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The Clinical Usefulness of Measuring Apolipoproteins in Diabetic Patients: A Preliminary Report

J. David Fachnie, MD, Janet McGill, MD, Craig Foreback, PhD, and Dorothy M. Kahkonen, MD

A commercial assay for apolipoproteins A-I and B as well as total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol was applied to 12-hour fasted serum from 24 insulin-dependent and 19 noninsulin-dependent diabetic persons. Women with noninsulin-dependent diabetes mellitus (NIDDM) had the highest levels of total cholesterol and apolipoprotein B. Apolipoprotein B values fell within the normal range in all patients except the NIDDM females, where four of the ten (40%) samples were elevated. When apolipoprotein B was elevated, total cholesterol was also elevated, over 220 mg/dL. Apolipoprotein A-I values fell within or above the normal range in all subjects, and a considerable discordance was observed between apolipoprotein A-I elevations and HDL cholesterol elevations.

The divergence between HDL cholesterol and apolipoprotein A-I supports the altered composition of HDL in the diabetic persons studied. Further study should elucidate the clinical usefulness of apolipoprotein measurements in diabetic patients. (Henry Ford Hosp Med J 1986;34:113-6)

When a commercial assay for apolipoproteins A-I and B became available, we wondered how this additional information could be used to evaluate risk for atherosclerosis in our diabetic patients. In nondiabetic persons, apolipoprotein A-I was claimed to better discriminate those with and without coronary-artery disease than high-density lipoprotein (HDL) cholesterol (1). However, in persons with noninsulin-dependent diabetes mellitus (NIDDM), a divergence between apolipoprotein A-I and HDL cholesterol suggested that such comparisons may yield different results in diabetic subjects (2). Our preliminary experience with measuring apolipoproteins A-I and B in diabetic patients and relating these measurements to commonly measured triglyceride, cholesterol, and HDL cholesterol is described.

Materials and Methods

In our clinic, the 43 ambulatory patients with NIDDM and IDDM (insulin-dependent diabetes mellitus) who donated 12-hour fasted blood specimens had the following characteristics: 1) IDDM female, mean age 30 years, mean BMI (body mass index) 26.6, N = 9; 2) IDDM male, mean age 30 years, mean BMI 26.7, N = 15; 3) NIDDM female, mean age 58 years, mean BMI 33.0, N = 9; 4) NIDDM male, mean age 56 years, mean BMI 27.6, N = 9; and 5) nondiabetic persons, N = 15. Fasting serum was assayed for triglyceride, cholesterol, and HDL cholesterol using standard methods (3-5). Glycosylated hemoglobin was run by high pressure liquid chromatography by American Bio-Science Laboratories (Farmington Hills, MI). Apolipoproteins A-I and B were assayed with a commercial assay by ligand combined with nephelometry. In this assay, apolipoproteins A-I and B in serum react with specific antisera prepared against purified apolipoproteins A-I and B. The resulting antigen-antibody complex increases the turbidity in the solution. A beam of collimated, monochromatic light from a laser source is passed through the solution, and the antigen-antibody complexes produce light scatter. The forward light scatter is measured quantitatively in relative light scatter units and is proportional to the amount of antigen in the sample (6,7).

The data were subjected to statistical analysis including computation of mean, standard deviation, analysis of variance, paired comparison for significant results, and correlation coefficients (Pearson Product Moment) (8).

Further evaluation of the data for clinical usefulness was made by comparing the total serum cholesterol, HDL cholesterol, and apolipoprotein A-I and B values with available reference ranges. Total serum cholesterol values were compared to the "moderate risk" for coronary-artery disease category of the Consensus Conference on Lowering Blood Cholesterol, ie, 75th to 90th percentile for the Lipid Research Clinic’s Prevalence Study (9). Levels greater than 220 mg/dL were considered to be elevated. HDL cholesterol levels were compared with Framingham values.
Lower HDL cholesterol was shown to increase the risk for coronary-artery disease. Depressed HDL cholesterol was defined as less than 45 mg/dL for males and less than 55 mg/dL for females. Desired ranges for apolipoproteins were defined by our clinical laboratory as follows: 35 to 147 mg/dL for apolipoprotein B and 95 to 152 mg/dL for apolipoprotein A-1. Higher levels of apolipoprotein A-1 and lower levels of apolipoprotein B are supposedly related to a decreased risk for coronary-artery disease in nondiabetic persons (11-14).

