Effects of Insulin Resistance and Type 2 Diabetes on Lipoprotein Subclass Particle Size and Concentration Determined by Nuclear Magnetic Resonance

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The insulin resistance syndrome (IRS) is associated with dyslipidemia and increased cardiovascular disease risk. A novel method for detailed analyses of lipoprotein subclass sizes and particle concentrations that uses nuclear magnetic resonance (NMR) of whole sera has become available. To define the effects of insulin resistance, we measured dyslipidemia using both NMR lipoprotein subclass analysis and conventional lipid panel, and insulin sensitivity as the maximal glucose disposal rate (GDR) during hyperinsulinemic clamps in 56 insulin sensitive (IS; mean ± SD: GDR 15.8 ± 2.0 mg · kg^{-1} · min^{-1}, fasting blood glucose [FBG] 4.7 ± 0.3 mmol/l, BMI 26 ± 5), 46 insulin resistant (IR; GDR 10.2 ± 1.9, FBG 4.9 ± 0.5, BMI 29 ± 5), and 46 untreated subjects with type 2 diabetes (GDR 7.4 ± 2.8, FBG 10.8 ± 3.7, BMI 30 ± 5). In the group as a whole, regression analyses with GDR showed that progressive insulin resistance was associated with an increase in VLDL size ($r = -0.40$) and an increase in large VLDL particle concentrations ($r = -0.42$), a decrease in LDL size ($r = 0.42$) as a result of a marked increase in small LDL particles ($r = -0.34$) and reduced large LDL ($r = 0.34$), an overall increase in the number of LDL particles ($r = -0.44$), and a decrease in HDL size ($r = 0.41$) as a result of depletion of large HDL particles ($r = 0.38$) and a modest increase in small HDL ($r = -0.21$; all $P < 0.01$). These correlations were also evident when only normoglycemic individuals were included in the analyses (i.e., IS + IR but no diabetes), and persisted in multiple regression analyses adjusting for age, BMI, sex, and race. Discontinuous analyses were also performed. When compared with IS, the IR and diabetes subgroups exhibited a two- to threefold increase in large VLDL particle concentrations (no change in medium or small VLDL), which produced an increase in serum triglycerides; a decrease in LDL size as a result of an increase in small and a reduction in large LDL subclasses, plus an increase in overall LDL particle concentration, which together led to no difference (IS versus IR) or a minimal difference (IS versus diabetes) in LDL cholesterol; and a decrease in large cardioprotective HDL combined with an increase in the small HDL subclass such that there was no net significant difference in HDL cholesterol. We conclude that *1*) insulin resistance had profound effects on lipoprotein size and subclass particle concentrations for VLDL, LDL, and HDL when measured by NMR; *2*) in type 2 diabetes, the lipoprotein subclass alterations are moderately exacerbated but can be attributed primarily to the underlying insulin resistance; and *3*) these insulin resistance–induced changes in the NMR lipoprotein subclass profile predictably increase risk of cardiovascular disease but were not fully apparent in the conventional lipid panel. It will be important to study whether NMR lipoprotein subclass parameters can be used to manage risk more effectively and prevent cardiovascular disease in patients with the IRS. *Diabetes* 52:453–462, 2003

The insulin resistance syndrome (IRS) is a trait cluster composed of risk factors for the future development of atherosclerosis and/or type 2 diabetes (1–3). A key component of the IRS is dyslipidemia. When assessed by the conventional lipid panel, the dyslipidemia is characterized by high triglycerides and low HDL cholesterol, whereas total cholesterol and calculated LDL cholesterol are not consistently altered in the IRS (1–4). However, there is heterogeneity in particle size and density within the major lipoprotein classes, which is not measured by the conventional lipid panel. For example, in the general population, increased levels of small, dense LDL (5–7) and a preponderance of the small subfraction of HDL (8–10) have been shown to be associated with increased risk for atherosclerosis. The IRS has been most extensively studied for its effects on LDL particle subclasses (11–23) and is known to be associated with smaller, denser LDL particles that may be more atherogenic. Less information is known regarding the effects of insulin resistance on VLDL and HDL subclasses.

Until recently, methods to determine lipoprotein subclass distribution have been relatively laborious and time-consuming, which limited their applicability in epidemiology and in the study of large sample populations. These techniques have depended on physical separation of lipoprotein subclasses, typically using gradient gel electrophoresis or density gradient ultracentrifugation. Recently, nuclear magnetic resonance (NMR) spectroscopy has

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apoB, apolipoprotein B; GDR, glucose disposal rate; IDL, intermediate-density lipoprotein; IR, insulin resistant; IS, insulin sensitive; IRS, insulin resistance syndrome; NMR, nuclear magnetic resonance.

TABLE 1
Demographic, anthropometric, and biochemical characteristics in IS, IR, and type 2 diabetic subjects

Variables	All (n = 148)	IS (n = 56)	IR (n = 46)	Diabetes (n = 46)	P IR vs. IS	P DM vs. IS
Age (years)	36.8 ± 11.8	34.8 ± 11.2	36.5 ± 10.3	39.5 ± 13.7	0.4594	**0.0438**
Male (%)	43	34	53	37	**0.0124**	0.7502
Caucasian (%)	66	82	63	50	**0.0296**	**0.0006**
BMI (kg/m^2)	28.1 ± 5.2	25.8 ± 4.5	29.2 ± 5.3	29.9 ± 5.1	**0.0006**	**0.0001**
WHR	0.86 ± 0.08	0.80 ± 0.06	0.88 ± 0.07	0.90 ± 0.08	**<0.0001**	**<0.0001**
% Body fat	30.3 ± 10.8	29.8 ± 12.4	29.4 ± 10.0	31.6 ± 9.5	0.8393	0.4152
Insulin (pmol/l)	44 ± 35	27 ± 11	60 ± 46	50 ± 31	**<0.0001**	**0.0007**
Fasting glucose (mmol/l)	6.7 ± 3.5	4.7 ± 0.3	4.9 ± 0.5	10.8 ± 3.7	0.5012	**<0.0001**
GDR (mg · kg^{-1} · min)	11.4 ± 4.2	15.8 ± 2.0	10.2 ± 1.9	7.4 ± 2.8	**<0.0001**	**<0.0001**

