Inverse Relationship Between Serum Lp(a) Levels and First-Phase Insulin Secretion

MANDEEP SIDHU, DAVID CROOK, IAN F. GODSLAND, CHRISTOPHER WALTON, VICTOR WYNN, AND MICHAEL F. OLIVER

Relationships between serum Lp(a) levels and insulin metabolism were investigated in 147 healthy nonobese men attending an executive health-screening program. Each subject received an IVGTT with measurement of plasma levels of glucose, insulin, and C-peptide. An inverse relationship was seen with the first-phase plasma insulin response when subjects were stratified into quartile ranges of the serum Lp(a) distribution. This relationship was supported by mathematical modeling analyses of these data, which revealed an inverse relationship between serum Lp(a) levels and first-phase pancreatic insulin secretion and plasma insulin responsiveness to glucose. *Diabetes* 41:1341–45, 1992

Elevated serum Lp(a) levels often are seen in individuals at risk of CHD (1,2). Recently, raised Lp(a) levels have been reported in people with insulin-dependent diabetes mellitus (3–5) and NIDDM (6–7), conditions associated with increased risk of CHD (8). Consequently, it has been suggested that elevated serum levels of Lp(a) may contribute to the increased risk of CHD in these patients.

These relationships suggest an association between serum Lp(a) and insulin metabolism. To investigate whether such a link exists, we measured serum Lp(a) levels in a group of 147 healthy nonobese men in whom insulin metabolism had been assessed with an IVGTT.

RESEARCH DESIGN AND METHODS

Experimental subjects. We studied 147 healthy white men aged 24–77 yr who participated in an executive health-screening program. None were obese (ideal body weight <120%; Metropolitan Life Tables), and none took any drugs known to affect insulin or Lp(a) metabolism. Ethical committee approval was obtained for the study, and written, informed consent was given in all cases. This group was the subject of a previous study of insulin metabolism and body fat distribution (9).

Procedures. The patients were instructed to consume >200 g/day carbohydrate for the 3 days before the IVGTT and to fast overnight (>12 h). After the subjects rested for 15 min in a semirecumbent position, an indwelling cannula was inserted under local anesthesia into an antecubital vein in each arm. Blood for measurement of lipids, lipoproteins, and apo was taken from the cannula into tubes containing plastic granules. These tubes stood at room temperature for 30 min, after which serum was prepared by low-speed centrifugation. Serum samples for lipid and lipoprotein assays were stored at 4°C and analyzed within 4 days. Apo and Lp(a) assays were performed on contingency samples stored at −20°C. These samples did not undergo more than a single freeze-thaw cycle.

For measurement of basal plasma glucose, insulin, and C-peptide levels, two successive blood samples (10 min apart) were drawn into tubes containing lithium-heparin and plasma, prepared by low-speed centrifugation. Immediately after basal sampling, subjects were given an i.v. injection of glucose (0.5 g of glucose/kg

From the Wynn Institute for Metabolic Research, St John's Wood, London, United Kingdom.

Address correspondence and reprint requests to David Crook, PhD, Wynn Institute for Metabolic Research, 21 Wellington Road, St John's Wood, London NW8 9SQ, UK.

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Lp(a), lipoprotein(a); CHD, coronary heart disease; NIDDM, non-insulin-dependent diabetes; IVGTT, i.v. glucose-tolerance test; HDL, high-density lipoprotein; HDL_3, high-density lipoprotein subfraction 3; HDL_2, high-density lipoprotein subfraction 2; LDL, low-density lipoprotein; apo, apolipoprotein; ELISA, enzyme-linked immunosorbent assay; S_i, sensitivity of insulin-dependent glucose elimination; S_g, sensitivity of non-insulin-dependent glucose elimination; ϕ_1, first-phase hepatic insulin delivery to glucose; ϕ_2, second-phase hepatic insulin delivery to glucose; n_1, insulin elimination constant; f, fractional hepatic throughput of insulin; K_1, insulin elimination constant; IS_1, first-phase pancreatic insulin secretion; IS_2, second-phase pancreatic insulin secretion; IS_b, net basal insulin secretion; IAUC, incremental area under the curve; ANOVA, analysis of variance; NS, not significant; IAUC1, first-phase incremental area under the curve; IAUC2, second-phase incremental area under the curve.

body weight as a 50% [wt/vol] solution of dextrose, given over 3 min) by the cannula in the other arm. Further samples were taken at 3, 5, 7, 10, 20, 30, 45, 60, 75, 90, 120, 150, and 180 min, and plasma was prepared as before. Plasma samples for glucose assay were measured within 6 h; aliquots for insulin and C-peptide assays were stored at −20°C.

