


Association of Cerebrospinal Fluid (CSF) Insulin with Cognitive Performance and CSF Biomarkers of Alzheimer's Disease

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Abstract.

Background: Abnormal insulin signaling in the brain has been linked to Alzheimer's disease (AD).

Objective: To evaluate whether cerebrospinal fluid (CSF) insulin levels are associated with cognitive performance and CSF amyloid- β and Tau. Additionally, we explore whether any such association differs by sex or APOE ϵ 4 genotype.

Methods: From 258 individuals participating in the Parelnoer Institute Neurodegenerative Diseases, a nationwide multicenter memory clinic population, we selected 138 individuals (mean age 66 ± 9 years, 65.2% male) diagnosed with subjective cognitive impairment ($n = 45$), amnesic mild cognitive impairment ($n = 44$), or AD ($n = 49$), who completed a neuropsychological assessment, including tests of global cognition and memory performance, and who underwent lumbar puncture. We measured CSF levels of insulin, amyloid- β_{1-42} , total (t-)Tau, and phosphorylated (p-)Tau.

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Results: CSF insulin levels did not differ between the diagnostic groups ($p = 0.136$). Across the whole study population, CSF insulin was unrelated to cognitive performance and CSF biomarkers of AD, after adjustment for age, sex, body mass index, diabetes status, and clinic site (all $p \geq 0.131$). Importantly, however, we observed effect modification by sex and APOE $\epsilon 4$ genotype. Specifically, among women, higher insulin levels in the CSF were associated with worse global cognition (standardized regression coefficient -0.483 ; $p = 0.008$) and higher p-Tau levels (0.353 ; $p = 0.040$). Among non-carriers of the APOE $\epsilon 4$ allele, higher CSF insulin was associated with higher t-Tau (0.287 ; $p = 0.008$) and p-Tau (0.246 ; $p = 0.029$).

Conclusion: Our findings provide further evidence for a relationship between brain insulin signaling and AD pathology. It also highlights the need to consider sex and APOE $\epsilon 4$ genotype when assessing the role of insulin.

Keywords: Alzheimer's disease, cerebrospinal fluid, cognition, epidemiology, insulin

INTRODUCTION

Over the last decades, a growing body of evidence from *in vitro* experiments, animal models, and observations in patients has linked abnormal insulin signaling to Alzheimer's disease (AD) [1–4]. Multiple pathways may account for this, which have been extensively reviewed elsewhere [1–4]. Insulin is, for example, believed to interfere with the metabolism of amyloid- β ($A\beta$) and tau [3].

At present, brain insulin signaling cannot be measured directly in humans *in vivo*. Evidence of deficient insulin signaling in patients with AD therefore stems from postmortem brain studies showing fewer insulin receptors and decreased downstream signaling activity in the brains of individuals with AD as compared to age-matched controls [5]. To overcome the lack of direct measures, previous population-based studies used blood-derived markers of peripheral insulin resistance as a possible indicator of deficient brain signaling. These studies generally showed that hyperinsulinemia [6–8] and insulin resistance [6, 9, 10] are associated with cognitive impairment [8–10] and risk of AD [6–8, 10], although these associations might be limited to, or most prominent in, women [9] and non-carriers of the apolipoprotein E $\epsilon 4$ (APOE $\epsilon 4$) allele [7, 9, 10]. More recently, blood-derived markers of insulin resistance have also been related to cerebrospinal fluid (CSF) biomarkers of AD in cognitively asymptomatic individuals, but these associations depended on APOE $\epsilon 4$ genotype [11, 12].

Evidently, peripheral insulin levels and insulin sensitivity may not accurately reflect the cerebral situation [13]. Transport of insulin across the blood-brain barrier decreases with increasing insulin resistance [14] and there appears to be local production of insulin in the brain [15]. This may explain why there is no clear correlation between plasma and CSF insulin levels [15]. Published data on CSF insulin in relation to AD are, however, scarce, show variable results, and

generally (with one exception [16]) did not account for the potential modifying effects of sex and APOE $\epsilon 4$ genotype [17–21]. At the same time, an increasing number of clinical trials have shown that intranasal insulin administration enhances memory function (in AD) (e.g., [22–26]), particularly in women and those without APOE $\epsilon 4$ [24–26]. Here, we made use of a standardized multicenter memory clinic population to assess the associations of CSF insulin with cognitive performance and CSF biomarkers of AD [i.e., $A\beta_{1-42}$, Tau, and phosphorylated Tau (p-Tau)] and explored whether any such association differed by sex or APOE $\epsilon 4$ genotype. We hypothesized that higher CSF insulin levels, likely reflecting cerebral insulin resistance, would be associated with AD pathology.

