Nutrition and metabolic profiles in Alzheimer's disease

de Leeuw, F.A.

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Chapter 3.2

Circulating metabolites are associated with brain atrophy and white matter hyperintensities

Francisca A. de Leeuw*
Hata Karamujić-Čomić*
Betty M. Tijms
Carel F.W. Peeters
Maartje I. Kester
Philip Scheltens
Shahzad Ahmad
Dina Vojinovic
Hieab H.H. Adams
Thomas Hankemeier
Daniel Bos
Aad van der Lugt
Meike W. Vernooij
M. Arfan Ikram
Najaf Amin
Frederik Barkhof
Charlotte E. Teunissen
Cornelia M. van Duijn#
Wiesje M. van der Flier#
*Shared first authorship
Shared last authorship

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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 to develop future intervention trials.

Introduction

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 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 currently be investigated 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 multi-center 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.

Methods

Cohorts 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.

MRI measures

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 5-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.**

Rotterdam Study and Erasmus Rucphen Family Study

Brain MRI scans were obtained at 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, brain stem, ventricles, CSF, and choroid plexus [20]. Hippocampus volume was defined as the mean of right and left hippocampal volumes.

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 dataset included 150 absolute metabolite measures. Six

metabolites with >10% missing in one of the cohorts were excluded from data-analysis. Pyruvate was excluded as 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 dataset included 143 metabolites.

Covariates

In ADC, Rotterdam Study and ERF Study apolipoprotein E (APOE) genotype was measured as previously described [24-26]. Subjects were classified as APOE epsilon4 (ε4) 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.

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 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 way that direction of visual scores in the ADC cohort were the same as for WMH and the volumetric data in the Rotterdam Study and ERF Study (i.e. higher scores means less brain/hippocampal atrophy or more WMH).

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 (ANOVA) 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, 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 datasets using Rubin's 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 and 2) stratified for a diagnosis of diabetes mellitus (DM) (yes/no) 3) stratified for a short (≤6 months) and long (>6 months) time interval between blood sampling and MRI. Since metabolites are highly correlated we used the method of Li and Ji [28] 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 [28]. In our study, 143 metabolites corresponded to 27 independent tests (p for significance=0.05/27=1.85x10^{-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 I^2 statistic. A heatmap was used to visualize the distribution of effects found between each metabolite and MRI measures using the "heatmap.2" R package.

Results

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 ADC. The proportion APOE ε4 carriers was highest in ADC and lowest in the Rotterdam Study.

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

Table 1 Continued.

Cohort	ADC	Rotterdam Study	ERF Study	p-value
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 hyperintensities 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 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; ERF, Erasmus Rucphen Family; GCA, global cortical atrophy; MTA, medial temporal atrophy; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; N/A, not applicable; SD, standard deviation.

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 associated with more brain and hippocampal atrophy and more WMH. Moreover, higher levels of very low density lipoprotein (VLDL) 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. Last, 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.

