Plasma $\alpha\beta 42$ correlates positively with increased body fat in healthy individuals

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Abstract. Obesity and overweight, well known risk factors for cardiovascular disease and type 2 diabetes, are now associated with Alzheimer’s disease (AD). It remains to be determined if obesity and overweight contribute to the risk of developing AD through modulating levels of amyloid-beta ($\alpha\beta$), a key molecule in AD pathogenesis. Thus, we investigated whether there were any associations between plasma $\alpha\beta$ levels and body mass index (BMI) or fat mass (FM) in a group of 18 healthy adults. A statistically significant correlation was found between BMI, FM, and plasma levels of $\alpha\beta 42$ ($\text{BMI } r = 0.602, P = 0.008; \text{FM } r = 0.547, P = 0.019$), the longer, more pathogenic form of $\alpha\beta$, but not with plasma levels of the shorter, less pathogenic $\alpha\beta 40$.

Although not significant, positive correlations between plasma levels of $\alpha\beta 42$ and levels of insulin and the inflammatory marker C-reactive protein (CRP), along with an inverse trend between plasma $\alpha\beta 42$ levels and levels of high density lipoprotein (HDL) were answered. These results suggest that proteins implicated in inflammation, cardiovascular disease and type 2 diabetes, which in turn are risk factors for AD, may contribute to the associations between BMI/FM and plasma $\alpha\beta 42$ levels. Longitudinal studies involving larger cohorts are required to determine if elevated body fat may predispose individuals to AD through increasing $\alpha\beta 42$ levels throughout early to late adulthood.

Keywords: Abbreviations: AD, Alzheimer’s disease; $\alpha\beta$, amyloid-beta protein; $\text{APOE}^4$, Apolipoprotein E epsilon 4 allele; BMI, body mass index; CRP, C-reactive protein; FM, Fat Mass; HDL, High density lipoprotein; IL, interleukin; LDL, Low density Lipoprotein; TNFa/\beta, tumour necrosis factor alpha or beta.

1. Introduction

The prevalence of overweight and obesity continues to increase in western societies at an alarming rate which may be attributed to high saturated fat diets and physical inactivity. In Australia, for example, almost 60% of men and women are overweight or obese with the prevalence almost doubling in the past 20 years [7].
Over weight is defined as a body weight that exceeds the normal or standard weight based on height and frame size and obesity refers to the condition of having excess amount of body fat. An individual can be classified as normal weight, overweight and classification I, II or III obesity.

The most prominent risk factors associated with overweight and obesity are type 2 diabetes and cardiovascular disease, which are in turn themselves, well known risk factors for Alzheimer’s disease (AD) [27, 58]. In overweight and obese individuals, insulin clearance is reduced [3, 71] contributing to hyperinsulinaemia, a condition that has been associated with an increased risk of developing AD [39, 45]. The cardiovascular risk factors, including high levels of plasma cholesterol, low density lipoprotein (LDL) and low plasma levels of high density lipoprotein (HDL) have all been shown to increase the risk of developing AD [36, 56]. Interestingly some of these factors, including low HDL levels have also been reported in patients with type-2 diabetes in the form of diabetic dyslipidemia (reviewed in [35]), providing further evidence for a close association between cardiovascular disease, diabetes and AD.

These risk factors appear to be exacerbated in individuals carrying the apolipoprotein E4 (APOE ε4) allele such that they have an increased risk of developing type – 2 diabetes, hypertension, hypercholesterolaemia, cardiovascular disease and Alzheimer’s disease (reviewed in [6, 42]). This increase risk in APOE ε4 carriers maybe partly attributed to diet. It has been reported that a higher intake of calories and fats may be associated with increased risk of AD in individuals carrying this allele [44]. Furthermore, individuals with type – 2 diabetes possessing the APOE ε4 allele, are at twice the risk of developing AD compared to ε4 carriers who do not have diabetes [60].

