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Oxidative LDL modification is increased in vascular dementia and is inversely associated with cognitive performance

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Abstract

It is not known whether the association between increased plasma homocysteine (Hcy) associated with LDL modification and propensity for LDL uptake by macrophages in cardiovascular disease patients holds true in vascular dementia (VaD). Plasma from 83 subjects diagnosed with Alzheimer's disease (AD), VaD, mild cognitive impairment (MCI) and from controls was analysed to examine (1) whether LDL isolated from the plasma of VaD is biochemically and functionally distinct from that isolated from AD, MCI or controls; and (2) whether such biomarkers of LDL phenotype are related to plasma folate levels, Hcy levels and/or to disease severity. Folate and vitamin B12 levels were significantly lower in VaD subjects than in controls. VaD-LDL showed increased protein carbonyl content ($p < 0.05$) and was more susceptible to scavenging by macrophages ($p < 0.05$) than AD- or control-LDL. Patients from the VaD cohort were more prevalent in the lowest tertile for HDL:LDL and the upper tertile for LDL oxidation; the combined parameters of HDL cholesterol, LDL oxidation and scavenging by macrophages show 87% sensitivity towards VaD detection. The association between folate deficiency, LDL modification and dysfunction in VaD but not in AD may provide a novel biomarker assessment to discriminate between the diseases.

Keywords: *Alzheimer's dementia, vascular dementia, mild cognitive impairment, LDL oxidation, homocysteine, folate, inflammation.*

Introduction

Folic acid is an essential vitamin for health, for example in DNA synthesis [1]. Together with vitamins B12 and B6, folic acid is involved in promoting intracellular homocysteine (Hcy) turnover. Therefore, folate inadequacy leads to elevated plasma Hcy [2]. High plasma Hcy levels are recognized as an independent risk factor for cardiovascular disease (CVD) and also, more recently, for vascular dementia (VaD) [3–5]. VaD is the second most common form of dementia in the elderly after Alzheimer's disease (AD) and is characterized by a step-wise progression of dementia that is closely associated with cerebral infarcts [6].

Elevated Hcy and low folate levels have also been associated with radiological markers (especially magnetic resonance imaging, MRI) of cognitive impairment, but in some cases patients with MRI markers of disease are not clinically deficient in folate status [7]. Conventional techniques for diagnosing folate deficiency may therefore be inadequate for identifying people who may benefit from dietary supplementation. Thus, it is important to re-evaluate the definition of functional folate deficiency and explore what this implies for different age and health state groups. Identification of sub-populations of patients who are most likely to benefit from nutritional intervention is

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enhanced by the identification of suitable biomarkers as intermediate endpoints which relate to clinical outcome [8]. To achieve this, knowledge of the possible mechanism of vascular dysfunction and identification of suitable intermediate endpoint biomarkers is essential. The auto-oxidation of Hcy in the presence of metal ions and oxygen has been shown to result in the generation of ROS such as hydrogen peroxide [9], where the rate of the auto-oxidation process and therefore the rate of generation of ROS available for oxidative reactions depends in part upon the concentration of the Hcy and also on trace metal ions. Elevated homocysteine, either via ROS or through independent pathways, triggers proinflammatory responses within the vasculature, resulting in monocyte recruitment, adhesion and transmigration through the vessel wall and in the differentiation of monocytes into cholesterol-scavenging macrophages [10].

In addition to hyperhomocysteinemia, the main risk factors for VaD include age, hypertension, CVD and inflammation [11]. The discovery that the apolipoprotein E4 allele and certain genetic variants of IL-1 are linked to dementia supports the involvement of both inflammation and dysregulation of lipid metabolism in the development of dementia [12,13]. Both age and inflammation are associated with increased levels of oxidative damage to biomolecules [14] that include variable modifications to lipids, proteins and DNA [15]. Moreover, oxidative modifications are increased further in patients with dementias [16–19].

