Low and High Density Lipoprotein Metabolism in Atherothrombotic Brain Infarction

Rita Alam, PhD; Frank M. Yatsu, MD; Ranganna Kasturi, PhD; and Georgina Bui, BS

Background and Purpose: Elevated low density lipoprotein and reduced high density lipoprotein cholesterol may increase the risk of atherothrombotic brain infarction, but the metabolic mechanisms accounting for this relation are poorly understood.

Methods: The kinetic parameters of low density and high density lipoprotein were studied in nine subjects with atherothrombotic brain infarction or identifiable (by noninvasive testing) extracranial occlusive disease and in 12 control subjects. Autologous iodine-125-labeled lipoproteins were injected intravenously. Blood samples were drawn 10 minutes after injection and periodically thereafter for 10 days. Kinetic parameters were calculated from the decay curves.

Results: The stroke-risk group showed significantly higher triglyceride ($p<0.05$), total cholesterol ($p<0.02$), and low density lipoprotein cholesterol ($p<0.01$). The fractional catabolic rate of low density lipoprotein was significantly lower ($p<0.001$) and the high density lipoprotein rate higher ($p<0.02$) in the stroke-risk group than in the control group. Regression analysis (using all subjects) of serum lipoproteins and their respective fractional catabolic rates correlated significantly (for low density lipoprotein, $r=0.684$, $p<0.001$; for high density lipoprotein, $r=0.595$, $p<0.002$). Mean percent stenosis showed a significant relation with triglyceride level ($r=0.678$, $p<0.01$) and low density lipoprotein cholesterol ($r=0.535$, $p<0.02$) but not with high density lipoprotein cholesterol. Mean percent stenosis also showed correlation with both fractional catabolic rate of low density lipoprotein ($r=0.667$, $p<0.002$) and with serum high density lipoprotein levels ($r=0.504$, $p<0.02$).

Conclusions: Our study provides insights into the role of altered low and high density lipoprotein metabolism in the pathogenesis of carotid stenosis. The statistically significant association of serum lipoprotein metabolic rates with carotid stenosis, rather than their respective serum concentrations, implies that metabolic parameters may be more important in predicting stroke risk. (Stroke 1992;23:1265–1270)

KEY WORDS • cerebral infarction • cholesterol • lipoproteins • risk factors

A therothrombotic brain infarction (ABI), or stroke due to atherosclerosis, remains the major cause of neurological disability, yet its pathophysiological mechanisms, while likely similar to coronary heart disease, remain unclear. Abnormalities in the complex metabolism of both low and high density lipoproteins (LDL and HDL) are believed, however, to play a critical role in ABI pathogenesis. In ABI, lipoproteins and serum cholesterol have been evaluated, and in a review of 26 series, all but three showed abnormalities.1 Abnormal associations that have been reported include elevated total serum cholesterol, increased LDL, reduced HDL, or elevated LDL/HDL ratios.2-4 Many studies on the abnormal metabolic kinetics in vivo of LDL, apoprotein B (apo B), HDL, and apoprotein A-I (apo A-I) have been reported on coronary heart disease and accelerated atherosclerosis,5-11 but no data are available on these parameters in carotid bifurcation athereosclerosis, a common prelude to ABI. Because many studies correlate lipoprotein abnormalities with ABI, we believe an assessment of the dynamic indexes of LDL and HDL synthesis and fractional catabolic rates were warranted to shed light on ABI pathophysiology. For HDL metabolism, we used the smaller HDL$_3$ particle, which is more abundant than and a precursor to the larger, cholesterol-laden HDL$_2$, to clarify its complex interconversions and clearance rates.

In the present study, we compared the in vivo metabolism of LDL and HDL and their respective apoproteins, B and A-I, in a group of patients with carotid bifurcation stenosis ranging from 30% to 80%, or with overt ABIs, to age- and sex-matched controls. To minimize the variability of lipoproteins from dietary influences, the subjects were on a metabolic diet low in cholesterol and saturated fats and did not ingest alcohol. We found that the degree of carotid stenosis correlated directly with increasing fractional catabolic rates (FCR) of both HDL$_3$ and its apoprotein, apo A-I, but not with their synthesis rates (SR). Contrariwise, FCR of LDL (apoprotein B) were inversely related to carotid stenosis and the occurrence of ABIs. The degree of carotid bifurcation stenosis paralleled the serum triglyceride and LDL concentrations.

