Neuropsychological performance is positively associated with plasma albumin in healthy adults

Short title: Neuropsychology and plasma albumin

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ABSTRACT

**Background:** Albumin serves a range of physiological functions that are vital to overall brain and cognitive health. Indeed, associations between cognitive performance and albumin have been demonstrated in individuals with chronic liver, or kidney disease and in patients with high urinary excretion of albumin. However, an association of plasma albumin with cognitive performance has not been reported in otherwise healthy participants with clinically acceptable plasma albumin concentration. **Method:** This study utilized a wide-ranging neuropsychological test battery to investigate the relationship between cognitive performance and plasma albumin homeostasis in 222 healthy participants (143 female) between the ages of 43 and 84 years (mean = 65 years). **Results:** Albumin both with and without the covariates of age, sex and premorbid ability was positively associated with enhanced performance on a range of neuropsychological domains including perceptual speed measure, Stroop and verbal ability. Albumin manifested generally positive but less robust associations with secondary and primary memory. **Conclusion:** The results indicate that there is a positive association between albumin and cognitive performance in physiologically healthy participants free of chronic renal or liver disease.

**KEYWORDS:** Plasma albumin, neuropsychological performance, neurovascular inflammation, Bayesian analysis
Introduction

Albumin is a major component in blood accounting for 50-60% of total protein [1]. Albumin’s abundance, relatively small size and negative charge makes it the pivotal protein for maintaining oncotic pressure homeostasis and micro-vascular permeability [1-3]. Albumin serves as a chaperone-transporter for a range of exogenous and endogenous substances, particularly those with hydrophobic properties [4]. The chaperone function of albumin provides reservoir functionality for important active biological compounds and facilitates its role as a scavenger for potentially toxic compounds [4,5]. Through mechanisms that may involve heightened inflammation and aberrant capillary vascular permeability [6], low levels of plasma albumin have been associated with frailty [7,8] depression and apathy [9], increased morbidity [10], mortality [11] and poorer recovery post-surgery [12].

An association of plasma albumin with cognitive capacity is a reasonable proposition given its critical physiological function. On clinical diagnostic status and brief measures of global neuropsychological performance such as the Mini-Mental State Exam (MMSE), a number of studies have reported a positive association with plasma albumin (or a negative association with albuminuria) [6,13-21]. Clinical findings in patients with cirrhotic or alcoholic liver disease [22-29] and in patients with renal failure [30-32] suggest a positive association between plasma albumin and performance on the Digit Symbol Coding test, reflecting potential enhancements of albumin on perceptual speed. In other cognitive domains, the association between albumin and cognitive function has been less robust, but also generally positive. Performance on secondary memory [15,19,22,25,30], Trails A & B [15,18,22-24,27,30,33], the Rey Copy Figure Test [15], animal and category fluency [18,19,23,26], primary memory [19,23,28,30] and the Stroop test [26] support positive associations between plasma albumin and cognitive functioning. However, further studies are needed, as several papers have failed to find evidence for a statistically significant association between plasma/urinary albumin and cognitive functioning [27,29,34-38]. Moreover, a limitation concerning the interpretation of extant findings is that the majority of studies reported to date may
have been confounded by comorbidities, including kidney or liver diseases (e.g. Fontana et al., 2005; Griva et al., 2004; Radic et al., 2011). Other relevant confounders include the age and sex of the participant, both of which are known to influence cognition and normal physiology. Therefore, it is important to take into account the influence of these factors on any relationship found between albumin and neuropsychological performance.

As with other complex biological phenomena, cognition is a multifactorial entity that reflects the composite of many different elements, including the domains of perceptual speed, primary memory, secondary memory, verbal ability, linguistic abilities and executive functioning. In measuring cognitive capacity in healthy subjects with plasma albumin in the normal reference range, we used in this study, a battery of tests that specifically evaluated each of the aforementioned domains. Age and sex were used as covariates to avoid potential confounding effects on cognitive performance. The fractionation of different elements of cognition assessed against important biomarkers such as plasma albumin provides a powerful approach to modeling the putative relationship between cognitive outcomes and regulatory biological factors.

