1   **Elevated blood glucose levels are associated with the progression of brain hypometabolism, and HDL-C**

2   **and *APOE4* add to this association**

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5

6   **Abstract**

7   **Background:** Brain glucose hypometabolism has consistently been found in neurodegenerative disorders,

8   including Alzheimer's disease (AD). High blood glucose and HDL cholesterol (HDL-C) levels have also been

9   linked to neurodegeneration and AD. However, there is limited understanding of the relationships

10   between dementia-related risk factors in the brain and blood.

11   **Methods:** A linear mixed model was used to examine the relationship between blood glucose and HDL-C

12   levels and the progression of brain hypometabolism, adjusting for *APOE4* and other clinical covariates.

13   The hypometabolic convergence index (HCI) was measured by fluorodeoxyglucose-18 (FDG) positron

14   emission tomography (PET) in participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI).

15   Data visualizations were generated to understand the joint effects of plasma glucose, HDL-C, and *APOE4*

16   on HCI.

17   **Results:** There were 336 individuals (781 observations), of whom 22.62% had AD. The majority were male

18   (63.98%) and of white race, and 48.51% were carriers of *APOE4*. Over time, high blood glucose level was

19   associated with the progression of brain glucose hypometabolism (β=0.33, 95% CI: 0.02, 0.64, p<0.05). A

20   high plasma HDL-C level (β=1.22, 95% CI: 0.09, 2.35, p<0.05), more study visits (β=1.67, 95% CI: 1.37, 1.98,

21   p<0.001), and being an *APOE4* allele carrier (β=1.29, 95% CI: 0.15, 2.42, p<0.05) were also significant

22   predictors of brain hypometabolism progression. *APOE4* carrier status and number of visits account for

23   the largest proportion of the variance from the fixed effects model. Random effects due to participant

24   characteristics and fixed effects together accounted for 95.2% of the model variance. Subgroup analysis

25   revealed that these effects were observed only in those without AD.

26   **Conclusion:**

27   High plasma glucose levels facilitated the progression of brain hypometabolism. The effect was more

28   prominent in the *APOE4* double-carriers with elevated HDL-C. Elevated blood glucose may reflect systemic

29   insulin resistance, which could impair brain glucose uptake, resulting in brain hypometabolism.

30   Controlling blood glucose and HDL-C levels in *APOE4* carriers may improve brain metabolism, potentially

31   delaying the onset of dementia.

32   **Keywords**

33   Brain hypometabolism, Blood glucose, HDL cholesterol, *APOE4,* Alzheimer's disease, Hypometabolic

34   Convergence Index

35

36   **Background**

37   Glucose usage in the brain has been finely tuned throughout evolution.[1] Brain cells are highly energy-

38   dependent and require a constant supply of glucose for optimal functioning.[2] Glucose meets the energy

39   demands for a diverse range of activities, including brain signaling, neurotransmitter production, and

40   maintaining homeostasis.[3] Due to this, multilayered mechanisms, including sensors, glucose transporters,

41   enzymes, and specific cell pathways, work together to ensure the availability of glucose.[2] Complex

42   learning processes in neurons and astrocytes are correlated with brain metabolism, which is directly

43   dependent on glucose usage.[4] Exposure to insufficient glucose supply can harm memory and learning, and

44   prolonged insufficiency can potentially cause permanent brain alterations.[3] Thus, any deviation from the

45   normal glucose uptake pattern in the brain might indicate a serious medical illness.[5]

46   Earlier neuroimaging studies have considerably expanded our knowledge of metabolic alterations in

47   dementia.[6,7] Neuroimaging outcomes are superior to traditional cognitive assessments in detecting

48   related changes and correlate well with neuropathological changes in individuals with dementia.[8] A

49   pattern of glucose deficit in the brain is noted for several neurodegenerative disorders, which can be

50   distinguished among the relevant conditions.[9] In the case of Alzheimer's disease (AD), hypometabolism

51   begins much earlier than the actual onset of clinical symptoms and contributes to the further progression

52   of the disease.[10] The $[^{18}F]$-2-fluoro-2-deoxy-2-glucose (FDG) tracer-based positron emission tomography

53   (PET) is a widely used diagnostic method to ascertain the metabolic rate in different tissues. FDG mimics

54   glucose absorption and remains in the body longer than glucose. Studying its buildup in tissues helps

55   quantify the metabolic rate.[11] The FDG PET-derived hypometabolic convergence index can accurately

56   distinguish the AD signature brain hypometabolic pattern through automated brain image analysis.

57   Dementia subtypes, such as AD, have a complex, multifactorial etiology, stemming from an interplay

58   between aging, genetics, and the environment.[12] In this context, among the risk factors, hyperglycemia,

59   and particularly diabetes, are major concerns due to its rising global prevalence.[13] Clinical features of

60   diabetes, such as abnormal insulin signaling and insulin resistance, are also pathological features of

61   dementia.[14] Lipids are another risk factor that deserves attention in the control of dementia.[15]

62   Until recently, it was believed that elevated levels of HDL cholesterol were beneficial for health. [16] Indeed,

63   numerous studies demonstrated protective associations between elevated HDL cholesterol levels and

64   reduced risk of heart disease, inflammatory conditions, and even cognitive decline.[17] The protective

65   effects of HDL may be attributed to its antioxidant and anti-inflammatory properties, as well as its ability

66   to remove excess 'bad' cholesterol.[18] Based on several such studies, it has even suggested that increasing

67   HDL levels or restoring its functions could be explored as a therapeutic option to combat inflammation

68   and AD.[19,20]

69 Emerging evidence, however, has now challenged this established understanding.[21] Despite these

70 findings, there is a lack of evidence regarding the connections between dementia risk factors in the brain

71 and in the blood. The combination of risk factors might drive individual differences in dementia

72 progression.[10] Therefore, examining the combination of hypometabolism risk factors, such as blood

73 glucose and HDL-C levels, may provide more insights into individual differences in dementia progression.

74 The presence of the *APOE4* allele significantly increases the risk of developing AD.[22] Despite numerous

75 research studies, many aspects of the role of *APOE4* in AD remain unclear, including its interaction with

76 dementia risk factors.[23] Due to these reasons, we also sought to explore how the effects were modified

77 when these risk factors were present in carriers of *APOE4.*

78

79 **Methods:**

80 **Data Source**

81 We conducted this analysis using the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The

82 ADNI project was started in 2004 with Michael Weiner as the chief investigator. This project is a part of

83 public-private collaboration that includes major institutions across North America. ADNI encourages the

84 cost-free sharing of deep genotyped and phenotyped datasets with interested researchers worldwide.

85 ADNI collects biomarkers, brain scans, clinical data, and cognitive assessments from volunteer participants

86 based on a preset inclusion criteria. ADNI seeks to improve clinical prediction of AD and its treatment by

87 leveraging the wide variety of available longitudinal neuroimages, biomarkers, and cognitive scales.

