LDL Particle Size in Relation to Insulin, Proinsulin, and Insulin Sensitivity

The Insulin Resistance Atherosclerosis Study

ANDREAS FESTA, MD
RALPH D'AGOSTINO JR., PHD
LEENA MYKKÄNEN, MD, PHD
RUSSELL P. TRACY, PHD
C. NICK HALES, MD, PHD
BARBARA V. HOWARD, PHD
STEVEN M. HAFFNER, MD

OBJECTIVE — LDL particles are heterogeneous in terms of size and density; small dense LDL particles are considered more atherogenic than larger LDL particles. The aim of this study was to investigate the interrelationships among LDL size, insulin, proinsulin (intact and split), and insulin sensitivity in a tri-ethnic population with varying degrees of glucose tolerance (n = 1,549) in the Insulin Resistance Atherosclerosis Study.

RESEARCH DESIGN AND METHODS — Insulin sensitivity was assessed by a frequently sampled intravenous glucose tolerance test with minimal model analysis. Proinsulin levels were measured using highly sensitive assays without detectable cross-reactivity with insulin, and LDL size was determined by gradient-gel electrophoresis.

RESULTS — In univariate analyses, LDL size was related to various features of the insulin resistance syndrome, including fasting insulin ($r = -0.18$), intact proinsulin ($r = -0.24$), split proinsulin ($r = -0.24$), the proinsulin-to-insulin ratio ($r = -0.14$), and insulin sensitivity ($r = 0.21$; all $P < 0.0001$). In a multivariate regression model (adjusted for age, BMI, ethnicity, and clinic), triglyceride levels ($P = 0.0001$), HDL cholesterol ($P = 0.0001$), sex ($P = 0.002$), and proinsulin ($P = 0.01$) were significantly related to LDL size. In the same model stratified by sex, LDL size was significantly inversely related to proinsulin in men ($P = 0.005$ and $P = 0.04$ after further adjustment for the glucose tolerance status), but not in women ($P > 0.15$).

CONCLUSIONS — We found an inverse relation of proinsulin to LDL particle size in a large tri-ethnic population with varying degrees of glucose tolerance. This relation was independent of age, BMI, and triglyceride and HDL cholesterol concentrations, and was more pronounced in men than in women.

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From the Department of Medicine (A.F., L.M., S.M.H.), Division of Clinical Epidemiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; the Department of Public Health Sciences (R.D.), Wake Forest University School of Medicine, Winston Salem, North Carolina; the Department of Pathology (R.T.), University of Vermont School of Medicine, Burlington, Vermont; the Medlantic Research Institute (B.V.H.), Washington, DC; and the Department of Clinical Biochemistry (C.N.H.), University of Cambridge, Cambridge, U.K.

Address correspondence and reprint requests to Andreas Festa, MD, Department of Medicine, Division of Clinical Epidemiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, Texas 78284-7873. E-mail: festa@uthscsa.edu.

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Abbreviations: CV, coefficient of variation; FSIGT, frequently sampled intravenous glucose tolerance test; IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study; NGT, normal glucose tolerance; PI/I ratio, proinsulin-to-insulin ratio; S_I, insulin sensitivity index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

LDL particles are heterogeneous in terms of size and density (1–3). Small dense LDL particles are considered to be more atherogenic than larger LDL particles (3–5) and have been associated with most individual components of the insulin resistance syndrome, including hypertriglyceridemia (3–9), low serum HDL cholesterol (3,6–9), hypertension (7,8), and diabetes (7,9,10). However, studies dealing with the relation of LDL size and insulinemia (6–9,11–17) or direct measures of insulin sensitivity (8,13,15,17,18) have yielded controversial results. Fasting insulin was a significant contributor to LDL size in nondiabetic subjects (6,7,11,12), whereas in type 2 diabetes, no association could be shown (13,14). An association of insulin sensitivity, as determined by clamp techniques (8,13,15,17) or by a frequently sampled intravenous glucose tolerance test (FSIGT) (18), to LDL size was found in three studies (8,13,15), while two others failed to demonstrate such a relationship (17,18).

With the availability of highly specific insulin assays recognizing proinsulin, it has become possible to study the contribution of "true" insulin relative to its precursors (intact and split proinsulin) to various components of the insulin resistance syndrome. Under physiological conditions, only small amounts of intact and split proinsulin are co-secreted with insulin from the pancreatic β-cell; in type 2 diabetes, however, proinsulin and proinsulin split products can be markedly elevated (19). The biological significance of these insulin conversion intermediates is still a matter of debate (20). The glucose-lowering potency of proinsulin is considerably less compared with that of insulin (20); however, a greater effect of proinsulin on hepatic than on peripheral tissue relative to insulin has been reported (21). In a small study, treatment with proinsulin resulted in an unexplained high rate of fatal and nonfatal cardiovascular events (22). Data on the relation between insulin precursors and LDL size are scarce and conflicting. In a previous study, we have shown in nondiabetic subjects an association of LDL size with proinsulin that was somewhat stronger than that of specific fasting

insulin (11). In a study from Tan et al. (14), 32-33 split proinsulin, but not fasting insulin or intact proinsulin, was significantly related to LDL size in patients with NIDDM. Finally, sex-related differences in LDL size and contributing factors have been shown previously (8,10,11,16).

