Lipoprotein Concentrations and Carotid Atherosclerosis by Diabetes Status

Results from the Insulin Resistance Atherosclerosis Study

From the Department of Public Health Sciences (D.C.G., R.B.D’A., L.E.W.), Wake Forest University School of Medicine, Winston-Salem, North Carolina; the Division of Clinical Epidemiology (S.M.H.), Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas; and the Division of Endocrinology (M.F.S.), University of California at Los Angeles, California. Address correspondence and reprint requests to David C. Goff Jr., MD, PhD, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157. E-mail: dgoff@wfubmc.edu. Received for publication 27 September 1999 and accepted in revised form 7 April 2000.

Abbreviations: IGT, impaired glucose tolerance; IMT, intimal-medial thickness; IRAS, Insulin Resistance Atherosclerosis Study; NGT, normal glucose tolerance.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

OBJECTIVE — Lipoprotein concentrations are associated with the development of atherosclerosis in people with and without diabetes. The relative strength of these associations could differ by diabetes status as a result of diabetes-related lipoprotein modifications.

RESEARCH DESIGN AND METHODS — The associations between lipoprotein concentrations and internal and common carotid artery intimal-medial thickness (IMT) assessed by B-mode ultrasonography were examined by diabetes status in a cross-sectional analysis among 1,391 participants in the Insulin Resistance Atherosclerosis Study. Participants included 442 individuals with type 2 diabetes, 308 with impaired glucose tolerance, and 641 with normal glucose tolerance.

RESULTS — The differences in internal and common carotid IMT between the highest and lowest tertiles of LDL were 58.1 μm (P = 0.054) and 51.0 μm (P < 0.001), respectively. The differences in internal and common carotid IMT between the lowest and highest tertiles of HDL were 56.2 μm (P = 0.07) and 37.8 μm (P = 0.003), respectively. Triglycerides and VLDL were not associated with IMT. These associations did not differ significantly because of diabetes status.

CONCLUSIONS — These results support the importance of dyslipidemia as a major risk factor for atherosclerosis in people with diabetes. Future research in humans should measure lipoprotein oxidizability, glycation, size, and composition directly in people of differing glucose tolerance status to address the importance of diabetes-related lipoprotein modifications more conclusively.

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Lipoproteins are associated with risk of coronary heart disease in people with diabetes (1–8), as well as in people without diabetes. The possibility that the relative strength of this association may differ between people with and without diabetes has been raised (3,4), but rarely examined (5,8). The degree of modification of lipoproteins (e.g., oxidation, glycation, size, and composition) may differ between people with and without diabetes (3,4,7,9). Modification (e.g., oxidation and glycation) of lipoproteins, especially of LDL, has been implicated as a potentially atherogenic process (7,10) that could underlie the aggressive nature of atherosclerosis in people with diabetes (11). Modified LDL increases the macrophage uptake of the LDL particle and may increase migration of monocytes into the subintimal space, in part, through stimulation of the expression of cytokines and cellular adhesion molecules. In addition to lipoprotein modification, lipoprotein size and composition may be important modulators of risk for atherosclerosis. Smaller denser LDL particles appear to be more atherogenic than larger less dense particles (12,13). In comparison with people without diabetes, people with diabetes have LDL particles that are smaller and relatively depleted of core lipids (7,14–16). The resulting change in apoprotein conformation may make the lipoprotein particle more susceptible to oxidative modification. If it is important to the pathogenesis of atherosclerosis, the differences in the degree of lipoprotein modification (and particle size and composition) between people with and without diabetes should combine to lead to differences in the relationship between lipoprotein concentrations and atherosclerosis according to diabetes status.

In this article, we addressed this question in the Insulin Resistance Atherosclerosis Study (IRAS) population. We hypothesized that because of the aforementioned diabetes-associated modifications of lipoprotein particles, LDL and VLDL cholesterol would be more strongly associated with atherosclerosis in people with diabetes than in those without diabetes.