88

89 **Details of FDG PET Imaging**

90 FDG PET imaging was performed on a subset of participants based on a standard protocol. Data gathered

91 within ADNI are allocated to different cores, each comprising experts specializing in corresponding

92   domains, tasked with efficiently managing the data.[24] There are two imaging cores, with PET imaging

93   falling under the jurisdiction of Banner Institute, which specifies parameters to maintain optimal imaging

94   quality. To ensure comparability and quality across various scanners, a 3D correction is applied to the

95   acquired images. For more information on PET measurements in the ADNI study, refer to Mueller et al.'s

96   publication.[8]

97

98   **Generation and Interpretation of Hypometabolic Convergence Index Scores**

99   We investigated the longitudinal changes in hypometabolic convergence index (HCI) as the study

100   outcome.   HCI   values   were   accessed   from   the   BAIPETNMRCFDG   dataset

101   (https://adni.bitbucket.io/reference/baipetnmrc.html). The HCI was generated from comprehensive

102   whole-brain image analysis rather than regional examination of FDG PET brain images, and summarizes

103   the extent of brain hypometabolism in the form of z-scores.[25] These scores were obtained through voxel-

104   wise analysis of the images using Statistical Parametric Mapping (SPM) software.[26] An increasing HCI was

105   interpreted as indicative of greater brain hypometabolism.[25]

106

107   **Covariate Extraction, Measurements, and Data linkage**

108   We extracted glucose and lipid biomarkers from the ADNINIGHTINGALELONG dataset. In ADNI, *APOE4*

109   was measured using DNA extracted by Cogenics from a 3 mL aliquot of EDTA blood collected during

110   participant screening visits.[27] Only rows containing values for all the variables were further considered for

111   the analysis. Information on smoking and alcohol use was obtained from the medical history file. Data

112   regarding systolic and diastolic blood pressure, sex, marital status, race, and education were derived from

113   the vitals and demographics datasets. Age was calculated as the difference in years between the

114   examination date and the date of birth for each participant over time. Diabetes medications were

115   extracted from the medication data using the Anatomical Therapeutic Classification (ATC) codes

116   (https://www.who.int/tools/atc-ddd-toolkit/atc-classification). When applicable, we used the visit code

117   and distinct participant identifier to link datasets. Once linked, a participant was considered to be taking

118   diabetes medication for all subsequent data following the initial prescription. We utilized the tidyr

119   package's *fill* function with the 'direction=down' option to propagate the diabetes medication use labels.

120

121   **Statistical Analysis**

122   We performed data analysis and visualizations using the R programming language (R version 4.3.2). [28] At

123   first, variable summaries measured at baseline were computed. For this, continuous variables were

124   presented as mean with standard deviation. Categorical variables were summarized as frequencies and

125   percentages. To depict correlations between continuous covariates at the baseline, we employed a

126   correlation heatmap. To assess longitudinal variations in plasma glucose and HDL-C levels, we pooled all

127   observations from all visits and calculated the coefficient of variation percentage (CV%). CV% was

128   computed by dividing the standard deviation by the mean and multiplying by 100. We generated a scatter

129   plot of the CV% for glucose and HDL-C distributions to assess their relationship. Additionally, we plotted

130   the CV% for these measures stratified by *APOE4* allele status and determined if the differences were

131   statistically significant using the Kruskal-Wallis test.

132   To examine the relationship between blood glucose, HDL-C levels, *APOE4* status, and the progression of

133   brain hypometabolism, and to account for the correlated data structure, we conducted a linear mixed

134   model analysis using the lme4 package.[29] Variations between participants and visits were accounted for

135   by specifying random intercepts and varying slopes in the random effects part of the model. We analysed

136   multiple models with different combinations of terms and then selected the best model with the lowest

137   Akaike Information Criterion (AIC) values. This model was deemed the model with the minimum set of

138   variables that best explains the data. Model comparisons were performed using the anova built-in

139   function. For unbiased regression estimates in the optimal model, we employed restricted maximum

140   likelihood estimation. The Nelder-Mead optimizer was used to ensure model convergence.

141   To analyze the conditional and marginal variable contributions of the covariates in the parsimonious

142   mixed model, we utilized the hierarchical partitioning method.[30] This approach helps to understand how

143   *APOE4*, HDL-C, and glucose modify the HCI. We computed marginal means and corresponding 95%

144   confidence intervals (CI) to quantify the average change in HCI for these variables.

145   We also checked the functional relationship between glucose and HDL-C with HCI using generalized

146   additive mixed effect models (GAMM). The advantage of GAMM is that it is able to incorporate the

147   benefits of generalized additive models, i.e., nonlinear effects modeling, while also accounting for

148   correlations due to repeated measures.[31] The AIC from both linear and nonlinear models were compared

149   to determine the most suitable functional relationship between the variables. A two-tailed p-value <0.05

150   was considered statistically significant. Additionally, we quantified and visualized individual heterogeneity

151   for the random effects terms specified in the optimal mixed effects model. Lastly, to assess the HCI

152   reduction associated with varying values of glucose and HDL-C, we generated a partial effect plot.

153   **Results**

154   **Sample description**

155   Data on 336 individuals (781 observations) were available for analysis after data linkage, with 22.62% of

156   participants diagnosed with AD (Supplementary Figure 1). Table 1 describes the baseline demographic

157   and clinical characteristics of the participants in this study. The participants were, on average, 75.43 years

158   old and had 15.6 years of education. The majority were male (63.98%) and White (93.45%). Over a third

159   (38%) had a smoking history, and 97.6% reported being ever married. The mean HbA1C and HDL-C levels

160   were 5.42 and 1.52, respectively. The mean systolic blood pressure (SBP) was relatively high at 135 mm

161   Hg, whereas the mean diastolic blood pressure (DBP) was below the normal reference level at 73.77 mm

162   Hg. Almost half (48.51%) were carriers of the *APOE4* allele. Figure 1 shows the distribution of longitudinal

163   CV% for glucose and HDL-C, as well as their relationship. In Figure 2, the distributions of longitudinal CV%

164   for glucose and HDL-C, stratified by *APOE4* allele status, are shown. Non-linear modeling (spline fit) of the

165   relationship between CV% for glucose and HDL-C supports a nonlinear relationship. P-values from the

166   Kruskal-Wallis test to see the influence of *APOE4* alleles on the CV% for glucose and HDL-C were non-

167   significant (0.78 and 0.56, respectively). Brain hypometabolism, measured by HCI, was higher in this

168   sample, with a mean of 15.06 (range 4.34 - 47.40). Initially, only 2 participants (0.60%) were using anti-

169   diabetes medication, but by the end of the follow-up, this number had risen to 10 (2.97%). As regards to

170   the baseline correlations, *APOE4* was positively correlated to HCI (Supplementary Figure 2). *APOE4* had

171   no strong correlations with either lipid subgroups and blood glucose. In those with AD, blood glucose and

172   HDL-C modelled using splines seems to favor a more non-linear relationship in comparison to those not

173   diagnosed with AD (Supplementary Figures 3-4).

