Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity

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Abstract **Reduced plasma HDL cholesterol concentration has been associated with an increased risk of coronary heart disease. However, a low HDL cholesterol concentration is usually not observed as an isolated disorder because this condition is often accompanied by additional metabolic alterations. The objective of this study was to document the relevance of assessing HDL particle size as another feature of the atherogenic dyslipidemia found among subjects with visceral obesity and insulin resistance. For that purpose, an average HDL particle size was computed by calculating an integrated HDL particle size using nondenaturing 4–30% gradient gel electrophoresis. Potential associations between this average HDL particle size versus morphometric and metabolic features of visceral obesity were examined in a sample of 238 men. Results of this study indicated that HDL particle size was a significant correlate of several features of an atherogenic dyslipidemic profile such as increased plasma TG, decreased HDL cholesterol, high apolipoprotein B, elevated cholesterol/HDL cholesterol ratio, and small LDL particles as well as increased levels of visceral adipose tissue (AT) ($0.33 \leq |r| \leq 0.61$, $P < 0.0001$). Thus, men with large HDL particles had a more favorable plasma lipoprotein-lipid profile compared with those with smaller HDL particles. Furthermore, men with large HDL particles were also characterized by reduced overall adiposity and lower levels of visceral AT as well as reduced insulinemic-glycemic responses to an oral glucose load. In conclusion, small HDL particle size appears to represent another feature of the high TG-low HDL cholesterol dyslipidemia found in viscerally obese subjects characterized by hyperinsulinemia.**—Pascot, A., I. Lemieux, D. Prud'homme, A. Tremblay, A. Nadeau, C. Couillard, J. Bergeron, B. Lamarche, and J-P. Després. **Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity.** ***J. Lipid Res.*** **2001.** 42: **2007–2014.**

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The inverse relationship between plasma HDL cholesterol concentration and the incidence of coronary heart disease (CHD) is a well-documented phenomenon (1, 2). Plasma HDL cholesterol levels are determined by numerous environmental (3, 4) and genetic (5) factors. We and others have shown that low HDL cholesterol levels are often accompanied by elevated TG concentrations (6–8). This high TG-low HDL cholesterol dyslipidemia is a salient feature of the insulin resistance syndrome, which is strongly related to abdominal obesity, especially when accompanied by high levels of visceral adipose tissue (AT) (9). Furthermore, the high TG-low HDL cholesterol dyslipidemic phenotype has clearly been associated with an increased CHD risk in several prospective studies (7, 10, 11).

HDL particles are heterogeneous, and several approaches, such as ultracentrifugation, precipitation, immunoaffinity chromatography, and various types of electrophoresis (12, 13), have been used to isolate and characterize HDL subpopulations. Among these methods, HDL have been characterized on the basis of size using nondenaturing polyacrylamide gradient gel electrophoresis (14, 15). Different subclasses have been identified with this method, namely, HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b} (small, dense HDL particles to large HDL particles). However, this classification is subjective because the subclasses were determined only by arbitrary particle sizes without regard to their composition. Thus, the interrelationships among HDL particle size, HDL function, and HDL lipid content are not yet fully defined.

Previous studies using this classification have reported relationships between HDL subclasses and different metabolic and anthropometric parameters (3, 4, 16, 17). Williams et al. (17) have reported high levels of HDL_{3b} to be associated with CHD risk factors, suggesting that low HDL_{3b} levels may contribute in part to the low CHD risk in subjects who have high HDL cholesterol. Furthermore,

Abbreviations: AT, adipose tissue; BMI, body mass index; CHD, coronary heart disease; OGTT, oral glucose tolerance test; TG, triglycerides.

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an increased body mass index (BMI) has been associated with higher levels of HDL_{3b} and lower levels of HDL_{2b} (4), and fasting plasma insulin concentrations have been inversely correlated with plasma HDL_{3a}, HDL_{2a}, and HDL_{2b} (3). In addition, Syvänne et al. (16) have reported that a high hepatic lipase (HL) activity, hyperinsulinemia, and hypertriglyceridemia were independently associated with low levels of HDL_{2b} and generally with small HDL particle size.

