NIH Public Access
Author Manuscript
Alzheimers Dement. Author manuscript; available in PMC 2010 January 1.

Published in final edited form as:
Alzheimers Dement. 2009 January ; 5(1): 18–29. doi:10.1016/j.jalz.2008.10.004.

Aβ peptides in human plasma and tissues and their significance for Alzheimer's disease

Alex E. Roher[1], **Chera L. Esh**[1], **Tyler A. Kokjohn**[1,2], **Eduardo M. Castaño**[3], **Gregory D. Van Vickle**[1], **Walter M. Kalback**[1], **R. Lyle Patton**[1], **Dean C. Luehrs**[1], **Ian D. Daugs**[1], **Yu-Min Kuo**[4], **Mark R. Emmerling**[5,*], **Holly Soares**[5], **Joseph F. Quinn**[6], **Jeffrey Kaye**[6], **Donald J. Connor**[7], **Nina B. Silverberg**[7,#], **Charles H. Adler**[8], **James D. Seward**[7,¶], **Thomas G. Beach**[9], and **Marwan N. Sabbagh**[7]

1 *The Longtine Center for Molecular Biology and Genetics, Sun Health Research Institute, Sun City, AZ 85351*

2 *Department of Microbiology, Midwestern University, Glendale, AZ 85308*

3 *Fundacion Instituto Leloir, Buenos Aires, Argentina C1405-B1*

4 *Department of Cell Biology and Anatomy, National Cheng Kung University, Tainan, Taiwan 70101*

5 *Pfizer Global Research and Development, Groton, CT 06340*

6 *Oregon Health and Science University, Department of Neurology, Portland, OR 97239*

7 *Cleo Roberts Center for Clinical Research, Sun Health Research Institute, Sun City, AZ 85351*

8 *Department of Neurology, Mayo Clinic, Scottsdale, AZ 85259*

9 *W. H. Civin Laboratory of Neuropathology, Sun Health Research Institute, Sun City, AZ 85351*

Abstract

Background—We evaluated the amounts of amyloid-beta (Aβ) peptides in the central nervous system (CNS) and in reservoirs outside the CNS and their potential impact on Aβ plasma levels and Alzheimer's disease (AD) pathology.

Methods—Amyloid-β levels were measured in: 1) The plasma of AD and non-demented (ND) controls in a longitudinal study 2) The plasma of a cohort of AD patients receiving a cholinesterase inhibitor 3) The skeletal muscle, liver, aorta, platelets, leptomeningeal arteries and in gray and white matter of AD and ND control subjects.

Results—Plasma Aβ levels fluctuated over time and among individuals suggesting continuous contributions from brain and peripheral tissues and associations with reactive circulating proteins.

Corresponding Author: Alex E. Roher, MD., PhD., 10515 W. Santa Fe Dr., Sun City, AZ 85351, Phone: (623)-876-5465, Fax: (623)-876-5698, E-mail: alex.roher@sunhealth.org.
*Mark R. Emmerling (present address), 1237 N. Freer Rd., Chelsea, MI 48118
#Nina B. Silverberg (present address), Neuroscience and Neuropsychology of Aging, National Institute on Aging, Bethesda, MD 20892
¶James D. Seward (present address), Banner Alzheimer's Institute, Phoenix, AZ 85006

Conflicts of Interest: The authors do not have any conflicts of interests with the expection of Dr. Marwan N. Sabbagh who receives support from Pfizer, Novartis, Elan, Wyeth, Medivation and Lilly. He is on the speaker's bureau for Eisai, Pfizer, Novartis and Forest and a consultant for Lilly.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Arteries with atherosclerosis had larger amounts of Aβ40 than disease-free vessels. Inactivated platelets contained more Aβ peptides than activated ones. Substantially more Aβ was present in liver samples from ND patients. Overall, AD brain and skeletal muscle contained increased levels of Aβ.

Discussion—Efforts to employ plasma levels of Aβ peptides as AD biomarkers or disease staging scales have failed. Peripheral tissues may contribute both to the circulating amyloid pool and AD pathology within the brain and its vasculature. The wide spread of plasma Aβ values is also due in part to the ability of Aβ to bind to a variety of plasma and membrane proteins. Sources outside the CNS must be accounted for as pharmacological interventions to reduce cerebral amyloid are assessed by monitoring Aβ plasma levels. Furthermore, the long-range impact of Aβ immunotherapy on peripheral Aβ sources should also be considered.

Keywords

plasma Aβ; Alzheimer's disease; peripheral Aβ; atherosclerotic vascular disease; Aβ immunotherapy

1. Background

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by dementia and an abundance of amyloid-beta (Aβ) peptides in the brain parenchyma and cerebral vasculature that are derived from the amyloid-beta precursor protein (AβPP). Genetic investigations strongly suggest that Aβ peptides have a central role in AD pathogenesis. However, genetically-determined AD is rare, while the sporadic form of the disease accounts for up to 98% of patients. The cause of sporadic AD is complex and multifactorial, with contributions from genetic as well as environmental factors. Amyloid-β peptides are considered an important pathological marker of AD due to their profuse extracellular deposition in senile and diffuse plaques and vascular walls. In addition, Aβ is present in neurons and glial cells and in soluble oligomeric forms that diffuse along the narrow extracellular space of the brain. During the last three decades, enormous resources have been expended to determine the chemical structure of the Aβ peptides in their diverse physical forms and to elucidate the multiple functional roles of Aβ. Most important, vast investments have been made to create therapeutic agents that either interfere with Aβ production or are capable of dispersing amyloid deposits.

Due to the omnipresence of Aβ peptides in familial and sporadic AD, the levels of these molecules in physiological fluids have been measured and characterized in order to establish their potential utility as reliable disease biomarkers. Unfortunately, despite the seemingly obvious association of Aβ with AD pathology, the efforts to validate plasma Aβ peptides as dementia biomarkers and correlate threshold concentration levels with disease stage have been fraught with frustration [1]. In the brain, Aβ peptides exist both within defined deposits (plaques and vascular) and as a diverse array of other forms including soluble, membrane associated and intracellular species that may play far more significant roles in the production of dementia than the molecules sequestered in extracellular plaques. In addition, the critical role that circulating Aβ peptides play in AD pathology cannot be ignored. Besides the brain, Aβ peptides are generated outside of the central nervous system (CNS) in appreciable quantities by the skeletal muscle, platelets and vascular walls [2–4]. Other non-neural tissues expressing AβPP include: pancreas, kidney, spleen, heart, liver, testis, aorta, lung, intestines, skin as well as the adrenal, salivary and thyroid glands [5–7]. These distinct reservoirs may allow for an active and dynamic interchange of Aβ peptides between the brain and periphery. These sources undoubtedly contribute to the pool of circulating Aβ, and must be considered when the success or failure of AD pharmacological interventions, intended to reduce cerebral amyloid, is assessed by monitoring Aβ plasma levels. Interestingly, Aβ peptides in the periphery fail to generate filamentous structures probably due to the presence of albumin, erythrocyte

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

membrane proteins and the other multiple circulating molecules that bind Aβ peptides avidly and thereby alter their apparent free-plasma levels [8–10].

In the present study, the potential significance of the different sources and pools of Aβ on the plasma levels of these peptides and on the general pathology of AD were evaluated in several complimentary ways. A comprehensive, longitudinal assessment of plasma Aβ levels in AD and age-matched non-demented (ND) control individuals was performed at baseline, 3, 6 and 12 months. An additional longitudinal study determined the potential effects of the cholinesterase inhibitor donepezil hydrochloride (Aricept) on plasma Aβ levels in AD patients and compared them to a control group. Amyloid-β levels were also estimated in inactivated platelets and collagen/thrombin activated platelets. Finally in an autopsy cohort, the levels of Aβ peptides were determined in skeletal muscle, in human aorta and leptomeningeal arteries with and without atherosclerotic vascular disease (AVD), human liver and independently in brain gray and white matter in AD and in ND matching age controls.

2. Materials and Methods

2.1 Human Subjects

All clinical protocols and experiments were carried out under the guidelines of Sun Health Corporation Institutional Review Board and Oregon Health and Science University Institutional Review Board. All living specimen donors signed a consent agreement for participation in the present investigation. Postmortem specimens were obtained from the Brain and Body Donation Program of Sun Health Research Institute (SHRI) [11].

2.2 Quantification of Aβ peptides in plasma

The inclusion criteria is as follows: subjects must be at least 65 years, fluent in English, accompanied by a collateral informant, educated (at least a 6th grade level), able to provide a work history, able to sign and date the informed consent and able to meet one of the diagnostic criteria (ND control, AD, mild cognitive impairment, vascular dementia, or non-AD dementia). Subjects were not restricted by gender, race or ethnic background. Subjects were excluded if they are diagnosed with delirium (DSM-IV), were not able to be assessed due to conditions such as blindness or deafness, had a history of alcohol or substance abuse/dependence (DSM-IV) within the past 10 years, were unable to undergo brain MR scanning or could not provide a collateral informant. The diagnosis was based on the NINDS-ADRDA criteria. Blood samples were obtained at baseline, 3 months, 6 months and 12 months, under fasting conditions, from 17 individuals with clinically-diagnosed AD (average age 81.4 years; 10 females and 7 males) and 21 ND subjects (average age 75.8 years;14 females and 7 males). Due to subject attrition, in the total of 38 enrolled individuals, 28 cases had their plasma analyzed at 4 time points and 10 cases at 3 time points. The average mini-mental state examination (MMSE) score for the ND control group was 29.0 (range 24–30) and 22.3 (range 15–30) for the AD group **(**Table 1).

