Metabolism of very low- and low-density lipoproteins isolated from normolipidaemic Type 2 (non-insulin-dependent) diabetic patients by human monocyte-derived macrophages

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Summary. The very low- and low-density lipoprotein fractions were isolated from 16 normolipidaemic Type 2 (non-insulin-dependent) diabetic patients in good to fair glycaemic control and from corresponding age-, sex-, and race-matched, non-diabetic control subjects. Rates of cholesteryl ester synthesis averaged 268 ± 31 vs 289 ± 40 pmol ^{14}C -cholesteryl oleate $\cdot \text{mg cell protein}^{-1} \cdot 20 \text{ h}^{-1}$ for very low- and 506 ± 34 vs 556 ± 51 pmol ^{14}C -cholesteryl oleate $\cdot \text{mg cell protein}^{-1} \cdot 20 \text{ h}^{-1}$ for low-density lipoproteins isolated from the Type 2 diabetic patients and control subjects, respectively, when they were incubated with human macrophages. A group of approximately one-third of the patients was selected for separate analyses because very low-density lipoproteins isolated from these patients did stimulate more cholesteryl ester synthesis when incubated with macrophages. There were no significant differences in the lipid composition of the lipoproteins isolated from the three groups of subjects. The relative proportion of apoprotein C to apoprotein E was sig-

nificantly decreased ($p < 0.002$) in the very low-density lipoproteins from diabetic patients and was further decreased in samples from these selected diabetic patients. The apoprotein C-I content of very low-density lipoproteins isolated from diabetic patients was increased compared to control subjects and was further increased in samples from the selected diabetic patients ($p < 0.02$). There were no significant differences in the proportions of apoproteins C-III-0, C-III-1, or C-III-2 among the three groups. These studies suggest that in normolipidaemic Type 2 diabetic patients, the apoprotein composition of VLDL is abnormal and this may alter VLDL macrophage interactions and thus contribute to the increased prevalence of atherosclerosis in diabetic patients.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, VLDL metabolism, LDL metabolism, human macrophages.

There is an increased prevalence of atherosclerosis in patients with diabetes mellitus, and vascular disease accounts for 70–80% of the deaths in diabetic patients [1–3]. It is known that for similar lipid levels, the morbidity and mortality is higher in diabetic than in non-diabetic subjects. Thus, factors other than quantitative alterations of lipid and lipoprotein concentrations are important in the acceleration of atherosclerosis in diabetes. 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.

The catabolism by human fibroblasts of low-density lipoproteins (LDL) isolated from Type 1 (insulin-dependent) diabetic patients is impaired [4], while studies using LDL isolated from Type 2 (non-insulin-dependent) diabetic patients have not documented any abnormality in LDL catabolism by fibroblasts except in patients who were markedly hypertriglyceridaemic [5, 6]. Both triglyceride enrichment [5, 6] and glycosylation [7–12] of the

LDL particle have been implicated in the impaired recognition of LDL by cell lipoprotein receptors. In addition, when very low-density lipoproteins (VLDL) were glycosylated in vitro and incubated with fibroblasts, VLDL catabolism was significantly reduced [13].

Macrophages, however, are thought to be the main precursors of the cholesterol-laden foam cells characteristic of the atheromatous lesion. We have shown recently that LDL glycosylated in vitro [14] and LDL isolated from Type 1 diabetic patients in fair to good glycaemic control [15] stimulate cholesteryl ester synthesis and accumulation in human macrophages. In contrast, studies performed in mouse peritoneal macrophages showed that the rates of degradation of LDL glycosylated in vitro [8, 9] or isolated from Type 2 diabetic patients [5] were not significantly faster than that of native LDL.

