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Postprandial Chylomicron Clearance Rate in Late Teenagers with Diabetes Mellitus Type 1

WILLIE M. VAN WAARDE, ROEL J. ODINK, CATRIENUS ROUWÉ, FRANS STELLAARD, MARISKA WESTERS, ROEL J. VONK, PIETER J.J. SAUER, AND HENKJAN J. VERKADE

Department of Pediatrics, Beatrix Children's Hospital, University Hospital Groningen, 9700 RB Groningen, The Netherlands.

ABSTRACT

A delayed chylomicron (CM) clearance rate, a known risk factor for atherosclerosis, has been described in adults with diabetes type 1 (DM1). We determined the CM clearance rate in late teenagers with DM1, and the relationship between CM clearance rate and elevated plasma lipid concentrations in DM1 teenagers in poor metabolic control (as characterized by HbA_{1c} percentage). Plasma lipids and CM clearance were determined in nine patients with DM1 (mean age ± SD: 17.5 ± 0.6 y) and four healthy controls (mean age ± SD: 20.1 ± 0.8 y), by measuring breath $^{13}CO_2$, plasma triglyceride, retinyl palmitate, and ^{13}C-labeled oleic acid concentrations, after oral administration of a fat-rich meal together with vitamin A and ^{13}C-oleic acid. In patients with DM1, fasting triglyceride and cholesterol concentrations were positively correlated with HbA_{1c} percentage ($p < 0.05$). Neither in DM1 patients, nor in controls, was an elevated triglyceride concentration (above 1.7 mmol/L) found. Yet, in 22% of DM1 patients, cholesterol concentration was above 5.2 mmol/L, but not in any of the controls. CM clearance rate in DM1 patients was similar to that in controls and did not significantly correlate with HbA_{1c} percentage. Fasting lipid concentrations in DM1 patients were not significantly correlated with CM clearance rate. Present data indicate that elevated lipid concentrations in late teenagers with DM1 are not attributable to a delay in CM clearance rate. A delayed CM clearance rate at late teenager age is not a risk factor contributing to the increased risk for atherosclerosis in DM1. **(*Pediatr Res* 50: 611–617, 2001)**

Abbreviations

DM1, diabetes mellitus type 1
LDL, low density lipoprotein
VLDL, very low density lipoprotein
CM, chylomicron
HDL, high density lipoprotein
BW, body weight
HPLC, high pressure liquid chromatography
GC, gas chromatography
GC-C-IRMS, gas chromatography-combustion isotope ratio mass spectrometry

Diabetes mellitus type 1 (DM1) is associated with a 2–4 fold increased risk for cardiovascular disease (1). Specific diabetes-related phenomena have been suggested to play a role in the observed association, such as glycosylation of proteins (2), presence of renal disease (3), elevated concentrations of plasma cholesterol, triglycerides, and low density lipoprotein (LDL) cholesterol (4), and altered composition of very low density lipoprotein (VLDL) and LDL (5, 6). In children and late teenagers, studies on risk factors associated with DM1 and atherosclerosis have mainly focused on cholesterol and triglyceride concentrations (7–9). Elevated concentrations of both cholesterol and triglycerides have been reported in children with DM1 in poor metabolic control, which tended to decrease to nondiabetic values upon improvement of metabolic control (7–9).

A delayed chylomicron (CM) clearance has been identified as a risk factor for atherosclerosis in patients with coronary heart disease, in patients with type 2 diabetes, and in *adult* patients with DM1 (10–12). Insulin enhances the metabolism of CM by stimulating the hydrolysis of CM triglycerides by lipoprotein lipase and the hepatic uptake of CM remnants (11). A relative deficiency of insulin, such as present in DM1, could therefore lead to a delay in CM clearance. A delay in CM clearance would imply a prolonged postprandial hyperlipidemia, which supposedly leads to increased deposition of CM contents into the arterial wall and to an unfavorable translocation of cholesteryl-esters from HDL to CM (13, 14). Delays in CM clearance are amenable to dietary intervention, by changing the intake of fish oil (long chain PUFA) (15), monounsaturated-fat (16), or carbohydrate (17). Yet, it is not known whether CM clearance is delayed in *late teenagers* with DM1. In nondiabetic individuals CM clearance rate appeared to be inversely correlated with age (*i.e.* a delayed clearance in older

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Correspondence: WM van Waarde, Department of Pediatrics, Beatrix Children's Hospital, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands; e-mail: w.m.van.waarde@bkk.azg.nl
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individuals) (18, 19). Specific dietary recommendations to late teenagers with DM1 are only warranted if CM clearance would be delayed at their age. In the present study, we investigated, whether a delayed clearance rate of chylomicrons (CM) can be a risk factor for atherosclerosis and may contribute to higher lipid concentrations, especially triglycerides, in late teenagers with DM1. We also determined whether CM clearance is correlated with the level of metabolic control, as characterized by HbA_{1c}. CM clearance was investigated by determination of plasma concentrations of three CM constituents (triglycerides, vitamin A, and ^{13}C-oleic acid) after their prior oral ingestion.