174

175   **Table 1**. Characteristics of participants measured at baseline (781 observations, n=336)

Variables	Mean (Frequency)	SD	Range
Age (Years)	75.43	6.69	55.24 -89.00
Sex (Male)	215 (63.98%)		
Education (Years)	15.66	2.96	6-20

Blood Glucose (mmol/L)	5.42	0.87	2.99-10.47
HDL-C (mmol/L)	1.52	0.39	0.73 – 3.29
Race (White)	314 (93.45%)		
Married (Ever)	328 (97.61%)		
Smoking (Ever)	126 (37.50%)		
Alcohol (Ever)	11 (3.27%)		
AD	76 (22.61%)		
APOE4			
0	173 (51.48%)		
1	131 (38.98%)		
2	32 (9.52%)		
SBP (n=335)	135.12	16.59	90-201
DBP (n=335)	73.77	9.23	43-98
NonHDL-C	3.49	1.60	1.21-6.16
LDL-C	2.01	0.46	0.61 – 3.47
HCI	15.06	7.54	4.34 - 47.40

176   Note. SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; NonHDL-C = Non-High-density

177   lipoprotein cholesterol; LDL-C = Low-density lipoprotein cholesterol; HDL-C = High-density lipoprotein
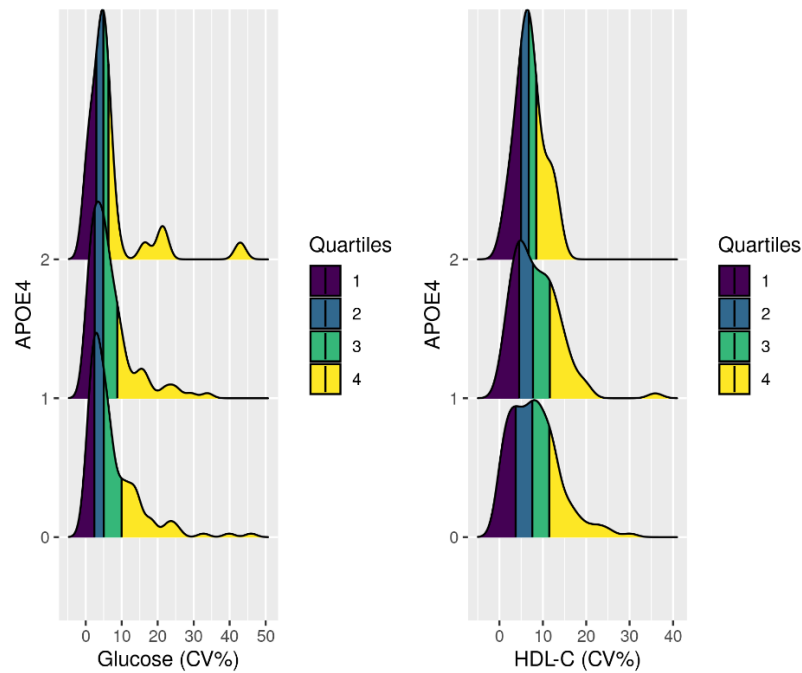
178   cholesterol; HCI = Hypometabolic Convergence Index.

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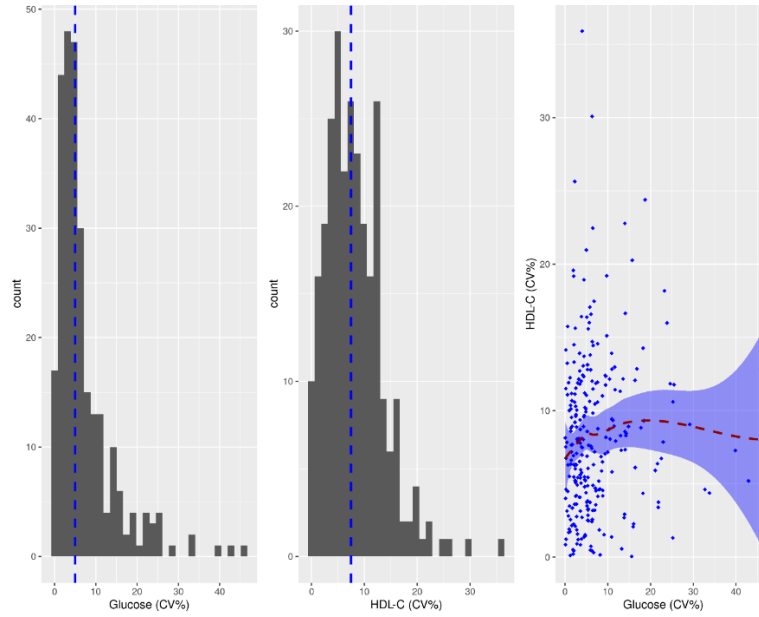
Figure 1. Histogram of longitudinal CV% for glucose and HDL-C, and a scatterplot with smoothed regression line showing their relationship

Figure 2. Distribution of longitudinal CV% for glucose and HDL-C stratified by *APOE4* allele status

10

187 **Effect of Elevated Blood Glucose and HDL-C Variability on Brain Hypometabolism**

188 As shown in the Supplementary Table 1, Model 2 was selected for detailed analysis. Table 2 shows the

189 adjusted regression estimates from the optimal linear mixed model. More number of clinical visits

190 (β=1.67, 95% CI: 1.37, 1.98, p<0.001), and *APOE4* carrier status were the strongest predictors for the

191 decline in brain metabolism in ascending order respectively (Supplementary Figure 5). The contributions

192 of these variables to variance from fixed effects in the best model were also the highest. Over time, an

193 increase in plasma glucose was significantly associated with an increased area of brain hypometabolism

194 (β=0.33, p<0.05). None of the cholesterol markers except HDL-C were statistically significant in the

195 multivariate analysis (β=1.22, p<0.05). Additionally, age, sex, smoking, blood pressure, and race were not

196 significant predictors. The percentage variance of the fixed effects (marginal R-squared) was estimated at

197 4.5%. Supplementary Figure 6 elucidates the random effects represented by the clinical visits and

198 individual variability. Variance, combining both the fixed and random effects (conditional R-squared),

199 accounted for 95.2% of the model variance.

200

201 **Table 2**. Adjusted model estimates corresponding to the optimal linear mixed model for the HCI trend

202 outcome

Variable	Coefficient	95% CI	p-value
Glucose	0.33	0.02, 0.64	0.034*
HDL-C	1.22	0.09, 2.35	0.032*
APOE4	1.29	0.15,2.42	0.023*
Race (White)	2.85	-0.09,5.79	0.060
Visits	1.67	1.37,1.98	0.000***

11

203   Note. *p<0.05; ***p<0.001

204

205   **Functional Relationship Between Glycemic Variability, HDL-C Levels, and Their Interaction with *APOE4***

206   **on Brain Hypometabolism**

207   A comparison of effects plots (Supplementary Figure 7, Figure 3, and Figure 4) generated from linear and

208   additive mixed models (GAMM) indicates that glucose has a linear relationship with HCI. Conversely, HDL-

209   C showed a nonlinear relationship, which was preferred over the linear model. Additive mixed models

210   work similarly to linear mixed models, with the difference being that they allow for modeling complex

211   non-linear relationships of variables by fitting smooth functions.[31] Partial effects here refer to the mean

212   effect in HCI due to the change in exposures while keeping the effects of other covariates held constant

213   in the model. The dose-response relationship between HDL-C and HCI was observed to decrease slightly,

214   stabilize and then decline after 2 mmol/L for higher values. According to Figure 4, elevated HDL-C reduces

215   brain metabolism even when glucose levels are optimal. Hypometabolism increases with each unit rise in

216   glucose, particularly above 7.5 mmol/L.

