Lipoprotein(a) in Android Obesity and NIDDM

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OBJECTIVE — To assess the level of serum lipoprotein(a) [Lp(a)] in nonobese and obese NIDDM subjects with android body distribution.

RESEARCH DESIGN AND METHODS — Serum Lp(a) levels were measured in 30 long-standing NIDDM patients (duration of diabetes 12.5 ± 3 years, mean ± SD), with 15 of the patients being obese of android distribution (BMI >30 kg/m^2 and waist-to-hip ratio >0.8). In addition, there were 15 android obese nondiabetic subjects and 10 healthy subjects serving as the control group.

RESULTS — All groups of patients in this study (diabetic, obese, and obese diabetic) showed significantly higher levels of Lp(a) than the healthy control group. Lp(a) concentrations were significantly higher in NIDDM patients with android type of obesity than in nondiabetic androids (24.1 ± 5.6 vs. 14.8 ± 2.4 mg/dl, $P < 0.001$). Significantly greater levels of Lp(a) were found in nonobese subjects with diabetes when compared with obese subjects without diabetes (22.3 ± 4.1 vs. 14.8 ± 2.4 mg/dl, $P < 0.001$). Furthermore, Lp(a) serum concentrations were not dependent on the degree of glycemic control (controlled NIDDM 23.6 ± 5.0 vs. uncontrolled NIDDM 21.4 ± 2.7 mg/dl, NS), but were much greater in subjects with diabetes complicated by vascular disease (complicated 26.3 ± 5.0 vs. uncomplicated 20.5 ± 2.7 mg/dl, $P < 0.001$). No correlation was found between Lp(a) and other lipid parameters in this study.

CONCLUSIONS — Lp(a) levels are significantly elevated in both android-obese and nonobese NIDDM patients regardless of the degree of glycemic control. Lp(a) is an independent risk factor showing greater elevations in those subjects complicated with diabetic vascular diseases.

The risk of cardiovascular disease is increased two- to fourfold in NIDDM patients compared with nondiabetic subjects. Furthermore, this excess risk is explained only partially by the patients' increased levels of standard risk factors (1). Obesity, one of these factors, is associated with many metabolic disturbances in lipid and lipoprotein metabolism, with these changes being more obvious in diabetic patients than in nondiabetic individuals and more obvious in patients with NIDDM than with IDDM (2).

Lipoprotein(a), a macromolecular complex found in human serum, is a genetically determined lipoprotein that is poorly influenced either by dietary measures or by hypolipidemic drugs (3). Numerous studies have suggested that lipoprotein(a) [Lp(a)] is an important independent predictor of risk for vascular disease (4–7). Substantially conflicting data are available on Lp(a) levels in NIDDM patients. Schernthaner et al. (8), for example, found no difference in mean or median Lp(a) concentrations between diabetic patients and nondiabetic subjects. In this study, no distinction was made between individuals with NIDDM and those with IDDM. In a preliminary report, Arauz et al. (9) found higher concentrations of Lp(a) in a combined group of patients with IDDM and NIDDM. Another study, by Haffner et al. (10), yielded even more conflicting results: NIDDM patients did not have increased levels of Lp(a) compared with control subjects, and in fact, Lp(a) levels were slightly lower in these patients.

Other investigations undertaken to link obesity and weight loss with serum Lp(a) concentrations revealed a decline of serum Lp(a) after weight loss in one study (11), in contrast to no significant change in Lp(a) concentrations in another study (12). However, both research parties observed no relationship between Lp(a) concentrations and adiposity at baseline.

In this study, we assess the level of Lp(a) in obese NIDDM subjects with an android type of body fat distribution. We aim at comparing this group of patients with their nonobese as well as their nondiabetic counterparts. Within the diabetic patients, we also examine the relationship of Lp(a) concentrations to degree of glycemic control and vascular complications.

