Plasma apolipoproteins and physical and cognitive health in very old individuals

Julia Muenchhoff[1], Fei Song[1], Anne Poljak[1,2,3], John D. Crawford[1], Karen A. Mather[1], Nicole A. Kochan[1,4], Zixuan Yang[1], Julian N. Trollor[1,5], Simone Reppermund[1,5], Kate Maston[1], Adam Theobald[1], Susanne Kirchner-Adelhardt[6], John B. Kwok[3,7]; Robyn L. Richmond[8], Mark McEvoy[9], John Attia[9,10], Peter W. Schofield[9,11], Henry Brodaty[1, 12], Perminder S. Sachdev[1,4,*]

[1]Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, Australia; [2]Bioanalytical Mass Spectrometry Facility, University of New South Wales, Sydney, Australia; [3]School of Medical Sciences, University of New South Wales, Sydney, Australia; [4]Neuropsychiatric Institute, Prince of Wales Hospital, Sydney, Australia; [5]Department of Developmental Disability Neuropsychiatry (3DN), School of Psychiatry, University of New South Wales, Sydney, Australia; [6]Department of Psychology, University of Washington, Seattle, USA; [7]Neuroscience Research Australia, Randwick, Australia; [8]School of Public Health and Community Medicine, University of New South Wales, Sydney, Australia; [9]School of Medicine and Public Health, University of Newcastle, Newcastle, Australia; [10]Hunter Medical Research Institute, Newcastle, Australia; [11]School of Psychology, University of Newcastle, Newcastle, Australia; [12]Dementia Collaborative Research Centre, School of Psychiatry, University of New South Wales, Sydney, Australia.

***Corresponding author:** Prof. Perminder S. Sachdev; Centre for Healthy Brain Ageing, UNSW Australia, Euroa Centre, Prince of Wales Hospital, Barker St., Randwick NSW 2031, Australia. E-mail: p.sachdev@unsw.edu.au; Phone: +61(2) 9382 3763; Fax: +61(2) 9382 3774

ABSTRACT

Apolipoproteins play a crucial role in lipid metabolism with implications in cardiovascular disease, obesity, diabetes, Alzheimer's disease and longevity. We quantified seven apolipoproteins in plasma in 1067 individuals aged 56–105 using immunoassays and explored relationships with *APOE* polymorphism ε2/3/4, vascular health, frailty and cognition. ApoA1, ApoA2, ApoB, ApoC3, ApoE, ApoH and ApoJ decreased from midlife, although ApoE and ApoJ had U-shaped trends. Centenarians had the highest ApoE levels and the lowest frequency of *APOE* ε4 allele relative to younger groups. Apolipoprotein levels trended lower in *APOE* ε4 homo- and heterozygotes compared to non-carriers, with ApoE and ApoJ being significantly lower. Levels of all apolipoproteins except ApoH were higher in females. Sex- and age-related differences were apparent in the association of apolipoproteins with cognitive performance, as only women had significant negative associations of ApoB, ApoE, ApoH and ApoJ in mid-life, whereas associations at older age were non-significant or positive. Our findings suggest levels of some apolipoproteins, especially ApoE, are associated with lifespan and cognitive function in exceptionally long-lived individuals.

Keywords: longevity; cognition; lipids; *APOE* phenotype; centenarian; frailty

ABBREVIATIONS

ACE-R: Addenbrooke Cognitive Examination – Revised

AD: Alzheimer's disease

APOC3: apolipoprotein C-III gene

APOD: apolipoprotein D gene

APOE: apolipoprotein E gene

ApoE: apolipoprotein E

ApoH: apolipoprotein H

BMI: body mass index

CRP: C-reactive protein

CSF: cerebrospinal fluid

eGFR: estimated glomerular filtration rate

HCS: Hunter Community Study

HDL: high-density lipoproteins

LDL: low-density lipoproteins

MAS: Sydney Memory and Ageing Study

MCI: mild cognitive impairment

MMSE: Mini–Mental State Examination

SCS: Sydney Centenarian Study

SNPs: single nucleotide polymorphisms

VLDL: very-low-density lipoproteins

1. INTRODUCTION

The population of the world is ageing, with individuals over the age of 90 being the fastest growing proportion of the population. By 2050, the number of centenarians is expected to reach 2.2 million individuals worldwide (Yang et al.). Exceptionally long-lived individuals may be regarded as models of longevity and successful ageing, and their study presents a unique opportunity to discover factors that are protective from age-related disease and cognitive decline. This is particularly important as assumptions based on findings from "younger" old cohorts around 70-85 years do not necessarily apply to exceptionally long-lived individuals (Sachdev et al.).

A protein family that has recently been suggested to be of particular relevance to the ageing process and longevity are the apolipoproteins. The majority of apolipoproteins are constituents of lipoprotein particles, such as chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), which transport lipids between tissues for fuel and cholesterol metabolism (Figure 1). The apolipoproteins serve as carrier, receptor-binding and regulatory proteins in these particles. They are therefore crucial components in lipid metabolism with implications for cardiovascular disease, obesity, diabetes mellitus and other diseases (for a review see (Dominiczak and Caslake, 2011)). Apolipoproteins also play roles in immune and vascular functions (Stoll and Bendszus, 2006).

There is evidence that apolipoprotein metabolism changes with age. For example, ApoJ mRNA and protein were found to be up-regulated in cellular senescence (Petropoulou et al., 2001; Trougakos et al., 2006), and the apolipoprotein D gene (*APOD)* is the most significantly up-regulated gene as a function of age in mice, apes and humans (de Magalhaes et al., 2009; Loerch et al., 2008; Zahn and Kim, 2007). *APOD* also contributes to an extended life span in the fruit fly (Muffat et al., 2008; Sanchez et al., 2006). The homozygote *CC* genotype in the promoter region of the apolipoprotein C-III gene (*APOC3)* is significantly more prevalent among centenarians (Bergman et al., 2007), and is associated with significantly lower serum levels of ApoC3 (Atzmon et al., 2006). Besides their influence on Alzheimer's disease (AD) risk, the ε2 and ε4 alleles of the apolipoprotein E gene (*APOE*) might also be associated with longevity but in opposing ways. Whereas ε2 has been associated with an increase in life span, ε4 has an adverse effect on life span in multiple studies (Shadyab and LaCroix, 2015).

Apolipoproteins have also been implicated in cognitive decline in the elderly and in AD. We have previously shown that individuals aged 70 to 90 years with mild cognitive impairment (MCI), a potential

prodrome of AD, have abnormal plasma levels of apolipoproteins, in particular apolipoprotein H (ApoH) and apolipoprotein J (ApoJ also known as clusterin), which were also associated with cognitive decline (Song et al., 2012). Plasma and cerebrospinal fluid (CSF) levels of ApoJ are emerging as meaningful biomarkers for AD and carriers of ApoJ gene (*CLU*) risk alleles show faster rates of cognitive decline (Thambisetty et al., 2013; Thambisetty et al., 2010; Yu and Tan, 2012). The *APOE* ε4 allele is the most significant genetic risk factor for late onset AD, and may be a greater risk factor in females than males (Altmann et al., 2014). This *APOE* polymorphism may also affect plasma levels of apolipoprotein E (ApoE) (Gupta et al., 2011; Slooter et al., 1998); however reports of its effects on levels of other apolipoproteins are limited (Henriques et al., 2014; Song et al., 2012). Furthermore, ApoJ, ApoE as well as apolipoprotein A-I (ApoA1) might interact with Aβ peptide to influence its neurotoxicity, aggregation or clearance from the brain (Narayan et al., 2012; Paula-Lima et al., 2009; Verghese et al., 2013). Methylation of a specific CpG in the *APOA1* gene was also associated with memory performance in a cohort of elderly community-dwelling individuals from the Sydney Memory and Ageing Study (MAS) (Lazarus et al.).

In this cross-sectional study, we set out to define age-related differences in plasma levels of these proteins and their potential contribution to physical and cognitive health, especially in the oldest old (≥95 years). Specifically, we measured seven plasma apolipoproteins (ApoA1, ApoA2, ApoB, ApoC3, ApoE, ApoH and ApoJ) in over 1000 individuals aged from 56 to 105 years to determine age-related differences in apolipoprotein levels. All participants were genotyped for *APOE* ε4 and ε2 carrier status to investigate the association of *APOE* polymorphism with plasma apolipoprotein levels. We have used a data driven (inductive) approach to investigate the relationship of apolipoprotein levels with sex, blood lipids, vascular health, frailty and cognition in individuals aged from 56 to 105 years with a particular focus on the oldest age group of ≥95 years.

2. MATERIALS AND METHODS

2.1. Study participants and blood collection

Samples were obtained from three independent, population-based studies of older adults conducted in New South Wales, Australia: 147, 575 and 345 EDTA plasma samples were available from the Sydney Centenarian Study (SCS), the second Wave of the Sydney Memory and Ageing Study (MAS) and the Hunter Community Study (HCS), respectively. HCS and MAS participants were included in order to better assess

the relationship between plasma apolipoprotein levels and age starting from mid-life. The HCS and MAS also provide a younger age group for comparison with centenarians in regard to cognition.

