Relationship Between Obesity and Concentration and Composition of Low-Density Lipoprotein Subfractions in Normoinsulinemic Men

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Obesity, insulin resistance (IR) with hyperinsulinemia, and a dyslipoproteinemia characterized by reduced high-density lipoprotein 2 (HDL_2) cholesterol and elevated levels of small, dense low-density lipoprotein (LDL) particles are risk factors for coronary artery disease (CAD). The impact of obesity independent of hyperinsulinemia on the concentration and composition of small, dense LDL subfractions is uncertain. The aim of this study was to investigate the relationship between obesity indices, namely body mass index (BMI), skinfold measurements (SF), and waist to hip ratio (WHR), and LDL-subfraction particle concentration and composition in 200 healthy men without evidence of IR. A precise analysis of the concentration of lipids and apolipoproteins and the composition of very–low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and two HDL- and six LDL-subfraction particles was obtained using the technique of density-gradient ultracentrifugation. Dividing the individuals according to BMI showed that those with a BMI greater than 27 kg/m² had significantly lower HDL_2 cholesterol and apolipoprotein (apo) A-I and higher VLDL and IDL cholesterol and apo B concentrations than those with a BMI less than 25 kg/m². Regarding LDL particles, we found that men with a BMI above 25 kg/m² had significantly more small, dense LDL particles (d 1.044 to 1.063 g/mL) and correspondingly fewer medium, dense LDL particles (d 1.031 to 1.037 g/mL) than leaner men; those with a BMI above 27 kg/m² had the highest concentration of circulating small, dense LDL particles. These findings were not influenced by fasting insulin concentrations, IR, or WHR. This study showed that obesity (BMI > 25 kg/m² associated with significantly higher SF, percent body fat, and waist circumference) is an important factor for the expression of a more atherogenic LDL-subclass phenotype even in normoinsulinemia. Characteristic differences in composition of LDL-subfraction particles between lean and obese men underlie the role of body weight in the metabolism of LDL-subfraction particles.

ELEVATED LEVELS of low-density lipoprotein (LDL) cholesterol and apolipoprotein (apo) B and decreased levels of high-density lipoprotein (HDL) cholesterol and apo A-I are associated with an increased risk of coronary artery disease (CAD).[1-3] Recently, the atherogenic lipoprotein profile has been better characterized, particularly, the association of distinct LDL-subfraction phenotypes with CAD.[4-6] A pattern of increased concentration of small, dense LDL particles, elevation of triglycerides, and reduction in HDL_2 cholesterol has been shown to increase the risk of ischemic cardiac events.[5,7-10] Epidemiologic studies indicate that obesity is an important risk factor for CAD, coronary death, and congestive heart failure independent of other standard risk factors for ischemic heart disease.[11,12] Furthermore, obesity is associated with hyperinsulinemia, hypertension, and hypercholesterolemia,[13] themselves important risk factors for CAD. There is also evidence that obesity correlates with increased levels of small, dense LDL particles.[14-16] An investigation involving female twins showed that this association is dependent on serum insulin levels.[17]

To investigate whether body mass, body composition, and subcutaneous fat distribution influences the LDL-subfraction phenotype in normoinsulinemia, we assessed body mass index (BMI), waist to hip ratio (WHR), and subcutaneous skinfold measurements (SF) and compared these obesity indices with the lipid profile by measuring lipid and apolipoprotein concentrations and the composition of very–low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL subfractions in normoinsulinemic men without evidence of CAD. In contrast to previous studies that have investigated the relation between obesity and lipoproteins by using gradient polyacrylamide gel electrophoresis or peak flotation rates from analytic ultracentrifugation, we used preparative density-gradient ultracentrifugation. With this technique, a precise analysis of the concentration and composition of VLDL, IDL, and LDL-subfraction particles was possible.

SUBJECTS AND METHODS

Subjects

Two hundred healthy men (age, 30 ± 9 years; weight, 74.6 ± 10.3 kg; height, 178 ± 7 cm) were recruited for the study. These subjects either were volunteers from the staff and students of Freiburg University Medical School or were randomly selected from the outpatient clinic of the Department of Physical Performance Medicine, where they had attended for an elective cardiopulmonary assessment. All participants consumed a normal Western diet without daily or excessive intake of alcohol. Exclusion criteria were drug therapy of any kind, diabetes mellitus, elevated fasting insulin (>17 μU/mL) and fasting glucose (>110 mg/dL), a history of gastrointestinal, hepatic, or endocrine disease, symptoms of CAD, or an abnormal physical examination. In addition, no pathological findings were detected in a stepwise exercise stress test.

