

**ATVB/2008/163998 R2****Low HDL cholesterol is a risk factor for deficit and decline in memory in midlife: the Whitehall II Study**Archana Singh-Manoux, PhD <sup>1,2,3\*</sup>David Gimeno, PhD <sup>2</sup>Mika Kivimaki, PhD <sup>2</sup>Eric Brunner, PhD, FFPH <sup>2</sup>Michael G. Marmot, MD, PhD, FRCP, FFPH <sup>2</sup>

\*Corresponding author &amp; address

<sup>1</sup>INSERM U687-IFR69

Hôpital Paul Brousse, Bât 15/16

16 Avenue Paul Vaillant Couturier

94807 VILLEJUIF CEDEX, France

Telephone: +33 (0)1 77 74 74 10

Fax: +33 (0)1 77 74 74 03

Email: [Archana.Singh-Manoux@inserm.fr](mailto:Archana.Singh-Manoux@inserm.fr)<sup>2</sup>Department of Epidemiology and Public Health

University College London, UK

<sup>3</sup>Centre de Gériatrie, Hôpital Ste Péline, AP-HP, France

Word count text: all text: 4987; abstract: 196; # tables: 5

## Abstract

**Objective:** To examine the relationship between fasting serum lipids and short term verbal memory in middle aged adults.

**Methods:** Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides and memory were measured twice, at mean ages 55 and 61, in 3673 male and female participants of the Whitehall II study. Short term verbal memory was assessed using a 20-word-list. Logistic regression was used to model associations between ATP-III categories of lipids and memory deficit (recall of  $\leq 4$  words) and decline (decrease of  $\geq 2$  words). Analyses were adjusted for education, occupational position, coronary heart disease, stroke, hypertension, use of medication, diabetes, smoking and alcohol consumption.

**Results:** Compared to high HDL-C ( $\geq 60$  mg/dL), low HDL-C ( $< 40$  mg/dL) was associated with greater odds of memory deficit at the first (OR=1.27; 95% confidence interval (CI)=0.91-1.77) and second wave of this study (OR=1.53; 95% CI=1.04-2.25) in fully adjusted analysis. Decrease in HDL-C over the 5-year follow-up period was associated with decline in memory in the adjusted analysis (OR=1.61; 95% CI=1.19-2.16), no interaction with APOE e4 status was present.

**Interpretation:** HDL-C levels are potentially modifiable and our results suggest that low HDL-C is associated with poor memory and decline in memory in middle aged adults.

## Condensed abstract

We examined the relationship between fasting serum lipids and short term verbal memory in middle aged adults. Low HDL cholesterol and decreasing levels over a five year follow-up period were associated with poor memory and decline in memory, respectively. No other lipid that was tested was associated with memory.

The distinction between vascular dementia and Alzheimer's disease has become blurred, partly because of similar associations of cardiovascular disease and its risk factors with different types of dementia<sup>1</sup> and lower cognitive functioning.<sup>2,3</sup> Among the many risk factors investigated, the association between cholesterol and cognition appears to be the most elusive. High cholesterol is a proven risk factor for cardiovascular disease,<sup>4</sup> but the association with cognition appears complicated. Some studies have shown high lipid levels to be risk factor for impaired cognition or dementia,<sup>3,5-14</sup> whereas others either show no association<sup>15-17</sup> or a protective association.<sup>18,19</sup> Findings from lipid lowering agents are also mixed, some studies show a protective effect on dementia<sup>20</sup> and others no effect.<sup>21</sup>

There is some consensus to suggest that dementia itself modifies lipid levels either through changes in diet or metabolism, leading to low total or low-density lipoprotein cholesterol levels (LDL-C) (i.e., a more favorable profile) among those with dementia.<sup>1,6,7,22</sup> Thus, examination of the effect of lipids on cognition in the elderly either in cross-sectional analysis or in analysis using short follow-up periods is likely to yield spurious results. The associations between midlife lipid levels and late life dementia appear to be robust.<sup>5-7,23</sup> However, the precise lipid that might be important remains unclear with studies implicating high levels of LDL-C,<sup>3,13</sup> or total cholesterol (TC)<sup>5-7,24</sup> or low levels of high-density lipoprotein cholesterol (HDL-C).<sup>8-12,14,25</sup> HDL-C is critical for the maturation of synapses and the maintenance of synaptic plasticity.<sup>26</sup> It can influence the formation of amyloid  $\beta$ , the main constituent of amyloid plaques.<sup>27</sup> Low HDL-C has also been shown to be associated with lower hippocampal volume.<sup>11</sup>

We investigate the association between lipids and short term verbal memory by examining the cross-sectional associations and associations between changes in lipids with changes in memory over 5 years in middle aged individuals. We examine whether these associations are independent of morbidities related to lipid levels, such as cardiovascular disease, stroke and hypertension, and inherited apolipoprotein E epsilon 4 status (APOE  $\epsilon$ 4), shown to be important in the association between lipids and cognition.<sup>1,5</sup>

## Methods

Data are drawn from the Whitehall II study, established in 1985 among 10,308 civil servants (6,895 men and 3,413 women), further details provided elsewhere.<sup>28</sup> All civil servants aged 35-55 years in 20 London based departments were invited to participate and 73% agreed. Data for our analysis come from phases 5 (1995-97) and 7 (2002-04) of the study. The University College London ethics committee approved the study.

