Plasma Total Cholesterol Level as a Risk Factor for Alzheimer Disease

The Framingham Study

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**Background:** Previous studies examining the association of plasma cholesterol levels with the risk for development of Alzheimer disease (AD) have been inconclusive. We examined the impact of baseline and lifetime plasma total cholesterol levels averaged across many years on the risk for AD in a large, population-based cohort.

**Methods:** Five thousand two hundred nine subjects from the Framingham Study original cohort underwent biennial evaluation for cardiovascular risk factors since 1950, with estimations of serum total cholesterol levels at 19 of these 25 biennial examinations. The study sample consisted of 1026 subjects from this cohort who were alive and free of stroke and dementia at examination cycle 20 (1988-1989) and had undergone apolipoprotein E (APOE) genotyping. The main outcome measure was incident AD diagnosed using standard criteria, according to average total cholesterol levels across biennial examination cycles 1 to 15 and baseline total cholesterol level measured at the 20th biennial examination cycle.

**Results:** Alzheimer disease developed in 77 subjects from 1992 to 2000. After adjustment for age, sex, APOE genotype, smoking, body mass index (calculated as weight in kilograms divided by the square of height in meters), coronary heart disease, and diabetes, we found no significant association between the risk for incident AD and average cholesterol level at biennial examination cycles 1 to 15 (hazard ratio per 10-mg/dL [0.3-mmol/L] rise, 0.95; 95% confidence interval, 0.87-1.04) or baseline total cholesterol level at examination 20 (hazard ratio, 0.97; 95% confidence interval, 0.90-1.05).

**Conclusion:** In this large, population-based cohort, baseline and long-term average serum total cholesterol levels were not associated with the risk for incident AD.

Arch Intern Med. 2003;163:1053-1057

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The apolipoprotein E (APOE) ε4 allele is a cholesterol transport protein that is a well-established risk factor for late-onset Alzheimer disease (AD). The presence of an APOE ε4 allele increases the lifetime risk for development of AD and is also associated with an earlier age of onset. However, it is an incomplete predictor of disease susceptibility, as AD does not develop in all subjects with the APOE ε4 allele and not all patients with AD have the ε4 allele.

The underlying pathophysiology that explains the relationship between APOE ε4 and AD remains speculative, but may involve an APOE ε4-mediated increase in serum cholesterol levels. Cholesterol has been linked to the development of AD by studies showing an association between lipoprotein(a) levels and AD and between low-density lipoprotein receptor–related protein and AD and by epidemiological studies implicating cardiovascular risk factors in the development of dementia.

Recently, several studies have suggested that the use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) to lower cholesterol levels reduces the risk for AD. These studies are observational, and results from randomized, clinical trials are not yet available.

Several observational epidemiological studies examining the association between cholesterol and AD have yielded conflicting results. Possible explanations for the heterogeneity of results from these studies may be the use of single rather than cumulative, time-averaged cholesterol levels, the restriction of the study population to men, and the brief follow-up in some studies. The Framingham Heart Study data provide an opportunity to examine the association between lifetime cholesterol levels and the risk for development of AD in a large population-based cohort of men and women during a 25-year follow-up.
The Framingham Study is a longitudinal population-based cohort study of 5209 participants (2336 men and 2873 women) who have undergone evaluation for cardiovascular risk factors since 1948. In 1975, at the start of the 14th biennial examination cycle, TC1-15, total cholesterol levels across biennial examination cycles 1 to 15; ΔTC15-20, change in total cholesterol levels between examination cycles 15 and 20. SI conversion factor: To convert cholesterol to millimoles per liter, multiply by 0.0259.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 380)</th>
<th>Women (n = 646)</th>
<th>Total (n = 1026)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>75.7 (5.2)</td>
<td>76.4 (5.4)</td>
<td>76.1 (5.3)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>205.9 (38.4)</td>
<td>221.6 (38.4)</td>
<td>215.7 (37.9)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>41.2 (11.8)</td>
<td>53.5 (16.2)</td>
<td>48.9 (15.8)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.1 (3.9)</td>
<td>26.4 (5.0)</td>
<td>26.7 (4.6)</td>
</tr>
<tr>
<td>Mean TC1-15</td>
<td>229.6 (34.2)</td>
<td>233.1 (33.7)</td>
<td>231.8 (33.9)</td>
</tr>
<tr>
<td>ΔTC15-20</td>
<td>-15.3 (30.2)</td>
<td>-16.1 (32.3)</td>
<td>-15.8 (31.5)</td>
</tr>
<tr>
<td>APOE-genotype, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/ε2+ε2/ε3, ε3/ε3</td>
<td>297/380 (78.2)</td>
<td>513/646 (79.4)</td>
<td>810/1026 (78.9)</td>
</tr>
<tr>
<td>ε2/ε4+ε3/ε4, ε4/ε4</td>
<td>83/380 (21.8)</td>
<td>133/646 (20.6)</td>
<td>216/1026 (21.1)</td>
</tr>
<tr>
<td>CHD, No. (%)</td>
<td>103/380 (27.1)</td>
<td>99/646 (15.3)</td>
<td>202/1026 (19.7)</td>
</tr>
<tr>
<td>Diabetes, No. (%)</td>
<td>47/380 (12.4)</td>
<td>45/646 (8.1)</td>
<td>92/1026 (8.9)</td>
</tr>
<tr>
<td>Smoking ≥5 pack-years, No. (%)</td>
<td>121/338 (35.8)</td>
<td>374/589 (63.5)</td>
<td>495/927 (53.4)</td>
</tr>
</tbody>
</table>

