

RESEARCH ARTICLE

Insulin resistance is related to cognitive decline but not change in CSF biomarkers of Alzheimer's disease in non-demented adults

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Abstract

Introduction: We investigated whether insulin resistance (IR) was associated with longitudinal age-related change in cognition and biomarkers of Alzheimer's disease (AD) pathology and neurodegeneration in middle-aged and older adults who were non-demented at baseline.

Methods: IR was measured with homeostatic model assessment of insulin resistance (HOMA2-IR). Core AD-related cerebrospinal fluid (CSF) biomarkers and cognition were assessed, respectively, on $n = 212$ (1 to 5 visits) and $n = 1299$ (1 to 6 visits). Linear mixed models tested whether HOMA2-IR moderated age-related change in CSF

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biomarkers and cognition. Linear regressions tested whether HOMA2-IR x apolipoprotein E ϵ 4 allele (*APOE* ϵ 4) carrier status predicted amyloid beta [$A\beta$] chronicity (estimated duration of amyloid positron emission tomography [PET] positivity) ($n = 253$).

Results: Higher HOMA2-IR was associated with greater cognitive decline but not with changes in CSF biomarkers. HOMA2-IR x *APOE*4 was not related to $A\beta$ chronicity but was significantly associated with CSF phosphorylated tau (P-tau)₁₈₁/ $A\beta$ ₄₂ level.

Discussion: In non-demented adults IR may not be directly associated with age-related change in AD biomarkers. Additional research is needed to determine mechanisms linking IR to cognitive decline.

KEYWORDS

*APOE*4, cognition, CSF biomarkers, insulin resistance, neurodegeneration, p-tau181, amyloid beta

1 | INTRODUCTION

Insulin resistance (IR) is a condition of reduced tissue sensitivity to the action of insulin and is often accompanied by hyperinsulinemia to control glucose levels.¹ IR increases risk for type 2 diabetes (T2D)¹ and has been related to worse episodic memory and executive function,^{2,3} cognitive domains affected by Alzheimer's disease (AD), and aging. In samples of older adults where the majority were not diabetic, IR was associated with an increased risk for Alzheimer's clinical syndrome.^{4,5}

Although potential molecular mechanisms that link IR to AD are unclear,⁶ some animal studies suggest that peripheral IR facilitates dysregulation of insulin signaling in the central nervous system, which in turn promotes tau hyperphosphorylation⁷ and synaptic dysfunction.^{8,9} Peripheral IR may also be related to amyloid beta ($A\beta$)₄₂ ($A\beta$ ₄₂) aggregation and/or accumulation through islet amyloid polypeptide (IAPP). Released by the pancreas with insulin, IAPP can be increased in IR and cross the blood-brain barrier.^{10,11} Misfolded forms of IAPP are hypothesized to act as a seed to activate the formation of $A\beta$ ₄₂ fibrils or slow the clearance of $A\beta$ ₄₂ protein from the brain.¹¹⁻¹⁴ In addition, hyperinsulinemia may cause substrate inhibition of insulin-degrading enzyme, one of the enzymes responsible for $A\beta$ degradation.¹⁵

Results from investigations relating IR to measures of AD pathology and neurodegeneration have been mixed. Although increased risk for neuritic plaques was predicted by 10-year antemortem IR,¹⁶ results examining the relationship between IR and $A\beta$ detected by Pittsburgh Compound B (PiB) positron emission tomography (PET) have been inconsistent.^{17,18} In addition, significant relationships have not been found between IR and $A\beta$ ₄₂ measured in cerebrospinal

fluid (CSF).^{2,19} In contrast, significant associations have been found between IR and elevated CSF phosphorylated tau₁₈₁ (P-tau₁₈₁)² and markers of neurodegeneration, specifically elevated CSF total tau (T-tau)^{2,19} and decreased cerebral glucose metabolism.²⁰ However, 10-year ante-mortem IR was not related to post-mortem tau tangle pathology,¹⁶ and IR has not been consistently related to decreased cerebral glucose metabolism.²¹ Several studies have been cross-sectional,^{2,18-21} thereby limiting the ability to investigate whether IR exacerbates aging-related changes in biomarkers of AD pathology and neurodegeneration.

We investigated if IR moderated aging-related change in biomarkers of AD pathology and neurodegeneration in a sample of middle-aged and older adults who were non-demented at baseline. We examined whether age-related declines in CSF $A\beta$ ₄₂/ $A\beta$ ₄₀ ratio and increases in CSF P-tau₁₈₁/ $A\beta$ ₄₂ ratio, P-tau₁₈₁, and markers of neurodegeneration (T-tau, neurogranin, and neurofilament light chain [NFL]) were worsened in individuals with higher IR. Given prior studies indicating an increased risk for dementia due to IR, we also examined whether individuals with higher IR had greater age-related decline in cognition.

In addition, we examined apolipoprotein E ϵ 4 allele (*APOE* ϵ 4) as a moderator of the relationship between IR and age-related change in CSF amyloid outcome variables. *APOE* ϵ 4 carriers have been found to accumulate amyloid sooner and possibly at a faster rate relative to non-carriers,²² and prior studies suggest a potential interaction between IR and *APOE* ϵ 4.¹⁶ Higher IR was related to increased odds of neuritic plaques in *APOE* ϵ 4 carriers relative to non-carriers.¹⁶ We also tested whether an interaction between IR and *APOE* ϵ 4 was related to $A\beta$ chronicity, a novel measure of the estimated length of time that an individual has been PiB-PET positive.²³

2 | METHOD

2.1 | Participants

Participants were enrolled in the Wisconsin Registry for Alzheimer's Prevention (WRAP), a longitudinal observational study of middle-aged and older adults who were non-demented at baseline.²⁴ The cohort is enriched for parental family history of AD; thus there is a relatively high proportion of APOE ϵ 4 carriers. Diagnosis of mild cognitive impairment and dementia was determined through National Institute on Aging–Alzheimer's Association (NIA-AA) criteria²⁵ and consensus conference.²⁴ Recruitment procedures have been described previously.²⁴ The University of Wisconsin (UW)–Madison Health Sciences Institutional Review Board approved WRAP; all participants provided written informed consent prior to enrollment.