## **Lipids at Phases 5 & 7**

Blood samples were collected after either an 8-h fast (participants presenting to the clinic in the morning) or at least 4 h after a light fat-free breakfast (participants presenting in the afternoon). Venepuncture of the left antecubital vein was performed with tourniquet. Blood was collected into plain and fluoride Sarstedt (Neumbrecht, Germany) monovettes. Serum for lipid analyses was refrigerated at  $-4^{\circ}\text{C}$  and assayed within 72 hours. Cholesterol and triacylglycerols were measured with the use of a Cobas Fara centrifugal analyzer (Roche Diagnostics System, Nutley, NJ). HDL-C was measured by precipitating non-HDL cholesterol with dextran sulfate-magnesium chloride with the use of a centrifuge and measuring cholesterol in the supernatant fluid. Technical error was estimated by assaying blinded duplicate samples for 5% of subjects. Coefficients of variation were 2.0 to 6.6 percent.

The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program guidelines were used to categorize all lipids for this study.<sup>29</sup> The categories were: TC: <200, 200-239, and  $\geq 240$  mg/dL; HDL-C: <40, 40-59, and  $\geq 60$  mg/dL; triglycerides: <150, 150-199,  $\geq 200$  mg/dL. Note 1mg/dL cholesterol = 0.0259 mmol/L, 1mg/dL Triglycerides = 0.0113 mmol/L

**Short term verbal memory at Phases 5 & 7** was assessed with a 20-word free recall test. Participants were presented a list of 20 one or two syllable words at two second intervals and were then asked to recall in writing as many of the words in any order and had two minutes to do so. Memory deficit, for the cross-sectional analyses, and decline, for the longitudinal analysis, was defined as performance in the worst quintile. This corresponded to a recall of up to 4 words for defining deficit at Phases 5 and 7 and a decline of 2 or more words between the two phases for defining decline.

## **Confounders at Phases 5 & 7**

Possible confounders included age, sex, education (no or primary, lower secondary, higher secondary, first university degree, higher degree) and occupational position (high, intermediate and low employment grade). Prevalent diseases such as coronary heart disease (CHD), hypertension, stroke and medication use (lipid lowering and oral contraceptive (OC) or hormone replacement therapy (HRT)). CHD prevalence was based on clinically verified events and included fatal and non-fatal myocardial infarction (MI) and definite angina. MI was defined as a coronary death (ICD 9 codes 410–414 or ICD 10 codes I20-25) or non-fatal MI using the MONICA criteria.<sup>30</sup> Angina included participants who reported symptoms of

angina,<sup>31</sup> with corroboration in clinical records or abnormalities on a resting ECG, exercise ECG, or coronary angiogram. Hypertension was classified as systolic and diastolic blood pressure >140/90 mm/Hg or treatment for hypertension. Stroke diagnosis and medication use (lipid lowering drugs, OC/HRT) were self-reported. Diabetes status was determined on the basis of self-report of doctor diagnosis, use of diabetes medication or the oral glucose tolerance test (2h glucose $\geq$ 200mg/dL fasting glucose $\geq$ 126mg/dL). Finally, health behaviors were assessed with a measure of smoking (yes/no) and units of alcohol consumed in a week, logged in the analysis to correct for skewness.

### Statistical analysis

The association between lipids (TC, HDL-C and triglycerides) and memory deficit was examined using logistic regression first in cross-sectional analysis at Phase 5 with all covariates also drawn from Phase 5. The reference group for TC and triglycerides was the “low” category and for HDL-C the “high” category. The analysis was first adjusted for age and sex (Model 1), then the lipids (TC, HDL-C, triglycerides) were mutually adjusted (Model 2). Further adjustments were made for education and employment grade (Model 3) and finally for prevalent disease and health behaviors (Model 4). This whole procedure was repeated using data on all measures from Phase 7 on the same individuals but five years after Phase 5.

The longitudinal analysis examined the association between change in lipids with decline in memory between Phases 5 and 7. Change in lipid levels was characterized as remaining in the same risk category over the two phases of data collection (low-low, intermediate-intermediate, high-high) or decreasing (increasing for HDL-C) or increasing lipid levels (decreasing for HDL-C). The reference category in these analyses was the “low-low” category, except for HDL-C which was the “high-high” category. This analysis was adjusted for confounders measured at Phase 7 with the four levels of adjustment described previously.

Sensitivity analysis for the analysis of change were carried out on two subsets of data to assess the robustness of the change analysis. First, the analyses were repeated among those who were APOE e4 negative. APOE genotype was determined using a standard PCR assay of DNA extracted from whole blood using the salting out method<sup>32</sup>. We also examined the interaction term between APOE e4 status and change in HDL-C. Second, we examined whether the results were robust to adjustment for LDL-C. LDL-C was calculated using the Friedwald method which results in the LDL-C being highly correlated with total cholesterol

(correlation coefficient =0.91,  $p<0.0001$ ). Due to collinearity issues we chose not to use it in the analysis except in the sensitivity analysis where it replaced total cholesterol.

