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Circulating metabolome and White Matter hyperintensities in women and men

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Author contributions

SS and ZP conceptualized the study. ES, JS, and ZP contributed to the analysis plan. ES, JS, SA, DMW, SF, FG, SEH, AKH, YHH, RW, and KW analyzed the data and generated results. ES and ZP interpreted the results and wrote the original manuscript. QY, HV, JMS, MR, MN, LL, MAI, NH, HG, IJD, SRC, JMW, MRL, BJ, CS, MWV, PP, MG, JB, MVH, NC, JIR, MF, TP, SS, and ZP obtained funding, provided additional study resources, and contributed to metabolic and/or MRI data. All authors contributed to revising the content and approved the final version.

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Abstract

Background: White matter hyperintensities (WMH) are identified on T2-weighted magnetic resonance images of the human brain as areas of enhanced brightness; WMH are a major risk factor of stroke, dementia, and death. Currently, there are no large-scale studies testing associations between WMH and circulating metabolites.

Methods: We studied up to 9,290 individuals (50.7% females, average age 61 years) from 15 populations of 8 community-based cohorts. WMH volume was quantified from T2-weighted or fluid-attenuated inversion-recovery images or as hypointensities on T1-weighted images. Circulating metabolomic measures were assessed with mass spectrometry and nuclear magnetic resonance spectroscopy. Associations between WMH and metabolomic measures were tested by fitting linear regression models in the pooled sample, and in sex-stratified and statin treatment-stratified subsamples. Our basic models were adjusted for age, sex, age*sex, and technical covariates, and our fully adjusted models were additionally adjusted for statin treatment, hypertension, type 2 diabetes, smoking, body mass index, and estimated glomerular filtration rate. Population-specific results were meta-analyzed using the fixed-effect inverse variance-weighted method. Associations with false discovery rate (FDR)-adjusted *p*-values (p_{FDR})<0.05 were considered significant.

Results: In the meta-analysis of results from the basic models, we identified 30 metabolomic measures associated with WMH (p_{FDR}<0.05), 7 of which remained significant in the fully adjusted models. The most significant association was with higher level of hydroxyphenylpyruvate in males ($p_{FDR.full.adj}=1.40\times10^{-7}$) and in both the pooled sample ($p_{FDR.full.adj}=1.66\times10^{-4}$) and statin-nontreated ($p_{FDR.full.adj}=1.65\times10^{-6}$) subsample. In males, HPP explained 3–14% of variance in WMH. In males and the pooled sample, WMH were also associated with lower levels of lysophosphatidylcholines and hydroxysphingomyelins, and a larger diameter of low-density lipoprotein particles, likely arising from higher triglyceride-to-total-lipids and lower cholesteryl ester-to-total-lipids ratios within these particles. In females, the only significant association was with higher level of glucuronate ($p_{FDR}=0.047$).

Conclusions: Circulating metabolomic measures, including multiple lipid measures (e.g., lysophosphatidylcholines, hydroxysphingomyelins, low-density lipoprotein size and composition) and non-lipid metabolites (e.g., hydroxyphenylpyruvate, glucuronate) associate with WMH in a general population of middle-aged and older adults. Some metabolomic measures show marked sex specificities and explain sizable proportion of WMH variance.

Keywords

brain; glucuronic acid; hydroxyphenylpyruvate; lipid ratios; lipidomics; lipids; lysophosphatidylcholines; metabolomics; sphingomyelins; white matter

INTRODUCTION

White matter hyperintensities (WMH) are among the most commonly encountered signal alterations on brain magnetic resonance imaging (MRI) images – they are areas of high intensity in the periventricular and deep cerebral white matter on T2-weighted (T2W) or T2 fluid-attenuated inversion recovery (FLAIR) images. The exact neuropathological

mechanisms are not fully understood, but the proposed fundamental features of WMH are loss of myelin and axons, and mild gliosis (reviewed in[1,2]). These features might arise due to chronic ischemia and dysfunction of the blood-brain barrier (BBB) related to cerebral small vessel disease[2].

Evidence from a recent meta-analysis of prospective cohort studies indicates that WMH are of major clinical significance[3] – data obtained in 14,529 participants show that higher volume of WMH is associated with higher risk (hazard ratio, HR) of incident stroke (HR=2.45 [1.93–3.12], 95% confidence interval in square brackets), intracerebral hemorrhage (HR=3.17 [1.54–6.52]), ischemic stroke (HR=2.39 [1.65–3.74]), dementia (HR=1.84 [1.40–2.43]), Alzheimer's disease (HR=1.50 [1.22–1.84]), and all-cause mortality (HR=2.00 [1.69–2.36])[3]. WMH are common in the general population: the prevalence is ~20% at the age of 60 years and >90% at the age of 80 years and higher[4]. Hypertension, type 2 diabetes, and smoking are the key cardiometabolic disease risk factors for WMH[5–7].

We and others have shown that aberrations in the blood metabolome are associated with brain health: circulating levels of multiple lipid measures are associated with Alzheimer's disease[8,9] and cognitive functioning[10], as well as structural properties of the brain, such as T1-weighted (T1W) signal intensity of white matter[11,12] and thickness of the cerebral cortex[13]. The metabolomic associations of WMH, however, are incompletely understood and, to date, only two small studies have been published[14,15]. Here we used metabolomics technologies to provide a comprehensive characterization of variations in circulating metabolomic measures as a function of WMH volume assessed in general population. We further explored whether these associations are independent of the key risk factors of WMH, including hypertension, type 2 diabetes, and smoking.

METHODS

The authors declare that the summary-level results are available within the article and its online-only Data Supplement. The individual-level data analyzed in this study are available by application to the respective cohort committees.

Study populations

This is a large collaborative work incorporating data from 8 population-based cohort studies: Age, Gene/Environment Susceptibility-Reykjavik Study[16], Framingham Heart Study[17,18], Insight 46 (a sub-study of the British 1946 birth cohort)[19], Lothian Birth Cohort 1936[20,21], Rotterdam Study[22–24], Saguenay Youth Study[25], Study of Health in Pomerania[26,27], and the Southall And Brent Revisited study[28]. Study-specific descriptions are given in the Supplemental Methods. After excluding individuals with dementia, stroke, multiple sclerosis, brain surgery, or gross morphological abnormalities of the brain (e.g., cysts, brain tumors), and individuals with poor quality of MRI scans, we analyzed up to 9,290 individuals of mostly European ancestries with brain MRI and blood metabolomic data. All cohort studies were approved by local ethics committees, and all participants have provided their written informed consent (Supplemental Methods).

