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APOE ε4 carriers have a greater propensity to glycation and sRAGE which is further influenced by RAGE G82S polymorphism.

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Abstract

APOE ε4 allele is an established risk factor for Alzheimer’s disease and hypercholesterolemia. However, its association with metabolic and genetic risk factors related to glycation is not clear. We tested the hypothesis that, apart from high plasma cholesterol, APOE ε4 carriers may also have higher advanced glycation end products (AGEs) and total soluble extracellular domain of RAGE (sRAGE) and that these biomarkers may be modified by the common Gly82Ser (G82S) polymorphism (rs2070600) in the RAGE gene. To test this, we measured these biomarkers in 172 healthy cognitively normal individuals, of which 32 were APOE ε4 carriers and 140 non-carriers. APOE ε4 carriers showed higher levels of cholesterol (p< 0.001), glyoxal (p< 0.001), fluorescent AGEs (p< 0.001), Nε-carboxymethyllysine (p< 0.001) and sRAGE (p= 0.018) when compared to non-carriers. Furthermore, sRAGE was also higher in those that did not carry the A allele of the RAGE gene that codes for serine instead of glycine (p = 0.034).

Our study indicates that APOE ε4 carriers have a greater propensity to glycation than non-carriers which may further increase their risk for diabetes and dementia. The increased sRAGE levels in APOE ε4 carriers suggests a defensive response against AGEs that may be further influenced by the RAGE G82S polymorphism.

Keywords: cholesterol, advanced glycation end products, Nε-carboxymethyllysine, APOE ε4
Introduction.

Alzheimer’s disease (AD), the most common form for dementia, has recently become a worldwide health concern, with a current estimate of 44 million people living with dementia and the number predicted to reach 135 million by 2050 (1). In Australia, there are more than 376,000 individuals living with dementia with a projected growth to 1 million by 2050 (2).

Diet, lifestyle and genetic profile, in particular the \( APOE \) genotype, are recognized as the key risk factors attributed to AD. There are three common alleles of \( APOE \): \( APOE \varepsilon3 \) is the most common allele with a 78% prevalence in the general population, \( APOE \varepsilon4 \) is at 15%; and \( APOE \varepsilon2 \) is at 7% prevalence (3). The presence of \( APOE \varepsilon4 \) allele, despite low allelic frequency, is a major genetic risk factor for AD later in life and accounts for 50-60% of late onset AD cases(4). Individuals who have a single copy of the \( APOE \varepsilon4 \) allele have approximately 4 times increased likelihood of developing AD compared with carriers of the \( APOE \varepsilon3 \) allele, whereas double copy of \( APOE \varepsilon4 \) allele increases the risk of developing AD by 12-20 fold (5, 6). Carriage of the \( \varepsilon4 \) allele may advance the age of onset by 7-9 years (5).

The \( APOE \varepsilon4 \) allele is also associated with hyper-lipidemia, atherosclerosis, coronary heart disease, stroke and Type-2 diabetes mellitus (T2DM) (3, 7). The molecular mechanisms by which dyslipidaemias are associated with an increased risk of AD remain unclear however the critical role of membrane cholesterol has been proposed (8). Cholesterol, an electrical insulator, is an important component of cellular membranes required to maintain membrane structure and function (8). Genetic studies indicated that the expression of \( APOE \varepsilon4 \), the key protein in lipid metabolism, is a risk factor for AD, suggesting that cholesterol may be involved in the aetiology of AD (3). \( APOE \) plays an essential role in the delivery of lipids, and cholesterol from the liver to all the cells of the body, via specific receptor-based mechanism in plasma membranes. It has been shown that the \( APOE \varepsilon4 \) allele is associated with reduced cholesterol uptake by astrocytes.
in the hippocampus (9). *APOE* genotype, has been shown to account for up to 7% of the variance observed in total cholesterol and low-density lipoprotein (LDL-cholesterol) (10). Previous studies have also reported that *APOE* ε4 carriers have higher plasma concentrations of total cholesterol and LDL-cholesterol (11, 12).

