Insulin Resistance Is Not a Major Determinant of Low-Density Lipoprotein Particle Size

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The relationship between low-density lipoprotein (LDL) peak particle diameter and insulin sensitivity, very-low-density lipoprotein (VLDL) + intermediate-density lipoprotein (LDL) triglyceride, cholesterol, and apoprotein B, postprandial lipemia, and LDL + high-density lipoprotein (HDL) triglyceride was assessed. The subjects were 101 healthy males aged 15 to 45 years. Sixty-one subjects (60.4%) were offspring of a parent with coronary artery disease before age 60, and 40 subjects (39.6%) had no parental history of coronary artery disease. LDL peak particle diameter was measured following polyacrylamide gradient gel electrophoresis. An insulin sensitivity index (S_I) was determined from a frequently sampled intravenous glucose tolerance test using a minimal modeling method. A fat tolerance test was performed with a test meal containing 70 g/m^2 fat, with triglyceride concentrations measured hourly for 12 hours. LDL peak particle diameter was significantly correlated with body mass index (BMI) ($r = -.282$, $P < .01$), waist to hip ratio ($r = -.291$, $P < .01$), fasting triglyceride (logarithmically [log] transformed) ($r = -.566$, $P < .001$), area under the postprandial triglyceride curve (log transformed) ($r = -.562$, $P < .001$), VLDL + IDL triglyceride (log transformed) ($r = -.462$, $P < .001$), VLDL + IDL cholesterol (log transformed) ($r = -.477$, $P < .001$), VLDL + IDL apoprotein B (log transformed) ($r = -.321$, $P < .001$), LDL + HDL triglyceride (log transformed) ($r = .583$, $P < .001$), and HDL cholesterol ($r = .347$, $P < .001$), but there was no significant correlation with S_I. Using stepwise regression analysis, LDL + HDL triglyceride showed the strongest relationship to LDL peak particle diameter, accounting for 34% of the variation in size. S_I was not an independent predictor of LDL particle size. In conclusion, insulin sensitivity appears to have little influence on LDL particle size. The importance of LDL + HDL triglyceride should be considered a preliminary finding warranting verification in this and other populations.

LOW-DENSITY LIPOPROTEIN (LDL) particles are nonhomogeneous in terms of size, density, and composition. A useful means of assessing LDL particle size involves nondenaturing polyacrylamide gradient gel electrophoresis.[1] A much-used classification divides LDL into two major categories based on peak particle diameter: type A pattern, with LDL peak particle diameter greater than 25.5 nm, and type B pattern, the smallest, most dense particles, with peak particle diameter less than 25.5 nm.[2] An intermediate pattern with a maximum particle diameter of 25.2 to 25.5 nm has also been suggested. LDL peak particle diameter can also be expressed as a continuous rather than dichotomous variable, and this more readily permits examination of the relationships to particle size.

An association between LDL particle size and coronary artery disease incidence is suggested by the observation that survivors of coronary artery disease show a significant increase in small, dense LDL particles.[2-6] A 55% incidence of the type B pattern was noted by Austin et al[2] in 366 post–myocardial infarction men and women, compared with a 31% incidence in age- and sex-matched controls. Adjusting for the use of β-blockers, Campos et al[3] found a 48% incidence of small LDL particles (diameter <26.0 nm) in 280 male coronary artery disease survivors younger than 60 years of age, compared with a 30% incidence in controls. There is also an increase in small LDL particles in many conditions predisposing to atherosclerosis, such as non–insulin-dependent diabetes mellitus,[7] familial combined hyperlipidemia,[8] and familial dyslipidemic hypertension.[9]

However, the precise determinants of LDL particle size are poorly understood. The type B LDL subclass pattern is one of several lipoprotein abnormalities comprising the insulin resistance syndrome.[10] This could suggest a role for insulin resistance in the regulation of LDL particle size. In addition, a strong correlation between fasting triglyceride concentration and LDL particle size and density has been invariably noted.[10-18] Changes in LDL particle size are also strongly correlated with changes in fasting triglyceride concentration occurring over time,[19] and as a result of lipid-lowering therapy.[20] However, the association with fasting triglyceride could be a reflection of an association with another closely correlated variable such as postprandial lipemia, very–low-density lipoprotein (VLDL) triglyceride, cholesterol, or apoprotein B, or non-VLDL triglyceride. In this study, we examined the relationship of insulin sensitivity and these other lipoprotein variables to LDL particle size.

