

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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Figures: 4

Total word count:

1 Abstract

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

11
12 **Objective:** The objective of the current study was to examine whether IR is associated with
13 increased AD pathology and decreased memory performance in asymptomatic middle-aged
14 adults enriched for AD (i.e. with a parental family history of sporadic late-onset AD). We
15 postulated that IR would be associated with greater AD pathology, particularly in carriers of the
16 *APOE* ϵ 4 allele, and poorer memory performance.

17
18 **Method:** Asymptomatic middle-aged adults with a parental family history of AD (N=70, mean
19 age=57.7 years) from the Wisconsin Alzheimer’s Disease Research Center underwent lumbar
20 puncture, blood draw and neuropsychological testing. Cerebrospinal fluid (CSF) samples were
21 assayed for AD related biomarkers including soluble amyloid precursor protein β (sAPP- β),
22 abeta₄₂ (A β ₄₂) and phosphorylated tau (P-tau₁₈₁). The ratio P-tau₁₈₁/A β ₄₂ was also examined as a
23 more sensitive measure of AD pathology with respect to *APOE* ϵ 4 status, IR and memory
24 function. IR was indexed by the Homeostatic Model Assessment for Insulin Resistance (HOMA-
25 IR). Data were analyzed using linear regression models to determine significant effects of IR and
26 *APOE* ϵ 4 on CSF biomarkers of AD and memory performance.

27
28 **Results:** Both *APOE* ϵ 4 carriage and higher IR were associated with higher sAPP- β . *APOE* ϵ 4
29 carriers were observed to have significantly higher levels of AD pathology (P-tau₁₈₁/A β ₄₂)
30 compared to non-carriers. Investigation of memory performance revealed that higher IR and the

31 concurrent presence of AD pathology (P-tau₁₈₁/Aβ₄₂) were associated with worse delayed
32 memory performance.

33
34 **Conclusion:** The current study provides evidence that IR in middle age is associated with higher
35 sAPP-β, a soluble marker of amyloidogenic APP-processing. Further, these results reveal that IR
36 and AD pathology concomitantly result in subclinical memory impairment in asymptomatic
37 middle-aged individuals enriched for AD. Lastly, these findings converge with prior studies
38 indicating that *APOE* ε4 carriage is associated with increased AD pathology that can be observed
39 in early aging prior to the onset of clinical memory impairment. This study has implications for
40 development of interventions targeted at reducing the prevalence of IR through modifiable
41 lifestyle factors, particularly in individuals who harbor the ε4 allele.

42
43 Keywords: insulin resistance, CSF AD biomarkers, *APOE*ε4, memory function

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62 1. Introduction

63 Epidemiological studies have provided mounting evidence that insulin resistance (IR) is
64 associated with an increased risk for Alzheimer's disease (AD) (Arvanitakis et al., 2004; Ott et
65 al., 1999; Peila et al., 2002; Schrijvers et al., 2010). Over the past fifty years, the rate of type 2
66 diabetes mellitus (a non-insulin dependent form of diabetes mellitus) has increased alarmingly
67 with approximately 387 million individuals diagnosed with the disease as of 2014. Recent
68 evidence indicates that cognitively healthy individuals in late-middle life with higher IR harbor
69 more AD-like neuropathology (Starks et al., 2014, Willette et al., 2015). Such studies provide
70 support that IR may be an underlying mechanism driving an increased risk for development of
71 AD pathology and that neurodegenerative changes can be observed prior to the onset of marked
72 cognitive or behavioral impairment. While several animal studies suggest a mechanistic link
73 between IR and neuropathology (for review see de la Monte and Wands, 2005), the effect of IR
74 on the development of AD pathology among humans remains poorly understood, particularly at
75 the preclinical stage of the disease. Identification of modifiable risk factors in midlife may be
76 particularly relevant to individuals who harbor known genetic risk factors for AD (e.g., *APOE*
77 $\epsilon 4$) or who have parental family history of AD. Provided the increasing prevalence of Type 2
78 diabetes and a growing incidence of dementia, elucidation of neuropathologic processes that
79 arise from IR that may be associated with increased risk of AD is of paramount importance.

