Cardiovascular Disease and Arterial Calcification in Insulin-Dependent Diabetes Mellitus: Interrelations and Risk Factor Profiles

Pittsburgh Epidemiology of Diabetes Complications Study-V

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Cardiovascular disease is a frequent complication of insulin-dependent diabetes mellitus (IDDM), but the prevalence, interrelations, and risk factors of its principal components (coronary, cerebrovascular, and lower-extremity arterial disease) and of medial arterial wall calcification are not well understood. To address these issues, data from the Epidemiology of Diabetes Complications Study (n=657) baseline examination were examined. The term coronary heart disease (CHD) was applied to those with myocardial infarction or angina, whereas lower-extremity arterial disease (LEAD) was applied to those who had undergone amputation of a lower limb or who had an ankle to arm blood pressure ratio less than 0.8 at rest or after exercise. Calcification of the lower-extremity arteries was considered to be present if ankle pressure was more than 100 mm Hg higher than brachial pressure. Although the prevalence of CHD was low, LEAD was significantly more common in women than in men (p<0.01), whereas calcification was more frequent in men than in women (p<0.01). Ten percent of those with LEAD also had CHD, and 8% with LEAD had calcification. Modeling of potential risk factors (e.g., diabetes duration and glycosylated hemoglobin) revealed that duration, female gender, fibrinogen, low density lipoprotein cholesterol, high density lipoprotein cholesterol, and triglyceride to apolipoprotein A-I ratio were independent predictors of LEAD, whereas for CHD only, diabetes duration and hypertension contributed to CHD. Calcification revealed a mixed pattern, with duration, hypertension, and low density lipoprotein cholesterol, high density lipoprotein cholesterol, and high density lipoprotein cholesterol to apolipoprotein A-I ratio being the statistically significant associated factors. The results suggest that although LEAD, CHD, and calcification often coexist, their risk factor profiles differ. (Arteriosclerosis and Thrombosis 1991;11:958-965)

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ower-extremity arterial disease (LEAD), coronary heart disease (CHD), and cerebrovascular disease (CBVD) are frequent complications of insulin-dependent diabetes mellitus (IDDM). The atherogenic process involved in these vascular diseases appears to proceed at a more rapid rate in diabetic than in nondiabetic subjects but does not appear to differ either in its morphological appearance or in the types of vessels affected. However, some reports suggest that a greater number of vessels are likely to be affected in subjects with diabetes. Although this accelerated atherogenic process may logically relate to long-term glycemic control, the evidence for this is weak, and disturbed lipoprotein metabolism appears to be a more likely candidate. It has also been suggested that the determinants of arterial disease of the limbs and heart may differ in the diabetic subject.

The prevalence, interrelations, and risk factors for LEAD, CHD, and CBVD in pure IDDM populations are largely unknown. It appears that, as in non-
insulin-dependent diabetes mellitus (NIDDM), the sex differential in CHD is much reduced.7 To study the varied manifestations of macrovascular diseases and their risk factor associations, data from the baseline examination of the Epidemiology of Diabetes Complications (EDC) Study are presented. In addition, because medial wall calcification may be a potent complication risk factor in diabetes and can interfere with the measurement of LEAD (by causing a seriously elevated arterial pressure in the legs), a proxy measure of calcification is included in an attempt to account for this condition.

Methods

Subjects

Individuals in this study were participants in the EDC Study, a prospective study that is based on the Children’s Hospital of Pittsburgh (CHP) IDDM Registry.8 To be eligible for the EDC Study, subjects had to have been diagnosed (or seen within a year of diagnosis) at CHP between January 1, 1950 and May 30, 1980 and to be living within 100 miles of Pittsburgh. All cases were diagnosed at an age of less than 17 years. One thousand three hundred thirty-six subjects met these criteria. One hundred forty-seven subjects had died before the baseline examination (1986–1988); according to death certificates, 38% of these deaths (i.e., 56) were due to renal disease, 18% (26) were due to coronary artery disease, and 5% (seven) were due to stroke. A further 11% were untraced or refused an earlier survey. The CHP registry has been shown to be representative of the population-based Allegheny County IDDM registry.8 Both the CHP registry and the EDC Study population have been described in detail previously.8,9 This report focuses on the final examined cohort (n=657) seen at baseline. There were 325 women and 332 men with a mean age (±SD) of 27.6±7.9 years and a mean duration (±SD) of diabetes of 19.4±7.5 years.