VLDL isolated from Type 1 diabetic patients in fair to good glycaemic control stimulate cholesteryl ester synthesis and accumulation in human macrophages [16] and VLDL isolated from Type 2 diabetic patients are also taken up more avidly by mouse peritoneal macrophages

Table 1. Clinical characteristics of Type 2 (non-insulin-dependent) diabetic and control groups

Group ^a	Sex (male:female)	Race (black:white)	Age (years)	Body mass index	Duration of diabetes (years)	Diabetes treatment ^c (D:O:I)
Control	13:3	2:14	56.5 ± 2.6 ^b (36 – 73)	25.8 ± 1.0 (21.2 – 35.6)	–	–
Diabetic	8:3	2:9	55.0 ± 2.9 (38 – 68)	28.9 ± 1.1 (21.7 – 36.8)	7.2 ± 2.3 (1 – 23)	4:5:2
Selected diabetic	5:0	0:5	57.3 ± 2.1 (52 – 61)	29.3 ± 1.4 (22.0 – 35.1)	10.5 ± 9.5 (1 – 20)	2:2:1

^a Data for control group derived from all non-diabetic subjects. Diabetic group is composed of those patients whose VLDL did not stimulate more cholesteryl ester synthesis in human macrophages. Selected diabetic group composed of those patients whose VLDL did stimulate more cholesteryl ester synthesis in macrophages. ^b Values shown are mean ± SEM and (range) for each group. ^c Type of treatment regimen followed to maintain glycaemic control: D, diet only; O, oral hypoglycaemic agents; I, insulin

[17]. No studies have investigated, however, the interaction of VLDL or LDL isolated from normolipidaemic, Type 2 diabetic patients with human monocyte-derived macrophages. Therefore, we decided to investigate whether VLDL and LDL isolated from normolipidaemic, Type 2 diabetic patients in good to fair glycaemic control would interact abnormally with human macrophages and whether that abnormal interaction, if present, results from an altered composition of the VLDL and LDL due to the diabetic state.

Subjects and methods

Subjects

Sixteen patients with Type 2 diabetes were recruited from the Private Diagnostic Clinic of the Medical University of South Carolina and were diagnosed according to the criteria established by the National Diabetes Data Group [18]. For every diabetic patient, an age-, sex-, and race-matched, non-diabetic control subject was recruited and blood was drawn on the same day from each member of the matched pair. Only normolipidaemic subjects were accepted for inclusion in the study in order to exclude the potentially confounding effects of hypertriglyceridaemia on cell-lipoprotein interactions.

Three patients had evidence of background retinopathy while none had proteinuria. One patient was taking a β -blocker, one, digoxin, and three, nitrates. Two patients and one control subject were taking aspirin. Two patients reported having had a myocardial infarct more than six months prior to the study. No participants were receiving hypolipidaemic medications. The diabetic patients were treated by diet ($n = 6$), oral hypoglycaemic agents (no biguanides) ($n = 7$) or insulin ($n = 3$). Additional clinical characteristics and the

parameters of glycaemic control and plasma lipid levels of the diabetic and control groups are shown in Tables 1 and 2, respectively. Informed consent, as approved by the Institutional Review Board of the Medical University of South Carolina, was obtained from all subjects involved in the study.

Protocol

A 60 ml sample of blood was collected in ethylenediamine-tetraacetic acid (EDTA) (1 mg/ml blood) from all subjects after a 12–14 h fast. This sample was used to isolate VLDL and LDL for metabolic studies and for the determination of lipoprotein composition. Cholesteryl ester (CE) synthesis was measured in human monocyte-derived macrophages incubated with the VLDL or LDL from the diabetic and respective control subjects. For each experiment, the monocytes used were obtained from an individual, non-diabetic donor. Different donors were used for different experiments. Matched samples from diabetic and control subjects were always studied in the same experiment and each sample was incubated in duplicate. The lipid composition (free and esterified cholesterol, triglycerides and phospholipids) and apoprotein composition were determined for each lipoprotein sample. Fasting plasma glucose concentration, HbA_{1c} level, fasting lipid profiles and the extent of glycosylation of serum proteins and LDL were determined for each subject.