217   At this threshold, risk is evident even for low HDL-C values. Hence, it could be deduced that elevated

218   glucose values are an important risk factor for brain hypometabolism, and that its effects with HDL-C on

219   brain metabolism are non-linear. Supplementary Figure 8 complements this finding, showing substantial

220   differences in predicted HCI across stratified HDL-C categories of low, intermediate, and high levels.

221   Similar trends were evident in the interaction effects of blood glucose levels, HDL-C, and the *APOE4* allele

222   on HCI (Figure 5). The plot shows a dramatically pronounced decline in brain metabolism for increased

223   blood glucose levels and HDL-C in *APOE4* homozygous carriers compared to non-carriers and

224   heterozygous carriers. In addition, for better interpretation of the slopes, the marginal means for the HCI,

225   computed for the different combinations of these risk factors, are shown in Supplementary Table 2.

12

226   Compared to non-carriers with low HDL-C and high glucose, there was a more than 10-point increase in

227   predicted mean HCI for double *APOE4* carriers with high HDL-C.

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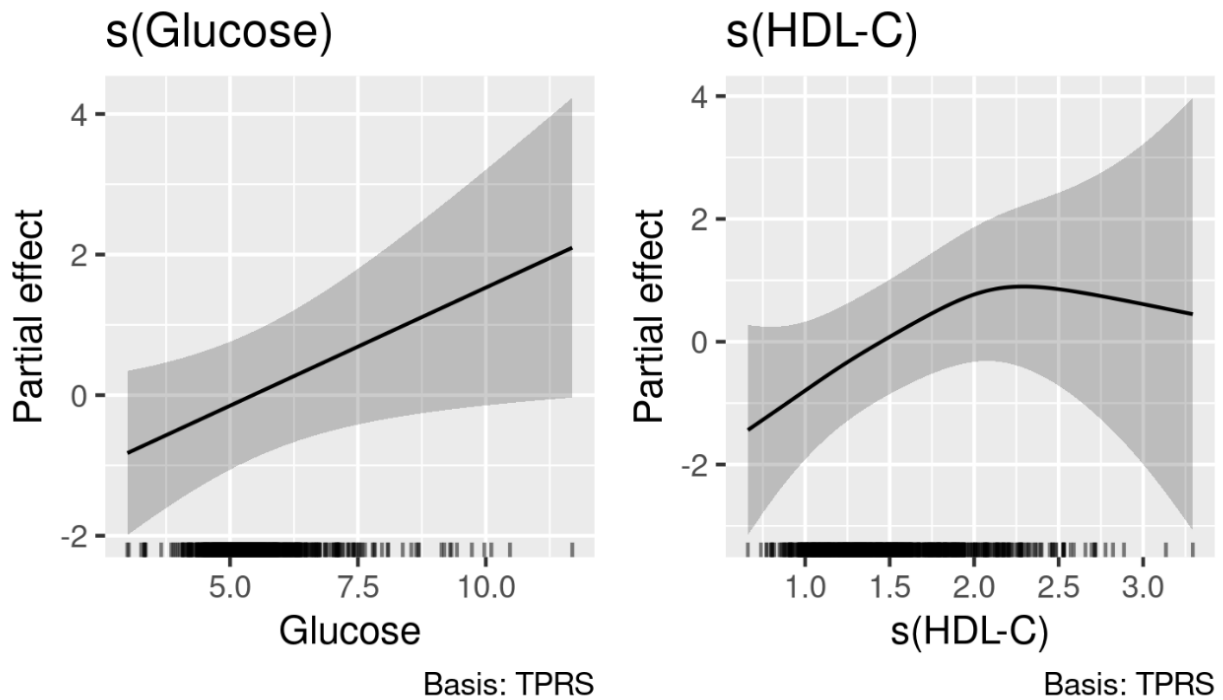
230   **Figure 3.** Partial effects of Glucose and HDL-C levels on the HCI from the GAMM model. Note. TPRS (thin

231   plate regression splines) are basis functions that allow for model fitting of local segments of the exposure-

232   outcome relationship, which are then connected to provide a complete picture of the overall relationship.

233   Thin plate splines automatically determine the location and number of knots based on changes in the

234   values of the covariate.

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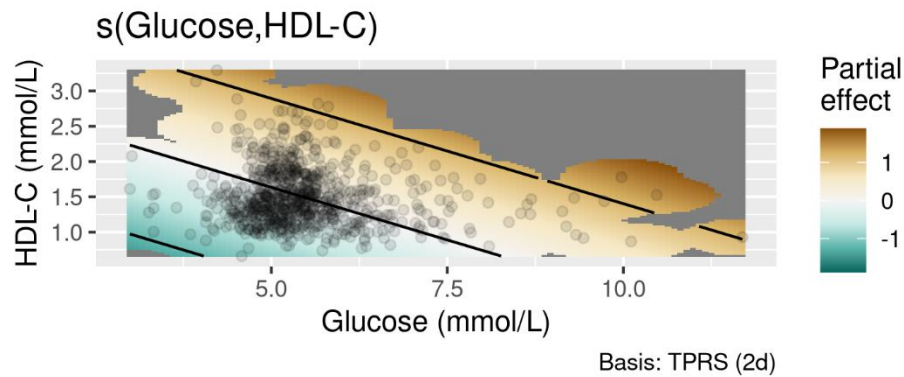
Figure 4. The partial effect plot illustrates the combined effects of HDL-C and glucose levels on HCI. The variable relationships are shown as a smoothed relationship as a function of these variables and are estimated from the additive mixed model. In the plot, the black lines and black dots represent the contour lines and observed data points, respectively. A change in color from green to orange reflects how the relationship changes, i.e., from a negative to a positive partial effect. A partial effect of -1 means that a one-unit increase in the predictor variable is associated with a one-unit decrease in the outcome variable, and vice versa. It is evident that the partial effects vary at different levels of the predictor variables.

