Original Article

Glycemic control and high-density lipoprotein characteristics in adolescents with type 1 diabetes

Background: Recent evidence suggests that high-density lipoprotein (HDL) physicochemical characteristics and functional capacity may be more important that HDL-C levels in predicting coronary heart disease. There is little data regarding HDL subclasses distribution in youth with type 1 diabetes.
Objective: To assess the relationships between glycemic control and HDL subclasses distribution, composition, and function in adolescents with type 1 diabetes.
Methods: This cross-sectional study included 52 adolescents with type 1 diabetes aged 12–16 years and 43 age-matched non-diabetic controls. Patients were divided into two groups: one in fair control [hemoglobin A1c (HbA1c) < 9.6%] and the second group with poor glycemic control (HbA1c ≥ 9.6%). In all participants, we determined HDL subclasses distribution, composition, and the ability of plasma and of isolated HDL to promote cellular cholesterol efflux. Levels of soluble adhesion molecules were also measured.
Results: Although both groups of patients and the control group had similar HDL-C levels, linear regression analyses showed that compared with non-diabetic subjects, the poor control group had a lower proportion of HDL2b subclass (p = 0.029), triglyceride enriched (p = 0.045), and cholesteryl ester depleted (p = 0.028) HDL particles. Despite these HDL changes, cholesterol efflux was comparable among the three groups. The poor control group also had significantly higher intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 plasma concentrations.
Conclusions: In adolescents with type 1 diabetes, poor glycemic control is associated with abnormalities in HDL subclasses distribution and HDL lipid composition, however, in spite of these HDL changes, the ability of HDL to promote cholesterol efflux remains comparable to that of healthy subjects.

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Cardiovascular disease has been recognized as the major cause of death in patients with type 1 diabetes (1). An important part of the cardiovascular risk in patients with type 1 diabetes may be mediated by atherogenic lipid abnormalities (2). Compared to non-diabetic subjects, children with type 1 diabetes have higher levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (apoB), and higher proportions of small, dense LDL particles (3). These lipid disorders are highly dependent on glycemic control (4, 5).

Although epidemiological studies have shown that low plasma levels of high-density lipoprotein cholesterol (HDL-C) is an independent risk factor for coronary heart disease (CHD) (6), recent evidence suggests that HDL physicochemical characteristics (7) and functional capacity (8) may be more important than HDL-C levels in predicting CHD. HDL is a heterogeneous group of large (HDL2b and HDL2a) and small (HDL3a, HDL3b, and HDL3c) lipoprotein particles (9) that differ in size, lipid composition, density, and function (10). The HDL protection against atherosclerosis may be mediated, among many other actions (11), by its ability to inhibit the expression of intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1), that reduces the recruitment of blood monocytes into the artery wall (12), and also by its central role in reverse cholesterol transport, a process by which cholesterol from peripheral tissues is returned to the liver for excretion or recycling (13).

Increased levels of large HDL, reduced levels of small HDL, and a higher mean particle size have been reported in adults with type 1 diabetes. However, in those with poor glycemic control higher levels of small HDL, lower levels of large HDL, and a reduced HDL size were observed (14). This latter pattern in HDL subclasses distribution has also been reported in adults with type 1 diabetes who subsequently developed CHD (15). Recently, Gallo et al. (16) showed that youths with type 1 diabetes have increased large HDL particles concentration and higher mean HDL particle size compared to non-diabetic subjects. However, HDL function and the effect of glycemic control on HDL related characteristics were not assessed in that study. Accordingly, the aim of this study was to investigate the HDL subclasses distribution, HDL composition, and HDL capacity to promote cellular cholesterol efflux in adolescents with type 1 diabetes with fair and poor glycemic control. Plasma levels of soluble adhesion molecules ICAM-1 and VCAM-1 were also measured.

