Effect of Metabolic Control on Lipid, Lipoprotein, and Apolipoprotein Levels in 55 Insulin-dependent Diabetic Patients

A Longitudinal Study

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SUMMARY

Plasma lipid, lipoprotein, and apolipoprotein levels were measured in 55 insulin-dependent diabetics (20 males and 35 females) before and after 2–3 wk of intensive insulin therapy in a metabolic unit.

At the time of discharge from the metabolic unit the levels of total, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) cholesterol as well as triglycerides, VLDL triglycerides, and apolipoprotein B (Apo B) were significantly decreased. Conversely, the levels of high-density lipoprotein (HDL) cholesterol, and apolipoprotein A_1 (Apo A_1) were significantly increased.

The data were further analyzed after subdividing the patients into two subgroups: (1) patients admitted in poor glycemic control ($HbA_{1c} > 11\%$) and (2) patients admitted in fair control ($HbA_{1c} < 11\%$). In the group admitted in poor control the changes in lipid and lipoprotein levels were similar to the ones found in the group of patients as a whole, while in patients admitted in fair control only the levels of total and VLDL triglycerides showed significant changes with control.

Patients' sex appeared to influence the magnitude of changes observed in HDL cholesterol and Apo A_1 levels. In males a significant increase in both HDL cholesterol and Apo A_1 was achieved after glycemic control either in the whole group or in the subgroup admitted in poor control. In the subgroup admitted in fair control HDL cholesterol levels rose but no significant change was observed in the Apo A_1 levels. In females no significant change was observed with improved control in HDL cholesterol and Apo A_1, either in the whole group or in the group admitted in fair control. A small but significant increase was detected in the HDL cholesterol levels of the female patients admitted in poor control, but no change was observed in the Apo A_1 levels.

In conclusion, in insulin-dependent diabetics, normalization in plasma lipid, lipoprotein, and apolipoprotein levels was obtained after intensive insulin therapy.

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An increased incidence of atherosclerosis in insulin-dependent diabetic patients has long been recognized.[1-2] Hyperlipemia, a common finding in these patients, may be one of the contributing factors.[3-6]

It is generally accepted that improved diabetic control will result in a reduction in plasma lipid levels.[7-8] Effects on lipoprotein concentrations, however, have been controversial. While metabolic control leads to a reduction in very-low-density lipoprotein (VLDL) levels, its effect on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels is not clear.[9-12] Furthermore, the effect of glycemic control on apolipoprotein levels in diabetes is not fully characterized.[13-16]

We postulated that lipoprotein and apolipoprotein responses to glycemic control in diabetic patients might depend on the degree of initial control and might differ in males and females. We therefore examined the effect of 2–3 wk of intensive insulin therapy on lipid, lipoprotein, and apolipoprotein levels in 55 insulin-dependent diabetic patients of both sexes. Results were analyzed according to the levels of diabetic control upon entry into the study.

SUBJECTS AND METHODS

Patients. Fifty-five patients (20 males and 35 females) with insulin-dependent diabetes were studied. Forty-nine percent were black, 51% Caucasian. The age of the group ranged from 5 to 52 yr with a mean of 17 ± 9 (mean ± SD) yr. Only 3 patients were above the age of 30 yr. The duration of diabetes was 7 ± 6 (mean ± SD) yr. The body weight of the whole population was 103% ± 8% (mean ± SD) of ideal

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body weight (growth charts, National Center for Health Statistics and Center for Disease Control, U.S. Public Health Service for less than 18 yr of age and table of desirable weights for men and women according to height and frame, prepared by the Metropolitan Life Insurance Company for 18 yr and above).

Twelve patients (22%) had retinopathy (nine background and three proliferative). Three patients had proteinuria between 250 and 500 mg/24 h and two had nephropathy (urinary protein greater than 500 mg/24 h).[17] Four patients drank moderately and another four smoked 1–1½ packs of cigarettes/day.

Ten patients (18%) were taking medications other than insulin, including β-blockers, diuretics, antifungal agents, contraceptives, phenobarbital, and chloropropamide. The latter drug was used in two siblings for treatment of concomitant diabetes insipidus. None of the medications mentioned above was used by more than two patients during this study.

All studies were carried out either at General Clinical Research Center or at the Pediatric Metabolic Unit of the Medical University Hospital. Informed consent was obtained from all adult subjects involved in the study. Parental consent was obtained for minors.

