Running title: Alzheimer's disease, insulin resistance, CSF biomarkers

Insulin resistance is associated with increased Alzheimer disease pathology and reduced memory function in at risk healthy middle-aged adults

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Abstract

Background: Metabolic disorders in midlife increase risk for Alzheimer's disease (AD). Dysfunction in insulin signaling is suspected to increase the formation of $A\beta_{42}$ peptides and is thought to be associated with impaired memory function. Insulin resistance (IR) observed in midlife prior to indication of neurodegeneration and cognitive decline is a candidate risk factor that can be modified by targeted interventions. Regulation of normal insulin function, as a modifiable risk factor, may be important in reducing the prevalence of AD, particularly in individuals who harbor a genetic risk or have a parental family history of AD. The relationship between IR and AD pathology remains poorly understood, particularly at the preclinical stage of the disease.

Objective: The objective of the current study was to examine whether IR is associated with increased AD pathology and decreased memory performance in asymptomatic middle-aged adults enriched for AD (i.e. with a parental family history of sporadic late-onset AD). We postulated that IR would be associated with greater AD pathology, particularly in carriers of the *APOE* ε4 allele, and poorer memory performance.

Method: Asymptomatic middle-aged adults with a parental family history of AD (N=70, mean age=57.7 years) from the Wisconsin Alzheimer's Disease Research Center underwent lumbar puncture, blood draw and neuropsychological testing. Cerebrospinal fluid (CSF) samples were assayed for AD related biomarkers including soluble amyloid precursor protein β (sAPP-β), abeta$_{42}$ ($A\beta_{42}$) and phosphorylated tau (P-tau$_{181}$). The ratio P-tau$_{181}$/$A\beta_{42}$ was also examined as a more sensitive measure of AD pathology with respect to *APOE* ε4 status, IR and memory function. IR was indexed by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Data were analyzed using linear regression models to determine significant effects of IR and *APOE* ε4 on CSF biomarkers of AD and memory performance.

Results: Both *APOE* ε4 carriage and higher IR were associated with higher sAPP-β. *APOE* ε4 carriers were observed to have significantly higher levels of AD pathology (P-tau$_{181}$/$A\beta_{42}$) compared to non-carriers. Investigation of memory performance revealed that higher IR and the

concurrent presence of AD pathology (P-tau$_{181}$/Aβ_{42}) were associated with worse delayed memory performance.

Conclusion: The current study provides evidence that IR in middle age is associated with higher sAPP-β, a soluble marker of amyloidogenic APP-processing. Further, these results reveal that IR and AD pathology concomitantly result in subclinical memory impairment in asymptomatic middle-aged individuals enriched for AD. Lastly, these findings converge with prior studies indicating that *APOE* ε4 carriage is associated with increased AD pathology that can be observed in early aging prior to the onset of clinical memory impairment. This study has implications for development of interventions targeted at reducing the prevalence of IR through modifiable lifestyle factors, particularly in individuals who harbor the ε4 allele.

Keywords: insulin resistance, CSF AD biomarkers, *APOE*ε4, memory function

1. Introduction

Epidemiological studies have provided mounting evidence that insulin resistance (IR) is associated with an increased risk for Alzheimer's disease (AD) (Arvanitakis et al., 2004; Ott et al., 1999; Peila et al., 2002; Schrijvers et al., 2010). Over the past fifty years, the rate of type 2 diabetes mellitus (a non-insulin dependent form of diabetes mellitus) has increased alarmingly with approximately 387 million individuals diagnosed with the disease as of 2014. Recent evidence indicates that cognitively healthy individuals in late-middle life with higher IR harbor more AD-like neuropathology (Starks et al., 2014, Willette et al., 2015). Such studies provide support that IR may be an underlying mechanism driving an increased risk for development of AD pathology and that neurodegenerative changes can be observed prior to the onset of marked cognitive or behavioral impairment. While several animal studies suggest a mechanistic link between IR and neuropathology (for review see de la Monte and Wands, 2005), the effect of IR on the development of AD pathology among humans remains poorly understood, particularly at the preclinical stage of the disease. Identification of modifiable risk factors in midlife may be particularly relevant to individuals who harbor known genetic risk factors for AD (e.g., *APOE* ε4) or who have parental family history of AD. Provided the increasing prevalence of Type 2 diabetes and a growing incidence of dementia, elucidation of neuropathologic processes that arise from IR that may be associated with increased risk of AD is of paramount importance.