Obesity Indices

BMI (weight in kilograms divided by the square of height in meters) was calculated for all participants in the study. WHR and SF were only determined in 85 individuals. Waist circumference was obtained at the level of the umbilicus, and hip circumference at the level of the greater trochanters.[18] SF were obtained with a manual caliper (Cambridge Scientific Industries, Cambridge MD).

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A minimum of three measurements each were obtained at the midposterior point between the tip of the acromion and olecranon processes ($SF_{triceps}$), at the level of the xiphoid process in the midaxillary line (SF_{chest}), 1 cm below the inferior tip of the scapula ($SF_{scapula}$), and adjacent to the umbilicus ($SF_{umbilicus}$). All measurements were performed by the same investigator (M.H.) and always on the right side of the body.

Body density and percent body fat were calculated according to the formulae reported by Brozek et al.[19,20] *body density* = $1.1017 - (2.82 \cdot 10^{-4} \cdot SF_{umbilicus}) - (7.36 \cdot 10^{-4} \cdot SF_{chest}) - (8.83 \cdot 10^{-4} \cdot SF_{triceps})$, and *percent body fat* = $(4.57/\text{density} - 4.142) \cdot 100$.

According to their BMI,[21,22] all 200 men were divided into five groups (1 to 5): BMI < 21 kg/m^2, BMI 21-23 kg/m^2, BMI 23-25 kg/m^2, BMI 25-27 kg/m^2, and BMI >27 kg/m^2. Obesity was defined as BMI greater than 25 kg/m^2, ie, BMI groups 4 and 5, with individuals in group 4 (BMI 25-27 kg/m^2) considered mildly obese and those in group 5 (BMI > 27 kg/m^2) markedly obese.

Insulin Resistance

Insulin resistance (IR) was calculated from fasting blood glucose in millimolars and serum insulin concentrations using a computer-solved homeostasis model assessment method.[23,24] IR = fasting insulin/$22.5e^{-\ln(\text{fasting glucose})}$, which is equal to IR = fasting insulin · fasting glucose/22.5.

Density-Gradient Ultracentrifugation

EDTA plasma was obtained after an overnight fast. VLDL (d < 1.006 g/mL), IDL (d 1.006 to 1.019 g/mL), LDL (d 1.019 to 1.063 g/mL), and HDL (d 1.063 to 1.210 g/mL) were prepared by sequential flotation according to the method reported by Lindgren.[25] Total LDLs were separated into six density classes and HDLs into two density classes by equilibrium density-gradient centrifugation.[26] The density ranges of LDL subfractions as determined by precision refractometry[25] of blank gradients were as follows: LDL-1, 1.019 to 1.031 g/mL; LDL-2, 1.031 to 1.034 g/mL; LDL-3, 1.034 to 1.037 g/mL; LDL-4, 1.037 to 1.040 g/mL; LDL-5, 1.040 to 1.044 g/mL; LDL-6, 1.044 to 1.063 g/mL; HDL_2, 1.063 to 1.125 g/mL; and HDL_3, 1.125 to 1.210 g/mL. All centrifugation steps were performed at a temperature of 18°C using partially filled 6-mL polycarbonate bottles in a 50Ti rotor (Beckman, Munich, Germany).

Chemical Analysis

In all HDL and LDL subfractions, levels of total cholesterol, free cholesterol (FC), triglycerides, and phospholipids were measured by automated (EPOS; Eppendorf, Hamburg, Germany) enzymatic methods (Boehringer, Mannheim, Germany, and bioMérieux, Nürtingen, Germany). Esterified cholesterol was calculated as the molar difference between total cholesterol and FC. Apo A-I and apo B levels were measured by kinetic nephelometry (Beckman ICS Analyzer II; Munich, Germany). Apo A-II level was measured by end point nephelometry (Behring, Marburg, Germany). Insulin concentrations were determined by an enzyme-linked immunosorbent assay (Boehringer), and free or nonesterified fatty acids (FFA) by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany).

