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Short Communication

# Association of Peripheral Insulin Resistance and Other Markers of Type 2 Diabetes Mellitus with Brain Amyloid Deposition in Healthy Individuals at Risk of Dementia



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**Abstract**. We explored the association of type 2 diabetes related blood markers with brain amyloid accumulation on PiB-PET scans in 41 participants from the FINGER PET sub-study. We built logistic regression models for brain amyloid status with12 plasma markers of glucose and lipid metabolism, controlled for diabetes and *APOE ε*4 carrier status. Lower levels of insulin, insulin resistance index (HOMA-IR), C-peptide, and plasminogen activator (PAI-1) were associated with amyloid positive status, although the results were not significant after adjusting for multiple testing. None of the models found evidence for associations between amyloid status and fasting glucose or HbA1c.

Keywords: Amyloid-β, apolipoprotein E, plasminogen activator, positron emission tomography, type 2 diabetes

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## INTRODUCTION

The association of type 2 diabetes (DM2) and insulin resistance (IR) with formation of amyloid-β plaques in Alzheimer's disease (AD) is still unclear. DM2 is a risk factor for cognitive impairment and for the clinical diagnosis of probable AD dementia [1]. However, autopsy studies have consistently found that DM2 diagnosis is associated with small vessel pathology and not with AD pathological hallmarks (amyloid-β plaques and neurofibrillary tangles) [2, 3]. IR, an indicator of a pre-diabetic state and a hallmark of DM2, has been associated with amyloid accumulation in middle-aged [4] and late-middle-aged [5], but not in older [6] cognitively healthy individuals. A study of older individuals reported no relation of lifelong IR exposure with either *in vivo* or postmortem measures of brain amyloid pathology [7]. The mechanisms linking amyloid-β accumulation and insulin resistance are unclear, and hypotheses include, e.g., dysregulated hormonal signaling and "type 3 diabetes" of the brain [8]. We explored the association of peripheral blood markers of IR and DM2 with amyloid-β accumulation on PET scans in older individuals at risk for dementia, but without dementia or substantial cognitive decline. Our hypothesis was that we would find associations between exploratory diabetes blood markers and an amyloid positive status in PET.

## METHODS

The study population included 41 participants in the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) exploratory PET sub-study who had available data on IR and DM2-related blood markers (fasting blood glucose, insulin, HbA1c, and a 12-itemBio-Plex Pro Human Diabetes assay). The FINGER main study [9] and PET sub-study [10] have previously been described in detail. Briefly, FINGER investigated the cognitive benefits of a 2-year multi domain lifestyle intervention versus regular health advice in 1,260 individuals aged 60–77 years from the general population. Inclusion criteria were elevated dementia risk based on Cardiovascular Risk Factors, Aging and Dementia (CAIDE) score [11], and cognitive performance at mean level or slightly lower than expected for age according to Finnish population norms for the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test. $^{11}$C-Pittsburgh compound B (PiB) PET scans were conducted at the Turku PET Centre. MR-based attenuation corrected images with summated PET data over 60–90 min were visually assessed as amyloid-β positive or negative by two-party consensus agreement. Positive individuals showed cortical accumulation in at least one AD-typical region, and negative individuals showed only nonspecific retention in white matter [10]. The FINGER study was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa. All participants gave written informed consent at the screening and baseline visits, and for the exploratory neuroimaging sub-study.

In the FINGER study, venous blood samples were taken at baseline in fasting status and using EDTA tubes. Plasma aliquots were stored at –80°C until analysis. Twelve markers related to glucose and lipid metabolism were analyzed using the multiplex suspension array system Bio-Plex Luminex® 200 instrument, (Bio-Rad Laboratories, Hercules, CA, USA), with the Bio-Plex Pro Human Diabetes 10-plex panel (C-peptide; ghrelin; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; glucagon; insulin; leptin; PAI-1; resistin; visfatin) and 2-plex panel (adiponectin, adipsin) (Bio-Rad Laboratories, Hercules, CA, USA). The assays were performed in one batch, and samples preparation and setting of the system running protocol were done following the manufacturer's instructions (www.Biorad.com).