Efficient utilization of insulin by neurons underlies normal cellular and cognitive function. Insulin signaling regulates processes critical for neuronal survival and synaptic plasticity moderating processes such as glucose uptake and energy production through glucose oxidation (Cersosimo and DeFronzo, 2006). A growing body of evidence has shown that insulin plays an integral role in learning and memory by modulating excitatory and inhibitory receptors such as glutamate and GABA receptors, respectively (see Zhao et al., 2004a). Insulin homeostasis is believed to be an instrumental component in signal transduction cascades that underlie memory consolidation and long-term memory function (Cardoso et al., 2009; Zhao et al., 2004a; Zhao and Aklon 2001). It is suspected that brain regions dense with insulin receptors, such as the medial temporal and frontal lobes, are preferentially sensitive to insulin signaling. Interestingly, frontal and medial temporal lobe regions, particularly the hippocampus, are some of the first to be adversely affected in AD (Craft & Watson, 2004; Henneberg & Hoyer, 1995; Zhao et al.,

2004). Cognitively healthy individuals with reduced peripheral insulin function show subtle cognitive deficits and reduced cerebral glucose metabolism and diminished cerebrocortical activity (Baker et al., 2011; Tschritter et al., 2006). Among cognitively intact older adults, reduced glucose tolerance has been associated with poor memory performance and hippocampal atrophy (Convit et al., 2003). Further, abnormal glucose metabolism—believed to arise from desensitization of cerebral insulin receptors—is a core feature of sporadic late-onset AD (Henneberg & Hoyer, 1995) and a number of human studies have provided evidence for a linkage between Type 2 diabetes and greater risk for developing AD (Li et al., 2015; Peila et al., 2002). Histology from human postmortem studies has provided evidence that insulin pathways are severely degraded in AD brains showing a vast reduction in insulin receptor expression and insulin receptor binding (de la Monte & Tong, 2014). These findings are suggestive of an integral role of insulin dysfunction in the pathogenesis of AD.

The proteolytic processing of amyloid precursor protein (APP) is believed to be one of many neurobiological processes negatively affected by IR that may contribute to AD pathology. APP is processed by two competing pathways: the amyloidogenic β-secretase-mediated and the nonamyloidogenic α-secretase-mediated pathways. Cleavage of APP by α-secretase is thought to mitigate the formation of extracellular amyloid plaques by preventing the formation of amyloid beta (Aβ). APP processed through the α-secretase pathway is cleaved within the Aβ sequence producing soluble peptides (Nunan and Small, 2000). By contrast, APP cleavage by β-secretase produces sAPP-β that consequently results in generation of Aβ peptides by endoproteolytic processing (Vassar et al., 1999). In the β-secretase pathway cleavage by γ-secretase adjacent to residue 42 is the last step in formation of the 42 amino acid species $A\beta_{42.}$ Among the Aβ peptides, $A\beta_{42}$ is implicated in the production and proliferation of amyloid plaques that arise from the aggregation and oligomerization of $A\beta_{42.}$ Amyloid plaques are a hallmark of AD pathology and amyloidogenic processes such as $A\beta_{42}$ oligomerization is suspected to be involved in neuronal dysfunction and cell death. Over the past decade a number of studies have provided evidence that $A\beta_{42}$ oligomers have neurotoxic effects that include degradation of synaptic structure (Lacor et al., 2004, 2007) and disruption of molecular and cellular mechanisms integral to memory formation such as synaptic transmission and plasticity (Rowan et al., 2003; Shankar et al., 2008; Lambert et al., 1998). Transgenic mouse models provide evidence that IR promotes

activation of the amyloidogenic pathway via cleavage of amyloid precursor protein by β-secretase (sAPP-β) (Farris et al., 2003; Gasparini et al., 2001) and that overproduction of sAPP-β is associated with disrupted synaptic transmission and plasticity in the hippocampus (Rowan et al., 2003). Congruent with these findings, a study of diet-induced IR in Tg2576 transgenic mice showed that IR promoted amyloidogenic Aβ production, increased AD-type amyloid plaque burden, and impaired performance on hippocampal-dependent memory tasks (i.e., allocentric spatial water maze) (Ho et al., 2004).

