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Featured Article

Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies

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Abstract

Introduction: Identifying circulating metabolites that are associated with cognition and dementia may improve our understanding of the pathogenesis of dementia and provide crucial readouts for preventive and therapeutic interventions.

Methods: We studied 299 metabolites in relation to cognition (general cognitive ability) in two discovery cohorts (N total = 5658). Metabolites significantly associated with cognition after adjusting for multiple testing were replicated in four independent cohorts (N total = 6652), and the associations with dementia and Alzheimer's disease (N = 25,872) and lifestyle factors (N = 5168) were examined.

Results: We discovered and replicated 15 metabolites associated with cognition including subfractions of high-density lipoprotein, docosahexaenoic acid, ornithine, glutamine, and glycoprotein acetyls. These associations were independent of classical risk factors including high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and apolipoprotein E (*APOE*) genotypes. Six of the cognition-associated metabolites were related to the risk of dementia and lifestyle factors.

Discussion: Circulating metabolites were consistently associated with cognition, dementia, and lifestyle factors, opening new avenues for prevention of cognitive decline and dementia.

Keywords: Cognitive function; General cognitive ability; Alzheimer's disease; Dementia; Metabolites; Metabolomics; NMR; Lifestyle factors

1. Introduction

Cognitive function is an important determinant of health and well-being and a key component of the dementia spectrum, including Alzheimer's disease (AD), the most common cause of dementia [1]. Vascular dysfunction and metabolic dysregulation contribute to impairment in cognitive performance [2]. Clinical and population-based studies suggest a relationship of cognitive function with midlife hypertension, high blood levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides and glucose, and low levels of high-density lipoprotein cholesterol (HDL-C) [3–5]. The recent decrease in incidence of dementia in longitudinal studies has been attributed to improved control of vascular and metabolic factors [6–9]. These findings have fueled speculation that discovery of other circulating metabolites influencing cognition and future dementia may not only improve our understanding of the determinants of cognition but may also facilitate prevention through interventions on lifestyle factors and dedicated medication [10]. Previous studies have shown circulating metabolites in blood (e.g., lipoproteins, amino acids, fatty acids, and other small molecules) to be associated with cognitive function and conversion from normal cognition to dementia or AD [11–17]. However, these studies were relatively small and findings have not been replicated [15,18], emphasizing the need for studies in large well-characterized populations where findings are replicated [10,19].

We performed a comprehensive metabolic analysis to study the role of circulating metabolites in cognitive function. Discovery of novel measures associated with cognitive function was performed in two large population-based studies in the Netherlands—the Rotterdam Study (RS) and the Erasmus Rucphen Family (ERF) study. We determined whether the associations were independent of known vascular and metabolic risk factors. Metabolites independently associated with cognition were replicated in independent cohort studies, and their relation to the risk of dementia and AD was validated in eight cohort studies. Finally, we assessed whether lifestyle factors, including dietary fish intake, smoking, and physical activity, were associated with the identified metabolites.

2. Methods

For a schematic overview of the analysis setup, see Fig. 1.

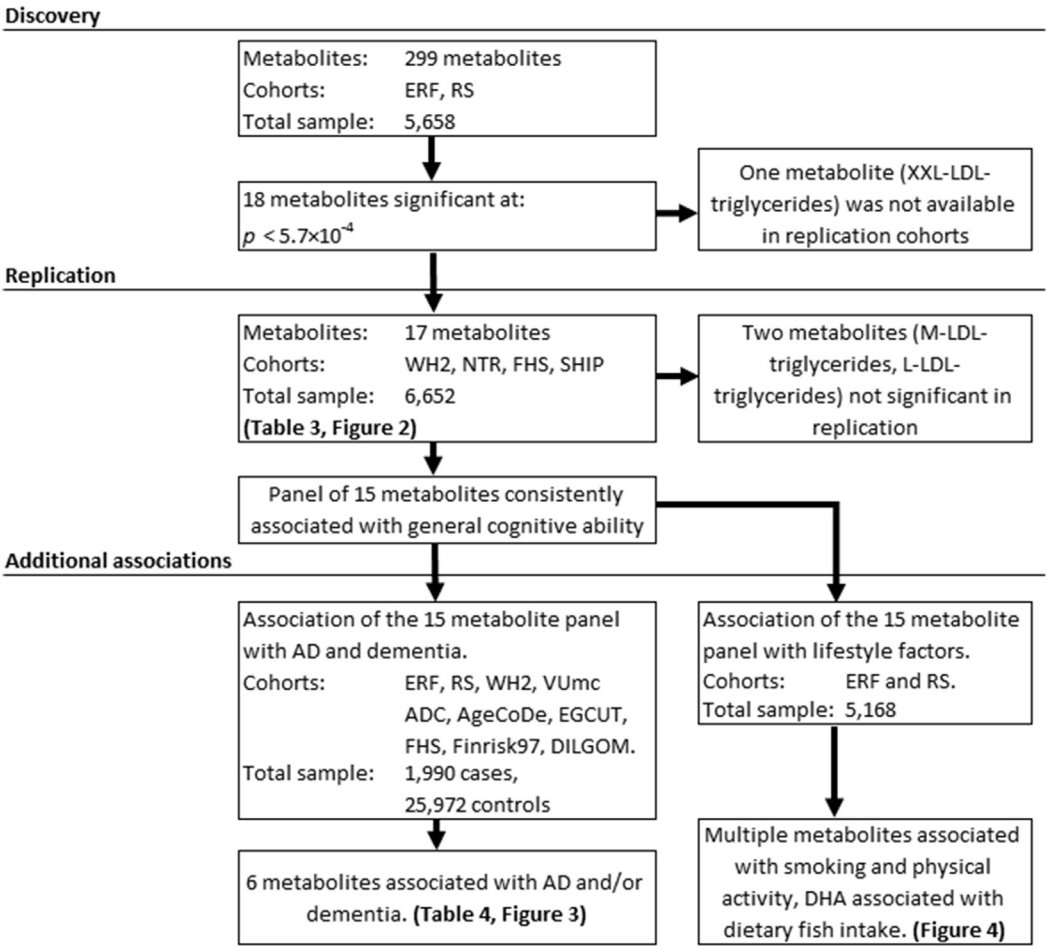

Fig. 1. Flowchart of analyses. Study names: ERF, RS, WHII, NTR, SHIP-Trend, FHS, VUmc ADC, Finrisk97, DILGOM, EGCUT, and AgeCoDe. Abbreviations: AgeCoDe, German Study on Ageing, Cognition, and Dementia; DHA, docosahexaenoic acid; EGCUT, Estonian Biobank; DILGOM, Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic Syndrome; ERF, Erasmus Rucphen Family; FHS, Framingham Heart Study; Finrisk97, National FINRISK Studies 1997; LDL, low-density lipoprotein; NTR, Netherlands Twin Register; RS, Rotterdam Study; SHIP-Trend, Study of Health in Pomerania–Trend; VUmc ADC, VUmc Amsterdam Dementia Cohort; WHII, Whitehall II.