The cohorts are described in detail in previous publications (McEvoy et al., 2010; Sachdev et al., 2010; Sachdev et al., 2013). Briefly, the SCS is a population-based study of the exceptionally long-lived, enrolling individuals aged 95 and over, with the aim to examine the determinants of healthy ageing and longevity (Sachdev et al., 2013). Extensive neuropsychiatric, medical, nutritional and lifestyle data are gathered for participants in multiple sessions and informants are also interviewed. Participants to date number 349 individuals who were examined biannually in the first 1.5 years with annual follow-ups thereafter. Subsets of participants also donated blood. The Sydney MAS is a longitudinal study of 1,037 individuals aged 70-90 at baseline, who receive comprehensive medical, neuropsychological, and dietary assessments every two years (Sachdev et al., 2010). The HCS comprises 3,253 randomly selected participants aged 55-85, residing in Newcastle, New South Wales, Australia (McEvoy et al., 2010; Sachdev et al., 2010; Sachdev et al., 2013). It has a comprehensive range of physical and biological measures assessing factors important to the aging population, such as health, well-being, social functioning, and economic status. Over 90% of participants gave blood samples, which were stored for future analyses.

The SCS and MAS were approved by the Ethics Committees of the University of New South Wales and the South Eastern Sydney and Illawarra Area Health Service (ethics approval HC12313 and HC14327, respectively). The HCS was approved by the University of Newcastle and Hunter New England Human Research Ethics Committees (HREC 03/12/10/3.26). All work involving human subjects conformed to the principles of the Declaration of Helsinki of the World Medical Association.

EDTA plasma samples from SCS, MAS and HCS were aliquoted with a minimum of freeze-thaw cycles (generally ≤2) and stored at -80°C at all times prior to analysis. Transport of frozen plasma samples was carried out within 24 hours on dry ice and samples were stored at -80°C immediately upon arrival.

2.1.1. APOE genotyping

Standard methods were used for *APOE* genotyping and are described in the supplementary section.

2.2. Blood chemistry

Blood samples from SCS and MAS participants were assayed for lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), creatinine, estimated glomerular filtration rate (eGFR) C-reactive protein (CRP) and glucose at the clinical diagnostics laboratory of the South Eastern Area Laboratory Services, Randwick NSW, using standard procedures (Sachdev et al., 2013). For HCS, samples were assayed by the Hunter Area Pathology Service, using standard procedures described previously (30).

2.3. Quantitation of apolipoproteins in plasma

The concentrations of seven apolipoproteins (ApoA1, ApoA2, ApoB, ApoC3, ApoE, ApoH and ApoJ) in EDTA plasma were determined using multiplex fluorescent immunoassay kits (WideScreenTM Human CVD Panel 1; Novagen, EMD Chemicals Inc, WI). The xMAP platform used was based on the Rules Based Medicine (RBM) fluorescent beads and antibody pairs. Plasma samples (5 µl) were diluted 1:2500 in dilution buffer, and manufacturer's instructions were followed, with the additional precaution of handling the fluorescent beads in a darkened room. Samples were run in singlicate using a Bioplex system (Luminex 100, BioRad, Hercules, CA) Bio-Plex Manager 4.0 and a five parameter logistic regression model. The range of standard concentrations is provided in Supplementary Table 1. The plasma samples from HCS, MAS Wave 2 and SCS cohorts were assayed in random order across 28 kits. Two controls of known protein concentration were provided with the kit and together with an in-house control consisting of a pooled plasma sample were included in all assays in duplicate. The average inter- and intra-assay CVs were 18.4% and 6.3%, respectively. The individual CV values for each protein and control are given in Supplementary Table 2.

2.4. Neuropsychological testing

Methods for obtaining the measures of global cognitive function used here for each of the three cohorts are described in detail in previous publications (McEvoy et al., 2010; Sachdev et al., 2010; Sachdev et al., 2013). Briefly, SCS participants underwent an in-depth neuropsychological assessment that was usually administered in one sitting with breaks but if necessary was broken up into two sittings. Test materials were adapted for sensory deficits, for example enlargement of printed visual materials. The test battery administered has been described in detail previously (Sachdev et al., 2013) and included the Addenbrooke Cognitive Examination – Revised (ACE-R) as a measure of global cognition (Mathuranath et al., 2000). For MAS participants, a composite global cognition score was obtained as a combination of standardised scores

on ten neuropsychological tests measuring the cognitive domains of memory, attention/processing speed, language, visuo-spatial and executive function (Sachdev et al., 2010). In the HCS, the neuropsychological assessment included the Audio Recorded Cognitive Screen (Schofield et al., 2010). All three studies also included the Mini–Mental State Examination (MMSE) as a measure of global cognitive function (Crum et al., 1993).

2.5. Computation of a frailty score in MAS

A frailty score was only calculated in MAS, since missing data prevented the calculation of a frailty score for a significant proportion of SCS participants and the majority of HCS participants were deemed too young to apply the frailty concept in a meaningful way.

The frailty score was derived based on Fried's criteria (Fried et al.). MAS participants were scored positively or negatively for each of the five criteria shown below. The frailty score was then obtained as the total number of items that were scored positively.

1. Unintentional low weight: Individuals with a body mass index (BMI) of ≤18.5.
2. Weakness: the individuals who scored in the lowest 20% for Timed Sit-to-Stand test, adjusted for sex and BMI.
3. Exhaustion: Self-report of exhaustion based on answering in the negative to the question "Do you feel full of energy?" from the Geriatric Depression Scale (Yesavage, 1983).
4. Slowness: the individuals who were in the slowest 20% of the sample for the timed 6-metre walk, adjusted for sex and height.
5. Low physical activity: the self-reported number of hours of activity per week was calculated and individuals in the lowest quintile were scored as positive.

Individuals who scored positively for none, 1-2 or 3-5 of the criteria above were categorised as robust, prefrail or frail, respectively.

2.6. Statistical analyses

All statistical analyses were performed using IBM Statistics SPSS v22 and v23. When parametric tests were applied, distributions of variables were inspected for normality and, if necessary, variables were log or square-root transformed to achieve normality. After transformation any outliers greater than 3 standard

deviations from the mean were winsorised to 3 standard deviations from the mean. Group differences were examined using *t*-tests, Mann Whitney U-tests or in case of categorical variables, χ^2 tests. One-way analysis of variance (ANOVA) was used to examine differences between more than two groups, and analysis of covariance (ANCOVA) was used when comparisons required the inclusion of covariates. Effects with $p < 0.05$ are regarded as statistically significant.

Raw data from the quantitation of all seven apolipoproteins were inspected manually for outliers and trends over the 28 kits used. Linear regression revealed negative or positive associations for some of the apolipoproteins (e.g. ApoB and ApoE) with the time of use of each of the 28 kits. Hence, it was decided to adjust levels for all apolipoproteins for time of kit use. The adjusted values of each apolipoprotein were calculated as the residuals of the regression of apolipoprotein level on time of kit use, plus the mean value of apolipoprotein.

Ordinary least squares regression analysis to assess the association of age with plasma apolipoprotein levels was carried out with age as the independent variable and plasma concentration of apolipoproteins as the dependent variable, including sex, and in the case of ApoE also *APOE* ε4 carrier status, as control variables. As samples from all cohorts were assayed together for apolipoprotein concentrations, cohort was not included as a covariate, particularly as it is highly confounded with age. ApoA1, ApoC3, ApoE and ApoJ data were square-root transformed and any outliers winsorised. To examine non-linear effects regression analysis was carried out as above but with inclusion of the quadratic term for age (age^2). For these analyses age was centred by subtracting its mean value in order to reduce collinearity between the variables age and age^2.

Partial correlations were used to examine the relationships between plasma levels of apolipoproteins and lipid profile. Age and sex were control variables. As above, variables with non-normal distributions were transformed, and outliers winsorised.

The association of apolipoprotein levels with the occurrence of heart attack and stroke was investigated using binary logistic regression, including age, sex, *APOE* ε4 carrier status, HDL-cholesterol, LDL-cholesterol and triglyceride levels as control variables.

To assess the relationship of apolipoprotein levels with frailty, regression analysis was carried out with plasma concentrations of apolipoproteins as the independent variable and the frailty score as the dependent variable including age, sex, *APOE* ε4 carrier status, HDL-cholesterol and LDL-cholesterol as covariates.

To assess the association of apolipoprotein levels with global cognition, separate regression analyses were carried out on each of the three different cohorts (HCS, MAS and SCS) with plasma concentrations of apolipoproteins as the independent variable and standardised neuropsychological measures as the dependent variables. Analyses were carried out separately in the three cohorts rather than in a combined sample, since different measures of global cognition were used in each cohort, as shown in Table 3. Age, sex, *APOE* ε4 carrier status, years of education, HDL-cholesterol and LDL-cholesterol were included as covariates. β values for the three age groups were compared and denoted as significantly different if $(\beta_A - \beta_B)/SE_{Diff} > 1.96$. Potential sex differences in the association of apolipoprotein levels with cognition were investigated within each group by repeating the regression analyses as above but with the inclusion of a product term representing an interaction between apolipoprotein levels and sex. For these analyses, apolipoprotein levels were mean centered to avoid multicollinearity between the interaction term and its component variables.

