RESEARCH ARTICLE

Circulating metabolites are associated with brain atrophy and white matter hyperintensities

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Abstract

Introduction: Our aim was to study whether systemic metabolites are associated with magnetic resonance imaging (MRI) measures of brain and hippocampal atrophy and white matter hyperintensities (WMH).

Methods: We studied associations of 143 plasma-based metabolites with MRI measures of brain and hippocampal atrophy and WMH in three independent cohorts (n = 3962). We meta-analyzed the results of linear regression analyses to determine the association of metabolites with MRI measures.

Results: Higher glucose levels and lower levels of three small high density lipoprotein (HDL) particles were associated with brain atrophy. Higher glucose levels were associated with WMH.

Discussion: Glucose levels were associated with brain atrophy and WMH, and small HDL particle levels were associated with brain atrophy. Circulating metabolites may aid in developing future intervention trials.

KEYWORDS
Alzheimer's disease, cholesterol, glucose, lipids, magnetic resonance imaging (MRI), metabolism

1 | BACKGROUND

Dementia, including Alzheimer's disease (AD), is a rapidly growing health care problem. Vascular disease is an important contributor to AD pathology.[1] Moreover, adequate treatment of cardiovascular risk factors has been associated with a reduced risk of dementia.[2] Low density lipoprotein cholesterol (LDL) is an important risk factor for cardiovascular disease, and lowering LDL improves cardiovascular outcomes.[3] This has fueled research on metabolic factors that are potentially involved in the etiology of AD. Brain atrophy, hippocampal atrophy, and white matter hyperintensities (WMH) measured on magnetic resonance imaging (MRI) are neurodegenerative and vascular imaging markers characteristic of AD.[4–6] Detailed understanding of metabolic factors related to imaging markers of AD can provide insight into biological pathways.

Metabolic processes can be investigated currently by large high throughput platforms for simultaneous analysis of many metabolites.[7] Previous studies highlight that altered lipid metabolism and decreased levels of amino acids are associated with cognitive decline and dementia.[8,9] Moreover, in a recent multicenter study, we found 15 metabolites associated with cognitive function including higher high-density lipoprotein (HDL) subclasses and docosahexaenoic acid and lower ornithine, glutamine, and glycoprotein acetyls.[10] These studies, however, only associated metabolite concentrations with clinical signs and symptoms. To study possible underlying mechanisms of metabolic dysregulation in AD, studies should include biological measures, such as brain MRI features of brain atrophy and WMH.

We aimed to investigate the association between blood-based metabolites and global brain atrophy, hippocampal atrophy, and WMH across the clinical spectrum of AD in almost 4000 participants from three different Dutch cohort studies, a memory-clinic study, a population-based study, and a family-based study.

2 | METHODS

2.1 | Cohort description

The study population included 3962 participants from three prospective cohort studies; the memory-clinic–based Amsterdam Dementia Cohort (ADC; n = 980), the population-based Rotterdam Study (n = 2918), and the family-based Erasmus Rucphen Family (ERF) Study (n = 64). All studies were part of the BioBanking for Medical Research Infrastructure of the Netherlands (BBMRI) metabolomics consortium. Participants were included if they underwent brain MRI and metabolite data were available. In addition, in the ADC, participants were only included with a clinical diagnosis of mild cognitive impairment (MCI) (n = 130) or AD dementia (n = 523), and controls with subjective cognitive decline (n = 327).[11] In the Rotterdam Study (n = 2918) and ERF Study (n = 64), participants were only included if they had no dementia or stroke.[12,13] All studies have been approved by a medical ethics committee. All participants provided written informed consent to participate in the study.

2.2 | MRI measures

2.2.1 | Amsterdam Dementia Cohort

MRI scans were obtained at 1.0, 1.5, or 3.0 T scanners. Details on scanners and acquisition parameters can be found in Supplementary Table 1.

The scan protocol essentially remained the same over the years. Visual ratings were performed by a trained rater and subsequently evaluated in a consensus meeting together with an experienced neuroradiologist.[14,15] Global cortical atrophy (GCA) was visually rated on axial fluid-attenuated inversion recovery sequence (FLAIR) sequence images (range 0-3).[16] Medial temporal lobe atrophy (MTA) was rated using a five-point rating scale (0-4)[17] on coronal T1-weighted images; the mean of left and right MTA scores was used for data analysis. WMH were assessed on the FLAIR images using the Fazekas scale, with scores from 0 to 3 (none, punctuate, early confluent, and confluent).[18] More information about the visual rating scales can be found in Supplementary Table 2.