The primary predictor in our study was IR; therefore, analyses focused on WRAP participants who had glucose and insulin values available from at least one visit ($n = 1384$), because both measures are required for calculating homeostatic model assessment of insulin resistance (HOMA2-IR).²⁶ Participants were excluded if they did not have any useable glucose and/or insulin values whether due to: (1) failure to fast and abstain from caffeine for a minimum of 8 hours ($n = 11$) or (2) treatment with insulin ($n = 21$), a confound for the measurement of HOMA2-IR. We excluded participants who did not have APOE ϵ 4 data ($n = 49$) so that APOE ϵ 4 carriership could be included in analyses. This resulted in a sample of $n = 1303$ participants. Three subsamples were selected based on availability of relevant outcomes, specifically CSF biomarkers, PiB PET for calculation of A β chronicity, and cognitive data (see Figure S1).

In the CSF biomarker subsample ($n = 211$), 15 (7.1%) met cut-off criteria for CSF amyloid and P-tau₁₈₁ positivity (see Section 2.3) at baseline; that number increased to 26 (12.3%) by study end. No participant in the CSF biomarker subsample converted to dementia by last lumbar puncture (LP). In the A β chronicity subsample ($n = 253$), 58 participants (22.9%) had a positive PiB-PET (see Section 2.2) at the time of their most recent PiB-PET. One participant in this subsample had dementia. In the cognitive subsample ($n = 1299$), four participants were diagnosed with dementia subsequent to first cognitive assessment. Sample characteristics for all three subsamples can be found in Table 1. Descriptive statistics of dependent variables and longitudinal follow-up data for CSF biomarker and cognitive subsamples can be found in Table 2.

2.2 | Procedures

2.2.1 | Blood collection and testing

Participants fasted and abstained from caffeine for a minimum of 8 hours prior to having their blood collected during a scheduled biennial WRAP visit. The majority (70.4%) of participants had blood collected and tested within the UW Hospital and Clinics at Madison. The remaining had their blood collected and tested at the Mayo Clinic

RESEARCH IN CONTEXT

- 1. Systematic review:** The authors reviewed the published literature on insulin resistance (IR) and Alzheimer's disease (AD). Although IR has been related to an increased risk of Alzheimer's clinical syndrome, results from studies associating IR with AD biomarkers have been mixed and largely limited by assessing outcomes once. We investigated the association of IR with longitudinal age-related change in cognition and biomarkers of AD and neurodegeneration in adults non-demented at baseline. Apolipoprotein E ϵ 4 allele (APOE ϵ 4) carrier status was tested as a moderator of the relationship between IR and amyloid beta (A β) measures.
- 2. Interpretation:** Our findings suggest that IR is associated with cognitive decline but not age-associated change in core AD-related cerebrospinal fluid (CSF) biomarkers in non-demented adults. APOE ϵ 4 status moderated the relationship between IR and one A β measure.
- 3. Future directions:** Mechanisms mediating the relationship between IR and cognitive decline, prior to dementia, require elucidation. Whether APOE ϵ 4 and IR interact to influence AD pathology warrants further study.

Health System in LaCrosse, WI (22.4%) or at Advocate Aurora Health in Milwaukee (7.2%). WRAP visit procedures have been described previously.²⁴

2.2.2 | CSF collection

Participants in the CSF biomarker subsample had one to five LPs (see Table 2 for count and percentage of participants with one to five LPs). Follow-up LPs were collected on average every 2.6 years (SD = 1.3) and were available in 55.7% of the participants. The mean follow-up period for these participants was 4.8 years. Participants fasted for 8–12 hours before receiving an LP in the morning for CSF collection using a Sprotte 24- or 25-gauge atraumatic spinal needle. Approximately 22 mL of CSF was collected through gentle extraction using polypropylene syringes and combined in a 30 mL polypropylene tube. Samples were gently mixed, centrifuged, and aliquoted into 1.5 mL polypropylene tubes. Aliquot tubes were stored at -80°C within 30 minutes of collection.

2.2.3 | PiB-PET imaging

Participants in the A β chronicity subsample underwent T1-weighted magnetic resonance imaging (MRI) for anatomical delineation and [C-11]PiB-PET for the quantification of cerebral A β . Details regarding

TABLE 1 Descriptive statistics of demographic, health characteristics, and study variables of participants

	CSF biomarker study ^a (n = 212) ^b	Amyloid beta chronicity study (n = 253)	Cognitive study ^a (n = 1299)
Age (y)	63.1 (6.6)	67.1 (6.1)	58.8 (6.5)
Sex (female)	138 (65.1%)	174 (68.8%)	915 (70.4%)
Race (White)	206 (97.6%)	242 (95.7%)	1215 (93.5%)
Education (y)	16.1 (2.1)	16.1 (2.2)	15.8 (2.2)
APOE ε4 carrier	74 (35.1%)	101 (39.9%)	505 (38.9%)
Systolic blood pressure (mm Hg)	126.9 (16.6)	125.3 (12.9)	124.7 (15.9)
Homeostatic model assessment of insulin resistance (HOMA2-IR)	1.1 (0.7)	within-person mean ^c : 1.1 (0.7) within-person SD ^c : 0.3 (0.3)	1.2 (0.9)
Pre-diabetes ^d	53 (25.0%)	61 (24.1%)	324 (24.9%)
Diabetes ^d	10 (4.7%)	26 (10.3%)	78 (6.0%)
Mild cognitive impairment (MCI) ^e	6 (2.8%)	12 (4.7%)	17 (1.3%)
Cognitive impairment (not MCI) ^e	0 (0.0%)	2 (0.8%)	12 (0.9%)
Pittsburgh Compound B (PiB) PET			
PiB positivity ^f		58 (22.9%)	
PiB chronicity ^g (y)		PiB pos = 9.7 (7.9) PiB neg = -18.4 (6.3)	

Data presented are means (SD) or counts (%)

^aData represent values collected at time of baseline HOMA2-IR.

