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## Insulin resistance is associated with higher cerebrospinal fluid tau levels in asymptomatic *APOE* $\epsilon$ 4 carriers

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### Abstract

**Background**—Insulin resistance (IR) is linked with the occurrence of pathological features observed in Alzheimer's disease (AD), including neurofibrillary tangles and amyloid plaques. However, the extent to which IR is associated with AD pathology in the cognitively asymptomatic stages of preclinical AD remains unclear.

**Objective**—To determine the extent to which IR is linked with amyloid and tau pathology in late-middle-age.

**Method**—Cerebrospinal fluid (CSF) samples collected from 113 participants enrolled in the Wisconsin Registry for Alzheimer's Prevention study (mean age = 60.6 years), were assayed for AD-related markers of interest:  $A\beta_{42}$ , P-Tau<sub>181</sub>, and T-Tau. IR was determined using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR). Linear regression was used to test the effect of IR, and *APOE*  $\epsilon$ 4, on tau and amyloid pathology. We hypothesized that greater IR would be associated with higher CSF P-Tau<sub>181</sub> and T-Tau, and lower CSF  $A\beta_{42}$ .

**Results**—No significant main effects of HOMA-IR on P-Tau<sub>181</sub>, T-Tau, or A $\beta$ <sub>42</sub> were observed; however, significant interactions were observed between HOMA-IR and *APOE*  $\epsilon$ 4 on CSF markers related to tau. Among *APOE*  $\epsilon$ 4 carriers, higher HOMA-IR was associated with higher P-Tau<sub>181</sub> and T-Tau. Among *APOE*  $\epsilon$ 4 non-carriers, HOMA-IR was negatively associated with P-Tau<sub>181</sub> and T-Tau. We found no effects of IR on A $\beta$ <sub>42</sub> levels in CSF.

**Conclusion**—IR among asymptomatic *APOE*  $\epsilon$ 4 carriers was associated with higher P-Tau<sub>181</sub> and T-Tau in late-middle age. The results suggest that IR may contribute to tau-related neurodegeneration in preclinical AD. The findings may have implications for developing prevention strategies aimed at modifying IR in mid-life.

### Keywords

Insulin Resistance; Cerebrospinal Fluid; Tau protein; Glucose; Insulin; Apolipoprotein  $\epsilon$ 4; Alzheimer's disease

## INTRODUCTION

Type 2 diabetes and insulin resistance (IR) have been linked to cognitive decline[1] and increased risk for developing Alzheimer's disease (AD)[2]. In transgenic mouse models of AD mice, IR promotes the generation of A $\beta$ <sub>42</sub> peptides and amyloidosis [3], part of a pathway instigated by cleavage of amyloid precursor protein by  $\beta$ -secretase. IR is also linked with amyloidosis by increasing  $\gamma$ -secretase activity and decreasing insulin degrading enzyme (IDE) activity[3]. IDE regulates A $\beta$  levels in neuronal cells, and IDE knockout mice have hyperinsulinemia, glucose intolerance, and increased cerebral accumulation of A $\beta$  [4]. Diabetic phenotypes in mice are also associated with increased tau phosphorylation [5]. The brain, previously thought to be insulin insensitive, has insulin receptors distributed in several regions including hippocampus, an important site for AD pathology [6]. Furthermore, insulin receptor density decreases in aging, insulin signaling is altered in AD [6, 7] and decreased insulin mRNA in post mortem brain tissue has been observed in AD patients compared to controls [8]. Additionally, prevalence of impaired fasting glucose and type 2 diabetes is increased in AD patients [9]. Some human studies suggest IR and type 2 diabetes are linked with abnormal phosphorylation of tau protein [10] and greater amyloid burden [11], although other studies suggest otherwise [12-14].

The pathological process in AD is now known to begin decades before clinically significant symptoms of cognitive impairment manifest [15]. For many adults, this coincides with midlife. Interestingly, risk factors for AD such as type 2 diabetes and the associated condition of IR at midlife increase the relative risk of developing AD, even when controlling for obesity and other vascular risk factors [16]. Possibilities for little or modest association between type 2 diabetes and postmortem assessment of AD-associated pathology may be due to the use of diabetes medication which may confer neuroprotective effects, development of diabetes in later life which is less associated with dementia risk, or the selective early death of severe diabetic patients attributed to co-morbid conditions such as heart disease and stroke.