METHODS

Subjects. Chylomicron clearance was studied in nine late teenagers with DM1 (four boys, five girls; mean age ± SD: 17.5 ± 0.6 y) and four healthy controls (two boys, two girls; mean age ± SD: 20.1 ± 0.8 y). Patients were studied at late teenager age to rule out the effect of puberty on lipid metabolism, as puberty is associated with a relative insulin resistance (20). DM1 patients were selected from our outpatient clinic population with fair or poor metabolic control (5 with HbA_{1c} <8.7%, 4 with HbA_{1c} >9.5%). Patients were selected on basis of their HbA_{1c} (<8.7%, >9.5%) and differed only in metabolic control. HbA_{1c} ± SD at the test day were 7.8 ± 0.5% and 10.9 ± 1.5%, respectively. Mean HbA_{1c} in the year preceding the test were 7.6 ± 0.4% and 11.2 ± 1.9%, respectively. Patients with microvascular complications like microalbuminuria (defined as albumin excretion rate >30 mg/24 h in 24 h urine collection), or retinopathy (ophthalmoscopy through dilated pupils by ophthalmologist) were excluded from the study. Other exclusion criteria were severe insulin resistance (insulin dosages above 1.5 U/kg/d), with diabetes associated diseases like hypothyroidism and celiac disease, renal or liver disease, obesity (weight for height >90th percentile), anorexia nervosa (weight for height <10th percentile), first grade relatives with lipid disturbances, or cardiovascular disease under the age of 60. Patients, who had had diabetes for less than one year were also excluded. No medication known to affect lipoprotein metabolism was used by the subjects, except for oral contraceptives (3 of DM1, 2 of controls) and insulin. In control subjects, the absence of microalbuminuria was tested. They had none of the above mentioned diseases. At palpation control subjects did not have a goiter, and they had no known first grade relatives with lipid disturbances or cardiovascular disease under the age of 60. Mean insulin dosage was not significantly different between patients with HbA_{1c} below 8.7% or above 9.5% (0.9 ± 0.2 U/kg/d in each group). Patients with DM1 did not have ketones in their urine nor any sign of illness during the three days preceding the test. Informed consent was obtained from all patients and controls over 18 y of age. Parental informed consent was obtained for minors. The study was approved by the medical ethical committee of our hospital.

Study design. In DM1 patients and controls, CM metabolism was investigated after an overnight fast. CM metabolism was investigated by analyzing plasma disappearance of 3 CM constituents. First, the disappearance rate of the main CM core component, triglycerides, was measured after ingestion of a high-fat meal. Second, a classic test to quantitate uptake of CM remnants by the liver, the vitamin A test, was applied (21, 22). After oral ingestion, vitamin A is taken up by enterocytes and incorporated into the core of CM in the form of retinyl esters. Retinyl esters remain associated with the CM in the circulation until the remnant stage, after which they are taken up by the liver (21). Third, hydrolysis of CM triglycerides by lipoprotein lipase was investigated by ingestion of a stable isotopically labeled fatty acid (^{13}C-oleic acid). Orally ingested ^{13}C labeled fatty acid will be incorporated in CM triglycerides. After appearance in plasma, ^{13}C-triglycerides will be hydrolyzed by lipoprotein lipase and the hydrolyzed ^{13}C-fatty acid will be taken up into body cells and metabolized. The metabolism of ^{13}C labeled CM fatty acids can be quantified by measuring the disappearance of ^{13}C labeled fatty acid from plasma and, indirectly, by determining the appearance of $^{13}CO_2$ in breath, reflecting oxidation of the parent compound. Subjects were instructed to avoid food naturally enriched in ^{13}C, such as corn, pine apple, corn flour, cane sugar, and millet for three days before the test.

