Low-Density Lipoprotein Particle Size, Insulin Resistance, and Proinsulin in a Population Sample of 58-Year-Old Men

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The relationship between insulin sensitivity and low-density lipoprotein (LDL) peak particle size was examined in 104 clinically healthy 58-year-old men recruited from the general population. Insulin sensitivity was measured by the euglycemic hyperinsulinemic clamp method with adjustment for lean body mass. LDL peak particle size was determined by gradient gel electrophoresis, and insulin, proinsulin, and 32,33 split proinsulin were determined by 2-site immunoradiometric assays. The results showed that 16 subjects (15%) had pattern B, with a predominance of small LDL particles. These cases and a small LDL peak particle size were characterized by the features of the insulin resistance syndrome, ie, general and central obesity, elevated diastolic blood pressure, low serum concentrations of high-density lipoprotein (HDL) and apolipoprotein A_1 (apoA_1), increases in serum triglycerides and circulating insulin peptides, and low insulin-mediated glucose uptake. The correlation between insulin sensitivity and LDL peak particle size was significant ($r = .33$, $P = .001$) and independent of obesity. In a traditional multiple regression analysis, LDL peak particle size was independently associated not with insulin-mediated glucose uptake but with circulating triglycerides and HDL cholesterol, which together explained 67% of the variability in LDL particle size ($P = .000$). Of all insulin peptides, only proinsulin showed an independent relation to LDL peak particle size, but it disappeared after adjustment for other variables. We conclude that a small LDL particle size was associated with insulin resistance among these clinically healthy men, but this was not independent of serum triglycerides and HDL cholesterol. Serum proinsulin was more directly related to LDL particle size than insulin.

LOW-DENSITY LIPOPROTEIN (LDL) particles consist of a number of subspecies that vary in size, density, and composition. A classification of LDL subclasses based on gradient gel electrophoretic patterns has been established during recent years.[1] Pattern A is mainly characterized by a predominance of large buoyant LDL particles, whereas pattern B has a predominance of small LDL particles. Subjects with a preponderance of such small LDL particles seem to have an increased risk of coronary heart disease.[2,3]

The LDL particle size is both genetically influenced[4,5] and related to life-style factors such as diet and exercise.[3] Small dense LDL particles have been associated with most individual components in the insulin resistance syndrome such as hypertriglyceridemia, low serum high-density lipoprotein (HDL) cholesterol, hypertension, diabetes, visceral obesity, and hyperinsulinemia.[6,7]

The early observation by Reaven et al[8] that small LDL particles may be related to insulin resistance has not been a consistent finding in later studies. Of 8 studies, 4 were positive,[8-11] 1 showed statistically significant positive results in a healthy subgroup,[12] and 3 were negative.[13-15] The study populations have varied from diabetics and healthy controls,[12-14] to healthy subjects,[9-11,15] to randomly selected population samples.[9] Several studies have excluded subjects with serum triglycerides or blood pressure above a specified level[9,12-14] or with obesity.[12] Four studies used the euglycemic-hyperinsulinemic clamp method[9,10,12,14] to determine insulin sensitivity, whereas other methods were used in the other studies.[8,11,13,15]

Hyperinsulinemia is both a feature of insulin resistance and a factor related to small LDL particles.[6,16] The pancreatic β cell cosecretes C-peptide, intact proinsulin and its conversion intermediates, ie, 31,32 split proinsulin, together with insulin. The biologic activity is lower for proinsulin versus insulin, but proinsulin has a longer half-life and may have a stronger impact on risk factors for cardiovascular disease than intact insulin.[16-20] The increased β-cell secretory demand due to insulin resistance could result in disproportionate changes in circulating proinsulin levels, which may have pathophysiologic effects.[18-22] The fact that proinsulin cross-reacts with insulin in conventional insulin radioimmunoassays may have confounded the results from studies based on such methods. A previous study has indicated that proinsulin and 32,33 split proinsulin are associated with a small LDL particle size.[21] The proinsulin to insulin ratio, indicating a β-cell defect, also has been found to be related to small LDL particles in nondiabetic subjects.[6]

The aims of the present study in a sample of 58-year-old men from the general population were to examine whether small LDL particle size is associated with insulin resistance as measured with the euglycemic-hyperinsulinemic clamp technique and to explore the association between insulin metabolites and LDL particle size.

SUBJECTS AND METHODS

Subjects

The inclusion criteria were as follows: age 58 years, male sex, and Swedish ancestry. Exclusion criteria were cardiovascular disease, clinical diabetes mellitus or other clinically overt disease, treatment with cardiovascular medications that might disturb the measurements performed in the study, or an unwillingness to participate. The subjects were randomly selected among men in the County Council register and

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were invited to a screening examination. Of 818 screened men, 104 men were randomly selected and invited into the present examination.