Serum total cholesterol and triglyceride levels were measured by fully enzymatic procedures. HDL cholesterol and HDL_3 cholesterol concentrations were measured after sequential precipitation with heparin, manganese ions (10), and dextran sulfate (11), respectively. HDL_2 cholesterol was calculated as the difference between the cholesterol content of HDL and HDL_3. LDL cholesterol content was estimated using the Friedewald formula (12). Because the LDL level obtained using this formula incorporates Lp(a), we also corrected these values by subtracting 30% of the Lp(a) levels (13). Apo A-I and apoB were measured by immunoturbidimetry (14). Plasma glucose was measured by a glucose oxidase procedure (15). Plasma levels of insulin and C-peptide were measured by the radioimmunoassay procedure of Albano et al. (16) and the radioimmunoassay kit supplied by Guildhay Ltd, Surrey, UK, respectively. Serum Lp(a) levels were measured by an ELISA method (Biopool AB, Umea, Sweden). This assay measures the total mass of the Lp(a) particle; human plasminogen does not interfere with this assay at concentrations <1 mg/ml in the undiluted sample. Coefficients of variation for these assays were 1–2% (glucose), 4–6% (insulin), 7–9% (C-peptide), 1–2% (total cholesterol), 2–3% (HDL cholesterol), 5–7% (HDL_3 cholesterol), 6–8% (HDL_2 cholesterol), 2–4% (apoA-I and apoB), and 4–7% [Lp(a)].

Modeling analyses. Mathematical modeling analyses of glucose, insulin, and C-peptide concentrations during the IVGTT were used to quantify insulin resistance, delivery, and secretion. Modeling analyses were conducted with programs written in Fortran 77 and run on a PDP 11/83 minicomputer. The sensitivity of glucose elimination to insulin (S_i, inversely proportional to insulin resistance) and of non-insulin-dependent glucose elimination (S_g) were estimated with the minimal model technique of Bergman et al. (17). The plasma insulin concentration profile during the IVGTT reflects distinct first and second phases of pancreatic insulin secretion; these concentration changes were quantified according to the minimal model of posthepatic insulin delivery attributed to Toffolo et al. (18). This model provided measures of the responsiveness of net first-phase and second-phase hepatic insulin delivery to glucose (ϕ_1 and ϕ_2) and the insulin elimination constant (n_i, equal to ln (2)/insulin half-life).

Estimates of pancreatic insulin secretion were derived from insulin and C-peptide concentrations during the IVGTT by the model of Vølund et al. (19) and the model identification system of Watanabe et al. (20). Values obtained by this procedure include an index of the fractional hepatic throughput of insulin (f, inversely related to hepatic uptake of insulin), the insulin elimination constant (K_i), net basal insulin secretion (IS_b), and net incremental first- and second-phase pancreatic insulin secretion (IS_1 and IS_2, respectively).

Variables derived from these analyses were transformed (logarithmic or square root) to normalize their distribution before analysis. For a modeling analysis to be acceptable, variable estimates were required to have fractional standard deviations <100% and to be positive (17). Results were excluded if a model identification resulted in a value >4 SD from the mean. A further exclusion criterion (ratio between first-phase insulin and C-peptide areas >1, together with f >2) was used for the pancreatic secretion model. This ensures that only those cases are analyzed in which the assumptions underlying the pancreatic secretion model have not been violated.

For the minimal model of glucose disappearance, 5 cases failed the model, and 2 sets of results were considered statistical outliers. A further 6 cases failed the minimal model of posthepatic insulin delivery, and 2 sets of results were considered statistical outliers. For the pancreatic insulin secretion model, 9 cases were rejected because of missing or incomplete C-peptide data, 17 cases failed the model exclusion criterion, and one was considered a statistical outlier. In total, the number of acceptable data sets from the three models (minimal model of glucose disappearance, the minimal model posthepatic insulin delivery, and pancreatic insulin secretion model) was 140, 134, and 120, respectively.

Data analysis. The total area under the plasma glucose, insulin, and C-peptide profiles (area under curve) was calculated by the trapezoidal rule. From this, we derived IAUC, i.e., areas between the fasting concentration and the IVGTT concentration profile, because these provide measures of response unconfounded by changes in the fasting level. Insulin response areas were distinguished as first-phase (0–10 min) and second-phase (10–180 min) postinjection (21).