We have previously shown that plasma Hcy correlates with nitration but not oxidation of LDL in CVD patients [20]. LDL isolated from CVD patients is scavenged by macrophages to a greater extent than LDL isolated from control patients, suggesting that it may be more likely to increase foam cell formation which is seen in atherosclerosis [20]. This work suggested a molecular mechanism that may contribute to the clinical link between CVD, low plasma folate and elevated plasma Hcy in the absence of sufficient folate, modification to LDL and increased propensity for foam cell formation. Atherosclerotic occlusion of small brain vessels is the main pathology of VaD, but it is to date unknown whether LDL from VaD patients possesses biochemical and functional traits similar to those previously shown for CVD [20].

To investigate the possible association between folate deficiency, increased plasma Hcy and vascular disease which may be important in VaD development, we have examined (1) whether LDL isolated from the plasma of VaD patients is modified to a greater degree than in age-matched AD patients as well as in subjects with mild cognitive impairment (MCI) without dementia and control subjects and (2) whether the degree of altered LDL relates to plasma levels of the micronutrients folate and vitamin B6, to plasma total Hcy and to the degree of cognitive impairment.

Patients and methods

Subject recruitment and clinical characteristics

Eighty-three community dwelling subjects were recruited from the Unit of Cognitive Frailty, Neurology Outpatient Clinic, Cologne, Germany. Patients were recruited after diagnosis with AD using NINCDS-ADRDA criteria, VaD using the NINDS-AIREN criteria and with mild cognitive impairment (MCI) [21]. Control subjects showed no evidence of cognitive impairment. Informed consent was obtained from the patients or their caregivers according to severity of disease. Exclusion criteria included malnutrition, blood cell count alterations, smoking, alcohol abuse and ongoing drug treatment of any type including antioxidant/vitamin/iron supplementation. Patients with secondary dementias or with ongoing acute diseases including infections and inflammation were also excluded. Patients with cardiovascular comorbidities such as mild hypertension or mild diabetes were only included if non-pharmacologic treatment was sufficient. The patient demographics and clinical characteristics are reported in Table I.

Table I. Demographic, clinical characteristics and main laboratory values of subjects studied.

	Controls (n = 22)	AD (n = 28)	VaD (n = 16)	MCI (n = 17)
Age (years)	77.0 ± 4.6	81.0 ± 4.3	80.0 ± 4.0	75.0 ± 5.7
Gender (% male)	41	35.7	43.7	58.8
MMSE score	29.4 ± 0.8	17.4 ± 8.0	19.7 ± 1.8	27.9 ± 1.8
Albumin (mg/dL)	4.1 ± 0.6	4.1 ± 0.6	4.0 ± 0.7	4.1 ± 0.7
HDL Cholesterol (mg/dL)	60.8 ± 15.8	56.9 ± 16.5	46.1 ± 7.3*	51.7 ± 15.6
LDL Cholesterol (mg/dL)	98.8 ± 17.1	92.6 ± 16.4	83.7 ± 14.9*	91.9 ± 18.8
CRP (mg/L)	1.8 ± 0.8	5.0 ± 4.7	3.5 ± 3.1	5.0 ± 4.2
BMI	23.8 ± 1.4	23.8 ± 2.0	24.9 ± 1.5	24.2 ± 1.9
Hypertension, n (%)	5 (23)	8 (28)	9 (56)	5 (29)
Type 2 diabetes, n (%)	6 (27)	8 (28)	4 (25)	5 (29)

CRP = C-reactive protein, MMSE = mini-mental state examination. Data are presented as mean ± SD unless otherwise specified. *$p < 0.05$ compared to the other groups.

Subjects underwent a full neurological examination as well as collection of medical history to assess clinical conditions and of nutritional status by means of BMI and a qualitative food-frequency questionnaire modified to assess daily intake of fruits and vegetables [22]. An ECG, two consecutive measurements of blood pressure, as well as the Mini-Mental State Examination (MMSE) [23] were performed in all subjects.

