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Effect of APOE ϵ 4 genotype on metabolic biomarkers in aging and Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) may have heterogeneous pathophysiological underpinnings, with risk factors including apolipoprotein ϵ 4 (APOE4) genotype and insulin resistance. We hypothesized that distinct phenotypes exist within AD. We examined APOE4 and metabolic biomarkers in 338 subjects (n=213 nondemented (ND), n=125 AD). We further characterized steady state free fatty acid (FFA) levels in a subset of 45 participants who had also participated in a hyperinsulinemic-euglycemic clamp. Insulin resistance (HOMA-IR) was elevated in AD vs ND ($p=0.04$) and in APOE4 noncarriers vs. carriers ($p<0.01$). This was driven by increased fasting insulin in AD vs. ND ($p<0.01$) and in APOE4 non-carriers vs carriers ($p=0.01$). Fasting glucose was not different. In subjects who underwent a clamp, there was a group x genotype interaction on FFA levels during hyperinsulinemia ($p=0.03$). APOE4 non-carriers with AD had higher FFA levels, while APOE4 carriers with AD exhibited lower FFA levels. Metabolic dysfunction is overrepresented in individuals with AD dementia who do not carry the APOE4 allele. This suggests that important subsets of AD phenotypes may exist that diverge metabolically.

Keywords

Alzheimer's disease; insulin; apolipoprotein E; hyperinsulinemia; insulin resistance

Introduction

Genetic, neuroimaging, and autopsy data suggest that Alzheimer's disease (AD) dementia syndrome is caused by heterogeneous pathologies [1-3]. In fact, recent work has shown that over half of subjects with clinical AD have mixed pathology, and approximately half of subjects with pathologically defined AD exhibit mixed pathology. [4] In addition, recent

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advances in molecular neuroimaging have demonstrated that a significant percentage of those with AD dementia do not have the hallmark AD pathology of cerebral amyloid deposits, especially Apolipoprotein ε4 (APOE4) non-carriers [5, 6]. Insulin resistance, diabetes, and vascular disease are also recognized risk factors for dementia [7-10]. However, the relationship of such risk factors with AD pathology is unclear, with studies showing both no association and positive associations with neuropathological markers of AD [11-14]. In addition, it was recently shown that in subjects with low AD pathology (but not higher levels of AD pathology), cardiovascular risk factors made an AD clinical diagnosis more likely [15], underscoring the complexity of the relationship between environmental factors, clinical diagnosis, and neuropathology.

On the contrary, APOE4, the primary risk gene for AD, is strongly and consistently linked with amyloid pathology in AD [16]. APOE is known to play a role in lipid metabolism and transport [17, 18] and differences in peripheral glucose disposal have been observed between APOE4 carriers and non-carriers. [19, 20] Interestingly, APOE genotype may also impact the effect of insulin resistance on AD risk; hyperinsulinemia was associated with AD risk only in non-carriers, but not in APOE4 carriers. [21] In addition, in a prior study we observed increased regional cerebral amyloid in nondemented (ND) APOE4 non-carriers only if they also had impaired fasting glucose, while APOE4 carriers exhibited higher cerebral amyloid regardless of glucose levels.[14] Although these relationships are complicated, they are important; some investigational therapies for AD have been shown to elicit memory benefits that may be APOE4 genotype-specific.[22-25] This may be due to vascular or metabolic pathophysiology, which likely plays a role for many individuals with clinical AD. The relationship between vascular (i.e. insulin resistance, heart disease, and stroke), amyloid, and other protein pathologies (TDP-43, Tau, etc.) remains imprecisely defined.

We hypothesize that AD dementia is heterogeneous and that important subsets of AD dementia exist and can be identified by altered metabolism. The goal of the current study was to assess insulin and related metabolic biomarkers in a large, well-characterized cohort of elderly ND and AD subjects and to parse out metabolic differences based on cognitive diagnosis and APOE4 genotype. In a subset of individuals, we also analyzed peripheral free fatty acid (FFA) levels during hyperinsulinemia to characterize insulin's antilipolytic effect (also termed adipose insulin sensitivity), which is strongly related to insulin resistance [26]. We hypothesized that we would observe differences in metabolic biomarkers between diagnostic groups and based on genetic AD risk, which would support insulin resistance as a potential independent, non-genetic risk factor for AD.

