Association of Lipoprotein(a) With Puberty in IDDM

JENNIFER J. COUPER, MD
DAVID J. BATES, PHD
ROBERT COCCIOLONE, BAPPSC
ANTHEA M. MAGAREY, BSC (HONS)
T. JOHN C. BOULTON, MD
JAMES L. PENFOLD, MBBS
RICHARD G. RYALL, PHD

OBJECTIVE — To determine serum lipoprotein(a) in a large sample of IDDM and control children and to examine a possible association with puberty.

RESEARCH DESIGN AND METHODS — Serum lipoprotein(a), apoB-100, and apoA-I were measured under identical conditions in 170 Caucasian children with IDDM aged 12.3 ± 3.59 yr and 233 Caucasian control children aged 13.6 ± 1.12 yr. Patients with persistent microalbuminuria were excluded. Lipoprotein(a), apoB-100, and apoA-I were measured by nephelometry using a specific monoclonal antibody. Pubertal assessment was performed using Tanner staging and testicular volume measurement.

RESULTS — Lipoprotein(a) was higher in the IDDM than control group (geometric mean 237 mg/L, 25–75th percentile 134–465 vs. 172 [99–316] mg/L, $P = 0.0008$). When analyzed according to pubertal stage, only pubertal and postpubertal patients had higher levels than control subjects (265 [148–560] vs. 174 [101–320] mg/L, $P = 0.0001$), with prepubertal patients showing no difference. Pubertal and postpubertal patients showed both higher lipoprotein(a) ($P = 0.01$) levels and higher albumin excretion rates ($P = 0.02$) than prepubertal patients, correcting for the other variable. Lipoprotein(a) was not related to HbA_{1c}, albumin excretion rate, duration, age, sex, mean arterial pressure, or a family history of premature coronary artery disease in the IDDM group. Lipoprotein(a) was not higher in patients with overnight albumin excretion rate above the 95th percentile but below the microalbuminuric range. ApoB-100 did not differ between IDDM and control children. ApoA-I was significantly lower in the IDDM group (1.04 [0.94–1.17] vs. 1.21 [1.10–1.31] g/L; $P < 0.0001$).

CONCLUSIONS — Pubertal and postpubertal IDDM patients have higher serum lipoprotein(a) than Caucasian control subjects. Our findings suggest a rise in lipoprotein(a) may occur during puberty in IDDM. Longitudinal studies are required to clarify the relationship between lipoprotein(a), albumin excretion rate, and puberty.

FROM THE DEPARTMENT OF ENDOCRINOLOGY AND DIABETES, THE UNIVERSITY OF ADELAIDE DEPARTMENT OF PEDIATRICS, THE DEPARTMENT OF CHEMICAL PATHOLOGY, AND THE DEPARTMENT OF NUTRITION AND DIETETICS, ADELAIDE CHILDREN'S HOSPITAL, NORTH ADELAIDE, SOUTH AUSTRALIA; AND THE DEPARTMENT OF PEDIATRICS, UNIVERSITY OF NEWCASTLE, NEWCASTLE, AUSTRALIA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO JENNIFER J. COUPER, MD, UNIVERSITY DEPARTMENT OF PEDIATRICS, ADELAIDE CHILDREN'S HOSPITAL, NORTH ADELAIDE, SOUTH AUSTRALIA 5006.

RECEIVED FOR PUBLICATION 28 SEPTEMBER 1992 AND ACCEPTED IN REVISED FORM 4 MARCH 1993.

IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; Lp(a), LIPOPROTEIN(A); APO, APOLIPOPROTEIN; CAD, CORONARY ARTERY DISEASE; MA, MICROALBUMINURIA; AER, ALBUMIN EXCRETION RATE; CVD, CEREBROVASCULAR DISEASE; RIA, RADIOIMMUNOASSAY; ELISA, ENZYME-LINKED IMMUNOSORBENT ASSAY; CV, COEFFICIENT OF VARIATION; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; CI, CONFIDENCE INTERVAL; MAP, MEAN ARTERIAL PRESSURE; HDL, HIGH-DENSITY LIPOPROTEIN; DCCT, DIABETES CONTROL AND COMPLICATIONS TRIAL; IGF-I, INSULINLIKE GROWTH FACTOR I; NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; BMI, BODY MASS INDEX.

Lp(a) was first described in 1963 (1). Since then, epidemiological studies have shown Lp(a) to be an independent risk factor for premature CAD (2,3) and stroke (4) in the normal population.

Lp(a) contains apo(a) linked to apoB-100 by a disulfide bond. The sequence of apo(a) shows close homology to plasminogen (5), and the apo(a) gene accounts for considerable intersubject variability in Lp(a) concentrations (6). The frequency distribution of Lp(a) is highly skewed and differs among Caucasian, Asian, and African black populations (7). Lp(a) concentrations increase in the first 6 mo of life but do not vary with age or sex thereafter (8).

Patients with IDDM are at increased risk of CAD, females are not protected, and the greatest risk is seen in those with diabetic nephropathy (9). Lp(a) levels are raised in adults with diabetes and MA, but not in those without MA (10,11). The timing of this rise is not clear.

A previous study in children and adolescents with IDDM showed a weak association between Lp(a) and HbA_{1c} in a small group of white but not black children (12). We aimed to determine Lp(a) and investigate a possible association with puberty within a large group of Caucasian children with IDDM and schoolchildren controls.

RESEARCH DESIGN AND METHODS

— Patients attending Adelaide Children's Hospital Diabetes Clinic who were Caucasian and aged 3–18 yr entered the study over a 3-mo period. Patients were excluded if they had persistent MA, defined as AER >20 μg/min on at least 66% of overnight urine collections ($n = 5$) or familial hypercholesterolemia ($n = 2$), factors known to be associated with higher Lp(a). The study group represented 74% of the clinic's population. The control group included normal Caucasian Adelaide schoolchildren; 196 were participants in a longitudinal study of nutrition and growth, and

Table 1—*Clinical characteristics of study subjects*

	IDDM PATIENTS	CONTROL SUBJECTS
n	170	233
AGE (YR)	12.3 ± 3.69	13.6 ± 1.12
SEX (M/F)	84/86	119/114
DURATION OF DIABETES (YR)	4.0 ± 3.20	
HbA_{1c} (%)	9.0 ± 2.64	
AER (μG/MIN)	3.5 (2.0–7.1)	
BMI (KG/M^2)	20.5 ± 3.82	
MAP (MMHG)	88.7 ± 12.06	
INSULIN (U/KG)	1.01 ± 0.40	
FAMILY HISTORY		
PREMATURE CAD	42/166	69/233
PREMATURE CVD	12/166	
HYPERTENSION	28/166	

37 had had a blood investigation with a normal result at Adelaide Children's Hospital. The study was approved by the Adelaide Children's Hospital Ethics Committee.

Capillary blood was collected while the nonfasted subject was sitting for measurement of Lp(a), apoA-I, apoB-100, and HbA_{1c}. Blood pressure was measured sitting at rest after 9 min by using an automated monitor (Dinamap, Critikon, Tampa, FL). Pubertal assessment using Tanner staging and testicular volume assessment was performed by one of three pediatric endocrinologists. Girls were assessed according to breast Tanner stage; boys according to Tanner stage and testicular volume of >4 ml for stage 2 and >15 ml for stage 5. Family history was taken for first-degree relatives and grandparents for premature CAD (angina requiring treatment or myocardial infarction before 60 yr), hypertension (requiring treatment before 60 yr), and premature CVD (presenting before 60 yr). AER was measured on timed overnight urine collections. Patients collected 2.3 ± 0.2 timed collections during the study period.