Protocol. All patients were placed on a eucaloric diet consisting of 20% calories as protein, 35% fat, and 45% carbohydrate. Activity was maintained as close to prehospital levels as possible. Regular walking schedules were used in most patients with a few utilizing a stationary bicycle.

Twenty-two patients recruited to participate in the study were studied in the General Clinical Research Center and treated with a closed-loop insulin-delivery system (Biostator glucose controller, Miles Laboratories, Elkhart, Indiana) for 2 days followed by continuous subcutaneous insulin infusion administered by portable infusion pump (AS2C and AS6C Autosyringe, Autosyringe Incorporated, Hooksett, New Hampshire). Thirty-three patients, admitted as part of their routine clinical care, were studied in the Pediatric Metabolic Unit and treated with multiple doses of regular insulin given subcutaneously. The mean daily dose of insulin for all patients (U/kg body weight) was 1.03 ± 0.64 (mean ± SD). Twenty-four-hour urinary glucose excretion was determined daily in all patients. Mean plasma glucose levels were obtained 4 times/day before meals during the first 48 h and on the 4 days preceding discharge in the patients on the pediatric unit and daily in pump-treated patients. Their mean period of hospitalization was 14 ± 6 (mean ± SD) days. Hemoglobin A_{1c} (HbA_{1c}) was measured in all patients on admission and at discharge. In all patients fasting blood samples were collected for lipoprotein studies within 24 h of admission and within 48 h of discharge.

Methods. Plasma and 24-h urinary glucose were assayed using the glucose-oxidase method as adapted for use in the Beckman glucose analyzer.[21] HbA_{1c} was measured by isoelectric focusing of erythrocyte hemolysates over a pH gradient of 6–8, according to a modification of the method of Spicer et al.[22] The relative concentration of HbA_{1c} was expressed as a percentage of the total hemoglobin. Normal levels in our laboratory ranged from 4% to 6.7%. The interassay coefficient of variation of the method is 11%.

Blood samples for lipid and lipoprotein assay were collected in EDTA (1 mg/ml of blood) after an overnight fast, and the plasma was refrigerated immediately. The separation of lipoproteins was performed by ultracentrifugation[18] on a preparative ultracentrifuge (Beckman L5-50) using a fixed-angle rotor (type 50) with appropriate adaptors. Plasma (3–4 ml) was layered under a 0.199-mol/L saline solution containing 1 mmol/L of EDTA (density: 1.006 g/ml) and spun for 18 h at 40,000 rpm at 16°C.

Very-low-density lipoproteins (VLDL) were sliced from the top layer and the infranatant (HDL + LDL) was adjusted to the initial volume and used to determine cholesterol and triglycerides. VLDL cholesterol was determined by subtracting from the total cholesterol level the value obtained for HDL + LDL cholesterol. Recovery studies were performed by adjusting VLDL to 1–2 ml of volume and measuring cholesterol and triglycerides in VLDL. Dilution and concentration factors were taken into consideration.

HDL was determined in whole plasma, after precipitation of LDL and VLDL with 4% of sodium phosphotungstate in the presence of 2 M $MgCl_2$ as previously described by us.[19] LDL cholesterol was obtained by subtracting the previous level from the value obtained for HDL + LDL cholesterol. Cholesterol and triglyceride levels were measured using the semiautomated method standardized by the Lipid Research Clinics Program.[20]

Apolipoprotein B (Apo B) levels were measured using a slightly modified version[23] of Laurell's electroimmunodiffusion technique.[24] Anti-Apo B antisera (1.5 ml) per liter of an Agarose/Dextran 10 solution were used. LDL isolated from a normal donor by ultracentrifugation (density > 1.030 and < 1.050) was used to calibrate the assay. LDL to be assayed was diluted from 1/40 to 1/240. All samples were run in duplicate in at least two dilutions. Apolipoprotein A_1 (Apo A_1) levels were measured as previously described.[25]

Data were analyzed using an analysis of variance (Statistical Analysis System). Age, sex, race, duration of the disease, and duration of hospitalization were accounted for by considering these factors as covariants in the analysis.

RESULTS

Table 1 summarizes the laboratory studies in all patients studied, on admission and discharge from the hospital. At discharge, the levels of total, LDL, and VLDL cholesterol as well as triglycerides, VLDL triglycerides, and Apo B were significantly decreased when compared with the admission levels. Conversely, the levels of HDL cholesterol and Apo A_1 were significantly increased.