The subjects received both written and oral information before they provided consent to participate. The study was approved by the Ethics Committee at Sahlgrenska University Hospital.

Study Protocol

Established questionnaires were used to evaluate the history of previous and current disease.[23] Body weight was measured on a balance scale with the subject dressed in underwear. Blood pressure was measured twice using the appropriate cuff size with the subject having rested in the supine position for 5 minutes. Diastolic blood pressure was determined as Korotkoff phase V. The whole blood glucose level was measured with the glucose oxidase technique. Blood samples were drawn, and serum and plasma were frozen in aliquots at −70°C within 4 hours.

A euglycemic-hyperinsulinemic clamp examination was performed ad modum de Fronzo, slightly modified as previously published.[24] During the 2 days preceding the day of the examination, subjects were to avoid unusual physical exercise, alcohol consumption, or any major change in caloric intake. They had to fast and to avoid nicotine (smoking or snuff-taking) from midnight of the preceding day; subjects were allowed to drink water in the morning on the day of the examination. Before the examination started, a questionnaire was completed to verify that the subject had followed the instructions and that there were no signs of respiratory infection or fever. After a priming dose, the insulin infusion rate was 1 mU/min/kg body weight, continuing for 120 minutes until the end of the examination. During the clamp, the target whole blood glucose concentration was 5 mmol/L and the glucose infusion rate was adjusted in connection with each determination of whole blood glucose if necessary. After the clamp examination, fat-free mass was measured using the dual-energy x-ray absorptiometry body composition model (Lunar DPX-L, Madison, WI).[25] Insulin sensitivity was calculated as the glucose infusion rate per minute adjusted for fat-free mass during the final hour of the examination.[24]

Laboratory Procedures

Plasma insulin was determined on the Access Immunoassay System (Sanofi Pasteur Diagnostics, Chaska, MN) using a 1-step chemiluminescent immunoenzymatic assay. Cross-reactivity with intact proinsulin is less than 0.2% at 400 pmol/L, and with 32,33 proinsulin, less than 1% at 400 pmol/L. The between-assay coefficient of variation is 6.6% at 28.6 mmol/L (n = 99), 4.8% at 153.1 pmol/L (n = 102), and 6.0% at 436.7 pmol/L, respectively. Intact proinsulin and 32,33 split proinsulin were determined in duplicate using a time-resolved fluorometric assay (Delfia, MD). The solid-phase antibody bound to a microtiter plate was the same in each case.[26] The labeled antibody used in the 32,33 split proinsulin assay (CPT-3F11) was produced by Dako Diagnostics (Copenhagen, Denmark). The intact proinsulin assay shows less than 1% cross-reactivity with insulin and 32-33 split proinsulin at a concentration of 2,500 and 400 pmol/L, respectively. The between-batch coefficient of variation is 8.5% at 20 pmol/L. Plasma C-peptide was assessed by radioimmunoassay (Guildhay, Guilford, UK).

LDL particle size was assessed on commercially available nondenaturing 2% to 16% polyacrylamide gradient gels (Alamo, San Antonio, TX) as previously described.[27] A pooled plasma standard (kindly supplied by R.M. Krauss, Lawrence Berkeley Laboratory, University of California) with 3 peaks of known size, 31.69 ± 0.22, 27.77 ± 0.18, and 25.06 ± 0.14 nm, respectively, was used along with apo-ferritin 12.2 nm to calibrate a standard serum developed at the Wallenberg Laboratory from 1 patient with 3 distinct peaks (30.58 ± 0.14, 26.33 ± 0.11, and 25.38 ± 0.09 nm, respectively). This standard serum was then used in the present study. The coefficient of variation (same serum analyzed on different gels on different days) for LDL peak particle size was 0.3%, with a correlation coefficient (r) of .99. To minimize the reading error from the gels, each lane was scanned twice. An automated computerized analyzing system reduces subjective influences in the measurement procedure. Mean values from the two readings are used in the present study. For the present study, the main variables were (1) LDL peak particle size (nm), (2) percentage of particles under the curve with a size less than 25.5 nm, called B%, and (3) percentage of particles under the curve with a size between 27.5 and 30 nm, intermediate-density lipoprotein (IDL).

Cholesterol and triglyceride levels were determined by fully enzymatic techniques.[28,29] HDL was determined after precipitation of apolipoprotein B (apoB)-containing lipoproteins with manganese chloride and dextran sulfate. LDL cholesterol was calculated as described by Friedewald et al.[30] $ApoA_1$ and apoB concentrations were measured by a rate-nephelometric method.[31] The blood glucose level was measured with the glucose oxidase technique.