In brief, plasma samples (10 μl) were first diluted (1 : 400 for adipsin adiponectin; 1 : 4 for all the other compounds) using Serum Based Diluent provided by the manufacturer. Assay beads (50 μl) were transferred in the 96-well plates and washed twice with wash buffer. Then, standards and plasma samples (50 μl) were added to the appropriate wells. Plates were incubated for 1 h in a dark room, with mild agitation at room temperature. The fluid was then removed by vacuum and wells were washed three times with wash buffer. Detection antibodies (25 μl) were added to each well, and plates were incubated for 30 min in a dark room, with mild agitation at room temperature. The fluorescent conjugate Streptavidin-Phycoerythrin (60 μl) was then added to each well and plates incubated for 10 min at room temperature. Finally, plates were washed with wash buffer and assay beads were resuspended in assay buffer (125 μl in each well), and plate reading was done with the Bio-Plex Luminex® 200 instrument.

All samples and standards were run in duplicate and were measured as pg/ml. Quality controls were

performed according to the manufacturer guidelines to ensure accuracy of measurements. The intra-assay coefficient of variability (CV) for all compounds ranged from 1 to 2%. The detection limits (in pg/ml) were the following: 32.7 for Adiponectin; 17 for Adipsin; 14.5 for C-peptide; 1.2 for ghrelin; 0.8 for GIP; 5.3 for GLP-1; 4.9 for glucagon; 1 for insulin; 3.1 for leptin; 2.2 for PAI-1; 1.3 for resistin; 37.1 for visfatin). All sample results below the lower limit of quantitation were classified as missing data.

After the plate reading, the results files were generated using Bio-Plex Manager software 4 (Bio-Rad Laboratories, Hercules, CA, USA).

Data from the FINGER baseline visit, before the start of the intervention, was used in this study. Diabetes diagnoses were self-reported at the screening visit interview with the study physician. The homeostatic model assessment of insulin resistance index (HOMA-IR) was calculated based on fasting blood insulin and glucose measures. *APOE* genotype was determined as described previously [12]. We built a logistic regression model for brain amyloid-β status based on log-transformed diabetes markers, and diabetes status (yes/no) and *APOE* ε4 carrier status (yes/no) as confounders. Goodness of model fit was assessed by calculating the percentage of deviance of the fit explained. We corrected for multiple comparisons using the false discovery rate (FDR) method [13]. All analyses were performed using MATLAB R2017b (function mnrfit).

## RESULTS

The participants' mean age (SD) was 71.1 (5.0) years, 51% were female, 15% had diabetes, 29% were *APOE* ε4 carriers, and 39% had amyloid positive PiB-PET scans. All participants diagnosed with diabetes were receiving treatment: five were using oral glucose-lowering medication and one was using a combination of oral glucose-lowering medication and insulin. The individual receiving combination therapy was amyloid negative. Amyloid-β prevalence was 37% in non-diabetics and 50% in diabetics (*p*-value for difference 0.57). *APOE4* prevalence was 31% in non-diabetics and 17% in diabetics (p-value 0.48). One person with diabetes had a history of stroke (amyloid positive), and another person with diabetes had a history of myocardial infarction (amyloid negative).

Table 1 summarizes population characteristics by amyloid-β status. *APOE* ε4 prevalence was significantly higher in the amyloid positive group (56% versus 12%, $p = 0.003$). No statistically significant differences in age, sex, body mass index (BMI), diabetes status, or history of stroke or myocardial infarction were observed. IR and DM2-related markers by amyloid-β status are shown in Table 2. Insulin and plasminogen activator inhibitor-1 (PAI-1) concentrations were significantly lower in amyloid positive compared with negative individuals ($p < 0.05$). C-peptide and HOMA-IRwere also lower in the amyloid positive groups, but differences were significant only at the 90% confidence level. Other markers showed no significant between-group differences.

In the logistic regression model adjusted for diabetes status and *APOE* genotype (Table 2), higher levels of insulin, HOMA-IR, C-peptide, and PAI-1were significantly associated with lower odds of amyloid positivity. After FDR correction, these four markers were significant only at the 90% confidence level. Models with either BMI, age, or sex as additional confounders showed a similar pattern, including after FDR correction. Goodness of fit of the LR model was also highest for models including insulin, HOMA-IR, C-peptide, and PAI-1. Fit was worse in all cases when APOE was removed from the model.

A further exploratory model was built to assess the joint effect of PAI-1 and IR related markers.