After an overnight fast, a standardized high-fat meal (composition: 1 g fat/kg body weight (BW), 60% (wt/wt) saturated fat; 1 g carbohydrate/kg BW, 0.5 g protein/kg BW), together with vitamin A (50.000 IU/m^2) and [1- ^{13}C]-oleic acid (5 mg/kg BW) was ingested between 8:30 and 9:00 AM (time 0). The high-fat meal consisted of a milkshake and wheat bread with butter and cheese. For 6 h after ingestion of the test-meal, no other food was ingested. DM1 patients administered their insulin in an adjusted dosage 30 min before the test-meal. The dosage of short acting insulin was adjusted for the carbohydrate composition in the test-meal (compared with their normal carbohydrate intake at breakfast and at 10:30 AM) and the glucose value at 8.00 AM. The dosage of long acting insulin was lowered in view of the fasting period of 6 h after the test-meal. Before, and for 6 h after ingestion of the test-meal, every half hour breath samples were collected to quantitate breath $^{13}CO_2$, and every hour blood samples were collected to quantitate plasma concentrations of retinyl palmitate, triglycerides, cholesterol, ^{13}C-oleic acid, glucose, and FFA. In the first blood sample taken, HbA_{1c} concentration was determined.

Analytical methods. Glucose levels were measured on a calibrated Companion 2 glucometer (Medisense, Abbott laboratories, Abbott Park, IL). Triglyceride and cholesterol concentrations were determined in plasma by enzymatic methods using commercial kits (Triglycerides/GB and Chol respectively, Boehringer, Mannheim, Germany), as were FFA in plasma (NEFA C, Wako, Neuss, Germany). Plasma retinyl palmitate concentrations were determined by high-pressure liquid chromatography (HPLC), according to Bui and to Zaman *et al*. (23, 24). HbA_{1c} was determined by ion-exchange HPLC (VARIANT^{TM} HbA_{1c} Program with Bio-Rad VARIANT Hb Testing System, Bio-Rad, Hercules, CA). Normal range of HbA_{1c} in nondiabetic individuals in our hospital is 4.6–6.1%.

^{13}C-oleic acid content was measured in total plasma lipid. To determine ^{13}C-oleic acid, plasma lipids were methylated and extracted according to Lepage and Roy (25). Resulting fatty acid methyl esters were analyzed by gas chromatography

(GC) to quantitate oleic acid concentration (26) and by GC combustion isotope ratio mass spectrometry (GC-C-IRMS) to determine ^{13}C-enrichment of oleic acid. Fatty acids were quantified using heptadecanoic acid (17:0) as internal standard. ^{13}C-enrichment of the oleic acid methyl esters was determined by using a Finnigan MAT Delta S isotope ratio mass spectrometer interfaced to a Varian 3400 gas chromatograph via a capillary oxidation furnace (Finnigan MAT, Bremen, Germany). Separation of the methyl esters and determination of [^{13}C] abundance was performed according to the method described by us previously (27). The concentration of ^{13}C-oleic acid in plasma is expressed as the percentage of the dose administered per liter plasma (% Dose/L).

^{13}C-enrichment in the breath samples was determined by means of continuous flow isotope ratio mass spectrometry (Finnigan Breath MAT, Finnigan MAT Gmb, Bremen, Germany). The ^{13}C abundance of breath CO_2 was expressed as the difference (‰) compared with the reference standard Pee Dee Belemnite limestone ($\delta\ ^{13}C_{pdb}$,‰). The proportion of ^{13}C-label excreted in breath CO_2 was expressed as the percentage of administered ^{13}C-label recovered per hour (%^{13}C Dose/h), and was corrected for individual CO_2 production as determined every hour for 5 min by indirect calorimetry (Oxycon, model ox-4, Dräger, Breda, The Netherlands).

Statistical analysis. Results are expressed as means ± SD. Group means were compared by Student-*t* test, or, if SDs were significantly different, by Welch's alternate-*t* test. The postprandial clearance rates of retinyl palmitate and triglycerides were calculated in individual subjects by calculating the slope of the linear regression line, using the peak concentration and plasma concentrations up to 3 h after peak concentration as dependent variables and corresponding time points as independent variables in each individual. The slopes in DM1 and control late teenagers (means ± SD) were compared by Student-*t* test. The postprandial clearance of retinyl palmitate was also investigated by calculating the area under the retinyl palmitate curves of each individual and comparing the two group means and SDs by Student-*t* test.