RESEARCH DESIGN AND METHODS

Study population

The study was conducted on 55 Egyptian subjects. Their mean age (± SD) was 52 ± 7.5 years. Thirty of these subjects were NIDDM patients attending the Ain Shams University Hospital Diabetes Clinic in Cairo, Egypt. Diabetes was diagnosed according to WHO criteria (fasting glucose level ≥7.8 mmol/l and/or 2-h glucose level ≥11.1 mmol/l [13]). All patients had long-standing diabetes, with a duration of 12.5 ± 3 years (mean ± SD), and were under treatment with oral antidiabetic agents. None were treated with insulin therapy. Of these patients, 15 were specifically selected as being obese and diabetic with upper-body fat distribution, having a BMI of >30 kg/m^2 and a waist-to-hip ratio (WHR) of >0.8. All diabetic patients were subjected to a diabetes complications examination that included a complete ophthalmologic examination using slitlamps with three mirror glasses and assessment of retinopathy by retinal angiography. Urinary albumin excretion, estimated by rate nephelometry, was taken as the mean of two 12-h overnight urine collections performed on consecutive days. A urinary albumin excretion rate of >20 mg/24 h was defined as nephropathy. Neuropathy evidenced by neurological examination accord-

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Received for publication 20 March 1997 and accepted in revised form 29 July 1997.
Abbreviations: Lp(a), lipoprotein(a); WHR, waist-to-hip ratio.

Table 1—*Anthropometric and metabolic characteristics of study subjects*

Variable	Control	NIDDM	Android NIDDM	Android
n	10	15	15	15
Age (years)	52 ± 7.5	52 ± 7.2	52 ± 6.5	52 ± 7.5
Sex (M/F)	5/5	7/8	7/8	7/8
BMI (kg/m²)	<25	<25	>30	>30
WHR	—	—	>0.8	>0.8
HbA_{1c} (%)	7.0 ± 0.3	8.7 ± 3.0	8.8 ± 3.5	6.9 ± 0.6
Triglycerides (mg/dl)	85.0 ± 12.9	128.2 ± 32.3*	335.5 ± 42.1†‖	231.4 ± 38.0†§‖
Total cholesterol (mg/dl)	163.8 ± 15.8	159.5 ± 8.7	336.5 ± 24.1†‖	289.2 ± 31.0†‡‖
LDL cholesterol (mg/dl)	78.8 ± 7.4	80.8 ± 5.8	280.8 ± 15.8†‖	209.9 ± 29.5†‡‖
Lp(a) (mg/dl)	11.8 ± 2.6	22.3 ± 4.1†	24.1 ± 5.6†	14.8 ± 2.4*§‖

Data are expressed as means ± SD. *$P < 0.05$ vs. control; †$P < 0.001$ vs. control; ‡$P < 0.05$ vs. android NIDDM; §$P < 0.001$ vs. android NIDDM; ‖$P < 0.001$ vs. NIDDM.

ing to the Diabetes Control and Complications Trial criteria (14) was also determined. The cardiovascular screen included an electrocardiogram at rest, a treadmill test, and coronary angiography when indicated. Of the 30 diabetic subjects, 14 revealed complications after thorough examination. Six patients had background retinopathy, three of whom also had concomitant nephropathy. Neuropathy was diagnosed in two patients. The remaining six diabetic patients showed evidence of ischemic heart disease.

Another 15 subjects were chosen from the adjacent "Outpatient Clinic for the Obese" in the same hospital. Otherwise healthy obese patients who were about to join the weight-loss program were very carefully chosen after complete clinical and laboratory investigations to exclude any disease. Anthropometric measurements (height, weight, waist and hip circumference) were made after subjects had removed their shoes and upper garments and donned an examining gown. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. WHR was used as a measure of upper-body adiposity (15). All obese subjects in this study were chosen with a BMI >30 kg/m² and WHR >0.8 (i.e., android body distribution).

To provide a control group, 10 healthy, nonobese, nondiabetic subjects of matched age, sex, and socioeconomic level were carefully chosen from the hospital staff members (five women, five men, mean ± SD age 52 ± 7.5, BMI <25 kg/m²). The study was approved by the Review Board of the Ministry of Health in Cairo. None of the patients or control subjects was affected by conditions or medications known to influence Lp(a) levels (16). In addition, pregnant women and subjects with intercurrent illness were excluded from this study.