^bn = 212 had CSF neurodegeneration biomarkers; n = 211 had CSF AD pathology biomarkers.

^cAverage of within-person mean and SD of 3-6 HOMA2-IR values.

^dPre-diabetes defined as fasting glucose between 100 and 125 mg/dL (American Diabetes Association [ADA], 2010); Diabetes defined as self-report of taking oral antidiabetic medication or, if no self-report, fasting glucose \geq 126 mg/dL (ADA, 2010).

^eDiagnosed using NIA-AA criteria (McKhann et al., 2011) and consensus conference (Johnson et al., 2018).

^fPiB positivity: global PiB DVR $>$ 1.19 at participant's most recent PiB-PET.

^gPositive values = estimated years of PiB-PET positivity. |Negative values| = estimated years until PiB positivity or estimated life expectancy for those with no evidence of PiB accumulation.

radioligand synthesis, image acquisition, processing, and analysis of MRI and PiB-PET images have been described previously.²⁷ Amyloid burden was quantified as the average cortical PiB distribution volume ratio (DVR; Logan graphical analysis, cerebellum gray matter reference region) using dynamic PiB data acquired 0-70 minutes post-injection with either a Siemens Biograph Horizon PET/CT or Siemens EXACT HR+ tomograph. MRI and PET image processing and quality control were performed using a pipeline that uses MATLAB (The Mathworks, Inc., Natick, MA) and SPM12 (www.fil.ion.ucl.ac.uk/spm). The cut-point for PiB positivity was a global DVR of 1.19.²⁸

2.2.4 | Cognitive testing

Participants completed cognitive tests during biennial WRAP visits. In the cognitive subsample, participants had one to six cognitive assessments (see Table 2 for count and percentage of participants with one to six cognitive assessments). Follow-up cognitive testing occurred on average every 2.6 years (SD = 0.5) and was available in 91.4% of the participants. The mean follow-up period for these participants was 7.1 years.

2.3 | Measures

2.3.1 | IR

IR was measured using HOMA2-IR (i.e., the computer model),²⁶ which was calculated by entering fasting glucose and insulin into the HOMA calculator version 2.2.3 (University of Oxford, 2013: <https://www.dtu.ox.ac.uk/homacalculator/>). HOMA2-IR provides an indication of IR in the fasting or basal state, with higher values reflecting higher IR. It has been shown to correlate strongly with clamp-derived whole-body insulin sensitivity.²⁶ Although there is no reference range, a level of 1.0 is thought to approximate normal.²⁶ In the study with the largest number of participants (ie, cognitive study), the average HOMA2-IR in non-diabetics was 1.1 (SD = 0.8) and in diabetics (defined as self-report of taking oral antidiabetic medication or, if no self-report, fasting glucose \geq 126 mg/dL²⁹) the average level was 2.4 (SD = 1.5).

HOMA2-IR values were calculated from insulin and glucose collected together closest in time to outcome data. For the CSF biomarker study, blood for HOMA2-IR was collected within \pm 1 year (mean = 0.25, SD = 0.39) of the baseline LP. In the cognitive study, blood for HOMA2-IR was collected at the same visit as the earliest available cognitive

TABLE 2 Descriptive statistics of dependent variables for cerebrospinal fluid (CSF) biomarker and cognitive study

CSF biomarker study (n = 212) ¹			
1-5 Lumbar Punctures (LPs) collected			
	Participant		
Number of LPs:	count (%):		
1	94 (44.3%)		
2	41 (19.3%)		
3	45 (21.2%)		
4	26 (12.3%)		
5	6 (2.8%)		
LP collection period (years) for subsample with >1 LP:			
Mean = 4.8, range 1–8.2			
CSF biomarker means (SD) at baseline and last visit			
	Baseline	Last visit	
Aβ ₄₂ /Aβ ₄₀ ratio	Raw	.06 (.02)	.06 (.02)
	Raw	.02 (.02)	.03 (.02)
P-tau ₁₈₁ /Aβ ₄₂ ratio	Ln	−3.9 (0.5)	−3.8 (0.6)
	Raw	18.1 (6.8)	19.1 (7.4)
P-tau ₁₈₁ (pg/mL)	Ln	2.8 (0.4)	2.9 (0.4)
	Raw	206.7 (72.3)	215.3 (76.5)
T-tau (pg/mL)	Ln	5.3 (0.3)	5.3 (0.4)
	Raw	829.1 (330.4)	850.5 (336.5)
Neurogranin (pg/mL)	Ln	6.6 (0.4)	6.7 (0.4)
	Raw	94.2 (50.2)	105.5 (64.1)
NfL (pg/mL)	Ln	4.5 (0.4)	4.6 (0.4)
Amyloid and P-tau status ^b : count (%) at baseline and last visit			
	Baseline	Last visit	
Amyloid+/P-Tau+	15 (7.1%)	26 (12.3%)	
Amyloid+/P-Tau-	27 (12.8%)	26 (12.3%)	
Amyloid-/P-Tau+	11 (5.2%)	12 (5.7%)	
Amyloid-/P-Tau-	158 (74.9%)	147 (69.7%)	
Cognitive study (n = 1299)			
1-6 PACC-3 tests			
	Participant		
Number of PACC-3 tests:	count (%):		
1	112 (8.6%)		
2	151 (11.6%)		
3	270 (20.8%)		
4	412 (31.7%)		
5	351 (27.0%)		
6	3 (0.2%)		
PACC-3 collection period (years) for subsample with >1 PACC-3 tests:			
Mean = 7.1, range 1.3-12.3			

(Continues)

TABLE 2 (Continued)

Cognitive performance means (SD) at baseline and last visit		
PACC-3	Baseline	Last visit
	.006 (0.8)	−.09 (0.8)

Abbreviations: Aβ: amyloid beta; NFL: neurofilament light chain; PACC-3: Preclinical Alzheimer's Cognitive Composite (three-test version); P-tau: phosphorylated tau; T-tau: total tau.