The study group was divided into quartile ranges of serum Lp(a) level. Relationships between quartile ranges of Lp(a) and other variables were examined with one-way ANOVA with linear contrasts to explore trends (22). We also wished to compare subjects above and below the serum Lp(a) level associated with increased CHD risk. Kostner et al. (13) and Armstrong et al. (23) suggested that a limit of 30 mg/dl is discriminatory. Accordingly, we used Student's *t* test (unpaired) to compare subjects above and below this value, representing high and low risk, respectively. Because other studies (24, 25) proposed the use of a lower level (20 mg/dl), corresponding to the top quartile range of the Lp(a) distribution in our study group (21.0–118.0 mg/dl), we also compared subjects in this group with subjects pooled from the lower three quartile ranges.

All statistical analyses were carried out with BMDP Statistical Software (Los Angeles, CA).

RESULTS

We found no significant differences in age, height, body weight, or blood pressure between quartile ranges of Lp(a) (Table 1), nor did these variables differ significantly when subjects in the top quartile range were compared

TABLE 1
Study group characteristics and serum lipid and lipoprotein levels in 147 healthy nonobese men classified by serum Lp(a) level

	Quartile range of Lp(a) distribution (mg/dl)				*P*		
	1 (0–0.8)	2 (0.8–5.3)	3 (5.3–21.0)	4 (21.0–118.0)	Trend	Quartile 4 vs. 1–3	High vs. low*
Age (yr)	49.8 ± 10.6	49.7 ± 10.6	48.6 ± 12.0	50.7 ± 10.0	NS	NS	NS
Height (cm)	179 ± 8	178 ± 6	178 ± 7	180 ± 7	NS	NS	NS
Weight (kg)	83.3 ± 15.3	82.2 ± 11.1	80.1 ± 11.9	81.3 ± 11.8	NS	NS	NS
Ideal body weight (%)	111.6 ± 18.9	110.7 ± 10.2	108.4 ± 14.1	107.9 ± 12.2	NS	NS	NS
Body mass index (kg/m)	25.9 ± 4.1	25.9 ± 2.5	25.3 ± 3.3	25.2 ± 2.9	NS	NS	NS
Systolic blood pressure (mmHg)	127 ± 17	123 ± 18	125 ± 19	124 ± 16	NS	NS	NS
Diastolic blood pressure (mmHG)	80 ± 9	78 ± 9	79 ± 11	78 ± 7	NS	NS	NS
Total cholesterol (mM)	5.3 ± 0.9	5.5 ± 0.7	5.3 ± 0.9	6.0 ± 0.9	<0.01	<0.001	<0.001
Triglycerides† (mM)	1.0 (−0.5, +0.9)	1.1 (−0.4, +0.6)	0.9 (−0.4, +0.7)	1.1 (−0.4, +0.7)	NS	NS	NS
LDL cholesterol (mM)	3.3 ± 0.8	3.7 ± 0.7	3.3 ± 0.8	4.1 ± 0.9	<0.01	<0.001	<0.001
Corrected LDL cholesterol‡ (mM)	3.3 ± 0.8	3.6 ± 0.7	3.2 ± 0.8	3.7 ± 0.9	NS	<0.05	NS
HDL cholesterol (mM)	1.3 ± 0.4	1.3 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	NS	NS	NS

Values are mean ± SD, except where noted.
* Comparison of groups with Lp(a) levels < and >30 mg/dl.
† Data derived after log-transformation; the minus and plus values are given in parentheses.
‡ Corrected for contamination by Lp(a).

with those in lower ranges, or when subjects with low (<30 mg/dl^{-1}) and high (>30 mg/dl^{-1}) serum Lp(a) levels were compared. A significant positive relationship was seen between quartile ranges of serum Lp(a) and total cholesterol ($P < 0.01$; Table 1). This was attributable to higher levels of LDL cholesterol (calculated with the Friedewald equation) in these subjects. When LDL-cholesterol values were corrected for contamination by Lp(a), this trend was diminished. Serum levels of apoB, present in both LDL and Lp(a), were elevated in subjects in the top quartile range of Lp(a) ($P < 0.001$, data not shown). No relationships were seen between serum levels of Lp(a) and those of triglycerides or HDL cholesterol (Table 1) or between serum Lp(a) and HDL subfractions or apoA-I (data not shown).