Statistical Analysis

ANOVA was used to test the hypothesis that lipoprotein values of the BMI groups were equal. A conservative multiple comparison test (Scheffé test) was chosen for pairwise comparisons of means between BMI groups.

Insulin concentrations, the IR factor, SF, WHR, and lipoprotein values for each BMI group are expressed as the mean ± SD.

The composition of apo B–containing particles (VLDL, IDL, and LDL) is expressed as lipid molecules per apo B (lipids/apo B), since one VLDL, IDL, or LDL particle contains exactly one apo B molecule.[27,28] Calculation of lipid molecules per LDL particle apo B is superior to percentage calculations, because with the latter, differences in concentration of only one component influence the percentage values of all other components of that particle.

To investigate whether concentrations of fasting insulin or glucose or IR influence lipoprotein subfractions even in normoinsulinemia, a Pearson univariate correlation analysis was performed between parameters of IR (serum concentrations of insulin, glucose, FFA, and the IR factor) and concentrations of cholesterol and apolipoproteins of HDL and LDL subfractions. In addition, a stepwise multiple regression analysis was performed in which parameters indicating IR were entered according to level of significance, with serum lipoproteins and lipoprotein subfractions being the dependent variable.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS/PC+; SPSS Inc, Chicago, IL). All *P* values less than .05 were considered to indicate statistical significance.

RESULTS

The subjects had been divided into five BMI groups: group 1, BMI < 21 kg/m^2 (20.1 ± 0.9 kg/m^2, n = 34); group 2, BMI 21 to 23 kg/m^2 (22.0 ± 0.6 kg/m^2, n = 67); group 3, BMI 23 to 25 kg/m^2 (23.8 ± 0.6 kg/m^2, n = 45); group 4, BMI 25 to 27 kg/m^2 (25.9 ± 0.6 kg/m^2, n = 29); group 5, BMI > 27 kg/m^2 (29.0 ± 1.6 kg/m^2, n = 25). BMI ranged from 17.2 to 31.8 kg/m^2, with a mean of 23.5 ± 2.8 kg/m^2. In a subset of 85 individuals (BMI group 1, n = 15; group 2, n = 22; group 3, n = 19; group 4, n = 17; and group 5, n = 12), circumferences of the waist and hips and SF at four sites were obtained. Mean values for WHR were less than 1.0 in all groups and did not significantly differ between BMI groups (Table 1). Nonetheless, men with a BMI above 25 kg/m^2 had significantly greater circumferences of the waist and hips than lean men. SF produced similar results. With an increment in BMI, SF at all four sites increased continuously. In particular, those with a BMI above 25 kg/m^2 had significantly thicker SF than those with a BMI less than 25 kg/m^2 (Table 1). Values for insulin, glucose, IR, and FFA determined in all 200 men were within normal limits and did not vary significantly between BMI groups (Table 1).

Mean cholesterol and apolipoprotein concentrations of lipoprotein subfractions of all men and of BMI groups are listed in Table 2. There were no differences between BMI groups with respect to serum cholesterol or LDL or HDL cholesterol concentrations. Serum triglycerides and VLDL cholesterol were higher in men with a BMI greater than 25 kg/m^2 than in those with a BMI less than 25 kg/m^2; IDL cholesterol was only significantly higher in markedly obese men (BMI > 27 kg/m^2) as compared with lean men (BMI < 23 kg/m^2). LDL and HDL cholesterol concentrations did not differ between BMI groups. However, when dividing LDL and HDL particles into subfractions, it became evident that BMI groups differed significantly in the cholesterol-subfraction profile. In particular, markedly obese men with a BMI greater than 27 kg/m^2 had significantly higher concentrations of small LDL cholesterol (LDL-6, d 1.044 to 1.063 g/mL) than lean men (BMI < 25

Table 1. Mean Values (±SD) for WHR, SF at Four Sites, Body Density, Body Fat, Glucose, Insulin, FFA, and IR of Five BMI Classes

