Metabolism
Clinical and Experimental

VOL 49, NO 3 MARCH 2000

Impact of Insulin Resistance on Lipoprotein Subpopulation Distribution in Lean and Morbidly Obese Nondiabetic Women

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The purpose of this study was to examine the effects of insulin resistance on the lipoprotein subpopulation distribution of very–low-density, low-density, and high-density lipoproteins (VLDL, LDL, and HDL) in lean and morbidly obese nondiabetic women. Lean women (body mass index [BMI], 20 to 27 kg/m^2) stratified by BMI were divided into insulin-sensitive (SL, n = 12) and insulin-resistant (RL, n = 8) groups according to Bergman's minimal model, S_I. A group of obese women (BMI, 30 to 53 kg/m^2), also stratified by BMI, were divided into insulin-sensitive (SO, n = 10) and insulin-resistant (RO, n = 11) groups in a similar fashion. Resistant groups were similar to sensitive groups (SL *v* RL and SO *v* RO) in age, weight, percent body fat, and waist circumference, ie, total and regional adiposity. VLDL, LDL, and HDL subpopulation distributions were determined in fasting plasma samples by nuclear magnetic resonance (NMR) spectroscopy. The average particle sizes of all 3 classes of lipoproteins were similar for the SL and RL groups. In contrast, RO subjects had larger VLDL, smaller LDL, and smaller HDL, than SO subjects ($P < .05$). Lower concentrations of large LDL and large HDL were found in RO compared with SO subjects ($P < .05$). In obese women, but not in lean women, VLDL size was associated with plasma insulin ($r = .60$, $P < .005$), while LDL size and HDL size were negatively correlated with plasma insulin ($r = -.39$, $P < .05$ and $r = -.38$, $P < .05$) and positively correlated with S_I ($r = .54$, $P < .01$ and $r = .42$, $P < .05$). These results suggest that in obese women, insulin resistance may be involved in the formation of lipoprotein subpopulation distributions that are associated with vascular disease.

THE INSULIN RESISTANCE syndrome is a metabolic disorder with a cluster of aberrations, including resistance to insulin-mediated glucose uptake, glucose intolerance, abdominal obesity, and dyslipidemia (for review, see Krauss[1] and Reaven[2]). The accompanying dyslipidemia involves changes in plasma lipid concentrations, as well as alterations in the subpopulation distribution of each major class of plasma lipoprotein. Larger very–low-density lipoprotein (VLDL), smaller high-density lipoprotein (HDL), and smaller low-density lipoprotein (LDL) all have been associated with components of the insulin resistance syndrome[3-22] and the incidence and severity of vascular disease.[23-31] Small, dense LDL particles, in particular, have a putative atherogenic role[32-37] and are thought to mediate part of the relationship between the metabolic abnormalities of the insulin resistance syndrome and the accompanying high rate of vascular disease.[38-40] As a result, several investigations have focused on how individual components of the insulin resistance syndrome relate to the prevalence of small, dense LDL, with the goal of identifying the underlying causes of the high rate of vascular disease in insulin-resistant patients. While most studies have focused on LDL, recent evidence indicates that abnormalities in the subpopulation distribution of VLDL and HDL are also strong predictors of vascular disease,[23] and investigating the underlying causes of these abnormalities is similarly justified. Understanding the etiology of vascular disease in patients with the insulin resistance syndrome will focus the proper therapeutic regimens on high-risk patients.[38]

Studies that have examined the relationship between the resistance to insulin-stimulated glucose uptake and small, dense LDL have reported conflicting results. Using various indices of insulin resistance, some researchers have related insulin resistance to the subpopulation distribution of LDL,[8,11-16] while others have not.[17-19] When estimating insulin resistance by the minimal model of Bergman (S_I),[41] one study reported a significant but weak relationship, which was independent of the

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Submitted February 25, 1999; accepted August 17, 1999.

Supported by a grant from the National Institutes of Health (Department of Health and Human Services-Public Health Service DK45029).