Phenotype quantifications

Brain MRI and WMH assessment—The brain MRI was carried out in each cohort study separately, and the details are provided in Table S1. In the present analyses, the total volume of WMH (or hypointensities on T1W images) was considered as a continuous variable.

Metabolomic quantifications—We used nuclear magnetic resonance (NMR)-based techniques (Nightingale Health Ltd, Helsinki, Finland; National Phenome Centre, London, UK; Bruker Biospin, Rheinstetten, Germany) and mass spectrometry (MS)-based technologies (Metabolon, Morrisville, North Carolina, USA; Biocrates Life Sciences AG, Innsbruck, Austria; Broad Institute, Cambridge, Massachusetts, USA) that are commonly employed in epidemiological research and are described elsewhere[29–33] (Table S1). Using these aforementioned platforms, it was possible to measure a total of 2,217 different metabolomic measures; of these, 1,174 metabolomic measures were quantified in 2 or more of the study populations. We did not include unknown metabolites in our study. Metabolomic quantifications were completed using serum or plasma samples, typically extracted from blood samples drawn after overnight fasting (Table S1). Blood samples were drawn before or at the time of MRI in all cohorts except for the Rotterdam Study, in which MRI scans have been conducted at multiple time-points and metabolomic data that were from closest in time to the MRI scans were analyzed (Table S2).

Statistical analyses

Linear regression models—The cross-sectional associations between log-transformed WMH and circulating metabolomic measures were studied using linear regression. Prior to model fitting, the log-transformed WMH and metabolomic measures were scaled to standard deviation (SD) units, which enables the comparison and meta-analysis of data in different units and varying numerical ranges that originate from the multiple quantification methods used (Table S1). We fitted the regression models in five analytical samples (*i.e.*, pooled sample and sex- or statin treatment-stratified subsamples) using a simple covariate structure (basic models) and a more complete covariate structure (fully adjusted models) to test if the associations are independent of key risk factors. The study models were as follows:

Pooled sample

basic models: logWMH ~ metabolic measure + age + sex + age*sex + time (if applicable) + fasting duration (if applicable) + intracranial volume or brain size + cohort-specific covariates

fully adjusted models: as above + statin treatment + hypertension + type 2 diabetes + BMI + eGFR + current smoking status

Sex-stratified subsamples

basic models: logWMH ~ metabolic measure + age + time (if applicable) + fasting duration (if applicable) + intracranial volume or brain size + cohort-specific covariates

fully adjusted models: as above + statin treatment + hypertension + type 2 diabetes + BMI + eGFR + current smoking status

Statin treatment-stratified subsamples

basic models: logWMH ~ metabolic measure + age + sex + age*sex + time (if applicable) + fasting duration (if applicable) + intracranial volume or brain size + cohort-specific covariates

fully adjusted models: as above + hypertension + type 2 diabetes + BMI + eGFR + current smoking status

Here, 'time' indicates the time in years between blood sampling and brain MRI, and 'fasting duration' denotes the time in hours between the last meal and blood sampling. Moreover, to investigate possible differences in the associations between the analytical subsamples, we fitted models to test for 'metabolomic measure*sex' and 'metabolomic measure*statin use' interactions; this was done in both basic and fully adjusted models. In the Southall And Brent Revisited study where multiple major ethnicities were present, all models were fitted separately in each ethnic group.

Meta-analyses and multiple testing correction—The association results for metabolomic measures that were present in two or more cohorts (N=1,173) were meta-analyzed. We used inverse variance-weighted fixed-effect meta-analysis to combine the effect estimates and standard errors from each cohort. In case a cohort reported multiple association results for the same metabolic measure (*i.e.*, the metabolic measure was quantified using more than one metabolomic platform within the same cohort), the result that was obtained using a larger number of individuals was included in the meta-analysis.

Many metabolic measures are highly correlated and, thus, the number of independent tests is lower than the number of metabolomic measures tested. To correct for multiple testing, we estimated false discovery rate (FDR)-adjusted *p*-values using a method developed by Benjamini and Hochberg[34]. Here, all original *p*-values from the meta-analyses (including all analytical samples and both basic and fully adjusted models, and all metabolomic measures analyzed, including all lipid ratios) were included in the numeric vector of p-values used for estimating FDR-adjusted *p*-values (p_{FDR}); all associations with $p_{FDR}<0.05$ were considered significant. Testing for 'metabolomic measure*sex' and 'metabolomic measure*statin treatment' interactions were considered exploratory and no correction for multiple comparisons was applied.

All statistical analyses were conducted using R[35].

RESULTS

Characteristics of the study populations are given in Table 1, Table S2 and Figures S1–S2. In the meta-analysis, we identified 416 metabolomic measures showing nominally significant associations with WMH in at least one of the study models. Out of these, 30 (basic models) and 7 (fully adjusted models) associations remained significant after correction for multiple testing ($p_{FDR}<0.05$). An overview of the associations between circulating metabolomic measures and WMH in all basic and fully adjusted models is given in Figure 1. The relative importance metrics for the fully adjusted model of the most significant metabolite, hydroxyphenylpyruvate, are given in Table S3. All meta-analyzed results from the basic

models and fully adjusted models are tabulated in Tables S4 and S5, respectively, and the results for 'metabolomic measure'-by-sex and 'metabolomic measure'-by-'statin treatment' interactions are given in Tables S6 and S7. Cohort-specific associations results are given in Tables S8–S22. Figure S3 illustrates cohort-specific results of the metabolomic measures showing FDR-significant association with WMH. Figure S4 shows a comparison of the meta-analyzed results reported here versus the meta-analyzed results obtained in participants of European ancestries only. We found the cohort-specific results to be highly similar across the cohorts, with very little heterogeneity observed (Figure S3, Tables S4 and S5).