Other risk factors for AD include non-modifiable risk factors such as genetic risk, parental family history of AD and age. It is known that carriage of one or two ε4 alleles of apolipoprotein E (*APOE* ε4) is a major genetic risk factor for AD. Several studies on asymptomatic *APOE* ε4 carriers compared to non-carries have provided evidence that ε4 allele carriers harbor features characteristic of AD pathology such as hypometabolism (Reiman et al., 2004, 2005; Small et al., 2000), higher amyloid burden (Corder et al., 1993) and gray matter atrophy in AD-sensitive regions (Lemaitre el a., 2005). Animal studies have provided evidence that *APOE* ε4 carriage may be particularly deleterious to the hippocampus showing that *APOE* ε4 mice are impaired on hippocampal-dependent tasks such as the Morris Water Maze, object recognition and context fear conditioning (Salomon-Zimri et al., 2013). Thus *APOE* ε4 has been implicated in several AD-relevant pathways, including amyloid accumulation, brain hypometabolism and gray matter degradation.

Several studies have shown that asymptomatic late-midlife individuals with parental family history of AD (FH+) show early pathological changes in AD-sensitive regions (Adluru et al., 2014; Honea et al., 2010, 2011; Johnson et al., 2006, 2014; Mosconi et al., 2010; Okonkwo et al., 2012). Neurodegeneration and neuropathology observed in these individuals is consistent with, although substantially less severe, pathology observed in mild cognitive impairment (MCI) and AD patients. As genetic and familial risk factors are invariable, modifiable behaviors that may decrease the risk of developing AD are of clinical interest. Development of intervention-based strategies aimed at preventing or reversing IR in midlife may help to significantly reduce one's risk of incipient neuropathological changes that may underlie or contribute to the etiopathogenesis of AD. Midlife is a critical time period during which lifestyle factors that

confer risk for AD may be altered. While carriage of the ε4 allele and parental family history of AD are known to increase Alzheimer's disease risk moderating effects of these factors on molecular mechanisms alone are not sufficient to cause the disease.

To investigate the association between IR and AD pathology observed in healthy middle-aged adults enriched for AD [i.e. parental family history of AD (FH+)], the current study examined the effect of IR on cerebrospinal fluid (CSF) biomarkers of AD and a hippocampal-dependent memory task (i.e., delayed recall). Specifically we examined the effects of IR and *APOE* ε4 status on CSF sAPP-β, $A\beta_{42}$, P-tau_{181} and P-$tau_{181}/A\beta_{42}$, and performance on the Rey Auditory Verbal Learning Test (RAVLT), delayed recall. *APOE* ε4 status was also examined as an invariable risk factor for AD pathology in midlife. We hypothesized that both IR and *APOE* ε4 would be associated with 1) increased CSF biomarkers of neural injury and amyloid burden and 2) decreased memory performance. Given prior studies showing that the effects of IR may depend on *APOE* ε4 carriage, we also tested for interactions between IR and *APOE* ε4 on CSF biomarkers and memory function.

2. Methods and Materials

2.1. Participants

The current study examined 70 asymptomatic middle-aged adults (mean age= 57.7 years, SD= 5.11, range= 46-66, 78.6% female) from the Wisconsin Alzheimer's Disease Research Center (ADRC) Investigating Memory in People At risk, Causes and Treatments (IMPACT) cohort (Table 1). All participants had a parental family history of sporadic AD (FH+) defined as one or both biological parents meeting AD clinical diagnosis criteria (McKhann et al., 1984, 2011). Parental family history was determined by a validated interview reviewed by a multidisciplinary diagnostic consensus panel (Kawas et al., 1994) or postmortem neuropathological diagnosis of AD. Individuals underwent lumbar puncture, fasting blood draw and comprehensive neuropsychological testing. Inclusion criteria entailed no history of a clinical diagnosis of diabetes or current diabetes treatment. Fasting plasma glucose (FPG) was evaluated to exclude individuals who met FPG criteria for diabetes diagnosis (i.e. FPG > 125 mg/dL). All participants were required to have normal cognitive function, as determined by neuropsychological evaluation and consensus review, and no current diagnosis of major psychiatric illness.

