Influence of Glycemic Control on Interaction of Very-Low- and Low-Density Lipoproteins Isolated From Type I Diabetic Patients With Human Monocyte-Derived Macrophages

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The VLDL and LDL fractions were isolated from 29 patients with type 1 diabetes at the time of admission to the hospital to restore glycemic control and again at discharge. These lipoprotein fractions were incubated with human monocyte-derived macrophages, and the rates of macrophage CE synthesis were determined. The rates of CE synthesis in human macrophages were significantly greater ($P < 0.005$) when incubated with VLDL isolated from type I diabetic patients before compared with after glycemic control was attained and averaged 1.84 ± 0.52 and 1.09 ± 0.27 nmol (1.20 ± 0.34 and 0.71 ± 0.18 μg) [^{14}C]cholesteryl oleate synthesized · mg cell protein^{-1} · 20 h^{-1}, respectively. In contrast, when LDL isolated from the same patient during the same period was incubated with human macrophages, the rates of cellular cholesteryl ester synthesis did not differ significantly and averaged 4.23 ± 1.26 and 3.91 ± 0.96 nmol (2.75 ± 0.82 and 2.55 ± 0.63 μg) [^{14}C]cholesteryl oleate synthesized · mg^{-1} cell protein · 20 h^{-1}, respectively. There was a significant increase in the total cholesterol content of VLDL isolated before glycemic control compared with that isolated after glycemic control was attained ($P < 0.05$) resulting from a significant increase in the FC and CE ($P < 0.05$) contents of these VLDL particles. There was a significant decrease in the ratio of FC to PL in VLDL, but not LDL, isolated after glycemic control ($P < 0.05$). The percentage of apoE in VLDL was significantly decreased ($P < 0.05$) after glycemic control was attained. In LDL isolated after glycemic control was achieved, a significant decrease ($P < 0.005$) in the percentage of triglycerides was observed. In addition, there was a significant decrease ($P < 0.0001$) in the extent of glycation of LDL isolated after glycemic control. These studies suggest that during periods of poor glycemic control, the composition of VLDL isolated from type 1 diabetic patients is altered. These modified VLDLs stimulate CE synthesis rates in human macrophages and, thus, may contribute to the increased prevalence of atherosclerosis in diabetic patients. ***Diabetes*** **41:1301–07, 1992**

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VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; CE, cholesteryl ester; *d*, density; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; type 1 diabetes, insulin-dependent diabetes mellitus; type 2 diabetes, non-insulin-dependent diabetes mellitus; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; apo, apolipoprotein; FC, free (unesterified) cholesterol; PL, phospholipid; S_f, svedberg flotation units; TC, total cholesterol; TG, triglyceride; BMI, body mass index; FPG, fasting plasma glucose.

Atherosclerosis is more prevalent in patients with diabetes mellitus, and vascular disease accounts for 70–80% of the deaths in diabetic patients (1–3). Recently, several studies have investigated the metabolism of lipoproteins isolated from diabetic patients with cells involved in the atherosclerosis process to determine if altered lipoprotein-cell interactions contribute to increased atherogenesis in diabetic patients. Macrophages are thought to be the main precursors of the cholesterol-laden foam cells characteristic of the atheromatous lesion. We showed previously that LDL glycated in vitro (4) and LDL isolated from type 1 diabetic patients in fair to good glycemic control (5) stimulate CE synthesis and accumulation in human macrophages. In contrast, LDL isolated from type 2 diabetic patients were not degraded significantly faster than LDL isolated from nondiabetic subjects when LDL was incubated with mouse peritoneal macrophages (6), and LDL isolated from normolipidemic type 2 diabetic patients did not stimulate CE synthesis in human macrophages (7). We also showed that, similar to LDL, VLDLs isolated from type 1 diabetic patients in fair to good glycemic control stimulate CE synthesis and accumulation in human macrophages (8). In contrast, VLDL isolated from normo-