80
81 Efficient utilization of insulin by neurons underlies normal cellular and cognitive function.
82 Insulin signaling regulates processes critical for neuronal survival and synaptic plasticity
83 moderating processes such as glucose uptake and energy production through glucose oxidation
84 (Cersosimo and DeFronzo, 2006). A growing body of evidence has shown that insulin plays an
85 integral role in learning and memory by modulating excitatory and inhibitory receptors such as
86 glutamate and GABA receptors, respectively (see Zhao et al., 2004a). Insulin homeostasis is
87 believed to be an instrumental component in signal transduction cascades that underlie memory
88 consolidation and long-term memory function (Cardoso et al., 2009; Zhao et al., 2004a; Zhao
89 and Akon 2001). It is suspected that brain regions dense with insulin receptors, such as the
90 medial temporal and frontal lobes, are preferentially sensitive to insulin signaling. Interestingly,
91 frontal and medial temporal lobe regions, particularly the hippocampus, are some of the first to
92 be adversely affected in AD (Craft & Watson, 2004; Henneberg & Hoyer, 1995; Zhao et al.,

93 2004). Cognitively healthy individuals with reduced peripheral insulin function show subtle
94 cognitive deficits and reduced cerebral glucose metabolism and diminished cerebrocortical
95 activity (Baker et al., 2011; Tschritter et al., 2006). Among cognitively intact older adults,
96 reduced glucose tolerance has been associated with poor memory performance and hippocampal
97 atrophy (Convit et al., 2003). Further, abnormal glucose metabolism—believed to arise from
98 desensitization of cerebral insulin receptors—is a core feature of sporadic late-onset AD
99 (Henneberg & Hoyer, 1995) and a number of human studies have provided evidence for a
100 linkage between Type 2 diabetes and greater risk for developing AD (Li et al., 2015; Peila et al.,
101 2002). Histology from human postmortem studies has provided evidence that insulin pathways
102 are severely degraded in AD brains showing a vast reduction in insulin receptor expression and
103 insulin receptor binding (de la Monte & Tong, 2014). These findings are suggestive of an
104 integral role of insulin dysfunction in the pathogenesis of AD.

105
106 The proteolytic processing of amyloid precursor protein (APP) is believed to be one of many
107 neurobiological processes negatively affected by IR that may contribute to AD pathology. APP is
108 processed by two competing pathways: the amyloidogenic β -secretase-mediated and the
109 nonamyloidogenic α -secretase-mediated pathways. Cleavage of APP by α -secretase is thought to
110 mitigate the formation of extracellular amyloid plaques by preventing the formation of amyloid
111 beta ($A\beta$). APP processed through the α -secretase pathway is cleaved within the $A\beta$ sequence
112 producing soluble peptides (Nunan and Small, 2000). By contrast, APP cleavage by β -secretase
113 produces sAPP- β that consequently results in generation of $A\beta$ peptides by endoproteolytic
114 processing (Vassar et al., 1999). In the β -secretase pathway cleavage by γ -secretase adjacent to
115 residue 42 is the last step in formation of the 42 amino acid species $A\beta_{42}$. Among the $A\beta$
116 peptides, $A\beta_{42}$ is implicated in the production and proliferation of amyloid plaques that arise
117 from the aggregation and oligomerization of $A\beta_{42}$. Amyloid plaques are a hallmark of AD
118 pathology and amyloidogenic processes such as $A\beta_{42}$ oligomerization is suspected to be involved
119 in neuronal dysfunction and cell death. Over the past decade a number of studies have provided
120 evidence that $A\beta_{42}$ oligomers have neurotoxic effects that include degradation of synaptic
121 structure (Lacor et al., 2004, 2007) and disruption of molecular and cellular mechanisms integral
122 to memory formation such as synaptic transmission and plasticity (Rowan et al., 2003; Shankar
123 et al., 2008; Lambert et al., 1998). Transgenic mouse models provide evidence that IR promotes