In order to examine the contribution of IR to development of AD pathology at the critical juncture of midlife and presumed preclinical phase of AD, this study examined the relationship between IR and cerebrospinal fluid (CSF) markers of AD pathology in asymptomatic late-middle-aged adults with risk factors for AD. Cerebrospinal fluid (CSF) biomarkers show utility for predicting conversion to AD [17] and provide an indirect, but close marker of tissue pathology [18, 19]. Phosphorylated tau (P-tau<sub>181</sub>) and total tau (T-tau) are elevated in mild cognitive impairment (MCI) and AD while CSF A $\beta$ <sub>42</sub> has been shown to be inversely proportional to amyloid in the brain [20] and is decreased in MCI and AD [21]. Participants were from the Wisconsin Registry for Alzheimer's Prevention (WRAP) study, a cohort enriched for AD risk factors, including *APOE*  $\epsilon$ 4 genotype (40%) and parental family history of AD (75%). Based on evidence from animal models and human epidemiological studies suggesting IR contributes to AD risk and pathology, we hypothesized that higher IR would be associated with higher P-Tau<sub>181</sub> and T-Tau and lower levels of A $\beta$ <sub>42</sub> in this preclinical cohort of adults.

## METHODS

### Participants

This study examined 113 asymptomatic late-middle-aged adults (mean age = 60.6 years, range= 47-75 years, 70% female) from WRAP who had undergone lumbar puncture and fasting blood draw. This continuing study assesses genetic and biological factors postulated to contribute to the development of dementia-related cognitive decline and neural dysfunction. Inclusion criteria for this study entailed no clinical diagnosis of a memory disorder, no current diagnosis of major psychiatric disease or other major medical conditions (e.g., myocardial infarction, or recent history of cancer), and no history of head trauma. Participants were also required to have available fasting insulin and glucose levels, in addition to CSF assays for AD-relevant biomarkers.

Parental family history (FH) of AD classification was based on probable or confirmed AD of one or both parents [22]. A positive family history was defined as having one or both parents with autopsy-confirmed or probable AD as outlined by research criteria [23, 24], reviewed by a multidisciplinary diagnostic consensus panel. Detailed medical history and phone interviews were conducted to confirm absence of parental FH, where absence required that the participant's father survive to at least age 70 and the mother to age 75 without incurring a formal diagnosis of dementia or exhibiting cognitive deterioration. Participants were classified using a binary variable as FH positive or FH negative.

Fasting blood samples were collected following vital sign measurement and processed at the University of Wisconsin Clinical Research Unit and fasting glucose and insulin values were determined at the UW Hospital laboratory. Subjects' weight and height were measured without shoes. Weight was measured to the nearest tenth of a kilogram using a medical scale balance beam model, Health-O-Meter. Height was measured to the nearest centimeter using a wall-mounted ruler. Body mass index (BMI) was calculated based on height and weight.

Insulin resistance was measured by HOMA-IR (glucose\*insulin/405) using fasting blood glucose and insulin from each subject. Currently, cut-off points of HOMA-IR for insulin

resistance are not well established. For example, studies in American Indians, American Mexican, Spanish, Japanese, and European populations demonstrate great variability in HOMA-IR cut-offs and cut points have not been determined for a sample comparable to ours [25-28]. Thus, HOMA-IR was analyzed as a continuous variable. Because the raw data were skewed, all analyses used log transformed HOMA-IR. Presence of type 2 diabetes in this sample was assessed by reviewing medication records, self-report, and using American Diabetes Association criteria, where participants with fasting blood glucose over 125 mg/dL were flagged as diabetic. Four participants had previously been diagnosed with type 2 diabetes and were taking Metformin. One participant not diagnosed with diabetes had elevated fasting glucose according to ADA criteria. Medications were also reviewed to identify additional participants with diabetes. In order to test for relationships in the pre-diabetic range, the analyses were repeated excluding participants with diagnosed or possible diabetes. The results did not differ, thus, these 4 participants were retained.