Table 1
Baseline population characteristics by amyloid status

| | Amyloid negative | Amyloid positive | *p* *p* |
|---|---|---|---|
| N | 25 | 16 | |
| Sex (% female) | 52% | 50% | 0.914 |
| *APOE* ε4 carrier (%) | 12% | 56% | 0.003 |
| Age | 70.2 (5.6) | 72.4 (3.0) | 0.234 |
| BMI | 28.1 (3.8) | 26.1 (2.8) | 0.080 |
| Diabetic (%) | 12% | 19% | 0.570 |
| History of stroke (%) | 12% | 6% | 1.000 |
| History of myocardial infarction (%) | 8% | 13% | 0.637 |

Table 2
Associations of IR and DM2-related markers with amyloid status

| Metabolic marker | Mean concentration (SD) | | | Logistic regression model | | | | |
|---|---|---|---|---|---|---|---|---|
| | Amyloid negative | Amyloid positive | $p^a$ | B[b] (95% confidence interval) | $p$ | Adjusted $p$ | Goodness of fit[c] with *APOE* | Goodness of fit[c] without *APOE* |
| C-Peptide ($10^3$ pg/ml) | 1.31 (0.61) | 0.95 (0.45) | 0.056 | -5.70 (-10.35 – -1.05) | 0.016 | 0.072 | 33.4 % | 9.8 % |
| Ghrelin ($10^3$ pg/ml) | 1.57 (0.51) | 1.55 (0.39) | 0.936 | 0.11 (-6.07 – 6.29) | 0.972 | 0.998 | 19.0 % | 0.6 % |
| GIP ($10^3$ pg/ml) | 0.29 (0.12) | 0.29 (0.16) | 0.479 | -1.53 (-5.39 – 2.32) | 0.436 | 0.689 | 20.1 % | 1.4 % |
| GLP-1 ($10^3$ pg/ml) | 0.59 (0.11) | 0.58 (0.08) | 0.224 | 0.01 (-8.79 – 8.81) | 0.998 | 0.998 | 19.0 % | 0.7 % |
| Glucagon ($10^3$ pg/ml) | 1.07 (0.23) | 1.00 (0.17) | 0.530 | -2.14 (-11.30 – 7.01) | 0.646 | 0.808 | 19.4 % | 2.8 % |
| Insulin ($10^3$ pg/ml) | 0.27 (0.17) | 0.17 (0.09) | 0.036 | -4.54 (-8.26 – -0.81) | 0.017 | 0.072 | 33.2 % | 13.8 % |
| Leptin ($10^3$ pg/ml) | 7.55 (4.79) | 6.06 (5.15) | 0.145 | -1.63 (-4.07 – 0.81) | 0.191 | 0.569 | 22.2 % | 3.6 % |
| PAI-1 ($10^3$ pg/ml) | 5.31 (1.32) | 4.16 (0.62) | 0.003 | -13.27 (-23.93 – -2.61) | 0.015 | 0.072 | 36.0 % | 20.0 % |
| Resistin ($10^3$ pg/ml) | 2.22 (0.56) | 2.03 (0.46) | 0.316 | -3.67 (-10.13 – 2.79) | 0.266 | 0.569 | 21.3 % | 2.2 % |
| Visfatin ($10^3$ pg/ml) | 4.83 (2.25) | 4.43 (2.30) | 0.224 | -2.03 (-6.77 – 2.71) | 0.401 | 0.689 | 20.3 % | 2.8 % |
| Adiponectin ($10^6$ pg/ml) | 5.45 (3.44) | 6.03 (3.82) | 0.548 | -0.25 (-2.30 – 1.79) | 0.808 | 0.932 | 19.1 % | 1.2 % |
| Adipsin ($10^6$ pg/ml) | 1.21 (0.46) | 1.45 (0.89) | 0.925 | 1.08 (-2.06 – 4.22) | 0.500 | 0.689 | 20.1 % | 2.1 % |
| fP-Glucose (mmol/l) | 5.92 (0.88) | 6.30 (1.17) | 0.303 | 4.80 (-9.33 – 18.93) | 0.505 | 0.689 | 19.8 % | 2.4 % |
| B-HbA1c (mmol/mol) | 36.72 (4.11) | 37.25 (4.63) | 0.957 | 14.96 (-10.94 – 40.86) | 0.258 | 0.569 | 21.4 % | 0.7 % |
| HOMA-IR (mmol·mU/l²) | 2.06 (1.24) | 1.33 (0.71) | 0.060 | -4.52 (-8.31 – -0.74) | 0.019 | 0.072 | 32.4 % | 11.9 % |

Regression model: $\ln(Y_{A+}/Y_{A-}) = C + B_{DM} \cdot X_{DM} + B_{APOE} \cdot X_{APOE} + B \cdot \log(X)$. [a]Significance tested using the Mann-Whitney U test. [b]Coefficient of log-transformed values. [c]Goodness of fit expressed in percentage of deviance explained. fP-Glucose, fasting plasma glucose; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; PAI-1, plasminogen activator inhibitor-1; B-HbA1c, blood glycated hemoglobin.