Monocyte isolation and maturation

Monocytes were isolated from leukapheresis specimens by counter-current centrifugal elutriation [19, 20]. The purity of the monocyte preparations was found to be 93% by checking morphology on Wright's stained cytocentrifuge preparations, 92% by non-specific

Table 2. Parameters of glycaemic control and plasma lipid levels in Type 2 (non-insulin-dependent) diabetic and control groups

Group	FPG (mmol/l)	HbA _{1c} (%)	Gly-PP ³ H-HAA cpm/ μ g	Gly-LDL ³ H-HAA cpm/ μ g	Total Chol (mmol/l)	Total TG (mmol/l)	Lipoprotein Chol (mmol/l)		
							VLDL	LDL	HDL
Control	5.0 ± 0.1 ^a (4.1 – 5.5)	5.5 ± 0.1 (5.0 – 6.4)	118.6 ± 3.2 (94 – 143)	73.0 ± 3.8 (47 – 112)	5.06 ± 0.16	1.11 ± 0.14	0.51 ± 0.09	3.40 ± 0.15	1.07 ± 0.09
Diabetic	8.7 ± 0.6 ^b (6.1 – 13.0)	6.9 ± 0.3 ^b (5.5 – 8.1)	154.7 ± 8.7 ^b (113 – 214)	87.9 ± 5.6 (59 – 112)	5.02 ± 0.18	1.38 ± 0.20	0.62 ± 0.13	3.41 ± 0.27	0.99 ± 0.06
Selected diabetic	10.9 ± 5.1 ^b (5.5 – 18.9)	8.1 ± 1.0 ^b (6.1 – 12.0)	193.8 ± 24.2 ^c (127 – 236)	104.8 ± 7.1 ^d (85 – 126)	5.58 ± 1.1	1.70 ± 0.37	0.41 ± 0.10	4.01 ± 0.10	1.16 ± 0.10

^a Values shown are mean ± SEM and (range) for each group. ^b $p < 0.001$ vs control group; ^c $p < 0.005$ vs control group; ^d $p < 0.01$ vs control group.

FPG – fasting plasma glucose; Gly-PP – glycosylated plasma proteins; Gly-LDL – glycosylated LDL; ³H-HAA – tritiated hexitol amino acids; Chol – cholesterol; TG – triglycerides

esterase staining and 93% by observing their ability to ingest latex particles [21]. The average viability of the cells was 99%. The isolated monocytes were matured by incubation at 37°C for eight days in a humidified incubator with a 5% CO₂ atmosphere. The medium used to induce maturation was a supplemented Iscove's modified Dulbecco's medium (IMDM) containing 30% volume/volume (V/V) of whole human serum (Whittaker MA Bioproducts, Walkersville, Md., USA) as described previously [14].

Lipoprotein isolation and composition

VLDL (density = d ; $d < 1.006$ g/ml) were isolated from plasma at 10°C by ultracentrifugation at 60 000 rev/min for 18 h in a Beckman 60 Ti rotor (Beckman Instruments, Inc, Palo Alto, Calif., USA). The floating VLDL were removed after tube-slicing and washed and concentrated by ultracentrifugation at their isolation density at 10°C in a SW41 rotor (Beckman Instruments, Inc), spun at 40 000 rev/min for 24 h. The LDL fraction ($1.019 < d < 1.063$ g/ml) was isolated after preliminary ultracentrifugation of the $d > 1.006$ g/ml plasma fraction at $d = 1.019$ g/ml. The floating intermediate density lipoprotein fraction (IDL) was discarded and the plasma solvent density was increased to $d = 1.063$ g/ml. LDL was isolated by tube-slicing after ultracentrifugation at 60 000 rev/min at 10°C for 22 h. The isolated LDL solution was washed and concentrated by centrifugation in a SW41 rotor as described above. The isolated and washed VLDL and LDL were dialyzed against saline (0.9% NaCl, 0.01% weight/volume (W/V) EDTA, pH 7.4). Salt solutions used to adjust solvent densities also contained 0.1% (W/V) EDTA, pH 7.4. The dialysed lipoprotein solutions were sterilized by passage through a 0.2 µm filter (Gelman Sciences, Ann Arbor, Mich., USA) and stored at 4°C.