Methods

Participants

A total of 250 participants (96 males, 154 females) over the age of 40 (range = 43 years to 84 years) were recruited for this study. The study was approved by the Curtin University Human Research Ethics Committee (approval number HR97/2011). Potential participants completed a medical history and medications questionnaire and were interviewed to confirm the information provided. Participants were excluded from the study if any of the following applied: major surgery or a clinical event ‘in the last six months’; current diagnosis with a psychiatric disorder or taking psychotropic medications; haemophilia; cancer/chemotherapy; head injury within 5 years; diagnosis with HIV. In addition, data from participants suggesting renal impairment (estimated glomerular filtration rate <55 ml/min/1.73m²), or liver dysfunction (abnormal alanine aminotransaminase
(ALT), bilirubin, alkaline phosphatase (ALP) or gamma glutamyl transferase (GGT)) were not included in the statistical analyses.

**Serum Albumin**

Participants arrived for provision of fasting blood samples between 8-10am in collection rooms adjacent to an accredited diagnostic centre following an overnight fast (minimum 8 hours). Participants were asked to avoid prolonged strenuous exercise and to limit alcohol intake to 2 standard drinks over the 24 hour period prior to blood sampling. A venous sample was drawn from each subject (after being seated for 10 minutes) into lithium heparin coated Vacutainer™ tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 3800 rpm for 7 minutes at room temperature for analysis. Blood albumin was measured on the Abbott Diagnostics c16000 analyser (Abbott Diagnostics, Abbott Laboratories, Abbott Park, Illinois, USA) using the Bromcresol Green (BCG) dye binding method (CV 0.48 to 0.55%).

**Neuropsychological Measures**

Trained staff under the supervision of a registered clinical neuropsychologist administered the cognitive battery. Tests were selected based upon their widespread use within the field, their reliability and validity, and to cover the main domains of cognitive performance affected in age-related cognitive decline. The battery of cognitive tests included the Mini Mental State Examination (MMSE) [39], Rey Auditory Verbal Learning Test (RAVLT) [40], Delis-Kaplan Executive Function System (D-KEFS) verbal fluency subtests [41], 60-item Boston Naming Test (BNT) [42], National Adult Reading Test (NART) [43], Digit Span and Digit-Symbol Coding subtests of the Wechsler Adult Intelligence Scale-Third edition (WAIS-III) [44,45] and the Stroop test (Victoria version) [44].

**Statistical Analysis**

We used a nested domain Bayesian mixed-model approach, similar to that described by Thurston et
al. (2009), to estimate the relationship between plasma albumin and cognitive performance [46]. The method accounts for multiple correlated outcomes that are nested within cognitive domains, e.g. the domain Secondary Memory in this study is measured by the RAVLT, which is composed of several correlated outcome measurements, including short delay and long delay recall which are moderately correlated. The hierarchical nature of the nested domain model significantly increases power by partially pooling outcome estimates from within a cognitive domain towards each other, i.e., the coefficients for each individual outcome level coefficient take into account the information that is present within each domain and the overall influence of the variable. The property of hierarchical models also aids in reducing Type S (sign) and Type M (magnitude) errors through shrinkage towards common estimates [47], e.g., by partially shrinking the coefficient obtained for RAVLT short delay recall towards a common overall estimate for episodic memory. We included the covariates of age, sex and NART within a series of models to account for possible shared variance of these variables on cognitive performance with albumin. Most importantly, age and sex are two factors that are known to influence both cognitive performance and a range of physiological attributes that may offer an alternative explanation for the relationship between serum albumin concentration and cognitive performance.

The domains (and associated outcome measures) assessed in the present study were D1 - verbal ability (BNT and D-KEFS fluency [letter fluency, category fluency, and switching fluency]); D2 - Stroop (Dots, Words, Colours, and interference [Colours/Dots] response time); D3 - secondary memory (total items recalled across learning trial, interference list, short-delay free recall, long-delay free recall, recognition 'hits', short delay decay [learning trial 5 – short delay], and long delay decay [learning trial 5 – long delay]); D4 - primary memory (digits forward, digits backwards, and sum of digits recalled both backwards and forwards); D5 - perceptual speed (digit-symbol coding). The selection of outcome measurements to be nested within each domain was chosen a priori based upon the instrument used and the cognitive domain that the measure tested.