Apo and Lp(a) were measured on serum samples stored at 2–8 °C using end point nephelometry (Hyland laser nephelometer PDQ) and addition of specific monoclonal antibodies. Commercially available antisera, calibrators, controls, and dilutents were used (INCSTAR, Stillwater, OK). Antibodies to Lp(a) from this source recognize 11 polymorphic forms of Lp(a), which comprise 66 described phenotypes (13), and do not cross-react with plasminogen to concentrations of 2.3 g/L and apoB to concentrations of 4.36 g/L (14). Immunoturbidimetric procedures using these antibodies closely correlate with RIA and ELISA methods (14). Sensitivity to turbidity of the sample, which arises when triglyceride concentrations exceed 2.26 mM (15), was overcome by centrifuging serum samples at 20,000 *g* for 30 min and diluting before analysis (16). Intraassay CV was 4% at 216 mg/L and 4% at 530 mg/L; interassay CV was 7.9 and 6.5%, respectively.

Albumin was measured using nephelometry and addition of a specific monoclonal antibody (INCSTAR). Intraassay CV was 4.1% at 8 mg/L and 4.9% at 45 mg/L; interassay CV was 13 and 7.7%, respectively. Normal range for different age-groups (5–18 yr) for AER on overnight urine collections was obtained from 690 Adelaide schoolchildren. The 95th percentile was 7.2 μg/min. Borderline elevation of AER was defined as a mean overnight AER in the range of 7.2–20 μg/min.

HbA_{1c} was measured by HPLC with a normal range of 4–6% (17).

Statistical analysis

Statistical analysis was performed using Origin and CSS-Statistica data analysis programs. Variables with a skewed distribution, Lp(a), apoA-I, and apoB-100, were normalized by logarithmic conversion. Lp(a), apoA-I, and apoB100 are shown as geometric means and 75% CIs. Other variables are shown as percentage means ± SD. Comparisons between groups and relationships between variables were made with two-tailed Student's *t* test, χ^2 test, or multiple regression analysis.

RESULTS — Patient characteristics are shown in Table 1. Serum Lp(a) was higher in the IDDM group (n = 170) than in the Caucasian control group (n = 233) (geometric mean 237 mg/L, 25–75th percentile 134–465 vs. 172 [99–316] mg/L, respectively, P = 0.0008). Lp(a) distribution in both groups showed a positive skew (Fig. 1).

When Lp(a) was analyzed according to pubertal stage, prepubertal IDDM patients (Tanner 1) did not show different levels from prepubertal control subjects, but pubertal and postpubertal (Tanner 2–5) patients showed significantly higher levels than matched control subjects (265 [148–560] vs. 174 [101–320] mg/L, P = 0.0001) (Fig. 2). Lp(a) for prepubertal, pubertal, and postpubertal subjects in both groups is shown in Table 2. Within the IDDM group we noted a significant rise from prepubertal to postpubertal Lp(a) levels, controlling for AER (P = 0.01) (Fig. 3). No change according to pubertal status was seen within the control group. Pubertal status was not obtained in 4 IDDM patients and 5 control subjects.

Lp(a) was not related to AER. A total of 23 patients with borderline elevation of overnight AER on a mean of three collections (11.6 [8.5–14.6] μg/

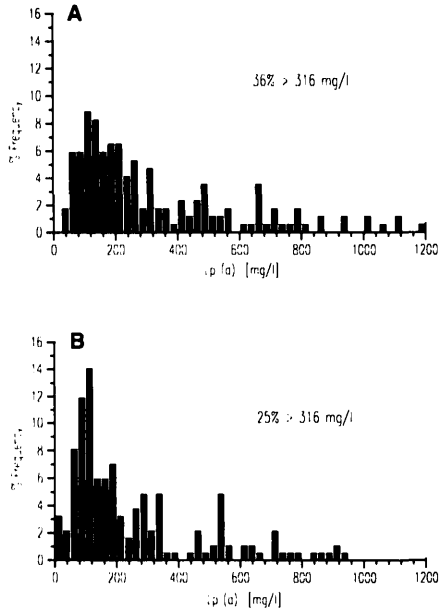

Figure 1—*Frequency distribution of Lp(a) in IDDM patients (A; n = 170) and control subjects (B; n = 233).*