Statistics

All statistics were analyzed using SPSS for Windows 9.0 (SPSS, Chicago, IL). The results are presented as the mean ± SD. Skewed variables were logarithmically transformed. ANOVA, Dunnett's test (post hoc analyses), and the Mann-Whitney U test were used for comparison of continuous variables, and the χ^2 test was used for categorical data. The nonparametric Spearman rank correlation test was used in the analysis with relationships illustrated with Pearson's correlation coefficient. Partial correlation coefficients and forward stepwise multiple regression were used in the analyses examining associations between the study variables. LDL peak particle size showed a bimodal distribution, and the glucose infusion rate showed a nonlinear relationship to LDL peak particle size. Therefore, dummy techniques were also used for these variables: the LDL peak particle size was ranked as below, at, or above 25.5 nm and the glucose infusion rate was dichotomized as the first, second, or third tertile. A 2-sided P value less than .05 was considered statistically significant.

RESULTS

A LDL peak particle size less than 25.5 nm was found in 16 subjects (15%). These men were characterized by obesity, elevated diastolic blood pressure, hypertriglyceridemia, low serum HDL cholesterol and $apoA_1$, and a low glucose infusion rate in comparison to the subjects with a larger LDL particle size. The former also had higher concentrations of circulating insulin and insulin metabolites (Table 1).

Correlations With LDL Peak Particle Size

The variables that showed a statistically significant association with LDL peak particle size are demonstrated in Table 2. In a multiple regression analysis with LDL peak particle size as the dependent variable and weight, total body fat, waist circumference, and body mass index as regressors, only the body mass index showed a statistically significant association (β-coefficient = −.28, P = .005). Correspondingly, insulin and insulin metabolites were regressed on LDL peak particle size. Log proinsulin was independently related to LDL particle size (β-coefficient = −.32, P = .001).

LDL peak particle size correlated with the glucose infusion rate independently of the body mass index (partial correlation coefficient = .23, P = .018). However, the association was not independent of serum triglycerides or plasma proinsulin (data not shown).

Table 1. Characteristics of the Subjects by LDL Peak Particle Size

Characteristic	LDL Peak Particle Size (nm) <25.5 (n = 16)	LDL Peak Particle Size (nm) ≥25.5 (n = 88)	P
Weight (kg)	89.2 ± 15.0	81.7 ± 14.2	.028
Body mass index (kg/m^2)	28.6 ± 3.8	25.7 ± 4.3	.005
Total body fat (kg)	26.0 ± 6.9	20.7 ± 8.4	.011
Waist circumference (cm)	101.8 ± 9.4	94.5 ± 12.3	.012
Blood pressure (mm Hg)			
Systolic	128 ± 18	126 ± 20	.39
Diastolic	82 ± 8	77 ± 10	.025
Heart rate (bpm)	63 ± 9	62 ± 8	.67
Serum cholesterol (mmol/L)			
Total	6.00 ± 1.00	6.03 ± 1.13	.75
HDL	0.94 ± 0.20	1.31 ± 0.35	.000
LDL	3.82 ± 1.08	4.14 ± 1.01	.59
Serum triglycerides (mmol/L)	3.13 ± 1.93	1.28 ± 0.50	.000
ApoB (g/L)	1.28 ± 0.22	1.20 ± 0.28	.11
ApoA$_1$ (g/L)	1.27 ± 0.20	1.44 ± 0.24	.014
LDL particle size (nm)*	24.86 ± 0.36	26.55 ± 0.42	—
<25.5 nm (B %)*	43 ± 12	12 ± 6	—
27.5-30 nm (IDL %)*	20 ± 8	25 ± 7	—
Blood glucose (mmol/L)	5.0 ± 0.9	4.9 ± 0.5	.34
Plasma insulin (pmol/L)	53.5 ± 35.2	33.8 ± 23.7	.041
Plasma proinsulin (pmol/L)	11.6 ± 7.9	6.5 ± 4.6	.004
Plasma 32,33 split proinsulin (pmol/L)	15.2 ± 13.5	8.3 ± 6.9	.016
Plasma C-peptide (pmol/L)	811 ± 424	554 ± 236	.016
Plasma proinsulin/insulin ratio	0.27 ± 0.14	0.22 ± 0.09	.26
Glucose infusion rate (mg/kg/min)	6.8 ± 4.5	8.5 ± 2.8	.019

*Statistical analysis was not performed, as the variable is associated with the definition of the groups.