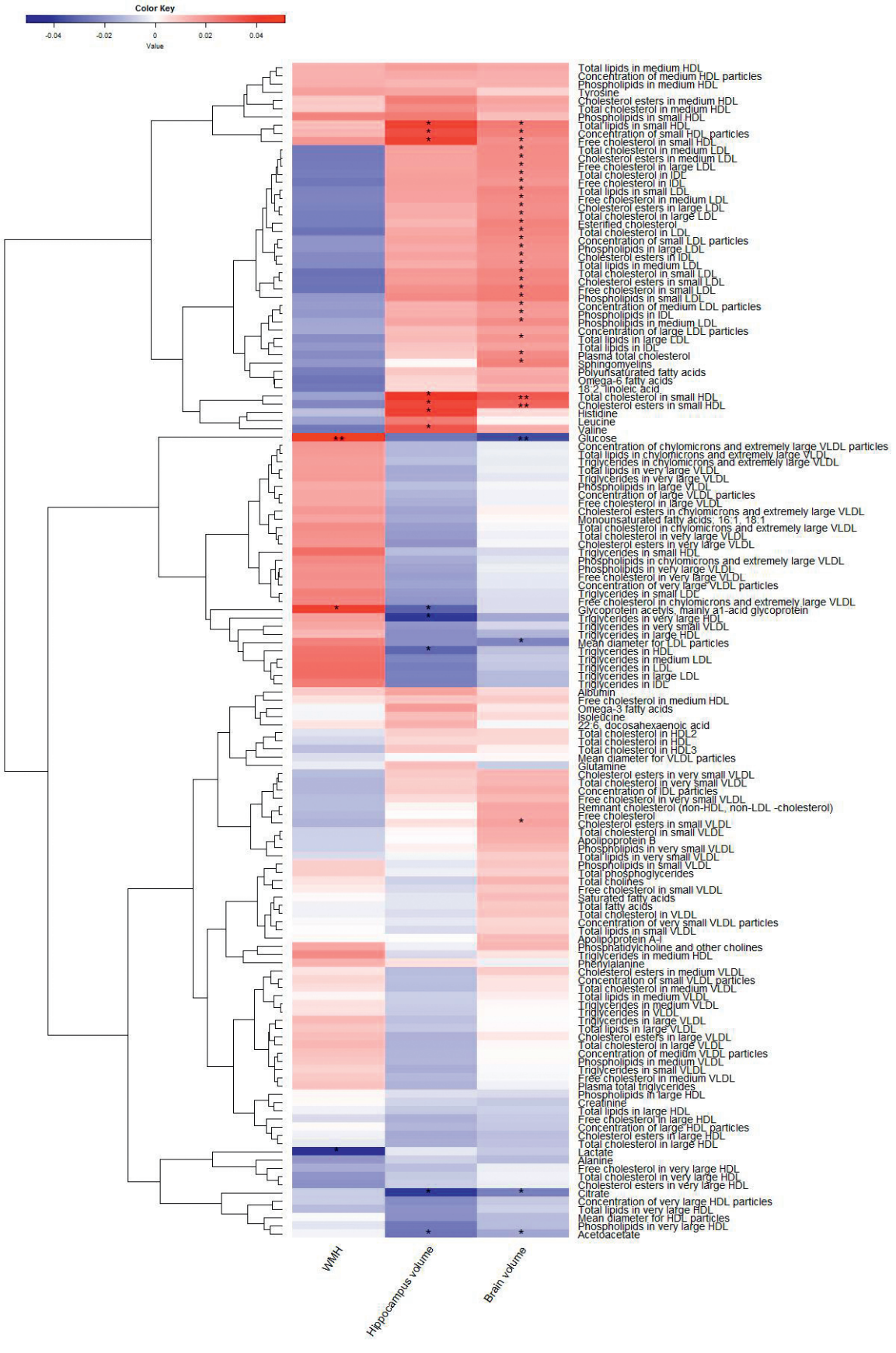
Brain atrophy and hippocampal atrophy

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), $p=1.4x10^{-4}$, 0.031(0.008), $p=4.9x10^{-5}$, 0.028(0.008), $p=2.6x10^{-4}$, 0.025(0.008), $p=1.4x10^{-3}$). In model 2 these associations remained significant, except for the association between total lipids in small HDL (**Figure 2**). The associations between higher glucose levels and lower total cholesterol in small HDL, cholesterol esters in small HDL also surpassed the more stringent Bonferroni correction for significance ($p=0.05/143<3.5x10^{-4}$). In separate analyses of ADC and meta-analyses of Rotterdam Study and ERF Study, direction of effects were 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 that surpassed the threshold for multiple testing either (data not shown).

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), $p=1.5x10^{-6}$, 0.051(0.014), $p=4.0x10^{-4}$). In model 2, these effects attenuated and only effects for glucose remained significant (B(SE) 0.051 (0.016), $p=1.5x10^{-3}$). In separate analyses of ADC and meta-analyses of Rotterdam Study and ERF Study, same direction of effects were found as in the meta-analyses for glucose (**Supplementary table 3**).