Materials and Methods

This study was approved by the University of Kansas Medical Center's Institutional Review Board. All participants in this study provided informed consent according to institutional guidelines and this project was performed in accordance with the Declaration of Helsinki. Inclusion criteria included participation in the University of Kansas Alzheimer's Disease Center clinical cohort, stable medication dose, post-menopausal, ability to provide informed consent, and diagnosis of either no dementia, mild cognitive impairment due to AD or

Dementia due to AD. Exclusion criteria included type 1 diabetes, neurodegenerative disorders other than AD with the potential to impair cognition, and clinically significant depression.

This cross-sectional study included 338 subjects (n=213 ND, n=125 AD). All subjects included in this study were part of the University of Kansas Alzheimer's Disease Center Clinical Cohort. All individuals were assessed clinically and underwent neuropsychometric testing and a fasting blood draw. The severity of dementia was assessed by a clinician using the Clinical Dementia Rating (CDR) scale. Diagnosis was determined at consensus conference based on clinical information and neuropsychometric performance. Subjects were considered ND if they were CDR 0 and there were no clinically significant deficits on neuropsychometric testing. Subjects were included in the AD group if they met criteria for Mild Cognitive Impairment due to AD or Dementia due to AD. All AD subjects had a CDR of 0.5 or higher.

After an overnight fast, blood was collected via venipuncture into EDTA vacutainer tubes and processed for plasma. For the main study, individuals were allowed to take their normal medications unless the medication needed to be taken with food, in which cases subjects were asked to withhold taking the medication until after the fasting blood draw. For the hyperinsulinemic clamp sub-study, diabetic subjects (n=1 ND, n=1 AD) were asked to withhold diabetic medication until after the procedure. Glucose and lactate were measured using a YSI 2300 Glucose and Lactate analyzer. Insulin (Genway) and amylin (Millipore) were quantified using ELISA. Insulin sensitivity was computed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Body composition was assessed using dual energy x-ray absorptiometry (DEXA, Lunar Prodigy, version 11.2068).

A subset of 45 subjects (n=34 ND (21 APOE4 noncarriers, 11 APOE4 carriers), n=11 AD (7 APOE4 noncarriers, 4 APOE4 carriers) from the main analysis also underwent a hyperinsulinemic-euglycemic clamp. Methods and initial results from these analyses have been previously published, and show that AD subjects were more insulin resistant, an effect that was modified by genotype.[20] To expand on the previous finding that AD subjects were more insulin resistant and to investigate potential mechanisms, we measured plasma FFA concentrations on samples collected during the hyperinsulinemic steady state (140min, 160min, and 180min), a condition when lipolysis should be inhibited and FFA should be suppressed. FFA concentration was determined by high-performance liquid chromatography using [$^2\text{H}_3$] palmitate as an internal standard.[27] An average of the steady state FFA values was computed for each participant.

DNA was isolated from whole blood for APOE genotyping. Whole blood was collected into EDTA coated vacutainer tubes, which were inverted 10x to mix prior to centrifugation and sent at ambient temperature for next-day delivery to the National Cell Repository for Alzheimer's Disease (NCRAD). Genotyping was performed on NCRAD samples by LGC Genomics (Beverly, MA) using KASP proprietary genotyping technology. Participants were classified into two genetic groups based upon the presence of a single APOE4 allele (APOE4 non-carriers vs. APOE4 carriers).

Statistical Analyses

Metabolic biomarker data was assessed using Shapiro-Wilk tests of normality and non-normally distributed variables were log-transformed prior to statistical analyses. Two-way analysis of Variance (ANOVA) with diagnosis and genotype as factors was used to determine significance. For interaction effects, the Least Significant Difference (LSD) post-hoc test was used to distinguish differences between groups. Categorical variables were analyzed using Chi-Square tests. Analyses were controlled for age and sex. Cognitive analyses (MMSE) were also controlled for education.