SUBJECTS AND METHODS

Subject Selection

There were 101 subjects aged 15 to 45 years, all of whom were healthy males. Sixty-one subjects were the offspring of a parent with documented evidence of major coronary artery disease: either coronary angioplasty, coronary bypass surgery, or myocardial infarction. The remaining 40 subjects had no history of parental heart disease. Subjects with a family history of coronary heart disease had significantly higher diastolic blood pressure and LDL cholesterol. Nevertheless, the associations under study had a similar order of magnitude in the two groups, and these groups were therefore combined. Subjects were without evidence of gastrointestinal, renal, cardiac, endocrine, or other signifi-

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Submitted September 27, 1996; accepted May 12, 1997.

Supported in part by a Grant-in-Aid from the American Heart Association, Wisconsin Affiliate; the Children's Hospital Foundation of the Children's Hospital of Wisconsin, Milwaukee, WI; the R.D. and Linda Peters Research Endowment, Medical College of Wisconsin; General Clinical Research Center Grant No. 5M01RR00058, and a grant from the Helen Bader Foundation, Milwaukee, WI (to S.Z.).

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0026-0495/97/4611-0007$03.00/0

cant chronic disease. Sixteen subjects were less than 20 years of age. All adolescents were at Tanner stage V for pubertal development, with a testicular length of 4 cm or greater. Subject characteristics and fasting lipoprotein concentrations are summarized in Table 1. All but one subject had a fasting plasma glucose level less than 115 mg/dL, with one subject having a fasting plasma glucose of 116.7 mg/dL. Ten subjects had the type B LDL subclass pattern, six had an intermediate pattern, and the remainder were type A.

Study Protocol

The study was approved by the Institutional Review Board of the Medical College of Wisconsin. Informed consent was obtained from each subject, and parental consent was also obtained for subjects less than 18 years of age. Height, weight, and blood pressure were measured at a preliminary examination. The blood pressure recorded was the average of three readings from the right arm after the subject had rested for 10 minutes in the supine position. The waist to hip ratio was calculated from a waist measurement taken at the level of the umbilicus and hip measurement at the level of the greater trochanter.

A frequently sampled intravenous glucose tolerance test was performed on each subject as previously described.[21] Three days before the test, the subject was placed on a weight-maintaining diet containing at least 150 g carbohydrate. No alcohol was permitted for 3 days before the test, and smoking was prohibited from the prior evening. Following a 12-hour overnight fast, the subject was admitted to the Clinical Research Center and intravenous catheters were inserted into both arms. Three baseline samples were taken for serum insulin and plasma glucose at −20, −15, and −10 minutes. At time zero, 50% glucose 0.3 g/kg was injected over 1 minute. Blood was taken from the contralateral arm at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes. At time 20 minutes, 300 mg tolbutamide diluted in 10 mL sterile water was injected over 20 seconds. For adolescents less than 17 years of age, the dose of tolbutamide was 5 mg/kg, to a maximum dose of 300 mg, and the test was terminated at the 90-minute specimen. Each sample was centrifuged and stored immediately at −20°C.

A fat tolerance test was performed within 6 weeks of the frequently sampled intravenous glucose tolerance test. As before, the subject was instructed on an adequate carbohydrate intake to be taken 3 days before the test. No alcohol was permitted for 3 days before the test or smoking from the prior evening. Following a 12-hour overnight fast, the subject was admitted to the Clinic Research Center, an intravenous catheter was inserted, and a baseline triglyceride sample was taken. At this time, a blood specimen was also taken into an EDTA-containing tube for fasting lipoproteins. The fat load was 70 g/m^2 provided as heavy whipping cream without any additives. The test meal was administered over 10 minutes together with a glass of water. Subjects remained fasting for the duration of the test, and blood samples for triglyceride were drawn at hourly intervals for 12 hours.