TABLE 1
Clinical characteristics of type 1 (insulin-dependent) diabetic patients

Sex (M:F)	Race (black:white)	Age (yr)	Age at onset (yr)	Duration of diabetes (yr)	BMI (kg/m^2)
10:19	17:12	14.1 ± 0.5 (9–18)	9.4 ± 0.8 (2–15)	4.6 ± 0.7 (0.1–12)	20.0 ± 0.7 (14.8–29.4)

Values are means ± SE (range) for patient population (except ratios).

lipidemic type 2 diabetic patients do not stimulate CE synthesis in human macrophages (7), although they are taken up more avidly by mouse peritoneal macrophages (9). However, VLDL isolated from diabetic patients in poorer glycemic control did stimulate more CE synthesis in macrophages than did VLDL isolated from diabetic patients with better glycemic control or from control subjects (7). At present, no information is available about the effect of short-term glycemic control on the interaction of VLDL and LDL isolated from type 1 diabetic patients with human monocyte-derived macrophages. Thus, we isolated VLDL and LDL from type 1 diabetic patients before and after a period of intensive management of glycemic levels and investigated the interaction of these lipoproteins with human macrophages. Furthermore, we investigated whether the abnormal interaction, if present, resulted from an altered composition of the lipoproteins and whether the altered composition could be normalized by improved glycemic control.

RESEARCH DESIGN AND METHODS

Subjects. We recruited 29 patients with type 1 diabetes, as diagnosed according to the criteria established by the National Diabetes Data Group (10), for this study. One patient was admitted on three occasions and another on two separate occasions; thus, a total of 32 observations are reported. Each patient was admitted to the Medical University of South Carolina Hospital to restore satisfactory glycemic control. The mean period of hospitalization was 11 days (range, 10–12 days). During this period, each patient received intensive management using multiple daily insulin injections. Dietary therapy during hospitalization was similar to that recommended to the patient for home use. None of the patients studied had proteinuria >150 mg/24 h or evidence of renal impairment. One patient had background retinopathy; none had proliferative retinopathy. No patient reported taking any medication except insulin. Additional clinical characteristics of the patient population, including age, race, sex distribution, BMI, and duration of diabetes, are shown in Table 1. Of the 19 female patients studied, 14 were postpubertal. Informed consent, as approved by the Institutional Review Board for Human Research of the Medical University of South Carolina, was obtained from each patient in the study, and parental consent was obtained for minors.

Protocol. A 30-ml sample of blood was collected in EDTA (1 mg/ml blood) on admission and again at discharge. All samples were collected after at least a 10-h fast within 24–36 h of admission. This sample was used to isolate VLDLs and LDLs for metabolic studies and for determination of lipoprotein composition. The isolated VLDLs and LDLs (100 μg of lipoprotein protein/ml, final concentration) were incubated with human monocyte-derived macrophages, and the rates of CE synthesis in the cells were determined. The monocytes used for each experiment were obtained from a healthy nondiabetic donor. Lipoproteins isolated before glycemic control was attained were always studied in the same experiment with lipoproteins isolated after glycemic control. Monocytes were isolated from different donors for each experiment.

Monocyte isolation and maturation. Monocytes were isolated from leukapheresis specimens by countercurrent centrifugal elutriation, as described previously (11,12). The monocyte preparations were found to be 93% pure by checking morphology on Wright's stained cytocentrifuge preparations, 92% pure by nonspecific esterase staining, and 93% pure by observing their ability to ingest latex particles (13). The average viability of the cells was 99%. The isolated monocytes were transferred to 35-mm tissue culture dishes (1×10^6 cells/dish) and incubated in specially formulated medium (4) that contained 30% (vol/vol) whole human serum (Whittaker Bioproducts, Walkersville, MD). The cells were incubated for 8 days at 37°C in a humidified incubator (5% CO_2), and the medium was changed every 3 days. Metabolic experiments were performed with medium that did not contain serum or cholesterol (serum-free medium; 4).

