

Presence of the *APOE* $\epsilon 4$ allele modifies the relationship between type 2 diabetes and cognitive performance: the Maine–Syracuse Study

G. A. Dore · M. F. Elias · M. A. Robbins · P. K. Elias · Z. Nagy

Received: 13 July 2009 / Accepted: 16 July 2009
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Abstract

Aims/hypothesis The primary aim of this study was to determine whether the presence of one or more *APOE* $\epsilon 4$ alleles modifies the association between diabetes (defined by glucose ≥ 7 mmol/l or treatment) and cognitive function. **Methods** Diabetic status and *APOE* genotype interactions were assessed cross-sectionally for 826 community-dwelling, stroke-free, non-demented individuals (526 non-diabetic non-*APOE* $\epsilon 4$ carriers, 174 non-diabetic *APOE* $\epsilon 4$ carriers, 87 diabetic *APOE* $\epsilon 4$ non-carriers, 39 diabetic *APOE* $\epsilon 4$ carriers) ranging in age from 50 to 98 years. Cognitive function was assessed using the Mini-Mental State Examination (MMSE), the similarities subtest from the Wechsler Adult Intelligence Scale, and four composite scores derived from 17 additional neuropsychological tests. Multiple linear regression analyses were employed to relate diabetes and *APOE* genotype to cognitive performance and to examine the interaction between these two risk factors as

they relate to cognitive performance. Multiple cardiovascular disease risk factors were statistically controlled.

Results With adjustment for age, education, sex, race/ethnicity and *APOE* genotype, performance level was lower for the diabetic than for the non-diabetic group for the MMSE, the similarities subtest and each of the cognitive composites with the exception of the verbal memory composite. Interactions ($p < 0.05$) between diabetes and *APOE* genotype were found for all but the visual–spatial memory/organisation composite. The negative association between diabetes and cognitive performance was of a higher magnitude for individuals who carry one or more *APOE* $\epsilon 4$ alleles. Results were similar with additional adjustment for cardiovascular disease and associated risk factors.

Conclusions/interpretation The presence of one or more *APOE* $\epsilon 4$ alleles modifies the association between diabetes and cognitive function.

Keywords Apolipoprotein E · Cognition · Type 2 diabetes

Electronic supplementary material The online version of this article (doi:10.1007/s00125-009-1497-2) contains supplementary material, which is available to authorized users.

G. A. Dore (✉) · M. F. Elias · M. A. Robbins · P. K. Elias
Department of Psychology, University of Maine,
5742 Little Hall,
Orono, ME 04469-5742, USA
e-mail: gregory.dore@maine.edu

M. F. Elias · M. A. Robbins
Graduate School of Biomedical Sciences, University of Maine,
Orono, ME, USA

Z. Nagy
School of Clinical and Experimental Medicine,
College of Medical and Dental Sciences,
University of Birmingham,
Birmingham, UK

Abbreviations

3MSE	Modified Mini-Mental State Examination
A β	β -Amyloid
AGE	Advanced glycation endproducts
CES-D	Center for Epidemiological Studies Depression Scale
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
IDE	Insulin degrading enzyme
MMSE	Mini-Mental State Examination
MSLS	Maine–Syracuse Longitudinal Study
RF	Risk factor
SBP	Systolic blood pressure
tHcy	Plasma homocysteine

Introduction

Diabetes mellitus has been associated with decrements in cognitive performance and dementia in cross-sectional and longitudinal studies [1–4]. A recent review indicates that individuals with diabetes have a greater risk of cognitive decline and a greater risk of developing dementia than do non-diabetic individuals [3]. Among others, possible mechanisms for this association include oxidative stress, accelerated ischaemic brain damage [5] and impaired use of glucose during cognitive tasks [6].

The presence of at least one apolipoprotein E (*APOE*) ϵ 4 allele—another important risk factor for cardiovascular disease (CVD)—is associated with dementia and lowered levels of cognition [7, 8]. While some studies have indicated that the presence of at least one *APOE* ϵ 4 allele is associated with lowered cognitive performance [9, 10], negative findings have been reported in a number of investigations, particularly those in which persons with dementia or preclinical dementia have been excluded from the study samples [11–15].

Aside from serving as a CVD risk factor in its own right, the *APOE* ϵ 4 allele may modify the effects of other CVD risk factors, including diabetes, on performance on the Modified Mini-Mental State Examination (3MSE) [16]. That is, the negative association between diabetes and mental status may be more pronounced in diabetic individuals who carry at least one *APOE* ϵ 4 allele. Effect modification by the presence of the *APOE* ϵ 4 allele has been reported in investigations of the association of diabetes mellitus and dementia. Individuals with both diabetes and an *APOE* ϵ 4 allele are more likely to develop dementia than either those with diabetes alone or non-diabetic individuals who carry the *APOE* ϵ 4 allele. The risk of dementia for diabetic *APOE* ϵ 4 carriers is greater than would be predicted by the simple additive effect of the two risk factors [17, 18].