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0026-0495/00/4903-0001$10.00/0

body mass index (BMI), between S_I and LDL size in a nondiabetic population that included both lean and severely obese individuals.[16] Another study that primarily examined nondiabetic lean patients found no such relationship.[19] While no study has examined the relationship between S_I and VLDL size or HDL size, studies investigating the relationship between plasma insulin and HDL subpopulation distribution also have been inconsistent.[9,20-22]

We speculated that the degree of adiposity may influence the effect of insulin resistance on the subpopulation distribution of lipoproteins. To date, no study has investigated the role of the resistance to insulin-stimulated glucose uptake in the formation of atherogenic profiles of VLDL, LDL, and HDL in separate comparisons of nondiabetic lean and nondiabetic severely obese patients. Therefore, the purpose of this study was to examine the subpopulation distribution differences for VLDL, LDL, and HDL between insulin-sensitive and insulin-resistant subjects categorized by S_I in a group of lean women (BMI, $\approx$24 kg/m^2) and a group of severely obese women (BMI, $\approx$37 kg/m^2). We limited our study to nondiabetic women, and unlike previous studies that statistically adjusted for the BMI and/or body fat distribution, we examined insulin-sensitive and -resistant groups stratified by age and BMI and similar in weight, percent body fat, waist circumference, and maximal O_2 consumption ($\dot{V}O_2$max). This study design provides an examination of how S_I, without the complicating factors of age, adiposity, physical fitness, and diabetes, relates to aberrations not only in LDL profiles but also in VLDL and HDL profiles, and how the presence of obesity may influence these relationships.

SUBJECTS AND METHODS

Patient Characteristics and Treatment

Subjects were recruited from a database of patients examined at the Human Performance Laboratory at East Carolina University. Only caucasian women were recruited, as it has been suggested that the relationship between insulin sensitivity and lipoprotein subpopulation distribution may be more pronounced in women versus men[18] and may vary between ethnic groups.[16] The subjects were sedentary nonsmokers who were free of cardiovascular disease, diabetes, and orthopedic problems. All subjects were weight-stable ($\pm$2 kg) for the 6 months preceding the study, and none were using any medications known to affect lipid metabolism. Ten obese women who showed a significant improvement in insulin sensitivity from gastric bypass surgery[42] performed 24 to 30 months prior to the study were selected for the insulin-sensitive obese (SO) group. Eleven insulin-resistant obese women (RO) without surgical intervention were stratified with surgery patients based on age and BMI. RO and SO groups were subsequently found to be similar in weight, percent body fat, waist circumference, and $\dot{V}O_2$max. In a separate comparison, 12 insulin-sensitive lean (SL) women were stratified with 8 insulin-resistant lean (RL) women according to age and BMI. RL and SL groups were subsequently found to be similar in weight, percent body fat, waist circumference, and $\dot{V}O_2$max. Patients classified as resistant in this study had S_I[41] less than 3.5 min^{-1}/μU/mL and patients classified as sensitive had values greater than 4.2 min^{-1}/μU/mL, consistent with a population-based study.[43] The difference in insulin sensitivity between sensitive and resistant groups was justified in that a minimum difference of 3 min^{-1}/μU/mL in S_I between age and BMI–stratified counterparts was a prerequisite for inclusion in the study. None of the patients were using oral contraceptives. Postmenopausal women were equally represented in resistant and sensitive groups in each comparison (SL *v* RL, n = 4/3; SO *v* RO, n = 2/2), and none were using any form of hormone replacement therapy. Written consent was obtained from the patients after they were informed of the nature and potential risks of the study. The Institutional Review Board for research on human subjects approved the protocols used in this study.

Insulin sensitivity was determined by an intravenous glucose tolerance test according to the method of Bergman et al.[41] Tests were initiated at 7 AM following an overnight (12-hour) fast. Blood samples were analyzed spectrophotometrically for glucose (16-UV; Sigma, St Louis, MO) and by microparticle enzyme immunoassay for insulin (IMx; Abbott Laboratories, Abbott Park, IL). S_I was calculated using the MINMOD program.[41]

Body density was determined by hydrostatic weighing following expiration to residual volume, as determined by oxygen dilution.[44] Body density was used to calculate percent body fat using the Siri equation.[45] Body mass and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively. The BMI was determined as mass/height^2 (kg/m^2). The umbilicus minimal waist and maximal hip circumferences were obtained in duplicate as previously described.[46] The waist circumference was obtained with spring-tension, stretchless Gulick tape (Lafayette Instruments, Lafayette, IN) to the nearest 1 mm. All anthropometric measurements were performed by the same researcher, who is qualified with several years of experience in the field of human exercise physiology. $\dot{V}O_2$max and time to exhaustion were determined during a physician-supervised incremental treadmill test. Expired gases were continuously monitored (model 2900; Sensormedics, Anaheim, CA) to determine oxygen consumption.