Case-control and angiographic studies have suggested that CHD risk may be increased when HDL_{2b} concentration is decreased relative to HDL_{3c} and HDL_{3b} (18, 19). In the Québec Cardiovascular Study, the cholesteryl ester-rich HDL_2 particles appeared to have a greater contribution to the cardioprotective effects of increased HDL cholesterol than did smaller HDL_3 particles (20). On the other hand, abdominal obesity has been associated with decreased levels of HDL_2 cholesterol (9). Furthermore, subjects with type 2 diabetes and CHD have been characterized by small-sized HDL particles with a low cholesterol content (16). These results suggest that the HDL particles of insulin-resistant viscerally obese subjects with high TG-low HDL cholesterol dyslipidemia were likely to be reduced in size. However, this issue has never been examined.

Therefore, the objective of the present study was to examine the potential relationships of obesity, visceral AT accumulation, glucose tolerance, plasma insulin, and lipoprotein-lipid concentrations to an average HDL particle size (a cumulative marker of the distribution of the sizes of HDL particles) obtained by nondenaturing 4–30% gradient gel electrophoresis in a sample of 238 men. Furthermore, because we had previously reported that the presence of some features of the insulin resistance syndrome (hyperinsulinemia, elevated apoB, and small LDL particles, defined as the "atherogenic metabolic triad") were associated with a substantial increase in CHD risk (21), we also examined the potential relationships between HDL size and the features of this atherogenic metabolic triad.

METHODS

Subjects

Two hundred and thirty-eight men were recruited from the Québec City metropolitan area by solicitation through the media between 1987 and 1998. Subjects were between 19 and 68 years of age. Participants covered a wide range of BMI values (18–42 kg/m^2). All subjects were healthy, nonsmoking volunteers and were not under treatment for CHD, diabetes, dyslipidemias, or endocrine disorders. All participants signed an informed consent document approved by the Laval University Medical Ethics Committee.

Anthropometry

The hydrostatic weighing technique (22) was used to measure body density, which was obtained from the mean of six measurements. Pulmonary residual volume was measured before immersion in the hydrostatic tank, using the helium dilution method of Meneely and Kaltreider (23). Percent body fat was derived from body density using the equation of Siri (24). Height and body weight were measured according to the procedures recommended at the Airlie Conference (25), whereas waist circumference was measured as previously mentioned (26). Measurements of abdominal AT areas were performed by computed tomography with a Siemens Somatom DHR scanner (Erlangen, Germany), as previously described (27).

Plasma lipoprotein-lipid profile

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after a 12-h overnight fast for the measurement of plasma lipid and lipoprotein levels. Cholesterol and TG levels were determined in plasma and lipoprotein fractions using a Technicon RA-500 analyzer (Bayer Corporation, Tarrytown, NY), and enzymatic reagents were obtained from Randox Laboratories Ltd. (Crumlin, UK). Plasma VLDL ($d < 1.006$ g/ml) were isolated by ultracentrifugation (28). The HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$ (29). HDL_2 was then precipitated from the HDL fraction (30) with a 4% solution of low-molecular-weight dextran sulfate (15–20 kDa) obtained from SOCHIBO (Boulogne, France). Apolipoprotein B and A-I concentrations were measured by the rocket immunoelectrophoretic method of Laurell (31), as previously described (32).

Oral glucose tolerance test (OGTT)

A 75-g OGTT was performed in the morning after an overnight fast. Blood samples were collected in EDTA-containing tubes through a venous catheter placed in an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min for the determination of plasma glucose and insulin concentrations. Plasma glucose was measured enzymatically (33), whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation (34). The total glucose and insulin areas under the curve during the OGTT were determined with the trapezoid method.