Participants were defined as *APOE*ε4 positive if they harbored at least one copy of the ε4 allele. Hetero- and homozygous ε4 allele carriers constituted 47% of the sample.

2.2. Design and Procedure

To assess insulin resistance and biomarkers of CSF AD pathology, venous blood and CSF were collected in the morning after a 12h fast. Blood samples were collected in 9ml polypropylene tubes, allowed to clot for 30mins and centrifuged at 4*C at 3000 rpm for 10mins. Cell-free plasma/serum was aliquoted into 1.5mL micro centrifuge tubes and frozen at -80 degrees Celsius. Plasma and serum samples were analyzed at the University of Wisconsin Hospital and Clinics Hospital Laboratory (Madison, WI). To assess fasting glucose, plasma was assayed using hexokinase glucose method (Siemens Dimension Vista). To assess fasting insulin, serum was assayed using chemiluminescent immunoassay on an ADVIA Centaur XP Immunoassay System (Siemens Corporation, Washington DC, USA). Insulin resistance was calculated from fasting serum insulin and fasting plasma glucose using the homeostatic model assessment of insulin resistance (HOMA-IR) method (Matthews et al., 1985) calculated as HOMA-IR = Insulin (mg/dL) x Glucose (uIU/mL) / 405.

CSF was collected through gentle extraction via lumbar puncture using a Sprotte 25- or 24-gauge spinal needle at the L3/4 or L4/5 level of the spinal column. Approximately 22mL of CSF was extracted, gently mixed and centrifuged at 2000g for 10mins. Supernatants were frozen in polypropylene tubes in 0.5mL aliquots and stored at -80 degrees Celsius. CSF was assayed for P-tau_{181} and $A\beta_{42}$ using commercially available enzyme-linked immunosorbent assay (ELISA) methods (INNOTEST assays, Fujiurebio, Ghent Belgium) as previously described in detail (Palmqvist et al., 2014). CSF sAPP-β was measured using the MSD Multiplex Soluble APP assay (Meso Scale Discovery, Rockville, MD), as described by the manufacturer. Board-certified laboratory technicians blinded to clinical information analyzed all samples in accordance to protocols approved by the Swedish Board of Accreditation and Conformity Assessment (SWEDAC). One batch of reagents was used yielding intra-assay coefficients of <10 % variation. The P-tau_{181}/$A\beta_{42}$ ratio was examined as a marker of multiple pathological processes that occur in AD.

APOE ε4 status was determined using genetic testing by the Wisconsin Alzheimer's Disease Research Center. Genotyping was performed using non-fasting blood sample collected at baseline visit, using standard polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA) sequencing techniques. DNA extracted from whole blood was genotyped with the use of a homogenous Florescent Resonance Energy Transfer technology coupled to competitive allele specific PCR (LGC Genomics; Beverly, MA). The National Cell Repository for Alzheimer's disease (NCRAD) also performed genotyping. There was 100% concordance for APOE genotyping between these analyses.

Participants underwent a comprehensive neuropsychological battery that included the Rey Auditory Verbal Learning Test (RAVLT) (Rey, 1941) a widely used and well-validated assessment of memory function. The delayed component of the RAVLT is designed to examine long-term memory function and is a well-known measure that assesses cognitive changes from intact, to MCI to AD patients (Estevez-Gonzalez et al., 2003; Ewers et al., 2012). In the current study the RAVLT delayed task was chosen as a focal index of long-term hippocampal-dependent memory function.

2.7. Statistical Analyses

Linear regression analyses were conducted in SPSS (Version 21.0). Analyses tested main effects of IR as indexed by HOMA-IR and *APOE* ε4 status, and the interaction between HOMA-IR and *APOE*ε4 on CSF biomarkers of AD and memory performance. A secondary analysis tested the interaction between HOMA-IR and CSF biomarkers of AD on delayed memory performance. Age, sex and body mass index (BMI) were included as covariates in all analyses. Education was added as a covariate in analyses that included neuropsychological test measures (i.e., RAVLT delayed) as a dependent variable.