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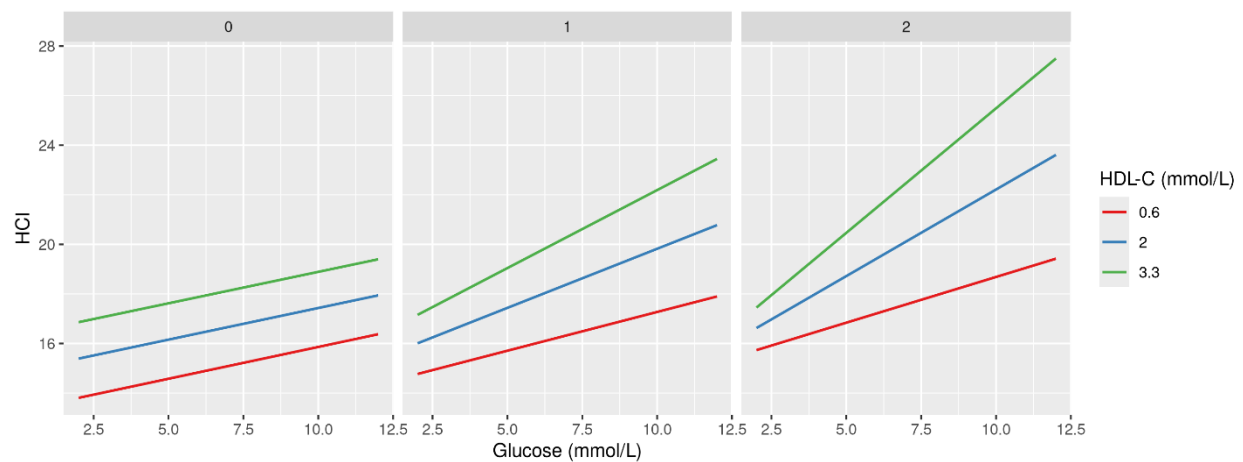
14

245   **Figure 5**. Plot showing the synergistic effects of blood glucose levels, HDL-C, and *APOE4* allele on HCI. The

246   predictions were generated from a model containing interaction terms for blood glucose levels, HDL-C,

247   and *APOE4*. Each window corresponds to the effects of the *APOE4* allele (0, 1, 2) and is shown specifically

248   for HDL-C categories (low, high, and intermediate, arbitrarily selected based on the data)

249

250   **Comparison of Subgroup Analysis to Identify Heterogeneity in Effects**

251   Given that the visualizations indicated a possible difference in the effects of the exposures in AD and non-

252   AD individuals, we prepared the data accordingly. Upon further investigation, we noted that the statistical

253   associations were limited to the non-AD subgroup (Supplementary Tables 3-4). Remarkably, the strength

254   of the associations for White race, HDL-C, and *APOE4* with HCI was much stronger in the stratified analysis

255   for this group. Overall, the effect size for glucose did not differ for the non-AD individuals compared to the

256   main analysis, whereas the clinical visits exhibited a diluted effect. For the AD group, both *APOE4* and visits

257   were still significantly associated with HCI. While the impact of visits was considerably stronger than in the

258   aggregated model, the effects of *APOE4* were strongly reversed, indicating a protective association.

259

260   **Discussion**

261   In this study of older adults, plasma glucose and HDL-C variability were significantly associated with a

262   reduction in brain metabolism, but only in individuals without AD. Importantly, these effects were

263   independent of *APOE4* and common confounders such as age, sex, and other lipids profiles. We also found

264   that the relationship of plasma glucose and HDL-C with brain metabolism operates independently of each

265   other. *APOE4* status and measurement visit were the strongest predictors of brain metabolism at the

266   population level. This is not surprising, as we demonstrated in our previous work using the ADNI data that

267   measurement visits provide more information than age alone.[32] Measurement visits may indirectly reflect

268   patient characteristics, response, and observation time.[33]

15

269   A hyperglycemic milieu can cause widespread systemic effects, thereby modulating physiological

270   responses.[34] It is important to note that the diabetes burden was quite low in our data. However, diabetes

271   is not an absolute requirement for the glucose to impact brain. Our findings are consistent with a previous

272   study which demonstrated that even midlife increase in glucose can accelerate dementia.[35] Although

273   hyperglycemia contributes to the development of dementia, its role in certain types of dementia such as

274   in AD remains to be established.[36] In our case, one possible reason could be that participants had AD at

275   baseline and, therefore, already had much lower brain metabolism. Therefore, it is much less likely that

276   glucose and HDL-C variations would have any impact. Also, *APOE4* is no more a risk factor since they had

277   already developed AD. Hyperglycemia-induced physiological changes are quite complex and usually do not

278   conform to the normal dose-response framework.[37] However, in our study, an increase in blood glucose

279   levels was linearly related to a decline in brain metabolism. This contradicted a previous study in which

280   cognitive decline was observed with both high and low blood sugar levels, and it worsened with age.[38]

281   Nevertheless, high blood sugar-driven outcomes at the individual level are highly heterogeneous and also

282   depend on the combination of other risk factors and genes.[39,40]

283   In our data, elevated HDL-C was stronger risk factor for brain hypometabolism than plasma glucose levels.

284   The role of lipids in dementia remains controversial. A meta-analysis of multiple studies published on lipid

285   subgroups shows that LDL-C could be a likely candidate risk factor for dementia, but there was no evidence

286   of involvement of HDL-C or other lipids.[41] On the other hand, it is worth noting that high HDL-C levels could

287   negatively impact health and survival, including increased all-cause and cardiac-related mortality.

288   However, individuals in the mid-range levels of HDL-C seem to be protected.[42] This was observed in the

289   'U-shaped' relationship between HDL-C levels and cognitive outcomes, with individuals having HDL-C

290   values above 2.50 mmol/L experiencing more than a two-fold increased risk of poor cognitive outcomes.[43]

291   Such a relationship was also reported in a large-scale survival analysis of health insurance data exploring

16

292   dementia outcome. Similar to our study, LDL-C in this study did not influence dementia risk, except for a

293   minor increase in risk observed among statin users.[44]

294   A study published in Lancet, that investigated whether HDL-C is a risk factor for incident dementia reported

295   a risk above 3.3 mmol/L. [45] Consistent with the previous findings, individuals aged above 75 years with

296   high HDL-C were at substantial risk for dementia. In stark contrast, lower HDL-C values were shown to be

297   protective against dementia.[45] This was in line with our finding. Based on the partial effects plots in our

298   study, HDL-C associated risk appeared to diminish beyond 2.25 mmol/L. However, these findings require

299   further validation, as there were only a few HDL-C values above this threshold in the data. It might be that

300   the HDL-C effects seen were due to the presence of AD, co-morbidities, or other age-related factors. In

301   such scenarios, high HDL would be merely reflective of these conditions rather than providing any real

302   health benefits.[20]

303   *APOE4* carriage was associated with a greater metabolic decline compared to non-carriers for the

304   concomitant values of glucose and HDL-C. Notably, this interaction was particularly strong in individuals

305   with HDL-C levels above 2 mmol/L. It is intriguing that the direction of the predicted slope for brain

306   hypometabolism with glucose elevation was similar at lower HDL values across all *APOE4* isoforms, with

307   carriers experiencing a slightly higher metabolic decline. The observed interaction effects appear plausible

308   as *APOE4* have a direct link with cholesterol metabolism and lower HDL synthesis.[46–48] In addition it has

309   been observed that mice with *APOE4* risk alleles exhibit a poor response to glucose spikes and inadequate