Data are means ± SD. WHR, waist-to-hip ratio. Boldface data signify statistical significance at $P < 0.05$. DM, diabetes.

been used to measure lipoprotein size and subclass particle concentration in whole sera (24–29). This method exploits that lipoprotein particles emit spectral signals that vary in a characteristic manner as a function of particle diameter. The measured amplitude of these signals provide a novel and efficient means for quantifying lipoprotein subclasses, differing from traditional methods that quantify lipoproteins on the basis of their lipid (cholesterol or triglyceride) or apolipoprotein content. Thus, NMR lipoprotein subclass analysis readily provides measures of lipoprotein subclass particle size and concentration. In addition, by using calculations based on the diameters of particles emitting the spectral signals and known relations between lipoprotein particle diameter and lipid content, the measured NMR spectral data may be transformed to give subclass concentrations expressed in particle concentrations units (nanomoles of particles per liter) or, alternatively, in lipid mass concentration units (millimoles of cholesterol or triglycerides in a specific particle per liter of serum).

The effects of insulin resistance on lipoprotein subclasses quantified by NMR have not been rigorously studied. To define the impact of insulin resistance, we measured the NMR profile in nondiabetic individuals over a wide range of insulin sensitivity as defined by the hyperinsulinemic-euglycemic clamp and in patients with type 2 diabetes. We observed specific effects on lipoprotein particle size and subclass concentrations that encompassed all of the major lipoprotein classes: VLDL, LDL, and HDL. It became apparent that the conventional lipid panel may not provide critical information needed for comprehensive assessment of the effects of insulin resistance and the accompanying risks of cardiovascular disease.

RESEARCH DESIGN AND METHODS

Clinical characterization. We studied subjects with and without type 2 diabetes (30), and the clinical characteristics of the study group are listed in Table 1. Before the study, all patients with type 2 diabetes were being treated with diet or sulfonylurea and/or metformin oral hypoglycemic agents but were withdrawn from therapy for at least 3 weeks and followed on an outpatient basis. All subjects were allowed to equilibrate on a weight-maintenance diet (28–32 kcal · kg^{-1} · day^{-1}) consisting of 50% carbohydrate, 30% fat, and 20% protein. Nondiabetic and untreated subjects with type 2 diabetes were then admitted to the General Clinical Research Center, where they remained active, and the isocaloric diet was maintained throughout. Weight had to be stable (±3%) for at least 3 months before study, and none of the study subjects engaged in regular exercise. None of the volunteers had cardiovascular, renal, or hepatic disease, and all were chemically euthyroid. No subjects were ingesting any pharmacological agents known to affect carbohydrate homeostasis, lipids, or lipoprotein metabolism. Protocols were approved by the Medical University of South Carolina Institutional Review Board, and written informed consent was obtained from every subject.

Standard 75-g oral glucose tolerance tests were performed (30) after a 12-h overnight fast. All nondiabetic subjects had normal fasting plasma glucose concentrations (all were ≤5.7 mmol/l); however, 14 of 102 were found to have impaired glucose tolerance (30) on the basis of the 2-h glucose measurement during the oral glucose tolerance test. The nondiabetic subjects were categorized as insulin sensitive (IS) or insulin resistant (IR) on the basis of the maximally insulin-stimulated glucose uptake rates more than or ≤12.8 mg · kg^{-1} · min^{-1}, respectively, during hyperinsulinemic-euglycemic clamp studies. This cutoff placed 45% of the randomly recruited, nondiabetic patients in the IR category and was selected because logistic analyses indicated that values <12.8 mg · kg^{-1} · min^{-1} tended to be associated with the trait cluster of the IRS (31). Of the 14 subjects with impaired glucose tolerance, 10 of these were in the IR subgroup and 4 were in the IS subgroup.

Percentage of body fat, regional percentage of body fat, and lean body mass were measured by dual energy X-ray absorptiometry (Lunar Radiation, Madison, WI), as previously described (32).

Insulin sensitivity. In vivo insulin sensitivity was assessed using the euglycemic-hyperinsulinemic glucose clamp technique as previously described (32–34). After a 12-h fast, a catheter was inserted into the brachial vein to administer insulin, glucose, and KPO_4. A dorsal hand vein was cannulated in a retrograde manner and kept in a warming device (65°C) to provide arterialized venous blood for sampling. For maximally stimulating glucose uptake and suppressing hepatic glucose production, regular insulin (Humulin; Eli Lilly, Indianapolis, IN) was administered at a rate of 200 mU · m^{-2} · min^{-1}, producing a mean steady-state insulin concentration of 3,480 ± 138 pmol/l that is maximally effective for stimulating glucose uptake into skeletal muscle (33). Plasma glucose was clamped at 5.0 mmol/l for at least 3 h, and maximal glucose uptake for each individual was calculated from the mean glucose infusion rate over the final three 20-min intervals. Whole-body glucose uptake was calculated on the basis of the glucose infusion rate corrected for changes in the glucose pool size, assuming a distribution volume of 19% body weight and a pool fraction of 0.65. Glucose uptake was normalized per kilogram of lean body mass (excluding bone mass) determined by dual energy X-ray absorptiometry to yield the glucose disposal rate (GDR) per kilogram of lean body mass.

Nuclear magnetic resonance lipoprotein subclass profile. Fasting blood for the nuclear magnetic resonance (NMR) lipoprotein subclass profile was obtained from the same draw as the conventional lipid panel. Venipuncture did not involve intravenous fluids or heparin administration. Serum was isolated by centrifugation (3,000 rpm, 20 min, 4°C) promptly after blood clotting and stored at −80°C until assay. The NMR lipoprotein subclass profile was determined using a 400-MHz proton NMR analyzer at LipoScience (Raleigh, NC). In brief, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different size as the basis of their quantification (24,25). Each measurement produces the signal amplitudes of 16 VLDL, LDL, and HDL subclasses. Conversion factors relating these signal amplitudes to either particle concentration or lipid mass concentration units were obtained from NMR and chemical lipid analyses of a set of purified subclass standards. These standards were isolated from a diverse group of normo- and dyslipidemic individuals by a combination of ultracentrifugation and agarose gel filtration chromatography and characterized for size distribution by electron microscopy (VLDL and LDL subclasses) or polyacrylamide gradient gel electrophoresis (HDL subclasses). Particle concentrations (nanomoles of particles per liter) were derived for each subclass standard by

measuring the total concentration of core lipid (cholesterol ester plus triglyceride) and dividing the volume occupied by these lipids by the core volume per particle calculated from knowledge of the particle's diameter. From lipid measurements performed on each subclass standard, conversion factors were generated to estimate lipid mass concentrations of the VLDL subclasses (millimoles per liter triglyceride) and LDL and HDL subclasses (millimoles per liter cholesterol). Average particle sizes (diameter, nanomole) of VLDL, LDL, and HDL were determined by weighting the relative mass percentage of each subclass according to its diameter. The technique for NMR lipoprotein determination has previously been described in detail (24–29).