Abbreviations: APOE, apolipoprotein E; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CHD, coronary heart disease; HDL, high-density lipoprotein; TC1-15, total cholesterol levels at examination cycle 20; AD, Alzheimer disease; TC1-15, change in total cholesterol levels between examination cycles 15 and 20. SI conversion factor: To convert cholesterol to millimoles per liter, multiply by 0.0259.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AD, No./Study Population</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean TC1-15</td>
<td>77/1016</td>
<td>0.99 (0.92-1.06)</td>
</tr>
<tr>
<td>TC20</td>
<td>76/978</td>
<td>0.98 (0.91-1.04)</td>
</tr>
<tr>
<td>HDL at examination 20</td>
<td>74/976</td>
<td>1.12 (0.97-1.31)</td>
</tr>
<tr>
<td>ΔTC15-20</td>
<td>80/951</td>
<td>1.01 (0.93-1.10)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; TC1-15, total cholesterol levels across examination cycles 1 to 15; TC20, total cholesterol level measured at the 20th examination cycle; ΔTC15-20, change in cholesterol levels from the 15th to the 20th examination cycles.

Methods

The Framingham Study is a longitudinal population-based cohort study of 5209 participants (2336 men and 2873 women) who have undergone evaluation for cardiovascular risk factors since 1948. In 1975, at the start of the 14th biennial examination cycle, 3330 subjects from the original cohort were still alive. Of these, 2828 (85%) attended the 14th biennial examination. Of those, 203 subjects were excluded due to prevalent stroke (n = 75) or lack of APOE data (n = 128). The remaining 1026 subjects (646 women and 380 men) constituted our study sample. Baseline characteristics of subjects at examination cycle 20 are presented in Table 1 and Table 2.

Cholesterol Levels

During the 40 years of follow-up (examination cycles 1-20), nonfasting cholesterol levels in the Framingham Study subjects were measured at 19 examinations. Nonenzymatic laboratory methods were used until the 20th biennial examination. Plasma cholesterol level was determined according to the Abell-Kendall method, and high-density lipoprotein cholesterol (HDL-C) level was measured after precipitation of plasma with a combination of heparin-manganese. Previous studies have shown that in comparing fasting and nonfasting cholesterol measurements, a 6% increase would be found in one third of the population; no change, in one third; and a 7% decrease, in one third, for a negligible population net difference attributable to fasting status.

We used the following 3 different measures of total cholesterol level: the mean of the total cholesterol measurements obtained at or before the 15th examination cycle (mean TC1-15) as an estimate of time-averaged cholesterol level in subjects undergoing total cholesterol estimates at 4 or more examination cycles by cycle 15; the total cholesterol level measured at examination cycle 20 (TC20) as an estimate of baseline cholesterol level; and the change in cholesterol levels between examination cycles 15 and 20 (ΔTC15-20) as a measure of change in cholesterol levels, excluding subjects taking medications to lower lipid levels (n = 83). The ΔTC1-15 was taken as an absolute difference between the cholesterol levels in the 2 examination cycles and treated continuously in the regression. In addition, we examined the relation of HDL-C levels at the 20th biennial examination to the risk for incident AD.

APOE Genotypes

We determined APOE genotypes using an isoelectric focusing of plasma and confirmed the findings by subsequent genotyping. Participants were dichotomized into one group consisting of persons with APOE ε2/ε2, ε2/ε3, or ε3/ε3 genotypes, and the other consisting of those with APOE ε2/ε4, ε3/ε4, or ε4/ε4 genotypes.

