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Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study

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Abstract

Background: Insulin resistance and obesity are associated with a dyslipidemia composed of high levels of triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), and no change in level of low-density lipoprotein cholesterol (LDL-C). We examined the association of insulin resistance and adiposity with lipoprotein particle size, concentration, and subclass concentrations.

Methods: The Insulin Resistance Atherosclerosis Study is a multicenter cohort study of middle-aged men and women. Lipoprotein lipid concentrations were determined using standard methods. Lipoprotein size, particle concentration, and subclass concentrations were determined using nuclear magnetic resonance technology. Insulin resistance (S_I) was determined based on the frequently sampled intravenous glucose tolerance test and the MINMOD program. A higher S_I represents less insulin resistance. Fasting insulin, body mass index, waist circumference, and waist/hip ratio were assessed.

Results: Among the 1371 participants were 754 women and 617 men; 459 Hispanics, 383 African Americans, and 529 non-Hispanic whites; 437 with type 2 diabetes, 301 with impaired glucose tolerance, and 633 with normal glucose tolerance. The mean (SD) age was 55.5 (8.5) years, body mass index was 29.3 (5.8) kg/m^2, and S_I was 1.6 (1.8) units. Adjusted for age, sex, and ethnicity, S_I was not associated with LDL-C ($r = 0.01$); however, S_I was associated with LDL size ($r = 0.34$, $P < .001$), LDL particle concentration ($r = -0.28$, $P < .001$), small LDL ($r = -0.34$, $P < .001$), intermediate LDL ($r = -0.37$, $P < .001$), and large LDL ($r = 0.21$, $P < .001$). In addition, S_I was associated with TG ($r = -0.36$, $P < .001$), VLDL particles ($r = -0.08$, $P < .01$), large VLDL ($r = -0.32$, $P < .001$), VLDL size ($r = -0.38$, $P < .001$), HDL-C ($r = 0.37$, $P < .001$), HDL particles ($r = 0.09$, $P < .001$), large HDL ($r = 0.31$, $P < .001$), and HDL size ($r = 0.33$, $P < .001$). A factor analysis revealed a factor that accounted for 41.4% of the variance across the lipoprotein measures and that was correlated with S_I ($r = -0.33$, $P < .001$). Similar results of opposing direction were observed for analyses of lipoprotein measures with fasting insulin and adiposity.

Conclusions: The dyslipidemia associated with insulin resistance and obesity includes effects on lipoprotein metabolism that are missed when traditional lipoprotein cholesterol and total TG are examined. Lipoprotein size and subclasses should be examined in studies investigating the roles of insulin resistance and obesity in the pathogenesis and prevention of atherosclerosis.

1. Introduction

Insulin resistance, adiposity, and diabetes are associated with a dyslipidemia composed of high concentrations of triglycerides (TG), low concentrations of high-density lipoprotein cholesterol (HDL-C), and no change in concentration of low-density lipoprotein cholesterol (LDL-C) [1-7]. Although LDL-C concentrations are unaffected, insulin resistance, adiposity, and diabetes influence LDL size [1,8,9]. Previous investigations of these relationships have been based most often on results regarding lipoprotein size and subclass quantification from gradient gel electrophoresis [1,8,9]. Recently, nuclear magnetic resonance (NMR) techniques have been developed that enable the high volume assessment of lipoprotein size, concentration, and subclass concentration [10,11]. The automated nature of this NMR method may lead to increased clinical use of this information. Garvey and colleagues [12] reported the association of insulin resistance and diabetes with lipoprotein subclass, particle size, and concentration from a small group (n = 148) of patients examined using the hyperinsulinemic clamp. In this report, we examined the association of insulin resistance, measured directly using the frequently sampled intravenous

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glucose tolerance test [13], and adiposity with lipoprotein size, particle concentration, and subclass concentrations measured using NMR technology in a large and ethnically diverse cohort.

2. Methods

2.1. Design and population

The objectives, design, and methods of the Insulin Resistance Atherosclerosis Study (IRAS) have been published previously [14]. Briefly, the major objective of IRAS was to assess the relationship between insulin resistance and atherosclerosis. This cross-sectional epidemiologic study was conducted at 4 clinical centers. African Americans and non-Hispanic whites were studied in center in Oakland and Los Angeles, Calif, and Hispanics and non-Hispanic whites were studied in centers in San Luis Valley, Colo, and San Antonio, Tex. In Los Angeles and Oakland, participants were recruited from members of a nonprofit health maintenance organization. In Colorado and Texas, participants were recruited from ongoing population-based epidemiologic studies of the risk factors for type 2 diabetes mellitus (DM) and coronary heart disease in Hispanics and non-Hispanic whites, the San Luis Valley Diabetes Study [15] and the San Antonio Heart Study [16], respectively. Sampling strategies were used to identify sufficient numbers of persons in different ethnic, age, sex, and glucose tolerance groups to allow an efficient study of relationships among and within these groups. Persons taking insulin were excluded. The IRAS was approved by the institutional review boards of all 4 clinical centers and the coordinating center, and informed consent was obtained for all participants.

2.2. Baseline clinic examination

The IRAS baseline clinical examination consisted of two 4-hour visits scheduled approximately 1 week apart [14,17]. Before each visit, participants were asked to refrain from alcohol and heavy exercise for 24 hours, from food for 12 hours, and from smoking on the day of the examination. The first visit included a 75-g oral glucose tolerance test; blood was collected for fasting and 2-hour glucose samples. Glucose tolerance status was classified according to World Health Organization criteria as either normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or DM [18]. To assess the presence and extent of atherosclerosis, participants underwent B-mode real-time ultrasound of the carotid artery [19,20]. Race and ethnicity were self-reported. Cigarette smoking status was assessed by self-report as current, past, or never. Anthropometry included weight, height, and waist circumference.

Insulin sensitivity was assessed by the frequently sampled intravenous glucose tolerance test (FSIGT) with minimal model (MINMOD) analyses. The protocol has been previously described in detail [14,17]. FSIGT (insulin-modified with 12 time points) protocol used in the IRAS study has been compared to the hyperinsulinemic euglycemic clamp and shown to be a valid measure of insulin resistance [21].

Plasma glucose was measured with the glucose oxidase technique on an automated autoanalyzer (Yellow Springs Equipment Co, Yellow Springs, Ohio). Insulin was measured using the dextran-charcoal radioimmunoassay that has considerable cross-reactivity with proinsulin.

Plasma lipoprotein cholesterol measurements were obtained from single fresh plasma samples using Lipid Research Clinics methods at the Penn Medical Laboratories of Medlantic Research Institute (Washington, DC). Low-density lipoprotein and HDL were isolated by isokypnic ultracentrifugation, and VLDL (top) and bottom fractions were measured for cholesterol and TG concentrations [22]. High-density lipoprotein cholesterol was measured in the presence of manganese chloride and heparin in which non-HDL were precipitated, leaving HDL in the supernatant. The supernatant was removed after centrifugation, and the cholesterol content was measured on a separate autoanalyzer channel set to measure low cholesterol values. Low-density lipoprotein cholesterol was calculated as the difference between the HDL-C and the bottom cholesterol. Triglycerides were measured enzymatically after correction for free glycerol.

Lipoprotein subclasses were determined using NMR spectroscopy (LipoScience, Raleigh, NC). The methodology has been described in detail [10,11]. In brief, each lipoprotein subclass is quantified using the NMR signals, which differ in frequency and shape depending on the diameter of the lipoprotein particles. The individual signals are derived from the total recorded signal using data from previously modeled reference lipoprotein subclasses. The intensity of each signal is proportional to the quantity of the subclass, which is reported in particle concentration units (nanomoles of particles per liter for VLDL and LDL and micromoles per liter for HDL). The NMR spectroscopy method reported 3 VLDL subclasses, intermediate-density lipoprotein (IDL), 3 LDL subclasses, and 3 HDL subclasses: large (60-200 nm), medium (35-60 nm), and small (27-35 nm) VLDL; IDL (23-27 nm); large (21.3-23.0 nm), medium (19.8-21.2 nm), and small (18.3-19.7 nm) LDL; and large (8.8-13 nm), medium (8.2-8.8 nm), and small (7.3-8.2 nm) HDL. Mean VLDL, LDL, and HDL particle sizes (nanometer diameter) were computed as the sum of the diameters of the individual subpopulations multiplied by their relative mass percentages as estimated from the amplitudes of their methyl NMR signals. The coefficients of variation for the mean particle sizes were 2% or less and for the particle concentrations of VLDL, LDL, and HDL were 4% or less; whereas, the coefficients of variation of the individual subclasses were less than 10% with 2 exceptions: IDL and intermediate HDL (27%).

2.3. Statistical analysis

Descriptive summary statistics were generated for the study population using means (and SEs) for continuous

Table 1
Baseline characteristics of IRAS participants and subgroup with NMR lipoprotein data

Characteristic	IRAS cohort		NMR subgroup	
	N = 1625	Prevalence (%)	n = 1371	Prevalence (%)
Average age (SD)	55.6 (8.5)		55.5 (8.5)	
Sex				
Female	906	55.8	754	55.0
Male	719	44.2	617	45.0
Ethnicity				
African Americans	464	28.6	383	27.9
Hispanic Americans	548	33.7	459	33.5
Non-Hispanic whites	613	37.7	529	38.6
Clinic				
Los Angeles, Calif	405	24.9	371	27.1
Oakland, Calif	405	24.9	309	22.5
San Antonio, Tex	408	25.1	346	25.2
San Luis Valley, Colo	407	25.1	345	25.2
Glucose tolerance status				
NGT	719	44.2	633	46.2
IGT	369	22.7	301	21.9
DM	537	33.1	437	31.9

variables and proportions for dichotomous variables. The associations between measures of insulin sensitivity and adiposity and lipoprotein measures were examined in using Spearman (nonparametric) correlation coefficients for continuous independent variables and analysis of variance for categorical independent variables, adjusting for age, sex, ethnicity, and clinic. Factor analysis was performed to identify underlying multivariable correlational structures reflective of unifying influences on lipoprotein metabolism as represented in the NMR data. Scores were calculated for factors that had eigenvalues exceeding 1 and associations between these scores and measures of insulin sensitivity and adiposity were examined using Spearman (nonparametric) correlation coefficients adjusted initially for age, sex, ethnicity, and clinic and subsequently for additional measures of insulin sensitivity or adiposity.

3. Results

The IRAS cohort included 1625 participants. Data were available for the present analyses for 1371 participants with mean (SD) age of 55.5 (8.5) years. Other characteristics are shown in Table 1. The study population was slightly more than half women and approximately one third each were African Americans, Hispanic Americans, and non-Hispanic whites. Almost half had NGT, approximately one fifth had IGT, and three tenths had diabetes. There were no substantive differences between persons with and without NMR data.

Measures of insulin sensitivity, adiposity, lipids, and lipoproteins are shown overall and by glucose tolerance status in Table 2. All measures of insulin sensitivity and adiposity worsened across categories of increasing glucose intolerance. Concentrations of TG and VLDL particles, including all VLDL subfractions except small VLDL, and VLDL particle size increased across categories of increasing glucose intolerance. The concentration of LDL-C did not

Table 2
Insulin sensitivity (S_I), fasting insulin, BMI, lipid and lipoprotein measures by glucose tolerance status in middle-aged adults

Variable	Total		NGT		IGT		DM		P[a]
	Mean	(SD)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	
S_I	1.6	(1.8)	2.6	(0.1)	1.3	(0.1)	0.5	(0.04)	<.0001
Fasting insulin (μU/mL)	18.2	(15.2)	13.8	(0.4)	20.0	(1.2)	23.2	(0.7)	<.0001
BMI (kg/m^2)	29.3	(5.8)	27.4	(0.2)	30.6	(0.4)	31.3	(0.3)	<.0001
Waist circumference (cm)	93.2	(13.1)	88.3	(0.5)	95.5	(0.8)	98.9	(0.6)	<.0001
Waist/hip ratio	0.88	(0.09)	0.85	(0.003)	0.88	(0.005)	0.91	(0.004)	<.0001
TG (mg/dL)	149.5	(101.8)	124.1	(3.3)	157.4	(5.3)	180.8	(5.8)	<.0001
Large VLDL (nmol/L)	3.5	(3.8)	2.6	(0.1)	3.5	(0.2)	4.7	(0.2)	<.0001
Intermediate VLDL (nmol/L)	18.4	(14.1)	17.7	(0.5)	18.5	(0.7)	19.4	(0.8)	.02
Small VLDL (nmol/L)	44.1	(20.1)	43.9	(0.8)	45.2	(1.1)	43.8	(0.9)	.47
Total VLDL particles (nmol/L)	66.0	(31.4)	64.2	(1.3)	67.2	(1.7)	67.8	(1.5)	.03
VLDL size (nm)	51.1	(11.4)	48.1	(0.4)	51.3	(0.6)	55.2	(0.6)	<.0001
LDL-C (mg/dL)	141.5	(35.3)	139.8	(1.4)	144.6	(2.1)	141.7	(1.7)	.40
IDL (nmol/L)	45.2	(26.5)	41.9	(1.0)	46.9	(1.5)	48.6	(1.3)	.004
Large LDL (nmol/L)	476.0	(230.3)	510.3	(8.4)	479.0	(13.4)	424.1	(11.8)	<.0001
Intermediate LDL (nmol/L)	175.8	(98.0)	151.0	(3.6)	183.5	(5.7)	206.5	(4.7)	<.0001
Small LDL (nmol/L)	523.7	(392.7)	433.1	(14.5)	550.8	(22.7)	636.3	(19.1)	<.0001
Total LDL particles (nmol/L)	1220.1	(401.7)	1136.4	(15.2)	1260.1	(22.8)	1315.6	(19.6)	<.0001
LDL size (nm)	21.2	(0.8)	21.4	(0.03)	21.2	(0.05)	20.9	(0.04)	<.0001
HDL-C (mg/dL)	44.9	(14.5)	47.5	(0.6)	45.6	(0.8)	40.6	(0.6)	<.0001
Large HDL (μmol/L)	4.6	(2.6)	5.1	(0.1)	4.5	(0.1)	3.9	(0.1)	<.0001
Intermediate HDL (μmol/L)	2.8	(3.8)	2.6	(0.1)	3.3	(0.2)	2.8	(0.2)	.03
Small HDL (μmol/L)	23.8	(5.4)	23.7	(0.2)	24.0	(0.3)	23.8	(0.3)	.46
Total HDL particles (μmol/L)	31.2	(5.4)	31.4	(0.2)	31.9	(0.4)	30.5	(0.3)	.001
HDL size (nm)	8.9	(0.5)	9.0	(0.02)	8.9	(0.02)	8.8	(0.02)	<.0001

[a] For difference between glucose tolerance groups adjusted for age, sex, and ethnicity.

Table 3
Partial Spearman correlations of lipid and lipoprotein measures with insulin sensitivity (S_I), fasting insulin, BMI, waist circumference, and waist/hip ratio adjusted for age, sex, and ethnicity in middle-aged adults

Variable	S_I	Fasting insulin	BMI	Waist	Waist/hip ratio
Fasting insulin	−0.67*				
BMI	−0.54*	0.56*			
Waist	−0.58*	0.58*	0.88*		
Waist/hip ratio	−0.50*	0.43*	0.43*	0.65*	
TG (mg/dL)	−0.36*	0.36*	0.26*	0.29*	0.32*
Large VLDL (nmol/L)	−0.32*	0.30*	0.22*	0.25*	0.30*
Intermediate VLDL (nmol/L)	−0.04	0.06**	0.00	0.03	0.12*
Small VLDL (nmol/L)	−0.03	0.04	0.07**	0.11*	0.15*
Total VLDL particles (nmol/L)	−0.08***	0.09*	0.06**	0.11*	0.19*
VLDL size (nm)	−0.38*	0.33*	0.23*	0.24*	0.24*
LDL-C (mg/dL)	0.01	0.03	0.02	0.05	0.09*
IDL (nmol/L)	−0.07***	0.09*	0.14*	0.13*	0.09*
Large LDL (nmol/L)	0.21*	−0.16*	−0.09*	−0.13*	−0.16*
Intermediate LDL (nmol/L)	−0.37*	0.33*	0.25*	0.31*	0.34*
Small LDL (nmol/L)	−0.34*	0.30*	0.22*	0.28*	0.32*
Total LDL particles (nmol/L)	−0.28*	0.26*	0.21*	0.26*	0.30*
LDL size (nm)	0.34*	−0.29*	−0.21*	−0.27*	−0.31*
HDL-C (mg/dL)	0.37*	−0.37*	−0.29*	−0.33*	−0.33*
Large HDL (μmol/L)	0.31*	−0.32*	−0.27*	−0.31*	−0.34*
Intermediate HDL (μmol/L)	−0.02	0.01	0.02	0.01	−0.03
Small HDL (μmol/L)	−0.04	0.05**	0.07***	0.09***	0.08***
Total HDL particles (μmol/L)	0.09*	−0.10*	−0.05**	−0.06**	−0.09***
HDL size (nm)	0.33*	−0.33*	−0.27*	−0.32*	−0.35*

* $P < .001$.
** $P < .05$.
*** $P < .01$.

differ by glucose tolerance status; however, this pattern masked potentially important changes in LDL particles. Concentrations of IDL and LDL particles, including small and intermediate LDL subfractions, increased across categories of increasing glucose intolerance; whereas, the concentration of large LDL particles decreased across categories of increasing glucose intolerance. Consequently, LDL particle size decreased across categories of increasing glucose intolerance. The concentrations of HDL-C, HDL particles, and large HDL particles and HDL size decreased across categories of increasing glucose intolerance.

Correlations between measures of insulin sensitivity and adiposity and measures of lipids and lipoproteins are shown in Table 3. Measures of insulin sensitivity and adiposity were strongly interrelated, with absolute values of correlation coefficients ranging from 0.43 between body mass index (BMI) and waist/hip ratio to 0.88 between BMI and waist. Triglycerides, large VLDL, total VLDL, and VLDL size were inversely correlated with S_I and positively correlated with fasting insulin and measures of adiposity. Small VLDL was also positively correlated with adiposity. The concentration of LDL-C was correlated only with waist/hip ratio; however, this pattern masked potentially important relationships. Concentrations of IDL and LDL particles, including small and intermediate LDL subfractions, were inversely associated with S_I and positively associated with fasting insulin and measures of adiposity. The concentration of large LDL particles and LDL size were positively correlated with S_I and inversely associated with fasting insulin and measures of adiposity. Concentrations of HDL-C, HDL particles, large HDL, and HDL size were positively correlated with S_I and inversely correlated with fasting insulin and measures of adiposity. Small HDL was positively correlated with fasting insulin and adiposity.

Results from a factor analysis used to identify metabolic influences on the underlying structures within the NMR lipoprotein data are shown in Table 4. As 16 variables were entered into the analysis, each component would be expected to partition 6.25% of the variance if the variables were completely independent. The first 5 factors partitioned at least 6.5% of the variance and had Eigenvalues in excess of 1. The first component partitioned 41.4% of the variance in these 16 measures and had positive loadings from VLDL particles, including all VLDL subfractions, total LDL particles, including small and intermediate LDL, and negative loadings from large LDL, LDL size, large HDL particles, and HDL size. The second component partitioned 13.8% of the variance and had positive loadings from small VLDL, large LDL, and small HDL, and negative loadings from large VLDL, VLDL size, and intermediate HDL particles. The third component partitioned 11.4% of the variance and had positive loadings from IDL and total HDL, including large and small HDL.

Table 4
Factor loadings for the major PCs of lipoprotein measures in middle-aged adults

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	6.63	2.21	1.83	1.55	1.05
Variance (%)	41.4	13.8	11.4	9.7	6.5
Variable					
Large VLDL	71[a]	−41*	24	10	−29
Intermediate VLDL	74[a]	0	17	29	−37
Small VLDL	55[a]	60[a]	15	41[a]	−7
Total VLDL particles	77[a]	33	20	40	−25
VLDL size	33	−66[a]	18	−26	−21
IDL	28	13	45[a]	27	45[a]
Large LDL	−59[a]	43[a]	28	20	−16
Intermediate LDL	92[a]	−4	−4	−15	24
Small LDL	91[a]	−1	−6	−8	32
Total LDL particles	80[a]	23	12	1	31
LDL size	−89[a]	20	16	16	−23
Large HDL	−67[a]	−15	45[a]	13	33
Intermediate HDL	20	−70[a]	34	32	−6
Small HDL	12	53[a]	42[a]	−70[a]	−14
Total HDL particles	−6	−5	87[a]	−40	−2
HDL size	−72[a]	−21	24	35	23

PC indicates principal component.
[a] |Factor loadings| greater than 40, taken as an indicator of significant loading of a variable into the factor.

