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Short Communication

Effect of type 2 diabetes on various electrophoretic characteristics of low-density lipoprotein particles in women

P. Blackburn[1, 2] · **I. Lemieux**[1] · **B. Lamarche**[2, 3] · **J. Bergeron**[4] · **P. Perron**[5] · **G. Tremblay**[5] · **D. Gaudet**[5] · **J.-P. Després**[1, 2, 4]

[1] Québec Heart Institute, Laval Hospital Research Center, Pavilion Marguerite-D'Youville, Ste-Foy, Québec, Canada
[2] Department of Food Sciences and Nutrition, Laval University, Ste-Foy, Québec, Canada
[3] Institute on Nutraceuticals and Functional Foods, Laval University, Ste-Foy, Québec, Canada
[4] Lipid Research Center, CHUL Research Center, Ste-Foy, Québec, Canada
[5] Community Genomic Medicine Center, University of Montréal, Chicoutimi Hospital, Chicoutimi, Québec, Canada

Abstract

Aims/hypothesis. Coronary heart disease represents the leading cause of death in type 2 diabetic patients. As the small, dense LDL phenotype is a typical feature of the dyslipidaemic state found in type 2 diabetes, this characteristic could be an important mediator of the elevated coronary heart disease risk in this condition. We have therefore studied the effect of type 2 diabetes on various electrophoretic characteristics of LDL particles.
Methods. Potential differences in LDL peak particle size and in concentration of LDL cholesterol in small (<255 Å) and large (>260 Å) LDL particles were assessed by polyacrylamide gradient gel electrophoresis among 183 non-diabetic and 56 type 2 diabetic women.
Results. LDL peak particle size was significantly smaller in type 2 diabetic women than in non-diabetic women (p<0.0001). In addition, the proportion of small LDL particles (<255 Å) was higher in type 2 diabetic women, whereas the proportion of large LDL particles (>260 Å) was lower than in non-diabetic women (p<0.0002). Type 2 diabetic women also had the highest waist circumference and triglyceride levels (p<0.03). When subgroups of non-diabetic and type 2 diabetic women were individually matched (n=41) for similar waist circumference and triglyceride levels, the differences initially found in LDL peak particle size and in the proportion of small and large LDL particles remained significantly different between the two groups (p<0.01).
Conclusions/interpretation. These results provide evidence that type 2 diabetes may have an independent effect on LDL peak particle size and on the proportion of small and large LDL particles.

Keywords LDL particles · Triglycerides · Type 2 diabetes · Waist circumference · Women

Introduction

It is well documented that type 2 diabetes is associated with an increased risk of CHD [1]. However, it has been shown that hyperglycaemia only partially explains the relationship between type 2 diabetes and CHD, suggesting that other risk factors could be responsible for the higher cardiovascular risk found in type 2 diabetic patients [2]. Subjects with type 2 diabetes also display an altered fasting lipoprotein–lipid profile compared with non-diabetic subjects, largely explained by a higher accumulation of visceral adipose tissue in type 2 diabetic patients than in non-diabetic subjects [3].

Among the metabolic alterations observed in type 2 diabetic patients, there is solid evidence that the LDL particle size is smaller in type 2 diabetic patients than in normoglycaemic controls [4, 5]. Even after adjustment for confounding variables, LDL particle size has been reported to remain lower in type 2 diabetic wom-

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J.-P. Després (✉)
Québec Heart Institute, Laval Hospital Research Center,
Pavilion Marguerite-D'Youville, 4th floor, 2725,
chemin Ste-Foy, Ste-Foy, Québec, G1V 4G5, Canada
E-mail: jean-pierre.despres@crhl.ulaval.ca

en than in non-diabetic women [4]. Thus, type 2 diabetes itself could be an important contributing factor in the variation of LDL particle size, especially in women.

Recently, it has been suggested that the measurement of the absolute and relative concentrations of LDL cholesterol in the small and large LDL subfractions may represent a better approach to evaluating the risk of ischaemic heart disease in men than the LDL peak particle size [6, 7]. However, to the best of our knowledge, no study has examined the impact of type 2 diabetes on LDL peak particle size and on the absolute and relative concentrations of LDL cholesterol in LDL particles of differing sizes in a sample of non-diabetic and type 2 diabetic women.