2.1. Discovery and replication populations for research of cognitive function

Metabolomics profiling in multiple cohorts from the Netherlands was done as part of the BioBanking for Medical Research Infrastructure of the Netherlands (BBMRI) metabolomics consortium. These include the two discovery cohorts (ERF and RS). A short description of the cohort studies included in this article can be found in Supplementary Table 1. ERF is a prospective family-based study (ERF, N = 2683) from the southwest of the Netherlands, and the RS is a prospective population-based cohort study that started in 1990 in Ommoord, a district of Rotterdam. In this analysis, we used the fourth wave of the baseline cohort (N = 2975). Replication cohorts included the Netherlands Twin Register (NTR, N = 338; also part of the BBMRI Metabolomics Consortium), the Whitehall II (WHII, N = 4612) study [20], the Framingham Heart Study (FHS, N = 2356), and the Study of Health in Pomerania–Trend (N = 944).

2.2. Cohorts for extrapolation to dementia and AD

Dementia and AD was assessed in eight cohorts; the ERF study, RS, a series of dementia patients and controls from the VUmc Amsterdam Dementia Cohort metabolically characterized as part of the BBMRI Metabolomics Consortium (N = 1303) [21], two cohorts used in the replication of cognitive findings (WHII = 4,612 and FHS = 2356), the National FINRISK Studies 1997 (Finrisk97; N = 7517), Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic Syndrome (DILGOM; N = 4788), the Estonian Biobank (EGCUT; N = 2572), and the German Study on Ageing, Cognition, and Dementia (AgeCoDe, N = 310).

2.3. Assessment of cognitive function and dementia

Participants underwent cognitive tests using a highly variable battery of assessments, which varied across studies; details on the cognitive tests used can be found in Supplementary Table 2. Cognitive function tests were

assessed at the same time point in all studies. To enable meta-analyses of results from the heterogeneous set of tests efficiently, we constructed a general cognitive ability score to capture information from a wide variety of cognitive tests reliably into a single cognitive measure [22,23]. General cognitive ability was calculated by principal component

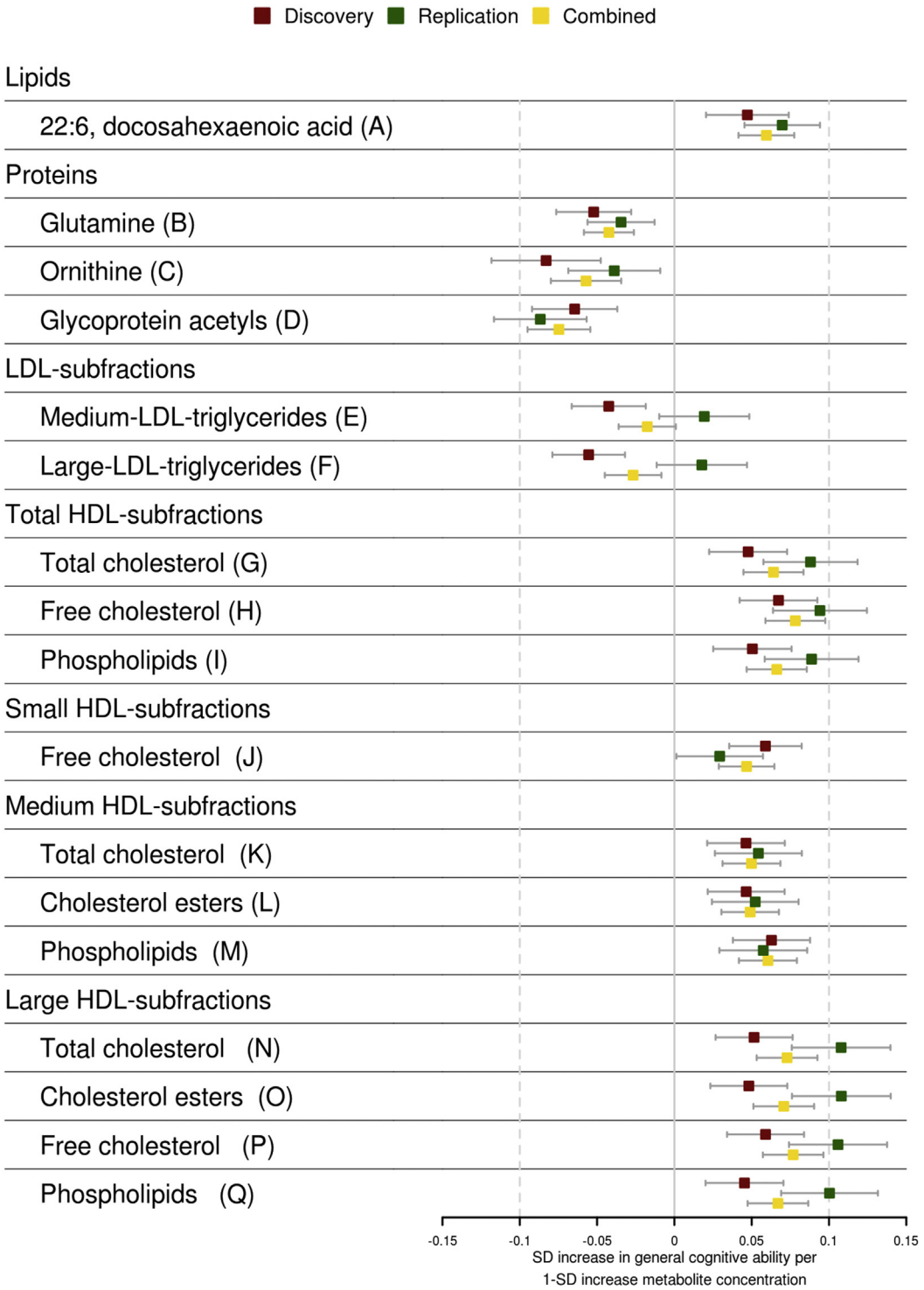

Fig. 2. Associations of metabolites with general cognitive ability. The standardized effect estimates on general cognitive ability of metabolites adjusted for age, sex, body mass index, and lipid-lowering medication use are shown. The estimates are shown for the discovery (red), replication (green), and the combined (yellow) analysis. Point estimates are shown as boxes with whiskers denoting the 95% confidence interval of the effect estimates. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