310   insulin production.[49] Individuals with *APOE4* risk alleles and high blood glucose were more likely to

311   experience greater risk for severe dementia and features of AD in late life.[35] First of all, carrying two

312   *APOE4* alleles is now considered a genetic form of AD by itself.[50] Our finding regarding the synergistic

313   effect modulated by *APOE4* double carriers is in firm agreement, showing a vastly different pattern of

314   hypometabolism compared to its other isoforms. Therefore, the effects in those without AD might actually

315   be congruent with preclinical AD.[51] This should be kept in mind, especially in the context that the

17

316   diagnostic criteria for AD are still evolving.[51] *APOE4* carrier status favors AD and dementia mainly through

317   promoting amyloid beta, increased phosphorylated tau, and contributing to neurodegeneration.[27,52,53] It

318   is also recognized that *APOE4* variation can negatively impact mitochondrial respiration and energy

319   production, consequently leading to brain hypometabolism.[54] Other potential pathways include

320   neuroinflammation, blood-brain barrier dysfunction, gliosis, brain structural and functional changes,

321   demyelination and impaired clearance of toxic substances.[55–60]

322   In relevance to our work, it has already been demonstrated that *APOE4* variation adversely impacts the

323   ability of HDL-C to effectively sequester cholesterol by modifying HDL-C structurally. Not only does the

324   concentration of HDL matter, but also its size. For instance, individuals with AD and dementia tend to have

325   relatively smaller HDL particle sizes.[61] We presume that *APOE4*-induced changes in the brain may make it

326   more vulnerable to the negative physiological effects of elevated glucose and other risk factors. As

327   corroborating evidence, we found no indication that longitudinal variability in glucose and HDL-C can be

328   attributed to *APOE4* alleles.

329   This study has several strengths including the availability of serial participant data and a well-characterized

330   cohort. Our study is among the first to illuminate the joint contributions of glucose, HDL-C, and *APOE4*

331   allele variations on brain hypometabolism. We were able to clearly demonstrate the change in relative

332   importance of these major risk factors through visualizations. This approach provides a more

333   comprehensive understanding of the potential pathophysiology, which may not be fully revealed when

334   examining these factors individually.

335   Our study had limitations. Blood glucose and HDL-C collection was not timed according to disease

336   pathology. The study did not account for comorbidity status or medication use as covariates, with the

337   exception of blood pressure, and diabetes. We have not specifically investigated the possibility of sexual

338   dimorphism in our results, nor have we considered the involvement of potential mechanisms such as

339   amyloid pathways or other inflammatory markers. Another constraint was the limited representation of

340   non-white samples, which restricted our ability to compare and explore differences across racial groups.

341   This, combined with the smaller sample size and selective recruitment in the cohort, may further limit the

342   generalizability of the findings. Lastly, there could be unmeasured confounding. Therefore, we

343   recommend replicating our findings in large cohorts and across multiple ethnicities to ascertain the

344   benefits of glucose and HDL reduction in diverse populations. Further studies may be conducted using

345   genetic variants that influence these exposures to gather causal evidence.

346

347   **Conclusion**

348   High blood glucose levels facilitated progression of cerebral hypometabolism in ADNI participants. The

349   negative impact of blood glucose on brain hypometabolism was aggravated by elevated HDL-C levels and

350   *APOE4* carrying status. High blood glucose levels may reflect systemic insulin resistance, which, in turn,

351   might impair brain glucose uptake, resulting in brain hypometabolism. While further validation is

352   warranted, controlling for plasma glucose/insulin resistance and HDL-C levels in *APOE4* carriers may

353   attenuate the decline in brain metabolism, potentially delaying dementia clinical onset.

354

355   **Abbreviations**

AD	Alzheimer's Disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
ATC	Anatomical Therapeutic Classification
CV%	Coefficient of Variation Percentage
DBP	Diastolic Blood Pressure (DBP)

19

FDG	Fluorodeoxyglucose-18 (FDG)
GAMM	Generalized Additive Mixed Effect Models
HCI	Hypometabolic Convergence Index
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
NonHDL-C	Non-High-density lipoprotein cholesterol
PET	Positron Emission Tomography
SBP	Systolic Blood Pressure (SBP)
SPM	Statistical Parametric Mapping
TPRS	Thin Plate Regression Splines

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358 **References**

359 1.   Harris JJ, Jolivet R, Attwell D. Synaptic Energy Use and Supply. Neuron. 2012;75(5):762–77.

360

361 2.   Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: The role of glucose in

362   physiological and pathological brain function. Vol. 36, Trends Neurosci. 2013. 587–97.

363

364 3.   Hertz L, Gibbs ME. What learning in day-old chickens can teach a neurochemist: focus on astrocyte

365   metabolism. J Neurochem. 2009;109(Suppl 1):10–6.

366

367   4.   Turner DA, Adamson DC. Neuronal-Astrocyte Metabolic Interactions: Understanding the Transition

368   into Abnormal Astrocytoma Metabolism. J Neuropathol Exp Neurol. 2011 ;70(3):176.

369

370   5.   Dienel GA. Fueling and Imaging Brain Activation. ASN Neuro. 2012;4(5).

371

372   6.   Benson DF, Kuhl DE, Hawkins RA, Phelps ME, Cummings JL, Tsai SY. The Fluorodeoxyglucose 18F

373   Scan in Alzheimer's Disease and Multi-infarct Dementia. Arch Neurol. 1983;40(12):711–4.

374

375   7.   Friedland RP, Budinger TF, Ganz E, Yano Y, Mathis CA, Koss B, et al. Regional Cerebral Metabolic

376   Alterations in Dementia of the Alzheimer Type: Positron Emission Tomography with [18F]

377   fluorodeoxyglucose. J Comput Assist Tomogr . 1983;7(4):590–8.

378

379   8.   Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack C, Jagust W, et al. The Alzheimer's Disease

380   Neuroimaging Initiative. Neuroimaging Clin N Am. 2005;15(4):869–77.

381

382   9.   Han R, Liang J, Zhou B. Glucose metabolic dysfunction in neurodegenerative diseases—new

383   mechanistic insights and the potential of hypoxia as a prospective therapy targeting metabolic

384   reprogramming. Int J Mol Sci. 2021;22:5887.

385

386   10. Zilberter Y, Zilberter M. The vicious circle of hypometabolism in neurodegenerative diseases: Ways

387   and mechanisms of metabolic correction. J Neurosci Res. 2017;95:2217–35.

388

389   11.   Pauwels EKJ, Coumou AW, Kostkiewicz M, Kairemo K. [18F] Fluoro-2-deoxy-D-glucose positron

390   emission tomography/computed tomography imaging in oncology: Initial staging and evaluation

391   of cancer therapy. Med Princ Pract. 2013;22(5):427–37.

392

393   12.   Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. The Lancet. 2006;368(9533):387–403.

394

395   13.   Sims-Robinson C, Kim B, Rosko A, Feldman EL. How does diabetes accelerate Alzheimer disease

396   pathology? Nat Rev Neurol. 2010;6(10):551–9.