An objective Bayesian approach to setting the priors was used, i.e. all priors could be described as
being weakly informative, or uninformative for the scale of the data. Priors for the overarching coefficients were described by a normal distribution with a mean = 0 and SD = 100 (relatively flat for standardised data). The remaining coefficients for the outcomes and domains were described as being derived from a normal distribution centred on 0 and a SD estimated from a half-Cauchy distribution centred on 0, and scale set to 25 (again, diffuse for the scale) [48]. Outcome level errors were modelled as being derived from a $t$-distribution to render the analysis robust [49,50]. The prior for the SD for each outcome was described by a uniform distribution between 0 and 100 and the degrees of freedom parameter was estimated from the inverse of a uniform distribution with lower and upper limits of 0.001 and 0.5. A large estimate for the degrees of freedom parameter indicates that the residuals can be described by normal distribution, while a smaller degrees of freedom parameter indicates that the data have fatter tails and data points in this region are appropriately down-weighted.

Before entering the data into the analysis, each dependent variable was scaled to a mean of 0 and a standard deviation of 1. If better performance was indicated by a smaller score, the variable was inverted by subtracting each score from the maximum. Similarly, albumin and the covariates age and NART error score were also centred at 0 and scaled.

In total, 5,000 steps were used to tune the samplers, 50,000 steps were used to burn in the chains, and a total of 50,000 Markov Chain Monte Carlo (MCMC) samples thinned every 10th step were saved across 3 chains for the final parameter estimates. Convergence was confirmed by examining plots of the posterior and using the Gelman-Rubin convergence diagnostic [51]. All posterior distributions used for inference had a minimal effective sample size of at least 1000 (usually ~10000). The means ± 95% highest density intervals (HDI) of the posterior distribution were used to describe the credibility interval for each of the estimates and contrasts [49].

All statistical analyses were conducted in the open source statistical package R version 2.15.3 (R Development Core Team, 2013) using the “rjags” package to link with the Gibbs sampler “JAGS”
Results

A total of 222 (79 men and 143 women) met the key inclusion criteria for participation in this study (Table 1) out of 250 individuals in the full cohort. Nine participants were excluded because they had an estimated glomerular filtration rate <55 ml/min/1.73m$^2$, suggesting impaired renal function. All participants had plasma albumin concentrations within the normal reference range (35-50 g/L). Sixteen participants were excluded because their score on the MMSE was less than 24, suggesting significant cognitive impairment. Three individuals were excluded due to liver dysfunction (abnormal ALT, Bilirubin, ALP or GGT).

Relationship between Albumin and the covariates age and sex

For the selected cohort, there was a negative relationship between age and plasma albumin concentration, with a mean reduction of 0.079 g/mL per year (HDI = -0.12, -0.04) (Table 2). However, albumin concentrations were not credibly different between males and females.

Relationship between albumin and neuropsychological performance

Figure 1 presents the standardised slope coefficients (mean ± 80%, 95% HDI) for the association between albumin and neuropsychological performance both with and without the covariates of age, sex and acute-phase proteins as a marker of significant inflammation (APP: indicated by plasma CRP and white-blood cells). Albumin both with and without these covariates was positively associated with enhanced performance on a range of neuropsychological domains and their outcome measures. In the models that excluded covariates, albumin was positively associated with all domains and their outcome measurements, with the exception of the primary memory domain (D4) and some measures within the secondary memory domain (D3). After relevant covariates were included in the model, the relationship between albumin and cognitive performance was modestly
reduced. However, the relationship between albumin and Perceptual Speed (D5), Stroop (D2), and verbal ability (D1) domains all remained credibly larger than 0 when age and sex were included as covariates, with the perceptual speed measure Digit Symbol Coding (D5) demonstrating the most robust association. While all 95% credible intervals for secondary memory (D3) and primary memory (D4) covered 0 after including the covariates, the relationship between albumin on each domain was predominantly positive and not substantially different to the Stroop (D2) and verbal ability (D1) domains.