2.2.2 | Rotterdam Study and Erasmus Rucphen Family Study

Brain MRI scans were obtained with a 1.5-T scanner.[19] Details on scanners and acquisition parameters can be found in Supplementary Table 1. Brain volume, gray matter volume, white matter volume, WMH volume, and intracranial volume (ICV) (in milliliters) were estimated using automated segmentation using the FreeSurfer software.[19] Total

brain volume was defined as the sum of all voxels within the skull, except cerebellum, brainstem, ventricles, cerebrospinal fluid (CSF), and choroid plexus.[20] Hippocampus volume was defined as the mean of right and left hippocampal volumes.

2.3 | Metabolites

Metabolites were quantified from non-fasted (in ADC) and fasted (in Rotterdam Study and ERF Study) ethylenediaminetetraacetic acid (EDTA) plasma samples using high-throughput proton nuclear magnetic resonance metabolomics (Nightingale Ltd, Helsinki, Finland).This metabolite platform enables simultaneous quantification of 231 lipoprotein subclasses and metabolites including amino acids, ketone bodies, and gluconeogenesis-related metabolites.[10,21,22] The data set included 150 absolute metabolite measures. Six metabolites with >10% missing in one of the cohorts were excluded from data analysis. Pyruvate was excluded because this measurement is not reliable in EDTA plasma.[23] All included metabolites were measured as concentrations ([m]mol/L or g/L), except for albumin reported as signal area and three metabolite derivatives measuring lipid particle volume in nanometer. The final data set included 143 metabolites.

2.4 | Covariates

In ADC, Rotterdam study, and ERF Study apolipoprotein E (*APOE*) genotype was measured as described previously.[24–26] Subjects were classified as *APOE ε*4 carrier or non-carrier. Use of lipid-lowering medication (yes/no) was assessed in all cohorts. Body mass index (BMI) was calculated as kg/m^2. In the ADC, 125 subjects (13%) missed BMI measurement. Missing values were estimated by five times imputation using the predictive mean matching method as implemented in the R package MICE.

2.5 | Data pre-processing

All metabolites were transformed using natural logarithmic transformation (ln[x+1]), and next, both metabolites and MRI measures were Z-transformed. For Z-transformation we used the mean and SD of each (sub)cohort in the Rotterdam Study and ERF Study. For the ADC Z-transformation of metabolites and MRI measures was done by calculating SD units with controls as a reference group in order to increase comparability of effects between cohorts. For the Rotterdam Study and ERF Study the measurement of brain MRI measurements was transformed using ln(x+1) before Z-transformation. GCA and MTA were inversed in such a way that direction of visual scores in the ADC cohort was the same as for WMH and the volumetric data in the Rotterdam Study and ERF Study (ie, higher scores means less brain/hippocampal atrophy or more WMH).

HIGHLIGHTS

- Multiple metabolites were related to brain atrophy and white matter hyperintensities (WMH).
- These metabolites are promising for further validation.
- Predominantly lower small high-density lipoprotein (HDL) particle levels were associated with more brain atrophy.
- Higher glucose levels were associated with more brain atrophy and WMH.
- These metabolites might be of interest for future studies exploring biological pathways in neurodegenerative diseases, such as Alzheimer's disease (AD).

RESEARCH IN CONTEXT

1. **Systematic review**: Previous studies have discovered circulating metabolites as determinants of cognitive function and dementia. However, underlying biological mechanisms between metabolites and Alzheimer's disease (AD) have not been studied. Examining the associations of metabolites with brain magnetic resonance imaging (MRI) measures characteristic of AD might provide a deeper understanding of the metabolic determinants of AD.
2. **Interpretation**: In this multi-cohort study we found that higher glucose levels were associated with brain atrophy and WMH, and lower small HDL particle levels were associated with brain atrophy.
3. **Future directions**: Our findings have emphasized that circulating metabolites are related to neurodegenerative and neurovascular brain changes that are characteristic for AD. Future studies should be performed to validate our metabolite findings and indicate if metabolites can be used as treatment targets or to stratify patients for intervention trials.