METHODS

Study population

This study was based on data from the Parelsnoer Institute: Neurodegenerative Diseases, a collaboration between the eight Dutch University Medical Centres (<http://www.parelsnoer.org>) [27]. In short, the Pearl Neurodegenerative Diseases focuses on the role of biomarkers in the early diagnosis, differential diagnosis, and prognosis of neurodegenerative diseases, in particular AD [27]. Eligible for inclusion are individuals referred to one of the eight academic memory clinics for the evaluation of cognitive problems, with a Clinical Dementia Rating scale of 0, 0.5, or 1, and a Mini-Mental State Examination (MMSE) of 20 or higher. Harmonized protocols are used to collect clinical data and biomaterials from patients who provide written informed consent [27]. Individuals are classified as having subjective cognitive impairment (SCI), mild cognitive impairment (MCI), or dementia, using a diagnostic procedure that is harmonized across the participating centers [27].

For the present cross-sectional analyses, we used data from the first 258 patients who were enrolled in the database between March 2009 and December 2013 and successfully underwent lumbar puncture, which is optional for participants in this study. From these, we selected those patients diagnosed with AD or its potential prodromal stages (i.e., amnesic MCI or SCI), thereby excluding individuals with non-amnesic MCI ($n=26$) or other types of dementia ($n=37$). To distinguish amnesic from non-amnesic MCI, objective memory deficits were defined as age-, sex-, and educational level-adjusted immediate or delayed recall scores on the 15 Word-Auditory Verbal Learning Test (AVLT) [28, 29] that were at least 1.5 standard deviation below the normative mean [30]. Individuals in whom the presence of objective deficits could not be verified due to missing data on the AVLT or educational level were excluded ($n=3$).

CSF insulin could be determined for 160 out of the 192 patients (83.3%) who underwent lumbar puncture and were eligible for the present study. In the other 32 patients, there was too little CSF left after $A\beta_{1-42}$, Tau, and p-Tau assays. Of the 160 patients, 5 (3%) had no data on APOE $\epsilon 4$ genotype and were therefore excluded from the study sample. An additional 17 individuals were excluded because of missing data on demographic and clinical variables, primarily body mass index ($n=13$) and diabetes status ($n=8$; Supplementary Table 1), resulting in a final study population of 138 individuals ($n=133$ for analyses with cognitive performance). Individuals who were excluded because of missing data did not differ from those who were included in the present analyses (Supplementary Table 1).

The Pearl Neurodegenerative Diseases was approved by the Medical Ethics Review Committee of the VU University Medical Centre, and written informed consent was obtained from patients enrolled in the dataset.

Cerebrospinal fluid measures

Lumbar puncture was performed at the L3/L4 or L4/L5 intervertebral space and 3 ml of the collected CSF was aliquoted in 0.5 ml samples, which were stored at -80°C [27]. From these samples, $A\beta_{1-42}$, Tau, and p-Tau were measured using Innostest[®] Enzyme-Linked Immunosorbent Assays (Innogenetics, Ghent, Belgium) [27]. These assays were performed at the Neurochemistry Laboratory of the department of Clinical Chemistry of the VU Medical Centre Amsterdam according to described

procedures [27]. The samples were analyzed all at once, using the same batch of reagents.

CSF levels of insulin were measured at the same department, in the Endocrine Laboratory, in the CSF volume that remained after aliquots had been taken for $A\beta_{1-42}$, Tau, and p-Tau assays, a leftover volume that ranged between 75 and 150 μL per patient. CSF insulin levels were measured using an ultrasensitive radioimmunoassay (EMD Millipore, Bellerica, MA) with a detection limit of 1.2 pmol/L. The limited amount of CSF available prevented us from measuring insulin in duplicate. Note that patients were not required to fast prior to lumbar puncture.

Cognitive performance

As part of the diagnostic workup, patients completed a standardized battery of cognitive tests [27]. We selected neuropsychological tests that reflect global cognition (i.e., MMSE [31], a screening test) and memory performance (i.e., AVLT [28, 29]), the latter because insulin signaling has been primarily related to memory performance [32, 33]. Total immediate and delayed recall scores of the ALVT were combined into one composite memory score. To this end, raw test scores were transformed into z-scores and averaged.

Covariates

A detailed description of the covariates (i.e., educational level, medical history, and lifestyle factors) is provided in the Supplementary Material.

Statistical analyses

Differences in demographic and clinical characteristics across the diagnostic groups (i.e., SCI, aMCI, and AD) were evaluated using analysis of variance for continuous variables and chi-square tests for categorical variables. Because there are currently limited data on variables that determine CSF levels of insulin, we used univariate linear regression to explore the association between CSF insulin and the available set of demographic and clinical variables known to be associated with cognitive performance (i.e., age, sex, educational level, blood pressure, body mass index, diabetes, cardiovascular or cerebrovascular disease, smoking behavior, and alcohol consumption). In these analyses, CSF insulin was taken as the dependent variable and assumptions of linear regression were verified prior to analyses. Variables that

showed a significant association with CSF insulin (p -value < 0.05) were considered as potentially confounding factors in subsequent multivariate analyses.