^an = 212 had CSF neurodegeneration biomarkers; n = 211 had CSF AD pathology biomarkers.

^bAmyloid+: CSF Aβ₄₂/Aβ₄₀ ratio ≤.046; P-Tau+: CSF P-Tau₁₈₁ ≥24.8 pg/mL.

assessment in the longitudinal series. In the Aβ chronicity study, a within-person HOMA2-IR mean and SD, calculated using three or more values out of all available HOMA2-IR, was assessed in order to relate chronic exposure and variability in IR to Aβ chronicity. Timeframe of collection for HOMA2-IR values used in the Aβ chronicity study was 8.2 years (SD = 2.0).

2.3.2 | Apolipoprotein E ε4 (APOE ε4)

APOE ε4 was genotyped using competitive allele-specific polymerase chain reaction (PCR)-based KASP genotyping assays for rs7412 and rs429358 (LGC Genomics, Beverly, MA). Individuals with one or two C alleles for rs429358 were coded as APOE ε4 carriers.

2.3.3 | CSF biomarkers of AD pathology and neurodegeneration

CSF biomarkers of AD pathology included Aβ₄₂/Aβ₄₀ ratio, P-tau₁₈₁/Aβ₄₂ ratio, and P-tau₁₈₁. CSF biomarkers of neurodegeneration included T-tau and markers of synaptic and axonal degeneration, neurogranin, and NfL, respectively. CSF biomarker levels were measured with the Roche NeuroToolKit panel using either the Elecsys β-Amyloid (1-42), Total-Tau, and Phospho-Tau (181P) CSF immunoassays, or robust prototype assays (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). T-tau, P-tau₁₈₁, and Aβ₄₂ were assayed on the cobas e 601; Aβ₄₀, neurogranin, and NfL were assayed on the cobas e 411. All analyses were performed at the Clinical Neurochemistry Laboratory at the University of Gothenburg. Previously determined thresholds for amyloid (Aβ₄₂/Aβ₄₀) and P-tau₁₈₁ positivity were used to describe the sample.³⁰

2.3.4 | Amyloid beta chronicity

Aβ chronicity was defined as the estimated time in years that an individual had been PiB positive at the time of last HOMA2-IR assessment. It was calculated as the age at last HOMA2-IR minus the estimated age at PiB positivity. To avoid missing values for individuals with no evidence of PiB accumulation, estimated age at PiB positivity was

estimated as age at PET scan plus life expectancy from a sex-specific life-expectancy table. Information regarding the estimation of the age at PiB positivity and amyloid chronicity has been described previously.²³ Previous findings indicate that A β chronicity is a valid predictor of core AD-related outcomes. Higher A β chronicity was significantly associated with greater odds of mild cognitive impairment/AD dementia (defined by consensus conference) and tau burden in the entorhinal cortex (assessed by MK-6240 PET).²³

2.3.5 | Preclinical Alzheimer's cognitive composite (PACC-3)

A modified preclinical Alzheimer's cognitive composite³¹ was derived from the Rey Auditory Verbal Learning (RAVLT; Trials 1-5),³² Logical Memory II,³³ and Digit Symbol Substitution.³⁴ Scores were standardized using the mean from first cognitive assessment of cognitively unimpaired participants. Details on the derivation of this metric have been described previously.³¹

2.4 | Statistical approach

Primary, sensitivity, and exploratory analyses for each study are discussed first prior to a discussion of secondary analyses and model fit and assumptions applicable to more than one study. Covariates included age, APOE ϵ 4 carriership, sex, and education (a social determinant of health). Systolic blood pressure was also controlled because hypertension has been associated with IR³⁵ and at mid-life increases dementia risk.³⁶

2.4.1 | CSF biomarker study

Primary analyses: Linear mixed effects (LMEs) tested an Age x HOMA2-IR interaction to determine whether HOMA2-IR moderated age-associated change in CSF biomarkers of AD pathology and neurodegeneration. Age at each LP visit was centered using average age at baseline LP. **Exploratory analyses:** Because APOE ϵ 4 has been shown to influence amyloid accumulation,²² LME tested an Age x HOMA2-IR x APOE ϵ 4 interaction to explore whether HOMA2-IR interacted with APOE ϵ 4 carrier status (ϵ 4 carrier vs non-carrier) to predict age-related change in CSF A β_{42} /A β_{40} and P-tau₁₈₁/A β_{42} ratios. If the three-way interaction was not significant, the lower-order two-way interactions were subsequently tested.

2.4.2 | Amyloid beta chronicity study

Primary analyses: Multiple linear regression was used to test whether chronic exposure and variability in IR interacted with APOE ϵ 4 carrier status to predict A β chronicity at age of last HOMA2-IR. Within-person HOMA2-IR means and SDs were used to measure chronic

exposure and variability in IR, respectively. Age at last HOMA2-IR was controlled. **Sensitivity analyses** were also conducted using PiB positivity as an outcome in two binary logistic regressions where HOMA2-IR (within-person -mean or -SD) x APOE ϵ 4 was a predictor. PiB positivity was determined from the participant's most recent PiB-PET. HOMA2-IR within-person mean and SD were calculated using a minimum of three HOMA2-IR values, with the last value being collected within an average of 1.0 year (SD = 1.1) prior to the most recent PiB-PET (n = 219). Age at most recent PiB-PET was controlled.

2.4.3 | Cognitive study

Primary analyses: LME was used to examine whether baseline HOMA2-IR moderated age-related decline in PACC-3 scores. Age at each visit was centered using average age at baseline PACC-3. **Exploratory analyses:** LME tested an Age x HOMA2-IR x APOE ϵ 4 interaction to explore whether HOMA2-IR interacted with APOE ϵ 4 carrier status to predict age-related change in PACC-3 scores. If the three-way interaction was not significant, the lower-order two-way interactions were subsequently tested.