For simplifying data analysis, six individual VLDL, three individual LDL, and five individual HDL subclasses were each grouped into three size categories (large, intermediate, and small). This resulted in the following 10 lipoprotein subclasses: large VLDL (60–200 nm), intermediate VLDL (35–60 nm), small VLDL (27–35 nm), intermediate-density lipoprotein (IDL; 23–27 nm), large LDL (21.3–23 nm), intermediate LDL (19.8–21.2 nm), small LDL (18.3–19.7 nm), large HDL (8.8–13 nm), intermediate HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Weighted average VLDL, LDL, and HDL particle sizes (nanomole diameter) were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its NMR signal. LDL and HDL subclass distributions determined by NMR and gradient gel electrophoresis are highly correlated (24,25,27,29). Although NMR-derived LDL subclass diameters are uniformly ~5 nm smaller than those estimated by gradient gel electrophoresis, they compare with gradient gel-based subclasses when assessed by both electron microscopy (35) and lipid compositional data (36).

Other assays. Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald equation from the total and HDL cholesterol levels measured by a colorimetric oxidase technique, and triglycerides were measured by a colorimetric glycerophosphate technique, using Vitos autoanalyzers (Johnson and Johnson, Rochester, NY).

Statistical analyses. Two approaches were used to examine the association of NMR lipoprotein profile and insulin resistance. First, the correlations between various lipoprotein measures and GDR (as continuous measures) were examined using Spearman correlation coefficients. These coefficients were calculated with and without adjustment for age, sex, race, and BMI. Then, the patients were classified into three distinct subgroups: IS, IR, and type 2 diabetes. Two indicator (dummy) variables representing insulin resistance and diabetes, respectively, were tested by linear regression models with insulin sensitive as the reference group. The SAS program version 8.0 (SAS Institute, Cary, NC) was used for analyses. Differences were accepted as significant at $P < 0.05$.

For major lipoprotein class measurements (i.e., VLDL, LDL, and HDL) obtained by summation of individual subclass levels, strengths of association with insulin resistance may differ depending on whether levels are expressed as particle concentrations or lipid mass concentrations. The reason is that lipoprotein levels expressed in lipid mass concentration units are weighted most heavily by contributions from the largest particles in the group (because larger particles contain more lipid than smaller particles), whereas those expressed in particle concentration units are weighted most heavily by contributions from the smallest particles. Any differences in the degree to which insulin resistance is associated with the larger compared with smaller subclass constituents within a lipoprotein class will cause different associations to be observed when the level of the lipoprotein class is expressed in particle versus lipid mass concentration terms. We therefore conducted separate evaluations of relations with insulin resistance using both particle and lipid mass concentration data.

RESULTS

Table 1 shows mean clinical and metabolic characteristics of the study sample. The data are presented for all 148 subjects, including 102 individuals without diabetes and 48 untreated individuals with type 2 diabetes. In addition, mean data are shown separately for IS and IR subgroups of individuals with normal fasting plasma glucose levels, as well as for the subgroup with diabetes. Compared with IS, the IR subgroup was composed of more men and ethnic minorities and exhibited higher BMI and waist-to-hip ratio. Except for overt hyperglycemia in diabetes, the clinical and metabolic variables were similar in the IR and diabetes subgroups.

To study the impact of insulin resistance on lipoproteins, we assessed the NMR lipoprotein subclass profile in fasting serum from all subjects. We used maximally stimulated GDR during hyperinsulinemic-euglycemic clamps, normalized for the amount of lean body mass, as the measure of insulin sensitivity. Because the GDR and characterizations of lipoprotein subclasses are continuous variables, we first emphasized continuous relationships in our analyses of the data. Accordingly, Table 2 and Fig. 1 show the correlations between the insulin-stimulated GDR and lipoprotein subclass particle concentration and size. In the study group as a whole, increasing GDR was negatively correlated with large VLDL ($P < 0.0001$), small LDL ($P < 0.0001$), and small HDL ($P = 0.0103$) particle concentrations and positively correlated with large LDL and large HDL particle concentrations (both $P < 0.0001$). Importantly, these relationships persisted at a highly significant level even after adjustment for age, sex, race, and BMI. The one exception is that the correlation between GDR and small HDL particle concentration became nonsignificant after adjustment for covariables; however, the correlation with the ratio of small to large HDL particles remained statistically significant. Thus, worsening insulin resistance (i.e., decreasing GDR) was associated with a progressive increase in large VLDL particle concentrations, and a shift toward lower concentrations of large LDL and HDL particles to increased concentrations of small, dense LDL and HDL particles. Consistent with these observations, GDR was negatively correlated with mean VLDL particle size and positively correlated with LDL and HDL particle size in Fig. 1; these relationships with particle size were highly significant regardless of whether adjusted for age, sex, race, and BMI (all $P \leq 0.0003$).

Table 3 shows correlations between the GDR and both lipoprotein subclass mass measurements from NMR analyses and conventional lipid panel parameters. The data on lipoprotein subclass mass (millimoles cholesterol/l for LDL and HDL or millimoles triglyceride/l for VLDL) parallel the results obtained with lipoprotein subclass particle concentrations (Table 2). Specifically, the GDR is highly negatively correlated with large VLDL, small dense LDL, and small HDL and positively correlated with large LDL and HDL. With respect to the conventional lipid panel, the GDR was negatively correlated with total cholesterol ($P = 0.0003$) and triglycerides ($P < 0.0001$) and exhibited weaker correlations with HDL cholesterol ($r = 0.20$; $P < 0.013$) and LDL cholesterol ($r = -0.19$; $P = 0.025$).