**APOE  $\epsilon 4$** —*APOE*  $\epsilon 4$  extraction and isoform classification have been described previously [29]. Participants were categorized using a binary variable as being non-carriers (zero  $\epsilon 4$  alleles) or *APOE*  $\epsilon 4$  carriers (one or two  $\epsilon 4$  alleles).

**Cerebrospinal Fluid**—CSF was collected in the morning after a 12hr fast with a Sprotte 25-or 24-gauge spinal needle at the L3/4 or L4/5 using gentle extraction into propylene syringes. CSF (~22mL) was then combined, gently mixed and centrifuged at 2000g for 10 minutes. Supernatants were frozen in 0.5mL aliquots in polypropylene tubes and stored at -80°C. Samples were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) methods (INNOTEST assays, Fujiirebio, Ghent Belgium) as described previously in detail [30]. Board-certified laboratory technicians who were blinded to clinical information performed all analyses. All samples were analyzed according to protocols approved by the Swedish Board of Accreditation and Conformity Assessment (SWEDAC) using one batch of reagents (intra-assay coefficients of variation <10%).

## Statistics

A multiple regression analysis was conducted using SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) software to examine the effects of HOMA-IR, *APOE*  $\epsilon 4$  genotype and FH status on P-Tau<sub>181</sub>, T-Tau and A $\beta$ <sub>42</sub>. Separate analyses were performed for each CSF measure of interest. A secondary analysis also tested the effect of IR on A $\beta$ <sub>42</sub>/P-Tau<sub>181</sub> a ratio that takes into account both facets of pathology with a smaller ratio implying higher AD pathology [31]. Interactions between HOMA-IR and *APOE*  $\epsilon 4$  genotype, and HOMAIR and FH status were also examined. Covariates were age, sex and BMI.

**Outliers and collinearity**—BMI and IR are positively correlated with one another, which could lead to collinearity artifacts in the regression analyses. We assessed for collinearity effects of BMI and the log of HOMA-IR by calculating the variance inflation factor (1.5), which revealed that the variables were not highly co-linear. Our original N was 115, however two participants were identified as significant outliers (> 3 SD from the mean) on

CSF A $\beta$ <sub>42</sub> (one participant) and HOMA-IR (one participant), even after log transforming HOMA-IR, and thus were removed from further analysis for a total N of 113.

## RESULTS

### Demographics

Demographics and clinical characteristics of the participants are shown in Table 1. Mean MMSE across all participants was well within the normal range ( $29.36 \pm .80$ ). Mean HOMA-IR was 2.2 (SD = 1.6), with untransformed values ranging from 0.45-7.65.

### Effects of HOMA-IR on P-Tau<sub>181</sub>, T-Tau and A $\beta$ <sub>42</sub>

A multiple regression analysis was conducted to test the effect of HOMA-IR on A $\beta$ <sub>42</sub>, T-Tau, and P-Tau<sub>181</sub> including the covariates of sex, age, BMI, FH, *APOE*  $\epsilon$ 4 status, HOMA-IR, and the interaction of *APOE*  $\epsilon$ 4 status and HOMA-IR. Each CSF measure was tested individually as a dependent variable in a multiple regression analysis. There was no main effect of HOMA-IR on the CSF markers.

Our regression model indicated a significant interaction between *APOE*  $\epsilon$ 4 and HOMA-IR on CSF P-tau<sub>181</sub> after adjusting for covariates, ( $\beta = 0.369$ ,  $t(112) = 2.422$ ,  $p = 0.017$ ) where there was a positive relationship between HOMA-IR and P-Tau<sub>181</sub> among *APOE*  $\epsilon$ 4 carriers, and a negative relationship between HOMA-IR and P-Tau<sub>181</sub> among non-carriers (Figure 1, Table 2). Likewise, there was a significant interaction between HOMA-IR and *APOE*  $\epsilon$ 4 status on CSF T-Tau ( $\beta = 0.367$ ,  $t(112) = 2.391$ ,  $p = 0.002$ ), whereby there was a positive relationship between HOMA-IR and T-Tau among *APOE*  $\epsilon$ 4 carriers, but HOMA-IR was negatively associated with T-Tau<sub>181</sub> among *APOE*  $\epsilon$ 4 non-carriers (Figure 2, Table 3). There was no significant effect of HOMA-IR, *APOE*  $\epsilon$ 4, or the interaction term on CSF A $\beta$ <sub>42</sub> alone.