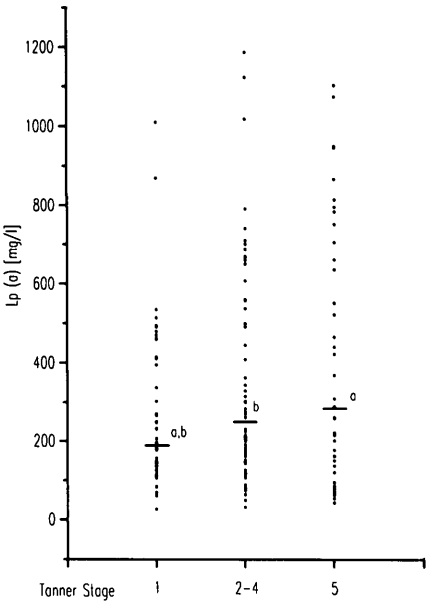

Figure 3—*Serum Lp(a) in IDDM patients according to pubertal stage. (———), geometric means; (a), P = 0.01; (b), P = 0.05.*

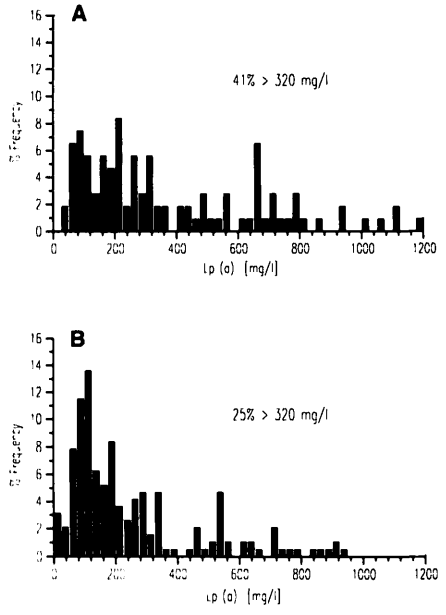

Figure 2—*Frequency distribution of Lp(a) in pubertal and postpubertal IDDM patients (A; n = 107) and pubertal and post pubertal control subjects (B; n = 191).*

min) did not show higher Lp(a) than the group with normal AER (n = 107). No patient had persistent MA. A significant relationship was found between AER and Tanner stage in the IDDM group when controlling for Lp(a) (r = 0.50, P = 0.007). Tanner 2–5 patients had higher AER than Tanner 1 patients, controlling for Lp(a) (4.7 [2.6–8.3] vs. 2.1 [1.3–3.3] μg/min, P = 0.02). Ten patients with background retinopathy on fundoscopy did not have higher Lp(a).

Lp(a) was not related to HbA_{1c} (r = 0.11), duration, age, sex, MAP, insulin dose, or a family history of premature CAD, premature CVD, or hypertension in the IDDM group. Lp(a) did not differ between patients with HbA_{1c} >8% and those with HbA_{1c} ≤8%, nor was there a significant difference in HbA_{1c} between patients with Tanner 1 and Tanner 2–5 pubertal development (8.8 ± 2.04 vs. 9.1 ± 2.79%). Lp(a) was not related to age, sex, or a family history of premature CAD in the control group.

ApoB-100 did not differ between IDDM and control groups (0.53 [0.45–0.61] vs. 0.53 [0.46–0.62] g/L). It did not alter with age, sex, or pubertal stage in either group. A weak association was found between apoB-100 and HbA_{1c} (r = 0.27, P = 0.0004).