Relatively few studies have examined the interaction of objectively defined diabetes and the *APOE* ϵ 4 allele as it relates to complex cognitive abilities in non-demented individuals. Small et al. [13], using self-report of diabetes, failed to find an interaction between diabetes and the *APOE* ϵ 4 allele in multiple cognitive abilities. However, Haan et al. [16], using objective measures of diabetes, found that individuals with both an *APOE* ϵ 4 allele and diabetes showed a greater decline on the 3MSE than those with neither of these risk factors. Given the fact that the combined influence of diabetes and the *APOE* ϵ 4 allele may be greater than the influence of either risk factor alone, it is important to examine the combined influence of diabetes and the *APOE* ϵ 4 allele in a study assessing a variety of domains of cognitive performance.

Our hypotheses were as follows: (1) consistent with the literature, type 2 diabetes will be associated with perfor-

mance decrement in multiple cognitive domains even with adjustment for CVD risk factors and events; (2) however, there will be an interaction between the presence or absence of *APOE* ϵ 4 allele and type 2 diabetes such that the difference in cognitive performance between diabetic and non-diabetic individuals will be larger within *APOE* ϵ 4 carriers (one or two alleles) compared with non-carriers.

Methods

Sample and design Cross-sectional data were taken from the sixth serial repetition (wave 6) of the Maine–Syracuse Longitudinal Study (MSLS), a community-based study of CVD risk factors and cognition begun in Syracuse, New York, in 1974. Recruitment and data collection procedures for wave 6 have been described in detail previously [19]. The MSLS is an open-enrolment longitudinal study in which new individuals are recruited at each wave. Wave 6 (2001 to 2006) was the first and only wave for which diabetes was determined by objective methods (see below) for all individuals.

Of the 1,060 participants (23 to 98 years of age) eligible for the study at wave 6, participants were excluded in the following sequence: (1) history of stroke ($n=27$); (2) probable dementia ($n=7$); and (3) under 50 years of age ($n=200$). The final sample consisted of 826 participants. Persons under 50 years of age were excluded in the primary analysis because several studies indicate that *APOE* genotype does not relate to cognitive function in younger individuals [20, 21]. A secondary set of analyses was done with the individuals under 50 years of age included. We did not exclude persons with mild cognitive impairment because we wished to retain the full range of variation in continuously distributed cognitive test scores, while eliminating persons who showed major decrements in performance level and were often unable to complete few, if any, of the tests in our battery (i.e. those with stroke and/or dementia).

Stroke, defined as a focal neurological deficit of acute onset persisting more than 24 h, was based on self-report and was confirmed by a record review indicating a diagnosis of acute stroke. The clinical diagnosis of dementia was based on cognitive data and medical records, using the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association criteria [22].

Procedures Participants completed the Center for Epidemiological Studies Depression Scale (CES-D) [23] within 1 week prior to neuropsychological testing. Following a fast from midnight, a blood sample was drawn in the morning and followed by a light breakfast and interview (including medical history). Subsequently, after supine rest

for 15 min, five reclining, five standing, and five sitting automated blood pressure measurements (GE DINAMAP 100DPC-120XEN; GE Healthcare, Chalfont St Giles, UK) were obtained sequentially with a 5 min interval between each set of measurements. Neuropsychological testing followed the BP measurements. Tests were presented in the same order for each individual because of the necessity of uniform sequencing and standard presentation of the Wechsler Adult Intelligence Scale subtests, the Wechsler Memory Scale subtests, trails A and B and other measures. Brief rest periods were given whenever participants appeared to be in need of a rest before continuing. The order of presentation for all of the individual tests is given in Electronic supplementary material (ESM) Table 1. All assay methods used to derive data on independent variables and covariates have been described previously [19]. Diabetes mellitus was defined by treatment with insulin, oral glucose-lowering agents, or by fasting glucose level of 7 mmol/l or higher. Objective data on duration of diabetes were not available to the study, but persons with diabetes at wave 6 (objectively defined) were asked to estimate the duration of their diabetes and this was used as a descriptive variable. For diabetic participants, glycaemic control was defined as 3.9–7.2 mmol/l preprandial or <10 mmol/l postprandial plasma glucose, in accordance with glycaemic recommendations outlined by the American Diabetes Association [24].

Standard *APOE* genotyping used polymerase chain reaction and restriction enzyme digest with HhaI [25]. Serum creatinine was determined using a two-point rate test type on a Johnson and Johnson VITROS instrument (Ortho Clinical Diagnostics, Rochester, NY, USA). Coefficients of variation for these procedures were less than 5.0%. Estimated glomerular filtration rate was derived from the four-variable (serum creatinine, age, sex and ethnicity) Modification of Diet in Renal Disease study equation [26, 27]. Chronic renal disease (yes/no) was defined as estimated glomerular filtration rate <60 ml min⁻¹ (1.73 m²)⁻¹. Determinations of high sensitivity C-reactive protein (CRP), plasma homocysteine (tHcy), triacylglycerols and glucose were performed as recently described [19]. Mean systolic BP (SBP) and diastolic BP (DBP) were determined by taking the average of 15 BP measurements (described previously).

Additional covariates used in various analyses included: BMI (kg/m²), self-report of number of cigarettes smoked per week, alcohol consumption (g/week), and self-reported presence of CVD confirmed by medical records and/or treatment. As in the Framingham Heart Study [28], CVD was defined as the presence of any one of the following: (1) myocardial infarction (4.5%); (2) coronary artery disease (8.8%); (3) heart failure (2.4%); (4) angina pectoris (5.9%); (5) transient ischaemic attack (4.1%).