Gradient gel electrophoresis of plasma samples

HDL size. Nondenaturing 4–30% polyacrylamide gel electrophoresis was performed for the measurement of HDL size using whole plasma kept at −80°C, as recently described (35). Briefly, gels were casted in the laboratory using acrylamide and bis-acrylamide (40:1.1) obtained from Bio-Rad (Hercules, CA). A volume of 10 μl of plasma samples was applied onto the gel in a final concentration of 15% sucrose and 0.2% bromophenol blue. Electrophoresis was performed at 4°C for a prerun of 15 min at 125 V before the entry of samples and at 70 V for 20 min for the entry of samples into stacking gel, followed by migration at 100 V for 6 h, at 150 V for 12 h, and finally at 200 V for 1–3 h. Gels were stained for lipids overnight with Sudan black B (Lipostain, Paragon electrophoresis system, Beckman, Montréal, Canada) in 55% ethanol. Gels were restored in a 9% acetic acid, 20% methanol solution and subsequently analyzed using the Imagemaster 1-D Prime computer software (version 3.01; Amersham Pharmacia Biotech, Baie d'Urfé, Québec, Canada). The mean HDL particle size was obtained with the migration of lipid-stainable plasma standards of known diameters (35). The lipid-stainable standards used were calibrated by computing a log-linear standard curve of the protein-stainable Pharmacia HMW standards as a function of their relative migration distance (Rf). A similar approach was used to assess HDL particle size using the calibrated lipid-stainable bands. The average HDL particle size represents the overall distribution of HDL subspecies and is calculated as a continuous variable using the migration distance of each peak multiplied by its relative area under the densitometric scan, as recently reported (35). A higher average HDL size indicated a greater proportion of "large" HDL particles, whereas a low average HDL size suggested an increased prevalence of "small" HDL particles. Inter- and intra-assay coefficients of variation for the average

HDL size assessed by this method were <3% (n = 59) and <1% (n = 20), respectively.

LDL size. Nondenaturing 2–16% polyacrylamide gel electrophoresis was performed on whole plasma according to the procedure described by Krauss and Burke (36) and McNamara et al. (37) and as previously reported (38).

Statistical analyses

A general linear model was used to compare the groups divided on the basis of average HDL particle size tertiles, and the Duncan post hoc test was used in situations in which a significant group effect was observed. Pearson correlation coefficients were calculated to quantify the univariate associations between variables. Stepwise multiple regression analyses were computed to sort out the independent contribution of metabolic variables to the variance of the average HDL particle size. An unpaired Student's *t*-test was performed to compare men of this study with low HDL cholesterol levels further divided into two groups on the basis of the 50th percentile of HDL particle size (low vs. high HDL size). All analyses were performed using the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Correlations between HDL parameters and anthropometric indices are presented in **Table 1**. HDL particle size was inversely related to BMI and waist girth as well as to the levels of fat mass and abdominal visceral and subcutaneous AT areas ($-0.26 \leqslant r \leqslant -0.32$, $P < 0.0001$). Relationships were found between HDL particle size and plasma HDL cholesterol and HDL_2 cholesterol levels ($r = 0.61$ and 0.64, respectively, $P < 0.0001$), and significant but weak correlations were noted between HDL particle size and HDL_3 cholesterol and apolipoprotein A-I levels. Overall, HDL particle size followed the same pattern of correlations with body fat and body fat distribution indices as HDL cholesterol and HDL_2 cholesterol, whereas HDL_3 cholesterol and apolipoprotein A-I showed weak or even no correlation with body fat indices. On the other hand, no association was found between HDL particle size and LDL-cholesterol (**Fig. 1**), whereas a significant relationship between LDL peak particle size and average HDL particle size was observed (Fig. 1, $r = 0.55$, $P < 0.0001$).

To further explore the relationship of HDL particle size to the metabolic features of abdominal obesity, the sample of 238 men was subdivided into subgroups using the 33rd and 66th percentiles of the average HDL particle size distribution as cutoff values. **Table 2** shows subjects' physical characteristics and plasma lipoprotein-lipid profiles across tertiles of HDL particle size. Men in the upper tertile of HDL particle size (large HDL particles) were characterized by reduced adiposity as reflected by a lower cross-sectional area of visceral abdominal AT and decreased BMI and total body fat mass compared with subjects in the lower and middle tertiles ($P < 0.0001$). For the plasma lipoprotein-lipid profile, men in the middle tertile were characterized by decreased plasma levels of VLDL cholesterol, TG, VLDL TG, LDL TG, and apo-