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

131
132 Other risk factors for AD include non-modifiable risk factors such as genetic risk, parental
133 family history of AD and age. It is known that carriage of one or two $\epsilon 4$ alleles of apolipoprotein
134 E (*APOE* $\epsilon 4$) is a major genetic risk factor for AD. Several studies on asymptomatic *APOE* $\epsilon 4$
135 carriers compared to non-carriers have provided evidence that $\epsilon 4$ allele carriers harbor features
136 characteristic of AD pathology such as hypometabolism (Reiman et al., 2004, 2005; Small et al.,
137 2000), higher amyloid burden (Corder et al., 1993) and gray matter atrophy in AD-sensitive
138 regions (Lemaitre et al., 2005). Animal studies have provided evidence that *APOE* $\epsilon 4$ carriage
139 may be particularly deleterious to the hippocampus showing that *APOE* $\epsilon 4$ mice are impaired on
140 hippocampal-dependent tasks such as the Morris Water Maze, object recognition and context
141 fear conditioning (Salomon-Zimri et al., 2013). Thus *APOE* $\epsilon 4$ has been implicated in several
142 AD-relevant pathways, including amyloid accumulation, brain hypometabolism and gray matter
143 degradation.

144
145 Several studies have shown that asymptomatic late-midlife individuals with parental family
146 history of AD (FH+) show early pathological changes in AD-sensitive regions (Adluru et al.,
147 2014; Honea et al., 2010, 2011; Johnson et al., 2006, 2014; Mosconi et al., 2010; Okonkwo et al.,
148 2012). Neurodegeneration and neuropathology observed in these individuals is consistent with,
149 although substantially less severe, pathology observed in mild cognitive impairment (MCI) and
150 AD patients. As genetic and familial risk factors are invariable, modifiable behaviors that may
151 decrease the risk of developing AD are of clinical interest. Development of intervention-based
152 strategies aimed at preventing or reversing IR in midlife may help to significantly reduce one's
153 risk of incipient neuropathological changes that may underlie or contribute to the
154 etiopathogenesis of AD. Midlife is a critical time period during which lifestyle factors that

155 confer risk for AD may be altered. While carriage of the $\epsilon 4$ allele and parental family history of
156 AD are known to increase Alzheimer's disease risk moderating effects of these factors on
157 molecular mechanisms alone are not sufficient to cause the disease.

158
159 To investigate the association between IR and AD pathology observed in healthy middle-aged
160 adults enriched for AD [i.e. parental family history of AD (FH+)], the current study examined
161 the effect of IR on cerebrospinal fluid (CSF) biomarkers of AD and a hippocampal-dependent
162 memory task (i.e., delayed recall). Specifically we examined the effects of IR and *APOE* $\epsilon 4$
163 status on CSF sAPP- β , $A\beta_{42}$, P-tau₁₈₁ and P-tau₁₈₁/ $A\beta_{42}$, and performance on the Rey Auditory
164 Verbal Learning Test (RAVLT), delayed recall. *APOE* $\epsilon 4$ status was also examined as an
165 invariable risk factor for AD pathology in midlife. We hypothesized that both IR and *APOE* $\epsilon 4$
166 would be associated with 1) increased CSF biomarkers of neural injury and amyloid burden and
167 2) decreased memory performance. Given prior studies showing that the effects of IR may
168 depend on *APOE* $\epsilon 4$ carriage, we also tested for interactions between IR and *APOE* $\epsilon 4$ on CSF
169 biomarkers and memory function.