Blood sampling and measurements

The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the local Ethical Committee. After informed consent, fasting blood was collected from the antecubital vein into EDTA tubes and kept on ice until centrifugation within 2 h of collection. Plasma was frozen at –80°C until analysis and buffy coat was removed from the interface between the plasma and erythrocytes. Plasma samples were coded and analysis was carried out in a blind fashion.

ApoE polymorphism

Genomic DNA was extracted from buffy coat using the Wizard® Genomic DNA Purification Kit from Promega. ApoE polymorphism was determined using the LightCycler Apo E Mutation Detection Kit (Roche Diagnostics, Mannheim, Germany). From 5µl genomic DNA, a 265-bp fragment containing exon 4 of the Apo E gene was amplified with the detection probes covering codons 112 and 158, which were 5′ labelled with LightCycler (LC)-Red 640 and LC-Red 705, respectively. The resulting melting peaks allowed discrimination between homozygous and heterozygous genotypes.

Determination of micronutrients

Plasma vitamin B6 levels were measured using HPLC by a kit (Immunodiagnostik, Germany). Folate and vitamin B12 were determined in the laboratory of Professor J. Scott, Trinity College, Dublin using a microbiological assay as previously reported [24]. Active vitamin B12 bound to transcobalamin was determined by using the non-isotopic the AxSYM *xtra* progamme, Axis-Shield (Axis-Shield, Cambridgeshire, UK).

Cholesterol analysis

Total, HDL and LDL cholesterols were determined in plasma samples by using Randox colourimetric kits.

Lipoprotein fractionation

Lipoproteins were isolated from plasma by potassium bromide density gradient ultracentrifugation as described previously [25]. Plasma (2 ml) was mixed with 50 mg sucrose, 770 mg KBr and 200 µl ethylene glycol and layered into the bottom of a Beckman centrifuge tube. A discontinuous potassium bromide density gradient was prepared in ultracentrifugation tubes using potassium bromide solutions as follows (from bottom to top): plasma (2 ml; d = 1.24 g/ml); heavy solution (2 ml; d = 1.125 g/ml); light solution (4 ml; d = 1.01 g/ml); and MilliQ H_2O (3 ml). LDL was isolated after 24 h ultra-centrifugation at 32 000 rpm at 4°C and desalted using Econo10DC columns using the minimal dilution protocol (Bio-Rad, Hemel Hempstead, UK) into phosphate buffered saline (0.01 M phosphate, pH 7.4) (PBS). Protein in LDL fractions was determined by the method of Smith et al. [26]. Bovine serum albumin was used as a standard. For all LDL uptake experiments, LDL concentration was adjusted to 200 µg protein/ml in PBS.

Competition for oxidized LDL (oxLDL) scavenging by macrophages

LDL was minimally oxidized by incubation with 100 µM copper sulphate for 1 h. The reaction was terminated using 400 µM EDTA. Oxidized LDL (oxLDL, 1 mg/ml) was labelled with 1,1V-dioctadecyl-3,3,3V, 3V,-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Invitrogen, Paisley, UK) (300 µg/mg LDL) by incubation overnight at 37°C, as previously described [20]. DiI-labelled oxLDL (DiI-oxLDL, 10 µg/well) was added to THP1 macrophages (0.7 million cells per well (1 ml) and differentiated with 160 nM phorbol-12-myristate-13-acetate (PMA) for 5 days, together with 10 µg/well LDL from each subject in the study. Cells were incubated for 20 h with 10 µg/ml DiI-oxLDL. Cells were then washed twice with HBSS containing 0.4% BSA, then twice with HBSS alone. Monocytes were centrifuged at 600 rpm for 10 min to form a cell pellet and re-suspended during each washing stage. Cells were then incubated for 1 h with lysis buffer (1 g/L SDS in 0.1 M NaOH) in the dark with regular gentle mixing. The fluorescence of each well was measured using a plate reader, with an excitation wavelength of 520 nm and an emission wavelength of 580 nm.

