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Plasma Insulin Predicts Early Amyloid-β Pathology Changes in Alzheimer's Disease

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Abstract.

Background: Evidence suggests that type 2 diabetes (T2D) is an independent risk factor for Alzheimer's disease (AD), sharing similar pathophysiological traits like impaired insulin signaling.

Objective: To test the association between plasma insulin and cerebrospinal fluid (CSF) AD pathology.

Methods: A total of 304 participants were included in the Alzheimer's Disease Neuroimaging Initiative, assessing plasma insulin and CSF AD pathology. We explored the cross-sectional and longitudinal associations between plasma insulin and AD pathology and compared their associations across different AD clinical and pathological stages.

Results: In the non-demented group, amyloid-β (Aβ)+ participants (e.g., as reflected by CSF $A\beta_{42}$) exhibited significantly lower plasma insulin levels compared to non-demented Aβ– participants ($p < 0.001$). This reduction in plasma insulin was more evident in the A+T+ group (as shown by CSF $A\beta_{42}$ and pTau181 levels) when compared to the A–T– group within the non-dementia group ($p = 0.002$). Additionally, higher plasma insulin levels were consistently associated with more normal CSF $A\beta_{42}$ levels ($p < 0.001$) across all participants. This association was particularly significant in the Aβ– group ($p = 0.002$) and among non-demented individuals ($p < 0.001$). Notably, baseline plasma insulin was significantly correlated with longitudinal changes in CSF $A\beta_{42}$ ($p = 0.006$), whereas baseline CSF $A\beta_{42}$ did not show a similar correlation with changes in plasma insulin over time.

Conclusions: These findings suggest an association between plasma insulin and early Aβ pathology in the early stages of AD, indicating that plasma insulin may be a potential predictor of changes in early Aβ pathology.

Keywords: Alzheimer's disease, amyloid, diabetes, plasma insulin, pTau

[1]These authors contributed equally to this work.

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INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is characterized by two main pathological features, including the extracellular aggregation of amyloid-β (Aβ) plaques and the intraneuronal formation of tau-related neurofibrillary tangles [1–3]. There is mounting evidence from epidemiological, clinical, and neuropathology studies that suggests individuals with type 2 diabetes (T2D) are at higher risk of developing AD [4, 5], particularly when diagnosed at a younger age [6]. Subjects with AD were found to have a greater severity and longer duration of T2D [7]. Additionally, AD and T2D share many similar characteristics. For instance, patients with T2D exhibit brain structural alterations that are similar to those observed in patients with AD, such as reduced volumes of grey matter, white matter, hippocampus, and whole brain when compared to individuals without diabetes [8, 9]. In addition, one typical characteristic of AD is glucose hypometabolism, which is associated with dysfunction of insulin signaling, which is also a key feature of T2D. Nonetheless, the mechanisms underlying the relationship between T2D and AD remain unclear.

Several studies indicate that the brain is an insulin-responsive organ and that insulin resistance (IR) is the key factor between T2D and AD [10–12]. Dysfunction in peripheral insulin signaling can worsen brain IR [13], resulting in reduced glucose metabolism in the hippocampus, decreased volume of grey matter, impaired cognitive performance [8, 9], reduced clearance of Aβ [14], and exacerbated AD-like symptoms [15, 16]. Autopsy studies have shown that reduced insulin and IR in AD brains are associated with neuropathology [17], particularly in the hippocampus and cerebellar cortex, which is inversely correlated with cognitive function [18]. However, the relationship between IR and AD pathology, particularly amyloid pathology, has been inconsistently reported in population-based studies [19–21]. Similarly, the association between plasma insulin levels and AD pathology has shown varying results. Some studies have found that higher peripheral insulin is associated with lower cerebrospinal fluid (CSF) $A\beta_{42}$/tau [22], indicating more severe amyloid pathology, while others have linked increased peripheral insulin to reduced amyloid positivity in positron emission tomography (PET) scans [23, 24]. These conflicting results highlight the need for a thorough investigation into the complex relationships between insulin and AD pathology, especially to explore how insulin changes in different stages of AD and its effect on AD pathology.

To systematically assess the impact of plasma insulin on AD pathology, we aimed to test the changes in plasma insulin levels across diverse clinical and pathological AD stages, examine the cross-sectional and longitudinal associations between plasma insulin and CSF AD key pathologies, and explore the diagnostic value of plasma insulin on AD pathology. We hypothesized that plasma insulin would be significantly associated with AD pathology and could serve as an important biomarker for AD pathology.

MATERIALS AND METHODS

Study participants

The data used in this study was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). The ADNI project was established in 2003 as a public-private partnership to validate and develop early diagnostic measures for AD by integrating clinical, genetic, imaging, and biochemical biomarkers. ADNI received approval from the institutional review boards of all participating institutions. All participants have provided written informed consent following the Declaration of Helsinki before enrollment.