Clinical Evaluation and Procedures

Both a standardized medical history and clinical examination were performed by a trained internist to document cardiovascular disease. A 12-lead electrocardiogram was also obtained, and blood pressures were measured with a random-zero sphygmomanometer according to a standard protocol (Hypertension Detection and Follow-up Program)10 after a 5-minute rest period. Subjects were considered to be hypertensive if they were taking blood pressure medication and/or if they had a blood pressure greater than 140 mm Hg systolic and/or 90 mm Hg diastolic. Height was measured with the clinic stadiometer.

Stable glycosylated hemoglobin (HbA1c) was originally measured in saline-incubated samples by microporous column-exchange chromatography (Isolab, Akron, Ohio). On October 26, 1987 the method was changed to high-performance liquid chromatography (Diamat, Bio-Rad Laboratories, Hercules, Calif.). Readings with the two methods were shown to be almost identical (r=0.95; Diamat HbA1c = −0.18+1.00 Isolab HbA1c). The difference between the means of the two methods was 0.158% (normal range, 4.9–7.3% HbA1c). High density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a modification11 of the Lipid Research Clinics method.12 The concentration of HDL3 was measured after precipitation of HDL2 by dextran sulfate. Cholesterol was measure enzymatically,13 as were triglycerides.14 Low density lipoprotein (LDL) cholesterol levels were calculated from measurements of the levels of total cholesterol, triglycerides, and HDL cholesterol.15 Apolipoproteins (apos) A-I and B were determined via immunoelectrophoresis,16 and apo A-II was determined by an enzyme-linked immunosorbent assay methodology.17

Serum fibrinogen levels were determined via a biuret colorimetric procedure and a clotting method. White blood cell (WBC) counts were determined by use of the Counter S-Plus IV. Spontaneous whole-blood platelet aggregation was determined immediately after the blood was drawn by calculating the percent fall in a single platelet count after 15 minutes’ shaking of a citrated sample in a hot water bath (37°C). A Clay Adams Ultraflo 100 platelet counter was used for the determinations, and the values were subtracted from the previously determined platelet count in an EDTA sample. Details of this methodology have been published previously.18

Diagnosis of Arterial Disease

Resting ankle/arm systolic blood pressures were taken in the supine position by use of a Doppler blood-flow detector. The right and left ankle pressures were compared with the arm pressure, and ankle to arm ratios were calculated using the arm pressure measurement taken closest in time to the ankle pressure. Subjects without contraindications for exercise were exercised for 5 minutes on an 8% incline (30 seconds at 1.5 mi/hr followed by 4½ minutes at 2 mi/hr). Ankle to arm pressure ratios were calculated at 1, 3, and 5 minutes after exercise. An ankle to arm ratio of less than 0.8 at rest or after exercise was considered to be evidence of LEAD. This cutoff ratio of 0.8, used by others,19 approximates the mean±2SD (0.8) for 82 non-diabetic control subjects. Arterial calcification was presumed if the ankle pressure was more than 100 mm Hg greater than the arm pressure.20,21

CHD comprised clinic physician–diagnosed angina or confirmed myocardial infarction (i.e., either pathological Q waves at the time of examination or review of previous hospital records by use of standardized criteria).9 Stroke was recognized on the basis of reported history.