3. Results

3.1. HOMA-IR, *APOE* ε4 and CSF biomarkers of AD

Linear multiple regression analysis revealed that HOMA-IR and *APOE* ε4 status were significant predictors of CSF sAPP-β. Higher HOMA-IR was significantly associated with increased levels of CSF sAPP-β ($F_{[1,63]} = 4.21$, $p = 0.044$) (Figure 1). Further, carriage of the ε4 allele significantly

predicted higher levels of CSF sAPP-β ($F_{[1,63]}$ = 7.74, p = 0.007) (Figures 2). The interaction between HOMA-IR and *APOE* ε4 on CSF sAPP-β was not significant.

Significant relationships between CSF $A\beta_{42}$ and predictor variables and CSF P-tau_{181} and predictor variables were not found, however, a significant effect of *APOE* ε4 status on the ratio of CSF P-$tau_{181}/A\beta_{42}$ was observed ($F_{[1,63]}$ = 5.21, p = 0.026). CSF P-$tau_{181}/A\beta_{42}$ was significantly greater in *APOE* ε4 carriers compared to non-carriers (Figure 3).

3.2. HOMA-IR, *APOE* ε4, CSF biomarkers of AD and Memory Performance

To investigate independent and concomitant effects of IR, genetic risk and AD pathology on memory function, HOMA-IR, *APOE* ε4 and P-$tau_{181}/A\beta_{42}$ were examined with respect to delayed memory score on the RAVLT. Analyses yielded a significant interaction between HOMA-IR and P-$tau_{181}/A\beta_{42}$ on memory performance ($F_{[1,60]}$ = 6.14, p = 0.016). Higher HOMA-IR and greater P-$tau_{181}/A\beta_{42}$ predicted impaired delayed memory performance (Figure 4). No other significant interactions were observed.

4. Discussion

IR is associated with an increased relative risk for developing sporadic late-onset AD. Dysregulation of insulin signaling is thought to contribute to a cascade of neuropathological changes that promote amyloidogenic processing, neurotoxicity, and brain amyloidosis (Devi et al., 2012; Henneberg and Hoyer, 1995; Ho et al., 2004; Willette et al., 2015; Zhao et al., 2009). Brain changes associated with IR that may underlie or contribute to the pathogenesis of AD are poorly understood, particularly in midlife prior to clinical disease onset. IR is a modifiable risk factor and thus regulation of normal insulin signaling is an important target for early intervention, one that may be particularly relevant to persons who harbor genetic risk or have a parental family history of AD. The current study aimed to investigate the relationship between IR, AD pathology and memory performance in middle-aged individuals enriched for AD. As a subsidiary aim, we examined *APOE*ε4 genotype relative to IR, AD pathology and memory function, as it is a well-established genetic risk factor for AD.

Our findings revealed that higher IR and carriage of the ε4 allele were both predictors of increased sAPP-β in CSF, suggesting preferential cleavage of APP through the amyloidogenic β-

secretase pathway. Evidence from animal studies shows that IR promotes amyloidogenic β-amyloid peptide production and upregulated levels of β-site APP cleaving enzyme 1 (BACE1), a process that generates $A\beta_{42}$ (Devi, et al., 2012). Although higher IR was associated with an increase in sAPP-β, a relationship between IR and $A\beta_{42}$ was not observed. It is known that cleavage of APP through the β-secretase pathway results in the formation of sAPP-β and the eventual generation and deposition of Aβ. While the generation of $A\beta_{42}$ is complex and the relationship between sAPP-β and $A\beta_{42}$ is not 1:1, a reasonable prediction would be that an association between higher IR and increased levels of sAPP-β would yield an inverse relationship between IR and $A\beta_{42}$. Prior research from our group has shown that IR predicts brain amyloid deposition in late middle-aged adults at risk for AD as indexed by [C-11] Pittsburg compound B (PiB) positron emission tomography (Willette et al., 2015). Given that we did not find an effect of IR on Aβ42, one could postulate that in the case of IR, $A\beta_{42}$ is overproduced and begins depositing in the brain but mechanisms of clearance through the CSF remain intact and appear to be within normal range during subclinical stages of the disease. The participants studied here were also younger than in Willette et al (2015). Longitudinal follow-up on CSF and amyloid-PET will provide further information for mapping out longitudinal trajectories of amyloid deposition.