Results

There were no differences in age between groups. Overall, there was no difference in sex between APOE4 carriers (42% male) and noncarriers (also 42% male). However, the AD cohort had more males (56% male) than the ND cohort (35% male, $p<0.01$). AD subjects exhibited greater insulin resistance, as estimated by higher HOMA-IR, compared to ND subjects (Table 1). These differences were driven by elevated fasting insulin; glucose levels were not different between groups. Fasting insulin was higher in both AD subjects ($p=0.009$) and in APOE4 non-carriers ($p=0.012$; Fig 1A). Similarly, APOE4 non-carriers exhibited greater insulin resistance compared to APOE4 carriers (HOMA-IR, $p=0.005$; Fig 1B).

No significant differences were observed with any other metabolic biomarkers (i.e. glucose, lactate, amylin), although there was a trend for higher fasting amylin in AD subjects (Table 1). Measures of body weight, total and regional (android and gynoid) fat mass, total lean mass, BMI, and blood pressure were also not different between groups. As expected, AD subjects had lower MMSE scores compared to ND subjects ($p<0.001$, Table 1).

Given the relationship of diagnosis and genotype with fasting insulin, we analyzed samples from a subset of 45 participants who also underwent a hyperinsulinemic-euglycemic clamp. The insulin stimulated glucose disposal rate (GDR) from this subgroup of 45 participants has been published and shows that AD subjects exhibit lower rates of glucose disposal (more insulin resistance) compared to ND subjects [20]. Here, a novel analysis of free fatty acid (FFA) metabolism in the samples collected following steady state insulin infusion of this procedure showed a significant interaction effect of diagnosis and genotype on insulin-stimulated antilipolysis ($p=0.03$, Figure 2A). Least significant difference (LSD) post-hoc analyses revealed that higher FFA levels were observed in AD compared to ND APOE4 non-carriers during the hyperinsulinemic steady state ($p=0.03$). FFA levels are normally suppressed during hyperinsulinemia through insulin's well-characterized anti-lipolytic effect. Loss of this effect in AD APOE4 non-carriers is a sensitive indication of insulin resistance, which was not observed in AD APOE4 carriers or ND APOE4 non-carriers. To verify that FFA levels are indicative of insulin resistance in aging and AD cohorts, we regressed FFA with the steady state glucose disposal rate (GDR). We observed a strong correlation between steady state FFA and insulin stimulated GDR ($p<0.01$, Fig 2B).

Discussion

APOE4 is the most common risk gene for sporadic AD [28] but still only accounts for approximately half of AD cases. Although other genes are being investigated, genetic risk does not fully account for the disease and a metabolic contribution to AD has been suggested (reviewed in [29]). In this large cross-sectional analysis of well-characterized AD subjects and ND controls, our main finding was an effect of both diagnosis (AD vs. ND) and genotype (APOE4 carrier vs. APOE4 non-carrier) on fasting insulin. Insulin was increased in AD subjects compared to ND subjects and also increased in APOE4 non-carriers relative to APOE4 carriers. This difference in insulin levels also drove differences in fasting insulin resistance (estimated by HOMA-IR), which previous work has shown is related to lower brain glucose metabolism [30]. These effects were observed in the absence of differences in body composition, blood pressure, or fasting glucose. Our secondary finding, a loss of suppression of adipose tissue lipolysis during hyperinsulinemia in AD APOE4 non-carriers, underscores metabolic differences. AD subjects who did not carry APOE4 exhibited a more insulin resistant metabolic phenotype compared to both AD subjects who do carry APOE4 and ND subjects who do not carry APOE4. This suggests that insulin resistance may be involved in a non-APOE pathway to AD.