3. RESULTS

3.1. Study populations and plasma apolipoprotein levels in males and females

Participant demographic and health information for HCS, MAS and SCS cohorts are listed in Table 1. As expected, due to the longer life span of women, the proportion of females was significantly higher in the SCS cohort than in MAS and HCS. The general decline in cognition with age was reflected in significantly lower MMSE scores of centenarians compared to MAS and HCS participants. The proportion of *APOE* ε4 carriers was significantly lower in the centenarian population. Centenarians were leaner than MAS and HCS participants, but the HCS group had the lowest blood glucose levels. The centenarians had worse kidney function (creatinine, glomerular filtration rate) and higher levels of inflammation (C-reactive protein) than both younger groups. MAS and SCS participants had lower total and LDL cholesterol than HCS participants, and slightly higher HDL cholesterol. Interestingly, the rate of previous or current high cholesterol diagnosis and correspondingly, medication with hypolipidaemic drugs, was significantly lower in centenarians than in MAS and HCS participants, by about 50%.

Plasma levels of all apolipoproteins except ApoH were higher in females than in males, although for ApoA2 this did not reach statistical significance ($p = 0.054$) (Supplementary Table 3). Levels of ApoH were not significantly different between males and females.

3.2. *APOE* polymorphism affects plasma apolipoprotein levels

After adjusting for age and sex, apolipoprotein mean levels were generally lower in *APOE* ε4 homozygotes relative to non-ε4 carriers, with ApoE and ApoJ being significantly lower, and ApoB and ApoC3 approaching statistical significance testwise ($p \sim 0.07$) (Table 2). Furthermore, ApoE and ApoJ levels were significantly lower in ε4 homozygotes relative to heterozygotes, with ApoE mean levels being the lowest (Table 2). In *APOE* ε2 carriers, ApoE levels were significantly higher compared to ε2 non-carriers with the highest levels in ε2 homozygotes (Table 2). Although not statistically significant, ApoB and ApoJ showed trends towards lower and higher levels, respectively, in ε2 carriers compared to ε2 non-carriers (Table 2). When ε3 homozygotes were compared to *APOE* ε2 and ε4 carriers (ε2/4 genotypes were excluded) no significant differences were observed across the genotype groups for all proteins except ApoE (Bonferroni corrected $p = 0.05/7 = 0.007$), although ApoB was significant testwise ($p = 0.045$) (Supplementary Table 4).

3.3. Age-related differences in plasma apolipoprotein levels

The association of age with apolipoprotein levels was examined with linear and non-linear regression analyses for all seven apolipoproteins quantified in plasma from 1067 individuals spanning from 56 to 105 years of age (mean age 77.9, standard deviation 11.4). For all apolipoproteins except ApoE, there were modest but statistically significant negative linear effects of age on plasma levels (Figure 2). For ApoE, the linear effect was near zero. Linear regression analyses showed that there were lower levels of plasma apolipoproteins in increasingly older age bands by 1.21, 28.54, 35.24, 0.30 and 6.75 µg/ml per decade for ApoA1, ApoA2, ApoB, ApoC3 and ApoH, respectively.

The non-linear effect of age on apolipoprotein levels was non-significant except for ApoE and ApoJ (Figure 2E & 2G). For these proteins, the introduction of the quadratic term (age^2) produced a slight upwards bend at older age in the fit lines and small increases in R^2 of 3.17% and 4.12%, respectively (Figure 2). Mean apolipoprotein levels per decade are also presented in Supplementary Table 5. Consistent with the regression analysis results, Supplementary Table 5 shows the initial decrease in levels of ApoE and ApoJ with increasing age, followed by higher levels in the oldest age group. Although not statistically significant, a similar trend is also evident for the other apolipoproteins.

3.4. Plasma apolipoprotein levels and the lipid profile

Inter-correlations between apolipoprotein levels were positive and moderate to strong (0.29-0.78, $p < 0.0001$) (Supplementary Table 6). The association of apolipoprotein levels with the lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) reflected the composition of lipoprotein particles. Levels of ApoA1, the major constituent of HDL, were positively associated with levels of HDL-cholesterol and negatively associated with triglyceride levels. ApoB, the major constituent of LDL, was positively associated with total cholesterol and LDL-cholesterol, ApoC3 was positively correlated with triglycerides and to a lesser extent with total cholesterol. ApoE had modest positive associations with total cholesterol, LDL-cholesterol and triglycerides. ApoH and ApoJ were weakly positively associated with triglyceride levels (Supplementary Table 6).

3.5. Plasma apolipoprotein levels and vascular disease indicators

Since ApoA1, ApoB and the lipid profile are established indicators for vascular disease risk, we investigated the potential association of apolipoprotein levels with the occurrence of heart attack (self-reported in 58 out of 1067 participants) and stroke (self-reported in 33 out of 1067 participants). An increase of ApoA2, ApoC3, ApoH and ApoJ levels by 1 standard deviation resulted in a slightly increased risk of heart attack (OR 1.38-1.55, $p < 0.05$) (Supplementary Table 7). A rise in ApoA2 levels by 1 standard deviation was also associated with a slightly increased risk of stroke (OR 1.52, $p < 0.05$) (Supplementary Table 7).

3.6. Plasma apolipoprotein levels and physical frailty

To investigate any potential association of plasma apolipoprotein levels with physical health in old age, a frailty score based on the frailty phenotype conceptualised by Fried *et al.* (Fried et al.) was computed for MAS Wave 2 participants. Data were available for computation of a frailty score for 513 participants resulting in scoring of 182 (35.5%) individuals as robust, 276 as prefrail (53.8%) and 55 as frail (10.7%). The potential association of apolipoprotein levels with frailty was then examined using ordinary least squares regression with age, sex, *APOE* ε4 carrier status, HDL-cholesterol and LDL-cholesterol as covariates. However, no significant associations were found, either with the frailty score or the five individual variables contributing to the overall score (data not shown). Due to missing data, it was not possible to compute a frailty score for a significant proportion of the SCS cohort.

3.7. Plasma apolipoprotein levels and cognition

The association of plasma apolipoprotein levels with global cognition was examined using linear regression with age, sex, years of education, *APOE* ε4 carrier status, LDL-cholesterol and HDL-cholesterol as covariates, separately in the three age groups, 56 to 86, 72 to 92 and 95 to 105 years, corresponding to HCS, MAS and SCS participants, respectively (Table 3). Significant negative associations were observed for two proteins (ApoE and ApoJ) in the youngest age group, whereas in the oldest age group ApoB, ApoC3 and ApoE were positively associated with global cognition scores ($p < 0.05$). In the latter group, HDL-cholesterol levels were also positively associated with cognition in conjunction with ApoB and ApoE, whereas high LDL-cholesterol levels were negatively associated with cognition in conjunction with ApoA2, ApoB, ApoC3 and positively with ApoJ. Omitting LDL-cholesterol and HDL-cholesterol levels from the analysis had no effect in both younger age groups, but did affect outcomes of the regression analysis in 95 to 105 year olds, increasing the strength of association for ApoA1 ($\beta = 0.22$, $p = 0.018$), whilst decreasing it for ApoB ($\beta = 0.11$, $p = 0.25$). Including triglyceride levels as an additional covariate had no effect and did also not yield any significant associations of triglyceride levels with global cognition scores, hence triglyceride levels were omitted from the final analyses. Interestingly, the effect sizes for the association of a number of apolipoprotein levels with cognition varied significantly between the three age groups, consistent with a general trend for the negative effects in the youngest group disappearing in 72-92 year olds before turning into positive effects in the oldest age group (Table 3). Positive regression coefficients for ApoC3, ApoE, ApoH and ApoJ in the oldest cohort differed significantly from near-zero or negative coefficients in either of the two younger cohorts. However, none of the observed associations survived Bonferroni correction.

To explore potential sex differences in the association of apolipoprotein levels with cognition, the regression analyses were repeated with the inclusion of a product term representing an interaction between apolipoprotein level and sex. Significant sex differences were observed for 56 to 86 year olds for ApoB, ApoE and ApoH. Females had significant negative associations of ApoB, ApoE, ApoH and ApoJ with cognition. The remaining three apolipoproteins (ApoA1, ApoA2 and ApoC3) showed similar trends in females approaching significance, whereas associations for males were non-significant (Table 4). There were no significant sex differences or associations with cognition in 72 to 92 year olds (Table 4). For the oldest age group of 95 to 105 year olds, sex differences were not significant and results were overall similar to the regression analysis controlling for sex with positive associations of ApoB, ApoC3, ApoE and ApoH with

cognition, although only for females (Table 4). For males, significant associations were not evident, however, this could potentially be due to the low number of males (n=36) in this age group. After Bonferroni correction, only the association of ApoE levels with cognition in 56-86 year old females remained significant.

In a secondary exploratory analysis, the association of apolipoprotein levels with cognitive domain scores (attention, fluency, language, memory and visuo-spatial) from the ACE-R in 95 to 105 year olds was investigated (Supplementary Table 8). ApoE was significantly ($p < 0.05$) positively associated with four out of the five domains, namely attention, fluency, memory and visuo-spatial function. ApoC3 was positively associated with memory and visuo-spatial domains. ApoB, ApoH and ApoJ were significantly positively associated with visuo-spatial, memory and visuo-spatial domains, respectively.