RESULTS

Baseline characteristics of DM1 patients and controls. Table 1 shows the clinical data and fasting cholesterol and triglyceride concentrations in DM1 patients and controls. For triglyceride concentrations in children and teenagers, a cut-off concentration of 1.7 mmol/L is used (as defined by the European Non-Insulin-Dependent Diabetes Policy Group) (7). In none of the DM1 patients or controls were fasting triglyceride concentrations higher than 1.7 mmol/L. According to the National Cholesterol Education Program (NCEP), fasting cholesterol concentrations in healthy children and teenagers are classified as "borderline increased" when between 4.4 and 5.2 mmol/L and as "high" when exceeding 5.2 mmol/L (28). Fasting cholesterol concentrations were higher than 5.2 mmol/L in 22% (2 of 9) of the DM1 patients and none of the controls. Borderline increased cholesterol concentrations were found in 1 of 9 DM1 patients and 1 of 4 controls. DM1 patients and controls had similar body mass indices. As expected, HbA_{1c} percentage was significantly higher in patients with DM1 compared with controls ($p < 0.01$). The controls were slightly older than the patients ($p < 0.01$). In DM1 patients, fasting triglyceride and cholesterol concentrations were positively correlated to HbA_{1c} percentage ($p < 0.05$). Fasting plasma triglyceride concentration was correlated with HbA_{1c}, with the following equation: [fasting plasma triglyceride concentration] = 0.164[HbA_{1c}]-0.794 (r = 0.80, $p < 0.01$). Fasting plasma cholesterol concentration was correlated with HbA_{1c} with the following equation: [fasting plasma cholesterol concentration] = 0.45[HbA_{1c}]-0.147 (r = 0.76, $p < 0.05$).

Table 1. *Clinical data and fasting lipid concentrations in late teenagers with DM1 and controls*
Values represent means ± SD

	DM1 patients (n = 9)	Controls (n = 4)	Significance
Age (y)	17.6 ± 0.6	20.1 ± 0.8	$p < 0.01$
Body mass index (kg/m^2)	22.3 ± 2.0	22.1 ± 2.3	NS
Duration of diabetes (y)	8.8 ± 3.6		
Insulin dosage (IU/kg BW/day)	0.9 ± 0.2		
HbA_{1c} (%)	9.2 ± 1.9	5.1 ± 0.2	$p < 0.01$
Plasma cholesterol (mM)	4.0 ± 1.1	4.1 ± 0.5	NS
Plasma triglycerides (mM)	0.7 ± 0.4	0.6 ± 0.3	NS

NS = not significant.

Postprandial response: glucose, FFA, and triglycerides. Fasting and postprandial glucose concentrations were significantly higher in patients with diabetes compared with controls ($p < 0.01$, Fig. 1A). In patients with diabetes, maximal glucose concentrations were found at 2 h after ingestion of the high-fat meal. FFA concentrations were significantly higher in patients with diabetes compared with controls, before (time point 0) and at 1 and 2 h after administration of the test-meal ($p < 0.01$), but were similar thereafter (Fig. 1B).

Postprandial triglyceride concentrations showed maximal values at 2 h (DM1) or 3 h (controls) after ingestion of the test-meal (Fig. 2A). Fasting and postprandial triglyceride concentrations were not significantly different between the two groups at any time point. Also, the rate of decrease in triglyceride concentration, after reaching its maximum, was not significantly different between the two groups, as calculated from individual decay regression lines ($p > 0.05$; slope of regression line in DM1, and controls -0.32 ± 0.19, and -0.14 ± 0.08 mmol L^{-1} h^{-1}, respectively). If postprandial triglyceride concentrations were compared between DM1 patients with high and moderate HbA_{1c} (Fig. 2B), the patients with high HbA_{1c} (>9.5%) had significantly higher plasma triglyceride concentrations at time point 2 and 6 h compared with the patients with moderate HbA_{1c} (<8.7%)($p < 0.05$). Yet, these significant differences disappeared when the percentage increase in triglyceride concentration from time point 0 was calculated; indicating that higher postprandial triglyceride concentrations in DM1 patients with $HbA_{1c} > 9.5\%$ were due to higher fasting triglyceride concentrations.

Vitamin A test. Figure 3 shows that in diabetic and in control late teenagers, mean concentrations of retinyl palmitate were maximal at 2 h after the ingestion of the vitamin A-containing high-fat meal. Except for the 6-h time point, no significant differences were observed between the two groups in plasma

