Atherosclerosis, vascular amyloidosis and brain hypoperfusion in the pathogenesis of sporadic Alzheimer's disease

Walter Kalback*, Chera Esh*, Eduardo M. Castaño*#, Afroza Rahman*, Tyler Kokjohn†, Dean C. Luehrs*, Lucia Sue‡, Raquel Cisneros‡, Francoise Gerber§, Claudia Richardson§, Bernd Bohrmann§, Thomas G. Beach‡ and Alex E. Roher*

*Longtine Center for Molecular Biology and Genetics and ‡ W. Harold Civin Laboratory of Neuropathology, Sun Health Research Institute, Sun City, AZ, USA
†Department of Microbiology, Midwestern University, Glendale, AZ, USA
§Department of Pharma Research Biology Discovery, F. Hoffmannn-La Roche Ltd, Basel, Switzerland
#Dr Eduardo Castaño is a 'SHRI Visiting Professor' from the Department of Biological Chemistry, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina

We postulate that severe atherosclerotic occlusion of the circle of Willis and leptomeningeal arteries is an important factor in the pathogenesis of some sporadic Alzheimer's disease (AD) cases. These arterial stenoses are complicated by an overwhelming amyloid accumulation in the walls of leptomeningeal and cortical arteries resulting in a significant decrease in perfusion pressure and consequent ischemia/hypoxia of the brain tissue. We also propose that the distal areas of the white matter (WM) will be the first affected by a lack of oxygen and nutrients. Our hypotheses are supported by the following observations: (1) the number of stenoses is more frequent in AD than in the control population ($p = 0.008$); (2) the average index of occlusion is greater in AD than in the control group ($p < 0.00001$); (3) the index of stenosis and the total number of stenoses per case are positively correlated ($R = 0.67$); (4) the index of stenosis correlates with the neuropathological lesions of AD and with the MMSE psychometric test; (5) the number and degree of atherosclerosis of the anterior, middle and posterior cerebral arteries is more severe in cases of AD than in the control population; (6) atherosclerosis severity is apparently associated with the severity of the vascular amyloidosis; (7) the WM rarefaction correlates with the severity of the atherosclerosis and vascular amyloidosis; (8) the total cell count and microvessel count in the areas of WM rarefaction correlate with the neuropathological lesions of AD and with the MMSE score. Our data strongly suggest that severe hemodynamic disturbances contribute to sporadic AD and support the numerous observations indicating cardiovascular system participation in the pathogenesis of these dementias. [Neurol Res 2004; **26**: 000–000]

Keywords:

1

'*A man is as old as his arteries*'
Thomas Sydenham, British physician (1624–1689)

INTRODUCTION

Genetic studies on familial Alzheimer's disease (FAD) etiology have generated a great deal of information regarding the amyloid-β precursor protein (AβPP) and presenilin 1 and 2 gene mutations that result in amyloid-beta (Aβ) deposits in cerebrovascular walls and senile plaques, neurofibrillary tangles (NFT), neuronal injury and early onset of dementia[1]. However, FAD is only observed in an extremely limited number of individuals while the precise pathogenic mechanism underlying the most common sporadic forms of this dementia, which affects about 98% of AD patients, remains to be established. Sporadic Alzheimer's disease (AD) results from the complex interaction of susceptible gene combinations and multiple protein pathways strongly affected by environmental, lifestyle and personality factors that collectively culminate in a unique aging pattern. Intriguingly, the multiple forms of sporadic AD share the neuropathological lesions observed in FAD, but because APP and presenilin mutations are not present in the sporadic forms of AD, other additional pathogenetic events, some primary to the central nervous system (CNS) and others originating from peripheral influences or in combination, probably precede amyloid and NFT deposition. However, it is well recognized that the presence of amyloid and tangle deposits alone, by displacing normal tissue and altering intraneuronal homeostasis, strongly disrupt brain metabolism and function. We postulate that amyloid accumulation in senile plaques represents

Correspondence and reprint requests to: Alex E. Roher, MD, PhD, Sun Health Research Institute, 10515 W. Santa Fe Dr., Sun City, AZ 85351, USA. [alex.roher@sunhealth.org] Accepted for publication March 2004.

10.1179/01616410422501XXXX

4

a defense mechanism whereby obnoxious soluble oligomeric Aβ peptides are concentrated into insoluble amyloid fibrils surrounded by glial cells[2]. By this means, a large amount of soluble Aβ in the interstitial fluid may be prevented from reaching the walls of cortical and leptomeningeal arteries where the fibrillar amyloid can totally destroy the structure and function of these vessels. From a functional point of view, these deposits drastically alter the control of regional cerebral blood flow and brain perfusion. On the other hand, vascular amyloid deposition may represent a critical defense mechanism by which insoluble fibrils act to seal a hemodynamically injured and leaky blood–brain barrier (BBB)[3]. The deposition of intracellular NFT may also represent an effort to preserve neuronal architecture and integrity as a response to physical and/or chemical injury to the neuron by creating an indestructible fibrillar scaffold. It is possible that therapeutic interference with NFT and amyloid deposition may have several undesirable effects and produce a modified form of dementia without these neuropathological lesions.

It is unfortunate that knowledge of the exact character of the autosomal dominant genetic mutations that cause FAD has not been translated into an efficient therapeutic intervention that enhances the quality of life of the demented patient. It is obvious that AD dementias share basic pathophysiological trends. Among these, disturbances in neuronal energy metabolism due to drastic hemodynamic alterations resulting from chronic arterial disease that, in the end, result in a myriad of neuronal and glial homeostatic distresses. Auspiciously, some of the pathological arterial alterations can be prevented or alleviated by changes in life-style and existing medications that may delay the onset and course of AD. In this paper we describe the pathological changes that affect the brain's arterial tree, in both sporadic AD patients and non-demented control individuals, and correlate these alterations with the neuropathological lesions of AD.

BRAIN HYPOPERFUSION AND CIRCLE OF WILLIS ATHEROSCLEROSIS IN AD

In the search for pathogenetic events in AD numerous studies have demonstrated a remarkable cerebral blood flow reduction in this dementia[4–13]. In addition, radiological studies have clearly demonstrated wide pathological alterations in the white matter (WM) of demented individuals which are morphologically described as leukoaraiosis[14, 15] and have been related to brain ischemia and hypoperfusion[5,16–23]. Interestingly, cerebral hypoperfusion and leukoaraiosis along with gray matter (GM) atrophy have been associated with the cognitive deficits observed in AD[5,15–32]. It comes as no surprise that surgical procedures that enhance brain perfusion such as greater omentum transposition improve, at least temporarily, AD patient cognitive function[33–34]. In addition, magnetic resonance (MR) perfusion and single photon emission computed tomography (SPECT) studies have visibly demonstrated that in AD there is a decrease in cerebral blood flow, and these techniques have aided the diagnosis of dementia[25,35]. Functional magnetic resonance imaging (MRI) has also been used in the evaluation of AD dementia[36] to determine blood volume distribution. A comparison of the dynamic contrast susceptibility observations to fluorodeoxyglucose positron emission tomography (PET) in the same patients demonstrated a high degree of concordance. Thus, the decrease in temporo-parietal perfusion seen in AD by PET–SPECT can also be identified by dynamic contrast susceptibility MR[36,37]. Using a spin-labeling technique, cerebral perfusion was evaluated by MR revealing a significant hypoperfusion in frontal, temporal, parietal and cingulate regions in AD[38]. These studies, conducted with living patients, demonstrate a substantial and widespread pathologic perturbation in AD patient brain hemodynamics. Based on these observations we speculate that extensive atherosclerotic occlusion of the a rteries of the circle of Willis and of the arteries of the convexities of the brain, in addition to severe amyloidosis of the leptomeningeal and cortical vessels, may represent some of the physical bases for sporadic AD-associated brain hypoperfusion.

We serendipitously observed, during the course of examining the brains of deceased AD patients, that some exhibited abundant circle of Willis atherosclerosis. These vascular lesions were less extensive and less common in non-demented (ND) subjects. A review of the recent literature concerning risk factors for sporadic AD yielded a large number of articles supporting a close relationship between this neurodegenerative disorder and cardiovascular disease. Myocardial infarction[39], critical coronary artery disease[40,41], cardiac arrest[42], cardiovascular inflammation[43], hypertension[44–55], hypotension[56], hypercholesterolemia[55,57–60], hyperhomocysteinemia[61] and vascular pathology associated with diabetes mellitus[62–65], alone or in combination represent significant risk factors for sporadic AD. Moreover, the brains of ND individuals with severe heart disease were found to contain considerable numbers of senile plaques and Aβ deposits similar to those observed in AD[66]. Subjects with neuropathologically diagnosed AD have also demonstrated high serum cholesterol levels compared with ND controls[67]. Individuals with AD have also shown a positive correlation between the brain Aβ*n*–42 levels and total serum cholesterol, LDL-cholesterol and Apo B-100, and a negative correlation with HDL-cholesterol levels[67]. Likewise, patients with probable or possible AD, who are in the first stages of this dementia, also have significantly elevated total cholesterol values from those observed in age- and gender-matched ND control subjects[68]. In addition to these associations, an increasing body of experimental, neuropathological and clinical evidence strongly suggests the participation of the brain vasculature in sporadic AD pathogenesis[69].

In this paper, we present rigorous experimental data that strongly implicate cerebrovascular disease as a primary pathogenetic episode in the development of some cases of sporadic AD. Severe atherosclerosis of the circle of Willis and of the major cerebral arteries causes chronic brain hypoperfusion, hypoxia/ischemia and

severe neuronal damage. Further harm to cerebral blood flow is engendered by the concurrent deposition of amyloid in the vascular walls of leptomeningeal and cortical arteries. We propose that these deposits compromise the drainage of the brain's interstitial fluid that results in dilation of the WM periarterial spaces (e'tat crible') and in WM edema with retention of obnoxious metabolites, destruction of the capillary network, WM demyelination, irreparable axonal injury and astrogliosisve[70].

Recently, we demonstrated that the atherosclerosis of the circle of Willis is quantitatively more advanced in sporadic AD than in ND age-matched individuals. Fifty-four consecutive autopsy cases, 32 sporadic AD and 22 ND controls, were studied to establish the degree of arterial stenosis. The diagnosis of AD was made according to published consensus criteria established by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and by the National Institute on Aging and Reagan Institute (NIA-R)[71,72]. Cases were defined as AD if they met CERAD criteria for 'definite' or 'probable' AD as well as NIA-R criteria for 'intermediate' or 'high' probability for AD. These individuals were voluntary participants enrolled in the Brain Donation Program of Sun Health Research Institute (Sun City, AZ). The time elapsed between the removal of their brains and fixation of the circle of Willis in 4% paraformaldehyde was on the average 2.5 h. The leptomeningeal membranes carrying the arteries of the convexity of the brain (anterior, middle and posterior cerebral arteries and their leptomeningeal perforating branches) were carefully removed from the surface of the brain, snap frozen and preserved at –86°C. All subjects were Caucasians. The 32 AD individuals, 18 women and 14 men, averaged 84.4 and 86.4 years of age, respectively (combined average age: 85.4 years). The ND cohort was composed of 14 women and eight men, averaging 87.1 and 82.6 years of age, respectively (combined average age: 84.8 years). There were no significant differences between the ages of the AD and ND populations (unpaired 2-tailed, $p=0.91$), but when divided by gender, there was a significant difference between the average ages of males and females in the ND group (82.6 and 87.1 years, respectively; unpaired, 2-tailed $p=0.004$). In the AD group the differences in average age between the males and females were not significant (86.4 and 84.4 years, respectively: unpaired, 2-tailed $p=0.46$).