Non-lipid measures

The most robust association – in terms of both effect size and *p*-value – from across all studied metabolites was observed between WMH and higher circulating concentration of an amino acid derivative hydroxyphenylpyruvate (HPP; Figure 2): this association was most significant in the male subsample ($beta_{basic}$=0.20, $p_{FDR.basic}=1.65\times10^{-6}$; $beta_{full.adj}$=0.22, $p_{FDR.full.adj}=1.40\times10^{-7}$), but it was also significant in the pooled sample ($beta_{basic}$=0.13, $p_{FDR.basic}$=0.002; $beta_{full.adj}$=0.15, $p_{FDR.full.adj}=1.66\times10^{-4}$) and in statin-nontreated subsample ($beta_{basic}$=0.15, $p_{FDR.basic}=7.04\times10^{-5}$; $beta_{full.adj}$=0.18, $p_{FDR.full.adj}=1.65\times10^{-6}$). The HPP-by-sex interaction reached nominal significance in both the basic and fully adjusted models ($p_{sexINT.basic}$=0.0094, $p_{sexINT.full.adj}$=0.0077), suggesting that the positive effect size is larger in males than females.

In females, the only significant association, after correction for multiple testing, was observed between WMH and higher circulating concentration of glucuronate ($beta_{basic}$=0.11, $p_{FDR.basic}$=0.047; $beta_{full.adj}$=0.11, $p_{FDR.full.adj}$=0.047; Figure 2); this association was significant also in statin-nontreated subsample ($beta_{basic}$=0.10, $p_{FDR.basic}$=0.025; $beta_{full.adj}$=0.10, $p_{FDR.full.adj}$=0.040). The glucuronate-by-sex interaction did not reach statistical significance ($p_{sexINT.basic}$=0.053, $p_{sexINT.full.adj}$=0.129).

Lipid measures

WMH volume was associated with lower circulating concentrations of lysophosphatidylcholines (LPCs) and hydroxylated sphingomyelins (SM-OHs) (Figure 3). Among the studied LPCs, the strongest association was seen with LPC(22:6), which was significant in the pooled sample ($beta_{basic}$=−0.078, $p_{FDR.basic}$=0.028; $beta_{full.adj}$=−0.082, $p_{FDR.full.adj}$=0.047) and in the male subsample ($beta_{basic}$=−0.11, $p_{FDR.basic}$=0.025; $beta_{full.adj}$=−0.12, $p_{FDR.full.adj}$=0.046). Among the studied SM-OHs, the strongest association was observed with SM (OH) C22:2, and this association was significant in the male subsample only ($beta_{basic}$=−0.11, $p_{FDR.basic}$=0.017; $beta_{full.adj}$=−0.10, $p_{FDR.full.adj}$=0.042). Typically, the interactions between LPC measures and sex or statin treatment did not reach statistical significance (Tables S5 and S6); the exceptions were nominally significant interaction with sex for LPC(17:0) in the basic and fully adjusted models ($p_{sexINT.basic}$=0.027, $p_{sexINT.fully.adj}$=0.045, respectively), and with statin treatment for LPC(20:4) in the fully adjusted model ($p_{statinINT.fully.adj}$=0.015) (Figure 3). The SM-OH species-by-sex interactions were nominally significant in both basic and fully adjusted models for SM (OH) C14:1 ($p_{sexINT.basic}$=0.018, $p_{sexINT.fully.adj}$=0.033), SM (OH) C16:1 ($p_{sexINT.basic}$=0.027, $p_{sexINT.fully.adj}$=0.044), and SM (OH) C22:2 ($p_{sexINT.basic}$=0.0061,

$p_{sexINT.fully.adj}$=0.013). Also, SM (OH) C14:1-by-statin treatment interaction was nominally significant in the fully adjusted model ($p_{statinINT.fully.adj}$=0.047).

A sizable proportion of the studied metabolomic measures were circulating concentrations of lipoproteins and lipoprotein lipids (approximately 24% of the meta-analyzed measures). Out of the 30 metabolomic measures showing FDR-significant associations with WMH in the basic models, 12 were with measures of the lipid composition of the intermediate density and low-density lipoprotein (IDL, LDL) particles (lipid composition calculated as a ratio of a lipid concentration against total lipids concentration within individual lipoprotein subfractions). Specifically, higher WMH volume was associated with higher TG-to-total-lipids ratio and lower cholesteryl ester (CE)-to-total-lipids ratio in the pooled sample and male subsample (Figure 4). The effect sizes were attenuated in the fully adjusted models (Figure 4). Concomitantly, higher WMH volume was associated with larger LDL-particle diameter in the male subsample, and this association remained FDR-significant in the fully adjusted model ($beta_{basic}$=0.074, $p_{FDR.basic}$=0.049; $beta_{full.adj}$=0.078, $p_{FDR.full.adj}$=0.047). We observed a nominally significant LDL diameter-by-sex interaction ($p_{sexINT.basic}$=0.0093, $p_{sexINT.full.adj}$=0.036). Higher WMH volume was also associated with lower free cholesterol-to-total-lipids-ratio within medium-sized very-low-density lipoprotein (VLDL) subfraction in the statin-treated subsample (Figure 1), but no other significant association with either VLDL or high-density lipoprotein (HDL) subfraction measures was seen (Tables S3 and S4).

DISCUSSION

In this study, we investigated associations between WMH volume and circulating metabolomic measures in up to 9,290 individuals. As discussed in the following text, several aspects of our metabolomic findings support a possible vessel-related pathophysiology of WMH, and some suggest the involvement of myelin disruption and neuron injury. Further, a number of the observed associations showed sex specificities indicating that distinct metabolomic features accompany WMH in males and females.

In the present study, the most robust association was the positive association between WMH volume and HPP in males ($p_{FDR.full.adj}=1.40\times10^{-7}$); the association was also significant in the pooled sample ($p_{FDR.full.adj}=1.66\times10^{-4}$) and statin-nontreated individuals ($p_{FDR.full.adj}=1.65\times10^{-6}$). HPP is a potentially toxic compound derived from the catabolism of phenylalanine and tyrosine[36] (Figure 2). Higher circulating levels of phenylalanine and tyrosine have been associated with higher risk of cardiovascular disease (CVD) in prior studies[37,38], but, to our knowledge, HPP was not examined in those studies. We did not see strong associations with phenylalanine or tyrosine and, thus, the association between WMH and HPP likely arises downstream from phenylalanine hydroxylase (PAH) (Figure 2). The enzymatic alterations possibly contributing to higher level of HPP could be a higher activity of tyrosine aminotransferase (TAT) or lower activity of hydroxyphenylpyruvate dioxygenase (HPD). In tyrosine breakdown, TAT converts tyrosine and α-ketoglutarate to HPP and glutamate[39] and, thus, higher TAT activity could contribute to lower α-ketoglutarate and, at the same time, to higher glutamate. In males and statin-treated participants, however, we found only a nominal association between WMH and circulating α-ketoglutarate that was