Lipoprotein isolation. VLDL ($d < 1.006$ g/ml) were isolated from plasma by ultracentrifugation at 10°C in a Beckman 60 Ti rotor (Palo Alto, CA), spun at 362,000 g_{max} for 18 h. The floating VLDL was removed after tube slicing, and this VLDL solution was washed through a layer of saline solution by ultracentrifugation at 10°C in an SW41 rotor (Beckman) spun at 288,000 g_{max} for 24 h. LDL ($1.019 < d < 1.063$ g/ml) was isolated from each plasma sample after preliminary ultracentrifugation of the $d > 1.006$ g/ml plasma fraction at $d = 1.019$ g/ml. The floating IDL fraction was discarded, and the plasma solvent density was increased to $d = 1.063$ g/ml. LDL was isolated by tube-slicing after ultracentrifugation at 10°C at 362,000 g_{max} for 22 h. The isolated LDL solution was washed and concentrated by ultracentrifugation at $d = 1.063$ g/ml in an SW41 rotor, as described above. The isolated and washed VLDLs and LDLs were dialyzed against 0.9% (154 mM) NaCl, 0.01% (0.3 mM) (wt/vol) EDTA, pH 7.4. Salt solutions used to adjust the solvent densities also contained 0.01% (0.3 mM) (wt/vol) EDTA, pH 7.4. The lipoprotein preparations were sterilized by passage through a 0.2-μm filter (Gelman Sciences, Ann Arbor, MI) and stored at 4°C under an N_2 atmosphere.

Lipoprotein composition analysis. Aliquots of the isolated lipoprotein fractions were extracted with chloro-

TABLE 2
Glycemic control and plasma lipid levels in type 1 (insulin-dependent) diabetic patients before and after glycemic control

	FPG (mM)	HbA_{1c} (%)	Plasma cholesterol (mM)	Plasma triglycerides (mM)	Cholesterol (mM)		
					VLDL	LDL	HDL
Before	17.3 ± 2.4* (2.7–40.2)†	10.4 ± 0.5* (5.7–16.2)‡	5.7 ± 0.3§ (3.4–10.4)	2.6 ± 0.5* (0.6–11.7)	1.0 ± 0.2‖ (0.1–4.8)	3.6 ± 0.2 (1.4–4.8)	1.1 ± 0.1 (0.8–1.7)
After	6.4 ± 0.7 (2.1–14.3)	9.1 ± 0.4 (5.7–12.4)	4.9 ± 0.3 (3.2–9.3)	1.2 ± 0.3 (0.4–7.6)	0.5 ± 0.1 (0.1–2.3)	3.3 ± 0.3 (1.6–6.0)	1.1 ± 0.1 (0.7–1.7)

Values are means ± SE (range) for each group.
*$P < 0.0001$ vs. after.
†The plasma glucose level of 2.7 mM was obtained in 1 patient who exhibited an HbA_{1c} level of 13.8%. Blood sample was collected 20 h after admission.
‡HbA_{1c} level of 5.7% was obtained in a newly diagnosed patient with a plasma glucose level of 405 mg/dl.
§$P < 0.001$ vs. after.
‖$P < 0.05$ vs. after.

form/methanol (2:1, vol/vol; 14). The FC and TC (15), TG (16), PL phosphorus (17), and protein (18) concentrations of the lipoprotein samples were determined as described previously (4).

The apolipoprotein composition of VLDL was examined by quantitative immunoelectrophoresis and SDS-PAGE, as described previously (8). VLDL apoB concentrations were determined by quantitative immunoelectrophoresis, with LDL ($1.03 < d < 1.05$) as the standard (19). The relative proportions of apoE and apoC were estimated by densitometric scanning of apo-VLDL proteins separated by electrophoresis with 12% polyacrylamide gels. After electrophoresis, the gels were fixed and stained overnight in a solution of 50% (vol/vol) methanol, 7.5% (vol/vol) acetic acid, and 0.1% (wt/vol) Coomassie blue G-250. The gels were destained in a solution of 7.5% (vol/vol) acetic acid and 5% (vol/vol) methanol. The destained gels were scanned in a Beckman scanning densitometer. Densitometric scanning was validated and its reproducibility was assessed by the scanning linearity observed with gels loaded with different amounts of protein. The concentrations of apoE and apoC in VLDL were estimated using the following calculations. The concentration of non–apolipoprotein B proteins (apoE and apoC) was calculated as the difference between the total protein concentration of VLDL (apoB, apoE, and apoC) minus the concentration of apoB in VLDL as determined by immunoelectrophoresis. The relative concentrations in VLDL of apoE and apoC were estimated by distributing the concentration of the non–apoB proteins in VLDL in proportion to the relative densitometric areas of apoE and apoC determined by scanning the polyacrylamide gels.