These relationships between insulin sensitivity and lipoprotein/lipid measurements all remain statistically significant when patients with diabetes are removed from the analyses (data not shown). When only individuals without diabetes are considered (IS plus IR subgroups in Table 1), the GDR is correlated with large VLDL particle concentration ($r = -0.287$, $P = 0.0034$) and mass ($r = -0.313$, $P = 0.0014$), small dense LDL particle concentration ($r = -0.244$, $P = 0.0136$) and mass ($r = -0.244$, $P = 0.0136$), large HDL particle concentration ($r = 0.292$, $P = 0.0029$) and mass ($r = 0.295$, $P = 0.0026$), and small/large HDL ratio for particle concentrations ($r = -0.269$, $P = 0.0067$) and mass ($r = -0.273$, $P = 0.0059$). In the individuals

TABLE 2
Correlations between GDR and NMR lipoprotein subclass particle concentration and lipoprotein size

	Unadjusted		Adjusted	
	r	*P*	*r*	*P*
Particle concentration				
VLDL				
Total	−0.20	**0.0154**	−0.07	0.4040
Large	−0.42	**<0.0001**	−0.35	**<0.0001**
Intermediate	−0.11	0.2050	−0.02	0.8032
Small	−0.15	0.0629	−0.06	0.5068
IDL	−0.11	0.1683	−0.08	0.3551
LDL				
Total	−0.44	**<0.0001**	−0.31	**0.0001**
Large	0.34	**<0.0001**	0.26	**0.0020**
Intermediate	−0.14	0.0998	−0.12	0.1423
Small	−0.34	**<0.0001**	−0.24	**0.0031**
Intermediate + small	−0.49	**<0.0001**	−0.36	**<0.0001**
HDL				
Total	−0.03	0.6937	0.04	0.6123
Large	0.38	**<0.0001**	0.29	**0.0004**
Intermediate	0.03	0.6963	−0.04	0.6586
Small	−0.21	**0.0103**	−0.04	0.5965
Small-to-large ratio	−0.38	**<0.0001**	−0.22	**0.0073**
Particle size				
VLDL	−0.40	**<0.0001**	−0.40	**<0.0001**
LDL	0.42	**<0.0001**	0.30	**0.0003**
HDL	0.41	**<0.0001**	0.30	**0.0003**

*Adjusted for age, sex, race, and BMI. Boldface data signify statistical significance at $P < 0.05$.

without diabetes, the GDR was also correlated with VLDL size ($r = -0.372$, $P = 0.0001$), LDL size ($r = 0.249$, $P = 0.0117$), and HDL size ($r = 0.322$, $P = 0.0010$). Regarding the conventional lipid panel, GDR was correlated with triglycerides ($r = -0.394$, $P < 0.0001$) and HDL cholesterol ($r = 0.238$, $P = 0.0164$) in the individuals without diabetes, whereas the relationship with LDL cholesterol did not reach statistical significance.

Importantly, the regression analyses in individuals without diabetes demonstrate that the effects of insulin resistance on lipoprotein subclasses are independent of any effects of diabetes or hyperglycemic metabolic milieu. To examine this issue further, we performed discontinuous analyses of lipoprotein subclasses in discrete subgroups of IS, IR, and diabetic subjects. When compared with IS, mean values for large VLDL and small LDL particle concentrations are higher, whereas large HDL particle concentrations are lower, in the IR (all $P < 0.05$) and diabetes (all $P < 0.01$) subgroups, as shown in Table 4. Similarly, Fig. 2 demonstrates that in both IR and diabetes subgroups, mean particle sizes for VLDL (larger), LDL (smaller), and HDL (smaller) are significantly different from that observed in IS (all $P < 0.05$). Table 5 shows the lipoprotein subclass mass measurements (in millimoles of triglyceride for VLDL or cholesterol for IDL, LDL, and HDL), and the results correspond entirely to the differences observed for lipoprotein subclass particle concentrations among the subgroups (i.e., Table 4). Results from the conventional lipid panel are also depicted in Table 5. In comparing IS and IR, only the difference in triglycerides achieves statistical significance ($P = 0.03$), whereas total cholesterol ($P = 0.004$), LDL cholesterol ($P = 0.034$), and triglyceride ($P < 0.001$) values are elevated in diabetes compared with IS.

From these data, it is apparent that the conventional lipid panel did not reflect profound differences in lipoprotein subclasses in comparing the subject groups. One example is shown in Fig. 3, which compares IS, IR, and diabetes subgroups with respect to calculated LDL cholesterol from the conventional lipid panel and NMR LDL subclass particle concentrations. Compared with IS, the large LDL particle concentration is reduced in IR (NS) and diabetes ($P < 0.001$), whereas small LDL particles are markedly increased ($P < 0.001$ for both IR and diabetes). As a result of these counterbalancing effects, the calculated LDL cholesterol level in the lipid panel is not significantly different between the IR and IS subgroups (NS) and is only modestly increased in diabetes ($P < 0.05$).

DISCUSSION

Our data provide a comprehensive examination of the effects of insulin resistance on lipoprotein subclasses. In subjects who exhibit a broad range of insulin sensitivity (with and without type 2 diabetes), we assessed insulin sensitivity using the gold standard hyperinsulinemic-euglycemic clamp and lipoprotein subclass particle concentrations and particle size by NMR. This is the first study to directly assess the impact of insulin resistance on lipoproteins using these methods. NMR generates unique spectra for quantifying different lipoprotein subclasses, and measurements of all lipoprotein subclass particle sizes and concentrations are determined simultaneously in less than a minute by deconvolution analyses of the complex NMR spectrum (24–29). Thus, subclass quantification does not require physical separation of lipoprotein subclass as is necessary for more laborious approaches, such as gradient gel electrophoresis and density gradient ultracentrifuga-

