Accelerated cholesteryl ester transfer and altered lipoprotein composition in diabetic cynomolgus monkeys

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Abstract To determine whether nonhuman primates demonstrate the same alterations in transport of cholesteryl ester (CE) in plasma observed in diabetic humans, cholesteryl ester transfer (CET) was measured in cynomolgus monkeys with chronic spontaneous diabetes mellitus (glycated hemoglobin: diabetics 10.7 ± 4.1%; controls 3.8 ± 0.8%, $P < 0.005$). Among the plasma lipids, only triglycerides were significantly increased in diabetic monkeys (diabetics 303 ± 294 mg/dl; controls 85 ± 34 mg/dl; $P < 0.05$); total plasma, LDL, HDL_2, and HDL_3 cholesterol concentrations did not differ significantly from those of control animals. Similar to human beings with insulin-dependent and non-insulin-dependent diabetes mellitus, CET estimated both as the mass of cholesteryl ester transferred from HDL to the apoB-containing lipoproteins (VLDL + LDL) and as the loss of radiolabeled cholesteryl ester from HDL was significantly greater ($P < 0.001$) in diabetic compared to control monkeys. Glycated hemoglobin levels in the combined control and diabetic groups correlated directly with both the mass of cholesteryl ester transferred at 2 h ($r = 0.75$; $P < 0.001$) and the isotopic transfer (k) ($r = 0.64$; $P < 0.005$). The mass of cholesteryl ester transfer protein (CETP) tended to be higher in the diabetic animals (diabetic 4.06 ± 0.73 μg/ml versus control 3.05 ± 0.93; $P < 0.1$). Consistent with CET being enhanced in vivo in the diabetic animals, compositional studies revealed that the triglyceride:cholesteryl ester core lipid ratio of their VLDL tended to be lower and LDL and HDL_2 significantly higher than in controls ($P < 0.001$); and like human beings with non-insulin-dependent diabetes mellitus, the free cholesterol:lecithin ratio was reduced in their HDL_2 ($P < 0.05$). Moreover, the sphingomyelin:lecithin ratio was significantly reduced in the diabetic monkeys' VLDL and LDL ($P < 0.05$ and $P < 0.005$, respectively), indicating that a disturbance also was present in lipoprotein surface phospholipid composition. Thus, diabetic cynomolgus monkeys have abnormalities in CET and disturbances in lipoprotein composition that resemble those in human beings with diabetes mellitus. Cynomolgus monkeys may be useful models for studying the mechanism(s) that underlie the acceleration of CET and altered lipoprotein composition in diabetic patients.—**Bagdade, J. D., J. D. Wagner, L. L. Rudel, and T. B. Clarkson.** Accelerated cholesteryl ester transfer and altered lipoprotein composition in diabetic cynomolgus monkeys. *J. Lipid Res.* 1995. **36:** 759–766.

Supplementary key words diabetes mellitus • lipoproteins • phospholipids

Cholesterol ester transfer (CET) is a central step in reverse cholesterol transport. During CET, cholesteryl ester generated on HDL by lecithin:cholesterol acyltransferase (LCAT) is redistributed to the apoB-containing lipoproteins VLDL and LDL. Under normal conditions, the cholesteryl ester transferred to VLDL and LDL in this way can be recycled for use by other tissues or delivered to the liver, where it can be catabolized and excreted as bile acids in the final step of reverse cholesterol transport. When cholesteryl ester is transferred from HDL to VLDL during CET, there is a concomitant equimolar transfer of triglyceride from VLDL to HDL, resulting in the enrichment of VLDL with cholesteryl ester and of HDL with triglyceride. CET is abnormally increased in a number of conditions in which atherogenesis is accelerated, such as hypercholesterolemia (1), hypertriglyceridemia (2), dyslipidemia (3), and in both the insulin-dependent (4, 5) and non-insulin-dependent (6) forms of diabetes mellitus. Moreover, it has been shown recently that concentrations of the protein that mediates CET, cholesteryl ester transfer protein (CETP), correlate with the extent of coronary artery atherosclerosis in nonhuman primates (7). For this reason, it is believed that the atherogenicity of both VLDL and LDL may be enhanced as a consequence of their interaction with CETP.

