.

CHD risk factors (Table 2; model 2), women in the highest RDMP quartile had higher risk of CHD (OR=1.51 [95% CI, 0.97–2.37];  $P_{\text{trend}}=0.02$ ), compared with women in the lowest quartile. When stratified by race and using race-specific RDMP quartiles, higher RDMP was not associated with risk of CHD in Black women in the WHI ( $P_{\text{trend}}=0.47$ ). Similar findings were observed among Black women from the JHS ( $P_{\text{trend}}=0.40$ ). However, among White women in the WHI, scoring in the highest RDMP quartile, compared with the lowest, was significantly associated with increased risk of CHD (OR=1.49 [95% CI, 1.02–2.17];  $P_{\text{trend}}=0.01$ ). Similar results were observed in White women from the NHS, although not statistically significant (OR<sub>Q4\_vs\_Q1</sub>=1.36 [95% CI, 0.81–2.28];  $P_{\text{trend}}=0.22$ ).

## DISCUSSION

Using data from the WHI and MESA, we observed substantial differences in a large number of metabolites, mostly lipid metabolites and amino acids, between Black

and White women after adjusting for baseline characteristics, socioeconomic status, lifestyle factors, baseline health conditions, and medication use. The observed differences did not change substantially after additional adjustment for blood lipids, dietary factors, and psychological characteristics. Several lipid metabolites that demonstrated large differences by race were associated with CHD risk in the WHI, independent of self-reported race and known CHD risk factors. Moreover, to estimate the impact of race on metabolomic profiles, we identified and validated an RDMP composed of 152 metabolites, which could reflect the impact of the cumulative exposure to both known and unmeasured differences in the lived experiences between Black and White women in the United States on metabolism. Additionally, higher RDMP was associated with increased risk of CHD in the WHI, independent of self-reported race and CHD risk factors, suggesting that metabolite differences between Black and White women might partially explain differences in CHD risk.

In the United States, racial disparities in CHD have persisted for decades.<sup>1,2</sup> Contributors to racial disparities