4. DISCUSSION

Members of the family of apolipoproteins are known to be associated with vascular health and have recently been linked to age-related cognitive decline, AD and longevity (Altmann et al., 2014; Bergman et al., 2007; de Magalhaes et al., 2009; Dominiczak and Caslake, 2011; Lazarus et al.; Loerch et al., 2008; Muffat et al., 2008; Narayan et al., 2012; Paula-Lima et al., 2009; Petropoulou et al., 2001; Sanchez et al., 2006; Shadyab and LaCroix; Song et al., 2012; Thambisetty et al., 2013; Thambisetty et al., 2010; Trougakos et al., 2006; Verghese et al., 2013; Yu and Tan, 2012; Zahn and Kim, 2007). However, it is not well-established how levels of these proteins differ with age, whether the level of protein is related to healthy ageing and cognition in later life and if so, whether associations with cognition and health remain stable, diminish or increase with age. This seems particularly relevant considering that the *APOE* ε2 and ε4 alleles, which are associated with beneficial and adverse effects on longevity respectively, have also been reported to affect plasma levels of apolipoproteins (Gupta et al., 2011; Slooter et al., 1998). Hence, we quantified plasma levels of seven apolipoproteins including ApoE in over 1000 individuals aged from 56 to 105 years to investigate age-related differences and the association of plasma apolipoprotein levels with the *APOE* ε2/3/4 polymorphism, sex, physical health and cognitive health. The results for all seven proteins are summarised in Table 5.

4.1. Apolipoprotein levels in the second 50 years of life

Plasma levels of all seven apolipoproteins modestly decreased with age from mid-life. However, for ApoE and ApoJ, this trend was non-linear with levels increasing again after a certain age. Indeed the oldest old (>95 years) had the highest levels of ApoE, reversing the initial decrease from mid-life and even surpassing mid-life levels. A similar trend was observed for ApoJ. Therefore, higher levels of these two apolipoproteins in exceptionally long-lived individuals seem related to an extended life span. Interestingly, this trend is also reflected in the other apolipoproteins when levels are compared across decades (Supplementary Table 5), although not as pronounced (or statistically significant) as for ApoE and ApoJ. Our previous study on MAS participants at baseline (aged from 70 to 90 years) reported a weak but significant positive correlation of ApoE and ApoJ with age (Song et al.). This could correspond to the upwards trajectory of ApoE and ApoJ levels at older age that was observed here as a result of the non-linear relationship. A previous study on ApoJ levels in individuals aged from 20 to 106 found that levels in 100-106 year olds did not differ significantly from levels in younger age groups (20-50, 60-75, 80-89 and 90-99 year olds). By contrast, ApoJ levels in the 60-75, 80-89 and 90-99 year old groups differed significantly from 20-50 year olds with the younger group having lower levels (Baralla et al., 2015). Other studies in younger age groups (generally 21-60 years) report constant or slightly increasing levels of ApoA1, ApoA2, ApoB, ApoC3, ApoE and ApoH (Albers et al., 1976; Avogaro et al., 1979; de Groot and Meijers, 2011; Dedonder-Decoopman et al., 1980; Noma et al., 1991; Takeuchi et al., 1983) with ApoA1, ApoA2, ApoC3 and ApoE reported to decrease after 60 years of age (Noma et al., 1991; Takeuchi et al., 1983), and our findings are consistent with the latter publications that report data for age groups >60 years.

Our finding of a more positive (or less negative) effect of age on some apolipoprotein levels in the oldest age group could possibly be due, at least in part, to a survivor effect. This would occur if persons in the oldest age range with lower levels of apolipoprotein were less likely to be included in the sample, either because of being deceased or not being included in the sample for some other reason. However, survivorship is an inevitable component of an aging study, and may even be considered a rationale for the study, since the mechanisms which underpin survivorship are under investigation. Importantly, our cohorts are representative of their population and age groups. In particular, the oldest cohort (SCS) represents about 5-10% of the Sydney population in this age range and was a representative sample, selected from the electoral roll and Medicare Australia data, and from seven distinct local government areas (Sachdev et al., 2013).

4.2. *APOE* genotype and plasma apolipoprotein levels

When comparing *APOE* ε4 carriers and non-carriers, the highest levels of ApoE are generally observed in *APOE* ε4 non-carriers (Davignon et al., 1988; Davignon et al., 1999; Gupta et al., 2011; Millar et al., 2001; Slooter et al., 1998; Song et al., 2012). Our observations that centenarians had the highest ApoE levels and the lowest frequency of the ε4 allele are consistent with such previously published work (Davignon et al., 1988; Davignon et al., 1999; Gupta et al., 2011; Millar et al., 2001; Slooter et al., 1998; Song et al., 2012). Assuming high levels of ApoE are protective in regard to life span and cognition, as we could infer from our data, this negative association might be mediated in part by the decrease in ApoE levels in ε4 carriers (besides or in addition to any effects conveyed by the amino acid changes in this isoform). Conversely, ApoE levels in carriers of the ε2 allele, which evidence suggests is protective in regard to life span and cognition, were the highest. The protective effect of ε2 on cognition may therefore be mediated by higher ApoE levels (Conejero-Goldberg et al., 2014; Shinohara et al., 2016). In general, the ε2 allele seems to have the opposite effect on apolipoprotein levels than ε4, since levels of ApoJ were decreased in ε4 homozygotes, but trended upwards in ε2 carriers. Levels of the other apolipoproteins were not significantly affected although there was a non-significant trend to higher ApoB levels in ε4 carriers and lower ApoB levels in ε2 carriers. Previous studies also report increased levels of ApoB and decreased levels of ApoC3 and ApoH in *APOE* ε4 carriers (Demant et al., 1991; Henriques et al., 2014; Song et al., 2012). Why expression levels of other apolipoproteins should be affected by genetic variants or isoforms of ApoE is not entirely clear, however it is possible that assembly or structure of lipoprotein particles is affected, which may in turn alter the half-lives of a variety of their apolipoprotein constituents. These findings have broad mechanistic implications indicating that deficits in cholesterol trafficking extend well beyond the ApoE ε4 protein.

4.3. Plasma apolipoprotein levels, cognition and sex in older age groups

ApoE and ApoJ were negatively associated with cognitive performance in the “youngest” old age group (56-86 years), whereas ApoB, ApoC3 and ApoE were positively associated with cognitive performance in the oldest old. There was no significant association in 72-92 year olds.. Significant sex differences were apparent in 56 to 86 year olds with females having negative associations with ApoB, ApoE, ApoH and ApoJ. In centenarians, statistically significant positive associations between apolipoprotein levels and cognition were evident for females (significant for ApoB, ApoC3, ApoE and ApoH), though not at the Bonferroni corrected

p value of 0.0024. The sex difference in association of apolipoproteins and cognition was particularly remarkable, in that no significant associations with cognition were observed for males, nor even approaching statistical significance. Hence for females, the associations of apolipoprotein levels with cognition are more pronounced than for males and also change dramatically with age: significant negative and positive regression coefficients were observed in the 55-85 year old group *versus* the centenarians, respectively. From these observations we infer that effects of apolipoproteins on cognition are much more pronounced in females than males in the 50+ age range. Interestingly, the association with cognition is differentially manifested with age, being negative in the younger old females (56-86 age group) and positive in centenarian females (95+). Differential associations of cognition and apolipoproteins with age have also been noted by others, albeit not at the protein level but *APOE* ε4 genotype. Jochemsen et al., (2012) found the *APOE* ε4 allele to be associated with increased immediate recall in younger subjects (≤57 years) as compared with decline in immediate recall in older (>57 years) individuals, consistent with the hypothesis of antagonistic pleiotropy. Liu et al. (2013) noted a differential effects of the *APOE* ε4 allele on memory with age, which they also suggested may be due to antagonistic pleiotropy. Since we observe that apolipoprotein levels vary depending on the presence of the *APOE* ε4 allele, then such antagonistic pleiotropy may in part be driven by variation in circulating and/or central apolipoprotein levels. Our own observations show a negative association of apolipoproteins with cognition in the 56-86 age group, consistent with the findings of Jochemsen et al. (2012). Then the positive association of apolipoproteins and cognition we observe in the centenarian group may reflect maintenance of a "younger" phenotype in this super-aging group. Sex- and age-related differences have also been reported in a previous longitudinal study of 816 participants aged 50 and older (mean age 63.8 ± 8.5), which found low ApoB levels beneficial for verbal ability in women, whereas high levels beneficial for perceptual speed in men with the associations for both diminishing by age 65 (Reynolds et al., 2010). Associations of *APOC3* polymorphism with impaired cognition have been reported in subjects with diabetes (Smith et al., 2009). Our previous study on MAS participants at baseline aged 70 to 90 years did not find a significant association of ApoB and ApoC3 levels with global cognitive performance, consistent with what is observed here for this age group, but did detect a negative association of ApoE levels with memory and verbal memory in this 70-90 year age group (Song et al.). The mechanism underlying the association of these apolipoproteins with cognitive performance in the oldest old is beyond the scope of this work. Yet it is tempting to speculate a link to lipid metabolism, since a favourable lipid

profile (i.e. high levels of HDL-cholesterol) is associated with preservation of cognitive function in centenarians presumably mediated *via* vascular health (Atzmon et al., 2002; Barzilai et al., 2006). But considering the multi-faceted roles of many apolipoproteins (e.g. in inflammation, blood coagulation) as well as their exchange between lipoprotein classes (e.g. ApoC3 is a major component of VLDL and chylomicrons but is also a minor component in HDL), the mechanism underlying the positive association of plasma apolipoprotein levels with cognition is bound to be more complex, nuanced, and different for each of the apolipoproteins (Dominiczak and Caslake, 2011). Further complexity is added by the observed age- and sex-related differences in the associations of apolipoprotein levels with cognition, with sex differences apparently more pronounced in midlife and "younger" old age. Interestingly, there might also be sex-related differences in the increased risk for AD conveyed by the *APOE* ε4 allele, which might be a greater risk factor in women than men (Altmann et al., 2014). Future studies should aim to further investigate these sex-related differences.