Lipoprotein composition analysis

Samples of the isolated lipoprotein fractions were extracted with chloroform/methanol (2:1, V/V) [22]. The free and total cholesterol [23], triglyceride [24], phospholipid phosphorus [25], and protein [26] concentrations of the lipoprotein samples were determined as described previously [14].

The apoprotein composition of VLDL isolated from both groups was examined using quantitative immunoelectrophoresis and analytical isoelectric focussing (IEF). VLDL apoprotein B concentration was determined using quantitative immunoelectrophoresis with LDL ($1.030 < d < 1.050$) used as the standard [27]. The relative proportions of apoprotein E and apoprotein C were estimated by densitometric scanning of VLDL apoproteins separated by IEF using 7.5% polyacrylamide gels containing 2% ampholine, pH 4–6 [28–30]. The relative proportions of the apoproteins were determined by scanning densitometry at 580 nm in a Beckman scanning densitometer (Beckman Instruments, Inc). 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 apoprotein E and C in VLDL were estimated using the following calculations. The concentration of non-apoprotein B proteins was calculated as the difference between total VLDL protein concentration and the concentration of apoprotein B determined by quantitative immunoelectrophoresis. The relative concentrations of apoprotein E and C were estimated by distributing the concentration of the non-apoprotein B proteins in VLDL in proportion to the relative densitometric areas of apoproteins E and C in the IEF gels. No compensation was made for the differential chromogenicity of apoprotein C-II [31, 32].

Incorporation of [$1\text{-}^{14}\text{C}$]-oleate into cholesteryl esters

Cholesteryl ester synthesis was determined after incubation of monocyte-derived macrophages for 20 h, at 37°C with serum-free medium containing 0.2 mmol ^{14}C -oleate/2.5 mg of bovine serum albumin [4] and 100 µg of VLDL or LDL isolated from diabetic and

respective control subjects. The cells were incubated for 20 h at 37°C and were harvested with a rubber policeman using 1 ml of phosphate-buffered saline and rinsed with 2 ml of the same solution. Lipids in the cell pellet were extracted with chloroform/methanol (2:1) (V/V) [22]. 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) (V/V/V). Lipids were visualized with I₂ vapour, and the spots which co-migrated with a cholesteryl oleate standard were marked and scraped into scintillation vials after the total disappearance of colour. Scintillation fluid (ScintiPrep, Fisher Scientific Co, Fairlawn, NJ, USA), was added and the samples counted in a liquid scintillation counter. Correction for procedural losses was made by adding ^3H -cholesteryl oleate as an internal standard to the chloroform/methanol extraction mixture. After the lipid extraction, the cell pellet was solubilized with 1 mol/l NaOH and the protein content determined [26].

Measurement of glycosylation of LDL

Glycosylation of LDL was determined by affinity chromatography as described previously [33]. Stored LDL samples were thawed and aggregated lipoproteins were dispersed by brief (< 5 s) sonication (Probe sonicator, Heat Systems-Ultrasonic Inc, Plainview, NY, USA) before assessing their degree of glycosylation. In every case both members of a diabetic-control pair were studied in the same experiment, and the same batch of tritiated sodium borohydride was used throughout.

Other methods

Plasma glucose was assayed by the glucose oxidase method, as adapted for use in the Beckman glucose analyser [34]. HbA_{1c} was measured by IEF of erythrocyte haemolysates in a gradient of pH 6–8 [35]. Plasma HDL cholesterol concentration was determined after precipitating VLDL and LDL with sodium phosphotungstate/magnesium chloride as described [36]. Total cholesterol and triglyceride were measured in whole plasma and lipoprotein fractions by the semi-automated methods standardized by the Lipid Research Clinics Program [24].

Statistical analysis

Data from paired observations was analysed with the Wilcoxon signed-rank test. Group data from non-paired observations was analysed with the Mann-Whitney U test. These analyses, as well as other correlation analyses, were determined with the Stat View SE + Graphics statistical package (Abacus Concepts, Berkeley, Calif., USA).