Our secondary analysis of A $\beta$ <sub>42</sub>/P-Tau<sub>181</sub> showed a significant interaction between *APOE*  $\epsilon$ 4 and HOMA-IR ( $\beta = -0.410$ ,  $t(112) = -2.80$ ,  $p = 0.006$ ) on the A $\beta$ <sub>42</sub>/P-Tau<sub>181</sub> ratio. Among *APOE*  $\epsilon$ 4 carriers only, there was a negative relationship between HOMA-IR and A $\beta$ <sub>42</sub>/P-Tau<sub>181</sub> as HOMA-IR increased, the ratio decreased.

We found no significant effects of family history on CSF biomarkers, nor were any significant interactions with family history observed.

## DISCUSSION

Higher IR in this sample of asymptomatic late-middle-aged adults was associated with increased T-Tau and P-Tau<sub>181</sub>, and decreased A $\beta$ <sub>142</sub>/P-Tau<sub>181</sub> in *APOE*  $\epsilon$ 4 carriers. These results suggest a possible preclinical mechanism that may contribute to increased risk for AD related neurodegeneration among late middle-aged adults with glucoregulatory impairment.

Insulin resistance potentially promotes abnormal phosphorylation of tau in cognitively healthy middle-aged *APOE*  $\epsilon$ 4 carriers. Phosphorylation of tau decreases binding to

microtubules and hyperphosphorylation eventually leads to formation of paired helical filaments aggregating to form neurofibrillary tangles. Impaired insulin signaling has been shown to enhance hyperphosphorylation of tau, which mediates dissociation of the protein from microtubules in the axons and association into tangles [32, 33]. IR has been implicated in tau hyperphosphorylation through upregulation of kinases, particularly downstream kinases that are known to phosphorylate tau protein, including glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) [34, 35].

The relationship between type 2 diabetes, IR, and brain pathology in humans has been mixed, but may in part be explained by *APOE*  $\epsilon$ 4. In a postmortem study conducted by Malek-Ahmadi et al [36], AD patients who were diagnosed with type 2 diabetes and who were *APOE*  $\epsilon$ 4 carriers, showed higher plaque and tangle burden compared to non-carriers, suggesting that type 2 diabetes may exacerbate AD neuropathology in the presence of *APOE*  $\epsilon$ 4. This is consistent with the current findings, where *APOE*  $\epsilon$ 4 carriers showed greater CSF tau levels. Conversely, the general prevalence of type 2 diabetes among AD patients is actually higher in *APOE*  $\epsilon$ 4 non-carriers compared to *APOE*  $\epsilon$ 4 carriers [37].

Underscoring the importance of *APOE*  $\epsilon$ 4 status specifically in relation to insulin, AD and MCI patients have shown differences in memory performance in response to intranasal insulin therapy based on *APOE*  $\epsilon$ 4 carriage. In one study, intranasal insulin therapy led to improved memory performance among *APOE*  $\epsilon$ 4 carriers [38], while in another study, facilitated memory function in *APOE*  $\epsilon$ 4 non-carriers [39]. Like intranasal insulin, intravenous insulin infusion shows differential effects among *APOE*  $\epsilon$ 4 carriers and non-carriers. Specifically, *APOE*  $\epsilon$ 4 non-carriers show memory facilitation in hyperinsulinemic conditions, an effect not observed among *APOE*  $\epsilon$ 4 carriers [40]. Basal levels of plasma and CSF insulin in AD patients also differ by genotype, with *APOE*  $\epsilon$ 4 homozygotes showing normal basal levels of insulin compared to heterozygotes and non-carriers [41], suggesting an importance of *APOE*  $\epsilon$ 4 load. Animal work suggests that insulin signaling may differ depending on *APOE*  $\epsilon$ 4 status. A study of *APOE*  $\epsilon$ 4 knock-in mice revealed altered insulin signaling when compared to *APOE*  $\epsilon$ 3 mice. Effector molecules including IRS-1, PI-3K and Akt phosphorylation were all decreased in the liver of *APOE*  $\epsilon$ 4 mice suggesting an *APOE*  $\epsilon$ 4 mediated alteration in insulin signaling [42]. More research is needed to determine whether the product of *APOE*, apolipoprotein E, is involved in molecular processes modulating the effect of insulin resistance on pathological markers of AD.