Basal plasma levels of glucose and glucose IAUC were similar throughout the serum Lp(a) distribution (Table 2). Basal plasma insulin levels also were similar in these groups. However, a significant relationship between Lp(a) and insulin IAUC was noted, with subjects

TABLE 2
Fasting and IVGTT concentration measures in 147 healthy nonobese men classified by serum Lp(a) level

	Quartile range of Lp(a) distribution (mg/dl)				*P*		
	1 (0–0.8)	2 (0.8–5.3)	3 (5.3–21.0)	4 (21.0–118.0)	Trend	Quartile 4 vs. 1–3	High vs. low*
Basal glucose (mM)	5.3 ± 0.3	5.3 ± 0.4	5.4 ± 0.4	5.4 ± 0.5	NS	NS	NS
Glucose IAUC (mM/min)	491 ± 168	493 ± 200	466 ± 159	495 ± 174	NS	NS	NS
Basal insulin† (pmol/ml)	0.03 (−0.04, +0.05)	0.03 (−0.02, +0.04)	0.03 (−0.02, +0.04)	0.03 (−0.02, +0.04)	NS	NS	NS
Insulin IAUC† (pmol · min · ml^{-1})	21.8 (−11.8, +25.8)	17.6 (−7.4, +12.8)	16.3 (−7.6, +14.2)	16.2 (6.37, +10.5)	<0.05	NS	NS
Insulin IAUC1† (pmol · min · ml^{-1})	3.2 (−1.6, +3.4)	3.0 (−1.4, +2.7)	2.7 (−1.4, +2.8)	2.4 (−1.2, +2.3)	<0.05	NS	<0.05
Insulin IAUC2† (pmol · min · ml^{-1})	17.5 (−10.4, +25.3)	13.8 (−6.3, +11.8)	13.4 (−7.1, +15.1)	13.1 (−6.1, +11.3)	NS	NS	NS
Basal C-peptide† (pmol/ml)	0.55 (−0.20, +0.40)	0.48 (−0.17, +0.25)	0.53 (−0.19, +0.30)	0.51 (−0.19, +0.20)	NS	NS	NS
C-peptide IAUC† (pmol · min · ml^{-1})	97 (−44, +57)	80 (−33, +41)	82 (−30, +36)	84 (−37, +47)	NS	NS	NS
C-peptide IAUC1† (pmol · min · ml^{-1})	5.2 (−3.1, +4.4)	5.1 (−2.7, +3.8)	5.0 (−2.9, +4.1)	3.9 (−2.0, +2.8)	NS	NS	<0.05
C-peptide IAUC2† (pmol · min · ml^{-1})	91.2 (−42.4, +55.6)	73.9 (−32.2, +41.3)	76.3 (−28.1, +34.6)	77.1 (−35.0, +45.7)	NS	NS	NS

Values are mean ± SD.
* Comparison of groups with Lp(a) levels > and <30 mg/dl.
† Data derived after log-transformation; the minus and plus values are given in parentheses.

TABLE 3
Selected model-derived parameters of insulin metabolism in 147 healthy nonobese men classified by serum Lp(a) level

	Quartile range of Lp(a) distribution (mg/dl)				P		
	1 (0–0.8)	2 (0.8–5.3)	3 (5.3–21.0)	4 (21.0–118.0)	Trend	Quartile 4 vs. 1–3	High vs. low*
Minimal model of glucose disappearance							
S_i† ($min^{-1} \cdot \mu U^{-1} \cdot ml$) $\times 10^4$	2.9 (−2.0, +3.3)	2.9 (−1.8, +2.6)	3.0 (−2.2, +3.5)	3.0 (−1.5, +1.9)	NS	NS	NS
Posthepatic insulin delivery model							
ϕ_1‡ ($\mu U \cdot ml^{-1} \cdot min \cdot mg^{-1} \cdot dl$) $\times 10^2$	4.7 (−2.5, +5.3)	3.9 (−3.5, +3.2)	3.9 (−1.7, +3.0)	3.3 (−1.6, +3.0)	<0.05	NS	<0.05
ϕ_2‡ ($\mu U \cdot ml^{-1} \cdot min^{-2} \cdot mg^{-1} \cdot dl$) $\times 10^4$	9.8 (−4.4, +8.0)	10.0 (−5.8, +13.8)	9.0 (−5.1, +11.9)	11.2 (−5.0, +8.9)	NS	NS	NS
Pancreatic insulin secretion model							
IS_1 (pmol/ml)	0.81 ± 0.48	0.75 ± 0.34	0.76 ± 0.39	0.60 ± 0.32	<0.05	<0.01	<0.01
IS_2† (pmol/ml)	2.2 (−1.3, +1.9)	1.6 (−0.8, +1.1)	1.5 (−1.0, +1.5)	1.6 (−0.9, +1.2)	NS	NS	NS

Values are means ± SD.
* Comparison of groups with Lp(a) levels > and <30 mg/dl.
† Data derived after square-root transformation; minus and plus values are given in parentheses.
‡ Data derived after log-transformation; minus and plus values are given in parentheses.

having the highest serum Lp(a) levels having the lowest insulin response ($P < 0.05$; Table 2). This was because of differences in first-phase insulin IAUC ($P < 0.05$), with no difference in second-phase response. The difference in first-phase insulin response was also significant ($P < 0.05$) when high and low serum Lp(a) groups were compared.