Parameter	BMI (kg/m²)				
	<21	21-23	23-25	25-27	>27
Waist circumference (cm)	81 ± 4	79 ± 5	85 ± 6	93 ± 6*†‡	102 ± 5*†‡§
Hip circumference (cm)	82 ± 13	87 ± 12	94 ± 3	99 ± 5*†	105 ± 2*†
WHR	0.98 ± 0.2	0.93 ± 0.2	0.90 ± 0.1	0.94 ± 0.1	0.96 ± 0.1
$SF_{triceps}$ (mm)	9 ± 3	11 ± 5	13 ± 5	16 ± 6	21 ± 7*†
SF_{chest} (mm)	7 ± 3	11 ± 7	18 ± 11	23 ± 10*†	28 ± 8*†
$SF_{scapula}$ (mm)	10 ± 2	13 ± 5	19 ± 7	24 ± 9*†	27 ± 9*†
$SF_{umbilicus}$ (mm)	12 ± 5	17 ± 8	27 ± 12	33 ± 10*†	40 ± 12*†
Body density	1.09 ± 0.01	1.08 ± 0.01	1.07 ± 0.02	1.06 ± 0.01*†	1.05 ± 0.01*†‡
Body fat (%)	6.7 ± 2.1	9.2 ± 4.2	13.2 ± 6.3	16.3 ± 5.2*†	20.6 ± 4.7*†‡
Insulin (μU/mL)	7.3 ± 3.4	7.3 ± 3.6	7.2 ± 3.9	7.3 ± 3.5	9.5 ± 3.3
Glucose (mg/dL)	92 ± 12	90 ± 11	89 ± 10	97 ± 10	95 ± 7
IR	1.57 ± 0.98	1.60 ± 0.85	1.59 ± 0.96	1.89 ± 1.0	2.2 ± 0.85
FFA (mmol/L)	0.37 ± 0.17	0.38 ± 0.22	0.43 ± 0.19	0.52 ± 0.33	0.51 ± 0.18

NOTE. WHR and SF were only determined in 85 of 200 men investigated.
*$P < .05$ *v* BMI < 21 kg/m².
†$P < .05$ *v* BMI 21-23 kg/m².
‡$P < .05$ *v* BMI 23-25 kg/m².
§$P < .05$ *v* BMI 25-27 kg/m².

Table 2. Serum Cholesterol, Triglycerides, and Cholesterol and Apolipoprotein Concentrations of VLDL, IDL, Total LDL, LDL Subfractions (LDL-1 to LDL-6), total HDL, and HDL Subfractions (HDL_2 and HDL_3) (mean ± SD) for Five BMI Classes

Parameter (mg/dL)	BMI (kg/m²)				
	<21 (n = 34)	21-23 (n = 67)	23-25 (n = 45)	25-27 (n = 29)	>27 (n = 25)
Serum cholesterol	216 ± 70	217 ± 59	209 ± 35	227 ± 39	236 ± 44
Serum triglycerides	81 ± 31	92 ± 39	104 ± 55	166 ± 105*†‡	195 ± 11*†‡
Cholesterol					
VLDL	12 ± 8	14 ± 10	16 ± 11	27 ± 19*†‡	35 ± 25*†‡
IDL	7 ± 5	8 ± 6	9 ± 5	11 ± 6	13 ± 10*†
LDL	127 ± 61	122 ± 42	112 ± 23	125 ± 33	120 ± 25
LDL-1	25 ± 14	25 ± 11	23 ± 9	24 ± 9	24 ± 7
LDL-2	21 ± 13	18 ± 8	16 ± 7	14 ± 6*	12 ± 6*
LDL-3	25 ± 16	21 ± 9	18 ± 6	17 ± 8*	14 ± 7*
LDL-4	25 ± 14	22 ± 10	20 ± 6	23 ± 11	17 ± 7
LDL-5	17 ± 9	19 ± 10	18 ± 7	23 ± 11	22 ± 11
LDL-6	15 ± 5	17 ± 8	17 ± 10	24 ± 13*	31 ± 19*†‡
HDL	51 ± 13	52 ± 12	51 ± 13	46 ± 10	43 ± 12
HDL_2	31 ± 14	32 ± 11	30 ± 11	24 ± 8†	21 ± 7*†‡
HDL_3	19 ± 5	20 ± 3	21 ± 4	21 ± 5	20 ± 4
Apo B					
VLDL	5 ± 2	5 ± 3	6 ± 3	9 ± 5*†	11 ± 7*†‡
IDL	3 ± 1	4 ± 2	4 ± 2	5 ± 2	5 ± 3*†
LDL	74 ± 34	70 ± 24	65 ± 14	76 ± 20	78 ± 15
LDL-1	12 ± 6	12 ± 5	11 ± 4	12 ± 4	12 ± 3
LDL-2	11 ± 7	10 ± 4	8 ± 3	7 ± 3*	7 ± 3*
LDL-3	14 ± 9	12 ± 5	10 ± 3*	10 ± 4*	8 ± 4*
LDL-4	15 ± 8	13 ± 6	12 ± 3	13 ± 6	11 ± 4
LDL-5	11 ± 5	12 ± 6	11 ± 4	15 ± 7	15 ± 8
LDL-6	10 ± 4	12 ± 5	12 ± 7	18 ± 11*†	24 ± 13*†‡
Apo A-I					
HDL	108 ± 32	115 ± 24	116 ± 26	111 ± 27	100 ± 22
HDL_2	45 ± 22	51 ± 19	47 ± 17	38 ± 17†	31 ± 14†‡
HDL_3	60 ± 15	61 ± 11	63 ± 14	65 ± 16	61 ± 11