4.4. Study limitations

Despite all methodological care, our study has potential limitations. Some of these are due to the cross-sectional nature of the study, and would be improved by a longitudinal design. As discussed in the last section some of the results could be influenced by survivorship bias, in particular the observed age-related differences in plasma apolipoprotein levels and in the association between apolipoprotein levels and cognition. However, survivorship may also be considered a rationale for an aging study, since the mechanisms of survivorship are being investigated. Also, samples for this study were obtained from three cohorts, hence cohort effects cannot be excluded. Furthermore, the measures of global cognition differed across the three cohorts, meaning a combined analysis of the association of apolipoprotein levels with cognition was not possible. In addition there are limitations inherent to the study of centenarians, for example, the reliability of interview data as well as incomplete data due to the inability of participants to complete all questionnaires or assessments (Sachdev et al., 2012). In the SCS, these issues were addressed by the use of informants, independent verification of birthdate, assistance with self-reporting questionnaires, home assessments and adaptation of psychometric tests to account for sensory deficits (Sachdev et al., 2013). The latter is also crucial to ensure sensory deficits do not influence scoring and interpretation of neuropsychological test questions, which have generally been developed for younger people.

Pre-analytical variation in the sample can lead to bias on the level of study design and technical analyses. On the design level, our sample might include participants with clinical disorders, as we have not screened for and excluded participants based on health status. However, data on apolipoprotein levels were screened for outliers and the sample number was large, leading us to believe the effect of clinical disorders would be minimal. All methodological care was taken to minimise technical bias, for example samples were assayed together in random order using kits from the same batch to minimise batch-to-batch variation. Nevertheless, it is impossible to eliminate all sources of technical bias, such as varying length of sample storage at -80°C and sample collection/processing by different phlebotomists.

Lastly, despite the relatively large number of samples for analyses relating to cognition, many results did not survive multiple hypothesis testing. For future studies, a larger sample size would be ideal to confirm the observations made here. With regard to age-related differences in plasma levels of apolipoproteins longitudinal study design would be desirable to delineate changes within individuals.

5. CONCLUSION

In summary, levels of all seven apolipoproteins had significant declines with age in the older age range studied here, but with a general turnaround towards higher levels in the oldest old. This may reflect a somewhat "younger" apolipoprotein profile for the centenarian age group which may be a factor contributing to their longevity. The benefit of higher apolipoprotein levels is also evident in the trend to higher plasma levels in carriers of the protective *APOE* ε2 allele and lower levels in *APOE* ε4 carriers. The oldest old in this study not only had the lowest frequency of *APOE* ε4 allele but also the highest ApoE plasma levels compared to individuals aged 56-94. Therefore, absence of the *APOE* ε4 allele and higher plasma levels of ApoE in old age seem conducive to a long life span. Observations for ApoJ were similar, as it also showed nonlinear age-related differences and differential plasma levels in *APOE* ε4 carriers and non-carriers. But an increase in apolipoprotein levels may not be beneficial in all circumstances as an increase in levels of some apolipoproteins was associated with a slightly increased risk of heart attack or stroke. Apart from these effects on vascular health, associations of plasma apolipoprotein levels with physical health were not evident. By contrast, associations with cognitive health were identified for ApoB, ApoC3, ApoE, ApoH and ApoJ and found to be complex with marked sex and age-related differences. From the data presented here, we cannot draw conclusions about the mechanisms giving rise to these associations, but it seems likely to be

more complex than just a favourable lipid profile benefiting vascular health. Therefore, this family of proteins poses a fascinating target for future studies aiming to elucidate the mechanisms that contribute to extended life span and healthy cognition in later life.

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DISCLOSURE STATEMENT

The authors have no conflict of interest to declare.

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TABLES

Table 1. Demographic and health information for participants from the Hunter Community Study (HCS, 56-86 years), Wave 2 of the Sydney Memory and Ageing Study (MAS, 72-92 years) and the Sydney Centenarian Study (SCS, 95-105 years). Parameters were compared between cohorts using statistically appropriate methods and significant results are highlighted in bold.

	HCS (n=345)	**MAS (n=575)**	**SCS (n=147)**	**Test statistic and p-values for effect of cohort***
Mean age, years (SD)	66.3 (8.0)[a]	79.9 (4.6)[a]	97.2 (1.9)[a]	**F = 1594.0 (<0.001)**
Male, n (%)	193 (55.6)[a]	262 (45.6)[a]	44 (29.9)[a]	**χ = 27.3 (<0.001)**
Mean years of education (SD)	11.3 (2.6)[a]	11.9 (3.6)[a]	10.5 (2.7)[a]	**F = 11.5 (<0.001)**
APOE ε2 carrier, n (%)	53 (15.4)	91 (15.8)	23 (15.6)	χ = 0.12 (0.941)
APOE ε3 carrier, n (%)	303 (87.8)	545 (94.8)	143 (97.2)	χ = 0.10 (0.608)
APOE ε4 carrier, n (%)	99 (28.8)[a]	137 (23.8)[b]	19 (12.9)[a,b]	**χ = 18.4 (<0.001)**
Mini mental state exam, mean (SD)	27.6 (1.7)[a]	28.8 (1.6)[a]	22.6 (5.4)[a]	**F = 271.2 (<0.001)**
Kidney function				
Creatinine, μM (SD)	83.7 (17.7)[a]	81.9 (24.3)[b]	102.5 (35.9)[a,b]	**F = 39.3 (<0.001)**
Estimated glomerular filtration rate, mL/min/1.73m^2, mean (SD)	63.4 (11.2)[a]	62.1 (14.6)[b]	50.8 (14.0)[a,b]	**F = 47.5 (<0.001)**
Diabetic indicators				
Glucose (fasting), mM, mean (SD)	5.1 (1.0)[a]	5.9 (1.1)[a]	5.7 (1.8)[a]	**F = 66.0 (<0.001)**
BMI	28.5 (4.9)[a]	27.2 (4.5)[a]	24.0 (4.3)[a]	**F = 36.3(<0.001)**
Cardiovascular health				
Total Cholesterol, mM, mean (SD)	5.1 (1.0)[a,b]	4.6 (1.0)[a]	4.7 (1.1)[b]	**F = 21.9 (<0.001)**
HDL cholesterol, mM, mean (SD)	1.3 (0.4)[a,b]	1.4 (0.4)[a]	1.4 (0.5)[b]	**F = 6.0 (0.003)**
LDL cholesterol, mM, mean (SD)	3.1 (0.9)[a,b]	2.7 (0.9)[a]	2.7 (1.0)[b]	**F = 20.1 (<0.001)**
Triglycerides, mM, mean (SD)	1.4 (0.9)[a]	1.1 (0.5)[a,b]	1.3 (0.6)[b]	**F = 22.6 (<0.001)**
Diagnosed with high cholesterol, n (%)	135 (38.9)[a]	293 (51.0)[a]	33 (22.8)[a]	**χ = 37.8 (<0.001)**
Hypolipidaemic medication, n (%)	102 (29.4)[a]	262 (45.6)[a]	28 (19.0)[a]	**χ = 39.3 (<0.001)**
Inflammation				
C-reactive protein, mg/L, mean (SD)	3.2 (4.4)[a]	2.8 (5.0)[a]	5.4 (8.7)[a]	**F = 38.6 (<0.001)**

*For continuous variables, F statistics were derived from ANOVA. If necessary, variables (MMSE, creatinine, glucose, triglycerides and C-reactive protein) were square root or log transformed to approximate a normal distribution. For categorical variables, p-values were derived from Fisher's Exact test.

[a,b]Values marked with the same superscript are significantly different to each other in ANOVA *post hoc* pairwise comparisons.

Table 2. Mean plasma apolipoprotein levels (µg/mL) in different *APOE* ε4 and ε2 genotype groups. *APOE* ε4/ε2 heterozygotes were omitted form the analysis. Mean levels for the entire cohort are also shown. The standard deviations are given in brackets and values significant testwise ($p < 0.05$) are highlighted in bold.