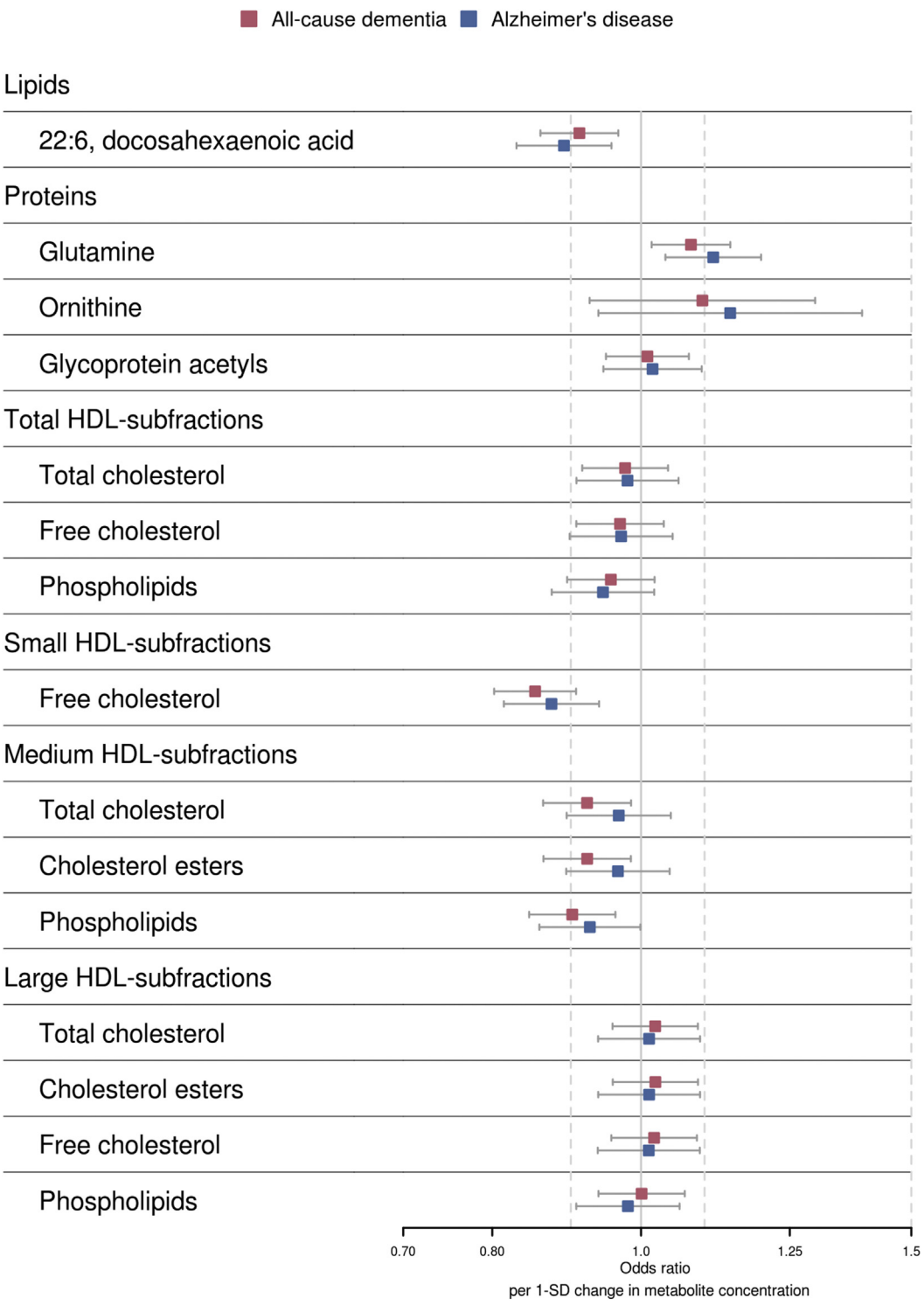

Fig. 3. Association of metabolites with all-cause dementia and Alzheimer's disease. The standardized OR of metabolites with all-cause dementia (red) and Alzheimer's disease (blue) shown as point estimates with whiskers denoting the 95% confidence interval of the OR. Associations shown are adjusted for age (at entry), sex, and if available body mass index and lipid-lowering medication. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; SD, standard deviation.

analysis of the different cognitive tests, the first principal component being the measure representing general cognitive ability [22,24,25]. General cognitive ability can be reliably estimated over the life course [26] and is very similar when derived from different cognitive test batteries in the same individuals [22,23]. To ensure comparability for the general cognitive ability in our study, only studies that had cognitive tests covering at least three different

cognitive domains were included. The domains covered are shown in Supplementary Table 3. General cognitive ability accounted for between 35% and 58% of variance in cognitive tests in various studies (Supplementary Table 3). Correlations between the individual test measures and the derived principal component (loadings) by study are shown in Supplementary Table 3. In all studies, the general cognitive ability had a high correlation with multiple single cognitive measures, showing the factor was not driven by a single measure.

Details on the ascertainment of dementia and AD for the various cohorts can be found in Supplementary Table 2. The diagnosis of dementia is based on continuous follow-up health records in the ERF, WHII, EGCUT, Finrisk97, and DILGOM. Studies that additionally used data on periodic visits to a research center were the RS, FHS, and AgeCoDe. The ascertainment of dementia and AD in the VUmc Amsterdam Dementia Cohort was done by clinical visits of participants.

2.4. Assessment of genetic and environmental factors

In the two discovery cohorts, ERF and RS, apolipoprotein E ε4 (*APOE* ε4) genotypes were determined by direct genotyping [27,28]. In both studies, lifestyle factors, including smoking (current vs. past and never smokers), physical activity (yes/no), and dietary fish (oil) intake [29] were ascertained using questionnaires as described previously [30,31]. Glucose, TC, HDL-C, LDL-C, and triglycerides were measured in mainly fasting blood samples by standard procedures [32,33]; further details are provided in Supplementary Table 1. Multiple metabolites associated with smoking and physical activity, and docosahexaenoic acid (DHA) associated with fish intake (Fig. 4).

2.5. Assessment of blood metabolites

In the ERF and RS, the metabolic biomarkers were quantified from fasted ethylenediaminetetraacetic acid (EDTA) plasma samples using high-throughput proton nuclear magnetic resonance (NMR) metabolomics (Nightingale Ltd, Helsinki, Finland). This method provides simultaneous quantification of metabolites, that is, routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular weight metabolites including amino acids, ketone bodies, and gluconeogenesis-related metabolites in molar concentration units. Details of the experimentation and applications of this NMR metabolomics platform have been described previously [34,35]. Metabolomics measurements of the ERF study further included two NMR experiments [35,36]. If a metabolite was measured in a study by multiple experiments, the experiment measuring the largest number of samples would be used. In total, 299 unique metabolite concentrations were measured in ERF; and of these, 242 metabolites were also available in the RS. The summary statistics of metabolomics measurements in the discovery cohorts are shown in Supplementary Table 4. The cohorts used for replication of the cognitive findings and extrapolation to dementia used NMR-based platforms or mass spectrometry techniques (Supplementary Table 1). The Nightingale NMR platform was also used in NTR, VUmc, EGCUT, WHII, Finrisk97, and DILGOM. In NTR, additional NMR experiments [35,36] were performed, and again the experiment with the largest number of observations was used. Measurements of cognitive function and blood drawn for metabolite measurements were concurrent in all metabolite measurements from our discovery and 73.6% of the

