Low- and High-Density Lipoprotein Cholesterol and Ischemic Cerebrovascular Disease

The Bezafibrate Infarction Prevention Registry

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Background: Despite increasing evidence that \(\beta\)-hydroxy-\(\beta\)-methylglutaryl coenzyme A reductase inhibitors reduce the incidence of stroke in patients with coronary heart disease (CHD), the associations between blood lipid levels and cerebrovascular disease (CVD) are not clear.

Objective: To evaluate whether blood cholesterol level and its fractions are risk factors for stroke in a large group of patients with CHD.

Methods: We followed up 11177 patients with documented CHD who were screened for but not included in the Bezafibrate Infarction Prevention study, a secondary prevention randomized clinical trial of lipid modification, and had no history of stroke for subsequent CVD. During a 6- to 8-year follow-up period, 941 patients were identified as having nonhemorrhagic CVD, of whom 487 had verified ischemic stroke or transient ischemic attack (TIA).

Results: Increases in age-adjusted rates of both nonhemorrhagic CVD and verified ischemic stroke or TIA were identified with increasing cholesterol and low-density lipoprotein cholesterol levels, decreasing high-density lipoprotein cholesterol levels, and decreasing percentage of total serum cholesterol contained in the HDL moiety. In logistic regression models, adjusting for clinical covariates, the following odds ratios (95% confidence intervals) were identified for lipid values in the upper vs lower tertile for the end point of nonhemorrhagic CVD: total cholesterol, 1.43 (1.20-1.70); low-density lipoprotein cholesterol, 1.52 (1.27-1.81); high-density lipoprotein cholesterol, 0.84 (0.70-1.00); and percentage of serum cholesterol contained in HDL, 0.69 (0.58-0.83). Similar trends appeared for the end point of verified ischemic stroke or TIA.

Conclusion: These findings clearly support the role of total cholesterol and its fractions in prediction of ischemic CVD among patients with established CHD.

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PATIENTS AND METHODS

STUDY PARTICIPANTS

The Bezafibrate Infarction Prevention (BIP) study was a placebo-controlled, secondary prevention randomized clinical trial that assessed the effect of bezafibrate retard. The design and rationale of the BIP study have been previously published.14 A total of 15,524 patients with documented CHD were screened for inclusion in the BIP study between February 1990 and October 1992. Patients 40 to 74 years old, with a diagnosis of CHD, were eligible for inclusion in the BIP study if they had evidence of myocardial infarction occurring 6 months or more but 3 years or less before enrollment or coronary insufficiency observed either at rest or during effort, as manifested by typical pain and dynamic electrocardiographic changes or both. Coronary insufficiency episodes must have occurred between 6 months and 2 years before enrollment. In addition, specific serum lipid ranges were required because the study examined the efficacy of intervening to reduce serum triglyceride levels and increase HDL-C levels. The limits to enter an initial 2-month dietary period were as follows: total cholesterol, 270 mg/dL or less (≤6.88 mmol/L); HDL-C, 45 mg/dL or less (≤1.16 mmol/L); and serum triglycerides, 300 mg/dL or less (≤3.39 mmol/L). In approximately 7000 patients who fulfilled these criteria at visit 1, there were duplicate laboratory measures that permitted the estimation of the regression dilution bias. For randomization into the study, after the 2-month diet, the following levels were required: serum total cholesterol, 180 mg/dL (4.65 mmol/L); HDL-C, 45 mg/dL or less (≤1.16 mmol/L); and triglycerides, 300 mg/dL or less (3.39 mmol/L). Main exclusion criteria other than serum lipid levels outside the preset limits were current use of lipid-modifying drugs, severe heart failure, unstable angina pectoris, insulin-dependent or poorly controlled diabetes mellitus, or refusal to participate.

For the current analysis, we excluded the actual BIP study participants, since the use of bezafibrate may have modified the association between blood lipid levels and stroke. We also excluded patients with a history of prior stroke or transient ischemic attack (TIA) to assess the risk of first-ever stroke associated with blood lipid levels. The total number of patients in the present analysis was 11,177.

During the first physician visit, records were obtained on medical history, conventional risk factors, and medications used, and a complete physical examination was performed. Mortality data were obtained through January 1999 from the Israel Population Registry, with cause of death coded according to the International Classification of Diseases, Ninth Revision (ICD-9). The participants in the BIP study (3122 patients) were of similar age to the other BIP study screenees but were more often men (92%), had a lower proportion of diabetes mellitus (10%), had a higher proportion of a prior myocardial infarction (78%), and had a selected lipid profile, as required for inclusion in this study.

