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Research Article

Metabolic Signature of Insulin Resistance and Risk of Alzheimer's Disease

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Abstract

Background: Substantial evidence supports the relationship between peripheral insulin resistance (IR) and the development of Alzheimer's disease (AD)-dementia. However, the mechanisms explaining these associations are only partly understood. We aimed to identify a metabolic signature of IR associated with the progression from mild cognitive impairment (MCI) to AD-dementia.

Methods: This is a case-control study on 400 MCI subjects, free of type 2 diabetes, within the ACE cohort, including individuals ATN + and ATN−. After a median of 2.1 years of follow-up, 142 subjects converted to AD-dementia. IR was assessed using the homeostasis model assessment for insulin resistance (HOMA-IR). A targeted multiplatform approach profiled over 600 plasma metabolites. Elastic net penalized linear regression with 10-fold cross-validation was employed to select those metabolites associated with HOMA-IR. The prediction ability of the signature was assessed using support vector machine and performance metrics. The metabolic signature was associated with AD-dementia risk using a multivariable Cox regression model. Using counterfactual-based mediation analysis, we investigated the mediation role of the metabolic signature between HOMA-IR and AD-dementia. The metabolic pathways in which the metabolites were involved were identified using MetaboAnalyst.

Results: The metabolic signature comprised 18 metabolites correlated with HOMA-IR. After adjustments by confounders, the signature was associated with increased AD-dementia risk (HR = 1.234; 95% CI = 1.019–1.494; $p < .05$). The metabolic signature mediated 35% of the total effect of HOMA-IR on AD-dementia risk. Significant metabolic pathways were related to glycerophospholipid and tyrosine metabolism.

Conclusions: We have identified a blood-based metabolic signature that reflects IR and may enhance our understanding of the biological mechanisms through which IR affects AD-dementia.

Keywords: Biomarkers, Blood, Dementia, Metabolomics

Background

Alzheimer's disease (AD)-dementia is the most common neurodegenerative disease, with an estimated 55 million people worldwide affected, and expected to reach 139 million in 2050 (1). Although there are new promising drugs for treating AD-dementia (2), there is no effective cure for the disease yet. This underscores the importance for early detection and prevention, as well as the search for efficient treatments to delay or slow onset or progression of the disease. To achieve this, understanding AD-dementia pathophysiological mechanisms and identifying novel and noninvasive biomarkers for disease may contribute to further designing potential strategies to slow the rates of cognitive decline more effectively.

The higher prevalence of mild cognitive impairment (MCI) in subjects with obesity (3), type 2 diabetes, or metabolic syndrome (4) pointed out insulin resistance (IR) as one of the most remarkable metabolic dysfunction in the early stages of AD-dementia. Insulin plays an important role in the brain influencing neuronal function and synaptic plasticity (5), and

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its progressive dysregulation contributes to structural and functional deficits in synaptic plasticity, as well as in worsening cognition (6). Experimental studies in rodent models of AD have also observed the presence of hippocampal IR, thus suggesting that IR dysregulation in the brain may negatively affect cognition (7). Although insulin locally released by neurons provides a rapid modulation of neural networks (8), most of the insulin used by the brain is produced at peripheral level and transported through the blood-brain barrier (BBB). Peripheral dysregulation of insulin metabolism has been implicated in AD pathogenesis because IR contributes to the accumulation of Aβ plaques, phosphorylated Tau protein, inflammation, and oxidative stress (9). Furthermore, alterations in the levels of certain metabolite species have been observed in both IR and AD. For instance, elevated levels of branched-chain amino acids in the blood have been positively associated with both IR and AD (10,11). However, the molecular mechanisms linking IR and progression to AD-dementia are not well understood yet. Metabolic profiling can uncover molecular signatures that underlie the relationship between IR and AD-dementia and help identify intermediate biological mechanisms and potential biomarkers. Although previous research has identified individual metabolites and metabolic pathways associated with either IR or AD (12–15), no studies have explored a comprehensive metabolic profile that could offer a more holistic view of the early metabolic dysregulations driving disease progression. In the present study, we tested the hypothesis that early dysregulations of insulin metabolism may contribute to the progression from MCI to AD-dementia. We aimed to identify a metabolic signature associated with homeostasis model assessment for insulin resistance (HOMA-IR) and examine its association with the progression from MCI to AD-dementia. To provide deeper insights into the mechanisms that link IR with AD-dementia, we further examined whether the identified signature mediates the association between IR and AD-dementia risk.