2.6 | Statistical analyses

All analyses were performed in R (version 3.5.2 [2018-07-02]). Cohort differences in participant characteristics were tested using one-way analysis of variance with post-hoc Bonferroni adjusted *t* tests for continuous variables or χ^2 tests for categorical variables. Linear regression analyses were used to assess the association of each of the 143 metabolites with brain atrophy, hippocampal atrophy, and WMH in separate models. All associations were assessed in two models: a first model, adjusted for age and sex. In the Rotterdam Study and ERF Study the first model was additionally adjusted for ICV. In model 2,

TABLE 1 Characteristics of the study population by cohort

Cohort	ADC	Rotterdam Study	ERF Study	P-value
N	980	2918	64	
Age, years	64 ± 9	69 ± 9[a]	64 ± 4[b]	<0.001
Female	449 (46)	1664 (57)[a]	35 (55)	<0.001
Diagnosis	Controls 327 (33) MCI 130 (13) AD dementia 523 (53)	No dementia 2918 (100)	No dementia 64 (100)	N/A
APOE ε4 carrier	519 (54)	762 (27)[a]	23 (40)[a]	<0.001
Lipid-lowering medication	209 (21)	745 (25)[a]	16 (25)	0.03
Time difference scan date and date blood withdrawal, years	0.0 ± 0.0	2.0 ± 3.4[a]	3.7 ± 0.7[a,b]	<0.001
Stroke	24 (2)	0 (0)	0 (0)	N/A
MRI				
GCA scale	0 (0-1)			
MTA scale	1 (0-1.5)			
Fazekas scale	1 (0-1)			
Intracranial volume, mm^3		1,469,849 (1,366,670-1,584,694)	1,419,618 (1,291,443-1,544,271)	
Total brain volume, mm^3		889,950 (826,270-959,679)	878,054 (815,299-958,440)	
Hippocampal volume, mm^3		3,852 (3,544-4,131)	3,810 (3,524-4,155)	
White matter hyperintensity volume, mm^3		1,952 (1,282-3,405)	1,710 (1,005-2,854)	

Data are presented as mean ± SD, median (interquartile range) or n (%). Differences were tested with one-way analysis of variance (ANOVA) with post hoc Bonferroni adjusted *t* tests for continuous variables and with chi-square tests for categorical variables. Significant difference upon post hoc testing to
[a] ADC
[b] Rotterdam Study.
Abbreviations: AD, Alzheimer's disease; ADC, Amsterdam Dementia Cohort; *APOE*, apolipoprotein E gene; ERF, Erasmus Rucphen Family; GCA, global cortical atrophy; MCI, mild cognitive impairment; MMSE, mini-mental state examination; MTA, medial temporal atrophy; N/A, not applicable; SD, standard deviation.

we adjusted for age, sex, ICV (Rotterdam Study and ERF Study), use of lipid lowering medication, BMI, and *APOE ε4* presence. For model 2 with adjustment for (imputed) BMI values in the ADC, results were pooled over imputed data sets using Rubin rules as implemented in the R package MICE.[27] Effect estimates of the linear regression analyses by cohorts (ADC, three Rotterdam Study subcohorts and, ERF Study) were combined with inverse variance-weighted fixed-effects meta-analysis using the "rmeta" package (version 3.0). In addition, we present three sensitivity analysis: (1) excluding subjects with a clinical AD dementia diagnosis, (2) stratified for a diagnosis of diabetes mellitus (DM) (yes/no), and (3) stratified for a short (≤6 months) and long (>6 months) time interval between blood sampling and MRI. Because metabolites are highly correlated we used the method of Li and Ji (4) to correct for multiple testing using R (version 3.5.2 [2018-07-02]) and the R package "Hmisc". With this method, we calculated the "effective number"' (Meff) of independent tests. The full formulas are explained in detail by Li and Ji (4). In our study, 143 metabolites corresponded to 27 independent tests (P for significance = 0.05/27 = 1.85 × 10^{-3}). The association magnitudes are reported in units of SD per 1 SD increase in each metabolite. We used METAL (version 2011-03-25) to check whether heterogeneity plays a role in the variation in results between our different studies by calculating the Iš statistic. A heatmap was used to visualize the distribution of effects found between each metabolite and MRI measures using the "heatmap.2" R package.