Analysis of LDL carbonyls

Protein carbonyls were assessed by ELISA following the method of Carty et al. [27]. Protein carbonyl standards were prepared by reductive and oxidative modification to bovine serum albumin with details of the method standardization reported in Anlasik et al. [22]. Levels of protein carbonyl detected by dinitrophenylhydrazine and measured by ELISA correlated with aminoadipic semialdehyde concentration, a recognized carbonyl oxidation product of lysine. Carbonyl content was calculated from a

standard curve and expressed as nmol carbonyl per mg of LDL.

Determination of C-reactive protein (CRP)

Plasma CRP was determined by ELISA using a high sensitivity kit from RnD Systems (UK). Assay sensitivity was 0.2 μg CRP/ml plasma and the lowest plasma CRP concentration detected in the samples was 0.35 μg/ml.

Homocysteine analysis

Plasma Hcy was determined in duplicate by ion-pair reverse phase HPLC analysis [28]. The internal standard confirmed less than 10% inter-batch variability against a standard curve of Hcy.

Statistical analysis

Statistical analysis was performed with the program Statistical Package for the Social Sciences (SPSS version 11.5, Chicago, IL). A general linear model of ANOVA was used to test the differences between categorical variables or analytes (known as factors) and the subject group diagnoses. Age and ApoE genotype were introduced in the model as covariates, as both are expected to influence parameters of oxidation, based on existing literature that shows protein oxidation to increase with age and ApoE genotype. A stepwise, discriminant analysis method was also adopted to identify the possible contribution of the LDL modification variables analysed within this pilot project to the presence of dementia, where the Wilks' lambda statistic was used for entering or removing new variables into the model. Correlations between parameters were examined by Spearman's correlation. Significance was accepted if the null hypothesis was rejected at the $p < 0.05$ level.

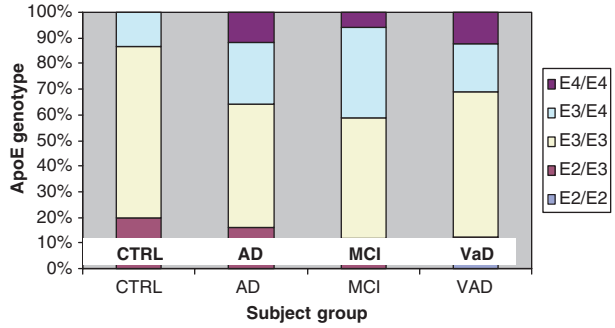

Figure 1. Apolipoprotein E genotype distribution in control subjects ($n = 22$) and patients with AD ($n = 26$; insufficient DNA was retrieved from two samples), MCI ($n = 17$) or VaD ($n = 16$). ApoE genotype was determined using genomic DNA extracted from buffy coat cells and analysed by the LightCycler Apo E Mutation Detection Kit from Roche.

Results

In agreement with existing data, the distribution of ApoE genotype was significantly skewed towards increased presence of the ApoE4 allele in demented patients irrespective of cause within the population studied. No E4 homozygotes were present among controls and the E3/E4 heterozygote frequency was