Serum and cellular proteins exposed to excessive glucose, dicarbonyls and reactive oxygen species become impaired due to glycation, leading to the formation of advanced glycation end products (AGEs). Glycation is a non-enzymatic set of reactions between the carbonyl group of reducing sugar (and/or sugar oxidation products) and free amino group of proteins, peptides or amino acids which lead to the formation of Schiff bases and their rearrangements to produce reversible Amadori products and ultimately a group of heterogeneous compounds collectively known as AGEs (13, 14). Most of the AGEs exhibit heterocyclic ring structures and fluorescence (e.g., pentosidine) whereas others are non-fluorescent and do not cross-link with macromolecules (e.g., Nε-carboxymethyllysine (CML), Nε-carboxyethyllysine) (13, 15). In addition, *APOE* is highly susceptible to glycation, with *APOE* ε4 showing a 3-fold greater AGE-binding activity than *APOE* ε3 (16). This suggests that glycation of *APOE* ε4 may be an early step in the Alzheimer’s cascade and that an increased affinity of *APOE* ε4 and AGEs may be a significant contributor to increased risk of AD.

The receptor for AGEs (RAGE), a multiligand signal transduction receptor, has the ability to bind with AGEs, S100 and β-amyloid (Aβ) (13). RAGE and these ligands have been implicated in the amplification of pro-inflammatory and neurotoxic reactions in AD brains. In contrast, the soluble form of RAGE, a proteolytically cleaved form of RAGE that lacks the transmembrane domain, has the ability to inhibit the RAGE-mediated pathological effects by acting as a decoy receptor and thus preventing signal transduction by AGEs within cells (13, 17). The genetic contribution of the RAGE gene G82S polymorphism to the amount of soluble
form of RAGE levels in AD patients has been recently reported (18, 19). However, the association between AGE levels and G82S polymorphism with the concentration of the soluble form of RAGE in APOE genotype is lacking. Thus, in the present study, the association between cholesterol levels, protein glycation biomarkers and the G82S polymorphism of RAGE gene in APOE ε4 allele carriers versus non-carriers were investigated. We hypothesized that APOE ε4 carriers have increased cholesterol in plasma due to increased glycation that disables lipoprotein transport of cholesterol.

Methods

Volunteer recruitment. One hundred and seventy two healthy volunteers, aged 35-65 years completed the study as per protocol previously published (20). Briefly the following inclusion criteria were used: i) non-smokers, ii) non-diabetic, iii) not currently diagnosed with MCI or AD, iii) mini-mental state examination (MMSE) score ≥ 20, iv) not on medication for life-threatening diseases (e.g., chemotherapy for cancer treatment), v) not taking daily minerals, fish oil and/or vitamin supplements above the RDA level, vi) able to understand the study protocol and vii) not on cholesterol-lowering medication or diabetic medications. All participants were found to be cognitively normal (29.16 ± 0.12) as per the mini-mental state examination (MMSE) scores. The MMSE status of APOE ε4 carriers was 29.25 ± 0.16 whereas in the non-carriers it was 29.06 ± 0.08.

Blood collection. Fasted (12 h) whole blood samples (~18 mLs) were collected at the Adelaide CSIRO clinic via venipuncture into lithium-heparin (LH) (Greinder Bio-One) vacutainers by trained nurses. All blood vacutainers were first spun at 1500 x g for 20 mins at 4 ±1°C, and the plasma removed and stored at -80 °C for further analysis.

DNA isolation and genotyping for APOE ε and RAGE G82S gene polymorphisms. Genomic DNA was isolated from peripheral blood mononuclear cells using a Qiagen DNeasy
Blood and Tissue kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany) and detailed in the supplementary file.

**Lipid biomarkers.** Plasma total cholesterol, triglycerides and HDL-cholesterol were measured on the Hitachi 902 Automated Clinical Analyser at the CSIRO Analytical and Clinical Chemistry Laboratory in Adelaide.