Methods

This cross-sectional study included patients with type 1 diabetes aged 12–16 years, attending the outpatient diabetic clinic at the Hospital Infantil de Mexico Federico Gomez (Mexico City, Mexico). All patients were diagnosed according to de American Diabetes Association criteria (17), and were on multiple dose insulin regimens. Exclusion criteria were evidence of thyroid dysfunction, hepatic, renal or liver disease, chronic or acute infections, micro or macrovascular complications, history of smoking, and use of vitamin supplements. The healthy control subjects were matched for age, sex, and body mass index (BMI) with type 1 diabetes patients and were selected from a public junior high school of Mexico City. All control subjects included were normoglycemic, had no clinical evidence of diabetes, thyroid, renal, or liver disease, and were not receiving vitamin supplementation or a specific diet at the time of the study. No patients or controls were receiving drug therapy to modify lipid metabolism. Parental informed written consent and child assent were obtained from all participants. The protocol was approved by the Institutional Review Board of the Hospital Infantil de Mexico Federico Gomez (HIM-2009-019). Sample size was calculated to detect differences in size of HDL subclasses based on a previous study by Gallo et al. (16), with 80% power and a 5% significance level. The required sample size was 24 subjects in each group. This number of patients was also suitable to detect a difference in cholesterol efflux.

Measurements

In all participants a questionnaire was applied and weight (kg), height (m), waist circumference (cm), and blood pressure (BP) were measured. BP was measured using the auscultatory method. Systolic and diastolic blood pressure (SBP and DBP) were assessed after subjects had been seated for at least 10 min, using an appropriate cuff size for each participant. Three readings were recorded for each individual and the average of the second and third measurements was used for analyses. Waist circumference was measured with a non-stretching fiberglass tape to the nearest 0.5 cm at the midpoint between the bottom of the rib cage and above the top of the iliac crest after a normal exhalation. BMI was calculated by dividing weight by height squared (kg/m^2). Pubertal stage was assessed by physical examination performed by a trained pediatric endocrinologist using Tanner classification (18).

Venous blood samples were obtained after 10 h overnight fast. Ethylenediaminetetraacetic acid plasma was prepared by centrifugation at 4°C at 2500 rpm for 20 min, and used for glucose, lipids, and lipoprotein measurements or stored frozen at −70°C until their analysis. Plasma glucose, TC, TG, and HDL-C were measured using standard enzymatic procedures in a Hitachi 902 analyzer (Hitachi, LTD, Tokyo, Japan). In the group with type 1 diabetes, hemoglobin A1c

(HbA1c) levels were also measured at the same time. Accuracy and precision of lipid measurements in our laboratory are under periodic surveillance by the Centers for Disease Control and Prevention service (Atlanta, GA, USA). LDL-C was estimated by using the Friedewald formula as modified by DeLong et al. (19). ApoB and apoAI, were determined by immunonephelometry on a BN Pro Spec nephelometer (Dade Behring Marburg GmbH, Schwalbach, Germany) according to the manufacturer method. Interassay coefficients of variation were less than 6%.

For HDL subclasses, total HDL were isolated from plasma of each adolescent by sequential ultracentrifugation in a density of 1.21 g/mL at 4°C in a Beckman TL-100 ultracentrifuge. The resulting total HDL were dialyzed against phosphate buffer (pH 7.4) and loaded into a native 4–25% polyacrylamide gel electrophoresis (PAGE) gels. Gels were stained for proteins with Coomasie brilliant blue R-250, scanned and digitalized in a GS-670 Bio-Rad densitometer, and the software MOLECULAR ANALYSTTM was used for the analysis. Migration distance intervals of each gel were calculated by computing a standard curve of protein stainable high molecular weight (HMW) standards (thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm; and albumin, 7.1 nm) as a function of their relative migration distance. The relative proportion of each HDL subclass was estimated with the following size intervals: HDL3c 7.21–7.76 nm; HDL3b 7.76–8.17 nm; HDL3a 8.17–8.77 nm; HDL2a 8.77–9.71 nm; and HDL2b 9.71–12.93 nm (20, 21). The coefficient of variation for each subclass was less than 10%. The average HDL particle size represents the overall distribution of the HDL subclasses, and was calculated as the average size of each HDL subclass interval (nm), multiplied by its relative area under the densitometric scan. Coefficient of variation for this determination was less than 1%.

Total protein, TC, free cholesterol (FC), phospholipid (PL) and tryglyceride (TG) contents of isolated HDL were determined using commercially available enzymatic assays in a Hitachi 902 analyzer. Cholesteryl esters (CE) were calculated by multiplying the difference between TC and FC by 1.67 (22). Total lipoprotein mass was calculated as the sum of total protein, CE, FC, PL, and TG. Apolipoprotein content (AI, AIV, and E) was evaluated semiquantitatively by sodium dodecyl sulfate PAGE (22, 23). Samples were frozen for approximately 6–8 months. The effects of long-term storage on HDL subclasses have been investigated, and no significant changes in the values obtained after the measurement of the same samples fresh and from short-term and long-term storage were observed. This implies that the chemical composition has not been altered (24).

The serum concentrations of adhesion molecules ICAM-1 and VCAM-1 were determined by enzyme-linked immunosorbent assay (Biosource, San Jose, CA, USA). Coefficient of variation for this determination was 5.7%.

Cellular cholesterol efflux was determined using Fu5AH rat hepatoma cells following the procedure described by de la Llera Moya et al. with slight modifications (25). Briefly, Fu5AH cells were maintained in minimal essential medium (MEM) containing 5% of bovine serum, 250 000 cells/well were placed on 24-well plates and 24 h after plating radiolabeled cholesterol (1,2,6,7-^{3}H cholesterol; American Radiolabeled Chemicals, INC. St Louis, MO) was added (0.5 μCi/well). Cells were then washed and incubated for 18 h in MEM with 0.5% of bovine serum albumin. Finally, cells were washed and incubated at 37°C for 4 h with 2.0% diluted serum, or isolated total HDL fractions (20 μg HDL-protein/mL or 100 μg HDL-PL/mL, as indicated), prepared by sequential ultracentrifugation, as previously described. Radioactivity was measured in medium and cells, and the percentage of cholesterol efflux calculated. All determinations were made in triplicate. As an internal control, a total serum and isolated total HDL were included in each plate, obtaining CVs of 3.3 and 5.3%, respectively.

Data analysis

Data are expressed as mean ± SD or median (maximum and minimum value) if the data were skewedly distributed. Statistical analyses were performed using analysis of variance or Kruskall Wallis test for comparison. Multiple regression analysis was performed to determine the independence of the association between glycemic control and HDL subclasses distribution, composition, and functionality. All analyses were conducted using the STATA V. 11. All p-values ≤0.05 were considered statistically significant.

Results

To assess the effect of glycemic control in HDL subclasses distribution, composition, and function, patients with type 1 diabetes were divided into two groups using the median value of HbA1c (9.6%) at the time of study. Group 1 with fair glycemic control (HbA1c < 9.6%) included 25 patients, and group 2 with poor glycemic control (HbA1c ≥ 9.6%) was formed with 27 patients. Clinical, biochemical, and anthropometric characteristics of patients with type 1 diabetes according to glycemic control and non-diabetic subjects are shown in Table 1. There were no differences among the groups with respect to age,

Table 1. Clinical, biochemical, and anthropometric characteristics of non-diabetic adolescents and patients with type 1 diabetes according to glycemic control

| | | Type 1 diabetes | | |
	Non-diabetic (n = 43)	Group 1 Fair glycemic control (n = 25)	Group 2 Poor glycemic control (n = 27)	p-Value
Age (yr)	12.88 ± 1.25	13.02 ± 1.69	13.34 ± 1.65	0.471
Sex (M/F)	17/26	8/17	12/15	0.652
Weight (kg)	47.43 ± 8.44	44.30 ± 8.73	46.33 ± 8.99	0.360
Height (m)	1.54 ± 0.09	1.50 ± 0.08	1.53 ± 0.11	0.213
BMI (kg/m^2)	19.68 ± 2.32	19.50 ± 2.53	18.80 ± 4.41	0.485
WC (cm)	66.33 ± 6.92	68.00 ± 6.36	69.49 ± 6.43	0.179
Tanner stage (II−III/IV−V)	11/32	17/8*	15/12	0.015
Disease duration (yr)	N/A	1.4 (0.5−10)*	5.0 (0.58−13)	0.012
Insulin dose (UI/kg/day)	N/A	0.72 ± 0.33*	1.02 ± 0.25	0.002
SBP (mmHg)	100.05 ± 8.12	86.61 ± 9.82*†	94.88 ± 12.63	<0.001
DBP (mm/Hg)	62.15 ± 6.92	56.68 ± 7.39*†	63.30 ± 10.24	0.013
Glucose (mg/dL)	85.0 (70.0−99.0)	178.4 (69.0−414.0)	243.0 (52.0−506.0)	0.001
HbA1c%	N/M	7.32 ± 1.17	11.55 ± 2.16	<0.001
Total cholesterol (mg/dL)	144.76 ± 23.73	144.94 ± 33.31	153.90 ± 36.72	0.436
Triglycerides (mg/dL)	88.13 ± 22.19	62.63 ± 15.63*†	85.45 ± 42.42	0.001
LDL-C (mg/dL)	78.22 ± 18.98	74.86 ± 27.05	84.63 ± 31.23	0.375
HDL-C (mg/dL)	52.41 ± 11.33	55.01 ± 12.05	55.72 ± 17.5	0.563
Non-HDL-C (mg/dL)	92.34 ± 19.64	89.92 ± 32.68	98.18 ± 35.23	0.557
ApoAI (mg/dL)	123.35 ± 20.22	119.95 ± 16.74	122.91 ± 24.31	0.815
ApoB (mg/dL)	47.64 ± 12.15	47.00 ± 15.46*	60.25 ± 21.24†	0.008
ApoB/apoAI ratio	0.39 ± 0.11	0.39 ± 0.12*	0.50 ± 0.19†	0.011