Figure 1 (right page) 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 $p<1.85x10^{-3}$. Abbreviations HDL, high density lipoprotein; (V)LDL, (very) low density lipoprotein; WMH, white matter hyperintensities.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Value
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Concentration of medium HDL particles
Phospholipids in medium HDL
Tyrosine
Cholesterol esters in medium HDL
Total cholesterol in medium HDL
Phospholipids in small HDL
Total lipids in small HDL
Concentration of small HDL particles
Free cholesterol in small HDL
Total cholesterol in medium LDL
Cholesterol esters in medium LDL
Free cholesterol in large LDL
Total cholesterol in IDL
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Total lipids in small LDL
Free cholesterol in medium LDL
Cholesterol esters in large LDL
Total cholesterol in large LDL
Esterified cholesterol
Total cholesterol in LDL
Concentration of small LDL particles
Phospholipids in large LDL
Cholesterol esters in IDL
Total lipids in medium LDL
Total cholesterol in small LDL
Cholesterol esters in small LDL
Free cholesterol in small LDL
Phospholipids in small LDL
Concentration of medium LDL particles
Phospholipids in IDL
Phospholipids in medium LDL
Concentration of large LDL particles
Total lipids in large LDL
Total lipids in IDL
Plasma total cholesterol
Sphingomyelins
Polyunsaturated fatty acids
Omega-6 fatty acids
18:2, linoleic acid
Total cholesterol in small HDL
Cholesterol esters in small HDL
Histidine
Leucine
Valine
Glucose
Concentration of chylomicrons and extremely large VLDL particles
Total lipids in chylomicrons and extremely large VLDL
Triglycerides in chylomicrons and extremely large VLDL
Total lipids in very large VLDL
Triglycerides in very large VLDL
Phospholipids in large VLDL
Concentration of large VLDL particles
Free cholesterol in large VLDL
Cholesterol esters in chylomicrons and extremely large VLDL
Monounsaturated fatty acids, 16:1, 18:1
Total cholesterol in chylomicrons and extremely large VLDL
Total cholesterol in very large VLDL
Cholesterol esters in very large VLDL
Triglycerides in small HDL
Phospholipids in chylomicrons and extremely large VLDL
Phospholipids in very large VLDL
Free cholesterol in very large VLDL
Concentration of very large VLDL particles
Triglycerides in small LDL
Free cholesterol in chylomicrons and extremely large VLDL
Glycoprotein acetyls, mainly a1-acid glycoprotein
Triglycerides in very large HDL
Triglycerides in very small VLDL
Triglycerides in large HDL
Mean diameter for LDL particles
Triglycerides in HDL
Triglycerides in medium LDL
Triglycerides in LDL
Triglycerides in large LDL
Triglycerides in IDL
Albumin
Free cholesterol in medium HDL
Omega-3 fatty acids
Isoleucine
22:6, docosahexaenoic acid
Total cholesterol in HDL2
Total cholesterol in HDL
Total cholesterol in HDL3
Mean diameter for VLDL particles
Glutamine
Cholesterol esters in very small VLDL
Total cholesterol in very small VLDL
Concentration of IDL particles
Free cholesterol in very small VLDL
Remnant cholesterol (non-HDL, non-LDL -cholesterol)
Free cholesterol
Cholesterol esters in small VLDL
Total cholesterol in small VLDL
Apolipoprotein B
Phospholipids in very small VLDL
Total lipids in very small VLDL
Phospholipids in small VLDL
Total phosphoglycerides
Total cholines
Free cholesterol in small VLDL
Saturated fatty acids
Total fatty acids
Total cholesterol in VLDL
Concentration of very small VLDL particles
Total lipids in small VLDL
Apolipoprotein A-I
Phosphatidylcholine and other cholines
Triglycerides in medium HDL
Phenylalanine
Cholesterol esters in medium VLDL
Concentration of small VLDL particles
Total cholesterol in medium VLDL
Total lipids in medium VLDL
Triglycerides in medium VLDL
Triglycerides in VLDL
Triglycerides in large VLDL
Total lipids in large VLDL
Cholesterol esters in large VLDL
Total cholesterol in large VLDL
Concentration of medium VLDL particles
Phospholipids in medium VLDL
Triglycerides in small VLDL
Free cholesterol in medium VLDL
Plasma total triglycerides
Phospholipids in large HDL
Creatinine
Total lipids in large HDL
Free cholesterol in large HDL
Concentration of large HDL particles
Cholesterol esters in large HDL
Total cholesterol in large HDL
Lactate
Alanine
Free cholesterol in very large HDL
Total cholesterol in very large HDL
Cholesterol esters in very large HDL
Citrate
Concentration of very large HDL particles
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Mean diameter for HDL particles
Phospholipids in very large HDL
Acetoacetate
WMH
Hippocampus volume
Brain volume

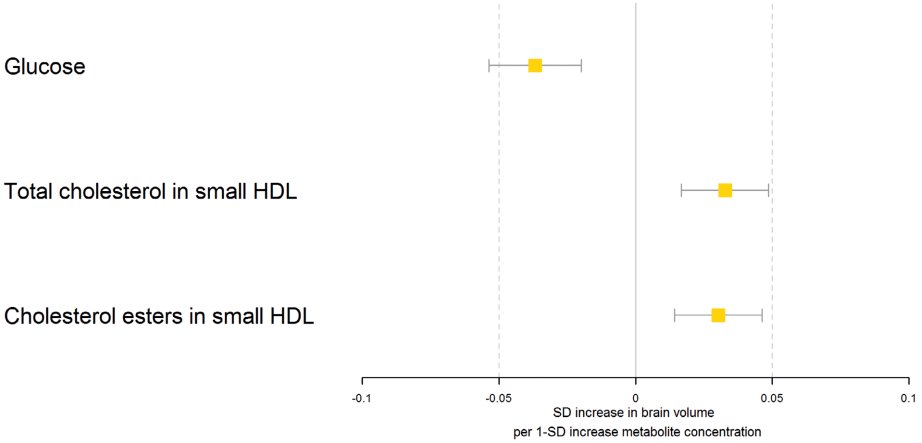

Figure 2 Associations of metabolites with brain volume. The standardized effect estimates of metabolites on brain volume adjusted for sex, age, lipid lowering medication, body mass index and apolipoprotein ε4 status are shown. Point estimates are shown as boxes with whiskers denoting the 95% confidence interval of the effect estimates. Abbreviations HDL, high density lipoprotein; SD, standard deviation.