3 | RESULTS

3.1 | Descriptives

Characteristics of each cohort and diagnosis group are presented in Table 1. The Rotterdam Study included more females and older subjects than those in the ERF Study and the ADC. The proportion *APOE ε4* carriers was highest in the ADC and lowest in the Rotterdam Study.

3.2 | Metabolic patterns of neurodegeneration and vascular brain changes

Figure 1 shows a heatmap of all associations of metabolites with MRI measurements (model 2). Although only a limited number of associations pass the threshold for significance (as reported in 3.3 and 3.4) some global patterns can be observed. Overall, lower levels of LDL cholesterol particles and higher levels of triglycerides and glucose were
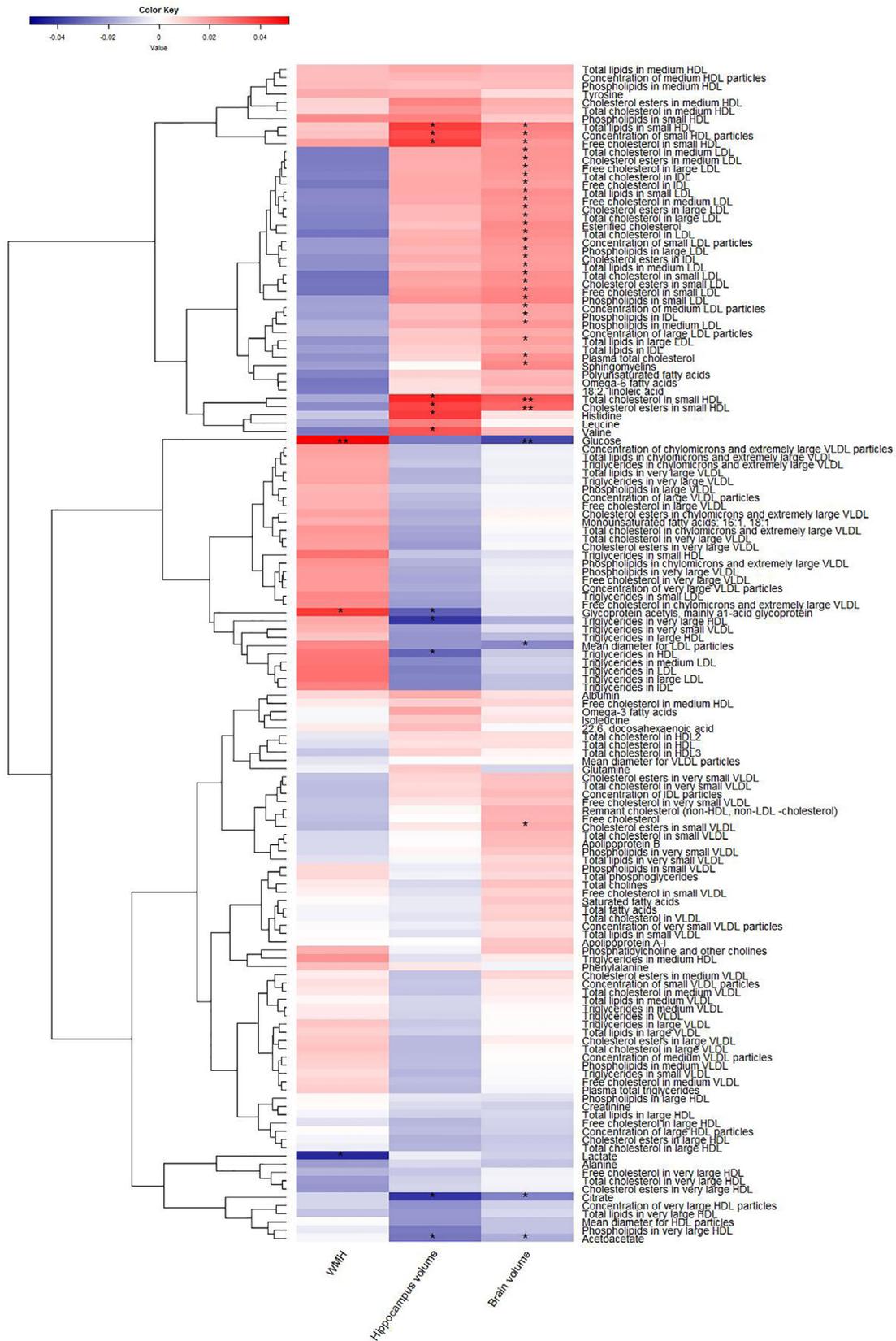
FIGURE 1 Associations of metabolites with MRI measures. Colors represent the standardized effect estimates of metabolites with brain volume, hippocampus volume, and white matter hyperintensities (WMH) adjusted for sex, age, lipid-lowering medication, body mass index, and apolipoprotein ε4 status. Red, high; blue, low; white, in between. * Stands for P-value < 0.05 and ** stands for P-value below the threshold for multiple testing $P < 1.85 \times 10^{-3}$. Abbreviations: HDL, high density lipoprotein; (V)LDL, (very) low density lipoprotein; WMH, white matter hyperintensities