Plasma Analysis

Fasting blood samples were collected and treated with the addition of sodium azide (10 KIU/mL) and aprotinin (0.1 mg/mL). The plasma was separated by low-speed centrifugation (2,500 $\times$ *g*) for 30 minutes at 4°C and stored at −80°C until analysis. Lipid concentrations and lipoprotein subpopulation distributions were determined by nuclear magnetic resonance (NMR) spectroscopy.[23,47-49] As described by Freedman et al,[23] NMR analysis of lipoprotein subclasses is based on each lipoprotein particle within a given diameter range exhibiting a distinct lipid NMR signal, the intensity of which is proportional to its bulk lipid mass concentration. NMR data are collected in 3 steps: (1) acquisition of the 250-MHz proton NMR spectra of the plasma sample; (2) deconvolution of the lipid methyl group signal envelope appearing in these spectra at about 0.8 ppm, yielding signal amplitudes for 16 lipoprotein subclasses; and (3) conversion of the signal amplitudes into subclass concentrations with experimentally derived factors based on chemically determined isolated subclass standards. The estimated diameters (in nanometers) for the 16 subclasses (chylomicrons, >200; 6 VLDL: V6, 150 $\pm$ 70; V5, 70 $\pm$ 10; V4, 50 $\pm$ 10; V3, 38 $\pm$ 3; V2, 33 $\pm$ 2; and V1, 29 $\pm$ 2; 4 LDL: IDL, 25 $\pm$ 2; L3, 22 $\pm$ 0.7; L2, 20.5 $\pm$ 0.7; and L1, 19 $\pm$ 0.7; and 5 HDL: H5, 11.5 $\pm$ 1.5; H4, 9.4 $\pm$ 0.6; H3, 8.5 $\pm$ 0.3; H2, 8.0 $\pm$ 0.2; and H1, 7.5 $\pm$ 0.2) have been previously reported.[48] The particle size for each class of lipoprotein was determined by weighting the relative percentage of each subclass by its diameter. The 16 subclasses were then grouped into 9 subclasses based on the relationship of NMR subclasses to particle size estimates of gradient gel electrophoresis or electron microscopy measurements.[48,49] The grouping of the subfractions is as follows: large VLDL (V6 + V5), intermediate VLDL (V4), small VLDL (V3 + V2 + V1), large LDL (IDL + L3), intermediate LDL (L2), small LDL (L1), large HDL (H5, $\sim$HDL$_{2b}$), intermediate HDL (H4 + H3, $\sim$HDL$_{2a,3a}$), and small HDL (H2 + H1, $\sim$HDL$_{3b,3c}$). Otvos et al[47-49] found close agreement between chemically determined lipid concentrations and those determined with NMR (HDL-C, r = .93; LDL-C, r = .91; and VLDL-triglyceride [TG], r = .98). We confirmed these findings in a limited number of our patients. LDL and HDL subclass determinations by gradient gel electrophoresis and NMR have also been shown to be closely re-

lated.[47,49] In a limited number of patients, we also found that LDL size determined by NMR was closely correlated with LDL size determined from polyacrylamide gradient gel electrophoresis ([PGGE] $r = .77$, $P < .0001$). LDL size determined by NMR was approximately 15% lower than our PGGE determinations, a consistent finding when comparing the 2 methods. The reason for this methodological difference is unknown. However, LDL size determinations by light-scattering techniques, like NMR and electron microscopy, also report LDL sizes considerably lower than those determined by PGGE, while results from the 2 methods correlate strongly.[50] O'Neal et al[50] suggested that the differences in the absolute values may be due to the possibility that polystyrene latex particle standards in PGGE may behave differently in gel media than LDL, or the charged surface of LDL particles may react differently in the different suspension media. While there appear to be consistent methodological differences in absolute LDL size determinations, the methods agree in the variation of size in their respective ranges. Thus, researchers and clinicians should be aware of the method used before determining vascular disease risk. As a general reference, patients with the PGGE classification of the less atherogenic, type A profile have a NMR LDL size greater than 20.5 nm, while those with type B have a NMR LDL size less than 20.5 nm (NMR Lipoprofile; Lipomed, Raleigh, NC).