2.4.4 | All studies

Secondary analyses: Simple slopes analysis was performed if an interaction was significant. If Age x HOMA2-IR in the cognitive and CSF biomarker study or HOMA2-IR x APOE4 in the A β chronicity study was not significant, the main effect of HOMA2-IR was tested without the interaction term.

Model fit and assumptions: Natural log transformation of CSF biomarkers, except for A β_{42} /A β_{40} , and log10 transformation of A β chronicity were necessary to meet the homogeneity of variance assumption. Convergence criteria were not met for a random intercept plus age slope model when CSF A β_{42} /A β_{40} and CSF ln(P-tau₁₈₁/A β_{42}) were outcomes; thus a random intercept only model was used instead. Random intercept plus age slope models were used for remaining analyses in the CSF biomarker and cognitive studies. HOMA2-IR values were transformed to z-scores using the HOMA2-IR mean and 1 SD unique to each study. A P < .05, uncorrected, was interpreted as significant.

3 | RESULTS

3.1 | CSF biomarker study

Primary analyses: When controlling for covariates and at the mean of HOMA2-IR, age was significantly related to decreased A β_{42} /A β_{40} and increased ln(P-tau₁₈₁/A β_{42}), ln(P-tau₁₈₁), ln(T-tau), ln(NfL), and ln(neurogranin) (Table 3). HOMA2-IR did not moderate the relationship of age to CSF biomarkers (Table 3 and Figure 1). **Secondary analyses:** Without the Age x HOMA2-IR interaction term, HOMA2-IR

TABLE 3 Results from linear mixed-effects models investigating homeostatic model assessment of insulin resistance (HOMA2-IR) as a moderator of the relationship between age and CSF biomarker outcomes

(a) CSF biomarkers of AD pathology (n = 211)									
	A β ₄₂ /A β ₄₀ ratio			Ln(P-tau ₁₈₁ /A β ₄₂ ratio)			Ln(P-tau ₁₈₁)		
	β	P	95% CI	β	P	95% CI	β	P	95% CI
Intercept	.08	<.0001	.06 to .10	-4.46	<.0001	-5.0 to -3.9	2.74	<.0001	2.38 to 3.10
Sex (0 = female)	.0005	.82	-.004 to .005	-.01	.86	-.15 to .13	-.03	.55	-.13 to .07
Education (y)	-.0009	.09	-.002 to .0002	.03	.07	-.002 to .06	.006	.62	-.02 to .03
Systolic blood pressure (z-scores)	-.0006	.57	-.003 to .002	-.01	.69	-.08 to .05	-.006	.82	-.05 to .04
APOE ϵ 4 status (0 = non-carrier)	-.01	<.0001	-.02 to -.007	.28	<.0001	.15 to .42	.03	.50	-.06 to .13
Age (y)	-.0006	<.0001	-.0009 to -.00	.02	<.0001	.02 to .03	.02	<.0001	.01 to .02
HOMA2-IR (z-scores)	.00008	0.94	-.002 to .002	.001	.97	-.06 to .06	.03	.14	-.01 to .08
Age x HOMA2-IR (z-scores)	.0001	.31	-.0001 to .0001	-.001	.67	-.008 to .005	-.0007	.71	-.005 to .003
(b) CSF biomarkers of neurodegeneration (n = 212)									
	ln(T-tau)			ln(NfL)			ln(neurogranin)		
	β	P	95% CI	β	P	95% CI	β	P	95% CI
Intercept	5.14	<.0001	4.80 to 5.49	4.29	<.0001	3.98 to 4.61	6.68	<.0001	6.27 to 7.09
Sex (0 = female)	-.03	.53	-.13 to .06	.10	.02	.01 to .19	-.09	.10	-.20 to .02
Education (y)	.008	.47	-.01 to .03	.009	.37	-.01 to .03	-.0008	.95	-.03 to .02
Systolic blood pressure (z-scores)	-.003	.89	-.05 to .04	.02	.46	-.03 to .06	-.002	.93	-.05 to .05
APOE ϵ 4 status (0 = non-carrier)	.04	.45	-.06 to .13	-.06	.14	-.15 to .02	.02	.71	-.09 to .13
Age (y)	.01	<.0001	.01 to .02	.03	<.0001	.03 to .04	.01	<.0001	.006 to .02
HOMA2-IR (z-scores)	.03	.12	-.009 to .07	.02	.27	-.02 to .06	.04	.15	-.01 to .08
Age x HOMA2-IR (z-scores)	-.0006	.74	-.004 to .003	-.004	.13	-.009 to .001	-.002	.42	-.007 to .003

Notes: Random intercept model was used for CSF amyloid outcomes (A β ₄₂/A β ₄₀ ratio and Ln(P-tau₁₈₁/A β ₄₂ ratio); random intercept and age slope model was used for remaining CSF biomarker outcomes. Age was centered using baseline age in all models.

Abbreviations: A β = amyloid beta; CI = confidence interval; NfL = neurofilament light chain; P-tau = phosphorylated tau; T-tau = total tau.

did not explain significant variability in any CSF outcome (Table S1). **Exploratory analyses:** The Age x HOMA2-IR x APOE ϵ 4 interaction was not a significant predictor of A β ₄₂/A β ₄₀ or Ln(P-tau₁₈₁/A β ₄₂); however, the lower order HOMA2-IR x APOE ϵ 4 interaction was significantly associated with Ln(P-tau₁₈₁/A β ₄₂) in the model with the three-way interaction term. Thus HOMA2-IR x APOE ϵ 4 was tested, along with Age x HOMA2-IR and Age x APOE ϵ 4 as a predictor of Ln(P-tau₁₈₁/A β ₄₂) without the three-way interaction. In the reduced model, HOMA2-IR x APOE ϵ 4 was significant, Age x HOMA2-IR was not significant, and as expected,²² Age x APOE ϵ 4 was significant. When tested without the Age x HOMA2-IR interaction and when controlling for Age x APOE ϵ 4, HOMA2-IR x APOE ϵ 4 was a significant predictor of Ln(P-tau₁₈₁/A β ₄₂) (Table 4). Simple slopes analysis of this interaction suggested that for every 1 SD increase in HOMA2-IR, Ln(P-tau₁₈₁/A β ₄₂) was .10 higher in APOE ϵ 4 carriers ($P = .06$) compared with .04 lower ($P = .24$) in non-carriers (Figure 2).