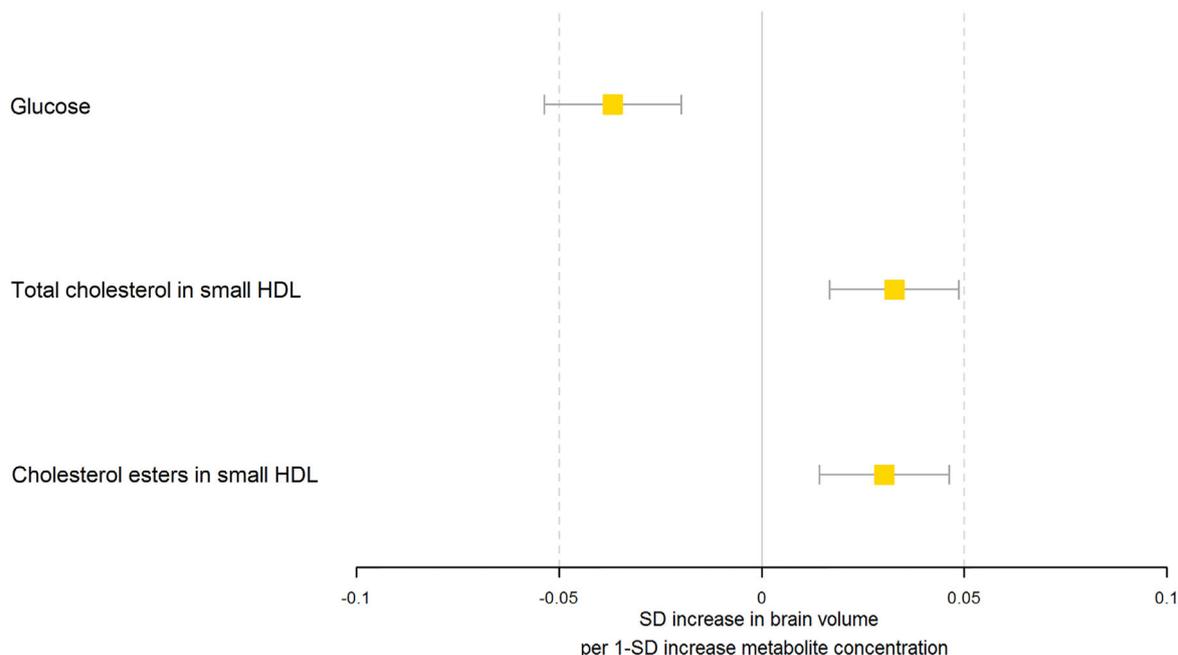
FIGURE 2 Associations of metabolites with brain volume. The standardized effect estimates of metabolites on brain volume adjusted for sex, age, lipid-lowering medication, body mass index, and apolipoprotein ε4 status are shown. Point estimates are shown as boxes with whiskers denoting the 95% confidence interval of the effect estimates. Abbreviations: HDL, high density lipoprotein; SD, standard deviation

associated with more brain and hippocampal atrophy and more WMH. Moreover, higher levels of very-low-density lipoprotein particles were associated with more hippocampal atrophy and more WMH. More brain and hippocampal atrophy was additionally associated with lower small HDL particles and higher citrate levels. Finally, more hippocampal atrophy was associated with lower histidine, leucine, and valine levels. Together this suggests some overlapping and some separate metabolic patterns associated with neurodegenerative and vascular brain changes.