**Table 2. Association Between Quartiles of the RDMP and CHD Risk in the WHI, JHS, and NHS**

	OR or HR (95% CI) for per SD	OR or HR (95% CI) for quartiles of RDMP				P value for trend
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
<b>All women in WHI*</b>						
Case	717	168	169	192	188	
Control	719	191	190	167	171	
Model 1	1.14 (0.96–1.36)	1.00 (reference)	0.93 (0.68–1.28)	1.35 (0.97–1.88)	1.33 (0.86–2.05)	0.09
Model 2	1.20 (1.01–1.44)	1.00 (reference)	0.97 (0.71–1.34)	1.46 (1.04–2.06)	1.51 (0.97–2.37)	0.02
Model 3a	1.19 (0.99–1.42)	1.00 (reference)	0.98 (0.71–1.35)	1.45 (1.02–2.04)	1.48 (0.95–2.32)	0.03
Model 3b	1.19 (1.00–1.43)	1.00 (reference)	0.96 (0.69–1.32)	1.46 (1.03–2.05)	1.45 (0.92–2.27)	0.03
Model 4	1.19 (0.99–1.43)	1.00 (reference)	0.96 (0.70–1.33)	1.45 (1.02–2.06)	1.48 (0.94–2.33)	0.03
<b>Black women in WHI**</b>						
Case	109	26	26	31	26	
Control	108	29	28	23	28	
Model 1	0.68 (0.40–1.17)	1.00 (reference)	0.97 (0.33–2.91)	1.48 (0.57–3.85)	0.43 (0.14–1.32)	0.36
Model 2	0.64 (0.36–1.14)	1.00 (reference)	1.22 (0.39–3.86)	1.71 (0.61–4.81)	0.47 (0.14–1.54)	0.47
Model 3a	0.65 (0.36–1.17)	1.00 (reference)	1.24 (0.39–3.97)	1.77 (0.62–5.05)	0.48 (0.14–1.61)	0.52
Model 3b	0.59 (0.32–1.07)	1.00 (reference)	1.23 (0.37–4.03)	1.75 (0.60–5.07)	0.42 (0.12–1.42)	0.39
Model 4	0.92 (0.42–2.00)	1.00 (reference)	1.70 (0.43–6.80)	2.29 (0.62–8.40)	1.42 (0.28–7.05)	0.44
<b>Black women in JHS††</b>						
Person-year	16 262.12	4337.13	4435.67	4057.51	3431.81	
CHD case	107	35	26	20	26	
Model 1	0.94 (0.76–1.10)	1.00 (reference)	0.83 (0.50–1.40)	0.57 (0.32–1.00)	0.93 (0.54–1.60)	0.47
Model 2	0.92 (0.75–1.10)	1.00 (reference)	0.84 (0.50–1.40)	0.57 (0.32–1.00)	0.89 (0.52–1.50)	0.40
Model 3a	0.93 (0.75–1.10)	1.00 (reference)	0.86 (0.51–1.40)	0.53 (0.30,0.96)	0.94 (0.54–1.60)	0.46
<b>White women in WHI*†</b>						
Case	608	140	140	165	163	
Control	611	165	165	139	142	
Model 1	1.22 (1.01–1.48)	1.00 (reference)	0.87 (0.62–1.21)	1.46 (1.03–2.08)	1.28 (0.89–1.83)	0.07
Model 2	1.31 (1.08–1.59)	1.00 (reference)	0.93 (0.66–1.31)	1.53 (1.07–2.19)	1.49 (1.02–2.17)	0.01
Model 3a	1.30 (1.06–1.58)	1.00 (reference)	0.93 (0.66–1.32)	1.52 (1.06–2.18)	1.44 (0.98–2.10)	0.02
Model 3b	1.30 (1.07–1.58)	1.00 (reference)	0.91 (0.64–1.28)	1.52 (1.06–2.18)	1.45 (1.00–2.12)	0.02
Model 4	1.29 (1.06–1.58)	1.00 (reference)	0.92 (0.65–1.31)	1.52 (1.05–2.19)	1.42 (0.97–2.08)	0.02
<b>White women in NHS††</b>						
Person-year	50333.58	11 857.25	12 446.08	13 098.42	12 931.83	
CHD case	136	33	37	34	32	
Model 1	1.08 (0.90–1.30)	1.00 (reference)	1.04 (0.64–1.68)	1.09 (0.66–1.80)	1.16 (0.69–1.93)	0.56
Model 2	1.14 (0.95–1.38)	1.00 (reference)	1.05 (0.65–1.71)	1.19 (0.72–1.96)	1.36 (0.81–2.28)	0.22
Model 3b	1.15 (0.95–1.38)	1.00 (reference)	1.04 (0.64–1.68)	1.18 (0.72–1.96)	1.37 (0.82–2.30)	0.20

Model 1: adjusted for substudies (OS/estrogen-alone trial/estrogen-plus-progestin trial; WHI only), batch (JHS only), age (JHS and NHS), body mass index, menopausal status (JHS and NHS), hormone therapy use status, smoking status, diabetes, antihyperglycemic medication use, systolic blood pressure, antihypertensive medication use, aspirin use, and lipid-lowering medication use. Model 2: adjusted for variables in Model 1 plus plasma levels of total and high-density lipoprotein cholesterol. Model 3a (WHI and JHS): adjusted for variables in Model 2 plus education and family income. Model 3b (WHI and NHS): adjusted for variables in Model 2 plus Healthy Eating Index-2005 (WHI) or Alternative Healthy Eating Index-2010 (NHS). Model 4 (WHI only): adjusted for variables in Model 3a plus psychosocial characteristics, including emotional well-being, hostility, general health, optimism, social functioning, social strain, social support, and sleep disturbance. CHD indicates coronary heart disease; HR, hazard ratio; JHS, Jackson Heart Study; NHS, Nurses' Health Study; OR, odds ratio; RDMP, racial difference metabolomic pattern; WHI, Women's Health Initiative; WHI-HT, WHI-Hormone Therapy; and WHI-OS, Women's Health Initiative-Observational Study.

\*In the WHI, CHD cases were matched to healthy controls on age, self-reported race, hysterectomy, and enrollment window. Conditional logistic regressions were used to estimate associations between RDMP and CHD risk. Women from the WHI-OS and placebo arms of the WHI-HT were combined in this analysis.

†In race-stratified analysis, race-specific quartiles of the RDMP were used in the association analyses.