IR may also be linked to pathological processes via amyloidosis by decreasing availability of insulin-degrading enzyme (IDE), a large zinc-binding protease that binds to and degrades insulin. IDE preferentially binds to insulin but also has an affinity for Aβ proteins. In the absence of IR, IDE is available to bind to and degrade Aβ proteins. In the case of IR, IDE preferentially binds to the elevated levels of insulin as it aggregates in extracellular space, resulting in less availability for IDE to be allocated toward degradation of Aβ proteins. Excess amyloid peptides consequently lead to the formation of amyloid plaques, brain degeneration and neuronal dysfunction. Animal studies have provided evidence that selective removal of the IDE gene results in more than a 50% decrease in Aβ degradation and a significant increase in Aβ deposition (Farris et al., 2003; Ho et al., 2004). Transgenic mouse models of IR have provided evidence that IDE may not only be affected by IR through interference of IDE-mediated degradation of Aβ but may also decrease IDE expression and activity (Ho et al., 2004). Further, recent research has provided evidence that IDE also degrades sAPP-β in the intracellular domain

(Edbauer et al., 2002) and that elevated levels of sAPP-β were observed in homozygous deletions of the IDE gene (Farris et al., 2003). These results are aligned with the notion that IR may lead to increased leaves of sAPP-β through mechanisms that involve diminished IDE availability and reduced sAPP-β degradation.

Carriage of the ε4 allele of *APOE* was also a significant predictor of CSF markers of AD pathology. *APOE* ε4 status showed a positive relationship with sAPP-β and the ratio of P-tau$_{181}$/Aβ_{42} although significant relationships between *APOE* ε4 carriage and Aβ_{42} or P-tau$_{181}$ were not observed. These results validate the notion that P-tau$_{181}$/Aβ_{42} may be a more sensitive index of AD pathology in preclinical populations compared to a single marker of disease and provide further evidence that multiple pathological processes associated with AD occurs at a subclinical threshold in asymptomatic midlife persons, particularly ε4 allele carriers. *APOE* ε4 status is a strong predictor of AD pathology and is believed to act on the Aβ pathway, leading to a reduction in clearance and increased Aβ aggregation and deposition (Schmechel et al., 1993). Our findings provide further support that ε4 allele carriage is associated with AD pathology, and extends these findings to increased upstream amyloidogenic processing (i.e., sAPP-β) in healthy middle-aged persons enriched for a family history of AD.

Interestingly, we found that high IR and P-tau$_{181}$/Aβ_{42} concomitantly act to impair memory performance on the RAVLT delayed memory. Long-term memory tasks—such as the one examined here—are dependent on the hippocampus, a medial temporal lobe structure negatively affected by IR (Benedict et al., 2012; Rasgon et al., 2011; Stranahan et al., 2008) and among the first regions to show structural and functional changes in AD (Convit et al., 2000; Du et al., 2001; Pennanen et al., 2004, Wang et al., 2006). Research from our group has shown that IR in midlife is associated with hypometabolism in brain regions involved in episodic memory, including the hippocampus (Willette et al., 2015). Taken together, our findings suggest that dysregulation of the insulin system in midlife may have deleterious effects on functional integrity of the hippocampus that precede or act in concert with early pathological AD changes leading to subclinical memory dysfunction. It is worth noting that while we found that high IR and AD pathology interacted to impair memory function, we did not observe an interaction with ε4 allele carriage. This finding suggests that while AD pathology is more prominent in *APOE* ε4

carriers in midlife, the combined deleterious effects of IR and AD pathology on memory performance is observed across carriers and non-carriers alike.