3.3 | Brain atrophy and hippocampal atrophy

In the meta-analysis for model 1, four metabolites passed the threshold for significance for brain atrophy. Higher glucose levels and lower total cholesterol in small HDL, cholesterol esters in small HDL, and total lipids in small HDL levels were associated with more brain atrophy (B[SE] $-0.030[0.008]$, $P = 1.4 \times 10^{-4}$, $0.031[0.008]$, $P = 4.9 \times 10^{-5}$, $0.028[0.008]$, $P = 2.6 \times 10^{-4}$, $0.025[0.008]$, $P = 1.4 \times 10^{-3}$). In model 2 these associations remained significant, except for the association between total lipids in small HDL (Figure 2). The associations of higher glucose levels and lower total cholesterol in small HDL and lower cholesterol esters in small HDL with brain atrophy also surpassed the more stringent Bonferroni correction for significance ($P = 0.05/143 < 3.5 \times 10^{-4}$). In separate analyses of ADC and meta-analyses of Rotterdam Study and ERF Study, direction of effects was the same as in the meta-analyses for the three metabolites associated with brain atrophy (Supplementary Table 3). No associations between metabolites and

hippocampus atrophy passed the threshold for multiple testing. An exploratory analysis in the MCI and AD group (n = 653) showed no associations between metabolites and hippocampus atrophy that surpassed the threshold for multiple testing either (data not shown).

3.4 | White matter hyperintensities

In the meta-analysis for model 1, higher glucose and glycoprotein acetyls were associated with more WMH (B[SE] 0.071[0.015], $P = 1.5 \times 10^{-6}$, $0.051[0.014]$, $P = 4.0 \times 10^{-4}$). In model 2, these effects attenuated, and only effects for glucose remained significant (B[SE] 0.051 [0.016], $P = 1.5 \times 10^{-3}$). In separate analyses of ADC and meta-analyses of Rotterdam Study and ERF Study, the same direction of effects was found as in the meta-analyses for glucose (Supplementary Table 3).

3.5 | Heterogeneity of results

Next, heterogeneity analysis was used to assess the variety in results between studies. I^2 reflects the percentage of variation across studies due to heterogeneity (Supplementary Table 3). The association between total cholesterol in small HDL and cholesterol esters in small HDL and brain atrophy were consistent among the different studies, with an I^2 value of 21.8 ($P = 2.8 \times 10^{-1}$) and 2.9 ($P = 3.9 \times 10^{-1}$) in model 2. For the association between glucose and brain atrophy and WMH, the I^2 was 41.1 ($P = 1.5 \times 10^{-1}$) and 45.8 ($P = 1.2 \times 10^{-1}$).

3.6 | Sensitivity analyses

We examined three sensitivity analyses to determine effects of: (1) clinical AD dementia diagnosis, (2) DM, and (3) time interval between blood sampling and brain MRI scanning. To determine whether the observed effects may be driven by disease effects of AD dementia, we reanalyzed the data excluding the subjects with a clinical diagnosis of AD dementia (n = 523, ADC). In model 2, the associations between total cholesterol in small HDL, cholesterol esters in small HDL levels, and glucose with brain atrophy remained similar; the association between glucose and WMH showed a similar effect size as in the total cohort but lost significance (B[SE] 0.051 [0.017], $P = 2.0 \times 10^{-3}$) (Supplementary Table 4). Next, we hypothesized that the association of higher glucose levels with more brain atrophy and more WMH might be different for subjects with DM (n = 373) and those without DM (n = 3552). For the association of glucose with brain atrophy, effect sizes diminished and became non-significant in both subgroups (B[SE] −0.023[0.030], $P = 4.4 \times 10^{-1}$ DM, −0.020[0.009], $P = 2.1 \times 10^{-2}$ no DM, model 2). Effect sizes for the association of glucose with WMH were substantially larger in the DM subgroup (B[SE] 0.136[0.057], $P = 1.7 \times 10^{-2}$) versus the subgroup without DM (B[SE] 0.023[0.017], $P = 1.7 \times 10^{-1}$, model 2) (Supplementary Tables 5 and 6). Furthermore, we performed stratified analyses based on the time difference between blood withdrawal and brain MRI scanning (≤6 months [n = 2432] vs >6 months [n = 1530]) (Supplementary Tables 7 and 8). Lower total cholesterol in small HDL and cholesterol esters in small HDL levels remained associated with more brain atrophy in the subgroup with a short time interval (≤6 months) (B[SE] 0.041[0.011], $P = 2.2 \times 10^{-4}$, 0.037[0.011], $P = 5.8 \times 10^{-4}$), but effect sizes were smaller and lost significance in the long time interval group (>6 months) (B[SE] 0.026[0.012], $P = 3.6 \times 10^{-2}$, 0.016[0.012], $P = 2.0 \times 10^{-1}$, model 2). The association of high glucose levels with more brain atrophy and more WMH was slightly weaker in the subgroup with a short time interval (≤6 months) (B[SE] -0.028[0.012], $P = 2.0 \times 10^{-2}$, 0.044[0.021], $P = 3.8 \times 10^{-2}$), in comparison to the long time interval group (>6 months) (B[SE] −0.045[0.013], $P = 2.8 \times 10^{-4}$, 0.058[0.025], $P = 2.0 \times 10^{-2}$).