The aim of this study was to investigate the interrelationships of LDL size, insulin, proinsulin (intact and split), and insulin sensitivity in a tri-ethnic population with varying degrees of glucose tolerance (n = 1,549) in the Insulin Resistance Atherosclerosis Study (IRAS).

RESEARCH DESIGN AND METHODS

— IRAS is a multicenter epidemiologic study aiming to explore relationships between insulin resistance, cardiovascular risk factors, and disease across different ethnic groups and varying states of glucose tolerance. A full description of the design and methods of the IRAS has been published (23). In brief, this study was conducted at four clinical centers. Clinical centers in Oakland and Los Angeles, CA, studied non-Hispanic whites and African-Americans recruited from Kaiser Permanente, a nonprofit health maintenance organization. Clinical centers in San Antonio, TX, and San Luis Valley, CO, studied non-Hispanic whites and Hispanics recruited from two ongoing population-based studies (the San Antonio Heart Study [24] and the San Luis Valley Diabetes Study [25]). Recruitment was tailored to yield approximately equal numbers of participants by ethnicity, sex, and glucose tolerance categories (type 2 diabetes, impaired glucose tolerance [IGT], and normal glucose tolerance [NGT]).

A total of 1,625 individuals participated in the IRAS. This report includes data on 1,549 subjects, in whom LDL particle size and proinsulin concentrations were available for analyses. Demographic and metabolic data are shown in Table 1. Race and ethnicity were assessed by self-report. Hispanic ethnicity was defined by the U.S. census question "Are you of Spanish or Hispanic descent?" Height, weight, and girths (minimum waist and hips) were measured following a standardized protocol. BMI (weight in kilograms divided by the square of height in meters) was used as an estimate of overall adiposity. The ratio of waist-to-hip circumferences was used as an estimate of body fat distribution. The IRAS examination required two visits. Patients were asked to fast for 12 h before each visit, to abstain from heavy exercise and alcohol for 24 h, and to refrain from smoking on the morning of the examination.

For the oral glucose tolerance test, a 75-g glucose load (Orangedex; Customs Laboratories, Baltimore, MD) was administered over a period of <10 min. Blood was drawn immediately before ingestion and 2 h after the glucose load. Glucose tolerance status was based on World Health Organization criteria (26). Glucose and insulin levels in all samples were measured at the central IRAS laboratory at the University of Southern California, Los Angeles, CA. Plasma glucose was measured with the glucose oxidase technique on an automated autoanalyzer (Yellow Springs Equipment, Yellow Springs, OH). Insulin was measured using the dextran-charcoal radioimmunoassay (27). This insulin assay cross-reacts with proinsulin. The split pair coefficient of variation (CV) for the insulin radioimmunoassay was 19% (n = 163).

Fasting serum intact proinsulin and split proinsulin were determined from samples stored at −70°C for an average of 3.3 years (35–44 months) by means of highly specific two-site monoclonal antibody–based immunoradiometric assays (28). The split-pair CV was 14% for proinsulin (n = 98) and 18% for split proinsulin (n = 98). There was no detectable cross-reactivity of insulin or split proinsulin in the intact proinsulin assay. Insulin did not significantly cross-react in the assay for split proinsulin, and the cross-reactivity of intact proinsulin in this assay was 84%. Assay values of split proinsulin were corrected for this by subtracting the corresponding proinsulin cross-reactivity. The assay of split proinsulin cross-reacts equally with 32-33, des-32, and des-31-32 split proinsulins. We used the term split proinsulin to indicate the sum of these three molecules, the majority of which are des-31-32 split proinsulin (29). The sensitivity limit of the intact proinsulin and split proinsulin assays was 1.25 pmol/l (3 SDs from zero). Intact proinsulin and split proinsulin were determined at the laboratory of the Department of Clinical Biochemistry at Addenbrook's Hospital, Cambridge, U.K.