Overall, our findings provide evidence that pathological processes associated with AD are observed early in aging, prior to the onset of memory difficulty or manifestation of clinically significant symptoms of cognitive impairment. IR and carriage of the ε4 allele are predictors of early changes in AD-related CSF biomarkers in persons enriched for parental history of late-onset sporadic AD. We show that IR and AD pathology interact to impair long-term memory function in middle age. IR is a modifiable risk factor that may be corrected with targeted interventions. Insulin homeostasis plays an integral role in maintaining healthy neuronal function, normal cerebral glucose metabolism and reducing or ameliorating pathological processes associated with AD. Targeted interventions designed to maintain normal insulin signally may be particularly affective in delaying or ameliorating AD disease onset in persons with invariable risks for AD.

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Table 1

Demographic, glucoregulatory, and genetic data	
Sex	55 female, 15 male
Age (years)	57.7 ± 5.1
Education (years)	15.9 ± 2.5
BMI (kg/m^2)	28.66 ± 5.08
HOMA-IR	2.26 ± 1.24
Diabetes status	
Normoglycemic (fasting glucose < 100 mg/dL)	61
Prediabetic (fasting glucose < 126 mg/dL)	9
APOE genotype	
*APOE*ε4 (hetero- or homozygous)	33 (47%)

Include sAPP-beta, Abeta42 and P-tau data in this table, and perhaps also have another table with the same data comparing IR with non-IR groups?

Figures

Figure 1. Association between HOMA-IR (log) and CSF sAPP-β across all participants. Units are adjusted for age, sex and BMI.

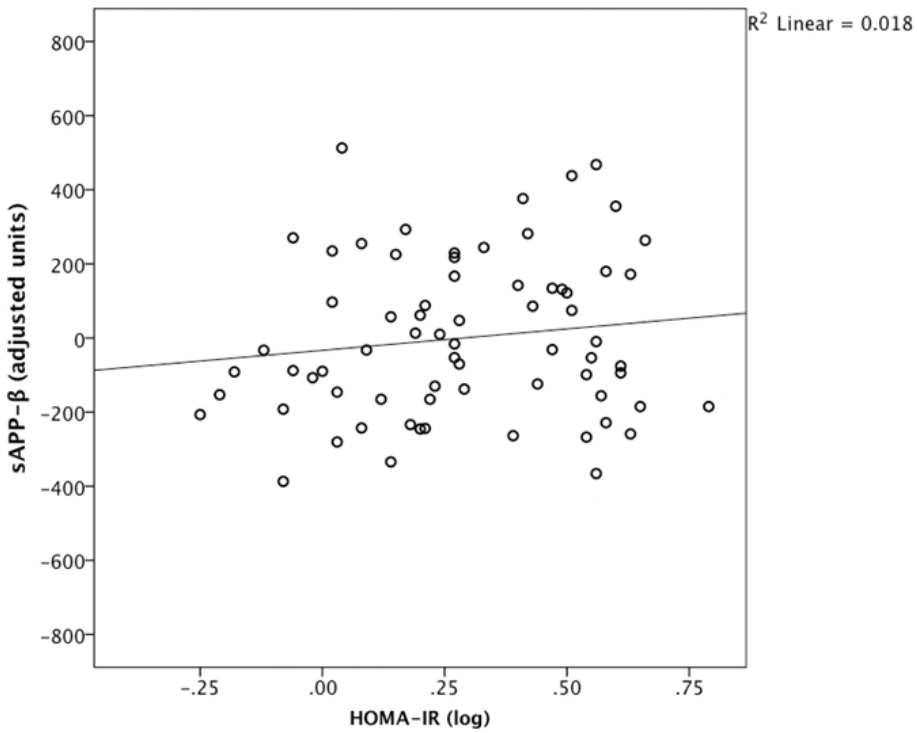

Figure 2. Comparison of CSF sAPP-β in *APOE*ε4 carriers compared to non-carriers. Error bars represent the standard error of the mean. *Significant at $p < 0.01$.