The plasma levels of Aβ1-40 and Aβ1-42 peptides were immunoassayed, in duplicate, by enzyme-linked immunosorbent assays (ELISA) that were obtained from Immunobiological Laboratories (Minneapolis, MN) and from Innogenetics (Gent, Belgium), respectively. The ELISAs were carried out following the manufacturer's instructions and executed by the same investigator who was blind to the identity of the specimens. The high sensitivity method was used for the Aβ1-42 kit. Aβ1-40 had a measurement range of 7.81 to 500 pg/ml and a sensitivity of 5.00 pg/ml. The coefficient of variation (CV) values for inter-assay measurements were < 7%. The CV values for intra-assay measurements were < 8%. Using the high sensitivity method, the Aβ1-42 ELISA had a measurement range of 7.81 to 1000 pg/ml and the sensitivity

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

was 5.00 pg/ml. The CV for inter-and intra-assay measurements were < 10% and < 5%, respectively [12].

In a second study, the potential disease-modifying effects of donepezil, a cholinesterase inhibitor, were evaluated by quantifying the levels of Aβ in plasma. The inclusion criteria for this study were diagnosis of probable Alzheimer's disease according to NINDS-ADRDA criteria, MMSE > 10, and willingness to undergo serial blood draws. The "donepezil-initiation" and the "stable-donepezil" groups were well matched in age **(**Table 2). The Aβ peptide levels were determined in a population of 28 individuals at baseline and 12 weeks later. All subjects had a diagnosis of probable AD. Twenty subjects had never been exposed to cholinesterase inhibitors at baseline. After the baseline plasma collections, these subjects initiated donepezil at a dose of 5 mg PO per day for one month and then increased to 10 mg PO per day and remained on this dose until the follow-up plasma sampling. This group was compared to a second group of 8 subjects who were on stable doses of donepezil 10 mg per day for at least 6 months prior to baseline, and who remained on donepezil through the time of follow-up to plasma collection. At both time stages, the plasma Aβ levels were quantified by ELISA by taking 3 plasma samples per individual on Monday, Wednesday and Friday, under fasting conditions, at base time and 12 weeks afterward. The "donepezil-initiation" cohort was composed of 15 females and 5 males, average age 76.4 years and the "stable-donepezil" cohort was composed 6 females and 2 males, average age 75.8 years. At baseline, the average MMSE of the donepezil-initiation cohort was 23.5 (range 14–28) and the stable-donepezil group was 18.5 (range 11–25). All individuals completed the study. All immunoassay evaluations of Aβ40 and Aβ42 were performed using the same ELISA techniques as described above.

2.3 Quantification of Aβ peptides in brain gray matter

Amyloid-β peptides were quantified in the superior frontal gyrus of 23 AD neuropathologically-diagnosed cases (average age 85.6 years; 11 females and 12 males) and 20 ND individuals (average age 77.7 years; 5 females and 15 males). All brain specimens were obtained from the rapid autopsy Brain Bank of SHRI and had an average postmortem delay time of 2.5 h [11]. All AD and ND brains were rated following the CERAD criteria (Consortium to Establish a Registry for Alzheimer's Disease) and the Braak stage classification. All 43 AD and ND cases were apolipoprotein E (Apo E) genotyped. Briefly, the cerebral frontal gyrus cortices were homogenized with glass-distilled formic acid (GDFA) and the acid-soluble fraction submitted to fast protein liquid chromatography (FPLC) on a Superose 12 column (Amersham/GE Healthcare) using 80% GDFA as the mobile phase [13]. The fractions containing the Aβ peptides were collected and neutralized and the Aβ40 and Aβ42 quantified by Europium immunoassay using antisera against Aβ40 (Aβ residues 34–40) and Aβ42 (Aβ residues 36–42) as capture antibodies and the Europium labeled anti Aβ 17–24 (4G8) as the reporter antibody (Zenetec Maryland Heights, MO) and the Europium enhancement solution (Wallac Inc., Gaithersburg MD), as previously described [2;10].

2.4 Quantification of Aβ peptides in white matter

The Aβ peptides were quantified in the white matter (WM) of 10 cases of neuropathologically-diagnosed AD (average age 84.0 years; 6 females and 4 males) with moderate to severe WM rarefaction and from 13 ND controls (average age 82.8; 7 females and 6 males) with none to mild WM rarefaction. The GDFA-soluble Aβ peptides were separated by FPLC and after neutralization submitted to europium immunoassay as described in detail in a previous publication [13].

2.5 Quantification of Aβ peptides from inactivated and activated human platelets

Fresh human platelets were obtained from 5 Red Cross donors by platelet-pheresis. The experimental processing of the platelets was performed following the protocols published by

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Li QX, et al. [3;14] with minor modifications. The platelets were suspended in a final plasma volume of 220–300 ml, which included 32–50 ml of Anticoagulant Citrate Dextrose Solution-Formula A (ACD-A) and maintained at room temperature (~25° C). The platelet suspensions were centrifuged (type 19 rotor, Beckman Coulter) at 3,800 × *g* for 30 min at 25° C in 250 ml-capacity polyallomer bottles. Following removal of the plasma, the pelleted platelets were suspended in a total volume of 200 ml of 0.38% sodium citrate, 0.6% glucose and 0.72% NaCl, pH 7.0 (washing buffer: WB) and this washing and centrifugation step was repeated twice. The supernatants were eliminated and each of the individual platelet preparations divided into 3 equal fractions and washed once more with 32 ml of WB. The supernatants were discarded. Two ml of the inactivated pelleted platelets were lysed by the addition of 10 ml of 98% GDFA using a 30 ml-capacity glass homogenizer. The platelet homogenate was centrifuged in polyallomer tubes at 250,000 × *g* for 1 h at 25° C in a SW41 rotor (Beckman Coulter, Fullerton, CA). The top layer of lipids and small pellet of insoluble material were eliminated and the intermediate supernatant fraction collected and apportioned into 500 μl samples that were submitted to FPLC size-exclusion Superose 12 columns (Amersham Biosciences) using 80% GDFA as the mobile phase to isolate Aβ peptides, as previously described [13].

For the preparation of activated platelets, the platelets were separated from plasma as described above. A volume of 2 ml of the pelleted platelets was suspended in 20 ml of Tyrode's buffer (137 mM NaCl, 2.68 mM KCl, 11.9 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5.5 mM glucose, pH 7.4) containing 1 unit per ml of human thrombin (Calbiochem, San Diego, CA) and 20 μg/ml of human collagen (Sigma, St. Louis, MO). The activated clumped platelets were dispersed and after 30 min of stirring, the whole preparation was freeze-dried. To the recovered lyophilized powder, 10 ml of 80% GDFA was added and the suspension thoroughly homogenized (Tenbroeck glass homogenizer), centrifuged at 250,000 × *g* (SW41 rotor) for 1 h using polyallomer tubes. The top layer of lipids and insoluble pellet were discarded and the supernatant submitted in 500 μl aliquots to FPLC separation [13]. Both the activated and inactivated platelet fractions containing the Aβ peptides were submitted to ELISA as in Section 2.2.

2.6 Quantification of Aβ peptides from aorta

The amounts of Aβ peptides present in the aortic walls of 6 elderly individuals (mean age 83 years) with severe AVD were quantified. The atherosclerotic specimens (~9 g of tissue) had extensive zones of calcification and multiple complicated lesions with ulceration and rupture of the fibrous caps showing areas of thrombosis. These complicated aortic atheromatous lesions also showed large crater-like morphology with hemorrhagic areas. For the control, we utilized a pool of two aortic specimens (~1.5 g each) from individuals with a mean age of 82 years, without atherosclerotic lesions and minimal fatty streaks. All specimens were extensively rinsed with cold distilled water to remove all traces of blood. The adventitial connective tissue, easily dissected from the subjacent tunica media, was removed. The aortic tissues were pooled, minced and pulverized in liquid nitrogen using a mortar and pestle, suspended in 20 ml of 98% GDFA and stirred overnight at 4° C. The suspension was centrifuged at 250,000 × *g* for 1 h at 4° C using a SW-41 Ti rotor (Beckman). The acid-soluble phase was collected and filtered (Whatman # 1 paper). Five hundred μl aliquots of GDFA samples were submitted to FPLC [13].

The fractions containing Aβ peptides were pooled, the volume reduced to 500 μl and dialyzed (Spectrapor # 6 membrane 1,000 Da cut off) against deionized water, followed by dialysis against 5 M guanidine-hydrochloride, 50 mM Tris-HCl, pH 8.0 at room temperature. For quantification the dialyzed samples were submitted to Aβ40 and Aβ42 ELISAs as described in Section 2.2.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2.7 Quantification of Aβ peptides from leptomeningeal arteries

Two grams of leptomeningeal arteries, representing the anterior, middle, and posterior cerebral arteries showing advanced AVD lesions were dissected and pooled from 4 individuals with AD (average age 82.0 years; 2 males and 2 females) and from a group of 5 ND individuals without AVD (average age 86.6 years; 3 females and 2 males). The two separate leptomeningeal arterial pools were minced, extensively rinsed with cold distilled water to remove blood cells and thoroughly homogenized in 10 ml of GDFA. The leptomeningeal vessels, with and without AVD, were centrifuged and the acid supernatant collected and submitted to size-exclusion FPLC (Superose 12) in 80% GDFA [13]. The Aβ containing fractions were pooled, the volume reduced and the samples dialyzed as described for the aorta specimens. The Aβ peptides were quantified by ELISA as described in Section 2.2.