**Glucose and glyoxal analysis.** Plasma glucose was measured using an automated spectrophotometric analyser (Konelab 20Xti, Thermo Electron, Massachusetts, MA, USA). Glyoxal concentration in plasma samples was determined using Girard’s reagent T as reported previously (21) and detailed in the supplementary file.

**Total fluorescent AGEs, CML levels and sRAGE.** Fluorescent AGEs were measured as previously reported (22) and the results are presented as AU/mL. CML content in plasma was determined by RP-HPLC with an o-phthalaldehyde derivatisation step as previously reported (23) and the results are presented as µmol CML/mol Lysine. Total soluble extracellular domain of RAGE (sRAGE) concentration in plasma was determined using a commercial ELISA kit (BioVendor, Karasek, Czech Republic). The assay measures total sRAGE resulting from both cleavage (cRAGE) and endogenous secreted (esRAGE) of RAGE and measured as per the manufacturer’s instructions. The intra-assay and inter-assay coefficients of variation values were <5% and <10%, respectively. The detailed method is provided in the supplementary file.

**Statistical analysis**

All data was analysed after performing an ANCOVA test with adjustment for age, gender and BMI using IBM SPSS 23.0 Statistics software. Parametric or non-parametric tests were used depending on whether the results had a normal Gaussian distribution or a non-Gaussian distribution, respectively. A t-test was performed to compare the mean of age and BMI in
APOE ε4 carriers and non-carriers. Non-parametric statistical methods were used for comparisons of lipid profiles and glycation biomarkers between APOE genotypes. Correlation analysis was performed by non-parametric Spearman’s (r_s) test. Two-way ANOVA was performed to determine the percent variance and interaction explained by APOE genotype and RAGE genotype using Prism 7.0 (Graphpad Inc., USA).

Results

Volunteer characteristics and genotype frequency. This study involved 172 volunteers with 36 males and 136 females with the combined age ranging from 35-65 years (Table 1). Genotype and allele frequency according to TaqMan® SNP are shown in Table 1. Among these 172 volunteers, APOE ε4+ haplotypes (ε3/ε4 (12.8%), ε2/ε4 (2.3%), ε4/ε4 (3.5%)) were detected in 32 (18.6 %) individuals whereas 140 (81.4%) individuals carried APOE ε4- haplotypes (ε3/ε3 (66.3%), ε2/ε3 (15.1%), ε2/ε2 (0%). No significant difference (p=0.531) in mean age was recorded in the APOE ε4 carriers versus non-carriers (Table 1). The BMI details are included in Table 1. No significant difference in BMI (p=0.656) was seen between APOE ε4 carriers versus non-carriers (Table1). With regards to RAGE polymorphism, 152 (88.3%) were found to have the GG (glycine) genotype, 18 (10.5%) had the heterozygous (AG) genotype and only 2 (1.2%) had the homozygous AA (serine) type genotype (Table 1).

Cholesterol levels. The total cholesterol and the LDL-cholesterol levels were significantly higher in APOE ε4 carriers, by 13% and 20% respectively, after correcting for age, gender and BMI compared to non-carriers (Table 2, Supplementary Figure 1). Triglycerides and HDL-cholesterol were not significantly different in APOE ε4 carriers when compared with non-carriers (Table 2, Supplementary Figure 1). No statistically significant differences were
observed when APOE ε4+ or APOE ε4- genotypes were compared for triglycerides, total cholesterol and HDL-cholesterol after adjusting for age, gender and BMI (Supplementary Table 1). Furthermore, amongst APOE ε4 carriers, a significant increase in LDL-cholesterol was seen in the ε4/ε4 sub-group when compared to those with the ε2/ε4 genotype (Supplementary Table 1).