397

398   14.   Nguyen TT, Ta QTH, Nguyen TKO, Nguyen TTD, Giau V Van. Type 3 Diabetes and Its Role

399   Implications in Alzheimer's Disease. Int J Mol Sci. 2020;21(9):3165.

400

401   15.   Zhu Y, Liu X, Zhu R, Zhao J, Wang Q. Lipid levels and the risk of dementia: A dose–response meta-

402   analysis of prospective cohort studies. Ann Clin Transl Neurol. 2022;9(3):311.

403

404   16.   Rajagopal G, Suresh V, Sachan A. High-density lipoprotein cholesterol: How High. Indian J

405   Endocrinol Metab. 2012;16(2).

406

407   17.   Bates KA, Sohrabi HR, Rainey-Smith SR, Weinborn M, Bucks RS, Rodrigues M, et al. Serum high-

408   density lipoprotein is associated with better cognitive function in a cross-sectional study of aging

409   women. International Journal of Neuroscience. 2017;127(3):243–52.

410

411   18.   Barochia A V., Gordon EM, Kaler M, Cuento RA, Theard P, Figueroa DM, et al. High density

412   lipoproteins and type 2 inflammatory biomarkers are negatively correlated in atopic asthmatics. J

413   Lipid Res. 2017;58(8):1721.

414

415   19.   Button EB, Robert J, Caffrey TM, Fan J, Zhao W, Wellington CL. HDL from an Alzheimer's disease

416   perspective. Curr Opin Lipidol. 2019;30(3):234.

417

418   20.   Trakaki A, Marsche G. High-Density Lipoprotein (HDL) in Allergy and Skin Diseases: Focus on

419   Immunomodulating Functions. Biomedicines. 2020;8(12):558.

420

421   21.   Gong J, Harris K, Peters SAE, Woodward M. Serum lipid traits and the risk of dementia: A cohort

422   study of 254,575 women and 214,891 men in the UK Biobank. EClinicalMedicine. 2022;54(101695).

423

424   22.   Pires M, Rego AC. Apoe4 and Alzheimer's Disease Pathogenesis—Mitochondrial Deregulation and

425   Targeted Therapeutic Strategies. Int J Mol Sci. 2023;24(1):778.

426

23

427   23.   Mosconi L, Herholz K, Prohovnik I, Nacmias B, Cristofaro MTR De, Fayyaz M, et al. Metabolic

428   interaction between ApoE genotype and onset age in Alzheimer's disease: implications for brain

429   reserve. J Neurol Neurosurg Psychiatry. 2005;76:15–23.

430

431   24.   Jack CR, Bernstein MA, Fox NC, Thompson P, Alexander G, Harvey D, et al. The Alzheimer's Disease

432   Neuroimaging Initiative (ADNI): MRI Methods. J Magn Reson Imaging. 2008;27(4):691.

433

434   25.   Chen K, Ayutyanont N, Langbaum JBS, Fleisher AS, Reschke C, Lee W, et al. Characterizing

435   Alzheimer's disease using a hypometabolic convergence index. Neuroimage. 2011;56:52–60.

436

437   26.   Caroli A, Prestia A, Chen K, Ayutyanont N, Landau SM, Madison CM, et al. Summary Metrics to

438   Assess Alzheimer Disease–Related Hypometabolic Pattern with 18F-FDG PET: Head-to-Head

439   Comparison. J Nucl Med. 2012;53(4):592–600.

440

441   27.   Sudre CH, Cardoso MJ, Frost C, Barnes J, Barkhof F, Fox N, et al. APOE ε4 status is associated with

442   white matter hyperintensities volume accumulation rate independent of AD diagnosis. Neurobiol

443   Aging. 2017;53:67–75.

444

445   28.   R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical

446   Computing, Vienna, Austria. 2021.

447

448   29.   Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat

449   Softw [Internet]. 2015;67(1):1–48.

450

451   30.   Lai J, Zou Y, Zhang S, Zhang X, Mao L, Zhang WH. glmm.hp: an R package for computing individual

452   effect of predictors in generalized linear mixed models. Journal of Plant Ecology [Internet]. 2022

453   Dec 1 [cited 2023 Jul 2];15(6):1302–7.

454

455   31.   R package version 0.2-6. gamm4: Generalized Additive Mixed Models using "mgcv" and "lme4"

456   [Internet]. 2020 [cited 2024 Jul 16]. Available from: https://CRAN.R-project.org/package=gamm4.

457

458   32.   Rajendrakumar AL, Arbeev KG, Bagley O, Yashin AI, Ukraintseva S, Initiative for the ADN. The SNP

459   rs6859 in NECTIN2 gene is associated with underlying heterogeneous trajectories of cognitive

460   changes in older adults. BMC Neurol. 2024;24(78).

461

462   33.   Coulombe J, Moodie EEM, Shortreed SM, Renoux C. Estimating individualized treatment rules in

463   longitudinal studies with covariate-driven observation times. Stat Methods Med Res.

464   2023;32(5):868–84.

465

466   34.   Mcmurry HS, Mendenhall E, Rajendrakumar A, Nambiar L, Satyanarayana S, Shivashankar R.

467   Coprevalence of type 2 diabetes mellitus and tuberculosis in low - income and middle - income

468   countries : A systematic review. Diabetes Metab Res Rev. 2019;35(2018).

469

470　35.　Bangen KJ, Himali JJ, Beiser AS, Nation DA, Libon DJ, Fox CS, et al. Interaction between midlife blood

471　　　glucose and APOE genotype predicts later Alzheimer pathology. J Alzheimers Dis.

472　　　2016;53(4):1553–62.

473

474　36.　Doney ASF, Bonney W, Jefferson E, Walesby KE, Bittern R, Trucco E, et al. Investigating the

475　　　Relationship Between Type 2 Diabetes and Dementia Using Electronic Medical Records in the

476　　　GoDARTS Bioresource. Diabetes Care. 2019;42(10):1973–80.

477

478　37.　Rajendrakumar AL, Hapca SM, Nair ATN, Huang Y, Chourasia MK, Kwan RSY, et al. Competing risks

479　　　analysis for neutrophil to lymphocyte ratio as a predictor of diabetic retinopathy incidence in the

480　　　Scottish population. BMC Med. 2023;21(1):304.

481

482　38.　Chakrabarty R, Yousuf S, Singh MP. Contributive Role of Hyperglycemia and Hypoglycemia Towards

483　　　the Development of Alzheimer's Disease. Mol Neurobiol. 2022;59(7):4274–91.

484

485　39.　Nair ATN, Wesolowska-Andersen A, Brorsson C, Rajendrakumar AL, Hapca S, Gan S, et al.

486　　　Heterogeneity in phenotype, disease progression and drug response in type 2 diabetes. Nat Med.

487　　　2022;28:982–8.

488

489   40.   Lathika Rajendrakumar A, Narayanan Nair AT, Chourasia MK, Nangia C, Srinivasan S, Radha V, et

490   al. Multi-ancestry genome-wide association study of neutrophil-lymphocyte ratio and polygenic

491   risk score development to explore causal association with diabetic retinopathy. medRxiv. 2024 Jan

492   1;2024.06.19.24309194.