The following paraformaldehyde-fixed arteries of the circle of Willis were investigated: right and left internal carotid arteries, right and left anterior cerebral arteries, anterior communicating artery, right and left middle cerebral arteries, right and left posterior communicating arteries, right and left posterior cerebral arteries, basilar artery and right and left vertebral arteries. The anatomical variations of the circle of Willis were recorded. From the leptomeninges the anterior, middle and posterior cerebral arteries were dissected, rinsed several times in cold distilled water until there were no traces of entrapped blood cells and plasma and fixed with 4% paraformaldehyde. Sections, 3 mm in length, from the circle of Willis and leptomeningeal arteries were cross-cut and examined with a Leica S8APO dissecting microscope and the areas of appreciable stenosis photographed with an Optronics Magnafire SP camera (Model s99805) and processed by the Optronics software program. Measurements of the external and lumenal areas were obtained using the calibrated ImagePro Express software, version 4.0 (Media Cibernetics). The index of stenosis was calculated for each artery by subtracting the lumenal area from the outer area, dividing the difference by the outer area and multiplying the quotient by 100. *Figure 1A* illustrates the morphological conditions of a ND case, while *Figure 1B* represents an AD case.

In *Figure 2A*, the average index of arterial stenosis for each of the arteries of the circle of Willis in AD and ND individuals is represented in the form of a histogram. The larger degree of stenosis in AD over the ND control group is evident. The mean number of stenoses observed in the circle of Willis of AD subjects was 30.2 and in the ND population 19.9 (unpaired, 2-tailed $p=0.007$). The degree of cerebral hypoperfusion is directly proportional to the number of atheroma plaques along the arterial tree and the extent of stenosis determined by the size of the remaining arterial lumen. *Figure 2B* represents a correlation plot between the index of stenosis and the total number of stenoses per individual case ($R=0.67$). The average index of stenosis per case in the ND group was 53.7, whereas in the AD cohort it was 67 ($p<0.00001$).

In this investigation, the degree of arterial stenosis due to atherosclerosis positively correlated with the five typical neuropathological lesions of AD (*Figure 3A–E*). The neuropathologic diagnosis and scoring for AD-related pathology have been recently described[69]. All correlations were evaluated using Spearman's rank test. When the mean stenosis score (average stenoses of all blood vessels for each case) was compared with the brain total plaque score (sum of plaque scores of all brain regions) there was a positive correlation ($R_S=0.43$; $p<0.01$; *Figure 3A*), with very little difference between males ($R_S=0.45$, $p<0.05$) and females ($R_S=0.42$, $p<0.05$). The average arterial stenosis score and total brain NFT score (*Figure 3B*) was also positive ($R_S=0.44$, $p<0.01$). However, there were important differences between genders (males $R_S=0.32$, $p>0.05$; females $R_S=0.50$, $p<0.01$) with a more robust female correlation. The average arterial stenosis score and Braak stage (*Figure 3C*) were also correlated ($R_S=0.51$, $p<0.001$) with large differences when the genders were separately compared (males $R_S=0.36$, $p>0.05$; females $R_S=0.60$, $p<0.001$). Similar correlations were also obtained between the average stenosis score and the CERAD neuritic plaque score: $R_S=0.59$, $p<0.01$ (*Figure 3D*). When divided by gender: males $R_S=0.58$, $p=0.01$; females $R_S=0.59$, $p<0.01$. The mean stenosis score and the WM score (*Figure 3E*) demonstrated a positive correlation which was more obvious in females than in males (males $R_S=0.32$, $p>0.05$; females $R_S=0.60$, $p=0.001$; males and females together $R_S=0.47$, $p=0.005$). The Mini Mental Score Examination (MMSE) was available for some of the individuals under investigation (AD $n=10$ and ND $n=13$). *Figure 3F* demonstrates a clear correlation between MMSE and the index of stenosis; the higher the score the less atherosclerosis of the circle of Willis

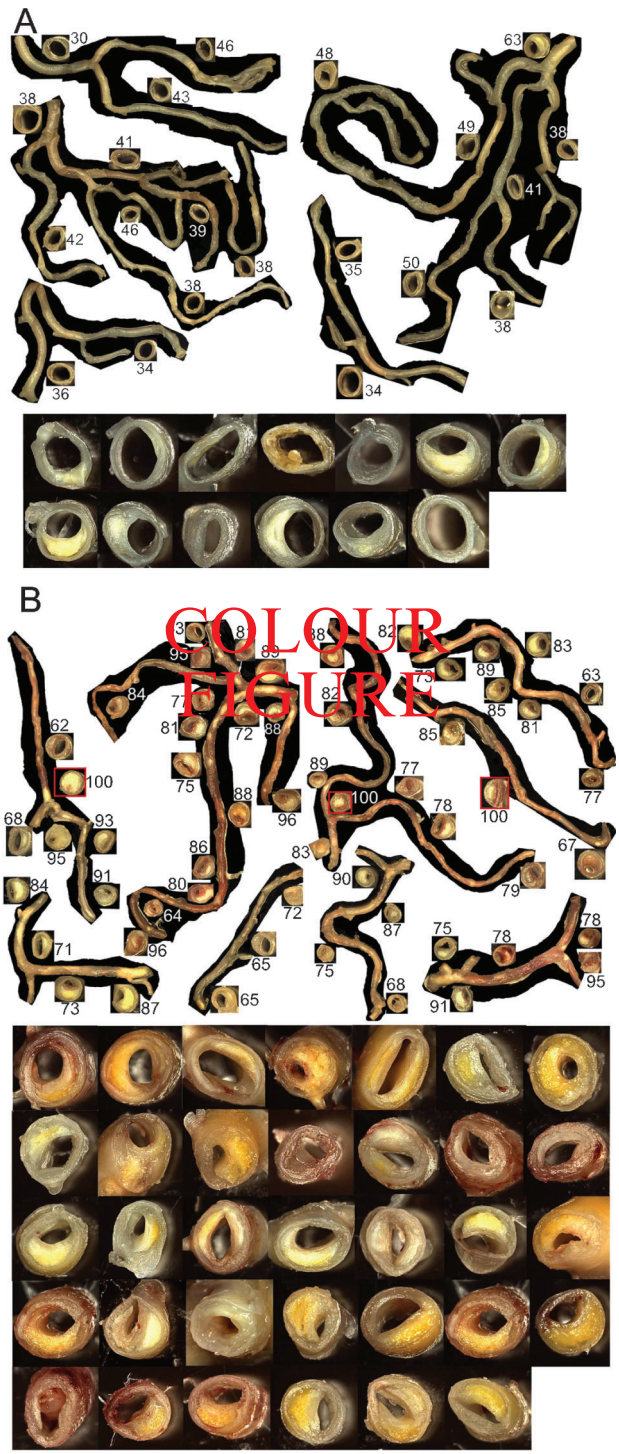

Figure 1: Cross-sections of the arteries of the circle of Willis and their leptomeningeal branches from ND and AD cases. (A) The bottom panel shows 13 sections of the arteries of the circle of Willis from a ND control individual. There is a minimal amount of atherosclerosis. The lumen of the arteries remain patent with an overall index of stenosis of 42%. The leptomeningeal branches as shown in the top panel demonstrate an index of stenosis of 41%. (B) In the bottom panel the arteries of the circle of Willis from an AD individual are represented. The 34 atherosclerotic plaques showed a mean index of stenosis of 84%. The top panel shows the leptomeningeal arteries of the same individual. There were 57 regions of atherosclerosis with a mean index of stenosis of 81%. Three of these arteries, boxed in red, are 100% occluded

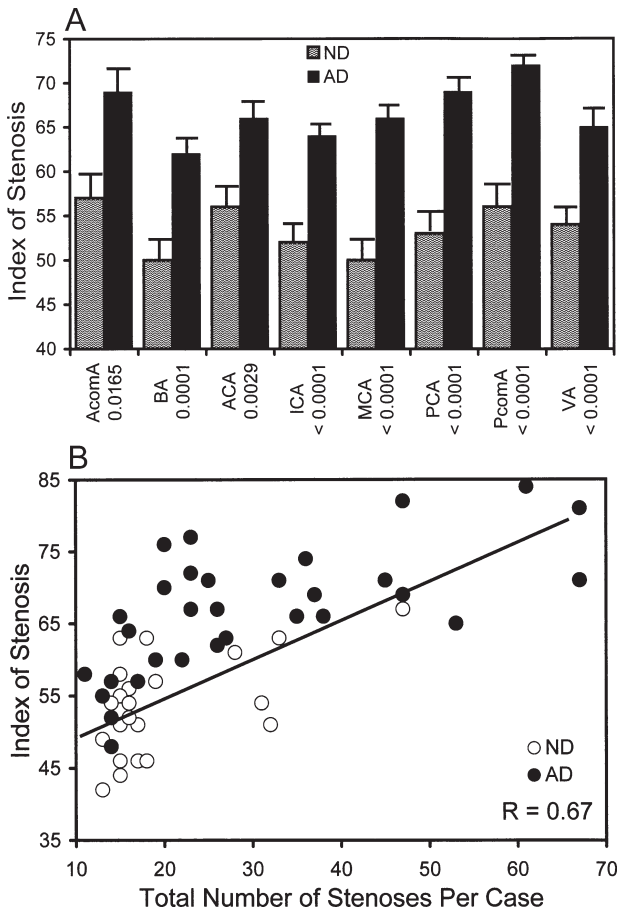

Figure 2: Average percentage stenosis of the arteries of the circle of Willis. (A) The mean percentage of stenosis of all the arteries of the circle of Willis from 32 AD and 22 ND control individuals. At the bottom of each histogram the *p* values (unpaired, 2-tailed *t* test) for each artery are shown. To obtain these data almost 1000 arterial sections were measured. In the AD group 22.3% of the examined arteries were more than 80% occluded while in the ND cohort only 4.7% were as occluded (χ^2, $p<0.001$). AcomA = anterior communicating artery; BA = basilar artery; ACA = anterior cerebral arteries; ICA = internal carotid arteries; MCA = medial cerebral arteries; PCA = posterior cerebral arteries; PcomA = posterior communicating arteries; VA = vertebral arteries. (B) A correlation between the average index of stenosis and the total number of stenosis per each case of AD and ND controls. This figure was first published in *Arterioscler Thromb Vasc Biol* and is reproduced here with permission

($R=0.73$; $p<0.0001$). The mean number of stenoses per each artery was calculated and averaged (2.33 for the AD and 1.47 for the ND; unpaired, 2-tailed $p=0.004$). The total number of vascular stenoses in the AD cohort was always greater than that of the ND population.

The apolipoprotein E (Apo E) allelic frequencies in the ND collection were $\varepsilon2=0.09$, $\varepsilon3=0.73$, $\varepsilon4=0.18$, and for the AD cohort they were $\varepsilon2=0.02$, $\varepsilon3=0.72$ and $\varepsilon4=0.26$. We found no association between the Apo E genotype and the index of stenosis in either the AD and ND groups. Neuropathological assessment of the brain coronal sections showed no statistically significant differences in the numbers for ischemic stroke between AD (38%) and control individuals (36%). Interestingly, other cardiovascular pathology such as coronary artery