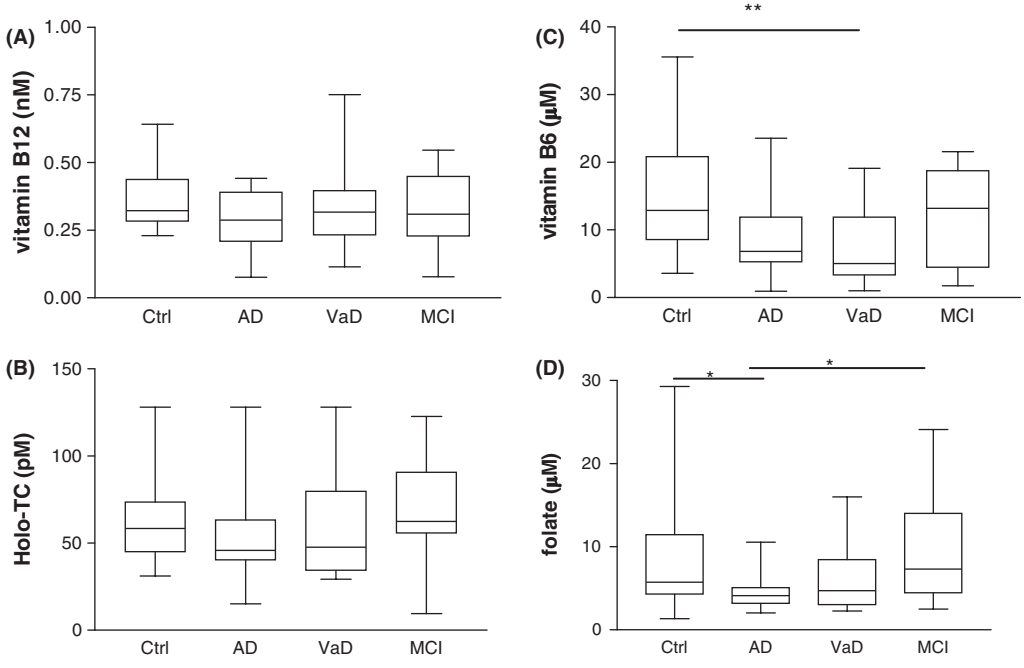

Figure 2. Plasma levels of total vitamin B12 (A), of active vitamin B12 (bound to transcobalamin, holo-TC, (B), vitamin B6 (C) and folate (D) in subjects with VaD, AD and MCI compared to control subjects. Data were analysed by ANOVA where *represents $p < 0.05$ and **represents $p < 0.01$.

lower in control subjects than in either dementia or MCI groups (Figure 1). In contrast, ApoE2/E2 was only detected in VAD subjects.

Neither levels of total nor levels of active vitamin B12 (bound to transcobalamin; holo-TC) were significantly different between controls and subjects with dementia (Figures 2A and B). Vitamin B6 levels were significantly lower in the plasma of VaD subjects compared to controls ($p < 0.01$) and a trend to lower vitamin B6 was observed in AD and MCI groups (Figure 2C). In contrast, plasma folate was significantly reduced in subjects with VaD, AD or MCI compared to control subjects ($p < 0.05$) (Figure 2D).

As displayed in Figure 3, circulating plasma Hcy levels in AD ($p < 0.01$) and VaD ($p < 0.05$) subjects were significantly higher than in controls. In addition, plasma Hcy levels exceeded the range of control subjects in 30% of MCI patients (Figure 3).

Figure 4 shows that protein carbonyl levels are significantly increased in LDL isolated from VaD ($p < 0.05$) but not MCI, AD or control subjects. In contrast, plasma CRP concentration was not different between any patient group and controls, although there was a trend towards increased CRP in all disease groups (Table I).

When isolated LDL was examined for its propensity to compete for scavenger receptor-mediated uptake of copper-oxidized LDL, only LDL isolated from VaD patients showed any significant competition ($p < 0.05$) (Figure 5).

Finally, analysis of total plasma cholesterol levels showed that there were no significant differences between dementia patients and control subjects. However, LDL cholesterol levels were significantly lower in the VaD subjects compared to other groups (Table I). Similarly, in VaD subjects, the plasma concentrations of HDL cholesterol were significantly diminished when compared to control subjects ($p = 0.002$) and to AD subjects ($p = 0.034$) (Table I). Neither low HDL cholesterol levels nor low fruit and vegetable intake were significantly associated with oxidation or uptake of LDL by macrophages.

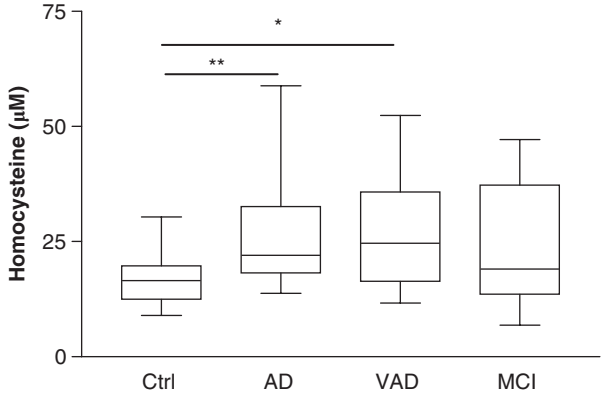

Figure 3. Plasma levels of Hcy in subjects with VaD, AD and MCI compared to control subjects. Data were analysed by ANOVA where *represents $p < 0.05$ and ** represents $p < 0.01$.