Method

Study Population and Design

The ACE cohort consists of MCI men and women aged >48 years recruited and assessed between 2016 and 2023 at the Memory Disorders Unit (ACE Alzheimer Center, Spain) using the Spanish version of the Mini-Mental State Examination (MMSE) and an extensive neuropsychological battery (N-BACE), among other tests. Participants were classified according to the ATN criteria when CSF markers were available. The cutoff values used to dichotomize each CSF biomarker into positive or negative within the ATN system were as follows: for ELISA, $A\beta_{1\text{-}42}$ < 676 pg/mL for A, p181-tau > 58 pg/mL for T, and T-Tau > 367 pg/mL for N; for CLEIA, $A\beta_{1\text{-}42}$ < 796 pg/mL for A; p181-tau > 54 pg/mL for T, and T-tau > 412 pg/mL for N (16). A detailed description of the study population and clinical diagnosis has been published elsewhere (17). From the initial cohort of 1 675 individuals, 632 participants had MCI, were free of type 2 diabetes, had ATN classification, available plasma samples, and follow-up data (Supplementary Figure S1). We designed a nested case-control study, randomly selecting 200 participants with MCI with positive CSF biomarkers of (A+(T|N)+) matched by sex with 200 MCI with negative CSF biomarkers (A-T-N-). The ACE cohort data set was downloaded on February 15, 2023. All procedures were implemented following the ethical standards of the Declaration of Helsinki. Informed consent was obtained from all the participants in the study for CSF and blood sample collection and for obtaining general information. The protocol was approved by the institutional ethics review board (Research Ethics Committee with medicines, CEIm Institute of Health Pere Virgili, Ref. 164/2019).

Data Collection

Demographic and lifestyle information such as medical history, smoking status, and education level were collected during the first visit. Weight and height were measured with calibrated scales and a wall-fixed stadiometer and body mass index (BMI) was calculated. Apolipoprotein E (APOE) was genotype using genomic DNA extracted from peripheral blood using the commercially available kit Chemagic system (Perkin Elmer, Waltham, United States) and fluorogenic allele-specific oligonucleotide probes (TaqMan assay; Life Technologies, Carlsbad, United States).

Blood samples were collected after overnight fasting using EDTA tubes and stored at −80°C until further analysis. Glucose and insulin concentrations were measured using the standard enzymatic method with hexokinase (Cobas 8000 Roche Diagnostics) and by Electrochemiluminescence immunoassay (Elecsys Roche Diagnostics), respectively. The HOMA-IR was estimated.

Participants were followed up and assessed annually by neurologists, neuropsychologists, and social workers. Cognitive evaluations incorporated the Spanish version of the MMSE, the memory component of the Spanish version of the 7 Minute test, the Spanish version of the Neuropsychiatric Inventory Questionnaire (NPI-Q), the Hachinski Ischemia Scale, the Blessed Dementia Scale, the Clinical Dementia Rating (CDR) scale, and the comprehensive neuropsychological battery of ACE (N-BACE). The MMSE and N-BACE were administered at all visits, with the NPI-Q assessed at baseline. AD-dementia conversion was assessed according to the 2011 NIA-AA for AD guidelines.

Metabolomic Profiling

We employed a multiplatform approach including liquid chromatography with tandem mass spectrometry (LC-MS and LC-MS/MS) and gas chromatography with tandem mass spectrometry (GC-MS and GC-MS/MS). Using targeted metabolomic approaches, we profiled several metabolites of different nature including fatty acids, organic acids, sugar metabolites, polar metabolites, amino acids and derivatives (acylcarnitines), serotonin, short-chain fatty acids, bile acids, and lipid species (lysophospholipids, sphingomyelins, phospholipids, diglycerides, triglycerides, and cholesteryl esters [CE]). Details of the metabolite profiling platforms and protocols have been described previously (Supplementary Methods S1) (18). After quality filtering and standardization, 621 metabolites were identified and quantified or semi-quantified. Information about the platform, concentration, retention time, precursor ion, product ion, repeatability, and reproducibility for each metabolite is shown in Supplementary Table S1.