Upon entry into the study the degree of diabetic control within the group of patients studied varied from fair to very poor. For this reason, we assessed whether the degree of control of the patients on admission would influence the magnitude of change in lipid, lipoprotein, and apolipoprotein levels obtained after intensive insulin therapy. Patients were subdivided into two groups: (1) patients admitted with levels of HbA_{1c} above 11% (poor control) and (2) patients admitted with levels of HbA_{1c} equal or below 11% (fair control). Group 1 included 41% of the patients admitted to the General Clinical Research Center and 82% of the patients admitted to the Pediatric Metabolic Unit. The body weight of the patients included in groups 1 and 2 was, respectively, 103 ± 7.6 and

TABLE 1
Fasting plasma lipid, lipoprotein, apolipoprotein, glucose, and HbA_{1c} levels in insulin-dependent diabetics at admission and discharge from the hospital

	Diabetic patients	
	At admission	At discharge
No. of subjects	55	55
Total cholesterol (mg/dl)	215 ± 61	180 ± 44*
LDL cholesterol (mg/dl)	144 ± 47	119 ± 43*
Apolipoprotein B (mg/dl)	143 ± 58	117 ± 54*
HDL cholesterol (mg/dl)	38 ± 10	44 ± 13*
Apolipoprotein A_1 (mg/dl)	104 ± 29	109 ± 28‡
Triglycerides	189 ± 201	88 ± 44*
VLDL triglycerides	148 ± 192	59 ± 38†
VLDL cholesterol	33 ± 35	16 ± 10*
Glucose	244 ± 127	115 ± 65*
HbA_{1c}	13.1 ± 3.7	10.3 ± 2.6*

*$P < 0.001$.
†$P < 0.01$.
‡$P < 0.05$.
The levels of Apo B and Apo A_1 were determined in only 49 and 48 patients, respectively. The data are expressed as mean ± SD. Paired *t* test and Wilcoxon signed rank test were used for the statistical analysis.

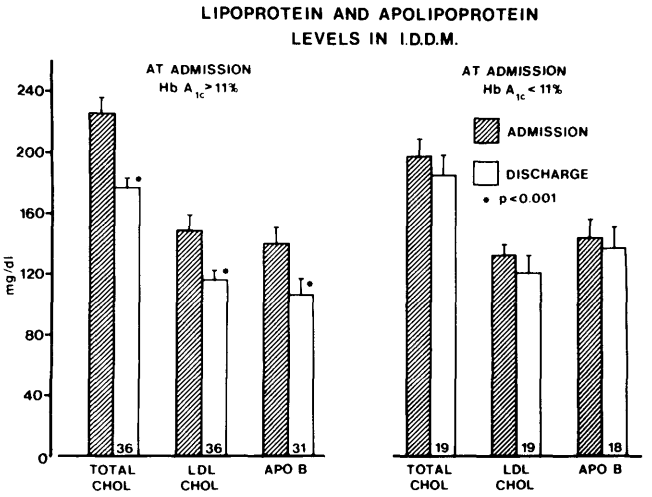


FIGURE 1. Levels of total and LDL cholesterol and apolipoprotein B in insulin-dependent diabetic patients. The total group of patients was subdivided into two subgroups: 1—patients admitted in poor metabolic control ($HbA_{1c} > 11\%$)—represented on the left, and 2—patients admitted in fair metabolic control ($HbA_{1c} < 11\%$)—represented on the right. In this figure and in the following ones the hatched bars represent the levels obtained at admission to the hospital, the open bars the levels obtained at discharge from the hospital, after appropriate metabolic control had been achieved.

104 ± 8.5 (mean ± SD) percent of ideal body weight. The mean levels of glucose and hemoglobin A_{1c} obtained on admission and at discharge in these two subgroups are shown in Table 2.

In the patients admitted in poor control the levels of Apo B, total cholesterol, LDL and VLDL cholesterol, total triglycerides, and VLDL triglycerides were significantly decreased at discharge when compared with admission levels. Conversely in the group of patients admitted in fair control no significant difference was found between admission and discharge levels, except for total and VLDL triglycerides (Figures 1, 2, and 3).