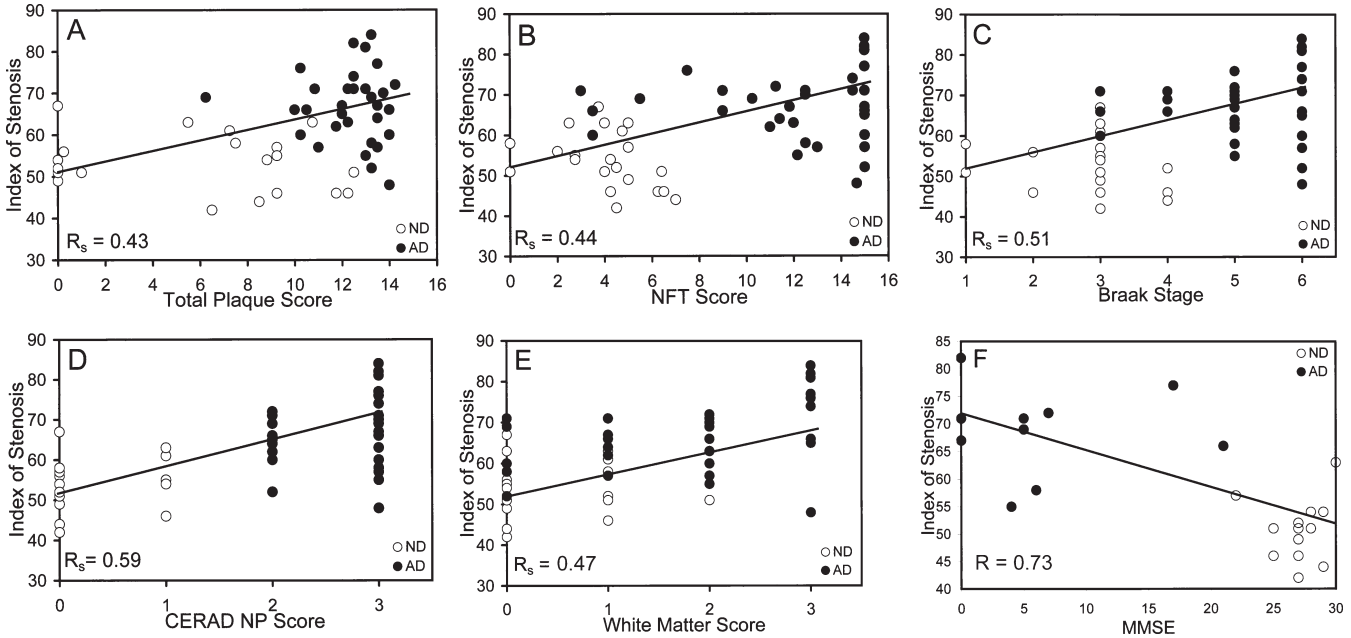

Figure 3: Distribution and correlations between the percentage of arterial stenosis of the circle of Willis in AD and ND controls and neuropathological and clinical scores. (A) Total plaque score. (B) NFT score. (C) Braak stage. (D) CERAD neuritic plaque score. (E) WM score. (F) MMSE score

disease was twice more prevalent in AD than ND patients (44% and 23%, respectively). Myocardial infarction was more common in AD than in ND (13% and 5%, respectively). Conversely, there was a higher incidence of valvular heart disease, disorders of rhythm and conduction and other peripheral vascular diseases in the ND group than in the AD cohort: 5% and 0%; 45% and 9%; and 23% and 3%, respectively. Other cardiovascular ailments such as hypertension, cardiomyopathy, stroke and lacunar infarcts and cardiorespiratory failure were almost equal in frequency between the AD and ND groups.

INDEPENDENT NEUROPATHOLOGICAL ASSESSMENT OF THE CIRCLE OF WILLIS IN AD

An independent neuropathological assessment of the circle of Willis atherosclerosis in AD and ND cohorts was established. These evaluations are routinely done as part of the mandatory neuropathology report. The population under investigation involved 215 consecutive cases that came to autopsy at Sun Health Research Institute between the years of 1999 and 2003. These cases were divided into two groups on the basis of AD diagnosis and the degree of atherosclerosis (none to mild versus moderate to severe). It was apparent that individuals with a greater degree of atherosclerosis occurred with greater frequency in the AD group. In the ND control group, 12 cases had moderate or severe atherosclerosis versus 49 lacking atherosclerosis or having mild atherosclerosis. In the AD group, 92 cases had moderate or severe atherosclerosis versus 62 without atherosclerosis or with mild atherosclerosis. This difference in proportions was significant on a chi-squared test ($\chi^2 = 26.50$; $p < 0.0001$). The degree of atherosclerosis was significantly correlated with the NFT score (Spearman Rank Correlation test; correlation coefficient = 0.15; 2-tailed $p = 0.03$) but not with total plaque score. Additionally, the mean circle of Willis atherosclerosis scores for AD cases differed significantly from those of the controls, with AD cases having a mean score of 1.81 as against 1.23 for ND control cases [Wilcoxon Rank Sum Test (WRST), 2-tailed $p = 0.0014$]. There was a non-significant trend for female AD cases to exhibit higher circle of Willis atherosclerosis scores than males. The mean atherosclerosis score for females with AD was 1.94, as compared with 1.68 for males. This same trend was observed in ND control cases, with females having a mean score of 1.31 versus 1.14 for males.

The Apo E ε4 allele was also unequally distributed between AD and ND control groups. There were 17 cases with ε4 alleles versus 44 without in the ND control group, while in the AD group there were 81 cases with an ε4 allele versus 73 without. This difference was significant ($\chi^2 = 2.17$; $p = 0.0005$). The Apo E allelic frequency in the control and AD populations was: ε2 = 0.08, ε3 = 0.77 and ε4 = 0.15, and ε2 = 0.03, ε3 = 0.65 and ε4 = 0.32, respectively. As the Apo E ε4 allele has been associated with increased coronary atherosclerosis[73], it was possible that the above-noted association of circle of Willis atherosclerosis with AD histopathology is secondary to the increased ε4 allele frequency in the AD group. However, this possible confounding linkage was disproved, as the correlation between AD histopathology and circle of Willis atherosclerosis is even stronger when cases possessing the ε4 allele are excluded. In this case, both total plaque and total tangle score have a correlation with the atherosclerosis score (correlation coefficient with plaque score = 0.29, 2-tailed $p = 0.0043$; correlation coefficient with tangle score = 0.25, 2-tailed $p = 0.013$). Likewise, in the smaller sample described above ($n = 54$) that was rigorously quantified, there was no correlation between the degree of stenosis and Apo E genotype in either the

AD or control cohorts. The circle of Willis atherosclerosis score was significantly correlated with the frequency of cerebral infarcts. For all cases together with infarcts, the mean atherosclerosis score was 2.02, while the cases without infarcts had a mean score of 1.29 (WRST 2-tailed $p<0.0001$). Non-demented control cases with infarcts had a mean atherosclerosis score of 1.5 versus 1.03 for control cases without infarcts and were non-significant (WRST). The overall frequency of infarcts was not significantly more prevalent in the AD cases when compared with controls as 46.5% of the AD cases had infarcts versus 41.9% of control cases.

ATHEROSCLEROSIS AND AMYLOID ANGIOPATHY

One of the most intriguing observations in AD is the close relationship that exists between atherosclerosis of the anterior middle and posterior cerebral arteries and the degree of vascular amyloidosis that affects the leptomeningeal and cortical arteries (*Figure 4*). We have observed a correlation between cortical Aβ concentration and the percentage of arterial stenosis of the circle of Willis. The higher the degree of stenosis, the higher the levels of Aβ ($R=0.40$) when comparing AD ($n=8$) and ND ($n=11$). Likewise, there is an apparent correlation between the Apo E phenotype and the amount of vascular amyloid load, being overwhelmingly severe in those individuals with Apo E ε4/ε4, moderate to severe in the Apo E ε3/ε4 and moderate in the Apo E ε3/ε3 and Apo E ε2/ε3. Furthermore, an association between aortic atherosclerosis and cerebral amyloidosis with learning deficits has been found in the B6Tg2576 transgenic mouse model[74]. The negative hemodynamic influence of the amyloid deposits on the structure of the arteries is devastating. The vascular myocytes disappear, leaving a

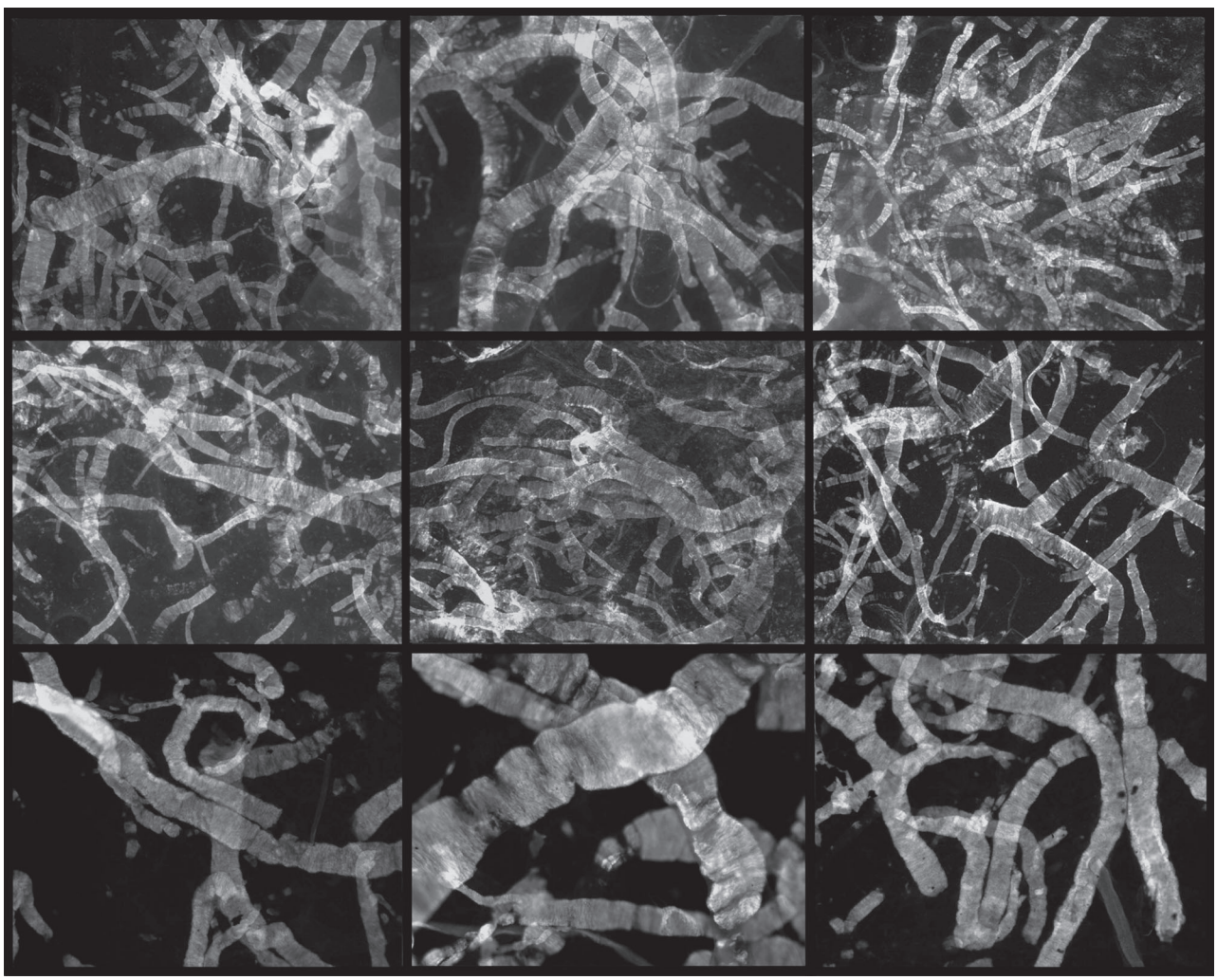

Figure 4: Leptomeningeal vascular amyloid deposits in AD. The mosaic of photos illustrate the overwhelming amount of fibrillar Aβ deposited in the leptomeningeal (top six panels) and cortical (bottom three panels) arteries of a patient with the Apo E ε4/ε4 genotype. The fibrillar amyloid is mostly deposited in concentric rings perpendicular to the main axis of the vessel transforming the walls into rigid structures. The leptomeningeal arteries and their perforating branches, measuring 500 μm in diameter or less, supplying the frontal, parietal, temporal and occipital lobes showed the same density of amyloid load that persists along the arteries of the cerebral cortex. Intriguingly, no vascular amyloid deposits are observed in the perforating medullary arteries supplying the WM in spite of being, in most cases, the continuation of the cortical perforating arteries, suggesting a cortical source of Aβ. Magnification: top six panels, ×25; bottom three panels, ×100