LABORATORY METHODS

Laboratory measurements were all performed at a central study laboratory (the Physiological and Hygiene Laboratory at the Wollson Medical Center, Holon, Israel). All analyses were performed with a random access analyzer (Boehringer-Hitachi 704; Boehringer-Mannheim, Mannheim, Germany) using Boehringer diagnostic kits. Blood samples were obtained after at least 12 hours of fasting. Precipitation of LDL and very low-density lipoprotein with phosphotungstate reagent and determination of cholesterol determined HDLC levels. Cholesterol levels were determined by the CHOD-PAP (cholesterol oxidase/p-amino phenozene) method (enzymatic colorimetric test), and LDL-C levels were approximated by the formula of Friedewald et al.15

ASSessment of CVD

We obtained computerized data files from hospitals participating in the BIP study screening process. Hospitalizations during the follow-up period, 941 cases of nonhemorrhagic CVD were identified. Patients who subsequently developed a nonhemorrhagic CVD were, as expected, older (62 vs 60 years) and had a higher frequency of diabetes mellitus (35% vs 20%), hypertension (42% vs 32%), peripheral vascular disease (8% vs 4%), angina syndrome (66% vs 59%), and current smoking (12% vs 10%; P<.05 for all). They had higher serum levels of total cholesterol and LDL-C, lower levels of HDL-C, and a smaller percentage of total serum cholesterol contained in the HDL moiety (%HDL) (Table 1). Patients identified as having the large vessel atherothrombosis subtype had significantly higher antecedent levels of LDL-C than patients developing ischemic stroke related to cardioembolism or small vessel occlusive disease (Table 1).

Age-adjusted rates of nonhemorrhagic CVD increased with increasing quintiles of total cholesterol and LDL-C (Figure 1A-B). Rates per 10,000 person-years increased for total cholesterol and LDL-C from 103 to 141 and 104 to 142, respectively. Age-adjusted rates of nonhemorrhagic CVD per 10,000 person-years decreased stepwise with increasing HDL-C and %HDL quintiles from 151 to 90 and 176 to 91, respectively (Figure 1C-D). Similar trends appeared with all lipid fractions for the end point of ischemic stroke or TIA.

MULTIVARIATE ANALYSIS

The ORs associated with total cholesterol level or its fractions, adjusted for clinical covariates for incident nonhemorrhagic CVD, verified ischemic stroke or TIA, and the subgroup of patients with the large vessel atherothrombosis subtype, are presented in Table 2. Total cholesterol level and its fractions were independent predictors of nonhemorrhagic CVD and verified ischemic stroke or TIA, after adjusting for clinical covariates. For the large vessel atherothrombosis subtype, LDL-C, but not HDL-C, emerged as a strong predictor. The ORs of incident nonhemorrhagic CVD associated with total cholesterol or LDL-C (per 40 mg/dL [1.03 mmol/
with a diagnosis of CVD (ICD-9 codes 430-438 or code 38.1) were identified. We also matched the patients (based on national identification number and name) against a registry of the Clalit Health Services, which insures more than 60% of Israeli citizens (Israel has an obligatory 100% national health insurance). This registry contains information on both Clalit-maintained hospitals participating in the BIP study and several who did not participate. A total of 1100 patients were identified, and attainable medical records and hospital discharge summaries were systematically reviewed. Data were collected on history, findings on neurologic examination, brain computed tomography (CT), and ancillary examinations as available to verify the diagnosis and determine stroke type and ischemic stroke subtypes. Two investigators, including a stroke neurologist (D.T.), reviewed all classifications.

Stroke was defined according to World Health Organization criteria. Events that resolved completely within less than 24 hours were diagnosed as TIA. Patients with subarachnoid hemorrhage or subdural hemorrhage and those not fulfilling the criteria for stroke or TIA after review were excluded. Ischemic stroke and intracerebral hemorrhage were differentiated by the results of brain CT performed at the acute stage. Ischemic stroke was diagnosed if the patient had an appropriate clinical event and had a brain CT that showed a compatible low-density lesion or was normal or had findings compatible with hemorrhagic conversion of a cerebral infarct. Ischemic stroke subtypes were determined based on the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification. Only 24 cases were identified with verified intracerebral hemorrhage by clinical findings and brain CT, and thus associations with intracerebral hemorrhage were not sought.

For the purpose of this study, we assessed 2 end points. The first end point was cases considered to have any nonhemorrhagic CVD, which totaled 941 cases. This end point included cases with ICD-9 codes of CVD other than hemorrhage, excluding cases considered to have had a nonvascular cause after medical record review.