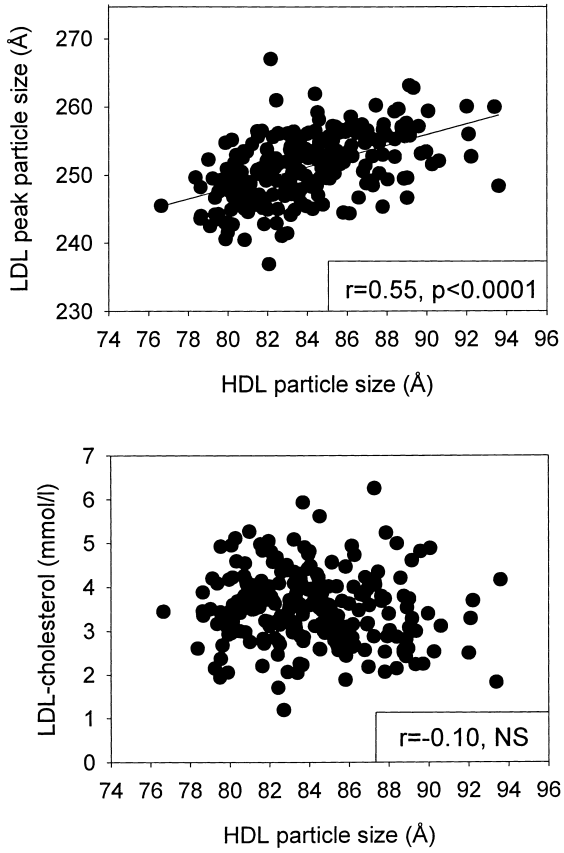

Fig. 1. Relationship between HDL particle size and LDL cholesterol concentration and LDL peak particle size.

TABLE 1. Correlations between HDL components and HDL particle size with body mass index (BMI), total body fatness, and visceral and subcutaneous adipose tissue (AT) accumulation measured by computed tomography and waist girth

	HDL-Cholesterol	HDL_2-Cholesterol	HDL_3-Cholesterol	Apolipoprotein A-I	HDL Size
BMI	-0.35^a	-0.40^a	-0.08	-0.01	-0.32^a
Fat mass	-0.36^a	-0.38^a	-0.13^b	-0.04	-0.26^a
Visceral AT	-0.32^a	-0.32^a	-0.14^b	0.02	-0.28^a
Subcutaneous AT	-0.36^a	-0.40^a	-0.10	-0.08	-0.27^a
Waist girth	-0.36^a	-0.38^a	-0.12	-0.03	-0.31^a
HDL size	0.61^a	0.64^a	0.21^b	0.31^a	—

[a] $P < 0.0001$.
[b] $P = 0.05$.

TABLE 2. Physical characteristics and lipoprotein-lipid profile of the sample of 238 men classified on the basis of HDL particle size tertiles

	HDL Particle Size		
	Tertile 1 (n = 79) (<82.3 Å)	Tertile 2 (n = 80) (82.3–85.1 Å)	Tertile 3 (n = 79) (>85.1 Å)
Physical characteristics			
BMI (kg/m^2)	29.5 ± 4.42	28.5 ± 4.63	25.3 ± 4.3[b]
Fat mass (kg)	25.1 ± 9.4	24.2 ± 10.2	17.6 ± 9.5[b]
Visceral AT area (cm^2)	154 ± 63	145 ± 69	98 ± 61[b]
Lipoprotein-lipid profile			
Cholesterol (mmol/l)			
Total	5.35 ± 0.81	5.22 ± 0.84	4.90 ± 0.95
VLDL	0.86 ± 0.37	0.67 ± 0.44[a]	0.41 ± 0.26[b]
LDL	3.63 ± 0.74	3.56 ± 0.83	3.35 ± 0.87
HDL	0.85 ± 0.15	0.97 ± 0.19[a]	1.14 ± 0.22[b]
HDL_2	0.21 ± 0.10	0.31 ± 0.14[a]	0.45 ± 0.17[b]
HDL_3	0.64 ± 0.13	0.67 ± 0.14	0.69 ± 0.13[a]
Cholesterol/HDL cholesterol ratio	6.47 ± 1.22	5.54 ± 1.30[a]	4.42 ± 1.12[b]
Triglycerides (mmol/l)			
Total	2.31 ± 0.83	1.84 ± 0.76[a]	1.25 ± 0.62[b]
VLDL	1.70 ± 0.75	1.29 ± 0.69[a]	0.78 ± 0.54[b]
LDL	0.35 ± 0.14	0.30 ± 0.10[a]	0.25 ± 0.09[b]
HDL	0.23 ± 0.05	0.22 ± 0.04	0.22 ± 0.05
Apolipoprotein B (g/l)	1.17 ± 0.21	1.08 ± 0.21[a]	0.95 ± 0.23[b]
Apolipoprotein A-I (g/l)	1.17 ± 0.15	1.21 ± 0.16	1.27 ± 0.15[b]
HDL particle size (Å)	80.6 ± 1.1	83.7 ± 0.8[a]	87.6 ± 2.0[b]
LDL particle size (Å)	248.1 ± 4.5	251.2 ± 4.3[a]	254.1 ± 4.0[b]

[a] Significantly different from tertile 1; $P < 0.03$.
[b] Significantly different from tertiles 1 and 2; $P < 0.005$.

lipoprotein B as well as by lower cholesterol/HDL cholesterol ratio and increased plasma levels of HDL cholesterol and HDL_2 cholesterol and increased LDL peak particle size compared with subjects in the lower tertile of HDL size ($P < 0.01$). Furthermore, men in the upper tertile for HDL size were characterized by decreased plasma levels of VLDL cholesterol, TG, VLDL TG, LDL TG, and apolipoprotein B and by a lower cholesterol/HDL cholesterol ratio as well as by increased plasma levels of HDL cholesterol, HDL_2 cholesterol, apolipoprotein A-I, and increased LDL peak particle size compared with subjects in both the lower and middle tertiles ($P < 0.01$). It is also relevant to point out that no difference in levels of total and LDL cholesterol was observed across tertiles of HDL particle size.

Figure 2 shows that men in the upper tertile of HDL particle size were also characterized by significantly lower fasting glucose and insulin concentrations as well as by reduced glycemic and insulinemic responses to a 75-g oral glucose load compared with men in the lower and middle tertiles of HDL particle size ($P < 0.05$).

Multiple regression analyses were also conducted to sort out the independent contribution of metabolic variables to the variance of HDL particle size (data not shown). Variables considered in the model included waist girth, visceral AT area, fat mass, log-transformed TG, HDL cholesterol, apolipoprotein B, apolipoprotein A-I, LDL peak particle size, and fasting insulin and glucose levels as well as insulin and glucose areas. About 37% of the variance in HDL particle size was explained by HDL cholesterol levels. The LDL particle size further explained 8% of the variance in HDL particle size, whereas log-transformed TG levels made a weak but significant (2%) contribution to its variance.

Previous results from the Québec Cardiovascular Study have revealed that men with the atherogenic metabolic triad (elevated insulin and apolipoprotein B concentrations and small, dense LDL particles) were characterized by a substantial increase in relative risk of ischemic heart disease (21). To identify carriers of this atherogenic metabolic triad, we have recently used cut-off values derived from a sample of normolipidemic healthy nonobese men (BMI <25 kg/m^2), these values corresponding to 48.5 pmol/l, 0.96 g/l, and 255.5 Å for insulin, apolipoprotein B, and LDL size, respectively (39). The frequency of men characterized by the atherogenic metabolic triad in each tertile of HDL particle size is illustrated in **Fig. 3**. Up to 66% of the men in the lowest tertile of HDL particle size (small HDL particles) were characterized by the presence of the metabolic triad (high fasting insulin, high apolipoprotein B, and small, dense LDL particles) compared with 53% and only 16% in the middle and upper tertiles of HDL size, respectively.

Finally, because there is a significant correlation between HDL cholesterol levels and HDL particle size ($r = 0.61$, $P < 0.0001$), we tested whether measuring HDL particle size could further discriminate features of an atherogenic "dysmetabolism" among men with equally reduced HDL cholesterol levels (HDL cholesterol ≤0.9 mmol/l). For that purpose, we conducted a subanalysis among 102 men in our study who had HDL cholesterol levels ≤0.9

