Visceral Adipose Tissue and Low-Density Lipoprotein Particle Size in Middle-Aged Versus Young Men

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Age is associated with increased deposition of visceral adipose tissue. We examined whether this age-related change in regional adipose tissue distribution had an impact on low-density lipoprotein (LDL) particle size. For this purpose, the plasma lipoprotein-lipid profile, including LDL peak particle diameter as determined by gradient gel electrophoresis, was assessed in 38 young men (aged 26.4 ± 4.2 years, mean ± SD) and compared with 40 middle-aged men (55.9 ± 6.2 years). Middle-aged men had higher values for total body fat and visceral adipose tissue area as measured by computed tomography than young men ($P < .001$). Although significant differences were noted between the two age groups for plasma cholesterol, triglyceride (TG), apolipoprotein B (apo B), LDL cholesterol, and LDL apo B, as well as the cholesterol to high-density lipoprotein (HDL) cholesterol ratio ($P < .001$), no difference was found for LDL peak particle size between young and middle-aged men. While visceral adipose tissue was a significant correlate of plasma lipoprotein levels, the fasting TG concentration was the best predictor of LDL particle size, and the regression of TG levels on LDL peak particle diameter was not different between the two age groups. These results suggest that middle-aged men are characterized by an increased concentration of LDL particles (reflected by increased LDL apo B levels) but not by a reduced LDL peak particle size compared with young men. It is therefore proposed that in the absence of an important age-related change in TG levels, age per se is associated with an increased concentration of atherogenic LDL particles rather than a reduction of LDL particle diameter.

LOW-DENSITY LIPOPROTEIN (LDL) particles exhibit considerable heterogeneity in density, size, and chemical composition.[1-5] Despite multiple approaches for defining LDL subclasses, most methods accurately differentiate relatively smaller, denser, and lipid-depleted particles from those that are larger, more buoyant, and lipid-enriched.

A growing body of epidemiologic evidence has shown a consistent association between small, dense LDL particles and the prevalence of coronary heart disease (CHD) in case-control studies.[6-9] Recent prospective studies have also confirmed that the presence of small, dense LDL particles is associated with a twofold to threefold increase in CHD risk.[10-12] The presence of small, dense LDL has also been associated with a concomitant variation in the plasma lipid-lipoprotein profile, such as elevated triglyceride (TG) and reduced high-density lipoprotein (HDL) cholesterol concentrations.[7,9,13-19] Furthermore, visceral obesity and insulin resistance are frequently found among subjects with small, dense LDL particles.[14,20] However, among the numerous variables associated with small, dense LDL particles, the fasting TG concentration appears to be the critical factor. Thus, we have reported that visceral adiposity is not an independent predictor of an increased proportion of small, dense LDL particles after controlling for TG levels.[14]

Age has also been associated with changes in body composition and in visceral adipose tissue accumulation, as well as with concomitant alterations in the plasma lipoprotein-lipid profile.[21,22] However, little is known about the respective contributions of total body fatness, visceral adipose tissue, and TG levels to the age-related variation in LDL concentration and composition.

Thus, the aim of the present study was to examine the contribution of age-related differences in TG levels and in regional adipose tissue accumulation to the LDL peak particle diameter as assessed by gradient gel electrophoresis in a sample of middle-aged and young men.

SUBJECTS AND METHODS

Subjects

The subjects who volunteered to participate in the present study are currently involved in phase 2 of the prospective Québec Family Study designed to investigate the role of genetic factors in the etiology of obesity.[23] Subjects included in the present study are of French Canadian descent and are fathers and adult male unrelated offspring in whom the body composition, visceral adipose tissue obtained by computed tomography, and plasma lipoprotein-lipid profile were measured. The fathers were, on average, in their mid-fifties (middle-aged men), whereas the sons were in their mid-twenties (young men). The 78 men participating in this study cam from 54 distinct families. The 40 middle-aged men (fathers) came from 40 distinct families, and only one young man per family was selected for the present analyses. Men with diabetes or cardiovascular disease or taking medication known to affect carbohydrate or lipid metabolism were excluded from the present analyses. Men with familial hypercholesterolemia or common French Canadian lipoprotein lipase gene mutations were also excluded. All participants signed an informed-consent document. The study was approved by the Medical Ethics Committee of Laval University.

Body Composition and Adipose Tissue Distribution

Body density was measured by the hydrostatic weighing technique,[24] and the mean of six measurements was used in the calculation of body density. Pulmonary residual volume was measured before immersion in the hydrostatic tank, using the helium dilution method of Meneely and Kaltreider.[25] The percentage body fat was derived from body density using the equation of Siri.[26] Body weight, height, and waist and hip

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Submitted January 22, 1999; accepted March 25, 1999.

Supported by the Medical Research Council of Canada (PG-11811, GR-15187, and MT-14014), the Quebec Heart and Stroke Foundation, and the Canadian Diabetes Association.

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0026-0495/99/4810-0002$10.00/0

circumferences were measured with standardized procedures.[27] Measurements of abdominal adipose tissue areas were performed by computed tomography with a Somatom DHR scanner (Siemens, Erlangen, Germany) as previously described.[28] Briefly, subjects were examined in the supine position with both arms stretched above the head. The scan was performed at the abdominal level (L4 and L5 vertebrae) using an abdominal scout radiograph to standardize the position of the scan to the nearest millimeter. Total adipose tissue area was calculated by delineating the abdominal scan with a graph pen and then computing the total abdominal adipose tissue area with an attenuation range of −190 to −30 Hounsfield units. The abdominal visceral adipose tissue area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous adipose tissue area was calculated by subtracting the visceral adipose tissue area from the total abdominal adipose tissue area.

Plasma Lipoprotein-Lipid Profile

Following a 12-hour overnight fast, blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA for measurement of plasma lipid and lipoprotein levels. Cholesterol and TG concentrations were determined in plasma and lipoprotein fractions using a Technicon RA-500 analyzer (Bayer, Tarrytown, NY). Plasma very–low-density lipoproteins ($d < 1.006$ g/mL) were isolated by ultracentrifugation,[29] and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$.[30] The cholesterol content of the infranatant fraction was measured before and after the precipitation step for measurement of LDL and HDL cholesterol. Apolipoprotein B (apo B) was measured in the plasma and LDL apo B in the infranatant by the rocket immunoelectrophoretic method of Laurell[31] as previously described.[32] Lyophilized serum standards for apo B measurements were prepared in our laboratory and calibrated with reference standards obtained from the Centers for Disease Control (Atlanta, GA).

Electrophoresis of Plasma Samples

Nondenaturing 2% to 16% polyacrylamide gel electrophoresis was performed on whole plasma kept at −80°C before use, according to the procedure described by Krauss and Burke[1] and McNamara et al.[15] Gels were cast in our laboratory using acrylamide and bis-acrylamide (30:0.8) obtained from Bio-Rad (Hercules, CA). A 7.5-μL volume of plasma samples was applied on the lanes in a final concentration of 20% sucrose and 0.25% bromophenol blue. Electrophoresis was performed in a refrigerated cell (10° to 15°C) for a prerun of 15 minutes at 125 V and for the entry of samples into stacking at 70 V for 20 minutes, followed by migration at 200 V for 12 to 16 hours and finally at 400 V for 2 to 4 hours. Gels were stained for lipids overnight with Sudan Black (Lipostain, Paragon electrophoresis system; Beckman, Montreal, Canada) in 55% ethanol. Gels were destained in a 45% ethanol solution, and the original gel size was restored in a 9% acetic acid and 20% methanol solution. A plasma pool was used as an internal standard. Gels were analyzed using an optical densitometric image-analyzer (Bio-Image Visage 110; Genomic Solutions, Ann Arbor, MI) coupled to a SPARC Station 2 Sun computer (Millipore, Ville St-Laurent, Canada) and using GEL 1D software. LDL peak particle size was obtained using the migration of standards of known diameter such as ferritin (122 Å), thyroglobulin (170 Å), and 380-Å Latex beads (Duke Scientific, Palo Alto, CA) and plasma standards of known diameter. Analyses of pooled plasma standards showed that identification of the major LDL peak was highly reproducible, with an interassay coefficient of variation less than 3% (unpublished data, April 1996).

Statistical Analyses

The unpaired Student t test was used to compare mean values between young and middle-aged men. Spearman correlation coefficients were used to quantify univariate associations among TG, LDL apo B, the cholesterol to HDL cholesterol ratio, and LDL peak particle diameter. After individual pairing of young and middle-aged men for TG concentrations (within a 0.1-mmol/L difference), anthropometric and lipoprotein-lipid variables were compared with a paired t test. Covariance analyses were also used to evaluate the effect of TG on the age-related differences in plasma lipoprotein-lipid variables. Subjects were carefully chosen to avoid the effects of familial aggregation; thus, a father was never matched with his son. According to these criteria, we were able to form 22 pairs of unrelated middle-aged versus young subjects. Furthermore, multiple regression analyses were performed to quantify the independent contribution of age, TG level, visceral adipose tissue area, fat mass, and cholesterol to HDL cholesterol ratio to the variance of LDL peak particle diameter. All statistical analyses were performed with the SAS package (SAS Institute, Cary, NC).

RESULTS

Table 1 lists physical characteristics for the 38 young and 40 middle-aged men of the study. Older men had a higher body mass index (BMI), body fat mass, waist circumference, waist to hip ratio, and total and visceral adipose tissue area than younger men ($P < .05$). Older men were characterized by higher levels of total and LDL cholesterol ($P < .0001$) and a substantially higher cholesterol to HDL cholesterol ratio compared with young men ($P < .001$). Furthermore, middle-aged men were characterized by considerably higher apo B and LDL apo B levels ($P < .0001$). However, LDL peak particle diameter showed no difference between middle-aged versus young men, despite a significant but small difference in fasting TG levels between the two age groups (Table 2).

Since the fasting TG concentration has been consistently reported as a good correlate of LDL particle size, we examined whether age could alter this relationship. Figure 1 shows that the correlation between the LDL peak particle size and TG level was statistically significant in both age groups ($P < .01$). A high TG concentration was associated with small, dense LDL particles in both age groups. It is also important to note that regression slopes were similar between young and middle-aged men, suggesting that age did not alter this relationship. Figure 1 also shows the correlation between LDL peak particle diameter and the cholesterol to HDL cholesterol ratio in both young and middle-aged men. There was a significant negative association between LDL peak particle size and the cholesterol to HDL

Table 1. Physical Characteristics of the Young and Middle-Aged Men of the Study

Characteristic	Young Men (n = 38)	Middle-Aged Men (n = 40)
Age (yr)	26.4 ± 4.2	55.9 ± 6.2‡
BMI (kg/m²)	25.1 ± 4.2	28.2 ± 4.3†
Fat mass (kg)	16.2 ± 10.3	23.3 ± 8.1†
Waist circumference (cm)	86.0 ± 12.2	97.6 ± 11.1‡
Waist to hip ratio	0.87 ± 0.06	0.96 ± 0.06‡
Abdominal adipose tissue area (cm²)		
Total	273.4 ± 173.0	400.0 ± 168.7*
Visceral	76.6 ± 43.1	154.1 ± 64.6‡
Subcutaneous	196.8 ± 133.7	245.9 ± 123.1

*$P < .05$.
†$P < .001$.
‡$P < .0001$.

Table 2. Metabolic Risk Profile of the Young and Middle-Aged Men of the Study

Variable	Young Men (n = 38)	Middle-Aged Men (n = 40)
Cholesterol (mmol/L)	4.7 ± 0.8	5.5 ± 0.8‡
TG (mmol/L)	1.4 ± 0.8	1.6 ± 0.7*
LDL cholesterol (mmol/L)	3.2 ± 0.8	4.0 ± 0.7‡
HDL cholesterol (mmol/L)	1.1 ± 0.2	1.0 ± 0.2
Non-HDL cholesterol (mmol/L)	3.6 ± 0.9	4.6 ± 0.8‡
Cholesterol/HDL cholesterol ratio	4.7 ± 1.5	5.8 ± 1.2†
Apo B (g/L)	0.93 ± 0.21	1.2 ± 0.2‡
LDL apo B (g/L)	0.84 ± 0.19	1.0 ± 0.2‡
LDL size (Å)	252.0 ± 5.3	252.1 ± 5.1

NOTE. TG levels were log arithmically transformed.
*$P < .05$.
†$P < .001$.
‡$P < .0001$.

cholesterol ratio in both young and middle-aged men ($P < .02$). In both age groups, no relationship was found between LDL peak particle size and the LDL concentration estimated by LDL apo B levels. However, there was a marked age-related difference in the plasma LDL apo B concentration, as indicated by the rightward shift of LDL apo B levels in middle-aged compared with young men.

Multiple regression analyses were performed to determine the independent contribution of age, TG level, visceral adipose tissue area, fat mass, and cholesterol to HDL cholesterol ratio to LDL peak particle diameter (data not shown). Approximately 25% of the variance in LDL peak particle diameter was explained by the fasting TG concentration in a regression model including age, TG concentration, visceral adipose tissue area, fat mass, and cholesterol to HDL cholesterol ratio ($P < .0001$). After inclusion of fasting TG in the model, no other variables had an independent contribution to the variance in LDL peak particle size.

To further investigate the contribution of TG to age-related differences in plasma lipoprotein-lipid variables, 22 young men were individually matched to 22 middle-aged men on the basis of fasting TG levels. As expected from the design of this analysis, TG concentrations were similar in both young and middle-aged men as a result of the pairing procedure (Table 3). Older men were characterized by a higher BMI, waist circumference, and visceral adipose tissue accumulation than younger men ($P < .05$). However, despite a lack of difference in TG levels, middle-aged men showed significant differences in the plasma lipoprotein-lipid profile, as they had higher cholesterol, LDL cholesterol, and LDL apo B levels compared with young men ($P < .05$). However, no difference in LDL peak particle size was found between the two age groups. Results of the covariance analysis after adjustment for TG levels showed similar findings, with no difference found in the LDL peak particle size between young and middle-aged men (not shown).

DISCUSSION

Since LDLs are involved in the development of CHD,[33,34] the study of factors contributing to a variation in their concentration and composition is of considerable interest. LDL particles have been shown to be a heterogeneous subclass of lipoproteins with respect to size, density, and chemical composition.[1-5] In this regard, the predominance of small, dense LDL subspecies has been associated with a significantly increased risk of CHD.[6-12] A portion of the increased CHD risk associated with the dense LDL phenotype may be related to the concomitant variation in the plasma lipoprotein-lipid profile,[13,15-17] which is also predictive of an increased CHD risk. In the present study, LDL peak particle diameter showed significant correlations with the TG concentration and cholesterol to HDL cholesterol ratio. Previous studies have reported essentially similar associations between these variables in other populations.[7,9,13-19]