170

171 2. Methods and Materials

172 2.1. Participants

173 The current study examined 70 asymptomatic middle-aged adults (mean age= 57.7 years, SD=
174 5.11, range= 46-66, 78.6% female) from the Wisconsin Alzheimer's Disease Research Center
175 (ADRC) Investigating Memory in People At risk, Causes and Treatments (IMPACT) cohort
176 (Table 1). All participants had a parental family history of sporadic AD (FH+) defined as one or
177 both biological parents meeting AD clinical diagnosis criteria (McKhann et al., 1984, 2011).
178 Parental family history was determined by a validated interview reviewed by a multidisciplinary
179 diagnostic consensus panel (Kawas et al., 1994) or postmortem neuropathological diagnosis of
180 AD. Individuals underwent lumbar puncture, fasting blood draw and comprehensive
181 neuropsychological testing. Inclusion criteria entailed no history of a clinical diagnosis of
182 diabetes or current diabetes treatment. Fasting plasma glucose (FPG) was evaluated to exclude
183 individuals who met FPG criteria for diabetes diagnosis (i.e. FPG > 125 mg/dL). All participants
184 were required to have normal cognitive function, as determined by neuropsychological
185 evaluation and consensus review, and no current diagnosis of major psychiatric illness.

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

188

189 2.2. Design and Procedure

190 To assess insulin resistance and biomarkers of CSF AD pathology, venous blood and CSF were
191 collected in the morning after a 12h fast. Blood samples were collected in 9ml polypropylene
192 tubes, allowed to clot for 30mins and centrifuged at 4°C at 3000 rpm for 10mins. Cell-free
193 plasma/serum was aliquoted into 1.5mL micro centrifuge tubes and frozen at -80 degrees
194 Celsius. Plasma and serum samples were analyzed at the University of Wisconsin Hospital and
195 Clinics Hospital Laboratory (Madison, WI). To assess fasting glucose, plasma was assayed using
196 hexokinase glucose method (Siemens Dimension Vista). To assess fasting insulin, serum was
197 assayed using chemiluminescent immunoassay on an ADVIA Centaur XP Immunoassay System
198 (Siemens Corporation, Washington DC, USA). Insulin resistance was calculated from fasting
199 serum insulin and fasting plasma glucose using the homeostatic model assessment of insulin
200 resistance (HOMA-IR) method (Matthews et al., 1985) calculated as $HOMA-IR = \text{Insulin}$
201 $(\text{mg/dL}) \times \text{Glucose (uIU/mL)} / 405$.

202

203 CSF was collected through gentle extraction via lumbar puncture using a Sprotte 25- or 24-gauge
204 spinal needle at the L3/4 or L4/5 level of the spinal column. Approximately 22mL of CSF was
205 extracted, gently mixed and centrifuged at 2000g for 10mins. Supernatants were frozen in
206 polypropylene tubes in 0.5mL aliquots and stored at -80 degrees Celsius. CSF was assayed for P-
207 tau₁₈₁ and Aβ₄₂ using commercially available enzyme-linked immunosorbent assay (ELISA)
208 methods (INNOTEST assays, Fujiirebio, Ghent Belgium) as previously described in detail
209 (Palmqvist et al., 2014). CSF sAPP-β was measured using the MSD Multiplex Soluble APP
210 assay (Meso Scale Discovery, Rockville, MD), as described by the manufacturer. Board-certified
211 laboratory technicians blinded to clinical information analyzed all samples in accordance to
212 protocols approved by the Swedish Board of Accreditation and Conformity Assessment
213 (SWEDAC). One batch of reagents was used yielding intra-assay coefficients of <10 %
214 variation. The P-tau₁₈₁/Aβ₄₂ ratio was examined as a marker of multiple pathological processes
215 that occur in AD.

216

217 *APOE* ϵ 4 status was determined using genetic testing by the Wisconsin Alzheimer's Disease
218 Research Center. Genotyping was performed using non-fasting blood sample collected at
219 baseline visit, using standard polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA)
220 sequencing techniques. DNA extracted from whole blood was genotyped with the use of a
221 homogenous Florescent Resonance Energy Transfer technology coupled to competitive allele
222 specific PCR (LGC Genomics; Beverly, MA). The National Cell Repository for Alzheimer's
223 disease (NCRAD) also performed genotyping. There was 100% concordance for *APOE*
224 genotyping between these analyses.