Statistical Analyses

Descriptive data are shown as median and interquartile range for quantitative variables, and percentages for categorical variables. The distribution of variables was assessed using the Anderson–Darling normality test (histograms provided in

Supplementary Figure S2). Group differences were assessed using the Mann–Whitney test and the chi-square test, for quantitative and categorical variables, respectively. Individual metabolites with ≥20% missing values were excluded (19), leaving 611 metabolites for further analyses. The remaining metabolites had an average percentage of missingness (min, max) equal to 4.51% (0.25, 19.75; Supplementary Figure S3). Missing values were imputed by applying the random forest approach using the missForest package (v1.5; Supplementary Figure S4). Missing values in the data set were attributed to determinations that fell below the limit of quantitation during the analysis process. The inverse normal transformation, which generates a rank-based standard normal distribution (mean = 0, *SD* = 1), was applied to 611 metabolites, whereas HOMA-IR was log-transformed.

To identify a metabolic signature for HOMA-IR, we followed a 2-steps approach (20). First, we performed feature selection regressing HOMA-IR (log-transformed) on the 611 metabolites using linear regression with elastic net penalty to account for high dimensionality and multicollinearity of the metabolomics data. Second, we evaluated the prediction ability of the selected metabolites for HOMA-IR using support vector machine (SVM) and performance metrics.

During feature selection, we performed linear regression with elastic net penalties that are implemented in the "glmnet" R package (v4.1.6) combining the Lasso and Ridge penalties. We randomly split 90% of the data into training set and the remaining 10% sample into the test set. Then, we applied the model with 10-fold cross-validation (CV) in a 20-iteration loop on the training set and estimated the optimal value for alpha (α) from 0.1 to 1 in 0.05 increments as well as for the tuning parameter [λ (lambda)], and we computed the root-mean squared error (RMSE) in the test set. We selected the optimal values of alpha and lambda based on the combination that yielded a low RMSE in the test set (RMSE = 0.863217318743199). We applied the selected alpha (0.4) and lambda (0.122766218278957) values to each elastic net regression for every training set in a 100-iteration loop. We selected those features (metabolites) that were consistently selected in 60 or more iterations. In the second step with SVM approach, we used the tune function of the "e1071" R package (v1.7-3) in a 20-iteration loop to optimize the model and then employed the best parameters and a radial kernel to build the SVM model. The coefficients from SVM were applied to the metabolites as weights (positive or negative) to estimate the metabolic signature of log-transformed HOMA-IR as the weighted sum. For each selected metabolite, we calculated the mean coefficient and the 95% CI. To evaluate the performance of the metabolic signature in assessing HOMA-IR, we calculated the RMSE, the Pearson correlation and R^2 using a 10-fold CV approach.

For the association between HOMA-IR and the metabolic signature with AD-dementia risk, HOMA-IR and the signature were standardized by *z* score (mean = 0, *SD* = 1) before Cox regression analysis. Furthermore, missing values of covariates (see 2.5; Supplementary Figure S5) were imputed by applying multiple imputation by chained equations (MICE) (R package mice, v.3.16.0; Supplementary Figure S6). For our analyses of AD-dementia incidence, the time-to-event variable was the interval between the date of enrollment and the date of an AD-dementia event. We examined associations of HOMA-IR and the metabolic signature with AD-dementia risk fitting 3 multivariable Cox proportional hazards models to estimate hazard ratio (HR) and 95% confidence interval (CI). The first multivariable Cox regression model included age, sex, BMI, APOE ε4, education, smoking status, MMSE score; in the second multivariable Cox regression model, we further adjusted for the use of antihypertensive drugs, and lipid-lowering medication; in the third model, we further adjusted for HOMA-IR and metabolic signature simultaneously to examine association independence. To investigate whether the associations were independent of biological biomarkers of AD-dementia, we further adjusted for $A\beta_{1\text{-}42}$, p181-tau, and t-tau in a sensitivity analysis.

Mediation Analysis

The mediating role of the metabolic signature on the relationship between HOMA-IR and the risk of AD-dementia was tested under a counterfactual framework (21). The total effect of HOMA-IR on the risk of developing AD-dementia can be decomposed into 2 components: a natural direct effect (NDE) of HOMA-IR on AD-dementia risk, and a natural indirect effect (NIE) of HOMA-IR on AD-dementia accounted by the effect of the metabolic signature. The mediated proportion was calculated by dividing the natural indirect log-incident rate ratios (IRR) by the total effect log-IRR. The 95% confidence intervals were estimated by bootstrapping. The analysis was performed using Poisson Generalized Linear Models by splitting the follow-up time every time an event was observed, and thereafter we estimated the effects of the model parameters. We added a time term to the Poisson model to approximate more the Cox results because it is not implemented in the "medflex" package (v0.6-10). The same covariates that were incorporated into the multivariable Cox model 2 were used in the mediation analysis.