With use of analysis of covariance, we examined whether CSF insulin levels differed between the diagnostic groups, independent of the identified potentially confounding variables. Multiple linear regression analyses were then conducted to examine the association of CSF insulin with cognitive performance (i.e., MMSE and composite memory score) and CSF biomarkers of AD (i.e., $A\beta_{1-42}$, Tau, and p-Tau). In these main analyses, which were performed across and within each diagnostic group, CSF insulin was the independent variable. Two predefined models of adjustment were used (model 1: clinic site; model 2: model 1 + age and sex). The third model also included those variables being associated with CSF insulin in the univariate analyses.

Multiple interaction terms (i.e., CSF insulin * sex and CSF insulin * APOE $\epsilon 4$ carriership) were used to explore whether any association between CSF insulin and cognitive performance or Alzheimer pathology differed by sex or APOE $\epsilon 4$ genotype. Likewise, we examined whether any association differed by the presence of diabetes given that diabetes is characterized by abnormal insulin signaling, at least in the periphery [34]. In case of significant interaction, stratified analyses were performed. We additionally evaluated whether any of the univariate associations observed between CSF insulin and demographic or clinical characteristics differed by sex or APOE $\epsilon 4$ genotype.

As a sensitivity analysis, we reanalyzed the data with exclusion of individuals previously diagnosed with diabetes.

All analyses were performed with use of SPSS for Windows, version 23.0 (IBM SPSS, IBM Corp, Armonk, NY, USA). Levels of CSF insulin, Tau, and p-Tau were transformed with the natural logarithm prior to the analyses. A two-sided p -value < 0.05 was considered significant, except for interaction analyses where the significance level was set at 0.10 to compensate for the loss of statistical power in these analyses [35]. No adjustments were made for multiple testing [36].

RESULTS

Table 1 shows the demographic and clinical characteristics of the study population as a whole, by sex,

and by diagnostic group [i.e., SCI ($n=45$); aMCI ($n=44$), and AD ($n=49$)]. Overall, patients were in late adulthood (66 ± 9 years), with a slight male predominance (65.2%). Men had somewhat unhealthier lifestyle variables than women and more frequently reported a history of cardiovascular disease. Cardiovascular disease was, however, equally distributed between the sexes. Of note, the cardiovascular risk profile was similar across the diagnostic groups, except for the presence of diabetes, which was least common in the SCI group.

CSF insulin in relation to demographic and clinical variables: Basic findings

Table 2 shows the univariate associations of clinical and demographic variables with CSF levels of insulin in the total study population. Diabetes status and higher body mass index were associated with higher CSF insulin levels, whereas carriers of the APOE $\epsilon 4$ allele had lower CSF insulin in comparison with non-carriers. There were no significant associations with the other variables.

Multiplicative interaction analyses showed that sex modified the univariate association between diabetes and CSF insulin in that diabetes was more strongly associated with higher CSF insulin levels in men than women. Sex also modified the association between APOE $\epsilon 4$ and CSF insulin. Specifically, APOE $\epsilon 4$ carriership was more strongly associated with lower CSF insulin levels in women than men, although the association between APOE $\epsilon 4$ and CSF reached statistical significance in neither of the sexes. APOE $\epsilon 4$ carriership itself, in turn, modified the univariate association between diabetes and CSF insulin in that diabetes was only associated with higher CSF insulin levels in non-carriers of the APOE $\epsilon 4$ allele. Please note that CSF insulin levels did not differ between sexes and diabetes was not more common among men (Table 1). Likewise, APOE $\epsilon 4$ genotype did not differ between sexes (Table 1).

CSF insulin in the diagnostic groups of cognitive impairment

Median (interquartile range) levels of CSF insulin in individuals with SCI, aMCI, and AD were 3.77 pmol/L (3.34–4.39), 3.61 pmol/L (3.22–4.36), and 3.98 pmol/L (3.57–4.77), respectively. Despite the seemingly higher levels of CSF insulin in AD, CSF insulin did not differ significantly between the groups ($p=0.136$). The linear trend across the

Table 1
Demographic and clinical characteristics of the study population as a whole and stratified by sex and diagnostic group