RESEARCH DESIGN AND METHODS

Design and population

The objectives, design, and methods of IRAS have been published previously (17). Briefly, the major objective of IRAS was to assess the relationship between insulin resistance and atherosclerosis. This cross-sectional epidemiologic study was conducted at 4 clinical centers. African-Americans and non-Hispanic whites were studied in centers in Oakland and Los Angeles, California and Hispanics and non-Hispanic whites were studied in centers in San Luis Valley, Colorado and San Antonio, Texas. In Los Angeles and Oakland, participants were recruited from members of a nonprofit health maintenance organization. In Col-
orlando and Texas, participants were recruited from ongoing population-based epidemiologic studies of the risk factors for type 2 diabetes and coronary heart disease in Hispanics and non-Hispanic whites, the San Luis Valley Diabetes Study (18) and the San Antonio Heart Study (19), respectively. Sampling strategies were used to identify sufficient numbers of people in different ethnic, age, sex, and glucose tolerance groups to allow an efficient study of relationships among and within these groups. People taking insulin were excluded. IRAS was approved by the institutional review boards of all 4 participating clinical centers, and informed consent was obtained for all participants.

Clinical examination
The IRAS clinical examination consisted of two 4-h visits scheduled 1 week apart (20). Before each visit, participants were asked to refrain from alcohol and heavy exercise for 24 h, from food for 12 h, and from smoking on the day of the examination. The first visit included a 75-g oral glucose tolerance test, and blood was collected for fasting and 2-h glucose samples. Glucose tolerance status was classified according to World Health Organization criteria, as either normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or diabetes (21). To assess the presence and extent of atherosclerosis, participants underwent B-mode real-time ultrasound of the carotid artery (22,23). Race and ethnicity were self-reported. Cigarette smoking status was assessed by self-report as current, past, or never. Hypertension was defined based on a systolic blood pressure of at least 140 mmHg, a diastolic blood pressure of at least 90 mmHg, or pharmaceutical treatment for hypertension.

Carotid ultrasonography
The ultrasound protocol used to assess carotid artery intimal-medial thickness (IMT) was identical to that used in the Cardiovascular Health Study (23). Briefly, a bilateral assessment of the IMT was made in the internal carotid artery and the common carotid artery. For the internal carotid artery, the sonographer sought the site of maximal IMT in the region between the dilatation of the carotid bulb and the internal carotid artery 1 cm distal to the tip of the flow divider. For the internal carotid artery, 3 images were obtained (bilaterally) at the site of maximal thickness at different interrogation angles (proximal, lateral, and anterior). For the common carotid artery, bilateral images were obtained 1 cm proximal to the dilatation of the carotid bulb at a single (lateral) angle.

Ultrasound images were recorded on videotape and transferred to a central reading facility (D.H.O., principal investigator) for measurement of the IMT. For each of the 8 available images, the maximal IMT was taken over a 1-cm segment of the arterial wall, distant from the skin surface (the far wall). Two summary statistics were calculated as follows: 1) the mean of the 6 internal carotid artery sites and 2) the mean of the 2 common carotid artery sites. To allow equal weighting of the left and right arteries in the presence of missing data, the mean value of the available measures on the left internal carotid artery and the mean value of the available measures on the right internal carotid artery were calculated, and then the mean of these 2 means was used in the analysis. This approach is similar to that used to provide an index of atherosclerosis in other epidemiologic studies (23–27) and clinical trials (28–30).