not in the anticipated direction and no association with glutamate (Figure 2). Therefore, in the view of these results, lower HPD activity appears as the most likely mechanism driving the association between WMH and circulating HPP. HPD requires oxygen to convert HPP to homogentisic acid (Figure 2) and, consistent with this requirement, hypoxia promotes accumulation of HPP[40]. The deep and periventricular white matter, which is the predilection site for WMH, is characterized by sparse vasculature consisting of long, narrow end arteries/arterioles that are vulnerable to oxygen desaturation[41]. Thus, higher circulating HPP in association with higher WMH may be an indicator of ischemic hypoxia promoting WMH. As indicated above, the association of HPP with WMH volume was present in the pooled sample, in males, and in the statin-nontreated subsample, but not in females. In males, HPP explained 3.3% and 14.3% of variance in WMH in Insight46 and the 3rd Generation of Framingham Heart Study (FHS-GEN3), respectively, the two cohorts providing results for HPP in the fully adjusted model (Table S3). Consistent with previous research demonstrating that vascular risk factors, including hypertension, explain only up to 2% of variance in WMH volume[7,42], we found the respective proportions of variance explained by hypertension, diabetes, and smoking to be 0.1%, 2.9%, and 0.03% in males from Insight46 and 1.1%, 1.0% and 0.1% in males from FHS-GEN3 (Table S3). Taken together, our results suggest that circulating HPP may be a strong biomarker of WMH in males, but its potential in clinical use requires further research.

Glucuronate was the only metabolite demonstrating a robust association with WMH in females ($p_{FDR.full.adj}$=0.047); the association was also significant in statin-nontreated participants ($p_{FDR.full.adj}$=0.040). Glucuronate is derived from glucose, and, in humans, it is involved in the elimination of toxic substances by making them more water-soluble in a process called glucuronidation[36]. In addition, hormones can be glucuronidated to enable easier transport[36]. A key enzyme of glucuronidation is UDP-glucuronosyltransferase, which is highly expressed in endothelial cells of the blood-brain barrier (BBB) and associated astrocytes, where the enzyme contributes to the protection of the brain from systemic toxic substances (reviewed by Ouzzine *et al.*[43]). Also, endothelial cells use glucuronate to synthesize glycosaminoglycans, which are constituents of the glycocalyx layer that tightens the endothelial barrier and limits vascular permeability[44]. The observed WMH association with glucuronate may involve altered glucose metabolism in endothelial cells, which might compromise the molecular mechanisms enabling the protective functions of the BBB. Of note, the association between WMH and glucuronate remained significant after adjusting for type 2 diabetes in the fully adjusted model. In our study, WMH association with (mostly fasting) glucose level did not reach statistical significance, which is in line with some[45] but not all[46] previous reports.

In addition to the above-discussed non-lipid measures, we found that WMH were associated with several circulating lipids – most notably with LPCs and SM-OHs. These associations were almost exclusively negative and reached statistical significance predominantly in males and, in some cases, also in the pooled sample. LPCs are phospholipids generated by partial hydrolysis of phosphatidylcholines, which are the building blocks of cell membranes, such as those of blood cells and endothelial cells. In circulation, LPCs modulate inflammation and oxidative stress[47,48], and they may alter the integrity of endothelial membranes, including the BBB[49,50]. Lower circulating levels of LPCs, such as LPC(18:2), have been

associated with higher CVD risk[51] and adverse cognitive outcomes[52]. Consistent with these previous reports, we found that lower LPC(18:2) is associated with higher WMH volume, but similar to most other tested LPCs, the association reached only nominal significance in the fully adjusted model. The only LPC that remained significantly associated with WMH in the fully adjusted model was LPC(22:6) (males: $p_{FDR.full.adj}$=0.046; pooled sample: $p_{FDR.full.adj}$=0.047). Docosahexaenoic acid (DHA, 22:6n-3) is one of the two predominant fatty acids in the human brain and a structural component of neuronal cell membranes[53,54]. DHA plays a crucial role in neuronal survival, neurogenesis, and synaptic function[53]. Evidence obtained in mice suggests that oral administration of LPC-DHA, but not free DHA, increases DHA content of the brain and improves spatial learning and memory[55]. Thus, the associations of circulating LPCs with WMH observed here suggest the BBB and neuron injury-related pathobiologies of WMH.

Similar to LPCs, the associations between WMH and SM-OHs were negative, with the most significantly associated SM-OH being SM (OH) C22:2 in males ($p_{FDR.full.adj}$=0.042). SM-OHs are important components of myelin sheaths[56], which are lipid-rich cell membranes wrapped around neuronal axons, protecting the axons physically and providing trophic support, as well as increasing the conduction speed of action potentials[57]. The negative associations between WMH and SM-OHs could point towards disruption of myelin sheaths[56], which is one of the key features of WMH[1]. In line with our findings, previous evidence suggests that low level of serum total SM is associated with cross-sectional memory impairment[58].

Regarding the associations with lipoprotein measures, we observed that WMH were associated with larger LDL-particle diameter ($p_{FDR.full.adj}$=0.047 in males), likely arising from altered LDL-lipid composition, namely relatively higher TG and lower CE content. Putatively, an altered interaction between lipoproteins and enzymes modulating their lipid composition could play a role in these associations. For example, the endothelium-bound lipoprotein lipase (LPL), which catalyzes the hydrolysis of lipoprotein TGs, interacts directly with LDL-receptor related protein[59] that is involved in the regulation of the BBB permeability[60]; as such, LPL displays functions related to both blood metabolome and WMH found in areas with enhanced permeability of the BBB[61]. Statin treatment may have a small confounding effect on these associations, as statin-treated individuals have the highest WMH volumes together with the lowest cholesterol levels (Table 1). But considering the facts that the effect estimates of these measures were consistent across all analytical subsamples, including statin-nontreated individuals, and, that the effect estimates were only marginally attenuated in the statin use-adjusted models compared with the basic models (Figure 4), statin use does not seem to be a key factor driving the negative associations between WMH and lower CE content in the LDL subfractions.