	Total (n = 138)	Men (n = 90)	Women (n = 48)	p-value for difference	SCI (n = 45)	aMCI (n = 44)	AD (n = 49)	p-value for trend
Age (y)	66 ± 9	66 ± 9	65 ± 10	0.564	62 ± 9	67 ± 9	68 ± 9	<0.001
Male	90 (65.2%)	30 (66.7%)	30 (68.2%)	30 (61.2%)	0.573
Educational level								
Low	32 (23.2%)	21 (23.3%)	11 (22.9%)		10 (22.2%)	11 (25.0%)	11 (22.4%)	
Middle	52 (37.7%)	27 (30.0%)	25 (52.1%)	0.020	16 (35.6%)	19 (43.2%)	17 (34.7%)	0.962
High	54 (39.1%)	42 (46.7%)	12 (25.0%)		19 (42.2%)	14 (31.8%)	21 (42.9%)	
SBP (mmHg)*	145 ± 19	145 ± 19	146 ± 20	0.933	142 ± 19	145 ± 17	149 ± 22	0.127
DBP (mmHg)*	84 ± 10	85 ± 10	83 ± 10	0.325	83 ± 11	84 ± 7	84 ± 12	0.470
BMI (kg/m ²)	25.2 ± 3.2	25.6 ± 3.2	24.7 ± 3.1	0.113	25.7 ± 3.2	25.3 ± 3.0	24.8 ± 3.4	0.194
Diabetes	16 (11.6%)	12 (13.3%)	4 (8.3%)	0.382	2 (4.4%)	5 (11.4%)	9 (18.4%)	0.036
Cardiovascular disease	43 (31.2%)	38 (42.2%)	5 (10.4%)	<0.001	17 (37.8%)	13 (29.5%)	13 (26.5%)	0.244
Cerebrovascular disease	17 (12.3%)	12 (13.3%)	5 (10.4%)	0.620	7 (15.6%)	3 (6.8%)	7 (14.3%)	0.877
APOE ε4 carrier	81 (58.7%)	52 (57.8%)	29 (60.4%)	0.766	20 (44.4%)	31 (70.5%)	30 (61.2%)	0.110
Smoking behavior*								
Never	58 (43.0%)	29 (32.2%)	29 (60.4%)		16 (35.6%)	19 (45.2%)	23 (47.9%)	
Former	56 (41.5%)	42 (46.7%)	14 (29.2%)	0.005	23 (51.1%)	15 (35.7%)	18 (37.5%)	0.454
Current	21 (15.6%)	17 (18.9%)	4 (8.3%)		6 (13.3%)	8 (19.0%)	7 (14.6%)	
Alcohol consumption*	110 (80.3%)	78 (86.7%)	32 (66.7%)	0.003	37 (82.2%)	35 (81.4%)	38 (77.6%)	0.568
CSF insulin (pmol/L)	3.79 [3.37–4.52]	3.85 [3.43–4.60]	3.65 [3.12–4.40]	0.234	3.77 [3.34–4.39]	3.61 [3.22–4.36]	3.98 [3.57–4.77]	0.059
CSF Aβ ₁₋₄₂ (ng/L)	678 ± 281	701 ± 301	636 ± 237	0.172	835 ± 244	656 ± 279	554 ± 250	<0.001
CSF Tau (ng/L)	421 [229-633]	397 [231-619]	494 [222-707]	0.418	229 [176-354]	485 [267-651]	544 [401-864]	<0.001
CSF p-Tau (ng/L)	55 [36-77]	54 [36-76]	57 [39-86]	0.663	36 [23-55]	58 [40-83]	71 [53-89]	<0.001
MMSE (score)*	26 ± 3	27 ± 3	26 ± 3	0.111	28 ± 2	27 ± 3	24 ± 3	<0.001
AVLT (words)								
Total immediate recall*	30 ± 11	30 ± 11	31 ± 13	0.408	41 ± 10	26 ± 7	25 ± 8	<0.001
Delayed recall*	4 ± 4	4 ± 3	5 ± 4	0.418	8 ± 3	2 ± 2	3 ± 3	<0.001

Data are presented as n (%), mean ± SD, or median [IQR]. *Data not available from all participants (n = 137 for alcohol consumption; n = 136 for AVLT total immediate recall; n = 135 for SBP, DBP, smoking behavior, MMSE, and AVLT delayed recall). SCI, subjective cognitive impairment; aMCI, amnesic mild cognitive impairment; AD, Alzheimer's dementia; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; AVLT, 15 Word-Auditory Verbal Learning Test.