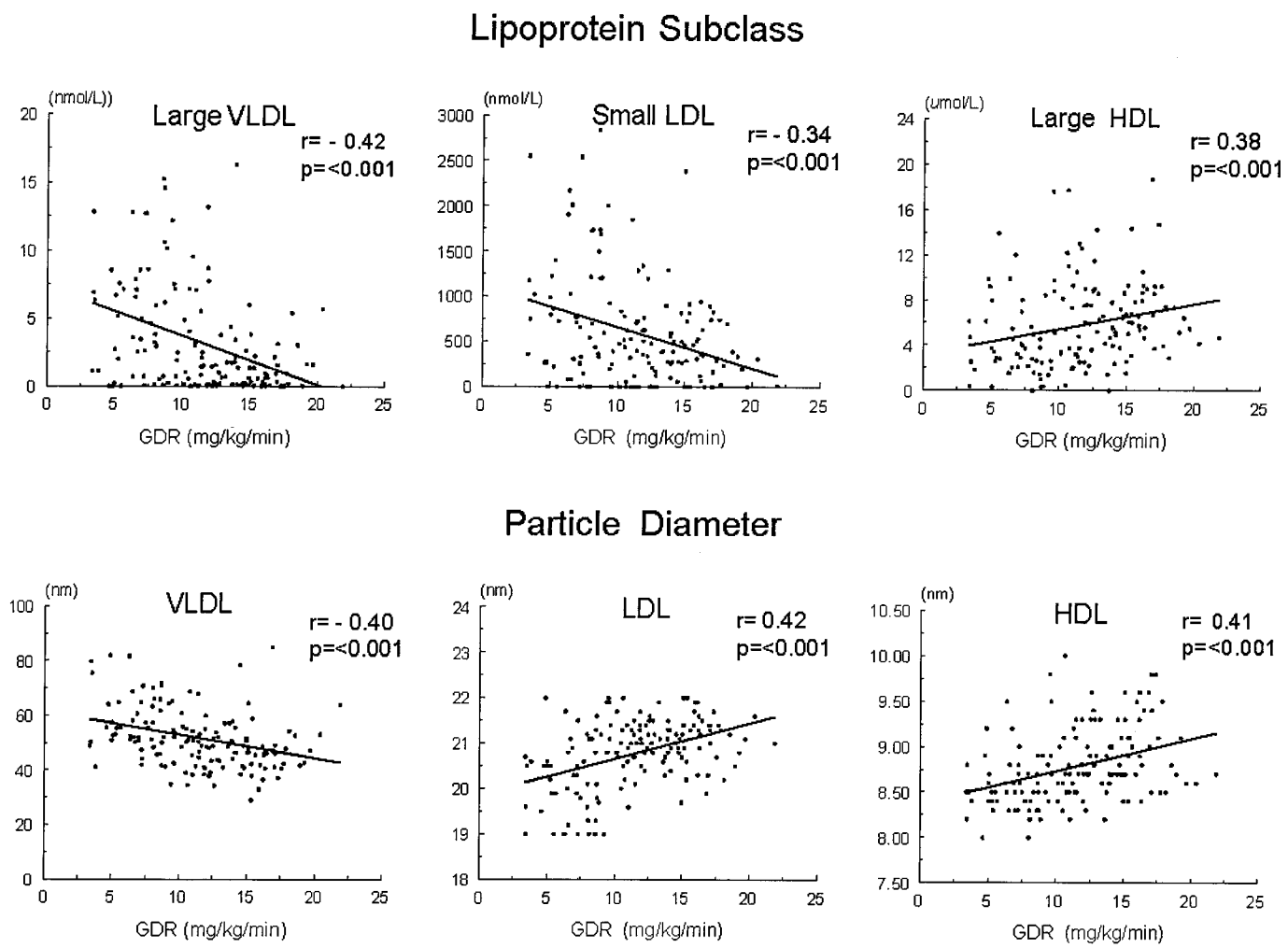

FIG. 1. Correlations between GDR (insulin sensitivity) and selected lipoprotein subclass particle concentrations and particle sizes. Insulin sensitivity was measured using the hyperinsulinemic-euglycemic clamp technique and lipoprotein subclass profiles were assessed by NMR in 148 individuals over a broad range of insulin sensitivity. The graphs show the relationship between maximally insulin-stimulated GDR normalized per kilogram of lean body mass with large VLDL, small LDL, and large HDL particle concentrations in the upper panels and VLDL, LDL, and HDL particle size (diameter in nanomoles) in the lower panels. The correlation coefficient (r) and P value are shown for each graph.

tion. For these reasons, analysis of lipoprotein subclasses using NMR has great potential for epidemiological and diagnostic applications.

We demonstrated that all three major lipoprotein classes were affected as a function of insulin sensitivity. As insulin resistance becomes more severe, the mean particle size of VLDL increased, whereas LDL and HDL particle sizes decreased. The progressive changes in lipoprotein sizes reflected alterations in particle concentrations for specific lipoprotein subclasses. The increase in VLDL size was entirely accounted for by an increase in the number of large VLDL particles without significant alterations in the concentrations of medium or small VLDL particles. Regarding LDL, both a decrease in the number of large particles combined with a marked increase in small LDL particle concentrations (without consistent changes in medium LDL) were responsible for the overall decrease in LDL size. Importantly, insulin resistance was also associated with an increase in the overall number of LDL particles. The decrement in HDL size was due primarily to a decrease in large HDL particles together with a modest increase in small HDL. These relationships with insulin resistance were clearly demonstrable whether insulin sensitivity was considered as a continuous variable or when mean lipoprotein subclasses were compared among discrete subgroups of IS and IR nondiabetic individuals and untreated patients with type 2 diabetes. The data regarding lipoprotein subclasses were similar whether expressed as particle concentration (nanomoles per liter) or after conversion to mass concentrations (millimoles per liter) of triglycerides in VLDL subclasses and cholesterol in IDL, LDL, and HDL subclasses. Moreover, the data remained statistically significant in multiple regression analyses controlling for any effects of age, race, sex, and BMI. Thus, the changes in lipoproteins were specifically and independently related to the degree of insulin resistance.