Insulin sensitivity was assessed by an FSIGT (30) with minimal model analysis (31). Two modifications of the original protocol were used. An injection of regular insulin, rather than tolbutamide, was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance (32) including diabetic patients because of their blunted or absent insulin response. In addition, the reduced sampling protocol (which required 12 rather than 30 plasma samples and shows results similar to the full protocol [33]) was used because of the large number of subjects. Glucose in the form of a 50% solution (0.3 g/kg body wt) and regular human insulin (0.03 U/kg body wt) were injected through an intravenous line at 0 and 20 min, respectively. Blood was collected at −5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 min for plasma glucose and insulin concentrations. Insulin sensitivity, expressed as the insulin sensitivity index (S_I), was calculated by mathematical modeling methods (MINMOD, version 3.0, 1994). This modified version of the FSIGT protocol used in the IRAS has been compared with the hyperinsulinemic-euglycemic clamp and shown to be a valid measure of insulin resistance (34).

Plasma lipoprotein measurements were obtained from fasting single fresh plasma samples using Lipid Research Clinic methods. Plasma lipoproteins were measured at the central IRAS laboratory at Medlantic Research Institute, Washington, DC. After preparative ultracentrifugation, VLDL ($d <$ 1.006 g/ml) and $d >$ 1.006 g/ml fractions were measured for cholesterol and triglyceride concentrations. HDL cholesterol was measured in the presence of $MnCl_2$ and heparin in which non-HDL lipoproteins were precipitated, leaving HDL in the supernatant (35). The supernatant was removed after centrifugation, and the cholesterol content was measured on a separate autoanalyzer channel set to measure low cholesterol values (Cholesterol/HP; Boehringer Mannheim, Mannheim, Germany). LDL cholesterol was calculated as the difference between the HDL cholesterol and the >1.006 g/ml fraction of cholesterol. In the present study, no attempt was made to separate LDL from IDL. Triglycerides were measured enzymatically after correction for free glycerol (Triglycerides/GB; Boehringer Mannheim).

LDL size distribution (i.e., distribution of diameter of the major LDL peak for each participant) was determined using the method of Krauss and Burke (36). Gradient gels were obtained from Isolab (Akron, OH). Measurement of the size of the predominant peak was calibrated using LDL subfractions whose molecular diameter was determined by analytical ultracentrifugation (courtesy of Dr. R. Krauss, Donner Laboratories, Berkeley, CA). The LDL size of the predominant peak for an individual was

Table 1—*Demographic and metabolic characteristics of study subjects by sex*

	Women	Men	*P* value
n	857	692	—
Ethnicity (NHW/AA/H) (%)	35/29/36	41/28/31	—
Glucose tolerance status (NGT/IGT/type 2 diabetes) (%)	43/25/32	46/20/34	—
Age (years)	55.6 ± 0.3	55.7 ± 0.3	NS
BMI (kg/m^2)	30.2 ± 0.2	28.6 ± 0.2	0.0001
Waist circumference (cm)	90.5 ± 0.4	97.2 ± 0.5	0.0001
Waist-to-hip ratio	0.83 ± 0.002	0.94 ± 0.002	0.0001
Fasting glucose (mmol/l)	6.7 ± 0.1	7.0 ± 0.1	0.038
Fasting insulin (pmol/l)	109.2 ± 3.0	111.0 ± 3.6	0.0001
Proinsulin (pmol/l)	9.31 ± 0.42	11.4 ± 0.46	0.0007
Split proinsulin (pmol/l)	12.5 ± 0.5	14.6 ± 0.6	0.008
PI/I ratio	0.093 ± 0.005	0.12 ± 0.005	0.0007
Insulin sensitivity $S_I \times 10^{-4}$ ($min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$)	1.66 ± 0.07	1.63 ± 0.07	NS
Triglyceride (mmol/l)	1.63 ± 0.05	1.85 ± 0.05	0.0011
Total cholesterol (mmol/l)	5.57 ± 0.04	5.40 ± 0.04	0.0025
HDL cholesterol (mmol/l)	1.27 ± 0.01	1.04 ± 0.01	0.0001
LDL cholesterol (mmol/l)	3.69 ± 0.04	3.61 ± 0.04	NS
LDL size (Å)	261.2 ± 0.3	257.8 ± 0.4	0.0001

Data are means ± SEM unless otherwise indicated. *P* values are from *t* test. AA, African-Americans; H, Hispanics; NHW, non-Hispanic whites.

defined as that person's LDL size (10). The CV for LDL size from 133 blind split duplicates was 2%.