Another link between obesity, cardiovascular disease, type-2 diabetes and AD may be through inflammatory processes. Several studies have suggested that inflammation may be associated with hyperinsulinaemia, insulin resistance, atherosclerosis and AD. Elevated levels of the C-reactive protein (CRP), a sensitive and stable marker of inflammation [63], has been associated with increased body mass index (BMI); a measure of obesity, serum lipids (indicator of cardiovascular disease risk) and increased fasting glucose levels (indication of diabetes) [10, 17, 25, 30, 34, 70]. In AD, an inflammatory state with altered cytokine production has been well characterised, and associations between AD risk and functional cytokine polymorphisms have also been demonstrated. Such markers include, interleukin-1 (IL-1) [22], interleukin-6 (IL-6) [72, 76], tumour necrosis factor alpha (TNF-α) [16] and transforming growth factor-β (TGF-β) [8, 68, 79]. Furthermore, cytokines are present in senile plaques, a neuropathological hallmark of AD [28]. These findings suggest that inflammation has a key role in AD pathogenesis that may be exacerbated by obesity, diabetes or cardiovascular disease.

The strongest support for the recent link between overweight, obesity and AD was provided by Gustafson and colleagues [23], who performed a longitudinal study examining the relationship between BMI and the subsequent risk of AD. Non-demented Swedish adults (n = 392) were recruited at age 70 and were followed through to age 88. The authors found a significant association between BMI and AD in women and not men. Those women who developed AD through the ages of 79–88 had a significantly higher BMI than aged matched controls. While it does seem that overweight and obesity appear to be risk factors for dementia, their role in AD pathogenesis remains unclear.

A key molecule in AD pathogenesis is the amyloid-beta (Aβ) protein. This molecule is central to the “amyloid hypothesis” in which the increased production of Aβ results in neurodegeneration and ultimately dementia through a cascade of events (reviewed in [73]). Furthermore, increased plasma levels of this protein have been associated with the increased risk of developing AD in the elderly [46, 47, 50]. It is conceivable that overweight and obesity may directly or indirectly, through its associated cardiovascular and diabetes risk factors or inflammatory processes, contribute to increased risk of AD by altering the levels of Aβ. To date there has been no investigation into whether direct indicators of obesity and overweight, such as BMI, body weight and percentage fat mass (FM), are associated with altered plasma Aβ levels. Thus, we sought to investigate if there was any relationship between BMI, FM and plasma Aβ levels in a small cohort of 18 adults. We found a significant correlation between the direct measures of obesity, (BMI and FM) and plasma levels of the longer more pathological form of Aβ, Aβ42. This study suggests that overweight and obesity may contribute to the risk of developing AD, by modulating plasma Aβ42 levels.

2. Materials and methods

2.1. Subjects

For this investigation, 18 healthy adults comprising of 10 males and 8 females between the ages of 23 and
Table 1
Measurements and descriptive statistics for Age, levels of total cholesterol, HDL, LDL, triglycerides (Tg), CRP, Insulin, Aβ40 and Aβ42 and BMI and FM (n = 18)

<table>
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<th>Cholesterol (mM)</th>
<th>Tg (mM)</th>
<th>HDL (mM)</th>
<th>LDL (mM)</th>
<th>CRP (mg/L)</th>
<th>Insulin (mU/L)</th>
<th>BMI (kg/m²)</th>
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Descriptive statistics

- Min 23 3.9 0.5 0.9 1.8 0.2 1.5 15.6 4.2 47.5 2.5
- Max 64 7.7 5.3 2.5 5.3 3.7 18.9 30.9 38.2 231.8 62.0
- Mean 36 5.4 1.7 1.3 3.3 1.0 6.3 24.6 20.0 146.0 29.1
- SD 14 1.2 1.2 0.4 1.0 0.9 4.1 3.9 10.0 43.3 18.1
- Median 29 5.0 1.5 1.1 3.2 0.6 4.9 24.3 19.2 143.0 27.0
- IQR 26 1.8 1.1 0.4 1.4 0.9 4.0 5.5 45.5 59.6 31.0

SD – standard deviation, IQR – inter quartile ratio, BMI – body mass index.

