Original Articles

Plasma Lipids and Microangiopathy in Insulin-dependent Diabetes Mellitus

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The relationship of plasma levels of high density lipoprotein (HDL) cholesterol, apolipoproteins A-I and A-II (the major apolipoproteins in HDL), low density lipoprotein (LDL) cholesterol, triglyceride, and glucose to microangiopathy was evaluated in 49 insulin-dependent diabetic subjects. Although the HDL cholesterol/LDL cholesterol ratio (a risk determinant for macroangiopathy) was lower in women with proteinuria, no other relationships between HDL cholesterol or the A apolipoproteins and renal microangiopathy were found. The only independent association between HDL and retinal microangiopathy was found in women, where an inverse correlation was found between the apo A-I/apo A-II ratio and the number of microaneurysms ($r_s = -0.561$, $P < 0.05$). Men showed strong relationships of glucose, triglyceride, cholesterol, and LDL cholesterol to renal microangiopathy whereas women, in general, had stronger correlations of these variables with retinal microangiopathy. Thus, several alterations in lipoprotein cholesterol distribution and HDL composition are associated with diabetic microangiopathy. In addition, differences between sexes suggest that previously undescribed hormonal factors may influence the severity of this process. DIABETES CARE 4: 447–453, JULY–AUGUST 1981.

Microvascular disease in the insulin-dependent diabetic person is a cause of substantial morbidity and mortality in a large percentage of patients.[1,2] Both the onset and severity of microangiopathy, however, are unpredictable and causative factors are unknown. An increased prevalence and progression of microvascular disease in patients with a longer duration of diabetes has been found by most investigators.[3–5] In studies of possible metabolic abnormalities that might be associated with the disorder, hypercholesterolemia[6–10] and hypertriglyceridemia[6,7,9] have been found in patients with more advanced microvascular disease.

Insulin-dependent diabetic patients also have a high incidence and accelerated development of atherosclerotic cardiovascular disease (ASCVD),[11,12] a disease associated not only with high levels of plasma cholesterol[13,14] and triglyceride,[15,16] but also low levels of high density lipoprotein (HDL) cholesterol.[17–20] Since plasma levels of HDL cholesterol have been reported to be low in non-insulin-dependent diabetes,[21–23] a population also prone to develop both atherosclerotic and microvascular disease, a contributory role of plasma HDL cholesterol, in addition to other lipids and glucose, to small vessel changes was tested in 49 insulin-dependent diabetic subjects. Because alterations in HDL composition have been recently found in insulin-dependent diabetes,[24] we also examined the relationship of plasma apolipoproteins (apo) A-I and A-II to the microvascular disease in this population. Although the ratio of HDL cholesterol/LDL cholesterol provides an index of cholesterol distribution in plasma, the apo A-I/apo A-II ratio is additionally helpful since it reflects the relative concentrations of the HDL subfractions, HDL_2 and HDL_3.[24–27] Available data strongly suggest that a higher apo A-I/apo A-II ratio indicates an increase in HDL_2, the subclass of HDL that is associated with a lower prevalence of ASCVD,[28,29] a relationship that could be extended to microvascular disease.

MATERIALS AND METHODS

Forty-nine patients (29 men, 20 women), age range 18–56 yr (mean 31 yr), with diabetes mellitus diagnosed before age 25 yr and present for greater than 10 yr, were recruited from the greater Seattle area by physician referral. All patients meeting the criteria for inclusion were accepted for study and informed consent obtained. Ketoacidosis had previously occurred in most, ketonuria in all. All patients were receiving insulin (mean dose: men 53 U/day, women 36 U/day). Patients were maintained on their usual diets. Alcohol intake

and exercise varied considerably between subjects. Patients with episodes of ketoacidosis within 3 mo of the study period were excluded. Also excluded were patients with recent changes in weight or insulin dose and patients with previously known renal disease or on drugs affecting lipid levels (e.g., oral contraceptives, clofibrate).

Plasma samples. Venous blood (15 ml) was obtained in Vacutainer tubes containing disodium EDTA, 1 mg/dl, three times over a 3-mo period, preferably 1 mo apart, after an overnight (12 h) fast. The closest interval between samplings was 10 days. The plasma was promptly separated by low-speed centrifugation and placed at 4°C. Aliquots were stored at −20°C for later measurements of glucose and the apolipoproteins.