We also considered whether significant correlations between lipoproteins and glucose uptake rates were primarily a function of changes that occur in patients with type 2 diabetes, perhaps a product of the hyperglycemia or the more profound degree of insulin resistance observed in these patients. We therefore excluded the patients with diabetes and repeated the analyses. The major portion of the effects of insulin resistance on lipoprotein particle size

TABLE 3
Correlations between GDR and NMR lipoprotein subclass mass and conventional lipid panel measurements

	Unadjusted		Adjusted*	
	r	*P*	*r*	*P*
Lipoprotein subclass				
VLDL				
Total	−0.31	**0.0001**	−0.22	**0.0095**
Large	−0.45	**<0.0001**	−0.40	**<0.0001**
Intermediate	−0.12	0.1377	−0.03	0.6869
Small	−0.13	0.1159	−0.05	0.5190
IDL	−0.11	0.1671	−0.08	0.3522
LDL				
Total	−0.30	**0.0002**	−0.20	**0.0179**
Large	0.34	**<0.0001**	0.26	**0.0020**
Intermediate	−0.14	0.0998	−0.12	0.1421
Small	−0.34	**<0.0001**	−0.24	**0.0032**
Intermediate + small	−0.48	**<0.0001**	−0.36	**<0.0001**
HDL				
Total	0.27	**0.0010**	0.22	**0.0090**
Large	0.39	**<0.0001**	0.31	**0.0002**
Intermediate	0.03	0.6971	−0.04	0.6546
Small	−0.20	**0.0132**	−0.03	0.7605
Small-to-large ratio	−0.39	**<0.0001**	−0.24	**0.0036**
Conventional lipid panel				
Total cholesterol	−0.30	**0.0003**	−0.21	**0.0130**
LDL	−0.19	**0.0250**	−0.13	0.1338
HDL	0.20	**0.0131**	0.16	0.0511
Triglycerides	−0.47	**<0.0001**	−0.40	**<0.0001**
Triglycerides/HDL	−0.43	**<0.0001**	−0.36	**<0.0001**

*Adjusted for age, sex, race, and BMI. Boldface data signify statistical significance at $P < 0.05$.

and subclass particle concentrations was evident in subjects without diabetes. The imposition of diabetes led to some exacerbation of the changes observed in IR individuals without diabetes (as well as some worsening of insulin resistance); however, the severity of the dyslipidemia in type 2 diabetes could be largely explained by insulin resistance alone.

It is widely accepted that the IRS is characterized by high triglycerides and low HDL cholesterol, without consistent changes in total or calculated LDL cholesterol in the conventional lipid panel (1–3). However, it was clear that the conventional lipid panel did not reflect important effects of insulin resistance on the NMR lipoprotein subclass profile for all three major lipoprotein classes. The first example relates to VLDL subclasses. Insulin resistance was correlated with an increase in total triglycerides in the conventional lipid panel. Although most circulating triglycerides are contained within VLDL, the NMR lipoprotein subclass profile indicated that the increase in VLDL is not due to a uniform increase in VLDL subclasses. Rather,

TABLE 4
NMR lipoprotein subclass particle concentrations in IS, IR, and type 2 diabetic subjects

				P values	
	IS	IR	Diabetes	IR vs. IS	DM vs. IS
VLDL (nmol/l)					
Total	79.5 ± 43.5	83.8 ± 41.1	99.2 ± 52.2	0.6437	**0.0319**
Large	1.7 ± 2.7	3.4 ± 4.0	4.8 ± 5.0	**0.0334**	**0.0002**
Intermediate	20.3 ± 15.7	21.4 ± 14.7	21.3 ± 17.9	0.7336	0.7690
Small	57.5 ± 40.6	58.9 ± 35.5	73.2 ± 45.7	0.8601	0.0550
IDL (nmol/l)	9.1 ± 28.6	19.7 ± 47.5	17.8 ± 38.1	0.1673	0.2560
LDL (nmol/l)					
Total	1201.5 ± 367.0	1435.0 ± 399.3	1593.4 ± 504.4	**0.0063**	**<0.0001**
Large	589.3 ± 324.5	533.6 ± 357.3	385.4 ± 417.8	0.4457	**0.0058**
Intermediate	237.5 ± 315.6	213.3 ± 270.7	385.2 ± 424.5	0.7218	**0.0312**
Small	365.6 ± 397.5	668.4 ± 599.1	805.0 ± 711.7	**0.0088**	**0.0002**
HDL (μmol/l)					
Total	26.6 ± 5.9	26.5 ± 5.7	26.2 ± 6.2	0.9550	0.7129
Large	6.9 ± 3.5	5.3 ± 3.8	4.7 ± 3.7	**0.0292**	0.1162
Intermediate	5.5 ± 4.0	6.0 ± 4.3	4.1 ± 4.9	0.6010	0.1162
Small	14.2 ± 6.7	15.2 ± 5.6	17.3 ± 6.8	0.3992	**0.0147**
Small/large	2.9 ± 2.8	6.1 ± 11.7	7.8 ± 11.4	0.0849	**0.0082**

Data are means ± SD. Boldface data signify statistical siginficance at $P < 0.05$. DM, diabetes.