In the present study, several of the FDR-significant associations in the fully adjusted models showed nominally significant metabolite-by-sex interactions suggesting that the effect sizes vary depending on sex. These associations included the amino acid-derivative HPP ($p_{sexINT.full.adj}$=0.0077), and two lipid measures, namely SM (OH) C22:2 ($p_{sexINT.full.adj}$=0.013) and LDL-particle size ($p_{sexINT.full.adj}$=0.036): for all three, the effect sizes were nominally greater in males than in females. These sex specificities were observed

despite similar sample sizes of males and females, and no major sex differences in the distributions of WMH and key traits, such as BMI, cigarette smoking or clinical lipid traits (Table 1). Also, the metabolomic measures did not show major sex differences, except for the circulating levels of SM (OH) C22:2, which tended to be lower in males than in females (Figure S1), as reported previously[62]. Considering that hydroxysphingomyelins are important for the maintenance of myelination[56], the lower levels of this lipid in males than in females could contribute to the nominally greater negative effect size of SM (OH) C22:2 on WMH in males than in females. Regarding HPP, the circulating levels were similar between males and females (Figure S1), and the biological mechanism of the nominally greater positive effect of HPP on WMH in males vs. females remains inconclusive. Nevertheless, it could be that circulating HPP is a marker of ischemic hypoxia that is of a similar magnitude in both males and females (as reflected by similar circulating levels of HPP in the two sexes), but the ischemic hypoxia is of a greater impact in males than in females. This is supported by previous research indicating that, in males compared with females, ischemic hypoxia in the brain induces greater oxidative stress and, thus, possibly greater tissue damage and more extensive WMH[63,64]. Finally, with respect to the LDL-particle size, this measure also did not show consistent sex differences across the analyzed cohorts (Figure S1), and the biological mechanisms of the nominally greater positive effect size of LDL-particle size on WMH in males than in females are not clear. Previous literature on sex differences in WMH is not extensive[65–67]. Sex hormones may play a role, as they show direct effects on the endothelium[68] and multiple brain cells, including oligodendrocytes producing myelin[69] and neurons[70]. In our study, female hormonal status was not likely a major factor in the observed sex specificities, as female participants were likely menopausal (average age >60 years). We cannot, however, exclude the possibility that the use of hormonal replacement therapy and a greater lifetime exposure to female sex hormones would modulate the associations examined in older age. Overall, our results indicating sex-specific associations between metabolomic measures and WMH add to the growing body of research reporting sex differences in the associations between risk factors and cerebrovascular outcomes[71–75].

The strengths and limitations of this study should be considered. We analyzed data from multiple population-based cohorts that enables a large sample size, yielding the statistical power required to detect reliably the small effect sizes and to replicate/meta-analyze the results. Although the study sample included individuals from multiple geographical areas and diverse ethnic backgrounds, the vast majority of participants were of European ancestries, and, thus, replication of the findings in other ethnicities would be of high value. The fact that we analyzed metabolomic data quantified with multiple platforms could be considered as both a strength and a limitation: while covering a wide spectrum of circulating lipids and metabolites, some of the metabolic measures are underrepresented and, therefore, we may lack statistical power to detect associations with these measures. Further, due to the multiple testing correction to minimize the risk of false positive associations, some biologically relevant associations may be missed. For instance, many of the associations between WMH and lipid concentrations within IDL and LDL subfractions were negative with non-null-containing 95% confidence intervals; however, only a minority of these associations reached FDR-significance. As lipoprotein metabolism is an interconnected

system, it may be more meaningful to look at the big picture instead of interpreting the associations on the level of individual metabolic measures. In most cohorts, WMH were quantified with automated image-analysis techniques. Therefore, we cannot exclude the possibility that small lacunar lesions (also a feature of small vessel disease[2,76]) were included in the quantified WMH volume. This potential misclassification is expected to be low, however, as lacune prevalence is not high in the general population[77] and, when present, lacunes usually occur as singular or in low numbers, and the volume of WMH surrounding small lacunes is low. Manual segmentation of WMH is extremely labor-intensive and, therefore, it is not frequently used in large-scale cohort studies including thousands of participants[6], such as the ones included here. In future studies, automatic segmentation of multi-modal MR images may allow one to compare metabolic profiles of different types of tissue abnormalities present in white matter. Additionally, it would be of high value to assess the causal inferences between circulating metabolites and WMH in future studies once suitable genetic instruments are available. Considering the results of our study, it is likely necessary to test the causality in sex-specific analyses. Finally, further insight into the pathophysiology of WMH awaits the results of LACI-2 trial, as this trial is testing two repurposed licenced drugs with effects on endothelium-dependent vasodilation and BBB integrity to prevent progression of cerebral small disease[78].

In summary, this is the first large-scale study to determine associations between WMH and circulating metabolomic measures. While no causal associations can be inferred from the present findings, our findings indicate that alterations in circulating metabolism are closely linked with WMH in a general population of middle aged and older adults. The present findings suggest that the metabolomic profiles accompanying WMH in males and females may differ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest disclosures

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Non-standard Abbreviations and Acronyms:

BBB	blood-brain barrier
CE	cholesteryl ester
FDR	false discovery rate
HPD	hydroxyphenylpyruvate dioxygenase
HPP	hydroxyphenylpyruvate
IDL	intermediate-density lipoprotein
LDL	low-density lipoprotein
LPC	lysophosphatidylcholines
LPL	lipoprotein lipase
MRI	magnetic resonance imaging
PAH	phenylalanine hydroxylase
SM-OH	hydroxylated sphingomyelin
T1W	T1-weighted
T2W	T2-weighted
TAT	tyrosine aminotransferase
TG	triacylglycero

WMH white matter hyperintensities

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CLINICAL PERSPECTIVE

What is new?

- This is the first large-scale study to identify circulating metabolomic measures associated with white matter hyperintensities (WMH) volume, which is the most common brain-imaging marker of small vessel disease and a major risk factor for incident stroke, dementia, and all-cause mortality.
- The metabolomic profile of WMH volume appears largely sex specific.
- The most significant metabolite, hydroxyphenylpyruvate, explains up to 6% and 14% of variance in WMH volume in the pooled sample and in males, respectively, which is comparatively more than the proportions of variance explained by hypertension (1%), type 2 diabetes (1–3%) or smoking (<0.1%).

What are the clinical implications?

- Hydroxyphenylpyruvate is a new potentially useful clinical biomarker explaining more variance in WMH volume than the established WMH risk factors.
- The marked sex specificities in the metabolomic associations of WMH volume add to the growing body of research suggesting that sex-specific molecular pathways underlie the associations between risk factors and cerebrovascular outcomes.
- Some associated metabolites support vessel-related pathophysiology of WMH, while others suggest the involvement of myelin disruption and neuron injury.