Discussion

Albumin was positively associated with neuropsychological performance across a range of cognitive domains. In particular, the perceptual speed measure, Digit Symbol Coding, showed the largest association with plasma albumin. Positive associations were also found with respect to performance on the Stroop and verbal ability domains, while weaker evidence was found for the secondary memory and primary memory domains. The results indicate a wide-ranging positive association between albumin and cognitive performance that was most evident on the perceptual speed domain.

*D5 - Perceptual speed*

The largest association between albumin and cognitive performance was found with the perceptual speed measure, Digit Symbol Coding. Mean estimates from the hierarchical models indicated a correlation between 0.2 and 0.3 (depending on covariates). The magnitude of this positive association is consistent with the literature showing correlations ranging between 0.07 and 0.36 between albumin and Digit Symbol Coding performance in cirrhotic and kidney patients [22-25,27,29]. More specifically, this finding suggests a positive association with psychomotor speed and ability. For example, Joy, Kaplan and Fein (2004) reported that 50% of the variance in performance on Digit Symbol Coding can be attributed to psychomotor speed and coordination,
while only approximately 7-15% is attributable to memory mechanisms [53]. Digit Symbol Coding is also sensitive to age related changes [54], dementia [55] and brain damage [56].

**D1 & D2 - Verbal ability and the Stroop**

Increased plasma albumin was also associated with better performance on the two other predominantly speeded task domains: verbal ability (which comprised D-KEFS fluency measures and the BNT) and the Stroop test. A positive association between the D-KEFS measures and albumin is consistent with Tarter et al. (1989) who found a statistically significant correlation of 0.43 for category fluency in participants with cirrhotic liver disease. Our reported correlation of between 0.10 and 0.15 (depending on the inclusion of covariates) was substantially lower than their estimate. The differences in strength of association may be because the positive association between plasma albumin and cognitive function is accentuated in patients with cirrhosis and the use of relevant covariates that share common variance between albumin and cognitive function (e.g., age) were not included in previous studies. Additionally, the hierarchical model used for our analysis partially shrinks estimates towards each other, which results in smaller effect differences between individual outcome measures.

The verbal ability and Stroop measures represent tests that are sensitive to damage to frontal and temporal brain regions. Better performance on these verbal fluency measurements indicates enhancements in retrieval strategies underpinning both lexical and semantic memories [57]. Better performance on the Stroop indicates enhancements in attention and executive functioning abilities [58]. Therefore, these findings suggest that albumin is a positive modulator of memory retrieval and elements of executive functioning and attention.

**D3 & D4 - Secondary and Primary memory**

A less robust association was found between albumin and the two memory domains: secondary memory and primary memory. Nevertheless, the mean parameter estimates were positive and the majority of the posterior densities were above 0 and small. Positive associations are consistent with
previous studies [22,25,29]. Furthermore, the influence of albumin on these two cognitive domains could plausibly be as large as the influence of albumin on the Stroop and verbal ability domains (given the overlap of the 95% credible intervals). Secondary memory and primary memory rely on distinct neural substrates, with the former dependent on hippocampal and medial temporal lobe regions [59], while the latter is more dependent on left temporo-parietal and frontal regions [60].

_Putative mechanisms for the relationship between albumin and cognitive performance_

Moderate plasma hypo-albuminemia has few, if any, associated clinical consequences. Hence, in an otherwise healthy cohort with plasma albumin within an accepted reference range, a positive association with measures of cognitive function may be indicative of other genetic, environmental or lifestyle factors that influence both cognition and albumin separately. For example, people with better nutritional status, greater dietary protein intake or who exercise more may manifest better cognitive performance and also higher plasma albumin. However, dietary intake of protein and other macronutrients has little effect on plasma albumin in subjects with good nutritional status [61]. Rather, hepatocytes have a substantial capacity to increase albumin biogenesis (three-fold), reflecting its critical function in maintaining oncotic pressure [3]. A positive association of plasma albumin with exercise (and by extension, exercise-enhanced cognitive performance) is also unlikely given that albumin degradation is increased in skeletal muscle and skin as a consequence of increased physical activity [62].