Table 5
Partial Spearman correlations of major PC scores with insulin sensitivity (S_I), fasting insulin, BMI, waist circumference, and waist/hip ratio in middle-aged adults

PC	S_I	Fasting insulin	BMI	Waist	Waist/hip ratio
Model 1					
PC1	−0.33*	0.31*	0.24*	0.29*	0.35*
PC2	0.13*	−0.07**	−0.01	0.00	0.02
PC3	0.11*	−0.10*	−0.06***	−0.07**	−0.08**
PC4	0.20*	−0.18*	−0.15*	−0.16*	−0.13*
PC5	−0.05	0.02	0.05	0.04	−0.01
Model 2					
PC1	−0.17*	0.16*			
PC2	0.17*	−0.09*			
PC3	0.08*	−0.07***			
PC4	0.13*	−0.10*			
PC5	−0.05	0.00			
Model 3					
PC1			0.04	0.10*	0.21*
PC2			0.07***	0.09*	0.10*
PC3			0.01	0.00	−0.02
PC4			−0.03	−0.04	−0.03
PC5			0.03	0.02	−0.04

Model 1: adjusted for age, sex, and ethnicity.
Model 2: adjusted for age, sex and ethnicity, BMI, waist and waist/hip ratio.
Model 3: adjusted for age, sex and ethnicity, S_I, and fasting insulin.
* $P < .001$.
** $P < .01$.
*** $P < .05$.

Correlations between the measures of insulin sensitivity and adiposity and the 5 factor scores are shown in Table 5. The factor 1 score was inversely correlated with S_I and positively correlated with fasting insulin and measures of adiposity after adjustment for age, sex, and ethnicity. Additional adjustment for measures of adiposity attenuated the associations of the factor 1 score with S_I and fasting insulin; however, the correlations remained significant ($P < .001$). Adjustment for measures of insulin sensitivity attenuated the associations of the factor 1 score with measures of adiposity. The factor 1 score was no longer associated with BMI, but remained associated with waist circumference and waist/hip ratio. The other scores showed weaker correlations with the measures of insulin sensitivity and adiposity.

Similar results were seen in analyses restricted to patients without diabetes (results not shown).

4. Discussion

Nuclear magnetic resonance technology reveals a complex array of relationships between lipoprotein particles (including size, concentration, and subfraction concentration), glucose tolerance status, insulin sensitivity, and adiposity.

The dyslipidemia associated with insulin resistance and obesity includes a derangement of LDL metabolism that is missed when only total LDL-C concentration is examined. The lack of association between LDL-C concentration and measures of insulin sensitivity and adiposity masks opposing associations between these measures and concentrations of LDL subfractions. Greater concentrations of small LDL particles and lower concentrations of large LDL particles are associated with impaired insulin sensitivity or greater adiposity; hence, greater total LDL particle concentration and smaller LDL size accompany impaired insulin sensitivity and greater adiposity. Previously, we and others have reported an inverse association between insulin sensitivity and LDL size, as measured using gradient gel electrophoresis [1,8,9]. These results extend our earlier findings to include results regarding relationships with LDL particle concentration and subfraction concentrations that could only be inferred in our previous report. These results are consistent with a recent report from Garvey et al [12] from a small group (n = 148) studied using the hyperinsulinemic clamp. The relationships reported here are likely to represent atherogenic influences, as in the presence of impaired insulin sensitivity or greater adiposity, the arterial wall will be exposed to a greater concentration of circulating LDL particles that may be able to penetrate the endothelial lining more readily because of their smaller size [23,24].

Low HDL-C concentrations have been reported in association with impaired insulin sensitivity or greater adiposity [1-7]. Our results extend these findings to HDL particle concentration and subfraction concentrations. The associations between HDL-C and insulin sensitivity and adiposity reflect the influence of impaired insulin sensitivity and adiposity leading to a substantial reduction in concentrations of large HDL particles that is not offset by increases in intermediate or small HDL particles. The net effect is a small reduction in total HDL particle concentration; however, the resulting HDL particles are smaller. Whether small HDL particles as detected by NMR technology are less antiatherogenic than large HDL particles is currently not known with certainty and deserves further study. Colhoun et al [25] reported that lower HDL size was associated with coronary artery calcium in persons without diabetes, but saw no association in persons with diabetes, and Mackey et al [26] found no association of small HDL particles with coronary artery calcium in a cohort of healthy women. However, Rosenson et al [24] reported that small HDL particles were predictive of angiographic progression of coronary artery disease in the PLAC-1 Trial.