††In the JHS and NHS, incident CHD cases were documented, and cox proportional-hazards models were used to estimate associations between RDMP and incident CHD risk.

in CHD include differences in structural factors, socio-economic status, individual level CHD risk factors, neighborhood factors, and unequal access to the medical care system and biases in treatment.<sup>6,9,29</sup> These contribute to differences by race in educational attainment, wealth, smoking, physical activity, obesity, hypertension,

diabetes, and blood lipids.<sup>6,7,29</sup> In this study, the observed differences between Black and White women in baseline characteristics in the WHI were in line with these previous publications. However, many other factors may influence how race and related exposures and environments impact health, including environmental factors,<sup>30,31</sup> racial discrimination, and lifestyle factors, but these factors have often been difficult to measure and quantify.

The human metabolome reflects an individual's metabolic status in response to the interaction of many factors that could impact human metabolism, including environmental exposures,<sup>13</sup> dietary factors,<sup>12,24</sup> and health status (eg, obesity and diabetes).<sup>11,14</sup> In our study participants, Black women had a higher prevalence of obesity and diabetes than White women at the study baseline, which could have a cumulative impact on metabolism and contribute to the observed differences in metabolomic profiles between Black and White women. Moreover, as a social and cultural construct, race has an impact on many individual exposures and other social/environmental determinants of health due to the exposure to inequitable systems,<sup>1,4,5</sup> which could influence the human metabolome through a variety of mechanisms and pathways. Thus, metabolomics may be a tool to sum the potential impact of factors related to the embodiment of the experience of race in the United States, including factors which are difficult to measure. However, only a few studies have reported racial differences in metabolites in US adults.<sup>18–20</sup> Using metabolomics data from 2 national cohorts in the United States (the WHI and MESA), we observed significant and substantial differences in metabolomic profiles between Black and White women; such differences in metabolomic profiles may reflect the cumulative impact of racial differences in a variety of endogenous and exogenous factors on metabolism, which could play a mechanistic role in the development of CVD and other health outcomes.

Black women had lower levels of total cholesterol and higher levels of HDL-cholesterol than White women in the WHI, which is consistent with prior data from the Million Veteran Program<sup>32</sup> and the National Health and Nutrition Examination Survey.<sup>2,29</sup> In this study, more than half of the metabolites included in the analysis were lipid metabolites, which also presented significant and substantial differences between Black and White women. Existing evidence indicates that Black individuals have lower total triacylglycerol levels than White individuals.<sup>33</sup> Consistently, in this study, most glycerolipids were lower in Black women; however, several long-chain polyunsaturated (C56–C60 with 8–12 double-bonds) triacylglycerols were higher in Black women. Of note, the observed racial differences in lipid metabolite levels persisted after adjusting for blood lipids as well as multiple baseline characteristics, including lifestyle factors, dietary intake, and lipid-lowering medication use. Further studies are

needed to reveal factors that influence racial differences in lipidomics, as well as the impact in explaining racial differences in CVDs and other health outcomes.

In addition to lipid metabolites, metabolites in several other categories also differed significantly between Black and White women, including amino acids, purines and pyrimidines, fatty acids, bile acids and bilirubins and acylcarnitines. Our findings are consistent with a recent study of 73 Black and White bladder cancer patients in the United States that reported significant racial differences in the abundance of 53 of 300 analyzed metabolites, most of which were related to the metabolism of amino acids, lipids, and nucleotides.<sup>20</sup> In our external validation analysis, the observed associations between self-reported race and metabolite levels in MESA were generally nonsignificant, possibly due to a small sample size. However, the magnitude of the regression coefficients had good concordance with those observed in the WHI-OS.

Levels of several metabolites have been associated with CHD risk in the WHI<sup>15</sup> and other populations.<sup>17</sup> However, no study has examined whether differences in the metabolomic profiles by race could partially explain the observed racial disparities in CHD. In this study, we observed that C53:1 triacylglycerol has a lower abundance in Black women, and higher levels of C53:1 triacylglycerol were associated with a decreased risk of CHD, indicating that C53:1 triacylglycerol may be involved in a pathway that were associated with higher risk of CHD in Black women. Moreover, the RDMP, which was validated in 2 separate cohorts, may act as a composite score that represents racial differences in the metabolome that are related to cumulative differences in exogenous factors. As such, the RDMP could reflect the impact of cumulative exposure to known and unmeasured differences in the lived experiences between Black and White women on metabolism, and further act as an innovative tool to assess to what degree the differences in social experiences between races contribute to racial disparities in health outcomes.