64 (mean age 36.4) provided written consent to participate in this study. Ethics approval was provided by Hollywood Private Hospital Human Ethics Committee. BMI, and FM was estimated for all participants prior to blood collection between the hours of 0800 and 0900 after subjects have fasted overnight. Participants were also asked to avoid vigorous physical activity within 12 hours of the assessment. As bioelectrical impedance to measure FM directly depends on body water content for its calculation [32], participants were asked to abstain from drinking 3–4 hours before the assessment, abstain from alcohol consumption and ingestion of diuretics, including caffeine, 48 hours prior to the assessment.

2.2. Measurement of body mass index and fat mass

Body mass was measured through the use of an electronic scale (UM-018, Tanita Corporation, Japan). Participants were instructed to wear light clothing to minimise error. Height was measured through the use of a wall chart and the BMI (kg/m²) was calculated by dividing mass (kg) by the height squared (m²). FM was determined by using the same scale as used for the mass measurement, which measures percentage body fat based on the bioelectrical impedance, age, sex and height of each subject. FM (kg) was calculated as the product of body mass (kg) and percentage body fat. Normal weights, overweight and different stages of obesity were classified according to the following: Healthy weight; BMI = 18.5 – 24.9, Overweight; BMI = 25.0 – 29.9; Obesity; Stage I – BMI = 30.0 – 34.9, Stage II – BMI = 35.0 – 39.9, Stage III – BMI = > 40.0 [14,15,55].

2.3. Blood collection and analysis

Blood samples were collected using standard venipuncture techniques into serum, EDTA and heparin blood collection tubes (Interpath services, Australia) containing Prostaglandin E to prevent platelet activation. Plasma and serum from the blood samples were separated by centrifugation at 130 g, aliquoted and stored at −20°C. Plasma Aβ40 and Aβ42 levels were determined using a double antibody sandwich enzyme immunoassorbent (ELISA) as previously described [49, 54]. Measurement of other parameters including levels of insulin, CRP, cholesterol, triglycerides, HDL, and LDL was performed by the Clinical Biochemistry department, Path Centre (Perth, Western Australia). Insulin (normal range 6–20 mU/L) was measured on
To initially explore the relationship between variable sets, scattergram plots were created using Microsoft Excel (2000) (Microsoft Corporation). Where a relationship was observed, SPSS Version 11.5.0 (SPSS Inc) was used to verify the statistical significance of the association. Simple linear regression was initially used, and then multiple linear regression was used to control for covariates. Associations were considered significant if linear regression provided a $p$-value of less than 0.05.

### 3. Results

A study was performed in a group of 18 healthy adults to investigate if there was any association between body mass index (BMI) or fat mass (FM) and plasma Aβ40 or Aβ42 levels. Of the 18 subjects (10 male and 8 female), 8 were found to carry an APOE ε4 allele (48%). The levels and descriptive statistics for age, total cholesterol, HDL, LDL, insulin CRP and insulin levels, FM, BMI, plasma Aβ40 and Aβ42 levels for the 18 subjects are presented in Table 1. The data from this study followed a normal distribution where the mean age of the participants was 36 years, and BMI values ranged from 18.7 kg/m² (healthy weight) to 33.0 kg/m² (obese – stage I obesity).

#### 3.1. Plasma Aβ40 levels was not correlated with either FM or BMI

Linear regression was used to explore the relationship between plasma Aβ40 level and FM or BMI. No significant correlation between plasma Aβ40 levels and either FM ($r = -0.183$, $\beta = 0.855$, $P = 0.466$) or BMI ($r = -0.099$, $\beta = -1.014$, $P = 0.696$) was observed. Separately, age, sex and APOE ε4 genotype were examined by linear regression in relation to plasma Aβ40 levels. None of these co-variates were associated with plasma Aβ40 levels (data not shown). To further control for these covariates, multiple linear regression was used, and FM or BMI, age, sex and the presence of the APOE ε4 allele were then all included in the regression model on Aβ40. However, with the inclusion of these covariates, neither FM nor BMI had a significant effect on plasma Aβ40 levels (Tables 2 and 3). Overall these results indicate that no significant association between plasma Aβ40 levels and BMI or FM was observed in our cohort.
3.2. Plasma $\text{A}42$ levels were positively correlated with both FM and BMI