The second end point was patients with verified ischemic stroke (350 cases) or TIA (137 cases) after review of medical records, which totaled 487 cases. For the remaining patients, brain CT was not performed or medical records were not available for review so that stroke type could not be determined.

STATISTICAL ANALYSIS

Age-adjusted rates of nonhemorrhagic CVD per 10000 person-years were calculated using an SAS statistical software macro (SAS Institute Inc, Cary, NC). Multivariate analyses were performed using the logistic regression models. Levels of lipids were introduced into the model once as continuous variables and once as tertiles. For the remaining patients, brain CT was not performed or medical records were not available for review so that stroke type could not be determined.

The regression dilution correction factors for total cholesterol, LDL-C, HDL-C, and %HDL (per 5%) were 1.14, 1.19, 0.93, and 0.86, respectively (Table 2). The ORs were similar when patients taking lipid-lowering drugs at baseline were excluded from analyses, and 0.89, respectively. The Hosmer-Lemeshow goodness-of-fit test was conducted for assessing overall model fit for each of the models.

Odds ratios (ORs) were also computed to apply correction for regression dilution, using the repeated measurements for 7000 patients 2 months after the first visit. The regression dilution factors were calculated by dividing the difference in mean lipid levels between the lowest and highest quintiles computed from the first measurement by the difference in mean lipid level at the second measurement in similarly defined lowest and highest quintiles.

Nonhemorrhagic CVD-free survival of patients in the first, mid, and top tertile of cholesterol and HDL-C was assessed through December 1998. Survival curves were adjusted for sex, age, lipid-lowering medications, previous myocardial infarction, diabetes mellitus, New York Heart Association class, hypertension, chronic obstructive pulmonary disease, peripheral vascular disease, angina pectoris, current smoking, and body mass index.

Figure 2 shows the curve divergence of the nonhemorrhagic CVD-free survival functions (at mean of covariates) by tertiles of lipids. Longer survival time free of nonhemorrhagic CVD was evident in the lowest tertiles of total cholesterol and LDL-C and in the highest tertiles of HDL-C and %HDL (P<.01 for all).
COMMENT

Epidemiologic findings have indicated little or no association between cholesterol and its fractions and incident stroke in persons free of CHD. An apparent conflict arose when strong evidence was forwarded that HMG-CoA reductase inhibitors (statins) and more recently the fibrate gemfibrozil reduced the risk of both myocardial infarction and ischemic stroke in patients with CHD.7-13 However, statins also have important nonlipid effects that may reduce the risk of stroke.21

Because atherosclerosis is a main underlying process for both myocardial and atherothrombotic ischemic stroke, it is not unreasonable to expect a similar pattern of lipid abnormalities in both diseases. Yet, a review of 45 prospective cohorts6 found no association between total cholesterol and the risk of stroke. Furthermore, in the Framingham Study, no clear association was found between cholesterol and vascular disease of the brain,22 and most case-control studies were consistent with an absence of an association.23

In this context, our study found clear evidence for the role of cholesterol and its fractions in prediction of subsequent ischemic stroke or TIA among a large group of patients with established CHD. How could our findings be explained in light of the substantial amount of...
older negative studies? First, our study patients had established CHD, similar to the randomized clinical trials showing the benefit of statins and fibrates in stroke prevention. Second, most of the older studies did not record nonfatal strokes and types of strokes were not assessed. Lack of any overall relationship might conceal a positive association with ischemic stroke together with a negative association with hemorrhagic stroke, as shown in the Multiple Risk Factor Intervention Trial for stroke mortality and in several other studies. When patients in the Honolulu Heart Study were followed up for 15 or more years, increases in both CHD and thromboembolic stroke rates were seen with higher levels of serum cholesterol. In the Copenhagen City Heart Study, an increased risk of ischemic stroke was found for total cholesterol, but only for levels above 309 mg/dL (8 mmol/L).

In the current study, we assessed specifically incident ischemic stroke or TIA during a relatively long follow-up in an especially large group of patients. All lipid measurements have been performed at one central study laboratory and assessed before the event. Because of additional lipid measurements in a large proportion of patients, we were able to estimate and correct for the regression dilution bias, thus providing a more accurate estimate of ORs.

Although myocardial infarction is typically caused by in situ thrombosis associated with a coronary artery plaque, the pathogenesis of ischemic stroke is more complex with multiple potential mechanisms that often coexist, complicating the assessment of the role of lipids. In a population-based study from Rochester, Minn, the main identifiable subtype of ischemic stroke was cardioembolic, whereas large vessel cervical and intracranial atherosclerosis with stenosis together constituted about 16% of cases and small vessel occlusive disease a similar proportion. Furthermore, about 40% of ischemic strokes are of undetermined cause.