**AGE biomarkers.** Plasma glyoxal concentration was significantly increased by 36% (p<0.001; Table 2) in the APOE ε4+ carriers relative to non-carriers. There were no differences in glyoxal concentration amongst APOE ε4+ or APOE ε4- sub-groups (Table 3). Plasma glucose concentration in APOE ε4 carriers was not different when compared with non-carriers (Table 2). Fluorescent AGEs were significantly increased by 32% (p<0.001) in APOE ε4 carriers when compared with the non-carriers (Table 2, Supplementary Figure 2). When sub-groups amongst APOE ε4+ or APOE ε4- genotypes were compared for fluorescent AGEs, no significant difference were seen (Table 3). CML levels in APOE ε4- and APOE ε4+ were 48.09 ± 0.54 µmol CML/mol Lys and 53.71 ± 1.14 µmol CML/mol Lys, respectively (Table 2, Supplementary Figure 2). The 12% increase in plasma CML levels in the APOE ε4+ cohort was statistically significant (p< 0.001) when compared with APOE ε4- cohort. Within the APOE ε4+ cohort, no significant difference in CML levels was evident among the ε3/ε4, ε2/ε4 and ε4/ε4 genotypes.

In APOE ε4 carriers, the increased total cholesterol did not appear to correlate with either fluorescent AGES ($r_s$=0.129; p=0.481) or CML levels ($r_s$=-0.075; p =0.684) (Supplementary Table 3).

sRAGE level was significantly (p=0.018) higher by 21% in the APOE ε4 carriers when compared with the non-carriers (Table 2, Supplementary Figure 2). Among the APOE ε4+
cohort, no significant difference were observed among the ε2/ε4, ε3/ε4 and ε4/ε4 genotypes in the levels of sRAGE (Table 3).

Plasma sRAGE levels were significantly higher in the APOE ε4 carriers relative to non-carriers (p=0.018) and remained higher even after stratifying for polymorphism in RAGE gene (G82S) (AG+AA; p=0.002; GG; p= 0.034) (Table 2, Supplementary Figure 2). In this cohort, the sRAGE levels were significantly higher (p= 0.008) in the sRAGE GG genotype compared to the sRAGE AA+AG genotype group. Despite the significant effect of APOE ε4 and sRAGE genotypes on plasma sRAGE levels, the interaction effect of these two genes was not significant (% variance explained = 0.046; p=0.770) (Figure 1).

Supplementary Tables 2, 3 and 4 provide the correlation matrix data for all parameters measured in this study based on all subjects, APOE ε4 carriers and non-carriers respectively. The most notable significant and consistent correlation was that between glucose and fluorescent AGEs (r_s = 0.290, p<0.001; r_s=0.551, p<0.001; r_s=0.422, p<0.001 in all subjects, APOE ε4 carriers and non-carriers, respectively). In addition, a significant correlation between glucose and CML (r_s = 0.358, p<0.05) was observed in APOE ε4 carriers but not in non-carriers. A significant correlation between fluorescent AGEs versus CML (r_s = 0.224, p<0.01) and sRAGE (r_s = 0.173, p<0.05) was observed in the non-carriers but not in APOE ε4 carriers. The sRAGE GG genotype showed a significant correlation with fluorescent AGEs (r_s = 0.211, p<0.05) in all subjects. In addition, sRAGE GG genotype was significantly correlated with triglyceride (r_s = -0.187, p<0.05), HDL-cholesterol (r_s = 0.252, p<0.01) and fluorescent AGEs (r_s = 0.184, p<0.05) in the non-carriers but not in APOE ε4 carriers.
Discussion

To our knowledge, this is the first study to examine the association between hypercholesterolemia, protein glycation biomarkers and RAGE G28S polymorphism in APOE ε4 carriers.

Our results support previous studies showing increased levels of total cholesterol and LDL-cholesterol in APOE ε4 carriers (11, 12, 24). In a systematic review, Khan et al (25) reported a positive association between APOE genotype and total cholesterol with highest total cholesterol shown with APOE ε4+ in the LIPGENE cohort. An increase in total cholesterol and LDL-cholesterol in APOE ε4+, appears to be due to preferential association of the ε4 protein with triacylglycerol-rich protein, thus increasing competition with other protein isoforms binding to the LDL-receptor, resulting in less uptake of LDL and increased circulating plasma cholesterol (26, 27).