Intermediate-density lipoprotein cholesterol in this analysis did not differ between the groups and comprises less than 10% of the large LDL fraction. As this is a postabsorptive study, the concentrations of chylomicrons in our patients were minimal, did not differ between groups, and are not reported.

Statistical Analysis

Separate models were used to identify differences in lean (SL *v* RL) and obese (SO *v* RO) patients. For each comparison, significant differences were determined with 2-tailed independent *t* tests. Results are expressed as the mean ± SEM. Pearson correlation coefficients were calculated to examine the relationship between parameters of insulin resistance and lipoprotein subpopulation distribution. Statistical significance was inferred at a *P* level less than .05 (Systat; SPSS, Chicago, IL).

RESULTS

Table 1 shows physical and biochemical characteristics of the 2 groups of lean and 2 groups of obese women. No differences in age were observed between SL and RL or between SO and RO subjects. SL and RL women were similar in body weight, BMI, percent body fat, and waist circumference, ie, both total and regional adiposity. SL and RL women were also similar in aerobic capacity, as suggested by a similarity in $\dot{V}O_2$max. However, the RL group had higher plasma glucose, higher plasma insulin, and lower S_I compared with the SL group ($P < .05$). Likewise, SO and RO women did not differ in total and regional adiposity or $\dot{V}O_2$max, but the RO group had higher plasma glucose, higher plasma insulin, and lower S_I compared with the SO group ($P < .05$).

Plasma lipid concentrations in the 4 groups of women are shown in Table 2. No statistical differences were observed in lipid concentrations between SL and RL or between SO and RO groups. The average particle size and subpopulation distribution of the 3 major classes of lipoproteins are also shown in Table 2. No statistical differences were observed between SL and RL groups in the average particle size of VLDL, LDL, or HDL. In contrast, RO women had a significantly larger average VLDL size than SO women ($P < .05$). In addition, RO women had a smaller average LDL size and a smaller average HDL size ($P < .05$) than SO women.

Table 1. Physical and Biochemical Characteristics of the Lean and Obese Women

Characteristic	Lean		Obese	
	SL	RL	SO	RO
No. of subjects	12	8	10	11
Age (yr)	35 ± 4	36 ± 7	40 ± 5	41 ± 2
	(18-60)	(19-63)	(27-57)	(31-59)
Body weight (kg)	66 ± 4	62 ± 2	107 ± 4	101 ± 6
	(55-79)	(56-73)	(82-126)	(85-144)
BMI (kg/m²)	24 ± 1	23 ± 1	39 ± 2	36 ± 2
	(21-27)	(21-27)	(30-50)	(30-53)
Body fat (%)	26 ± 2	24 ± 2	40 ± 2	40 ± 1
	(19-37)	(15-34)	(32-46)	(35-52)
Waist (cm)	83 ± 4	80 ± 2	118 ± 4	119 ± 4
	(70-104)	(70-88)	(99-146)	(102-146)
$\dot{V}O_2$max (mL/kg/min)	30.0 ± 2.7	26.5 ± 2.7	21.6 ± 1.6	20.1 ± 2.0
	(16-42)	(17-39)	(16-27)	(11-29)
Glucose (mg/dL)	87 ± 4	92 ± 5*	85 ± 2	94 ± 2†
	(60-107)	(74-108)	(71-105)	(81-107)
Insulin (pmol/L)	29 ± 2	55 ± 9*	32 ± 6	74 ± 10†
	(16-43)	(24-103)	(17-71)	(28-136)
S_I ($min^{-1}/\mu U/mL$)	6.5 ± 0.6	1.9 ± 0.3*	5.6 ± 0.3	1.9 ± 0.3†
	(4.2-10.5)	(1.0-3.5)	(4.2-6.7)	(0.5-3.4)

NOTE. Values are the mean ± SEM (range). Data were analyzed separately for lean and obese patients.

*$P < .05$ *v* SL.

†$P < .05$ *v* SO.