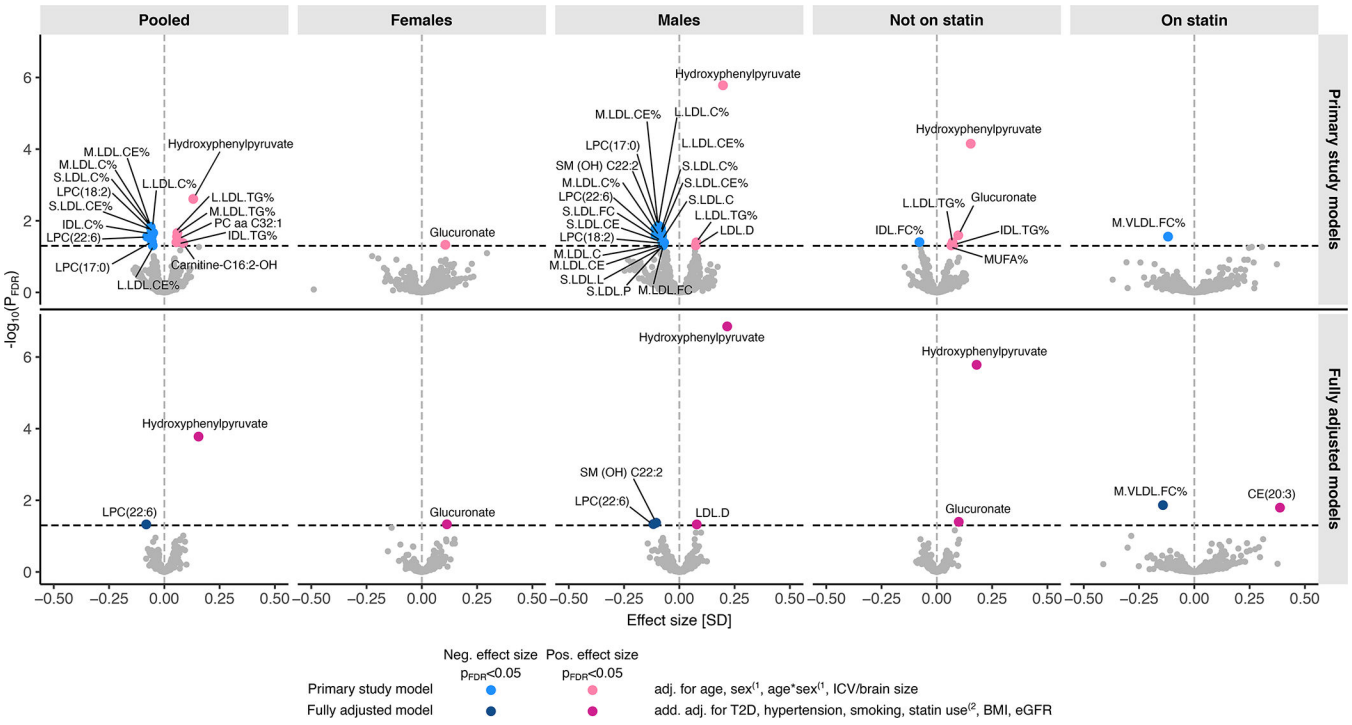

Figure 1. Metabolomic associations of white matter hyperintensities.

The plot shows the effect sizes (x-axis) and statistical significance (y-axis) of the metabolomic associations of WMH as obtained in the meta-analysis of up to 9,290 individuals from 15 populations of mostly European ancestry. Metabolomic associations of log-transformed WMH were determined by fitting linear regression models separately in a pooled sample, and in sex or statin treatment-stratified subsamples (columns). Population-specific effect sizes and standard errors were combined with inverse variance-weighted fixed effect meta-analysis. The metabolomic measures showing a significant (p_{FDR}<0.05) association with WMH are labelled. *Primary study models (top row)*: The associations were adjusted for age and intracranial volume or brain size, and also for sex and age-by-sex interaction in the pooled sample and in statin treatment-stratified subsamples[1]. *Fully adjusted models (bottom row)*: The associations were additionally adjusted for type 2 diabetes (T2D), hypertension, current smoking status, body mass index (BMI), estimated glomerular filtration rate (eGFR), and hypertension, and for statin treatment in the pooled sample and in sex-stratified subsamples[2]. Where relevant, all models were also adjusted for fasting duration, time between blood sampling and brain MRI, and cohort study-specific covariates. C indicates cholesterol; CE, cholesteryl ester; FC, free cholesterol; FDR, false discovery rate; ICV, intracranial volume; IDL, intermediate-density lipoprotein; L, large; LDL, low-density lipoprotein; LPC, lysophosphatidylcholines; M, medium; MUFA, monounsaturated fatty acid; S, small; SM-OH, hydroxylated sphingomyelin; TG, triglycerides; and VLDL, very-low-density lipoprotein.