**Results**

The degree of adiposity, as measured by BMI (kg/m²) and analyzed by Student’s t-test, was not significantly different between our IDDM and NIDDM subjects of the same sex. The glycosylated hemoglobin, an index of blood sugar control, also was not significantly different between groups when subjected to analysis of variance.

Using analysis of variance, the mean cholesterol and apolipoprotein B were significantly different, \( p < 0.05 \), between diabetic subgroups (Table). A paired comparison for significant results revealed that cholesterol was significantly greater in NIDDM females than in NIDDM males. Also, apolipoprotein B was significantly greater in NIDDM females than in NIDDM males, IDDM males, and IDDM females.

Correlation coefficients between commonly measured cholesterol, triglyceride, and HDL cholesterol and the apolipoprotein measures were strongly positive: cholesterol and apolipoprotein B (0.832), low-density lipoprotein (LDL) cholesterol and apolipoprotein B (0.650), HDL cholesterol and apolipoprotein A-1 (0.786), and triglyceride and apolipoprotein B (0.806).

Total serum triglycerides were within the laboratory normal range (40 to 160 mg/dL) for IDDM males and females. Elevations were observed in four of eight (50%) NIDDM males and four of seven (57%) NIDDM females. Total serum cholesterol values were elevated in four of nine (44%) IDDM males, three of five (60%) IDDM males, and four of eight (50%) NIDDM females.

HDL cholesterol was low, less than 55 mg/dL, in two of four (50%) IDDM females and in five of seven (71%) NIDDM females. HDL cholesterol was less than 45 mg/dL, in six of ten (60%) IDDM males and in four of eight (50%) NIDDM males (Fig 1).

Apolipoprotein B values fell within the normal range in almost all subjects except for NIDDM females, where four of ten (40%) values were elevated over 147 mg/dL. When apolipoprotein B was elevated, total cholesterol was also elevated, over 220 mg/dL.

All apolipoprotein A-1 values fell within or above the normal range (8-14 mg/dL) for IDDM males and females. Elevations were observed in 11 of 15 (73%) IDDM males, three of eight (38%) IDDM females, seven of nine (78%) NIDDM males, and seven of ten (70%) NIDDM females. An elevation in apolipoprotein A-1 was frequently found in the same subject when HDL cholesterol was not elevated. This discordance was not seen in IDDM females, but was observed in six of nine (66%) and two of nine (22%) NIDDM females.

**Table**

| Serum Cholesterol, Triglyceride, HDL Cholesterol, and Apolipoproteins* |
|---------------------------------|-----------------|-----------------|--------------|-----------------|-----------------|
|                                | Number of Subjects | Glycosylated Hemoglobin | Cholesterol | Triglycerides | HDL Cholesterol | Apolipoproteins A-1 |
| IDDM Male                       | 15               | 10.9 (4)‡           | 199 ± 43    | 107 ± 68      | 41.7 ± 6.0      | 78 ± 36          |
| IDDM Female                     | 9                | 12.2 (5)            | 211 ± 46    | 76 ± 17       | 62.0 ± 30.0     | 73 ± 31          |
| NIDDM Male                      | 9                | 8.8 (4)             | 189 ± 40    | 160 ± 104     | 45.7 ± 17.5     | 78.5 ± 44        |
| NIDDM Female                    | 10               | 10.6 (5)           | 246 ± 31‡   | 180 ± 91      | 42.9 ± 13.7     | 142.4 ± 56       |
| Nondiabetic sample              | 15               | 10.6 (5)           | 246 ± 31‡   | 180 ± 91      | 42.9 ± 13.7     | 142.4 ± 56       |
| Reference normal ranges         |                  | 6-8.8              | 140-220     | 40-160        | 45 male         | 55 female        |

*All numbers are mean ± 1 standard deviation in mg/dL except glycosylated hemoglobin, which is a percentage.

‡Number inside parentheses indicates number of subjects.

\( p < 0.05 \). See text for explanation.

IDDM = insulin-dependent diabetes mellitus.