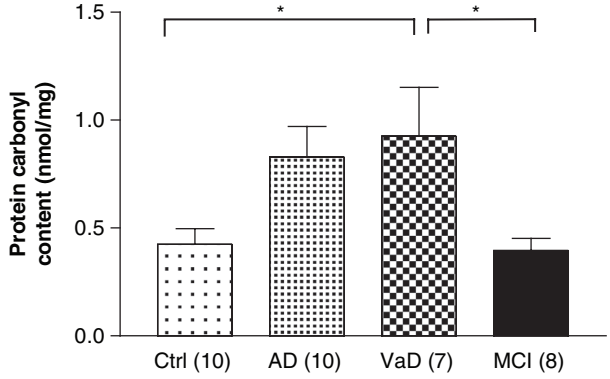

Figure 4. Plasma LDL from AD and VaD subjects shows increased oxidative modifications, determined by carbonyl ELISA, compared to MCI and control subjects. Numbers in parenthesis on the *x*-axis indicate number of subjects analysed. Data were analysed by ANOVA where *represents $p < 0.05$.

Further investigation of relationships between the variables analysed was undertaken to support the hypothesis of altered nutrient status and dementia development and correlations are reported in Table II. Of the principal nutritional analytes tested, Hcy concentration was found to have the strongest associations with cognitive ability measured by MMSE and was inversely associated with vitamins B6 and folate.

In order to identify the possible contribution of the LDL modification variables analysed within this pilot project to the presence of dementia, discriminant analysis was undertaken using ApoE genotype as a covariate. LDL precipitates formed after storage at –80°C for some preparations, therefore the numbers of each variable available for correlation were reduced. LDL precipitation was independent of subject group. Together, these analyses confirmed that oxLDL level is predictive of MMSE score across all subject groups ($p = 0.011$); however, no single variable was predictive of diagnosis. Combined analysis of HDL-cholesterol levels and LDL uptake by scavenger cells showed a significant interaction with VaD ($p = 0.038$). The definition of the presence of VaD was improved by the combined measurement of HDL cholesterol level and the propensity for isolated LDL to be scavenged by macrophages.

Discussion

The principal outcome from this study is that plasma LDL from subjects with VaD shows a greater degree of oxidative modification than plasma LDL in normal subjects or in subjects with AD or MCI. Dementias arise from neuronal loss over decades of life and cross-sectional associations cannot provide an indication of whether a biomarker is present as a cause or a consequence of disease. This study highlights a

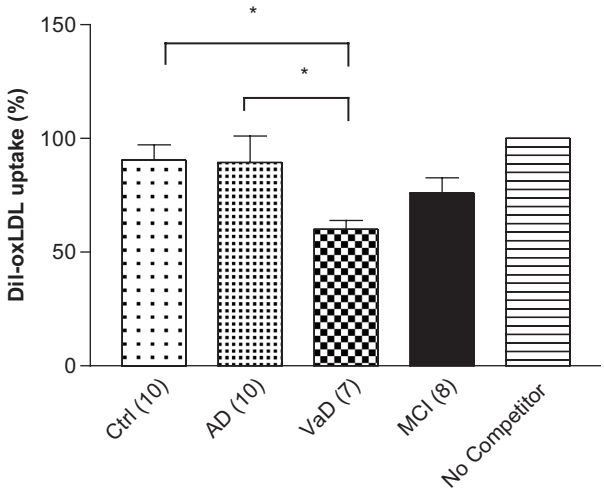

Figure 5. Receptor-mediated uptake of Dil-oxLDL in AD, VaD, MCI and control subjects; data are expressed as percentage uptake relative to 'no competitor' (No comp) control. Numbers in parenthesis on the *x*-axis indicate number of subjects analysed. Data were analysed by ANOVA where *represents $p < 0.05$.