*$P < .05$ *v* BMI < 21 kg/m².
†$P < .05$ *v* BMI 21-23 kg/m².
‡$P < .05$ *v* BMI 23-25 kg/m².

kg/m^2); medium, dense LDL-particle cholesterol concentration (LDL-2 and LDL-3, d 1.031 to 1.037 g/mL) was correspondingly lower in markedly obese men than in lean men (Table 2). The inverse was observed for the HDL-subfraction profile. Men with a BMI greater than 27 kg/m^2 had significantly lower HDL_2 cholesterol concentrations than those with a BMI less than 25 kg/m^2. No difference was found in HDL_3 cholesterol values between BMI groups (Table 2).

For apo B concentrations (Table 2), which give an estimation of the number of circulating apo B–containing particles,[27,28] similar results regarding LDL-subfraction distribution were observed as for the cholesterol-subfraction profile. Although LDL apo B values did not differ among BMI groups, LDL-subfraction analysis showed that particularly a BMI greater than 25 kg/m^2 was associated with increased small-LDL apo B concentrations (LDL-6, d 1.044 to 1.063 g/mL; Table 2). The highest concentrations were observed in the group with a BMI between 27 and 32 kg/m^2, indicating that these men have the highest concentration of circulating small, dense LDL particles. Mean values for small, dense LDL particles (d > 1.044 g/mL) were twice as high in markedly obese men (BMI > 27 kg/m^2) versus lean men (BMI < 25 kg/m^2; Table 2). These data show that men with a BMI greater than 27 kg/m^2, with the greatest waist circumference and elevated percent body fat (Table 1), have the least favorable lipoprotein pattern in terms of the ratio of the number of atherogenic small, dense LDL particles to cardioprotective HDL_2 apo A-I concentrations. This association is even present in normoinsulinemia. Multiple regression analyses showed that this relationship seems to be independent of fasting insulin and glucose levels, FFA, and IR. In a univariate correlation analysis, only FFA concentrations correlated with the concentration of small, dense LDL apo B ($r = .16$, $P < .05$), and fasting glucose concentrations correlated with HDL_2 cholesterol concentrations ($r = -.19$, $P < .05$). However, multiple regression analysis showed that only BMI significantly influenced concentrations of small, dense LDL (d > 1.044 g/mL) cholesterol ($r = .36$, $R^2 = .13$, $P < .001$), small, dense LDL apo B ($r = .40$, $R^2 = .16$, $P < .001$), and HDL_2 cholesterol ($r = -.28$, $R^2 = .1$, $P < .001$) in this population of healthy normoinsulinemic men. Concentrations of FFA, glucose, or other parameters indicating IR such as fasting insulin concentrations and the IR factor were not included in the regression equation for small, dense LDL particles and HDL_2 subfractions. However, mean values for insulin and IR were approximately 25% higher in men with a BMI greater than 27 kg/m^2 as compared with leaner men (Table 1). Although this was not statistically significant, a mild peripheral IR cannot be excluded for these normoinsulinemic obese subjects.