ApoA-I was significantly lower in the IDDM group compared with the control group (1.04 [0.94–1.17] vs. 1.21 [1.10–1.31] g/L, P < 0.0001). No association was found with age, sex, pubertal stage, or HbA_{1c}.

CONCLUSIONS— We have shown higher serum Lp(a), specifically apo(a), in pubertal and postpubertal patients with IDDM. Prepubertal patients were not different from control subjects. Our diabetes population did not include those known to have higher levels of Lp(a), i.e., patients with persistent MA (10,11) or familial hypercholesterolemia (14). The association with puberty was confined to the IDDM group and was seen despite there being no association between Lp(a) and AER, HbA_{1c}, or age.

We had anticipated that the higher Lp(a) in pubertal and postpubertal patients would be explained by small

Table 2—*Serum Lp(a) according to pubertal stage*

	IDDM patients	Control subjects	P
n	59	37	0.63
Tanner 1 (mg/L)	193 (125–303)	178 (88–443)	
n	64	142	0.002
Tanner 2–4 (mg/L)	252 (145–495)	170 (101–294)	
n	43	49	0.03
Tanner 5 (mg/L)	286 (150–660)	181 (95–389)	

increases in AER during this time. However, the association remained when correcting for AER. Given the intraindividual variation in AER and the significance of Lp(a) phenotype, it is likely that longitudinal study will be required to clarify the contribution of AER to changes in Lp(a) during puberty.

Clear cross-sectional evidence now indicates that adults with early diabetic nephropathy (persistent MA) have higher Lp(a) levels, whereas those without MA have normal levels (10,11). It could be speculated that this rise occurs at the same time as the development of MA, because patients with renal disease show alterations of Lp(a) with fluctuations in proteinuria (17). However, a rise in Lp(a) may precede the development of MA. Longitudinal studies are required to determine this and its relationship with the rise in AER that precedes MA (20).

ApoB-100 did not differ between IDDM patients and control subjects. ApoA-I, the apo of HDL cholesterol, was significantly lower in the IDDM patients. This last finding is consistent with recent findings in children with IDDM (21), and the DCCT Research Group has reported lower HDL cholesterol in adolescent girls and young adolescent boys (22).

Puberty is recognized as an important time in the development of the earliest signs of diabetic complications. The onset of clinical complications has been related more closely to duration since puberty than total duration of disease (23). Therefore, it is of considerable interest that only the pubertal and postpubertal patients with IDDM showed higher Lp(a). Previous studies of children and adolescents have been too small to analyze the effect of puberty (12). Puberty is characterized by increased growth hormone secretion and insulin resistance, both of which are more marked in the diabetic adolescent (24,28). Serum IGF-I is disproportionately low, which may imply a resistance to growth hormone perhaps mediated at the receptor level (26). However, the exact mechanism for the development of microvascular complications during puberty is not clear.

We found no association between Lp(a) and HbA_{1c}, nor did patients with poorer metabolic control have higher Lp(a) levels. We believe this is the largest study of Lp(a) in IDDM patients. Previous cross-sectional studies indicating a relationship between Lp(a) and HbA_{1c} in IDDM have been smaller (12,27), and this has not been a consistent finding (10,28). A recent large study in NIDDM found no relationship between Lp(a) and fasting glycemia (29). Reported longitudinal studies, although small, control for phenotype. They have shown a relationship with metabolic control in IDDM (30,31), but not consistently (32), and not in NIDDM (33).

Higher Lp(a) is an independent risk factor for CAD in the normal population (2,3). A recent suggestion that this may not hold for the diabetic population was limited by small sample size, but cautions us in extrapolating from the normal to the diabetic population (34). The marked skew and wide range of distribution of Lp(a) in both normal and diabetic populations requires large numbers for accurate analysis. A similar percentage of our subjects in the IDDM and control groups had a family history of premature CAD. However, a positive family history was not associated with higher Lp(a) in either group. This was in contrast to the reported association between parental history and Lp(a) in middle-aged males (35).