Munch and co-workers initially proposed that excess levels of free radicals and reactive carbonyl compounds (eg., glyoxal and methylglyoxal) lead to the formation of AGEs that could be responsible for extensive protein crosslinking and inflammation in AD (28, 29). Our research examined glyoxal, AGEs and CML levels in APOE ε4 carriers versus non-carriers and showed that APOE ε4 carriers had higher AGEs than non-carriers which may further aggravate their health risks. It is significant that the heightened glycation in our cohort is occurring at a stage in their life when cognitive decline is not evident with the MMSE test suggesting that increased AGEs may be useful to explore and validate as an additional early indicator for AD risk in APOE ε4 carriers.

The association of APOE ε4 genotype with AGEs and with risk of AD is of current interest. Our observation that glyoxal, total fluorescent AGEs and CML levels were significantly higher
in the APOE ε4+ compared to the APOE ε4- group suggests an important mechanism that can further explain the coincident cardiovascular and AD pathology in APOE ε4 carriers. Increased glucose, dicarbonyls, and oxidative stress contribute to the formation of AGEs. Some AGEs include, CML, imidazolones and lysine-residue modified products that commonly occur in AD (30, 31). In our study, APOE ε4+ carriers showed a slight but nonsignificant increase in glucose levels whereas the glyoxal level was significantly higher. Higher glyoxal levels in the APOE ε4+ carriers could contribute to the generation of Amadori-modified proteins which in turn are associated with formation of lysine-residue products (including pentosidine) and CML. Previous studies showed an association between methylglyoxal, its reactive derivatives and AD (32, 33). In addition, increased methylglyoxal has been associated with enhanced aggregation of amyloidβ (Aβ) (34) and increasing rate of cognitive decline in non-demented elderly individuals (35). In contrast there was no association between increased glyoxal levels and MMSE in the APOE ε4 carriers in our study (data not shown) which, however, was limited by the narrow range of MMSE in cognitively normal subjects. The average age of APOE ε4 carriers in our cohort was 54.56 ±7.58 years which may be a relatively young group to observe any changes in MMSE status. Beeri et al (35) also showed no relationship between increased methylglyoxal and MMSE when adjusted for APOE ε4 genotypes. Higher serum AGEs levels have been reported in AD patients previously (31, 36). Accumulation of fluorescent AGEs, which was evident in APOE ε4 carriers in our cohort, was reported to be significantly higher in participants with mild cognitive impairment based on skin auto fluorescence examination (37). Previous studies indicate that AGEs may be involved in the transformation of soluble Aβ into insoluble forms, aggregation of microtubule associate protein tau and act as a catalyst in the acceleration and stabilization of amyloid plaques in the AD brain (38, 39). The accumulation and deposition of AGEs, may continue to develop over time and could play an important role in the loss of cognitive function.
It has been hypothesized that sRAGE present in human plasma functions as a ‘decoy’, binds to a number of ligands including Aβ, AGEs and contributes to AGE clearance (13, 17). A number of polymorphic sites have been identified in human RAGE gene, including G82S located in the exon3 of chromosome 6p31.3, which causes a glycine to serine substitution at codon 82 within the ligand binding domain of the receptor (40). Previous studies have shown that the G82S polymorphism enhances ligand binding affinity and has an independent effect on inflammation, oxidative stress, insulin resistance and plasma sRAGE levels, all of which are considered high-risk factors for AD (18, 41). In addition, other studies showed that the plasma levels of sRAGE in AD were significantly lower than in normal healthy controls that they correlated with the severity of disease (18, 42, 43). In a Turkish population, a non-significant (p = 0.2) decrease in sRAGE was observed when either the AD or mild cognitive impairment patients were compared with healthy controls (44). In addition, in the same study there were no significant differences seen when the genotype for RAGE G28S was compared between the groups (44).