High-resolution ultrasonography has clearly established that thickening of the carotid artery intima and media is a predictor for strokes. An association between carotid atherosclerosis and LDL-C has been found in several studies, and randomized clinical trials with statins have demonstrated plaque regression or reduced progression of the carotid arteries. Furthermore, the Scandinavian Simvastatin Survival Study trial showed that the relative risk of cerebrovascular events was reduced by 37%, similar to the reduction in subsequent coronary events, but the benefit was confined to nonembolic stroke and TIA. This finding emphasizes that strokes with a basis of large vessel atheroma are most likely to be reduced by statins. In the current observational study, we have identified strong associations between LDL-C levels and a selected group of patients with cerebral large vessel atherosclerosis, supporting these results from randomized interventional trials.

We have previously found an independent inverse association between serum HDL-C levels and ischemic stroke mortality in a 21-year observational study of nearly 10,000 apparently healthy men included in the Israeli Ischemic Heart Disease study. In the current study, we have found similar inverse associations with HDL-C level and %HDL for incident ischemic stroke or TIA. Although HDL-C was an independent predictor for ischemic stroke or TIA, it is not a strong predictor compared to LDL-C and total cholesterol. The odds ratio for HDL-C was 0.72 (0.56-0.93) for ischemic stroke or TIA, compared to 1.27 (1.00-1.63) for LDL-C. This finding is consistent with previous studies showing that HDL-C has a more protective effect on ischemic stroke than LDL-C.

### Table 2. Odds Ratios Associated With Levels of Lipids for All Incident Nonhemorrhagic CVD, Ischemic Stroke or TIA, and Large Vessel Atherothrombosis Subtype*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Any Nonhemorrhagic CVD</th>
<th>Verified Ischemic Stroke or TIA</th>
<th>Large Vessel Atherothrombosis Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (per 40 mg/dL [1.03 mmol/L])</td>
<td>1.14 (1.07-1.21)</td>
<td>1.12 (1.03-1.23)</td>
<td>1.49 (1.18-1.89)</td>
</tr>
<tr>
<td>LDL-C (per 40 mg/dL [1.03 mmol/L])</td>
<td>1.19 (1.10-1.28)</td>
<td>1.14 (1.00-1.26)</td>
<td>1.68 (1.23-2.30)</td>
</tr>
<tr>
<td>HDL-C (per 10 mg/dL [0.26 mmol/L])</td>
<td>0.93 (0.86-0.99)</td>
<td>0.89 (0.81-0.98)</td>
<td>0.92 (0.67-1.26)</td>
</tr>
<tr>
<td>%HDL (per 5%)</td>
<td>0.86 (0.80-0.92)</td>
<td>0.83 (0.75-0.91)</td>
<td>0.65 (0.45-1.08)</td>
</tr>
</tbody>
</table>

*Number of patients on which the multivariate analysis is based varied according to the different models applied because of patients with at least one variable with unknown values. The number of events varied between 854 and 890 for nonhemorrhagic cerebrovascular disease (CVD), between 443 and 464 for ischemic stroke or transient ischemic attack (TIA), and between 40 and 41 for large vessel atherothrombosis. Each model included one lipid fraction and was adjusted for sex, age, lipid-lowering medications, previous myocardial infarction, diabetes mellitus, New York Heart Association class, hypertension, chronic obstructive pulmonary disease, peripheral vascular disease, angina pectoris, current smoking, and body mass index. CI indicates confidence interval; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and %HDL, percentage of HDL-C of total cholesterol.

### Table 3. Odds Ratios Associated With Tertiles of Lipids for All Incident Nonhemorrhagic CVD and Ischemic Stroke or TIA*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonhemorrhagic CVD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.00 (Referent)</td>
<td>1.22 (1.02-1.46)</td>
<td>1.43 (1.20-1.70)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.00 (Referent)</td>
<td>1.23 (1.03-1.48)</td>
<td>1.52 (1.27-1.81)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.00 (Referent)</td>
<td>0.93 (0.78-1.11)</td>
<td>0.84 (0.70-1.00)</td>
</tr>
<tr>
<td>%HDL</td>
<td>1.00 (Referent)</td>
<td>0.83 (0.70-0.98)</td>
<td>0.69 (0.58-0.83)</td>
</tr>
<tr>
<td>Ischemic stroke or TIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.00 (Referent)</td>
<td>1.30 (1.02-1.66)</td>
<td>1.50 (1.18-1.90)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.00 (Referent)</td>
<td>1.27 (1.00-1.63)</td>
<td>1.43 (1.12-1.82)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.00 (Referent)</td>
<td>0.94 (0.75-1.18)</td>
<td>0.72 (0.56-0.93)</td>
</tr>
<tr>
<td>%HDL</td>
<td>1.00 (Referent)</td>
<td>0.77 (0.61-0.97)</td>
<td>0.68 (0.53-0.98)</td>
</tr>
</tbody>
</table>