Metabolite Set Pathway Analysis

The online version of Metaboanalyst 5.0 (https://www.metaboanalyst.ca/) was used to explore the metabolic pathways in which the signature's metabolites were involved. The KEGG library, which consists of 84 reference metabolic pathways, was used as reference. The metabolites in the signature were assigned to their corresponding metabolic pathway and compared to the KEGG reference sets to identify overrepresented pathways, that is, if the group of metabolites in the signature occurred more frequently in a pathway than would be expected by chance. Pathways were considered statistically significant when p value < .05.

Results

Of the 400 participants with MCI included, 142 converted to AD-dementia during a follow-up of 2.12 years (interquartile range (IQR) = 1.10, 3.18; Table 1). AD converters were more likely to be older, have lower BMI, to be carriers of *APOE* ε4, had lower baseline scores of MMSE, take more medication, and had a shorter median follow-up period compared to non-converters. The correlation matrix of the 611 metabolites showed clustering patterns due to the correlations between metabolites (Supplementary Figure S7). Using elastic net regression analysis, 18 metabolites associated with IR were selected >60 times, 8 with negative coefficients, and 10 with positive coefficients (Supplementary Table S2). Final coefficients after SVM are shown in Figure 1 and Supplementary Table S3. The RMSE for this metabolic signature was 1.067 (95% CI = 1.030 to 1.103), the Pearson coefficient was

Table 1. Baseline Characteristics of the Study Participants

Variable	ACE Cohort		
	AD-Dementia Non-Converters	AD-Dementia Converters	*p* Value
n	258	142	—
Age (years)	70.7 (62.8, 76.4)	76.6 (72.3, 80.0)	<.001***
Women [*N* (%)]	139 (53.9)	76 (53.5)	.946
Body mass index (kg/m^2)	26.8 (24.2, 29.1)	25.8 (23.5, 28.5)	.045*
APOE ε4 carriers [*N* (%)]	61 (23.6)	78 (54.9)	<.001***
Education (years)	8 (6, 12)	8 (6, 10.8)	.065
Smoking [*N* (%)]			.166
Never	167 (64.7)	100 (70.4)	
Former	57 (22.1)	32 (22.5)	
Current	34 (13.2)	10 (7.0)	
MMSE at baseline (score)	27 (25, 29)	25 (23, 27)	<.001***
Medication [*N* (%)]			
Antihypertensives	105 (40.7)	73 (51.4)	.039*
Lipid-lowering	80 (31.0)	59 (41.5)	.034*
$A\beta_{1\text{-}42}$ (pg/mL)	10.0 (9.4, 10.3)	9.1 (8.8, 9.3)	<.001***
p181-Tau (pg/mL)	5.4 (5.0, 5.9)	6.6 (6.1, 7.0)	<.001***
t-Tau (pg/mL)	8.2 (7.7, 8.6)	9.1 (8.8, 9.6)	<.001***
Follow-up (years)	2.47 (1.3, 3.6)	1.2 (0.8, 2.2)	<.001***

Notes: AD = Alzheimer's disease; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination.
Continuous data are presented as median (interquartile range), and categorical variables are presented as number (%). The Mann–Whitney test was used for comparison of nonnormally distributed continuous variables, and the χ^2 test was used for comparison of categorical variables.
*$p < .05$, ***$p < .001$.

0.3433 (95% CI = 0.3430 to 0.3436, p value < .001), and the coefficient of determination was 0.1181 (95% CI = 0.1179 to 0.1183, p value < .001). The metabolic signature comprised several groups of metabolites including bile acids, acylcarnitines, CE, sphingomyelins, carbohydrates, medium-chain hydroxy acids, dicarboxylic acids, glycosphingolipids, sulfated steroids, eicosanoids, phosphatidylcholines, glycerophosphocholines, and phenols.

HOMA-IR was associated with a higher risk of AD-dementia (adjusted HR = 1.239; 95% CI = 1.039 to 1.477; p value = .017; Table 2). After further adjustment for the metabolic signature (Table 2) or CSF biomarkers of AD-dementia (Supplementary Table S4), these associations were attenuated and became insignificant. Instead, the metabolic signature was significantly associated with AD-dementia incidence (adjusted HR = 1.296; 95% CI = 1.084 to 1.549; p value = .004), even after further adjustment for HOMA-IR (HR = 1.234; 95% CI = 1.019 to 1.494; p value = .031; Table 2) or for CSF biomarkers of AD-dementia (HR = 1.242; 95% CI = 1.033 to 1.495; p value = .021; Supplementary Table S4).

