Lipid and Apolipoprotein Compositions of Two Species of ApoA-I Containing Lipoproteins in Young Girls with Insulin-Dependent Diabetes Mellitus

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ABSTRACT. Two species of lipoproteins containing apoA-I (A-I_{Lp}), lipoprotein containing apoA-I and apoA-II (LpA-I/A-II), and lipoprotein containing apoA-I but no apoA-II were isolated from 12 girls with insulin-dependent diabetes mellitus (IDDM) and from 19 healthy controls using affinity chromatography. When characterizing the lipid and apolipoprotein compositions, we noted compositional changes. In A-I_{Lp}, the levels of lipid, except for triglyceride, and the level of apoC-III were significantly higher in IDDM. In LpA-I/A-II, the levels of lipids, except for triglyceride, the levels of apoC-III, and the ratio of apoA-I to apoA-II were significantly higher in IDDM. In lipoprotein containing apoA-I but no apoA-II, the levels of all lipids and apolipoproteins in IDDM were similar to those in the controls. The percent phospholipid in A-I_{Lp} and LpA-I/A-II was significantly higher in IDDM. All of these changes of A-I_{Lp} are similar to those associated with the reduced risk in the nondiabetic population. However, apolipoprotein changes of LpA-I/A-II may possibly be related to the accelerated atherosclerotic processes noted in patients with IDDM. (*Pediatr Res* 28: 42-45, 1990)

Abbreviations

A-I_{Lp}, lipoprotein containing apoA-I
LpA-I/A-II, lipoprotein containing apoA-I and apoA-II
LpA-I, lipoprotein containing apoA-I but no apoA-II
HDL-C, HDL-cholesterol
TC, total cholesterol
TG, triglyceride
PL, phospholipid
CE, cholesteryl ester
IDDM, insulin-dependent diabetes mellitus
NIDDM, noninsulin-dependent diabetes mellitus

such data related to IDDM. Differing from NIDDM with lower HDL-C levels, plasma HDL-C levels in IDDM are either normal or elevated (6, 7). These lines of evidence suggest that factors other than HDL are responsible for the accelerated atherosclerotic process seen in IDDM. Should compositional changes of HDL be present in IDDM, then HDL would lose physiologic functions such as reverse cholesterol transport. There are data on the HDL composition of IDDM (8-10); however, most workers use HDL isolated by ultracentrifugation, a method that inevitably leads to a significant loss of apolipoproteins and lipids (11). We attempted to isolate a more native HDL than that obtained by conventional ultracentrifugation.

We developed antiapoA-I and antiapoA-II immunoaffinity columns using a formylated derivative of cellulose gel (12-14) and we isolated and characterized two species of A-I_{Lp} from 12 girls with IDDM: LpA-I/A-II, and LpA-I. These lipoproteins are assumed to be the more native form of HDL than is the HDL isolated by ultracentrifugation (12-15). The results were compared with findings in age- and sex-matched controls.

MATERIALS AND METHODS

Subjects. The clinical data on the 12 Japanese girls with typical IDDM are summarized in Table 1. The ages ranged from 6-15 y. The diagnosis of IDDM was based on the criteria of National Diabetes Data Group (16). All had been diagnosed 0.5-5 y earlier and were clinically stable at the time of the study. Insulin (a combination of intermediate and rapid human insulin) was given s.c. twice a day. All patients were on adequate diets (caloric intake: 50% from carbohydrate, 30% from fat, 20% from protein). Nineteen nondiabetic healthy female subjects from the same part of Japan (Kumamoto-City) were the controls. They were volunteers from children of the staff at Kumamoto University. Age distribution in the control group was similar to that of the patients. Their lipid and apolipoprotein levels (Table 2) were all within mean $\pm$ SD of those of healthy female children at Kumamoto-City. None of the study subjects had renal failure or hepatic or thyroid abnormalities and no child was taking any drug (except for insulin in the IDDM subjects). Blood samples were obtained from all of these subjects after an overnight fast, then the insulin was injected and breakfast provided. Informed consent was obtained from each subject and from parents, as required. Venous blood (10 mL) was drawn into vacutainer tubes containing disodium EDTA (1.5 mg/mL). Upon separation of the plasma at 4°C, 1500 $\times$ g for 15 min, sodium azide and gentamicin were added as preservatives in final concentration of 2.0 μ g/mL and 5.0 μ g/mL, respectively.