Mechanistically, fasting insulin is affected by several factors, such as pancreatic insulin release, insulin degradation, and autonomic changes that affect hepatic glucose release (which could lead to compensatory insulin secretion). In fact, incretins that increase pancreatic insulin release have shown promising effects on cognition in animal models [31] and are being investigated in humans [32]. Insulin is primarily degraded by insulin degrading enzyme (IDE), a metalloprotease that also degrades amyloid beta, [33] and has reduced activity in AD brain [34]. It is possible that changes in either insulin secretion, insulin degradation, or both are occurring in AD. It is also possible that hepatic dysregulation is occurring, resulting in changes in gluconeogenesis or triglycerides. Mechanistic explanation of the observed differences in insulin warrants further study.

One important role of insulin that is understudied in AD is suppression of lipolysis [35]. Thus, in addition to measuring fasting insulin levels, in a subset of individuals we assessed insulin's antilipolytic effect by measuring FFA levels during hyperinsulinemia. In metabolically healthy individuals, insulin suppresses fat mobilization, resulting in lower FFA levels after meals when insulin is elevated and higher concentrations of FFA when insulin is reduced such as after an overnight fast [36]. Here, insulin-suppressed FFA levels were measured during the steady-state of a hyperinsulinemic-euglycemic clamp on a subset of individuals we had previously characterized for insulin stimulated GDR. FFA levels did not differ between groups at baseline, but we observed a significant interaction effect between group and genotype on FFA levels during hyperinsulinemia. AD APOE4 negative subjects had higher steady-state FFA levels compared to ND APOE4 negative subjects. This may be due to impaired insulin signaling, and although we did not measure insulin signaling markers, we did observe a strong inverse relationship between glucose disposal rate and steady-state FFA levels, indicating that more insulin resistance results in higher steady-state FFA levels. These are subtle changes, as the low steady-state FFA levels observed in our

study suggest that these subjects still display relatively high insulin-sensitivity in adipose tissue, yet these changes may still be physiologically relevant.

Our finding of increased fasting insulin in both AD subjects and APOE4 non-carriers is partially consistent with recent work, which showed higher fasting insulin in cognitively impaired subjects but no effect of APOE4 status [37]. Differences in assay type, cohort age, and diagnostic characterization of subjects could contribute to these differences. Strengths of this study included a comprehensive diagnostic assessment (clinical dementia rating, neurological exam, neuropsychometric testing and consensus diagnosis conference), large sample size, and comprehensive metabolic assessment, including a very sensitive subset analysis of FFA release during hyperinsulinemia. Weaknesses of this study include the difference in the sex composition of groups, although sex was included as a covariate in statistical analyses and insulin levels were not different between sexes, and the small sample size of the AD group in the hyperinsulinemic clamp sub-analysis. Additional work to parse out the mechanism for these relationships is needed.

Conclusion

Insulin resistance appears to be consistently observed in some form in the AD population. However, the relationship between metabolic and genetic risk factors in AD is not straightforward. This cross-sectional study suggests that both AD diagnosis and APOE4 genotype affect fasting insulin levels and insulin resistance. These factors may partly explain the heterogeneous pathologies observed in AD, with insulin resistance present in AD but potentially playing a larger role in AD subjects who are APOE4 noncarriers. The composition of study groups in terms of both diagnosis and genotype may affect metabolic outcomes and should be an important consideration in study design.

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Figure 1.

A) Fasting insulin is significantly lower in both ND subjects and APOE4 carriers. B) Insulin drives differences in HOMA-IR. Bars are shown as means $\pm$ SE. * $p < 0.05$ ND vs. AD group
$p < 0.05$ between APOE ϵ 4 carriers vs. noncarriers.

Figure 2.

A) In a subset of 45 subjects who also participated in a hyperinsulinemic-euglycemic clamp, there was an interaction effect between diagnosis and genotype. Steady-state FFA levels were higher during hyperinsulinemia in AD APOE4 non-carriers compared to ND APOE4 non-carriers, indicating of insulin resistance. D) There was a strong inverse correlation between steady state glucose disposal rate and FFA levels. Bars are shown as means $\pm$ SE.

* $p < 0.05$ for diagnosis x genotype interaction.

Table 1

Participant characteristics.