Apo, apolipoprotein; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N/A, not applicable; N/M, not measured; SBP, systolic blood pressure; WC, waist circumference.
Data are expressed as mean ± SD, median (minimum and maximum value), and frequencies.
*p ≤ 0.05 vs. group 2.
†p ≤ 0.05 vs. non-diabetic.

sex, BMI, and WC. Tanner stage was more advanced in non-diabetic controls compared to adolescents with type 1 diabetes. Disease duration was longer and insulin dose was higher in type 1 diabetes group 2 compared to group 1. Type 1 diabetes group 1 had significantly lower levels of SBP, DBP, and TG than those of group 2 and non-diabetic adolescents. The highest values of apoB and of the apoB/apoAI ratio were observed in the poor glycemic control group.

Although HDL-C levels were comparable in the three groups (Table 1), the unadjusted analysis (Table 2) showed that patients with fair glycemic control were characterized by the highest proportions of large (HDL2b and HDL2a) and lowest proportions of small HDL subpopulations (HDL 3b and HDL3c) and, as a result of the shift toward large HDL particles, by the highest mean HDL particle size, with the differences being significant particularly when compared with the poor glycemic control group. HDL subclasses distribution was similar in group 2 patients and non-diabetic adolescents. Except for a significantly higher proportion of TG in HDL from group 2 compared with that in HDL from group 1, the unadjusted analysis showed no differences in percent content of

HDL lipids (FC, PL, CE, and TC) or apolipoproteins (AI, AII, AIV, and E) among the three groups.

In spite of differences in HDL subclasses distribution and HDL triglyceride content between patients with fair and poor glycemic control, no differences in the ability of HDL to promote cholesterol efflux from Fu5AH cells to total serum or to isolated HDL, adjusted either for protein content or for PL content, were observed among the groups. Compared with non-diabetic subjects and patients from group 1, patients from group 2 had increased plasma levels of soluble adhesion molecules ICAM-1 (p < 0.001) and VCAM-1 (p = 0.05) (Table 2).

To better assess the effect of glycemic control on the HDL subclasses distribution and composition, cholesterol efflux capacity, and adhesion molecules levels, we performed a multiple linear regression analyses in which non-diabetic adolescents were considered as a reference. Poor glycemic control in adolescents with type 1 diabetes was associated with a lower proportion of HDL2b subclass (p = 0.029), TG enriched (p = 0.045) and CE depleted (p = 0.028) HDL particles (Table 3), and to increased ICAM-1 levels (p = 0.031) (data no shown). These associations were independent of age, sex, Tanner stage, diabetes

Table 2. HDL subclasses distribution, HDL chemical composition, and functionality in non-diabetic subjects and adolescents with type 1 diabetes according glycemic control