Albumin is a critical transporter of a range of biochemical elements and compounds, including calcium, copper, cysteine, fat soluble vitamins, fatty acids, free radicals, glucocorticoids, thyroxine, tryptophan and bilirubin [63]. Cognitive deficits associated with reduced plasma albumin may therefore be a consequence of greater exposure to unconjugated bioactive proteins that influence cognition. However, this is unlikely in the cohort studied here, because plasma albumin is within a normal reference range (36-49 g/L).
Heightened inflammation exerts a negative effect on albumin synthesis [64-66], and many studies have reported an association between inflammation and cognitive decline [67,68]. Conversely, a change in micro-vascular permeability, which is principally modulated by plasma albumin (a negative acute-phase marker), promotes cytokine production and activation of inflammatory cells. A variety of inflammatory cytokines have been found to impair long-term potentiation (LTP) [69], a fundamental neuronal learning mechanism that is strongly linked with learning and memory. There is therefore, a plausible link between albumin and the physiological mechanisms underlying a central component of cognitive functioning, namely secondary memory. However, in this study group, the acute-phase inflammatory cytokines C-reactive protein and interleukin-6 were not associated with cognitive performance testing. Moreover, subjects with aberrant white-blood cell counts were also excluded from analysis.

A relationship between albumin and cognitive functioning may also be associated with vascular sequelae, specifically with respect to neurovascular inflammation. Modest reductions in colloid oncotic pressure can result in extravascular fluid accumulation [3,61]. Of potential relevance for cognitive outcomes, integrity of the blood-brain barrier may be compromised resulting in the parenchymal extravasation of plasma proteins and macromolecules, activation of astroglial cells and heightened redox state [70-73]. Therefore, a subtle but heightened state of neurovascular inflammation could be a relevant mechanism underlying the relationship between albumin and cognitive performance observed in this study.

Caveats

From this research, it cannot be determined whether plasma albumin is causally associated with cognitive performance in healthy adult men and women. Plasma albumin only measures the intravascular pool (40% of total endogenous pool) and may not accurately reflect extravascular concentration of albumin. However, there is flux between the two pools at approximately 4% per hour and, generally, a positive association between the two compartments is indicated.
Consideration should also be given to the substantial disulphide binding heterogeneity of plasma albumin that exists among individuals, and which may be of functional significance. Though less well characterized, disulphide-binding domains have the potential to modulate the chaperone transporter function and microvascular regulatory properties of albumin. The latter is a consequence of altered albumin kinetics between intravascular and extravascular pools.

**Conclusion**

The findings of this study extend the findings of previous studies conducted in participants with plasma hypoalbuminemia and demonstrate for the first time a positive association of plasma albumin with global measures of cognitive performance in healthy participants with unimpaired renal and liver function.

**LEGENDS TO TABLES AND FIGURES:**

**Table 1.** Mean and range of age, albumin concentration and gender of study population of 222 otherwise healthy participants.

**Table 2.** Neuropsychological Measure scores (MMSE, RAVLT, D-KEFS Letter Fluency, category fluency and switching, BNT, NART, digit span, digital symbol coding and the Stroop test) and the relationship between age, sex and NART error scores as covariates for the entire study cohort of
Figure 1. Standardised slope coefficients (± 80, 95% credible intervals) for the association between albumin and neuropsychological performance. The domains (D1-D5) indicate the nesting structure of the Bayesian nested domain model. Black circles – the effect of albumin without covariates, Grey circles – the effect of albumin with the covariates age and sex, White circles – effect of albumin with the covariates age, sex and acute-phase protein markers. N = 222.

TABLE 1: Measures of age, albumin concentration and gender of study cohort of 222 participants.

<table>
<thead>
<tr>
<th>Biological Measures</th>
<th>Mean± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.9 ± 7.3</td>
<td>43.6 – 84.2</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>42.1 ± 2.3</td>
<td>36 – 49</td>
</tr>
<tr>
<td>Sex (F</td>
<td>M)</td>
<td>143</td>
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</tbody>
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TABLE 2: Neuropsychological measures of sample cohort.