Statistical analysis

Statistical analyses were performed using the SAS statistical software system (SAS, Cary, NC). Descriptive statistics by sex are shown in Table 1; differences by sex were calculated with Student's *t* test. Unadjusted Spearman rank correlations of LDL size with measures of body fat and metabolic variables were calculated for the overall population (Table 2). Additionally, correlations of LDL size were stratified by sex (Table 3), glucose tolerance status, and ethnicity. Furthermore, we tested for interactions between LDL size and sex, ethnicity, and glucose tolerance status on the association with fasting insulin, intact proinsulin, split proinsulin, the proinsulin-to-insulin ratio (PI/I ratio), and insulin sensitivity (S_I) by calculating the respective interaction terms (sex × fasting insulin, sex × proinsulin, etc.). The interaction models were performed on ranked data analogous to the correlation analyses. Interaction was adjusted for age, clinic and/or sex, and/or ethnicity, and/or diabetic status and, in a second model, additionally for metabolic variables (BMI, waist-to-hip ratio, triglyceride, and HDL cholesterol). We found no significant interaction of ethnicity or glucose tolerance status on the relation of LDL size with any of the variables of interest; therefore, analyses with ethnic groups as well as diabetic and nondiabetic subjects pooled together are presented in this report. Stepwise multiple linear regression models were performed with LDL size as the dependent variable and with independent variables of interest (triglyceride, HDL cholesterol, fasting insulin, proinsulin [intact and split], PI/I ratio, and insulin sensitivity). These models were adjusted for age, BMI, ethnicity, and clinic (Table 4). Because intact proinsulin and split proinsulin were highly correlated, we fit separate models that included one or the other variable (but not both) to avoid collinearity problems. *P* values <0.05 (two-sided) were considered statistically significant.

RESULTS — LDL size was smaller in men compared with women (Table 1) and was correlated with measures of body fat and most metabolic variables included in the insulin resistance syndrome (Table 2). LDL size was, furthermore, more strongly related to measures of central obesity, such as waist-to-hip ratio ($r = -0.25$) and waist circumference ($r = -0.19$) than to general obesity, as measured by BMI ($r = -0.09$). In this univariate analysis, the association was most pronounced with triglycerides ($r = -0.47$) and HDL cholesterol ($r = 0.38$).

In men, LDL size was more closely associated with proinsulin (intact and split as well as the PI/I ratio) than it was in women (Table 3). When testing for sex interactions, the relationship between the PI/I ratio and LDL size was significantly stronger in men than in women ($P = 0.03$ for interaction). This interaction was more pronounced ($P = 0.007$) when accounting for confounding variables previously shown to be associated with LDL size (BMI, waist-to-hip ratio, triglyceride, HDL cholesterol). In subjects with type 2 diabetes (LDL size vs. PI/I ratio $r = -0.06$, NS in NGT; $r = -0.03$, NS in IGT; and $r = -0.12$, $P < 0.007$ in type 2 diabetes) and in African-Americans (data not shown), LDL size was somewhat more closely associated with proinsulin (intact and split as well as the PI/I ratio) compared with fasting insulin. However, there was no significant interaction of ethnicity or glucose tolerance status on the relation of LDL size with any of the variables of interest, including proinsulin.

In a stepwise multivariate regression model (after adjusting for age, BMI, ethnicity, and clinic), triglyceride ($P = 0.0001$), HDL cholesterol ($P = 0.0001$), sex ($P = 0.002$), and proinsulin ($P = 0.01$) were significantly related to LDL size in the overall population. Results were similar with split proinsulin instead of intact proinsulin in the model. Regression analysis stratified by sex (Table 4) showed that triglyceride and HDL cholesterol levels were significantly related to LDL size in both women

Table 2—*Spearman correlation analysis of LDL size with measures of body fat and metabolic variables in the overall population*

	LDL size
BMI	−0.09*
Waist-to-hip ratio	−0.25†
Waist circumference	−0.19†
Triglyceride	−0.47†
Total cholesterol	−0.07‡
LDL cholesterol	0.01
HDL cholesterol	0.38†
Fasting glucose	−0.17†
Fasting insulin	−0.18†
Intact proinsulin	−0.24†
Split proinsulin	−0.24†
PI/I ratio	−0.14†
S_I	0.21†

$n = 1,549$. *$P < 0.001$, †$P < 0.0001$, ‡$P < 0.01$.

Table 3—*Spearman correlation analysis of LDL size stratified by sex*

	Female	*P*	Male	*P*
n	857	—	692	—
Fasting insulin	−0.18	0.0001	−0.18	0.0001
Intact proinsulin	−0.19	0.0001	−0.27	0.0001
Split proinsulin	−0.21	0.0001	−0.27	0.0001
PI/I ratio	−0.06	NS	−0.19	0.0001
S_I	0.24	0.0001	0.19	0.0001

and in men ($P = 0.0001$). Additional independent variables significantly related to LDL size were S_I in women ($P = 0.026$) and proinsulin in men ($P = 0.0052$). Further adjustment for the glucose tolerance status diminished the effect of S_I on LDL size in women ($P = 0.09$), whereas the inverse relation of proinsulin to LDL size in men remained statistically significant ($P = 0.04$).