Cognitive tests and domains We employed the MMSE, the similarities subtest from the Wechsler Adult Intelligence Scale, and four composite test scores derived from a previous factor analysis of individual tests in the MSLS battery for this study population [19]. The four composite scores were visual–spatial memory and organisation (visual reproductions—immediate and delayed, matrix reasoning, block design, object assembly, and the Hooper Visual Organization Test), scanning and tracking (trails A and B, digit symbol substitution, and symbol search), verbal episodic memory (logical memory—immediate and delayed, and the Hopkins Verbal Learning Test) and working memory (digit span forward and backward, letter–number sequencing, and controlled oral word associations). The similarities subtest was used as a separate measure because it loaded on multiple composite scores in the previous factor analysis. In addition to the factor analyses, reducing the number of outcome variables, we followed a protection rule in which none of the results for individual tests would be interpreted in the absence of a significant result for the global composite score.

More detailed descriptions of the individual tests are given in Table 1. To construct the composite scores, the individual tests related to each composite were expressed in *z* scores and added [19]. The composite scores were again transformed to *z* scores. Composite scores were used to decrease error associated with analyses involving multiple related cognitive outcomes and to permit us to examine theoretically relevant cognitive domains.

This linear transformation results in a mean of zero and an SD of 1.00 for each test and enables expression of regression coefficients for the cognitive measures in terms of SD units. The previously identified composites (factors) [19] were confirmed via replication of the factor analysis for the present sample. In addition to composite scores, a global composite score was calculated by averaging the *z* scores for all individual tests (excluding the MMSE). The MMSE was considered to be a separate measure of mental status.

The University of Maine Institutional Review Board approved the protocol for this investigation. Informed consent for data collection was obtained from all participants.

Statistical analyses The independent variables were *APOE* genotype (classified as $\epsilon 4$ carrier or $\epsilon 4$ non-carrier) and diabetes. Covariates employed in the primary regression models were age (years), education (years), sex, race/ethnicity (African-American/other), systolic blood pressure (mmHg), smoking (cigarettes per week), triacylglycerols (mmol/l), mild renal dysfunction (yes/no), BMI (kg/m²), alcohol consumption (g/week), depressed mood (CES-D score), CRP (nmol/l), prevalent CVD (yes/no), and tHcy (μ mol/l).

These covariates were selected based on their theoretical relevance to the predictors and outcomes of interest (e.g. the association between diabetes and cognitive function may be partially explained by comorbid CVD risk factors). All of these covariates have been shown to predict cognitive function in previous studies. All of the covariates except for age and sex differed across the *APOE*/diabetes groups.

Using the general linear model (SAS Version 9.1), multivariable regression analyses were performed for the categorical and continuously distributed variables. All covariates within a covariate set entered into the regression equations simultaneously with the independent variable.

The following multivariable regression models were used: (1) basic model = diabetes + *APOE* + age(years) + sex + education + race/ethnicity + diabetes × *APOE*; (2) basic + risk factor(RF) + CVD model = basic model + SBP + smoking + triacylglycerols + chronic kidney disease + BMI + alcohol consumption + depressed mood + CRP + prevalent CVD + tHcy. The distributions for CRP

and tHcy were skewed, and were normalised with a natural log transformation.

After observing significant diabetes by age interactions ($p < 0.05$), we stratified by diabetes and *APOE* $\epsilon 4$ groups and performed multiple comparisons of *APOE* groups within diabetic groups and diabetic groups within *APOE* groups. We again followed a protection rule in which none of the individual tests or composite scores would be interpreted in the absence of a statistically significant effect ($p < 0.05$) for the global composite.

Results

Demographic and health information for the current sample and the results of analyses of study covariates comparing cell means or proportions between the *APOE* groups for diabetic and non-diabetic participants are provided in Table 2. Significance levels for the remaining contrasts are given in ESM Table 2. Stratification by presence/

Table 1 Descriptions of the cognitive tests contributing to each composite score indexing a cognitive domain^a

Test composite/tests included in the composite	Cognitive ability measured
Verbal episodic memory	
Logical memory—immediate recall ^a	Immediate memory, verbal
Logical memory—delayed recall ^a	Delayed memory, verbal
Hopkins verbal learning test	Verbal learning and memory
Visual-spatial memory/organisation	
Visual reproductions—immediate recall ^a	Immediate recall, visual memory, and visual-spatial problem solving
Visual reproductions—delayed recall ^a	Delayed recall, visual memory and visual-spatial problem solving
Matrix reasoning ^b	Abstract reasoning and pattern recognition
Block design ^c	Visual-spatial perception, organisation and construction
Object assembly ^c	Speed of visual-spatial organisation
Hooper Visual Organization	Visual-spatial organisation; some demands on executive function
Scanning and tracking	
Trail making A ^d	Visual scanning and tracking; concentration and attention
Trail making B ^d	Trails A plus demands on executive function abilities
Digit symbol substitution ^c	Psychomotor performance
Symbol search ^b	Visual processing speed
Working memory	
Digit span forward ^c	Attention and concentration
Digit span backward ^c	Attention, concentration, and working memory
Letter-number sequence ^b	Information processing while holding information in memory
Controlled oral word associations	Verbal fluency and executive functioning
Similarities ^c	Verbal intelligence and abstract reasoning