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 a I^2 value of 21.8 (p=2.8x10^{-1}) and 2.9 (p=3.9x10^{-1}) in Model 2. For the association between glucose and brain atrophy and WMH, the I^2 was 41.1 (p=1.5x10^{-1}) and 45.8 (p=1.2x10^{-1}).

Sensitivity analyses

We examined three sensitivity analyses to determine effects of: 1) clinical AD dementia diagnosis, 2) DM, 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 re-analyzed 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=2.0x10^{-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=4.4x10^{-1} DM, -0.020(0.009), p=2.1x10^{-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=1.7x10^{-2}) vs. the subgroup without DM (B(SE) 0.023(0.017), p=1.7x10^{-1}, Model 2) **(Supplementary table 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 table 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=2.2x10^{-4}, 0.037(0.011), p=5.8x10^{-4}), but effect sizes were smaller and lost significance in the long time interval group (>6 months) (B(SE) 0.026(0.012), p=3.6x10^{-2}, 0.016(0.012), p=2.0x10^{-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=2.0x10^{-2}, 0.044(0.021), p=3.8x10^{-2}), in comparison to the long time interval group (>6 months) (B(SE) -0.045(0.013), p=2.8x10^{-4}, 0.058(0.025), p=2.0x10^{-2}).

Discussion

In this multi-cohort study low 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 [29]. 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 [30]. 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 [31-33]. 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 [34-36]. These studies are consistent with our findings and underline 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 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 [37-39]. For example, small HDL has been suggested to have more anti-oxidant and anti-inflammatory properties in comparison to lipid-rich large HDL [40]. 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 x10^{-1} for brain atrophy, B(SE) 0.006 (0.017) p=7.2x10^{-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 [37, 41]. 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 large HDL particles are protective for cardiovascular disease [39, 42]. 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 [43]. 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, while 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 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). Since 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, while visual ratings can be reliably applied to different magnetic field strengths and scanners [44]. Previous studies have shown a similar validity of visual ratings and volumetric measures [45, 46]. 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 underlines 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 were 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, while the ADC had only non-fasting samples. This might have influenced (consistency of) our results, however direction of metabolites for the top candidates were 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 [47, 48]. 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. Further longitudinal studies should unravel whether these metabolites are related to disease etiology or are merely a consequence of disease. We did however show in a sensitivity analysis that our main findings were not driven by AD dementia subjects only. Last, 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 [49].

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 cohort 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 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

F.L., H.K.C., B.T., C.P., M.K., S.A., D.V., H.A., T.H., D.B., A.L., M.V., M.I., N.A., F.B., C.D. report no disclosures relevant to the manuscript. P.S. has received consultancy/speaker fees (paid to the institution) from Lilly, GE Healthcare, Novartis, Sanofi, Nutricia, Probiodrug, Biogen, Roche, Avraham, and EIP Pharma. PS has acquired grant support (for the institution) from GE Healthcare, Danone Research, Piramal, and MERCK. CT received grants from the European Commission, the Dutch Research Council (ZonMW), Association of Frontotemporal Dementia/Alzheimer's Drug Discovery Foundation, The Weston Brain Institute, Alzheimer Netherlands. C.T. has a collaboration contract with ADx Neurosciences, performed contract research or received grants from Probiodrug, Biogen, Esai, Toyama, Janssen prevention center, Boehringer, AxonNeurosciences, Fujirebio, EIP farma, PeopleBio, and Roche. Research programs of W.F. have been funded by ZonMW, NWO, EU-FP7, EU-JPND, Alzheimer Nederland, CardioVascular Onderzoek Nederland, Health~Holland, Topsector Life Sciences & Health, stichting Dioraphte, Gieskes-Strijbis fonds, stichting Equilibrio, Pasman stichting, Biogen MA Inc, Boehringer Ingelheim, Life-MI, AVID, Roche BV, Janssen Stellar, Combinostics. W.F. has performed contract research for Biogen MA Inc and Boehringer Ingelheim. W.F. has been an invited speaker at Boehringer Ingelheim and Biogen MA Inc. All funding is paid to her institution. F.B. is a consultant for Biogen-Idec, Janssen Alzheimer Immunotherapy, Bayer-Schering, Merck-Serono, Roche, Novartis, Genzyme, and Sanofi-Aventis; has received sponsorship from European Commission–Horizon 2020, National Institute for Health Research–University College London

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Supplementary tables

Supplementary tables for this chapter can be viewed by scanning the code below: