Whole-Body Insulin Sensitivity, Low-Density Lipoprotein (LDL) Particle Size, and Oxidized LDL in Overweight, Nondiabetic Men

Richard C. Ho, Kevin Davy, Brenda Davy, and Christopher L. Melby

Insulin resistance is often accompanied by elevated plasma triglycerides (TG) and a preponderance of small, dense low-density lipoprotein (LDL) particles. However, it remains unclear whether or not insulin resistance is related to LDL particle size, independent of plasma TG. We sought to determine the strength of the relationships among these variables in a group of overweight, nondiabetic men (N = 34; body mass index [BMI], 25 to 35 kg/m^2; age, 50 to 75 years), as well as to examine the possible relation between insulin sensitivity and oxidized LDL (oxLDL). We also examined the strength of the relationships between these lipid variables and estimates of insulin sensitivity using calculated indices based on fasting insulin and glucose concentrations. Insulin sensitivity (Si) was significantly associated with total TG (r = -0.61, P < .001), very–low-density lipoprotein (VLDL)-TG (r = -0.60, P < .001), and LDL size (r =.414, P < .05). LDL size was also significantly associated with TG (r = -0.73, P < .001), VLDL-TG (r = -0.73, P < .001), high-density lipoprotein-cholesterol (HDL-C) (r = 0.65, P < .001), the quantitative insulin sensitivity check index (QUICKI) (*rho* = 0.46, P < .01), and the homeostatic model for the assessment of insulin resistance (HOMA-IR) (*rho* = -0.45, P < .01). Si was a significant predictor of LDL size, with age and BMI also independent contributors to the variance in LDL size (R^2 = 0.172). However, when TG and HDL-C were added to the model, Si was no longer a significant predictor of LDL size. The correlation between Si and oxLDL was weak, but stastically significant (*rho* = -0.40, P = .02). These data suggest that the relation between Si and LDL size is largely mediated by plasma TG, and that Si is only weakly related to oxLDL in overweight, nondiabetic men.

SEVERAL LINES of evidence suggest that insulin resistance may be one of the most important defects contributed to the clustering of risk factors (hypertension, dyslipidemia) associated with the metabolic syndrome.[1,2] These metabolic abnormalities are associated with an increased risk for atherogenesis, although the mechanisms explaining these comorbitities are not entirely clear. Whole-body insulin resistance is associated with increases in plasma nonesterified fatty acids (NEFA). Elevations in plasma NEFA increase hepatic synthesis of triglyceride-rich particles, primarily very–low-density lipoprotein (VLDL). Initially, via exchange of cholesterol ester for VLDL-triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles become TG-enriched, but later have substantial amounts of TG removed by the action of hepatic TG lipase, resulting in higher circulating concentrations of small, dense LDL and HDL particles.

Small, dense LDL particles appear to be more atherogenic than larger, less dense LDL particles,[3,4] and individuals with a predominance of plasma small, dense LDL (pattern B lipoprotein phenotype) carry a 3-fold higher risk for coronary heart disease (CHD) as compared to those with a predominance of large LDL particles (pattern A phenotype).[3] An elevated LDL particle number, often recognized by high apoprotein B concentrations, has also been linked to an increased CHD risk.[5,6] Small, dense LDL particles are cleared from plasma at a slower rate compared to larger LDL particles,[7] and appear to be more susceptible to oxidative damage and accumulation in the artery wall. It has recently been suggested that circulating oxidized LDL (oxLDL) may be a marker for CHD risk.[8,9] Individuals with pattern B phenotype are typically more insulin-resistant and hyperinsulinemic compared to those with pattern A phenotype.[10,11]

An association between whole-body insulin sensitivity (Si) and LDL particle size has been shown in some studies,[10,12-15] but not all.[16-19] Howard et al[20] found a significant relation between Si and LDL size among nondiabetic men and women. Mykkanen et al[13] found an association between insulin resistance and the preponderance of small, dense LDL particles in normoglycemic middle-aged men. However, others have reported that neither fasting insulin concentrations nor Si was related to LDL size independently of circulating plasma TG and HDL-cholesterol (HDL-C) levels.[14,19] It remains unclear, then, whether or not insulin resistance results in higher circulating concentrations of small, dense LDL particles, independent of elevated plasma TG. If plasma TG rather than Si were found to be more predictive of small, dense LDL concentrations, this would be of substantial clinical relevance given the convenience of TG measurement compared to the difficulty in obtaining measures of Si and LDL particle size in clinical practice.

The possibility also exists that insulin resistance directly contributes to the oxidative modification of LDL. After adjusting for age, gender, body mass index (BMI), and waist-to-hip ratio (WHR), Carantoni et al[21] found that the steady-state plasma glucose concentration, and plasma glucose and insulin responses to oral glucose, remained significantly correlated with oxLDL. They reported a significant and independent correlation between insulin resistance and compensatory hyperinsulinemia and the amount of oxLDL in nondiabetic patients. Previous studies have suggested that a preponderance of small,

From the Department of Food Science and Human Nutrition, Nutrition and Metabolic Fitness Laboratory, and the Department of Health and Exercise Science, Colorado State University, Fort Collins, CO.

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Address reprint requests to Christopher L. Melby, DrPH, Colorado State University, Department of Food Science and Human Nutrition, 226 Gifford, Fort Collins, CO 80523.

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dense LDL particles is related to increased oxLDL in obesity and in individuals with type 2 diabetes mellitus.[11,22,23]

Obesity and insulin resistance are strong risk factors in the development of both type 2 diabetes mellitus and atherosclerosis, yet there is a paucity of data regarding the relationship between insulin sensitivity, LDL size, and oxLDL in overweight individuals. Therefore, the purpose of this study was to determine whether Si, as determined by the insulin-augmented frequently sampled intravenous glucose tolerance test (FSIGTT), is related to LDL particle size and circulating oxidized LDL, independent of total plasma TG in overweight, nondiabetic males. Given the difficulty in measuring Si in clinical practice, we further sought to determine the strength of the relations among LDL particle size, oxLDL, and several different surrogate estimates of Si that depend on only a single fasting measurement of plasma glucose and insulin.

MATERIALS AND METHODS

Male subjects aged 50 to 75 years with a BMI between 25 and 35 kg/m^2 and elevated blood pressure (systolic 130 to 160 mm Hg and/or diastolic 85 to 99 mm Hg) and fasting blood glucose less than 110 mg/dL were recruited from the surrounding community. Only subjects who were sedentary or minimally physically active (fewer than two 30-minute aerobic exercise sessions per week) were included. Individuals were excluded if they reported or were observed to have any of the following: overt cardiovascular, metabolic, or pulmonary disease, or use of any medications known to affect any of the dependent variables in this study. The Human Research Committee at Colorado State University approved the study. All subjects provided written informed consent prior to participation.

Anthropometric Measurements

Body weight was determined to the nearest 0.1 kg using a balance scale (Detecto, Webb City, MO) and height (cm) (without shoes) was measured using a wall-mounted stadiometer. Abdominal skinfold thickness measurement (mm) was obtained using calipers (Lange, Cambridge Scientific Industries, Cambridge, MD). Waist circumference was measured (cm) at the level of the umbilicus using a Gulick II measuring tape (Country Technology, Gays Mills, WI). Hip circumference was measured in a similar manner at the point of widest hip girth. The percentage of body fat, absolute fat mass, and fat-free mass was measured in all subjects using dual-energy x-ray absorptiometry (DEXA) (Model DPX-IQ Lunar Corp, Madison, WI). Medium length scans (20 minutes) were used for all subjects except for those with an anteriorposterior thickness greater than 27 cm, for whom the slow (40-minute) speed was used.

Insulin Sensitivity

An FSIGTT was administered with the subjects in the supine position following an overnight, 12-hour fast, and after a 30-minute relaxation period. An intravenous (IV) catheter was placed in each antecubital vein, one for the administration of insulin and glucose, and one for collecting blood samples. Two blood samples were obtained at time (t) = -10 and -5 minutes, with these 2 samples used for determination of the mean baseline insulin and glucose concentrations. A bolus of glucose (0.3 g/kg in a 50% dextrose solution) was infused over a 90-second period at t = 0. At t = 20 minutes, a bolus of insulin (0.03 U/kg) was injected. Blood samples were obtained at t = 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes and immediately centrifuged at 4°C and analyzed for glucose concentrations by the glucose oxidase method using a glucose autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). A sample of plasma was stored at –20°C for later determination of insulin concentrations by the enzyme-linked immunosorbent assay (ELISA) method (Diagnostic Systems Laboratories, Webster, TX). The MINMOD program (version 3.0, R. Bergman, University of Southern California) was used for determination of Si. This model uses measurements of plasma glucose and insulin concentrations over the 3-hour period to deduce in vivo whole-body Si.[24] Whole-body Si was also estimated by fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI).[25-27]

Plasma Lipid Determinations

Following an overnight fast, blood was obtained from an indwelling IV catheter into tubes containing EDTA. Samples were inverted, and then centrifuged to obtain plasma, which was then stored at –70°C until analysis. Plasma lipid and lipoprotein profiles and characteristics were determined from plasma samples using nuclear magnetic resonance (NMR) spectroscopy (LipoMed, Raleigh, NC). This process allows the simultaneous measurement of 15 lipoprotein subclasses. Individual lipoprotein lipid concentrations are then calculated based on the average measured lipid values for each of the lipoproteins, based on values previously determined from spectrophotometric analysis of lipids from reference samples.[28] Total plasma cholesterol, TG, LDL-C, and HDL-C concentrations are then determined by summing the lipid concentrations of the various subclasses. Values were expressed in mmol/L of cholesterol (LDL-C and HDL-C) and TG. Strong correlations between NMR and chemical analysis for VLDL-TG, LDL-C, and HDL-C have been reported (r = 0.98, 0.91, and 0.93, respectively).[28] LDL subclass distribution between NMR and gradient gel electrophoresis (GGE) also correlate well.[28] Average LDL particle size (diameter, nm) was also determined. Reference standards for LDL subclass diameter for the NMR method are based on electron microscopy.[29] LDL subclass diameters obtained with the NMR method are approximately 5 nm smaller than those obtained using GGE, but are in agreement with electron microscopy data and calculations based on LDL chemical composition.[29] Coefficients of variation using the NMR procedure have been reported to be 1.5% to 2.9% for standard lipid panel variables and about 0.5% for average LDL particle size.[29] A more detailed treatment of the use of NMR for measuring plasma lipid and lipoprotein concentrations and lipoprotein particle size has been described previously.[28-30] Oxidized LDL was measured by ELISA (Mercodia, Uppsala, Sweden). This method is based on the direct sandwich technique in which 2 monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule.[9,31-33]

Statistical Analysis

All variables were subjected to normality testing. Pearson product-moment correlations and partial correlation coefficients were used to determine relationships between variables. Because the insulin concentrations were not normally distributed in the sample, Spearman's rank order correlation coefficient for nonparametric data (*rho*) was determined for the simple correlations involving fasting plasma insulin and for those involving the estimates of Si derived from fasting plasma insulin and glucose concentrations. For the partial correlations, the insulin values were log-transformed. Multiple stepwise regression analyses were also performed to determine how well metabolic variables predict LDL size and oxLDL. Data were analyzed with SPSS for Windows 10.0 statistical software (SPSS, Inc, Chicago, IL) and are expressed as the mean ± SEM.

RESULTS

Subject characteristics are listed in Table 1. The sample was composed of overweight and obese middle-aged and elderly

Table 1. Clinical and Metabolic Characteristics

Variable	Mean ± SEM (range)
Age (yr)	59.2 ± 1.1 (50-75)
Weight (kg)	91.8 ± 2.1 (70.7-123.9)
BML (kg/m^2)	29.4 ± 0.5 (25.0-39.1)
% Fat	28.1 ± 0.7 (14.3-37.8)
WHR	0.985 ± 0.005 (0.87-1.07)
Si × 10^4 (μU × mL^{-1} × min^{-1})	2.07 ± 0.23 (0.21-5.63)
Fasting insulin (pmol × L^{-1})	62.3 ± 9.4 (16.2-271.2)
Fasting glucose (mmol × L^{-1})	5.33 ± 0.07 (4.51-6.19)
Total TG (mmol × L^{-1})	1.66 ± 0.12 (0.58-3.35)
Total cholesterol (mmol × L^{-1})	5.08 ± 0.12 (3.52-6.38)
LDL-C (mmol × L^{-1})	3.42 ± 0.11 (1.84-4.62)
HDL-C (mmol × L^{-1})	0.88 ± 0.03 (0.60-1.51)
LDL size (nm)	20.26 ± 0.13 (19.0-21.7)
oxLDL (U/L)	51.15 ± 2.31 (10.14-83.46)
VLDL-TG (mmol × L^{-1})	1.28 ± 0.12 (0.21-3.00)

NOTE. N = 36.

Table 2. Relationship Between LDL Size, oxLDL, and Anthropometric and Metabolic Variables.

Variable	LDL Size		oxLDL	
	r	Partial *r*†	*r*	Partial *r***
Si	0.41*	0.44*	−0.30	−0.33
Fasting insulin	−0.43*‡	−0.33§	0.33*†	0.23§
Fasting glucose	−0.21	−0.21	0.01	0.01
Total TG	−0.73*	−0.72*	0.13	0.20
VLDL-TG	−0.73*	−0.72*	0.10	0.17
Total cholesterol	−0.37*	−0.41*	0.25	0.26
LDL-C	−0.22	−0.30	0.34*	0.35
HDL-C	0.65*	0.67*	−0.01	0.01

*Correlation coefficient significant at $P < .05$.

†Partial *r* adjusted for age (yr), % body fat, and abdominal circumference.

‡Spearman's *rho* nonparametric correlation coefficient.

§ Insulin log-transformed.

men with a wide range of values for the anthropometric and metabolic variables.

Si was significantly associated with total TG (r = -0.61, $P <$ .001) and with VLDL-TG (r = -0.60, $P <$ .001) (Fig 1). Simple and partial correlations used to determine the relations among LDL size, oxLDL, and specific anthropometric and metabolic variables are shown in Table 2. LDL size was significantly associated with Si (r = 0.41, $P <$ .02) (Fig 2), total TG (r = -0.73, $P <$ 0.001), VLDL-TG (r =-0.73, $P <$ .001), total cholesterol (r = -0.37, P = .026), and HDL-C (r =0.65, $P <$ .001). After adjusting for variables commonly associated with the metabolic syndrome (age, percent body fat, and abdominal circumference), these associations remained statistically significant. Oxidized LDL was significantly associated with LDL-C (r =0.34, P = .04), with a trend for an association with Si (r = -0.30, P = .09). When the relation between Si and oxLDL was analyzed using Spearman's rank order correlation, the relationship was significant (*rho* = -0.40, P = .02). After adjusting for age, percent body fat, and abdominal circumference, the relation between Si and oxLDL approached statistical significance (r = -0.33, P = .07). LDL size was not significantly associated with oxLDL.

Fasting glucose and insulin concentrations were used to calculate 2 different estimates of Si. HOMA-IR was significantly associated with Si (*rho* = -0.61, $P <$ .001), as was QUICKI (*rho*= 0.61, $P <$ .001). Table 3 shows that QUICKI and HOMA-IR were both significantly associated with LDL size (with the correlation coefficients similar to that of Si and LDL size) and with plasma TG. Neither HOMA-IR nor QUICKI was associated with oxLDL, although the correlation coefficient (*rho*) approached significance at $P <$ .10.

Stepwise regression was performed to determine whether estimates of insulin resistance could predict LDL size independent of total TG and HDL-C. Table 4 shows that Si alone accounted for 17% of the variance in LDL size. When plasma TG was added to the model, the explained variance in LDL size reached 53%, but Si was no longer a significant predictor of LDL size. When the 3 variables, Si, plasma TG, and HDL-C,

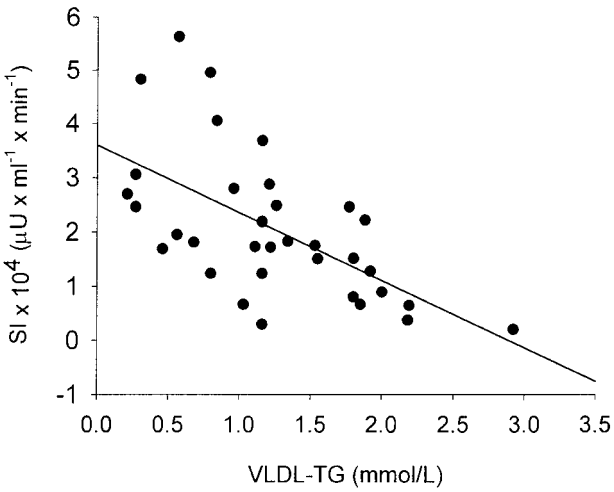

Fig 1. Relation between plasma concentrations of VLDL-TG and Si estimated by the FSIGTT. $r = -0.61$, $P < .001$.

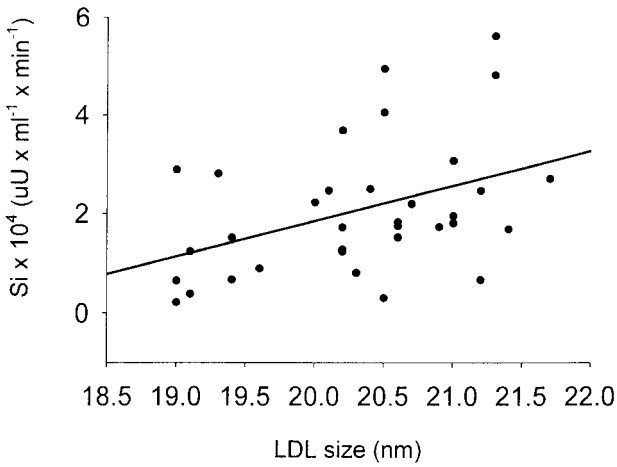

Fig 2. Relation between LDL particle size and Si estimated by the FSIGTT. $r = 0.41$, $P < .02$.

Table 3. Spearman's Rank Order Correlations (*rho*) Between LDL Size/oxLDL and Surrogate Measures of Insulin Sensitivity

	HOMA-IR	QUICKI
LDL size		
rho	−0.45	0.46
P value	<.01	<.01
oxLDL		
rho	0.29	−0.29
P value	.09	.09
Total TG		
rho	0.54	−0.55
P value	<.001	<.001

were all included in the model, 65% of the variance in LDL size was explained, but only plasma TG and HDL-C contributed significantly to the R^2 value. In a similar fashion, although QUICKI and HOMA-IR were significantly related to LDL size in the univariate analysis, they no longer contributed to explaining variance in LDL size when plasma TG was included in the regression analysis (data not shown).

DISCUSSION

The major findings in this study in overweight and obese nondiabetic mean are: (1) Si and LDL particle size are significantly correlated, but the ability of this particular index to explain the variance in LDL particle size is apparently mediated, to a large extent, by total plasma TG; (2) the QUICKI and the HOMA-IR, estimates of Si based on fasting glucose and insulin concentrations obtained from a single sample of blood, are as strongly correlated with LDL particle size as is the much more labor-intensive and expensive FSIGTT; and (3) Si is weakly correlated with plasma oxLDL concentrations in middle-aged and older overweight, nondiabetic men.

Si and LDL Particle Size

Insulin resistance is associated with elevations in plasma NEFAs, which, when cleared by the liver, stimulate the hepatic synthesis of TG-rich VLDL particles. Circulating VLDL exchanges TG for LDL/HDL-C esters via the action of cholesterol ester transfer protein. Hepatic lipase cleaves TG-rich LDL and HDL particles, producing small, dense lipoproteins. Additionally, insulin promotes the degradation of LDL through interaction with the LDL receptor. Some have suggested that larger LDL particles are preferentially cleared by insulin-stimulated LDL receptor endocytosis,[34] while small, dense LDL particles remain in the circulation longer.[35-38] Our results show a positive relation between Si and LDL size and an inverse relation between Si and both total TG and VLDL-TG as previously reported by Rainwater[39] and Ambrosch et al.[15] Contrary to these findings, Slyper et al[19] did not find a significant correlation between LDL size and Si among healthy, nondiabetic males.

While Si may be an important correlate of LDL size, in the present study Si contributed little to explaining the variance in LDL size when plasma TG were included in the multiple regression model. These findings are in agreement with those reported by Mykkanen et al[13] and Festa et al,[14] who found that whole-body glucose uptake and Si were not independently associated with LDL size after controlling for VLDL-TG and HDL-C.