Table 1. Characteristics for the 101 Subjects of the Study

Variable	Mean ± SD	Range
Age (yr)	31.8 ± 9.0	15-46
BMI (kg/m^2)	25.2 ± 3.5	18.6-34.6
Waist-to-hip ratio	0.898 ± 0.066	0.770-1.090
Systolic blood pressure (mm Hg)	126.8 ± 13.4	98.7-168.3
Diastolic blood pressure (mm Hg)	77.9 ± 12.5	40-105
Fasting triglyceride (mg/dL)	101.1 ± 62.3	38.1-354
Total cholesterol (mg/dL)	168.7 ± 36.8	92.3-271.8
LDL cholesterol (mg/dL)	92.2 ± 26.9	32.4-170.0
VLDL + IDL cholesterol (mg/dL)	20.7 ± 13.1	3.4-77.1
VLDL + IDL triglyceride (mg/dL)	62.1 ± 43.1	16.8-271.6
VLDL + IDL apoprotein B (mg/dL)	18.5 ± 7.2	7.4-53.6
LDL + HDL triglyceride (mg/dL)	44.0 ± 23.7	11.8-114.1
HDL cholesterol (mg/dL)	46.0 ± 12.3	24.5-81.1
Fasting glucose (mg/dL)	88.9 ± 7.2	71.3-116.7
Fasting insulin (μU/mL)	9.6 ± 4.9	3.4-28.4

Laboratory Procedures

Lipoproteins were removed from plasma by low-speed ultracentrifugation. VLDL together with intermediate-density lipoprotein (IDL), LDL, and high-density lipoprotein (HDL) were isolated by preparative ultracentrifugation at densities less than 1.019, 1.019 to 1.063, and greater than 1.063 g/mL, respectively, as described by Schumaker and Puppione[22] using a 50.3 Ti Beckman (Palo Alto, CA) rotor at 18°C. Lipoprotein cholesterol levels were measured using a standard kit (Boehringer Mannheim, Indianapolis, IN). Interassay and intraassay coefficients of variation for cholesterol measurements were 3.8% and 3.2%, respectively. Triglyceride levels were measured by a standard kit (Stanbio Laboratory, San Antonio, TX). Interassay and intraassay coefficients of variation were 3.2% and 2.0%, respectively. VLDL apoprotein B concentration was measured by a double-antibody sandwich enzyme-linked immunosorbent assay. Affinity-purified goat anti-human apoprotein B immunoglobulin (Ig), goat anti-human apoprotein B Ig alkaline phosphatase conjugate, and apoprotein B standard were kindly provided by Dr Anh Le (Division of Arteriosclerosis and Lipid Metabolism, Emory University School of Medicine). The interassay coefficient of variation for VLDL apoprotein B was 6.8%, and the intraassay coefficient of variation was 6.2%. Concentrations of LDL + HDL triglyceride were obtained by subtracting VLDL + IDL triglyceride from fasting triglyceride. The area under the postprandial triglyceride curve was estimated using the trapezoid method.

An aliquot of LDL was subjected to nondenaturing polyacrylamide gradient gel electrophoresis using 2.5% to 16% polyacrylamide gradient gels (Isolab, Akron, OH), Tris-borate buffer at pH 8.35, and an electrophoresis time of 24 hours at 175 V as previously described.[23] Gels were stained for protein using 0.05% Coomassie R-250. LDL particle size was measured using high–molecular-weight protein standards (Electrophoresis Calibration Kit; Pharmacia, Piscataway, NJ) and an LDL standard kindly calibrated by Dr Ronald Krauss (Lawrence Berkeley National Laboratory, Berkeley, CA), and these were both analyzed on each gel. An internal protein standard (apoferritin), which was part of the Electrophoresis Calibration Kit, was used to monitor the reproducibility of the assay. Another LDL specimen previously measured in the laboratory of Dr Krauss was also added to each analysis as a control, and was used to measure the coefficients of variation. The gels were scanned at 600 nm with a computer-assisted densitometer using I-D Analyst II data analysis software adapted for the Model 620 Densitometer (Bio-Rad Laboratories, Hercules, CA). The area under each LDL peak was calculated, and the peak particle diameter recorded was that of the peak with the maximum area. This peak was also used to classify samples as type A (peak particle diameter > 25.5 nm), type B (<25.2 nm), or intermediate (25.2 to 25.5 nm). Interassay and intraassay coefficients of variation for LDL particle size were 2.2% and 1.7%, respectively.