Following linear regression analysis, a significant positive correlation was found between plasma levels of $\text{A}42$ for both FM ($r = 0.602, \beta = 1.176, P = 0.008$, Fig. 2A) and BMI ($r = 0.547, \beta = 2.349, P = 0.019$, Fig. 2B). Age, sex and the presence of the $\text{APOE} \varepsilon 4$ allele were then separately examined in relation to plasma $\text{A}42$ levels, but these covariates were not significantly associated with altered $\text{A}42$ levels (data not shown). To further control for these covariates, multiple linear regression was used, and FM or BMI, age, sex and the presence of the $\text{APOE} \varepsilon 4$ allele were then all included in the regression model on $\text{A}42$. The association between plasma $\text{A}42$ levels and both FM and BMI remained significant (Tables 4 and 5). The $\beta$-coefficient in Tables 4 and 5 indicate that for every 1 unit increase in FM or BMI there was an increase of 1.471 pg/ml and 3.75 pg/ml respectively, in plasma $\text{A}42$ levels.

3.3. Association between insulin and plasma levels of $\text{A}42$

As type 2 diabetes mellitus has been established as a risk factor for AD, it was investigated as to whether there was an association between plasma insulin levels, and plasma $\text{A}40$ and $\text{A}42$ levels. Linear regression analysis revealed no correlation between plasma lev-
Fig. 2. Positive correlations between FM, BMI and plasma Aβ42 levels A) Scattergram plot with trendline of fat mass vs plasma Aβ42 levels (n = 18, r = 0.602, β = 1.176, P = 0.008). Bottom B) Scattergram plot with trendline of body mass index vs plasma Aβ42 levels (n = 18, r = 0.547, β = 2.349, P = 0.019).

Overall these results indicate that in our cohort there is a trend towards an association between plasma Aβ42 levels and insulin. Similar results were observed for plasma levels of C-peptide, a molecule produced from the same precursor molecule as insulin, proinsulin, is more stable than insulin, and directly reflects insulin secretion.

3.4. Association between Cardiovascular risk factors and plasma levels of Aβ42

We next investigated whether parameters used to assess for risk of cardiovascular disease, including, plasma levels of triglycerides, total cholesterol, low-density lipoprotein (LDL) levels, high-density lipoprotein (HDL), were correlated with plasma Aβ40 or 42 levels. Upon linear regression analysis, these metabolic parameters were not significantly correlated with either Aβ40 (Table 6) or Aβ42 levels (Table 7). However, although not significant, an inverse trend was observed between HDL and Aβ42 levels (r = −0.454,
3.5. Associations of an inflammatory marker with Aβ42 levels and BMI

We next investigated if there was an association between levels of the inflammatory marker CRP, used to determine risk of cardiovascular disease and type 2 diabetes (reviewed in [24,69]), and plasma Aβ levels. CRP levels did not correlate with plasma Aβ40 levels ($r = -0.165, \beta = -7.558, P = 0.513$). Although a positive trend was observed, the levels of CRP did not significantly correlate with plasma Aβ42 levels, ($r = 0.425, \beta = 8.151, P = 0.079$; see Fig. 5A). Interestingly, a significant association was found between CRP and BMI ($r = 0.831, \beta = 3.714, P = 0.000$; see Fig. 5B). Multiple linear regression analysis of CRP and BMI with plasma Aβ42 levels as the dependent variable was performed, revealing that the association between CRP and Aβ42 remained non-significant ($P = 0.807$; see Table 8).

4. Discussion

It is now becoming apparent that risk factors for obesity, such as type 2 diabetes and cardiovascular disease are also risk factors for Alzheimer’s disease. Direct
Evidence for obesity as an AD risk factor has come from one report which has shown that compared to age-matched controls, individuals who developed AD had a higher BMI, a measure of overweight and obesity [23]. However, to date an association between BMI and plasma levels of $A\beta_42$, a protein that is implicated in AD pathogenesis, has not been reported. In this study we investigated whether there was an association between BMI and plasma levels of $A\beta_40$ and $A\beta_42$.