The effects of age, race, sex, duration of diabetes, and duration of hospitalization on the changes in lipid, lipoprotein, and apolipoprotein levels, induced by improved glycemic control, were assessed by an analysis of variance and covariance. No effect of age, race, duration of diabetes, or duration of hospitalization was found. The analysis did, however, demonstrate an effect of sex on the magnitude of the changes observed in HDL cholesterol and Apo A_1. For this reason we analyzed our data subdividing the patients into males and females. Our results are summarized in Figures 4 and 5. In males, a significant increase in both HDL cholesterol and Apo A_1 levels was achieved after glycemic control either in the whole group of patients or in the subgroup admitted in poor control. In the subgroup admitted in fair control the HDL cholesterol levels rose but no significant change was observed in the Apo A_1 levels.

In females our findings were quite different. With improved glycemic control, no significant change in HDL cholesterol and Apo A_1 levels, either in the whole group or in the group admitted in fair control, was observed. In the group of patients admitted in poor control a small but significant increase in HDL cholesterol levels was detectable. No change, however, was observed in the Apo A_1 levels.

DISCUSSION

It is generally accepted that lipid and lipoprotein abnormalities are found in insulin-dependent diabetic patients in poor glycemic control,[7–10] but there is still no agreement concerning the predominant characteristics of these abnormalities.

The effect of improved diabetic control on lipid and lipoprotein levels is also a controversial point, particularly regarding HDL cholesterol levels. Chase and Glasglow[13] reported decreased levels of HDL in a group of children apparently in good control. Lopes-Virella et al.[9] and several other groups[26–28] found an increase in HDL cholesterol levels

TABLE 2
Plasma glucose and HbA_{1c} levels in 55 insulin-dependent diabetic patients

	Glucose (mg/dl)		HbA_{1c} (% total Hb)	
	Group 1 (HbA_{1c} > 11% at admission)	Group 2 (HbA_{1c} < 11% at admission)	Group 1 (HbA_{1c} > 11% at admission)	Group 2 (HbA_{1c} < 11% at admission)
No. of subjects	36	19	36	19
Admission	274 ± 141	187 ± 67	15.1 ± 2.8	9.3 ± 1.3
Discharge	111 ± 72	123 ± 51	11.4 ± 2.5	8.4 ± 1.6

Levels expressed as mean ± SD.

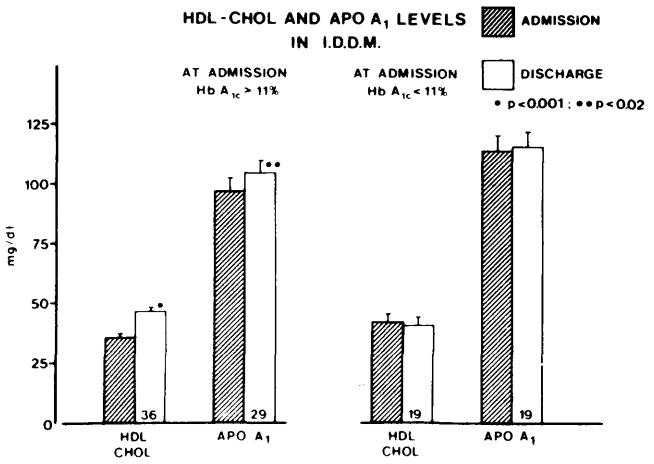


FIGURE 2. Levels of HDL cholesterol and apolipoprotein A_1 in insulin-dependent diabetic patients. The total group of patients was subdivided into two subgroups: 1—patients admitted in poor metabolic control (HbA_{1c} > 11%)—represented on the left, and 2—patients admitted in fair metabolic control (HbA_{1c} < 11%)—represented on the right.