tunica media completely replaced by amyloid deposits (*Figure 5*) that transform the arterial wall into rigid structures that impinge upon cerebral blood flow. The inability of these arteries to distend and contract during the systolic and diastolic cycles increases the peripheral resistance and consequently elevates the blood pressure. Not infrequently, particularly in the vicinity of the amyloid-loaded leptomeningeal arteries of those individuals carrying the Apo E ε4 isoform, the membranes revealed areas of brownish deposits of hemosiderin, the vestiges of previous hemorrhages. The amyloid accumulation also prompts the formation of microaneurisms that weaken the arterial wall. The composition of the vascular amyloid fibrils revealed a preponderance of Aβ *n*–40 over Aβ *n*–42. This imbalance could be attributed to the solubility factor that enables the more soluble Aβ *n*–40 to diffuse more into the periarterial spaces that drain the interstitial fluid into the lymphatics of the head and neck. The biochemical association between specific Apo E phenotype and the abundance of vascular Aβ remains to be established. However, in addition to the link between atherosclerotic cardiovascular disease and AD, a significant number of epidemiological, pathological and biochemical studies suggest an interaction among diet, cholesterol metabolism and Aβ production. As mentioned, individuals carrying the Apo E ε4/ε4 genotype are at high risk of developing AD at an earlier age[75], and have a shorter duration of the disease. Interestingly, large amounts of cholesterol are associated with the amyloid deposits of AβPP transgenic (Tg) mice and with the amyloid cores in the senile plaques of AD patients[76]. In the APP23 and tg2576 Tg mice the ever-increasing expression of the APP transgene is accompanied by an elevation in the synthesis of Apo E. In the absence of amyloid deposits, cholesterol is significantly decreased in AD patient WM[77]. Cholesterol-fed rabbits demonstrate a time-dependent elevation in intraneuronal Aβ immunoreactivity[78] and Tg AβPP mice fed a cholesterol-rich diet develop a more dramatic amyloid accumulation than their non-Tg littermates[79]. Tissue culture experiments have shown that cholesterol addition increases Aβ production while the reduction of cholesterol results in decreased Aβ synthesis[80–82]. Acyl-coenzyme A cholesterol transferase modulates the generation of Aβ peptides[83]. Reduction of cholesterol synthesis by inhibitors of the hydroxymethylglutarylCoA reductase (statins), the limiting enzyme in cholesterol synthesis, decreases Aβ production *in vitro* and *in vivo*[84,85].

ATHEROSCLEROSIS, AMYLOID ANGIOPATHY AND ISCHEMIC WM LESIONS

Chronic brain ischemia is characterized by an imbalance between oxygen supply and demand that may be caused by a reduction in blood flow and oxygen delivery due to cardiac or respiratory failure or to severe stenosis of the cerebral arteries. This ischemia not only deprives the brain of oxygen and nutrients, but also results in inadequate removal of obnoxious metabolites. The limitations imposed by atherosclerosis of the circle of Willis and its branches are commensurate to the physical features of the stenosis such as the severity and length, the degree of calcification, the stiffness or partial distensibility of the arteries and the presence of thrombosis. The stenosis extent has an important hemodynamic effect since most stenoses have sudden transitions instead of tapering gradually, causing the conversion of laminar flow into countercurrents and vortices with a significant loss of energy, increased vascular trauma and a fall in perfusion pressure at the distal end of the stenosis[86]. In most cases, the sheer number of stenotic arterial segments encountered along

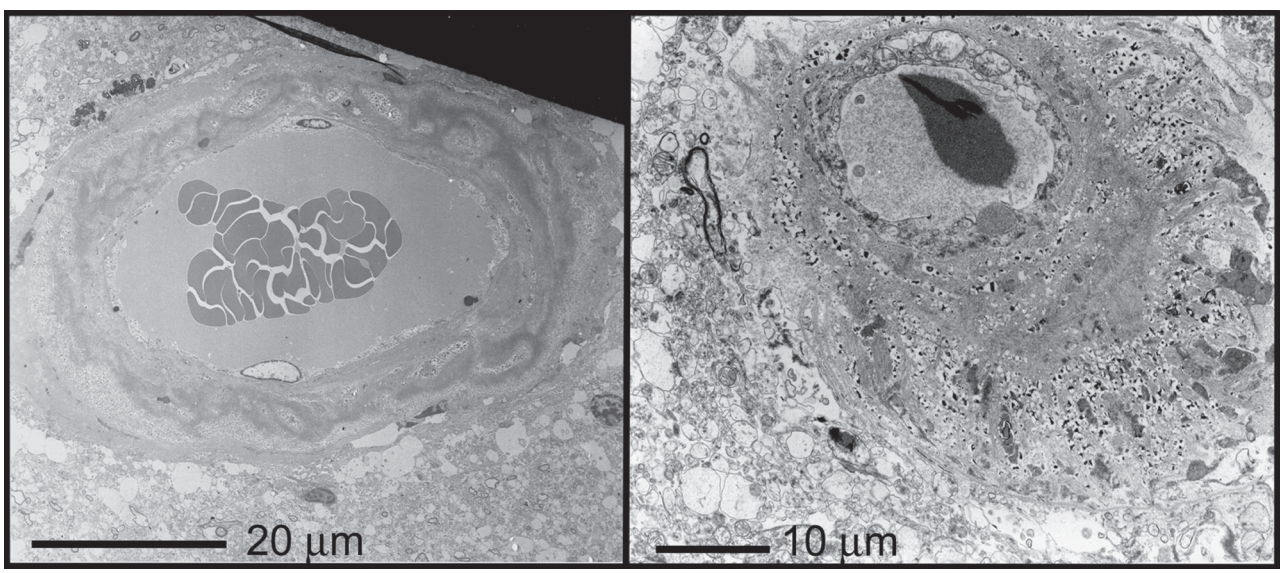

Figure 5: Electron micrographs of cerebral arteries laden with amyloid deposits. The endothelial cells are swollen, contain enlarged vesicles and show signs of degeneration. The myocytes of the tunica media are totally substituted by bundles of amyloid fibrils that extent beyond the limits of the tunica adventitia and into the surrounding neuropil totally obliterating the periarterial spaces. The destruction of elastic fibrils and smooth muscle cells results in total loss of vascular compliance

the path of the arteries that supply the brain severely compound the above-mentioned hemodynamic effects. Thus, given the extent of the atherosclerotic plaques observed in the arteries that supply the brain, in some cases of AD gravely compromised brain perfusion is inevitable.

From a physical point of view, the WM tissue of AD cases is soft, friable and easily deformed under pressure. In contrast, the WM from ND elderly individuals is firmer, denser and, when pressed, has a relatively resilient consistency. In addition, the remarkable WM atrophy and enlarged ventricles are both impressive features in AD. Another major difference between ND and AD brains is the more frequent and noticeable enlargement of the WM perivascular spaces in the latter condition (*Figure 6A*). We suggest that these enlargements result from the blockage of the interstitial fluid pathways by amyloid deposits in the walls of cortical and leptomeningeal arteries[70]. We have recently found a very strong relationship between the ratio of the dilated perivascular space diameter and the blood vessel diameter (*Figure 6A*) to the extent of arterial stenosis of the circle of Willis in AD ($n=8$; $R=0.77$). Interestingly, the same does not hold true for the ND cases ($n=11$; $R=0.01$).

Our interest in studying the pathological alterations of the WM in AD originated from the observation that in approximately two-thirds of these cases the WM demonstrates areas of rarefaction (*Figure 6B,C*). This condition refers to WM regions in which there is a significant loss of myelin, axons and oligodendroglia and a considerable deficit of microvessels resulting in severe ischemia/hypoxia of the WM. The normal WM tissue in AD is largely substituted by numerous swollen astrocytes and edema. The WM lesions have been traditionally attributed to sustained high blood pressure. We contend that, in addition to a potential hypertensive component, damage to the brain blood supply is compounded by severe atherosclerosis of the circle of Willis and its branches and by a heavy amyloidosis of leptomeningeal and cortical arteries that set the stage for a relentless injury of the long medullary vessels that supply the distal areas of the WM. From a hemodynamic point of view, both atherosclerosis and amyloidosis reduce cerebral blood flow and increase the pulsatility index, a parameter that reflects arterial wall compliance and peripheral resistance, as measured by transcranial Doppler ultrasonography[87,88]. As expected, the most severe microvascular and BBB damage would be localized at the most distal regions of the brain's arterial network, i.e. the deep WM. In the following subsections we describe a series of parameters that define the pathological conditions of the WM in AD ($n=10$; six females and four males; mean age = 84 years) and compare them to an ND ($n=13$; seven females and six males; mean age = 82.8 years) cohort. In the ND group, seven individuals were Apo E ε3/ε3, five individuals were Apo E ε3/ε4 and one subject was Apo E ε4/ε4. In the AD group five subjects were Apo E ε3/ε3 and five subjects were Apo E ε4/ε4.

We contend that WM disturbances in AD are as important as those observed in the GM, since the axons are extensions of the neuronal bodies and represent the means of communication between cells. Recent

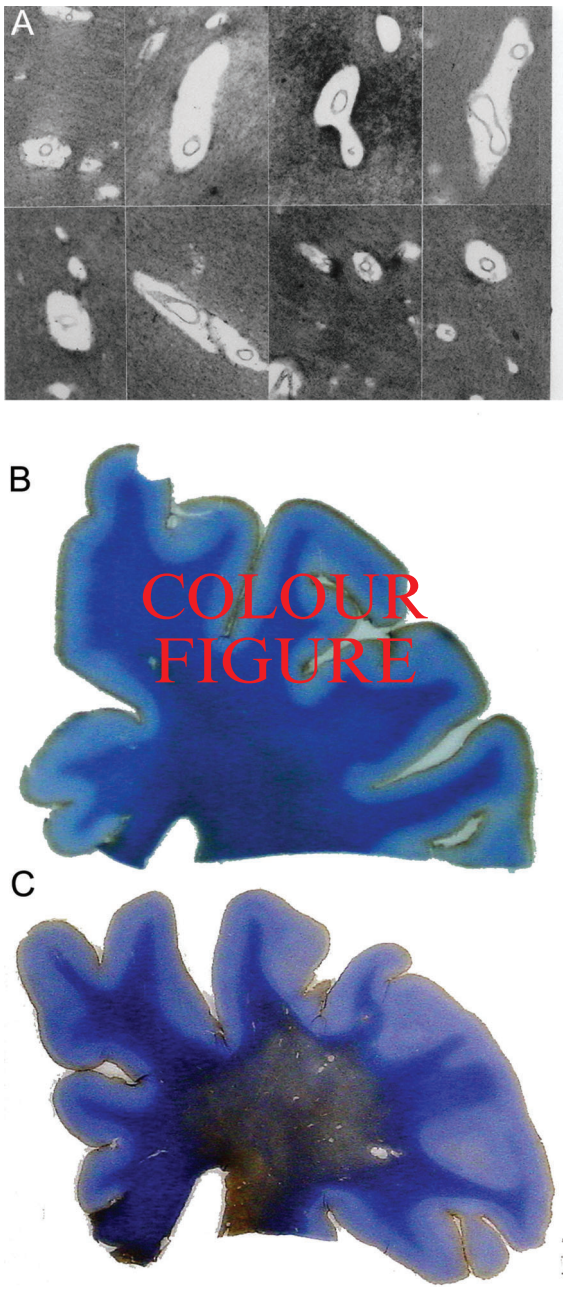

Figure 6: Alzheimer's disease and ND brain sections showing WM rarefaction and dilated periarterial spaces. (A) Blockage of interstitial fluid drainage, by amyloid deposition in the cortical and leptomeingeal arteries, would result in dilation of the WM periarterial spaces shown at a higher magnification. This feature known as *e'tat crible'* is more frequently seen in severe cases of leptomeningeal and cortical vascular amyloidosis suggesting an interruption in the flow of interstitial fluid along the periarterial spaces. These spaces are continuous with the WM periarterial spaces. We propose that stagnation of the interstitial fluid may result in severe hemodynamic alterations, WM tissue damage, and edema with interstitial fluid probably draining into the ventricles of the brain. (B) One half of hemisphere coronal section from a ND subject demonstrating a homogeneous WM in which the nuclei and myelin are stained in intense blue color (hematoxylin). (C) The large paler brownish area in the WM, from an AD patient, demonstrates an extensive area of rarefaction in which the myelin and WM cells have mostly disappeared and are replaced by a large number of astrocytes immunocytochemically stained (brown color) using an antibody against glial fibrillary acid protein (GFAP). The image also shows numerous and visibly enlarged periarterial spaces in the WM. Magnification: ×100

studies have demonstrated that the integrity of the myelin sheaths is essential for neural transmission and that the oligodendrocytes can control the function of neurons. Interestingly, some investigators have considered the possibility of WM alterations as primary events in the pathogenesis of sporadic AD[89–93]. This suggestion has recently been underscored by genomic studies indicating a progression of AD along myelinated axons. Transcriptional studies suggest that disturbances in axons and myelin sheaths incite the oligodendrocytes to secrete growth factors that activate neurons and glial cells eliciting a compensatory tumor suppressor response that affects axonal–myelin interactions[94]. Thus, we decided to investigate to which extent the biochemistry and morphology of the WM is disturbed in AD. Surprisingly, no one has ever determined the quantity of Aβ peptides in the WM despite the fact that these molecules are so central to the prevalent amyloid cascade hypothesis to explain the pathogenesis of AD.