2.8 Quantification of Aβ peptides in skeletal muscle

Temporalis skeletal muscles were obtained from the same population in which brain Aβ was quantified in the superior frontal gyrus (see above). A detailed extraction, purification and europium immunoassay of Aβ peptides present in skeletal muscle are fully described elsewhere [2].

2.9 Quantification of Aβ peptides from liver

Liver specimens (1 g each) were obtained from 6 neuropathologically diagnosed AD cases (average age 83 years; 3 females and 3 males) and from 6 ND individuals (average age 87 years; 2 females and 4 males). The liver tissues were minced and thoroughly washed to eliminate entrapped blood as much as possible. Each liver specimen was homogenized in 90% GDFA, centrifuged (250,000 × *g*, 1 h at 4°C) and the acid soluble fraction collected and submitted to FPLC [13]. After volume reduction and dialysis (see aorta preparation), Aβ peptides were quantified by ELISA as described in Section 2.2.

3. Results

3.1 Plasma Aβ

All mean values in this study were compared using two-independent sample t-tests. The differences in Aβ40 and Aβ42 levels, the total Aβ levels (Aβ40+Aβ42) and the Aβ42/Aβ40 ratios between the AD and ND groups were not statistically significant **(**Table 1 and Figure 1). The AD (mean age 81.4, SD = 5.3) and ND (mean age 75.8, SD = 7.1) groups differed significantly in age (p = 0.01), but age did not correlate with plasma concentrations of Aβ40, Aβ42 or Aβ40/42 ratio, within either the ND or AD groups (r squared values ranging from 0.00006 to 0.04). T-tests were performed without correction for multiple comparisons (e.g. Bonferroni), as this would have biased the results towards an increased rate of false positive findings. As the results with respect to plasma Aβ concentrations were all negative, we did not consider it necessary to apply a correction for multiple comparisons. The same situation was observed in the donepezil therapeutic study in which the high range of variability was confirmed and no statistically significant differences were found between the donepezil-initiation and stable-donepezil populations **(**Table 2 and Figure 2) and no significant correlations were evident between ADAS-Cog, MMSE score, age or gender and *Apo E* genotype.

Amyloid-β40 and Aβ42 levels fluctuated widely among individuals over time in both AD subjects and ND controls **(**Figure 1). The amounts of Aβ40 among individuals over the year of the study fluctuated from 659 pg/ml to 16 pg/ml and Aβ42 levels varied between 149 pg/ml and 4.0 pg/ml. The average Aβ42/40 ratios also varied substantially among individuals (see Tables 1 and 2).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3.2 Brain Aβ (gray matter)

Quantifying Aβ peptides in the cerebral cortex demonstrated the prevalence of Aβ42 over Aβ40 in AD individuals [2], with a high level of variability **(**Table 3). The mean value of Aβ42 was 6,096 ng/g tissue (range: 1,047–19,680 ng/g; SD: 1,099) with Aβ40 averaging 608 ng/g tissue (range: 26–3,434 ng/g; SD: 154). By contrast, in the ND control population the average amount of Aβ42 was 784 ng/g tissue (range: 42–3,402; ng/g; SD 221) and for Aβ40 was 209 ng/g tissue (range: 0–731 ng/g; SD: 59). There were significant differences in the amounts of cortical Aβ peptides observed between AD and ND (Aβ42 $p < 0.001$; Aβ40 $p = 0.022$) as well as between total Aβ ($p = < 0.001$). In those individuals with AD carrying the *Apo E*ε4 allele the mean value for total Aβ was 8,373 ng/g of cortex while in those without the *Apo E*ε4 the total Aβ mean was 5,631 ng/g ($p = 0.223$). In the case of the ND cohort, those individuals carrying the *Apo E*ε4 had a total Aβ average of 2,178 ng/g of cortex whereas those without the *Apo E*ε4 allele had a mean of 783 ng/g of tissue ($p = 0.043$). However, this marginally significant value may reflect the fact that only 3 heterozygous individuals out of 20 harbored an *Apo E*ε4 allele.

3.3 Brain Aβ (white matter)

Investigation of the Aβ peptide levels in WM revealed that in AD the mean values for Aβ42 and Aβ40 were 1,135 ng/g of tissue (range: 323–3,313 ng/g; SD: 895) and 1,069 ng/g of tissue (5.0–4,149 ng/g; SD: 1601) [13], respectively **(**Table 3). The highest values of Aβ among the 10 AD subjects were exhibited in two individuals carrying the *Apo E*ε4/ε4 genotype with Aβ42 levels of 1,624 ng/g and 3,313 ng/g and Aβ40 4,149 ng/g and 4,030 ng/g of WM. The ND cohort, on the other hand, had an Aβ42 mean of 418 ng/g of tissue (range: 122–880 ng/g; SD: 233) and for Aβ40 111 ng/g of tissue (range: 0–272 ng/g; SD: 77). The differences between the AD and ND groups were significant for both: Aβ42, $p = 0.05$ and Aβ40, $p = 0.02$. The difference between the total Aβ values in AD and ND groups was also statistically significant ($p = 0.02$).

3.4 Platelet Aβ

The Aβ40 and Aβ42 peptides were quantified in a pool of platelets in their quiescent and activated forms from 5 healthy individuals. In the latter case, the levels of Aβ were measured in the platelets and their medium, since activation is followed by immediate secretion of by-products into the medium. The Aβ40 and Aβ42 peptides in the quiescent platelets yielded 83.8 ng/ml and 1.7 ng/ml of packed platelets, respectively. In the case of the activated platelets these peptides represented 56.8 ng/ml and 1.6 ng/ml, respectively **(**Table 3).

3.5 Aortic Aβ

In the aortic walls with advanced atherosclerotic lesions, Aβ40 was present at 75.3 ng/g of tissue and Aβ42 at 0.7 ng/g of tissue, higher levels than those observed in aorta samples with minimal atherosclerotic lesions (fatty streaks) which contained 31.4 ng/g of tissue and 1.0 ng/g of tissue for Aβ40 and Aβ42, respectively **(**Table 3).

3.6 Leptomeningeal vascular Aβ

There were significant differences between the amounts of Aβ peptides present in the leptomeningeal arteries with and without atherosclerotic plaques. In the former, the Aβ40 levels were 113.1 ng/g of tissue and Aβ42 amounted to 28.0 ng/g of tissue, while in the latter these corresponded to 17.3 ng/g and 5.3 ng/g of tissue, respectively **(**Table 3).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

3.7 Skeletal muscle Aβ

Amyloid-β peptides in the AD and ND groups from skeletal muscle demonstrated a lesser degree of variability [2]. The differences between AD and ND control regarding the Aβ40 were borderline significant ($p = 0.067$) while there was a statistically significant difference with respect to the Aβ42 ($p = 0.010$) and total Aβ ($p = 0.019$) **(**Table 3). However, values between the AD and ND groups and between individuals exhibited substantial variance. The *Apo E*ε4 alleles, although more numerous in the AD group (10 out of 46 alleles) than in the ND cohort (3 out of 40 alleles) were not directly correlated with the levels of Aβ40 or Aβ42 peptides in skeletal muscle.

3.8 Liver Aβ

Amyloid-β40 and Aβ42 in the postmortem liver of ND individuals were on the average 67.5 ng/g of tissue (range: 18.8–94.6 ng/g) and 15.5 ng/g of tissue (range: 6.4–20.6 ng/g) while the Aβ40 and Aβ42 in AD subjects averaged 8.6 ng/g of tissue (range: 6.7–11.7) and 1.7 ng/g (range: 0–2.6) of tissue, respectively. The ND control patients' liver tissue contained 8-fold more total Aβ than AD liver with a total mean value for the ND control group of 83 ng/g of tissue and 10.3 ng/g of tissue in the AD group **(**Table 3).

4. Discussion

Longitudinal studies of plasma Aβ40 and Aβ42 levels demonstrated wide temporal variation within and among the individuals involved in the present investigation. The overall mean values for Aβ40 and Aβ42 in the longitudinal study were 384 pg/ml and 132 pg/ml, respectively. The overall mean values for Aβ40 and Aβ42 in the therapeutic study were 319 pg/ml and 179 pg/ml, respectively. These two independent plasma analyses showed virtually equivalent levels for total Aβ peptides: longitudinal = 517 pg/ml and therapeutic = 497 pg/ml. In both investigations there were no statistical correlations noted between Aβ values and MMSE scores, diagnoses, age or gender. In support of our observations are several previous biomarker studies in which the plasma Aβ levels were not correlated with the diagnosis, medications or with Apo E genotype [15;16] (reviewed in [1]).

The spread of plasma Aβ values may be due to the amphoteric and amphipathic structure of Aβ peptides that results in their avid binding to a variety of plasma and membrane proteins. At physiological concentrations of human serum albumin (approximately 40 mg/ml), this molecule can bind more than 95% of Aβ40/42 peptides at a stochiometry of 1:1. Kinetic studies have demonstrated that 1 ml of fresh plasma from healthy individuals, spiked with 5 ng or 20 ng Aβ40 or Aβ42 results in the recovery of 36% of Aβ1-40 and 26% of Aβ1-42 after a 1 h incubation [10]. When variable amounts of washed erythrocytes are diluted with PBS to hematocrit concentrations of 10% to 50% and are incubated with 5 ng/ml of Aβ40 or Aβ42, the amount of free Aβ peptides decreased as the hematocrit values increased. A large number of plasma proteins including immunoglobulins, apolipoprotein J, Apo E, complement C1q, C4, C3, C5 and C6, transthyretin, apoferritin, amyloid-P component and α2-macroglobulin bind and mask Aβ peptides [9;10;17]. Freshly purified plasma lipoproteins are capable of sequestering 94% of circulating Aβ peptides [9]. In particular Apo E, a ligand for various lipoprotein receptors bound to the surface of circulating low density lipoprotein (LDL), has high affinity for Aβ peptides [18;19].