The study involved 304 participants in the ADNI database who received measurements of CSF $A\beta_{42}$ and CSF pTau181, plasma insulin, serum fasting glucose, and serum creatinine. For the longitudinal analyses, each participant completed at least one follow-up assessment of CSF samples or plasma insulin samples (baseline plus one follow-up visit). We also obtained information on the history of alcohol abuse and smoking for each participant. Furthermore, we included information on the baseline history of diabetes (type 2 diabetes and other type diabetes) and the use of diabetes-related medications, including commonly used hypoglycemic agents and therapeutic insulin. The participants underwent clinical assessments, which included the Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR). The assigned MMSE and CDR scores describe the clinical diagnosis as cognitively normal (CN, MMSE > 24, CDR = 0), mild cognitively impaired (MCI, MMSE > 24, CDR = 0.5), or dementia based on anticipated criteria [25]. We included participants with CN and MCI as non-dementia and then divided all participants into non-dementia and dementia groups.

Measurements of CSF biomarker

CSF $A\beta_{42}$ and CSF pTau181 levels were measured using Elecsys electrochemiluminescence immunoassays conducted on a cobas 601 instrument [26]. For more information on analyte measurements, please refer to the ADNI LONI Data & Image Archive (http://adni.loni.usc.edu). In addition, we defined the positive status of CSF $A\beta_{42}$ and pTau181 using previously suggested thresholds: Aβ± (CSF $A\beta_{42} < 976.6$ pg/ml or ≥ 976.6 pg/ ml) and pTau± (pTau181 > 21.8 pg/ml or ≤ 21.8 pg/ml) [26].

Measurements of plasma biomarker

The blood samples were according to the ADNI protocol [27]. Plasma samples were obtained using EDTA tubes after an overnight fasting period and were frozen within 120 minutes. Plasma insulin levels were analyzed using a multiplex immunoassay panel based on the Luminex xMAP platform, which was provided by Rules-Based Medicine (Austin, RBM, TX, USA). The Luminex xMAP technology is an immunological method that allows for the simultaneous quantification of multiple target proteins by detecting fluorescent microspheres. Further information on the quantification methods can be found at http://adni.loni.usc.edu/wpcontent/uploads/2010/11/BC_Plasma_Proteomics_Data_Primer.pdf. Additionally, serum glucose and serum creatinine were selected as covariates in this study due to their well-documented associations with plasma insulin levels [28–30]. Recognizing the potential impact of these factors on insulin dynamics, it was imperative to account for them in our analysis to ensure a more accurate interpretation of the results. Their levels were analyzed using a nuclear magnetic resonance based blood biomarker analysis assay. This advanced method allows for the quantification of over 220 metabolic biomarkers from a single blood sample. Detailed information about analyzed methods can be found at http://adni.loni.usc.edu.

Statistical analysis

The statistical analyses were conducted using R version 4.1.0 software. Statistical significance was defined as a two-sided p value < 0.05. We assessed the normal distribution of each biomarker was assessed using the Kolmogorov-Smirnov test. For variables that did not follow a normal distribution, we applied a log10 transformation for statistical analysis. To enable comparison across different scales and units, we standardized the data by converting them into Z-scores using the 'scale()' function in R. This function centers the data by subtracting the mean and then scales it by dividing by the standard deviation. The standardized values were used for subsequent statistical analyses and graphical representations.

Baseline characteristics of the A/T group were compared among all participants using one-way analysis of covariance for continuous variables and chi-squared tests for categorical variables. *Post-hoc* tests were conducted using Tukey HSD for continuous variables. For categorical variables, chi-squared tests were followed by Bonferroni corrections to adjust p-values for multiple testing. To investigate the associations between plasma insulin and serum glucose with AD biomarkers, we used multivariate linear regression (MLR) in different various clinical groups, A/T groups, and Aβ status plus clinical groups. We adjusted for sex, age, *apolipoprotein* (*APOE*) ε4 carrier status, education, smoking, alcohol use, serum glucose, and serum creatinine. In this study, subject-specific slopes for CSF $A\beta_{42}$, CSF pTau181, and plasma insulin were computed using linear mixed-effect (LME) models. The model used the year as the independent variable and the variable intended to calculate the rate of change as the dependent variable, adjusted for age and gender and incorporating random slopes and intercepts. The MLR model was used to assess associations involving baseline or slope values for CSF $A\beta_{42}$, CSF pTau181, and plasma insulin, adjusting for the same covariate as above. In sensitivity analyses, we further adjusted for the history of diabetes and diabetes medication use in the MLR model. Finally, we used receiver operating curve (ROC) statistics to evaluate the predictive accuracy of plasma insulin for AD pathology among clinical groups. We constructed three ROC models: (1) a baseline model (BM) including sex, age, and *APOE* ε4 status, (2) plasma insulin alone, and (3) a combination of BM and plasma insulin (BM + plasma insulin). The model of BM and BM + plasma insulin used multivariable binary logistic regression to extract the predicted probabilities. We used the DeLong test to compare the AUC of the two ROC curves.