CET's central role in atherogenesis is implied by the fact that species with CET activity (e.g., rabbits, most nonhuman primates, and humans) are susceptible to diet-induced atherosclerosis (8), while those with no or very

Abbreviations: CE, cholesteryl ester; CET, cholesteryl ester transfer; CETP, cholesteryl ester transfer protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; LCAT, lecithin:cholesterol acyltransferase; GHb, glycated hemoglobin; FC, free cholesterol.

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low levels of CET (e.g., rats, mice, dogs) are resistant. These interspecies differences in CET activity are attributable not only to differences in CETP concentrations, but also to the presence of proteins in plasma that inhibit CET (9). The accelerated CET observed in human beings with diabetes mellitus appears to result from abnormal behavior of the acceptor lipoprotein VLDL rather than an increase in CETP (4). To obtain an animal model that might yield insights into the mechanism(s) that underlie the disturbance we have observed in humans with diabetes, we undertook studies in cynomolgus monkeys (*Macaca fascicularis*) which are prone to develop spontaneous diabetes mellitus as they age that presents with hyperglycemia, with concurrent hypertriglyceridemia and hypercholesterolemia, and occasionally ketonuria and glucosuria (10).

METHODS

Experimental animals

Nine diabetic cynomolgus monkeys that had spontaneously developed hyperglycemia while in captivity (ages 12–19 yr) with an average of 3 years of treatment (range 0.5–10 yr), and nine nondiabetic age- and sex-matched monkeys were studied. The mean pretreatment weight of the diabetic animals was 6.6 ± 1.4 kg and for the control animals, 5.9 ± 1.6 kg. Seven of the diabetic animals required insulin and received a combination of short-acting (regular) and longer-acting (intermediate) insulin (Humulin 70/30, Eli Lilly, Indianapolis, IN) twice daily by i.m. injections before meals. Two diabetic animals were treated with calorically restricted diets after initial insulin therapy. At the time of study each had been weight-stable after having lost about 20% of their pretreatment weights. All monkeys consumed the same diet (High Protein Monkey Chow, Ralston Purina Co., St. Louis, MO). All blood was collected after an overnight fast in Na EDTA-containing tubes. Plasma was separated at 4°C by low-speed centrifugation and sent on ice by overnight courier service from Winston-Salem to Chicago, where the following studies were performed.

Analytical methods

VLDL (d < 1.006 g/ml) was isolated from an aliquot of each plasma sample by ultracentrifugation. LDL, HDL_2, and HDL_3 were separated from each other by differential precipitation with dextran sulfate (11). Triglyceride content in whole plasma and in each fraction was determined by an enzymatic method (Sigma Chemical, St. Louis, MO). Total cholesterol in the same fractions also was quantified using a kit (Boehringer Mannheim, Indianapolis, IN). Free cholesterol was estimated with the same kit components except that cholesteryl ester hydrolase was omitted. Phospholipids (lysolecithin, sphingomyelin, lecithin, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine) were extracted from another aliquot and then separated by thin-layer chromatography using activated silica gel plates (0.5 mm thickness) and a solvent system of chloroform–methanol–acetic acid–water 25:15:4:2 (by volume) (12). Each phospholipid spot was scraped into glass tubes and lipid phosphorus was determined by the modified Bartlett procedure (13). Glucose and glycated hemoglobin (GHb) were measured as described previously (10). CETP was measured by a solid-phase competitive radioimmunoassay (14) in a subset of five diabetic and five control animals.

CET in plasma

Two types of assays were used to estimate CET: one measures the chemical net mass transport of cholesteryl ester between HDL and VLDL + LDL as described by Fielding et al. (15) in the presence of the LCAT inhibitor 5,5' thiobis 2-nitrobenzoic acid (DTNB; 1.5 mM); the other is an isotopic method that quantitates the movement of labeled HDL-cholesteryl ester from a human control donor between labeled and unlabeled native lipoproteins. In the mass transfer assay, plasma (0.4 ml) was incubated at 37°C in a metabolic shaker. Aliquots were removed at 1, 2, and 4 h, chilled on ice, and the apoB-containing lipoproteins (VLDL + LDL) were then precipitated with 1 vol of heparin/$MnCl_2$ (16). The amount of cholesteryl ester remaining in the supernatant fraction (HDL) was determined in pentuplicate. The amount of cholesteryl ester transferred to VLDL + LDL was calculated from the magnitude of the decrease in HDL-cholesteryl ester.