Table 2

Univariate associations of clinical and demographic characteristics with CSF insulin levels in the whole study population and stratified according to sex and APOE ϵ 4 genotype where applicable

	<i>n</i> (<i>n</i> _{max} = 138)	β	<i>p</i> -value for interaction with sex	β	<i>p</i> -value for interaction with APOE ϵ 4 carriership	
Age	138	0.012 (−0.158; 0.181)	0.315	...	0.792	...
Sex						
male versus female	138	0.214 (−0.139; 0.566)	0.540	...
Educational level						
medium versus low	138	−0.253 (−0.698; 0.193)	0.886	...	0.904	...
high versus medium		0.068 (−0.317; 0.453)	0.477	...	0.432	...
high versus low		−0.185 (−0.627; 0.258)	0.453	...	0.569	...
SBP	135	0.101 (−0.070; 0.271)	0.240	...	0.562	...
DBP	135	0.039 (−0.132; 0.211)	0.369	...	0.779	...
BMI	138	0.261 (0.097; 0.425)	0.756	...	0.326	...
Diabetes	138	1.152 (0.662; 1.642)	<0.001	Men: 2.232 (1.401; 3.063) Women: 0.657 (0.053; 1.261)	0.012	Carriers: 0.528 (−0.230; 1.285) Non-carriers: 1.706 (1.065; 2.346)
Cardiovascular disease						
yes versus no	138	0.211 (−0.153; 0.574)	0.492	...	0.704	...
Cerebrovascular disease						
yes versus no	138	0.089 (−0.425; 0.603)	0.360	...	0.784	...
APOE ϵ 4 genotype	138	−0.547 (−1.078; −0.016)	0.092	Men: −0.192 (−0.698; 0.313) Women: −1.118 (−2.295; 0.059)
carriers versus non-carriers						
Smoking behavior						
former versus never	135	0.121 (−0.252; 0.494)	0.455	...	0.400	...
current versus former		−0.025 (−0.534; 0.484)	0.319	...	0.519	...
current versus never		0.096 (−0.410; 0.603)	0.615	...	0.520	...
Alcohol consumption						
yes versus no	137	−0.080 (−0.506; 0.347)	0.650	...	0.090	Carriers: 0.395 (−0.301; 1.091) Non-carriers: −0.365 (−0.910; 0.179)

Data are presented as standardized regression coefficients β (95% confidence intervals) for continuous variables and between-group differences (95% confidence intervals) for categorical variables, the latter expressed in standard deviations of cerebrospinal fluid insulin levels. *p*-values are derived from interaction analyses with sex and APOE ϵ 4 carriership. Insulin levels were transformed with the natural logarithm prior to analysis. CSF, cerebrospinal fluid; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Table 3
Association of CSF insulin levels with cognitive performance

	Total (n = 133)	SCI (n = 43)	aMCI (n = 44)	AD (n = 46)	Men (n = 86)	Women (n = 47)
MMSE						
Model 1	-0.023 (-0.196; 0.150)	0.084 (-0.238; 0.405)	0.103 (-0.203; 0.410)	0.021 (-0.288; 0.329)	0.144 (-0.072; 0.360)	-0.242 (-0.537; 0.053)
Model 2	-0.045 (-0.209; 0.120)	0.111 (-0.231; 0.454)	0.095 (-0.200; 0.390)	-0.017 (-0.322; 0.288)	0.121 (-0.092; 0.333)	-0.210 (-0.481; 0.061)
Model 3	-0.071 (-0.252; 0.111)	0.175 (-0.190; 0.540)	0.045 (-0.323; 0.413)	-0.014 (-0.352; 0.324)	0.123 (-0.108; 0.354)	-0.483* (-0.831; -0.135)
Composite memory score						
Model 1	-0.051 (-0.224; 0.122)	-0.324* (-0.619; -0.030)	0.164 (-0.143; 0.472)	0.210 (-0.091; 0.511)	0.120 (-0.085; 0.324)	-0.206 (-0.489; 0.078)
Model 2	-0.048 (-0.206; 0.109)	-0.251 (-0.549; 0.047)	0.170 (-0.103; 0.444)	0.162 (-0.143; 0.467)	0.078 (-0.108; 0.264)	-0.174 (-0.434; 0.086)
Model 3	-0.014 (-0.188; 0.159)	-0.092 (-0.381; 0.197)	0.162 (-0.177; 0.502)	0.251 (-0.078; 0.580)	0.150 (-0.048; 0.347)	-0.273 (-0.628; 0.081)

Data are presented as standardized regression coefficients, which reflect the standard deviation change in cognitive performance per standard deviation increase in cerebrospinal fluid insulin levels. Insulin levels were transformed with the natural logarithm prior to analysis. Stratified analyses according to sex were performed as interaction analyses revealed statistically significant interaction of this factor on the association between CSF insulin and cognitive performance. * $p < 0.05$. Model 1: adjusted for UMC; Model 2: additionally adjusted for age and sex (the latter not in case of stratified analysis according to sex); Model 3: additionally adjusted for BMI and the presence of diabetes. CSF, cerebrospinal fluid; SCI, subjective cognitive impairment; aMCI, amnesic mild cognitive impairment; AD, Alzheimer's disease; UMC, University Medical Center; BMI, body mass index.

diagnostic groups was borderline significant (p for trend = 0.059). There were no interactions with sex or APOE $\epsilon 4$ genotype (p for interaction = 0.707 and 0.120, respectively). When diabetes status and body mass index were included as covariates, pairwise comparisons between the diagnostic groups confirmed that CSF insulin did not differ significantly between the groups (all $p > 0.105$).