novel biomarker for VaD that should be further investigated in longitudinal studies for early diagnostic and prognostic value and against which to evaluate novel interventions. Of particular interest is the association between low MMSE score and high level of LDL oxidation and the independent association that exists between high Hcy levels and low MMSE score. These observations suggest that systemic lipoprotein modification arising from homocysteine metabolism may play a role in deterioration of the brain function. Using the standard statistical modelling tools described in the methods, the principal components that define the presence of VaD are HDL cholesterol, LDL oxidation and scavenging by macrophages, which allowed the specific identification of VaD in 87% of cases.

The observation of increased LDL oxidation in VaD appeared to be unrelated to plasma B vitamin status. These findings do not support our hypothesis that the decreases in plasma folate concentration are the cause of elevated plasma Hcy levels, which may generate ROS through redox cycling, nor that folate deficiency can directly contribute to LDL oxidation in VaD. Nevertheless, plasma folate levels do not always correlate with intracellular folate levels and thus may not represent the expected relationship of folate deficiency and elevated Hcy levels.

However, it is clear that Hcy can undergo auto-oxidation and generate oxidants which may modify LDL and render it a suitable ligand for uptake by scavenger receptors. Indeed, LDL from VaD patients was a better competitor for uptake by macrophages than labelled, oxidized LDL. The observed increase in LDL oxidation in VaD patients contrasts with our previous work on LDL isolated from patients with inflammatory joint diseases and cardiovascular co-morbidities, where LDL protein carbonyl levels did not associate with either inflammatory joint or cardiovascular disease. Instead, our previous work showed that LDL nitration associated with disease and we propose that the presence of persistent inflammation may be an important contributor to nitrative modification of LDL in this previous work [20]. Indeed, it has been previously reported that there is a higher incidence of small-dense LDL in VaD patients than in control and AD patients [29]. Independently it has been identified that small-dense LDL is more prone to oxidative modification [30] and oxidized LDL is more avidly taken up by macrophages than native LDL [31]. This may contribute to cerebrovascular plaque formation, restricted blood flow and localized cell death in VaD.

Closer examination of the lipid profiles from the subjects under study in this report has shown a lowering of both LDL and HDL cholesterol in VaD subjects where the ratio of HDL:LDL is further decreased compared to subjects with AD, MCI or age-matched controls. We can not rule out the possibility that patients with VaD have followed dietary and lifestyle advice to diminish their cholesterol levels and this

Table II. Correlation analyses between micronutrient levels, LDL modifications and cognitive performance as assessed by MMSE scores.

	MMSE	LDL-Chol	Vitamin B6	Folate	Hcy	LDL uptake	oxLDL	HDL chol	Vitamin B12	Active B12
LDL-Chol (80)	0.128									
Vitamin B6 (83)	0.32**	−0.025								
Folate (77)	0.27*	0.159	0.28*							
Hcy (80)	−0.31**	−0.132	−0.408***	−0.412***						
LDL uptake (35)	0.039	−0.193	0.39	−0.238	−0.045					
Ox-LDL (35)	−0.404*	−0.237	−0.215	−0.183	0.266	−0.114				
HDL-Chol (80)	0.142	−0.042	0.072	0.039	−0.324***	−0.004	−0.136			
Vitamin B12 (80)	0.19	0.055	0.545	0.202	−0.309***	−0.059	−0.167	0.102		
Active B12 (80)	0.102	0.055	0.113	0.05	−0.22	0.1	0.103	0.042	0.615**	
CRP (80)	−0.1810	−0.1060	−0.1498	−0.00833	0.1034	0.05069	−0.1193	−0.2050	−0.1102	

Correlations were significant at *$p < 0.05$ level, **$p < 0.01$ level and ***$p < 0.001$ level. Numbers in parentheses represent number of samples analysed.