<table>
<thead>
<tr>
<th>Neuropsychological Measures</th>
<th>Mean± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>MMSE</td>
<td>28.7 ± 1.4</td>
<td>24-30</td>
</tr>
<tr>
<td>NART Errors</td>
<td>14.6 ± 7.5</td>
<td>3-50</td>
</tr>
<tr>
<td><strong>D5 – Perceptual Speed</strong></td>
<td></td>
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<tr>
<td>Digit Symbol Coding</td>
<td>64.6 ± 13.8</td>
<td>23-95</td>
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<tr>
<td><strong>D4 – Primary Memory</strong></td>
<td></td>
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<tr>
<td>Digits Forward</td>
<td>10.4 ± 2.0</td>
<td>6-16</td>
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<tr>
<td>Digits Backwards</td>
<td>6.8 ± 2.2</td>
<td>3-18</td>
</tr>
<tr>
<td>Digits Total</td>
<td>16.8 ± 3.8</td>
<td>9-29</td>
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<td><strong>D3 – RAVLT</strong></td>
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<tr>
<td>Learning 1-5</td>
<td>44.5 ± 9.6</td>
<td>16-64</td>
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<tr>
<td>List B</td>
<td>5.1 ± 1.9</td>
<td>1-11</td>
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<tr>
<td>Short Delay</td>
<td>9.1 ± 3.1</td>
<td>0-15</td>
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<tr>
<td>Long Delay</td>
<td>9.1 ± 3.1</td>
<td>0-15</td>
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<tr>
<td>Recognition</td>
<td>13.4 ± 1.9</td>
<td>0-15</td>
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<tr>
<td>T5 – Short Delay</td>
<td>2.2 ± 2.0</td>
<td>-3-11</td>
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<tr>
<td>T5 – Long Delay</td>
<td>2.2 ± 2.0</td>
<td>-3-8</td>
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<tr>
<td><strong>D2 – Stroop</strong></td>
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<tr>
<td>Dots</td>
<td>13.9 ± 3.4</td>
<td>7-36</td>
</tr>
<tr>
<td>Words</td>
<td>18.5 ± 7.2</td>
<td>11-101</td>
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<tr>
<td>Colours</td>
<td>29.9 ± 10.1</td>
<td>14-80</td>
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<tr>
<td>Interference C/D</td>
<td>2.2 ± 0.7</td>
<td>0.83-4.8</td>
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<td><strong>D1 – Verbal Ability</strong></td>
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<tr>
<td>BNT</td>
<td>56.3 ± 4.2</td>
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<td>D-KEFS Letter Fluency</td>
<td>40.9 ± 11.9</td>
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<td>D-KEFS Category Fluency</td>
<td>44.5 ± 8.9</td>
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<tr>
<td>D-KEFS Switching</td>
<td>13.6 ± 2.9</td>
<td>4 – 21</td>
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**Albumin & covariate relationships**

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>95% HDI</th>
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</thead>
<tbody>
<tr>
<td>Albumin ~ Age</td>
<td>-0.08</td>
<td>-0.12, -0.04</td>
</tr>
<tr>
<td>Albumin ~ Sex (F-M)</td>
<td>-0.07</td>
<td>-0.74, 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.09,</td>
</tr>
<tr>
<td>NART ~ Albumin</td>
<td>-0.04</td>
<td>0.004</td>
</tr>
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</table>
FIGURE 1. Standardised slope coefficients for the association between albumin and neuropsychological performance.

- Digit Symbol Coding
- Digits Forward
- Digits Backward
- RAVLT Sum of Trials 1 – 5
- RAVLT Interference List
- RAVLT Short Delay Recall
- RAVLT Long Delay Recall
- RAVLT Recognition ‘Hits’
- RAVLT T5 – Short Delay
- RAVLT T5 – Long Delay
- Stroop Words Time
- Stroop Dots Time
- Stroop Colours Time
- Interference: Colours/Dots
- D–KEFS Letter Fluency
- D–KEFS Category Fluency
- D–KEFS Switching Total
- Boston Naming Test

- Albumin Alone
- Alb, Age & Sex
- Alb, Age, Sex & APP

Albumin – standardised slope coefficient
Acknowledgements

This research was funded by the National Health and Medical Research Council of Australia.

Disclosure statement

There are no conflicts of interest to our knowledge.
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