Analytic methods

For each subject described in this report, blood specimens were drawn by venipuncture after a 12- to14-h fast. A portion of the blood was collected into lavender top vacutainers (containing EDTA) for the determination of glycosylated hemoglobin (HbA_{1c}) levels by ion exchange chromatography (Stanbio Laboratory Inc., San Antonio, TX). With a cut-off value of 8% used to assess glycemic control, 20 of the NIDDM patients were well-controlled (HbA_{1c} <8%), and 10 revealed poor metabolic control (HbA_{1c} >8%). Serum extracted from clotted blood after centrifugation was used for the immediate enzymatic determination of triglycerides, total cholesterol, and LDL cholesterol using kits from Biomérieux (Marcy l'Etoile, Charbonnières-les Bains, France). Lp(a) was measured on fasting serum specimens, which had been stored at −70°C for an average of 2 months, using a solid phase enzyme immunoassay technique (COALIZA Lp(a) Chromogenix kit, Haemochrom Diagnostica, Germany). The kit assay uses monoclonal monospecific anti-Lp(a) antibodies which do not cross-react with plasminogen or LDL. The detection limit of the assay was 1.2 mg/dl. The intra-and interassay coefficients of variation of this assay were 4.7 and 7.8%, respectively.

Statistical analysis

Although Lp(a) distribution in many populations is not Gaussian, giving skewed values, results in the Egyptian population revealed normal Lp(a) dispersal (17). In this study, there was no necessity to carry out nonparametric tests such as the Mann-Whitney *U* test or log-transformation to improve skewness and kurtosis. The application of the Student's *t* test was more than sufficient to determine degrees of significance between individual groups. The normal range for serum Lp(a) in the Egyptian population was found to be 12.2 ± 3.5 mg/dl (17). In this study, the normal control subjects showed a mean of 11.8 ± 2.6 mg/dl. In addition, linear regression analysis was applied for the correlation studies. All analyses were computed with a Macintosh computer using Statview statistical packages. Results for the groups are expressed as means ± SD. Statistical significance was defined as $P < 0.05$.

RESULTS — Anthropometric and metabolic characteristics of the study subjects are shown in Table 1. Age and sex were statistically matched (no significant difference, $P > 0.05$) in all studied groups. BMI exceeded 30 kg/m² in both obese groups with an observed WHR greater than 0.8 (denoting upper-body fat distribution).

The android NIDDM patients demonstrated significantly higher levels of triglycerides, total cholesterol, and LDL cholesterol than did the nonobese diabetic and android obese subjects. The latter group, in turn, also revealed remarkably greater serum lipid parameters than did the nonobese NIDDM patients. Comparison between the nonobese diabetic patients and normal control subjects showed similar patterns of total cholesterol and LDL cholesterol, whereas serum triglycerides were significantly higher in the former group. Lp(a) serum levels were highest in the android diabetic subjects (24.1 ± 5.6 mg/dl) followed by the nonobese diabetic subjects (22.3 ± 4.1 mg/dl), the android obese subjects (14.8 ± 2.4 mg/dl), and lastly the normal control subjects (11.8 ± 2.6 mg/dl). All groups were significantly comparable to one another except for the two NIDDM groups, where no statistical difference ($P > 0.05$) was obtained between them despite the observed difference.

Linear regression analyses confined to the diabetic subjects (both obese and nonobese) were performed in a trial to correlate serum Lp(a) (the dependent variable) with the other lipid parameters of this study (data not shown). Neither triglycerides nor cholesterol (total and LDL

fraction) was significantly related to Lp(a) concentrations among diabetic patients (correlation coefficient $r < 0.4$, $P > 0.05$).

Glycosylated hemoglobin was determined in all diabetic subjects to consider the effect of metabolic control on Lp(a) levels. A cutoff level of 8% was established. Twenty of the NIDDM patients in this study were well controlled (HbA_{1c} <8%), and the other 10 showed poor control (HbA_{1c} >8%). No significant difference was statistically obtained for Lp(a) concentrations between controlled and uncontrolled diabetic subjects (23.6 ± 5.0 vs. 21.4 ± 2.7 mg/dl, $P > 0.05$).