	Nondemented (n=213)		Alzheimer's Disease (n=125)		p-value (dx)	p-value (E4)	p-value (E4+dx)
	No E4 (n=159)	E4 carrier (n=54)	No E4 (n=47)	E4 carrier (n=78)			
Age (y)	72 [6.9]	73 [6.7]	74 [9.1]	74 [7.8]	0.17	0.54	0.89
Sex (#,%male)	58 [37]	15 [28]	29 [62]	41 [53]	<0.01	0.97	0.01 *
Diabetes (#,%)	19 [12.0]	5 [11.1]	9 [19.1]	12 [15.4]	0.11	0.86	0.46
Insulin (μU/mL)	7.6 [6.7]	5.1 [3.8]	9.3 [6.5]	7.1 [5.7]	<0.01 *	0.01 *	0.81
Amylin (pM)	18.1 [58.9]	9.5 [12.7]	21.8 [52.3]	18.7 [60.4]	0.08	0.16	0.90
Glucose (mg/dL)	93.0 [27.8]	90.3 [18.0]	97.1 [46.8]	90.7 [18.8]	0.97	0.34	0.74
Lactate (mg/dL)	23.5 [10.6]	25.8 [17.6]	23.7 [11.9]	23.8 [11.2]	0.58	0.51	0.32
HOMA-IR	1.9 [2.1]	1.1 [0.8]	2.2 [1.7]	1.8 [1.9]	0.04	<0.01	0.60
BMI (kg/m ²)	27.7 [4.8]	26.9 [4.4]	27.3 [5.9]	26.2 [4.3]	0.19	0.16	0.85
Waist toHip ratio	0.90 [0.09]	0.88 [0.08]	0.93 [0.10]	0.92 [0.11]	0.95	0.33	0.89
Total Fat mass (kg)	29.0 [9.9]	27.1 [9.2]	27.3 [11.7]	25.8 [7.8]	0.32	0.18	0.95
Total lean mass (kg)	44.7 [9.1]	42.5 [9.6]	47.4 [9.7]	45.4 [10.2]	0.08	0.28	0.91
Android fat mass (kg)	2.7 [1.2]	2.4 [1.1]	2.7 [1.5]	2.4 [1.1]	0.41	0.15	0.82
Gynoid fat mass (kg)	4.7 [1.6]	4.5 [1.5]	4.2 [1.9]	4.1 [1.2]	0.20	0.31	0.61
BMD (g/cm ³)	1.2 [0.14]	1.1 [0.17]	1.2 [0.12]	1.2 [0.13]	0.10	0.98	0.52
Systolic (mmHg)	128.3 [15.7]	131.6 [16.3]	129.9 [12.6]	132.1 [16.3]	0.67	0.20	0.75
Diastolic (mmHg)	73.6 [8.1]	74.2 [8.0]	75.2 [7.6]	72.5 [8.0]	0.75	0.37	0.09
MMSE (raw score)	29.3 [1.0]	29.2 [1.0]	23.9 [5.4]	23.4 [4.8]	<0.01 *	0.68	0.54
Basal FFA (nmol/mL)	456.2 [169]	558.4 [222]	406.4 [147]	141.3 [154]	0.45	0.88	0.21
SSFFA (nmol/mL) [‡]	34.5 [15.6]	41.2 [15.0]	49.2 [11.6]	32.8 [12.2]	0.78	0.55	0.03 *

Values are given as means ± SD. p-value (dx) and p-value (e4) are the p-values for main effects of diagnosis (ND vs AD) and APOE4 (noncarrier vs. carrier) and the interaction effect. Post-hoc values for significant interactions given in the text.

[‡]Steady-state FFA data available in a subset of 45 participants.

* p<0.05 between groups.

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HOMA-IR: homeostatic model assessment of insulin resistance, BMI: body mass index, BMD: bone mineral density, SBP: systolic blood pressure, DBP: diastolic blood pressure, MMSE: Mini-mental state examination, Basal FFA: basal free fatty acids, SSFA: steady-state free fatty acids.