Subjects and Methods

The stroke-risk patient population consisted of nine adults with ABIs or identifiable (by noninvasive testing)
extracranial occlusive disease. Subjects exhibiting less than 30% mean carotid stenosis were used as the control group, and subjects with more than 30% stenosis were considered stroke-risk subjects. For the control population, 12 age- and sex-matched controls from senior citizens groups and volunteers from the University of Texas Health Science Center were used. No subjects had hepatic, renal, thyroid, or immunologic disorders; women were not taking estrogens; and subjects with associated secondary disorders known to affect lipid metabolism were excluded. To minimize the effect of diet, subjects were treated initially with a hypocaloric National Institutes of Health type V diet until they stabilized. This study was approved by the Institutional Review Board of the University of Texas Medical School, and informed consent was obtained from all subjects.

For carotid ultrasound, a Diasonics duplex B-mode imager with pulsed Doppler real-time spectral analysis was used in this study (DRF-400 RV, Diasonics, Milpitas, Calif.). Anatomic localization of the common, external, and internal carotid arteries was accomplished using the carotid B-mode imager verified by simultaneous evaluation of the frequency spectrum of the Doppler signal, but only the B-mode image was used for final analysis of stenosis, as described below. Measurement of percent diameter stenosis was made at the locus of maximum narrowing at three levels (distal common, bulb, and proximal internal) in both carotid arteries by transverse imaging, with one cursor measuring the wall-to-wall diameter. The six measures of carotid artery transverse imaging were summed, and their average, referred to as "mean percent stenosis," was used as a measure of the extent of disease. All studies were performed by the same fully trained vascular technologist and interpreted by the same physician. Preliminary studies to determine the accuracy of carotid ultrasound compared favorably with arteriography as reported by Grotta.5

Forty milliliters of venous blood was obtained 2 weeks before the injection for the isolation of lipoprotein for labeling. LDL and HDL were isolated between d=1.019-1.063 g/ml and d=1.125-1.210 g/ml as described by Havel et al.12 After dialysis against 0.15 mol/l sodium chloride, pH 7.4, radioiodination was performed by Bilheimer modification13 of the McFarlane method.14 Purification of iodine-125-labeled LDL or 125I-HDL was done by Sephadex G50 or GX25, respectively, and dialyzed as described previously.12 Final specific activity of radioiodinated lipoprotein was approximately 250 cpm/ng protein. Homogeneity of labeled lipoprotein was tested by ultracentrifugation. The labeling procedure did not change immunochromal and ultracentrifugual properties and apolipoprotein pattern of apo HDL by polyacrylamide gel electrophoresis. The trichloroacetic acid-precipitable radioactivity was more than 95% of total counts. The lipid-bound radioactivity following extraction with chloroform/methanol (2:1 vol/vol) in the radioiodinated LDL and HDL was less than 5% of total counts.

We sterilized 125I-labeled lipoprotein solution by passing it through a filter (0.22-mm pore size; R.A. Millipore, Bedford, Mass.) An aliquot of sterilized solutions was assessed for pyrogens by the limulus amoebocyte lysate using pyrogen test (Whittaker Bioproducts, Walkerville, Md.). The solution was checked for sterility by University of Texas Health Science Center (UTHSC) clinical lab. Potassium iodide (100 mg daily) was given to prevent uptake of radioiodine by thyroid gland for 4 days before injection and throughout the studies. After injection of autologous LDL or HDL (25 μCi each), 15 ml blood was obtained at 10 minutes, 1 hour, 3 hours, and 6 hours, and daily for 10 days.

From each blood sample, LDL (d=1.019-1.063 g/ml), HDL2 (d=1.063-1.125 g/ml), and HDL3 (d=1.125-1.210 g/ml) were isolated. An aliquot was counted for total radioactivity and the rest dialyzed against saline and separated by polyacrylamide gel electrophoresis in duplicate and triplicate using 10% gel containing 6M/1 urea according to Kane et al.16 After electrophoresis, gels were fixed with Coomassie blue R250, destained with 7% acetic acid. Then radioactivity associated with apo A-I band was measured. Deviation of replicate radioactivity from their mean was <5.0% for apo A-I. The radioactivity decay curves were drawn using percent initial radioactivity remaining in apo A-I, HDL, and LDL at each time point.

For the calculation of kinetic parameters, the radioactivity decay curves were plotted. The fractional catabolic rate was calculated from this curve by the Matthews method.17 Under steady state conditions, synthetic rate was calculated by FCR×plasma volume×plasma apoprotein concentration.18 Plasma volume was estimated by the isotope dilution technique and also as 4.5% body weight.19 Linear regression analysis was performed using the least squares method, and statistical significance was done by Student’s t test. Calculations were performed on the UTHSC CLINFO system.

