Published in final edited form as:

J Alzheimers Dis. 2015 ; 49(1): 129–137. doi:10.3233/JAD-150428.

Age and Its Association with Low Insulin and High Amyloid-β Peptides in Blood

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Abstract

Age is the major risk factor for developing Alzheimer's disease (AD), and modifying age-related factors may help to delay the onset of the disease. The goal of this study was to investigate the relationship between age and the metabolic factors related to the risk of developing AD. The concentrations of insulin, amylin, and amyloid-β peptide (Aβ) in plasma were measured. We further measured the activity of serum Aβ degradation by using fluorescein- and biotin-labeled $A\beta_{40}$. Apolipoprotein E4 allele (ApoE4) and cognitive impairment were characterized. Subjects were divided into three age groups: 60–70, 70–80, and ≥80 years old. We found that the older the subjects, the lower the concentration of insulin ($p = 0.001$) and the higher the concentration of $A\beta_{1–40}$ ($p = 0.004$) in plasma. However, age was not associated with the concentration of another pancreatic peptide, amylin, and only marginally with $A\beta_{1–42}$. These relationships remained in the absence of diabetes, cardiovascular disease, and stroke, and regardless of the presence of ApoE4 and cognitive impairment. Both age and ApoE4 were inversely associated with, while insulin was positively associated with, the activities of Aβ degradation in serum. Our study suggested that low concentration of insulin and high concentration of $A\beta_{40}$ are aging factors related to the risk of AD.

Keywords

Aβ; Aβ degradation; age; Alzheimer's disease; ApoE4; insulin

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Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/15-0428r1).

INTRODUCTION

Dementia is a clinical syndrome and caused by several pathologies and etiologies in the brain. The most common pathology is Alzheimer's disease (AD), which accounts for approximately 60%–70% of cases. The greatest risk for developing AD is age. At ages 60 to 65, only 1% of population suffers from AD dementia. By age 80, this percentage increases to 30% to 50%. The United States Census Bureau projects that between 2005 and 2025, the total population in the U.S. will grow by 20%, but the number of individuals age 65 and over will increase by nearly 50%. The number of Americans surviving to 100 years and more will triple in only 20 years. There are currently 4–5.5 million patients with AD in the U.S alone, and this number of AD patients will grow to 14–16 million by the year 2050 [1]. While overwhelming studies investigate the pathological factors for AD, it is remarkable that even those who carry AD genetic risk factors like apolipoprotein E4 (ApoE4) allele do not have the disease until an old age [2]. The aging factors that increase the risk of AD, if exist during aging process, have been understudied, but identifying them may help to delay the onset of the disease.

It is still unclear how the aging process accelerates AD pathogenesis. One defining hallmark of AD is the presence of extracellular amyloid-β peptide (Aβ); $A\beta_{42}$ is in the form of brain amyloid plaques and $A\beta_{40}$ is in the form of cerebral amyloid angiopathy [3]. While a large body of data has focused on Aβ in AD research, how the aging process affects Aβ is unclear. Type 2 diabetes is another age-related disease and diabetes increases the risk of AD [4]. It is possible that diabetes related molecules like insulin are the shared aging factors for AD. A clinical trial has shown that the nasal insulin treatment can delay cognitive decline in AD [5].

However, working with biomarkers like insulin in peripheral blood to study their relationships with neurodegenerative diseases including AD could be confounded by many common diseases outside the brain such as diabetes and cardiovascular disease. The aim of this study was to use a large elderly population to investigate the relationships between age and the biomarkers related to AD, including with and without other peripheral diseases.

METHODS

Study population and recruitment

We studied a group of 912 subjects of known age ≥60 from the *Nutrition, Aging and Memory in the Elderly (NAME)* study, a cross-sectional, population-based study [6]. All homebound elders aged 60 and older from each of the four agencies, including Central Boston Elderly Services, Ethos Care, Boston Senior Care Center, and Somerville-Cambridge Elder Services, were invited to participate in the study. Our sample consisted of 912 subjects whose serum and plasma samples were used for this study. The study was approved by both Institutional Review Boards (IRB] of Tufts Medical Center and Boston University School of Medicine. All the subjects signed the written consent.