To our knowledge, there are no studies reported on the association of AGEs and sRAGE in APOE ε4 carriers before the symptoms of AD become evident. Interestingly, in our study sRAGE level in the APOE ε4 carriers was significantly higher when compared to non-carriers. Previous studies indicate that serum AGE and sRAGE levels are correlated with each other and that ratio of AGEs to sRAGE, rather than each parameter alone, may be a sensitive biomarker that reflects the AGE-RAGE system in humans (45, 46). In our study while fluorescent AGEs and CML levels increased in APOE ε4 carriers, they did not correlate with MMSE. The lack of association with MMSE may be due to the relative young age and good health status of our cohort and relative insensitivity of MMSE to detect small differences in cognitive function (47).
The studies of Li et al. (18) and Daborg et al. (19) both reported that carriers of the APOE ε4 allele who were also homozygotes for the RAGE G allele (i.e., RAGE GG genotype) had a significantly reduced risk of AD. In our study sRAGE was highest in APOE ε4 carriers who also had the RAGE GG genotype. These results are consistent with our hypothesis that sRAGE protects against the risk of AD. The most plausible explanation is that a high sRAGE in body fluids somehow prevents the pathological effects of AGEs possibly by preventing engagement with the full length RAGE in the cytoplasmic membrane (13). The results also suggest that the G allele codes for a form of RAGE that has a higher propensity to become sRAGE.

Our study differs from that of Daborg et al. and Li et al. in that we only investigated healthy controls and that in our cohort of APOE ε4 carriers the frequency of the RAGE GG genotype was significantly lower compared to non-carriers which was not the case for the other studies (Supplementary Table 4).

In conclusion, APOE ε4 carriers showed higher levels of total cholesterol, LDL-cholesterol and glycation biomarkers (i.e., glyoxal, fluorescent AGEs and CML) and sRAGE. Thus, it may be reasonable to speculate that in healthy APOE ε4 carriers, the pathogenic effects of increased AGEs in plasma may be attenuated by binding with the increased levels of sRAGE which acts as a “decoy” and limits the AGEs-induced aggregation of Aβ, thus slowing down the brain pathology that leads to cognitive decline. The potential interaction among the cholesterol, glycation biomarkers including sRAGE on cognitive impairment could be represented in a mechanistic scheme as outlined in Figure 2. Nevertheless, these observations are preliminary because (i) there are only limited data on the association of APOE genotype and glycation (ii) our study did not determine the AGE-binding activity of APOE ε4 or sRAGE; and (iii) the MMSE assessment technique may have not been sensitive enough to detect small differences.
in cognitive function. Further studies are needed to investigate if increased sRAGE in \textit{APOE} \varepsilon 4 carriers prevents cognitive decline.

\textbf{Acknowledgement:}

PD analysed AGE biomarkers and the initial drafting of the manuscript; VSD did the \textit{APOE} genotyping and RAGE G28S polymorphism work; AC was involved in volunteer recruitment, blood collection and \textit{APOE} genotyping; PT was involved in volunteer recruitment and blood work; MF initiated the study, oversaw data analysis and was responsible for the overall study. All authors critically reviewed the manuscript.

\textbf{Conflict of Interest:} the authors report no conflict of interest.

\textbf{References}


Table 1. Gender and Genotypic frequencies in the Study Cohort

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<tr>
<td>Female</td>
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<td>79.1</td>
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<tr>
<td>Total</td>
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<td>-</td>
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</table>

| Age of Non-ε4 and ε4 carrier (mean ± SD)\(^a\) | ε4- | 53.56 ± 8.11 | - |
|                                               | ε4+ | 54.56 ± 7.58 | - |

| BMI of Non-ε4 and ε4 carrier (mean ± SEM)\(^b\) | ε4- | 26.49 ± 0.42 | - |
|                                               | ε4+ | 26.66 ± 0.77 | - |

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<th>Non-ε4 and ε4 carrier frequency</th>
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*p = 0.531, †p = 0.656*
Table 2. Plasma lipid profile and glycation biomarkers in APOE ε4 carriers and non-carriers.