Plasma triglyceride, total cholesterol, and LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) concentrations were determined by clinical laboratory of UTHSC by standard autoanalyzer II procedure. Protein was determined by Lowry’s method,20 apo A-I and apo B were measured by respective double antibody radioimmunoassay procedure.21,22

**Results**

Lipid, lipoprotein levels, and mean percent stenosis were determined for each subject (Table 1). Patients were selected to provide a wide range of mean percent carotid stenosis levels from 30% upward. Mean HDL-C

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean % stenosis</th>
<th>Triglycerides</th>
<th>Total cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Apoprotein A-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=12)</td>
<td>21.5±3.5</td>
<td>126.7±34.5</td>
<td>208.0±31.1</td>
<td>114.2±31.9</td>
<td>55.3±17.2</td>
<td>126.0±18</td>
</tr>
<tr>
<td>Stroke (n=9)</td>
<td>52.7±12.7</td>
<td>205.2±115.7</td>
<td>251.2±48.1</td>
<td>157.2±35.1</td>
<td>49.2±11.0</td>
<td>126.0±16</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LDL, low density lipoprotein; HDL, high density lipoprotein.

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1. Plasma Lipids, Lipoproteins, and Mean Stenosis in Control and Stroke Subjects
values ranged from 30 to 85 mg/dl, and LDL-C values ranged from 69 to 240 mg/dl. The plasma concentrations of triglycerides, total cholesterol, and LDL-C and HDL-C cholesterol were compared between the control and stroke-risk groups. The stroke-risk group had significantly higher triglyceride \( (p<0.05) \), total cholesterol \( (p<0.02) \), and LDL-C concentrations \( (p<0.01) \) than the control group. No significant differences with regard to HDL-C and apo A-I were noted. After intravenous bolus injection of radiolabeled LDL or HDL, over the ensuing 10 days, a dieaway curve of plasma LDL, HDL, and apo A-I radioactivity was plotted. The apo A-I and apo B levels were measured and the metabolic parameters such as FCR/SR calculated as described in “Subjects and Methods.” As with all turnover studies, interpretation of this data was based on the assumption that the metabolism of the labeled apoprotein is equal to that of its unlabeled counterpart. Given the wide spectrum of various values, we expressed interrelations among the various study parameters by linear regression analysis. Regression analysis of LDL level and LDL FCR showed significant negative correlation \( (r=0.684, p<0.001) \) (Figure 1). Similar results were observed with HDL level and HDL FCR \( (r=0.595, p<0.002) \) (Figure 1). Correlation analyses were performed to see which metabolic parameters best predicted the extent of carotid stenosis. Mean percent stenosis showed significant correlation with the triglyceride \( (r=0.678, p<0.01) \), and LDL-C levels \( (r=0.535, p<0.02) \), but the relation between HDL level and mean percent stenosis was not significant \( (r=0.249, p=NS) \) (Figure 2). Correlation analyses were also performed to see if metabolic parameters of LDL and HDL turnover correlate with mean percent stenosis. Mean percent stenosis correlated highly significantly with LDL FCR \( (r=0.667, p<0.002) \) and also significantly with HDL FCR \( (r=0.504, p<0.02) \) (Figure 3). Correlations between metabolic parameters of control and stroke or ABI group were examined next. HDL was catabolized faster in the stroke group than the control group \( (p<0.02) \). Similarly, LDL FCR between the two groups was significantly different \( (p<0.001) \). Patients with mean percent stenosis higher than 30% showed slower LDL catabolism than did controls. LDL and HDL apoaprotein synthetic rates were similar in both groups (Table 2). The inclusion of both male and female subjects allowed us to test for possible sex differences in LDL and HDL metabolism under our study conditions. Within this small number of subjects, there were no significant differences in the measured parameters (data not shown).

**Discussion**

Atherosclerosis is the primary pathological condition accounting for ABIs. While ABI is but one manifestation of generalized atherosclerosis, separate study of ABI is warranted because hyperlipidemia correlates with the risk for cerebrovascular atherosclerosis with advancing age and has important differences from coronary heart disease. Elevated serum LDL-C and low HDL-C levels have been shown to be a consistent risk factor for coronary heart disease, as well as for ABI. The mechanism of increased LDL provoking atherosclerosis remains uncertain but may relate to the development of oxidized LDL, which is taken up macrophages in arterial wall. HDL metabolism is known, however, to be complex and determined by its interaction with other lipoproteins, cell membranes, and processing proteins such as cholesterol-ester transfer proteins.