Glucose levels were measured enzymatically using a Beckman Glucose Analyzer 2. The interassay coefficient of variation was 5.4%, and the intraassay coefficient of variation was 1.3%. Insulin levels were measured by radioimmunoassay using a commercial kit (Incstar, Stillwater, MN). The interassay coefficient of variation for insulin was 5.4%, and the intraassay coefficient of variation was 5.8%.

Analysis of glucose and insulin values was performed by the modified minimal modeling method of Bergman.[15] The model assumes that injected glucose is distributed rapidly into a single compartment and that plasma glucose decreases by two components: a component that is independent of the incremental insulin response, and a second component that is dependent on insulin. The glucose effectiveness index (S_G) is a measure of the effect of glucose to enhance its own

disappearance at basal insulin, and the insulin sensitivity index (S_I) is a measure of the ability of insulin to diminish endogenous glucose production and to augment glucose utilization. First-phase insulin (AIR_G) was calculated as the area under the incremental insulin curve from 0 to 10 minutes after administration of intravenous glucose, using the trapezoid method. The area under the absolute glucose curve was also determined by the trapezoid method. In subjects aged less than 18 years, the glucose tolerance test was terminated at 120 minutes.[24] In all instances in which the glucose tolerance test was terminated before the 180-minute sample either because of the age of the subject or because of hypoglycemia, values were extrapolated to 180 minutes.

Statistics

Relationships between LDL peak particle diameter and fasting triglyceride, area under the triglyceride curve, VLDL + IDL triglyceride, cholesterol, and apoprotein B, and LDL + HDL triglyceride were tested for deviation from linearity with polynomial regression. Relationships between LDL peak particle diameter and triglyceride, area under the triglyceride curve, VLDL + IDL triglyceride, and LDL + HDL triglyceride were significantly curvilinear; however, it was noted that the polynomial models showed a significant lack of fit at higher levels of triglyceride, area under the curve, VLDL + IDL triglyceride, and LDL + HDL triglyceride. This lack of fit was eliminated using a curvilinear model that did not curve upward (the exponential) at the higher levels.[25] Multiple regression analysis was used to examine the simultaneous effects of different variables on LDL peak particle diameter using a forward stepwise approach. Statistical analyses were performed using the Minitab statistical package (Minitab, State College, PA).

RESULTS

Correlations With LDL Peak Particle Diameter

Fasting triglyceride, area under the triglyceride curve, VLDL + IDL triglyceride, and LDL + HDL triglyceride showed significantly nonlinear relationships to LDL peak particle diameter that were best described by exponential curves (Fig 1). Relationships between LDL peak particle diameter and VLDL + IDL cholesterol and VLDL + IDL apoprotein B were linear. Correlations with LDL peak particle size are shown in Table 2. There were significant correlations between LDL particle size and body mass index (BMI), waist to hip ratio, fasting triglyceride (logarithmically [log] transformed), area under the postprandial triglyceride curve (log transformed), VLDL + IDL triglyceride (log transformed), VLDL + IDL cholesterol (log transformed), VLDL + IDL apoprotein B (log transformed), LDL + HDL triglyceride (log transformed), and HDL cholesterol. There was no significant correlation between LDL peak particle diameter and S_I. S_I was significantly