CSF insulin and cognitive performance

Table 3 shows the association of CSF insulin with cognitive performance across the groups and within each diagnostic group. After adjustment for center, CSF insulin was not associated with cognitive performance in the overall study population, nor within the groups of individuals with aMCI or AD. Among individuals with SCI, higher CSF insulin levels were associated with worse memory function, but this association lost significance once adjustments were made for age and sex.

Multiplicative interaction analyses revealed that the null associations between CSF insulin and cognitive performance did not differ by the presence of diabetes (all p for interaction > 0.614) or APOE $\epsilon 4$ genotype (all $p > 0.425$), but did differ by sex (all $p < 0.059$). Subsequent stratified analyses (Table 3, right panel) showed that among women, but not among men, higher CSF insulin were associated with worse cognitive performance. These associations

were significant for MMSE performance in the fully adjusted model.

CSF insulin and CSF biomarkers of AD

Table 4 shows the associations of CSF insulin with CSF biomarkers of AD across and within each diagnostic group. In brief, CSF insulin was not associated with CSF A β_{1-42} , nor with levels of Tau or p-Tau.

Multiplicative interaction analyses showed that diabetes did not modify these null associations (all p for interaction > 0.170), while sex and APOE $\epsilon 4$ genotype did, at least for the association between CSF insulin and (p-)Tau (all $p < 0.017$ and < 0.096 , respectively). Subsequent stratified analyses (Table 4, right panel) showed that among women, but not among men, higher CSF insulin levels were associated with higher levels of tau and tended to be associated with higher levels of p-Tau in the fully adjusted model. In APOE $\epsilon 4$ non-carriers, but not in carriers, higher CSF insulin was associated with both higher tau and p-Tau levels in the fully adjusted model. Notably, associations were strongest among female patients who were non-carriers of the APOE $\epsilon 4$ allele (data not shown).

Sensitivity analysis

When the data were reanalyzed excluding individuals with diabetes, qualitatively similar results were obtained (Supplementary Tables 2 and 3).

Table 4
Associations of CSF insulin levels with levels of CSF A β ₁₋₄₂ and (p-)Tau

	Total (n = 138)	SCI (n = 45)	aMCI (n = 44)	AD (n = 49)	Men (n = 90)	Women (n = 48)	APOE ϵ 4 carriers (n = 81)	APOE ϵ 4 non-carriers (n = 57)
CSF A β ₁₋₄₂								
Model 1	-0.013 (-0.183; 0.157)	-0.006 (-0.320; 0.309)	0.057 (-0.258; 0.373)	0.118 (-0.167; 0.403)
Model 2	-0.023 (-0.185; 0.139)	-0.034 (-0.354; 0.285)	0.061 (-0.245; 0.366)	0.087 (-0.204; 0.378)
Model 3	-0.130 (-0.303; 0.043)	-0.001 (-0.355; 0.353)	-0.185 (-0.519; 0.149)	-0.059 (-0.362; 0.245)
CSF Tau								
Model 1	0.006 (-0.164; 0.177)	0.171 (-0.139; 0.481)	-0.200 (-0.495; 0.096)	-0.119 (-0.414; 0.177)	-0.184 (-0.394; 0.026)	0.236 (-0.055; 0.528)	-0.147 (-0.370; 0.077)	0.253 (-0.007; 0.513)
Model 2	0.012 (-0.140; 0.164)	0.097 (-0.165; 0.359)	-0.202 (-0.466; 0.062)	-0.114 (-0.412; 0.185)	-0.147 (-0.335; 0.040)	0.194 (-0.071; 0.459)	-0.137 (-0.347; 0.074)	0.232* (0.014; 0.450)
Model 3	0.059 (-0.104; 0.222)	0.189 (-0.089; 0.467)	-0.060 (-0.361; 0.241)	-0.133 (-0.462; 0.196)	-0.098 (-0.297; 0.101)	0.311 (-0.030; 0.653)	-0.189 (-0.441; 0.063)	0.287* (0.079; 0.495)
CSF p-Tau								
Model 1	0.037 (-0.131; 0.206)	0.165 (-0.140; 0.471)	-0.162 (-0.473; 0.150)	-0.067 (-0.358; 0.225)	-0.174 (-0.383; 0.035)	0.287* (0.003; 0.571)	-0.073 (-0.294; 0.148)	0.224 (-0.042; 0.490)
Model 2	0.039 (-0.116; 0.195)	0.097 (-0.184; 0.377)	-0.164 (-0.455; 0.127)	-0.069 (-0.369; 0.231)	-0.145 (-0.340; 0.051)	0.246 (-0.013; 0.505)	-0.066 (-0.282; 0.150)	0.201 (-0.028; 0.430)
Model 3	0.074 (-0.090; 0.239)	0.207 (-0.082; 0.497)	-0.036 (-0.370; 0.298)	-0.100 (-0.426; 0.225)	-0.088 (-0.290; 0.113)	0.353* (0.018; 0.689)	-0.105 (-0.360; 0.149)	0.246* (0.026; 0.467)