4 | DISCUSSION

In this multi-cohort study, lower levels of small HDL particles were associated with more brain atrophy. In addition, high glucose levels were associated with more brain atrophy and more WMH.

The present study suggests a harmful role of high glucose levels on brain atrophy and vasculature. DM has been associated with an increased risk of cognitive decline and dementia.[28] Sensitivity analyses showed that the association of glucose with WMH might be largely attributable to DM subjects, but our findings with brain atrophy were not specific for DM, suggesting that higher glucose levels might also be harmful in subjects without DM. Previous work in the Rotterdam Study shows that higher baseline insulin resistance is associated with

an increased risk of AD.[29] Recent studies also link systemic glucose levels with brain measures, showing that higher blood glucose levels are associated with aberrant functional brain connectivity, WMH, and cortical thinning in healthy subjects.[30–32] Glucose dysregulation and DM are strongly associated with diet and lifestyle. The Mediterranean diet has shown beneficial effects on DM risk, cognition, brain volumes, and WMH.[33–35] These studies are consistent with our findings and underscore the potential for lifestyle interventions in the prevention of AD.

Lower levels of small HDL particles were associated with more brain atrophy. In a previous study investigating the role of HDL subclasses on cognition and dementia risk, we found that higher levels of small, medium, and large HDL particles were associated with better cognitive ability, and that only higher levels of small and medium HDL particles were associated with decreased risk of dementia.[10] This in line with our findings, and together our studies suggest that the smaller HDL particles might be more specific for neurodegeneration. Protective effects of high levels of HDL are thought to rely on the promoting effects of HDL on the reverse cholesterol transport. The current knowledge on HDL subclasses is limited, but previous studies suggest that subclasses differ in function and ability to promote cholesterol efflux.[36–38] For example, small HDL has been suggested to have more anti-oxidant and anti-inflammatory properties in comparison to lipid-rich large HDL.[39] This could explain the association we found for higher levels of small HDL with less brain atrophy. Perhaps the anti-oxidative and anti-inflammatory properties of small HDL protect the brain for neurodegenerative brain damage. In our study, levels of total HDL as measured in clinical practice showed no associations with brain atrophy or hippocampal atrophy (B[SE] -0.006 [0.009], $P = 5.2 \times 10^{-1}$ for brain atrophy, B[SE] 0.006 [0.017] $P = 7.2 \times 10^{-1}$ for hippocampal atrophy, model 2) suggesting that HDL subclasses are more informative when studying the role of HDL in neurodegeneration. Furthermore, HDL has been studied widely in relation to cardiovascular disease, which is also an important risk factor for AD.[40,36] Considering the beneficial effect of HDL on vascular disease we might have expected an association between HDL and WMH instead of brain atrophy. Previous studies examining the role of HDL subclasses on cardiovascular outcomes, however, found that small HDL particles predict higher risk on cardiovascular disease and that large HDL particles are protective for cardiovascular disease.[38,41] This is in contrast with our findings for low small HDL particle levels associated with more brain atrophy, suggesting that the effects of small HDL we found might not be mediated by vascular pathology but might depend on other the anti-oxidative, anti-inflammatory properties of small HDL.