## Results

Data for the longitudinal analysis were available on 3673 individuals; the cross-sectional analyses are also shown on the same individuals. Table 1 shows the characteristics of these individuals at Phases 5 and 7.

The cross-sectional associations between the lipids and poor memory are presented in Table 2. At Phase 5, 34.8%, 11.9% and 9.9% of the sample was in the high risk category for total cholesterol, HDL-C and triglycerides, respectively. Women were less likely to be present in the high risk categories of HDL-C (9.8%) and triglycerides (13.0%). Nevertheless, men and women were combined in these analyses as the interaction term with TC ( $p=0.49$ ), HDL-C ( $p=0.07$ ) and triglycerides ( $p=0.37$ ) did not provide strong evidence for sex differences in the association between lipids and memory deficit. Table 2 shows no association between TC and memory deficit, the same is true for triglycerides. However, low level of HDL-C was associated with memory deficit in the fully adjusted model (OR=1.27; 95% confidence Interval (CI) = 0.91-1.77). Table 3 shows results from analysis using Phase 7 data when the participants were 5 years older. The interaction term for TC ( $p=0.44$ ), HDL-C ( $p=0.53$ ) and triglycerides ( $p=0.54$ ) again allowed us to combine men and women in the analysis. Here again, low HDL-C was associated with memory deficit (OR=1.53; 95% CI = 1.04-2.25).

Table 4 shows the results for the effect of change in lipid levels on decline in memory. For a majority of individuals, lipid risk levels remained the same over the two measures that were 5 years apart: 59.1% for TC, 69.8% for HDL-C and 78.8% for triglycerides. Men and women were again combined in these analyses as the interaction term with TC ( $p=0.63$ ), HDL-C ( $p=0.63$ ) and triglycerides ( $p=0.09$ ) did not provide strong evidence for sex differences. Results show only changes in HDL-C to be associated with decline in memory. Compared to those with high levels of HDL-C, individuals with decreasing HDL-C had a greater risk of memory decline in fully adjusted analysis (OR=1.61; 95% CI = 1.19-2.16). Changing the reference category to those with decreasing HDL-C (results not shown but available on request) revealed that all groups except the low-low HDL-C group had lower odds of decline in memory. Statin use was not associated with decline in memory (OR=0.98; 95% CI = 0.73-1.32). It should be noted that the decreasing HDL-C category contains individuals who decreased from high to low or intermediate level or from intermediate to low level.

## Sensitivity analysis

Of the 3673 participants in this study, APOE e4 data were available on 3326 individuals. The interaction term ( $p=0.53$ ) between APOE e4 status and change in HDL-C did not suggest any evidence of a stronger association in different APOE e4 groups. 2397 participants were APOE e4 negative and Table 5 (sub sample 1) shows the association between change in HDL-C levels and memory decline in this group. Decreasing HDL-C was associated with decline in memory (OR=1.73, 95% CI = 1.20-2.50) after adjustment for all covariates.

The results for sub sample 2 (N=3326) in Table 5 also show a robust association between change in HDL-C and decline in memory (OR=1.58; 95% CI = 1.17-2.13), revealing LDL-C not to change the association between change in HDL-C and decline in memory.

## Discussion

This study of middle aged adults suggests a robust association between low HDL-C and poor memory. Furthermore, decreasing HDL-C was associated with decline in memory over a 5-year follow-up. These associations remained after adjustment for the effects of education, occupational position, prevalent disease or medication use and they were independent of APOE e4 status. Serum concentrations of total cholesterol and triglycerides show no association with memory deficit or decline. Thus, our results identify HDL-C as being important for memory.

Many previous investigations into the association between lipids and memory in the elderly have focused on total or LDL-C,<sup>5,33</sup> perhaps because of their status as proven risk factors for cardiovascular disease. Our findings emphasize the need to expand the focus to HDL-C. In our study on middle aged adults, the associations of low levels of HDL-C with memory deficit and decline were independent of other lipids and robust to adjustments for a number of potential confounding factors. There are a number of plausible mechanisms connecting low levels of HDL-C and memory, as HDL-C is the prominent lipoprotein in the human brain<sup>27</sup> and is involved in the regulation of amyloid  $\beta$  protein metabolism and deposition in the brain.<sup>34</sup> Deficit in HDL-C could also affect memory through its influence on atherosclerotic disease and stroke,<sup>35</sup> or subclinical vascular injury not reflected in the covariates examined. Other possible mechanisms linking low levels of HDL-C to neurodegenerative processes might involve its anti-inflammatory<sup>36</sup> or antioxidant<sup>37</sup> properties.