In a forward stepwise multiple regression with LDL peak particle size as the dependent variable and the body mass index, diastolic blood pressure, serum HDL cholesterol, triglycerides, and apoB and apoA$_1$, plasma proinsulin, and glucose infusion rate as independent variables, triglycerides and HDL cholesterol showed a statistically significant association with LDL particle size in a traditional analysis (R^2 = 67%, P = .000; β-coefficient for log triglycerides and HDL cholesterol = −.54 [P = .000] and .39 [P = .000], respectively). With the LDL peak particle size and glucose infusion rate as dummy variables, only log triglycerides was associated with the dependent variable (R^2 = 34.5%, P = .000; β-coefficient for log triglycerides = −.53).

Subjects below and above the median (1.34 mmol/L) for serum triglycerides did not show any statistically significant correlations between the glucose infusion rate and LDL peak particle size (r = .23 and r = .12, respectively).

Table 2. Pearson Correlation Coefficients for LDL Peak Particle Size

Variable	LDL Peak Particle Size
Weight (kg)	−.30†
Body mass index (kg/m^2)	−.38†
Total body fat (kg)	−.36†
Waist circumference (cm)	−.37†
Diastolic blood pressure (mm Hg)	−.20*
Serum HDL cholesterol (mmol/L)	.68†
Serum triglycerides (mmol/L)	−.70†
ApoB (mmol/L)	−.29†
ApoA$_1$ (mmol/L)	.56†
Plasma insulin (pmol/L)	−.38†
Plasma proinsulin (pmol/L)	−.40†
Plasma 32,33 split proinsulin (pmol/L)	−.36†
Plasma C-peptide (pmol/L)	−.42†
Glucose infusion rate (mg/kg/min)	.33†

*P < .05.
†P < .01.

Glucose Infusion Rate and LDL Particle Size

LDL peak particle size was smaller in the low tertile for the glucose infusion rate (indicating insulin resistance) in comparison to each of the other tertiles. However, there was no difference in LDL peak particle size between the middle and high tertiles. There was no significant correlation between the proportion of LDL particles less than 25.5 nm (B%) and the glucose infusion rate (r = −.14, nonsignificant). The proportion of LDL particles sized 27.5 to 30.0 nm (IDL%) was smaller in the low versus the high tertile (Table 3).

DISCUSSION

The results from the present study show that the LDL peak particle size is inversely associated with insulin sensitivity as measured by the euglycemic hyperinsulinemic clamp method. This association was not linear, as the subjects in the lowest tertile of insulin sensitivity had smaller LDL particles than subjects in the middle and upper tertiles, who were almost identical in LDL peak particle size. The relation between small LDL particles and insulin resistance was independent of the degree of obesity. In concordance with results from previous studies, small LDL particles were also associated with other components of the insulin resistance syndrome such as hypertriglyceridemia, reduced serum HDL and apoA$_1$, high blood pressure, and hyperinsulinemia.[6-10] The multiple regression analysis yielded the same results as previous studies, ie, LDL peak particle size showed a strong inverse association with serum triglycerides but no independent relationship to insulin sensitivity.[6-10,21] There is a possibility that the power to detect associations between small LDL particle size and insulin resistance may have been limited by the fact that only 16 of the subjects had a pattern B phenotype. However, the results of the

Table 3. LDL Particle Size by Tertiles of Insulin Sensitivity as Measured by the Euglycemic-Hyperinsulinemic Clamp Technique

Variable	Tertiles of Glucose Infusion Rate Low	Middle	High
LDL particle size (nm)	25.9 ± 0.81	26.53 ± 0.57	26.43 ± 0.66*
<25.5 nm (B %)	21 ± 17	13 ± 8	15 ± 12
27.5-30 nm (IDL %)	21 ± 6	25 ± 8	25 ± 7†

*$P \leq$ .010, low v high or middle.
†P = .040, low v high.

present study are in accordance with data from most previous reports.[6-10,21]

In previous studies using the clamp technique, insulin-mediated glucose uptake has been adjusted for body weight instead of lean body mass.[9,10,12,14] Although such an adjustment will result in a systematic underscoring of insulin sensitivity among obese subjects,[24,32] the results generally accord with those of the present study. Mykkänen et al[9] studied 87 Finnish normoglycemic men and found a univariate correlation between insulin-mediated glucose uptake and LDL peak particle size, and serum VLDL triglycerides were the strongest determinant of LDL peak particle size. However, we could not verify their finding that the association between LDL particle size and insulin sensitivity was more pronounced among those who were more hypertriglyceridemic. The results of a German study of 50 young healthy men and women also demonstrated that insulin-mediated glucose uptake correlated univariately with LDL particle size and that this correlation did not remain in a multiple regression analysis.[10] Serum triglycerides explained about half of the variation in LDL particle size. In a Finnish study of 18 type 2 diabetics and 19 nondiabetics, insulin-mediated glucose uptake was associated with LDL peak particle diameter only among subjects without diabetes.[12] The results of a Japanese study did not demonstrate any association between insulin-mediated glucose uptake and LDL particle size in 17 patients with type 2 diabetes or impaired glucose tolerance.[14]