<table>
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<th>Biomarkers</th>
<th>ε4-</th>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.08 ± 0.04</td>
<td>1.18 ± 0.18</td>
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<td>Total Cholesterol (mmol/L)</td>
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<td>HDL-cholesterol (mmol/L)</td>
<td>1.65 ± 0.03</td>
<td>1.64 ± 0.06</td>
<td>0.930</td>
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<td>LDL-cholesterol (mmol/L)</td>
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<td>3.78 ± 0.14</td>
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<td><strong>Glycation biomarkers</strong></td>
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<tr>
<td>Glucose (mmol/L)</td>
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<td>4.62 ± 0.14</td>
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<tr>
<td>Glyoxal (nmol/L)</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Fluorescent AGEs (AU/mL)</td>
<td>2473.11 ± 65.54</td>
<td>3265.00 ± 137.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nε-carboxymethyllysine (μmol)</td>
<td>48.09 ± 0.54</td>
<td>53.71 ± 1.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CML/mol Lysine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sRAGE (pg/mL)</td>
<td>634.30 ± 24.03</td>
<td>767.72 ± 50.31</td>
<td>0.018</td>
</tr>
<tr>
<td>sRAGE (pg/mL) in AG* +</td>
<td>415.42 ± 38.85</td>
<td>642.00 ± 43.06</td>
<td>0.002</td>
</tr>
<tr>
<td>sRAGE (pg/mL) in GG*</td>
<td>650.41 ± 24.97</td>
<td>787.81 ± 59.07</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Data was analysed after performing an ANCOVA test with adjustment for age, gender and BMI and presented as mean ± SEM. * refers to genotype for RAGE G82S polymorphism.
Table 3. Glycation biomarkers of APOE genotypes present in APOE ε4 carriers and noncarriers

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>Glyoxal (nmol/L)</th>
<th>Fluorescent AGEs (AU/mL)</th>
<th>Nε-carboxymethyllysine (µmol CML/mol Lysine)</th>
<th>sRAGE (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε4-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>13.54 ± 0.51</td>
<td>2464.20 ± 53.46a</td>
<td>47.22 ± 1.27a</td>
<td>658.76 ± 56.33a</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>12.99 ± 0.24a</td>
<td>2475.16 ± 73.21a</td>
<td>48.28 ± 0.61a</td>
<td>628.72 ± 26.87a</td>
</tr>
<tr>
<td>ε4+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>20.64 ± 1.30b</td>
<td>3573.90 ± 81.35b</td>
<td>50.48 ± 3.27ab</td>
<td>788.07 ± 45.11b</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>17.38 ± 0.55b</td>
<td>3227.11 ± 56.45b</td>
<td>53.52 ± 1.38b</td>
<td>790.25 ± 60.42b</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>17.07 ± 1.06b</td>
<td>3211.66 ± 22.70ab</td>
<td>51.19 ± 2.67ab</td>
<td>791.95 ± 18.45b</td>
</tr>
</tbody>
</table>

None of the subjects in the study cohort were ε2/ε2. Data was analysed after performing an ANCOVA test with adjustment for age, gender and BMI and presented as mean ± SEM. Values within a column that do not share the same letter are significantly different (p<0.05) from each other when comparing data across all genotype.
**Figure 1:** Two-way ANOVA analysis of the effect of *APOE* genotype (*APOE* ε4 or Non-*APOE* ε4), RAGE genotypes (AA+AG or GG), on plasma sRAGE levels and their interactions.

**Figure 2.** Mechanistic scheme showing interaction among the cholesterol, glycation biomarkers and soluble RAGE (sRAGE) on cognitive impairment and AD risk. AGE-advanced glycation end products, RAGE – receptor for AGE. Aβ- beta amyloid, ROS-reactive oxygen species, TC-total cholesterol, LDL-C low-density lipoprotein. ApoEε4 - apolipoprotein Eε4.
Figure 1.

% variance explained
interaction = 0.046; p = 0.77
APOE = 3.9; p = 0.008
RAGE GG = 4.58; p = 0.004

p = 0.0093
p = 0.001
p = 0.0016

sRAGE (pg/mL)
Non ApoE-ε4 ApoE-ε4
AA+AG
GG
Figure 2.