The metabolic signature mediated 35% of the total effect of HOMA-IR on risk for AD-dementia (Figure 2). The NDE of IRR was 1.121 (95% CI = 0.927 to 1.381) and the NIE was 1.063.

Metabolic pathway analysis showed that the identified 18 metabolites were mainly related to 14 pathways, 4 of them with a p value less than .05; glycerophospholipid metabolism, tyrosine metabolism, ascorbate and aldarate metabolism, and arginine biosynthesis (Figure 3).

Discussion

In this prospective nested case-control study, we identified a plasma metabolic signature of IR associated with a higher risk of AD-dementia. Metabolites included in the signature are involved in metabolic pathways related to glucose/insulin metabolism and cognitive decline. Considering that metabolites, such as lipids, amino acids, and carbohydrates, reflect the downstream consequences of pathophysiological mechanisms driven by genes and proteins, the identified 18-metabolite signature provides novel information on the physiological mechanisms underlying the progression from MCI to AD-dementia.

Lipids play a crucial role in brain function because they preserve cell membrane architecture, participate in several signaling pathways, and contribute to the regulation of inflammation. In AD, depletion of structural lipids, such as phospholipids, is associated with atrophy and impaired signal transmission (22). Sphingolipids are a group of phospholipids that constitute the plasma membrane and regulate basic cellular functions, including senescence, inflammation, angiogenesis, or intracellular trafficking (23). Within the diverse group of sphingolipids, a number of studies have reported associations between higher circulating levels of hexosylceramides (HexCer), such as HexCer(d18:1/18:1) and HexCer(d18:1/24:1), and AD risk (24,25). In line with these results, plasma HexCer(d18:1/22:0) levels were increased in the signature obtained in the current study. Moreover, previous evidence showed increased levels of this particular HexCer in other neurodegenerative conditions such as Parkinson disease (26). In Zucker diabetic fatty rat and diet-induced obese mice, inhibition of hexosylceramide metabolism was associated with improved glucose tolerance and insulin sensitivity (27).

Regarding phospholipids, of particular interest are those lipids that play a role in glycerophospholipid metabolism, because the remodeling of glycerophospholipids in cell membranes negatively affects insulin signaling and decreases glucose tolerance in subjects with obesity, probably due to changing accessibility to insulin receptors or altering glucose transporter trafficking (28). The glycerophospholipid metabolism was the main pathway differentially regulated by our signature. Taking into account that the administration of lysophosphatidylcholine (lysoPC; 17:0) improved hyperglycemia and IR in high-fat diet mice (29), this might explain our findings that lower levels of LysoPC(1-18:3) and lysophosphatidylethanolamine (LysoPE; 16:0/0:0) are associated with IR, whereas the elevated levels of lysoPC(O-16:0) might probably be a counteracting mechanism in the initial stages of the disease. Disrupted lysoPC levels may also reflect impaired linoleic acid metabolism, because it is a constituent of neuronal membrane phospholipids (30). In a large longitudinal study with cognitively normal and MCI participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, a greater decrease of lysoPC and phosphatydilcholines (PC) PC(O) species was found in AD converters compared to non-converters (31). In addition, in a 5-year observational study with participants aged 70 years or older, reduced serum

Metabolite	β (95 CI)	Metabolite	β (95 CI)
Propionylcarnitine	-8.352 (-8.821, -7.883)	HexCer(d18:1/22:0)	8.970 (8.465, 9.475)
Taurodeoxycholic acid	-7.469 (8.024, -6.915)	Hexadecatrienoic acid	8.047 (7.459, 8.636)
LPE(16:0/0:0)	-5.548 (6.093, -5.003)	PC(O-16:0/16:0)	6.651 (6.130, 7.172)
1-18:3-LysoPC	-4.935 (-5.483, -4.388)	PC(P-16:0/16:1)	6.555 (6.113, 6.997)
12-HETE	-4.486 (-5.277, -3.695)	CE(17:1)	5.711 (5.065, 6.358)
Tauroursodeoxycholic acid	-3.993 (-4.548, -3.438)	Fumaric acid	5.167 (4.599, 5.734)
D-Xylose	-3.052 (-3.571, -2.532)	Vanillylmandelic acid	4.064 (3.550, 4.577)
Dihydroxyoctanoic acid	-2.613 (-3.146, 2.080)	Glucaric acid	2.976 (2.407, 3.545)
		Pipecolic acid	2.955 (2.453, 3.457)
		LPC(O-16:0)	0.977 (0.352, 1.602)