Composition of apo B particles (VLDL, IDL, and LDL) is expressed as the relative lipid content per apo B molecule.[27,28] Since no significant differences were observed between BMI groups 1, 2, and 3, these three lowest BMI groups (BMI < 25 kg/m^2) were combined in Table 3 for easier examination of the data. Composition analysis showed

Table 3. Composition of Apo B Particles (lipids/apo B) in BMI Groups

	BMI < 25 kg/m^2 (n = 146)				BMI 25-27 kg/m^2 (n = 29)				BMI > 27 kg/m^2 (n = 25)			
Parameter	FC/Apo B	CE/Apo B	PL/Apo B	TG/Apo B	FC/Apo B	CE/Apo B	PL/Apo B	TG/Apo B	FC/Apo B	CE/Apo B	PL/Apo B	TG/Apo B
VLDL	1,425 ± 369	1,934 ± 526	2,379 ± 571	6,309 ± 2,032	1,752 ± 499*	2,221 ± 848	2,553 ± 793	7,568 ± 3,038*	1,696 ± 346*	2,273 ± 511*	2,463 ± 396	7,177 ± 1,859
IDL	876 ± 163	2,056 ± 487	1,232 ± 218	1,298 ± 675	1,006 ± 237*	2,261 ± 461	1,393 ± 481*	1,963 ± 1,712*	949 ± 119	2,241 ± 388	1,298 ± 204	1,739 ± 797
LDL	598 ± 72	1,703 ± 157	750 ± 107	121 ± 30	541 ± 92*	1,651 ± 151	696 ± 110*	130 ± 39	493 ± 104*†	1,570 ± 232*	646 ± 116*†	138 ± 51
LDL-1	745 ± 85	1,989 ± 230	910 ± 120	196 ± 61	725 ± 85	2,004 ± 218	900 ± 103	245 ± 95*	716 ± 82	2,003 ± 248	889 ± 119	270 ± 113*
LDL-2	672 ± 76	1,893 ± 218	842 ± 110	129 ± 49	642 ± 72	1,900 ± 177	830 ± 83	163 ± 73*	604 ± 71*	1,828 ± 208	789 ± 77*	192 ± 104*
LDL-3	623 ± 104	1,804 ± 257	790 ± 115	105 ± 42	593 ± 83	1,799 ± 195	772 ± 120	128 ± 55	565 ± 77*	1,736 ± 200	740 ± 84*	162 ± 81*
LDL-4	585 ± 92	1,712 ± 250	742 ± 111	94 ± 34	549 ± 72	1,700 ± 142	717 ± 90	108 ± 46	520 ± 83*	1,660 ± 189	689 ± 82*	132 ± 59*
LDL-5	526 ± 73	1,619 ± 197	700 ± 91	93 ± 27	489 ± 74	1,607 ± 154	661 ± 92	96 ± 31	470 ± 77*	1,592 ± 158	642 ± 73*	117 ± 45*†
LDL-6	449 ± 57	1,439 ± 131	644 ± 88	118 ± 26	421 ± 75	1,449 ± 151	603 ± 106	112 ± 29	393 ± 74*	1,417 ± 130	558 ± 94*	117 ± 29

NOTE. For easier examination of the data, BMI groups 1 to 3 (BMI < 25 kg/m^2) were combined in one group.
Abbreviations: CE, cholesterol ester; PL, phospholipid; TG, triglyceride.
*$P < .05$ v BMI < 25 kg/m^2.
†$P < .05$ v BMI 25-27 kg/m^2.

that the composition of VLDL, IDL, and LDL particles differed significantly between obese men (BMI > 25 kg/m^2) and non-obese men (BMI < 25 kg/m^2; Table 3). VLDL and IDL particles of normoinsulinemic men with a BMI greater than 25 kg/m^2 were significantly richer in lipids of all classes, but particularly FC, esterified cholesterol, and triglycerides (Table 3). VLDL and IDL of mildly obese men (BMI 25 to 27 kg/m^2) had an even higher triglyceride content than those of markedly obese men. In contrast to VLDL and IDL particles, LDL particles of markedly obese (BMI > 27 kg/m^2) men had a lower content of FC and phospholipids than LDL particles of non-obese men (BMI < 25 kg/m^2). When comparing markedly obese men (BMI > 27 kg/m^2) with mildly obese men (BMI 25 to 27 kg/m^2), LDL particles of the markedly obese group had an even lower content of FC and phospholipids; this could not be attributed to a single LDL subfraction. As for VLDL and IDL particles, LDL triglyceride content was higher in LDL particles of obese versus lean men, whereas the esterified cholesterol content of LDL particles was similar in obese and non-obese subjects (Table 3).