Measurements

Blood samples were centrifuged immediately following blood draw to isolate plasma. Glucose concentrations were measured by the hexokinase method and plasma insulin concentration was measured by radioimmunoassay in the clinical laboratories of New England Medical Center as for the routines of clinical practice in the hospital. We used ELISA assay to measure amylin concentration in plasma according to the manufacture's instructions (LINCO Research, St. Charles, Missouri). All plasma samples were assayed in duplicate and averaged to give final values.

To measure Aβ a sandwich Aβ ELISA was used, as described previously [7]. Briefly, plates were coated with 2G3 (anti-$A\beta_{40}$) and 21F12 (anti-$A\beta_{42}$) anti-bodies overnight at 4°C. Samples were then loaded and incubated overnight at 4°C followed by incubation with a biotinylated monoclonal anti-N terminus Aβ antibody (3D6B) for 2 h. Finally, streptavidin-conjugated alkaline phosphatase (Promega, USA) was added and incubated, and the signal was amplified by adding alkaline phosphatase fluorescent substrate (Promega, USA), which was then measured.

ApoE genotyping was characterized by using a 244 bp fragment of the APOE gene including the two polymorphic sites was amplified by PCR using a robotic Thermal Cycler (ABI 877, Perkin-Elmer/Applied Biosystems), using oligonucleotide primers F4 (5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′) and F6 (5′-TAAGCTTGGCACGGCTGTCCAAGGA-3′). The PCR products were digested with 5 units of Hha I and the fragments separated by electrophoresis on 8% polyacrylamide non-denaturing gel. The specific allelic fragments were: E2, E3, and E4. ApoE4 was defined by E4/4, E3/4, or E2/4 [8].

Medical conditions

Weight and height were measured using standardized instruments and protocol during the home visit. Body mass index (BMI) was calculated as measured weight in kilograms divided by measured height in meters squared. Diabetes was defined as the use of anti-diabetic medication or fasting glucose greater than 126 mg/dl [9]. Subjects were asked to disclose all medications they were taking. Blood pressure was assessed twice at the home visit, and the averages of the two measurements for systolic and diastolic blood pressure (mmHg) were used. Hypertension was defined by self-report, systolic pressure >140 or diastolic pressure >90, or reported use of hypertension medication. Cardiovascular diseases and stroke were assessed by self-report.

Cognition was assessed using a two-phase approach. The population was screened for severe cognitive impairment using the Mini-Mental State Examination (MMSE) and for estimated verbal IQ using the North American Adult Reading Test. Those with MMSE <10 or verbal IQ <75 were not eligible to continue in the study that eliminated severe dementia shown by another study [10]. Then subjects were divided into two subgroups by a cut-off MMSE score of 24.

FAβB degradation assay using fluorescence polarization

Double-labeled fluorescein-$A\beta_{1-40}$-biotin (FAβB) was purchased from AnaSpec (Fremont, CA). Pierce avidin agarose was purchased from Thermo Scientific (Rockford, IL). To characterize the activity of serum FAβB degradation in the presence of different protease inhibitors, an alternative, fluorescence polarization [FP]-based version of the FAβB degradation assay was used as described previously [11].

The assay as described above was conducted with some modifications to characterize the serum Aβ degradation in a large sample. Briefly, 24 μL of serum was mixed with 10 μL of freshly prepared 200 nM FAβB and diluted with PBS to a final concentration of 20 nM of FAβB. The mixtures were covered in aluminum foil and then incubated at 37° C for different time periods up to 1 h. The avidin beads were prepared by washing them with PBS six times and diluting them 1:5.7 with PBS. After the serum mixture incubation, samples were placed in dry ice for 5 min to stop the reaction, after which 200 μL of prepared avidinagarose beads were added to a total volume of 500 μL for each sample. These samples were then rotated at room temperature for 30 min on a rotating shaker, after which they were centrifuged at 13,200 rpm for 10 min. Duplicate 160 μL aliquots from each supernatant were then withdrawn and placed in separate wells of a 96-well black plate reader for analysis. The protease activity was measured by monitoring the quantity of fluoresceinated cleavage products present in the supernatant after removal of the residual intact fluorescein-and biotin-labeled peptide with avidin-agarose beads. The resulting fluorescence was measured at 535 nm, after excitation at 468 nm, using a Synergy MX fluoroscopy system (Biotek Inc., Winooski, VT) running Gen 5 software (v.1.08).