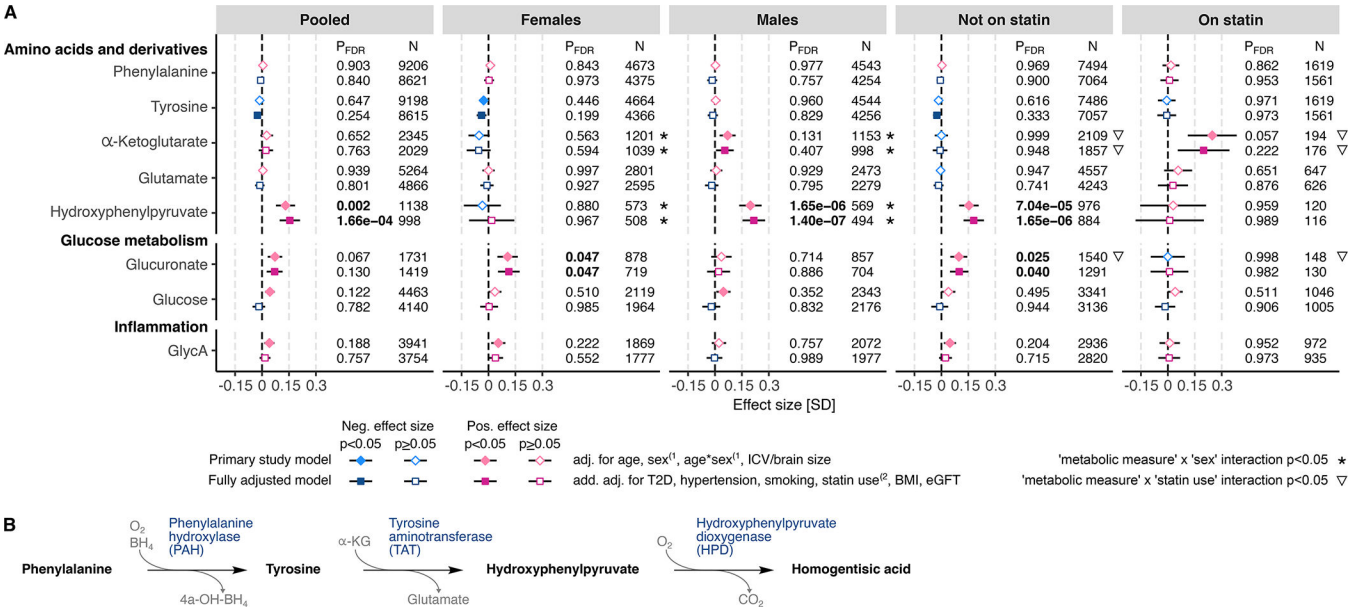

Figure 2. White matter hyperintensities associations with circulating levels of selected nonlipid metabolites.

A) The associations between WMH and circulating metabolomic measures were determined using linear regression. The primary study models (diamonds) were adjusted for age and intracranial volume or brain size, and, in the pooled sample and in statin treatment-stratified subsamples also for sex and age-by-sex interaction(1. The fully adjusted models (squares) were additionally adjusted for type 2 diabetes (T2D), hypertension, current smoking status, body mass index (BMI), and estimated glomerular filtration rate (eGFR), and, in the pooled sample and in sex-stratified subsamples, also for statin treatment(2. Where relevant, all models were adjusted for fasting duration, time between blood sampling and brain MRI, and possible cohort study-specific covariates. Blue color indicates negative effect size and red color indicates positive effect size. Error bars indicate 95% confidence intervals. P-values below the threshold for multiple testing correction (p_{FDR}<0.05) are indicated with bold font. B) Catabolic pathway of phenylalanine and tyrosine into hydroxyphenylpyruvate and homogentisic acid by phenylalanine hydroxylase (PAH), tyrosine aminotransferase (TAT) and hydroxyphenylpyruvate dioxygenase (HPD). FDR indicates false discovery rate; and GlycA, glycoprotein acetylation.

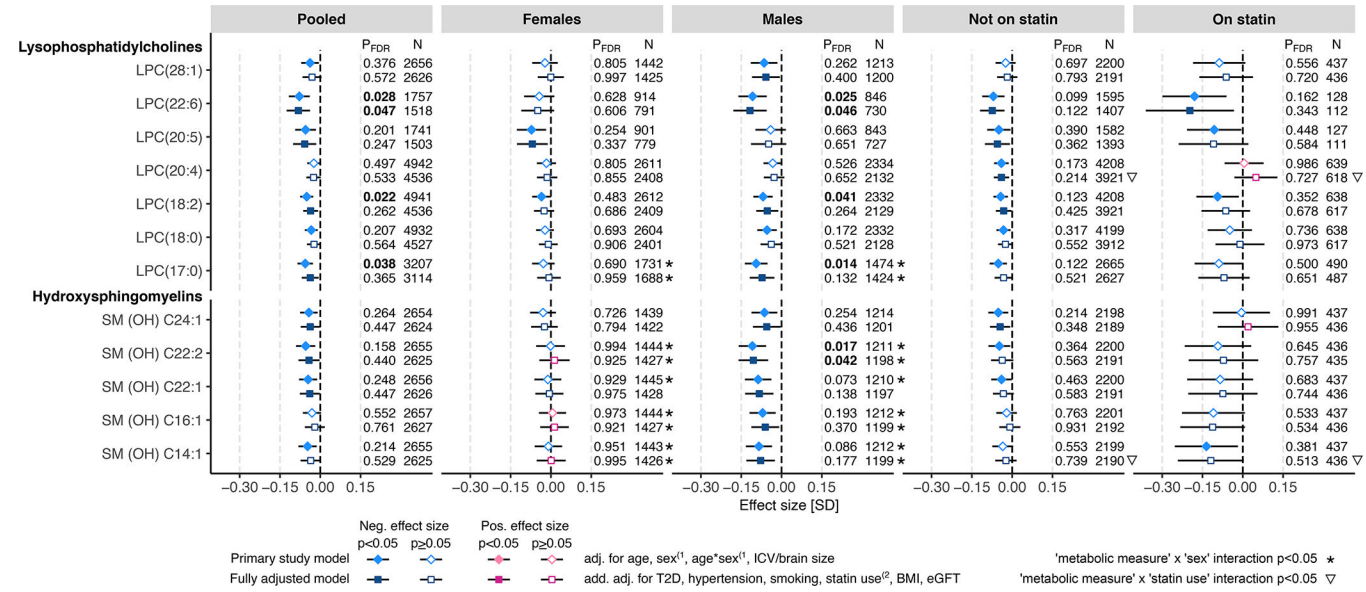

Figure 3. WMH associations with circulating levels of lysophosphatidylcholines (LPC) and hydroxylated sphingomyelins (SM (OH)).

The associations between WMH and circulating metabolomic measures were determined using linear regression. The primary study models (diamonds) were adjusted for age and intracranial volume or brain size, and, in the pooled sample and in statin treatment-stratified subsamples also for sex and age-by-sex interaction(1. The fully adjusted models (squares) were additionally adjusted for type 2 diabetes (T2D), hypertension, current smoking status, body mass index (BMI), and estimated glomerular filtration rate (eGFR), and, in the pooled sample and in sex-stratified subsamples, also for statin treatment(2. Where relevant, all models were adjusted for fasting duration, time between blood sampling and brain MRI, and possible cohort study-specific covariates. Blue color indicates negative effect size and red color indicates positive effect size. Error bars indicate 95% confidence intervals. P-values below the threshold for multiple testing correction (p_{FDR}<0.05) are indicated with bold font. FDR indicates false discovery rate; LPC, lysophosphatidylcholine; and SM (OH), hydroxylated sphingomyelin