| | | Type 1 diabetes | | |
| | | Group 1 | Group 2 | |
	Non-diabetic (n = 43)	Fair glycemic control (n = 25)	Poor glycemic control (n = 27)	p-Value
HDL subclasses distribution				
HDL2b (%)	13.45 ± 3.41	16.57 ± 3.21*,†	13.28 ± 3.29	0.001
HDL2a (%)	22.70 ± 2.55	24.32 ± 1.59†	22.59 ± 3.00	0.025
HDL3a (%)	27.70 ± 2.31	27.32 ± 2.56	27.52 ± 2.14	0.818
HDL3b (%)	22.82 ± 2.71	20.58 ± 1.95*,†	22.77 ± 2.94	0.003
HDL3c (%)	13.30 ± 2.82	11.15 ± 3.21†	13.81 ± 3.71	0.011
HDL size (nm)	8.82 ± 0.13	8.95 ± 0.12*,†	8.81 ± 0.15	<0.001
HDL composition				
Total protein (%)	46.75 ± 3.62	47.09 ± 5.68	47.76 ± 6.21	0.939
Phospholipids (%)	26.69 ± 2.00	26.94 ± 2.88	26.60 ± 3.48	0.887
Triglycerides (%)	2.93 ± 0.66	2.57 ± 0.67†	3.22 ± 1.08	0.018
Cholesteryl esters (%)	20.23 ± 1.92	19.73 ± 2.54	19.29 ± 2.79	0.435
Free cholesterol (%)	3.37 ± 0.45	3.65 ± 0.49	3.41 ± 0.70	0.072
HDL apolipoproteins				
ApoAI (%)	46.11 ± 7.15	47.73 ± 6.22	46.07 ± 4.51	0.575
ApoAII (%)	24.07 ± 4.40	23.41 ± 4.78	24.40 ± 4.61	0.662
ApoAIV (%)	6.55 ± 3.09	6.31 ± 2.57	5.85 ± 2.19	0.655
ApoE (%)	5.72 ± 1.60	6.18 ± 2.25	6.28 ± 2.55	0.678
Cholesterol efflux				
To total serum (%)	13.10 ± 3.45	14.12 ± 2.90	12.22 ± 3.39	0.286
To isolated HDL (%)‡	7.02 ± 2.60	6.55 ± 1.86	6.33 ± 1.77	0.499
To isolated HDL (%)§	6.61 ± 3.51	5.40 ± 1.93	5.33 ± 1.82	0.144
Adhesion molecules				
ICAM-1 (ng/mL)	288.09 ± 75.02	349.50 ± 98.33†	444.58 ± 141.36*	<0.001
VCAM-1 (ng/mL)	1048.96 ± 304.89	972.66 ± 346.76†	1218.25 ± 380.51	0.050

Apo, apolipoprotein; HDL, high-density lipoprotein; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular adhesion molecule-1.
Data are expressed as mean ± SD.
*$p \leq 0.05$ vs. non-diabetic.
†$p \leq 0.05$ vs. group 2.
‡Adjusted for protein content.
§Adjusted for phospholipid content.

duration, and insulin dose. No associations between glycemic control and the levels of VCAM-1 or the HDL capacity to induce cholesterol efflux were observed.

Discussion

This study showed that compared to adolescents with type 1 diabetes with fair glycemic control, patients with poor glycemic control had higher levels of SBP, DBP, and TG. However, this metabolic profile of adolescents with poorly controlled diabetes was not different from that of normoglycemic controls. In multivariate regression analyses, poor glycemic control was associated with lower levels of large HDL 2b, and abnormal HDL composition characterized by TG enrichment and CE depletion compared to normoglycemic controls. In spite of these HDL changes, cholesterol efflux was similar in normoglycemic controls and in the two groups of adolescents with type 1 diabetes. One additional finding was that adhesion molecule ICAM-1 was higher in patients with poor control type 1 diabetes.

As in our adolescent patients with fair glycemic control, increased level of large HDL, and higher HDL particle size have been previously reported in adults (14) and children (16) with type 1 diabetes, compared with non-diabetic subjects. The shift to larger HDL subclasses in these patients may be explained by the higher PL transfer protein activity in patients with diabetes (14), or by a slow catabolism of HDL in these patients (26). Our results in diabetic adolescents of group 2 also confirm previous reports on adult patients showing that poor glycemic control is significantly associated with higher levels of small HDL, reduced levels of large HDL, and lower HDL size (15, 27). Thus poor glycemic control is associated to abnormalities in HDL subclasses in both adults and children.