Interesting interactions have also been reported between insulin levels and cognitive status. Among non-demented older adults, higher serum insulin levels are associated with a deleterious effect on cognitive function in addition to greater global and hippocampal atrophy [43]. In contrast, among patients diagnosed with AD, higher insulin levels are associated with better cross-sectional and longitudinal outcomes, including less decline in cognitive function, and lower global and hippocampal atrophy [43, 44]. Taken together, these studies suggest the importance of considering both the non-linear relationships between IR and neural outcome across the spectrum of cognitive function, and genotype, in treatments that target insulin signaling.



With the exception of an interaction between IR and *APOE*  $\epsilon 4$  on  $A\beta_{42}/P\text{-Tau}_{181}$  ratio, we did not find a relationship between IR and CSF  $A\beta_{42}$ . Our group has previously found higher amyloid burden among normoglycemic WRAP participants with higher HOMA-IR, using Pittsburgh compound B position emission tomography (PiB-PET) scanning [11]; although at least one prior study has found a negative relationship between IR and amyloid levels measured with PiB-PET [11]. Further work will be needed to determine the extent to which CSF levels of  $A\beta_{42}$  correlate with regional amyloid deposition as shown with amyloid-PET in this preclinical population.

This study examined participants who were cognitively asymptomatic, and largely normal on fasting glucose and fasting insulin levels. Cut-offs for insulin resistance vary by study and the population under consideration, however, for comparison purposes, 85% of our sample fell below the cut-point of 3.8 for IR, while 75% of the sample was below the cut-point of 2.6 for IR [45-47]. Three participants were taking metformin for diabetes and one participant was identified who had elevated fasting glucose; however, removing these participants from the analyses did not change the relationship between IR and CSF markers of tau among *APOE*  $\epsilon 4$  carriers, suggesting these relationships can be observed within the relatively normal variation of IR. While we have framed IR as key player in the generation of AD pathology we must note that the observed variation in IR itself could be the result of preclinical AD having an effect on metabolic regulation, for example, due to AD pathology in brain regions which regulate metabolic pathways [48]. Alternatively, AD pathology and peripheral insulin resistance could be co-occurring manifestations of either brain, or systemic metabolic dysfunction. This notion is supported by studies showing commonalities among diabetes, insulin resistance, and AD, including mitochondrial dysfunction [49-51]. The fact that these relationships can be detected in preclinical stages of glucoregulatory impairment however, does suggest the potential importance of preclinical detection and prevention strategies for mitigating age and disease associated brain changes in later life.

It is important to note that while CSF tau levels are known to associate with postmortem tangle burden [52], T-Tau is a non-specific marker and we cannot conclude that an AD-specific process is at play. The vascular effects of insulin resistance also cannot be discounted. Cerebrovascular pathology could lead to neural injury that in turn could lead to elevated levels of tau protein in CSF, particularly T-Tau. Combined CSF and blood flow brain imaging studies are expected to shed further light on vascular contributions to AD pathology, as well as provide a method for localizing pathology. Furthermore, although P-Tau is more specific to AD, P-Tau levels were only assessed for tau phosphorylated at site 181. There are over 80 sites for phosphorylation of tau and some of these sites may be more closely linked with insulin signaling disruption [35].

Other potential limitations of this study include the small sample size and the cohort's demographics, particularly in this sub-sample that underwent lumbar puncture. Participants were largely Caucasian, female, and had high levels of educational attainment, and thus are not representative of the general population. Finally, it is important to note that longitudinal investigation will be necessary to assess the long-term impacts of insulin resistance on disease progression.