The subfraction concentrations of the 3 classes of lipoproteins are shown in Figs 1 to 3. No statistical differences were observed between SL and RL or between SO and RO groups in the concentration of large, intermediate, and small VLDL (Fig 1). Interestingly, the concentration of large and small VLDL tended to be higher in both RL and RO women compared

Table 2. Plasma Lipids and Average Lipoprotein Particle Sizes

Parameter	Lean		Obese	
	SL	RL	SO	RO
No. of subjects	12	8	10	11
Total cholesterol (mg/dL)	151 ± 13	177 ± 19	157 ± 14	156 ± 11
	(108-196)	(130-209)	(96-233)	(106-196)
HDL-C (mg/dL)	39 ± 2	42 ± 2	41 ± 5	37 ± 2
	(25-52)	(35-56)	(18-64)	(19-47)
LDL-C (mg/dL)	103 ± 11	119 ± 16	103 ± 11	102 ± 9
	(49-184)	(85-144)	(59-171)	(55-142)
TG (mg/dL)	82 ± 8	107 ± 23	99 ± 10	130 ± 23
	(27-117)	(57-265)	(52-174)	(52-325)
VLDL-TG (mg/dL)	49 ± 7	71 ± 20	64 ± 9	96 ± 23
	(13-81)	(26-204)	(26-129)	(13-291)
VLDL size (nm)	43.7 ± 1.9	42.7 ± 2.6	45.0 ± 1.7	53.5 ± 2.8*
	(35-54)	(34-55)	(35-56)	(44-70)
LDL size (nm)	21.2 ± 0.1	21.3 ± 0.1	21.1 ± 0.2	20.5 ± 0.2*
	(20.6-21.9)	(20.6-21.8)	(20.4-21.9)	(19.3-21.2)
HDL size (nm)	9.35 ± 0.15	9.33 ± 0.13	9.30 ± 0.14	8.96 ± 0.09*
	(8.5-10.2)	(8.7-9.9)	(8.6-10.0)	(8.5-9.3)

NOTE. Values are the mean ± SEM (range). Data were analyzed separately for lean and obese patients.

*$P < .05$ *v* SO.

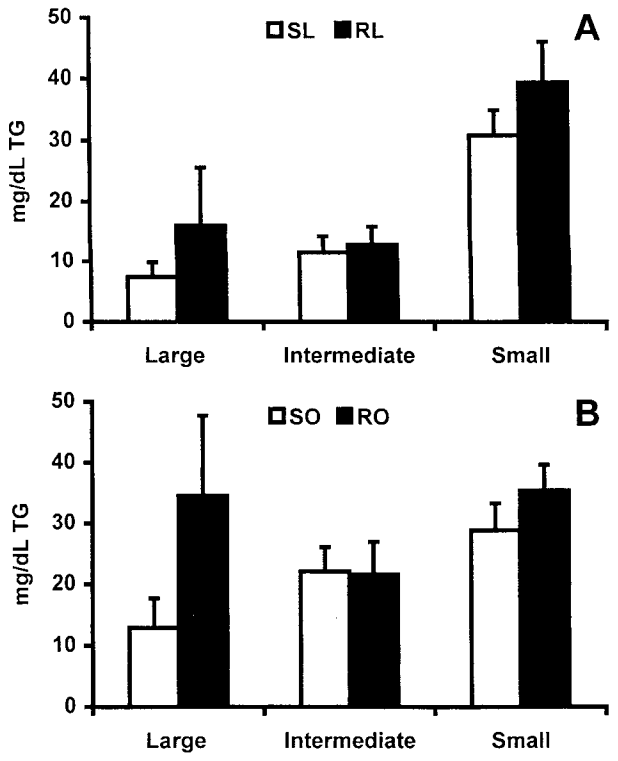

Fig 1. Subpopulation distribution of VLDL in (A) lean women (SL and RL) and (B) obese women (SO and RO). VLDL profiles determined by NMR spectroscopy are expressed as the concentration of TG in large (~60-200 nm), intermediate (~40-60 nm), and small (~30-40 nm) VLDL particles. Values are the mean ± SEM. Data were analyzed separately for lean and obese patients.

with their respective insulin-sensitive counterparts. SL and RL groups were similar with respect to the concentration of large, intermediate, and small LDL (Fig 2A). In contrast, the concentration of large LDL was lower in RO than in SO women ($P < .05$; Fig 2B). As with VLDL and LDL subpopulation distributions, no statistical differences were observed between SL and RL women in the concentration of large, intermediate, or small HDL (Fig 3A). However, in obese patients, while no differences were observed in intermediate or small HDL concentrations, RO women had a lower concentration of large HDL than SO women ($P < .05$; Fig 3B).