Despite an extensive search, we were unable to find studies on HDL composition in pediatric populations with type 1 diabetes. Studies in type 1 diabetes adult patients with poor glycemic control showed decreased esterified cholesterol to triglyceride ratio in HDL, indicating lower content of CE and higher content of TG in HDL particles (28, 29). Our findings of higher

Table 3. Effect of glycemic control on HDL subclasses distribution and composition in adolescents with type 1 diabetes

		Group 1 Fair glycemic control			Group 2 Poor glycemic control			
	Non-diabetic	Coefficient	95% CI	p-Value	Coefficient	95% CI	p-Value	**R²**
HDL subclasses								
HDL2b (%)	Referent	0.82	−1.49, 3.14	0.482	−3.40	−6.44, −0.36	0.029	**0.41**
HDL2a (%)	Referent	0.27	−1.49, 2.03	0.761	−1.97	−4.28, 0.33	0.092	**0.26**
HDL3a (%)	Referent	1.00	−0.68, 2.69	0.238	2.10	−0.10, 4.31	0.061	**0.11**
HDL3b (%)	Referent	−0.90	−2.68, 0.87	0.317	1.90	−0.42, 4.23	0.107	**0.39**
HDL3c (%)	Referent	−1.23	−3.49, 1.03	0.281	1.35	−1.60, 4.31	0.364	**0.15**
HDL size (nm)	Referent	0.16	0.05, 0.27	0.003	0.02	−0.08, 0.12	0.698	**0.40**
HDL composition								
Total protein (%)	Referent	1.00	−2.54, 4.55	0.574	2.52	−2.57, 7.62	0.328	**0.03**
Phospholipids (%)	Referent	−0.10	−0.95, 0.74	0.804	−0.36	−1.60, 0.88	0.561	**0.04**
Triglycerides (%)	Referent	−0.05	−0.59, 0.49	0.858	0.80	0.01, 0.58	0.045	**0.20**
Cholesteryl esters (%)	Referent	−1.31	−2.98, 0.34	0.121	−2.69	−5.09, −0.30	0.028	**0.20**
Free cholesterol (%)	Referent	0.18	−0.19, 0.55	0.342	−0.17	−0.71, 0.36	0.522	**0.13**

HDL, high-density lipoprotein.
Values were estimated from linear regression analyses adjusted for age, sex, Tanner stage, disease duration and insulin dose.
Regression coefficients are change in outcome variable (HDL subclasses or composition) in each group with type 1 diabetes compared to non-diabetic subjects.

TG proportion and less CE in HDL from adolescents with type 1 diabetes and poor glycemic control are consistent with the results in the those studies. This abnormal HDL composition may be attributed to an increased CE transfer from HDL to apoB-containing lipoproteins in patients with type 1 diabetes (29). However, the biological significance of these findings in terms of cardiovascular risk is uncertain, because in our study the changes in HDL subclasses distribution and lipid composition did not affect HDL function as assessed by their ability to efflux cholesterol.

Dysfunctional endothelial cells produce increased amounts of cellular adhesion molecules that mediate attachment and transmigration of leukocytes across the endothelial surface and represent an early stage in atherogenesis (30). Consistent with previous studies showing increased levels of adhesion molecules in adolescents with type 1 diabetes that were positively related to HbA1c (31–33), our data show that ICAM-1 and VCAM-1 concentrations are associated with hyperglycemia, which has been shown to increase formation of advanced glycation end products that impair endothelial function (34).

There are no previous studies on cholesterol efflux in children and adolescents with type 1 diabetes. In adult patients with type 1 diabetes and moderate hypercholesterolemia, de Vries et al. (35) reported increased cholesterol efflux out of Fu5AH rat hepatoma cells compared with healthy subjects. In that study no correlation was found between HbA1c and cholesterol efflux. In our adolescents with type 1 diabetes, the results show that cholesterol efflux to whole serum or to isolated total HDL using the same cellular system, were similar in patients with fair and poor glycemic control and in non-diabetic adolescents. A possible explanation for the differences between the two studies is the composition of HDL particles. It has been shown that the ability of plasma to promote cholesterol efflux mediated by the SR-BI in Fu5AH cells is largely dependent on mature PL rich HDL particles (36). Compared to controls, adults with type 1 diabetes had significantly higher HDL PLs (35), whereas in our adolescents no difference was observed in PL content of HDL between patients with diabetes and controls. Together, our results and those reported in adults (35) suggest that the higher CHD risk in patients with type 1 diabetes is not mediated by abnormalities in cholesterol efflux.