The tests employed in each composite score/domain define the abilities measured by that domain

^a Origin: Wechsler Memory Scale, revised

^b Origin: Wechsler Adult Intelligence Scale III

^c Origin: Wechsler Adult Intelligence Scale

^d Origin: Halstead-Reitan Neuropsychological Test Battery

Table 2 Demographic information and health characteristics ($n=826$)

Variable	Non-diabetic			Diabetic		
	No <i>APOE</i> $\epsilon 4$ ($n=526$)	<i>APOE</i> $\epsilon 4$ ($n=174$)	<i>p</i> value	No <i>APOE</i> $\epsilon 4$ ($n=87$)	<i>APOE</i> $\epsilon 4$ ($n=39$)	<i>p</i> value
Age (years), mean (SD)	66.2 (10.4)	65.4 (9.8)	0.39	67.0 (8.9)	63.0 (8.8)	0.02
Education (years), mean (SD)	14.7 (2.7)	14.7 (2.8)	0.95	13.4 (2.7)	13.5 (3.6)	0.83
Alcohol (g/week), mean (SD)	34.1 (58.8)	34.4 (62.3)	0.95	20.2 (41.8)	9.2 (20.7)	0.12
Cigarettes per week, mean (SD)	6.7 (35.9)	7.4 (30.2)	0.81	2.9 (13.1)	13.5 (40.6)	0.03
Total cholesterol (mmol/l), mean (SD)	5.3 (1.0)	5.3 (1.0)	0.97	4.8 (1.2)	5.0 (1.1)	0.32
LDL-cholesterol (mmol/l), mean (SD)	3.2 (0.9)	3.2 (0.8)	0.83	2.7 (0.9)	2.7 (0.8)	0.93
HDL-cholesterol (mmol/l), mean (SD)	1.4 (0.4)	1.4 (0.4)	0.94	1.2 (0.3)	1.2 (0.3)	0.20
Triacylglycerols (mmol/l), mean (SD)	1.5 (0.9)	1.6 (1.3)	0.50	1.9 (1.6)	2.7 (2.1)	0.03
Glucose (mmol/l), mean (SD)	5.2 (0.6)	5.1 (0.6)	0.46	7.9 (3.0)	8.2 (2.2)	0.62
C-reactive protein (nmol/l), mean (SD)	39.7 (44.2)	27.9 (26.9)	0.002	59.5 (60.4)	48.1 (56.4)	0.36
Plasma homocysteine (μ mol/l), mean (SD)	74.2 (27.3)	73.4 (23.1)	0.72	89.4 (44.2)	81.0 (24.4)	0.27
Serum creatinine (μ mol/l), mean (SD)	39.7 (44.2)	81.2 (18.8)	0.22	126.1 (145.7)	100.2 (51.6)	0.28
Systolic blood pressure (mmHg), mean (SD)	132.8 (20.9)	131.6 (21.5)	0.52	139.2 (22.5)	138.0 (20.3)	0.79
Diastolic blood pressure (mmHg), mean (SD)	71.1 (10.2)	69.7 (9.1)	0.11	72.5 (10.4)	72.2 (10.5)	0.87
Body mass index (kg/m^2), mean (SD)	28.7 (5.3)	28.7 (5.4)	0.91	32.6 (7.3)	34.3 (7.3)	0.24
Waist circumference (cm), mean (SD)	94.1 (13.9)	93.3 (13.4)	0.50	105.2 (16.9)	107.9 (13.9)	0.40
CES-D, mean (SD)	7.1 (6.5)	7.5 (6.9)	0.42	8.7 (8.1)	10.6 (7.5)	0.23
Duration of diabetes, mean (SD)	–	–	–	10.9 (9.5)	8.2 (6.7)	0.13
Glycaemic control, <i>n</i> (%)	–	–	–	37 (42.5)	10 (25.6)	0.07
Women, <i>n</i> (%)	326 (62.0)	114 (65.5)	0.40	44 (50.6)	23 (59.0)	0.38
African-American, <i>n</i> (%)	24 (4.6)	12 (6.9)	0.23	15 (17.2)	12 (30.8)	0.09
Depressed mood, <i>n</i> (%)	51 (9.7)	16 (9.3)	0.85	11 (12.6)	6 (15.4)	0.68
Drinker, <i>n</i> (%)	275 (52.3)	88 (50.6)	0.70	28 (32.2)	10 (25.6)	0.46
Smoker, <i>n</i> (%)	31 (5.9)	14 (8.1)	0.32	5 (5.8)	4 (10.3)	0.36
CVD, <i>n</i> (%)	73 (13.9)	25 (14.4)	0.87	30 (34.5)	10 (25.6)	0.32
Mild renal dysfunction, <i>n</i> (%)	76 (14.5)	32 (18.4)	0.21	29 (33.3)	10 (25.6)	0.39
Hypertensive, <i>n</i> (%)	337 (64.1)	111 (63.8)	0.95	77 (88.5)	33 (84.6)	0.54
<i>APOE</i> genotype, <i>n</i> (%)						
2/2	11 (2.09)	–	–	2 (2.3)	–	0.81
2/3	87 (16.5)	–	–	12 (13.8)	–	–
3/3	428 (81.4)	–	–	73 (83.9)	–	–
2/4	–	14 (8.1)	–	–	5 (12.8)	0.42
3/4	–	136 (78.2)	–	–	31 (79.5)	–
4/4	–	24 (13.8)	–	–	3 (7.7)	–