	All participants (n=1067)	**ε4 non-carrier (n=780)**	**ε4 heterozygote (n=236)**	**ε4 homozygote (n=19)**	**F (*p*)* comparing ε4 groups**	**ε2 non-carrier (n=868)**	**ε2 heterozygote (n=161)**	**ε2 homozygote (n=6)**	**F (*p*)* comparing ε2 groups**
ApoA1	2539.69 (1360.19)	2519.72 (1310.75)	2636.83 (1517.62)	2184.65 (1349.67)	1.23 (.292)	2558.39 (1404.81)	2445.38 (1115.64)	2448.42 (1094.22)	0.37 (0.694)
ApoA2	348.73 (135.98)	342.91 (131.58)	363.90 (147.65)	340.02 (176.99)	0.70 (.499)	349.97 (136.46)	336.44 (136.76)	306.08 (122.57)	1.01 (0.363)
ApoB	488.01 (162.04)	479.71 (160.39)	513.11 (167.79)	444.50 (146.60)	2.62 (.073)	491.24 (161.15)	465.98 (164.34)	381.79 (240.67)	2.86 (0.058)
ApoC3	36.57 (19.55)	36.16 (18.17)	37.66 (22.37)	29.13 (21.11)	2.65 (.071)	36.06 (19.48)	38.10 (18.21)	34.84 (16.95)	0.950 (0.387)
ApoE	31.80 (20.72)	**34.32 (20.90)[a]**	**24.65 (17.50)[a]**	**12.34 (8.62)[a]**	**39.07 (<.001)[#]**	**29.60 (19.21)[a]**	**41.35 (22.30)[a]**	**88.69 (27.34)[a]**	**38.43 (<0.001)[#]**
ApoH	123.11 (48.19)	123.00 (48.22)	123.92 (48.48)	105.78 (48.52)	1.46 (.233)	122.75 (48.43)	122.92 (46.97)	142.20 (67.92)	0.784 (0.457)
ApoJ	70.33 (36.25)	**69.91 (35.55)[a]**	**71.54 (38.09)[b]**	**51.26 (35.54)[a,b]**	**4.11 (.017)**	69.12 (35.57)	73.29 (38.24)	96.69 (58.82)	2.67 (0.070)

*ANCOVA comparing *APOE* groups adjusted for age and sex.

[#]Significant after Bonferroni correction ($p = 0.05/7 = 0.0071$)

[a,b]Values marked with the same superscript are significantly different to each other in *post hoc* pairwise comparisons for ApoE in ε4 and ε2 groups (Bonferroni corrected $p = 0.05/21 = 0.0024$) and ApoJ in the ε4 group ($p < 0.01$).

Table 3. Regression analyses for the effect of plasma apolipoproteins on global cognition, performed separately in each of the 3 age groups.

	Dependent variable = global cognition											
	56-86 year olds				72-92 year olds				95-105 year olds			
	t	β	SE_β	*p*	t	β	SE_β	*p*	t	β	SE_β	*p*
ApoA1	-1.1	-0.07	0.067	0.287	0.7	0.03	0.043	0.482	1.6	0.17	0.109	0.124
LDL-c	-1.0	-0.06	0.062	0.322	0.7	0.03[a]	0.040	0.513	-2.0	-0.18[a]	0.093	0.053
HDL-c	1.3	0.09	0.072	0.195	0.1	0.00	0.036	0.957	1.1	0.12	0.108	0.266
ApoA2	-1.3	-0.08	0.031	0.202	-1.6	-0.06	0.023	0.110	1.5	0.14	0.138	0.130
LDL-c	-1.1	-0.07	0.062	0.288	0.5	0.02[a]	0.040	0.583	**-2.0**	**-0.19[a]**	**0.092**	**0.044**
HDL-c	0.7	0.05	0.067	0.458	0.4	0.02	0.041	0.717	1.9	0.18	0.094	0.056
ApoB	-1.8	-0.12	0.045	0.077	-0.4	-0.02	0.042	0.687	**2.3**	**0.23**	**0.229**	**0.027**
LDL-c	-0.3	-0.02[a]	0.067	0.740	0.6	0.03[b]	0.043	0.562	**-2.8**	**-0.29[a,b]**	**0.103**	**0.006**
HDL-c	0.6	0.04	0.067	0.561	0.3	0.01	0.041	0.768	**2.0**	**0.19**	**0.092**	**0.047**
ApoC3	-1.0	-0.06[a]	0.062	0.314	-1.2	-0.05[b]	0.047	0.224	**2.8**	**0.26[a,b]**	**0.091**	**0.007**
LDL-c	-1.0	-0.06	0.062	0.337	0.4	0.01[a]	0.041	0.727	**-2.0**	**-0.18[a]**	**0.090**	**0.045**
HDL-c	0.8	0.06	0.068	0.413	0.5	0.02	0.041	0.587	1.7	0.15	0.092	0.097
ApoE	**-2.6**	**-0.17[a]**	**0.071**	**0.010**	-1.5	-0.06[b]	0.045	0.145	**2.7**	**0.25[a,b]**	**0.090**	**0.007**
LDL-c	-0.4	-0.02	0.064	0.715	0.8	0.03[a]	0.040	0.438	-2.0	-0.18[a]	0.092	0.052
HDL-c	0.2	0.01	0.070	0.876	0.3	0.01	0.038	0.755	**2.1**	**0.19**	**0.091**	**0.043**
ApoH	-1.4	-0.09[a]	0.044	0.151	-1.0	-0.04[b]	0.039	0.306	1.9	0.17[a,b]	0.087	0.063
LDL-c	-1.0	-0.06	0.061	0.301	0.4	0.02	0.039	0.664	-1.7	-0.16	0.093	0.089
HDL-c	0.5	0.03	0.069	0.621	0.3	0.01	0.042	0.792	1.9	0.18	0.093	0.057
ApoJ	**-2.5**	**-0.15[a]**	**0.077**	**0.012**	-0.3	-0.01	0.029	0.744	1.7	0.16[a]	0.095	0.086
LDL-c	-1.0	-0.06[a]	0.061	0.323	0.4	0.02	0.039	0.678	**2.0**	**0.19[a]**	**-0.096**	**0.047**
HDL-c	0.6	0.04[a]	0.065	0.564	0.3	0.01	0.039	0.775	-2.0	-0.18[a]	-0.091	0.054

Global cognition was measured by the Audio Recorded Cognitive Screen in 56 to 86 year olds from the Hunter Community Study (n=345), by composite score formed from a battery of ten neuropsychological tests in 72 to 92 year olds from the Sydney Memory and Ageing Study (n=568) and by the Adenbrooke Cognitive Exam - Revised in 95 to 105 year olds from the Sydney Centenarian Study (n=116).

Results of 7 separate multiple linear regression analyses are shown, one for each of the 7 apolipoproteins. Age, sex, years of education, *APOE* ε4 carrier status, LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c) levels were included in the models as covariates. Results for covariates LDL-c and HDL-c are shown, but those for the other covariates are not displayed.

[a,b]Values marked with the same superscript are significantly different to each other based on $(\beta_A-\beta_B)/SE_{Diff} > 1.96$. Values significant testwise are highlighted in bold ($p < 0.05$), and did not survive Bonferroni correction ($p = 0.05/21 = 0.0024$).

The inclusion of hypolipidaemic medication as covariate, or a quadratic term for age as covariate for ApoE and ApoJ, yielded virtually identical results.

Table 4. Association of plasma apolipoprotein levels with global cognition for males and females from the Hunter Community Study (56-86 year olds, males n=191, females n=154, Audio Recorded Cognitive Screen), the Sydney Memory and Ageing Study (72-92 year olds, males n=262, females n=313, composite score formed from a battery of ten neuropsychological tests) and the Sydney Centenarian Study (95-105 year olds, males n=36, females n=106, Addenbrooke Cognitive Examination – Revised). β values for males and females are shown for each of the 14 separate multiple linear regression analyses that included an interaction term for apolipoprotein levels and sex. Age, years of education, *APOE* ε4 carrier status, LDL-cholesterol and HDL-cholesterol were included as covariates. Values significant testwise are highlighted in bold ($p < 0.05$).

	Dependent variable = global cognition					
	56-86 year olds		**72-92 year olds**		**95-105 year olds**	
	β	**t (p)**	**β**	**t (p)**	**β**	**t (p)**
ApoA1 (male)	0.06	0.60 (0.549)	0.04	0.56 (0.576)	0.15	0.54 (0.590)
ApoA1 (female)	-0.17	-1.97 (0.050)	0.03	0.48 (0.631)	0.17	1.52 (0.132)
ApoA1 × sex	-0.17	-1.791 (0.075)	-0.01	-0.11 (0.916)	0.02	0.08 (0.938)
ApoA2 (male)	0.01	0.13 (0.900)	-0.03	-0.55 (0.585)	0.00	-0.01 (0.992)
ApoA2 (female)	-0.17	-1.92 (0.055)	-0.08	-1.63 (0.103)	0.20	1.85 (0.067)
ApoA2 × sex	-0.13	-1.44 (0.152)	-0.04	-0.64 (0.526)	0.17	1.05 (0.297)
ApoB (male)	0.02	0.18 (0.859)	-0.02	-0.27 (0.791)	0.14	0.74 (0.463)
ApoB (female)	**-0.26**	**-2.87 (0.004)**	-0.02	-0.33 (0.744)	**0.26**	**2.28 (0.025)**
ApoB × sex	**-0.19**	**-2.27 (0.024)**	0.00	-0.02 (0.984)	0.10	0.58 (0.561)
ApoC3 (male)	0.04	0.43 (0.670)	-0.03	-0.52 (0.606)	0.07	0.31 (0.756)
ApoC3 (female)	-0.16	-1.85 (0.065)	-0.06	-1.19 (0.234)	**0.29**	**2.86 (0.005)**
ApoC3 × sex	-0.14	-1.61 (0.109)	-0.03	-0.45 (0.650)	0.19	0.83 (0.408)
ApoE (male)	-0.01	-0.06 (0.955)	-0.03	-0.48 (0.635)	0.28	1.13 (0.260)
ApoE (female)	**-0.33**	**-3.71 (<0.001[#])**	-0.09	-1.59 (0.113)	**0.24**	**2.49 (0.015)**
ApoE × sex	**-0.23**	**-2.63 (0.009)**	-0.04	-0.73 (0.466)	-0.04	-0.15 (0.879)
ApoH (male)	0.03	0.33 (0.745)	-0.02	-0.26 (0.795)	-0.12	-0.52 (0.607)
ApoH (female)	**-0.23**	**-2.53 (0.012)**	-0.06	-1.14 (0.254)	**0.23**	**2.31 (0.023)**
ApoH × sex	**-0.17**	**-2.10 (0.037)**	-0.03	-0.57 (0.571)	0.32	1.41 (0.162)
ApoJ (male)	-0.06	-0.67 (0.506)	-0.06	-1.00 (0.317)	0.14	0.62 (0.534)
ApoJ (female)	**-0.27**	**-3.00 (0.003)**	0.02	0.41 (0.685)	0.17	1.62 (0.108)
ApoJ × sex	-0.15	-1.75 (0.082)	0.06	1.03 (0.304)	0.03	0.12 (0.904)

[#]Significant after Bonferroni correction ($p = 0.05/21 = 0.0024$)
Sex was coded as 0 for male and 1 for female.
β values for Apo (male) and Apo (female) represent estimated effects of apolipoprotein levels on global cognition within male and female subgroups.