Our study has some potential limitations. First, its cross-sectional nature does not allow us to establish a causal relationship between poor glycemic control and HDL abnormalities. Second, HbA1c was not measured in non-diabetic controls. However, their glucose levels were normal and they had no symptoms of diabetes before or at the time of the study. Also, our median value of HbA1c was high (9.6%) and the use of this cutoff point to divide patients into fair and poor glycemic control groups is arbitrary. However, taking into account that glycemic control is particularly difficult in adolescents and that some previous studies have used a similar HbA1c value to separate patients in good and poor glycemic control, we feel that the use of this cutoffs in acceptable.

In conclusion, in adolescents with type 1 diabetes, poor glycemic control is associated to increased levels of adhesion molecules and to abnormalities in HDL subclasses distribution and HDL lipid composition, but HDL function as assessed by cholesterol efflux is normal.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

References

1. MORRISH NJ, WANG SL, STEVENS LK, FULLER JH, KEEN H. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. Diabetiologia 2001: 44: S14–S21.
2. SOEDAMAH-MUTHU SS, CHATURVEDI N, TOELLER M et al. Risk factors for coronary heart disease in type 1 diabetic patients in Europe. Diabetes Care 2004: 27: 530–537.
3. GUY J, OGDEN L, WADWA P et al. Lipid and lipoprotein profiles in youth with and without type 1 diabetes. Diabetes Care 2009: 32: 416–420.
4. PETITTI DB, IMPERATORE G, PALLA SL et al. Serum lipids and glucose control: The SEARCH for Diabetes in Youth Study. Arch Pediatr Adolesc Med 2007: 161: 159–165.
5. NORDWALL M, AENQVIST H, BOJESTING M, LUDVIGSSON J. Good glycemic control remains crucial in prevention of late diabetic complications-the Linköping Diabetes Complications Study. Pediatr Diabetes 2009: 10: 168–176.
6. BODEN WE. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the veterans affairs high-density lipoprotein intervention trial. Am J Cardiol 2000: 86: 19–22.
7. ASZTALOS BF, COLLINS D, CUPPLES LA et al. Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent cardiovascular events in the veterans affairs HDL intervention trial. Arterioscler Thromb Vasc Biol 2005: 25: 2185–2191.
8. DE LA LLERA-MOYA M, DRAZUL-SCHRADER D, ASZTALOS BF, CUCHEL M, RADER DJ, ROTHBLAT GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. Arterioscler ThrombVac Biol 2010: 30: 796–801.
9. WILLIAMS PT, KRAUSS RM, NICHOLS AV, VRANIZAN KM, WOOD PD. Identifying the predominant peak diameter of high-density and low-density lipoproteins by electrophoresis. J Lipid Res 1990: 31: 1131–1139.
10. CHAHIL TJ, GINSBERG HN. Diabetic dyslipidemia. Endocrinol Metab Clin North Am 2006: 35: 491–510.
11. VERGEER M, HOLLEBOOM AG, KASTELEIN JJ, KUIVENHOVEN JA. The HDL hypothesis: does high-density lipoprotein protect from atherosclerosis? J Lipid Res 2010: 51: 2058–2073.
12. BARTER PJ, NICHOLLS S, RYE KA, ANANTHARAMAIAH GM, NAVAB M, FOGELMAN AM. Antiinflammatory properties of HDL. Circ Res 2004: 95: 764–772.
13. YANCEY PG, BORTNICK AE, KELLNER-WEIBEL G, DE LA LLERA-MOYA M, PHILLIPS MC, ROTHBLAT GH. Importance of different pathways of cellular cholesterol efflux. Arterioscler Thromb Vasc Biol 2003: 23: 712–719.
14. COLHOUN HM, OTVOS JD, RUBENS MB, TASKINEN MR, UNDERWOOD SR, FULLER JH. Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. Diabetes 2002: 51: 1949–1956.
15. SOEDAMAH-MUTHU SS, CHANG YF, OTVOS J, EVANS RW, ORCHARD TJ, Pittsburgh Epidemiology of Diabetes Complications Study. Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease in type 1 diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia 2003: 46: 674–682.
16. GALLO LM, SILVERSTEIN JH, SHUSTER JJ, HALLER MJ. Arterial stiffness, lipoprotein particle size, and lipoprotein particle concentration in children with type 1 diabetes. J Pediatr Endocrinol Metab 2010: 23: 661–667.
17. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012: 35: S64–S71.
18. MARSHALL WA, TANNER JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969: 44: 291–303.
19. DELONG DM, DELONG ER, WOOD PD, LIPPEL K, RIFKIND BM. A comparison of methods for the estimation of plasma low- and very low-density lipoprotein cholesterol. The Lipid Research Clinics Prevalence Study. JAMA 1986: 256: 2372–2377.
20. PÉRUSSE M, PASCOT A, DESPRÉS JP, COUILLARD C, LAMARCHE B. A new method for HDL particle sizing by polyacrylamide gradient gel electrophoresis using whole plasma. J Lipid Res 2001: 42: 1331–1334.
21. BLANCHE PJ, GONG EL, FORTE TM, NICHOLS AV. Characterization of human high-density lipoproteins by gradient gel electrophoresis. Biochim Biophys Acta 1981: 665: 408–419.
22. CHAPMAN MJ, GOLDSTEIN S, LAGRANGE D, LAPLAUD PM. A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. J Lipid Res 1981: 22: 339–358.
23. GUERIN M, LASSEL TS, LE GOFF W, FARNIER M, CHAPMAN MJ. Action of atorvastatin in combined hyperlipidemia :preferential reduction of cholesteryl ester transfer from HDL to VLDL1 particles. Arterioscler Thromb Vasc Biol 2000: 20: 189–197.
24. ASZTALOS BF, ROHEIM PS, MILANI RL et al. Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 2000: 20: 2670–2676.
25. DE LA LLERA MOYA M, ATGER V, PAUL JL et al. A cell culture system for screening human serum for ability to promote cellular cholesterol efflux. Relations between serum components and efflux, esterification, and transfer. Arterioscler Thromb Vasc Biol 1994: 14: 1056–1065.