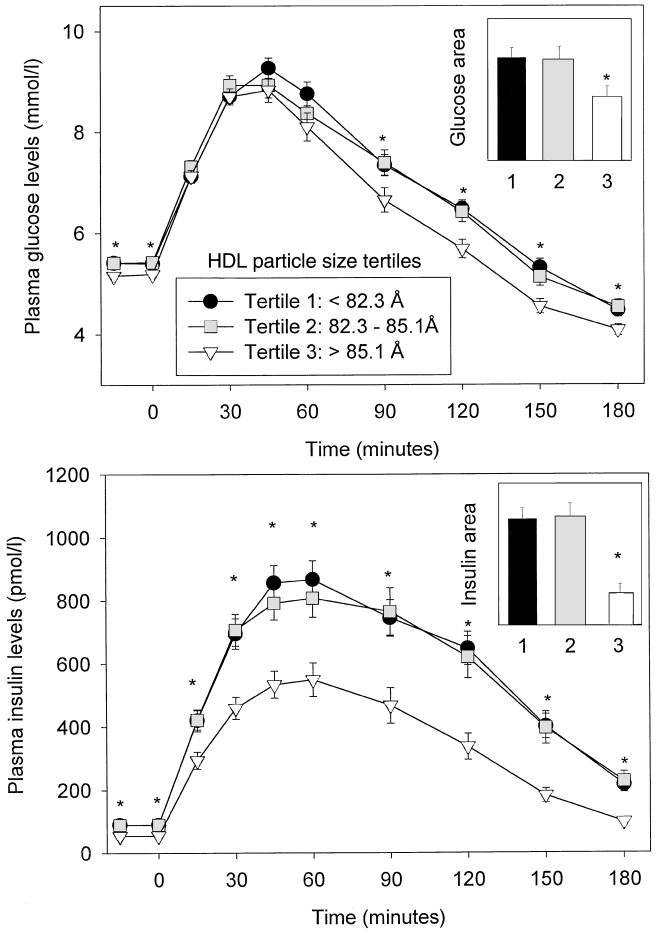

Fig. 2. Plasma glucose and insulin concentrations in the fasting state and after the oral glucose load among men divided on the basis of tertiles of HDL particle size. Bar charts show plasma glucose (mmol/l/min) $\times 10^{-3}$ and insulin areas (pmol/l/min) $\times 10^{-3}$ under the curve of their concentrations measured for 3 h after the oral glucose load. * Significant difference between tertile 3 versus tertiles 1 and 2, $P < 0.0001$.

mmol/l and further divided this subsample on the basis of the 50th percentile of the average HDL particle size into two subgroups with lower versus larger HDL particles. Covariance analysis adjusting for HDL cholesterol levels was also performed to adjust for the difference in HDL cholesterol levels, and adjusted variables are presented in **Table 3**. For the same levels of HDL cholesterol, men with the smallest particles (<50th percentile of HDL particle size) were characterized by decreased concentrations of HDL_2 cholesterol and by increased levels of HDL_3 cholesterol ($P < 0.01$). Furthermore, they were also characterized by decreased LDL particle size ($P < 0.03$), whereas a trend for increased apolipoprotein B concentrations was noted ($P = 0.06$). Men with low HDL cholesterol levels and with smaller HDL particles were also characterized by higher levels of TG and by an increased cholesterol/HDL cholesterol ratio ($P < 0.05$). Thus, results of this study suggest that measuring HDL particle size may possibly refine the evaluation of CHD risk among men with equally reduced HDL cholesterol levels.

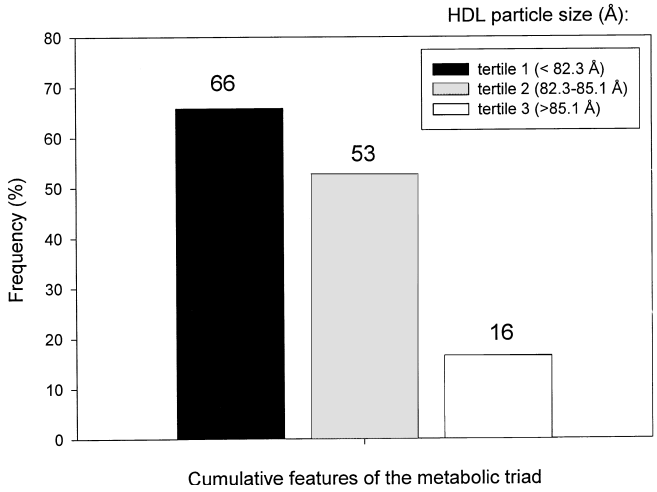

Fig. 3. Prevalence of the metabolic triad (hyperinsulinemia, elevated apolipoprotein B, and small, dense LDL) among tertiles of HDL particle size.