Plasma cholesterol appears to play a pivotal role in Aβ chemistry. The production of lipoproteins by liver and brain are likely to cause concomitant fluctuations in plasma levels of free and bound Aβ. Interestingly, individuals with higher midlife plasma cholesterol levels have an elevated risk of developing AD, and individuals with clinically or neuropathologically diagnosed AD have higher plasma cholesterol levels compared to ND controls [20;21].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Furthermore, in AβPP Tg mice it has been observed that hypercholesterolemia accelerates the evolution of amyloid pathology [22] and that cholesterol accumulates in senile plaques of AD patients as well as AβPP Tg mice [23]. In this context, a broad body of evidence derived from postmortem, epidemiologic, correlative and experimental studies links AVD with AD and multiple established risk factors for AVD have now been recognized to be risk factors for the development of AD [24–28]. Our studies also revealed that aortas with either mild fatty streaks or advanced AVD contained Aβ peptides, predominately Aβ40. Likewise, leptomeningeal arteries with AVD pathology have larger amounts of Aβ peptides than lesion-free arteries. A feature common to both AVD and vascular amyloidosis is a persistent degenerative pathology associated with chronic arterial wall inflammation [27;29]. Amyloid-β accumulation also induces microvascular inflammation mediated by proinflammatory molecules activated in glia, endothelium, smooth muscle cells and pericytes [30]. Circulating markers of inflammation are also increased in AVD, among them C-reactive protein [31]. Some of these proinflammatory molecules also represent risk factors for AD [32;33]. Cognitive decline in AD is apparently associated with the degree of cerebral amyloid angiopathy, arteriosclerosis and lipohyalinosis [34]. In particular, the capillary deposition of Aβ42 is highly correlated with AD pathology [35]. The severity of circle of Willis and leptomeningeal arterial stenosis caused by AVD significantly correlates with the neuropathological lesions of AD [25–28]. Inferring a functional, pathologic role for the Aβ peptides in atherosclerotic plaques is reasonably justified by the multiple associations between hypercholesterolemia and AVD [31], hypercholesterolemia and AD [36] and AVD and AD [25;28].

Amyloid-β in the brain is mostly synthesized by neurons, but other cells such as glia, vascular endothelia and myocytes also have the means to produce AβPP and Aβ peptides. Although there are multiple mechanisms for uptake and clearance of Aβ peptides through the cerebrovasculature, the functionality of these pathways during the aging process has not been well established [37]. The receptor for advanced glycation end-products (RAGE) and the LDL receptor and LDL receptor related protein (LRP) have been advocated as active mechanisms for uptake and clearance of Aβ, respectively [38–41]. Amyloid-β is also eliminated from the brain through the periarterial spaces of the cerebral vasculature that shunt Aβ peptides into the lymphatics of the head and neck and finally back into the systemic venous circulation [42; 43]. This suggests the clogging of the periarterial spaces in cortical and leptomeningeal arteries by fibrillar Aβ deposition is probably one of the more devastating pathological hemodynamic events in AD [44]. In AD, the WM contains 4-fold more ‘soluble’ total Aβ (mean 2,200 ng/g tissue) than ND controls (mean 520 ng/g tissue) [13]. It can be postulated that soluble Aβ in the WM normally drains through the periarterial spaces into the systemic circulation, as suggested by the gross dilation of the periarterial spaces observed in AD [44].

Platelets represent another significant source of Aβ peptides in the circulation. These structures mostly contain Aβ ending at residue 40, with a small amount of Aβ42, in both the quiescent and activated states. Our *in vitro*, thrombin-collagen activated platelets released Aβ40 and Aβ42. If this phenomenon occurs *in vivo*, platelets contribute to the pool of circulating Aβ peptides [45;46]. Intriguingly, our activated platelets apparently contain ~30% less Aβ40 than the quiescent ones. Although we do not have an explanation for this decay, it is possible that platelet activation and release of Aβ stimulates aggregation or sequestration of these peptides, thus escaping detection by the immunoassay. Alternatively, Aβ may intervene to facilitate platelet aggregation [47]. Platelets express $A\beta PP_{695}$, $A\beta PP_{751}$ and $A\beta PP_{770}$ isoforms, whose ratio appears to be altered in AD [48–50]. The latter two, also known as protease nexin-2 (PN2), have a serine (Kunitz) protease inhibitor (KPI) insert 56 amino acids long close to their N-termini [3;14;46;51]. This domain has an important role in the coagulation cascade where it functions as an inhibitor of factors, IXa, Xa, XIa and tissue factor VIIa complex [52]. AβPP and Aβ peptides have been observed in atherosclerotic plaque macrophages that have engulfed platelets in areas of neo-vascularization [53;54]. Although platelets and circulating Aβ may be

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

a significant source of these peptides in the atherosclerotic lesions, it is possible that additional Aβ peptides originate directly from the arterial tunica media myocytes and endothelial cells that also express the AβPP molecule [55]. During the process of aging, many aberrations occur in the vascular walls and endothelium as well as in platelets [56], hemostatic functions and fibrinolytic activity that lead to increased atherosclerosis and thrombosis [57;58]. Several studies have suggested that platelets are an important link between vascular repair and vascular amyloidosis and between atherosclerosis and AD [54;59].

The cholinergic neuromuscular junctions of skeletal muscle are another potentially rich source of Aβ peptides [2;60;61]. The production of Aβ peptides is exacerbated in inclusion body myositis, the most common skeletal muscle inflammatory disease among the elderly [61–63]. Our studies revealed significant differences in Aβ42 and total Aβ between AD and ND control populations. This observation suggests that AD might have systemic manifestations. Skeletal muscle also generates longer Aβ peptides ending at residues 44, 45 and 45 [2]. The potential contribution of skeletal muscle Aβ to the circulating pool should be considered since the total muscle mass represents about one third of the body weight.

The AβPP/Aβ peptides produced in different tissues may also have different half lives and degradation rates since these molecules are substrates for a large variety of proteolytic enzymes (reviewed in [64]). The liver is the major organ responsible for Aβ clearance from plasma, capable of capturing up to 90% of the circulating Aβ peptides, a fraction of which is degraded with the remainder removed through the bile [65]. A comparatively smaller amount of Aβ is eliminated through the kidneys and secreted in the urine [66]. Additionally, the liver endocytic uptake is mediated by the hepatocyte LRP-1 and enhanced by the presence of insulin [67]. The reduction in the amount of liver Aβ in the AD cases may be due to liver failutre in the advanced stages of the disease.

In summary, Aβ peptides are produced by a wide variety of tissues in both the CNS and periphery. Several lines of evidence suggest that a global assessment of Aβ sources and sinks may help to understand AD pathology and dementia. It will also help to elucidate the relationship between Aβ and cholesterol metabolism as well as the role of Aβ in AVD. Large individual fluctuations in plasma Aβ values are the most important observation in our longitudinal studies. When assessing Aβ therapeutic interventions, the potential multiple contributions to the plasma Aβ pool as well as the time and conditions of sampling physiological fluids for Aβ evaluation need to be considered. For example, CSF specimens demonstrated wide ranging Aβ levels within individuals along a period of 36 h [68]. We are aware that investigations of this type have limitations including small sample size due to difficulties in recruitment and attrition of participants and patient physical conditions. In addition, Aβ peptides fluctuations due to diet, medications, stress, circadian rhythm [68], metabolic conditions, etc. can affect the outcome.

Therapeutic vaccination trials in Tg animals and humans have revealed that senile plaques, a cardinal pathologic feature of AD, are dynamic structures [69] subject to dissolution by Aβ immunotherapy [42;70]. Peripheral Aβ production appears to contribute to brain amyloid through transport into the CNS and correspondingly the brain Aβ contributes to the pool in circulation [37]. Therefore, brain as well as plasma Aβ levels are the consequence of the intricate relationships that exist among several interacting Aβ peptides sources that are tempered by the natural physiologic decline accompanying aging and associated morbidities. The ultimate pathology of AD is focused within the brain, but neither the brain nor AβPP/Aβ exist as neatly isolated entities. The long term CNS and systemic consequences of Aβ immunization and secretase inhibitory treatments have the potential to disturb a wide range of cellular and systemic functions, in which AβPP metabolites and Aβ are essential [71–74]. As clinical trials advance, efforts should be undertaken to recognize adverse events both within

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and outside the brain proper. Although Aβ deposits are a logical therapeutic focus, it remains unclear whether the deposited or soluble forms of this molecule are the most toxic. Indeed, senile plaques may represent a mechanism of defense whereby excessive harmful levels of soluble Aβ peptides are inactivated into fibrillar core structures surrounded by glial cells [74–76]. Preventing or dissolving these deposits may be injurious to the brain. Understanding the dynamic balance between Aβ pools and their function may add clarity and suggest new routes to improve AD therapeutic strategies.

Acknowledgements

We are in debt to Dr. Douglas Walker for *apolipoprotein E* genotyping. This study was supported by the National Institute on Aging RO1-AG 19795 and AG11925, NIA Arizona Alzheimer Disease Core Center P30-AG19610, State of Arizona Alzheimer Disease Consortium, Arizona-ARC ADHS AGR2007-37, AZ-APDC AZPD-0011, Pfizer Global Research and Development and an Investigator initiated grant from Pfizer AG08017.