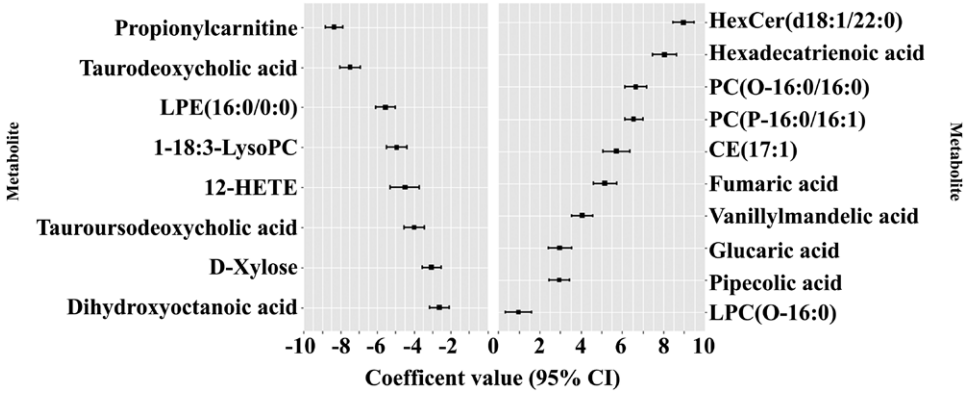

Figure 1. Metabolites ranked from the highest to the lowest positive and negative support vector machine regression coefficients for log HOMA-IR. On the left, the metabolites negatively associated; on the right, the metabolites positively associated with HOMA-IR. HOMA-IR = homeostasis model assessment for insulin resistance.

Table 2. Associations of HOMA-IR and Metabolic Signature With AD-Dementia Incidence

Analysis Model	HOMA-IR		Metabolic Signature	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Model 1	1.196 (1.013, 1.413)	.035*	1.194 (1.006, 1.417)	.042*
Model 2	1.239 (1.039, 1.477)	.017*	1.296 (1.084, 1.549)	.004**
Model 3	1.157 (0.958, 1.398)	.129	1.234 (1.019, 1.494)	.031*

Notes: AD = Alzheimer's disease; BMI = body mass index; CI = confidence interval; HOMA-IR = homeostasis model assessment for insulin resistance; HR = hazard ratio; MMSE = Mini-Mental State Examination.
Exposure contrast is per *z* score increase in the HOMA-IR and metabolic signature.
Model 1 included conventional risk factors (age, sex, BMI, APOE ε4, smoking status, education, and MMSE score); Model 2 further adjusted for the use of antihypertensive drugs, and lipid-lowering medication; Model 3 included both HOMA-IR and the metabolic signature of HOMA-IR simultaneously in Model 2 to examine independent associations from each other.
*$p < .05$, **$p < .01$.

Effect	Incident Rate Ratio (IRR)	Proportion mediated (%)
Total effect	1.192 (0.994, 1.452)	
Natural indirect effect	1.063 (1.006, 1.137)	34.79
Natural direct effect	1.121 (0.927, 1.381)	

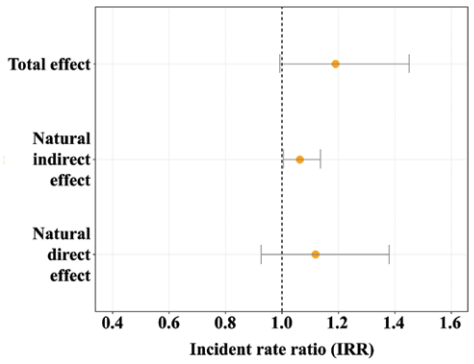

Figure 2. Medflex counterfactual mediation analysis for AD-dementia incident rate ratios (IRR) per 1 *SD* increment in HOMA-IR; proportion mediated by the metabolic signature with bootstrap 95% confidence intervals. AD = Alzheimer's disease; HOMA-IR = homeostasis model assessment for insulin resistance.

concentrations of PC in a 10-phospholipid biomarker signature predicted the conversion from healthy controls to amnestic MCI or AD with >90% accuracy (32). This is in line with the lower levels of lysoPC(1-18:3) and lysoPE(16:0/0:0) in the HOMA-IR signature in our study. Contrary to this, we observed higher levels of lysoPC(O-16:0), PC(O-16:0/16:0), and PC(P-16:0/16:1) in the signature. In a longitudinal study with 174 cognitively normal and mild cognitive adults without a diagnosis of dementia, lysoPC levels increased during normal aging and even more pronounced during AD conversion (33). Thus, our findings suggest that each glycerophospholipid may play a specific role in AD-dementia development. Further experimental studies are needed to elucidate how these molecules affect the development of AD-dementia.