In the isotopic assay, HDL (d 1.063–1.21 g/ml) from a control subject radiolabeled with cholesteryl ester according to the method of Quig and Zilversmit (17) was added (40 μg cholesteryl ester mass and 10,000 cpm cholesteryl ester radioactivity) to each plasma sample and incubated without DTNB for 1 h. The transfer of cholesteryl ester radioactivity at 15, 30, 45, and 60 min was calculated from the amount of radioactivity in the precipitated VLDL + LDL. The kCET was determined as the slope of the line comprised of the percent of the total added CE counts at zero time that had been transferred to the VLDL + LDL at each time point.

Statistics

Student's *t* test was used to determine the significance of the differences of mean values observed in CET between the control and diabetic monkeys. Linear relationships between variables were estimated by using Spearman rank correlation coefficients.

RESULTS

Table 1 shows the plasma lipid measurements and estimates of glycemic control in the diabetic and control mon-

TABLE 1. Plasma lipids, lipoprotein lipid composition, and estimates of glycemic control in nine diabetic and nine control cynomolgus monkeys

	Diabetic	Control
	mg/dl	
Plasma		
Total cholesterol	180.0 ± 83.0	140.0 ± 29.0
Cholesteryl ester (CE)	127.0 ± 60.0	101.0 ± 22.0
Free cholesterol	52.9 ± 25.7	39.4 ± 9.7
Triglyceride (TG)	303.0 ± 294.0[a]	85.0 ± 38.0
TG/CE ratio	2.1 ± 1.1[b]	.88 ± .46
Glucose	177.0 ± 90.0[c]	68.0 ± 11.0
Glycated hemoglobin (%)	10.7 ± 4.1[c]	3.8 ± 0.8
VLDL		
Total cholesterol	27.9 ± 40.2	2.40 ± 2.6
Cholesteryl ester	3.2 ± 3.7	0.61 ± 0.21
Free cholesterol	11.2 ± 15.0	0.92 ± 0.26
Triglyceride	124.0 ± 156.0	10.7 ± 3.4
TG/CE ratio	13.6 ± 8.5	16.1 ± 6.9
LDL		
Total cholesterol	77.3 ± 30.3	74.5 ± 11.2
Cholesteryl ester	54.1 ± 22.8	53.8 ± 7.9
Free cholesterol	23.2 ± 8.2	20.7 ± 5.6
Triglyceride	68.2 ± 60.5	28.2 ± 8.9
TG/CE ratio	1.19 ± 0.64[b]	0.53 ± 0.16
HDL_2		
Total cholesterol	12.2 ± 7.6	9.6 ± 6.3
Cholesteryl ester	7.5 ± 4.5	6.3 ± 4.1
Free cholesterol	4.7 ± 3.0	3.3 ± 2.4
Triglyceride	9.6 ± 10.4	3.2 ± 2.0
TG/CE ratio	1.22 ± 0.73[a]	0.58 ± 0.33
HDL_3		
Total cholesterol	26.9 ± 8.3	24.1 ± 7.6
Cholesteryl ester	17.9 ± 6.5	14.1 ± 5.6
Free cholesterol	9.0 ± 2.1	10.0 ± 3.0
Triglyceride	27.1 ± 5.0	24.6 ± 4.9
TG/CE ratio	1.74 ± 0.79	2.0 ± 0.92

Values given as mg/dl (mean ± SD) unless otherwise noted; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.005$.

keys. As expected, fasting glucose and GHb levels were increased significantly in the diabetic group ($P < 0.005$). Cholesterol (total, free, and unesterified) tended to be higher, but due to the wide variances observed, the differences were not statistically significant. Plasma triglyceride concentrations, however, were elevated significantly in the diabetic animals ($P < 0.05$), as well as the triglyceride:cholesteryl ester ratio ($P < 0.01$).

The CET responses of the diabetic and control monkeys differed markedly (Fig. 1 and 2). In the mass assay (**Fig. 1**), CET in controls was delayed initially and increased slowly in a curvilinear manner. In contrast, the response of the diabetic animals was characterized by a rapid initial increase that was significantly greater than that of controls at 1 and 2 h ($P < 0.005$ and $P < 0.001$, respectively).