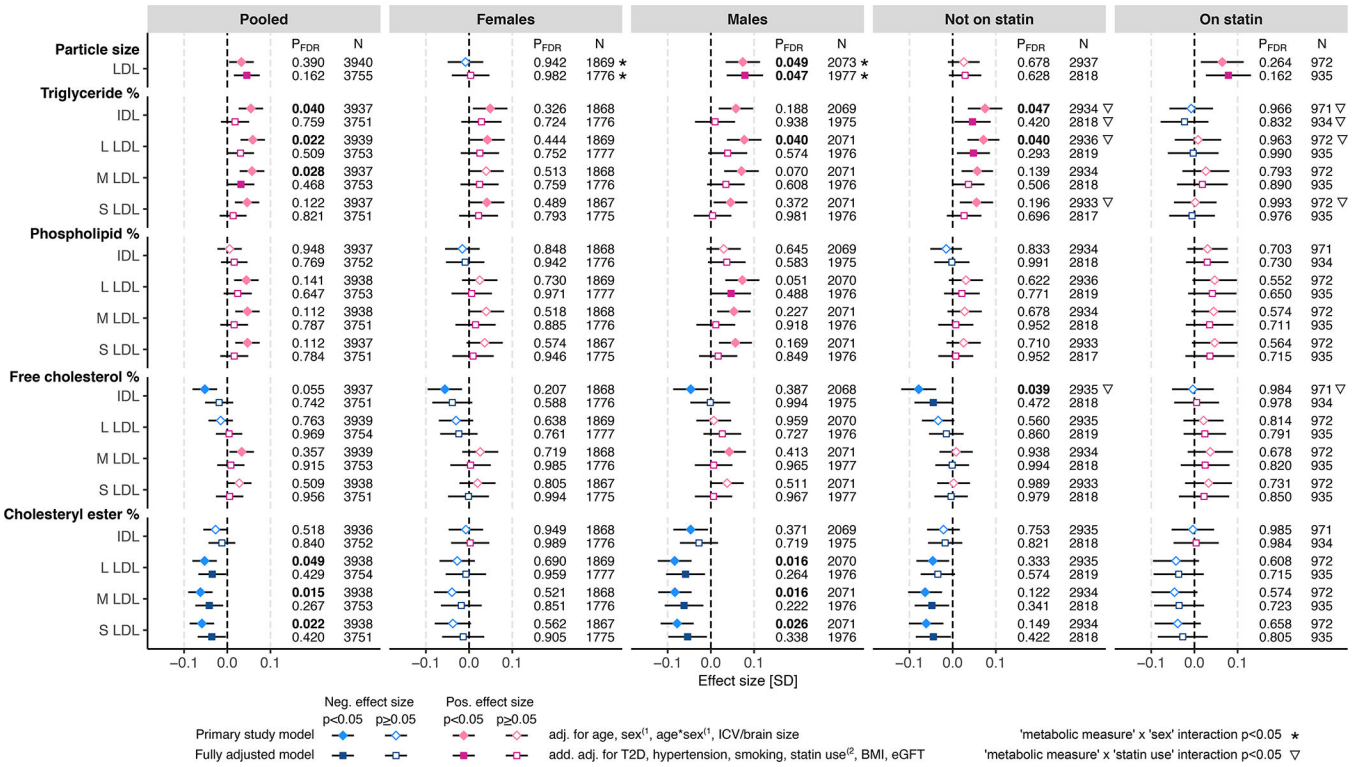

Figure 4. WMH associations with LDL particle size and lipid composition measures in IDL and LDL subfractions.

The associations between WMH and circulating metabolic measures were determined using linear regression. The primary study models (diamonds) were adjusted for age and intracranial volume or brain size, and, in the pooled sample and in statin treatment-stratified subsamples also for sex and age-by-sex interaction[1]. The fully adjusted models (squares) were additionally adjusted for type 2 diabetes (T2D), hypertension, current smoking status, body mass index (BMI), and estimated glomerular filtration rate (eGFR), and, in the pooled sample and in sex-stratified subsamples, also for statin treatment[2]. Where relevant, all models were adjusted for fasting duration, time between blood sampling and brain MRI, and possible cohort study-specific covariates. Blue color indicates negative effect size and red color indicates positive effect size. Error bars indicate 95% confidence intervals. P-values below the threshold for multiple testing correction (p_{FDR}<0.05) are indicated with bold font. FDR indicates false discovery rate; IDL, intermediate-density lipoprotein; L, large; LDL, low-density lipoprotein; M, medium; and S, small.

Table 1.

Sample characteristics.

Values are mean ± standard deviation of the pooled data of 15 populations from 8 cohort studies. The population-specific characteristics are given in Table S2.

Characteristic	Combined	Females	Males	Not on statin	On statin
Number of individuals	9,290	4,711	4,579	7,565	1,633
Males (%)	49.3	0	100	47.2	58.7
Smokers (%)	13.6	14.0	13.3	14.5	12.4
On statin (%)	17.7	14.4	21.0	0	100
Hypertension (%)	47.1	44.9	49.3	41.3	74.4
Type 2 diabetes (%)	7.0	6.1	7.9	4.0	20.7
Age (years)	61.0 ± 7.3	60.7 ± 7.4	61.4 ± 7.2	59.6 ± 7.5	67.9 ± 5.6
BMI (kg/m^2)	27.3 ± 4.4	27.1 ± 4.7	27.5 ± 4.0	27.1 ± 4.4	28.2 ± 4.1
logWMH	5.35 ± 0.95	5.17 ± 0.93	5.35 ± 0.95	5.05 ± 0.91	6.78 ± 1.07
Total-TG (mmol/L) *	1.38 ± 0.65	1.37 ± 0.62	1.39 ± 0.65	1.36 ± 0.63	1.45 ± 0.69
Total-C (mmol/L) *	4.95 ± 1.00	5.10 ± 0.97	4.81 ± 0.97	5.18 ± 0.93	4.17 ± 0.89
LDL-C (mmol/L) **	2.39 ± 0.67	2.49 ± 0.67	2.31 ± 0.66	2.57 ± 0.64	1.74 ± 0.54
HDL-C (mmol/L) **	1.51 ± 0.36	1.58 ± 0.34	1.43 ± 0.31	1.54 ± 0.63	1.39 ± 0.33

* Pooled data of 9 populations for which total-TG and total-C were available.

** Pooled data of 10 populations for which LDL-C and HDL-C were available.

BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and WMH, white matter hyperintensities.