The relationships of S_I and plasma insulin to the average particle size of the major lipoprotein classes are shown in Table 3. In lean patients, no significant associations were observed between the average particle size of the 3 lipoprotein classes and S_I or plasma insulin. In contrast, in obese patients, VLDL size correlated with plasma insulin, LDL size was associated with both S_I and insulin, and HDL size correlated with both S_I and plasma insulin. As expected with this study design, these relationships were independent of variations in age, BMI, and waist circumference.

DISCUSSION

This study provides the first direct evidence of an effect of resistance to insulin-mediated glucose uptake, as estimated by S_I, on the subpopulation distribution of VLDL, LDL, and HDL. We observed that nondiabetic obese women who are insulin-resistant have more atherogenic lipoprotein profiles than nondiabetic obese women who are more sensitive to insulin's actions, with the 2 groups being similar in age, weight, percent body fat, BMI, waist circumference, and $\dot{V}O_2$max. With respect to LDL profiles, our results agree with 2 large epidemiological studies in nondiabetic subjects that investigated the relationship between S_I and LDL size, statistically adjusting for BMI. Howard et al[16] found a relationship between S_I and LDL size in a large sample of men and women that included a significant number of obese and severely obese individuals (BMI, ~29 ± 7 kg/m^2). In a smaller study that examined primarily lean subjects (BMI, 25 ± 3 kg/m^2), Slyper et al[19] found no relationship between S_I and LDL size. Our data are consistent with both of these studies in that we observed this relationship in obese women, but not in lean women. Our results suggest that the inconsistent reports examining the relationship between insulin resistance and LDL profiles may be a result of variation in patient selection and/or sample size. In addition to our findings with LDL size, the present study extends this adiposity-dependent relationship between insulin resistance and lipoprotein subpopulation distribution to VLDL size and HDL size.

Tilly-Kiesi et al[8] examined the relationship between insulin resistance and VLDL subpopulation distribution in glucose-tolerant relatives of patients (BMI < 30) with type 2 diabetes. While estimates of VLDL particle size were not reported,

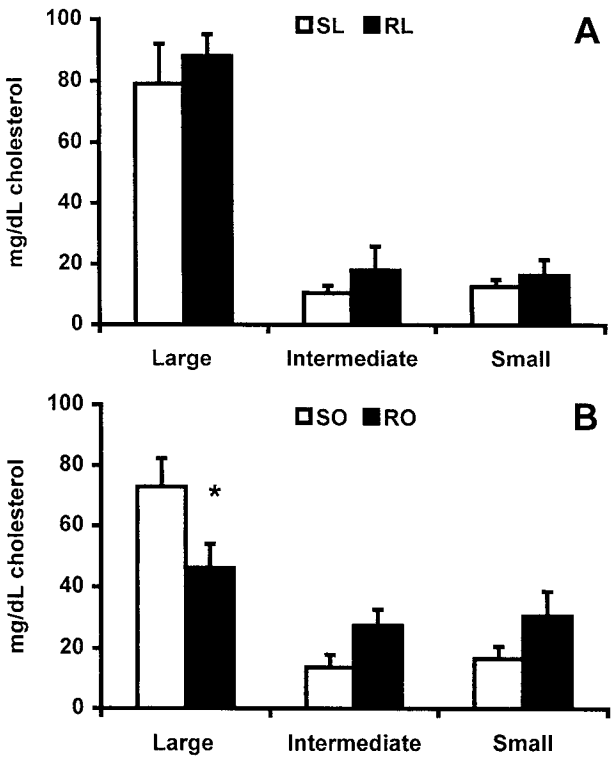

Fig 2. Subpopulation distribution of LDL in (A) lean women (SL and RL) and (B) obese women (SO and RO). LDL profiles determined by NMR spectroscopy are expressed as the concentration of cholesterol in large (~21.3-30 nm), intermediate (~20.2-21.3 nm), and small (~18-20.2 nm) LDL particles. Values are the mean ± SEM. Data were analyzed separately for lean and obese patients. *$P < .05$.