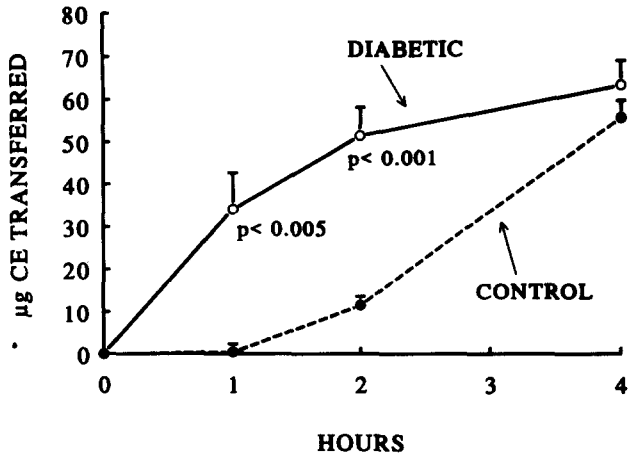

Fig. 1. Mass of cholesteryl ester (CE) transferred in intact plasma from HDL to the apoB-containing lipoproteins in diabetic (solid line) and control (dashed line) cynomolgus monkeys during incubation for 4 h.

Similar differences in transfer between diabetic and control monkeys were observed with the isotopic assay (**Fig. 2**). Whereas the rate at which isotopically labeled cholesteryl ester was transferred from HDL increased slowly in controls, transfer in the diabetic group was accelerated initially, increased with time, and was significantly greater than that of controls at all sampling intervals ($P < 0.001$). Consistent with the increased activity, CETP mass in the subset of animals examined tended to be higher in the diabetic group (diabetic 4.06 ± 0.73 µg/ml vs. control 3.05 ± 0.93 µg/ml; $P < 0.1$). Glycemic control expressed as the glycated hemoglobin concentration correlated positively with the magnitude of transfer estimated both by the cholesteryl ester mass transferred at 2 h ($r = 0.75$, $P < 0.001$; **Fig. 3**) and by k values for the isotopic assay ($r = 0.64$;

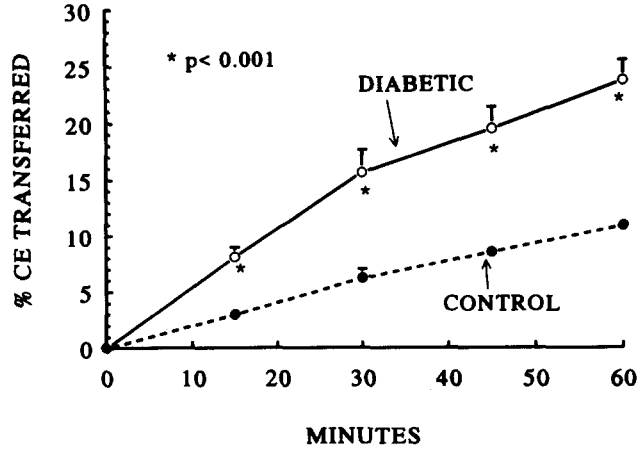

Fig. 2. Transfer of radiolabeled cholesteryl ester (CE) from HDL to the apoB-containing lipoproteins in diabetic (solid line) and control (dashed line) cynomolgus monkeys.

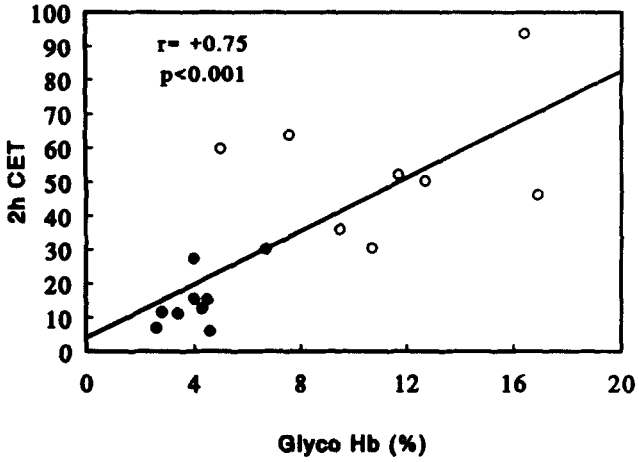

Fig. 3. Relationship between glycosylated hemoglobin concentrations (GHb) and mass of cholesteryl ester transferred from HDL to the apoB-containing lipoproteins at 2 h (2h CET) in diabetic (open circles) and control (closed circles) cynomolgus monkeys.