The total amount of fluorescence from added and undegraded FAβB at time 0 was treated as 100% FAβB degradation, the amount of released fluorescence after the incubation and avidin precipitation was treated as degraded FAβB. The remaining undegraded FAβB was calculated as the total amount of fluorescence from added FAβB minus the released amount of fluorescence. To measure the activity of serum FAβB degradation in the large sample set, one serum aliquot of a healthy male volunteer was used as an internal control for each time of measurement. Percent Aβ degradation in serum was calculated as follows: the average of two readings from the released fluorescence for each serum sample was normalized by expressing the values as a ratio to the amount of simultaneously measured proteolytic activity present in this internal control and the total amount of undegraded FAβB added and then times 100:

$$\frac{\text{Average released fluorescence from each sample} \times 100}{\text{The internal control} \times \text{total fluorescence from FA}\beta\text{B}}$$

All the measurements were conducted by a single researcher, Dr. Huajie Li. Coefficient of variation (CV) calculated from repetitive use of control serum in each set of measurements was 6.5%. Intraclass correlation coefficient (ICC) within each set of measurements was between 1.69 to 3.20%.

Statistical analysis

Statistical analysis was performed using SAS (version 9.1). Pearson analyses were performed to determine correlation coefficients between age and different factors. Mean + SD and ANOVA test were used for the variables to examine the differences on the continuous variables among different age subgroups. This analysis was also conducted in the absence and the presence of diabetes, cardiovascular diseases, stroke, and ApoE4 allele. Mean + SD and ANOVA were used for the variables with a normal distribution, and median (Q1, Q3) and Kurskal-Wallis test were used for the variables with a skewed distribution like Aβ. Insulin, $A\beta_{1–40}$, and $A\beta_{1–42}$ were transformed to $\log_{10}$ for multivariate regression. Linear regression was used to examine associations between insulin, $A\beta_{1–40}$ or $A\beta_{1–42}$ as an outcome and age, gender, race, BMI, diabetes, and ApoE4 allele. Linear regression was used to examine association between serum activity of FAβB degradation as an outcome and age, gender, race, BMI, and ApoE4 (Model I) and then plus insulin and diabetes (Model II). The two-sided significance level of <0.05 was used.

RESULTS

Characters of study population

A total of 912 serum samples from the NAME study with age and other well-characterized clinical and demographic information were used [6]. The average (mean ± SD) age of this population was 75.4 ± 8.4 years (Table 1). 77% of them were female, and 62% had at least a high school education. The population was multi-ethnic, with 62% Caucasian, 35% African American, and 3% other ethnicities. 23% of subjects carried at least one ApoE4 allele. The average BMI was 31.6 ± 8.7, and 37% of them had diabetes and 9% used insulin treatment. Glucose concentration was 114.9 ± 38.9 mg/L, insulin concentration was 109.5 ± 113.6 pM/L and amylin concentration was skewed with median (Q1, Q3) of 21.6 (11.0, 39.4).

Age and plasma insulin in the elderly

We divided the subjects into three age subgroups (60–69, 70–79, and >80) (Table 2). The older the subjects were, the lower concentrations of insulin (mean ± SD) they had, as follows: 133.8 ± 117.3 (aged 60–69), 109.6 ± 96.5 (aged 70–79), and 88.7 ± 123.4 (aged >80) ($p < 0.0001$). The relationship between age and insulin concentration remained in the absence of diabetes ($p = 0.001$) and in the absence of diabetes, cardiovascular disease, and stroke ($p = 0.003$) (Table 2). In the absence of diabetes, regardless of ApoE4 allele (Table 3) and of cognitive function (Table 4), the older the elderly, the lower concentration of plasma insulin they had. In contrast, another pancreatic peptide, amylin, was not found to be associated with age. The older the subjects were, the lower concentrations of glucose they had in the whole sample ($p = 0.0009$); however, unlike insulin, the relationship between age and glucose disappeared after removing those who had diabetes and other medical conditions (Table 2).