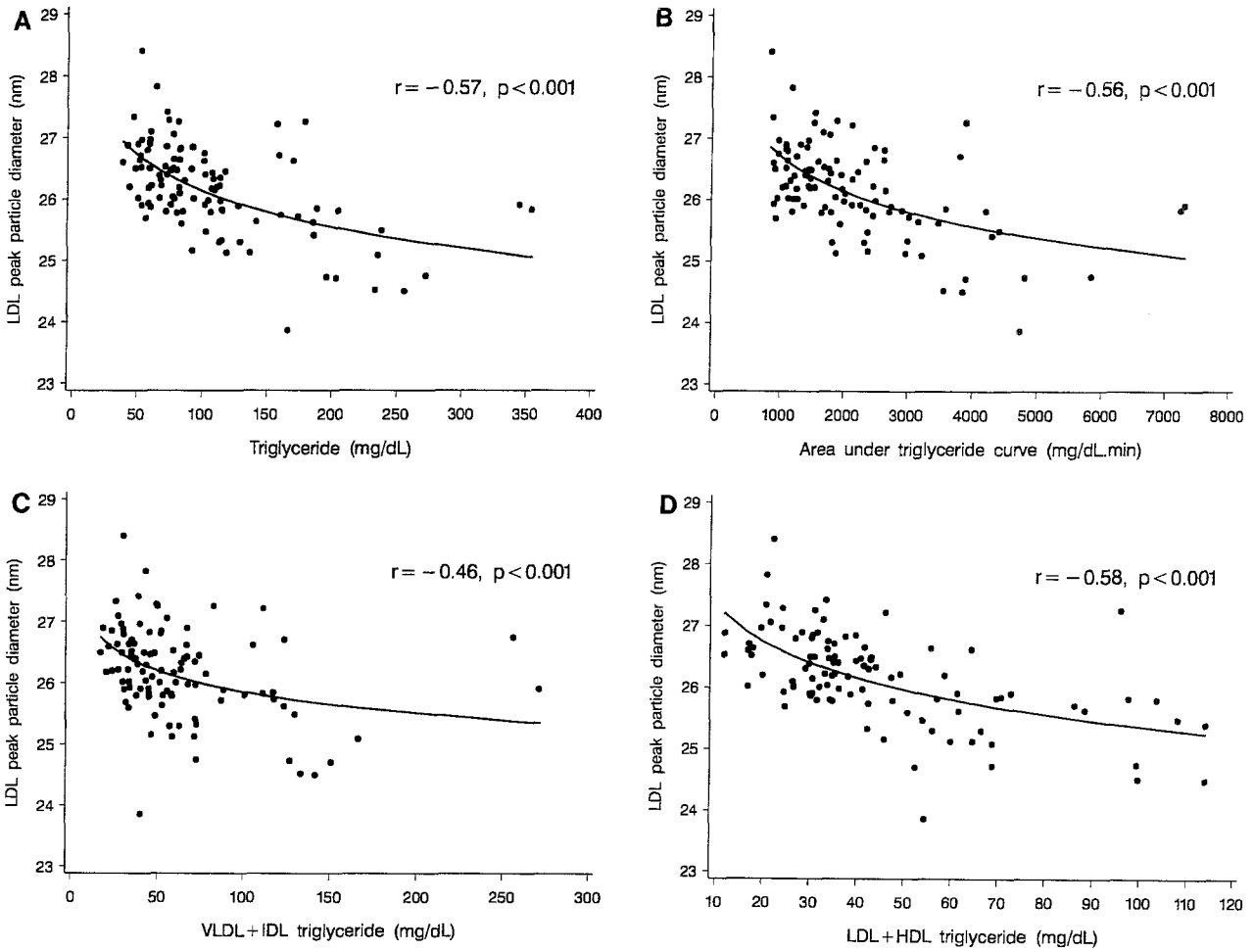

Fig 1. Scatterplots showing curvilinear relationships between LDL peak particle diameter and fasting triglyceride (A), area under the postprandial triglyceride curve (B), VLDL + IDL triglyceride (C), and LDL + HDL triglyceride (D).

Table 2. Pearson Correlation Coefficients for LDL Peak Particle Diameter, S_I, and Fasting Triglyceride

Variable	LDL Particle Size	S_I	Fasting Triglyceride§
BMI	−.282*	−.414†	.410†
Waist-to-hip ratio	−.291*	−.346†	.445†
Triglyceride‡	−.566†	−.330†	—
Area under postprandial triglyceride curve‡	−.562†	−.364†	.891†
VLDL + IDL cholesterol‡	−.477†	−.071	.640†
VLDL + IDL triglyceride‡	−.462†	−.295*	.930†
VLDL + IDL apoprotein B‡	−.321†	.005	.447†
LDL + HDL triglyceride‡	−.576†	−.290*	.865†
LDL cholesterol	−.011†	−.097	.179
HDL cholesterol	.347†	.141	−.134
S_I	.134	—	−.330†
S_G	.194	.165	−.139
Area under glucose curve	−.174	−.435†	.400†
AIR_G	−.039	−.373†	−.008