Laboratory analysis
Glucose was measured at the central IRAS laboratory at the University of Southern California. Plasma glucose was measured using the glucose oxidase technique on an automated autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma lipoprotein measurements were obtained from single fresh plasma samples using Lipid Research Clinics methods at the Penn Medical Laboratories of Medlantic Research Institute (Washington, DC). LDL and HDL were isolated by isokypnic ultracentrifugation, and VLDL (top) and bottom fractions were measured for cholesterol and triglyceride concentrations (31). HDL was measured in the presence of manganese chloride and heparin in which non-HDL lipoproteins were precipitated, leaving HDL in the supernatant. The supernatant was removed after centrifugation, and the

Table 1—Characteristics of 1,391 IRAS participants by glucose tolerance status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal</th>
<th>Impaired</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>641</td>
<td>308</td>
<td>442</td>
</tr>
<tr>
<td>Sex (% women)</td>
<td>54.6</td>
<td>61.7</td>
<td>53.6</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>26.4</td>
<td>27.9</td>
<td>33.7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>33.5</td>
<td>32.8</td>
<td>32.1</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>40.1</td>
<td>39.3</td>
<td>34.2</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>17.2</td>
<td>16.2</td>
<td>16.8</td>
</tr>
<tr>
<td>Past</td>
<td>38.1</td>
<td>36.0</td>
<td>43.5</td>
</tr>
<tr>
<td>Never</td>
<td>44.8</td>
<td>47.7</td>
<td>39.7</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>24.8</td>
<td>40.6</td>
<td>51.7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.6 ± 0.3</td>
<td>56.6 ± 0.4</td>
<td>56.9 ± 0.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 ± 0.2</td>
<td>30.5 ± 0.4</td>
<td>31.5 ± 0.3</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>139.1 ± 1.4</td>
<td>142.9 ± 2.1</td>
<td>139.9 ± 1.7</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>20.7 ± 0.7</td>
<td>26.6 ± 1.1</td>
<td>29.4 ± 1.2</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>119.9 ± 3.0</td>
<td>156.6 ± 5.2</td>
<td>183.3 ± 8.1</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>48.1 ± 0.6</td>
<td>45.6 ± 0.8</td>
<td>40.9 ± 0.6</td>
</tr>
</tbody>
</table>

Data are n, %, or means ± SEM.

Table 2—Ranges for lipoprotein and lipid tertiles among IRAS participants

<table>
<thead>
<tr>
<th>Lipoprotein/lipid</th>
<th>Lowest</th>
<th>Middle</th>
<th>Highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>37–123</td>
<td>124–154</td>
<td>155–299</td>
</tr>
<tr>
<td>VLDL</td>
<td>1–13</td>
<td>14–26</td>
<td>27–200</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>23–94</td>
<td>95–154</td>
<td>155–1,023</td>
</tr>
<tr>
<td>HDL</td>
<td>11–37</td>
<td>38–48</td>
<td>49–125</td>
</tr>
</tbody>
</table>
cholesterol content was measured on a separate autoanalyzer channel set to measure low cholesterol values. LDL cholesterol was calculated as the difference between the HDL cholesterol and the bottom cholesterol. Triglycerides were measured enzymatically after correction for free glycerol.

Statistical analysis
Descriptive summary statistics were generated for the study population by glucose tolerance status using means (and SEM) for continuous variables and proportions for dichotomous variables. General linear modeling was used to estimate the relationship between IMT and the predictor variables of lipoprotein, apolipoprotein, and lipid concentrations and glucose tolerance status. For each measure of IMT (internal carotid artery and common carotid artery), 4 models were constructed, one for each lipoprotein or lipid moiety (LDL, HDL, VLDL, and triglycerides). The purpose of the analysis was to determine whether or not the association between the selected lipoprotein or lipid concentration and IMT differed by glucose tolerance status. This question was assessed by examining the interaction of glucose tolerance status and the lipoprotein or lipid concentration on IMT. That is, we tested for glucose tolerance status-related differences in the slopes for the relationships between IMT and the lipoprotein or lipid concentrations examined as a continuous variable and after categorization into tertiles based on the distribution for the total study population. We examined differences in slopes because these differences, if present, would support the contention that the strength of the association between lipoprotein or lipid concentrations and IMT differed by glucose tolerance status. In contrast, glucose tolerance status-related differences in intercepts would support a different contention (i.e., the risk of atherosclerosis differs by glucose tolerance status independent of the lipoprotein of lipid moiety examined). All models include the following covariates: age, sex, ethnicity, clinic, cigarette smoking status (coded as current, past, or never), hypertension, and BMI. The overall models also included glucose tolerance status as a covariate. Since ethnicity and clinic are confounded in IRAS, the interaction term of ethnicity and clinic was also included. For ease of interpretation, results are displayed as the mean (with 95% CI) of IMT by tertile of lipoprotein or lipid concentration and glucose tolerance status.