Retinopathy was determined by examination of stereoscopic color fundus photographs of three standardized fields. Photographs were evaluated and graded at the Wisconsin Reading Center by use of a modification of the Airlie House Classification.22
Overt nephropathy was defined as an albumin excretion rate greater than 200 μg/min on at least two of three urine collections (24-hour, overnight, and postclinic [a 4-hour collection obtained on the morning of the participant’s clinic visit]) or end-stage kidney disease (renal dialysis or transplant). In the absence of two complete urine collections, a urinary albumin to creatinine (mg/mg) ratio greater than 0.31 was used to define nephropathy status as previously described. In the absence of any urine specimens, a serum creatinine greater than 176.8 μmol/l (>2 mg/dl) was considered to be indicative of nephropathy. Urinary albumin was determined immunonephelometrically.

Smoking histories and current alcohol use status were ascertained via questionnaire. In these analyses a smoker was defined as anyone who had smoked at least 100 cigarettes and a drinker as anyone consuming at least one alcoholic drink per week.

Statistical Methods

To examine the differences between those with and without disease, several univariate and multivariate analyses were performed. The χ² test was used for dichotomous variables. The Student’s t test was used for continuous variables unless a normal distribution could not be achieved despite data transformations; in that case, the Wilcoxon rank-sum test was used. Lipid parameters were adjusted for age, and the adjusted probability values are presented. Multiple logistic regression was used to examine the relation between the binary dependent variable (i.e., CHD, LEAD, or calcification) and the independent variables. Each full model was analyzed by backward stepping, where nonsignificant variables leave the model and the coefficients for the remaining variables are recomputed. Independent variables were chosen for the multivariate analysis on the basis of their univariate associations and their known or suspected associations from prior research and biologic plausibility. To distinguish between the importance of two correlated independent variables, the logarithmic values of the likelihood ratios of alternate models were compared.

Results

Four patients, three of whom also had CHD, reported a stroke. Thus, no analyses are presented for stroke alone. The prevalence of CHD, LEAD, and calcification in this cohort was 3.4%, 9%, and 4.6%, respectively. Table 1 shows the prevalence of CHD, LEAD, and calcification by gender, diabetes duration, and age group. Women generally had a higher prevalence of CHD and LEAD than did men, while men had a higher prevalence of calcification. The interrelations of CHD, LEAD, and calcification are shown in Table 2. As shown in the table, the degree of comorbidity is modest; however, more than...
TABLE 3. Univariate Risk Factor Comparisons for Those With and Without Coronary Heart Disease, Lower-Extremity Arterial Disease, and Calcification

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>CHD Yes</th>
<th>CHD No</th>
<th>LEAD Yes</th>
<th>LEAD No</th>
<th>Calcification Yes</th>
<th>Calcification No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (n)</td>
<td>22</td>
<td>632</td>
<td>59</td>
<td>594</td>
<td>30</td>
<td>623</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>36±5*</td>
<td>27±8</td>
<td>33±8*</td>
<td>27±8</td>
<td>36±5*</td>
<td>27±8</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>27±5*</td>
<td>19±7</td>
<td>24±7*</td>
<td>19±7</td>
<td>27±5*</td>
<td>19±7</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>59</td>
<td>49</td>
<td>66†</td>
<td>48</td>
<td>23†</td>
<td>51</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>10.8±2</td>
<td>10.4±2</td>
<td>10.4±2</td>
<td>10.4±2</td>
<td>10.6±2</td>
<td>10.4±2</td>
</tr>
<tr>
<td>White blood cell count x10^9/mm^3</td>
<td>7.1±2</td>
<td>6.6±2</td>
<td>7.7±2*</td>
<td>6.5±2</td>
<td>7.5±2†</td>
<td>6.6±2</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>311±97</td>
<td>289±90</td>
<td>338±125‡</td>
<td>285±84</td>
<td>328±103‡</td>
<td>288±89</td>
</tr>
<tr>
<td>Spontaneous whole-blood platelet aggregation (%)</td>
<td>7.1±5</td>
<td>8.3±7</td>
<td>8.0±7</td>
<td>8.3±7</td>
<td>8.4±6</td>
<td>8.3±7</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>46*</td>
<td>14</td>
<td>27†</td>
<td>14</td>
<td>57*</td>
<td>13</td>
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<tr>
<td>Smoking (%)</td>
<td>62†</td>
<td>37</td>
<td>54‡</td>
<td>36</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>Alcohol use (%)</td>
<td>25</td>
<td>33</td>
<td>25</td>
<td>33</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>Overt nephropathy (%)</td>
<td>59*</td>
<td>23</td>
<td>36†</td>
<td>23</td>
<td>80*</td>
<td>21</td>
</tr>
</tbody>
</table>