RESULTS

Participant characteristics

Table 1 shows the demographic, clinical, and biomarker data of 304 participants in our study,

Table 1
Participant characteristics at baseline by biomarker-defined groups

	A–T–	A+T–	A+T+	A–T+	*p*
N	67	41	159	37	–
Age, mean (SD), y	74.6 (7.0)	76.2 (5.7)	73.7 (7.8)	76.4 (7.3)	0.08
Sex, *n* (%) Female	40 (59.7)	30 (73.2)	97 (61.0)	24 (64.9)	0.48
Education, mean (SD), y	15.6 (2.9)	16.0 (3.0)	15.6 (3.0)	14.9 (3.5)	0.35
APOE ε4 carriers, *n* (%)	9 (13.4)	19 (46.3)	116 (73.0)	8 (21.6)	<0.001[a,b,c,d]
Dementia diagnosis, *n* (%)	4 (6.0)	10 (24.4)	59 (37.1)	8 (21.6)	<0.001[a,b,c,d]
Diabetes diagnosis, *n* (%)	5 (7.5)	1(2.4)	8(5.0)	3(8.1)	0.625
Diabetes medications use, *n* (%) Yes	4 (6.0)	0(0)	8(5.0)	3(8.1)	0.625
Ever smoker, *n* (%) Yes	37 (55.2)	18 (43.9)	62 (39.0)	16 (43.2)	0.17
History of alcohol abuse, *n* (%) Yes	4 (6.0)	2 (4.9)	6 (3.8)	3 (8.1)	0.70
CSF biomarkers, mean (SD), pg/ml					
CSF $A\beta_{42}$	1,473.8 (270.7)	588.4 (217.5)	586.1 (156.1)	1,735.2 (520.4)	<0.001[a,b,d,e]
CSF pTau181	16.8 (2.8)	16.4 (3.5)	37.3 (10.7)	32.0 (12.3)	<0.001[b,c,d,e,f]
Blood biomarkers, mean (SD)					
Plasma insulin, uIU/mL	0.40 (0.26)	0.26 (0.30)	0.26 (0.31)	0.36 (0.39)	0.007 [b]
Serum glucose, mmol/l	4.3 (0.7)	4.1 (0.6)	4.1 (0.8)	4.2 (0.6)	0.29
Serum creatinine, mmol/l	80.3 (20.0)	85.8 (20.6)	81.7 (19.1)	77.9 (15.5)	0.25

Aβ, amyloid-β; AD, Alzheimer's disease; CSF, cerebrospinal fluid; pTau, phosphorylated tau; [a]*p* value derived from comparison between A+T– and A–T–. [b]*p* value derived from comparison between A+T+ and A–T–. [c]*p* value derived from comparison between A+T+ and A+T–. [d]*p* value derived from comparison between A–T+ and A+T+. [e]*p* value derived from comparison between A–T+ and A+T–. [f]*p* value derived from comparison between A–T+ and A–T–. *p* values were computed using the one-way analysis of covariance test for age, education, CSF biomarkers, and blood biomarkers. The chi-squared test was used for sex, *APOE* ε4 status, dementia diagnosis, ever smoked, history of alcohol abuse, history of diabetes, and history of diabetes medication use. Tukey HSD *post-hoc* tests were employed for continuous variables. For categorical variables, chi-squared tests followed by Bonferroni corrections were applied to adjust *p*-values for multiple testing.

including 67 A–T–, 41 A+T–, 159 A+T+, and 37 A–T+ individuals. The average age was 75.2 years (±7.0), with 64.7% being female. Significant differences were observed among the A/T groups in plasma insulin ($F_{3,300} = 4.149$, $p = 0.007$), *APOE* ε4 carrier status ($p < 0.001$), dementia diagnosis ($p < 0.001$), and levels of CSF $A\beta_{42}$ ($F_{3,300} = 331.3$, $P < 0.001$) and pTau181 ($F_{3,300} = 112.6$, $p < 0.001$). The specific results of the post hoc tests comparing these groups are detailed in Table 1. However, there were no significant differences observed in age, sex, education, smoking history, alcohol abuse history, serum creatinine, or serum glucose. Table 2 details similar demographic, clinical, and biomarker characteristics for 229 participants in the longitudinal analysis, which were similar to the distribution observed in the baseline participants. In addition, there are no differences in plasma insulin between *APOE* ε4 carriers and *APOE* ε4 non-carriers ($F_{1,302} = 2.083$, $p = 0.15$) (Supplementary Figure 1).

Comparison of plasma insulin among different clinical and pathological stages of AD

Plasma insulin levels were compared among groups based on clinical and biological diagnoses. The non-demented Aβ+ group had significantly lower plasma insulin levels compared to the non-demented Aβ– group (adjusted $p < 0.001$; Fig. 1A). Additionally, within the non-dementia group, lower plasma insulin levels were observed in the A+T+ group compared to the A–T– group (adjusted $p = 0.002$; Fig. 1B). A similar pattern was found between the A+T+ and A+T– groups, but no significant differences were found in the A+T– group when compared to the A–T– group. However, there were no significant differences in plasma insulin levels between A/T groups within the dementia group (Fig. 1C).

Associations between plasma insulin and AD pathology

Regardless of clinical or Aβ status, higher plasma insulin levels were associated with increased levels of CSF $A\beta_{42}$ ($\beta = 0.17$, $p < 0.001$; Fig. 2A). To further explore whether the relationship between plasma insulin and AD pathology exists in early or late AD, we categorized participants into Aβ– or non-dementia groups as early stage and Aβ+ or dementia groups as late stages. The positive correlation between plasma insulin and CSF $A\beta_{42}$ was observed only in the Aβ– (β=0.34, $p = 0.002$) and non-dementia groups ($\beta = 0.21$, $p < 0.001$) when stratified by clinical and Aβ status (Fig. 2A, C).