CONCLUSIONS — In this study, we have shown an inverse relation of proinsulin concentration to LDL size in a large tri-ethnic population with varying degrees of glucose tolerance. The association was independent of ethnicity, glucose tolerance status, and confounding variables previously shown to be related to LDL size, such as serum triglyceride (3–16), HDL cholesterol (3,6–16), and measures of body fat (7,8,10,12,15,16) in men but not in women. LDL size was also associated with fasting insulin concentration and insulin sensitivity, but this association was largely explained by demographic and metabolic covariates.

Fasting insulinemia (37,38) as well as insulin resistance (39–41) has been linked to atherogenesis and/or its clinical sequelae; however, the underlying mechanisms are still poorly understood. It is not clear whether, in this context, fasting insulin stands as a proxy for insulin sensitivity, or whether the relationship with atherosclerosis is directly mediated by biological effects of insulin per se. With the advent of specific assays, it has become possible to discern effects of "true" insulin from those of its precursors (intact proinsulin and proinsulin split products) cosecreted with insulin from the pancreatic β-cell (20). The relative contribution of insulin, its precursors, and insulin sensitivity to various cardiovascular risk factors has therefore attracted much interest recently.

In the present study, neither fasting insulin nor insulin sensitivity, as measured by a FSIGT, were related to LDL size independently of confounding metabolic variables, namely serum triglyceride and HDL cholesterol levels. This is in accordance with previous studies (8,13,15,17,18). No independent relation between LDL size and insulin sensitivity was found in diabetic and nondiabetic subjects (17) or in healthy male subjects (18). Stewart et al. (13) found a relation in univariate analysis in diabetic subjects only, but no multivariate analysis was reported. In 100 nondiabetic subjects, the relation of LDL particle size to insulin resistance was significantly mediated by triglyceride concentration (8). Only in a distinct subgroup of individuals (middle-aged, nondiabetic men with mildly elevated triglyceride levels) has a relation of LDL size and insulin resistance independent of triglyceride and HDL cholesterol levels been reported (15). These results suggest that the effect of insulin resistance on LDL particle size is largely explained by prevailing serum triglyceride and HDL cholesterol levels. In the present study, the relation of insulin sensitivity to LDL size was somewhat more pronounced in women than in men. In previous studies, women were included in only two (n = 52 [8] and n = 3 [17]), and no subgroup analyses were reported.

Results of the present study further suggest that LDL size may be more strongly related to insulin precursors rather than insulin. Accordingly, split proinsulin, but not fasting insulin, was related to the presence of small dense LDL particles in type 2 diabetic men (14). In nondiabetic subjects from the San Antonio Heart Study, LDL size was also independently inversely associated not only to fasting insulin, but also to proinsulin and the PI/I ratio, the latter suggesting a predominant role of proinsulin relative to specific insulin (11).

The discussion of potential mechanisms linking insulin precursors to LDL size remains speculative. Even under physiological conditions, factors determining LDL particle size are not entirely understood. It has been suggested that LDL particle size results mainly from neutral lipid transfer and triglyceride hydrolysis (42). In states of hypertriglyceridemia, such as type 2 diabetes, transfer of cholesteryl ester and triglyceride between VLDL and LDL will be enhanced, resulting in triglyceride-enriched cholesteryl-ester depleted LDL particles. These LDL species are susceptible to the action of hepatic lipase, which hydrolyzes LDL triglycerides, resulting in the formation of small dense LDL particles. Hepatic and lipoprotein lipase activity are further determinants of LDL size (43), both of which are altered in diabetic patients (44). In these patients, lipoprotein lipase activity is commonly decreased, whereas hepatic lipase activity, which facilitates the formation of small dense LDL, is increased (44). Serum proinsulin concentrations are elevated in

Table 4—*Stepwise multiple regression analysis stratified by sex with LDL size as the dependent variable*

Sex	*n*	Independent variable	Regression coefficient	Standard error of regression coefficient	*P* value	Partial R^2 (%)
Female	857	Triglyceride	−3.26	0.44	0.0001	12.4
		HDL cholesterol	3.48	1.16	0.0001	2.20
		S_I	0.47	0.2	0.026	0.54
		R^2 for the model = 19.6%				
Male	692	Triglyceride	−2.55	0.35	0.0001	14.8
		HDL cholesterol	5.41	1.20	0.0001	2.71
		Proinsulin	−0.073	0.04	0.0052	1.01
		R^2 for the model = 21.9%				