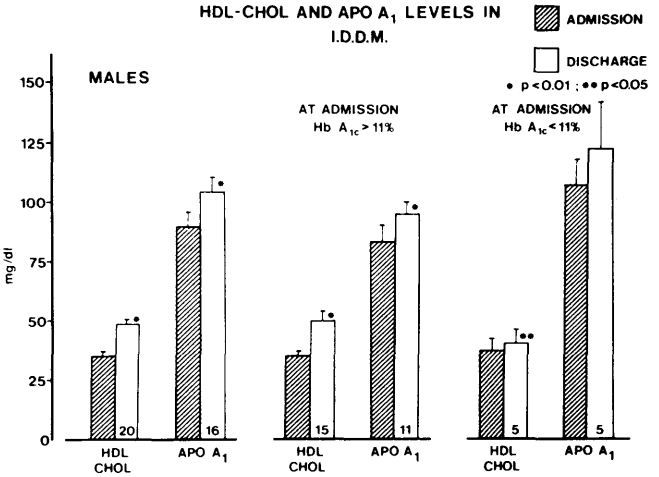


FIGURE 4. Levels of HDL cholesterol and apolipoprotein A_1 in male insulin-dependent diabetic patients. On the left the levels of the group as a whole are represented. In the middle and the right, respectively, the levels of the patients admitted in poor (HbA_{1c} > 11%) and in fair (HbA_{1c} < 11%) control are represented.

with improved glycemic control. Conversely no control-related changes were found in HDL cholesterol levels in a study performed by Sosenko and colleagues.[10] Nikkila et al.[12] reported high levels of HDL cholesterol in insulin-dependent diabetics, regardless of their degree of glycemic control, and Kennedy and associates[29] found normal HDL cholesterol levels in young insulin-dependent diabetics.

In recent studies comparing the effect of conventional and continuous subcutaneous insulin infusion therapy, Pietri et al.[30] found no change in the HDL cholesterol levels with improved glycemic control over a short-term therapy, but they reported a rise in HDL cholesterol levels when a longer course of therapy was used.[31]

Our present study shows that the magnitude of change in the lipid and lipoprotein levels observed in insulin-dependent diabetics during hospitalization is related to the degree of metabolic control of the patients on admission to the hospital. Only triglyceride and VLDL triglyceride levels changed significantly even with mild changes in the degree of metabolic control. In order to observe changes in total, LDL, VLDL, and HDL cholesterol levels a marked degree of improvement in control was necessary. These observations can help to explain some of the discrepancies found in the literature, since the degree of control of the patient upon entry into the study was usually not taken into consideration.

The effect of diet on the changes in lipid and lipoprotein levels observed during hospitalization cannot be excluded. We think, however, that this is an unlikely possibility since all patients were followed regularly by us in an outpatient diabetic clinic and they have been previously on a similar diet. Furthermore, with the same diet, no changes were ob-

FIGURE 3. Levels of total and VLDL triglycerides and VLDL cholesterol in insulin-dependent diabetic patients. The total group of patients was subdivided into two subgroups: 1—patients admitted in poor metabolic control (HbA_{1c} > 11%)—represented on the left, and 2—patients admitted in fair metabolic control (HbA_{1c} < 11%)—represented on the right.

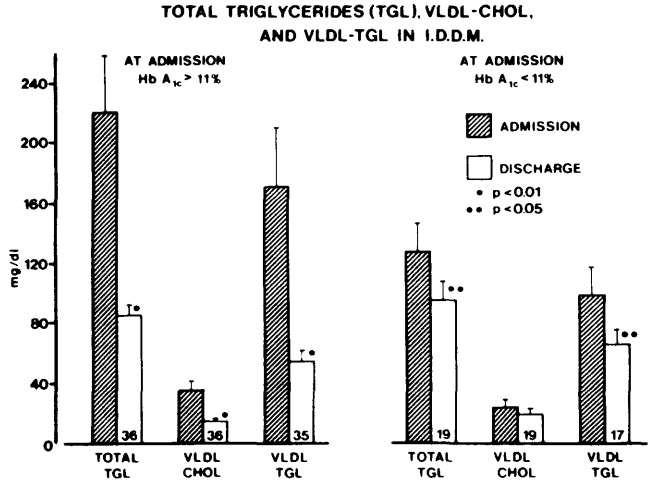
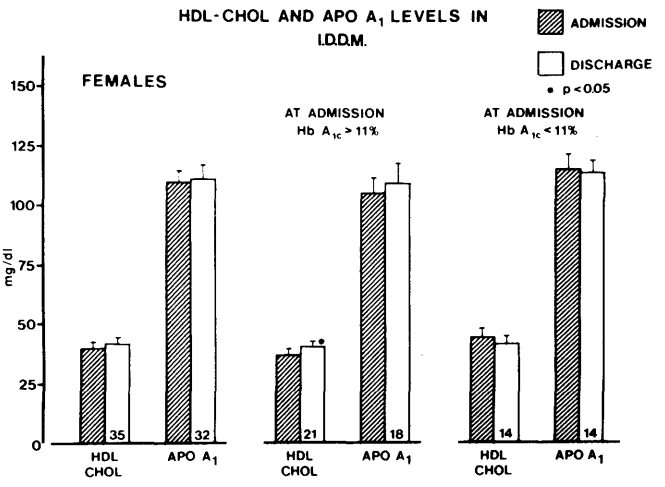