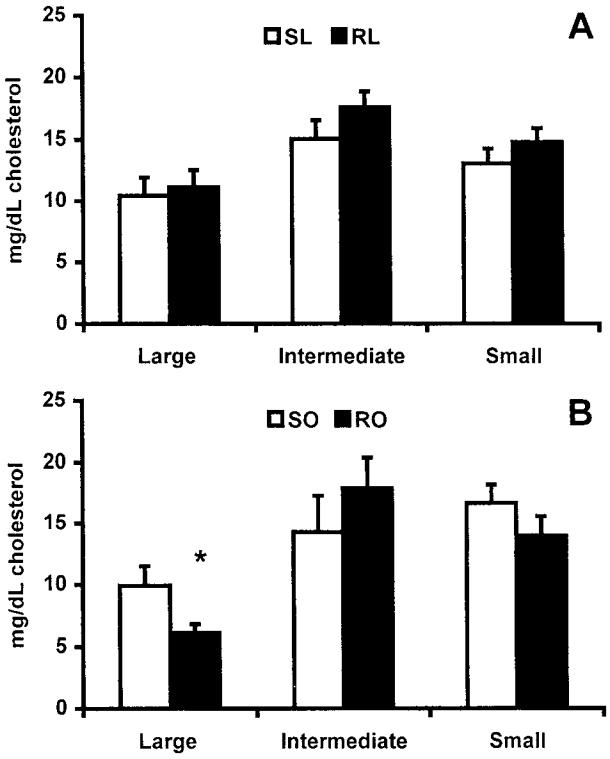

Fig 3. Subpopulation distribution of HDL in (A) lean women (SL and RL) and (B) obese women (SO and RO). HDL profiles determined by NMR spectroscopy are expressed as the concentration of cholesterol in large (~10-13 nm), intermediate (~8.2-10 nm), and small (~7.3-8.2 nm) HDL particles. Values are the mean ± SEM. Data were analyzed separately for lean and obese patients. *$P < .05$.

patients with high insulin levels (>60 pmol/L) had higher TG concentrations of both large and small VLDL particles than patients with low insulin levels (<24 pmol/L). We observed this trend in both lean and obese resistant patients, but we observed a difference in VLDL size between sensitive and resistant patients only in the obese group. Insulin resistance has been associated with a predominance of large VLDL particles in obese patients.[4] The present study confirms this effect of insulin resistance on VLDL size in 2 groups of obese patients who differ in S_I but are similar in total and regional adiposity.

Table 3. Pearson Correlations for Average Particle Size of VLDL, LDL, and HDL With S_I and Plasma Insulin

Group	S_I	Plasma Insulin
Lean subjects (n = 20)		
VLDL size	.13	.14
LDL size	−.34	.11
HDL size	.24	−.08
Obese subjects (n = 21)		
VLDL size	−.30	.60‡
LDL size	.54†	−.39*
HDL size	.42*	−.38*

Abbreviation: S_I, Bergman's minimal model estimation of insulin sensitivity.

*$P < .05$.

†$P < .01$.

‡$P < .005$.

However, a similar comparison of lean patients showed no significant effects on VLDL subpopulation distribution.

The association of plasma insulin with the subpopulation distribution of HDL has been shown in previous reports that included obese individuals.[9,20,21] These relationships were observed to be independent of total and/or regional adiposity. In addition to these cross-sectional approaches, Taskinen et al[3] demonstrated a redistribution of cholesterol from small to large HDL particles in men and women with diabetes in response to intensive insulin therapy. These patients were obese and remained weight-stable during the 4-week therapy, exhibiting a significant improvement in insulin sensitivity and glycemic control concurrent with the redistribution of cholesterol from small to large HDL particles. Our data support these findings in that we observed an effect of insulin resistance on HDL size in obese women. Interestingly, one study did not show a significant correlation between plasma insulin and HDL subpopulation distribution in lean women.[22] Consistent with that study, we did not observe HDL size perturbations in lean patients or any associations of HDL size with either measure of insulin resistance.

While S_I correlated strongly with TG (data not shown) as previously shown,[51] S_I was not related to VLDL size but was associated with both LDL and HDL size in obese women (Table 3). In contrast, plasma insulin associated with all 3 particle sizes reported in obese patients, providing a possible explanation for the differential effects of insulin resistance on VLDL size in lean and obese individuals. Insulin levels in the RL group did not appear to be as high as those in the RO group, even though the 2 groups appeared to have similar S_I values. However, RL women had elevated insulin levels compared with their insulin-sensitive counterparts with no apparent particle size perturbations, and no associations between particle size and insulin were observed in lean patients. There may be a cutoff point for plasma insulin above which the resistance related to the insulin level affects VLDL size. With little evidence to support this idea, the differential effects of insulin resistance on particle size in lean and obese patients are more likely due to some other complicating factor of obesity that was not measured in this study.