Models are adjusted for age, BMI, ethnicity, and clinic (forced into the model). Independent variables of interest (triglyceride, HDL cholesterol, fasting insulin, proinsulin [intact or split], PI/I ratio, and S_I) were entered in a stepwise approach. Only variables significant at the 0.15 level were finally left in the model, and variables significant at the 0.05 level are shown in the table.

subjects with type 2 diabetes (19,45). Therefore, proinsulin might contribute to one or several of the mechanisms controlling LDL particle size. Although the biological potency of proinsulin is only ~10% that of insulin in terms of its glucose-lowering effect (20), its potency may be considerably higher in terms of other metabolic or pathophysiologic pathways. A greater effect of proinsulin on hepatic than on peripheral tissue relative to insulin has been reported (21). Long-term treatment with proinsulin resulted in a disproportionately increased rate of fatal and nonfatal cardiovascular events in a small study (22). No sound explanation for this unexpected finding has yet been offered. Treatment with proinsulin, compared with human insulin, did not specifically modify serum lipids (46); however, no data on the effect of therapeutically used proinsulin on LDL size has been reported. Further, increased serum levels of proinsulin as well as an increased PI/I ratio have been associated with decreased insulin secretion, thus signaling defective β-cell function (47). An intact β-cell function might be crucial in regulating LDL size. In a previous study, insulin secretion, as determined by the 30-min increment of insulin during an oral glucose tolerance test, was closely related to the suppression of plasma nonesterified fatty acids, which was, in turn, the most important determinant of plasma triglyceride concentration (48). These findings provide a link between insulin secretion and triglyceride concentration, which is the most important single known factor determining LDL particle size.

We also found sex differences in variables associated with LDL size. Only in men, was proinsulin significantly related to LDL size independently of the glucose tolerance status. In women, the relation of S_I to LDL size diminished after accounting for the glucose tolerance status. The clinical implications of this finding, however, remain to be determined. In accordance with the present study, smaller LDL size in male subjects has been reported previously (8,10,11,16,49). The presence of diabetes seems to diminish this sex difference. In a large population of American Indians, LDL size was smaller in nondiabetic men compared with nondiabetic women, whereas there was no sex difference in subjects with diabetes (16). Likewise, in another study, there were no sex differences in the proportion of small dense LDL particles in the presence of type 2 diabetes (49). In a bi-ethnic population of Mexican Americans and non-Hispanic whites, only in women, and not in men, was LDL size associated with diabetes independently of obesity, body fat distribution, and triglyceride and HDL cholesterol levels (10). Thus, there is increasing evidence that there is indeed a sex difference in determinants of LDL size and the preponderance of small dense LDL. It has been suggested (10,49) that these differences might account for the greater relative risk of cardiovascular disease observed in women compared with men in type 2 diabetes (50,51).

In summary, we found an inverse relation of proinsulin to LDL particle size across the three ethnic groups of the IRAS (non-Hispanic whites, African-Americans, Hispanics) and also across varying degrees of glucose tolerance. This relation was independent of age, BMI, and triglyceride and HDL cholesterol concentrations and was more pronounced in men than in women. However, proinsulin was a substantially weaker determinant of LDL particle size than triglyceride concentrations, the most significant known determinant of LDL particle size.