Plus-minus values are mean±SD.
CHD, coronary heart disease; LEAD, lower-extremity arterial disease.
*p<0.001, †p<0.05, ‡p<0.01.

A quarter of those with CHD also have LEAD. Table 3 shows univariate comparisons of potential correlates for those with and without each of the cardiovascular disease entities. Those with disease were found to be older, to have had a longer duration of diabetes, and to have a higher prevalence of hypertension and overt nephropathy. No difference in glyceremic control, platelet aggregation, or alcohol consumption was seen between those with and without vascular disease. Those subjects with LEAD and calcification, however, were also found to have significantly higher WBC counts and fibrinogen levels. A higher prevalence of smoking was found for those with LEAD and CHD.

Table 4 shows univariate results of lipid and lipo-protein variables for those with and without CHD, LEAD, and calcification. Higher triglyceride concentrations and triglyceride to apo A-I ratios were the only lipid parameters that were significantly different for those with CHD. For subjects with LEAD, significantly higher concentrations of triglycerides and LDL cholesterol were seen, as were higher ratios of triglycerides to apo A-I and triglycerides to apo B. In subjects with calcification triglycerides, LDL cholesterol, apo B, and the ratios for triglycerides to apo A-I and triglycerides to apo B were significantly higher, while HDL cholesterol and HDL₃ cholesterol were significantly lower.

The results presented in Table 4 were reanalyzed (data not shown) by excluding subjects who had more
than one specific disease entity (e.g., had both CHD and LEAD). Significance levels for most risk factor differences were reduced, as might be expected with the resulting smaller sample size. However, the associations of triglycerides and LDL cholesterol levels with CHD were markedly reduced, suggesting that these associations may be secondary to the coexistence of LEAD.

Multiple logistic regression analyses (Table 5) showed that disease duration, female gender, serum fibrinogen, and lipid factors (i.e., LDL and HDL cholesterol and HDL cholesterol to apo A-I ratio) were independent predictors of the presence of LEAD. Because a substantial number (156) of subjects had overt renal disease, which has profound effects on lipids, blood pressure, and fibrinogen, nephropathy was added to the model as a further explanatory variable. This addition of nephropathy had no effect.

For subjects with calcification (Table 5), diabetes duration, hypertension, and the triglyceride to apo A-I ratio were found to be statistically significant (borderline significance) independent predictors, with gender (i.e., male sex) and LDL cholesterol remaining in the model. The addition of nephropathy, which was a strong predictor, to this model resulted in the removal of both hypertension and LDL cholesterol from the model, with the other variables remaining as independent predictors.

Similar analyses were also performed for CHD, wherein diabetes duration and hypertension were found to be independent predictors. The addition of nephropathy (a strong predictor) to this model resulted in the removal of hypertension as an explanatory variable.

**Discussion**

Studies of arteriosclerotic complications and risk factor associations in diabetes have usually been based on mixed populations of IDDM and NIDDM subjects but have not included measures of both lower-extremity and coronary arterial disease. This study of a representative cohort of IDDM subjects examined individuals with and without CHD, CBVD, LEAD, and calcification to determine if there were different risk factor associations for the development of these vascular complications. The small number of stroke cases (four) precludes any separate analysis by this entity alone.