Methods. Cholesterol and triglyceride concentrations were quantified by the Lipid Research Clinic continuous flow (AA-II) procedure.[30] Samples were mixed thoroughly before extraction into isopropyl alcohol in the presence of zeolite mixture. Cholesterol was measured by a Liebermann Burchard reagent method calibrated with a secondary serum standard to be equivalent to the reference method of Abell et al.[31] Triglycerides were determined by a fluorometric 24 pentanedione procedure calibrated with triolein.[32] All standard solutions and frozen serum quality control samples were provided by the Lipid Standardization Laboratory, Center for Disease Control, Atlanta, Georgia. The coefficient of variation of C.D.C. quality control pool for cholesterol was 2% and within 3 mg/dl of the target value. The coefficient of variation of triglyceride was 2% and within 3 mg/dl of the target value.

HDL cholesterol was determined by measurement of cholesterol in the supernatant after precipitation of very low density lipoproteins and low density lipoproteins in 2.0 ml of plasma with 0.15 ml of 1 M $MnCl_2$ and 0.12 ml of sodium heparin (35 mg/ml, Riker).[33] The total coefficient of variation of 5% represents both precipitation and cholesterol analytical variation for HDL determination. The LDL cholesterol was derived from the following equation:[34]

$$\text{LDL cholesterol} = \text{total cholesterol} - \left(\text{HDL cholesterol} + \frac{\text{triglyceride}}{5}\right)$$

Plasma apo A-I and A-II were determined by radial immunodiffusion assays as previously described.[28] Glucose was measured by the glucose-oxidase method using the Beckman Glucose Analyzer[35] (Beckman Instruments, Fullerton, California).

Assessment of microangiopathy. The extent of renal microvascular disease was assessed by measurement of serum creatinine in addition to creatinine clearance and the excretion of protein in a 24-h urine collection. Creatinine and protein were determined by standard autoanalyzer methods in the clinical laboratory. Retinal microangiopathy was determined in 47 patients with Kodachrome retinal photographs using a Zeiss fundus camera and by fluorescein angiography after the intravenous injection of 5.0 ml of 10% sodium fluorescein dye. All assessments of diabetic retinopathy were made by the ophthalmologist. The number of microaneurysms were quantified in field 2. This area, as defined by the Diabetic Retinopathy Study, is centered on the macula and included the optic disc and most of the superior and inferior temporal vascular arcades. Although photographs from both eyes were examined for microaneurysms, they were quantified only for the eye demonstrating more extensive retinopathy. Hard exudates were counted in field 2 of each eye and a number was assigned to the average of these two measurements. The exudative index was derived from an average of both eyes.

Microaneurysms were not enumerated in 13 patients (10 men, 3 women); 5 had received previous photocoagulation, and although some microaneurysms were present, it was felt that an accurate representation of microvascular disease activity was not offered by enumerating the lesions; 2 patients showed no discrete lesions but instead leaked fluorescein dye diffusely; 2 had extensive vitreous hemorrhages obscuring retinal visualization; and 4 had fluorescein angiograms that were inadequate for examination. Because of the invalidity of the measurement, the number of exudates were not quantified in 9 patients; 5 patients had previous photocoagulation (3 men, 2 women); 2 had vitreous hemorrhages (2 men); and 2 patients had photographs inadequate for interpretation.

Nonproliferative retinopathy was characterized by the presence of any or all of the following lesions: irregular caliber of retinal venules, microaneurysms, intraretinal punctate hemorrhages, hard exudates, or retinal edema. Proliferative retinopathy was considered present in those patients demonstrating proliferation of new vessels (neovascularization) at the vitreoretinal interface or elevated into the cortical vitreous. Fibrous proliferation was not present in any of the study patients.

Macroangiopathy, assessed by history, was present in only 3 patients (2, 25, and 27, Table 1) and was not further investigated as part of this study.

For all plasma samples, the mean of three measurements from plasma obtained over a 3-mo period was used for statistical comparisons. Statistical relationships were determined by nonparametric statistics (Spearman rank correlation coefficient). Multiple linear regression analysis was used to test the independence of these relationships. The nonpaired Student's *t* test was used for comparing metabolic parameters in patients with and without proliferative retinopathy or proteinuria.