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References

1. Shen MMS, Krauss RM, Lindgren FT, Forte TM: Heterogeneity of serum low density lipoproteins in normal human subjects. *J Lipid Res* 22:236–244, 1981
2. Krauss RM, Burke DJ: Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res* 23:97–104, 1982
3. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM: Low density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 260: 1917–1921, 1988
4. Campos H, Genest JJ Jr, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, Wilson PW, Schaefer EJ: Low density lipoprotein particle size and coronary artery disease. *Arterioscler Thromb* 12:187–195, 1992
5. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Després JP: Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men: prospective results from the Québec Cardiovascular Study. *Circulation* 95:69–75, 1997
6. Haffner SM, Mykkänen L, Valdez RA, Paidi M, Stern MP, Howard BV: Low density lipoprotein size and subclass pattern in a biethnic population. *Arterioscler Thromb* 13:1623–1630, 1993
7. Selby JV, Austin MA, Newman B, Zhang D, Quesenberry CP Jr, Mayer EJ, Krauss RM: LDL subclass pattern and the insulin resistance syndrome in women. *Circulation* 88:381–387, 1993
8. Reaven GM, Chen YDI, Jeppesen J, Maheux P, Krauss RM: Insulin resistance and hyperinsulinaemia in individuals with small dense low density lipoprotein particles. *J Clin Invest* 92:141–146, 1993
9. Barakat HA, Carpenter JW, McLendon VD, Khazanie P, Leggett N, Heath J, Marks R: Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition: possible link between hyperinsulinaemia and atherosclerosis. *Diabetes* 39:1527–1533, 1990
10. Haffner SM, Mykkänen L, Stern MP, Paidi M, Howard BV: Greater effect of diabetes on LDL size in women than in men. *Diabetes Care* 17:1164–1171, 1994
11. Haffner SM, Mykkänen L, Robbins D, Valdez R, Miettinen H, Howard BV, Stern MP, Bowsher R: A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non-diabetic subjects. *Diabetologia* 38:1328–1336, 1995
12. Tchernof A, Lamarche B, Prud'homme D, Nadeau A, Moorjani S, Labrie F, Lupien PJ, Després JP: The dense LDL phenotype: association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. *Diabetes Care* 19:629–637, 1996
13. Stewart MW, Laker MF, Dyer RG, Game F, Mitcheson J, Winocour PH, Alberti KGMM: Lipoprotein compositional abnormalities and insulin resistance in type II diabetic patients with mild hyperlipidemia. *Arterioscler Thromb* 13:1046–1052, 1993
14. Tan KCB, Cooper MB, Ling KLE, Griffin BA, Freeman DJ, Packard CJ, Shepherd J, Hales CN, Betteridge DJ: Fasting and postprandial determinants for the occurrence of small dense LDL species in non-insulin-dependent diabetic patients with and without hypertriglyceridaemia: the involvement of insulin, insulin precursor species and insulin resistance. *Atherosclerosis* 113:273–287, 1995
15. Mykkänen L, Haffner SM, Rainwater DL, Karhapää P, Miettinen H, Laakso M: Relationship of LDL size to insulin sensitivity in normoglycemic men. *Arterioscler Thromb Vasc Biol* 17:1447–1453, 1997
16. Gray RS, Robbins DC, Wang W, Yeh JL, Fabsitz RR, Cowan LD, Welty TK, Lee ET, Krauss RM, Howard BV: Relation of LDL size to the insulin resistance syndrome and coronary heart disease in American Indians: the Strong Heart Study. *Arterioscler Thromb*