RESULTS — The IRAS population comprised 1,625 individuals. In this analysis, the sample numbered 1,391, and 234 individuals were excluded for one or more of the following reasons: taking a lipid-lowering medication (130), and/or missing internal carotid artery IMT (114), missing common carotid artery IMT (113). A varying number of additional participants were excluded from one or more analyses because of missing data regarding the lipoprotein or lipid concentration of interest and the reasons are as follows: missing LDL concentrations (n = 50), missing HDL concentrations (n = 8), missing VLDL cholesterol concentrations (n = 165), and missing triglyceride concentrations (n = 7). Characteristics of the 1,391 participants in the study population are shown in Table 1. More than half were women, more than one-quarter were African-Americans, and almost one-third were Hispanic. Cigarette smoking status differed little by glucose tolerance status; however, the prevalence of hypertension and the mean BMI were both greater with glucose intolerance and diabetes than with normal glucose tolerance. The mean LDL cholesterol concentration was ∼140 mg/dl in all 3 glucose tolerance status groups. In addition, HDL concentrations were progressively lower and the VLDL and total triglyceride concentrations were progressively greater from the NGT to the IGT to the diabetic group. The ranges of the lipoprotein and lipid tertiles are shown in Table 2.

Overall, after multivariable adjustment, the internal carotid artery IMT was 58.1 µm thicker among participants in the top tertile of LDL compared with those in the bottom tertile (P = 0.054). Similarly, the common artery IMT was 51.0 µm thicker among participants in the top tertile compared with those in the bottom tertile (P < 0.001). The mean (95% CI) IMT by LDL tertile and glucose tolerance status are shown for the internal carotid artery and the common carotid artery in Fig. 1. For the internal carotid artery, the mean IMT increased in a stepwise fashion across increasing tertiles of LDL for all 3 glucose tolerance groups. For the common carotid artery, there was an apparent, but statistically nonsignificant, difference in the pattern of the association between IMT and LDL by glucose tolerance status.

Overall, after multivariable adjustment, the internal carotid artery IMT was 20.4 µm thicker among participants in the top tertile of VLDL compared with those in the bottom tertile (P = 0.75). The common artery IMT was 0.5 µm thinner among participants in the top tertile compared with those in the bottom tertile (P = 0.96). The mean (95% CI) IMT by VLDL cholesterol tertile and glucose tolerance status are shown for the internal carotid artery and the common carotid artery in Fig. 2. For the internal carotid artery, there was an apparent, but statistically nonsignificant, difference in the pattern of the association between IMT and VLDL by glucose tolerance status. No clear
pattern of association was observed for the common carotid artery.

Overall, after multivariable adjustment, the internal carotid artery IMT was 23.6 µm thicker among participants in the top tertile of triglycerides compared with those in the bottom tertile (P = 0.60). The common artery IMT was 2.8 µm thinner among participants in the top tertile compared with those in the bottom tertile (P = 0.95). The mean (95% CI) IMT by triglyceride tertile and glucose tolerance status are shown for the internal carotid artery and the common carotid artery in Fig. 3. No clear relationship was observed between triglycerides and IMT for either the internal carotid artery or the common carotid artery.