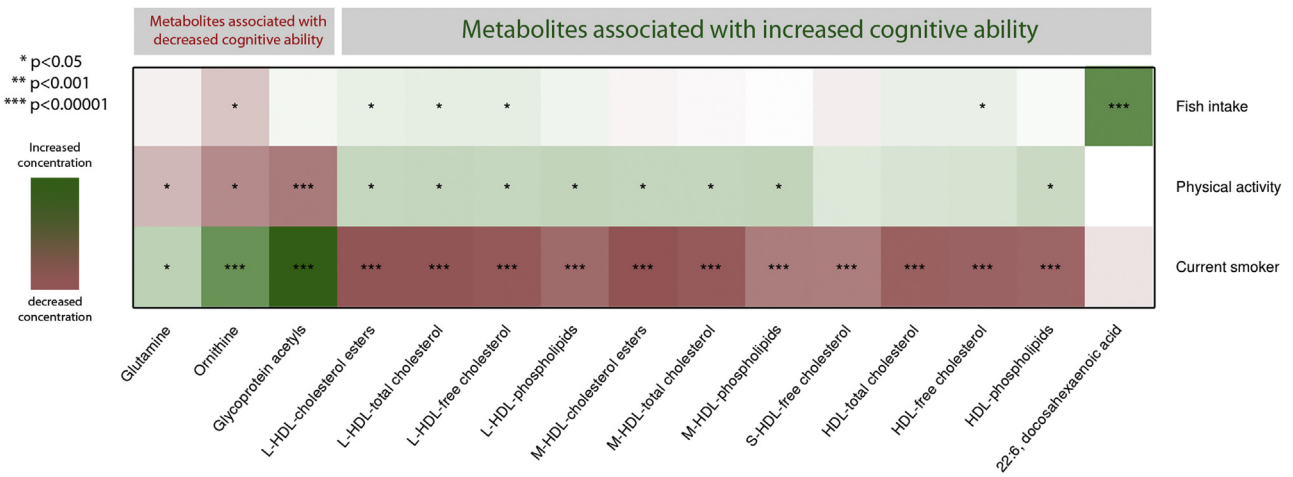

Fig. 4. Association of metabolites with lifestyle factors. Lifestyle factors including smoking (current vs. past and never smokers), physical activity (yes/no), and dietary fish (oil) intake. Metabolites are grouped based on their association with general cognitive ability and subfraction of HDL. Colors represent standardized effects estimates. Green shows the lifestyle factor associated with an increase in the metabolite concentration, and red shows the lifestyle factor associated with a decrease in the metabolite concentration. Significance of the associations is shown $*P < .05$, $**P < .001$, and $***P < 1 \times 10^{-5}$. Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; L, large particles; M, medium particles; S, small particles.

samples in the replication cohorts (Supplementary Table 3). Samples used in replication and extrapolation were collected after overnight fasting; except for the samples at the VUmc Alzheimer Center and Finrisk97, which were nonfasting or "semifasting" (participants were instructed to fast for 4 hours before the scheduled examination). The summary statistics of metabolomics measurements in the cohorts used for the replication of the cognitive findings and the cohorts used for extrapolation to dementia are shown in Supplementary Table 5.

2.6. Statistical analyses

Histograms of classical blood measurements and metabolites in the discovery cohorts were visually inspected for non-normality, if necessary natural logarithmic or rank-transformations were applied (Supplementary Table 4). Individuals who had suffered from a stroke or who were diagnosed with dementia at the time of cognitive assessment were excluded. Linear regression analyses were used to assess the relation of standardized measures of TC, HDL-C, LDL-C, triglycerides, and glucose with general cognitive ability, adjusting for age, sex, lipid-lowering medication (yes/no), and body mass index (BMI) as covariates. The effect of *APOE* ε4 on general cognitive ability was assessed using an additive model. The association of 299 standardized metabolites with general cognitive ability was assessed using linear regression with age, sex, BMI, and lipid-lowering medication as covariates (model 1). To test if the identified associations were independent of the classically measured and frequently studied circulating markers, we ran a second model (model 2) where we additionally included TC, HDL-C, LDL-C, triglycerides, and glucose as covariates to model. Finally, we tested if the identified metabolites–general cognitive ability association were confounded by *APOE* ε4 (model 3).

Because metabolites are highly correlated, we used the method of Li and Ji [37] to correct for multiple testing. The method calculates the number of independent tests in correlated measures. In this study, testing 299 metabolites corresponded to 87 independent tests (P for significance = $0.05/87 = 5.7 \times 10^{-4}$). To assess the relation of metabolites found to be associated with cognitive function to incident dementia and AD, we used Cox proportional hazard models when data came from prospective studies. Again, we standardized the metabolite levels and adjusted for age (at entry), sex, BMI, and lipid-lowering medication. For VUmc Alzheimer Center, we used logistic regression adjusted for age and sex. The relations with incident dementia and AD were evaluated in a second model additionally adjusting for *APOE* ε4 genotypes.

In the discovery cohorts, we used linear regression analysis to study associations of lifestyle factors (smoking, physical activity, and fish [oil] consumption) with metabolites and cognitive function, adjusting for age, sex, BMI, and lipid-lowering medication. All analyses were performed in R (version 3.2.1, 2015-06-18). Summary statistics by cohort were combined with inverse variance-weighted fixed-effects meta-analysis using the "*rmeta*" package (version 2.16). The association magnitudes are reported in units of standard deviation (SD) or odds ratio (OR) change per 1-SD increase in each metabolite [38,39] easing comparison of effects.

3. Results

Clinical characteristics of all cohorts analyzed in this study are provided in Table 1. Results of the association of general cognitive ability with baseline clinical characteristics in the discovery cohorts are shown in Table 2. As expected, general cognitive ability was higher in participants with higher education and was inversely associated with increasing age and the presence of *APOE* ε4 allele. Increased HDL-C was associated with higher general cognitive ability (0.034 SD higher general cognitive ability per 1 SD higher HDL-C concentration; $P = 6.4 \times 10^{-3}$), whereas fasting glucose levels were associated with lower cognitive ability (0.039 SD; $P = 2.2 \times 10^{-3}$).

3.1. The metabolic profile of general cognitive ability

We identified 18 metabolites that were significantly associated ($P < 5.9 \times 10^{-4}$ [model 1]) with general cognitive ability (listed as top 18 associations in Supplementary Table 6). Association results can be accessed through http://bbmri.researchlumc.nl/atlas. Of the 18 metabolites, XXL-LDL-triglycerides were not measured in the replication cohorts; therefore, 17 metabolites were tested for replication in independent cohorts (Supplementary Table 7 [model 1], $N_{max} = 6652$). Of these 17, we found 15 to be associated with general cognitive ability in the replication cohorts ($P_{replication} < .05$, Table 3). Thirteen metabolites surpassed the more stringent Bonferroni corrected threshold for significance in the replication ($P_{replication} < 2.9 \times 10^{-3}$). Combining discovery and replication data (Table 3), 12 metabolites were associated with higher general cognitive ability and three were associated with lower general cognitive ability. The metabolites associated with increased higher cognitive ability include 11 HDL subfractions, the most significant being free cholesterol in HDL (0.078 SD; $P = 2.3 \times 10^{-15}$) and docosahexaenoic acid (DHA or 22:6[n-3]) an omega-3-fatty acid (0.060 SD; $P = 9.8 \times 10^{-11}$). The three metabolites that were associated with lower general cognitive ability include glycoprotein acetyls (-0.075 SD; $P = 5.4 \times 10^{-13}$), glutamine (-0.042 SD; $P = 2.8 \times 10^{-7}$), and ornithine (-0.057 SD; $P = 8.5 \times 10^{-7}$). Of the 15 metabolites significantly associated with general cognition, only two metabolites, HDL-C esters ($P_{model2} = 9.9 \times 10^{-3}$) and medium HDL TC ($P_{model2} = 7.9 \times 10^{-3}$), lost their significance in the combined analysis when additionally adjusting for glucose, TC, HDL-C, LDL-C, and triglycerides (Supplementary Table 7 [model 2]). However, adjustment did not result in a major change in effect estimates for these two metabolites,