Abbreviations

Aβ
amyloid-beta

AβPP
amyloid-beta precursor protein

ACD-A
anticoagulant citrate dextrose solution – formula A

AD
Alzheimer's disease

ADAS-Cog
Alzheimer's Disease Assessment Scale-cognitive subscale

Apo E
apolipoprotein E

AVD
atherosclerotic vascular disease

CNS
central nervous system

CERAD
consortium to establish a registry for Alzheimer's disease

ELISA
enzyme-linked immunosorbent assay

FPLC
fast protein liquid chromatography

GDFA
glass distilled formic acid

GM
gray matter

KPI
Kunitz protease inhibitor

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

LDL
low-density lipoprotein

LRP
low-density lipoprotein receptor related protein

MMSE
mini-mental state examination

ND
non-demented

NINDS-ADRDA
National Institute of Neurological Disorders and Strokes, and Alzheimer's Disease and Related Disorders

PN2
protease nexin-2

PO
per os, orally

RAGE
receptor for advanced glycation end products

SD
standard deviation

SHRI
Sun Health Research Institute

Tg
transgenic

WB
washing buffer

WM
white matter

Reference List

1. Hansson O, Zetterberg H, Vanmechelen E, Vanderstichele H, Andreasson U, Londos E, et al. Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. Neurobiol Aging. 2008
2. Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, et al. Elevated abeta42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AbetaPP metabolism. Am J Pathol 2000;156:797–805. [PubMed: 10702395]
3. Li QX, Whyte S, Tanner JE, Evin G, Beyreuther K, Masters CL. Secretion of Alzheimer's disease Abeta amyloid peptide by activated human platelets. Lab Invest 1998;78:461–469. [PubMed: 9564890]
4. Van Nostrand WE, Melchor JP. Disruption of pathologic amyloid beta-protein fibril assembly on the surface of cultured human cerebrovascular smooth muscle cells. Amyloid 2001;8(Suppl 1):20–27. [PubMed: 11676286]
5. Selkoe DJ, Podlisny MB, Joachim CL, Vickers EA, Lee G, Fritz LC, et al. Beta-amyloid precursor protein of Alzheimer disease occurs as 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. Proc Natl Acad Sci U S A 1988;85:7341–7345. [PubMed: 3140239]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

6. Catteruccia N, Willingale-Theune J, Bunke D, Prior R, Masters CL, Crisanti A, et al. Ultrastructural localization of the putative precursors of the A4 amyloid protein associated with Alzheimer's disease. Am J Pathol 1990;137:19–26. [PubMed: 2372041]
7. Sandbrink R, Masters CL, Beyreuther K. Beta A4-amyloid protein precursor mRNA isoforms without exon 15 are ubiquitously expressed in rat tissues including brain, but not in neurons. J Biol Chem 1994;269:1510–1517. [PubMed: 8288617]
8. Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. J Biol Chem 1996;271:32916–32922. [PubMed: 8955133]
9. Kuo YM, Emmerling MR, Lampert HC, Hempelman SR, Kokjohn TA, Woods AS, et al. High levels of circulating Abeta42 are sequestered by plasma proteins in Alzheimer's disease. Biochem Biophys Res Commun 1999;257:787–791. [PubMed: 10208861]
10. Kuo YM, Kokjohn TA, Kalback W, Luehrs D, Galasko DR, Chevallier N, et al. Amyloid-beta peptides interact with plasma proteins and erythrocytes: implications for their quantitation in plasma. Biochem Biophys Res Commun 2000;268:750–756. [PubMed: 10679277]
11. Beach TG, Sue LI, Walker DG, Roher AE, Lue L, Vedders L, et al. The Sun Health Research Institute Brain Donation Program: Description and Experience, 1987–2007. Cell Tissue Bank 2008;9:229–245. [PubMed: 18347928]
12. De JC, Cras P, Vanderstichele H, Cruts M, Vanderhoeven I, Smouts I, et al. Evidence that Abeta42 plasma levels in presenilin-1 mutation carriers do not allow for prediction of their clinical phenotype. Neurobiol Dis 1999;6:280–287. [PubMed: 10448055]
13. Roher AE, Weiss N, Kokjohn TA, Kuo YM, Kalback W, Anthony J, et al. Increased A beta peptides and reduced cholesterol and myelin proteins characterize white matter degeneration in Alzheimer's disease. Biochemistry 2002;41:11080–11090. [PubMed: 12220172]
14. Li QX, Evin G, Small DH, Multhaup G, Beyreuther K, Masters CL. Proteolytic processing of Alzheimer's disease beta A4 amyloid precursor protein in human platelets. J Biol Chem 1995;270:14140–14147. [PubMed: 7775475]
15. Basun H, Nilsberth C, Eckman C, Lannfelt L, Younkin S. Plasma levels of Abeta42 and Abeta40 in Alzheimer patients during treatment with the acetylcholinesterase inhibitor tacrine. Dement Geriatr Cogn Disord 2002;14:156–160. [PubMed: 12218259]
16. Lopez OL, Kuller LH, Mehta PD, Becker JT, Gach HM, Sweet RA, et al. Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. Neurology 2008;70:1664–1671. [PubMed: 18401021]
17. Watson DE, Roher AE, Kim KS, Spiegel K, Emmerling MR. Complement interactions with amyloid-β1-42: a nidus for inflammation in AD brains. Amyloid: International Journal of Experimental and Clinical Investigation 1997;4:147–156.
18. Pillot T, Goethals M, Najib J, Labeur C, Lins L, Chambaz J, et al. Beta-amyloid peptide interacts specifically with the carboxy-terminal domain of human apolipoprotein E: relevance to Alzheimer's disease. J Neurochem 1999;72:230–237. [PubMed: 9886074]
19. Munson GW, Roher AE, Kuo YM, Gilligan SM, Reardon CA, Getz GS, et al. SDS-stable complex formation between native apolipoprotein E3 and beta-amyloid peptides. Biochemistry 2000;39:16119–16124. [PubMed: 11123940]
20. Kivipelto M, Helkala EL, Hanninen T, Laakso MP, Hallikainen M, Alhainen K, et al. Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. Neurology 2001;56:1683–1689. [PubMed: 11425934]
21. Notkola IL, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, et al. Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. Neuroepidemiology 1998;17:14–20. [PubMed: 9549720]
22. Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, et al. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis 2000;7:321–331. [PubMed: 10964604]
23. Mori T, Paris D, Town T, Rojiani AM, Sparks DL, Delledonne A, et al. Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. J Neuropathol Exp Neurol 2001;60:778–785. [PubMed: 11487052]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

24. Wolozin B, Bednar MM. Interventions for heart disease and their effects on Alzheimer's disease. Neurol Res 2006;28:630–636. [PubMed: 16945215]
25. Roher AE, Esh C, Kokjohn TA, Kalback W, Luehrs DC, Seward JD, et al. Circle of Willis atherosclerosis is a risk factor for sporadic Alzheimer's disease. Arterioscler Thromb Vasc Biol 2003;23:2055–2062. [PubMed: 14512367]
26. Beach TG, Wilson JR, Sue LI, Newell A, Poston M, Cisneros R, et al. Circle of Willis atherosclerosis: association with Alzheimer's disease, neuritic plaques and neurofibrillary tangles. Acta Neuropathol 2007;113:13–21. [PubMed: 17021755]
27. Kalback W, Esh C, Castano EM, Rahman A, Kokjohn T, Luehrs DC, et al. Atherosclerosis, vascular amyloidosis and brain hypoperfusion in the pathogenesis of sporadic Alzheimer's disease. Neurol Res 2004;26:525–539. [PubMed: 15265270]
28. Honig LS, Kukull W, Mayeux R. Atherosclerosis and AD: analysis of data from the US National Alzheimer's Coordinating Center. Neurology 2005;64:494–500. [PubMed: 15699381]
29. Ferreiro JA, Ansbacher LE, Vinters HV. Stroke related to cerebral amyloid angiopathy: the significance of systemic vascular disease. J Neurol 1989;236:267–272. [PubMed: 2760643]
30. Grammas P, Ovase R. Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. Neurobiol Aging 2001;22:837–842. [PubMed: 11754990]
31. Ridker, PM.; Libby, P. Risk Factors for Atherothrombic Disease. In: Zipes, DP.; Libby, P.; Bonow, RO.; Braunwald, E., editors. Braunwald's Heart Disease. Philadelphia: Elsevier Saunders; 2005. p. 939-958.
32. Licastro F, Pedrini S, Caputo L, Annoni G, Davis LJ, Ferri C, et al. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? J Neuroimmunol 2000;103:97–102. [PubMed: 10674995]
33. Zuliani G, Ranzini M, Guerra G, Rossi L, Munari MR, Zurlo A, et al. Plasma cytokines profile in older subjects with late onset Alzheimer's disease or vascular dementia. J Psychiatr Res 2007;41:686–693. [PubMed: 16600299]
34. Thal DR, Ghebremedhin E, Orantes M, Wiestler OD. Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. J Neuropathol Exp Neurol 2003;62:1287–1301. [PubMed: 14692704]
35. Attems J, Lintner F, Jellinger KA. Amyloid beta peptide 1-42 highly correlates with capillary cerebral amyloid angiopathy and Alzheimer disease pathology. Acta Neuropathol 2004;107:283–291. [PubMed: 14986026]
36. Sjogren M, Blennow K. The link between cholesterol and Alzheimer's disease. World J Biol Psychiatry 2005;6:85–97. [PubMed: 16156481]
37. Deane R, Zlokovic BV. Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. Curr Alzheimer Res 2007;4:191–197. [PubMed: 17430246]
38. Ito S, Ohtsuki S, Kamiie J, Nezu Y, Terasaki T. Cerebral clearance of human amyloid-beta peptide (1-40) across the blood-brain barrier is reduced by self-aggregation and formation of low-density lipoprotein receptor-related protein-1 ligand complexes. J Neurochem 2007;103:2482–2490. [PubMed: 17908238]
39. Wilhelmus MM, Otte-Holler I, van Triel JJ, Veerhuis R, Maat-Schieman ML, Bu G, et al. Lipoprotein receptor-related protein-1 mediates amyloid-beta-mediated cell death of cerebrovascular cells. Am J Pathol 2007;171:1989–1999. [PubMed: 18055545]
40. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, et al. Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. J Clin Invest 2000;106:1489–1499. [PubMed: 11120756]
41. Martel CL, Mackic JB, McComb JG, Ghiso J, Zlokovic BV. Blood -brain barrier uptake of the 40 and 42 amino acid sequences of circulating Alzheimer's amyloid beta in guinea pigs. Neurosci Lett 1996;206:157–160. [PubMed: 8710175]
42. Weller RO, Cohen NR, Nicoll JA. Cerebrovascular disease and the pathophysiology of Alzheimer's disease. Implications for therapy. Panminerva Med 2004;46:239–251. [PubMed: 15876980]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