With DM status, *APOE*, and PAI-1 as confounders, *p*-values were insignificant for insulin, HOMA-IR, C-peptide, fasting glucose, and HbA1c. The coefficient of PAI-1 was insignificant in models including insulin, HOMA-IR, and C-peptide, and significant for fasting glucose, HbA1c, and all other tested analytes.

## DISCUSSION

Results from the FINGER PET exploratory substudy suggested slightly better insulin homeostasis and related markers in amyloid positive older individuals at risk for dementia, but without dementia or substantial cognitive impairment. Although DM2 has been indicated as a risk factor for dementia and AD [1], it is not fully clear whether the underlying mechanisms are amyloid-related. Peripheral IR has been linked to IR in the brain, which may affect amyloid-β pathology through e.g. neuroinflammatory pathways or competitive cleavage of insulin and amyloid-β by the same enzyme [8]. However, previous studies investigating peripheral IR and brain amyloid-β in CSFor on PET scans in cognitively healthy individuals have reported no associations at older ages, and mixed findings in middle-aged populations [4–6, 14]. Ekblad et al. [4] found that midlife HOMA-IR, taken 15 years previous to the PET -scan, associated with greater brain amyloid-β accumulation in elderly individuals without dementia, in both carriers and noncarriers of *APOE* ε4 genotype, but the same association was not detected at the time of the scan in late-life. No associations have also been reported in people with mild cognitive impairment or AD [6].

Our results contrast with earlier no-association findings in the elderly, possibly due to the specific risk profile of the FINGER population. Peripheral IR and DM2 may also contribute to cognitive decline via vascular-related mechanisms. Interestingly, an autopsy study in the 85+ age group showed less amyloid-β pathology and more cerebrovascular pathology in people with diabetes, who also had an increased risk of AD dementia [15]. It was suggested that, in people with diabetes and vascular pathology, less amyloid-β pathology may be needed to trigger the onset of dementia. FINGER study participants had cognitive performance at the mean level or slightly lower than expected for age, and they were also at risk for dementia based on the CAIDE score including age, sex, education, BMI, systolic blood pressure, total cholesterol, and physical activity [11]. The cognitive performance of a participant could be hypothesized to be determined by a similar interplay of amyloid-β- and vascular-related mechanisms, e.g., poorer insulin homeostasis and higher vascular-related risk in amyloid negative individuals. It is also possible that FINGER participants with lower IR represent a selected group that has maintained better insulin homeostasis despite elevated cardiovascular risk. The mechanisms linking IR and amyloid-β accumulation may be different in this specific group.

To our knowledge, this is the first study investigating the association of peripheral blood PAI-1 level and *invivo* brain amyloid-βmarkers. Higher PAI-1 level seemed to be protective against amyloid-β accumulation, although not significantly after correction for multiple comparisons. The effect was not seen in models including both PAI-1 and IR-related markers. PAI-1 in CSF has in prior studies been reported to have no association with AD status [16, 17]. PAI-1 down regulates the activity of the protein-cleaving plasmin system, and it is considered a risk factor for atherosclerosis due to its prothrombotic effect. In the population-based Framingham Offspring Study, higher fasting insulin level was associated, among other things, with increased PAI-1 levels [18]. In the brain, however, PAI-1 and the plasmin system may interact with amyloid-β fibrils and possibly affect plaque formation [19], or be directly neuroprotective [20]. A previous study showed that increases in blood levels of PAI-1 were associated with white matter integrity loss in stroke-free, cognitively normal individuals aged 50–65 years and also reported an association of PAI-1 with lower performance in speed or visuomotor coordination [21]. It is unclear if the association suggested in the present study was mediated through effects in the brain, or if amyloid-β load was reduced due to effects in the cardiovascular system.

The main strength of this study is the assessment of a comprehensive assay of IR and DM2-related markers in relation to brain amyloid-β accumulation on PiB-PET, which has not been previously done. However, the small sample size is a key limitation, restricting statistical power and the ability to control for other potentially confounding factors. Additionally, overall amyloid-β status was determined visually from scans, and no region-specific analyses were conducted. Our exploratory study adds to the growing amount of data on the associations of IR and DM2-related markers with AD-related pathology. Future studies in larger populations and with longitudinal data should further investigate these associations, taking into account, e.g., *APOE* genotype, degree of vascular pathology, type of DM2 treatment, and level of glycemic control.

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