Incorporation of [1-^{14}C]oleate into CEs. CE synthesis was determined after incubation of monocyte-derived macrophages for 20 h at 37°C with serum-free medium containing 0.2 mmol of [^{14}C]oleate/2.5 mg of bovine serum albumin, as described previously (4). VLDL or LDL (100 μg of protein/ml) isolated from each patient before and after glycemic control was attained were added concomitantly to duplicate wells. After the incubation, the cells were harvested and cellular lipids were extracted with chloroform/methanol (2:1, vol/vol; 14). Cholesteryl-[^{14}C]oleate was isolated by thin-layer chromatography of the lipid extracts on silica gel plates developed in a solvent system of petroleum ether/ethyl ether/acetic acid (80:20:2, vol/vol/vol). Lipids were visualized with I_2 vapor, and material in the spot that comigrated with a cholesteryl oleate standard was scraped into scintillation vials; the samples were counted in a liquid scintillation spectrophotometer. Correction for procedural losses was made by including [^{3}H]cholesteryl oleate as an internal standard in the chloroform/methanol extraction mixture. After the lipid extraction, the cell pellet was solubilized with 1 mol/L (40 g/L) NaOH, and the protein content was determined by the method of Lowry as modified by Markwell et al. (18).

Other methods. Plasma glucose was assayed by the glucose oxidase method, as adapted for use in the Beckman glucose analyzer (20). HbA_{1c} was measured by isoelectric focusing of erythrocyte hemolysates in a gradient of pH 6–8 (21). Plasma HDL cholesterol concentration was determined after precipitating VLDL and LDL with sodium phosphotungstate/magnesium chloride, as described previously (22). TC and TG were measured in whole plasma and lipoprotein fractions by the semiautomated methods standardized by the Lipid Research Clinics Program (16). The extent of LDL glycation was determined as described previously (23).

Statistical analysis. Data from paired observations was analyzed with Wilcoxon's signed-rank test. These analyses were determined with the StatView SE+ Graphics statistical package (Abacus Concepts, Berkeley, CA).

RESULTS

There were statistically significant improvements in glycemic control and plasma lipid levels in the diabetic patients after intensive glycemic management compared with levels at time of admission (Table 2). FPG and HbA_{1c} levels were significantly ($P < 0.0001$) reduced after 2 wk of intensive insulin management. Total plasma cholesterol ($P < 0.001$) and TG ($P < 0.0001$) levels also were significantly reduced during the period. A significant decrease in VLDL cholesterol concentration ($P < 0.05$) was observed during this period; however, the concentrations of cholesterol in plasma LDL and HDL were unchanged.