RESULTS

The 29 diabetic men and 20 diabetic women were quite comparable for age and duration of disease (Table 1). Increasing plasma glucose was associated with increasing plasma cholesterol ($r_s = 0.513$, $P < 0.001$), plasma triglyceride ($r_s = 0.380$, $P < 0.01$), and LDL cholesterol ($r_s = 0.471$, $P < 0.001$) (Figure 1). Multiple linear regression confirmed the independence of these associations. Relationships between fasting plasma glucose and HDL cholesterol, apo A-I, apo A-

TABLE 1
Plasma glucose lipid* and A apolipoprotein concentrations in insulin-dependent diabetic subjects

	Patients	Age (yr)	Duration (yr)	Wt. (kg)	Ins. dose (U/day)	Glu. (mg/dl)	TG (mg/dl)	Chol. (mg/dl)	LDL chol. (mg/dl)	HDL chol. (mg/dl)	HDL-C/LDL-C	A-I (mg/dl)	A-II (mg/dl)	A-I/A-II
M	1	24	19	78.2	80	126	62	203	144	46	0.32	119	34.0	3.51
	2	45	20	84.1	50	141	89	171	103	50	0.49	142	32.1	4.43
	3	33	25	77.3	45	227	89	196	131	48	0.36	138	32.6	4.24
	4	47	22	68.6	25	279	139	222	134	60	0.45	164	33.5	4.89
	5	35	23	90.0	60	220	116	205	136	45	0.33	129	31.4	4.12
	6	24	11	90.9	70	155	69	171	116	41	0.36	114	28.8	3.96
	7	28	22	67.3	48	245	86	160	80	63	0.79	142	39.8	3.57
	8	29	24	83.6	62	145	64	165	110	44	0.40	122	34.1	3.57
	9	56	32	86.4	45	185	151	193	107	56	0.52	153	44.7	3.42
	10	27	12	72.7	48	154	118	213	152	37	0.24	116	30.6	3.80
	11	35	26	86.4	55	176	47	157	83	64	0.77	147	34.6	4.25
	12	18	10	75.9	55	129	57	168	98	59	0.61	129	29.9	4.31
	13	38	18	68.1	40	189	82	159	93	50	0.53	128	31.5	4.06
	14	17	12	70.0	60	257	82	147	90	41	0.45	124	26.9	4.61
	15	36	17	70.5	35	144	74	190	109	66	0.61	149	29.4	5.06
	16	23	18	52.2	50	196	94	136	84	33	0.39	100	28.8	3.48
	17	39	22	79.5	50	93	83	171	91	63	0.69	141	36.8	3.83
	18	29	12	77.7	74	275	128	240	170	44	0.26	123	30.1	4.08
	19	28	13	75.0	50	177	99	133	76	36	0.48	122	29.4	4.15
	20	30	25	72.7	76	202	103	183	118	44	0.38	137	36.0	3.81
	21	22	12	68.2	72	127	157	177	107	39	0.36	120	30.0	4.00
	22	33	17	85.5	62	216	89	163	89	55	0.62	146	35.6	4.11
	23	20	12	81.8	54	104	75	153	94	43	0.46	134	28.2	4.73
	24	33	14	62.7	40	237	106	199	125	53	0.43	120	26.2	4.59
	25	43	35	68.2	20	322	185	231	143	51	0.35	129	33.1	3.88
	26	25	18	83.2	82	167	62	148	93	43	0.47	128	26.3	4.85
	27	44	29	80.9	40	172	116	201	131	47	0.36	123	34.4	3.57
	28	34	19	82.7	56	277	251	271	180	41	0.23	113	31.6	3.58
	29	29	14	74.5	42	212	147	190	112	49	0.44	156	34.0	4.58
	$\bar{x}$ ± SD	32 ± 9	19.1± 6.6	76.4± 8.8	53± 15	229± 57	104± 44	183± 32	114± 27	48.8± 9.1	0.45± 0.14	131± 15	32.2± 4.0	4.10± 0.47
F	30	33	18	65.0	40	236	60	193	119	63	0.53	151	35.3	4.28
	31	30	26	60.0	22	175	72	209	136	59	0.43	139	33.4	4.17
	32	35	11	70.5	46	146	109	171	80	70	0.88	164	34.1	4.81
	33	30	20	69.5	35	248	100	241	142	79	0.56	152	34.1	4.46
	34	27	15	50.9	28	280	59	174	100	62	0.62	147	33.5	4.39
	35	37	14	55.5	38	140	60	152	93	47	0.50	132	24.6	5.38
	36	36	25	65.9	40	210	89	170	94	58	0.62	153	33.0	4.63
	37	42	17	50.0	42	193	66	195	110	72	0.65	160	32.3	4.95
	38	43	26	58.2	30	113	64	177	101	63	0.62	175	30.9	5.66
	39	28	17	59.1	25	176	84	171	103	50	0.49	106	25.9	4.08
	40	31	21	59.1	28	366	86	191	114	60	0.53	161	28.1	5.72
	41	18	12	56.8	30	297	105	215	141	49	0.35	143	28.1	5.10
	42	19	14	47.7	22	193	60	163	99	46	0.51	137	31.3	4.38
	43	42	21	63.2	42	194	50	146	74	62	0.84	120	28.1	4.26
	44	29	25	66.8	50	130	56	146	98	38	0.39	110	25.5	4.33
	45	30	20	76.4	45	156	48	154	89	55	0.62	149	32.8	4.53
	46	20	11	61.4	70	239	101	173	99	54	0.54	146	35.3	4.14
	47	21	13	50.9	34	354	199	272	164	68	0.42	98	32.2	3.03
	48	34	24	60.9	33	223	190	211	127	46	0.37	132	29.1	4.52
	49	27	15	56.8	30	272	621	388	220	45	0.20	131	30.9	4.24
	x ± SD	31 ± 7	18.3± 5.1	60.2± 7.4	37± 11	253± 71	114± 126	196± 55	115± 34	57.5± 10.3	0.53± 0.16	140± 20	30.9± 3.3	4.55± 0.61