FIGURE 5. Levels of HDL cholesterol and apolipoprotein A_1 in female insulin-dependent diabetic patients. On the left the levels of the group as a whole are represented. In the middle and on the right, respectively, the levels of the patients admitted in poor (HbA_{1c} > 11%) and in fair control (HbA_{1c} < 11%) are represented.



served in the group of patients admitted in fair diabetic control.

Another interesting observation that resulted from our study is the fact that the changes in HDL cholesterol levels induced by improved control are more marked in males than in females. This may help to explain some of the controversy in the literature regarding the relationship between HDL cholesterol levels and diabetic control. In the studies that reported a lack of relationship between HDL cholesterol levels and glycemic control[10,30] the data were not analyzed taking into consideration the sex of the patients studied. In our study male diabetics showed a significant increase in HDL cholesterol levels even with a mild improvement in their glycemic control. Conversely, the levels of HDL cholesterol in female diabetics are less sensitive to changes in the control of the patient. It is possible that, in females, the changes in HDL cholesterol levels induced by improved control are slower than in males, and that would explain why in some studies changes were found only after long-term therapy.[31]

We can speculate that the difference in behavior of male and female diabetics regarding HDL cholesterol levels is probably due to hormonal factors. If that is the case, prepubertal male and female patients would behave similarly. Unfortunately the number of prepubertal female patients in our group was too small (six patients) to perform a meaningful statistical analysis taking into consideration their hormonal status.

We did not find any significant difference between multiple-dose insulin therapy (MID) and continuous subcutaneous insulin infusion therapy.

The mechanisms responsible for the alteration of plasma lipoprotein levels in insulin-dependent diabetes are not entirely clear. An attractive hypothesis that may explain the HDL response was postulated by Tall and Small.[32] They suggested that accelerated formation of HDL takes place when increased levels of VLDL and/or chylomicrons are associated with normal or enhanced lipoprotein lipase. In insulin-deficient diabetes lipoprotein lipase activity is decreased while VLDL is increased. With improved glycemic control there is an activation of lipoprotein lipase due to insulin administration, and this may account for the increase of HDL levels seen in diabetic patients after appropriate insulin therapy.

Very few studies have been performed concerning levels of apolipoproteins in diabetic patients,[13–16] and until now no longitudinal studies examining the effect of diabetic control on apolipoprotein levels had been undertaken. In most of the studies performed a divergence between HDL cholesterol and Apo A_1 seems to emerge. The decrease in HDL cholesterol is not always accompanied by a decrease in the Apo A_1 levels. We found similar results in this and previous studies.[14] Thus, only in the subgroups with more marked changes in HDL cholesterol a difference was observed in the Apo A_1 levels (see Figure 5).

As pointed out by Taylor et al.[13] this observation may reflect a difference in the HDL composition. It has been shown that HDL is constituted of 2 subfractions, HDL_2 and HDL_3.[33] HDL_2, considered as the subfraction that protects against the development of atherosclerosis,[34] has a higher ratio of cholesterol to Apo A_1 than HDL_3.[35] If the HDL_2 subfraction is the one more commonly reduced in poorly controlled diabetic patients, that could explain why HDL cholesterol is relatively lower than Apo A_1 in these patients.

Unlike Apo A_1 levels Apo B levels follow closely the levels of LDL cholesterol. Since Apo B and LDL cholesterol levels are so closely related, the determination of Apo B levels could provide a useful clinical tool to evaluate LDL.

The association in insulin-dependent diabetics in poor glycemic control of increased LDL cholesterol and decreased HDL cholesterol, both well-known risk factors of coronary heart disease, may be one of the contributing factors to the greater incidence of atherosclerosis in diabetes. Improvement of glycemic control seems to favorably alter these parameters.

We do not know, however, whether the changes observed in lipid and lipoprotein levels with improved glycemic control will actually be able to delay the atherosclerotic process in these patients. Long-term studies are needed in order to provide some insight as to the biologic significance of these findings.

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