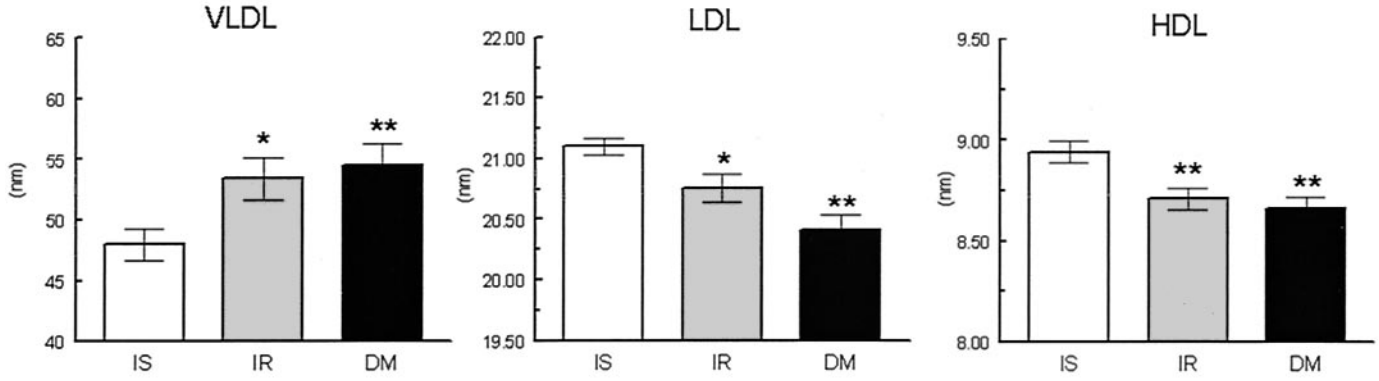

FIG. 2. Effects of insulin sensitivity and type 2 diabetes on lipoprotein particle sizes. Three subgroups were studied: individuals with normal fasting glucose levels categorized as IS or IR on the basis of maximally insulin-stimulated GDR and untreated patients with type 2 diabetes (DM). Data represent mean ± SE particle sizes (diameter in nanomoles) for VLDL (*left*), LDL (*middle*), and HDL (*right*) in the subgroups. Statistical significance was calculated compared with IS at *$P < 0.05$; **$P < 0.01$.

large VLDL particles, produced primarily by the liver, are markedly increased without consistent changes in medium or small VLDL, which are derived predominantly from the action of lipoprotein lipase on larger VLDL particles. Because large VLDL particles may confer more cardiovascular disease risk (28,37), the NMR profile provides a direct measure of this VLDL particle subclass that accounts for the increase in triglycerides. Second, in the conventional lipid panel, the IRS did not affect total and LDL cholesterol levels. The NMR data demonstrate why this is the case. Insulin resistance was associated with a decrement in the large LDL particle concentration in concert with a sizable increment in small LDL particles, and the net result is little or no change in overall LDL cholesterol. This is important because the increase in small LDL particle concentration has been shown to represent increased cardiovascular disease risk, independent of LDL cholesterol levels (5–7). Third, insulin resistance is known to be associated with lower circulating HDL cholesterol (1–3). The NMR data show that any decrease in HDL cholesterol is entirely explained by specific loss of the cardioprotective large HDL subclass, whereas noncardioprotective intermediate and small HDL particles may even be increased. Thus, the NMR lipoprotein subclass profile provides direct measurements relevant to cardiovascular risk that are either not provided or are obscured in the conventional lipid profile.

Another important aspect demonstrated only in the NMR data are that the total number of LDL particles was increased by insulin resistance. LDL particle concentra-

TABLE 5
NMR lipoprotein subclass mass and conventional lipid panel measurements in IS, IR, and type 2 diabetic subjects

				P values	
	IS	IR	Diabetes	IR vs. IS	DM vs. IS
NMR lipoprotein subclass					
VLDL (mmol/l)					
Total	0.76 ± 0.46	1.07 ± 0.78	1.24 ± 0.91	**0.0325**	**0.0011**
Large	0.18 ± 0.24	0.43 ± 0.57	0.57 ± 0.63	**0.0113**	**0.0001**
Intermediate	0.35 ± 0.26	0.39 ± 0.26	0.38 ± 0.31	0.4730	0.5712
Small	0.23 ± 0.15	0.25 ± 0.14	0.29 ± 0.18	0.5446	0.0647
IDL (mmol/l)	0.03 ± 0.08	0.06 ± 0.14	0.05 ± 0.11	0.1668	0.2560
LDL (mmol/l)					
Total	2.86 ± 0.85	3.20 ± 0.71	3.38 ± 0.96	**0.0415**	**0.0024**
Large	1.72 ± 0.95	1.56 ± 1.04	1.12 ± 1.22	0.4457	**0.0058**
Intermediate	0.53 ± 0.70	0.48 ± 0.60	0.86 ± 0.95	0.7214	**0.0311**
Small	0.61 ± 0.66	1.11 ± 1.00	1.34 ± 1.18	**0.0088**	**0.0002**
HDL (mmol/l)					
Total	1.10 ± 0.34	1.00 ± 0.33	0.93 ± 0.34	0.1127	**0.0119**
Large	0.56 ± 0.32	0.41 ± 0.33	0.37 ± 0.34	**0.0272**	**0.0049**
Intermediate	0.20 ± 0.15	0.22 ± 0.16	0.15 ± 0.18	0.6031	0.1154
Small	0.34 ± 0.15	0.37 ± 0.13	0.41 ± 0.15	0.4003	**0.0173**
Small/large	0.94 ± 0.98	1.71 ± 1.96	4.38 ± 12.96	0.6077	**0.0206**
Conventional lipid panel					
Total cholesterol (mmol/l)	4.33 ± 0.93	4.71 ± 0.87	4.99 ± 1.46	0.0863	**0.0036**
LDL (mmol/l)	2.74 ± 0.74	3.01 ± 0.74	3.12 ± 1.15	0.1282	**0.0341**
HDL (mmol/l)	1.11 ± 0.31	1.02 ± 0.30	1.04 ± 0.41	0.1987	0.3390
Triglycerides (mmol/l)	1.07 ± 0.57	1.49 ± 0.96	1.88 ± 1.29	**0.0299**	**<0.0001**
Triglycerides/HDL	1.03 ± 0.62	1.72 ± 1.49	2.18 ± 2.01	**0.0187**	**0.0001**

Data are means ± SD. Boldface data signify statistical significance at $P < 0.05$. DM, diabetes.