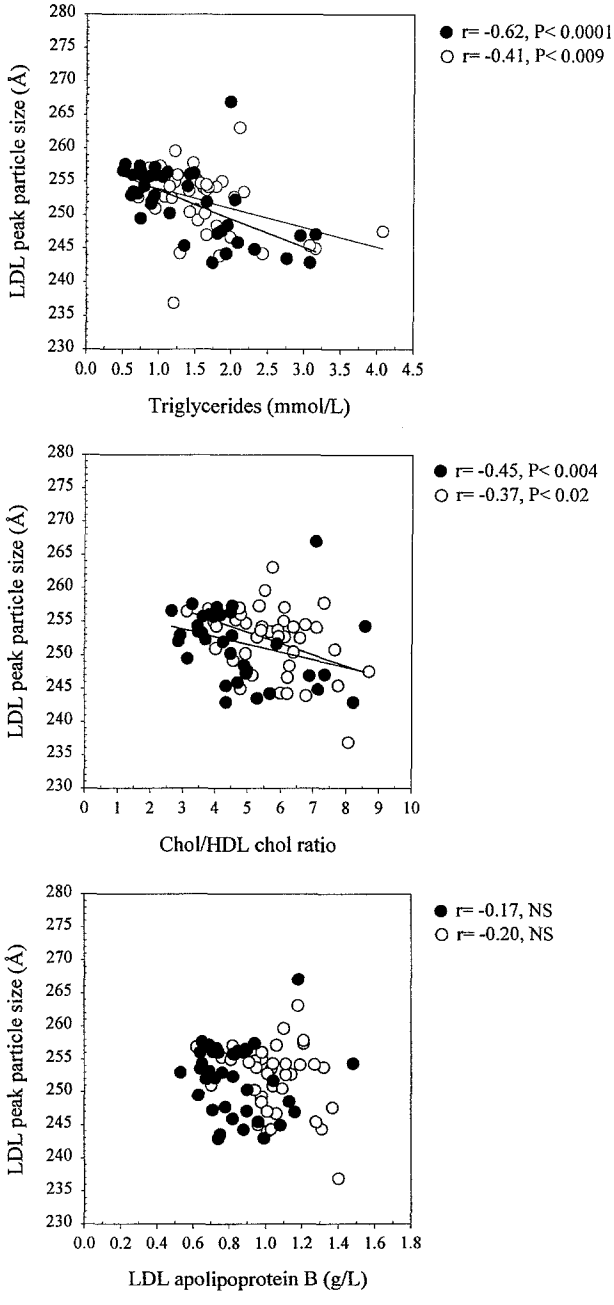

Fig 1. Spearman correlations between LDL peak particle diameter and TG, LDL apo B, and cholesterol to HDL cholesterol ratio in 38 young men (●) and 40 middle-aged men (○). NS, nonsignificant.

Table 3. Characteristics of Young and Middle-Aged Men Individually Matched on the Basis of TG Levels

Characteristic	Young Men (n = 22)	Middle-Aged Men (n = 22)
TG (mmol/L)	1.6 ± 0.7	1.6 ± 0.7
BMI (kg/m^2)	25.5 ± 4.3	28.0 ± 4.4*
Waist circumference (cm)	87.8 ± 12.7	96.5 ± 10.9*
Visceral adipose tissue area (cm^2)	81.2 ± 39.6	142.3 ± 55.5†
Cholesterol (mmol/L)	4.8 ± 0.7	5.4 ± 0.8*
LDL cholesterol (mmol/L)	3.3 ± 0.8	3.9 ± 0.8*
HDL cholesterol (mmol/L)	1.0 ± 0.2	1.0 ± 0.2
Cholesterol/HDL cholesterol ratio	5.0 ± 1.5	5.4 ± 1.1
LDL apo B (g/L)	0.88 ± 0.18	1.0 ± 0.2*
LDL size (Å)	251.7 ± 5.9	251.8 ± 4.6

*$P < .05$.
†$P < .001$.

On the other hand, numerous studies have shown that age, gender, hormonal status, abdominal obesity, physical deconditioning, diet, and other lifestyle variables could modulate the penetrance of the dense LDL phenotype,[13-16,35-38] which has also been suggested to have a genetic component.[39] Among these factors, the presence of dense LDL was associated with increased age.[15] Williams et al[35] reported a positive association between age and LDL subclass LDL-IIIA (247.0 to 255.0 Å) that was eliminated after adjustment for fasting TG levels, whereas there was no association of age with LDL peak particle diameter. In the present study, we examined whether there are age-related differences in LDL particle diameter when considering the concomitant variations in TG levels, body composition, and visceral adipose tissue accumulation that occur with age. In our cohort, no difference between young and middle-aged men was observed for LDL peak particle diameter, despite the fact that there were age-related differences in other variables of the plasma lipoprotein-lipid profile. These age-related differences in plasma lipid levels are concordant with previous findings.[21,22,40] Furthermore, HDL cholesterol levels were similar between the two age groups.