Case Ascertainment

Beginning at examination cycle 17 (1982), the Mini-Mental State Examination was administered biennially to the cohort. A Mini-Mental State Examination score below the education-specific cutoff score, a decline of 3 or more points between consecutive biennial examinations, or a decline of more than 5 points compared with any previous examination prompted more in-depth testing. For each subject with possible dementia, neuropsychological and neuropsychological examinations were performed. Subjects with mild cognitive impairment (Clinical Dementia Rating scale, 0.5) underwent reassessment at least biennially for progression to moderate or severe dementia. A panel consisting of 2 neurologists and a neuropsychologist...
gist reviewed all subjects designated as having significant dementia (Clinical Dementia Rating scale score, ≥1) by the examining neurologists. We used data from the neurologist’s examination, neuropsychological test performance, Framingham Study records, hospital medical records, information from primary care physicians, computed tomographic and magnetic resonance imaging records, and autopsy confirmation when available. All subjects identified as having dementia satisfied the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition,22 had dementia severity of at least 1 on the Clinical Dementia Rating scale, and exhibited symptoms of dementia for a period of at least 6 months. All subjects identified as having AD met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association23 for definite, probable, or possible AD.

All subjects who were alive and free of dementia and stroke and who attended examination cycle 20 (1988-1989) were followed up longitudinally from examination 20 onward for the development of dementia. Therefore, at least a 10-year interval occurred from the last measurement of cholesterol level at examination 15 to the start of longitudinal follow-up that began at examination 20. This 10-year interval and the baseline screening of all subjects for dementia in 1976 to 1978 significantly increased the probability of effectively screening out subjects with subclinical dementia at the time of estimation of cholesterol level.

DEFINITION OF ADDITIONAL RISK FACTORS

Risk factors that could potentially confound the relationship between cholesterol level and AD were defined using data collected at the 20th biennial examination. Educational status was dichotomized at the level of high school completion. Cigarette smoking was defined as a lifetime smoking exposure of at least 5 pack-years. Diabetes mellitus was defined as a recorded casual blood glucose level of at least 200 mg/dL (≥11.1 mmol/L), a previous diagnosis of diabetes mellitus, or use of a hypoglycemic agent, including insulin. Systolic blood pressure and body mass index (calculated as weight in kilograms divided by the square of height in meters) were treated as continuous variables. Coronary heart disease was defined to include angina pectoris, myocardial infarction, and coronary insufficiency.24

STATISTICAL ANALYSIS

We used Cox proportional hazards regression analyses to examine the association between each of the various measures of total cholesterol and HDL-C levels and the subsequent development of incident AD, with and without adjustment for potential confounders (age, sex, APOE genotype, coronary heart disease, smoking, therapy to lower lipid levels, diabetes, and body mass index).

RESULTS

From 1992 to 2000, AD developed in 77 subjects. The baseline characteristics of the subjects are presented in Table 1. We found no significant association between the risk for incident AD (Table 2) and mean TC1-15 or TC20. Adjusting for potential confounders did not change this lack of association between the dependent and independent variables (Table 3) (hazard ratios [HR] per 10-mg/dL [0.3-mmol/L] rise in serum total cholesterol level, 0.95 and 0.97, respectively; 95% confidence intervals [CIs] 0.87-1.04 and 0.90-1.05, respectively).

We also examined the relationship of HDL-C level measured at examination 20 and the risk for AD. No significant association was noted in this cohort (HR, 1.10; 95% CI, 0.93-1.31). Likewise, among subjects not receiving medication to lower lipid levels, the ΔTC15-20 was not significantly associated with AD risk (HR, 1.01; 95% CI, 0.92-1.11).

COMMENT

Epidemiological studies suggest that atherosclerosis is associated with an increased risk for Alzheimer dementia.4,25 Critical coronary artery disease has been associated with a 3- to 10-fold increased risk for cerebral β-amyloid plaques, the neuropathologic hallmark of AD, relative to control subjects free of coronary heart disease.26 Similarly, an in vivo animal study showed a graded hippocampal accumulation of immunolabeled β-amyloid with increasing duration of feeding with a high-cholesterol diet.27 A human postmortem study showed that subjects with neuropathologically diagnosed AD had significantly elevated levels of low-density lipoprotein cholesterol, apolipoprotein B, and brain β-amyloid and significantly lower levels of HDL-C compared with controls.28

A cholesterol-AD link is further suggested by recent epidemiological studies that demonstrate an association between the use of certain agents that lower cholesterol levels (statins) and a decreased risk for dementia.5,6 This observation appears to support earlier tissue culture studies that show complete inhibition of β-amyloid formation in hippocampal cell cultures treated with lovastatin and methyl-β-cyclodextrin, suggesting that β-amyloid formation is a cholesterol-dependent process.29 However, the observed protective effect of statins in the epidemiological studies was independent of the presence or absence of hyperlipidemia and was not seen in subjects treated with nonstatin agents to lower lipid levels.