$P < 0.005$) in the pooled group (**Fig. 4, Table 2**). The mass of cholesteryl ester transferred at 2 h and the isotopic transfer also were directly related ($r = 0.69$; $P < 0.001$).

To assess whether alterations in lipoprotein composition might contribute to enhanced CET in the diabetic monkeys and to determine whether this disturbance in CET altered the content of lipoprotein core lipids, the lipid (Table 1) and phospholipid (**Table 3**) composition of each major lipoprotein class was determined. In the diabetic animals, all VLDL lipids were present in greater concentrations than in VLDL of the control monkeys. However, relative to the mass of TG present in diabetic VLDL which was on the average more than 10-fold greater than in control VLDL, the content of CE was

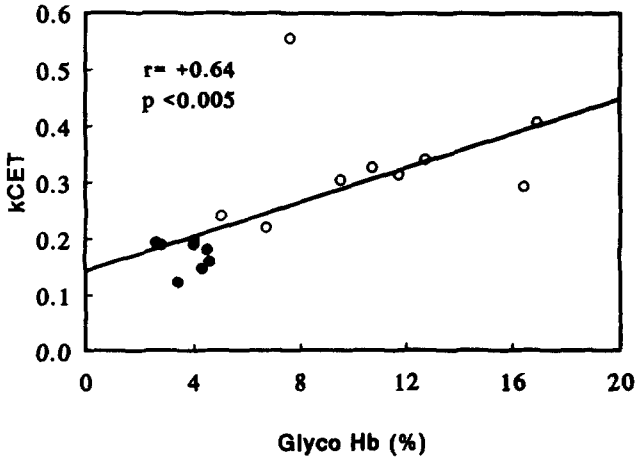

Fig. 4. Relationship between glycosylated hemoglobin concentrations (GHb) and the transfer of radiolabeled cholesteryl ester (kCET) from HDL to apoB-containing lipoproteins in diabetic (open circles) and control (closed circles) cynomolgus monkeys.

TABLE 2. Statistical correlations between glycemic control and cholesteryl ester transfer

Animals (n)	Fasting Glucose	GHb (%)	2-Hour CET	kCET
All (18)				
Fasting glucose		0.57[b]	0.38	0.42
GHb	0.57[b]		0.75[c]	0.64[c]
2-Hour CET	0.38	0.75[d]		0.69[c]
kCET	0.42	0.64[c]	0.69[d]	
Controls (9)				
Fasting glucose		0.08	0.72[a]	0.62
GHb	0.08		0.25	−0.22
2-Hour CET	0.72[a]	0.25		0.40
kCET	0.62	−0.22	0.40	
Diabetics (9)				
Fasting glucose		0.14	−0.42	−0.23
GHb	0.14		0.34	0.17
2-Hour CET	−0.42	0.34		0.17
kCET	−0.23	0.17	0.17	

CET, cholesteryl ester transfer; GHb, glycated hemoglobin; k, isotopic transfer.
[a] $P < 0.05$.
[b] $P < 0.025$.
[c] $P < 0.005$.
[d] $P < 0.001$.

somewhat higher than in controls, tending to decrease the VLDL triglyceride:cholesteryl ester ratio. The cholesterol (total, esterified, and free) content of LDL was very similar in diabetic and control animals. Although the LDL triglyceride content was considerably higher in the diabetic group, owing to the large variance, the difference did not reach statistical significance. Nevertheless, this increase in core triglyceride was sufficient to raise significantly the diabetic animals' LDL triglyceride: cholesteryl ester ratio ($P < 0.01$).

The profile of differences in the lipid content of the HDL subfractions was similar to that of LDL. Total, free, and esterified cholesterol and triglyceride concentrations in both HDL_2 and HDL_3 tended to be higher in the diabetic than in the control monkeys, although these differences were not statistically significant. In HDL_2 from diabetic monkeys, the increases in triglyceride and cholesteryl ester were disproportionate compared to control monkeys, so that the triglyceride:cholesteryl ester ratio was significantly increased ($P < 0.05$). Core lipids in HDL_3 were similar in the two groups.