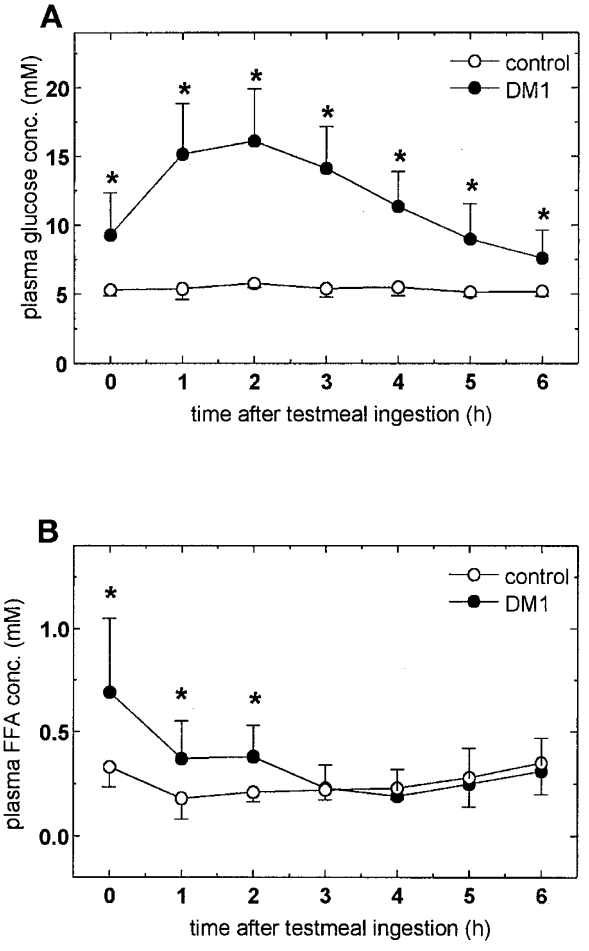

Figure 1. Fasting and postprandial plasma glucose concentrations (*A*) and FFA concentrations (*B*) in late teenagers with diabetes mellitus type 1 ($n = 9$, closed symbols), and nondiabetic controls ($n = 4$, open symbols), before and for 6 h after ingestion of a fat-rich meal at time point 0. Insulin dosage was adjusted for the carbohydrate composition of the test-meal, the glucose concentration at the beginning of the test, and the fasting period of 6 h after the test-meal. $*p < 0.05$, reflects a significant difference between the two groups.

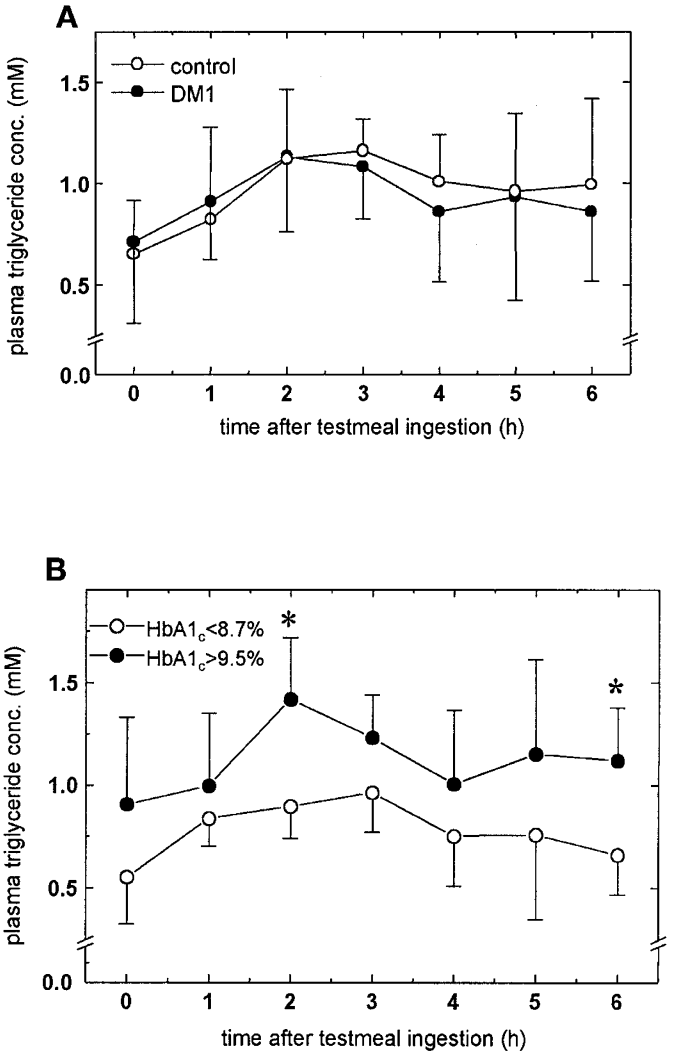

Figure 2. Fasting and postprandial plasma triglyceride concentrations in late teenagers with diabetes mellitus type 1 ($n = 9$, closed symbols), in nondiabetic controls ($n = 4$, open symbols) (*A*), and in DM1 patients with $HbA_{1c} > 9.5\%$ ($n = 4$, closed symbols) and $HbA_{1c} < 8.7\%$ ($n = 5$, open symbols) (*B*). A fat-rich meal was ingested at time point 0. Afterward no food was taken for 6 h. Results between the total diabetes and control group were not significantly different at any time point (*A*). In DM1 patients with $HbA_{1c} > 9.5\%$ plasma triglyceride concentrations were significantly higher at time point 2 and 6 h compared with DM1 patients with $HbA_{1c} < 8.7\%$ ($*p < 0.05$) (*B*).