Table 1
Baseline characteristics of all studied 11 cohorts

Variables	ERF	RS	WHII	NTR	SHIP-Trend	FHS	VUmc ADC	Finrisk97	DILGOM	EGCUT	AgeCoDe
Number of samples in cognitive analysis	2683	2505	4235	338	944	1508	-	-	-	-	-
Age (years)	48.9 ± 14.2	74.2 ± 6.2	55.8 ± 6.0	40.7 ± 12.4	50.1 ± 13.6	55.7 ± 9.8	64.1 ± 9.0	48.8 ± 13.5	52.3 ± 13.5	59.1 ± 12.4	84.1 ± 3.1
N-Women (%)	56.1	58.2	26.2	62.4	56.4	52.5	45.1	54.7	55.8	58.9	69.4
Education (1–4 scale)	2.1 ± 0.9	2.4 ± 0.9	2.0 ± 0.8	3.2 ± 0.8	2.4 ± 0.9	2.3 ± 0.6 *	5.0 ± 1.0†	2.0 ± 0.8*	2.1 ± 0.8*	3.0 ± 0.8	-
Body mass index (kg/m^2)	27 ± 4.7	27.4 ± 4.1	25.9 ± 3.8	24.7 ± 4	27.4 ± 4.6	27.5 ± 4.9	25.3 ± 3.8	26.7 ± 4.6	27.2 ± 4.9	28 ± 5.1	25.6 ± 3.7
Lipid-lowering medication (%)	12.7	22.8	3.0	7.0	7.4	7.6	19.5	3.49	15.6	24	21.6
APOE ε4 carriers (%)	37.7	27.6	27.7	26.6	22.5	22.5	51.7	35.1	24	23.6	20.3
Diastolic blood pressure (mm Hg)	80 ± 10	79 ± 11	77 ± 10.3	76 ± 9.8	76.7 ± 10	75 ± 10	86 ± 11	83 ± 11.24	79 ± 11	82 ± 10	78 ± 8.0
Systolic blood pressure (mm Hg)	140 ± 20	152 ± 21	122 ± 15	124 ± 12	124 ± 16	126 ± 18	141 ± 19	136 ± 20	137 ± 20	134 ± 17	136 ± 15
Established blood measures											
TC (mmol/L)	5.6 ± 1.1	5.6 ± 1.0	5.9 ± 1.0	5.1 ± 1.1	5.5 ± 1.1	5.3 ± 1.0	4.9 ± 1.0	5.5 ± 1.1	5.3 ± 1.0	5.8 ± 1.1	5.8 ± 1.1
LDL-cholesterol (mmol/L)	3.7 ± 1.0	3.5 ± 0.9‡	3.8 ± 0.9	3.1 ± 1.0	3.4 ± 0.9	3.3 ± 0.9	1.7 ± 0.5	3.5 + 0.9‡	3.2 ± 0.8‡	2.2 ± 0.7	3.5 ± 1.0
HDL-cholesterol (mmol/L)	1.3 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.3	1.5 ± 0.4	1.3 ± 0.4	1.5 ± 0.4	1.39 ± 0.4	1.44 ± 0.4	1.7 ± 0.4	1.6 ± 0.4
Triglycerides (mmol/L)	1.3 ± 0.8	1.49 ± 0.7	1.3 ± 0.8	1.4 ± 0.7	1.4 ± 0.9	1.7 ± 1.4	1.4 ± 0.7	1.51 ± 1.1	1.43 ± 0.9	1.8 ± 1.1	1.4 ± 0.6
Glucose (mmol/L)	4.7 ± 1.1	5.9 ± 1.5	5.2 ± 1	5.4 ± 0.7	5.4 ± 0.6	5.6 ± 1.5	5.7 ± 1.7	4.61 ± 1.1	4.12 ± 0.8	4.6 ± 1.7	-
Dementia analysis											
Number of samples	1532	2010	4612	-	-	2356	1303	7517	4788	2572	310
Follow-up time (years)	11.3 ± 1.7	7.6 ± 3.6	16.7 ± 1.6	-	-	15.7 ± 5	-	9.67 ± 1.35	7.68 ± 0.9	7.03 ± 2.22	4.5 ± 1.8
Maximum follow-up	13.6	11.7	17.9	-	-	22.6	-	10	7.9	12.9	6.4
Number of AD cases	28	346	35	-	-	81	665	100	75	-	75
Number of dementia cases	39	506	114	-	-	110	917	141	81	41	82

Abbreviations: AD, Alzheimer's disease; AgeCoDe, German Study on Ageing, Cognition, and Dementia; *APOE*, apolipoprotein E; DILGOM, Dietary, Lifestyle, and Genetic determinants of Obesity and Metabolic Syndrome; EGCUT, Estonian Biobank; ERF, Erasmus Rucphen Family; FHS, Framingham Heart Study; Finrisk97, The National FINRISK Studies 1997; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NTR, Netherlands Twin Register; RS, Rotterdam Study; SHIP-Trend, Study of Health in Pomerania–Trend; TC, total cholesterol; VUmc ADC, VUmc Amsterdam Dementia Cohort; WHII, Whitehall II.

NOTE. For VUmc ADC, Finnrisk97, DILGOM, EGCUT, and AgeCoDE the descriptive statistics are calculated based on the samples in the dementia analysis.

*Education in 1–3 scale.

†Education in 1–7 scale.

‡LDL-C estimated using the Friedewald estimation.

Table 2
Association of characteristics with general cognitive ability in discovery cohorts