43. Preston SD, Steart PV, Wilkinson A, Nicoll JA, Weller RO. Capillary and arterial cerebral amyloid angiopathy in Alzheimer's disease: defining the perivascular route for the elimination of amyloid beta from the human brain. Neuropathol Appl Neurobiol 2003;29:106–117. [PubMed: 12662319]
44. Roher AE, Kuo YM, Esh C, Knebel C, Weiss N, Kalback W, et al. Cortical and leptomeningeal cerebrovascular amyloid and white matter pathology in Alzheimer's disease. Mol Med 2003;9:112–122. [PubMed: 12865947]
45. Chen M, Inestrosa NC, Ross GS, Fernandez HL. Platelets are the primary source of amyloid beta-peptide in human blood. Biochem Biophys Res Commun 1995;213:96–103. [PubMed: 7639768]
46. Casoli T, Di Stefano G, Giorgetti B, Grossi Y, Balietti M, Fattoretti P, et al. Release of beta-amyloid from high-density platelets: implications for Alzheimer's disease pathology. Ann N Y Acad Sci 2007;1096:170–178. [PubMed: 17405928]
47. Shen MY, Hsiao G, Fong TH, Chen HM, Chou DS, Lin CH, et al. Amyloid beta peptide-activated signal pathways in human platelets. Eur J Pharmacol 2008;588:259–266. [PubMed: 18511035]
48. Di LM, Pastorino L, Bianchetti A, Perez J, Vignolo LA, Lenzi GL, et al. Differential level of platelet amyloid beta precursor protein isoforms: an early marker for Alzheimer disease. Arch Neurol 1998;55:1195–1200. [PubMed: 9740113]
49. Padovani A, Borroni B, Colciaghi F, Pettenati C, Cottini E, Agosti C, et al. Abnormalities in the pattern of platelet amyloid precursor protein forms in patients with mild cognitive impairment and Alzheimer disease. Arch Neurol 2002;59:71–75. [PubMed: 11790233]
50. Liu HC, Wang HC, Ko SY, Wang PN, Chi CW, Hong CJ, et al. Correlation between platelet amyloid precursor protein isoform ratio and cognition in Alzheimer's disease. J Alzheimers Dis 2007;11:77–84. [PubMed: 17361037]
51. Van Nostrand WE, Schmaier AH, Farrow JS, Cines DB, Cunningham DD. Protease nexin-2/amyloid beta-protein precursor in blood is a platelet-specific protein. Biochem Biophys Res Commun 1991;175:15–21. [PubMed: 1900151]
52. Xu F, Davis J, Miao J, Previti ML, Romanov G, Ziegler K, et al. Protease nexin-2/amyloid beta-protein precursor limits cerebral thrombosis. Proc Natl Acad Sci U S A 2005;102:18135–18140. [PubMed: 16330760]
53. De Meyer GR, De Cleen DM, Cooper S, Knaapen MW, Jans DM, Martinet W, et al. Platelet phagocytosis and processing of beta-amyloid precursor protein as a mechanism of macrophage activation in atherosclerosis. Circ Res 2002;90:1197–1204. [PubMed: 12065323]
54. Jans DM, Martinet W, Van De Parre TJ, Herman AG, Bult H, Kockx MM, et al. Processing of amyloid precursor protein as a biochemical link between atherosclerosis and Alzheimer's disease. Cardiovasc Hematol Disord Drug Targets 2006;6:21–34. [PubMed: 16724933]
55. Wisniewski HM, Frackowiak J, Mazur-Kolecka B. In vitro production of beta-amyloid in smooth muscle cells isolated from amyloid angiopathy-affected vessels. Neurosci Lett 1995;183:120–123. [PubMed: 7746469]
56. Shi C, Guo K, Yew DT, Yao Z, Forster EL, Wang H, et al. Effects of ageing and Alzheimer's disease on mitochondrial function of human platelets. Exp Gerontol 2008;43:589–594. [PubMed: 18359596]
57. Franchini M. Hemostasis and aging. Crit Rev Oncol Hematol 2006;60:144–151. [PubMed: 16860994]
58. Wilkerson WR, Sane DC. Aging and thrombosis. Semin Thromb Hemost 2002;28:555–568. [PubMed: 12536349]
59. Borroni B, Akkawi N, Martini G, Colciaghi F, Prometti P, Rozzini L, et al. Microvascular damage and platelet abnormalities in early Alzheimer's disease. J Neurol Sci 2002;203–204:189–193.
60. Schubert W, Prior R, Weidemann A, Dircksen H, Multhaup G, Masters CL, et al. Localization of Alzheimer beta A4 amyloid precursor protein at central and peripheral synaptic sites. Brain Res 1991;563:184–194. [PubMed: 1786532]
61. Askanas V, Engel WK. Molecular pathology and pathogenesis of inclusion-body myositis. Microsc Res Tech 2005;67:114–120. [PubMed: 16104000]
62. Finch CE. A perspective on sporadic inclusion-body myositis: the role of aging and inflammatory processes. Neurology 2006;66:S1–S6. [PubMed: 16432135]
63. Querfurth HW, Suhara T, Rosen KM, McPhie DL, Fujio Y, Tejada G, et al. Beta-amyloid peptide expression is sufficient for myotube death: implications for human inclusion body myopathy. Mol Cell Neurosci 2001;17:793–810. [PubMed: 11358479]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

64. Turner AJ, Nalivaeva NN. New insights into the roles of metalloproteinases in neurodegeneration and neuroprotection. Int Rev Neurobiol 2007;82:113–135. [PubMed: 17678958]

65. Ghiso J, Shayo M, Calero M, Ng D, Tomidokoro Y, Gandy S, et al. Systemic catabolism of Alzheimer's Abeta40 and Abeta42. J Biol Chem 2004;279:45897–45908. [PubMed: 15322125]

66. Ghiso J, Calero M, Matsubara E, Governale S, Chuba J, Beavis R, et al. Alzheimer's soluble amyloid beta is a normal component of human urine. FEBS Lett 1997;408:105–108. [PubMed: 9180278]

67. Tamaki C, Ohtsuki S, Terasaki T. Insulin facilitates the hepatic clearance of plasma amyloid beta-peptide (1 40) by intracellular translocation of low-density lipoprotein receptor-related protein 1 (LRP-1) to the plasma membrane in hepatocytes. Mol Pharmacol 2007;72:850–855. [PubMed: 17609417]

68. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology 2007;68:666–669. [PubMed: 17325273]

69. Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A, Rozkalne A, et al. Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature 2008;451:720–724. [PubMed: 18256671]

70. Hawkes CA, McLaurin J. Immunotherapy as treatment for Alzheimer's disease. Expert Rev Neurother 2007;7:1535–1548. [PubMed: 17997702]

71. Paris D, Townsend K, Quadros A, Humphrey J, Sun J, Brem S, et al. Inhibition of angiogenesis by Abeta peptides. Angiogenesis 2004;7:75–85. [PubMed: 15302999]

72. Plant LD, Boyle JP, Smith IF, Peers C, Pearson HA. The production of amyloid beta peptide is a critical requirement for the viability of central neurons. J Neurosci 2003;23:5531–5535. [PubMed: 12843253]

73. Pearson HA, Peers C. Physiological roles for amyloid beta peptides. J Physiol 2006;575:5–10. [PubMed: 16809372]

74. Heininger K. A unifying hypothesis of Alzheimer's disease. IV. Causation and sequence of events. Rev Neurosci 2000;(11 Spec No):213–328. [PubMed: 11065271]

75. Kuo YM, Emmerling MR, Vigo-Pelfrey C, Kasunic TC, Kirkpatrick JB, Murdoch GH, et al. Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. J Biol Chem 1996;271:4077–4081. [PubMed: 8626743]

76. Roher AE, Chaney MO, Kuo YM, Webster SD, Stine WB, Haverkamp LJ, et al. Morphology and toxicity of Abeta-(1-42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. J Biol Chem 1996;271:20631–20635. [PubMed: 8702810]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