Although memory deficits are critical to the diagnosis of mild cognitive impairment<sup>38</sup> and Alzheimer's disease, the association between lipids and memory remains little explored

in midlife. Dementia occurs late in life but it is increasingly recognized that there is a long preclinical phase characterized by progressive neuropathological changes that become clinically detectable later. The “life-long” view of dementia stresses the importance of risk factors in midlife.<sup>39</sup> Our findings on individuals aged 55 and 61 at the two phases of data collection suggest that low levels of HDL-C may be an important risk factor. Among the elderly, there is also some evidence of a link between HDL-C and poor memory,<sup>10</sup> and Alzheimer’s disease. However, some previous studies on the elderly have found low HDL-C to be associated with vascular dementia but not with Alzheimer’s disease.<sup>8,14</sup> The inconsistency in findings needs to be viewed in light of the fact that dementia itself modifies lipid levels;<sup>6,7</sup> necessitating further research where lipids are measured before the diagnosis of dementia.

The association between low levels of HDL-C and poor memory is unlikely to be simply an accidental finding in our data as the cross-sectional findings were consistently replicated across two study phases. There is some evidence of increase in association over time as the Phase 7 data show stronger associations between HDL-C and memory deficit. Furthermore, decreasing HDL-C level was also predictive of decline in memory. We undertook further analysis in sub samples in order first to assess whether this association held in APOE e4 negative subjects. APOE e4 is widely regarded as being implicated with adverse outcomes for dementia.<sup>1,5,23,24,40</sup> Our results on APOE e4 negative individuals show decreasing levels of HDL-C to be associated with greater odds of memory decline. In the second analysis on a sub sample we reran the longitudinal analysis by replacing total cholesterol with LDL-C. Here again, results were not much different.

There are a number of potential limitations to this study. First, causality cannot be inferred from observational data and a randomized controlled trial with treatment specifically targeted at elevating HDL-C levels and measurements of change in memory performance would be necessary to establish causality. A further possibility would be to examine this issue using brain imaging data. Second, despite extensive adjustments for a variety of potential confounding factors, it is possible that some unmeasured variable causes decline in both HDL-C and memory. Third, data here are drawn from the 5<sup>th</sup> and 7<sup>th</sup> phase of a study, implying both survival and selection effects. Therefore, it is possible that the association between lipids and cognition is underestimated in our sample.

In conclusion, our results show low levels of HDL-C (<40 mg/dL) to be associated with poor memory. Furthermore, decline in HDL-C was associated with declines in memory over a five-year period. The National Cholesterol Education Program<sup>29</sup> has stressed the



importance of lowering LDL-C in order to reduce the burden of cardiovascular disease. Our results suggest that increasing HDL-C might also be important, for cognitive outcomes in particular.

### **Sources of funding**

AS-M is supported by a “European Young Investigator Award” from the European Science Foundation. MK is supported by the Academy of Finland (grants 117604 and 124322). MM is supported by an MRC research professorship. The Whitehall II study has been supported by grants from the British Medical Research Council (MRC); the British Heart Foundation; the British Health and Safety Executive; the British Department of Health; the National Heart, Lung, and Blood Institute (grant HL36310); the National Institute on Aging (grant AG13196); the Agency for Health Care Policy and Research (grant HS06516); and the John D. and Catherine T. MacArthur Foundation Research Networks on Successful Midlife Development and Socioeconomic Status and Health.

### **Acknowledgements**

We thank all of the participating civil service departments and their welfare, personnel, and establishment officers; the British Occupational Health and Safety Agency; the British Council of Civil Service Unions; all participating civil servants in the Whitehall II study; and all members of the Whitehall II study team.

**Disclosure:** none

## References

1. Scarmeas N. Invited commentary: lipoproteins and dementia - is it the apolipoprotein A-I? *Am J Epidemiol* 2007;165:993-997.
2. Singh-Manoux A, Britton AR, Marmot M. Vascular disease and cognitive function: evidence from the Whitehall II Study. *J Am Geriatr Soc* 2003;51:1445-1450.
3. Yaffe K, Barrett-Connor E, Lin F, Grady D. Serum lipoprotein levels, statin use, and cognitive function in older women. *Arch Neurol* 2002;59:378-384.
4. Levine GN, Keaney JF, Jr., Vita JA. Cholesterol reduction in cardiovascular disease. Clinical benefits and possible mechanisms. *N Engl J Med* 1995;332:512-521.
5. Notkola IL, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, Tuomilehto J, Nissinen A. Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* 1998;17:14-20.
6. Stewart R, White LR, Xue QL, Launer LJ. Twenty-six-year change in total cholesterol levels and incident dementia: the Honolulu-Asia Aging Study. *Arch Neurol* 2007;64:103-107.
7. Solomon A, Kareholt I, Ngandu T, Winblad B, Nissinen A, Tuomilehto J, Soininen H, Kivipelto M. Serum cholesterol changes after midlife and late-life cognition: twenty-one-year follow-up study. *Neurology* 2007;68:751-756.
8. Reitz C, Tang MX, Luchsinger J, Mayeux R. Relation of plasma lipids to Alzheimer disease and vascular dementia. *Arch Neurol* 2004;61:705-714.
9. Razay G, Vreugdenhil A, Wilcock G. The metabolic syndrome and Alzheimer disease. *Arch Neurol* 2007;64:93-96.
10. Komulainen P, Lakka TA, Kivipelto M, Hassinen M, Helkala EL, Haapala I, Nissinen A, Rauramaa R. Metabolic syndrome and cognitive function: a population-based follow-up study in elderly women. *Dement Geriatr Cogn Disord* 2007;23:29-34.
11. Wolf H, Hensel A, Arendt T, Kivipelto M, Winblad B, Gertz HJ. Serum lipids and hippocampal volume: the link to Alzheimer's disease? *Ann Neurol* 2004;56:745-748.
12. van EE, de Craen AJ, Gussekloo J, Houx P, Bootsma-van der WA, Macfarlane PW, Blauw GJ, Westendorp RG. Association between high-density lipoprotein and cognitive impairment in the oldest old. *Ann Neurol* 2002;51:716-721.
13. Moroney JT, Tang MX, Berglund L, Small S, Merchant C, Bell K, Stern Y, Mayeux R. Low-density lipoprotein cholesterol and the risk of dementia with stroke. *JAMA* 1999;282:254-260.
14. Katzman R, Aronson M, Fuld P, Kawas C, Brown T, Morgenstern H, Frishman W, Gidez L, Eder H, Ooi WL. Development of dementing illnesses in an 80-year-old volunteer cohort. *Ann Neurol* 1989;25:317-324.