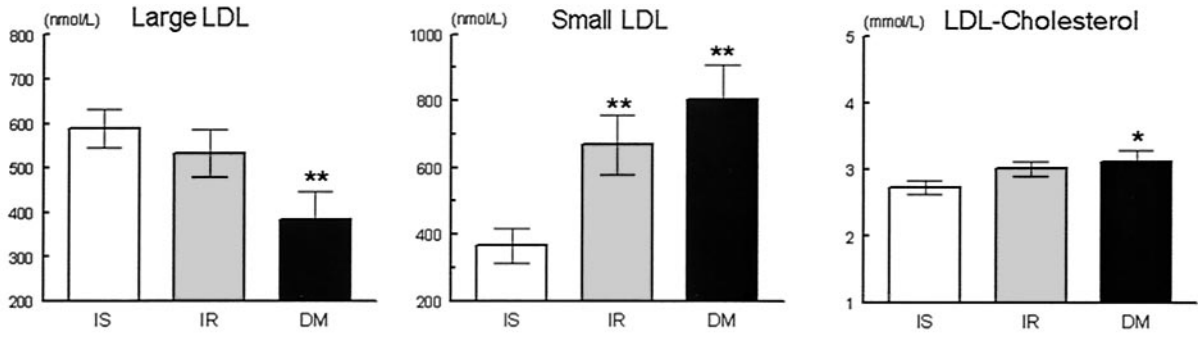

FIG. 3. Calculated LDL cholesterol values in the conventional lipid panel do not reveal effects of insulin sensitivity on LDL subclass particle concentrations. Three subgroups were studied: individuals with normal fasting glucose levels categorized as IS or IR on the basis of maximally insulin-stimulated GDR and untreated patients with type 2 diabetes (DM). Data represent mean ± SE values for large LDL subclass particle concentration (*left*), small LDL particle subclass concentration (*middle*), and calculated LDL cholesterol in the conventional lipid panel (*right*) in the subgroups. Statistical significance was calculated compared with IS at *$P < 0.05$; **$P < 0.01$.

tion has been shown to confer increased risk of cardiovascular disease (7) and is a stronger predictor of cardiovascular disease than LDL cholesterol in the few studies that have addressed this issue (27–29). Other methods do not directly assess LDL particle concentration, including serum apolipoprotein B (apoB) measurements (because apoB is not exclusively localized to LDL), gradient gel electrophoresis (which quantifies LDL size), and density gradient ultracentrifugation (unless apoB is measured in the isolated LDL fraction). The current article is the first to show by direct measurement that LDL particle concentration is increased as a function of insulin resistance.

The data are in general agreement and extend the observations of investigators using other methods to analyze lipoproteins and insulin sensitivity. Many of these studies have used gradient gel electrophoresis or density gradient ultracentrifugation to assess LDL size in subjects who were classified as IR using fasting serum insulin levels or the frequently sampled intravenous glucose tolerance test. These studies have demonstrated that LDL size is diminished in patients with type 2 diabetes (10,12,14,18,38) and the IRS (11,13–17,19–23). Small dense LDL has been shown to be associated with increased cardiovascular risk and is termed LDL subclass phenotype B (5,39–41).

In one of the few studies to address VLDL and HDL subclasses, Tilly-Kiesi et al. (23) demonstrated that hyperinsulinemia was associated with an increase in triglycerides as a result of increments in both large and small VLDL subclasses, increased small dense LDL without a change in LDL cholesterol, and lower HDL as a result of decrements in the larger cardioprotective HDL_2 subclass. One previous study has addressed the relationship between insulin resistance and the NMR lipoprotein subclass profile. MacLean et al. (22) assessed insulin sensitivity using the frequently sampled intravenous glucose tolerance test and found no difference in the NMR lipoprotein subclass profile between lean IS and lean IR women. This is in contrast to the current results: we observed significant relationships between VLDL, LDL, and HDL size and subclass particle concentrations independent of BMI or sex. These authors did find, however, that obese IR subjects had larger VLDL, smaller LDL as a result of a decrease in the large LDL subclass, and smaller HDL as a result of a decrease in the large HDL subclass when compared with obese IS individuals. These results in obese women are generally consistent with the current data.

The observed relationships between lipoprotein subclasses and insulin sensitivity have implications regarding mechanisms of dyslipidemia in the IRS. In IR individuals and individuals with diabetes, hypertriglyceridemia has been attributed to overproduction of triglyceride-rich VLDL particles combined with impaired VLDL clearance as a result, in part, of reduced plasma lipoprotein lipase activity (5,42–44). Our data indicate that the increased circulating triglycerides are carried predominantly in the large VLDL subclass. Increased large VLDL particle concentration could be a primary abnormality in generating the observed lipoprotein subclass pattern associated with insulin resistance. Increased large VLDL promotes cholesterol ester-triglyceride exchange between VLDL and LDL, resulting in LDL that is triglyceride enriched and cholesterol ester poor (44–47). Triglyceride-enriched LDL becomes a good substrate for hepatic lipase, and the ensuing triglyceride hydrolysis and structural remodeling lead to small dense LDL particles. This is consistent with previous studies indicating that plasma triglyceride concentration is a major determinant of LDL composition and particle size (44–49). Our data further demonstrate that the increase in small LDL particle concentration corresponds with an associated reduction in large LDL particles. An increase in triglyceride-rich large VLDL and in smaller LDL particles would also favor triglyceride exchange with HDL via lecithin-cholesterol acyltransferase and cholesterol ester transfer protein. Activities for cholesterol ester transfer protein and hepatic lipase have been negatively related to both HDL cholesterol levels and GDR in hyperinsulinemic glucose clamp studies (50). A reduction in large HDL2a and HDL2b subclasses, assessed by gradient gel electrophoresis, has been observed in IR subjects without pronounced effects on the concentration of smaller HDL3 particles (23). Although direct comparison with HDL subclasses assessed by NMR may not be fully precise, these previous results generally correspond (24,25) to our observation that large HDL subclasses (HDL4 and HDL5) are reduced, coupled with a small increase in small HDL subclasses (HDL1 and HDL2). This HDL subclass pattern is consistent with impaired reverse cholesterol transport in IR individuals.

In summary, progressive insulin resistance (i.e., decreasing GDR) was associated with increased VLDL size

and an increase in large VLDL particle concentrations, decreased LDL size reflecting a marked increase in small LDL particles and reduced large LDL, an overall increase in the number of LDL particles, and a decrease in HDL size as a result of depletion of large HDL particles and a modest increase in small HDL. These effects of insulin resistance are pronounced in subjects without diabetes and better define the dyslipidemia of the IRS. In type 2 diabetes, the lipoprotein subclass alterations are moderately exacerbated but can be attributed primarily to the underlying insulin resistance. These insulin resistance–induced changes in the NMR lipoprotein subclass profile predictably increased risk of cardiovascular disease but are not fully apparent in the conventional lipid panel. Because wide clinical utilization of the lipid panel may underestimate cardiovascular disease risk in the IRS, it will be important to study whether NMR lipoprotein subclass parameters can be used to manage risk more effectively and prevent cardiovascular disease in clinical trials.

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