absence of *APOE* $\epsilon 4$ allele resulted in 213 carriers of the $\epsilon 4$ allele and 613 non-carriers. In the non-diabetic participants, *APOE* $\epsilon 4$ carriers had significantly lower CRP levels. In the diabetic participants, *APOE* $\epsilon 4$ carriers had a significantly higher mean number of cigarettes smoked per week and higher triacylglycerol levels and were significantly younger than *APOE* $\epsilon 4$ non-carriers. It is important to note that self-reported duration of diabetes in years did not differ between diabetic *APOE* $\epsilon 4$ carriers and non-carriers, and the non-significant trend was for non-carriers to exhibit a longer duration of diabetes ($p=0.13$).

Glucose levels were not significantly different between diabetic *APOE* $\epsilon 4$ carriers and non-carriers (Table 2), although there was a trend for glucose levels to be higher for diabetic *APOE* $\epsilon 4$ carriers. However, a higher proportion of the diabetic *APOE* $\epsilon 4$ carriers were in the glycaemic control range ($p=0.05$). Therefore, glycaemic control was used as a covariate in secondary analyses (outlined below).

The number of persons classified into the possible *APOE* genotypes was as follows: 2/2 ($n=13$), 2/3 ($n=99$), 2/4 ($n=19$), 3/3 ($n=501$), 3/4 ($n=167$) and 4/4 ($n=27$). The *APOE* $\epsilon 4$ cohort (2/4, 3/4, 4/4) represented 25.8% of

our sample. This representation is within the range (24–30%) reported in other studies of *APOE* [16, 29, 30].

Mean cognitive scores for non-diabetic and diabetic individuals with adjustment for variables in the basic and basic+RF+CVD models are shown in Table 3. For both models, diabetic individuals performed significantly more poorly on all composite scores except for the verbal memory composite.

Cognitive score means adjusted for the basic and basic+RF+CVD models by *APOE* group are shown in Table 4. For the basic model, *APOE* $\epsilon 4$ carriers performed more poorly than non-carriers on the global and verbal memory composites and the MMSE. With adjustment for the basic+RF+CVD covariate set, no significant associations between *APOE* genotype and cognitive outcome variables were observed, although the trend was for *APOE* $\epsilon 4$ carriers to perform more poorly.

Significant diabetes \times *APOE* interactions were obtained for the global composite ($p=0.004$), the working memory composite ($p<0.001$), the verbal memory composite ($p=0.025$), similarities ($p=0.046$), and the MMSE ($p=0.008$) scores. A marginal interaction was also observed for the scanning and tracking composite ($p=0.088$). Therefore, the remaining analyses for these six variables were done with stratification by diabetes and *APOE* status.

Figure 1 illustrates the nature of the *APOE* $\epsilon 4$ by diabetes interaction with adjustment for the basic covariate set. For all cognitive outcomes with the exception of the

visual–spatial memory and organisation (Fig. 1), diabetic *APOE* $\epsilon 4$ carriers performed significantly worse than diabetic *APOE* $\epsilon 4$ non-carriers (p range= <0.001 – 0.02). In the non-diabetic subsample, no difference between the *APOE* groups was observed for any of the cognitive measures (all $p>0.35$). Within *APOE* $\epsilon 4$ carriers, participants with diabetes performed worse than those without diabetes on all cognitive measures shown in Fig. 1 (all $p<0.01$). Results were similar within *APOE* $\epsilon 4$ non-carriers; however, diabetic and non-diabetic participants did not differ in performance on the working memory ($p=0.23$) or the verbal memory ($p=0.95$) composites.

With adjustment for the basic+RF+CVD covariate set (data not shown), the pattern of significant results was the same with two exceptions: within *APOE* $\epsilon 4$ non-carriers, diabetic and non-diabetic participants did not differ significantly in performance on the similarities subtest ($p=0.22$) or the MMSE ($p=0.30$).

To address the possibility that diabetes \times *APOE* interactions may be due to poorer glycaemic control in the *APOE* carriers, we performed two secondary analyses within the diabetic sample using the definition of glycaemic control given above. The glycaemic control variable (yes/no) did not relate to any of the cognitive variables (all $p>0.47$). Further, with glycaemic control as a covariate, the relationship between *APOE* genotype and cognitive outcome variables within diabetic patients was significant ($p<0.05$) for all cognitive outcomes, with the exception of

Table 3 Adjusted means and standard errors illustrating the relationship between diabetes and cognitive outcome variables