Using Pearson analysis, we found that age was inversely associated with insulin concentration, both in the whole sample ($r = –0.151$, $p < 0.0001$) and in the absence of diabetes ($r = –0.167$, $p < 0.0001$). Using multivariate analyses, age remained to be negatively

associated with insulin concentration as an outcome (β = –0.011, SE = 0.003, $p = 0.0001$) after adjusting for gender, ethnicity, BMI, diabetes, and ApoE4 allele (Table 5).

Age and plasma Aβ in the elderly

In contrast to insulin in blood, the older the subjects were, the higher concentration of $A\beta_{1–40}$ (median) they had, as follows: 125.5 (aged 60–69), 141.6 (aged 70–79), and 138.6 (aged >80) ($p = 0.004$) (Table 2). The relationship between age and plasma $A\beta_{1–40}$ concentration remained in the absence of diabetes ($p = 0.004$) and in the absence of diabetes, cardiovascular disease and stroke ($p = 0.04$). Age was or tended to be positively associated with plasma $A\beta_{1–40}$ in the absence or the presence of ApoE4 allele (Table 3). While age was associated with plasma $A\beta_{1–40}$ among those with normal cognition, this relationship disappeared among those with cognitive impairment (Table 4). In multivariate regression analyses after adjusting for gender, ethnicity, BMI, diabetes and ApoE4 allele, age remained to be positively associated with plasma $A\beta_{1–40}$ (β = +0.005, SE = 0.002, $p = 0.01$), but only tended to be associated with plasma $A\beta_{1–42}$ (β = +0.005, SE = 0.003, $p = 0.15$) (Table 5).

Characterization of age and serum Aβ degradation in the elderly

Since age was associated with insulin and Aβ in opposite directions and these peptides are degraded by the same protease [12], we next studied the relationship between age and Aβdegrading activity in serum. Using a well-characterized fluorescence polarization-based assay [11], we measured the degradation of Aβ in the serum samples from this study population and found that the average serum Aβ degradation (mean ± SD) was 35.2 ± 12.7 (Table 1).

We first used Pearson analysis and found that age was inversely associated with the activity of serum Aβ degradation in the whole sample ($r = –0.129$, $p < 0.0001$) and in the absence of diabetes ($r = –0.144$, $p = 0.0006$). The older the subjects, the lower activity of serum Aβdegradation (mean ± SD) they had (Table 2), as follows: 36.7 ± 13.4 (aged 60–69), 35.7 ± 11.7 (aged 70–79), and 33.4 ± 13.2 (aged >80) ($p = 0.0002$) respectively, and the relationship between age and Aβ degradation remained after removing the medical conditions (Table 2) and regardless of the ApoE4 allele (Table 3). While age was associated with serum Aβ degradation among those with normal cognition, this relationship disappeared among those with cognitive impairment (Table 4). Using multivariate regression analysis, we found that serum Aβ degradation as an outcome was still inversely associated with age (β = –0.162, SE = 0.054, $p = 0.003$) after adjusting for gender, ethnicity, and BMI (Model I) (Table 6). Interestingly, serum Aβ degradation was also negatively associated with ApoE4 allele (β = –2.087, SE = 1.027, $p = 0.04$) (Model I) and positively associated with insulin (β = +0.015, SE = 0.004, $p = 0.0002$) but not with diabetes (Model II) after adjusting for the confounders.