Most phospholipid levels were significantly increased in whole plasma in the diabetic monkeys and paralleled the increases in plasma lipids in those animals (Table 3). The specific increases in plasma lysolecithin and phosphatidylethanolamine reflected elevations that were present in all of the lipoprotein fractions and reached significance in LDL, HDL_2, and HDL_3 for lysolecithin and in all fractions for phosphatidylethanolamine. Phosphatidylinositol and phosphatidylserine also tended to be

TABLE 3. Lipoprotein phospholipid composition in nine diabetic and nine control cynomolgus monkeys

	Lysolecithin	Sphingomyelin	Lecithin	PI + PS	PE	S/L	FC/L
			μmol/ml			mol/mol	
Plasma							
Diabetic	0.224 ± 0.08[c]	0.144 ± 0.04	2.70 ± 0.88[b]	0.112 ± 0.04[b]	0.212 ± 0.01[d]	0.054 ± 0.07[d]	0.50 ± 0.11
Control	0.122 ± 0.04	0.141 ± 0.03	1.90 ± 0.40	0.069 ± 0.01	0.107 ± 0.03	0.074 ± 0.00	0.54 ± 0.10
VLDL							
Diabetic	0.017 ± 0.02	0.015 ± 0.02	0.146 ± 0.13[b]	0.019 ± 0.02[b]	0.031 ± 0.03[a]	0.049 ± 0.05[a]	1.22 ± 0.79
Control	0.005 ± 0.004	0.004 ± 0.004	0.036 ± 0.04	0.006 ± 0.01	0.005 ± 0.01	0.235 ± 0.25	1.15 ± 0.50
LDL							
Diabetic	0.036 ± 0.02[a]	0.044 ± 0.01	0.687 ± 0.34	0.030 ± 0.01	0.049 ± 0.02[b]	0.073 ± 0.02[c]	0.94 ± 0.19
Control	0.020 ± 0.01	0.050 ± 0.01	0.476 ± 0.08	0.022 ± 0.01	0.028 ± 0.01	0.105 ± 0.02	1.13 ± 0.27
HDL_2							
Diabetic	0.023 ± 0.01[a]	0.021 ± 0.01	0.331 ± 0.23	0.026 ± 0.01	0.034 ± 0.02[a]	0.078 ± 0.03	0.37 ± 0.06[a]
Control	0.013 ± 0.01	0.018 ± 0.01	0.193 ± 0.16	0.021 ± 0.01	0.020 ± 0.01	0.113 ± 0.04	0.47 ± 0.11
HDL_3							
Diabetic	0.102 ± 0.04[b]	0.044 ± 0.01	0.978 ± 0.32	0.049 ± 0.02[b]	0.071 ± 0.03[a]	0.047 ± 0.02	0.27 ± 0.14
Control	0.063 ± 0.02	0.044 ± 0.01	0.908 ± 0.10	0.032 ± 0.00	0.048 ± 0.01	0.048 ± 0.01	0.29 ± 0.09

Values given as mean ± SD; FC, free cholesterol; L, lecithin; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; S, sphingomyelin.

[a] $P < 0.05$.
[b] $P < 0.025$.
[c] $P < 0.005$.
[d] $P < 0.001$.

higher in the diabetic than in the control group, but the differences were only significant in VLDL and HDL_3 ($P < 0.025$). There was a generalized increase in lecithin in the plasma ($P < 0.025$) as well as in all the lipoprotein fractions, which reached statistical significance only in VLDL ($P < 0.025$). By contrast, sphingomyelin differed little compared to the changes observed in lecithin in the diabetic group. As a result, the sphingomyelin:lecithin ratio was significantly decreased in plasma ($P < 0.001$), VLDL ($P < 0.05$), and LDL ($P < 0.005$). Free cholesterol tended to be higher in plasma and lipoproteins in the diabetic group, particularly in VLDL. As this increase was associated with a relatively greater increase in lecithin in HDL_2, the free cholesterol:lecithin ratio of the diabetic animals was actually lower than that of the control monkeys.