Cognitive outcome		Basic model ^a			Basic + RF + CVD model ^b		
		Non-diabetic	Diabetic	<i>p</i> value	Non-diabetic	Diabetic	<i>p</i> value
Global	Mean	0.028	−0.341	<0.001	0.040	−0.276	<0.001
	SEM	0.032	0.069		0.030	0.076	
Working memory	Mean	0.019	−0.301	<0.001	0.024	−0.225	0.02
	SEM	0.039	0.085		0.041	0.095	
Similarities	Mean	0.038	−0.345	<0.001	0.024	−0.213	0.01
	SEM	0.036	0.078		0.037	0.087	
Verbal memory	Mean	−0.013	−0.144	0.14	−0.010	−0.124	0.25
	SEM	0.038	0.082		0.040	0.092	
Visual–spatial memory/organisation	Mean	0.040	−0.301	<0.001	0.067	−0.251	<0.001
	SEM	0.034	0.074		0.035	0.082	
Scanning and tracking	Mean	0.024	−0.300	<0.001	0.037	−0.258	<0.001
	SEM	0.032	0.070		0.033	0.077	
MMSE	Mean	0.018	−0.339	<0.001	0.031	−0.199	0.01
	SEM	0.036	0.078		0.037	0.085	

^a Basic model: diabetes, *APOE* group, age, education, sex, race/ethnicity

^b Basic+RF+CVD model: diabetes, *APOE* group, age, education, sex, race/ethnicity, SBP, smoking, triacylglycerols, chronic kidney disease, BMI, alcohol consumption, depressed mood, CRP, prevalent CVD, tHcy

Table 4 Adjusted means and standard errors illustrating the relationship between *APOE* $\epsilon 4$ and cognitive outcome variables

Cognitive outcome		Basic model ^a			Basic+RF+CVD model ^b		
		No <i>APOE</i> $\epsilon 4$	<i>APOE</i> $\epsilon 4$	<i>p</i> value	No <i>APOE</i> $\epsilon 4$	<i>APOE</i> $\epsilon 4$	<i>p</i> value
Global	Mean	-0.098	-0.215	0.05	-0.077	-0.155	0.20
	SEM	0.040	0.056		0.042	0.059	
Working memory	Mean	-0.080	-0.202	0.09	-0.046	-0.156	0.15
	SEM	0.049	0.069		0.053	0.074	
Similarities	Mean	-0.111	-0.197	0.20	-0.044	-0.145	0.15
	SEM	0.045	0.064		0.049	0.068	
Verbal memory	Mean	-0.010	-0.144	0.05	-0.014	-0.120	0.15
	SEM	0.038	0.082		0.051	0.072	
Visual-spatial memory/organisation	Mean	-0.106	-0.154	0.45	-0.091	-0.093	0.97
	SEM	0.043	0.060		0.046	0.064	
Scanning and tracking	Mean	-0.085	-0.191	0.08	-0.079	-0.142	0.31
	SEM	0.040	0.057		0.043	0.060	
MMSE	Mean	-0.085	-0.236	0.02	-0.025	-0.143	0.09
	SEM	0.045	0.064		0.048	0.067	

^a Basic model: diabetes, *APOE* group, age, education, sex, race/ethnicity

^b Basic+RF+CVD model: diabetes, *APOE* group, age, education, sex, race/ethnicity, SBP, smoking, triacylglycerols, chronic kidney disease, BMI, alcohol consumption, depressed mood, CRP, prevalent CVD, tHcy

the visual-spatial memory and organisation composite ($p=0.09$).

Results were also the same regardless of the substitution of the various lipid subtypes for triacylglycerols in the models and when hypertensive diagnostic status (BP \geq 140/90 mmHg or treatment) was included in the model.

Given the relatively small number of African-American persons in the study and the possibility that statistical adjustment would not adequately control for race/ethnicity, we excluded all African-American individuals and repeated the analyses. The pattern of means for the diabetes/*APOE* groups was the same as that presented in Fig. 1, except that the diabetes \times *APOE* interaction was significant for only the following three composites: global ($p=0.05$), verbal memory ($p=0.04$) and working memory ($p=0.004$).

In a final analysis, the previously excluded individuals under the age of 50 were included in the analysis. As anticipated [20], no *APOE* main effects were observed, and there were fewer interactions, i.e. the diabetes \times *APOE* interaction was significant only for the global ($p=0.04$) and working memory composites ($p=0.01$), and the MMSE ($p=0.04$), and was marginal for similarities ($p=0.07$). ESM Fig. 1 shows the pattern of means for this analysis.

Discussion

APOE genotype was associated with decrements in cognitive performance for the global and verbal memory

composites and the MMSE. This finding is in agreement with previous studies reporting relations between *APOE* genotype and cognitive function [8, 31].

Also consistent with previous findings [2, 4, 32], and as hypothesised, mean performance scores were lower for the diabetic than for the non-diabetic group for MMSE, the similarities subtest and each of the cognitive composite scores, with the exception of the verbal memory composite.

Also as hypothesised, *APOE* status served as an effect modifier (i.e. the relationship between diabetes and lower cognitive performance was greater for individuals who carried one or more *APOE* $\epsilon 4$ alleles with adjustment for age, education, sex and race/ethnicity). The same result was observed with adjustment for the basic+CVD+RF covariate set and the alternative covariates introduced in the secondary analyses. Moreover, the same finding was observed when the participants excluded (those aged under 50 years) were admitted to the study, except that fewer cognitive measures were statistically significant.