Data are presented as standardized regression coefficients, which reflect the standard deviation change in cerebrospinal fluid levels of A β ₁₋₄₂ and (p-)Tau per standard deviation increase in cerebrospinal fluid insulin levels. Insulin and (p-)Tau levels were transformed with the natural logarithm prior to analysis. * $p < 0.05$. Stratified analyses according to sex and APOE ϵ 4 genotype were performed as interaction analyses revealed statistically significant interaction of these variables on the association between CSF insulin and CSF (p-)Tau. Model 1: adjusted for UMC; Model 2: additionally adjusted for age and sex (the latter not in case of stratified analysis according to sex); Model 3: additionally adjusted for BMI and the presence of diabetes. CSF, cerebrospinal fluid; SCI, subjective cognitive impairment; aMCI, amnesic mild cognitive impairment; AD, Alzheimer's disease; A β ₁₋₄₂, amyloid- β ₁₋₄₂; Tau, total Tau; p-Tau, phosphorylated Tau; UMC, University Medical Center; BMI, body mass index.

DISCUSSION

The present study shows that, among women and non-carriers of the APOE $\epsilon 4$ allele, higher levels of CSF insulin were associated with worse cognitive performance and/or higher levels of CSF (p-)Tau. CSF insulin did, however, not differ between diagnostic groups of cognitive impairment (i.e., SCI, aMCI, and AD). These findings add to the growing body of evidence indicating that abnormal insulin signaling is involved in AD [1–4] and also provide an explanation for why women and non-carriers of the APOE $\epsilon 4$ allele seem to benefit most from intranasal insulin administration to enhance cognitive performance (in AD) [24–26].

There are few earlier studies on the association between CSF insulin and AD [17–21]. Results on CSF insulin levels in patients with AD were inconsistent and reported CSF insulin to be increased [17], decreased [18, 20], or unchanged [19, 21] in individuals with AD as compared to healthy individuals [17–19, 21] or those with SCI [20]. The most probable explanation for these variable results across studies is the use of different study populations. For example, studies reporting decreased levels of CSF insulin in AD [18, 20] excluded individuals with diabetes, while the present study provides evidence that the presence of diabetes is associated with higher levels of CSF insulin. Other differences between the populations concern the severity of the dementia and the use of different reference groups.

Substantially more research has been conducted on the association between cerebral glucose metabolism, based on fluorodeoxyglucose positron emission tomography (FDG-PET) [36–42] or the CSF to plasma glucose ratio [43], and AD pathology. Collectively, these studies suggest that cerebral hypometabolism is more prominent in individuals with AD [42] and is associated with higher CSF (p-)Tau [36–39] and lower CSF $A\beta_{1-42}$ [36, 38, 40, 41, 43] levels. Although it has been suggested that cerebral hypometabolism may reflect cerebral insulin resistance [43], it is important to realize that glucose uptake in the brain is largely independent of insulin levels [44]. Even in the hippocampus, one of the brain regions with the highest concentrations of insulin sensitive GLUT-4 receptors, it is believed that glucose uptake does not necessarily require insulin [44]. The link between cerebral hypometabolism and AD does, thus, not directly support a role for insulin resistance in AD pathology. This is particularly the case because glucose itself may have a direct effect

on tau metabolism [45] and because it has been suggested that tau-related pathology may precede cerebral hypometabolism rather than being a consequence of it [46].