*$P < .01$.
†$P < .001$.
‡Log transformed.

correlated with BMI, waist to hip ratio, triglyceride (log transformed), VLDL + IDL triglyceride, LDL + HDL triglyceride, area under the glucose curve, and AIR_G.

Multivariate Stepwise Regression Analysis for LDL Peak Particle Diameter

To examine the influence of insulin sensitivity, VLDL constituents, LDL + HDL triglyceride, and postprandial lipemia on LDL particle size, we performed a stepwise regression analysis. Two models were examined (Table 3). In model 1, the following variables were entered into the analysis: BMI, waist to hip ratio, fasting triglyceride (log transformed), area under the postprandial triglyceride curve (log transformed), VLDL + IDL cholesterol (log transformed), VLDL + IDL apoprotein B (log transformed), HDL cholesterol, S_I, and S_G. In this model, fasting triglyceride was shown to be the strongest predictor of LDL peak particle diameter, accounting for 32% of the variation in size, with HDL cholesterol accounting for 7.8% of the variation. In model 2, fasting triglyceride was replaced by VLDL + IDL triglyceride (log transformed) and LDL + HDL triglyceride (log transformed). In this model, LDL + HDL triglyceride accounted for 34% of the variation, with HDL cholesterol, area under the triglyceride curve, and S_G accounting for 5.6%, 3%, and 2.6% of the variation, respectively.

Table 3. Stepwise Regression Analyses Showing Contributions to the Variance of LDL Peak Particle Diameter

Variable	Regression Coefficient (standard error)	*P*
Model 1		
Fasting triglyceride*	−0.695 (0.122)	<.001
HDL cholesterol	0.018 (0.005)	<.001
Model 2		
LDL + HDL triglyceride*	−0.392 (0.180)	.032
HDL cholesterol	0.015 (0.005)	.001
Area under triglyceride curve*	−0.456 (0.174)	.010
S_G	0.015 (0.007)	.034

*Log transformed.

DISCUSSION

We were unable to demonstrate in this young male population a relationship between LDL peak particle diameter and S_I, the latter being assessed by the minimal model method of Bergman. No significant correlation was noted between LDL particle size and S_I, and S_I did not appear as an independent predictor of LDL particle size on stepwise regression analysis.

It is well established that the type B LDL subclass pattern is associated with an "atherogenic" lipoprotein phenotype, with increased concentrations of triglyceride, apolipoprotein B, LDL, and IDL and decreased concentrations of HDL cholesterol, HDL_2, and apolipoprotein A-1.[26,27] These lipoprotein abnormalities are characteristic of the insulin resistance syndrome described by Reaven.[28,29] A relationship between LDL particle size and insulin resistance was suggested by Selby et al,[30] who showed that female twins aged 30 to 91 years with the type B LDL subclass pattern had a higher fasting and postload insulin, triglyceride, systolic blood pressure, and waist to hip ratio and a decreased concentration of HDL cholesterol. Insulin resistance was also demonstrated by Reaven et al[10] in normal men and women with the type B LDL subclass pattern, with these individuals showing higher steady-state glucose levels after a constant infusion of somatostatin, glucose, and insulin. This relationship persisted after correcting for gender, age, and degree of obesity. When expressing LDL peak particle diameter as a continuous rather than dichotomous variable, moderate correlations were also noted between LDL peak particle diameter and steady-state plasma glucose levels, area under the glucose curve, and area under the insulin curve.