White matter Aβ peptides are more abundant in AD

Prior to immunoassay quantification, we separated the Aβ peptides from other WM proteins by size-exclusion chromatography under denaturing conditions and collected the fraction containing the 3–8 kDa molecules. Aβ quantification by europium immunoassay demonstrated significant differences between AD and control groups. White matter tissue was obtained from the deep regions of the centrum semiovale far away from the subcortical regions that, in some cases, lodge a small number of amyloid plaques. On the average, the soluble Aβ *n*–40 and Aβ *n*–42 peptides were more abundant in AD than in the ND cases: 9.6 and 2.7 times, respectively. The total Aβ (*n*–40 plus *n*–42) was on the average 4.2 times greater in AD compared with the ND control group. In the WM, there were significant differences between the control and AD cohorts with respect to both Aβ *n*–40 levels (means: 111 ng/g and 1069 ng/g of tissue, respectively; $p=0.05$) and Aβ *n*–42 levels (means: 418 ng/g and 1135 ng/g of tissue, respectively, $p=0.01$). In reference to the Apo E ε3/ε3 genotype, a significant difference was evident between the ND and AD groups with respect to Aβ *n*–40 levels (means: 77 ng/g and 263 ng/g of tissue, respectively; $p=0.05$) . There was a slightly less significant difference between those ND individuals carrying at least one Apo E ε4 gene (mean: 145 ng/g of tissue) and those AD subjects with Apo E ε4/ε4 (mean: 1875 ng/g of tissue; $p=0.06$). In terms of significance, the results were reversed for Aβ *n*–42. The levels of Aβ *n*–42 peptide in the control and AD populations were not significantly different for the Apo E ε3/ε3 genotype (means: 371 ng/g and 552 ng/g of tissue, respectively; $p=0.16$). However, there was a significant difference in Aβ *n*–42 between the ND groups with at least one Apo E ε4 gene (mean: 473 ng/g of tissue) and the AD group with ApoE ε4/ε4 genotype (mean: 1719 ng/g of tissue; $p=0.013$). The Aβ *n*–40 and Aβ *n*–42 levels were also examined with respect to Braak stage score. For Aβ *n*–40, the average values for the Braak I&II and Braak VI groups were 87 ng/g and 1,175 ng/g, respectively. The Braak VI cohort had about 13.4 times more Aβ *n*–40 in the WM than did the Braak I&II group ($p=0.05$). For Aβ *n*–42, however, the mean values for the Braak I&II and Braak VI groups were 415 ng/g and 1,178 ng/g, respectively. Thus, the Braak VI group had about 2.8 times more WM Aβ *n*–42 than the Braak I&II group ($p=0.03$). From a pathological point of view, the presence of soluble Aβ in the WM remains to be thoroughly investigated. However, some preliminary results suggest that, in rodents, Aβ is toxic to oligodendrocytes by activating the sphingomyelinase that leads to the accumulation of ceramides[95]. In this study, we report a significant reduction in the numbers of microvessels in the rarefied WM of AD patients that may be instrumental in WM hypoxia. We have observed, in a preliminary investigation, that some angiogenesis promoting genes seem to be up-regulated in the WM of AD patients[96]. Interestingly, Aβ rich in β-sheet structure has been found to be a powerful inhibitor of angiogenesis in both *in vitro* and *in vivo* assays[97].

White matter protein levels are reduced in AD

An estimation of the total WM protein concentration was consistent with the physical nature of this tissue. The AD cohort had an average value of 23.3 mg/g WM tissue, whereas the ND subjects had an average value of 32.8 mg/g tissue. Thus, the WM total protein of the AD group was about 29% less than the control cohort ($p=0.029$).

White matter cholesterol is decreased in AD

The mean AD group WM cholesterol level was 28.6 mg/g tissue while the control subjects had an average value of 32.4 mg/g tissue ($p=0.0003$). Within the AD group, the extent of the decrease was gender specific with female AD subjects having a 12% lower WM cholesterol level than the male group ($p=0.006$). When the Apo E genotype was considered for AD and ND groups, the WM cholesterol was about 20% greater in the ND Apo E ε3/ε4 and ε4/ε4 subjects compared with AD subjects with the same genotypes ($p=0.006$). White matter cholesterol was examined in relation to age. If the Apo E genotype is ignored, a continuous decline in WM cholesterol occurred with increasing age in the ND cohort ($R=0.54$). For the AD cases, the WM cholesterol correlated with age, although this correlation was not nearly as strong as for the ND group ($R=0.14$). The correlation lines demonstrated that, at any age, the WM cholesterol levels would always be lower for the AD individuals.

White matter myelin and oligodendrocyte protein levels are diminished in AD

Myelin basic protein (MBP)

The major protein isoforms of MBP were separated and analyzed by Western blots. When the WM average levels of MBP were compared between AD and ND groups there was a decrease of about 16% in the demented group ($p=0.035$). Separation of the WM MBP values by Apo E genotype revealed 14% and 17% declines in the AD groups carrying no ε4 or one or two ε4 alleles ($p=0.17$ and $p=0.12$), respectively.

Myelin proteolipid protein (PLP)

In the WM there was a 10% decrease in PLP in the AD cases when compared with the ND group ($p=0.018$). The AD subjects homozygous for ApoE ε3 had 15% less PLP than the control group with the same genotype ($p=0.04$). The AD Apo E ε4-carrying individuals had a modest 4% decrease when compared with the control group with the same genotype ($p=0.32$). The average PLP values for the Braak I&II and Braak VI groups were 0.99 and 0.88, respectively, resulting in a decrease of about 10.5 % in the WM PLP level ($p=0.03$).

2',3'-Cyclic nucleotide-3'-phosphodiesterase (CNPase)

Western blots of CNPase showed that this protein was 17% less abundant in the WM of AD subjects than in the ND group ($p=0.017$). When Apo E genotype was considered, those AD individuals with ε3/ε3 had 20% less CNPase than the ND counterpart ($p=0.010$). Furthermore, AD subjects carrying Apo E ε3/ε4 and ε4/ε4 genotypes also showed a 16% CNPase decrease ($p=0.07$). The Braak VI group had 17% lower WM CNPase level than did the Braak I&II group ($p=0.05$).

White matter ultrastructure is altered in AD

By taking advantage of our brain bank facilities and our rapid autopsy program that gives us immediate access to fresh brain tissue, periventricular WM from AD and ND cases was dissected and processed for electron microscopic studies. Our preliminary ultrastructural observations demonstrated that the WM from AD cases has fewer and smaller axons, thinner myelin and more abundant and enlarged astrocytes than does the WM of ND cases (*Figure 7A,B*). Separation of lamellae was more pronounced in the AD cases than in the ND individuals, and therefore could be attributed to the disease. More striking, however, was a noticeable reduction in the number and caliber of the axons in the AD WM when compared with the control cases. In the AD ($n=3$) versus ND cases ($n=3$), there was an overall reduction of up to 25% in the number of axons. In addition the caliber of the axons is clearly reduced in AD WM. The pathologic pallor of the WM rarefactions, evident under the majority of histological stains and observed by radiological techniques, suggests a loss of the formed and densely packed elements common to normal WM. Oligodendrocytes, myelin and axons have been mostly replaced with interstitial water and swollen enlarged astrocytes.

White matter total cell count is reduced in AD

One of the most noticeable characteristics of the WM rarefaction is a reduction in the number of cells. As illustrated in *Figure 8A,B*, there is a remarkable difference in the number of surviving cells between the WM of AD and ND individuals. We counted the number of nuclei in the periventricular area of 12 individuals with AD and 12 ND controls. The average number of nuclei observed in six areas per individual at ×400 amounted to 331/

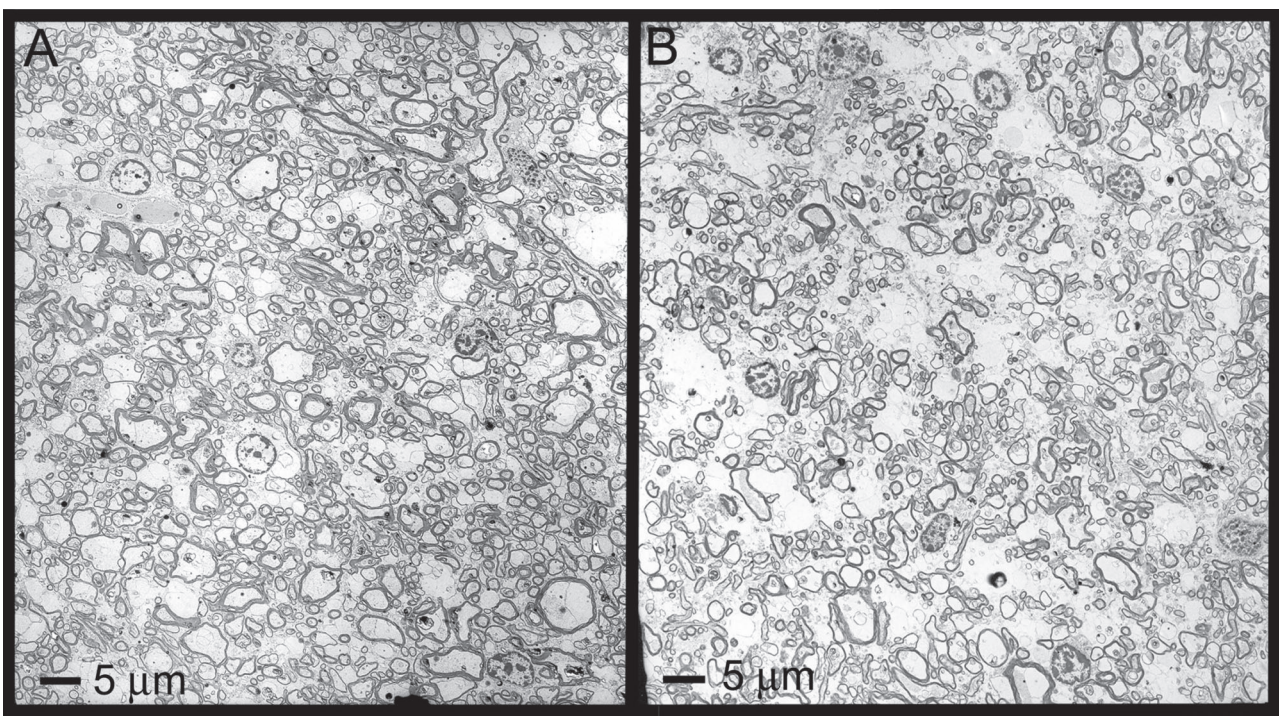

Figure 7: Electron micrographs of WM in AD and ND individuals. (A) The WM from a ND individual demonstrates a large number of myelinated small and large axons. Aging causes degenerative changes in myelin thickness and packing, alterations which in part are responsible for the motor, sensory and cognitive deficits observed in the elderly. (B) In the case of AD, the number of myelinated axons is drastically reduced. There are apparent empty areas representing accumulation of extracellular water or extensive swelling and vacuolation of astrocytes which are interpreted as incorporated edema fluid and cellular debris