DISCUSSION

Reduced concentrations of HDL and increased concentrations of LDL cholesterol have been shown repeatedly to accelerate the development of atherosclerosis in nondiabetic human (18, 19) and nonhuman (20, 21) primates. Because HDL and LDL cholesterol concentrations are often normal in diabetic patients (22), other factors likely play a role in promoting the development of macrovascular complications. The normal plasma lipid profile and normal or even increased HDL cholesterol concentrations in human beings with IDDM (23) have suggested that lipoprotein compositional abnormalities not apparent in routine lipid measurements may compromise lipoproteins functionally and thereby contribute to atherogenesis. Results of the current study indicate that cynomolgus monkeys that spontaneously developed diabetes mellitus have a plasma lipid profile like that of diabetic human beings with respect to normal HDL and LDL cholesterol concentrations. However, the composition of their plasma lipoproteins differs in a number of significant ways from that of diabetic humans. Although the lipolytic enzymes have not been measured in cynomolgus monkeys with diabetes, it is probable that the triglyceride elevation in those animals that were relatively more insulinopenic was attributable to an impairment in the activity of lipoprotein lipase which is insulin-dependent and plays a central role in the catabolism of triglyceride-rich lipoproteins (24).

The diabetic monkeys demonstrated an abnormal acceleration in CET similar to that described in human beings with IDDM and non-insulin-dependent diabetes mellitus (4–6). This recently recognized disturbance in lipoprotein function may provide new insight into why diabetic patients who have normal plasma lipid concentrations are still predisposed to lipid accumulation within cells of the arterial wall. An increase in CET may promote the development of macrovascular complications because CET generates lipoprotein particles that resemble atherogenic postprandial lipoproteins, which also are enriched in cholesteryl esters. This defect was documented with two experimental systems. With the mass transfer assay, which estimates the net amount of cholesteryl ester transferred from HDL to VLDL + LDL, the diabetic monkeys showed the same distinctive rapid initial acceler-

ation of CET reported in people with diabetes and dyslipidemia. Control monkeys, on the other hand, showed a nonlinear response and an early delay in net transfer identical to those in nondiabetic control subjects in other studies (1, 3, 4, 25). This lack of early net transfer in controls has been shown by VanTol, Sheek, and Groener (25) to be due to the fact that more cholesteryl ester is transferred from LDL to HDL than from HDL to VLDL. Despite these differences during the early portion of the CET reaction in vitro, however, by 4 h both groups had transferred the same net mass of CE. This finding suggests that gradients present among the lipoprotein core lipids that reflect differences in composition or their accessibility to CETP are important determinants of the nature of the CET response.

With the isotopic assay, the diabetic monkeys also demonstrated increased movement of radiolabeled cholesteryl ester from HDL to VLDL + LDL. Because results of assays that estimate the isotopic transfer of CET correlate closely with CETP mass in plasma (14), the increase found in isotopic transfer in the diabetic group suggested that their CETP mass may be increased, as in humans with IDDM (4). This was supported by the trend toward higher CETP concentrations observed in the subset of five diabetic and control animals studied. The 5-fold increase in both mass and isotopic transfer of cholesteryl ester in these diabetic cynomolgus monkeys, however, exceeds by many times the 30% increase we found in their CETP mass. This finding suggests that in both diabetic humans and nonhuman primates, other factors, such as the composition of the acceptor and donor lipoproteins (26) or reduced activity of an inhibitory protein (9) may influence CETP-lipoprotein interactions and modulate the extent of cholesteryl ester transfer. As we have recently observed in IDDM patients (27), we found in the present study that diabetic glycemic control expressed as glycated hemoglobin correlated closely with the magnitude of CET in each assay, although this important relationship became apparent only when the diabetic and control groups were combined. As Mann et al. (2) have previously reported that CETP activity correlates closely with plasma VLDL TG concentrations, a similar correlation between TG and CET might be expected in the diabetic and control animals in the present study. The fact that we were unable to demonstrate this relationship suggests that in this species diabetes and glycemic control may independently influence the regulation of CET and possible associations between CET and VLDL particle number.