APOE $\epsilon 4$ has been shown to modify the risk of longitudinal cognitive decline in 3MSE scores associated with other risk factors, including diabetes [16]. Further, previous investigations have demonstrated that individuals with both diabetes and an *APOE* $\epsilon 4$ allele are at higher risk for dementia [17, 18]. The current study indicates that the combination of these two risk factors leads to lower levels of cognitive function within the normal (i.e. non-demented) range of cognitive function and that this is true for measures of greater difficulty, including several measures

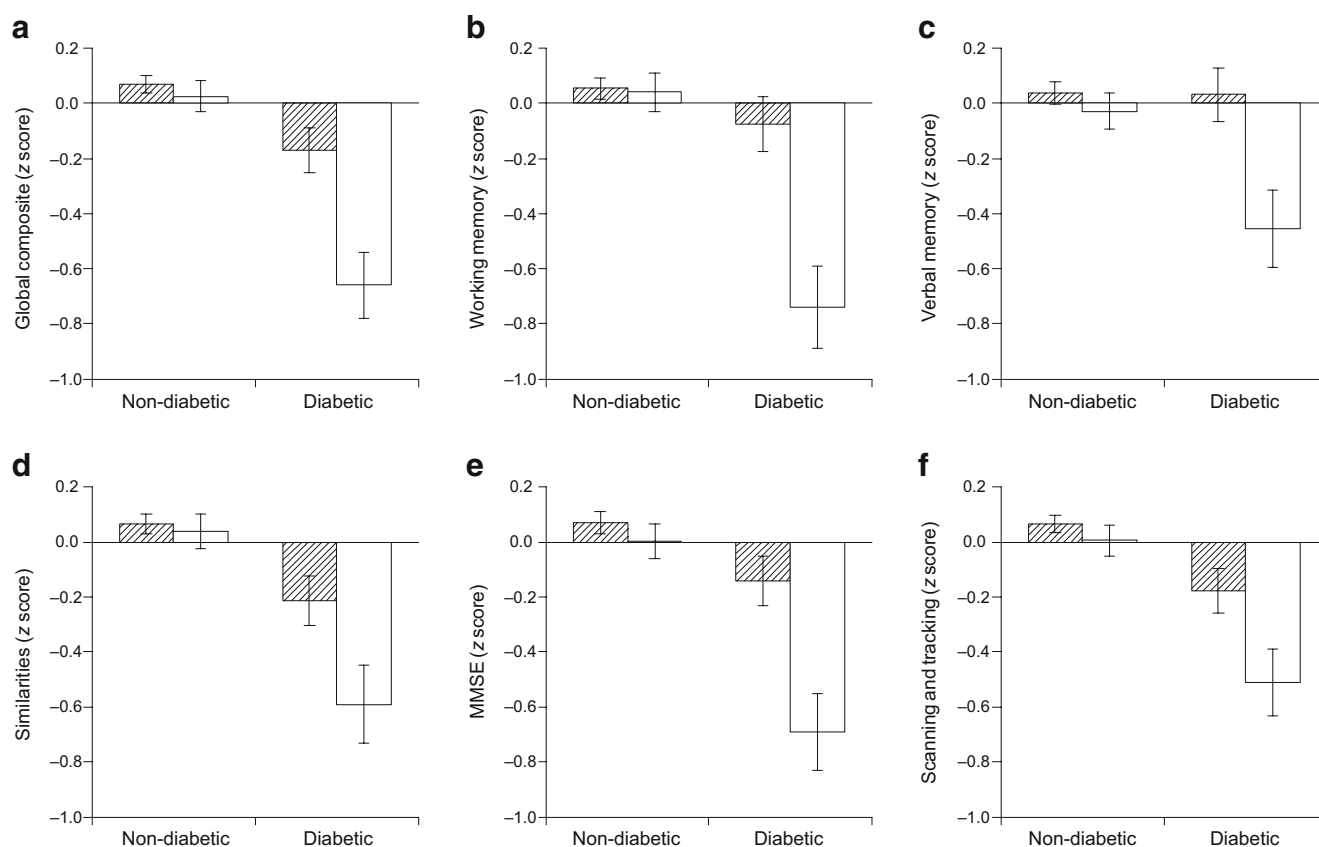


Fig. 1 Adjusted means for cognitive outcome measures by diabetic status and *APOE* group for: (a) the global composite; (b) the working memory composite; (c) the verbal memory composite; (d) the

similarities subtest; (e) the MMSE; and (f) scanning and tracking. Means are adjusted for age, education, sex and ethnicity. Cross-hatched bars, no *APOE* $\epsilon 4$; white bars, *APOE* $\epsilon 4$

that do not have the ceiling effects observed with MMSE measures. The finding of a significant interaction of *APOE* $\epsilon 4$ and diabetes parallels previous studies in which *APOE* genotype modified the association of CVD risk factors such as tHcy [33], peripheral vascular disease and atherosclerosis [16] with cognitive function.