225
226 Participants underwent a comprehensive neuropsychological battery that included the Rey
227 Auditory Verbal Learning Test (RAVLT) (Rey, 1941) a widely used and well-validated
228 assessment of memory function. The delayed component of the RAVLT is designed to examine
229 long-term memory function and is a well-known measure that assesses cognitive changes from
230 intact, to MCI to AD patients (Estevez-Gonzalez et al., 2003; Ewers et al., 2012). In the current
231 study the RAVLT delayed task was chosen as a focal index of long-term hippocampal-dependent
232 memory function.

233

234 2.7. Statistical Analyses

235 Linear regression analyses were conducted in SPSS (Version 21.0). Analyses tested main effects
236 of IR as indexed by HOMA-IR and *APOE* ϵ 4 status, and the interaction between HOMA-IR and
237 *APOE* ϵ 4 on CSF biomarkers of AD and memory performance. A secondary analysis tested the
238 interaction between HOMA-IR and CSF biomarkers of AD on delayed memory performance. Age,
239 sex and body mass index (BMI) were included as covariates in all analyses. Education was added
240 as a covariate in analyses that included neuropsychological test measures (i.e., RAVLT delayed)
241 as a dependent variable.

242

243 3. Results

244 3.1. HOMA-IR, *APOE* ϵ 4 and CSF biomarkers of AD

245 Linear multiple regression analysis revealed that HOMA-IR and *APOE* ϵ 4 status were significant
246 predictors of CSF sAPP- β . Higher HOMA-IR was significantly associated with increased levels
247 of CSF sAPP- β ($F_{[1,63]} = 4.21, p = 0.044$) (Figure 1). Further, carriage of the ϵ 4 allele significantly

248 predicted higher levels of CSF sAPP- β ($F_{[1,63]} = 7.74, p = 0.007$) (Figures 2). The interaction
249 between HOMA-IR and *APOE* $\epsilon 4$ on CSF sAPP- β was not significant.

250 Significant relationships between CSF A β_{42} and predictor variables and CSF P-tau $_{181}$ and predictor
251 variables were not found, however, a significant effect of *APOE* $\epsilon 4$ status on the ratio of CSF P-
252 tau $_{181}$ /A β_{42} was observed ($F_{[1,63]} = 5.21, p = 0.026$). CSF P-tau $_{181}$ /A β_{42} was significantly greater in
253 *APOE* $\epsilon 4$ carriers compared to non-carriers (Figure 3).

254

255 3.2. HOMA-IR, *APOE* $\epsilon 4$, CSF biomarkers of AD and Memory Performance

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

262

263 4. Discussion

264 IR is associated with an increased relative risk for developing sporadic late-onset AD.

265 Dysregulation of insulin signaling is thought to contribute to a cascade of neuropathological
266 changes that promote amyloidogenic processing, neurotoxicity, and brain amyloidosis (Devi et
267 al., 2012; Henneberg and Hoyer, 1995; Ho et al., 2004; Willette et al., 2015; Zhao et al., 2009).
268 Brain changes associated with IR that may underlie or contribute to the pathogenesis of AD are
269 poorly understood, particularly in midlife prior to clinical disease onset. IR is a modifiable risk
270 factor and thus regulation of normal insulin signaling is an important target for early
271 intervention, one that may be particularly relevant to persons who harbor genetic risk or have a
272 parental family history of AD. The current study aimed to investigate the relationship between
273 IR, AD pathology and memory performance in middle-aged individuals enriched for AD. As a
274 subsidiary aim, we examined *APOE* $\epsilon 4$ genotype relative to IR, AD pathology and memory
275 function, as it is a well-established genetic risk factor for AD.