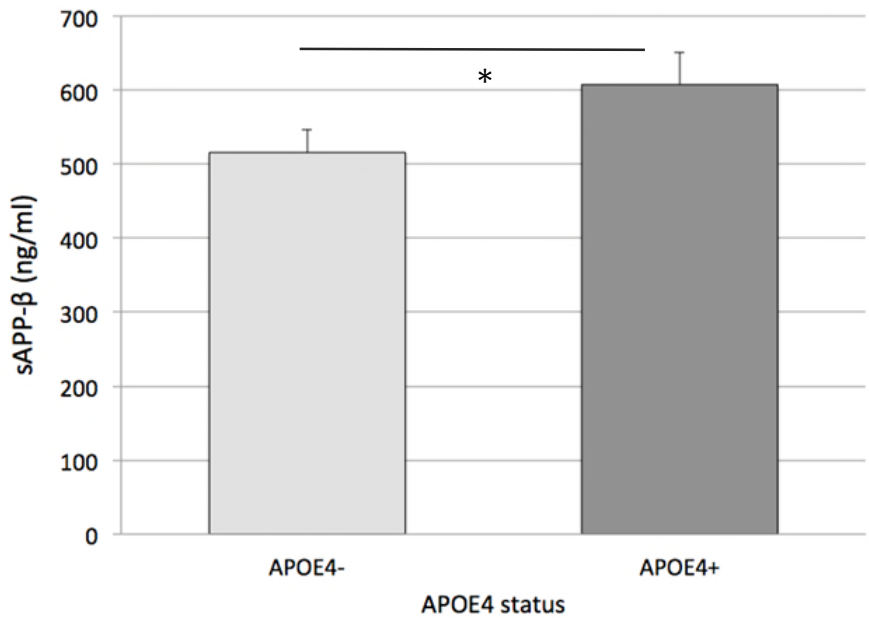

Figure 3. Comparison of CSF P-tau$_{181}$/Aβ_{42} in *APOE*ε4 carriers compared to non-carriers. Error bars represent the standard error of the mean. *Significant at $p < 0.01$.

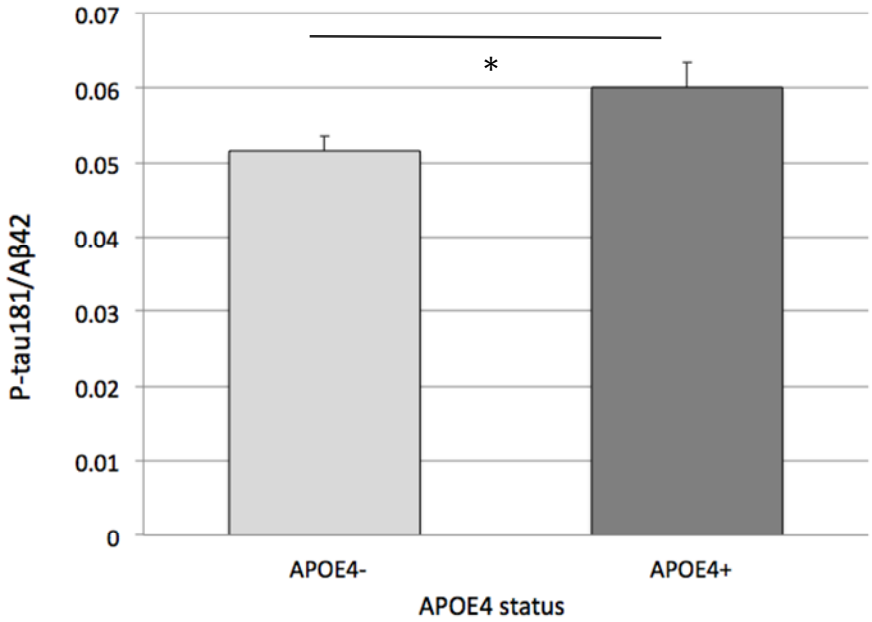

Figure 3. Comparison of CSF P-tau$_{181}$/Aβ_{42} in *APOE*ε4 carriers compared to non-carriers. Error bars designate standard error of the mean. *Significant at $p < 0.01$.

Figure 4. Interaction between HOMA-IR and CSF P-tau$_{181}$/Aβ_{42} on delayed memory performance. Memory performance was adjusted for sex, age and education. Shown as the median split of HOMA-IR(log). Individuals with lower IR are shown in blue. Individuals with higher IR are shown in green.