Although BMI is the most commonly used measurement of overweight and obesity, it does not take into account the relative muscle mass of subjects [40]. Thus, the fat mass (FM) of subjects was also measured and correlated with plasma $A\beta$ levels. The percentage fat mass was assessed through bioelec-
Fig. 5. The relationship between CRP levels and Aβ42. A) Scattergram plot with trendline of CRP levels vs plasma Aβ42 levels. Although not significant a positive trend is observed between CRP and plasma Aβ42 levels ($n = 18$, $r = 0.452$, $\beta = 8.151$, $P = 0.079$). (B) Scattergram plot with trendline of CRP levels vs BMI shows a significant correlation ($r = 0.831$, $\beta = 3.714$, $P = 0.000$).

Although bioelectrical impedance analysis is not as sensitive as dual-energy X-ray absorptiometry (DEXA), which measures fat mass, lean body mass and bone mass, it is relatively cheap and a reliable alternative. A very recent study in a large cohort of 591 healthy adults showed that data collected from bioelectrical impedance correlated well with that collected from DEXA when subjects were within a normal body fat range [64], which is the case in the current study (FM 17.8%, BMI 23.5 Kg/m$^2$).

Our data showed that a higher BMI and increased FM were significantly correlated with plasma levels of the longer form of Aβ (Aβ42) and was independent of age, sex, and the presence of an APOE ε 4 allele. However, plasma levels of Aβ40, the shorter form of Aβ, were not associated with increased BMI or FM. This finding is of great interest as Aβ42 is considered the more pathogenic species in AD and can rapidly aggregate into oligomers which are now thought to be the neurotoxic form of Aβ, rather than the fibrillar or plaque form (reviewed in [73]). Furthermore increased plasma levels of Aβ42 (but not Aβ40), have
been associated with an increased risk of AD [46], Mayeux and colleagues [46] also reported in 530 elderly (>65 yrs) individuals, that plasma Aβ42 levels, but not Aβ40 levels were inversely related to BMI \( (r = -0.1, P = 0.05) \). Here, we report a stronger, more significant positive correlation between plasma Aβ42 and both BMI \( (r = 0.547, P = 0.019) \) and FM \( (r = 0.602, P = 0.008) \) which do not corroborate the inverse correlation reported by Mayeux and colleagues [46]). This could be explained by the age differences between the cohorts in the two studies. The population used by Mayeux and colleagues [46] was an elderly population, where BMI is generally lower and may not reflect BMI at a younger age. In addition the population used in the Mayeux study consisted of normal controls (without dementia), patients clinically diagnosed with AD (at baseline) and those that developed AD (after follow up) in which BMI levels were found to be lower compared to those observed for the control subjects.

Plasma Aβ42 levels have been shown to either remain similar to age matched controls, or decline after the onset of the disease [46,50,67]. The findings from our studies and those from Mayeux and colleagues [46] indicate that investigations undertaken by each laboratory correspond to evaluating associations between BMI and plasma Aβ42 levels at different ages. The positive association between BMI and plasma Aβ42 levels shown by our study was in a younger population, thus serving as a potential risk factor for AD. The negative association between BMI and plasma Aβ42 shown by Mayeux and colleagues [46] was only obtained following the onset of the disease, which represents the sequela of the disease process.