NIDDM patients were further subdivided according to presence or absence of vascular complications. Only 14 of the 30 patients examined revealed diabetic complications in the form of background retinopathy (6 subjects, 3 of whom were diagnosed with concomitant nephropathy), diabetic neuropathy (2 patients), and evidence of ischemic heart disease (6 patients). Lp(a) levels in patients with proven diabetic vascular complications were found to be significantly higher than in diabetic patients without complications (26.3 ± 5.0 vs. 20.5 ± 2.7 mg/dl, $P < 0.001$).

CONCLUSIONS — Despite the relatively restricted number of subjects in this study (a result of financial limitations), our results indicate that NIDDM patients show highly significant elevations in serum Lp(a) levels compared with normal control subjects. These findings were confirmed by Joven and Viella (18) and Hessen et al. (19). The mechanism by which Lp(a) levels in diabetes are increased is unclear, however. It has been hypothesized that a defect in clearance (20) or an increased synthetic rate (21) of apoprotein B-100 lipoprotein exists in diabetes. Yet, it does not seem likely that this is the mechanism of the observed elevation of Lp(a) levels in our study because no significant correlation between the levels of Lp(a) and LDL cholesterol was found. In addition, the nonobese diabetic subjects had near-normal total cholesterol and LDL cholesterol with an elevation in serum triglycerides only. In contradiction with the above findings, other researchers (10,22) found no elevations of Lp(a) concentrations in NIDDM patients. Possible factors confounding the relationship between Lp(a) and diabetes are numerous and include variations in sample size, methods of laboratory determination of Lp(a), type of therapy administered to achieve metabolic control, and, most importantly, differences in human genetic constitution in various countries.

Comparison of serum Lp(a) levels between controlled NIDDM patients and those with poor metabolic control revealed no significant difference. These findings are in accordance with previous studies (17,23). Thus, the preponderance of evidence suggests no effect of glycemia on Lp(a) concentrations in NIDDM (in both obese and nonobese patients), thereby possibly implicating genetic predisposition as the underlying cause of elevated Lp(a) in these diabetic patients.

In this study, we preferentially chose patients with android obesity because in Egypt gynoid distribution (lower-body fat deposition) occurs more commonly in women. Their inclusion would thus have biased this study. Obesity is associated with many metabolic disturbances in lipid and lipoprotein metabolism, including hypercholesterolemia, resulting from increased production of LDL and decreased hepatic cholesterol clearance, and hypertriglyceridemia due to increased hepatic VLDL production (24). These changes are more obvious in diabetic patients, especially those with NIDDM, than in nondiabetic subjects (25). Our results coincided with the above findings where the android NIDDM patients revealed significantly greater elevations in their serum lipid parameters than did nondiabetic obese subjects. Furthermore, the android NIDDM group showed significantly higher Lp(a) serum levels when compared with the nondiabetic android group, but the latter group still had significantly higher Lp(a) concentrations than did the normal control subjects in this study. On the other hand, there was no statistical difference obtained in Lp(a) concentrations between the two NIDDM groups (obese and nonobese).

Lp(a) has been established as a new risk factor for cardiovascular disease (26); however, most of the studies have been performed in patients without diabetes (27). In an attempt to link Lp(a) with vascular disease in people with diabetes, we further subdivided the NIDDM patients (both obese and nonobese) according to presence or absence of vascular complications in the form of diabetic retinopathy, neuropathy, nephropathy, and evidence of ischemic heart disease. We found Lp(a) serum levels in patients with proven diabetic vascular complications to be significantly higher than in the diabetic patients without complications. In addition, linear regression analyses indicated no significant correlation between Lp(a) and other lipid risk factors, thereby establishing Lp(a) as an independent risk factor for cardiovascular disease in NIDDM. This finding has been previously confirmed in a prospective study by Hiraga et al. (27). These results suggest that elevated Lp(a) could be implicated as an important link between diabetic microvascular and macrovascular diseases, where Lp(a) may favor thrombosis through attenuation of clot lysis or by direct ingress into the arterial wall (28) and induce microangiopathy via capillary occlusion.

In conclusion, our case-controlled cross-sectional study (despite the relatively small number of patients) showed that Lp(a) levels were significantly elevated in NIDDM patients, both the android obese and the nonobese, regardless of the glycemic control, with greater Lp(a) values being obtained in those subjects with diabetic vascular complications.

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