Subjects and methods

Study population. Non-diabetic (n=183) and type 2 diabetic (n=56) women aged 32 to 82 years (mean age ± SD: 56.1±9.0 years) were recruited from the Saguenay-Lac-St-Jean regional hospital in Chicoutimi, Canada. Menopausal women were included in the study (n=165). Patients gave their written consent to participate in the study, which was approved by the Chicoutimi Hospital Ethics Committee.

Anthropometric measurements and laboratory analyses. Anthropometric measurements were performed using standardised techniques [8]. Plasma total cholesterol, triglyceride and HDL cholesterol levels were measured using enzymatic assays [9]. Total cholesterol was determined in plasma and HDL cholesterol was measured in the supernatant, as previously described [10]. LDL cholesterol was calculated with the Friedewald formula when triglyceride levels were lower than 5.0 mmol/l [11]. When triglyceride levels were 5.0 mmol/l or higher, LDL cholesterol levels were measured using a Technicon RA-500 analyser (Bayer Corporation, Tarrytown, N.Y., USA) as previously described [12]. Total apolipoprotein B concentrations were measured by a nephelometric method using polyclonal antibodies on the Behring BN-ProsPect (Dade–Behring, Marburg, Germany). Fasting plasma glucose was enzymatically measured [13], whereas fasting plasma insulin was assessed by radioimmunoassay with polyethylene glycol separation [14]. Non-diabetic and type 2 diabetic women were classified according to previously established diagnosis of type 2 diabetes or by their fasting glucose concentrations (non-diabetic: fasting glucose <7.0 mmol/l; type 2 diabetic: fasting glucose ≥7.0 mmol/l).

LDL particle size characterisation. LDL characterisation was determined by non-denaturing 2 to 16% polyacrylamide gradient gel electrophoresis, as previously described [6]. LDL particle size was extrapolated from the relative migration of four plasma standards of known diameters. The estimated diameter for the major peak in each scan was identified as the LDL peak particle size. The relative proportion of LDL with a diameter smaller than 255 Å was determined by computing the relative area of the densitometric scan corresponding to LDL particles smaller than 255 Å. It has been documented that Sudan black stains mainly non-polar lipids [15]. The absorbance profile with Sudan black staining was also assumed to closely reflect the cholesterol distribution among LDL particles of different sizes [16]. The absolute concentration of cholesterol among particles smaller than 255 Å was calculated by multiplying plasma LDL cholesterol levels by the relative proportion of LDL with a diameter smaller than 255 Å. A similar approach was used to assess the relative and absolute concentrations of cholesterol in LDL particles with a diameter greater than 260 Å.

Statistical analyses. Spearman correlations were used to quantify associations between variables. Differences between non-diabetic and type 2 diabetic women were examined by Student's unpaired t tests. As values were not normally distributed, fasting triglyceride levels were log-transformed. Comparison of prevalence data among subgroups was performed by the likelihood chi square analysis. All statistical analyses were performed with the SAS package (SAS Institute, Cary, N.C., USA). In all analyses, a p value of less than 0.05 was considered statistically significant.

Results

Table 1 shows physical characteristics and fasting metabolic profile of non-diabetic and type 2 diabetic women. Type 2 diabetic women were significantly older than non-diabetic women (p<0.03). Adiposity indices such as BMI and waist circumference were higher in type 2 diabetic women than in non-diabetic women (p<0.0001). Although there were no significant differences in LDL cholesterol levels between the two groups, type 2 diabetic women were characterised by a more disturbed fasting lipoprotein–lipid profile, which included elevated triglyceride levels, reduced HDL cholesterol concentrations and a higher total cholesterol : HDL cholesterol ratio than in non-diabetic women (p<0.03). The proportion of smokers and menopausal women as well as the proportion of women on hormone replacement therapy and on antihypertensive or hypolipidaemic drugs was similar in non-diabetic and type 2 diabetic women.