One possibility for the modification of the association between diabetes and cognition by the presence of one or more *APOE* $\epsilon 4$ alleles relates to increased β -amyloid ($A\beta$) deposition in individuals with diabetes. Insulin-degrading enzyme (IDE) degrades β -amyloid. Thus, the increased insulin levels associated with diabetes may result in less IDE available for the regulation of $A\beta$ [34]. A recent neuroimaging study indicates that $A\beta$ levels are associated with cognitive impairment in non-demented individuals [35]. Furthermore, Alzheimer's disease patients with an *APOE* $\epsilon 4$ allele exhibit lower levels of IDE in the hippocampus than those without an *APOE* $\epsilon 4$ allele [36]. It has been suggested that increased $A\beta$ deposition may result both from the decreased expression of IDE in individuals with an *APOE* $\epsilon 4$ allele and the decreased IDE levels caused by increased use of IDE for insulin regulation in individuals with diabetes, thus leading to

higher levels of Alzheimer's disease pathology in participants with both diabetes and *APOE* [18]. This hypothesis as it relates to diabetes is weakened by postmortem studies that have found no association between diabetes and $A\beta$ load [37, 38].

However, diabetes has been associated with other brain changes, including white matter lesions [39] and the *APOE* $\epsilon 4$ allele has been associated with increased deposition of both $A\beta$ plaques and neurofibrillary tangles [38, 40]. Therefore, the decreased levels of cognitive function observed for the diabetic *APOE* $\epsilon 4$ carriers may be due to increased levels of vascular pathology in diabetic individuals and $A\beta$ plaque deposition in *APOE* $\epsilon 4$ carriers.

Another possible mechanism involves neuronal damage and repair. Diabetic encephalopathy is a recognised consequence of diabetes and is associated with neuronal damage in the central nervous system, as well as cognitive deficits [5]. The mechanism of neuronal damage is thought to be related to the effects of hyperglycaemia, namely oxidative stress and the accumulation of advanced glycation endproducts (AGE). The *APOE* $\epsilon 4$ genotype has been implicated in impaired neuronal repair [41] and in altered binding of AGE [42]. It is therefore possible that diabetes

may have a more pronounced effect or progress more rapidly in individuals with impaired neuronal repair mechanisms (i.e. *APOE* $\epsilon 4$ carriers). However, the possibility that poor glycaemic control explains our results loses plausibility by virtue of the fact that results were the same with and without glycaemic control in the model and the glycaemic control variable did not relate significantly to any of the outcome measures. However, we obtained only one measure of glycaemic control (i.e. glucose) at the time of cognitive testing. Consequently, the poor glycaemic control hypothesis should be considered in further research.

Our community-based study did not have the necessary data to explore the possibility that these underlying mechanisms explain our findings but, hopefully, will promote studies designed to examine these possibilities. Clearly, longitudinal studies are important to resolve inconsistent findings in the literature with regard to the relation between *APOE* $\epsilon 4$ genotype and cognitive performance in non-demented individuals.

Limitations of the current study are as follows. First, the design was cross-sectional, which does not allow for the study of change in cognition over time, an examination of incidence of diabetes or the objective measurement of diabetes duration. Second, relatively high levels of education in our sample may have resulted in an underestimation of the associations observed. Third, there was a lack of power to examine associations between diabetes and cognitive function for *APOE* $\epsilon 4$ carriers with one vs two alleles. Fourth, because this was not a planned clinical study of diabetes, we did not have data on standard clinical variables (e.g. HbA_{1c}), which would have been useful when relating diabetes to cognitive performance. Fifth, there were too few non-treated diabetic participants to permit adjustment of results for treatment of diabetes ($n=16$ and $n=6$ in the non-*APOE* $\epsilon 4$ and *APOE* $\epsilon 4$ groups, respectively).

Strengths of the current study include a community-based sample, a relatively large number of cardiovascular disease covariates available for the various models, objective measures of diabetes and a comprehensive cognitive test battery allowing the ability to examine multiple cognitive domains in relation to diabetes and *APOE* $\epsilon 4$ for persons free from dementia.

Further investigation into the mechanisms responsible for the observed modification of the relation between diabetes and cognition by presence of the *APOE* $\epsilon 4$ alleles is important, as are community-based studies examining longitudinal change in cognitive performance in relation to diabetes and the presence of one or more *APOE* $\epsilon 4$ alleles. Longitudinal studies will inform us as to whether cognitive deficits associated with the presence of the *APOE* $\epsilon 4$ allele in patients with diabetes result in progressive cognitive deficit. Our findings do not suggest that presence of an *APOE* $\epsilon 4$ allele in the absence of diabetes is

unimportant, but rather that the presence of one or more *APOE* $\epsilon 4$ alleles may raise the risk of cognitive dysfunction among diabetic persons. This finding has important clinical and public health implications. Information on *APOE* $\epsilon 4$ status is an important consideration in the treatment of diabetes as it relates to the prevention of cognitive deficit given that lowered cognitive performance is itself a predictor of dementia [43–45].

Acknowledgements This study was supported in part by the National Heart, Lung and Blood Institute (grant numbers HL67358, HL81290); and the National Institute on Aging (grant number AG03055). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung and Blood Institute, the National Institute on Aging, or the National Institutes of Health. The authors wish to thank S. Brennan (University of Maine, Orono, ME, USA) for assistance with data collection, and A. Goodell and D. Briggeman (University of Maine, Orono, ME, USA) for assistance with copyediting.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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