* Mean of three measurements.

II, or either the HDL cholesterol/LDL cholesterol or apo A-I/apo A-II ratios were not found.

As expected, plasma levels of HDL cholesterol, apo A-I/apo A-II, were higher in the diabetic women than men. Plasma glucose, cholesterol, LDL cholesterol, triglyceride, and apo A-II concentrations were similar between sexes. No relationship between alcohol consumption and plasma lipids was found. The difference in insulin dose between men and women was chiefly weight related since insulin dose/kg, 0.70–0.61, respectively, were not significantly different.

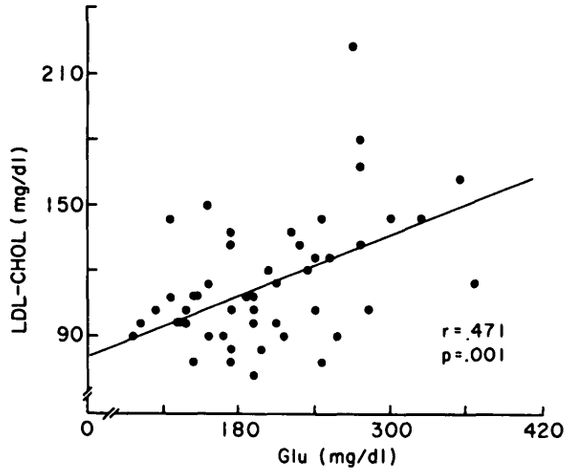

FIG. 1. The relationship of LDL cholesterol to glucose in 49 insulin-dependent diabetic subjects; each point represents the mean of three plasma samplings. r_s = Spearman rank correlation coefficient.

The distribution of renal and retinal microangiopathy is summarized in Table 2. Although a higher percentage of men had more than 1000 mg of urinary protein (25%; women 10%), no other obvious differences between men and women were seen. All but two insulin-dependent diabetic subjects had microaneurysms, while a minority had exudates (13/40).

Strong direct relationships were found between the amount of proteinuria in a 24-h urine collection and plasma glucose ($r_s = 0.463$, $P < 0.01$), cholesterol ($r_s = 0.463$, $P < 0.001$), and LDL cholesterol ($r_s = 0.506$, $P < 0.001$). Patients with proteinuria (N = 19) also had higher plasma glucose ($P < 0.01$), triglyceride ($P < 0.01$), cholesterol ($P < 0.01$), and LDL cholesterol ($P < 0.01$) concentrations than those without urinary protein (N = 29). Creatinine clearance in 48 subjects was inversely correlated with plasma glucose ($r_s = -0.334$, $P < 0.05$) and cholesterol ($r_s = -0.332$, $P < 0.05$), but not with LDL cholesterol or triglyceride. Multiple linear regression analysis showed that the correlation with glucose was independent, but the correlation with cholesterol was somewhat weakened when the interaction with triglyceride was considered ($P < 0.1$).