The principal goal of this study was to understand the mechanism responsible for accelerated carotid atherosclerosis and its relation to LDL and HDL turnover. The results show that patients with mean carotid stenosis greater than 30% have significant changes in HDL and LDL kinetics. Specifically, the FCR of apo B/LDL was 31.7% slower in stroke-risk group \((0.273±0.04\) versus \(0.362±0.06)\) than in the control group. In addition, LDL-C levels were significantly higher in the stroke-risk group \((152.7±35.7\) versus \(114±31.9)\). On the other hand, HDL catabolism was faster in the stroke-risk group \( (p<0.02) \), but it was somewhat surprising to find that plasma HDL cholesterol and apo A-I levels were similar in the two groups and no correlation existed between the
FCR and the HDL cholesterol level. Because of the compositional heterogeneity of HDL particles and continuous remodeling of HDL lipid composition by lipid transfer protein, it was difficult to explain these turnover data by this kind of analysis. Synthetic rates for both apo B and apo A-I were not significantly different between the two groups. Regression analysis showed a moderate relation ($r=0.44$) between plasma apo A-I and HDL FCR and a marginal relation between the apoprotein and its synthesis rate ($r=0.27$; data not shown). Under steady conditions, the absolute synthetic and catabolic rates of any plasma protein should be equal. The concentration of HDL and its apoproteins is controlled by synthesis, catabolism, or a combination of both. Experimental evidence indicates that apo A-I and A-II are synthesized in the intestine and the liver.$^{29-30}$ HDL is important to reverse cholesterol transport$^{31-32}$ and assists LDL in the transport of the cholesterol to the liver.$^{33}$ Further, apo A-I is important in this process, not only in free cholesterol uptake from cells$^{34}$ but also in cholesterol esterification.$^{35-36}$ Thus, increased HDL apo A-I FCR in patients with extracranial occlusive disease may be associated with decreased body cholesterol removal, assuming that HDL is involved in the efflux of cholesterol from tissues for excretion. The study may also provide insights into the role of LDL and HDL FCR in protecting against ABIs because the striking differences in LDL and HDL metabolic parameters between the groups implies that they determine ABI risk. This consideration also has important implications in our understanding of basic mechanisms by which lipid-regulating drugs may influence the atherosclerotic process.

The relation between triglyceride content and mean carotid stenosis was very significant ($r=0.678$, $p<0.01$). Metabolism of plasma high density lipoprotein is closely related to triglyceride-rich lipoproteins, chylomicrons, and very low density lipoproteins; hypertriglyceridemia and low HDL-C are common features of patients with atherosclerotic disease.$^{37-38}$ Faster HDL catabolism in association with elevated triglyceride levels and high carotid stenosis suggests that extracranial occlusive disease may be due in part to abnormal apo A-I metabo-
Changes in HDL apoprotein kinetics in patients with high carotid stenosis are similar to those described in patients with high blood pressure, patients with non–insulin-dependent diabetes mellitus, and hypertriglyceridemia. Similarly, slower LDL catabolism, a primary defect in type II hyperlipoproteinemia, may be secondary in our patients with carotid stenosis to an underlying abnormality in lipid metabolism in the stroke-risk group.

The finding that the FCR of LDL is decreased and HDL is increased significantly with carotid stenosis could be viewed in two ways. On one hand, the change could simulate type II hyperlipoproteinemia's mechanism in causing coronary heart disease. Alternately, changes in apo A-I/HDL FCR could be related to triglyceride metabolism due to alterations in lipolytic enzymes. It has been shown that changes in postheparin LPL, adipose tissue LPL, and hepatic lipase activity correlate positively (LPL) and negatively (HL) with plasma HDL-C concentrations. In addition, FCR of 125I-HDL correlates with both hepatic and adipose tissue LPL activity. It is possible that variations in the activity of either or both of these enzymes contributes to faster HDL apo A-I FCR in stroke. We are currently pursuing these possibilities. It seems likely that pathophysiological mechanisms common to patients with CHD, with high LDL, low HDL, and high TG accounts in part for extracranial occlusive disease and ABI. Furthermore, recent evidence from Hennerici et al on the reduction of LDL (using heparin-induced extracorporeal LDL elimination) being associated with carotid plaque regression suggests that dynamic evaluation of lipoprotein metabolism will provide further insights into prevention.

In summary, subjects with carotid stenosis had slower LDL FCR and faster HDL FCR, and higher TG and higher LDL levels. It is suggested that the changes in HDL metabolism are secondary to hypertriglyceridemia rather than to decreased synthesis of HDL apoprotein, but the role of lipids and lipoproteins in the etiology of atherosclerosis is not completely clear. Serum lipids or specific lipids subfractions may act differently on intracranial and extracranial arteries. Our studies support the view that abnormal metabolism of lipids in cerebral atherosclerosis, especially of the large vessels, is similar to their effects on coronary atherosclerosis.

**Acknowledgments**

This research was supported by the Clayton Foundation for Research, Houston. We acknowledge the expert statistical analysis of Roger Strong, MS, and expertly performed noninvasive tests by Dora Vital, RN.

**References**


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*Stroke*. 1992;23:1265-1270
doi: 10.1161/01.STR.23.9.1265

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/23/9/1265