276

277 Our findings revealed that higher IR and carriage of the $\epsilon 4$ allele were both predictors of
278 increased sAPP- β in CSF, suggesting preferential cleavage of APP through the amyloidogenic β -

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

296
297 IR may also be linked to pathological processes via amyloidosis by decreasing availability of
298 insulin-degrading enzyme (IDE), a large zinc-binding protease that binds to and degrades insulin.
299 IDE preferentially binds to insulin but also has an affinity for $A\beta$ proteins. In the absence of IR,
300 IDE is available to bind to and degrade $A\beta$ proteins. In the case of IR, IDE preferentially binds to
301 the elevated levels of insulin as it aggregates in extracellular space, resulting in less availability
302 for IDE to be allocated toward degradation of $A\beta$ proteins. Excess amyloid peptides
303 consequently lead to the formation of amyloid plaques, brain degeneration and neuronal
304 dysfunction. Animal studies have provided evidence that selective removal of the IDE gene
305 results in more than a 50% decrease in $A\beta$ degradation and a significant increase in $A\beta$
306 deposition (Farris et al., 2003; Ho et al., 2004). Transgenic mouse models of IR have provided
307 evidence that IDE may not only be affected by IR through interference of IDE-mediated
308 degradation of $A\beta$ but may also decrease IDE expression and activity (Ho et al., 2004). Further,
309 recent research has provided evidence that IDE also degrades sAPP- β in the intracellular domain

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

314

315 Carriage of the $\epsilon 4$ allele of *APOE* was also a significant predictor of CSF markers of AD
316 pathology. *APOE* $\epsilon 4$ status showed a positive relationship with sAPP- β and the ratio of P-
317 tau₁₈₁/A β ₄₂ although significant relationships between *APOE* $\epsilon 4$ carriage and A β ₄₂ or P-tau₁₈₁
318 were not observed. These results validate the notion that P-tau₁₈₁/A β ₄₂ may be a more sensitive
319 index of AD pathology in preclinical populations compared to a single marker of disease and
320 provide further evidence that multiple pathological processes associated with AD occurs at a
321 subclinical threshold in asymptomatic midlife persons, particularly $\epsilon 4$ allele carriers. *APOE* $\epsilon 4$
322 status is a strong predictor of AD pathology and is believed to act on the A β pathway, leading to
323 a reduction in clearance and increased A β aggregation and deposition (Schmechel et al., 1993).
324 Our findings provide further support that $\epsilon 4$ allele carriage is associated with AD pathology, and
325 extends these findings to increased upstream amyloidogenic processing (i.e., sAPP- β) in healthy
326 middle-aged persons enriched for a family history of AD.

327

328 Interestingly, we found that high IR and P-tau₁₈₁/A β ₄₂ concomitantly act to impair memory
329 performance on the RAVLT delayed memory. Long-term memory tasks—such as the one
330 examined here—are dependent on the hippocampus, a medial temporal lobe structure negatively
331 affected by IR (Benedict et al., 2012; Rasgon et al., 2011; Stranahan et al., 2008) and among the
332 first regions to show structural and functional changes in AD (Convit et al., 2000; Du et al.,
333 2001; Pennanen et al., 2004, Wang et al., 2006). Research from our group has shown that IR in
334 midlife is associated with hypometabolism in brain regions involved in episodic memory,
335 including the hippocampus (Willette et al., 2015). Taken together, our findings suggest that
336 dysregulation of the insulin system in midlife may have deleterious effects on functional
337 integrity of the hippocampus that precede or act in concert with early pathological AD changes
338 leading to subclinical memory dysfunction. It is worth noting that while we found that high IR
339 and AD pathology interacted to impair memory function, we did not observe an interaction with
340 $\epsilon 4$ allele carriage. This finding suggests that while AD pathology is more prominent in *APOE* $\epsilon 4$