Contrary to our results, 12-HETE was higher both in a model of obese and IR mice (34) and in CSF of patients with AD or MCI compared to aged-matched cognitively normal individuals (35). Levels of the fatty acyl 12-HETE have been suggested to follow an age-specific profile. In Tg2576 mice expressing APP and tau protein, 12-HETE concentration increased from 4 to 10 months of age and was then reduced at 15 months when compared to WT mice of the same ages. The fact that 12-HETE exerts anti-inflammatory action suggests that the concentration increases to protect against Aβ accumulation, but it later decreases as Aβ concentrations increase (36). This might explain the lower concentrations of 12-HETE associated with IR, and consequently with AD-dementia progression.

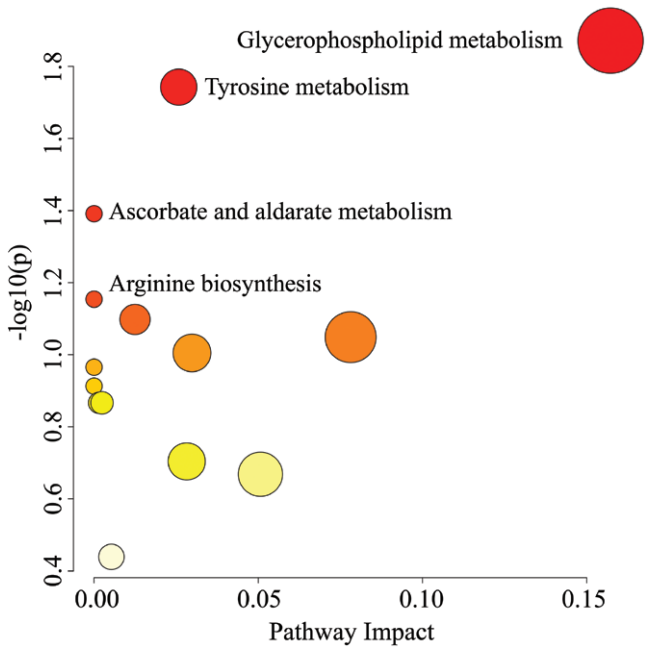

Figure 3. Metabolic pathway analysis. In red, the pathways with *p* value < .05.

Higher levels of propionylcarnitine (C3) have been associated with less Aβ accumulation and higher memory scores in MCI patients (37). Similarly, plasma levels of C3 were lower in patients who converted from MCI to AD compared to non-converters (32). In the same line, we have previously described a positive association between higher levels of short-chain acylcarnitines, including C3, and the risk of type 2 diabetes (T2D) (38). According to these previous findings, in our study, C3 was inversely associated with HOMA-IR, whereas lower C3 levels were associated with a higher AD-dementia risk, suggesting that it might be mediated by modulating amino acid metabolism, mitochondrial function, and fatty acid oxidation pathways (39).

Cholesteryl esters serve as the storage form of cholesterol, which is transported through the blood by lipoproteins and accumulate within cells. Consistent with our findings showing a positive association between CE and AD-dementia risk, CE were increased in plasma from APP/tau mouse models (36). CE delivered to the liver eventually undergoes conversion into bile acids (BA), including taurodeoxycholic acid (TDCA) and tauroursodeoxycholic acid (TUDCA) (40). BA such as TUDCA have been shown to enhance insulin sensitivity and clearance in obese mice and T2D mice (41). Importantly, TUDCA is able to cross the BBB, and it was demonstrated in a mouse model featuring APP/PS1 mutations that it could mitigate Aβ plaque accumulation, reduce synaptic loss, and ameliorate cognitive deficits (41). Similarly, a previous study from the AD Neuroimaging Initiative involving 1 562 subjects established that higher TDCA:cholic acid ratio in serum were associated with lower CSF $A\beta_{1\text{-}42}$ levels, hippocampal atrophy, and reduced FDG-PET uptake (42). In our study, TUDCA and TDCA were inversely associated with HOMA-IR, which suggests their role as potential mediators of IR on the progression to AD-dementia.