Results

There were no significant differences in the rates of cholesteryl ester synthesis in human macrophages incubated with VLDL or LDL isolated from hyperglycaemic, normolipidaemic, Type 2 diabetic patients compared with the same lipoprotein fractions isolated from their respective non-diabetic, control subjects. Rates of cholesteryl ester synthesis in human macrophages incubated with VLDL isolated from Type 2 diabetic patients and control subjects averaged 268 ± 31 and 289 ± 40 pmol ^{14}C -cholesteryl oleate synthesized $\cdot \text{mg cell protein}^{-1} \cdot 20 \text{ h}^{-1}$ (mean $\pm$ SEM), respectively. Rates of cholesteryl ester synthe-

Table 3. Chemical composition of VLDL and LDL isolated from Type 2 (non-insulin-dependent) diabetic patients and control subjects

Lipoprotein	Group	Ratio of constituents (mg/mg)			
		TC/Pro	FC/Pro	CE/Pro	TG/Pro
VLDL	Control	1.23 ^a ±0.10	0.57 ±0.05	1.12 ±0.11	7.11 ±1.51
	Diabetic	1.11 ±0.09	0.54 ±0.04	0.97 ±0.10	6.34 ±0.49
	Selected diabetic	1.36 ±0.18	0.63 ±0.06	1.24 ±0.28	6.69 ±0.37
LDL	Control	1.87 ±0.05	0.49 ±0.01	2.36 ±0.06	0.43 ±0.05
	Diabetic	1.83 ±0.03	0.48 ±0.09	2.31 ±0.06	0.38 ±0.06

^a Values are shown as mean ± SEM.

TC, total cholesterol; Pro, protein; FC, free cholesterol; CE, cholesteryl ester = 1.7 × esterified cholesterol; TG, triglyceride

Table 4. Apoprotein composition of VLDL isolated from control subjects and Type 2 (non-insulin-dependent) diabetic patients

Group ^a	%			Ratio of constituents		
	Apo B	Apo E	Apo C	B/E	B/C	C/E
Control	51.0 ±1.4 ^b	15.5 ±1.0	33.4 ±1.0	3.50 ±0.28	1.57 ±0.09	2.21 ±0.10
Diabetic	48.8 ±3.0	17.6 ±1.1	33.6 ±2.5	2.84 ±0.29	1.52 ±0.21	1.93 ±0.19 ^c
Selected diabetic	52.1 ±2.1	18.1 ±1.3	29.8 ±1.5	3.09 ±0.28	1.84 ±0.18	1.70 ±0.11

^a Data for control group resulted from analyses of VLDL from 11 control subjects from whom sufficient material was available for analysis. Diabetic subjects are those patients whose VLDL did not stimulate more cholesteryl ester synthesis than VLDL from non-diabetic control subjects when incubated with human macrophages. Selected diabetic subjects are the five diabetic patients whose VLDL did stimulate more cholesteryl ester synthesis when incubated with macrophages. ^b Values are shown as mean ± SEM. ^c $p < 0.002$ vs control subjects.

Apo B, apolipoprotein B; Apo E, apolipoprotein E; Apo C, apolipoprotein C; B/E, apolipoprotein B mass ÷ apolipoprotein E mass; B/C, apolipoprotein B mass ÷ apolipoprotein C mass; C/E, apolipoprotein C mass ÷ apolipoprotein E mass

Table 5. Apoprotein C composition of VLDL isolated from control subjects and Type 2 (non-insulin-dependent) diabetic patients

Group	% of apoprotein C densitometric area			Ratio of areas		
	C-I	C-II	C-III	C-II/C-I	C-III/C-I	C-III/C-II
Control	9.9 ±1.1 ^a	17.0 ±0.8	73.4 ±0.9	2.0 ±0.3	8.4 ±0.9	4.5 ±0.3
Diabetic	10.9 ±1.4	18.8 ±0.7	72.3 ±1.9	2.0 ±0.4	7.4 ±1.0	3.9 ±0.3
Selected diabetic	16.5 ±3.1 ^b	16.8 ±1.6	66.7 ±3.1 ^c	1.3 ±0.3	5.2 ±1.6 ^d	4.1 ±0.4

^a Values are shown as mean ± SEM. ^b $p < 0.02$ vs control subjects; ^c $p = 0.059$ vs control subjects; ^d $p < 0.05$ vs control subjects.