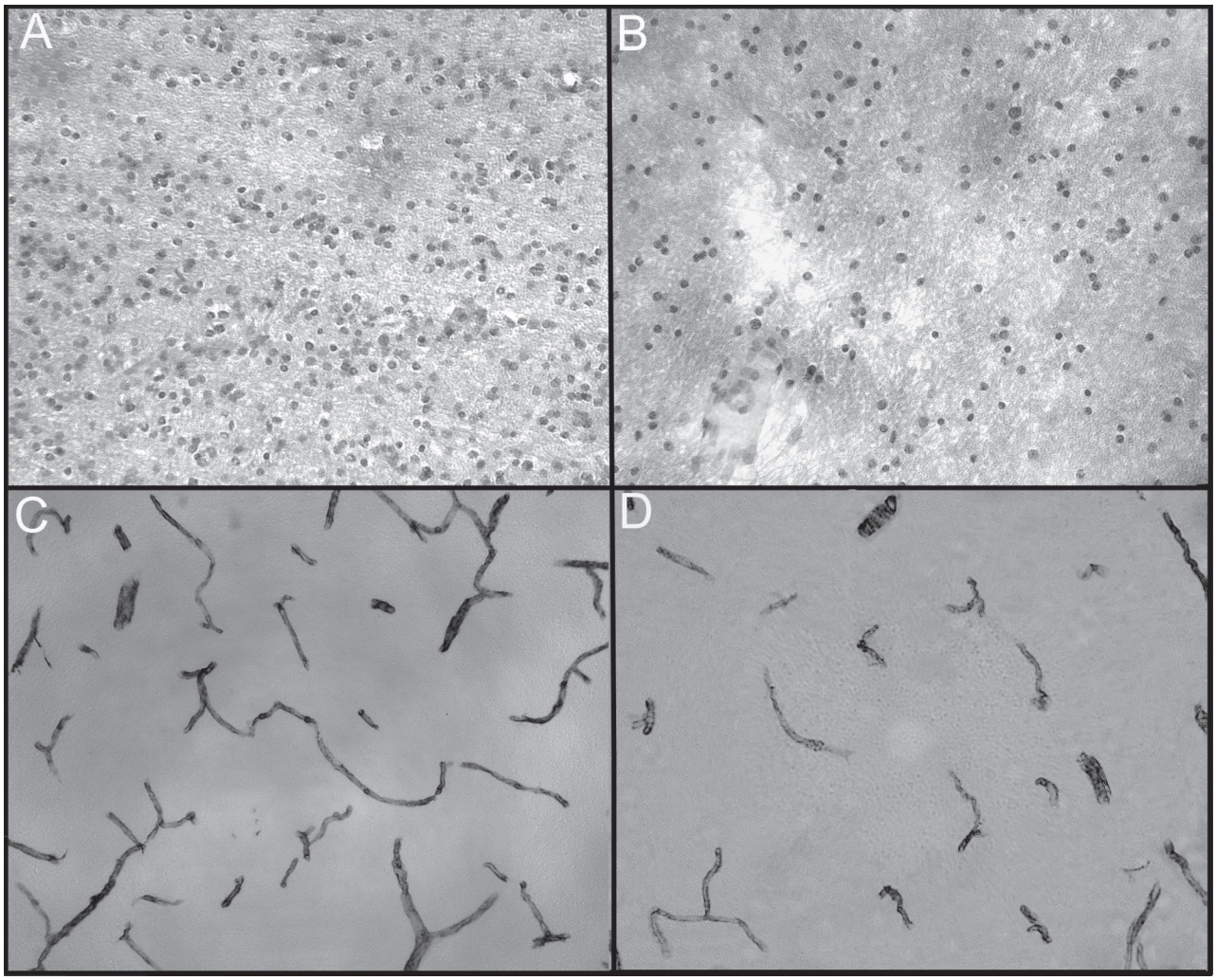

Figure 8: White matter cell density and microvessel density. (A) Cell density in ND control tissue. (B) Cell density in AD tissue. (C) Microvessel density in ND control tissue. (D) Microvessel density in AD tissue. Magnification: A and B, ×200; C and D, ×100

0.058 mm^2 (range 307–355) in the ND population, while in the AD cases at ×400 the count was 269/0.058 mm^2 (range 228–310). These differences were statistically significant ($p=0.025$). Interestingly, the total cell count correlated with the NFT score ($R_s=0.48$, $p=<0.001$). As can be seen in *Figure 9A* the higher the number of nuclei the lower the NFT score. Negative cell count correlations were also observed for the Braak score (*Figure 9B*). Higher cell counts were associated with lower (stages I–III) Braak scores and vice versa ($R_s=0.52$, $p<0.001$). A comparison between the total cell count and the plaque score was less robust ($R_s=0.24$, $p<0.01$) as seen in *Figure 9C*.

White matter microvessels are significantly reduced in AD

It is generally believed that the most important pathogenic factor underlying WM rarefaction in the AD is a vascular deficit that results in hypoxia/ischemia, oligodendrocyte damage, demyelination and astrocytosis. To examine this contention, we counted the number of blood vessels immunocytochemically stained by collagen IV (*Figure 8C,D*) in the periventricular WM rarefied areas of AD ($n=12$) and normal areas of ND controls ($n=12$). In the former cases the average number of vessels at ×200 was 31/0.23 mm^2, while in the latter group the average number at ×200 was 18/0.23 mm^2 ($p=0.0001$). In the areas of rarefaction, the distribution of the blood vessels displayed a clear quantity gradient. Blood vessels were less abundant in the deeper areas contiguous to the ventricles (ND=26; AD=16) and more numerous in the more superficial areas of the WM (ND=37; AD=19.5). The area between the deep and superficial WM contained 30 and 19.5 blood vessel counts for ND and AD cases, respectively. As in the case of the WM cell count, the NFT and Braak scores demonstrated a strong correlation with the number of vessels: $R_s=0.68$, $p<0.001$ (*Figure 9D*) and $R_s=0.68$, $p<0.001$ (*Figure 9E*), respectively. The correlation between blood vessel count and plaque score was less apparent ($R_s=0.38$), as seen in *Figure 9F*. We observed a positive correlation when the AD and ND total cell count were plotted against the blood vessel count ($R_s=0.38$,

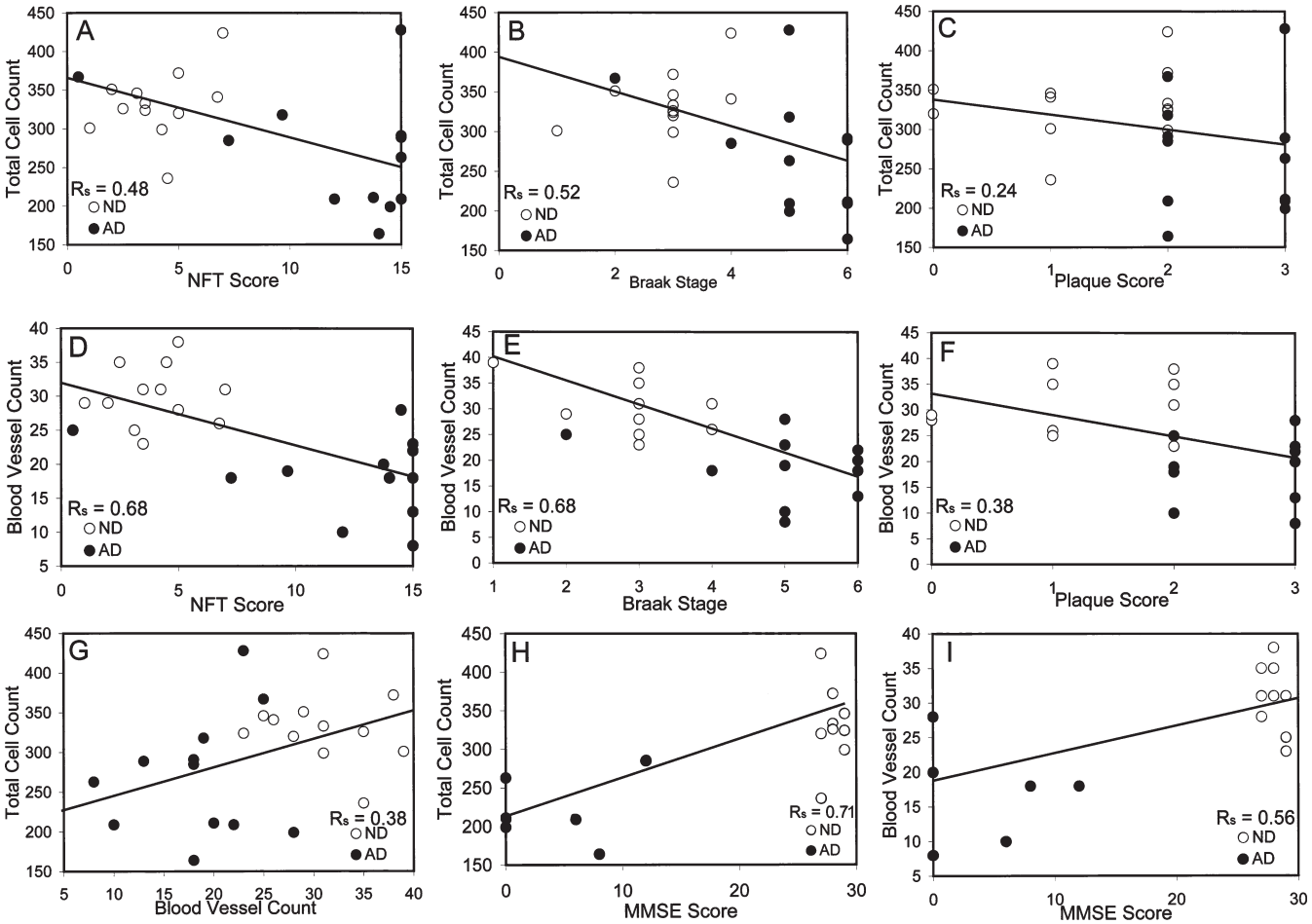

Figure 9: White matter total cell count and total blood vessel count correlated against the neuropathological scores of AD and MMSE. (A) Total cell count versus NFT score. (B) Total cell count versus Braak stage. (C) Total cell count versus plaque score. (D) Blood vessel count versus NFT score. (E) Blood vessel count versus Braak stage. (F) Blood vessel count versus plaque score. (G) Total cell count versus blood vessel count. (H) Total cell count versus MMSE score. (I) Blood vessel count versus MMSE score

$p<0.001$, *Figure 9G*). This association suggests that, in the areas of WM rarefaction in AD, both cell and vessels are depleted when compared with ND elderly controls.

MMSE score correlates with total cell and blood vessel counts

Individuals with AD ($n=6$) had, as expected, the lowest MMSE scores which correlated with the lowest number of cells in the areas of rarefaction. Conversely, those ND control individuals ($n=9$) with the highest MMSE scores contained higher numbers of cells ($R_s=0.71$, *Figure 9H*). This trend was also observed when the number of blood vessels was compared with the MMSE scores ($R_s=0.56$, *Figure 9I*). Altogether, the data indicate that the average number of remaining cells and vessels in the rarefied WM of AD is directly correlated with the degree of psychometric performance, suggesting a contribution to the clinical manifestation of dementia.

CONCLUSION

The recognition of cardiovascular disease involvement in sporadic AD pathogenesis represents a departure from the traditional emphasis placed on the amyloid cascade to explain the etiology of this dementia. It is possible that sporadic AD Aβ deposition and tau neurofibrillary accumulation represent the end results of a primary breakdown in neuronal and glial energy metabolism, as well as BBB malfunction caused by the extensive brain hypoperfusion resulting from chronic and sustained vascular failure. A contemporary, rigorous and extensive investigation of brain arterial atherosclerotic damage and its relationship to sporadic AD had not been conducted, possibly because the prevailing opinion is that the role of atherosclerosis in this dementia, if any, is minor. In our preliminary study, using quantitative techniques with fine-scale resolution, we found that, in AD patients, the circle of Willis and its related vessels possessed a significant degree of arterial stenosis as a consequence of multiple and severe atherosclerotic lesions. Measures of these atherosclerotic lesions showed that they were more severe in AD cases than in age-matched ND cases and significantly correlated with measures of AD neuropathological lesions in GM and WM[69].