With aging, there is a selective accumulation of abdominal visceral fat,[21,41-43] which has been suggested to be an important contributing factor to the age-related deterioration in the cardiovascular disease risk profile.[21,44-46] In accordance with these previous reports, we found that middle-aged men were characterized by a higher BMI and a larger fat mass compared with young men. We also found that the cross-sectional area of abdominal visceral adipose tissue measured by computed tomography was higher in middle-aged men versus young controls. To further investigate the contribution of visceral adipose tissue to potential age-related differences in LDL peak particle diameter and in the lipoprotein-lipid profile, 16 young men were matched to 16 middle-aged men for similar levels of visceral adipose tissue (within 10 cm^2; data not shown). Plasma LDL cholesterol, apo B, and LDL apo B levels remained significantly higher in middle-aged versus young men even after pairing for visceral adipose tissue accumulation. These results suggest that factor other than visceral adiposity and increased body fatness are responsible for the age-related increase of LDL cholesterol and LDL particle concentrations. Thus, the main conclusion of the present study is that age *per se* is associated with an increased LDL particle concentration but not with changes in LDL particle size.

A previous study by McNamara et al[17] examined changes in LDL particle size in a cohort of adults over an interval of 3 to 4 years. They reported that changes in LDL particle diameter were a reflection of the fasting lipid status. Indeed, in stepwise regression analyses, a variation in the TG concentration was the best single predictor of age-related variation in LDL size. They therefore concluded that the amount of circulating TG appears to be the single most important factor affecting LDL particle size and that changes in LDL particle diameter could be estimated through changes in plasma TG concentration. In agreement with this study,[17] almost 25% of the variance in LDL peak particle size in the present sample was explained by the fasting TG concentration, and no other variable could further explain the variation in LDL particle size. Thus, despite the fact that middle-aged men had a slightly higher TG concentration than young men, this small (1.4 *v* 1.6 mmol/L) difference was not sufficient to impact the LDL peak particle diameter.

However, pairing subjects by TG concentration only resulted in a trivial attenuation of the age-related differences in other plasma lipoprotein-lipid variables such as LDL cholesterol and LDL apo B levels. Thus, the age-related increase in TG levels cannot explain the age-associated increase in LDL particle concentration (as estimated by LDL apo B levels) that has been well documented in several studies.[21,47]

In the present study, we found no association between LDL peak particle size and LDL particle concentration as estimated by LDL apo B levels. Thus, it appears that the size and the number of LDL particles are two distinct metabolic markers. According to our previous prospective results from the Quebec Cardiovascular Study, the dense LDL phenotype increases the risk of ischemic heart disease only with the presence of a concomitant elevation in the apo B concentration.[12] Thus, in the present study, age *per se* may not alter the risk of CHD via an increase in the LDL concentration rather than the LDL composition and size, unless hypertriglyceridemia occurs.

In conclusion, the increasing prevalence of the small, dense LDL phenotype with age appears largely mediated by the age-related increase in TG levels. Moreover, the increased levels of abdominal visceral adipose tissue found in middle-aged men compared with young men were not associated with smaller LDL particles but rather with an increased LDL particle concentration (20% increase in LDL particles as estimated by LDL apo B levels). It is therefore proposed that in the absence of a change in TG levels, age is associated with an increased concentration of atherogenic LDL particles rather than with a change in LDL size.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the Quebec Family Study subjects for their excellent collaboration, and to the staff of the Lipid Research Center and the Physical Activity Sciences Laboratory for their contribution to this study. We especially want to thank L. Allard, S. Brulotte, L. Bargone, G. Fournier, H. Bessette, and R. Maheux for their help in the collection of data.

I.L. is the recipient if a fellowship from the Heart and Stroke Foundation of Canada, A.T. is the recipient of a postdoctoral fellowship from the Canadian Diabetes Association, and J.B. is a clinical research scholar from the Fonds de la Recherche en Santé du Québec.

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