	ERF study			Rotterdam Study			Meta-analysis		
Phenotype	Effect (±SE)	*P* value	N	Effect (±SE)	*P* value	N	Effect (±SE)	*P* value	N
Age	−0.042 (±0.001)	2.1×10^{-280}	2699	−0.078 (±0.003)	1.1×10^{-154}	2483	−0.046 (±0.001)	$<1 \times 10^{-500}$	5182
Sex (male vs. female)	−0.020 (±0.029)	0.49	2699	0.142 (±0.035)	3.8×10^{-5}	2483	0.048 (±0.022)	3.1×10^{-2}	5182
BMI	0.005 (±0.003)	0.15	2694	−0.015 (±0.004)	3.1×10^{-4}	2483	−0.003 (±0.003)	0.30	5177
Education	0.410 (±0.017)	1.4×10^{-117}	2699	0.277 (±0.020)	1.5×10^{-41}	2483	0.355 (±0.013)	$<1 \times 10^{-500}$	5182
APOE ε4	−0.046 (±0.028)	0.09	2342	−0.157 (±0.035)	9.0×10^{-6}	2378	−0.088 (±0.022)	5.2×10^{-5}	4720
Lipid-lowering medication	−0.131 (±0.047)	5.8×10^{-3}	2690	0.013 (±0.042)	0.76	2397	−0.050 (±0.031)	0.11	5087
Classical blood measures									
TC	−0.011 (±0.016)	0.50	2635	0.022 (±0.019)	0.24	2481	0.003 (±0.012)	0.8	5116
LDL-C*	−0.026 (±0.016)	0.10	2621	0.016 (±0.019)	0.41	2265	−0.009 (±0.012)	0.45	4886
HDL-C	0.037 (±0.016)	2.3×10^{-2}	2635	0.029 (±0.019)	0.13	2401	0.034 (±0.012)	6.4×10^{-3}	5036
Triglycerides	−0.014 (±0.016)	0.36	2637	−0.017 (±0.018)	0.35	2345	−0.015 (±0.012)	0.19	4982
Glucose	−0.047 (±0.017)	6.2×10^{-3}	2623	−0.029 (±0.019)	0.12	2401	−0.039 (±0.013)	2.2×10^{-3}	5024

Abbreviations: *APOE*, apolipoprotein E; BMI, body mass index; ERF, Erasmus Rucphen Family; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation; SE, standard error; TC, total cholesterol.

NOTE. Multivariate analysis of general cognitive ability with age was adjusted for sex and with sex was adjusted for age. Association of general cognitive ability with BMI, educational level, *APOE*, and lipid-lowering medication use adjusted for age and sex. Associations of general cognitive ability with blood measures (TC, LDL-C, HDL-C, triglycerides, and glucose) were adjusted for age, sex, and lipid-lowering medication use. The association magnitudes are reported in units of SD change (±SE) per 1-SD increase in each metabolite [38,39].

*LDL-C in the Rotterdam Study was estimated using the Friedewald estimation.

suggesting an independent effect (Supplementary Fig. 1 [model 2]). Adjusting for *APOE* ε4 did not change any of the 15 associations (Supplementary Table 7 [model 3] and Supplementary Fig. 1 [model 3]). In Box 1, we summarize the functions of the metabolites we found associated with general cognitive ability.

3.2. Association of the metabolic profile with dementia and AD

Next, we examined whether the 15 metabolites associated with general cognitive ability were associated with dementia. We compared (maximum) 1990 dementia patients, of whom 1356 were AD cases, with 23,882 controls. Six metabolites were associated with dementia, and three of these were also associated with AD ($P < .05$; Table 4, for all association results, see Supplementary Table 8 and Fig. 3). Free cholesterol in small HDL associated most significantly with a lower risk of dementia (OR = 0.85 per 1-SD increase in metabolite concentration; 95% CI = 0.80–0.91; $P = 6.3 \times 10^{-7}$) and AD (OR = 0.87; 95% CI = 0.81–0.94; $P = 2.3 \times 10^{-4}$). Other metabolites associated with a lower dementia risk were DHA (OR = 0.92; 95% CI = 0.86–0.97; $P = 3.4 \times 10^{-3}$; AD, $P = 1.5 \times 10^{-3}$) and subfractions of medium size HDL particles (phospholipids $P = 2.5 \times 10^{-3}$, TC $P = .025$, and cholesterol esters $P = .025$). Higher glutamine levels were associated with an increased risk of dementia (OR = 1.08; 95% CI = 1.02–1.15; $P = .011$) and AD (OR = 1.11; 95% CI = 1.04–1.20; $P = 3.0 \times 10^{-3}$). The association of free cholesterol in small HDL and DHA surpassed the more stringent Bonferroni corrected threshold for significance ($P < 1.5 \times 10^{-3}$). After additionally adjusting for the number of *APOE* ε4 alleles, the associations of dementia and AD with subfractions of medium size HDL particles were no longer significant ($P > .05$; Supplementary Table 8 and Supplementary Fig. 2).

3.3. Association of the metabolic profile with lifestyle factors

The analyses of the association of lifestyle factors with metabolites and general cognitive ability are shown in Supplementary Table 9 and summarized in Fig. 4. Fish (oil) intake was strongly associated with DHA blood concentrations ($P = 9.9 \times 10^{-53}$). Physical activity was associated with increased ($P < .05$) levels of metabolites that were associated with higher cognitive function (medium and large HDL subfractions) and decreased levels of metabolites that were associated with lower cognitive function (glycoprotein acetyls, ornithine, and glutamine). Smokers had decreased concentrations of all HDL subfractions associated with higher cognitive function and increased concentrations of metabolites associated with decreased cognitive function (Fig. 4).

4. Discussion

In this study, we discovered and replicated 15 metabolites associated with general cognitive ability. This metabolic profile includes subfractions of HDL, DHA, ornithine, glutamine, and glycoprotein acetyls. We show that metabolites in the profile are independent of classical cardiometabolic blood correlates of cognitive function. Of the 15 replicated metabolites, six were associated with dementia and three of these also with AD. Furthermore, we show that lifestyle factors, such as diet, smoking, and physical activity, have strong effects on metabolites in the profile.