concentrations of retinyl palmitate. The areas under the curve were 7.0 ± 3.1 and 8.9 ± 2.9 in DM1 patients and controls, respectively ($p > 0.05$). Based on individual measurements, the rates of decrease in retinyl palmitate concentrations, after reaching its maximum, were not significantly different between the 2 groups ($p > 0.05$; slope of regression line in DM1, and controls, -0.67 ± 0.45, and -0.75 ± 0.37 μmol L^{-1} h^{-1}, respectively).

^{13}C-oleic acid test. Whereas the vitamin A test mainly reflects the uptake of CM remnants in the liver, the ^{13}C-oleic acid test provides information, at least theoretically, on the lipolysis of CM-triglycerides. In case of a decreased activity of lipoprotein lipase during insulin deficiency, one would expect increased plasma^{13}C-oleic acid concentrations, a delayed disappearance of ^{13}C-oleic acid from plasma, and a delayed appearance of $^{13}CO_2$ in breath. However, plasma ^{13}C-oleic acid concentrations were not significantly different between DM1 and control late teenagers after ingestion of ^{13}C-oleic acid together with the high-fat meal ($p > 0.05$, Fig. 4A). Unlike retinyl palmitate concentrations (Fig. 3), plasma ^{13}C-oleic acid concentrations did not reach maximal values at time point 2 h, but rather continuously increased throughout the 6 h study period.

Detection of $^{13}CO_2$ in breath samples implies oxidation of the administered ^{13}C-oleic acid, indicating lipolysis of CM-triglycerides and cellular uptake of ^{13}C-FFA. Increase in breath $^{13}CO_2$ concentrations was not delayed in DM1 patients, and breath $^{13}CO_2$ concentrations were not significantly different between the 2 groups at any time point (Fig. 4B). Breath $^{13}CO_2$- and plasma ^{13}C-oleic acid concentrations appeared significantly correlated ($r = 0.93$, $p < 0.01$; data not shown).

Chylomicron clearance rate related to HbA_{1c}, gender, and fasting triglycerides. CM clearance in DM1 patients in poor metabolic control (arbitrarily chosen at $HbA_{1c} > 9.5\%$) was

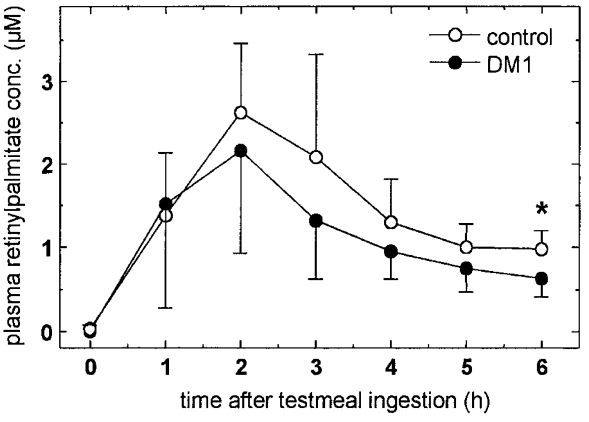

Figure 3. Plasma retinyl palmitate concentrations before and for 6 h after ingestion of a fat-rich meal and retinyl palmitate in late teenagers with diabetes mellitus type 1 ($n = 9$, closed symbols) and nondiabetic controls (n = 4, open symbols). *$p < 0.05$ reflects a significant difference between the two groups.