The composition of the apoB-containing lipoproteins in fasting plasma reflects the post-secretory modifications that take place in plasma as a consequence of the lipoprotein lipase, LCAT, and neutral lipid transfer system. No disturbances in the core lipid composition of newly secreted apoB-containing lipoproteins have yet been described in insulin-requiring humans or experimental animals. While it is still possible that the diabetic cynomolgus monkeys secrete VLDL with altered composition, we believe that the specific alterations we found in lipoprotein core lipid composition also are consistent with increased CET activity occurring in vivo, as we have shown in our ex vivo assay system. If the heteroexchange of neutral lipids between VLDL and HDL was increased, as we believe our data demonstrate, one would expect to find evidence of relative enrichment of HDL_2 with triglyceride, and conversely of VLDL with cholesteryl ester. Indeed, a profile of the directional changes in the triglyceride:cholesteryl ester ratios in these lipoprotein fractions is consistent with this predicted profile, although the change in VLDL was not statistically significant. In our studies in humans we found a similar trend to a reduced triglyceride:cholesteryl ester ratio in the whole VLDL fraction (4). However, when we examined the CET-promoting activity of the VLDL subfractions, we observed differences in their behavior (4): the activity of IDDM $VLDL_1$ was increased and that of $VLDL_2$ and $VLDL_3$ was normal. In related studies (M. C. Ritter and J. D. Bagdade, unpublished observations), we have found that IDDM $VLDL_1$ had a significant reduction in the triglyceride:cholesterol ester ratio, while that of IDDM $VLDL_2$ and $VLDL_3$ did not differ from those of control subjects. Further studies need to be done to determine whether the selective changes in VLDL subfraction core lipids we have observed in IDDM humans are also found in cynomolgus monkeys, as the trend in their core lipid composition suggests. While the significant increase in the LDL triglyceride:cholesteryl ester ratio of the diabetic monkeys was presumably a reflection of increased neutral lipid transfer between VLDL and LDL, this same change in their LDL and HDL_2 could also reflect the fact that monkeys have less hepatic lipase (28).

In contrast to the resemblance noted in the core lipid compositions of nonhuman primates and humans apparently related to increased CET, distinct differences were present in surface lipid composition. Compared to humans, monkeys have less lipoprotein free (unesterified) cholesterol relative to the major phospholipid lecithin. This relationship does not appear to be altered by diabetes and insulin treatment in cynomolgus monkeys, because the free cholesterol:lecithin ratios in plasma were similar in the diabetic and control groups. In contrast, in humans with IDDM, the plasma free cholesterol:lecithin ratio is abnormally increased (29, 30). This specific compositional disturbance was shown to be a risk factor for cardiovascular disease in a nondiabetic Lipid Research Clinic population (31).

In the current study, despite the similarity of free cholesterol:lecithin ratios in whole plasma in the two groups, noteworthy differences were still present among the lipoproteins. In humans with IDDM, the free cholesterol:lecithin ratio is increased in both VLDL and

HDL_2 and reduced in LDL (4, 30). Although the diabetic monkeys' free cholesterol:lecithin ratio also tended to be lower in LDL, it was significantly decreased in HDL_2. Gradients for free cholesterol between the lipoproteins in plasma, and between lipoproteins and cells expressed by free cholesterol:lecithin ratios, are believed to be an important determinant of cholesterol directional flux (32). Indeed, our prior studies in human subjects showing that VLDL from IDDM subjects was enriched in free cholesterol and that CET was accelerated were consistent with earlier studies by Morton (33) that showed that lipoprotein FC content was a positive regulator of CET. The fact that lipoproteins in these monkeys were not FC-enriched but still were abnormally engaged in CET suggests that mechanisms other than lipoprotein FC content drive CET in this species. One possibility is that diabetes decreases the activity of a protein in plasma that has been shown to inhibit lipid transfer protein (34).

In conclusion, diabetic cynomolgus monkeys have changes in CET and core lipid lipoprotein composition that are similar to those seen in diabetic patients. As recently reported in humans (27), the severity of the defect in CET was directly related to glycemic control. Consistent with an increase in CET activity in vivo in diabetic monkeys, the triglyceride:cholesteryl ester core lipid ratio was decreased in VLDL and increased in LDL and HDL_2. These changes in lipid composition resulting from CET may produce more atherogenic lipoprotein particles, which may explain the increased risk of macrovascular complications in diabetic patients despite a general lack of significant changes in lipoprotein concentration.

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