Table 3
Association of metabolites with general cognitive ability

	Discovery			Replication			Meta-analysis				
Metabolite	Effect (±SE)	*P* value	N	Effect (±SE)	*P* value	N	Effect (±SE)	*P* value	N	I^2	*P*-I^2
HDL-free cholesterol	0.067 (±0.013)	1.5×10^{-7}	4791	0.094 (±0.015)	1.2×10^{-9}	4542	0.078 (±0.010)	2.3×10^{-15}	9333	63	6.7×10^{-2}
L-HDL-free cholesterol	0.059 (±0.013)	3.4×10^{-6}	4793	0.106 (±0.016)	6.2×10^{-11}	4542	0.077 (±0.010)	1.5×10^{-14}	9335	80	7.3×10^{-3}
L-HDL-total cholesterol	0.052 (±0.013)	5.0×10^{-5}	4792	0.108 (±0.016)	3.5×10^{-11}	4542	0.073 (±0.010)	3.5×10^{-13}	9334	79	8.2×10^{-3}
Glycoprotein acetyls	−0.064 (±0.014)	4.5×10^{-6}	3778	−0.087 (±0.015)	1.4×10^{-8}	4542	−0.075 (±0.010)	5.4×10^{-13}	8320	0	0.60
L-HDL-cholesterol esters	0.048 (±0.013)	1.5×10^{-4}	4792	0.108 (±0.016)	3.4×10^{-11}	4542	0.071 (±0.010)	1.6×10^{-12}	9334	80	7.6×10^{-3}
L-HDL-phospholipids	0.045 (±0.013)	4.2×10^{-4}	4791	0.100 (±0.016)	3.3×10^{-10}	4542	0.067 (±0.010)	2.2×10^{-11}	9333	78	9.7×10^{-3}
HDL-phospholipids	0.050 (±0.013)	9.5×10^{-5}	4790	0.089 (±0.015)	9.8×10^{-9}	4542	0.066 (±0.010)	2.5×10^{-11}	9332	68	4.3×10^{-2}
HDL-total cholesterol	0.048 (±0.013)	2.1×10^{-4}	4796	0.088 (±0.016)	1.5×10^{-8}	4542	0.064 (±0.010)	9.8×10^{-11}	9338	66	5.4×10^{-2}
22:6, docosahexaenoic acid	0.047 (±0.014)	5.4×10^{-4}	3772	0.070 (±0.012)	2.1×10^{-8}	5480	0.060 (±0.009)	9.8×10^{-11}	9252	67	4.6×10^{-2}
M-HDL-phospholipids	0.063 (±0.013)	8.2×10^{-7}	4799	0.057 (±0.014)	7.2×10^{-5}	4542	0.060 (±0.010)	2.5×10^{-10}	9341	0	0.48
M-HDL-total cholesterol	0.046 (±0.013)	3.0×10^{-4}	4799	0.054 (±0.014)	1.5×10^{-4}	4542	0.050 (±0.010)	1.8×10^{-7}	9341	0	0.62
M-HDL-cholesterol esters	0.046 (±0.013)	2.5×10^{-4}	4799	0.052 (±0.014)	2.6×10^{-4}	4542	0.049 (±0.009)	2.4×10^{-7}	9341	0	0.63
Glutamine	−0.052 (±0.012)	2.5×10^{-5}	4715	−0.034 (±0.011)	1.8×10^{-3}	6652	−0.042 (±0.008)	2.8×10^{-7}	11,367	74	9.8×10^{-3}
S-HDL-free cholesterol	0.059 (±0.012)	8.4×10^{-7}	4796	0.029 (±0.014)	4.0×10^{-2}	4542	0.047 (±0.009)	3.5×10^{-7}	9338	0	0.55
Ornithine	−0.083 (±0.018)	4.5×10^{-6}	2228	−0.039 (±0.015)	1.0×10^{-2}	2750	−0.057 (±0.012)	8.5×10^{-7}	4978	43	0.18
L-LDL-triglycerides	−0.055 (±0.012)	3.7×10^{-6}	4797	0.018 (±0.015)	2.3×10^{-1}	4542	−0.027 (±0.009)	4.2×10^{-3}	9339	90	3.6×10^{-5}
M-LDL-triglycerides	−0.042 (±0.012)	5.1×10^{-4}	4800	0.019 (±0.015)	1.9×10^{-1}	4542	−0.018 (±0.009)	6.3×10^{-2}	9342	85	1.3×10^{-3}

Abbreviations: *APOE*, apolipoprotein E; HDL, high-density lipoprotein; I^2, measure for heterogeneity in the meta-analysis in percent; LDL, low-density lipoprotein; L, large particles; M, medium particles; *P*-I^2, *P* value for heterogeneity; S, small particles; SD, standard deviation; SE, standard error.

NOTE. Glycoprotein acetyls are mainly α-1-acid glycoprotein. The association magnitudes are reported in units of SD change (±SE) per 1-SD increase in each metabolite [38,39]. Shown associations of the metabolites with general cognitive ability are adjusted for age, sex, body mass index, and lipid-lowering medication.

Box 1
Description of pathways of metabolites in the context of cognitive function

High-density lipoprotein subfractions

The specific lipoprotein subfractions could point to the specific functions of lipoprotein subfractions. High-density lipoprotein (HDL) fractions are well known to be individually tasked for different functions across lipid metabolism, inflammation, anti-oxidation, and host defense [40,41]. In addition, specific protein pairs on specific HDL subspecies exist that maintain stable compositions [42]. Previous research reported links between HDL cholesterol profiles and changes in vascular health with plaque accumulation in arteries of the brain, damage to the blood brain barrier [43], and occurrence of thrombosis. All are possibly leading to progressive vascular brain damage resulting in loss of white matter microstructural organization.

Docosahexaenoic acid

Docosahexaenoic acid (DHA) levels in blood are highly associated with omega-3-fatty acid intake through diet [39], and it cannot be de novo synthesized in the brain and is therefore actively transported over the blood-brain barrier through the Mfsd2a [44,45]. DHA is essential for normal brain development in early life and is frequently associated with cognition [46]. High intake might also be beneficial in late life as DHA and fish oil intake associated with less Alzheimer's disease pathology [47]. The evidence of the attributed beneficial effects of DHA on the brain in literature is inconsistent [48].

Glutamine

In the brain, glutamine is not only used for energy production and protein synthesis, as in other cells, but is also an essential precursor for biosynthesis of amino acid neurotransmitters. It is involved in the glutamine-glutamate/GABA cycle, a well-studied concept in excitatory signaling in the brain [49]. The cycle involves transfer of glutamine from astrocytes to neurons and neurotransmitter glutamate or GABA from neurons to astrocytes. The leading opinion in the field is that in the brain an excess of glutamate, excitotoxicity, is seen as detrimental and glutamine in the brain as beneficial [50].

Glycoprotein acetyls

The measured glycoprotein acetyls is mainly α-1-acid glycoprotein (AGP) [34], also called orosomucoid, which is an acute phase plasma α-globulin glycoprotein. The protein is widely studied and has previously been found to predict 10-year mortality [51]. Increased plasma levels of glycoprotein acetyls as reaction to various diseases (cancer and inflammatory diseases) or following trauma (surgery) might explain the association with increased mortality and could partially explain the association with general cognitive ability as chronic diseases decrease cognitive abilities. Another function of AGP is to carry mainly neutrally charged medications in blood, for example, antidepressants [52]. The plasma concentration of AGP is relatively low, and there is only one drug-binding site in each AGP molecule [53], leading to lower antidepressant response in higher AGP concentrations [54].

Ornithine

Ornithine as a non–proteinogenic amino acid is an important intermediate product in arginine degradation and urea cycle. Hyperornithinemia is also the biochemical hallmark of an inherited metabolic disease, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome [55]. This disease is clinically characterized by mental retardation whose pathogenesis is still poorly known.

The most interesting metabolite in the profile is DHA, a long-chain omega-3 polyunsaturated fatty acid. As the largest cross-sectional study to date studying DHA in relation to cognitive function, the present study showed compelling evidence that DHA levels in blood were associated with higher cognitive function ($P = 9.8 \times 10^{-11}$). This finding is

Table 4
Metabolite concentrations associated with dementia and AD

	AD				Dementia			
Metabolite	OR	*P* value	N cases	N total	OR	*P* value	N cases	N total
S-HDL-free cholesterol	0.87 [0.81–0.81]	2.3×10^{-4}	1276	22,880	0.85 [0.80–0.80]	4.1×10^{-7}	1881	25,868
M-HDL-phospholipids	0.93 [0.86–0.86]	4.7×10^{-2}	1276	22,884	0.90 [0.85–0.85]	1.8×10^{-3}	1881	25,872
22:6, docosahexaenoic acid	0.89 [0.83–0.83]	1.5×10^{-3}	1334	22,466	0.91 [0.86–0.86]	1.9×10^{-3}	1938	25,417
Glutamine	1.11 [1.04–1.04]	3.1×10^{-3}	1356	25,181	1.08 [1.02–1.02]	1.3×10^{-2}	1990	25,640
M-HDL-cholesterol esters	0.97 [0.89–0.89]	0.38	1276	22,884	0.92 [0.86–0.86]	1.6×10^{-2}	1881	25,872
M-HDL-total cholesterol	0.97 [0.89–0.89]	0.40	1276	22,884	0.92 [0.86–0.86]	1.6×10^{-2}	1881	25,872

Abbreviations: AD, Alzheimer's disease; BMI, body mass index; HDL, high-density lipoprotein; L, large; M, medium; OR, odds ratio for the increase or decrease in AD or dementia risk per 1-SD increase of metabolite concentration.