## Conclusion

This study demonstrates for the first time that pre-diabetic levels of IR are associated with elevated levels of CSF tau protein in an asymptomatic population with genetic risk for AD. This study suggests that insulin resistance may enhance tau pathology, especially in *APOE*  $\epsilon 4$  carriers. Understanding modifiable factors during the preclinical stage is expected to contribute to treatment and prevention strategies in this devastating disease.

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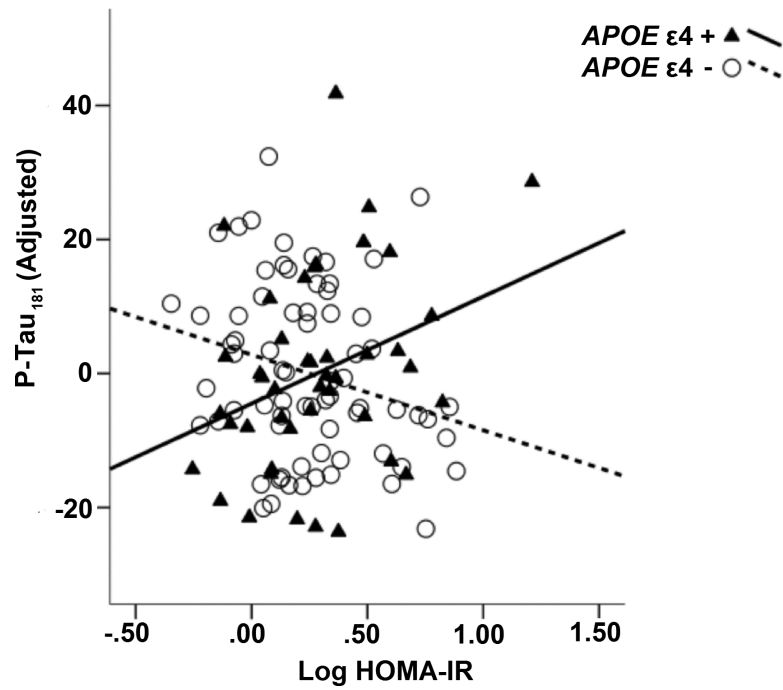
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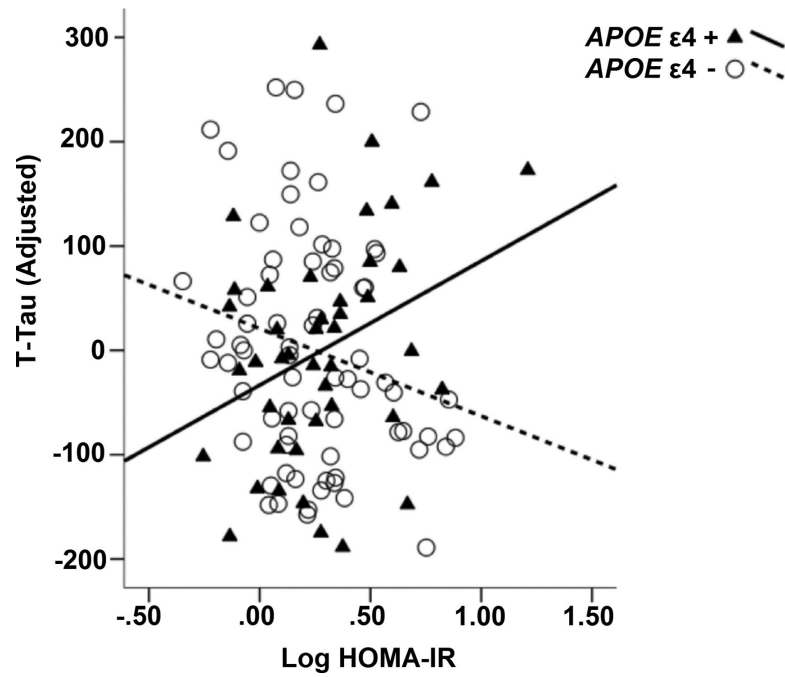
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**Figure 1. Interaction between insulin resistance and *APOE*ε4 predicts P-Tau<sub>181</sub>**

We found a significant interaction between HOMA-IR and *APOE* ε4 on P-Tau<sub>181</sub>, adjusting for age, sex, family history, and BMI. Carriers are shown with triangles and solid black line. Plotted P-Tau values are adjusted for covariates.