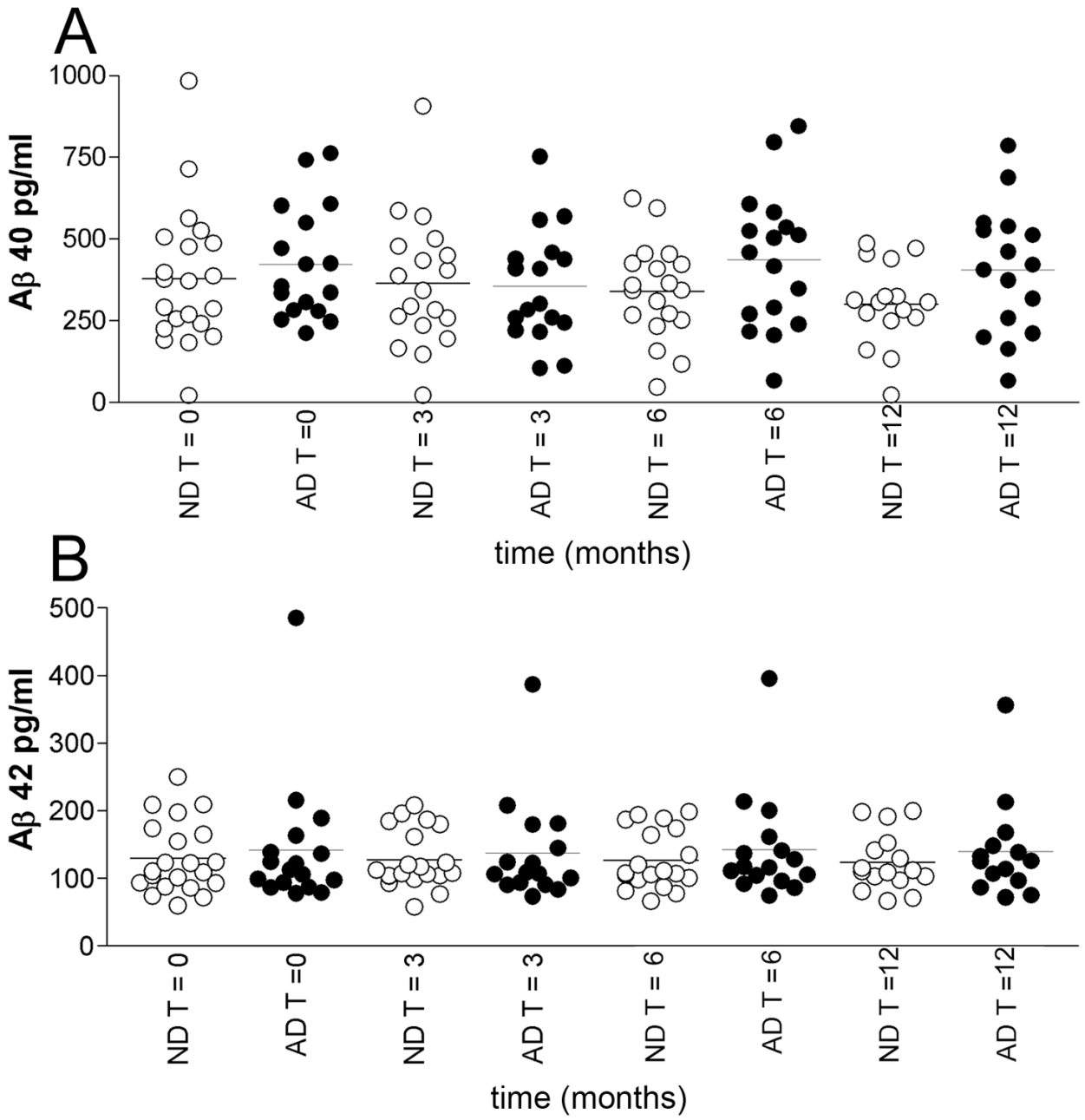

Figure 1.

Longitudinal comparison of AD (black) and ND control (white) plasma Aβ levels as measured by ELISA. Measurements were taken at baseline (T=0) and at 3, 6 and 12 months. The horizontal bars represent the mean values. **A)** Aβ40 pg/ml **B)** Aβ42 pg/ml.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

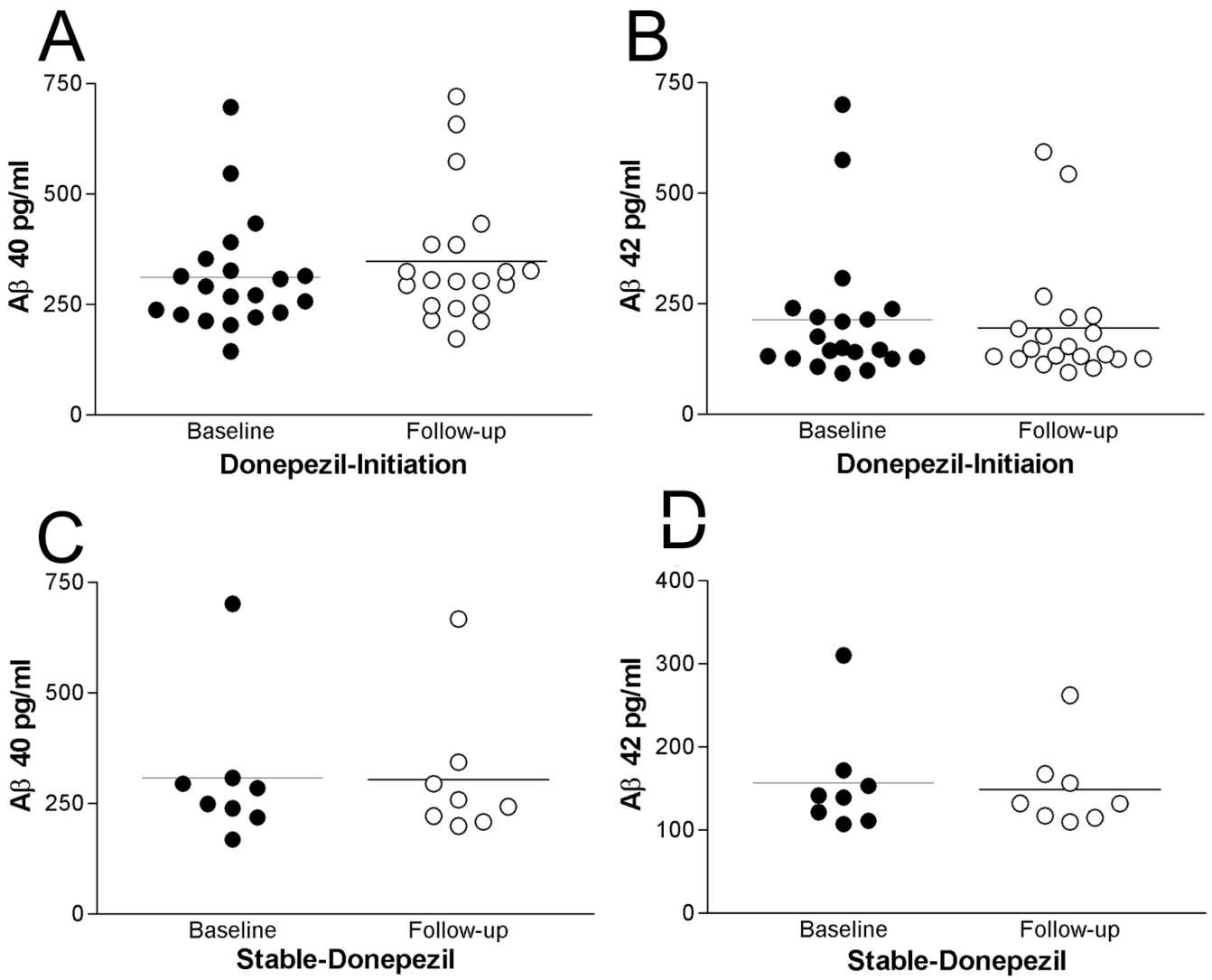

Figure 2.

Plasma Aβ levels measured by ELISA in the therapeutic study with donepezil. Measurements were taken at baseline and 12 weeks later (follow-up). **A)** Aβ40 pg/ml in donepezil-initiation group **B)** Aβ42 pg/ml in donepezil-initiation group **C)** Aβ40 pg/ml in stable-donepezil group **D)** Aβ42 pg/ml in stable-donepezil group.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Alzheimers Dement. Author manuscript; available in PMC 2010 January 1.

Table 1

Longitudinal study of plasma individual Aβ values and patient demographics

The Aβ amounts represent the average of those individuals completing 3 and 4 time points: baseline, 3, 6, 12 months.