DISCUSSION

Despite the fact that age is a major risk factor of AD, we have limited understanding on how aging process influences AD pathogenesis. Aging factors and disease factors are distinct and could have different impacts on the development of AD. For example, genetic mutations and

genetic risk polymorphisms for AD are carried after birth, but the disease does not occur until an old age after 60 years old and even the development of early-onset AD is at 30–40 years old. Thus understanding the aging factors contributing to AD pathogenesis will help delaying the onset of the disease. While many basic researches demonstrate that insulin is the key regulator of glucose metabolism of neurons in the brain [13], using human samples our study found that increased age was significantly associated with a low concentration of insulin even among the healthy elders (Tables 2 and 4). As Aβ is a major component of AD pathology in the brain, $A\beta_{40}$ contributes to AD pathology in the cerebrovasculature to cause cognitive decline [14]. We found that age was positively associated with $A\beta_{40}$ probably through decreased Aβ degradation activity in blood (Tables 2 and 5), suggesting a possible aging process leading to the risk of AD.

Our study shows that age was negatively associated with insulin, but not with amylin, in healthy elderly (Table 2). Several studies in humans suggest that aging impairs β cell function to reduce the production and secretion of insulin [15–17]. Since both insulin and amylin are co-secreted by pancreatic β cells [18], our study suggests that aging process also affects the metabolism of insulin probably through increased activity of insulin degrading enzyme (IDE) during aging process [19]. As insulin and its signal transduction are vital for neuronal homeostasis [20, 21], synapsis formation [22], and survive/repair [23], age-associated low levels of insulin increase the vulnerability to develop neurodegenerative diseases including AD as well as to develop type 2 diabetes in the elderly [24]. Since low levels of insulin during aging process were independent of a major AD risk factor, ApoE4, and cognitive function (Tables 3 and 4), it suggests that low concentration of insulin is an aging factor leading to cognitive decline rather than a disease factor for AD. Giving exogenous insulin may be useful for prevention and intervention of AD to delay and slow-down the process of the disease. Indeed, a clinical trial has shown that the nasal insulin treatment can delay cognitive decline in AD [5].

In contrast to insulin, age was positively associated with $A\beta_{1–40}$ and marginally with $A\beta_{1–42}$ (Tables 2 and 4), probably through decreasing ability of Aβ degradation during aging process (Tables 2 and 5). Our data plus others [25, 26] suggest that high $A\beta_{40}$ is probably an aging factor that damages cerebrovasculature [27]. On the other hand, $A\beta_{42}$, which is the major Aβ peptide depositing and more neurotoxic in the AD brain, impairs insulin signaling [28] and further induces $A\beta_{42}$ accumulation in the AD brain [29]. The majority of AD patients are sporadic, having a late onset (age =65) related to the aging process and often do not have significant genetic determinants for the disease. Sporadic AD patients have been shown to have decreased clearance of Aβ from the brain, rather than increased A β production [30]. It is thought that the aging process may derive from imperfect clearance of oxidatively damaged, relatively indigestible material, the accumulation of which further hinders cellular catabolic and anabolic functions [31]. The actual amount of neurotoxic Aβ in the brain is determined by the balance between the production of Aβ from the amyloid-β protein precursor and the degradation and clearance of Aβ after it is produced [32].

As age and the aging factor, low insulin, were associated with low $A\beta_{1–40}$ degradation (Tables 1 and 5), having the disease risk factor, ApoE4 allele, for AD and probably ethnicity were also negatively associated with low Aβ degradation (Tables 3 and 5). While several

studies reported that ApoE4 reduces Aβ degradation (reviewed by Zlokovic et al. [33]), our study shows that in the oldest group, ApoE4 carriers had more significantly lower ability to degrade Aβ than ApoE4 non-carriers (Table 3). Given that Aβis the key element in the AD pathogenesis [34], both aging process and the disease process may merge to impair the Aβ degradation/clearance to cause AD. While insulin resistance is associated with amyloid deposits in the brain [35], it is unclear how insulin influences Aβ degradation *in vivo* during aging process. However, an *in vitro* experiment shows that insulin can bind to β2-macroglobulin (β2M) [36], and insulin treatment increases the endocytic rate constant of β2M/LRP-1 complex [37]. Our previous study identified a serine protease that binds to β2M to form a stable high molecular weight complex capable of efficiently cleaving Aβ in serum [38]. It is possible that high $A\beta_{40}$ in blood through low Aβ degrading activity in aging process causes the damage of cerebrovasculature, cerebral amyloid angiopathy, to increase the risk of AD [39].