Table 1. Physical characteristics and fasting metabolic profile of the non-diabetic and type 2 diabetic women of the study

	Non-diabetic women n=183	Type 2 diabetic women n=56
Age (years)	55.4±9.0	58.4±8.6*
BMI (kg/m^2)	26.1±4.8	29.7±6.0*
Waist circumference (cm)	84.6±11.5	95.9±14.1*
Cholesterol (mmol/l)	5.75±1.17	5.71±0.96
LDL cholesterol (mmol/l)	3.58±1.16	3.58±1.02
HDL cholesterol (mmol/l)	1.27±0.41	1.10±0.35*
Total cholesterol: HDL cholesterol ratio	5.05±1.89	5.70±2.14*
Triglycerides (mmol/l)†	1.90±1.01	2.17±0.97*
Apolipoprotein B (g/l)	0.79±0.28	0.84±0.24
Fasting insulin (pmol/l)	96.1±55.2	133.3±77.4*
Fasting glucose (mmol/l)	5.17±0.61	7.80±3.13*

Data are means ± SD. * p<0.03 vs non-diabetic women; † $\log_{10}$ transformed

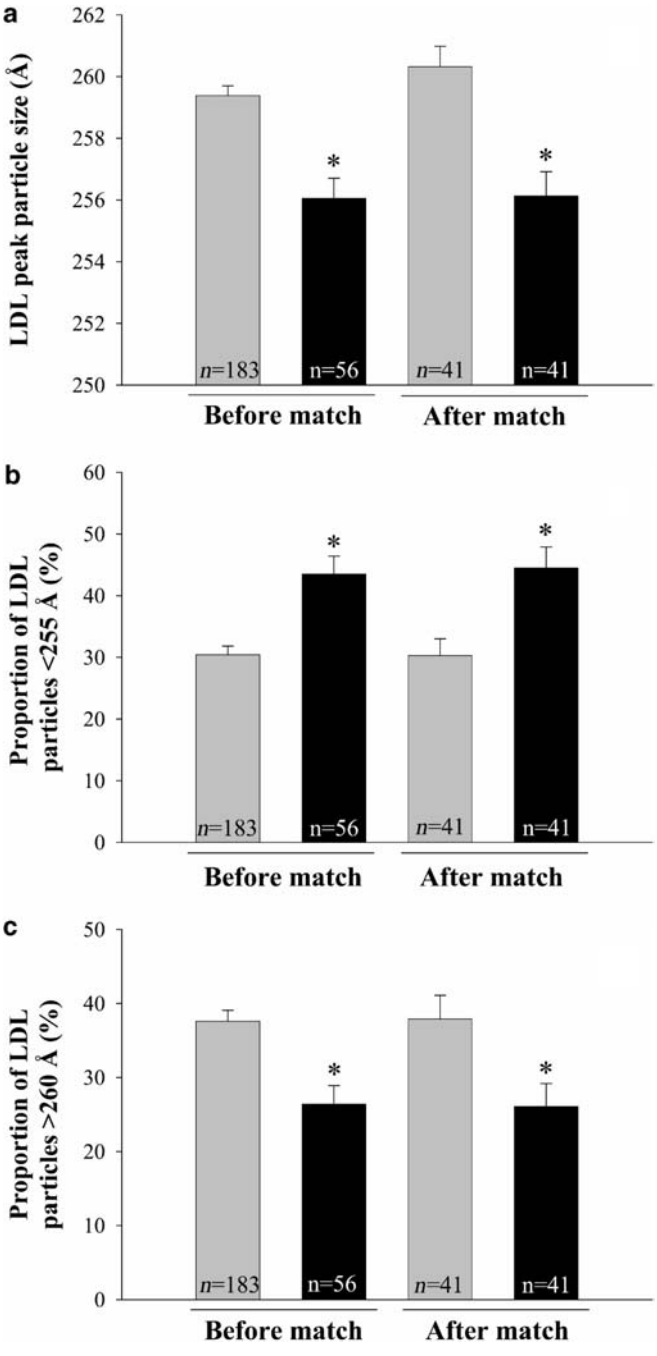

Fig. 1. LDL peak particle size (**a**), proportion of small LDL particles (<255 Å) (**b**) and proportion of large LDL particles (>260 Å) (**c**) among non-diabetic (grey) and type 2 diabetic (white) women before and after the matching procedure. * $p<0.01$ vs non-diabetic women