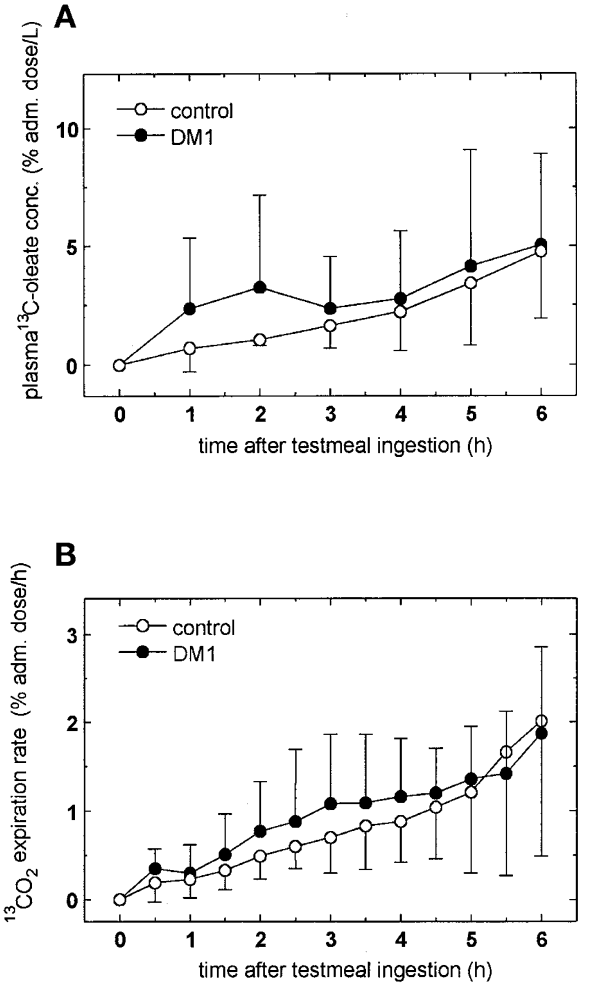

Figure 4. (*A*) Plasma ^{13}C-oleic acid concentrations before and for 6 h after ingestion of ^{13}C-oleic acid and a fat-rich meal in late teenagers with diabetes mellitus type 1 ($n = 9$, closed symbols) and nondiabetic controls ($n = 4$, open symbols). Results between the two groups were not significantly different at any time point. (*B*) Breath $^{13}CO_2$ concentration before and for 6 h after ingestion of ^{13}C-oleic acid and a fat-rich meal in late teenagers with diabetes mellitus type 1 ($n = 9$, closed symbols) and nondiabetic controls ($n = 4$, open symbols). Results between the two groups were not significantly different at any time point.

not delayed in any of the three tests (triglycerides, vitamin A, or ^{13}C-oleic acid), compared either with DM1 patients with moderate good metabolic control ($HbA_{1c} < 8.7\%$) or with nondiabetic controls (data not shown). When the slope of the regression lines of triglyceride clearance and of retinyl palmitate clearance were plotted against HbA_{1c}, no significant correlation was found ($p > 0.05$; r = 0.10, r = 0.12, respectively). Also, the area under the retinyl palmitate curves were not significantly correlated with HbA_{1c} (r = 0.22, $p > 0.05$). There was no significant difference between boys or girls with DM1 in CM clearance rates for any of the three tests applied and no significant correlation was found between CM clearance rates and fasting triglyceride concentrations.

DISCUSSION

In the present study we aimed to identify if a delayed CM clearance rate, as a known risk factor for atherosclerosis, is present in late teenagers with DM1 and if a delayed CM clearance could contribute to the higher plasma lipid concentrations in DM1 teenagers in poor control. In DM1 patients, fasting triglyceride and cholesterol concentrations were positively correlated with the level of metabolic control, as characterized by HbA_{1c}. Our results show that the clearance rate of CM was not delayed in late teenagers with DM1 compared with controls. CM clearance rate was not related with either metabolic control (HbA_{1c}), gender, fasting cholesterol, or triglyceride concentrations in late teenagers with DM1, indicating that relatively higher triglyceride and cholesterol concentrations in late teenagers with DM1 cannot be attributed to a delayed clearance of chylomicrons.

The observed correlation between HbA_{1c} and high plasma triglyceride and cholesterol concentrations is in agreement with other studies in children and late teenagers with DM1 (7–9, 29). The pathophysiological mechanisms of elevated lipid concentrations in diabetes type 1 are not fully understood. It has been hypothesized that lipid absorption from the intestine is increased in DM1. Studies in streptozotocin-induced diabetic rats showed an increased intestinal triglyceride production (30) and cholesterol synthesis (31) and an increased intestinal absorption of cholesterol (32). Another possible mechanism is an increased hepatic VLDL production, as a consequence of increased free-fatty acid release from adipose tissue and as a consequence of inefficient suppression of hepatic VLDL release by insulin. In poorly controlled adult patients with DM1 the antilipolytic effect of insulin is diminished, leading to a higher free-fatty acid flux from adipose tissue (33). Hepatic VLDL release in patients with type 2 diabetes was less inhibited by insulin compared with control subjects (34). Finally, a delayed metabolism and clearance of lipoproteins could lead to hypertriglyceridemia and hypercholesterolemia. A delayed chylomicron and VLDL clearance has been identified in patients with type 2 diabetes and in *adult* patients with DM1 (11, 12) and this could play a role in hypertriglyceridemia. Increased production of LDL from elevated VLDL and delayed clearance of (glycosylated) LDL could lead to hypercholester-

olemia (35, 36). A reduced hepatic uptake of LDL particles from patients with type 2 diabetes was associated with an altered lipid composition of the LDL particle and glycosylation of LDL protein (36). Insulin stimulates the hydrolysis of CM and VLDL triglycerides by lipoprotein lipase (37) and the hepatic uptake of chylomicron-, VLDL-remnants, and LDL in the liver, probably by stimulating the LDL receptor (38, 39).