Overall, after multivariable adjustment, the internal carotid artery IMT was 56.2 µm thinner among participants in the top tertile of HDL compared with those in the bottom tertile (P = 0.07). The common artery IMT was 37.8 µm thinner among participants in the top tertile compared with those in the bottom tertile (P = 0.003). The mean (95% CI) IMT by HDL tertile and glucose tolerance status are shown for the internal carotid artery and the common carotid artery in Fig. 4. For the internal carotid artery, there was an apparent, but statistically nonsignificant, difference in the pattern of the association between IMT and VLDL by glucose tolerance status. For the common carotid artery, there was no difference in the association between IMT and HDL by glucose tolerance status.

CONCLUSIONS — In this population, the overall relationships between lipoprotein and lipid concentrations and atherosclerosis were consistent with the literature published over the past several decades. LDL was positively associated and HDL was inversely associated with carotid atherosclerosis. The associations of VLDL cholesterol and triglycerides were weaker and possibly due to chance. The fact that our overall results are consistent with the body of literature concerning lipoproteins and atherosclerosis supports the contention that this population is appropriate for examining the possibility that the relative strength of these associations might differ according to glucose tolerance status as a reflection of diabetes-related differences in the degree of lipoprotein modification size and composition.

In this population, the association between LDL and carotid artery IMT did not differ significantly by glucose tolerance status. The observed pattern of association of VLDL and internal carotid artery IMT was suggestive of a greater role for VLDL in the pathogenesis of atherosclerosis in people with diabetes; however, this finding was not statistically significant in this sample. If diabetes-related modifications of LDL and VLDL particles were important mechanisms contributing to the aggressive atherosclerosis observed in patients with diabetes, we would have expected to see a more consistent pattern of relationships between LDL and VLDL and carotid IMT that differed systematically by glucose tolerance status. Although little supportive evidence was seen, lipoprotein modifications were not assessed directly and this indirect analysis had little power to detect modest differences. It is important to note that our findings are consistent with the literature showing a greater prevalence of dyslipidemia, especially hypertriglyceridemia and low HDL, in people with IGT or diabetes compared with people with NGT (1–9). Given the observation of associations between dyslipidemia and atherosclerosis that are at least as strong in people with diabetes as in people with normal glucose...
tolerance, our findings support the importance of dyslipidemia as a major risk factor for atherosclerosis in people with diabetes. This study has several limitations. As mentioned above, lipoprotein modifications were not directly measured; rather, the presence of diabetes-related effects on lipoprotein characteristics was inferred from glucose tolerance status, a surrogate marker. The degree of lipoprotein changes may differ importantly according to the degree of glycemic control, the duration of diabetes, and other factors not examined in this analysis. The most likely effect of this use of a surrogate measure of lipoprotein modifications is to reduce the power of this analysis to detect an effect of diabetes on the relationship between lipoproteins and atherosclerosis. Second, the study population was not population-based. The use of a population-based sample would provide greater support for generalizability; however, this apparent limitation could also be considered a strength for this analysis. Because the major interest was to detect an effect of glucose tolerance status on the relationships between lipoprotein measures and atherosclerosis, the overrepresentation of people with IGT and diabetes improved the power to detect a difference. Likewise, the overrepresentation of African-Americans and Hispanics could be considered a strength.

Previously, Kannel (8) reported, from the Framingham Heart Study, that total cholesterol had the same impact on incidence of coronary heart disease in people with diabetes as in people without diabetes. Stamler et al. (5) reported, from the Multiple Risk Factor Intervention Trial, that a lower relative risk, but greater absolute risk, of cardiovascular disease mortality associated with total cholesterol was observed in people with diabetes compared with those without diabetes. Our results extend this work to the level of lipoproteins and a more direct measurement of atherosclerosis. These results support the importance of dyslipidemia as a major risk factor for the development of atherosclerosis in people with diabetes. Given the wealth of laboratory and animal model data supportive of a role for lipoprotein modification in the pathogenesis of atherosclerosis, future research in humans should measure lipoprotein oxidizability, glycation, size, and composition directly in people of differing glucose tolerance status to address the importance of diabetes-related lipoprotein modifications more conclusively.

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