Table 2
The characteristics of longitudinal participant

	A–T–	A+T–	A+T+	A–T+	*p*
N	48	33	123	25	
Age, mean (SD), y	75.1 (6.6)	76.8 (4.2)	74.1 (7.3)	76.3 (7.9)	0.154
Sex, *n* (%) Female	17 (35.4)	10 (30.3)	45 (36.6)	8 (32.0)	0.906
Education, mean (SD), y	15.4 (2.9)	15.8 (3.1)	15.8 (3.0)	15.2 (2.9)	0.752
APOE ε4 carriers, *n* (%)	4 (8.3)	16 (48.5)	88 (71.5)	6 (24.0)	<0.001[a,b,d]
Dementia diagnosis, *n* (%)	2 (4.2)	8 (24.2)	42 (34.1)	4 (16.0)	<0.001[a,b,c,d,e,f]
Diabetes diagnosis, *n* (%)	14 (6.1)	5 (10.4)	1 (3.0)	6 (4.9)	0.462
Diabetes medication use, *n* (%) Yes	12 (5.2)	4 (8.3)	0 (0.0)	6 (4.9)	0.367
Ever smoker, *n* (%) Yes	26 (54.2)	16 (48.5)	49 (39.8)	11 (44.0)	0.375
History of alcohol abuse, *n* (%) Yes	3 (6.2)	2 (6.1)	5 (4.1)	3 (12.0)	0.475
CSF biomarkers, mean (SD), pg/ml					
CSF $A\beta_{42}$	1,491.2 (252.4)	610.4 (215.9)	586.6 (159.3)	1,677.1 (509.2)	<0.001[a,b,d,e]
CSF pTau181	17.2 (2.8)	16.8 (3.3)	37.2 (10.6)	31.1 (11.9)	<0.001[b,c,d,e,f]
CSF $A\beta_{42}$ per y	11.8 (23.6)	–3.5 (10.4)	–5.7 (8.2)	9.8 (35.8)	<0.001[a,b,d,e]
CSF pTau181 per y	–0.2 (0.2)	–0.2 (0.2)	0.1(0.6)	0.1 (0.4)	<0.001[b,c,f]
CSF follow up, y	3.0 (2.6)	2.2 (1.9)	2.2 (2.0)	2.7 (2.6)	0.136
Blood biomarkers, mean (SD)					
Serum glucose, mmol/l	4.3 (0.8)	4.1 (0.6)	4.1 (0.8)	4.0 (0.6)	0.246
Serum creatinine, mmol/l	83.2 (21.7)	84.6 (22.0)	82.3 (18.3)	79.4 (16.1)	0.775
Plasma insulin, uIU/mL	0.43 (0.27)	0.27 (0.32)	0.24 (0.32)	0.42 (0.29)	0.001[b,d]
Plasma insulin, uIU/mL per y	–0.04 (0.26)	0.01 (0.20)	0.03 (0.33)	–0.05 (0.24)	0.467
Plasma insulin follow up, y	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	0.84

Aβ, amyloid-β; AD, Alzheimer's disease; CSF, cerebrospinal fluid; pTau, phosphorylated tau; [a]*p* value derived from comparison between A+T– and A–T–. [b]*p* value derived from comparison between A+T+ and A–T–. [c]*p* value derived from comparison between A+T+ and A+T–. [d]*p* value derived from comparison between A–T+ and A+T+. [e]*p* value derived from comparison between A–T+ and A+T–. [f]*p* value derived from comparison between A–T+ and A–T–. *p* values were computed using the one-way analysis of covariance test for age, education, CSF biomarkers, and blood biomarkers. The chi-squared test was used for sex, *APOE* ε4 status, dementia diagnosis, ever smoked, history of alcohol abuse, history of diabetes, and history of diabetes medication use. Tukey HSD *post-hoc* tests were employed for continuous variables. For categorical variables, chi-squared tests followed by Bonferroni corrections were applied to adjust *p*-values for multiple testing.