3.2 | Amyloid beta chronicity study

Primary analyses: HOMA2-IR x APOE ϵ 4 was not a significant predictor of A β chronicity (Table S2). **Secondary analyses:** In multiple linear

regression models without the HOMA2-IR x APOE ϵ 4 interaction term, within-person HOMA2-IR mean and SD were not significant predictors of A β chronicity (Table S2). **Sensitivity analyses:** HOMA2-IR x APOE ϵ 4 was not a significant predictor of PiB positivity (Table S2).

3.3 | Cognitive study

Primary analyses: Baseline HOMA2-IR was a significant moderator of age-related decline in PACC-3 scores when controlling for sex, education, APOE ϵ 4 carrier status, and baseline systolic blood pressure (Table 4 and Figure 1). Participants with higher baseline HOMA2-IR experienced faster cognitive decline than participants with lower HOMA2-IR. Simple slopes for age at 1 SD above and below the HOMA2-IR mean were significant ($P < .0001$) and equaled $-.024$ and $-.017$, respectively. The Age x HOMA2-IR interaction remained significant ($\beta = -.004$, $P = .02$) when controlling for diabetes at baseline and after removal of four participants who subsequently developed dementia. **Exploratory analyses:** The Age x HOMA2-IR x APOE ϵ 4 interaction was not significantly associated with PACC-3 scores, indicating that the relationship between Age x HOMA2-IR and cognition was not significantly moderated by APOE ϵ 4 carrier status. In the model without the

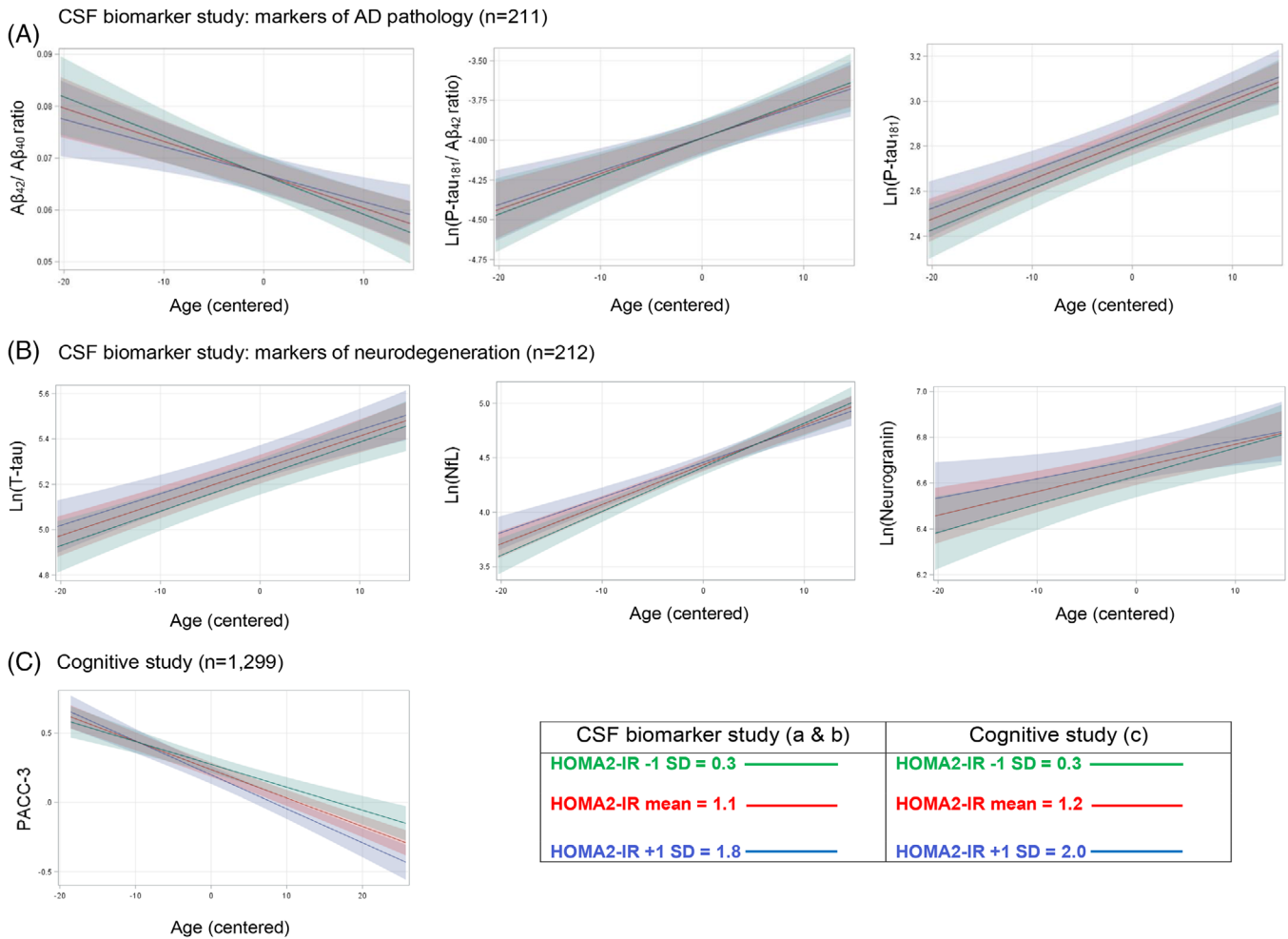


FIGURE 1 Graphs demonstrating relationship between Age x HOMA2-IR and CSF biomarker (A and B) and cognitive (C) outcomes determined by linear mixed effects models. Age was centered at average baseline age. Notes: Random intercept model was used for CSF amyloid outcomes ($A\beta_{42}/A\beta_{40}$ ratio and $\ln(P\text{-tau}_{181}/A\beta_{42}$ ratio); random intercept and age slope model was used for remaining CSF biomarker outcomes. The Age x HOMA2-IR interaction was significantly related to PACC-3 but not to any outcome in the CSF biomarker study. $A\beta$ = amyloid beta; HOMA2-IR = homeostatic model assessment of insulin resistance; NfL = neurofilament light chain; PACC-3 = Preclinical Alzheimer's Cognitive Composite (version 3); P-tau = phosphorylated tau; T-tau = total tau