15. Reitz C, Luchsinger J, Tang MX, Manly J, Mayeux R. Impact of plasma lipids and time on memory performance in healthy elderly without dementia. *Neurology* 2005;64:1378-1383.
16. Zimetbaum P, Frishman WH, Ooi WL, Derman MP, Aronson M, Gidez LI, Eder HA. Plasma lipids and lipoproteins and the incidence of cardiovascular disease in the very elderly. The Bronx Aging Study. *Arterioscler Thromb* 1992;12:416-423.
17. Romas SN, Tang MX, Berglund L, Mayeux R. APOE genotype, plasma lipids, lipoproteins, and AD in community elderly. *Neurology* 1999;53:517-521.
18. Elias PK, Elias MF, D'Agostino RB, Sullivan LM, Wolf PA. Serum cholesterol and cognitive performance in the Framingham Heart Study. *Psychosom Med* 2005;67:24-30.
19. Swan GE, LaRue A, Carmelli D, Reed TE, Fabsitz RR. Decline in cognitive performance in aging twins. Heritability and biobehavioral predictors from the National Heart, Lung, and Blood Institute Twin Study. *Arch Neurol* 1992;49:476-481.
20. Rockwood K, Kirkland S, Hogan DB, MacKnight C, Merry H, Verreault R, Wolfson C, McDowell I. Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. *Arch Neurol* 2002;59:223-227.
21. Li G, Higdon R, Kukull WA, Peskind E, Van Valen MK, Tsuang D, van BG, McCormick W, Bowen JD, Teri L, Schellenberg GD, Larson EB. Statin therapy and risk of dementia in the elderly: a community-based prospective cohort study. *Neurology* 2004;63:1624-1628.
22. Kivipelto M, Solomon A. Cholesterol as a risk factor for Alzheimer's disease - epidemiological evidence. *Acta Neurol Scand Suppl* 2006;185:50-57.
23. Saczynski JS, White L, Peila RL, Rodriguez BL, Launer LJ. The relation between apolipoprotein A-I and dementia: the Honolulu-Asia aging study. *Am J Epidemiol* 2007;165:985-992.
24. Evans RM, Hui S, Perkins A, Lahiri DK, Poirier J, Farlow MR. Cholesterol and APOE genotype interact to influence Alzheimer disease progression. *Neurology* 2004;62:1869-1871.
25. Muckle TJ, Roy JR. High-density lipoprotein cholesterol in differential diagnosis of senile dementia. *Lancet* 1985;1:1191-1193.
26. Michikawa M. Cholesterol paradox: is high total or low HDL cholesterol level a risk for Alzheimer's disease? *J Neurosci Res* 2003;72:141-146.
27. Olesen OF, Dago L. High density lipoprotein inhibits assembly of amyloid beta-peptides into fibrils. *Biochem Biophys Res Commun* 2000;270:62-66.
28. Marmot M, Brunner E. Cohort Profile: the Whitehall II study. *Int J Epidemiol* 2005;34:251-256.

29. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486-2497.
30. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation* 1994;90:583-612.
31. Rose G, Hamilton PS, Keen H, Reid DD, McCartney P, Jarrett RJ. Myocardial ischaemia, risk factors and death from coronary heart-disease. *Lancet* 1977;1:105-109.
32. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1998;16:1215.
33. Evans RM, Emsley CL, Gao S, Sahota A, Hall KS, Farlow MR, Hendrie H. Serum cholesterol, APOE genotype, and the risk of Alzheimer's disease: a population-based study of African Americans. *Neurology* 2000;54:240-242.
34. Reiss AB, Siller KA, Rahman MM, Chan ES, Ghiso J, de Leon MJ. Cholesterol in neurologic disorders of the elderly: stroke and Alzheimer's disease. *Neurobiol Aging* 2004;25:977-989.
35. Sacco RL, Benson RT, Kargman DE, Boden-Albala B, Tuck C, Lin IF, Cheng JF, Paik MC, Shea S, Berglund L. High-density lipoprotein cholesterol and ischemic stroke in the elderly: the Northern Manhattan Stroke Study. *JAMA* 2001;285:2729-2735.
36. Cockerill GW, Huehns TY, Weerasinghe A, Stocker C, Lerch PG, Miller NE, Haskard DO. Elevation of plasma high-density lipoprotein concentration reduces interleukin-1-induced expression of E-selectin in an in vivo model of acute inflammation. *Circulation* 2001;103:108-112.
37. Paterno R, Ruocco A, Postiglione A, Hubsch A, Andresen I, Lang MG. Reconstituted high-density lipoprotein exhibits neuroprotection in two rat models of stroke. *Cerebrovasc Dis* 2004;17:204-211.
38. Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de LM, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B. Mild cognitive impairment. *Lancet* 2006;367:1262-1270.
39. Kivipelto M, Ngandu T, Laatikainen T, Winblad B, Soininen H, Tuomilehto J. Risk score for the prediction of dementia risk in 20 years among middle aged people: a longitudinal, population-based study. *Lancet Neurol* 2006;5:735-741.
40. Sparks DL, Connor DJ, Sabbagh MN, Petersen RB, Lopez J, Browne P. Circulating cholesterol levels, apolipoprotein E genotype and dementia severity influence the benefit of atorvastatin treatment in Alzheimer's disease: results of the Alzheimer's Disease Cholesterol-Lowering Treatment (ADCLT) trial. *Acta Neurol Scand Suppl* 2006;185:3-7.

**Table 1. Sample characteristics of participants.**

	<b>Phase 5 (1997-1999)</b>	<b>Phase 7 (2002-2004)</b>
N	3673	3673
% women	26.8%	26.8%
Age (M (SD))	55.4 (5.9)	60.9 (5.9)
% with $\leq$ primary education	11.1%	11.1%
% low employment grade	19.9%	8.5%
Total cholesterol mg/dL <sup>†</sup> (M (SD))	229.31 (40.21)	227.74 (39.06)
% in high risk category	34.8%	29.5%
HDL cholesterol mg/dL <sup>†</sup> (M (SD))	56.84 (15.08)	61.48 (17.01)
% in high risk category	11.9%	7.3%
Triglycerides mg/dL <sup>†</sup> (M (SD))	116.03 (73.51)	118.69 (77.95)
% in high risk category	9.9%	9.4%
% Coronary Heart Disease	5.1%	7.8%
% Stroke	0.6%	2.1%
% Hypertension	25.8%	36.5%
% Lipid lowering drugs	2.6%	9.7%
% Oral contraceptive/Hormone Replacement Therapy	6.1%	4.7%
% Diabetes/Impaired Glucose Tolerance	12.6%	20.7%
% Smokers	8.2%	6.1%
Alcohol consumption GM(SDL) <sup>‡</sup>	8.89 (3.20)	8.10 (3.10)
Short term verbal memory (Range 0-20) (M (SD))	7.00 (2.41)	6.95 (2.40)

M: Mean; SD: Standard Deviation. HDL: high-density lipoprotein.

<sup>‡</sup> GM: Geometric mean; SDL: Standard deviation of logged values.

<sup>†</sup> 1mg/dL cholesterol = 0.0259 mmol/L, 1mg/dL Triglycerides = 0.0113 mmol/L

Table 2. Cross sectional associations between lipids and poor memory performance\* at Phase 5, 1997-1999 (N=3673).

Lipids at Phase 5		M (SD)	N (%)	Model 1 <sup>†</sup>		Model 2 <sup>‡</sup>		Model 3 <sup>§</sup>		Model 4 <sup>#</sup>	
				OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
Total Cholesterol											
Low	(<200mg/dL)	179.14 (16.26)	844 (23.0%)	1		1		1		1	
Intermediate	(200-239 mg/dL)	220.49 (11.85)	1550 (42.2%)	0.90 (0.70-1.14)		0.89 (0.70-1.14)		0.91 (0.71-1.17)		0.91 (0.71-1.16)	
High	(≥240 mg/dL)	271.88 (27.05)	1279 (34.8%)	0.89 (0.69-1.14)		0.87 (0.67-1.14)		0.90 (0.69-1.17)		0.88 (0.67-1.16)	
HDL-Cholesterol											
High	(≥60 mg/dL)	72.82 (10.94)	1329 (36.2%)	1		1		1		1	
Intermediate	(40-59 mg/dL)	50.27 (5.38)	1906 (51.9%)	1.07 (0.86-1.32)		1.03 (0.83-1.29)		0.98 (0.79-1.22)		0.92 (0.74-1.15)	
Low	(<40mg/dL)	35.69 (3.75)	438 (11.9%)	1.61 (1.19-2.17)		1.49 (1.08-2.07)		1.41 (1.01-1.96)		1.27 (0.91-1.77)	
Triglycerides											
Low	(<150mg/dL)	86.50 (28.61)	2880 (78.4%)	1		1		1		1	
Intermediate	(150-199 mg/dL)	168.70 (14.14)	431 (11.7%)	1.23 (0.93-1.64)		1.16 (0.87-1.57)		1.10 (0.82-1.49)		1.12 (0.83-1.52)	
High	(≥200 mg/dL)	283.61 (101.61)	362 (9.9%)	1.23 (0.91-1.68)		1.14 (0.81-1.59)		1.11 (0.79-1.56)		1.13 (0.79-1.60)	