We contend that the association of severe atherosclerosis of the circle of Willis and cerebral arteries with a severe vascular amyloidosis and destruction of the

microvasculature leads to serious hemodynamic deficiencies and reduced cerebral blood flow. In the elderly, a sustained and relentless reduction in perfusion pressure damages brain function, thus contributing to the pathological manifestations of this dementia. While it is true that some AD cases do not have atherosclerosis, this does not necessarily mean that atherosclerosis has no role in AD. This discordance could be due to the etiologic heterogeneity of sporadic AD, with only some cases being caused by atherosclerosis. In other individuals, atherosclerosis may not be entirely sufficient to cause AD, but may act as a catalytic event to accelerate or worsen the dementing process. Regardless of which of these alternatives is ultimately proved correct, our preliminary studies strongly suggest that atherosclerosis of the circle of Willis is substantially more common and more severe in AD cases than it is in the ND elderly.

We also anticipate a positive correlation between the severity of the atherosclerosis and the quantity of leptomeningeal amyloidosis based on the assumption that both degenerative processes are interrelated. This is supported by ample experimental evidence demonstrating vascular inflammation at all stages of atherosclerosis, a reaction also persistently observed in vascular amyloidosis. In addition, the leptomeningeal arteries are the target of inexorable trauma produced by the relentless pounding of the circulating blood that can be compounded by increased systolic pressure and countercurrents created by escalating atherosclerosis and amyloidosis, as well as by loss of arterial compliance. Aging and the increasing inability to repair accumulated vascular damage are the ultimate factors. Interestingly, by age 80 years the arterial walls have built up the sustained trauma of about 3 billion hemodynamic cardiac cycles. We suggest that the common denominators of these arterial disturbances are brain hypoperfusion and failure in the repair and maintenance of the BBB. If vascular inflammation is the forerunner of atherosclerosis, a leaky BBB may be the harbinger of vascular amyloidosis. Amyloid fibrils may represent, as we proposed[3], a plastering material that strongly and abundantly enmeshes with the molecules of the basal lamina of endothelial and smooth muscle cells in an attempt to re-seal a failing BBB. Leptomeningeal atherosclerosis and amyloidosis may be to a certain extent preventable, or at least delayed, by controlling cardiovascular disease through medication and changes in lifestyle. A positive correlation between atherosclerosis and amyloidosis and between these factors and the lesions of AD, in terms of the number of plaques, tangles and white matter rarefaction, will underscore the importance of the cerebral circulation in the pathogenesis of AD.

Sporadic AD is an emerging disease. While long recognized to afflict the elderly, the recent increased case rate leads to a potentially important unifying hypothesis regarding atherosclerosis as a critical underlying AD pathogenic mechanism. The historical disagreements on the participation of atherosclerosis in AD may be explained by recent economic, technological and scientific changes in our culture that need be considered in relation to AD epidemiology. During the last 50 years, the dietary habits and physical activity patterns have drastically changed in the USA[98–101]. The economic and technologic successes achieved in the last 50 years have provided abundant food, convenient access to vehicular transportation, and have led to the large-scale promotion of home entertainment and the cybernetic sciences. These factors have encouraged sedentary lifestyles which when combined with high calorie diets promote atherosclerosis. In this vein, one can appreciate as prescient Dr Robert Bell, a 19th century physician, who stated that 'A man's arteries are as old as he makes them'. With the outstanding advances in the medical field, the average life expectancy in the USA has significantly increased from about 50 years in 1900 (the time of Alois Alzheimer) to almost 80 years in 2004. A longer life expectancy allows more atheroma deposits, increased circle of Willis stenosis, more severe brain hypoperfusion, and a higher ultimate AD incidence. In summary, our preliminary data strongly suggest that, in addition to coronary artery disease and atherosclerosis of the carotid arteries, two well-recognized risk factors for AD, severe atherosclerosis of the circle of Willis and related arteries must also be considered a major risk factor in the pathogenesis of sporadic AD.

Coronary artery disease has commanded a great deal of medical attention because of the excruciating pain and the menace of sudden death that occur due to cardiac hypoperfusion. As a consequence, modern medicine has devised surgical procedures and pharmacological interventions that successfully prevent or delay cardiac failure. With the exception of stroke, substantially less attention has been given to the involvement of severe arterial stenosis associated with circle of Willis and to brain hypoperfusion in AD. Perhaps this is due to the fact that, unlike the situation for the heart, chronic brain ischemia does not manifest physical pain, but rather may be expressed as an anguishing and relentless trek into dementia. On the positive side, changes in life-style, in addition to medications designed to reduce hyperlipidemia, may diminish cardiovascular disease and by extension prevent or delay the onset of sporadic Alzheimer's disease. Understanding the full vascular pathological basis and the altered hemodynamic sequence of events that underlie this dementia will aid in the development of optimal therapeutic interventions that may delay the onset of AD and enhance the quality of life of these patients.

ACKNOWLEDGEMENTS

This work was partially supported by the NIA grants: AG-19795, AG-17490 and NS-38674 and by the State of Arizona Alzheimer's Disease Research Center

REFERENCES

1 Selkoe DJ, Podlisny MB. Deciphering the genetic basis of Alzheimer's disease. *Annu Rev Genomics Hum Genet* 2002; **3**: 67–99

2 Roher AE, Ball MJ, Bhave SV, *et al*. β-Amyloid from Alzheimer disease brains inhibits sprouting and survival of sympathetic neurons. *Biochem Biophys Res Commun* 1991; **174**: 572–579

3 Roher AE, Lowenstein JD, Clarke S, *et al*. β-Amyloid-(1–42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer's disease. *Proc Natl Acad Sci USA* 1993; **90**: 10836–10840

4 O'Brien JT, Eagger S, Syed GM, *et al*. A study of regional cerebral blood flow and cognitive performance in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1992; **55**: 1182–1187
5 Kawamura J, Meyer JS, Ichijo M, *et al*. Correlation of leuko-araiosis with cerebral atrophy and perfusion in elderly normal subjects and demented patients. *J Neurol Neurosurg Psychiatry* 1993; **56**: 182–187
6 Waldemar G, Bruhn P, Kristensen M, *et al*. Heterogeneity of neocortical cerebral blood flow deficits in dementia of the Alzheimer type. *J Neurol Neurosurg Psychiatry* 1994; **57**: 285–295
7 Minoshima S, Giordani B, Berent S, *et al*. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Ann Neurol* 1997; **42**: 85–94
8 De Jong GI, De Vos RA, Steur EN, *et al*. Cerebrovascular hypoperfusion: A risk factor for Alzheimer's disease? Animal model and postmortem human studies. *Ann NY Acad Sci* 1997; **826**: 56–74
9 de la Torre JC. Critically attained threshold of cerebral hypoperfusion: Can it cause Alzheimer's disease? *Ann NY Acad Sci* 2000; **903**: 424–436
10 de la Torre JC. Cerebral hypoperfusion, capillary degeneration, and development of Alzheimer disease. *Alzheimer Dis Assoc Disord* 2000; **14**: S72–S81
11 de la Torre JC. Impaired cerebromicrovascular perfusion. Summary of evidence in support of its causality in Alzheimer's disease. *Ann NY Acad Sci* 2000; **924**: 136–152
12 Kalaria RN. The role of cerebral ischemia in Alzheimer's disease. *Neurobiol Aging* 2000; **21**: 321–330
13 Bokde AL, Pietrini P, Ibanez V, *et al*. The effect of brain atrophy on cerebral hypometabolism in the visual variant of Alzheimer disease. *Arch Neurol* 2001; **58**: 480–486
14 Hachinski VC, Potter P, Merskey H. Leuko-araiosis: An ancient term for a new problem. *Can J Neurol Sci* 1986; **13**: 533–534
15 Hachinski VC, Potter P, Merskey H. Leuko-araiosis. *Arch Neurol* 1987; **44**: 21–23
16 Janota I, Mirsen TR, Hachinski VC, *et al*. Neuropathologic correlates of leuko-araiosis. *Arch Neurol* 1989; **46**: 1124–1128
17 Pantoni L, Garcia JH. Cognitive impairment and cellular/vascular changes in the cerebral white matter. *Ann NY Acad Sci* 1997; **826**: 92–102
18 Pantoni L, Garcia JH. The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review. *Stroke* 1995; **26**: 1293–1301
19 Prencipe M, Marini C. Leuko-araiosis: Definition and clinical correlates – an overview. *Eur Neurol* 1989; **29**: S27–29
20 Diaz JF, Merskey H, Hachinski VC, *et al*. Improved recognition of leukoaraiosis and cognitive impairment in Alzheimer's disease. *Arch Neurol* 1991; **48**: 1022–1025
21 Mirsen TR, Lee DH, Wong CJ, *et al*. Clinical correlates of white-matter changes on magnetic resonance imaging scans of the brain. *Arch Neurol* 1991; **48**: 1015–1021
22 Kobari M, Meyer JS, Ichijo M, *et al*. Leukoaraiosis: Correlation of MR and CT findings with blood flow, atrophy, and cognition. *Am J Neuroradiol* 1990; **11**: 273–281
23 Kawamura J, Meyer JS, Terayama Y, *et al*. Leuko-araiosis and cerebral hypoperfusion compared in elderly normals and Alzheimer's dementia. *J Am Geriatr Soc* 1992; **40**: 375–380
24 Brun A, Englund A. A white matter disorder in dementia of the Alzheimer type: A pathoanatomical study. *Ann Neurol* 1986; **19**: 253–262
25 Johnson KA, Mueller ST, Walshe TM, *et al*. Cerebral perfusion imaging in Alzheimer's disease. Use of single photon emission computed tomography and iofetamine hydrochloride I 123. *Arch Neurol* 1987; **44**: 165–168
26 Prohovnik J, Mayeux R, Sackeim HA, *et al*. Cerebral perfusion as a diagnostic marker of early Alzheimer's disease. *Neurology* 1988; **38**: 931–937
27 Besson JA, Crawford JR, Parker DM, *et al*. Neuroimaging, cognitive, and pathological changes in dementia of the Alzheimer type (DAT). *Psychiatry Res* 1989; **29**: 447–448
28 Bladin CF, Chambers BR. Clinical features, pathogenesis and computed tomographic characteristics of internal watershed infarction. *Stroke* 1993; **24**: 1925–1932
29 Obara K, Meyer JS, Mortel KF, *et al*. Cognitive declines correlate with decreased cortical volume and perfusion in dementia of Alzheimer type. *J Neurol Sci* 1994; **127**: 96–102
30 Ishijima M, Imazu O, Kitamura S, *et al*. Clinico-pathological evaluation of leukoaraiosis in Alzheimer's disease. *J Geriatrics* 1996; **33**: 774–753
31 Nagata K, Muruya H, Yuya H, *et al*. Can PET data differentiate Alzheimer's disease from vascular dementia? *Ann NY Acad Sci* 2000; **903**: 252–261
32 Nagata K, Sato M, Satoh Y, *et al*. Hemodynamic aspects of Alzheimer's disease. *Ann NY Acad Sci* 2002; **977**: 391–402
33 Goldsmith HS. Omental transposition to the brain for Alzheimer's disease. *Ann NY Acad Sci* 1997; **826**: 323–336
34 Goldsmith HS. Treatment of Alzheimer's disease by transposition of the omentum. *Ann NY Acad Sci* 2002; **977**: 454–467
35 Johnson KA, Jones K, Holman BL, *et al*. Preclinical prediction of Alzheimer's disease using SPECT. *Neurology* 1998; **50**: 1563–1571
36 Gonzalez RG, Fischman AJ, Guimaraes AR, *et al*. Functional MR in the evaluation of dementia. *Am J Neuroradiol* 1995; **16**: 1763–1770
37 Harris GJ, Lewis RF, Satlin A, *et al*. Dynamic susceptibility contrast MR imaging of regional cerebral blood volume in Alzheimer disease: A promising alternative to nuclear medicine. *Am J Neuroradiol* 1998; **19**: 1727–1732
38 Alsop DC, Detre JA, Grossman M. Assessment of cerebral blood flow in Alzheimer's disease by spin-labeled magnetic resonance imaging. *Ann Neurol* 2000; **47**: 93–100
39 Aronson MK, Ooi WL, Morgenstern H, *et al*. Women, myocardial infarction, and dementia in the very old. *Neurology* 1990; **40**: 1102–1106
40 Sparks DL, Hunsaker JC, Scheff SW, *et al*. Cortical senile plaques in coronary artery disease, aging and Alzheimer's disease. *Neurobiol Aging* 1990; **11**: 601–607
41 Soneira CF, Scott TM. Severe cardiovascular disease and Alzheimer's disease: Senile plaque formation in cortical areas. *Clin Anat* 1996; **9**: 118–127
42 Wisniewski HM, Maslinska D. β-Protein immunoreactivity in the human brain after cardiac arrest. *Folia Neuropathol* 1996; **34**: 65–71
43 Schmidt R, Schmidt H, Curb JD, *et al*. Early inflammation and dementia: A 25-year follow-up of the Honolulu-Asia Aging Study. *Ann Neurol* 2002; **52**: 168–174
44 Swan GE, Carmelli D, Larue A. Systolic blood pressure tracking over 25 to 30 years and cognitive performance in older adults. *Stroke* 1998; **29**: 2334–2340
45 Skoog I, Lernfelt B, Landahl S, *et al*. A 15-year longitudinal study of blood pressure and dementia. *Lancet* 1996; **347**: 1141–1145
46 Sparks DL, Scheff SW, Liu H, *et al*. Increased incidence of neurofibrillary tangles (NFT) in nondemented individuals with hypertension. *J Neurol Sci* 1995; **131**: 162–169
47 Tzourio C, Dufouil C, Ducimetiere PAA. Cognitive decline in individuals with high blood pressure. *Neurology* 1999; **53**: 1948–1954
48 Launer LJ, Ross GW, Petrovich H, *et al*. Midlife blood pressure and dementia: The Honolulu-Asia aging study. *Neurobiol Aging* 2000; **21**: 49–55
49 Petrovich H, White LR, Izmirilian G, *et al*. Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: The HAAS Honolulu-Asia aging study. *Neurobiol Aging* 2000; **21**: 57–62
50 Kilander L, Nyman H, Boberg M, *et al*. Hypertension is related to cognitive impairment: A 20-year follow-up of 999 men. *Hypertension* 1998; **31**: 780–786
51 Forette F, Seux ML, Staessen JA, T *et al*. Systolic Hypertension in Europe Investigators. The prevention of dementia with antihypertensive treatment: New evidence for the Systolic Hypertension in Europe (Syst-Eur) study. *Arch Intern Med* 2002; **162**: 2046–2052
52 Havlik RJ, Foley DJ, Sayer B, *et al*. Variability in midlife systolic blood pressure is related to late-life brain white matter lesions: The Honolulu-Asia Aging Study. *Stroke* 2002; **33**: 26–30
53 Peila R, White LR, Petrovich H, *et al*. Joint effect of the APOE gene and midlife systolic blood pressure on late-life cognitive impairment: The Honolulu-Asia aging study. *Stroke* 2001; **32**: 2882–2889