Previous prospective epidemiological studies exploring the relationship between serum cholesterol levels and the risk for AD have yielded varying results. A study of survivors of the Finnish cohort of the Seven Countries Study found that a high serum total choles-

### Table 3. Multivariate Adjusted Hazard Ratios of AD in Relation to Cholesterol Measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>AD, No./Study Population</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean TC1-15</td>
<td>60/853</td>
<td>0.95 (0.87-1.04)</td>
</tr>
<tr>
<td>TC20</td>
<td>60/853</td>
<td>0.97 (0.90-1.05)</td>
</tr>
<tr>
<td>HDL at examination 20</td>
<td>60/849</td>
<td>1.10 (0.93-1.31)</td>
</tr>
<tr>
<td>ΔTC15-20†</td>
<td>53/741</td>
<td>1.01 (0.92-1.11)</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; TC1-15, total cholesterol levels across examination cycles 1 to 15; TC20, total cholesterol level measured at the 20th examination cycle; ΔTC15-20, change in total cholesterol levels between examination cycles 15 and 20.

†Includes subjects who were not receiving medications to lower lipid levels.

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terol level (mean level, >251 mg/dL [>6.5 mmol/L]) at 40 to 59 years of age predicted AD in later life. The Finnish and Framingham Study cohorts are both white populations comparable in age and were studied during a similar period. Some differences between the 2 studies are the inclusion of subjects with prevalent dementia and the lack of women in the Finnish cohort. However, in our study population, the inclusion of subjects with prevalent dementia did not change our results (data not presented), and we did not find a sex-specific effect. Other possible explanations include the varying case definitions of dementia in the 2 studies (Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition, in the Finnish cohort and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition in the Framingham cohort), the use of fasting rather than nonfasting plasma cholesterol levels in the Finnish cohort, and ethnic variations within white populations. Mean plasma cholesterol levels in Finnish populations are higher than in other ethnic white populations. 20 

Romas et al 9 found that subjects with the lowest (<177 mg/dL [<4.6 mmol/L]) baseline total cholesterol level had an increased risk for incident dementia after 2.5 years of follow-up. This inverse relationship between cholesterol levels and incident AD may be accounted for by the short follow-up. When the follow-up is short, subjects with subclinical dementia at the start of the study may be erroneously classified as cognitively intact. This is important because, even in the early stages, dementia may cause a decrease in food intake, weight, and plasma cholesterol levels. 31 Moreover, since cholesterol levels decline with age, the age of the subject at the time of cholesterol level determination can be a significant factor, when using single rather than time-averaged cholesterol levels. 20 32 Older subjects are more likely to have low plasma cholesterol levels and also a greater risk for development of AD. This may explain why the association observed by Romas et al 9 was no longer significant after adjustment for age.

In our study, the 10-year interval between the last cholesterol level (examination 15) and the start of longitudinal follow-up for dementia (examination 20) significantly increased the probability of screening out subclinical dementia. We also used the average of multiple serial cholesterol levels estimated biennially for a 30-year period to achieve a more accurate reflection of lifetime cholesterol levels. Additional strengths of the present study are the ongoing surveillance for incident stroke in the Framingham cohort (thus reducing the chances that subjects with mixed or vascular dementia are inaccurately classified as having AD), long follow-up, and ability to control for multiple potential confounders. The primarily white American study population limits the applicability of our conclusions to other population groups.

The lack of association between TC20, mean TC1-15, or recent ΔTC15-20 and the risk for AD in the Framingham cohort supports the postulate that an alternative explanation exists for the observed association of statin use with a lower risk for AD. In addition to their lipid-lowering activity, statins have other putative beneficial effects, including capillary dilation, increased blood flow, blockage of macrophage and platelet activation, immunosuppression, and anti-inflammatory actions. These mechanisms, independent of the lowering of lipid levels, are hypothesized to be responsible for some of the beneficial effect of statins on coronary artery disease, renal failure, stroke prevention, and cerebral infarct size reduction. 33-35 A similar mechanism may exist for the relationship between statin use and the risk for Alzheimer dementia. Inflammatory mechanisms involving complement, acute-phase reactants, cytokines, and proteases have been implicated in the pathogenesis of AD. 36 Further studies are warranted to confirm the beneficial effect of statins in the risk for AD and to elucidate the mechanism by which they may exert this effect.

Despite plausible biological mechanisms and a remarkable convergence of data linking cholesterol levels to the pathophysiology of and the corresponding risk for AD, our study found that elevated serum total cholesterol level was not a significant risk factor for development of Alzheimer dementia.

Accepted for publication July 31, 2002.

This study was supported by grants 5R01-AG08122-11 and 5R01-AG16495-02 from the National Institute of Aging, Bethesda, Md; grant 5R01-NS17950-19 from the National Institute of Neurological Disorders and Stroke, Bethesda; and contract N01-HC-38038 from the National Institutes of Health, Bethesda.

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REFERENCES