*Odds ratios adjusted for sex, age, lipid-lowering medications, previous myocardial infarction, diabetes mellitus, New York Heart Association class, hypertension, chronic obstructive pulmonary disease, peripheral vascular disease, current smoking, CVD indicates cerebrovascular disease; TIA, transient ischemic attack; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and %HDL, percentage of HDL-C of total cholesterol.
mic stroke or TIA, it did not emerge as a predictor for the large vessel atherothrombosis subtype. Recently, Sacco and colleagues have shown in a population-based, incident case-control study that increased HDL-C levels are associated with reduced risk of ischemic stroke in elderly patients and among different racial or ethnic groups. High LDL-C levels had, however, a major predictive role in particular for large vessel atherothrombosis.

The diagnosis of large vessel atherothrombosis was derived from medical records of patients undergoing evaluation for their cause of stroke or screening for carotid endarterectomy. Large vessel atherothrombosis was therefore probably underdiagnosed, and our findings on this issue should be regarded as hypothesis generating. They demonstrate, however, that in a disease with a pathogenesis as heterogeneous as in ischemic CVD, the role of lipids may differ between subtypes. In the Atherosclerosis Risk in Communities study, Sharrett and colleagues recently reported that LDL-C was a major predictor for both carotid atherosclerosis and CHD, whereas HDL-C was strongly associated with CHD incidence but only weakly with carotid atherosclerosis.

Several potential sources of bias have been considered in our study. First, a common limitation to many observational studies is the absence of information concerning potential spontaneous or therapy-induced changes in cholesterol level and its fractions during the follow-up period. At baseline, only a small fraction of patients were treated with lipid-lowering medications and exclusion of these patients did not affect our results. Statins were introduced in increasing percentage toward the latter period of our follow-up period that started in 1990, following the results of the pivotal clinical trials. Despite the compelling evidence from these trials, recent CHD prevention surveys between 1994 and 1998 have unveiled a wide therapeutic gap between scientific evidence and practice in the secondary prevention of CHD. Second, CHD tends to occur at a substantially higher rate and earlier age than ischemic stroke. Because of shared risks between these conditions, total cholesterol and its fractions, which are powerful risk factors for CHD, may be underestimated as predictors of ischemic stroke. These shortcomings may have diluted the reported associations between blood lipid levels and ischemic CVD.

The screening process for the BIP study took place in most hospitals in Israel (18 of the 25 cardiology departments). In identifying incidence cases, we may have missed a few patients admitted to hospitals outside the BIP collaboration. This would occur only if the latter hospital did not belong to the comprehensive Clalit Health Services. We assumed that only a few cases were missed in this way. In addition, patients with minor stroke events who were not admitted to a hospital may have been missed.

Finally, the predictive role of cholesterol and its fractions for ischemic CVD in this study was found in a group of patients with established CHD. These findings are in agreement with the recent data on the beneficial role of statins for stroke prevention, specifically in patients with CHD. When primary and secondary prevention statin trials have been analytically segregated, a nonsignificant 15% to 20% reduction in stroke events has been observed.

**Figure 2.** Nonhemorrhagic cerebrovascular disease–free survival curves by tertiles of total cholesterol (A), low-density lipoprotein cholesterol (LDL-C) (B), high-density lipoprotein cholesterol (HDL-C) (C), and percentage of total serum cholesterol contained in the HDL moiety (%HDL) (D).
in the former studies, compared with a 31% to 32% reduction in the latter.10-13 The nonsignificant 11% stroke reduction observed in the West of Scotland Coronary Prevention Study,57 an investigation of the effects of pravastatin on cardiovascular outcome in patients with moderate hypercholesterolemia without prior myocardial infarction, would also seem to underscore this difference. Thus, generalization of our results to broader populations at lower risk of stroke, such as those without clinically manifest CHD, is unwarranted.

In conclusion, this large observational study of patients with CHD demonstrates for the first time to our knowledge strong evidence for the role of total cholesterol, LDL-C, and HDL-C in prediction of incident ischemic CVD. These findings corroborate the results of clinical trials with statins and fibrates and emphasize the role of serum cholesterol and its fractions as important risk factors for ischemic stroke.

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