No significant associations were observed between serum Lp(a) levels and basal C-peptide levels, C-peptide IAUC, or first- and second-phase C-peptide IAUC (Table 2). However, subjects with high-risk serum Lp(a) levels had lower first-phase C-peptide IAUC than did subjects at low risk ($P < 0.05$), consistent with the observed difference in first-phase insulin IAUC.

No relationships were seen between Lp(a) levels and S_i (Table 3). Net ϕ_1, derived from the posthepatic insulin delivery model, showed a significant negative association ($P < 0.05$) with Lp(a) quartile ranges. Furthermore, subjects with high-risk Lp(a) levels had lower ϕ_1 levels than subjects with low risk levels ($P < 0.05$). No significant differences were seen for ϕ_2.

IS_1 was reduced in subjects with elevated Lp(a) levels ($P < 0.01$; Table 3). This pattern resembled that seen with first-phase insulin IAUC (Fig. 1). No significant associations were seen between serum Lp(a) levels and IS_2. n_i was higher in high-risk subjects than in low-risk subjects ($P < 0.05$, data not shown). No significant associations were seen between serum Lp(a) and other model-derived values (data not shown). Data analyses omitting the exclusion criterion for the pancreatic insulin secretion model gave essentially identical results.

DISCUSSION

In this population of nonobese healthy men, serum Lp(a) levels were related inversely to first-phase plasma insulin response during an IVGTT. This relationship suggests either an interrelationship between Lp(a) and insulin metabolism, or that elevated Lp(a) levels and reduced first-phase pancreatic insulin secretion are common features of a single condition.

Little is known about the metabolic control of serum Lp(a) levels, although the weight of evidence suggests a strong genetic component (1, 26). We know of no experimental evidence for an effect of insulin on Lp(a) metabolism, or vice versa. However, several studies suggest that serum Lp(a) levels are raised in people with diabetes (4–7). A low first-phase insulin response to glucose may be a predictor of subsequent development of NIDDM (27–29), representing a prediabetic stage in otherwise healthy individuals. Genetic factors appear to be important determinants of first-phase insulin response (30). Therefore, we suggest that predisposition to development of NIDDM and the presence of high serum Lp(a) levels are linked.

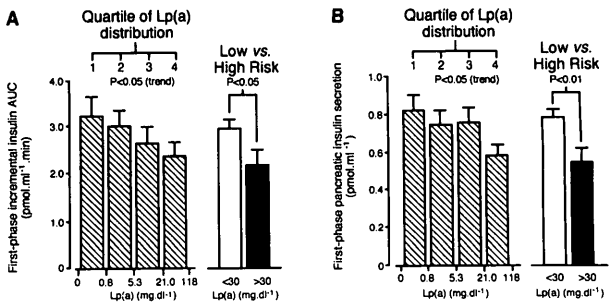

FIG. 1. Mean values for first-phase incremental insulin area under curve (*A*) and first-phase pancreatic insulin secretion (*B*) in 147 healthy men classified by quartile range of serum Lp(a) level or low-risk or high-risk serum Lp(a) level. Error bars represent standard error of the mean.

Insulin resistance, a predictor of NIDDM, is central to the constellation of metabolic disturbances associated with CHD (31). In our study, we found no evidence of a relationship between insulin sensitivity and serum Lp(a) levels. If this observation is confirmed, it would suggest that elevated serum Lp(a) levels are not part of the cluster of metabolic disturbances associated with insulin resistance. It is noteworthy that Sundell et al. (32) found no relationship between serum Lp(a) levels and mean insulin/glucose ratio during an oral glucose tolerance test, proposed by these authors as a measure of peripheral tissue insulin resistance.

Our observation that first-phase insulin responses were reduced in subjects with elevated serum Lp(a) levels suggests a role of Lp(a) in the development of chronic atherosclerotic complications in NIDDM, in addition to increasing CHD risk in otherwise healthy individuals. The mechanisms underlying atherogenic potential of Lp(a) in both diabetic and nondiabetic individuals remain to be defined.

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