C-I, apolipoprotein C-I; C-II, apolipoprotein C-II; C-III apolipoprotein C-III; C-II/C-I, apolipoprotein C-II mass ÷ apolipoprotein C-I mass; C-III/C-I, apolipoprotein C-III mass ÷ apolipoprotein C-I mass; C-III/C-II, apolipoprotein C-III mass ÷ apolipoprotein C-II mass

sis averaged 506 ± 34 and 556 ± 51 pmol ^{14}C -cholesteryl oleate · mg cell protein $^{-1}$ · 20 h $^{-1}$ when macrophages were incubated with LDL isolated from the Type 2 diabetic and control groups, respectively. When acetylated LDL was incubated with the same macrophage preparations, rates of synthesis averaged 7067 ± 207 pmol ^{14}C -cholesteryl oleate · mg cell protein $^{-1}$ · 20 h $^{-1}$. In incubations without added lipoproteins, synthesis rates averaged 86 ± 27 pmol ^{14}C -cholesteryl oleate · mg cell protein $^{-1}$ · 20 h $^{-1}$.

We observed, however, that VLDL isolated from five Type 2 diabetic patients, almost one-third of the population studied, did stimulate more cholesteryl ester synthesis when incubated with macrophages. The clinical characteristics and parameters of glycaemic control and plasma lipid levels of this group of five diabetic patients (selected diabetic) are shown in Tables 1 and 2, respectively. There were no significant differences in the clinical characteristics of the subjects in each of the three groups (Table 1). The concentrations of fasting plasma glucose and HbA_{1c} for both the diabetic and selected diabetic patients were significantly greater than those for the control group but were not statistically different between the diabetic and selected diabetic patients (Table 2). Plasma proteins were significantly more glycosylated in the diabetic ($p < 0.001$) and selected diabetic ($p < 0.005$) patients compared to control subjects, but the levels did not differ significantly between the two groups of diabetic patients. The extent of glycosylation of LDL did not differ significantly between control subjects and diabetic patients; however, LDL isolated from the selected diabetic patients was significantly more glycosylated than that isolated from control subjects ($p < 0.01$) but not from the other diabetic patients. There were no significant differences in the lipid profile of either group of diabetic patients compared to the control subjects or between the two groups of diabetic patients. The rate of cholesteryl ester synthesis in macrophages incubated with VLDL isolated from these selected patients was not significantly correlated with the patients' HbA_{1c} level ($p = 0.052$) or the extent of glycosylation of their LDL ($p = 0.101$) or plasma proteins ($p = 0.310$), although data from only five patients was used for this analysis. The rate of cholesteryl ester synthesis in macrophages incubated with LDL isolated from the selected diabetic patients did not differ significantly from that in cells incubated with LDL isolated from control subjects ($p = 0.051$). The correlation of the rate of cholesteryl ester synthesis in cells incubated with LDL isolated from selected diabetic patients with the extent of glycosylation of the LDL was not statistically significant ($p = 0.071$); however, again, data from only five patients was used for this analysis.

To determine if the increase in rate of cholesteryl ester synthesis in macrophages incubated with VLDL isolated from the selected diabetic patients resulted from alterations in VLDL composition, we determined the lipid and apoprotein composition of the VLDL isolated from each subject. The results of the analysis of VLDL lipid composition are shown in Table 3. There were no significant differences in the free cholesterol or cholesteryl ester contents of VLDL isolated from these selected diabetic patients compared to control subjects or the remaining

diabetic patients. The lipid composition of LDL isolated from both groups is also shown for reference. There were no significant differences in the lipid composition of LDL isolated from the selected diabetic patients compared to the two other groups; therefore, only the means for all diabetic patients are shown.