High TG concentrations have also been reported in association with impaired insulin sensitivity and adiposity [1-7]. Our results extend these findings to VLDL concentration, including VLDL subfraction concentrations. Impaired insulin sensitivity and adiposity were associated primarily with greater large VLDL particle concentration, and a shift to larger VLDL size, though greater waist/hip ratio was associated with greater concentration of all VLDL subfractions. The atherogenicity of large VLDL is not known; however, Castelli has suggested that large (“fluffy puffy”) VLDL may represent a less atherogenic pattern than small VLDL. Castelli [27] suggested that large VLDL is

converted to large LDL and small VLDL is converted to small LDL. The results of our factor analysis do not support that hypothesis. In factor 1, the concentration of small LDL was positively associated with the concentrations of all VLDL subfractions; whereas, large LDL and large VLDL were inversely associated with each other in both factors 1 and 2. Finally, VLDL and LDL size were not strongly correlated as demonstrated by their lack of strong simultaneous loading in any of the factors. It appears that greater VLDL concentrations are associated with greater concentrations of small LDL and smaller LDL size. On the contrary, Colhoun et al [25] and Mackey et al [26] have reported independently that the concentration of large VLDL particles was associated with coronary artery calcification. Our findings support the hypothesis that impaired insulin sensitivity and adiposity may contribute to the risk of atherosclerosis through increases in VLDL concentration, including especially the large VLDL subfraction.

According to recent lipoprotein transport modeling studies conducted by Chan et al [28], these insulin resistance–associated differences in lipoprotein concentrations could result from overproduction of VLDL apolipoprotein B-100 (apoB), decreased catabolism of apoB–containing particles, and increased catabolism of HDL apoA-I particles. Chan speculated that these abnormalities could be because of metabolic effects partly mediated by depressed plasma adiponectin levels that collectively increase the flux of fatty acids from adipose tissue to the liver, the accumulation of fat in the liver and skeletal muscle, the hepatic secretion of VLDL, and the remodeling of both LDL and HDL particles in the circulation. These lipoprotein changes could also relate to changes in both lipolytic enzymes and lipid transfer proteins [28].

The use of factor analysis enabled the identification of a factor that appeared to represent the influence of insulin sensitivity based on previous knowledge, that partitioned more than 40% of the variance across 16 measures of lipoproteins, and that was strongly correlated with measures of insulin sensitivity and adiposity. This finding underscores the importance of insulin sensitivity and adiposity in lipid metabolism and provides additional support for the contention that interventions that improve insulin sensitivity may improve lipid metabolism, thereby reducing risk of atherosclerosis.

This study has several limitations. First is the cross-sectional nature of the design, making determination of the temporal nature of these associations impossible. Second, the population is not truly population-based; however, the overrepresentation of African Americans, Hispanic Americans, and persons with either IGT or diabetes might be viewed as strengths.

This study also has several important strengths, including a large and diverse population, high-quality assessments of lipids in a standardized laboratory, and standardized methods for assessing insulin sensitivity and adiposity. All measurements of lipids and lipoproteins were made without knowledge of the insulin sensitivity or adiposity of the participants. These aspects of study design and conduct strengthen the inferences that can be drawn from these findings.

The clinical utility of this new technology is not yet clear. The results reported to date and summarized above provide some evidence of predictive utility; however, results from additional cohort studies and randomized trials are needed to determine whether the NMR measures are better predictors of risk of disease and response to therapy than the traditional measures of lipids.

In summary, NMR technology enables the assessment of lipoprotein particle size and concentrations, including subfractions. The use of this technology reveals potentially important information regarding the influences of insulin sensitivity and adiposity on lipoprotein metabolism. These measures should be examined in future studies investigating the roles of insulin sensitivity and obesity in the pathogenesis and prevention of atherosclerosis.

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References

[1] Howard BV, Mayer-Davis EJ, Goff D, et al. Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Metabolism 1998;47:1174-9.

[2] Reaven GM, Lerner RL, Stern MP, et al. Role of insulin in endogenous hypertriglyceridemia. J Clin Invest 1967;46:1756-67.