54 Skoog I, Gustafson D. Hypertension and related factors in the etiology of Alzheimer's disease. *Ann NY Acad Sci* 2002; **977**: 29–36
55 Kivipelto M, Laakso MP, Tuomilehto J, *et al*. Hypertension and hypercholesterolaemia as risk factors for Alzheimer's disease: Potential for pharmacological intervention. *CNS Drugs* 2002; **16**: 435–444
56 Qiu C, von Strauss E, Fastbom J, *et al*. Low blood pressure and risk of dementia in the Kungsholmen project: A 6-year follow-up study. *Arch Neurol* 2003; **60**: 223–228
57 Kivipelto M, Helkala EL, Laakso MP, *et al*. Apolipoprotein E ε4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. *Ann Intern Med* 2002; **137**: 149–155
58 Launer LJ, White LR, Petrovitch H, *et al*. Cholesterol and neuropathological markers of AD: A population-based autopsy study. *Neurology* 2001; **57**: 1447–1452
59 Kalmijn S, Launer LJ, Ott A, *et al*. Dietary fat intake and the risk of incident dementia in the Rotterdam study. *Ann Neurol* 1997; **42**: 776–782
60 Kivipelto M, Helkala E, Hanninen T, *et al*. Midlife vascular risk factors and late-life mid cognitive impairment; a population based study. *Neurology* 2001; **56**: 1683–1689
61 Seshadri S, Beiser A, Selhub J, *et al*. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Eng J Med* 2002; **346**: 476–483
62 Luchsinger JA, Tang MX, Stern Y, *et al*. Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. *Am J Epidemiol* 2001; **154**: 635–641
63 Launer LJ. Demonstrating the case that AD is a vascular disease: Epidemiologic evidence. *Ageing Res Rev* 2002; **1**: 61–77
64 Peila R, Rodriguez BL, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes* 2002; **51**: 1256–1262
65 Knopman D, Boland LL, Mosley T, *et al*. Cardiovascular risk factors and cognitive decline in middle-aged adults. *Neurology* 2001; **56**: 42–48
66 Sparks DL. Neuropathologic links between Alzheimer's disease and vascular disease. In: Iqbal K, Swaab DF, Winblad B, Wisniewski HM, eds. *Alzheimer's Disease and Related Disorders*. London: Wiley, 1999: pp. 153–163
67 Kuo YM, Emmerling MR, Bisgaier CL, *et al*. Elevated low-density lipoprotein in Alzheimer's disease correlates with brain Aβ 1–42 levels. *Biochem Biophys Res Commun* 1998; **252**: 711–715
68 Roher AE, Kuo Y.M., Kokjohn TA, *et al*. Amyloid and lipids in the pathology of Alzheimer disease. *Amyloid* 1999; **6**: 136–145
69 Roher AE, Esh C, Kokjohn TA, *et al*. Circle of Willis atherosclerosis is a risk factor for sporadic Alzheimer's disease. *Arterioscler Thromb Vasc Biol* 2003; **23**: 2055–2062
70 Roher AE, Kuo YM, Esh C, *et al*. Cortical and leptomeningeal cerebrovascular amyloid and white matter pathology in Alzheimer's disease. *Mol Med* 2003; **9**: 112–122
71 Mirra SS. The CERAD neuropathology protocol and consensus recommendations for the postmortem diagnosis of Alzheimer's disease: A commentary. *Neurobiol Aging* 1997; **18**: S91–94
72 The National Institute on Aging and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging* 1997; **18**: S1–2
73 Ilveskoski E, Perola M, Lehtimaki T, *et al*. Age-dependent association of apolipoprotein E genotype with coronary and aortic athersclerosis in middle-aged men: An autopy study. *Circulation* 1999; **100**: 608–613
74 Li L, Cao D, Garber DW, *et al*. Association of aortic atherosclerosis with cerebral β-amyloidosis and learning deficits in a mouse model of Alzheimer's disease. *Am J Pathol* 2003; **163**: 2155–2164
75 Corder EH, Saunders AM, Strittmatter WJ, *et al*. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; **261**: 921–923
76 Mori T, Paris D, Town T, *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
77 Roher AE, Weiss N, Kokjohn TA, *et al*. Increased Aβ peptides and reduced cholesterol and myelin proteins characterize white matter degeneration in Alzheimer's disease. *Biochemistry* 2002; **41**: 11080–11090
78 Sparks DL, Scheff SW, Hunsaker JC, *et al*. Induction of Alzheimer like β-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol* 1994; **126**: 88–94
79 Puglielli L, Konopka G, Pack-Chung E, *et al*. Acyl-coenzyme A: Cholesterol acyltransferase modulates the generation of the amyloid β-peptide. *Nat Cell Biol* 2001; **3**: 905–912
80 Refolo LM, Malester B, LaFrancois J, *et al*. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis* 2000; **7**: 321–331
81 Bodovitz S, Klein WL. Cholesterol modulates α-secretase cleavage of amyloid precursor protein. *J Biol Chem* 1996; **271**: 4436–4440
82 Racchi M, Baetta R, Salvietti N, *et al*. Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. *Biochem J* 1997; **322**: 893–898
83 Simons M, Keller P, De Strooper B, *et al*. Cholesterol depletion inhibits the generation of β-amyloid in hippocampal neurons. *Proc Natl Acad Sci USA* 1998; **95**: 6460–6464
84 Refolo LM, Pappola MA, LaFrancois J, *et al*. A cholesterol-lowing drug reduces β-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* 2001; **8**: 890–899
85 Fassbender K, Simons M, Bergmann C, *et al*. Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ 42 and Aβ 40 in vitro and in vivo. *Proc Natl Acad Sci USA* 2001; **98**: 5856–5861
86 Ganz P, Ganz W. Coronary blood flow and myocardial ischemia. In: Braunwald E, Zipes DP, Libby P, eds. *Heart Disease*, 6th edn, New York: Saunders, 2001: pp. 1087–1113
87 Roher AE. Unpublished data
88 Cacabelos R, Fernandez-Novoa L, *et al*. Cerebrovascular risk factors in Alzheimer's disease: Brain hemodynamics and pharmacogenomic implications. *Neurol Res* 2003; **25**: 567–580
89 Braak H, Braak E. Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. *Acta Neuropathol (Berlin)* 1996; **92**: 197–201
90 McGeer PL, McGeer EG, Akiyama H, *et al*. Neuronal degeneration and memory losss in Alzheimer's disease and aging. *Exp Brain Res* 1990; **21**: S411–426
91 Raz N. Aging of the brain and its impacts on cognitive performance. In: Craik FIM, Salthouse TA, eds. *Handbook of Aging and Cognition*, Mahway, NJ: Erlbaum, 1999
92 Reisberg B, Franssen EH, Hasan SM, *et al*. Retrogenesis: Clinical, physiologic, and pathologic mechanisms in brain aging, Alzheimer's and other dementing processes. *Eur Arch Psychiatry Clin Neurosci* 1999; **249**: III/28–36
93 Hyman BT. The neuropathological diagnosis of Alzheimer's disease: Clinical-pathological studies. *Neurobiol Aging* 1997; **18**: S27–32
94 Blalock EM, Geddes JW, Chen KC, *et al*. Incipient Alzheimer's disease: Microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci USA* 2004; **101**: 2173–2178
95 Lee JT, Xu J, Lee JM, *et al*. Amyloid-β peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. *J Cell Biol* 2004; **164**: 123–131
96 Paris D, Townsend K, Quadros A, *et al*. Inhibition of angiogenesis by Aβ peptides. *Angiogenesis* 2004, in press
97 Roher AE. Unpublished observations
98 Allison DB, Fontaine KR, Manson JE, *et al*. Annual deaths attributable to obesity in the United States. *JAMA* 1999; **282**: 1530–1538
99 Hahn RA, Teutsch SM, Rothenberg RB, *et al*. Excess deaths from nine chronic diseases in the United States. *JAMA* 1990; **264**: 2654–2659
100 Kuczmarski RJ, Flegal KM, Campbell SM, *et al*. Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960–1991. *JAMA* 1994; **272**: 205–211
101 Flegal KM, Carroll MD, Ogden CL, *et al*. Prevalence and trends in obesity among US adults, 1999–2000. *JAMA* 2002; **288**: 1723–1727

Author Query Sheet

Manuscript Information	
Journal Acronym	Neurol Res
Volume and issue	26:5
Author name	Kalbach
Manuscript No.	04/81

AUTHOR: The following queries have arisen during the editing of your manuscript. Please answer the queries when you e-mail your corrections.

QUERY NO.	QUERY DETAILS
Q1	Please supply keywords.
Q2	Ref. 30: please check page span
Q3	Ref. 96: more details available?
Q4	Please supply the DOI number.