We also analysed the VLDL apoprotein composition to determine if this may have contributed to the altered interaction of VLDL from Type 2 diabetic patients with macrophages which resulted in increased rates of cholesteryl ester synthesis in the cells. These results are presented in Table 4. There were no significant differences in the mass of apoprotein B relative to apoprotein E in VLDL isolated from the three groups (Table 4). In addition, the proportion of apoprotein B relative to apoprotein C in the three groups was similar. However, the relative proportion of apoprotein C compared to apoprotein E was decreased in VLDL from the diabetic patients ($p < 0.002$ vs control subjects). The mean apoprotein C to apoprotein E ratio value was further decreased in VLDL from the selected diabetic patients although this did not reach statistical significance either in relation to control subjects or to diabetic patients.

IEF was used to analyse VLDL apoprotein E and apoprotein C composition. This technique separates apoprotein C into the component apoprotein C isoforms. The distribution of apoprotein C isoforms in VLDL isolated from control subjects and diabetic patients is detailed in Table 5. There were no substantial differences in the percentage of apoprotein C-II in the VLDL isolated from each group. In contrast, there was an increase in the mean percentage of the densitometric area of VLDL apoprotein present as apoprotein C-I in VLDL isolated from the diabetic patients which was further increased in VLDL isolated from the group of selected diabetic patients ($p < 0.02$ vs control subjects). There was a concomitant decrease in the mean percentage of the densitometric area of VLDL apoprotein present as apoprotein C-III which was further decreased in VLDL isolated from the group of selected diabetic patients ($p = 0.059$ vs control subjects). There were no statistically significant differences in the proportion of the densitometric areas of apoprotein C-II relative to apoprotein C-I; however, the proportion of the densitometric area of apoprotein C-III relative to apoprotein C-I in VLDL from control subjects compared to VLDL from the selected diabetic patients was significantly decreased ($p < 0.05$). The relative proportions of the areas corresponding to apoprotein C-III compared to apoprotein C-II was similar for VLDL from the three groups. There were no differences in the proportions of the areas corresponding to apoprotein C-III-0, C-III-1, or C-III-2 among the three groups (data not shown).

A significant correlation was found between the extent of glycosylation of LDL isolated from the 16 diabetic patients and the extent of glycosylation of plasma proteins obtained from the same patients ($y = 1.41 \times + 36.8$, $r = 0.67$, $p < 0.01$). In addition, there was a significant correlation between HbA_{1c} concentration and the extent of glycosylation of both LDL ($y = 7.72 \times + 36.6$, $r = 0.66$, $p < 0.05$) and of plasma proteins ($y = 14.7 \times + 58.4$, $r = 0.57$, $p < 0.05$).

Discussion

The results of the present study suggest that the metabolism by human macrophages of VLDL and LDL isolated from normolipidaemic patients with Type 2 diabetes who are in good glycaemic control is not significantly altered. There were no significant differences in the rates of cholesteryl ester synthesis in human macrophages incubated with VLDL isolated from the Type 2 diabetic patients compared with VLDL isolated from the non-diabetic, control subjects when the results from all the diabetic patients were analysed. However, VLDL isolated from almost one-third of the Type 2 diabetic patients studied did stimulate cholesteryl ester synthesis in human macrophages. There were no significant differences in the lipid composition of VLDL isolated from the diabetic patients compared to that for VLDL from control subjects or to that from the group of selected diabetic patients (Table 3), which could have contributed to the observed differences in cholesteryl ester synthesis. Previous studies indicated that VLDL isolated from another group of non-insulin-dependent diabetic patients which was enriched in cholesterol and triglycerides, was degraded more rapidly by thioglycolate elicited mouse peritoneal macrophages [17]. In the present study, VLDL from neither of the diabetic populations were enriched in triglycerides (Table 3). Additional studies reported a significant increase in the free and esterified cholesterol concentrations in VLDL isolated from a group of Type 2 diabetic patients in good glycaemic control [37] and therapeutic intervention may influence these levels [38]. This increase in cholesterol concentration has been localized to the smallest sized VLDL particles [39] but our studies did not permit us to evaluate this possibility.