There are limitations of our study. We cannot determine changes of these factors during aging process without a longitudinal study. Without longitudinal biomarker data this analysis cannot rule out that differences in insulin and Aβ levels across the age groups are not subject to survival bias, including premature death, physical function, health status, and cognitive status. The NAME cohort consists of homebound elderly who may not be well representative of the larger population of community dwelling older adults. For instance, this homebound cohort appears to have a greater prevalence of diabetes with a lower socioeconomic status and more likely to have a frailty phenotype that the general population of older adults. While we did not have individual proteases involved in serum Aβ degradation, we are unable to conclude whether serum Aβ degradation activity is associated with the development of AD. Nevertheless, our results suggest that low blood insulin concentration and low ability of Aβ degradation, which are linked with the AD risk, are age-related phenomenon. Finally, it may be important to study whether exogenous insulin treatment can prevent or delay cognitive decline in aging population.

Acknowledgments

The authors thank the NAME study staff and the Boston homecare agencies for their hard work and acquisition of subjects. This work was supported by grants from NIA, AG-022476 and Ignition Award (W.Q.Q.) and BU ADC pilot grant (H.Z.).

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Table 1

Characterizations of a homebound elderly study

	Characterizations
Age, years, mean ± SD, *n* = 912	75.4 ± 8.4
Female, n/total (%)	700/912 (77%)
White, n/total (%)	561/908 (62%)
BMI, mean ± SD, *n* = 864	31.6 ± 8.7
ApoE4, n/total (%)	208/904 (23%)
Cardiovascular diseases, n/total (%)	362/880 (41%)
Stroke, n/total (%)	176/886 (20%)
Diabetes, n/total (%)	326/893 (37%)
Insulin, pM/L, mean ± SD, median, *n* = 897	109.5 ± 113.6, 79.6
Amylin, pM/L, median (Q1, Q2), *n* = 897	21.6 (11.0, 39.4)
Glucose, mg/L, mean ± SD, *n* = 907	114.9 ± 38.9
Insulin treatment, n/total (%)	78/892 (9%)
$A\beta_{1-42}$ pg/ml, median (Q1, Q3), *n* = 884	18.4 (12.2, 28.0)
$A\beta_{1-40}$ pg/ml, median (Q1, Q3), *n* = 884	133.8 (97.8, 175.7)
Serum Aβ degradation, mean ± SD, *n* = 886	35.2 ± 12.7

The homebound elderly sample was used and characterized to present with mean ± SD or n/total (%).

Table 2

Comparisons of metabolic factors, Aβ and Aβ degradation among different age groups in the elderly