The size of the apo(a) gene, specifically the number of kringle IV encoding sequences, correlates inversely with the concentration of Lp(a) (6). Our findings of significantly higher levels in pubertal and postpubertal IDDM patients justify both identification of the frequency of apo(a) alleles in the IDDM population and longitudinal study of Lp(a) in relation to microvascular complications during puberty.

Acknowledgments— We thank Dr. D. Corlis for providing patients to be studied.

References

1. Berg K: A new serum type system in man: the Lp system. *Acta Pathol Microbiol Scand* 59:369–82, 1963
2. Hoefler G, Harnoncourt P, Paschke E, Mirtl W, Pfeiffer KH, Kostner GM: Lipoprotein Lp(a): a risk factor for myocardial infarction. *Arteriosclerosis* 8:398–401, 1988
3. Rhoads GG, Dahlen G, Berg K, Morton NE, Dannenberg AL: Lp(a) lipoprotein as a risk factor for myocardial infarction. *JAMA* 256:2540–44, 1986
4. Woo J, Lau E, Lam CWK, Kay R, Teoh R, Wong HY, Prall WY, Creel L, Nicholls MG: Hypertension, lipoprotein(a), and apolipoprotein A-1 as risk factors for stroke in the Chinese. *Stroke* 22:203–208, 1991
5. McLean JW, Tomlinson JE, Kuang W-J, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM: cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* (Lond) 300:132–37, 1987
6. Lackner C, Boerwinkle E, Leffert L, Rahmig T, Hobbs H: Molecular basis of apolipoprotein(a) isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. *J Clin Invest* 87:2153–61, 1991
7. Utermann G: The mysteries of lipoprotein(a). *Science* 246:904–10, 1989
8. Albers JJ, Marcovina SM, Lodge MS: The unique lipoprotein(a): properties and immunochemical measurement. *Clin Chem* 36:2019–26, 1990
9. Borch-Johnsen K, Kreiner S: Proteinuria: value as predicator of cardiovascular mortality in insulin dependent diabetes mellitus. *Br Med J* 294:1651–54, 1987
10. Jenkins AJ, Steele JS, Janus ED, Best JD: Increased plasma apolipoprotein(a) levels in IDDM patients with microalbuminuria. *Diabetes* 40:787–90, 1991
11. Kapelrud H, Bangstad H-J, Dahl-Jorgensen K, Berg K, Hanssen KF: Serum lipoprotein(a) concentrations in insulin dependent diabetic patients with microalbuminuria. *Br Med J* 303:675–78, 1991