Although we did not study the mechanisms underlying the changes in lipoproteins, insight may be gained from what others have found. There are a number of mechanisms by which insulin can decrease large VLDL-TG.[3,5-6,52-54] All of these processes are diminished in insulin-resistant individuals,[3,5-7,55-57] leading to elevated VLDL-TG and larger VLDL particles. In light of these studies showing an inhibitory effect of insulin on plasma TG and large VLDL, the positive correlation between insulin and VLDL size in the present study is probably due to a component of insulin resistance that is reflected in elevated plasma insulin. It has been suggested that the overproduction of large TG-rich VLDL particles leads to elevated plasma TG. The higher plasma TG has been associated with the composition and subpopulation distribution abnormalities in HDL and LDL.[13,58] Elevated hepatic lipase (HL), elevated cholesteryl ester transfer protein (CETP), and depressed lipoprotein lipase (LPL) are also thought to contribute to this remodeling of HDL and LDL.[15,58-60] It has been suggested that obesity and/or abdominal adiposity may alter the expression of 1 or more of these enzymes,[58,61] and

these effects may explain the differential effect of insulin resistance in obese and lean women. Obese women in our study, irrespective of insulin sensitivity, had elevated levels of CETP activity in the plasma (data not shown). We did not measure LPL or HL activity in our patients, but the proposed effects of obesity and insulin resistance likely work together to contribute to the development of atherogenic lipoprotein profiles in severely obese insulin-resistant patients.

It should be noted that HDL-C levels in SL women were relatively low for lean healthy women, although the other lipid and lipoprotein subpopulation distribution parameters were relatively normal. This finding may be due to the number of postmenopausal women in this group or to variations in the stage of the menstrual cycle in premenopausal women, which was not documented in our patients. Interestingly, estrogen and progesterone appear to elevate HDL-C without significantly influencing LDL or VLDL subpopulation distribution.[62] The sedentary life-style and diet also may have contributed to the low HDL-C in the small number of patients that were studied. Even so, our findings confirm previous reports in that there does not appear to be an effect of insulin sensitivity on lipoprotein subpopulation distribution in lean patients, as both SL and RL groups had lipoprotein profiles that were relatively normal.

It should also be noted that SO subjects are a group of severely obese women who previously underwent gastric bypass surgery that resulted in improved insulin sensitivity. Whether other factors associated with this surgical intervention (ie, weight loss, caloric restriction, and vitamin supplementation) influenced the lipoprotein profiles independently from the improved insulin sensitivity is unknown. Future studies using alternative treatments to improve insulin sensitivity in obese patients should be able to clarify these issues. However, the strong relationships that we observed between insulin sensitivity indices and the average lipoprotein particle sizes suggest that the level of insulin sensitivity may influence lipoprotein subpopulation distribution, particularly in obese patients.

In summary, we observed VLDL, LDL, and HDL subpopulation distributions that have been associated with increased vascular disease in RO women compared with SO women of similar age, weight, BMI, percent body fat, waist circumference, and $\dot{V}O_2max$. These abnormalities were related to insulin resistance as estimated by plasma insulin (VLDL, LDL, and HDL) and S_I (LDL and HDL). Our data are consistent with the hypothesis that insulin resistance leads to the formation of large TG-rich VLDL particles which, together with the altered expression of key enzymes in the plasma, contribute to the remodeling of LDL and HDL. Our data suggest that the detrimental effects of insulin resistance on the subpopulation distribution of lipoproteins may be dependent on the degree of adiposity. The complicating factors of severe obesity that influence the relationship between insulin resistance and lipoprotein subpopulation distribution are unknown, warranting further study. Our findings are clinically relevant in that therapeutic regimens designed to improve insulin sensitivity in severely obese patients may improve atherogenic lipoprotein profiles even if the patient remains severely obese. Such improvements may result in fewer vascular disease complications.

ACKNOWLEDGMENT

We thank Dr Jim Otvos, Department of Biochemistry, North Carolina State University, for assistance in the processing and interpretation of NMR data. We thank Dr Lynis Dohm, Dr Don Holbert, Joe Bower, and Steven Pohnert for their review and editing of the manuscript.

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