three-way interaction term, the Age x HOMA2-IR interaction was significant ($\beta = -.004$, $P = .02$) when controlling for HOMA2-IR x APOE $\epsilon 4$ ($\beta = .04$, $P = .24$) and Age x APOE $\epsilon 4$ ($\beta = -.009$, $P = .005$) as well as sex, education, and baseline systolic blood pressure.

4 | DISCUSSION

Using longitudinal data from predominantly healthy, non-demented middle-aged and older adults, we found that IR was related to age-related change in cognition but not biomarkers of AD pathology. Specifically, higher IR was related to worse age-associated decline in PACC-3 scores. In the early stages of the AD continuum, IR may facilitate cognitive decline through mechanisms that are independent of AD pathology, similar to T2D, which has also been related to increased risk for Alzheimer's clinical syndrome but not post-mortem amyloid plaque and tau tangle pathology.³⁷

Previous studies indicate that IR increases risk for T2D,¹ which in turn increases risk for cerebrovascular disease, cerebral infarct, and subsequent cognitive dysfunction.³⁷ Because our sample was generally healthy, the relationship between IR and cognitive decline observed may have been due to cerebrovascular changes occurring prior to or independent of infarct development. Indeed, in the absence of brain infarction, IR and T2D have been associated with alterations in cerebrovascular as well as neural function that potentially worsen cognitive health. For example, neurovascular coupling (ie, the regulation of blood flow in response to neural activity) was found to be altered in people with T2D, with no evidence of brain infarct or vascular lesion.³⁸ IR has been related to lower cerebral arterial blood flow and microvessel perfusion.^{39,40} IR-associated hypoperfusion has been related to cognitive deficits in patients with T2D.³⁹ In predominantly stroke-free participants with T2D, IR was related to white matter hyperintensity severity and cognitive dysfunction.⁴¹ In a mouse model of IR, increased blood-brain barrier permeability,

TABLE 4 (a) Results from linear mixed effects model^a (LME) testing APOE ϵ 4 status as a moderator of the relationship between homeostatic model assessment of insulin resistance (HOMA2-IR) and CSF $\ln(\text{P-tau}_{181}/\text{A}\beta_{42})$ ratio ($n = 211$), and (b) results from LME^b testing HOMA2-IR as a moderator of age-related change in the preclinical Alzheimer's cognitive composite (PACC-3) ($n = 1299$)

(a) CSF biomarker study outcome: $\ln(\text{P-tau}_{181}/\text{A}\beta_{42})$ ratio			
	β	P	95% CI
Intercept	-4.50	<.0001	-5.00 to -4.00
Sex (0 = female)	-.003	.97	-.14 to .14
Education (y)	.03	.04	.002 to .06
Systolic blood pressure (z-scores)	-.009	.78	-.07 to .06
APOE ϵ 4 status (0 = non-carrier)	.25	.0003	.12 to .39
Age (y)	.01	.001	.005 to .02
HOMA2-IR (z-scores)	-.04	.24	-.12 to .03
Age x APOE ϵ 4	.02	.002	.009 to .04
HOMA2-IR x APOE ϵ 4	.14	.03	.02 to .27
(b) Cognitive study outcome: PACC-3			
	β	P	95% CI
Intercept	-1.23	<.0001	-1.49 to -.97
Sex (0 = female)	-.51	<.0001	-.59 to -.43
Education (y)	.09	<.0001	.08 to .11
Systolic blood pressure (z-scores)	-.02	.39	-.05 to .02
APOE ϵ 4 status (0 = non-carrier)	-.09	.02	-.16 to -.02
Age (y)	-.02	<.0001	-.023 to -.017
HOMA2-IR (z-scores)	-.04	.05	-.07 to -.0006
Age x HOMA2-IR	-.004	.02	-.007 to -.0006

Abbreviations: CI = confidence interval.

^aRandom intercept model when $\ln(\text{P-tau}_{181}/\text{A}\beta_{42})$ ratio was outcome.

^bRandom intercept and age slope model when PACC-3 was outcome. Age was centered at average baseline age.

concurrent with neuroinflammation, was associated with subsequent cognitive decline and neurodegeneration.⁴² Peripheral IR has been linked in some animal studies to central dysregulation of insulin signaling^{7,43} and subsequent abnormalities in synaptic function.^{8,9} In sum, multiple IR-associated changes, such as hypoperfusion, white matter hyperintensities, increased blood-brain barrier permeability, neuroinflammation, and dysregulation of central insulin signaling, may have contributed to cognitive decline in our participants.

IR has been associated with neurodegeneration: specifically, brain atrophy,⁴⁴ decreased cerebral glucose metabolism²⁰ and increased CSF T-tau.^{2,19} In our sample, IR did not predict age-associated increase of the CSF concentrations of T-tau, NFL, or neurogranin, markers of axonal and synaptic dysfunction/degeneration, respectively. Thus even though higher IR was related to worse age-related decline in cognitive performance, it was not similarly associated with age-associated increases in CSF biomarkers of neurodegeneration. We speculate that potential IR-related effects on cerebrovascular and neural function may not yet have resulted in detectable neurodegeneration given that participants were non-demented.