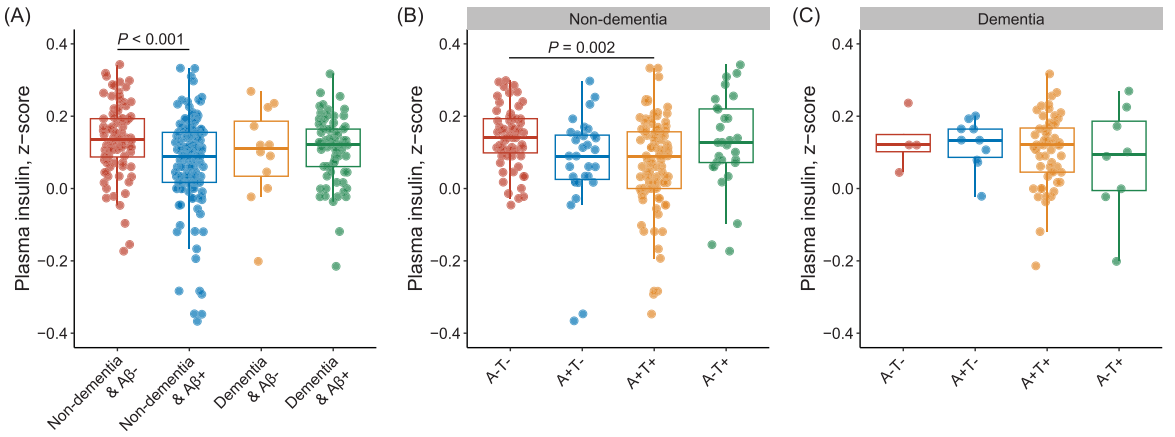

Fig. 1. **Comparison of plasma insulin in different clinical and pathological groups**. The panels showed group differences in plasma insulin among Aβ positive and clinical diagnosis (A), A/T scheme in the non-dementia group (B), and A/T scheme in the dementia group (C). The Box plots depicted the median (horizontal bar), interquartile range (IQR) (hinges), and 1.5 × IQR (whiskers). The Tukey honestly significant difference test was used for multiple group comparisons in different subgroups. The significant *p* values of group comparisons were shown at the top.

However, there was no significant association found between plasma insulin and CSF pTau181 in the whole cohort or its subgroups (Fig. 2B, D).

Longitudinal data were used to further investigate the relationship between plasma insulin and AD pathology. It is noteworthy that higher plasma insulin levels at baseline exhibited a positive association with annual changes in CSF $A\beta_{42}$ ($\beta = 0.19$, $p = 0.006$; Fig. 3A), but baseline CSF $A\beta_{42}$ did not correlate with annual changes in plasma insulin (Fig. 3B). No