12. Levitsky L, Scanu A, Gould S: Lipoprotein(a) levels in black and white children and adolescents with IDDM. *Diabetes Care* 14:283–87, 1991
13. Gaubatz JW, Ghanem KI, Guevara J, Nava JL, Patsch W, Morrisett JD: Polymorphic forms of human apolipoprotein (a): inheritance and relationship of their molecular weights to plasma levels of lipoprotein (a). *J Lipid Res* 31:603–607, 1990
14. Levine DM, Sloan B-J, Donner JE, Lorenz JD, Heinzerling RH: Automated measurement of lipoprotein (a) by immunoturbidimetric analysis. *Int J Clin Lab Res* 22:173–78, 1992
15. Labeur C, Shepherd J, Rosseneu M: Immunological assays of apolipoproteins in plasma: methods and instrumentation. *Clin Chem* 36:591–97, 1990
16. Cazzolato G, Prakasch G, Green S, Kostner GM: The determination of lipoprotein Lp(a) by rate and endpoint nephelometry. *Clin Chim Acta* 135:203–208, 1983
17. Philcock JC, Haywood MR, Rofe AM: HbA_{1c} by HPLC with a Pharmacia MonoS HR5/5 cation exchange column: influence of sample protein gradient on optimal chromatographic conditions. *Clin Chem* 38:1488–90, 1992
18. Utermann G, Hoppichler F, Dieplinger H, Seed M, Thompson G, Boerwinkle E: Defects in the low density lipoprotein receptor gene affect lipoprotein(a) levels: multiplicative interaction of two gene loci associated with premature atherosclerosis. *Proc Natl Acad Sci* USA 86: 4171–74, 1989
19. Short CD, Mallik NP, Durrington PN: Ischemic heart disease and lipoprotein abnormalities in human membranous nephropathy. *International Symposium on Lipid Abnormalities in Renal Disease, Porto Cervo, Oct 1–2, 1990*
20. Chase HP, Marshall G, Satish KG, Harris S, Oberg I: Borderline increases in albumin excretion rate and the relation to glycemic control in subjects with type 1 diabetes. *Clin Chem* 37:2048–52, 1991
21. Muhtaseb N, Yousuf A, Bajaj J: Apolipoprotein A-I, A-II, B, C-II, and C-III in children with IDDM. *Pediatrics* 89:936–41, 1992
22. DCCT Research Group: Lipid and lipoprotein levels in patients with IDDM. *Diabetes Care* 15:886–94, 1992
23. Kostraba JM, Dorman JS, Orchard TJ, Becker DJ, Ohki Y, Ellis D, Doft BH, Lobes LA, LaPorte RE, Drash AL: Contribution of diabetes duration before puberty to development of microvascular complications in IDDM subjects. *Diabetes Care* 12:686–93, 1989
24. Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV: Impaired insulin action in puberty: a contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 315:215–19, 1986
25. Edge JA, Dunger DB, Matthews DR, Gilbert JP, Smith CP: Increased overnight growth hormone concentrations in diabetes compared with normal adolescents. *J Clin Endocrinol Metab* 71:1356–62, 1990
26. Mercado M, Moliteh M, Bauman G: Low plasma growth hormone binding protein in IDDM. *Diabetes* 41:605–609, 1992
27. Ramirez L, Arauz-Pachelo C, Lackner C, Albright G, Adams B, Raskin P: Lipoprotein(a) levels in diabetes mellitus: relationship to metabolic control. *Ann Intern Med* 117:42–47, 1992
28. Guillausseau P-J, Peynet J, Chanson P, Legrand A, Altman J-J, Poupon J, N'Guyen M, Rousselet F, Lubetzki J: Lipoprotein(a) in diabetic patients with and without chronic renal failture. *Diabetes Care* 15:976–79, 1992
29. Haffner SM, Morales PA, Stern MP, Gruber MK: Lp(a) concentration in NIDDM. *Diabetes* 41:1267–73, 1992
30. Haffner SM, Tuttle RR, Rainwater DL: Decrease of lipoprotein(a) with improved glycemic control in IDDM subjects. *Diabetes Care* 14:302–307, 1991
31. Bruckert E, Davidoff P, Grimaldi A, Truffert J, Giral P, Doumith R, Therdet F, DeGennes J: Increased serum Lp(a) and their reduction with glycemic control. *JAMA* 263:35–36, 1990
32. Ritter MM, Richter WO, Lyko K, Schwardt P: Lp(a) serum concentrations and metabolic control. *Diabetes Care* 15: 1441–42, 1992
33. Haffner SM, Tuttle KR, Rainwater DC: Preliminary report: lack of change of Lp(a) concentration with improved glycemic control in subjects with type II diabetes. *Metabolism* 41:116–20, 1992
34. Haffner SM, Moss SE, Klein BEK, Klein R: Lack of association between lipoprotein(a) concentrations and coronary heart disease mortality in diabetes. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Metabolism* 41:194–97, 1992
35. Durrington PN, Ishola M, Hunt L, Arral S, Bhatnagar D: Apolipoproteins(a), A1 and B and parental history in men with early onset ischemic heart disease. *Lancet* i, 1070–73, 1988