The fact that Si did not remain an independent predictor of LDL size when fasting plasma TGs were included in the multivariate model should not detract from the role of insulin resistance in contributing to smaller, denser LDL particles. As discussed above, insulin resistance is strongly related to elevations in plasma TG, which in turn appears to contribute to the formation of small, dense LDL particles. However, these findings do suggest that in clinical practice, the measurement of plasma TG is more important in predicting LDL size than the measurement of Si.

Estimates of Si and LDL Size

The FSIGTT is relatively cost- and time-prohibitive for estimating Si in large-scale studies and in the clinical setting. Therefore, we sought to determine whether various estimates of Si that rely solely on fasting blood samples were associated with LDL size and oxLDL. Both surrogate measures of Si analyzed (QUICKI and HOMA-IR) were significantly associated with LDL particle size (based on Spearman's rank order correlations), with the strength of the association similar to that of Si and LDL size. However, as was true of the latter relationship, when TG were entered into the model, neither QUICKI nor HOMA-IR was a significant independent predictor of LDL size.

Insulin and oxLDL

In our study sample, we found a weak inverse relation between Si and oxLDL, and also a trend for fasting plasma insulin concentrations to be related to oxLDL. Several lines of evidence suggest that insulin resistance may directly affect the production of oxLDL. Studies have shown that physiologic hyperinsulinemia is associated with decreases in plasma vitamin E, as well as increases in H_2O_2 production.[40,41] Quinones-Galvan et al[35] reported that acute in vivo insulin administration increased the susceptibility of LDL-C to both copper-induced and cell-mediated oxidation. Carantoni et al[21] reported that insulin resistance was associated with increased serum levels of partially oxidized LDL in nondiabetic men and women. Individuals with type 2 diabetes mellitus exhibit increased free radical production, which is also associated with hyperglyce-

Table 4. Stepwise Regression: Contributions to the Variance in LDL Particle Size in 36 Overweight and Obese Men

Variable	Intercept	Standardized β	*P*	Partial R^2	Model R^2
Model 1	19.75				0.17
Si		0.414	.015	0.17	
Model 2	21.74				0.531
Si		−0.044	.718	0.001	
TG		−0.755	.000	0.530	
Model 3	19.777				0.651
Si		−0.029	.828	0.001	
TG		−0.571	.000	0.352	
HDL-C		0.413	.001	0.298	

*Si was significantly related to LDL particle size in model 1, but when TG was added (model 2) and TG and HDL-C were added (model 3), Si was no longer a significant correlate of LDL size.

mia.[42] Also, the susceptibility of LDL to oxidation may be higher with increasing levels of total and abdominal adiposity,[43] well-known concomitants of insulin resistance.

While insulin resistance may contribute to increased levels of oxLDL, the reverse may also be true. Chavakis et al[44] recently showed that oxLDL induced dephosphorylation and subsequent inactivation of Akt, a key signaling intermediate involved in insulin-stimulated glucose transport. Previous studies have reported that defects in Akt activity are associated with increases in the production of the sphingolipid, ceramide. Interestingly, oxLDL has been shown to increase the cellular ceramide content through the activation of acid sphingomyelinase.[45,46]

There are several aspects of the present study that deserve mention. While the relations among Si, LDL size, and oxLDL were not particularly strong, these associations should not be discounted. Stronger relations may have been masked by the lack of substantial heterogeneity in our study sample, given that all men were overweight or obese, and none exhibited type 2 diabetes. It is possible that had our sample included lean individuals as well as those with diabetes, a wider range of Si values, average LDL sizes, and oxLDL would have resulted in a stronger relation. Another possible caveat was that our sample included some individuals with stage I hypertension, which could be a confounding factor to some of the associations identified. However, none of the individuals with hypertension were taking blood pressure–lowering drugs, so measures of Si and plasma lipoproteins were unaffected by any medications.

In summary, the results of the current study suggest that among middle-aged and older, overweight, nondiabetic men, the relationship between Si and LDL particle size is highly dependent on plasma TG and HDL-C concentrations. Furthermore, estimates of Si, such as QUICKI and HOMA, which have more clinical utility because they rely on only a single fasting blood sample, are as strongly correlated to LDL size as the more burdensome FSIGTT. Finally, Si is only weakly associated with oxLDL.

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