In the present study we investigated the possibility that a delayed CM clearance rate is a possible contributor to the higher lipid values in diabetes in poor control. CM metabolism was studied by analyzing plasma disappearance of three different CM constituents. None of the three tests applied indicated significant differences in CM clearance between late teenagers with diabetes and controls, nor between DM1 boys or girls, nor between fairly or poorly controlled DM1 patients. Present data indicates that in patients with DM1, at this late teenager age, a delayed CM clearance rate is not an important atherosclerotic risk factor and does not contribute to the relatively higher triglyceride concentrations in late teenagers with DM1 in poor metabolic control. It therefore remains to be determined whether the observed correlation between metabolic control and triglyceride concentrations in late teenagers with diabetes is caused by one of the other hypothesized mechanisms, like increased hepatic VLDL production.

Several confounders could have influenced the outcome of the study. The relatively small study group could have confounded the results, however, because none of the three tests showed a trend toward delayed CM metabolism in DM1 patients, this possibility seems less likely. Due to the relatively small numbers of patients studied, we cannot exclude the possibility that a small difference in CM clearance exists, yet the clinical consequence of such a finding would then be questionable. Remarkably, studies performed in adult patients with type 1 or type 2 diabetes, who did show a difference in CM clearance rates, were based on patients studied comparable to the present study (12, 40).

It can be excluded that an acutely deteriorated metabolic control during the test confounded the results. The patients with diabetes did express a relative insulin deficiency compared with the nondiabetic controls, because fasting and postprandial glucose concentrations were increased in the former (Fig. 1A). Postprandial FFA concentrations decreased in both groups and were not significantly different between both groups from time point 3 h after ingestion of the fat-rich meal, indicating that during the test-meal, insulin levels were sufficient to inhibit lipolysis. It could be possible that the metabolic control of the DM1 patients was better during the test day compared with their daily practice, because of regular glucose control and adapted insulin concentrations. However, insulin concentrations were calculated according to the dosages the patients normally injected themselves, and this adaptation of insulin dosage (based on the normally injected insulin dosage) was comparable with other studies (12, 41). It is therefore unlikely that "over-regulation" has confounded our present study. Finally, postprandial lipoprotein metabolism is highly dependent on fasting plasma triglyceride concentration (42), and in the presented study group none of the subjects had fasting triglyceride concentrations $>$ 1.7 mmol/L. However, studies in normotriglyceridemic patients with type 2 diabetes and adults with DM1 did show a delayed postprandial chylomicron clearance (12, 43).

The presented results indicate that lipoprotein lipase activity and uptake of CM remnants in the liver are sufficiently stimulated in late teenagers in the diabetic state. The observed difference between the present results in late teenagers with DM1 (no delayed CM clearance rate) and previous data in adults with DM1 (delayed CM clearance rate) could be due to a worse metabolic regulation. Georgopoulos and Phair studied only adults with DM1 in poor control (HbA_1 12.8 $\pm$ 0.6%; normal range: 3.9–7.7%) (12), whereas in our study DM1 patients were in fair or poor control (mean HbA_{1c} 9.2 $\pm$ 1.9%; normal range: 4.6–6.1%). Comparison of metabolic regulation (HbA_{1c}) between the two studies is difficult however, because in the first study HbA_1 percentages were presented instead of HbA_{1c}, and HbA_1 was measured by agarose gel electrophoresis rather than by HPLC (44). Another explanation for the difference in CM clearance rate could be the age dependency of CM clearance. Studies in healthy individuals using the vitamin A test have shown that CM clearance rate delays with increasing age (18). It is tempting to speculate that DM1 patients do have an age dependency in CM clearance, similarly to subjects without DM1. However, the age-dependent delay in CM clearance rate is more pronounced in the former.

In conclusion, the present study showed that higher lipid concentrations in late teenagers with DM1 in poor control were not caused by a delay in CM clearance rate. Present data indicate that a delayed CM clearance rate at late teenager age is not a risk factor contributing to the increased risk for atherosclerosis in DM1.

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