Methods. Isolation of two species of A-I_{Lp} using immunosorbent column. Two species of A-I_{Lp} were isolated from plasma

The atherosclerotic process is accelerated in patients with IDDM, and atherosclerotic cardiovascular disease is one of the major causes of death in these patients (1, 2). Several research groups have shown that plasma levels of HDL inversely correlate with the incidence of atherosclerotic cardiovascular disease in cases of NIDDM, similar to findings in the nondiabetic populations (3-5). However, to the best of our knowledge, there are no

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Table 1. Clinical data on diabetic patients and controls*

	Age (y)	Sex	IBW (%)	Insulin dose (IU/kg)	Fasting glucose (mg/dL)†	HbA _{1c} (%)
Diabetics	12.3 ± 2.6	F	107.0 ± 5.5	1.0 ± 0.2	140 ± 32	8.2 ± 0.5
Controls	12.6 ± 3.0	F	109.3 ± 5.3	0	85 ± 9	4.7 ± 0.2

* Values are means ± SD. IBW, ideal body wt.

† To convert to mmol/L, multiply by 0.0556.

Table 2. Plasma lipid and apolipoprotein levels*

	Control	Diabetics
TC†‡	167.2 ± 3.8	187.5 ± 7.8
CE‡	119.4 ± 3.0	134.7 ± 5.9
TG§	77.7 ± 8.0	95.7 ± 11.2
PL	189.9 ± 3.1	212.9 ± 7.0
HDL-C†‡	57.7 ± 2.9	68.3 ± 2.4
ApoA-I	140.4 ± 3.9	145.8 ± 4.7
ApoA-II	32.6 ± 0.9	31.7 ± 1.9
ApoC-II	2.99 ± 0.29	3.62 ± 0.48
ApoC-III	7.66 ± 0.36	7.88 ± 0.58
ApoE	3.58 ± 0.17	3.96 ± 0.25

* Values are mean ± SEM (mg/dL).

† To convert to mmol/L, multiply by 0.0259.

‡ $p < 0.01$, significance between control and diabetics.

§ To convert to mmol/L, multiply by 0.0113.

|| $p < 0.005$, significance between control and diabetics.

by a combination of antiapoA-I and antiapoA-II immunosorbent columns, as described (12, 13). Briefly, fresh plasma (3 mL) was applied on an antiapoA-I immunosorbent column. After washing extensively with 0.01 M Tris, 0.5 M NaCl, 1 mM EDTA, pH 7.5 (buffer A), the column was eluted with 0.1 M acetic acid, 1 mM EDTA (pH 3.0). Each effluent was immediately adjusted to pH 7.4 with 1.0 M Tris solution and dialyzed against 0.15 M NaCl and 1 mM EDTA, pH 7.4 (buffer B). Finally, the sample was concentrated to 9 mL in an ultra-filtration cell (Amicon Corp., Danvers, ME) equipped with PM-10 membrane. A portion (3 mL) of this sample was used for analysis of apolipoproteins and lipids. The remaining portion (6 mL) was used to separate the A-IIp into LpA-I/A-II and LpA-I. The sample was applied on an antiapoA-II immunosorbent column. The column was washed with buffer A to obtain the unbound fraction (LpA-I). The bound fraction (LpA-I/A-II) was eluted with 0.1 M acetic acid, 1 mM EDTA, pH 3.0. Both the unbound and bound fractions were dialyzed and then concentrated with an ultrafiltration apparatus (Amicon) to 6 mL in buffer B, respectively.

Protein and lipid analysis. The apoA-I, apoA-II, apoC-II, apoC-III, and apoE concentrations of plasma, A-IIp, LpA-I/A-II, and LpA-I were measured by radial immunodiffusion assay, as described (17). Cholesterol and TG concentrations of these samples were analyzed on an ABA 100 Autoanalyzer (Abbott Laboratories, N. Chicago, IL), using enzymatic methods (18, 19). CE was measured by enzymatic methods and fluorometry (20). HDL-C was determined by the method of Lopes-Virella *et al.* (21) after precipitation with sodium phosphotungstate and magnesium chloride. Phospholipid was analyzed by the method of Bartlett (22). The protein content of each fraction from the immunosorbent columns was determined by the method of Lowry *et al.* (23).