Since HDL cholesterol levels are different in men and women with diabetes as well as in those without it, correlations were calculated separately for each sex. No relationships between urinary protein excretion of creatinine clearance and HDL cholesterol were found. Although women with proteinuria had a significantly lower ratio of HDL cholesterol/LDL cholesterol than those without proteinuria ($P < 0.01$), multiple linear regression analysis failed to demonstrate an independent correlation between the HDL cholesterol/LDL cholesterol ratio and the amount of proteinuria in both men and women. Independent relationships between apolipoproteins A-I, A-II or the apo A-I/apo A-II ratio and renal microangiopathy were not found.

Correlations between the manifestations of retinal microangiopathy and glucose or any of the plasma lipids were statistically significant only in the women. Here the relationship between the number of microaneurysms and glucose was especially impressive ($r_s = 0.698$, $P < 0.001$). Correlations with LDL cholesterol ($r_s = 0.514$, $P < 0.05$) and triglyceride ($r_s = 0.530$, $P < 0.05$) were weaker. Multiple linear regression confirmed the independence of these relationships. In addition, HDL cholesterol/LDL cholesterol ($r_s = -0.561$, $P < 0.01$) and apo A-I/apo A-II ($r_s = -0.561$, $P < 0.05$) ratios were lower in women with more microaneurysms. Although the relationship between the apo A-I/apo A-II ratio and microaneurysms was unaffected by other variables (e.g., glucose, triglyceride, LDL cholesterol), the HDL cholesterol/LDL cholesterol relationship was weakened when the effect of triglyceride was considered ($P < 0.1$).

DISCUSSION

Very little previous information on the relationship of HDL or LDL cholesterol to microangiopathy in insulin-dependent diabetes is available. Although Bhan et al. found increased beta/alpha lipoprotein levels in diabetic patients (some not on insulin) with vascular disease (retinopathy, nephropathy, or cardiovascular disease), only the beta/alpha lipoprotein ratios were reported, and it is impossible to distinguish increases in total or LDL cholesterol from decreases in HDL cholesterol.[9] In addition, the diabetic population contained a combination of insulin-dependent patients whose HDL cholesterol levels[21–23] are likely to be different from the non-insulin-dependent group.[24,36] Thus, the present study represents the first attempt to relate levels of HDL cholesterol, the HDL cholesterol/LDL cholesterol ratio, or levels of apo A-I, A-II or the apo A-I/apo A-II ratio to forms of microangiopathy in any defined diabetic population. Although several of the diabetic subjects in this study population had hyperlipidemia, the mean ± SD for triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol were no different from previously age- and sex-matched controls.[24] The HDL cholesterol/LDL cholesterol and apo A-I/apo A-II ratios were actually higher in diabetic men and diabetic subjects of both sexes, respectively, than the controls.[24]

The association of hyperlipidemia with proteinuria is well known;[37,38] however, despite the absence of proteinuria in 29 patients, a correlation is seen for the entire population. This could suggest that either the defect in lipoprotein metabolism that accompanies nephrotic syndrome occurs early in the process or that gradual increases in plasma lipids contribute to diabetic nephropathy. Since elevations in plasma glucose were independently related not only to proteinuria but to plasma triglyceride, cholesterol, and LDL cholesterol, it is impossible to conclude whether or not poor control leads to nephrosis and secondary defects in lipoprotein metabolism or that diabetic patients with poorer control have hyperlipidemia, which results in a higher prevalence of renal microangiopathy. A similar comment could be made for the in-