All subjects	**60 ≤ Age < 70 *n* = 261**	**70 ≤ Age < 80 *n* = 342**	**Age ≥80 *n* = 309**	***p* values**
Insulin, pM/L, mean ± SD	133.8 ± 117.3	109.6 ± 96.5	88.7 ± 123.4	<0.0001
Amylin, pM/L, median (Q1, Q3)	23.4 (12.1, 48.6)	19.4 (10.6, 39.6)	22.3 (11.8, 35.3)	0.36
Glucose, mg/L, mean ± SD	115.3 ± 36.8	118.8 ± 44.7	107.9 ± 32.5	0.0009
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	17.2 (12.0, 25.5)	18.9 (12.5, 28.1)	18.9 (12.4, 29.3)	0.46
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	125.5 (90.3, 157.5)	141.6 (100.3, 182.2)	138.6 (101.3, 185.1)	0.004
Serum Aβ degradation, mean ± SD	36.7 ± 13.4	35.7 ± 11.7	33.4 ± 13.2	0.0002
Those who did not have diabetes	60 ≤ Age < 70 *n* = 143	70 ≤ Age < 80 *n* = 198	Age ≥ 80 *n* = 226	*p* values
Insulin, pM/L, mean ± SD	101.7 ± 76.4	82.4 ± 54.5	76.8 ± 59.9	0.001
Amylin, pM/L, median (Q1, Q3)	20.0 (12.6, 32.7)	17.2 (10.4, 37.6)	23.0 (11.7, 34.4)	0.57
Glucose, mg/L, mean ± SD	96.8 ± 10.7	97.3 ± 10.2	97.2 ± 11.0	0.78
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	16.4 (11.6, 23.5)	18.6 (11.8, 27.5)	19.6 (12.7, 30.5)	0.05
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	117.7 (88.7, 150.4)	136.6 (98.7, 179.1)	134.5 (101.1, 175.2)	0.004
Serum Aβ degradation, mean ± SD	35.6 ± 13.1	35.1 ± 12.3	32.4 ± 11.4	0.05
Those who did not have diabetes, cardiovascular disease or stroke	60 ≤ Age < 70 *n* = 86	70 ≤ Age < 80 *n* = 107	Age ≥ 80 *n* = 118	*p* values
Insulin, pM/L, mean ± SD	104.8 ± 86.3	75.0 ± 51.4	73.1 ± 64.2	0.003
Amylin, pM/L, median (Q1, Q3)	19.9 (11.5, 32.8)	16.8 (10.1, 34.4)	20.6 (11.0, 29.7)	0.74
Glucose, mg/L, mean ± SD	97.8 ± 11.3	98.3 ± 10.2	97.9 ± 10.8	0.97
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	14.9 (11.5, 21.2)	18.4 (11.5, 28.2)	19.3 (12.3, 33.3)	0.08
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	117.2 (91.2, 153.5)	136.4 (99.3, 172.4)	138.1 (104.9, 175.8)	0.04
Serum Aβ degradation, mean ± SD	34.9 ± 13.2	35.8 ± 11.8	33.3 ± 12.1	0.13

Mean ± SD with ANOVA Test or n/total (%) with Chi-Square test is presented. *p* values for comparisons are shown for significance.

Table 3

Comparisons of insulin, Aβ and Aβ degradation in different age groups without diabetes and in the absence and the presence of ApoE4 allele

ApoE4 non-carriers	**60 ≤ Age < 70 *n* = 108**	**70 ≤ Age < 80 *n* = 145**	**Age ≥ 80 *n* = 181**	***p* values**
Insulin, pM/L, mean ± SD	100.0 ± 77.5	79.5 ± 52.1	79.6 ± 63.3	0.03
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	17.3 (11.3, 23.6)	20.2 (12.9, 28.2)	19.7 (12.7, 30.7)	0.11
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	121.3 (88.6, 149.2)	136.6 (100.9, 174.1)	132.4 (101.1, 172.5)	0.02
Serum Aβ degradation activity, mean ± SD	36.8 ± 13.2	36.1 ± 12.3	33.7 ± 13.1	0.003
ApoE4 carriers	60 ≤ Age < 70 *n* = 35	70 ≤ Age < 80 *n* = 53	Age ≥ 80 *n* = 44	*p* values
Insulin, pM/L, mean ± SD	107.1 ± 73.8	90.2 ± 60.1	63.3 ± 40.4	0.008
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	16.0 (11.3, 23.3)	17.4 (9.3, 26.6)	19.3 (12.8, 29.2)	0.35
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	110.5 (88.7, 168.6)	136.6 (98.4, 185.4)	143.8 (107.2, 204.2)	0.15
Serum Aβ degradation activity, mean ± SD	35.7 ± 13.6	34.0 ± 9.6	31.3 ± 10.3*	0.05

Mean±SD with ANOVA Test or n/total (%) with Chi-Square test is presented. *p* values for comparisons are shown for significance.

*Comparison between ApoE4 carriers and non-carriers with $p = 0.03$.