Electrophoretic analysis. Agarose gel electrophoresis was performed using a Pol-E Film system for lipoprotein electrophoresis (Corning Medical, Medfield, MA) at pH 8.6, followed by staining with Fat Red 7B. Slab gel electrophoresis of lipoproteins isolated in 15% polyacrylamide gels containing 0.1% SDS was performed according to Weber and Osborn (24). The Stokes diameters of the particles isolated were estimated by gradient PAGE on precast polyacrylamide 4/30 gels (Pharmacia, Inc., Piscataway, NJ) according to the procedure provided by the manufacturer. Thyroglobulin, apoferritin, catalase, lactate dehydrogenase, and bovine

albumin (Pharmacia) were used as the calibrating proteins. The Stokes diameters of these calibrating proteins are: thyroglobulin, 17.0 nm; apoferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm; and bovine albumin, 7.5 nm.

Statistical Evaluation. The Wilcoxon signed rank test was used to evaluate the data.

RESULTS

In a previous report (14), we found no compositional differences in lipoproteins containing apoA-I between prepubertal and pubertal girls. Thus, we treated our subjects studied as one group.

Plasma lipid and apolipoprotein levels. Plasma lipid and apolipoprotein levels are summarized in Table 2. The levels of TC, CE, PL, and HDL-C in IDDM were significantly higher than those in control subjects ($p < 0.01$ or 0.005). The TG and all apolipoprotein levels were similar in IDDM and control subjects.

Lipid and apolipoprotein compositions of A-IIp. The lipid and apolipoprotein compositions of A-IIp are shown in Table 3. TC, CE, PL, and apoC-III levels in IDDM were significantly higher than those in control subjects ($p < 0.05$ or 0.01). Other components were similar in IDDM and control subjects. The molar ratio of apoA-I to apoA-II was slightly higher in IDDM, albeit with no statistical significance.

Lipid and apolipoprotein compositions of LpA-I/A-II. The lipid and apolipoprotein components of LpA-I/A-II are summarized in Table 4. The levels of TC, CE, PL, and apoC-III were significantly higher in IDDM than in control subjects ($p < 0.05$, 0.01, or 0.005). TG and other apolipoprotein levels were similar in IDDM and control subjects. The molar ratio of apoA-I to apoA-II was significantly higher in IDDM than in control subjects ($p < 0.05$).

Lipid and apolipoprotein compositions of LpA-I. The lipid and apolipoprotein compositions of LpA-I are summarized in Table 5. The levels of all lipids and apolipoproteins were similar in IDDM and control subjects.

Percent lipid compositions of A-IIp, LpA-I/A-II and LpA-I. As shown in Table 6, percentages of PL in A-IIp and LpA-I/A-II were significantly higher in IDDM than those in control

Table 3. Lipid and apolipoprotein concentrations of A-IIp*

	Control	Diabetics
TC†‡	71.3 ± 3.9	80.3 ± 3.6
CE‡	52.4 ± 2.2	59.8 ± 2.6
TG§	18.3 ± 1.6	17.3 ± 1.8
PL	115.0 ± 4.0	132.5 ± 4.7
ApoA-I	140.3 ± 3.8	145.2 ± 4.6
ApoA-II	32.7 ± 0.9	31.7 ± 1.8
ApoA-I/ApoA-II	2.66 ± 0.06	2.91 ± 0.16
ApoC-II	1.49 ± 0.14	1.73 ± 0.17
ApoC-III‡	4.28 ± 0.26	5.34 ± 0.44
ApoE	1.49 ± 0.15	1.54 ± 0.18

* Values are mean ± SEM (mg/dL), except for apoA-I/apoA-II, which is a molar ratio (molecular wt of apoA-I and apoA-II are 28 300 and 17 400, respectively).

† To convert to mmol/L, multiply by 0.0259.

‡ $p < 0.05$, significance between control and diabetics.

§ To convert to mmol/L, multiply by 0.0113.

|| $p < 0.01$, significance between control and diabetics.