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

343 Overall, our findings provide evidence that pathological processes associated with AD are
344 observed early in aging, prior to the onset of memory difficulty or manifestation of clinically
345 significant symptoms of cognitive impairment. IR and carriage of the $\epsilon 4$ allele are predictors of
346 early changes in AD-related CSF biomarkers in persons enriched for parental history of late-
347 onset sporadic AD. We show that IR and AD pathology interact to impair long-term memory
348 function in middle age. IR is a modifiable risk factor that may be corrected with targeted
349 interventions. Insulin homeostasis plays an integral role in maintaining healthy neuronal
350 function, normal cerebral glucose metabolism and reducing or ameliorating pathological
351 processes associated with AD. Targeted interventions designed to maintain normal insulin
352 signally may be particularly affective in delaying or ameliorating AD disease onset in persons
353 with invariable risks for AD.

Acknowledgments

This research was supported by NIH grant P50 AG033514, University of Wisconsin Institute for Clinical and Translational Research, funded through a National Center for Research Resources/National Institutes of Health Clinical and Translational Science Award, 1UL1RR025011, a program of the National Center for Research Resources, United States National Institutes of Health, the Swedish Research Council, the Swedish Brain Foundation and Torsten Söderberg's Foundation to the University of Gothenburg. We want to thank the MRI staff at the Wisconsin Institute for Medical Research, Chuck Illingworth, the staff at the Wisconsin ADRC and above all, our dedicated participants.

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Table 1

Demographic, gluoregulatory, 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	
APOEε4 (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.

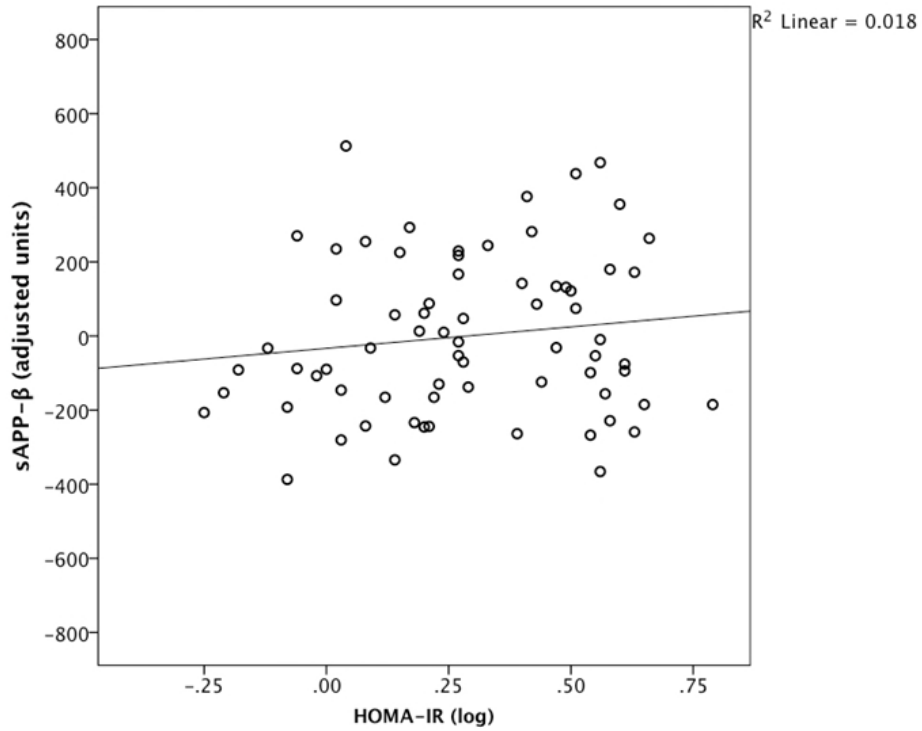


Figure 2. Comparison of CSF sAPP- β in *APOE* ϵ 4 carriers compared to non-carriers. Error bars represent the standard error of the mean. *Significant at $p < 0.01$.