DISCUSSION

Many epidemiological studies have demonstrated an inverse relationship between the concentration of HDL cholesterol and the risk of CHD (1, 2). HDL are heterogeneous lipoproteins made of different HDL subfractions, and it has been suggested that it is the large, cholesteryl

TABLE 3. Characteristics of the subsample of 102 men with low levels of HDL cholesterol (<0.9 mmol/l) divided on the basis of the 50th percentile of average HDL particle size[a]

Variable	Average HDL Particle Size	
	<50th Percentile (n = 51)	>50th Percentile (n = 51)
Age (years)	43.8 ± 12.0	43.5 ± 12.0
Body fatness and AT distribution indices		
BMI (kg/m^2)	30.2 ± 4.3	28.9 ± 4.3
Fat mass (kg)	26.8 ± 9.1	26.0 ± 9.1
Waist girth (cm)	101.8 ± 11.0	98.4 ± 11.0
CT Visceral AT area (cm^2)	160.6 ± 57.8	146.1 ± 57.8
Metabolic profile		
Total cholesterol (mmol/l)	5.40 ± 0.80	5.14 ± 0.80
LDL cholesterol (mmol/l)	3.68 ± 0.76	3.51 ± 0.76
HDL cholesterol (mmol/l)	0.79	0.79
HDL_2 cholesterol (mmol/l)	0.18 ± 0.09	0.23 ± 0.09[b]
HDL_3 cholesterol (mmol/l)	0.61 ± 0.09	0.56 ± 0.09[b]
Average HDL particle size (Å)	80.3 ± 1.3	83.9 ± 1.3[c]
Cholesterol/HDL cholesterol ratio	6.8 ± 1.0	6.5 ± 1.0[d]
Triglycerides (mmol/l)	2.39 ± 0.84	2.11 ± 0.84[d]
Apolipoprotein B (g/l)	1.19 ± 0.21	1.11 ± 0.21[e]
Apolipoprotein A-I (g/l)	1.15 ± 0.10	1.10 ± 0.10[b]
LDL peak particle size (Å)	247.6 ± 4.7	249.6 ± 4.7[b]
Fasting glucose (mmol/l)	5.45 ± 0.50	5.47 ± 0.50
Fasting insulin (pmol/l)	92.5 ± 64.3	98.2 ± 64.3

Values are mean ± SD. CT, computed tomography.
[a] Adjusted for HDL-cholesterol by covariance analysis.
[b] $P < 0.03$.
[c] $P < 0.0001$.
[d] log-transformed variables $P < 0.02$.
[e] $P = 0.06$.

ester-rich HDL_2 subfraction that may be cardioprotective (3, 4, 16, 17, 20, 40). Thus, it has been reported that high levels of HDL_{3b} are associated with CHD risk factors, suggesting that low HDL_{3b} levels may contribute in part to the low CHD risk in subjects who have high HDL cholesterol (17). Furthermore, an increased BMI has been associated with lower levels of HDL_{2b} (4). Hyperinsulinemia and hypertriglyceridemia, two features of atherogenic dyslipidemia, have also been associated with low levels of HDL_{2b} and generally small HDL particle sizes (16).

In this study, due to its correlations with plasma TG, HDL cholesterol and HDL_2 cholesterol levels, and the cholesterol/HDL-cholesterol ratio, the small, dense HDL phenotype appears to represent another feature of atherogenic dyslipidemia. We also noted significant correlations between HDL size and indices of adiposity, reinforcing the notion that a high accumulation of visceral AT is a major factor involved in the development of this atherogenic dyslipidemic profile. Indeed, obesity, especially abdominal obesity, has been associated with metabolic disturbances leading to the development of an atherogenic dyslipidemic state, which contributes to the increased risk of CHD (41), supporting the early clinical observations of Vague (42), who first documented in the 1940s that regional AT distribution was an important determinant of the health hazards of obesity (42). In this regard, we have previously reported that visceral obesity is a highly prevalent component of the high TG-low HDL cholesterol dyslipidemia associated with hyperinsulinemia and insulin resistance (9, 43, 44). Furthermore, hyperinsulinemia and the high TG-low HDL cholesterol dyslipidemic state have also been associated with an increased risk of CHD (10, 11). It has also been suggested that abdominal obesity per se may be a major cause of insulin resistance as a result of excess lipolysis of portally drained visceral AT, causing an increased flux of free fatty acids to the liver and an overproduction of hepatic TG-rich lipoproteins (43). The resulting hypertriglyceridemia promotes the transfer of TG to HDL and LDL through the action of lipid transfer proteins. TG-enriched HDL and LDL can then be subjected to further lipolysis by HL, leading to the formation of small, dense HDL and LDL particles. We also noted a positive relationship between LDL peak particle size and the average HDL particle size, suggesting that the synergistic reduction in the size of these two lipoprotein fractions may be the consequence of a common metabolic alteration leading to hypertriglyceridemia. It is also important to point out that no significant correlation was found between HDL particle size and LDL cholesterol, providing further support to our previously published notion that the measurement of LDL cholesterol alone does not allow the proper identification of individuals who are carriers of atherogenic dyslipidemia (21). Moreover, hyperinsulinemia was also a correlate of the average HDL particle size, supporting the notion that HDL particle size may be considered as another feature of the insulin resistance syndrome.