HDL: high-density lipoprotein. M: Mean; SD: Standard Deviation

\*Poor memory is performance in the worst quintile, here ≤ 4 words.

<sup>†</sup>Model 1: adjusted for age & sex.

<sup>‡</sup>Model 2: adjusted for age, sex and mutually adjusted.

<sup>§</sup>Model 3: + adjusted for education and employment grade.

<sup>#</sup>Model 4: + adjusted for CHD, stroke, hypertension, medication, diabetes, smoking, alcohol consumption.

Table 3. Cross sectional associations between lipids and poor memory performance\* at Phase 7, 2002-2004 (N=3673).

Lipids at Phase 7		M (SD)	N (%)	Model 1 <sup>†</sup>		Model 2 <sup>‡</sup>		Model 3 <sup>§</sup>		Model 4 <sup>#</sup>	
				OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
<b>Total Cholesterol</b>											
Low	(<200mg/dL)	176.95 (17.62)	1013 (27.6%)	1		1		1		1	
Intermediate	(200-239 mg/dL)	219.98 (12.03)	1575 (42.9%)	0.86 (0.69-1.08)		0.89 (0.71-1.11)		0.90 (0.72-1.13)		0.91 (0.72-1.16)	
High	(≥240 mg/dL)	268.50 (23.91)	1085 (29.5%)	0.84 (0.66-1.07)		0.86 (0.67-1.12)		0.89 (0.69-1.16)		0.90 (0.68-1.18)	
<b>HDL-Cholesterol</b>											
High	(≥60 mg/dL)	75.17 (13.45)	1718 (46.8%)	1		1		1		1	
Intermediate	(40-59 mg/dL)	50.93 (5.21)	1686 (45.9%)	1.20 (0.98-1.46)		1.16 (0.94-1.43)		1.08 (0.87-1.34)		1.04 (0.84-1.29)	
Low	(<40mg/dL)	36.19 (3.50)	269 (7.3%)	1.85 (1.31-2.60)		1.76 (1.21-2.56)		1.64 (1.12-2.40)		1.53 (1.04-2.25)	
<b>Triglycerides</b>											
Low	(<150mg/dL)	88.54 (27.76)	2810 (76.5%)	1		1		1		1	
Intermediate	(150-199 mg/dL)	169.01 (14.64)	519 (14.1%)	1.25 (0.97-1.61)		1.17 (0.90-1.54)		1.12 (0.85-1.47)		1.12 (0.85-1.48)	
High	(≥200 mg/dL)	288.51 (136.56)	344 (9.4%)	1.11 (0.80-1.53)		0.97 (0.68-1.38)		0.94 (0.65-1.34)		0.94 (0.65-1.35)	

HDL: high-density lipoprotein. M: Mean; SD: Standard Deviation

\*Poor memory is performance in the worst quintile, here ≤ 4 words.

<sup>†</sup>Model 1: adjusted for age & sex.

<sup>‡</sup>Model 2: adjusted for age, sex and mutually adjusted.

<sup>§</sup>Model 3: + adjusted for education and employment grade.

<sup>#</sup>Model 4: + adjusted for CHD, stroke, hypertension, medication, diabetes, smoking, alcohol consumption.



Table 4. Change in lipid levels and associated decline in memory\* between Phases 5 (1997-1999) and 7 (2002-2004), N=3673.