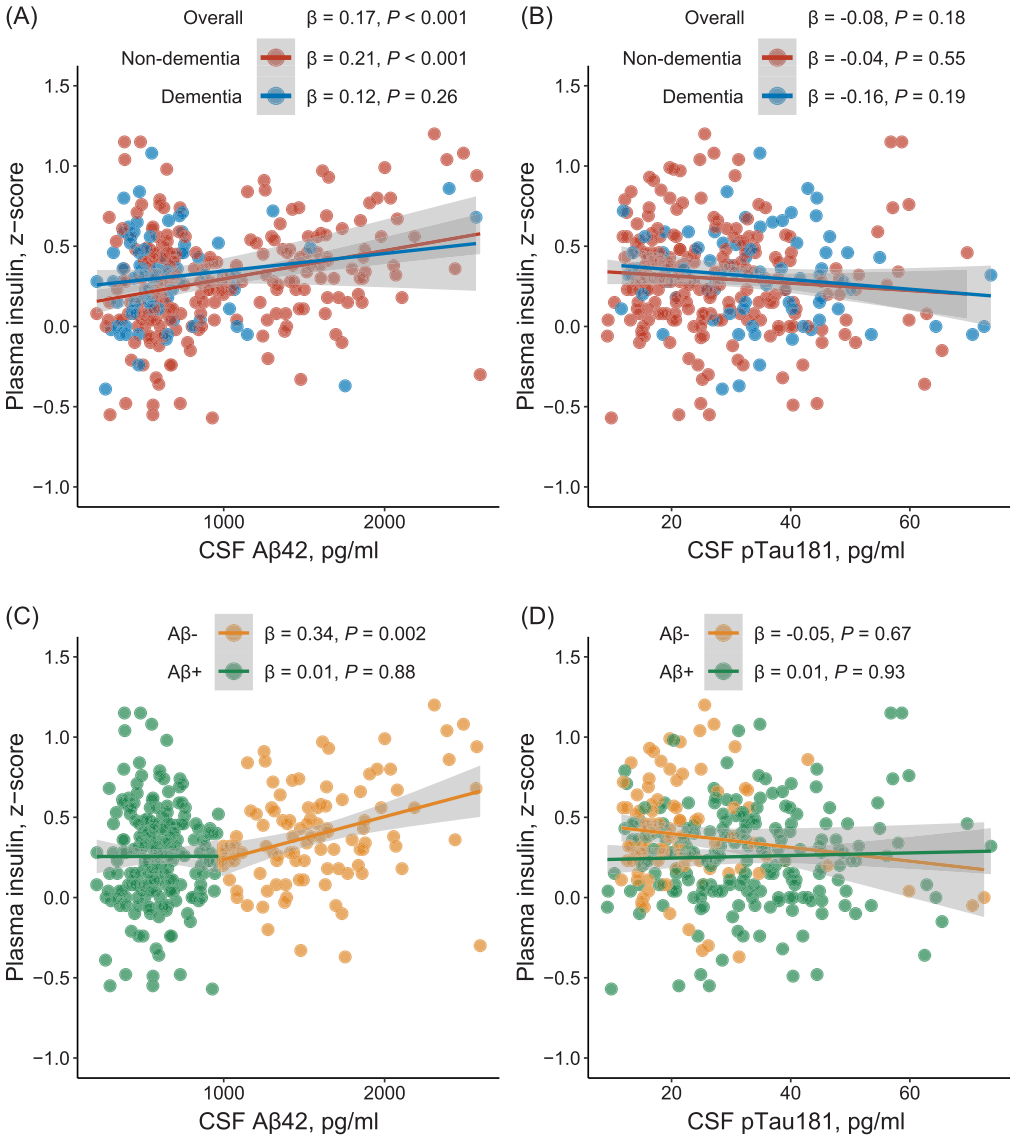

Fig. 2. **Cross-sectional relationship between plasma insulin and CSF AD pathology**. Scatter plots show the cross-sectional associations of plasma insulin with CSF $A\beta_{42}$ and CSF pTau181 in all participants and in different groups defined by clinical groups (A, B) and Aβ status (C, D). The normalized regression coefficients (β) and *p* values shown in scatter plots were derived from multiple linear regression. Linear model fits are indicated together with 95% confidence intervals. These models were adjusted for age, sex, *APOE* ε4 status, education, smoking, alcohol use, serum glucose, and serum creatinine.

significant associations were found between annual changes or baseline CSF pTau181 and plasma insulin (Supplementary Figure 2).

Accuracy of plasma insulin in predicting AD pathology

We evaluated the efficacy of plasma insulin in differentiating Aβ+ from Aβ– participants (Fig. 4). The results showed that plasma insulin alone had modest accuracy across all participants (AUC = 0.61), as well as in non-dementia (AUC = 0.65) but not in dementia groups (AUC = 0.52). Notably, adding plasma insulin to the baseline model (age, sex, *APOE* ε4 status) significantly enhanced its accuracy. The AUC improved from 0.75 to 0.80 (DeLong test $p = 0.014$) in the overall cohort and from 0.75 to 0.79 (DeLong test $p = 0.12$) in the non-dementia group. However, incorporating plasma insulin did not improve the model's accuracy in the dementia group (DeLong test $p = 0.8$).

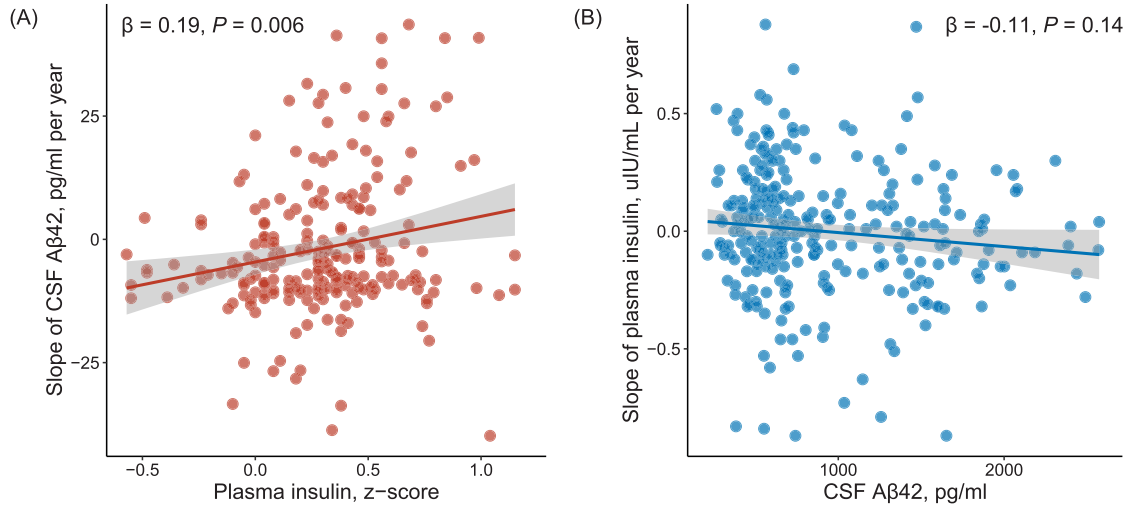

Fig. 3. **Longitudinal relationship between plasma insulin and amyloid pathology**. Scatterplots display the relationships between baseline plasma insulin and the slope of CSF $A\beta_{42}$ (A) and the relationships between baseline CSF $A\beta_{42}$ and the slope of plasma insulin (B). Linear model fits are indicated together with 95% confidence intervals. The normalized regression coefficients (β) and *p* values shown in scatter plots were derived from multiple linear regression, controlling for age, sex, *APOE* ε4 status, education, smoking, alcohol use, serum glucose, and serum creatinine.