Table 5. Summary of results. +/- and ++/-- indicate an increase or decrease in level or a positive or negative association of testwise significance or significance after Bonferroni correction, respectively.

	Higher levels in females[a]	*APOE* ε4 carriers *vs* non-carriers[b]	*APOE* ε2 carriers *vs* non-carriers[b]	Levels differ with age?[c]	Increased risk of vascular disease?[d]	Associated with frailty?	Associated with cognition?[e]
ApoA1	++			--			
ApoA2				--	+		
ApoB	+			--			+
ApoC3	++			--	+		+
ApoE	++	--	++	-/+			-/+
ApoH				--	+		
ApoJ	++			--/++	++		-

[a]Supplementary Table 3; [b]Table 2; [c]Figure 1; [d]Supplementary Table 7; [e]Table 3

FIGURE LEGENDS

Figure 1. Some plasma lipoprotein particles and their apolipoprotein constituents. VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein.

Figure 2. Association of age with plasma apolipoprotein levels. Participants spanned 56 to 105 years of age. Scatter plots and linear (panels A-D, F) or quadratic (panels E and G) fit line of participants' age *versus* plasma apolipoprotein levels. Untransformed, unadjusted data winsorised to 3 standard deviations are shown. Panel H, ordinary least squares regression analyses for age and plasma apolipoprotein levels adjusted for sex and in the case of ApoE also for *APOE* ε4 carrier status. Data was square-root transformed for ApoA1, ApoC3, ApoE and ApoJ. Significant values are highlighted in bold (Bonferroni corrected $p = 0.05/7 = 0.0071$).

SUPPLEMENTARY MATERIAL

Plasma apolipoproteins and physical and cognitive health in very old individuals

Julia Muenchhoff[1], Fei Song[1], Anne Poljak[1,2,3], John D. Crawford[1], Karen A. Mather[1], Nicole A. Kochan[1,4], Zixuan Yang[1], Julian N. Trollor[1,5], Simone Reppermund[1,5], Kate Maston[1], Adam Theobald[1], Susanne Kirchner-Adelhardt[6], John B. Kwok[3,7]; Robyn L. Richmond[8], Mark McEvoy[9], John Attia[9,10], Peter W. Schofield[9,11], Henry Brodaty[1, 12], Perminder S. Sachdev[1,4,*]

[1]Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Sydney, Australia; [2]Bioanalytical Mass Spectrometry Facility, University of New South Wales, Sydney, Australia; [3]School of Medical Sciences, University of New South Wales, Sydney, Australia; [4]Neuropsychiatric Institute, Prince of Wales Hospital, Sydney, Australia; [5]Department of Developmental Disability Neuropsychiatry (3DN), School of Psychiatry, University of New South Wales, Sydney, Australia; [6]Department of Psychology, University of Washington, Seattle, USA; [7]Neuroscience Research Australia, Randwick, Australia; [8]School of Public Health and Community Medicine, University of New South Wales, Sydney, Australia; [9]School of Medicine and Public Health, University of Newcastle, Newcastle, Australia; [10]Hunter Medical Research Institute, Newcastle, Australia; [11]School of Psychology, University of Newcastle, Newcastle, Australia; [12]Dementia Collaborative Research Centre, School of Psychiatry, University of New South Wales, Sydney, Australia.

APOE GENOTYPING

Standard methods were used to extract genomic DNA from peripheral blood leukocytes or saliva samples. Two *APOE* single nucleotide polymorphisms (SNPs rs7412, rs429358) were genotyped to determine the *APOE* haplotype of interest, which has three alleles (ε2, ε3, ε4) using Taqman genotyping assays (Applied Biosystems Inc., Foster City, CA). The SNP rs7412 distinguishes the ε2 allele from the ε3/ε4 alleles and the SNP rs429358 distinguishes the ε4 allele from the ε2/ ε3 alleles (Jorm et al., 2007). Further details of the method are described in (Poljak et al., 2016). An *APOE* ε4 carrier is defined as an individual with one or more *APOE* ε4 alleles.

References:

Jorm, A.F., Mather, K.A., Butterworth, P. et al. APOE genotype and cognitive functioning in a large age-stratified population sample. Neuropsychology 2007;21:1-8.

Poljak, A., Crawford, J.D., Smythe, G.A. et al. The Relationship Between Plasma Abeta Levels, Cognitive Function and Brain Volumetrics: Sydney Memory and Ageing Study. Curr Alzheimer Res 2016;13:243-55.

Supplementary Table 1. Range of standards (ng/ml) used in multiplex assay for quantification of apolipoproteins.

	ApoA1	ApoA2	ApoB	ApoC3	ApoE	ApoH	ApoJ
Concentration	3.1-6800	1.6-3600	8.2-18000	0.1-220	0.3-730	0.3-850	0.2-4700

Supplementary Table 2. Average intra- and inter-assay CVs of controls included across all 28 multiplex assay kits. Controls 1 and 2 were protein samples of known concentrations provided by the manufacturer with each kit, whereas control 3 consisted of a pooled plasma sample that was frozen in single-use aliquots at the beginning of this study.

	ApoA1	ApoA2	ApoB	ApoC3	ApoE	ApoH	ApoJ
Intra-assay CV							
Control 1	6.3	5.8	4.7	7.2	5.9	7.4	6.2
Control 2	5.0	6.9	3.1	6.1	4.0	6.3	3.9
Control 3	6.2	8.1	13.6	5.6	4.0	7.5	8.7
Inter-assay CV							
Control 1	20.6	14.4	8.8	21.8	16.0	16.5	7.5
Control 2	13.0	15.9	8.8	10.8	11.5	13.5	11.1
Control 3	37.2	13.0	32.5	13.4	67.2	10.0	22.3

Supplementary Table 3. Mean plasma apolipoprotein levels (µg/mL) in males and females from the Hunter Community Study (HCS), Wave 2 of the Sydney Memory and Ageing Study (MAS) and the Sydney Centenarian Study (SCS). The standard deviations are given in brackets and significant values are highlighted in bold.

Protein	Males (n=497)	Females (n=570)	F (*p*)*
ApoA1	2361.21 (1261.23)	2698.23 (1424.90)	**23.70 (<0.001)**
ApoA2	344.54 (135.17)	352.39 (136.69)	3.72 (0.054)
ApoB	482.04 (160.33)	493.23 (163.49)	**4.51 (0.034)**
ApoC3	34.43 (19.39)	38.45 (19.52)	**24.93 (<0.001)**
ApoE	29.60 (19.15)	33.69 (21.83)	**8.60 (0.003)**
ApoH	123.84 (48.39)	122.48 (48.06)	0.02 (0.892)
ApoJ	67.43 (35.28)	72.86 (36.92)	**11.38 (0.001)**

*ANCOVA adjusted for age, and in case of ApoE also *APOE* ε4 carrier status.

Supplementary Table 4. Mean plasma apolipoprotein levels (µg/mL) in *APOE* ε3 homozygotes, ε4 carriers and ε2 carriers from the Hunter Community Study, Wave 2 of the Sydney Memory and Ageing Study and the Sydney Centenarian Study. *APOE* ε2/ε4 heterozygotes were excluded from the analysis. The standard deviations are given in brackets and significant values are highlighted in bold (Bonferroni corrected $p = 0.05/7 = 0.007$).