The LDL peak particle size was significantly smaller in type 2 diabetic women than in non-diabetic women (256.1±4.9 vs 259.4±4.3 Å, $p<0.0001$; Fig. 1). Type 2 diabetic women were also characterised by an increased proportion (43.5%) of small LDL particles (<255 Å) and by a lower proportion (26.4%) of large particles (>260 Å) than non-diabetic women (30.4% and 37.6% for small and large LDL respectively, $p<0.0002$; Fig. 1). In addition, the concentration of LDL cholesterol in LDL particles smaller than 255 Å was higher in type 2 diabetic women (1.6±1.0 vs 1.0±0.7 mmol/l, $p<0.0003$) than in non-diabetic women, whereas the concentration of LDL cholesterol in larger particles (>260 Å) was smaller (0.9±0.7 vs 1.4±0.9 mmol/l, $p<0.0006$) in type 2 diabetic women.

Since triglyceride levels were correlated with the LDL peak particle size as well as with the proportion of small and large LDL particles (data not shown), we controlled for this confounder as well as for a marker of abdominal obesity (waist girth) in subsequent analyses. For this purpose, subgroups of non-diabetic or type 2 diabetic women were individually matched for similar waist circumference (within a variation of ±5 cm) and triglyceride levels (within a variation of ±0.3 mmol/l; Fig. 1). After such a procedure, apolipoprotein B concentrations were higher in type 2 diabetic women than in non-diabetic women ($p<0.03$). However, age, HDL cholesterol levels and total cholesterol : HDL cholesterol ratio were no longer different after matching non-diabetic and type 2 diabetic women for similar triglyceride levels and waist girth. However, the LDL peak particle size and the proportion of small and large LDL particles remained significantly different between the two groups after the matching procedure ($p<0.01$; Fig. 1). Similar results were obtained regarding differences in the concentration of LDL cholesterol in small (<255 Å) and large (>260 Å) particles between the two groups of women (data not shown).

Discussion

The presence of small LDL particles is increasingly recognised as a new and potentially important marker of CHD risk [17]. The LDL particle size may be influenced by many factors, which include type 2 diabetes [4]. In the present study, we found that LDL peak particle size was smaller in type 2 diabetic women than in non-diabetic women. These results are concordant with those of other studies which reported that the prevalence of the small LDL phenotype was increased in type 2 diabetic women [5]. We also found that type 2 diabetic women were characterised by an increased proportion of small LDL particles (<255 Å) and by a reduced proportion of large (>260 Å) LDL particles compared with non-diabetic women in the absence of significant differences in LDL cholesterol levels. To the best of our knowledge, this is the first study to investigate the impact of type 2 diabetes on these new markers of LDL heterogeneity which we found to be more discriminant markers of ischaemic heart disease in men than the commonly used LDL peak particle diameter [6, 7]. Thus, LDL cholesterol is not a reliable index of electrophoretic characteristics of LDL particles which could represent important features of the cardiovascular risk profile in type 2 diabetic patients.

The question of whether type 2 diabetes is an independent correlate of LDL particle size and of the proportion of small and large LDL particles remains an unresolved issue. Although in healthy subjects the role of dyslipidaemia (especially hypertriglyceridaemia) as the major determinant of LDL size is well recognised, in type 2 diabetic subjects this association is less clear [4, 18, 19]. In the present study, after matching non-diabetic and type 2 diabetic women for similar triglyceride levels and abdominal obesity (estimated by waist girth), type 2 diabetes appeared to have an independent association with LDL particle size and with the proportion of small and large LDL particles. Type 2 diabetes has also been associated with an increased prevalence of the small LDL phenotype, even in the absence of frank hyperlipidaemia in men [18]. This previous study suggested that additional features of type 2 diabetes may directly contribute to the formation of small LDL particles, although one of its limitations was the lack of control for the possible influence of body fat distribution [18]. However, it had been suggested that type 2 diabetes had an independent effect on LDL peak particle size in women (but not in men) beyond body fat distribution (assessed by the WHR) and plasma triglyceride and HDL cholesterol levels [4]. The present study extends this notion to the proportion of small (increased) and large (decreased) LDL particles in type 2 diabetic women. These findings could partly explain the accelerated atherosclerosis progression that occurs in some women with type 2 diabetes.

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