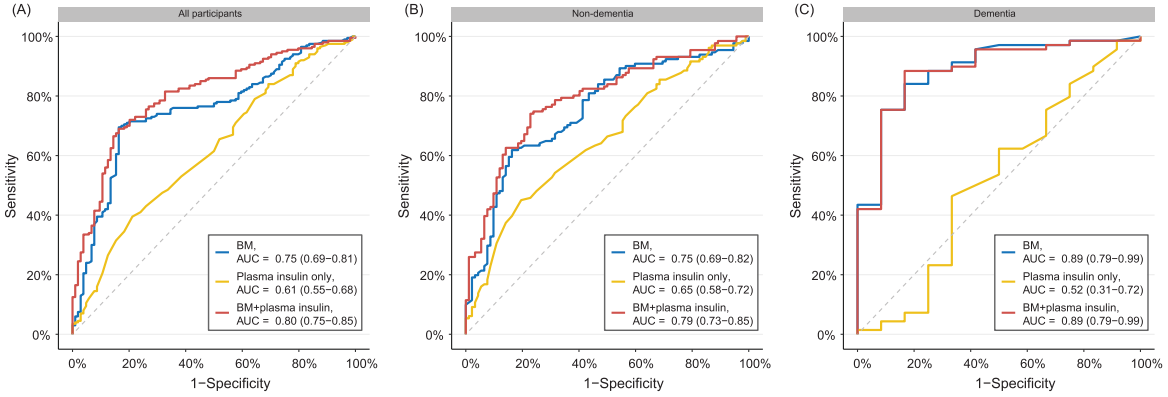

Fig. 4. **Accuracy of plasma insulin in predicting CSF Aβ status**. The receiver operating characteristic (ROC) curves were used to assess the predictive accuracy of plasma insulin for CSF Aβ positive versus CSF Aβ negative in all participants (A) and in individuals with non-dementia (B) and dementia (C). AUC statistic and 95% CI were calculated using predicted probabilities from multivariable binary logistic regression that included age, sex, and *APOE* ε4 status (0 = non-carriers, 1=ε4 carriers) (BM: baseline model) and based on biomarker alone (plasma insulin only), as well as using predicted probabilities from multivariable binary logistic regression that included plasma insulin, age, sex, and *APOE* ε4 status (BM + plasma insulin).

Sensitivity analysis

The primary analysis was further adjusted to consider the effects of diabetes history and diabetes medication use. Following the adjustment, the association between plasma insulin and CSF $A\beta_{42}$ remained significant. This association was consistent across all participant groups, including those in the non-dementia group and those in the Aβ– category, as detailed in Supplementary Tables 1 and 2.

DISCUSSION

In the present study, we examined changes in plasma insulin levels across different pathological and clinical stages of AD. We also tested the cross-sectional and longitudinal association between plasma insulin and AD pathology. The results suggest a significant decrease in plasma insulin levels in the non-demented Aβ+ group compared to the non-demented Aβ- group. In addition, the non-demented

A+T+ group had lower plasma insulin levels compared to the non-demented A–T– group. However, no significant differences were observed within the dementia group. Regression analyses showed a significant correlation between plasma insulin and CSF $A\beta_{42}$ but not CSF pTau181. It is worth noting that baseline plasma insulin levels were associated with longitudinal changes in CSF $A\beta_{42}$, whereas baseline CSF $A\beta_{42}$ did not affect longitudinal changes in plasma insulin. Plasma insulin, combined with AD risk factors such as age, sex, and *APOE* ε4 status, demonstrated high accuracy in discriminating between Aβ+ and Aβ– individuals. These findings provide valuable human evidence supporting plasma insulin as a potential biomarker for predicting early Aβ pathology in the early stages of AD.

Peripheral insulin signaling may affect the clearance and deposition of amyloid pathology in both the brain and blood [31]. Patients with AD exhibit impaired insulin function, which has been correlated with severe amyloid pathology [32] and a high risk of AD [13, 33]. Our study has identified a significant association between higher plasma insulin levels and reduced CSF amyloid pathology, which is evident in both cross-sectional and longitudinal analyses. However, these findings differ from a previous study that reported an inverse relationship between high plasma insulin levels and lower CSF $A\beta_{42}$/tau ratio [22], which is a better marker for reflecting amyloid pathology [34]. We hypothesize that there are several reasons that may account for the observed discrepancies in results [22]. Firstly, the focus of our study differs from that of previous research. While our study investigates the influence of plasma insulin on AD biomarkers, the previous study examined the impact of insulin resistance on AD biomarkers. Additionally, our study includes both male and female subjects, whereas the previous study only had male participants. Furthermore, our sample size is larger, with 304 participants and longitudinal repeated measurement data, compared to their relatively small sample of 58 cases. Finally, the statistical findings were limited and did not take into account the impact of covariates on the relationship between plasma insulin and AD biomarkers. In contrast, our study considered multiple factors that could potentially affect this relationship. Consistent with our study, two previous studies found a significant association between higher blood insulin and lower PET-based Aβ deposition positivity [23, 24].

Our study did not find an association between plasma insulin and CSF pTau181. A previous study explored the association with tau pathology and found a relationship between plasma insulin and CSF tTau [22]. However, there were several differences between that study and ours, as previously described. Most previous research has concentrated on the correlation between diabetes diagnosis and insulin resistance with AD pathology. These studies have identified an association between diabetes and higher insulin resistance with increased levels of CSF pTau181 and CSF tTau, but not CSF $A\beta_{42}$ [8, 35]. Other studies have suggested that Aβ mediates the relationship between insulin resistance and tau biomarkers [36, 37]. Additionally, there are reports linking diabetes and insulin resistance with brain Aβ pathology [20, 38]. It is worth noting that this correlation of insulin resistance may be induced by dietary factors rather than genetics [39]. In summary, these varying findings emphasize the need for further research to systematically investigate the relationship between blood glucose, plasma insulin, insulin resistance, diabetes, and AD pathology.