Table 4

Comparisons of insulin, Aβ and Aβ degradation in different age groups without diabetes and in the absence and the presence of cognitive impairment

MMSE Score ≥24	**60 ≤ Age < 70 *n* = 115**	**70 ≤ Age < 80 *n* = 153**	**Age ≥ 80 *n* = 172**	***p* values**
Insulin, pM/L, mean ± SD	94.4 ± 70.0	80.2 ± 50.6	77.0 ± 60.0	0.03
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	17.3 (11.8, 24.7)	18.4 (11.6, 28.2)	19.8 (12.7, 33.0)	0.18
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	120.0 (89.6, 149.2)	136.6 (99.3, 170.0)	136.0 (102.2, 175.5)	0.01
Serum Aβ degradation activity, mean ± SD	35.8 ± 12.5	34.8 ± 11.7	31.9 ± 11.1	0.003
MMSE Score <24	60 ≤ Age < 70 *n* = 28	70 ≤ Age < 80 *n* = 45	Age ≥ 80 *n* = 54	*p* values
Insulin, pM/L, mean ± SD	131.73 ± 94.0	86.6 ± 65.9	75.9 ± 59.0	0.004
$A\beta_{1-42}$ pg/ml, median (Q1, Q3)	12.2 (10.3, 19.1)	19.8 (12.9, 26.8)	19.1 (12.6, 26.6)	0.03
$A\beta_{1-40}$ pg/ml, median (Q1, Q3)	136.6 (74.5, 160.9)	136.6 (94.7, 210.7)	132.9 (87.8, 172.6)	0.18
Serum Aβ degradation activity, mean ± SD	35.1 ± 15.6	36.0 ± 14.0	36.7 ± 12.5	0.73

Mean ± SD with ANOVA Test or n/total (%) with Chi-Square test is presented. *p* values for comparisons are shown for significance.

Table 5

Multivariate analyses on the associations between age and the risk factors related to Alzheimer's disease

	Log Insulin (n = 851)			Log $A\beta_{1-42}$ (n = 851)			Log $A\beta_{1-40}$ (n = 837)		
	β Estimate	SE	*p* value	β Estimate	SE	*p* value	β Estimate	SE	*p* value
Age, years	– 0.011	0.003	0.0001	+0.005	0.003	0.15	+0.005	0.002	0.01
Female (0, 1)	– 0.049	0.056	0.38	– 0.001	0.065	0.99	– 0.018	0.043	0.67
White (0, 1)	– 0.020	0.016	0.21	+0.027	0.018	0.14	+0.019	0.012	0.11
BMI	+0.031	0.003	<0.0001	+0.002	0.003	0.65	+0.001	0.002	0.90
Diabetes (0, 1)	+0.304	0.051	<0.0001	+0.027	0.059	0.64	+0.075	0.038	0.05
ApoE4 (0, 1)	+0.028	0.054	0.61	– 0.121	0.063	0.06	+0.021	0.041	0.62

Multivariate regression analysis was used to examine the relationship between serum Aβ degradation as an outcome and other factors. Model I included age, gender, race, body mass index (BMI) and ApoE4. Model II included model I plus insulin and diabetes. Model III included model II plus albumin.

Table 6

The associations between serum Aβ degradation and the risk factors related to Alzheimer's disease in the elderly

	Model I Serum Aβ Degradation (*n* = 851)			Model II Serum Aβ Degradation (*n* = 851)		
	β Estimate	**SE**	***p* value**	**β Estimate**	**SE**	***p* value**
Age, years	– 0.162	0.054	0.003	– 0.139	0.054	0.01
Female (0, 1)	– 0.269	0.157	0.80	– 0.118	1,061	0.91
White (0, 1)	– 0.675	0.295	0.02	– 0.580	0.297	0.05
BMI	+0.026	0.053	0.63	– 0.043	0.056	0.44
ApoE4 (0, 1)	– 2.087	1.027	0.04	– 2.126	1.025	0.04
Insulin, pM/L	–	–	–	+0.015	0.004	0.0003
Diabetes (0, 1)	–	–	–	+1.128	0.962	0.24

Multivariate regression analysis was used to examine the relationship between serum Aβ degradation as an outcome and other factors. Model I included age, gender, race, body mass index (BMI) and ApoE4. Model II included model I plus insulin and diabetes. Model III included model II plus albumin.