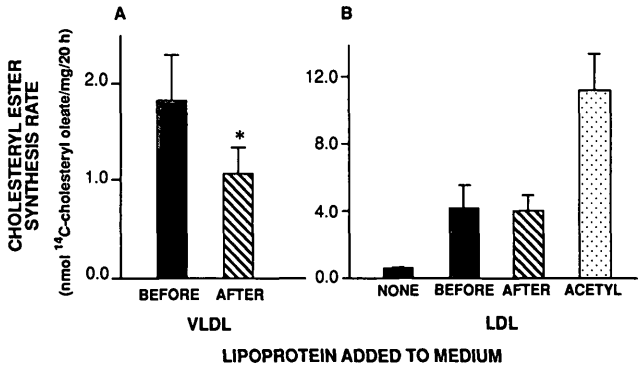

FIG. 1. Rates of synthesis of [^{14}C]cholesteryl oleate by human macrophages incubated with VLDL (*A*) or LDL (*B*) isolated from type 1 diabetic patients before (BEFORE) glycemic control and from the same patients after (AFTER) glycemic control was achieved. Lipoproteins (100 μg of protein/ml) were incubated with macrophages for 20 h after which rates of incorporation of [^{14}C]oleate into CEs were determined. Rates of CE synthesis in cells incubated without additional lipoproteins (NONE) or with acetylated LDL (ACETYL) are shown for comparison.
*** $P < 0.005$ vs. before VLDL.**

The rates of CE synthesis in human macrophages were significantly greater ($P < 0.005$) when incubated with VLDL isolated from 21 of the 29 type I diabetic patients before compared with after glycemic control was attained and averaged 1.84 ± 0.52 and 1.09 ± 0.27 nmol [^{14}C]cholesteryl oleate synthesized · mg cell protein^{-1} · 20 h^{-1}, respectively. These results are shown in Fig. 1. In contrast, when LDL isolated from the same patients during the same periods was incubated with human macrophages, the rates of cellular CE synthesis did not differ significantly and averaged 4.23 ± 1.26 and 3.91 ± 0.96 nmol of [^{14}C]cholesteryl oleate synthesized · mg^{-1} of cell protein · 20 h^{-1}, respectively. When acetylated LDL was incubated with the same macrophage preparations, rates of synthesis averaged 11.08 ± 2.13 nmol [^{14}C]cholesteryl oleate · mg cell protein^{-1} · 20 h^{-1}. In incubations without added lipoproteins, synthesis rates averaged 0.47 ± 0.11 nmol [^{14}C]cholesteryl oleate · mg cell protein^{-1} · 20 h^{-1}.

We showed previously that storage of LDL for 2 wk does not affect the interaction of the particle with cell lipoprotein receptors (19). We wished to determine, similarly, whether the 2-wk period of storage of the VLDL sample isolated before glycemic control was attained did affect the interaction of the particle with macrophage cell lipoprotein receptors. Therefore, we radiolabeled a VLDL sample isolated from a nondiabetic donor (freshly isolated VLDL) and compared its catabolism by macrophages with that of a VLDL sample isolated from the same donor 2 wk previously (aged VLDL). The rates of total degradation in duplicate incubations averaged 10.1 ± 0.9 and 10.5 ± 1.0 μg [^{125}I] VLDL degraded · mg cell protein^{-1} · 20 h^{-1} for aged and freshly isolated VLDL preparations, respectively. Similarly, the rates of receptor-mediated degradation averaged 6.9 ± 0.9 and 7.3 ± 1.9 μg [^{125}I]VLDL degraded · mg cell protein^{-1} · 20 h^{-1} for aged and freshly isolated VLDL preparations, respectively.

To determine whether the increased rate of CE synthesis in macrophages incubated with VLDL isolated from diabetic patients before glycemic control resulted from alterations in VLDL composition, we determined the lipid and apolipoprotein composition of the VLDL isolated from each patient. The results of the analyses of VLDL lipid composition are shown in Table 3. The TC of VLDL isolated before glycemic control was attained was significantly increased compared with that of VLDL isolated after glycemic control was achieved ($P < 0.05$). This increase was exhibited by a significant increase in both FC ($P < 0.05$) and CE (2.2-fold increase; $P < 0.05$) contents of these particles. There was, however, no significant change in the TG or PL contents of the VLDL obtained before compared with after glycemic control was achieved. Thus, as expected, there was a significant increase in the ratio of FC to PL in VLDL isolated before glycemic control ($P < 0.05$).