When dividing LDL particles into subfractions, it became evident that the LDL-subfraction composition of mildly obese and lean men was not significantly different with respect to cholesterol and phospholipids. Only the triglyceride content of LDL-1 to LDL-2 (d 1.019 to 1.034 g/mL) was higher in mildly obese subjects. The composition of LDL subfractions of markedly obese men (BMI > 27 kg/m^2) differed from that of leaner men, in so far as LDL with a density between 1.031 and 1.044 g/mL (LDL-2 to LDL-5) had significant surface-lipid (FC and phospholipids) depletion and a higher content of core lipids (particularly triglycerides; Table 3). Very small LDL particles (LDL-6, d > 1.044 g/mL) of markedly obese men (BMI > 27 kg/m^2) were depleted in surface lipids (FC and phospholipids), although the core lipid content was similar to those of mildly obese and lean men. These findings showed that with a BMI greater than 27 kg/m^2, significant changes in LDL-subfraction composition are to be expected.

DISCUSSION

Obesity and hyperinsulinemia have been shown to influence LDL-subfraction phenotype by increasing the concentration of small, dense LDL particles.[16,17,29] In this study, we have investigated whether body mass and subcutaneous fat are associated with an atherogenic lipoprotein profile in men with normal insulin levels as well. We found that BMI, SF, and relative body fat were closely associated with an atherogenic lipoprotein profile, despite the fact that parameters for IR such as fasting insulin, glucose, and FFA concentrations were within normal limits in our subjects. Multivariate regression analysis showed that the association between BMI and subfractions of LDL and HDL was independent of parameters indicating IR, namely WHR, insulin, glucose, and the IR factor. However, mean values for fasting insulin and the IR factor were almost 25% higher in men with a BMI greater than 25 kg/m^2 versus leaner men. Although this was not statistically significant, a mild peripheral IR might nonetheless be present in these normoinsulinemic obese subjects.

In nondiabetic women, Selby et al[17] showed that fasting insulin levels correlate well with serum triglycerides and the number of small, dense LDL particles determined by gradient gel electrophoresis. This association remained significant when adjusted for the women's BMI. Since we examined healthy men with normal fasting insulin levels and normal WHR, it is not surprising that the influence of insulin was not evident in our study. Nonetheless, it seems possible that gender is responsible for differences in the influence of obesity indices and insulin levels on lipoprotein-subfraction profiles.

Our study confirmed the previously reported positive association between BMI and serum triglycerides and the inverse association between BMI and HDL cholesterol for healthy men.[14,30,31] We found that men with a BMI greater than 27 kg/m^2 have higher concentrations of VLDL and IDL cholesterol and a higher number of circulating VLDL and IDL particles than lean men (BMI < 25 kg/m^2; Table 2). Since these lipoprotein particles have been shown to be prevalent in coronary heart disease patients,[32-34] obesity with a BMI greater than 27 kg/m^2 could increase the risk for CAD via this mechanism, as indicated by mortality studies.[35]

Regarding the composition of VLDL particles, we showed that VLDLs of mildly obese and markedly obese men are rich in lipids, particularly triglycerides and cholesterol. In obesity, there is an increased exchange of triglycerides for esterified cholesterol between VLDL and other lipoprotein fractions.[36] However, our data yield no evidence that such an exchange occurs in obese men with normal insulin levels, since the content of triglycerides and esterified cholesterol per VLDL particle increases in similar ways with increasing BMI. It must be hypothesized instead that in obesity VLDLs rich in cholesterol and triglycerides are directly excreted by the liver.