Tchernof et al,[11] on the other hand, expressing LDL particle size as a particle score in healthy men aged 29 to 53 years (N = 79), found no correlation between LDL particle size and the area under the insulin and glucose curves following an oral glucose tolerance test. However, there were weak but significant correlations between LDL particle size and fasting plasma insulin levels and between LDL particle size and visceral adipose tissue, the latter being assessed by computed tomography. Using a euglycemic clamp, Suehiro et al[31] studied Japanese men (N = 80), half of whom had either diabetes or impaired glucose tolerance, and found no difference in insulin sensitivity between individuals with small and normal-sized LDL. In total, these observations together with our own question a close relationship between LDL particle size and insulin sensitivity. They also suggest that LDL particle size is a poor marker for decreased insulin sensitivity, except perhaps at the extreme end of LDL particle size.

The influence of VLDL, postprandial lipemia, and non-VLDL triglyceride on LDL particle size was also examined using multiple stepwise regression analysis. In the first model, fasting triglyceride was shown to account for 32% of the variation in size. However, when fasting triglyceride was replaced by VLDL + IDL triglyceride and LDL + HDL triglyceride, LDL + HDL triglyceride showed the strongest relationship to LDL particle size, accounting for 34% of the variation. LDL + HDL triglyceride was estimated by subtracting VLDL + IDL triglyceride from fasting triglyceride. The

area under the postprandial triglyceride curve failed to appear when fasting triglyceride was included in the analysis, and accounted for only 3% of the variation in size when triglyceride was replaced by VLDL + IDL triglyceride and LDL + HDL triglyceride. In total, these analyses raise the possibility that the well-recognized association between LDL particle size and fasting triglyceride reflects a strong relationship to LDL + HDL triglyceride, with VLDL + IDL triglyceride and postprandial lipemia being more weakly related to LDL size.

It has been proposed that LDL particle size is influenced by a modeling process, linked in particular to the postprandial catabolism of triglyceride-rich VLDL and chylomicrons.[32] Under the influence of cholesterol ester transferase, triglyceride is transferred from triglyceride-rich lipoproteins to LDL and HDL in exchange for cholesterol. LDL particles thereby lose cholesterol and become enriched in triglyceride, and the action of lipases on LDL triglyceride leads to changes in LDL density. There are experimental data to support these concepts. A strong correlation has been noted between fasting triglyceride and the cholesterol ester to protein ratio of LDL.[32] The free-cholesterol content of LDL is strongly correlated with hepatic lipase activity.[33] Congenital hepatic lipase deficiency is associated with large buoyant LDL-like particles.[34] Individuals with the LDL subclass B phenotype have greater hepatic lipase activity than those with subclass A.[33,35] Nikkila et al[36] noted increased postprandial lipemia in subjects with the type B LDL subclass pattern. Furthermore, a study that examined LDL subclass distribution during a fat tolerance test found minor but significant changes in LDL subclass distribution 6 to 8 hours into the test, with a decrease in the percentage of smaller and larger particles and an increase in intermediate-sized particles in normotriglyceridemic non–insulin-dependent diabetics, and a decrease in smaller particles and an increase in intermediate-sized particles in matched nondiabetic controls.[37] However, other data cast doubt on this hypothesis. There is no correlation, for example, between cholesterol ester transferase activity and LDL subfraction distribution or apoprotein B content of dense LDL.[38,39] Using multivariate regression analysis, Jansen et al[35] found neither hepatic lipase nor lipoprotein lipase activities to be determinants of LDL particle size. Musliner et al[40] noted two distinct subpopulations of IDL in normal individuals. Based on this observation, it has been proposed that there is early channeling of IDL and VLDL subspecies into specific LDL subclasses.[41] The relevance of our findings to these mechanisms requires elucidation.

However, it should be noted that the variables fasting triglyceride, area under the triglyceride curve, VLDL + IDL triglyceride, and LDL + HDL triglyceride are highly intercorrelated. Furthermore, these variables show correlations of similar degree to LDL particle size. Therefore, the finding that LDL + HDL triglyceride has the strongest relationship to LDL particle size should be considered a preliminary observation warranting further study in this and other populations.

In conclusion, no association was demonstrated in this young male population between LDL peak particle diameter and insulin sensitivity. On multiple regression analysis, LDL + HDL triglyceride appeared to have the strongest relationship to LDL particle size. Confirmation of these findings in this and other populations may shed light on the nature of the association between LDL particle size and atherosclerosis.

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