ND controls (n = 21)	Age (years)	Gender	*ApoE* genotype	MMSE	Aβ40 pg/ml	Aβ42 pg/ml	Total Aβ pg/ml	ratio Aβ 42/Aβ 40
1	82	F	3/3	30	628.66	76.92	705.58	0.12
2	62	F	4/4	28	338.75	61.02	399.77	0.18
3	78	M	3/3	28	356.23	79.86	436.09	0.22
5	78	M	3/3	30	311.11	79.54	390.65	0.26
9	78	F	3/4	29	484.36	109.24	593.60	0.23
12	77	M	3/3	30	259.56	98.70	358.26	0.38
13	60	F	2/4	29	184.82	108.37	293.19	0.59
14	63	M	3/3	28	221.54	119.71	341.25	0.54
16	79	F	3/4	30	380.87	176.98	557.85	0.47
21	81	F	3/3	29	252.95	157.79	410.74	0.62
23	83	F	3/4	28	145.03	105.10	250.13	0.73
25	71	F	3/4	29	320.26	192.81	513.07	0.60
29	79	F	3/3	30	94.63	142.73	237.36	1.51
30	75	F	4/4	30	372.68	200.44	573.12	0.54
39	86	M	2/3	29	466.19	124.60	590.79	0.27
40	83	F	3/4	29	419.18	185.65	604.82	0.44
41	77	F	3/3	28	402.03	97.36	499.39	0.24
45	81	F	2/3	30	235.55	105.24	340.79	0.48
49	70	F	3/3	30	429.33	108.53	537.86	0.25
50	73	M	3/4	30	555.23	185.90	741.13	0.34
56	76	M	3/4	24	373.72	102.33	476.05	0.27
AVG	**75.81**	**14F/7M**		**28.95**	**344.41**	**124.71**	**469.12**	**0.44**
SD	**7.10**			**1.40**	**132.43**	**42.34**	**141.09**	**0.30**

AD (n = 17)	Age (years)	Gender	*ApoE* genotype	MMSE	Aβ40 pg/ml	Aβ42 pg/ml	Total Aβpg/ml	ratio Aβ 42/Aβ 40
4	77	F	3/4	19	236.43	92.13	328.56	0.39
8	80	M	3/3	24	565.46	136.05	701.51	0.24

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

ND controls (n = 21)	Age (years)	Gender	*ApoE* genotype	MMSE	Aβ40 pg/ml	Aβ42 pg/ml	Total Aβ pg/ml	ratio Aβ 42/Aβ 40
11	73	F	3/4	26	554.35	123.57	677.92	0.22
15	89	M	3/4	18	365.17	168.33	533.50	0.46
24	90	M	3/3	23	531.23	189.63	720.86	0.36
26	76	M	3/3	23	328.39	121.47	449.86	0.37
28	74	F	4/4	17	340.11	99.33	439.44	0.29
32	75	M	3/4	30	505.17	406.03	911.20	0.80
37	82	F	3/4	25	486.52	212.39	698.91	0.44
42	83	M	3/4	22	257.69	133.92	391.62	0.52
43	88	F	3/3	24	563.54	108.70	672.24	0.19
44	81	F	3/4	21	235.55	100.31	335.86	0.43
48	87	M	3/4	27	434.55	120.26	554.81	0.28
53	83	F	3/3	22	411.23	83.44	494.67	0.20
55	79	F	3/4	15	756.84	74.32	831.16	0.10
57	85	F	3/3	22	219.99	103.25	323.24	0.47
59	81	F	3/4	21	416.86	105.38	522.23	0.25
AVG	**81.35**	**10F/7M**		**22.29**	**424.06**	**139.91**	**563.98**	**0.35**
SD	**5.29**			**3.74**	**147.73**	**77.82**	**178.47**	**0.16**
ND vs AD *p* =	**0.011**			**<0.001**	**0.088**	**0.448**	**0.075**	**0.292**

ND = non-demented; ApoE = apolipoprotein E; MMSE = mini-mental state examination; avg = average; F = female; M = male; AD = Alzheimer's disease; SD = standard deviation; *p* = unpaired, 2-tailed, t-test

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

Therapeutic study of plasma Aβ values and patient demographics

All cases are probable AD. These values represent the average of 3 readings per week.

	Baseline Stable-Donepezil (n=8)							Follow-up Stable-Donepezil (n=8)			
	Age (years)	Gender	MMSE	Aβ 40 pg/ml	Aβ 42 pg/ml	Total Aβ pg/ml	ratio Aβ42/Aβ40	Aβ 40 pg/ml	Aβ 42 pg/ml	Total Aβ pg/ml	ratio Aβ42/Aβ40
1	85	M	11	701.76	121.46	823.22	0.173	667.35	114.92	782.27	0.17
2	84	F	15	307.98	310.00	617.98	1.007	294.73	262.14	556.87	0.89
3	70	F	22	249.25	153.13	402.39	0.614	258.74	132.07	390.81	0.51
4	78	F	19	218.32	107.05	325.37	0.490	208.62	117.33	325.95	0.56
5	76	F	21	294.78	111.31	406.09	0.378	199.18	109.86	309.04	0.55
6	57	M	18	238.95	139.09	378.04	0.582	343.57	131.89	475.45	0.38
7	77	F	25	168.23	141.66	309.90	0.842	221.38	156.49	377.87	0.71
8	79	F	17	284.72	171.83	456.55	0.604	242.81	167.59	410.39	0.69
AVG	**75.75**	**6F/2M**	**18.50**	**308.00**	**156.94**	**464.94**	**0.59**	**304.55**	**149.04**	**453.58**	**0.56**
SD	**8.91**		**4.34**	**165.43**	**65.51**	**173.33**	**0.26**	**154.17**	**49.97**	**154.88**	**0.22**
Baseline vs follow-up *p*=				**0.966**	**0.790**	**0.892**	**0.819**				

	Baseline Donepezil-Initiation (n=20)							Follow-up Donepezil-Initiation (n=20)			
	Age (years)	Gender	MMSE	Aβ 40 pg/ml	Aβ 42 pg/ml	Total Aβ pg/ml	ratio Aβ42/Aβ40	Aβ 40 pg/ml	Aβ 42 pg/ml	Total Aβ pg/ml	ratio Aβ42/Aβ40
10	82	F	23	390.82	209.63	600.45	0.536	385.39	193.45	578.84	0.50
11	75	M	28	314.91	144.20	459.11	0.458	326.65	153.49	480.14	0.47
12	73	F	28	290.91	150.43	441.35	0.517	303.57	125.99	429.56	0.42
13	75	F	27	231.84	129.83	361.67	0.560	305.57	125.44	431.00	0.41
14	80	F	25	271.05	575.01	846.06	2.121	302.41	543.31	845.71	1.80
15	77	F	14	143.75	125.48	269.23	0.873	171.99	135.00	306.99	0.79
16	79	F	27	221.14	219.88	441.02	0.994	293.23	219.31	512.53	0.75
17	56	F	22	212.57	141.17	353.74	0.664	215.08	130.99	346.07	0.61
18	83	M	24	237.59	107.95	345.54	0.454	247.17	112.53	359.69	0.46

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

	Baseline Stable-Donepezil (n=8)							Follow-up Stable-Donepezil (n=8)			
	Age (years)	**Gender**	**MMSE**	**Aβ 40 pg/ml**	**Aβ 42 pg/ml**	**Total Aβ pg/ml**	**ratio Aβ42/Aβ40**	**Aβ 40 pg/ml**	**Aβ 42 pg/ml**	**Total Aβ pg/ml**	**ratio Aβ42/Aβ40**
19	81	F	23	696.84	175.94	872.79	0.252	721.14	147.71	868.84	0.21
20	75	F	25	353.42	214.65	568.06	0.607	433.12	177.48	610.60	0.41
21	76	F	28	203.20	99.18	302.38	0.488	252.69	104.93	357.62	0.42
22	81	M	26	227.14	240.34	467.48	1.058	212.57	222.79	435.36	1.05
23	59	M	20	257.08	700.09	957.18	2.723	294.58	593.55	888.13	2.02
24	70	F	26	326.79	126.61	453.40	0.387	324.16	132.69	456.85	0.41
25	76	F	25	433.26	92.64	525.90	0.214	385.99	94.44	480.44	0.25
26	83	F	15	307.96	307.77	615.73	0.999	573.54	266.84	840.39	0.47
27	77	M	25	267.80	131.55	399.35	0.491	240.79	130.64	371.43	0.54
28	78	F	21	314.03	238.54	552.57	0.760	324.45	183.84	508.28	0.57
29	92	F	18	546.82	145.97	692.79	0.267	658.09	124.91	783.00	0.19
AVG	**76.4**	**15F/5M**	**23.5**	**312.45**	**213.84**	**526.29**	**0.771**	**348.61**	**195.97**	**544.57**	**0.64**
SD	**7.96**		**4.11**	**127.87**	**156.54**	**192.12**	**0.62**	**146.42**	**135.01**	**194.00**	**0.48**
Baseline vs follow-up *p* =				**0.411**	**0.701**	**0.766**	**0.433**				

MMSE = mini-mental state examination; avg = average; F = female; M = male; SD = standard deviation; *p* = unpaired, 2-tailed, t-test

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 3

Aβ Peptide Levels in Human Tissues

	Aβ 40 ng/g	Aβ 42 ng/g	Total Aβng/g
Brain-gray matter			
AD (n = 23)	608	6096	6704
ND (n = 20)	209	784	993
Brain-white matter			
AD (n = 10)	1069	1135	2204
ND (n = 13)	111	418	529
Platelets			
Q (n = 5)	83.8	1.7	85.5
A (n = 5)	56.8	1.6	58.4
Aorta			
severe AVD (n = 6)	75.3	0.7	76.0
minimal AVD (n = 2)	31.4	1.0	32.4
Leptomenigeal Arteries			
severe AVD (n = 4)	113.1	28.0	141.1
minimal AVD (n = 5)	17.3	5.3	22.6
Skeletal Muscle			
AD (n = 23)	37.8	15.7	53.5
ND (n = 20)	29.8	10.2	40.0
Liver			
AD (n = 6)	8.6	1.7	10.3
ND (n = 6)	67.5	15.5	83.0

AD = Alzheimer’s disease; ND = non-demented; Q = quiescent; A = activated; AVD = atherosclerotic vascular disease