may account for the lower concentration in these subjects. There was no significant difference in the genotype profile between VaD and AD, with increased prevalence of the E4 allele being observed in both subjects groups, consistent with previous reports. However, a significant decrease in HDL:LDL ratio may be attributed to lower expression of the principal apolipoprotein associated with HDL, apolipoprotein A1. Previous work has shown a clear correlation between hypomethylation of the 5'-end and expression of the Apo-AI gene [32]. It remains to be determined whether the abnormal Hcy levels and diminished plasma folate levels observed in dementia may contribute to altered ApoA1 expression through variable methylation. Similarly, we only found ApoE2 /E2 genotype in VAD subjects. This unexpected observation is likely to be a consequence of the small samples size for genetic analysis, when the frequency of this genotype within the population is expected to be in the order of 7%.

The importance of cholesterol in the development of dementias has recently been highlighted by the work of Cramer et al. [33], who have shown that treatment of elevated cholesterol in mid-life with statin drugs lowers the incidence of dementia and an association between previous statin use and neurofibrillary tangle burden post-mortem has also been reported [34]. Furthermore, a reduction in pathological changes, as β-amyloid accumulation, has been reported in the brains of TgCRND8 mice after treatment with hydrophilic, non-BBB permeant statin, pravastatin [35], supporting the hypothesis that peripheral metabolism/inflammation can contribute to brain pathology. Statins are potent inhibitors of HMG CoA-reductase and thereby contribute to a lowering of systemic cholesterol. However, there are many other potential effects of statins, including antioxidant activity, and the study of Cramer et al. [33] was not sufficiently powered to determine whether the benefit of statins is equal in those prescribed non-BBB permeant statins

Further studies are required to better characterize modification in LDL from VaD and to ascertain whether components of oxidized lipoproteins may damage or cross the BBB and exert selective neuronal toxicity. Elucidation of these aspects of pathogenesis could have important nutritional as well as therapeutic implications [36].

Patients were recruited to the study after diagnosis with AD using NINCDS-ADRDA criteria, VaD using the NINDS-AIREN criteria and controls with no evidence of cognitive impairment. In addition, a fourth MCI group was studied, which is particularly important to determine whether any of the markers of oxidation measured, either in isolation or in combination, may predict the progression and prognosis of the disease. In addition, if a reduction in micronutrients is evident prior to onset of symptoms it may prove possible to delay onset of symptoms with micronutrient supplements. It is of interest to evaluate whether subjects with elevated Hcy, lower B6 or lower B12 are more at risk of rapid disease development and thus whether intervention with micronutrients may be useful for particular subjects with lower B12. To further understand the contribution of vitamin B12 metabolism to dementias and oxidative damage biomarkers, we also evaluated the vitamin B12 bound to transcobalamin. This carrier protein mediates cellular delivery vitamin B12. Less than 30% of the vitamin B12 in plasma circulates as bound to transcobalamin (holoTC). A previous study has shown lower levels of holoTC in AD than in controls [37]; however, our sample size was smaller and whilst we observed a lower median holoTC level in AD plasma (45.8 pM) compared to control (58.4 pM) this did not achieve significance ($p = 0.06$). No associations between holoTC were observed with any other parameter studied across the whole cohort. While many existing intervention studies have not demonstrated significant benefit from B vitamin supplementation, these have largely been undertaken in patients with existing disease and have not considered whether the patient exhibited disturbed cobalamin status. Indeed, our study shows that folate deficiency was significantly associated with cognitive impairment.

In summary we have shown that LDL isolated from the plasma of VaD patients are biochemically and functionally distinct from those isolated from AD or control subjects; and that whilst biomarkers of LDL phenotype are not related to levels of the micronutrient folate in plasma, nor to plasma total Hcy, folate and LDL oxidation are independently related to disease activity measured by MMSE. It remains to be determined whether oxidized LDL or its low molecular weight products can damage and/or cross the blood–brain barrier and cause damage to specific neurons, thereby acting as a direct pathological risk factor for the development of VaD.

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The authors alone are responsible for the content and writing of the paper.

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