**Figure 2. Interaction between insulin resistance and *APOE*ε4 predicts T-Tau**

We found a significant interaction between HOMA-IR and *APOE* ε4 on T-Tau, adjusting for age, sex, family history, and BMI. Carriers are shown with triangles and solid black line. Plotted T-Tau values are adjusted for covariates.

**Table 1**

## Participant Characteristics

	All	APOE $\epsilon 4$		p
		Carrier (38%)	Non-carriers (62%)	
N	113	43	70	
Age	60.5 $\pm$ 5.9 (47-75)	60.5 $\pm$ 6.2	60.4 $\pm$ 5.8	.923
Sex (Female)	69% (78)	77%(33)	64% (45)	.164 <sup>#</sup>
BMI	28.5 $\pm$ 6.0 (18.9-52.7)	29.0 $\pm$ 5.9	28.12 $\pm$ 4.8	.378
Fasting plasma Glucose	95.3 $\pm$ 10.6 (74-132)	93.98 $\pm$ 10.5(74-124)	95.96 $\pm$ 10.2	.325
Fasting Plasma Insulin	9.38 $\pm$ 7.2	10.41 $\pm$ 8.9	9.0 $\pm$ 5.9	.225
Family History Positive	77%(89)	82%(36)	75%(53)	.312 <sup>#</sup>
HOMA-IR	2.2 $\pm$ 1.6	2.1 $\pm$ 1.4	2.2 $\pm$ 1.6	.941
Log of HOMA-IR	.25 $\pm$ .28	.28 $\pm$ .30	.24 $\pm$ .27	.437
<i>Cerebrospinal Fluid (pg/mL)</i>				
A $\beta$ <sub>1-42</sub>	756.2 $\pm$ 222.5	703.36 $\pm$ 199.5	769.0 $\pm$ 231.0	.092
P-Tau <sub>181</sub>	42.9 $\pm$ 13.9	43.2 $\pm$ 14.4	42.63 $\pm$ 13.6	.842
T-Tau	318.6 $\pm$ 112.3	331.9 $\pm$ 104.2	310.34 $\pm$ 116.9	.326
A $\beta$ <sub>1-42</sub> /P-Tau <sub>181</sub>		.067	.059	.162

<sup>#</sup> Chi-square



**Table 2**Summary of Multiple Regression Analysis for CSF P-Tau<sub>181</sub> (N = 113)

Dependent Variable	Predictor Variable	B	SE(B)	$\beta$	<i>t</i>	Sig.(p)
P-Tau <sub>181</sub>	Age	4.021	1.892	.211	2.126	.050
	Gender	-17.040	23.526	-.070	-.724	.628
	Family History	-3.763	25.727	-.014	-.146	.272
	BMI	-1.617	2.543	-.076	-.636	.520
	APOE $\epsilon$ 4	-31.076	29.296	-.135	-1.061	.117
	HOMA-IR	-111.865	55.039	-.281	-2.032	.005*
	(APOE $\epsilon$ 4 )X(HOMA-IR)	218.175	77.190	.447	2.826	.017*

Regression Model Statistics

 $R^2 = .120$  F(7,105) = 1.888  $p = .056$ 

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**Table 3**

Summary of Multiple Regression Analysis for CSF Total-tau (N = 113)

Dependent Variable	Predictor Variable	B	SE(B)	$\beta$	<i>t</i>	Sig.(p)
Total-tau	Age	.441	.232	.187	1.304	.032
	Gender	-1.093	2.884	-.036	-.379	.429
	Family History	-3.073	3.154	-.092	-.975	.842
	BMI	.109	.312	.041	.351	.645
	APOE $\epsilon$ 4	-6.722	3.591	-.236	-1.872	.365
	HOMA-IR	-17.899	6.747	-.364	-2.653	.030*
	(APOE $\epsilon$ 4 )X(HOMA-IR)	29.434	9.462	.488	3.111	.019*

Regression model Statistics

 $R^2 = .105$  F(7,105) = 1.768  $p = .101$