26. VALABHJI J, MCCOLL AJ, SCHACHTER M, DHANJIL S, RICHMOND W, ELKELES RS. High-density lipoprotein composition and paraoxonase activity in type 1 diabetes. Clin Sci 2001: 101: 659–670.
27. JENKINS AJ, LYONS TJ, ZHENG D et al. Serum lipoproteins in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Cohort. Diabetes Care 2003: 26: 810–818.
28. BAGDADE JD, BUCHANAN WE, LEVY RA, SUBBAIAH PV, RITTER MC. Effects of omega-3 fish oils on plasma lipids, lipoprotein composition, and postheparin lipoprotein lipase in women with IDDM. Diabetes 1990: 39: 426–431.
29. BAGDADE JD, RITTER MC, SUBBAIAH PV. Accelerated cholesteryl ester transfer in patients with insulin-dependent diabetes mellitus. Eur J Clin Invest 1991: 21: 161–167.
30. LIBBY P, RIDKER PM, MASERI A. Inflammation and atherosclerosis. Circulation 2002: 105: 1135–1143.
31. SECKIN D, ILHAN N, ILHAN N, ERTUGRUL S. Glycaemic control, markers of endothelial cell activation and oxidative stress in children with type 1 diabetes mellitus. Diabetes Res Clin Pract 2006: 73: 191–197.

32. GLOWINSKA B, URBAN M, PECZYNSKA J, FLORYS B. Soluble adhesion molecules (sICAM-1, sVCAM-1) and selectins (sE selectin, sP selectin, sL selectin) levels in children and adolescents with obesity, hypertension, and diabetes. Metabolism 2005: 54: 1020–1026.
33. MAGGIO AB, FARPOUR-LAMBERT NJ, MONTECUCCO F et al. Elevated E-selectin and diastolic blood pressure in diabetic children. Eur J Clin Invest 2012: 42: 303–309.
34. HARTGE MM, UNGER T, KINTSCHER U. The endothelium and vascular inflammation in diabetes. Diab Vasc Dis Res 2007: 4: 84–88.
35. DE VRIES R, KERSTENS MN, SLUITER WJ, GROEN AK, VAN TOL A, DULLAART RP. Cellular cholesterol efflux to plasma from moderately hypercholesterolaemic type 1 diabetic patients is enhanced, and is unaffected by simvastatin treatment. Diabetologia 2005: 48: 1105–1113.
36. FOURNIER N, PAUL JL, ATGER V et al. HDL Phospholipid content and composition as a major factor determining cholesterol efflux capacity from Fu5AH cells to human serum. Arterioscler Thromb Vasc Biol 1997: 17: 2685–2691.