The mechanism by which being overweight or obesity triggers increased plasma Aβ42 levels is unclear, and it is not known if the effects are a direct consequence of increased adiposity, or indirectly mediated through co-morbid states, such as diabetes or cardiovascular disease. To investigate this further, levels of insulin were measured in our cohort and compared with plasma Aβ42 levels to determine if there is an association. Although it did not reach statistical significance (most likely due to the small sample size), a positive trend was observed between plasma levels of insulin and Aβ42. This result is consistent with previous reports which showed that insulin infusion in healthy adults resulted in increased CSF or plasma levels of Aβ42 [75]. Insulin has been shown to increase the trafficking and extracellular secretion of Aβ [20]. Furthermore, insulin has been shown to increase extra-cellar Aβ levels by preventing Aβ degradation via competitive inhibition of the insulin degrading enzyme, (IDE) [16,37,38]. Thus the trend of increasing Aβ42 levels with increasing levels of insulin may be, in part, due to a reduction in the degradation and clearance of the Aβ peptide. Of particular note is the significant positive correlation that was observed between insulin and BMI, which is consistent with other studies [2,12], and suggests that the link between BMI and Aβ42 may be mediated to a certain extent through the effects of insulin, both on the increased production, and reduced degradation of Aβ.

Another potential mechanism by which being overweight and obesity may influence Aβ levels may be through changes to the lipid and cholesterol profile. High plasma cholesterol, triglyceride, and LDL levels, as well as low plasma HDL levels have long been known to be associated with overweight and obesity, and have all been identified as significant risk factors for AD [36,56]. Our study did not show significant associations between levels of cholesterol, triglycerides or LDL and Aβ levels. Increasing the number of participants in the study may show significant associations between these parameters. Nevertheless, our study revealed a negative trend between HDL and Aβ42 levels and a significant correlation between BMI and HDL levels. Plasma HDL has been shown to be lower in patients with AD and is highly correlated with lower cognitive scores from AD individuals [36,51]. Furthermore, HDL has been shown to interact with Aβ in plasma [52,53] and can regulate Aβ aggregation, degradation and toxicity [9,13,57] suggesting possible mechanisms by which HDL could modify the risk of developing AD. These cardiovascular risk factors may also contribute to AD through their modulatory effects on the proteolytic processing of the parent molecule to Aβ, the amyloid β protein precursor (AβPP) [1,62], generating more Aβ42.

It is interesting to note that obesity, cardiovascular disease, type 2 diabetes and AD all have inflammatory processes which play a role in disease process. We therefore investigated in our cohort whether there was an association between BMI, Aβ levels and the levels of CRP, a sensitive and stable marker of inflammation [63]. Elevated levels of CRP have been associated with increased BMI, (measure of obesity), serum lipids (indicator of cardiovascular disease risk) and increased fasting glucose levels (indicator of diabetes risk) [10, 17,25,30,34,70]. In AD, an inflammatory state has been well characterised, however CRP has yet to be associated with levels of Aβ40 or Aβ42.
Although not significant, an increasing trend in plasma Aβ42 levels with increasing levels of CRP was shown in our study. However, as previously reported [21,33,61,66], we found that increased levels of BMI were strongly associated with increases in CRP levels (r = 0.831, β = 3.714, P = 0.000). Furthermore, elevated levels of other inflammatory markers including interleukin 1 and 6 (IL-1, 6), tumour necrosis factor α and β (TNFα, β) have been reported in obese individuals [4,11,74]. Taken together, these data suggest that obesity and overweight may be associated with inflammatory processes. Some of these inflammatory markers including IL-1β, TNF-α and IL-6 have been shown to regulate the production of Aβ, particularly Aβ42 [5,43,59]. In light of these studies, our results showing a positive correlation between BMI and FM with plasma levels of Aβ42, suggest that this correlation may have associations with inflammatory processes. Furthermore, evidence exists that shows weight loss is associated with a fall in cytokine production [11,31]. Thus, it may be plausible that by losing weight, the risk of AD may be reduced in a manner somewhat analogous to the effect of non-steroidal anti-inflammatory drugs on AD risk [29,48,78].

This study is the first to report a positive association between plasma Aβ42 levels and the direct measures of overweight and obesity, BMI and FM, in non-demented adults. Although larger longitudinal studies are required, this pilot study suggests that elevated body fat may not only predispose individuals to cardiovascular disease, type 2 diabetes and associated inflammatory processes, but also to AD. Furthermore, these results suggest that by maintaining body weight at a normal level, individuals may be able to reduce their risk of developing AD.

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