NOTE. Sorted by the *P* values for dementia. Combined results from Cox proportional hazard models and logistic regression models are presented as OR. Associations shown are adjusted for age (at entry), sex, and if available BMI and lipid-lowering medication. N total is the sum of cases and controls.

in line with many previous studies, summarized by Cederholm et al. [46], suggesting a relation between nutritive DHA intake, or fish (oil) intake as its proxy, and better cognition. Blood levels of DHA are raised by eating fat fish, as also in our study. DHA from diet is most likely actively transported over the blood-brain barrier by Mfsd2a [44,56], where it is abundant in gray matter [57] and found in lower concentrations in brains of individuals with AD [58]. We showed for the first time that DHA in blood was associated with a lower risk of AD and dementia, using blood measures of DHA in up to 22,887 individuals. Taken together, our study implies that high levels of DHA could be beneficial for cognitive function, potentially also reducing the risk of dementia and AD.

Beyond the association of high HDL-C with better cognitive function [3–5], the present study points toward a role of cholesterol, free cholesterols, and phospholipids in small, medium, and large subclasses of HDL. However, current knowledge of the functions of HDL subclasses is limited; thus, we can only speculate on the pathways through which the metabolites that we observed exert their effect on cognitive function [59]. Phospholipids could have a direct effect as they are the main constituents of neuronal membrane structures, such as presynaptic and postsynaptic membranes, and neuronal membrane degeneration has been linked to synapse loss in AD [60]. Possibly, circulating phospholipids and free cholesterols in HDL form a buffer to repair damaged membranes. This is supported by the observation that both AD patients and patients with mild cognitive impairment have lower circulating levels of nutrients involved in phospholipid synthesis in blood and cerebrospinal fluid [61]. Alternatively, the free cholesterols in the phospholipid layer of HDL tag the presence of other important proteins that are transported to or are disposed from the brain. HDL contains up to 95 proteins and lipids that may segregate into distinct subclasses of HDL and lead to subclass-specific effects [42,62]. Regions in membranes of both neurons and astrocytes [63], where HDL-related free cholesterols, sphingomyelins, and free fatty acids (such as DHA) concentrate, are called lipid rafts. Changes in lipid raft composition may be an early marker of neurodegenerative diseases [64]. A hypothesis that requires further study is that increased free cholesterols in (small) HDL and DHA in blood affects lipid raft quantity, composition, or cell-signaling leading to beneficial effects on the brain.

In our study, levels of glycoprotein acetyls, mainly α-1-acid glycoprotein (also known as orosomucoid, an acute phase protein), were associated with lower cognitive function, smoking, and physical activity. Glycoprotein acetyl concentration has been shown to be a strong predictor of 10-year mortality [51,65]. A major genetic determinant of glycoprotein acetyl levels in blood is located close to the gene coding for haptoglobin (*HP*) [35]. This protein may link our findings of HDL subfractions to that of glycoprotein acetyls, as the *HP* protein has been found in specific HDL subfractions [62]. Furthermore, the *HP* gene was previously associated with the risk of cognitive impairment in type 2 diabetes with poor glycemic control [66].

Two nonessential amino acids that were associated with lower cognitive function were ornithine, which is part of the urea cycle, and glutamine. Ornithine accumulation causes hyperornithinemia-hyperammonemia-homocitrullinuria syndrome [55], a disease with a currently poorly known pathogenesis, which is clinically characterized by mental retardation [67]. Glutamine and its closely related neurotransmitter glutamate have been found to be differentially expressed in brains of AD patients [49]. In the brain, glutamate is considered harmful [49], and our population-based studies suggest that in the circulation, glutamine is associated with lower cognition. Both ornithine and glutamine are interesting targets for further studies.

A major strength of the present study is the large sample size, both in the discovery and replication. To our knowledge, this is the largest study exploring the association of a large array of blood-based metabolites with general cognitive ability to date. Other strengths are the similar methods across studies used to determine metabolites and the use of general cognitive ability to harmonize the studied cognitive outcome [23]. We chose to analyze the associations of metabolites with cognitive ability in the largest sample size available, accepting that subtle differences in cognitive testing, metabolite measuring, study design, and populations would introduce heterogeneity of effects and then followed by a replication in independent samples; this approach is modeled to the standard approach followed in genome-wide association studies [68]. A potential limitation of our cross-sectional study of cognition is that we cannot determine causality of the association with circulating metabolites. However, in our extrapolation to dementia and AD, we mostly studied incident cases with metabolites measured before the disease onset, suggesting that at least the six dementia-associated metabolites are most likely in a causal pathway. We note that the associations of the HDL subfractions associated with dementia were attenuated by the *APOE* ε4 genotype, suggesting they could be in the causal pathway of *APOE* ε4 to dementia or the associations found are pleiotropic effects of *APOE* ε4. Last but not least, we did not adjust for education because cognition and education are highly correlated [69] if measured at the same time. In fact, there is still debate on whether education determines cognitive ability [26,70] or vice versa [71,72]. In fact, there is a very high genetic correlation between educational attainment and cognitive ability (R^2 = 0.55 based on linkage disequilibrium (LD) score regression) [22,73,74]. This shared genetic background is probably the primary reason for the high correlation between education and cognitive ability [69,75]. Given the high genetic correlation, we decided that adjusting for education as a covariate in the model would lead to overadjustment and ultimately false-negative findings in the study.

In conclusion, we discovered and replicated the relation of 15 metabolites in blood to cognitive function in cognitively healthy individuals. We found that six metabolites

were associated with dementia and three with AD. The association of lifestyle factors to the metabolites associated with cognitive ability and dementia opens new avenues for targeted prevention.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2017.11.012.

RESEARCH IN CONTEXT

1. Systematic review: Cognitive function is an important indicator of brain health and a predictor of dementia. Metabolomics could provide valuable new insights into the determinants of cognitive function, but, to date, studies of blood metabolite measures and cognitive function are limited in size and findings are rarely replicated.
2. Interpretation: We undertook a large study on the associations of circulating metabolites with general cognitive ability and found a profile of 15 metabolites to be consistently associated with general cognitive ability, independently of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and *APOE* genotypes. Six of these metabolites were also associated with risk of dementia. The metabolites in the profile were associated with lifestyle factors.
3. Future directions: Future studies should examine the molecular mechanisms underlying the observed associations between metabolites, cognitive function, and dementia, whether metabolites can be used as readouts for preventive or therapeutic interventions, and whether selective interventions targeting metabolites would prevent dementia.

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