In 2018, the NIA-AA introduced the pathological AD classification, AT(N), which is useful for defining various stages of AD [34]. Our study reveals that plasma insulin levels are reduced exclusively in the non-dementia Aβ+ group compared to the dementia group. Even lower levels were observed in A+T– and A+T+ compared to A–T– in the non-dementia group, indicating changes in plasma insulin along the AD continuum. Furthermore, it was observed that the correlation between plasma insulin and CSF $A\beta_{42}$ was only present in the Aβ– and non-dementia groups. It is worth noting that the initial plasma insulin levels were associated with changes in CSF $A\beta_{42}$ over time, while the initial CSF $A\beta_{42}$ did not affect changes in plasma insulin over time. The study suggests that changes in plasma insulin may occur before the full deposition of amyloid pathology. This is supported by an *in vivo* study that indicates the presence of a pre-diabetic phenotype, characterized by compromised peripheral glucose tolerance, precedes the formation of Aβ plaques [15, 16]. Furthermore, our longitudinal data indicates no significant association between plasma insulin and CSF pTau. This suggests that plasma insulin signaling may primarily affect early amyloid pathology rather than later stages of AD pathology. These findings reinforce the role of peripheral insulin signaling in the development and progression of amyloid pathology in AD.

The amyloid cascade hypothesis, which is considered central to AD, suggests that Aβ accumulation triggers AD pathogenesis that leads to tau pathol-

ogy, synaptic loss, and cognitive decline [40]. Early detection of amyloid pathology is crucial. However, current detection methods, relying on CSF and PET, are costly, invasive, and technologically demanding, thus limiting their clinical use for amyloid pathology screening [41, 42]. Blood-based biomarkers offer a scalable, minimally invasive solution for amyloid pathology screening in AD patients. Plasma pTau species, including pTau181, pTau217, and pTau231, have demonstrated strong potential in identifying underlying amyloid pathology in AD [42–46]. Whilst plasma insulin alone has limited accuracy in identifying CSF Aβ status, it significantly enhances the predictive accuracy of established AD risk factors (age, sex, *APOE* ε4 status). Adding plasma insulin as a predictor offers valuable additional information (AUC = 0.8). Our longitudinal study further reveals that baseline plasma insulin levels effectively forecast future amyloid changes. These findings, coupled with our previous observations of early plasma insulin alterations preceding amyloid deposition, suggest its potential in predicting early amyloid deposition in the early stages of AD.

The precise mechanisms underlying the association between plasma insulin and Aβ pathology remain unclear. Potential mechanisms may include impacts on glucose metabolism, insulin signaling [10, 14], disruption of the insulin-like growth factor-1 (IGF-1) signaling pathway [47], and modulation of extracellular matrix (ECM) gene expression [47], among others. These mechanisms are commonly shared in both diabetes and AD. Enhancing our comprehension of the connection between plasma insulin and AD pathology is essential for uncovering the underlying links between diabetes and AD.

This study has several strengths, including being the first longitudinal investigation into the relationship between plasma insulin and CSF AD pathology. The incorporation of clinical and pathological AD diagnoses enhances our understanding of this relationship. However, it is important to consider several caveats when interpreting our findings. Firstly, our participants were predominantly of European descent, so our results need to be replicated in diverse ethnic populations for broader applicability. Secondly, our study primarily focused on the relationship between plasma insulin and AD pathology, specifically the biomarkers of $A\beta_{42}$ and pTau181. We acknowledge that other pTau species, such as MTBR-tau243 [47] and pTau217 [48–50], may offer superior performance for predicting tau pathology, but unfortunately, these were not available in the ADNI database at present. Thirdly, our study primarily included non-diabetic individuals, with only a small fraction having a history of diabetes (6%). Therefore, it is uncertain whether the correlation between plasma insulin and amyloid pathology applies to diabetic patients. Further studies are needed to validate our findings in larger cohorts, with longer follow-up durations, and using different designs, such as prospective designs. In particular, these studies should include participants with conditions that could affect insulin levels or AD biomarkers to fully consider the potential impact on the results.

In summary, this study provides new insights into the relationship between plasma insulin and CSF AD pathology. The findings suggest that plasma insulin could predict early Aβ pathology in the early stages of AD. These findings have implications for clinical settings, as plasma insulin may offer a promising target to slow the progression of AD pathology. The early detection of AD pathology through plasma insulin levels may lead to timely interventions, potentially altering the disease course. Furthermore, understanding the mechanistic links between insulin and Aβ pathology might open new avenues for treatment strategies. Importantly, incorporating plasma insulin measurements may enable more precise stratification of patients in clinical settings, thereby enhancing the efficacy of anti-Aβ therapies. This encourages further research into the connection between insulin signaling and AD pathology.

AUTHOR CONTRIBUTORS

Yuhan Chen (Conceptualization; Data curation; Investigation; Methodology; Software; Supervision; Visualization; Writing – original draft; Writing – review & editing); Zhibo Wang (Conceptualization; Data curation; Formal analysis; Methodology; Supervision; Writing – review & editing); Xipeng Liu (Supervision; Writing – review & editing); Zhiqi Mao (Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Writing – review & editing).

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available in the ADNI site, http://adni.loni.usc.edu/.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-240289.

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