Results of the present study indicate that carriers of the largest HDL particles (men in the top tertile of HDL size) were also characterized by a globally more favorable risk factor profile. The mechanism(s) by which HDL size could affect CHD risk remains to be established. However, it can be suggested that this relationship could be mediated by the association of dense HDL particles to concomitant alterations in plasma lipoprotein levels and glucose-insulin homeostasis. The possible relationship of dense HDL particles to CHD could also be explained by altered activities in lipases, proteins that are involved in the maturation and transformation of lipoproteins. Lipoprotein lipase (LPL) is the rate-limiting enzyme in the clearance of TG-rich lipoproteins from plasma and is involved in the formation of HDL, whereas HL plays a role in the catabolism of HDL. Therefore, abnormalities in the regulation of TG metabolism by LPL and HL may reduce levels of HDL (45), thereby increasing the risk for CHD, as previously suggested (16, 46, 47). Furthermore, CETP and phospholipid transfer protein (PLTP) are two others proteins involved in the metabolism of HDL. For instance, it has been reported that hypertriglyceridemia and CETP interact to drastically alter HDL levels and particle sizes because the presence of the CETP transgene in hypertriglyceridemic human apolipoprotein C-III transgenic mice lowered HDL cholesterol and apolipoprotein A-I, decreased HDL size, and increased HDL cholesterol ester fractional catabolic rate (FCR) (48). On the other hand, PLTP mediates conversion of an apparently homogeneous population of HDL_3 particles into a new population of particles with an increased average size, with concomitant release of apolipoprotein A-I. However, these proteins are not the only "regulator" of HDL particle size, because genetic (5, 49) and lifestyle (3, 4) factors also affect levels and distribution of HDL. Mechanistic studies have also shown that HDL size is an important determinant of its metabolic fate. Thus, it has been suggested that HDL size crudely estimated by the HDL cholesterol/apolipoprotein A-I + apolipoprotein A-II ratio could explain up to 70% of the variability in apolipoprotein A-I FCR (50).

On the other hand, the remaining variation in the metabolic risk profile observed for any given HDL particle size suggests that HDL particle size by itself cannot be considered as a single overall measurement of CHD risk. We would rather suggest that the average HDL particle size could possibly be considered as an additional parameter for further refinement of the evaluation of CHD risk. Therefore, this new and potentially relevant marker should not replace traditional lipid variables, such as TG, HDL cholesterol, or the cholesterol/HDL cholesterol ratio. We have already proposed a similar rationale for the use of LDL peak particle size, another marker that may help refine CHD risk assessment, as we have reported that LDL peak particle size had to be interpreted in combination with other features of the insulin-resistant dyslipidemic syndrome in order to improve our evaluation of CHD risk (21). Finally, we have to acknowledge the fact that we do not have prospective data with "hard" CHD end points. Prospective studies are clearly warranted to establish whether HDL particle size is an independent CHD risk factor or only another correlate of an atherogenic metabolic profile.

In summary, results of this study suggest that the high TG-low HDL cholesterol dyslipidemic state and an elevated cholesterol/HDL cholesterol ratio, abnormalities commonly found among viscerally obese individuals, are strong correlates of a reduced HDL particle size measured by gradient gel electrophoresis. Furthermore, the presence of small, dense HDL particles is associated with a high probability of finding the features of the atherogenic metabolic triad. Thus, the small, dense HDL phenotype is associated with a high risk of finding a cluster of atherogenic metabolic abnormalities. Therefore, HDL size may represent another relevant marker of an atherogenic "dysmetabolism." Prospective studies are clearly warranted to quantify the independent contribution of this new parameter to the evaluation of CHD risk.

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