There were substantial differences in the apoprotein composition of VLDL isolated from the Type 2 diabetic patients compared to control subjects. The percentage of VLDL apoprotein densitometric area present as apoprotein E was increased in the VLDL isolated from the diabetic patients whose VLDL did not stimulate cholesteryl ester synthesis in human macrophages and was further increased in the VLDL isolated from the selected diabetic patients (Table 4). Previous studies have reported an increase in plasma apoprotein E concentrations [39] and therapeutic intervention may decrease the apoprotein E content of VLDL isolated from Type 2 diabetic patients [38]. Additional studies have found no changes in the amount of apoprotein E relative to apoprotein B in VLDL isolated from Type 2 diabetic patients although the apoprotein E concentration was decreased in proportion to triglyceride in VLDL isolated from these patients [40].

The apoprotein C composition of VLDL also differed significantly among the three groups of subjects (Table 5). Only a few detailed studies have been reported concerning the VLDL apoprotein C composition in diabetic patients [38, 41–45]. The results of these studies are inconsistent but this may be related to the presence of hypertriglyceridaemia in the patient population or to differences in the techniques used to quantitate VLDL apoprotein composition.

The effects of alterations of VLDL total apoprotein composition or of apoprotein C composition on the inter-

actions of the VLDL with non-hepatic lipoprotein receptors are unknown. The results of the present study do not permit us to speculate on the potential effects of the altered VLDL apoprotein composition on the interaction of these particles with macrophage lipoprotein receptors. Sufficient VLDL was not available to conduct the necessary lipoprotein binding and degradation studies. Clearly, however, VLDL isolated from the selected diabetic patients exhibited an altered apoprotein composition and may be considered atherogenic since these particles stimulated cholesteryl ester synthesis rates in human macrophages.

There were no significant differences in the rates of cholesteryl ester synthesis in human macrophages incubated with LDL isolated from the Type 2 diabetic patients compared to LDL isolated from control subjects when the results from all the diabetic patients were analysed. This is in direct contrast to similar experiments conducted with human macrophages which utilized LDL isolated from Type 1 diabetic patients [15]. The cause of this apparent discrepancy between the two studies is not known. There were no significant differences between the chemical composition of LDL isolated from either the Type 1 [15] or Type 2 (Table 3) diabetic patients and their corresponding control subjects. However, the magnitude of difference in the extent of glycosylation of LDL may be a contributing factor. The LDL isolated from the Type 1 patients was significantly more glycosylated than LDL from control subjects and the rate of cholesteryl ester synthesis in human macrophages was proportional to the extent of glycosylation of the LDL [14]. Only the LDL isolated from the selected group of Type 2 diabetic patients in the present study was significantly more glycosylated than LDL isolated from control subjects (Table 2). The rate of cholesteryl ester synthesis in macrophages incubated with this LDL was not proportional to the extent of glycosylation of the LDL ($p = 0.071$); however, the number of subjects may have been too limited to assure statistical significance.

The extent of LDL glycosylation correlated well with commonly used indices of glycaemic control. There was a strong association between the extent of glycosylation of the LDL isolated from the selected diabetic patients and the increased rate of cholesteryl ester synthesis in macrophages incubated with these LDL. There was also a strong association between the rate of cholesteryl ester synthesis in macrophages incubated with VLDL isolated from the same patients and the extent of glycosylation of the LDL isolated from these patients. There was an even stronger association between the HbA_{1c} level in these patients and the rate of cholesteryl ester synthesis in macrophages incubated with their VLDL. These VLDL particles were also characterized by altered apoprotein compositions. These studies suggest that in normolipidaemic Type 2 diabetic patients, the level of glycaemic control may induce changes in lipoprotein composition which influence their interaction with macrophage lipoprotein receptors and alter cholesterol metabolism in these cells. This altered lipoprotein-macrophage interaction may contribute to the increased prevalence of atherosclerosis in diabetic patients.

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