The univariate and multivariate associations of diabetes duration and vascular disease imply that the longer a subject has diabetes, the greater the risk of arterial disease. The protection from the development of CHD that is normally found in the nondiabetic female population appears to be lost in this diabetic cohort, as evidenced by the lack of a male excess in CHD prevalence (Table 2). Although this has been seen previously with regard to the develop-
Learning of coronary disease, the significant excess of LEAD in the female population is a new finding. However, the prevalence of calcification was three times higher in men than in women, and although male gender was not a statistically significant predictor in the multivariate model, it did explain some of the variability. The regression models were reanalyzed excluding gender as an independent variable. In the LEAD model, the only model affected, HDL cholesterol was removed as an independent determinant, reflecting the fact that women usually have higher HDL cholesterol levels than do men.

Fibrinogen levels were found to be independently associated with LEAD. Hyperfibrinogenemia has previously been shown in prospective population surveys to be a risk factor for ischemic heart disease and stroke. Furthermore, it has been speculated, based on clinical observation of seven diabetic patients, that hyperfibrinogenemia may be one of the many factors involved in the pathogenesis of peripheral vascular disease. Our results in this much larger IDDM cohort provide some confirmation of this clinical observation. Another measure of hemostatic function, platelet aggregation, was marginally increased in LEAD, but an inverse pattern was seen for CHD and LEAD.

An interesting finding in the univariate analyses was the significantly higher WBC count found in subjects with LEAD and calcification. We are not aware of any previous reports in the diabetes literature of such a finding in IDDM. Although this increase in the WBC count was well within normal limits, the greater number of WBCs in those with vascular disease may reflect a physiological response to an injured vessel and may be of etiologic importance.

Significantly increased LDL cholesterol levels have previously been reported in IDDM subjects and in IDDM subjects with LEAD. Increased LDL cholesterol concentrations in IDDM may result from a number of mechanisms, including reduced LDL catabolism resulting from glycosylation of apo B and reduced receptor binding. As LDL cholesterol is a major risk factor for atherosclerotic disease in the general population, it is not surprising to find it to be associated with LEAD and calcification in this cohort. The lack of an association with CHD may reflect the smaller number of cases. In addition, it should be noted that 26 subjects had previously died of heart disease before recruitment and that both LEAD and calcification are diagnosed by examination as opposed to CHD, which was largely diagnosed by the presence of symptomatic disease (angina) or ischemic damage (myocardial infarction).

Apolipoproteins have been the focus of much attention lately because they serve both as a structural and as a functional component (i.e., cofactor for enzymes and ligands for the interaction of cell receptors and lipoprotein particles) of each lipoprotein particle. It has been postulated that apolipoproteins may be better discriminators than are lipids in atherosclerotic disease. Specifically, apo A-I is the main apolipoprotein of HDL cholesterol and is involved in the activation of lecithin:cholesterol acyltransferase and thereby reverse cholesterol transport. Our results have shown that apop A-I, A-II, and B do not vary much according to the different vascular end points; however, increases in the triglyceride to apo A-I ratio, suggesting lipoprotein particle compositional changes, may be important. The consistently high triglyceride to apo A-I ratio would be consistent with altered cholesterol ester transfer activity, as reported in IDDM. These findings, while suggestive of compositional changes, should not be interpreted definitively in view of the complex interrelations between lipids and apolipoproteins, which may not be fully accounted for in multivariate modeling.

Similarly, HDL subfractions do not seem to show a major effect. While HDL2 is thought to be the subfraction most often associated with vascular disease, HDL3 was significantly reduced in those with calcification. This finding would, however, be consistent with the results of the Speedwell Study that suggested that HDL2 is more important. Those contradictory findings may reflect methodological differences. Recent studies suggest that HDL particles containing only apo A-I (called LpA-I) are thought to be the antiatherogenic component. Consequently, the lack of a strong relation with HDL subfractions in this study does not preclude such an effect, as traditional measures (as used in this study) may not be the most appropriate.