493

494   41.   Sáiz-Vazquez O, Puente-Martínez A, Ubillos-Landa S, Pacheco-Bonrostro J, Santabárbara J.

495   Cholesterol and Alzheimer's Disease Risk: A Meta-Meta-Analysis. Brain Sci. 2020;10(6):386.

496

497   42.   Liu C, Dhindsa D, Almuwaqqat Z, Ko YA, Mehta A, Alkhoder AA, et al. Association Between High-

498   Density Lipoprotein Cholesterol Levels and Adverse Cardiovascular Outcomes in High-risk

499   Populations. JAMA Cardiol. 2022 Jul 1;7(7):672–80.

500

501   43.   Huang H, Yang B, Yu R, Ouyang W, Tong J, Le Y. Very high high-density lipoprotein cholesterol may

502   be associated with higher risk of cognitive impairment in older adults. Nutr J. 2024;23(79).

503

504   44.   Ferguson EL, Zimmerman SC, Jiang C, Choi M, Swinnerton K, Choudhary V, et al. Low- and High-

505   Density Lipoprotein Cholesterol and Dementia Risk Over 17 Years of Follow-up Among Members

506   of a Large Health Care Plan. Neurology. 2023;101(21):e2172–84.

507

508   45.   Monira Hussain S, Robb C, Tonkin AM, Lacaze P, T-J Chong T, Beilin LJ, et al. Association of plasma

509   high-density lipoprotein cholesterol level with risk of incident dementia: a cohort study of healthy

510   older adults. Lancet Reg Health West Pac. 2023;43(100963).

511

512   46.   Dunk MM, Li J, Liu S, Casanova R, Chen JC, Espeland MA, et al. Associations of dietary cholesterol

513   and fat, blood lipids, and risk for dementia in older women vary by APOE genotype. Alzheimer's

514   Dement. 2023;19:5742–54.

515

516   47.   Garcia AR, Finch C, Gatz M, Kraft T, Eid Rodriguez D, Cummings D, et al. APOE4 is associated with

517   elevated blood lipids and lower levels of innate immune biomarkers in a tropical Amerindian

518   subsistence population. Goldberg A, Perry GH, editors. Elife. 2021;10:e68231.

519

520   48.   Hopkins PCR, Huang Y, McGuire JG, Pitas RE. Evidence for differential effects of apoE3 and apoE4

521   on HDL metabolism. J Lipid Res. 2002;43(11):1881–9.

522

523   49.   Koren-Iton A, Salomon-Zimri S, Smolar A, Shavit-Stein E, Dori A, Chapman J, et al. Central and

524   Peripheral Mechanisms in ApoE4-Driven Diabetic Pathology. Int J Mol Sci. 2020;21(4):1289.

525

526   50.   Fortea J, Pegueroles J, Alcolea D, Belbin O, Dols-Icardo O, Vaqué-Alcázar L, et al. APOE4

527   homozygozity represents a distinct genetic form of Alzheimer's disease. Nat Med. 2024;30:1284–

528   91.

529

530 51. Jack CR, Andrews JS, Beach TG, Buracchio T, Dunn B, Graf A, et al. Revised criteria for diagnosis and

531   staging of Alzheimer's disease: Alzheimer's Association Workgroup. Alzheimer's Dement. 2024;1–

532   27.

533

534 52. Lehmann M, Ghosh PM, Madison C, Karydas A, Coppola G, O'Neil JP, et al. Greater medial temporal

535   hypometabolism and lower cortical amyloid burden in ApoE4-positive AD patients. J Neurol

536   Neurosurg Psychiatry. 2014;85(3):266–73.

537

538 53. Raulin AC, Doss S V., Trottier ZA, Ikezu TC, Bu G, Liu CC. ApoE in Alzheimer's disease:

539   pathophysiology and therapeutic strategies. Mol Neurodegeneration. 2022;17(72).

540

541 54. Chen HK, Ji ZS, Dodson SE, Miranda RD, Rosenblum CI, Reynolds IJ, et al. Apolipoprotein E4 Domain

542   Interaction Mediates Detrimental Effects on Mitochondria and Is a Potential Therapeutic Target

543   for Alzheimer Disease *. Journal of Biological Chemistry. 2011;286(7):5215–21.

544

545 55. Flowers SA, Rebeck GW. APOE in the normal brain. Neurobiol Dis. 2020;136:104724.

546

547 56. Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4 leads to

548   blood-brain barrier dysfunction predicting cognitive decline. Nature. 2020;581(7806):71–6.

549

550   57.   Matura S, Prvulovic D, Jurcoane A, Hartmann D, Miller J, Scheibe M, et al. Differential effects of the

551   ApoE4 genotype on brain structure and function. Neuroimage. 2014;89:81–91.

552

553   58.   Operto G, Molinuevo JL, Cacciaglia R, Falcon C, Brugulat-Serrat A, Suárez-Calvet M, et al. Interactive

554   effect of age and APOE-ε4 allele load on white matter myelin content in cognitively normal middle-

555   aged subjects. Neuroimage Clin. 2019;24:101983.

556

557   59.   Koutsodendris N, Blumenfeld J, Agrawal A, Traglia M, Grone B, Zilberter M, et al. Neuronal APOE4

558   removal protects against tau-mediated gliosis, neurodegeneration and myelin deficits. Nat Aging.

559   2023;3:275–96.

560

561   60.   Liao F, Yoon H, Kim J. Apolipoprotein E metabolism and functions in brain and its role in Alzheimer's

562   disease. Curr Opin Lipidol. 2017;28(1):67.

563

564   61.   Zheng JJ, Hong B V., Agus J, Guo F, Lebrilla CB, Jin L, et al. Alzheimer's Disease Patients, Especially

565   ApoE4 Carriers, Have Significantly Reduced High-density Lipoprotein Particle Size Revealed by

566   Negative-stained Transmission Electron Microscopy. Alzheimer's Dement. 2022;18(e063375).

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597   **Contributions**

598   KA, SU, and ALR were involved in conceptualization, design and wrote the paper. OB, ALR, MD conducted

599   data curation and formal analysis. KA, and AIY provided guidance on data analysis, interpreted the

600   findings, and critically revised the manuscript. All authors have read and agreed to the published version

601   of the manuscript.

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605

606   **Ethics declarations**

607   The studies involving human subjects were approved by the Duke University Health System Institutional

608   Review Board (Protocols Pro00105166 and Pro00105389). This publication includes only secondary

609   analyses of existing data available from ADNI, and does not include identifiable human data. Written

610   informed consent for ADNI participants was obtained by the ADNI in accordance with the local legislation

611   and ADNI requirements.

612

613   **Consent for Publication**

614   Not applicable

615   **Competing Interests**

616   The authors declare no competing interests.

617

618   **Additional information**

619   **Electronic Supplementary Material**

620   Supplementary Material 1

621