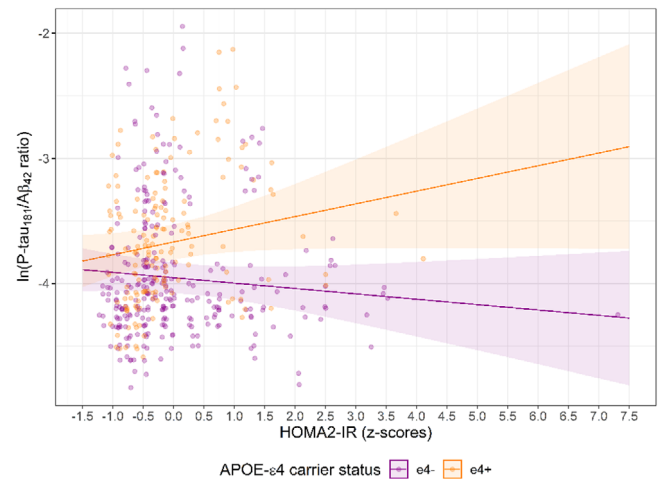


FIGURE 2 Scatterplot of HOMA2-IR x APOE ϵ 4 relationship with $\ln(\text{P-tau}_{181}/\text{A}\beta_{42})$ ratio determined by a random intercept model. Notes: HOMA2-IR mean = 1.1 (SD = 0.7); HOMA2-IR X APOE ϵ 4 interaction was significant and in same direction after removal of single case with extreme HOMA2-IR z-score (7.3), $\beta = .15$, $P = .04$, 95% CI = .008 to .28; A β = beta amyloid; HOMA2-IR = homeostatic model assessment of insulin resistance; P-tau = phosphorylated tau

We did not find a significant association between IR and the average level of CSF P-tau₁₈₁ in contrast with a previous cross-sectional study.² Differences in study design and sample characteristics may have contributed to the contrasting results. Our sample was younger on average and had more APOE ϵ 4 carriers. Whether the relationship between IR and P-tau₁₈₁ is manifested in cognitively unimpaired adults older than the participants that we studied deserves further investigation. Some research suggests that peripheral IR facilitates the dysregulation of neuronal insulin signaling kinases that mediate tau hyperphosphorylation.⁷ The potential interrelationship between central and peripheral IR requires further research⁴⁵ because it is unclear whether the central IR found in AD⁴⁶ precedes tau hyperphosphorylation.^{46,47}

APOE ϵ 4 moderated the relationship between IR and average level of P-tau₁₈₁/A β ₄₂ ratio. Results suggested that as IR increased, APOE ϵ 4 carriers had greater P-tau₁₈₁/A β ₄₂ than non-carriers. Similar moderating effects of APOE ϵ 4 have been found in some past research examining IR and biomarkers of AD pathology.¹⁶ Both insulin and A β ₄₂ are substrates for proteolytic degradation by insulin-degrading enzyme (IDE),¹⁵ a protein found to be lower in AD dementia cases who were APOE ϵ 4 carriers.⁴⁸ Elevated insulin associated with IR may reduce clearance of A β ₄₂ through competitive inhibition for degradation by IDE¹⁵ to a greater extent in APOE ϵ 4 carriers. Deficiency of IDE may also increase the IDE substrate IAPP,⁴⁹ whose misfolded forms may facilitate formation of A β ₄₂ fibrils or slow clearance of the protein.^{11,13,14}

In contrast to results for CSF P-tau₁₈₁/A β ₄₂, APOE ϵ 4 was not a significant moderator of the relationship between IR and A β chronicity or PiB positivity. Increases in CSF P-tau₁₈₁/A β ₄₂ may occur prior to A β detectable by PiB-PET.⁵⁰ However, because P-tau pathology is hypothesized to follow early A β accumulation,⁵¹ pathological increases

in CSF P-tau₁₈₁/Aβ₄₂ should correspond with prolonged duration of amyloid PET positivity. Indeed, Aβ chronicity was related in another study to higher entorhinal tau.²³ Further research is needed to investigate whether higher IR facilitates increased AD pathology in APOE ε4 carriers.

Our study has several strengths and limitations. Because we had longitudinal CSF core AD biomarker and cognitive data, we were able to assess age-related change in important variables related to AD. We had multiple measures of HOMA2-IR collected over several years, which allowed us to relate chronic exposure and variability in insulin sensitivity to a novel measure, Aβ chronicity. However, due to the correlational nature of our study, causative claims cannot be made. Although we controlled for potential confounds, we acknowledge the possibility that unmeasured factors may have influenced associations. The effect size for IR on cognition in our sample was modest. Simple slopes for age at 1 SD above and below the HOMA2-IR mean were $-.024$ and $-.017$ respectively, demonstrating that the difference between the two slopes, although statistically significant, was small. Due to sample size constraints, we may not have had adequate power to detect a similar small effect of IR on CSF biomarkers. Furthermore, there were few participants in the CSF biomarker study who were both amyloid and P-tau₁₈₁ positive at baseline ($n = 15$) and by study end ($n = 26$), indicating that most participants did not have AD as defined biologically. Nevertheless, there were 24.6% ($n = 52$) who were amyloid positive, and we did demonstrate that CSF amyloid and P-tau₁₈₁ changed in the expected direction with aging. The association between IR and biomarkers of AD pathology will be examined again after more participants within our cohort develop AD.

In conclusion, higher IR was associated with worse cognitive decline but not longitudinal age-related change in CSF biomarkers of AD pathology in non-demented adults. IR may not be related to change in amyloid and tau in the early stages of the AD continuum; whether IR contributes to AD pathology later in the disease trajectory deserves further study. APOE ε4 moderated the relationship between IR and level of P-tau₁₈₁/Aβ₄₂. That IR may act synergistically with APOE ε4 to influence AD pathology warrants investigation as do cerebrovascular and other mechanisms, which could be potential targets for treatment, mediating the relationship between IR and cognitive decline prior to dementia.

DECLARATION OF INTERESTS

IS is a full-time employee and shareholder of Roche Diagnostics International Ltd. GK is a full-time employee of Roche Diagnostics GmbH. HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, and CogRx; has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator

Program. COBAS and COBAS E are registered trademarks of Roche. All remaining authors have nothing to disclose.

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