Vasc Biol 17:2713–2720, 1997
17. Lahdenperä S, Sane T, Vuorinen-Markkola H, Knudsen P, Taskinen MR: LDL particle size in mildly hypertriglyceridemic subjects: no relation to insulin resistance or diabetes. *Atherosclerosis* 113:227–236, 1995
18. Slyper AH, Zvereva S, Schectman G, Hoffmann RG, Mueller RA, Walker JA: Insulin resistance is not a major determinant of low-density lipoprotein particle size. *Metabolism* 46:1275–1280, 1997
19. Temple RC, Carrington CA, Luzio SD, Owens DR, Schneider AE, Sobey WJ, Hales CN: Insulin deficiency in non-insulin-dependent diabetes. *Lancet* i:293–295, 1989
20. Galloway JA, Hooper SA, Spradlin CT, Howey DC, Frank BH, Bowsher RR, Anderson JH: Biosynthetic human proinsulin: review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience. *Diabetes Care* 15:666–692, 1992
21. Revers RR, Henry R, Schmeiser L, Kolterman O, Cohen R, Bergenstal R, Polonsky K, Jaspan J, Rubenstein A, Frank B, Galloway J, Olefsky JM: The effects of biosynthetic human proinsulin on carbohydrate metabolism. *Diabetes* 33:762–770, 1984
22. Spradlin CT, Galloway JA, Anderson JH: Apparent increase in coronary heart disease with human proinsulin (HPI): randomization failure or HPI effect? (Abstract) *Diabetologia* 33 (Suppl. 1):A60, 1990
23. Wagenknecht LE, Mayer EJ, Rewers M, Haffner SM, Selby J, Borok GM, Henkin L, Howard G, Savage PJ, Saad MF, Bergman RN, Hamman R: The Insulin Resistance Atherosclerosis Study (IRAS): objectives, design and recruitment results. *Ann Epidemiol* 5:464–471, 1995
24. Stern MP, Rosenthal M, Haffner SM, Hazuda HP, Franco LJ: Sex difference in the effects of sociocultural status on diabetes and cardiovascular risk factors in Mexican Americans: the San Antonio Heart Study. *Am J Epidemiol* 120:834–851, 1984
25. Hamman RF, Marshall JA, Baxter J, Kahn LB, Mayer EJ, Orleans M, Murphy JR, Lezotte DC: Methods and prevalence of non-insulin dependent diabetes mellitus in a biethnic Colorado population: the San Luis Valley Diabetes Study. *Am J Epidemiol* 129:295–311, 1989
26. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
27. Herbert V, Lau K, Gottlieb C, Bleicher S: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
28. Sobey WJ, Beer SF, Carrington CA, Clark PM, Frank BH, Gray IP, Luzio SD, Owens DR, Schneider AE, Siddle K, Temple RC, Hales CN: Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulin. *Biochem J* 260:535–541, 1989
29. Ostrega D, Polonsky K, Nagi D, Yudkin J, Cox LJ, Clark PMS, Hales CN: Measurement of proinsulin and intermediates: validation of immunoassay methods by high-performance liquid chromatography. *Diabetes* 44:437–440, 1995
30. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. *Endocr Rev* 6:45–86, 1985
31. Pacini G, Bergman RN: MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed* 23:113–122, 1986
32. Welch S, Gebhart SSP, Bergman RN, Phillips LS: Minimal model analysis of intravenous glucose tolerance test derived insulin sensitivity in diabetic subjects. *J Clin Endocrinol Metab* 71:1508–1518, 1990
33. Steil GM, Volund A, Kahn SE, Bergman RN: Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model: suitability for use in population studies. *Diabetes* 42:250–256, 1993
34. Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YDI, Sands RE, Pei D, Savage PJ, Bergman RN: A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Diabetes* 43:1114–1121, 1994
35. Bachorik PS, Albers JJ: Precipitation methods for quantification of lipoproteins. *Meth ods in Enz* 129:78–100, 1960
36. Krauss RM, Burke DJ: Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res* 23:97–104, 1982
37. Pyörälä K, Savolainen E, Kaukola S, Haapakoski J: Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during $9^{1/2}$ year follow-up of the Helsinki Policeman Study population. *Acta Med Scan* 701 (Suppl. 1):38–52, 1985
38. Després JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ: Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 334:952–957, 1996
39. Howard G, O'Leary DH, Zaccaro D, Haffner SM, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R: Insulin sensitivity and atherosclerosis. *Circulation* 93:1809–1817, 1996
40. Bressler P, Bailey SR, Matsuda M, DeFronzo RA: Insulin resistance and coronary artery disease. *Diabetologia* 39:1345–1350, 1996
41. Shinozaki K, Hattori Y, Suzuki M, Hara Y, Kanazawa A, Takaki H, Tsushima M, Harano Y: Insulin resistance as an independent risk factor for carotid artery wall intima media thickening in vasospastic angina. *Arterioscler Thromb Vasc Biol* 17: 3302–3310, 1997
42. Lagrost L, Gambert P, Lallemant C: Combined effects of lipid transfers and lipolysis on gradient gel patterns of human plasma LDL. *Arterioscler Thromb* 14:1327–1336, 1994
43. Lahdenperä S, Syvänne M, Kahri J, Taskinen M-R: Regulation of low-density lipoprotein particle size distribution in NIDDM and coronary disease: importance of serum triglycerides. *Diabetologia* 39:453–461, 1996
44. Taskinen M-R: Insulin resistance and lipoprotein metabolism. *Curr Opin Lipidol* 6:153–160, 1995
45. Yoshioka N, Kuzuya T, Matsuda A, Taniguchi M, Iwamoto Y: Serum proinsulin levels at fasting and after oral glucose load in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 31:355–360, 1988
46. Huisman J, Oolbekkink M, Heine RJ, van der Veen EA, Erkelens DW: Effects of biosynthetic human proinsulin on serum lipids and glycaemic control in non-insulin-dependent diabetes mellitus. In *Proinsulin: Pharmacokinetic and Clinical Aspects*. van der Veen EA, Erkelens DW, Eds. Utrecht, Tessel Offset BV, 1989, p. 83–94
47. Røder ME, Porte D Jr, Schwartz RS, Kahn SE: Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with non-insulin dependent diabetes mellitus. *J Clin Endocrinol Metab* 83:604–608, 1998
48. Byrne CD, Wareham NJ, Brown DC, Clark PMS, Cox LJ, Day NE, Palmer CR, Wang TWM, Williams DRR, Hales CN: Hypertriglyceridaemia in subjects with normal and abnormal glucose tolerance: relative contributions of insulin secretion, insulin resistance and suppression of plasma non-esterified fatty acids. *Diabetologia* 37: 889–896, 1994
49. Caixàs A, Ordóñez-Llanos J, de Leiva A, Payés A, Homs R, Pérez A: Optimization of glycemic control by insulin therapy decreases the proportion of small dense LDL particles in diabetic patients. *Diabetes* 46:1207–1213, 1997
50. Garcia MJ, McNamara PM, Gordon T, Kannel WB: Morbidity and mortality in diabetics in the Framingham population: sixteen-year follow-up. *Diabetes* 23: 105–111, 1974
51. Barrett-Connor E, Wingard DL: Sex difference in ischemic heart disease mortality in diabetics: a prospective population-based study. *Am J Epidemiol* 118:489–496, 1983