Higher RDMP was associated with an increased risk of CHD, independent of self-reported race and known CHD risk factors, and such associations were more pronounced in White women but were nonsignificant in Black women (using race-specific RDMP quartiles). Similar results were found in Black women from the JHS and White women from the NHS. These findings suggest that metabolomic patterns that were related to the social and lived experience of Black women may be related to higher risk of CHD and may thus help explain racial disparities in CVD in US women. We could not directly estimate the mediation effects of individual metabolites and the RDMP on racial differences in the risk of CHD, nor determine the causality of the observed associations, because of the matched nested case-control design in the WHI (race was one of the matching factors) and the

small number of documented incident CHD cases in the MESA. However, the RDMP could act as a surrogate, rather than a mediator, that reflects the impact of the social and lived experience of Black women on metabolism, and further contribute to racial disparities in cardiovascular outcomes in US women—a hypothesis that warrant further investigations.

This study has several strengths. First, women from the WHI were derived from a nested case-control study of CHD; thus, all women were free of CVD at the study baseline. Second, the statistical methods were robust; racial difference in metabolomic profiles observed in the WHI-OS were validated and replicated in independent population internally (WHI-HT) and externally (MESA). Third, the same well-validated metabolomic profiling method was used in the WHI and all replication cohorts (MESA, JHS, and NHS). Finally, detailed information on covariates was collected in all of the cohorts, and CHD end points were carefully adjudicated in the WHI, JHS, and NHS. All these strengths ensured the accuracy of the information used in this study and the robustness of our findings.

Our study has some limitations. First, the number of Black women in this study was relatively small. However, this study is the largest reported comparison, and we observed significant and substantial differences in the metabolomic profiles between Black and White women. Second, we did not have data on community-level exposures (eg, neighborhood characteristics and environmental exposures) nor on individual experience of racial discrimination or related exposures. Future studies are needed to investigate the degree to which these factors are associated with racial differences in the metabolome. Third, although we validated the associations between the RDMP and incident CHD risk that were discovered in the WHI in Black women (from the JHS) and White women (from the NHS) separately, we did not have another cohort in which the associations with CHD could be directly compared between races. Future cohort studies of larger sample sizes and consortium studies are needed to replicate our findings and to examine differences in metabolomic profiles between other racial and ethnic groups (ie, Asian and Hispanic/Latino women) and to estimate the impact of racial differences in the metabolome on racial disparities in CHD and other health outcomes. CHD cases in Black women may be under-diagnosed in the community, leading to diagnostic bias in CHD detection; however, once reported, all of the cases were carefully adjudicated, and our nested case-control design in the WHI, with self-reported race as one of the matching factors, should help ensure that the observed associations were valid. Finally, potential residual confounding is possible, which includes unmeasured exogenous factors that differ by race (eg, environmental exposures and medication use) and variations in metabolites that were related to baseline characteristics, disease

status, or the single time-point measurement of metabolomics profiles. Future studies with repeated measurements of exposures and metabolomics data are needed to examine the contribution of racial differences in these factors to racial differences in metabolites.

## CONCLUSIONS

Metabolomic profiles significantly and substantially differed between Black and White women. Several metabolites, especially lipid metabolites and amino acids, significantly differed by race. We identified and validated a composite metabolomic score (RDMP) that characterizes differences in metabolomic profiles between Black and White women, which might reflect the cumulative influence of racial differences in a variety of exogenous factors on individual metabolic processes. Furthermore, the RDMP, which may reflect the impact of differences in the social and lived experiences between Black and White women, was associated with incident CHD risk, independent of self-reported race and known risk factors for CHD, suggesting that differences in metabolomic patterns between Black and White women may partially explain racial disparities in CHD in the United States. Further studies are needed to replicate our findings and to estimate the impact of the cumulative exposure to the social experience of race on health disparities in the United States.

## ARTICLE INFORMATION

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## Disclosures

None.

## Supplemental Materials

Supplemental Methods  
Tables S1–S6  
Figures S1–S6

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