Despite the numerous reviews that have been published on the mechanisms through which cerebral insulin might interfere with $A\beta$ and tau metabolism (e.g., [4, 47]), only one previous study has actually evaluated CSF insulin in relation to CSF $A\beta_{1-42}$ and (p-)Tau levels [20]. The overall null results of that study are in line with the null associations we observed in our overall study population. We, however, now show that higher levels of CSF insulin were associated with higher levels of CSF (p-)Tau in women and non-carriers of the APOE $\epsilon 4$ allele, which reinforces data from experimental studies linking insulin to tau metabolism. From a pathophysiological viewpoint, higher levels of CSF insulin, potentially reflecting cerebral insulin resistance and thus deficient brain insulin signaling, may result in reduced activity of the phosphoinositide 3-kinase-protein kinase B pathway. This, in turn, leads to over activation of glycogen synthase kinase-3, and consequently, hyperphosphorylation of tau [47]. That the association between CSF insulin and tau differs with APOE $\epsilon 4$ genotype could be explained by a higher degree of insulin resistance among non-carriers relative to carriers of the APOE $\epsilon 4$ allele, as illustrated by differences in insulin-mediated glucose disposal rates [16]. Alternatively, a potential association between CSF insulin and tau in APOE $\epsilon 4$ carriers might have been simply overshadowed by the high AD risk associated with APOE $\epsilon 4$ carriership.

The sex differences observed in our study are in line with experimental data and clinical studies showing that the brains of men and women are differentially sensitive to the effects of insulin [23, 24, 48, 49]. More specifically, previous studies have shown that the anorexigenic effects of intranasally administered insulin are more prominent in men, while its memory enhancing effects are more prominent in women. It has been suggested [24] that these sex differences can be explained by the cognitive effects of estrogen [50]. A more recent study, however, showed that young and postmenopausal women are comparable sensitivity to the memory enhancing effects of intranasal insulin [51], which reiterates that estrogen explains the observed sex differences. What explains these differences should be explored in future studies. Perhaps it may relate to other physiological or anatomical factors that differ between sexes, for example the density of hippocampal insulin receptors.

Notably, we did not observe any association between CSF levels of insulin and A β , while a previous study noted that a single dose of intranasal insulin increased plasma levels of the short form of A β (i.e., A β ₄₀) [52]. A potential explanation for this finding are the complex and dual effects insulin (signaling) may have on A β clearance [4]. On the one hand, insulin may promote A β clearance by accelerating its intraneuronal transport and increasing protein levels of the insulin degrading enzyme (IDE), a metalloprotease that is able to degrade A β [4]. On the other hand, IDE also catabolizes insulin, for which it has a higher affinity, which means that insulin at the same time can inhibit A β degradation [4]. An alternative explanation is that cerebral insulin is involved in AD via pathways other than A β metabolism (reviewed in [33]).

The present study has limitations. First and foremost, CSF samples were not necessarily taken in the fasting state, nor was lumbar puncture performed at a fixed time of the day. The time of day at which lumbar puncture was performed was also not recorded in the database. Hence, we were unable to adjust our analyses for potential biorhythm effects, while the production and secretion of at least insulin [53] and A β [54] seem to follow a circadian rhythm. Although this may have led us to underestimate the association of CSF insulin with AD pathology, it is unlikely to explain the observed modulating effects of either sex or APOE ϵ 4 genotype. A second limitation that may have further affected our estimates of the association between CSF insulin and CSF p-Tau concerns the use of an antibody for p-Tau that was specific for phosphorylation at threonine 181, while deficient insulin signaling leads to a widespread phosphorylation of tau at multiple sites. Next, it can be questioned whether higher CSF insulin levels reflect cerebral insulin resistance the way hyperinsulinemia in the plasma reflects peripheral insulin resistance. Note, however, that individuals with diabetes, who are the most likely to have cerebral insulin resistance despite the fact that cerebral and peripheral resistance not necessarily concur [13], had higher levels of CSF insulin. Fourth, multiple analyses were performed without correction for multiple testing, though stratified analyses were only performed in case of statistically significant interaction with a clear *a priori* hypothesis. Fifth, in the current study, no blood samples were analyzed. Such data would have provided valuable information on how peripheral and cerebral insulin metabolism may interact in relation to cognitive performance and AD

pathology, although compartmentalization is also possible. Finally, it can be questioned whether the composition of the CSF is directly comparable to that of the brain's interstitial fluid [51]. Undoubtedly, however, CSF insulin is a better indicator of deficient brain signaling than blood-derived markers.

In conclusion, our results indicate that higher CSF insulin levels are related to impairment in cognitive performance and biomarkers of AD among women and non-carriers of the APOE ϵ 4 allele. As such, our data suggest that the potential involvement of cerebral insulin (resistance) in the pathogenesis of AD is not uniform across individuals. Future studies are needed to determine whether endogenous high CSF insulin levels indeed reflect cerebral insulin resistance, as this might have implications for selection of patients who may benefit from the intranasal use of insulin to improve or preserve cognitive performance. Longitudinal studies are required to strengthen the pathophysiological relevance of our findings.

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SUPPLEMENTARY MATERIAL

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