Change in lipid levels between Phases 5 and 7		Model 1 <sup>†</sup>	Model 2 <sup>‡</sup>	Model 3 <sup>§</sup>	Model 4 <sup>#</sup>
	N (%)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total Cholesterol		p=0.73	p=0.73	p=0.71	p=0.70
Low-Low	554 (15.1%)	1	1	1	1
Intermediate-Intermediate	900 (24.5%)	0.92 (0.72-1.17)	0.92 (0.72-1.18)	0.92 (0.72-1.18)	0.92 (0.72-1.18)
High-High	716 (19.5%)	1.01 (0.77-1.30)	1.00 (0.77-1.31)	1.01 (0.77-1.32)	1.01 (0.77-1.32)
Decreasing total cholesterol	876 (23.8%)	1.00 (0.79-1.28)	0.99 (0.77-1.27)	1.00 (0.78-1.29)	1.01 (0.78-1.31)
Increasing total cholesterol	627 (17.1%)	1.08 (0.83-1.40)	1.09 (0.84-1.42)	1.10 (0.85-1.43)	1.10 (0.84-1.43)
HDL-Cholesterol		p=0.02	p=0.03	p=0.03	p=0.03
High-High	1131 (30.8%)	1	1	1	1
Intermediate-Intermediate	1248 (34.0%)	1.16 (0.96-1.41)	1.16 (0.95-1.42)	1.16 (0.95-1.42)	1.14 (0.93-1.40)
Low-Low	182 (5.0%)	1.09 (0.75-1.58)	1.10 (0.74-1.63)	1.10 (0.74-1.62)	1.06 (0.71-1.58)
Increasing HDL	832 (22.7%)	1.09 (0.88-1.35)	1.09 (0.88-1.35)	1.08 (0.87-1.34)	1.06 (0.86-1.32)
Decreasing HDL	280 (7.6%)	1.63 (1.23-2.17)	1.63 (1.21-2.19)	1.62 (1.21-2.18)	1.61 (1.19-2.16)
Triglycerides		p=0.25	p=0.33	p=0.36	p=0.42
Low-Low	2542 (69.2%)	1	1	1	1
Intermediate-Intermediate	162 (4.4%)	1.39 (0.99-1.96)	1.33 (0.93-1.88)	1.31 (0.93-1.87)	1.31 (0.92-1.86)
High-High	190 (5.2%)	0.97 (0.69-1.37)	0.92 (0.64-1.33)	0.93 (0.65-1.34)	0.95 (0.66-1.37)
Decreasing triglycerides	365 (9.9%)	0.88 (0.68-1.14)	0.85 (0.65-1.12)	0.85 (0.65-1.12)	0.87 (0.66-1.14)
Increasing triglycerides	414 (11.3%)	1.08 (0.85-1.36)	1.00 (0.78-1.28)	1.00 (0.78-1.28)	1.00 (0.78-1.29)

HDL: high-density lipoprotein.

\*Decline scores in the worst quintile, corresponding to a decrease of memory score by 2 or more words.

<sup>†</sup>Model 1: adjusted for age & sex.

<sup>‡</sup>Model 2: adjusted for age, sex and mutually adjusted.

<sup>§</sup>Model 3: + adjusted for education and employment grade.

<sup>#</sup>Model 4: + adjusted for CHD, stroke, hypertension, medication, diabetes, smoking, alcohol consumption.

Table 5. Sensitivity analysis: change in HDL-C levels and associated decline in memory\* between Phases 5 (1997-1999) and 7 (2002-2004) on sub samples.

	N (%)	Model 1 <sup>†</sup>		Model 2 <sup>‡</sup>		Model 3 <sup>§</sup>		Model 4 <sup>#</sup>	
		OR ( 95%CI)		OR ( 95%CI)		OR ( 95%CI)		OR ( 95%CI)	
Subsample 1: APOE ε4 negative individuals (N=2397)									
High-High	774 (32.3%)		p=0.03		p=0.03		p=0.03		p=0.05
		1		1		1		1	
Intermediate-Intermediate	797 (33.2%)	1.14 (0.90-1.46)		1.16 (0.90-1.50)		1.18 (0.91-1.52)		1.12 (0.87-1.45)	
Low-Low	108 (4.5%)	0.99 (0.60-1.63)		1.06 (0.62-1.79)		1.06 (0.63-1.80)		1.00 (0.58-1.70)	
Increasing HDL	534 (22.3%)	1.24 (0.96-1.61)		1.26 (0.96-1.64)		1.26 (0.96-1.65)		1.21 (0.92-1.58)	
Decreasing HDL	184 (7.7%)	1.74 (1.22-2.58)		1.77 (1.23-2.56)		1.78 (1.24-2.57)		1.73 (1.20-2.50)	
Subsample 1: Adjustment for LDL-C instead of total cholesterol (N=3599)									
High-High	1131 (31.0%)		p=0.03		p=0.04		p=0.04		p=0.05
		1		1		1		1	
Intermediate-Intermediate	1238 (34.0%)	1.14 (0.94-1.39)		1.13 (0.93-1.39)		1.13 (0.92-1.38)		1.12 (0.91-1.37)	
Low-Low	171 (4.7%)	1.04 (0.71-1.52)		1.06 (0.71-1.58)		1.06 (0.71-1.58)		1.03 (0.69-1.55)	
Increasing HDL	825 (22.6%)	1.08 (0.88-1.34)		1.09 (0.88-1.35)		1.08 (0.87-1.35)		1.07 (0.86-1.33)	
Decreasing HDL	278 (7.6%)	1.61 (1.20-2.15)		1.60 (1.19-2.15)		1.60 (1.19-2.15)		1.58 (1.17-2.13)	

HDL: high-density lipoprotein.

\*Decline scores in the worst quintile, corresponding to a decrease of memory score by 2 or more words.

<sup>†</sup>Model 1: adjusted for age & sex.

<sup>‡</sup>Model 2: adjusted for age, sex and other lipids.

<sup>§</sup>Model 3: + adjusted for education and employment grade.

<sup>#</sup>Model 4: + adjusted for CHD, stroke, hypertension, medication, diabetes, smoking, alcohol consumption.