Other studies have been based on different methods to assess insulin sensitivity. Reaven et al[8] showed that LDL diameter was associated with insulin resistance as measured by the steady-state plasma glucose method after infusion of somatostatin, insulin, and glucose. This association was not independent of the serum triglyceride concentration. In the large Insulin Resistance Atherosclerosis Study from the United States in a triethnic population of both sexes (N = 931), there was a rather weak inverse correlation between LDL particle size and insulin sensitivity as assessed by the frequently sampled intravenous glucose tolerance test.[11] Slyper et al[15] used a similar method to measure insulin sensitivity but did not observe any association with LDL peak particle size in a young population of 101 healthy males. Stewart et al[13] reported that type 2 diabetics had smaller and denser LDL particles than controls, and insulin resistance, measured by a fixed insulin/glucose infusion method, showed a statistically significant correlation with LDL peak density but not with LDL size. These previous studies used gradient gel electrophoresis to measure LDL particle size.[8-15]

In conclusion, available data from the present and previous studies indicate a direct, although weak, relation between insulin sensitivity and LDL particle size, at least among nondiabetic subjects, and this relationship is dependent on the triglyceride metabolism. It should be kept in mind that LDL peak particle size is a crude measure of the LDL particle phenotype and does not capture all aspects of the distribution of different LDL subspecies. We also measured the percentage of small LDL particles (<25.5 nm) in each subject and observed no association with insulin-mediated glucose uptake.

The prevailing concept for the generation of small LDL particles is that the insulin-resistant state causes an increased hepatic production and a reduced degradation of very–low-density lipoprotein (VLDL) triglycerides.[11,33,34] Cholesteryl ester transfer protein mediates the exchange of cholesteryl esters from LDL to VLDL particles and triglycerides from VLDL to LDL particles.[35] Hence, LDL becomes enriched with triglycerides and serves as a good substrate for hepatic lipase, which hydrolyzes the triglycerides in LDL, resulting in small LDL particles.[36] In addition, hepatic lipase activity seems to be increased in insulin-resistant subjects.[21,37] Although insulin resistance is involved, other mechanisms may be even more important. In obesity, there is hepatic influx of free fatty acids from the splanchnic circulation, contributing to the increased production of VLDL triglycerides and maybe to the development of insulin resistance.[38] Isotope tracer techniques have shown that small dense LDL particles are largely derived from triglyceride-rich VLDL precursors,[39] explaining the invariable association between triglycerides and LDL particle size reported in all studies.

We observed that of all of the measured insulin-like molecules, only proinsulin showed an independent correlation with LDL particle size. This inverse association may be explained by the fact that insulin resistance is often accompanied by an increased secretion of insulin precursors, eg, proinsulin.[22] Thus, hyperproinsulinemia serves as a marker of insulin resistance. It is not clear whether this is a result of overstimulation of the pancreatic β cells or if it is an initiating factor in the development of insulin resistance.[21,40] Another insulin precursor, 32,33 split proinsulin, was shown to correlate univariately with small LDL particles in type 2 diabetics.[21] In a previous study, the proinsulin to insulin ratio, a measure of β-cell dysfunction, was associated with a small LDL particle size in nondiabetic subjects.[6] However, we could not verify this latter finding. Alternatively, proinsulin may be a better measure of insulin secretion than intact insulin since it has a longer half-life and a lower variability.[17,18] It is not clear if insulin precursors have any effects on LDL particle metabolism.

One limitation of this study is that we did not include women. Although women have larger LDL particles than men, previous studies have indicated that men and women show a similar association between insulin sensitivity and LDL particle size.[8-11]

In conclusion, among 58-year-old clinically healthy men, 15% had a phenotype with a predominance of small LDL particles. A small LDL particle size was associated with the components of the insulin resistance syndrome, including insulin resistance. However, the variability in LDL particle size was mainly explained by the serum triglyceride concentration, and insulin sensitivity did not show any independent contribution to this variability. For insulin and insulin precursors, only proinsulin showed an independent association with LDL particle size.

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