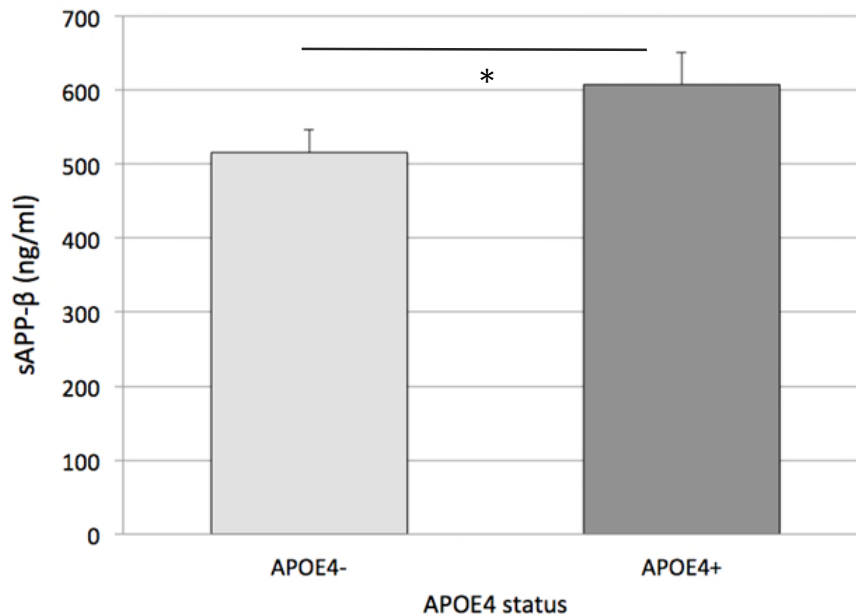


Figure 3. Comparison of CSF P-tau₁₈₁/Aβ₄₂ in *APOE*ε4 carriers compared to non-carriers. Error bars represent the standard error of the mean. *Significant at $p < 0.01$.

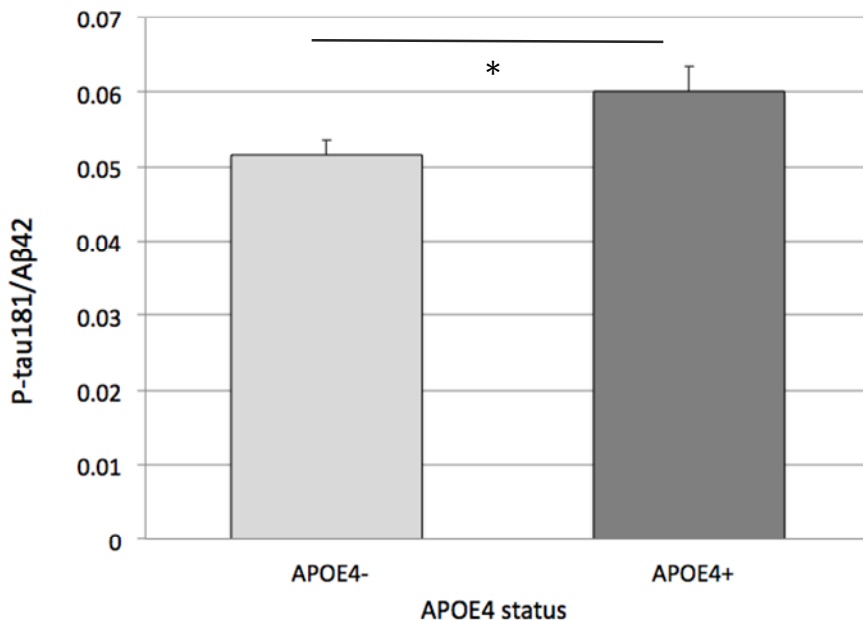


Figure 3. Comparison of CSF P-tau₁₈₁/Aβ₄₂ in *APOE*ε4 carriers compared to non-carriers. Error bars designate standard error of the mean. *Significant at $p < 0.01$.

Figure 4. Interaction between HOMA-IR and CSF P-tau₁₈₁/Aβ₄₂ 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.