We also analyzed the apolipoprotein composition of VLDL isolated from each patient to determine if it may have contributed to the altered interaction between macrophages and VLDL that were isolated before the period of glycemic control and that resulted in increased rates of CE synthesis in the cells. These results are presented in Table 4. No significant differences were observed between the percentage of apoB in VLDL isolated from each patient before glycemic control compared with after glycemia was controlled. In contrast, the percentage of apoE in VLDL was significantly decreased ($P < 0.05$) after glycemic control was attained. No difference was observed in the percentage of apoC in VLDL isolated from each patient during either period.

The chemical composition of LDL isolated from each patient also was analyzed. These results are shown in Table 5. We observed no significant differences in the contents of FC, CE, or PL in LDL isolated from each patient before compared with after glycemic control was attained. In contrast, there was a statistically significant decrease ($P < 0.005$) in the amount of TGs in LDL

TABLE 3
Chemical composition of VLDL isolated from diabetic patients before and after glycemic control

	Mass/mass					
	TC/protein	FC/protein	CE/protein	TG/protein	PL/protein	FC/PL
Before	2.02 ± 0.44*	0.82 ± 0.13*	2.03 ± 0.54*	7.22 ± 1.17	2.19 ± 0.23	0.38 ± 0.05*
After	1.07 ± 0.17	0.52 ± 0.07	0.94 ± 0.21	7.59 ± 0.26	2.06 ± 0.21	0.25 ± 0.02

Values are means ± SE
*$P < 0.05$ vs. after.

TABLE 4
Apo composition of VLDL isolated from type 1 (insulin-dependent) diabetic patients before and after glycemic control

	ApoB (%)	ApoE (%)	ApoC (%)
Before	46.3 ± 2.1	14.8 ± 2.4*	38.9 ± 2.9
After	47.2 ± 2.1	10.8 ± 4.2	42.0 ± 3.1

Values are means ± SE for 10 patients from whom sufficient VLDL was available for analysis.
*$P < 0.05$ vs. after.

isolated after glycemic control was reached. In addition, we noted a significant decrease ($P < 0.0001$) in the extent of glycation of the LDL isolated after glycemic control compared with that in LDL isolated from the same patient before glycemic control was achieved.

DISCUSSION

We have shown that VLDL, but not LDL, isolated from type 1 diabetic patients before a 2-wk period of intensive therapy to improve glycemic control stimulated the rates of CE synthesis in human macrophages more than did VLDL isolated from the same patients after glycemic control was attained. These results extend our previous observation that VLDL isolated from type 1 diabetic patients in good to fair glycemic control stimulate the rates of macrophage CE synthesis significantly more than that from age-, sex-, and race-matched nondiabetic control subjects (8).

The lipid composition of VLDL isolated before glycemic control was attained differed significantly from that of VLDL isolated after glycemia was controlled (Table 3). VLDLs isolated before glycemic control were significantly enriched in cholesterol in both the FC and CE forms. The apolipoprotein composition of VLDL isolated before glycemic control was attained also differed significantly from that of VLDL isolated after glycemic control was achieved (Table 4) in that VLDL isolated before glycemic control was achieved contained significantly more apoE. Recent studies have shown that the enrichment of normal VLDL with apoE significantly increases the catabolism of VLDL by macrophages (24,25). In addition, the CE/protein ratio of VLDL isolated before control was significantly increased (Table 3), and this ratio of VLDL chemical constituents has been shown to be a good indicator of the degree of VLDL abnormality and is highly correlated with the rate of VLDL degradation (26). Unfortunately, because of limited sample amounts, we were unable to determine rates of VLDL degradation by macrophages. However, even if the rates of degradation of the apoE–enriched VLDL isolated before glycemic control were similar to those of VLDL isolated after glycemic control, the amount of exogenous cholesterol delivered to the macrophage during the catabolism of VLDL isolated before glycemic control would have been greater because these particles were cholesterol enriched. Thus, during periods of poor glycemic control, VLDLs are potentially more atherogenic.