TABLE 2
Assessment of microangiopathy

	Renal			Retinal		
	Serum creatinine (mg/dl)	24-h creatinine clearance (ml/min)	24-h urine protein (mg)	Number of microaneurysms	Exudative index	Proliferative retinopathy
M 1	1.1	143	0	2	0	
2	0.9	157	0	24	1	‖
3	1.4	89	1850	54	0.5	
4	1.8	60	1240	*	1.5	
5	0.9	126	0	†	2	
6	0.9	151	0	61	0	
7	1.2	71	840	‡	‡	‡
8	1.3	122	0	40	0	
9	0.9	67	0	†	†	
10	0.9	135	1050	20	0	
11	1.2	140	0	14	0.5	
12	0.8	178	360	3	0	
13	1.0	129	0	12	0.5	
14	0.8	151	0	27	0.5	
15	1.0	113	0	2	0	
16	1.1	136	0	1	0	
17	1.0	113	0	50	0.5	
18	—	—	—	1	0	
19	1.0	143	0	†	0	
20	0.8	132	140	14	0	
21	1.0	104	0	3	0	
22	0.9	151	0	1	0	
23	1.0	105	0	†	†	
24	0.8	136	0	9	0	
25	0.9	96	2100	§	§	§
26	1.2	87	0	11	0	
27	1.4	95	3720	‡	‡	‡
28	1.7	50	3020	§	§	§
29	2.9	30	8210	§	§	§
x ± SD	1.1±	115±	805±	18±	0.3±	
	0.4	36	1759	19	0.5	
F 30	0.6	178	0	31	0	
31	0.8	113	0	16	0	
32	0.8	102	0	2	0	
33	1.0	121	0	8	0.5	
34	0.8	74	0	4	0	
35	0.9	92	320	0	0	
36	0.6	147	360	22	2	‖, ¶
37	0.7	86	0	0	0	
38	1.6	39	0	2	0	
39	0.8	142	0	6	0.5	
40	1.2	72	610	§	§	§
41	0.7	84	940	23	0	
42	0.8	81	0	26	0	
43	1.0	74	0	2	0	
44	0.8	123	110	§	§	§
45	0.7	124	0	2	0	
46	0.8	97	270	42	0	
47	1.0	50	3120	65	1	
48	0.9	85	110	31	0	
49	0.7	87	2100	*	1	
x ± SD	0.9±	99±	397±	17±	0.3±	
	0.3	34	814	18	0.5	

* Diffuse leakage on fluorescein angiography; †inadequate for examination; ‡vitreous hemorrhages; §previous photocoagulation; ‖NVD < 10A—new vessels on or within 1 disc diameter of the optic disc less than those in standard photo 10A; ¶NVE—new vessels elsewhere, i.e., more than 1 disc diameter from the optic disc, equal to or greater than ½ disc area in extent in one or more of the standard photographic fields.

verse relationship between plasma cholesterol and creatinine clearance.

In this insulin-dependent diabetic population, the lowered HDL cholesterol/LDL cholesterol ratio in women with proteinuria demonstrates that an alteration in lipoprotein cholesterol distribution is associated with the proteinuric state, at least in women. This lowered HDL cholesterol/LDL cholesterol ratio likely reflects both decreases in plasma HDL cholesterol and increases in LDL cholesterol. Altered glomerular pore size or charge, both of which have been shown to contribute to proteinuria,[39] might contribute to the decrease in HDL. There is evidence in rats that HDL cholesterol is lost in the urine in nephrotic syndrome and these particles are relatively deficient in cholesterol.[40]

Relationships between retinal microangiopathy and hyperlipidemia have been reported, but other than studies by Keiding et al.[6] and Bhan et al.,[9] the populations studied have either been non-insulin-dependent diabetic patients or a mixture of insulin-dependent and non-insulin-dependent subjects.[7,8,10] The much stronger correlations between retinal microangiopathy and lipid levels in women than in men has been shown previously for retinopathy and cholesterol by Lowy et al.[7] There is no evidence that the women in this study had more severe diabetes than the men. Although a possible role for lipid thrombi in the pathogenesis of retinal microaneurysm formation has been suggested,[41] in general, hyperlipidemia has not been considered to play a very important role in the pathogenesis of diabetic retinopathy.[42] Alterations in HDL cholesterol concentrations, however, have not previously been studied.

The inverse relationship between the HDL cholesterol/LDL cholesterol ratio and the number of microaneurysms in women may be partially explained by the correlation of triglyceride and microaneurysms. However, a role of HDL is not excluded, because an independent inverse correlation was also seen between the apo A-I/apo A-II ratio and the number of microaneurysms. This relationship might be important since higher apo A-I/apo A-II ratios reflect alterations in HDL composition, which are associated with a lower risk of atherosclerotic cardiovascular disease.[27,28] Other than this association, no other relationships between apo A-I or A-II and microangiopathy were found.

Relationships between HDL cholesterol and the extent of microangiopathy were not found. This lack of association does not agree with studies of macrovascular disease where a higher prevalence of atherosclerosis in populations with lower plasma levels of HDL cholesterol has been found.[17–20] The lower ratios of HDL cholesterol/LDL cholesterol in women with proteinuria could be secondary to rising levels of LDL cholesterol or a urinary loss of HDL. Since women with lower ratios also had more microaneurysms, a role for HDL in influencing the severity of microangiopathy cannot be excluded. An unexpected finding was the difference and strength of relationships of glucose and plasma lipids to microangiopathy among men and women. This would suggest that hormonal factors might also influence the severity of diabetic microvascular disease.

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