[3] Olefsky JM, Reaven GM. Decreased insulin binding to lymphocytes from diabetic subjects. J Clin Invest 1974;54:1323-8.

[4] Abbot WGH, Lillioja S, Young AA, et al. Relationships between plasma lipoprotein concentrations and insulin action in an obese hyperinsulinemic population. Diabetes 1987;36:897-904.

[5] Garg AJ, Helderman H, Koffler M, et al. Relationship between lipoprotein levels and in vivo insulin action in normal young white men. Metabolism 1988;37:982-7.

[6] Laakso M, Sarlund H, Mykkanen L. Insulin resistance is associated with lipid and lipoprotein abnormalities in subjects with varying degrees of glucose tolerance. Arteriosclerosis 1990;10:223-31.

[7] Godsland IF, Crook D, Walton C, et al. Influence of insulin resistance, secretion, and clearance on serum cholesterol, triglycerides, lipoprotein cholesterol and blood pressure in healthy men. Arterioscler Thromb 1992;12:1030-5.

[8] Reaven GM, Chen YDI, Jeppesen J, et al. Insulin resistance and hyperinsulinemia in individuals with small dense low density lipoprotein particles. J Clin Invest 1993;92:141-6.

[9] Austin MA, Selby JV. LDL subclass phenotypes and the risk factors of the insulin resistance syndrome. Int J Obes Relat Metab Disord 1995;19:S22-6.

[10] Otvos JD, Jeyarajah EJ, Bennett DW, et al. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clin Chem 1992;38:1632-8.

[11] Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. In: Rifai N, Wainick R, Cominazak M, editors. Handbook of lipoprotein testing. Washington (DC): AACC Press; 2000. p. 609-23.
[12] Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes 2003;52:453-62.
[13] Bergman RN, Finegod DT, Ader M. Assessment of insulin sensitivity in vivo. Endocrinol Rev 1985;6:45-86.
[14] Wagenknecht LE, Mayer EJ, Rewers M, et al. The Insulin Resistance Atherosclerosis Study (IRAS). Objectives, design and recruitment results. Ann Epidemiol 1995;5:464-71.
[15] Hamman RF, Marshall JA, Baxter J, et al. Methods and prevalence of non-insulin dependent diabetes mellitus in a biethnic Colorado population: the San Luis Valley Diabetes Study. Am J Epidemiol 1989;129:295-311.
[16] Stern MP, Rosenthal M, Haffner SM, et al. Sex differences in the effects of sociocultural status on diabetes and cardiovascular risk factors in Mexican Americans: the San Antonio Heart Study. Am J Epidemiol 1984;120:834-51.
[17] Haffner SM, D'Agostino Jr R, Saad MF, et al. Increased insulin resistance and insulin secretion in nondiabetic African Americans and Hispanics compared with non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Diabetes 1996;45:742-8.
[18] World Health Organization. Diabetes mellitus: report of a WHO study group. World Health Org Tech Rep Ser 1985;727.
[19] Howard G, et al, for the IRAS Investigators. Insulin sensitivity and atherosclerosis. Circulation 1996;93:1809-17.
[20] O'Leary DH, et al, on behalf of the CHS Collaborative Research Group. Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. Stroke 1991;22: 1155-63.
[21] Saad MF, Anderson RL, Laws A, et al. A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Diabetes 1994; 43:1114-21.
[22] Robbins DC, Welty TK, Wang WY, et al. Plasma lipids and lipoprotein concentrations among American Indians: comparisons with the US population. Curr Opin Lipidol 1996;7:188-95.
[23] Blake GJ, Otvos JD, Rifai N, et al. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002;106:1930-7.
[24] Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002;90:89-94.
[25] Colhoun HM, Otvos JD, Rubens MB, et al. Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. Diabetes 2002;51:1949-56.
[26] Mackey RH, Kuller LH, Sutton-Tyrrell K, Evans RW, Holubkov R, Matthews KA. Lipoprotein subclasses and coronary artery calcium in postmenopausal women from the healthy women study. Am J Cardiol 2002;90(8A):71i-6i.
[27] Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996;124:S1-S9.
[28] Chan DC, Barrett PH, Watts GF. Lipoprotein transport in the metabolic syndrome (part II): pathophysiological and interventional studies employing stable isotopy and modelling methods. Clin Sci (Lond) 2004;107:233-49.