An increased FC-lecithin ratio in plasma has been shown to be a potent predictor for cardiovascular risk (27). Bagdade et al. (28) have shown that this ratio of chemical constituents is increased in the VLDL + LDL fraction isolated from type 1 diabetic patients treated with either conventional insulin therapy or with continuous s.c. insulin infusion and remains elevated in the VLDL fraction isolated from type 2 diabetic patients treated with intensive insulin therapy for 2 mo (29). We did not directly measure lipoprotein phosphatidyl choline. However, phosphatidyl choline is the major PC in lipoproteins, and, thus, the FC/PL ratio approximates the FC-lecithin ratio. FC/PL was significantly decreased in VLDL isolated after glycemia was controlled compared with before (Table 3). This result differs somewhat from that observed by Bagdade et al., but this difference may reflect variations in the level of glycemic control between the two populations or reflect the determination of total PL rather than phosphatidyl choline specifically. However, the increased FC/PL ratio in VLDL isolated before control is consistent with these particles being more atherogenic.

The increase in FC/PL ratio of VLDL isolated before glycemia was controlled, which results from the significantly increased FC content of the particles (Table 3), is consistent with the significantly increased CE content of the particles. Morton (30) has shown that FC is a potent regulator of lipid transfer protein function. An increase in the FC content of VLDL causes a concentration-dependent decrease in CE transfer from these particles. Thus, VLDL CE content would increase because of decreased CE removal. VLDL CE content also may increase because of changes in the HDL FC content. Although not measured in this study, if HDL FC content also was increased, it would stimulate the transfer of CE to VLDL (30). This transfer also would be increased in diabetic patients because CE transfer activity is increased in type 1 diabetic patients (31), and this activity is positively

TABLE 5
Chemical composition of LDL isolated from type 1 (insulin-dependent) diabetic patients before and after glycemic control

	Mass/mass					
	TC/protein	FC/protein	CE/protein	TG/protein	PL/protein	Glycation
Before	1.70 ± 0.10	0.46 ± 0.04	2.10 ± 0.11	0.91 ± 0.17*	1.18 ± 0.04	130 ± 6†‡
After	1.75 ± 0.10	0.46 ± 0.02	2.20 ± 0.14	0.57 ± 0.10	1.15 ± 0.08	93 ± 8

Values are mean ± SE for 16 patients from whom sufficient LDL was available for analysis.
*$P < 0.005$ vs. after.
†$P < 0.0001$ vs. after.
‡Glycation reported as [H^3]hexitol lysine cpm/μg protein.

correlated with HbA_{1c} levels (32). Previous studies have shown that large VLDLs (S_f 100–400) are preferentially enriched with transferred CEs (33), and accelerated CE transfer has been associated only with this VLDL population (34). In type 2 (35) and type 1 (36) diabetic patients, there is a significant increase in the concentration of large (S_f 100–400) and medium (S_f 60–100) VLDL during poor glycemic control. These large, CE-enriched VLDL populations are not metabolized normally in hypertriglyceridemic patients, and lipolysis yields an abnormal, CE-rich remnant that can never become an LDL particle (37). In addition, recent studies have shown that the clearance of postprandial S_f 100–400 lipoproteins is impaired in type 1 diabetic patients (38), which may result in the formation of atherogenic remnant particles (39). Unfortunately, because of limited sample amounts, we did not analyze individual VLDL subfractions. Therefore, we cannot determine whether the altered VLDL composition and metabolism was localized to large particles only and whether remnant particles contributed to this lipoprotein fraction. However, periods of poor glycemic control do produce atherogenic VLDL, and the subsequent metabolism of these particles may produce an equally atherogenic CE-enriched remnant particle.

We have shown previously that plasma lipid and lipoprotein levels are highly correlated with the level of glycemic control in type 1 diabetic patients (40) and that the diabetic state may induce changes in VLDL and LDL that may be considered atherogenic (5,7,8). In this study, poor glycemic control was associated with chemical changes in VLDL, which resulted in more atherogenic VLDL particles. Thus, these studies reaffirm the need for the maintenance of tight glycemic control in type 1 diabetic patients.

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