We did not find any significant associations between metabolites and hippocampal atrophy. Hippocampal atrophy is a more AD-specific measure of neurodegeneration in comparison to global brain atrophy.[42] Only the ADC included AD dementia patients, which could have caused insufficient power to detect significant associations with hippocampal atrophy. In the meta-analysis, strongest associations with more hippocampal atrophy were found for lower small HDL particles, histidine, leucine, and valine levels, and higher citrate levels. This is consistent with previous studies that have associated small HDL particles

and branched-chain amino acids with lower dementia risk.[9,10] Moreover, the finding for small HDL particles overlaps with the associations we found between lower small HDL particle levels and more brain atrophy. Together, although our findings for hippocampus atrophy are not significant, it might be interesting to further investigate metabolite associations with hippocampal atrophy in cohorts with larger number of AD cases.

The associations between metabolites and brain measures we found in this study do indicate subtle effects with the moderate *P*-values. This is a common observation when studying associations between peripheral metabolites and brain diseases. AD is a multifactorial disease, and associations between peripheral metabolism and AD are likely to involve multiple metabolic pathways. Therefore, one should not strive to find strong single metabolic markers for clinical purposes, but subtle metabolic patterns can provide us valuable insight into the biological mechanisms for AD, as shown in our heatmap in Figure 1.

A potential limitation of our study is that different methods were used to estimate brain changes (volumetric in Rotterdam Study and ERF Study; and visual rating in ADC). Because visual ratings are very useful in daily clinical practice these were available for participants from the ADC, which is a memory-clinic cohort. Moreover, ADC participants were scanned on different scanners, which is a disadvantage for volumetric measures, whereas visual ratings can be reliably applied to different magnetic field strengths and scanners.[43] Previous studies have shown a similar validity of visual ratings and volumetric measures.[44,45] With transformation and standardization of our data, we made the measured outcomes of interest comparable between the different cohorts. Furthermore, our findings were robust, as the main findings show the same direction of effects in the separate cohort analysis as in the meta-analysis, which further emphasizes generalizability of the results. Another potential limitation is that the time between MRI and blood sampling in the Rotterdam Study and ERF Study varied from no time difference to multiple years. This might have mitigated our results as is shown in our sensitivity analysis where effects of small HDL particles were stronger in the subgroup with short time differences (≤6 months) than those with long (>6 months) time differences. Next, metabolites of the Rotterdam Study and ERF Study were measured in fasting plasma samples, whereas the ADC had only non-fasting samples. This might have influenced (consistency of) our results; however, direction of metabolites for the top candidates was in the same direction in all cohorts. Moreover, although fasting metabolite measurements are preferable, concentrations of amino acids, cholesterol, and several other metabolites have been shown to be relatively stable in non-fasting blood samples.[46,47] We note that our findings are difficult to interpret in terms of causality. Whether a found association is a cause or consequence of changes in the MRI measures cannot be studied with the cross-sectional design of this study. Furthermore, longitudinal studies should unravel whether these metabolites are related to disease etiology or are merely a consequence of disease. We did show, however, in a sensitivity analysis that our main findings were not driven by AD dementia subjects only. Finally, we used MRI measures as imaging endophenotypes of AD to investigate metabolite alterations in both cognitively healthy and memory-clinic patients. Imaging markers have the advantage to be more closely linked to pathologic effects in comparison to clinical outcomes and enable us to discover specific metabolic associations with neurodegeneration and vascular changes. MRI features are, however, not specific for AD and also associated with many other neurodegenerative diseases and aging.[48]

Among the strengths of this study is our large sample size and direct validation of our findings in three independent cohorts. Moreover, we investigated associations of metabolites with neurodegenerative imaging markers across the entire cognitive spectrum of AD. This makes our findings broadly applicable regardless of disease state. Furthermore, the same metabolite platform was used across the three cohorts to measure metabolites.

In summary, in a meta-analysis of three independent cohort studies we found that lower small HDL levels and higher glucose levels were associated with more brain atrophy and that higher glucose levels were associated with more WMH. Future studies are needed to pinpoint the role of these metabolites in neurodegenerative brain changes characteristic for AD.

CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.