In obese subjects, serum concentrations of LDL cholesterol and LDL apo B are often within normal limits.[31] For normoinsulinemia, we confirmed this finding; even the concentration of LDL apo B did not differ significantly between BMI groups, and mean values were not pathologically elevated (Table 2). Subfractionation of LDL particles, though, showed that obesity is associated with changes in the concentration of certain LDL particles. The concentration of small, dense LDL particles, particularly those with a density of 1.044 to 1.063 g/mL, is doubled in men with a BMI greater than 27 kg/m^2 versus lean men with a BMI less than 25 kg/m^2. Interestingly, no association was found between an elevated BMI and large LDL particles (d 1.019 to 1.031 g/mL), but an inverse association was seen between BMI and medium, dense LDL-subfraction particles (d 1.031 to 1.037 g/mL), which were particularly reduced in obese individuals (Table 2). Furthermore, LDL-subfraction analysis showed that small, dense LDL particles (d > 1.044 g/mL) were influenced by obesity indices in ways similar to triglyceride-rich particles, such as VLDL and IDL. These results confirm reports that levels of VLDL and IDL are closely related to small, dense LDL metabolism.[37] More-

over, we demonstrated that this association is even present for mildly obese men with a BMI between 25 and 27 kg/m^2, and moreover, that this is observed in a healthy normoinsulinemic population.

The lack of any association between obesity and total LDL can be explained via the following mechanism: due to higher hepatic VLDL-particle secretion in obesity,[38] LDL synthesis is more than 50% higher in obese individuals than in lean controls.[39] In addition, it is known that obesity is associated with a higher clearance of LDL particles by the liver and peripheral tissues, a finding that is also seen in metabolic states associated with obesity such as hypertriglyceridemia.[40] Simultaneously increased synthesis and conversion of LDL particles in obesity may explain why similar levels of LDL cholesterol and LDL apo B in obese and lean individuals were observed in this study. Furthermore, from our data, it may be suggested that catabolism of medium, dense LDL is increased in normoinsulinemic obese men, leading to elevated concentrations of circulating small, dense LDL particles (Table 2). Since a high turnover of LDL particles in itself seems to be an independent risk factor for CAD despite normal LDL cholesterol values,[39] obesity might be an additional coronary risk via increased LDL turnover and increased concentrations of circulating small, dense LDL particles.

With increasing BMI, LDL particles also change significantly in composition. In normoinsulinemic men with a BMI greater than 25 kg/m^2 and elevated percent body fat, large and medium, dense LDL particles (d 1.019 to 1.044 g/mL) are enriched in triglycerides as compared with those in leaner men. Indeed, in abdominal obesity, plasma LDL particles have been found to have a high triglyceride content.[41,42] High levels of LDL-subfraction particles rich in triglycerides were shown to be closely associated with severity of coronary atherosclerosis in young survivors of myocardial infarction.[9] Kesaniemi and Grundy[39] also observed that LDL particles of obese subjects had a lower cholesterol to protein ratio. We showed that this is due to a reduction in FC in most LDL-subfraction particles (LDL-2 to LDL-6), particularly in men with a BMI greater than 27 kg/m^2. Additionally, the concentration of phospholipids in medium and small, dense LDL (LDL-2 to LDL-6) declines with increasing BMI (Table 3). Therefore, small LDL particles of markedly obese men (BMI > 27 kg/m^2) have a lower content of surface lipids (FC and phospholipids) than small, dense LDL of leaner normoinsulinemic men. Since surface-lipid depletion with unchanged core lipids could only be observed in small, dense LDL subfractions (d > 1.044 g/mL), which are particularly elevated in markedly obese men, this finding might be characteristic of metabolic changes associated with a BMI greater than 27 kg/m^2 and with elevated abdominal and body fat. However, whether surface-lipid depletion in LDL particles, particularly in phospholipids, increases LDL-particle atherogeneity is uncertain.

Factors associated with BMI such as nutrition, sex hormone levels, lipid enzyme activities, IR, and physical fitness influence LDL-subfraction metabolism.[14,17,43-46] However, in men, a BMI greater than 25 kg/m^2 associated with increased body fat is a strong predictor for the expression of a more atherogenic lipoprotein phenotype characterized by increased small, dense LDL particles (d > 1.044 g/mL) with reduced surface lipids and reduced HDL_2 cholesterol and HDL_2 apoA-I levels even in normoinsulinemia. This might explain the increased CAD mortality of young individuals with a BMI higher than 25 kg/m^2.[35] Therefore, BMI should always be included in the assessment of a patient's coronary risk profile, especially in those with established CAD or dyslipoproteinemia. In these patients, a reduction of BMI to less than 25 kg/m^2 should be addressed as one of the major strategies for improving the lipid and coronary risk profile.

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