In the subjects with calcification, however, we did observe significantly lower levels of total HDL cholesterol. No statistical difference was found for those with CHD, although lower HDL cholesterol levels were independently associated with LEAD. Others, however, have not demonstrated a relation between HDL cholesterol and macrovascular disease (defined via noninvasive techniques of ankle/arm systolic blood pressure measurements both at rest and after exercise, together with Doppler velocimetry tracing patterns). It is possible that vascular disease can develop in the presence of normal HDL cholesterol levels due to altered constituents of HDL that may result in functional impairment.

Triglycerides have often been found to be strongly associated with atherosclerotic vascular disease. Although triglycerides were significantly higher in each of the disease groups, triglycerides were not shown to be an independent determinant for any disease entity, with the exception of triglyceride to apo A-I ratio for calcification. These results contrast with recent reports, although these studies did not include fibrinogen as a possible contributing factor. The interpretation of these multivariate models is difficult because many of the lipoprotein–lipid factors are interrelated, for example, triglycerides and HDL cholesterol. Although the models presented are the best statistical fit, it is possible that both triglycerides and HDL cholesterol are important, and both probably reflect in part the same
disturbance of lipoprotein metabolism, that is, very low density lipoprotein catabolism.

Hypertension was independently associated with CHD and calcification. The role of hypertension in the development of vascular disease may involve endothelial damage and may initiate the pathogenic pathway of atherosclerosis. Hypertension has been shown to be a major risk factor of atherosclerosis in both diabetic and nondiabetic populations.

Although the prevalence of smoking was significantly higher in those with LEAD, smoking was not independently associated with any of the disease entities. This is in contrast to reports by others. However, the high prevalence of smoking in this cohort may have reduced its discriminatory power. As we have recently shown that smoking is a strong risk factor for mortality in this cohort for women, the lack of an association in the current analyses may reflect a survivor bias resulting from early mortality in smokers.

Previous studies have suggested that individuals with persistent proteinuria may have different cardiovascular risk factors than do individuals without proteinuria. Elevations of blood pressure and hypercholesterolemia have both been shown to be associated with nephropathy. Because abnormalities of glomerular function may affect both lipid and blood pressure levels in IDDM subjects, nephropathy was added to each of the logistic models. After accounting for the presence of nephropathy, hypertension was no longer a contributor to the CHD model, although still allowing a marginal contribution by gender (female). In the calculation model, hypertension and LDL cholesterol were replaced by the presence of nephropathy. It should be emphasized that these results are analyses of cross-sectional data, and therefore the true relation of cause and effect is questionable. Furthermore, the full impact of renal disease on cardiovascular disease may be muted in this study because of the 56 renal and 26 coronary deaths before the start of this study. Nonetheless, it is possible that the changes in blood pressure and lipid parameters that result from renal disease are the mechanisms by which nephropathy increases risk; however, nephropathy is a more statistically efficient variable in the model. The addition of nephropathy to the logistic model of LEAD did not affect the selected predictors. Jensen et al have demonstrated that the concentrations of lipids and fibrinogen rise with increasing urinary albumin excretion. However, in our cohort 42% of the subjects with LEAD had a normal albumin excretion rate (<20 μg/min), suggesting that lipid parameters and fibrinogen are truly important predictors of LEAD despite an association with renal disease. Other mechanisms by which renal disease may be associated with CHD and calcification include a common genetically determined predisposition to altered heparan sulfate metabolism, which may be exacerbated in IDDM by hyperglycemia. However, it is also important to note that whatever effect diabetes duration and/or other diabetes-related factors have, age per se is a major risk factor, as shown in Table 4 and as reported by others.

In conclusion, the study of large-vessel disease in IDDM is complex, with three or four different and frequent manifestations that are neither clearly independent nor interdependent. Nonetheless, these data suggest that the pathogenesis of these three entities does differ, with each showing a different risk factor profile, although the smaller sample size available for CHD and calcification compared with that for LEAD may be partially responsible for some of the differences noted. As this report concerns cross-sectional data, a survival bias exists (26 subjects had previously died of coronary artery disease), which may weaken risk factor relations with heart disease. To better investigate the risk factor associations and the interaction noted between renal and vascular disease, prospective follow-up is clearly needed.

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