NIDDM = non-insulin-dependent diabetes mellitus.
nine (66%) IDDM males, three of five (60%) NIDDM females, and two of six (33%) NIDDM males.

Discussion

The prevalence of coronary-artery disease is 1.2 to 6.6 times higher in diabetic persons than the general population (15). Accurate determination of risk factors should help the physician to modify this poor outcome. Unfortunately, diabetes mellitus modifies the lipid phenotype in rather complex ways. Variables such as age, sex, type of diabetes mellitus, and state of diabetic control must be considered. Reference ranges for nondiabetic persons may not have the same meaning for diabetic persons. Our diabetic subgroups did not differ in degree of adiposity or estimate of diabetes control by glycosylated hemoglobin.

The present study represents a preliminary evaluation of apolipoproteins A-I and B in our metabolism clinic. The patients selected, although few, were more or less representative of our population of diabetic patients.

We evaluated our data with regard to three questions: 1) What is the prevalence of abnormal lipid values in our sample? 2) What differences exist between NIDDM, IDDM, and gender? 3) What additional information of clinical value is provided by measurement of apolipoproteins A-I and B?

Many of our diabetic subjects had abnormal lipid values. Triglycerides were elevated in 50% to 57% of NIDDM subjects. Cholesterol was elevated in 25% to 80% of all subjects. Depressed HDL cholesterol occurred in over half of all diabetic persons studied.

Regarding differences between subgroups, NIDDM females had elevation in both cholesterol and apolipoprotein B that reached statistical significance. Apolipoprotein B constitutes the major protein component of LDL. Elevations of apolipoprotein B are related to the development of coronary-artery disease in nondiabetic persons (16).

An excellent study by Walden et al, which included a large number of NIDDM and IDDM males and females compared to an age, sex, and weight matched control group, reported a similar profile of elevated cholesterol, triglyceride, and depressed HDL cholesterol in NIDDM females (17). Apolipoproteins were not available in that study. Our results are consistent with the view that NIDDM females have a particularly atherogenic lipoprotein profile and greater risk for coronary-artery disease (18).

When determining what additional information of clinical value was provided by apolipoprotein A-I and B measurement when combined with triglycerides, cholesterol, and HDL cholesterol, we found a high correlation coefficient between total cholesterol and apolipoprotein B. Furthermore, when apolipoprotein B was elevated, cholesterol was also elevated. We concluded that the apolipoprotein B measures reinforced but did not supplement the clinical impression available from cholesterol measures alone.

Apolipoprotein A-I is the major protein constituent of HDL. HDL cholesterol levels are inversely related to the risk for ischemic heart disease. We have previously reported lower HDL cholesterol levels in diabetic persons with vascular disease (19). Therefore, we would expect to see depressed HDL cholesterol levels and correspondingly depressed apolipoprotein A-I in a sample of putative high-risk diabetic persons. In fact, we observed a high correlation between HDL cholesterol and apolipoprotein A-I. However, the apolipoprotein A-I values were surprisingly high, while HDL cholesterol levels were normal or depressed. The frequent discordance between these two values presents a problem that demands further study. For example, we can no longer accept the conclusion that apolipoprotein A-I is more useful than HDL cholesterol in identifying persons with coronary-artery disease (1). The conclusion may be premature or may apply only to the nondiabetic patient.

A similar trend of elevated apolipoprotein A-I levels despite relatively low HDL cholesterol has been previously observed in NIDDM women (2). An increase in HDL triglyceride without alteration in HDL particle number was used to explain this divergence. Other authors have also observed a divergence between HDL cholesterol and apolipoprotein A-I in IDDM patients (20). We concluded that the divergence between HDL cholesterol and apolipoprotein A-I supports the concept of an altered composition of HDL in the diabetic persons studied. Further investigation should focus on the composition of HDL and its relationship to atherosclerosis and other complications of diabetes mellitus.

The available data suggest that the apolipoprotein B in diabetic persons will reinforce but not necessarily supplement the impression of coronary-artery disease risk provided by cholesterol alone. The measurement of apolipoprotein A-I, as an isolated value, may give a false impression of low risk. Accordingly, we cannot recommend the routine clinical measurement of apolipoproteins in diabetic persons at this time.

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