Protein	*APOE* ε3 homozygotes (n=632)	*APOE* ε4 carrier (n=236)	*APOE* ε2 carrier (n=148)	F (*p*)*
ApoA1	2526.40 (1352.15)	2642.60 (1534.73)	2491.12 (1120.13)	0.05 (0.950)
ApoA2	344.90 (131.29)	363.68 (148.99)	334.40 (132.94)	1.32 (0.269)
ApoB	483.92 (158.24)	510.88 (167.46)	461.79 (168.64)	3.10 (0.045)
ApoC3	35.74 (18.15)	36.92 (22.70)	37.97 (18.20)	1.22 (0.295)
ApoE	**32.00 (19.42)[a]**	**23.17 (17.08)[a]**	**44.39 (23.97)[a]**	**59.59 (<0.001)**
ApoH	122.79 (48.62)	122.65 (48.03)	123.90 (46.65)	0.23 (0.796)
ApoJ	69.06 (34.88)	69.28 (37.46)	73.49 (38.20)	1.35 (0.260)

*ANCOVA controlled for age and sex.
[a]Values marked with the same superscript are significantly different to each other in ANCOVA *post hoc* pairwise comparisons. (Bonferroni corrected $p = 0.05/3 = 0.017$)

Supplementary Table 5. Mean plasma apolipoprotein levels (µg/mL) over five decades in 1067 individuals from the Hunter Community Study (HCS), Wave 2 of the Sydney Memory and Ageing Study (MAS) and the Sydney Centenarian Study (SCS). The standard deviations are given in brackets and values with Bonferroni corrected $p < 0.007$ are regarded as significant.

Protein	56-65 years (n=178)	66-75 years (n=230)	76-85 years (n=424)	86-95 years (n=105)	96-105 years (n=129)	F (*p*)*
ApoA1	2675.52 (1385.67)	2830.54[3] (1622.25)	2391.91[2] (1168.38)	2377.37 (1281.59)	2450.58 (1389.25)	4.92 (<0.001)
ApoA2	414.13[3,4,5] (142.03)	378.21[3,4] (145.00)	316.47[1,2] (120.65)	323.68[1,2] (126.54)	331.92[1] (124.86)	22.20 (<0.001)
ApoB	562.70[3,4,5] (172.11)	522.48[3,4,5] (166.75)	451.93[1,2] (145.16)	458.99[1,2] (152.70)	464.16[1,2] (154.17)	20.59 (<0.001)
ApoC3	46.68[2,3,4,5] (24.95)	39.70[1,3,4] (21.37)	32.13[1,2] (14.72)	32.84[1,2] (18.35)	34.85[1] (16.87)	21.89 (<0.001)
ApoE	34.25[3,5] (22.85)	30.79[5] (20.37)	26.65[1,5] (16.73)	31.25[5] (18.86)	47.56[1,2,3,4] (23.43)	26.04 (<0.001)
ApoH	139.35[3,4] (51.44)	130.94[3] (52.41)	113.48[1,2] (42.44)	118.04[1] (49.02)	122.20 (45.20)	11.48 (<0.001)
ApoJ	86.56[3,4] (36.44)	76.56[3,5] (39.83)	61.41[1,2,5] (33.23)	60.10[1,2] (30.24)	73.8[3,4] (32.39)	23.00 (<0.001)

*ANCOVA adjusted for sex and in case of ApoE also *APOE* ε4 carrier status.
Superscripts indicate significant difference to other groups in *post hoc* pairwise comparisons following ANCOVA (Bonferroni corrected $p = 0.05/70 = 0.0007$): [1] different to 56-65 years olds; [2] different to 66-75 years olds; [3] different to 76-85 years olds; [4] different to 86-95 years olds; [5] different to 96-105 years olds.

Supplementary Table 6. Partial correlations amongst apolipoproteins, and between lipid profiles and apolipoproteins, with sex and age as covariates.

	ApoA1	ApoA2	ApoB	ApoC3	ApoE	ApoH	ApoJ
ApoA1	-	0.66 **(<0.0001)**	0.45 **(<0.0001)**	0.54 **(<0.0001)**	0.29 **(<0.0001)**	0.57 **(<0.0001)**	0.56 **(<0.0001)**
ApoA2	0.66 **(<0.0001)**	-	0.53 **(<0.0001)**	0.78 **(<0.0001)**	0.47 **(<0.0001)**	0.78 **(<0.0001)**	0.71 **(<0.0001)**
ApoB	0.45 **(<0.0001)**	0.53 **(<0.0001)**	-	0.52 **(<0.0001)**	0.46 **(<0.0001)**	0.53 **(<0.0001)**	0.60 **(<0.0001)**
ApoC3	0.54 **(<0.0001)**	0.78 **(<0.0001)**	0.52 **(<0.0001)**	-	0.58 **(<0.0001)**	0.74 **(<0.0001)**	0.68 **(<0.0001)**
ApoE	0.29 **(<0.0001)**	0.47 **(<0.0001)**	0.46 **(<0.0001)**	0.58 **(<0.0001)**	-	0.55 **(<0.0001)**	0.56 **(<0.0001)**
ApoH	0.57 **(<0.0001)**	0.78 **(<0.0001)**	0.53 **(<0.0001)**	0.74 **(<0.0001)**	0.55 **(<0.0001)**	-	0.70 **(<0.0001)**
ApoJ	0.56 **(<0.0001)**	0.71 **(<0.0001)**	0.60 **(<0.0001)**	0.68 **(<0.0001)**	0.56 **(<0.0001)**	0.70 **(<0.0001)**	-
Total cholesterol	0.11 **(0.0005)**	0.14 **(<0.0001)**	0.39 **(<0.0001)**	0.19 **(<0.0001)**	0.26 **(<0.0001)**	0.07 (0.032)	0.09 (0.003)
LDL-cholesterol	0.03 (0.283)	0.10 (0.002)	0.42 **(<0.0001)**	0.08 (0.014)	0.20 **(<0.0001)**	0.04 (0.183)	0.08 (0.019)
HDL-cholesterol	0.37 **(<0.0001)**	0.02 (0.462)	-0.01 (0.682)	-0.01 (0.706)	-0.07 (0.032)	-0.06 (0.071)	-0.05 (0.086)
Triglycerides	-0.18 **(<0.0001)**	0.17 **(<0.0001)**	0.07 (0.022)	0.42 **(<0.0001)**	0.35 **(<0.0001)**	0.18 **(<0.0001)**	0.15 **(<0.0001)**

The inclusion of hypolipidaemic medication as covariate, or age^2 as covariate for ApoE and ApoJ, yielded virtually identical results. *P*-values are given in brackets with significant values highlighted in bold (Bonferroni corrected $p = 0.05/77 = 0.0007$).

Supplementary Table 7. Binary logistic regression of apolipoprotein levels and indicators of cerebrovascular or coronary heart disease with age, sex, *APOE* ε4 carrier status and plasma levels of HDL-cholesterol, LDL-cholesterol and triglycerides as covariates in individuals from the Hunter Community Study (HCS), Wave 2 of the Sydney Memory and Ageing Study (MAS) and the Sydney Centenarian Study (SCS). Values significant testwise are highlighted in italics ($p < 0.05$), values significant after Bonferroni correction are in bold ($p = 0.05/14 = 0.004$).

Protein	Heart attack (n=58)		Stroke (n=33)	
	OR	Wald (p)	OR	Wald (p)
ApoA1	1.14	0.62 (0.432)	1.19	0.73 (0.391)
ApoA2	**1.53**	**8.52 (0.004)**	*1.52*	*5.36 (0.021)*
ApoB	1.08	0.17 (0.678)	0.92	0.13 (0.715)
ApoC3	*1.45*	*5.01 (0.025)*	0.91	0.13 (0.717)
ApoE	1.25	2.09 (0.149)	0.89	0.31 (0.580)
ApoH	*1.38*	*5.12 (0.024)*	1.14	0.46 (0.497)
ApoJ	**1.55**	**9.55 (0.002)**	1.31	2.09 (0.149)

Supplementary Table 8. Association of plasma apolipoprotein levels of participants from the Sydney Centenarian Study with cognitive domain scores from the Addenbrooke Cognitive Examination – Revised adjusted for age, sex, *APOE* ε4 carrier status, years of education, LDL-cholesterol and HDL-cholesterol. Values that are significant testwise ($p < 0.05$) are highlighted in bold.

Protein	Attention (n~118)		Fluency (n~111)		Language (n~108)		Memory (n~104)		Visuo-spatial (n~96)	
	β	t (*p*)	β	t (*p*)	β	t (*p*)	β	t (*p*)	β	t (*p*)
ApoA1	0.023	0.22 (0.823)	0.142	1.34 (0.184)	0.090	0.82 (0.414)	0.191	1.68 (0.097)	0.146	1.34 (0.184)
ApoA2	0.009	0.10 (0.917)	0.075	0.84 (0.405)	0.088	0.97 (0.336)	0.155	1.61 (0.110)	0.131	1.43 (0.155)
ApoB	0.110	1.12 (0.267)	0.083	0.82 (0.415)	0.082	0.80 (0.425)	0.215	1.96 (0.052)	**0.271**	**2.67 (0.009)**
ApoC3	0.169	1.88 (0.062)	0.150	1.62 (0.107)	0.176	1.87 (0.064)	**0.233**	**2.36 (0.020)**	**0.293**	**3.22 (0.002)**
ApoE	**0.179**	**2.02 (0.046)**	**0.189**	**2.09 (0.039)**	0.088	0.94 (0.348)	**0.202**	**2.080 (0.040)**	**0.198**	**2.15 (0.035)**
ApoH	0.086	0.98 (0.329)	0.039	0.43 (0.669)	0.122	1.32 (0.189)	**0.215**	**2.26 (0.026)**	0.123	1.33 (0.187)
ApoJ	0.075	0.85 (0.395)	0.080	0.87 (0.386)	0.050	0.54 (0.590)	0.137	1.40 (0.165)	**0.185**	**2.02 (0.046)**

The use of a quadratic rather than linear term for age as covariate for ApoE and ApoJ yielded virtually identical results.