Because brain IR is commonly observed in AD, glucose uptake and energy supply are compromised. To deal with, the brain uses ketone bodies, which are synthesized from medium-chain fatty acids (MCFA), although some MCFA can also act directly in the brain (43). MCFA supplementation has demonstrated beneficial to enhance insulin sensitivity (44) and cognitive function (45). We found the MCFA dihydroxyoctanoic acid inversely associated with HOMA-IR suggesting it might be a mediator between IR and AD-dementia.

Observational studies have suggested that polyunsaturated fatty acids (PUFAs) may decrease IR (46), whereas their role in AD is controversial (47). We observed a positive association between the PUFA hexadecatrienoic acid and HOMA-IR. Thus, this PUFA might contribute to AD by other mechanisms than IR. Further research is needed to understand the role of PUFAs in HOMA-IR and AD-dementia.

We found D-xylose inversely associated with HOMA-IR and AD-dementia progression. In line with our findings, D-xylose supplementation has been related with improved glucose and insulin metabolism both in animals (48) and humans (49), supporting its antidiabetic effect. D-Xylose is an inhibitor of acetylcholinesterase (AChE), and AChE inhibitors have demonstrated neuroprotective effects, becoming interesting therapeutic targets for neurodegenerative diseases (50).

Higher plasma amino acids have been associated with IR. In a 7.4-year longitudinal study including 5 181 participants, tyrosine was associated with an increased risk of T2D and IR (51). In our study, the tyrosine metabolism pathway was especially overrepresented by the 18-metabolites signature. Both fumaric acid and vanillylmandelic acid (VMA), end products of the catecholamine catabolism, trigger IR (52,53), and subsequent cognitive decline (54). Accordingly, in our study, fumaric acid and VMA were positively associated with HOMA-IR and with a higher risk of AD-dementia. Instead, pipecolic acid, which has been effective in reducing fasting glucose in obese subjects (55) and found reduced in AD (56), in our study was positively associated with HOMA-IR. We could speculate that pipecolic acid levels might have increased in an effort to maintain glucose homeostasis.

Within the organic acids, glucaric acid has been shown in silico to have anti-inflammatory and antioxidant effects (57). We observed higher glucaric acid levels associated with HOMA-IR. It is plausible that glucaric acid increases during IR to prevent inflammation and oxidation, 2 hallmarks of IR and AD. Further research is needed to clarify this mechanism.

The main strengths of the present study include its prospective design, large sample size, and the targeted analysis of a wide range of metabolites providing a broader understanding of the metabolic processes related to IR and AD-dementia. Although this study lacks an independent validation cohort, several factors support the robustness and value of our metabolomic signature as is common in many exploratory studies (58–61). First, we implemented rigorous quality control and preprocessing procedures to ensure data reliability, complemented with robust statistical methods, including CV, to assess the reproducibility of the signature. Additionally, results of the pathway enrichment analysis revealed that the identified metabolites cluster into biologically relevant pathways consistent with established disease mechanisms. Our findings offer a hypothesis-generating foundation for future research and open avenues for external validation in subsequent studies. These novel insights into insulin-related metabolic alterations hold significant potential. However, validation across diverse populations and clinical settings is necessary before the signature can be considered a reliable clinical biomarker for guiding diagnosis, treatment, or disease monitoring in clinical

practice. Additional limitations should be acknowledged. A longer follow-up period to capture AD-dementia onset, along with the inclusion of cognitively healthy participants would be beneficial for a more comprehensive assessment of disease progression over time.

Conclusion

The present study identified a metabolic signature of HOMA-IR consisting of lipid species, amino acids, and carbohydrates, which was associated with progression from MCI to AD-dementia. Furthermore, we demonstrated that the effect of IR on the risk of AD-dementia was partially mediated by the signature. In addition to providing a link between IR and AD-dementia, we also provide potential biomarkers for risk assessment of AD-dementia in peripheral biological fluids. This insight paves the way for personalized intervention, including the design of lifestyle and/or pharmacological interventions aiming to improve the early metabolic dysregulations involved in AD-dementia development, but also in other insulin-related diseases or conditions. Moreover, this approach would facilitate following the evolution of the disease over time to reduce the risk of AD-dementia development.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

A.R. is a member of the scientific advisory board of Landsteiner Genmed and Grifols SA. A.R. has stocks in Landsteiner Genmed. He reports grants/research funding from Araclon and Grifols. The other authors declare no conflict.

Data Availability

Further data will be provided under request (M.Bu.).

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