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Increased free cholesterol in plasma low and very low density lipoproteins in non-insulin-dependent diabetes mellitus: Its role in the inhibition of cholesteryl ester transfer

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ABSTRACT **Recombination of low and very low density lipoproteins (VLDL and LDL) from normal subjects with plasma from patients with non-insulin-dependent diabetes mellitus significantly increased the reduced rate of transfer of cholesteryl ester to these lipoproteins, which is characteristic of diabetic plasma, whereas diabetic VLDL and LDL reduced cholesteryl ester transfer rates in normal plasma. VLDL and LDL from diabetic plasma had an increased ratio of free cholesterol to phospholipid compared to normal, and unlike normal VLDL and LDL spontaneously lost free cholesterol to high density lipoprotein. These data suggest that the block to cholesteryl ester transfer to these lipoproteins in non-insulin-dependent diabetes is mediated by their increased free cholesterol content and may be related to the increased risk of these patients for developing atherosclerosis.**

Although diabetes mellitus is defined by abnormal carbohydrate metabolism, it has been apparent for some time that disturbances of lipid metabolism are also commonly seen in this syndrome. This is particularly true of patients with non-insulin-dependent diabetes mellitus (NIDDM). The plasma lipoprotein pattern of these individuals often shows increased levels of very low and low density lipoproteins (VLDL and LDL) and a decreased concentration of high density lipoprotein (HDL) (1–3). Since these changes have been identified in epidemiological studies as factors associated with increased risk of coronary artery disease (4, 5), they might be expected to play a role in the accelerated atherosclerosis associated with NIDDM (6). In this regard, we have recently described (3) a consistent pattern of abnormal cholesterol metabolic activity in the plasma of patients with NIDDM, which may provide a link between defects in lipid metabolism and atherogenesis in this form of diabetes. Specifically, the transfer of cholesteryl esters synthesized by lecithin:cholesterol acyltransferase (LCATase) to VLDL and LDL is inhibited, and these esters are transferred instead to HDL. Two further observations increase the potential significance of this abnormal pathway. First, a similar defect has now been identified in the plasma of several other patient groups at high risk for coronary artery disease (7). Second, the abnormal plasma cholesterol metabolism of the diabetic is normalized by the institution of good diabetic control with insulin (3). In this paper we provide evidence that the block in cholesteryl ester transfer activity in patients with NIDDM is secondary to a change in the properties of VLDL and LDL, which is the result of an increase in the free cholesterol content of these lipoprotein particles.

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MATERIALS AND METHODS

Subject Selection. Seven patients meeting the criteria for diagnosis of NIDDM (8) and eight normoglycemic controls were selected for this study. Mean glucose levels were 291 ± 16 and 93 ± 2 mg/dl in the two groups, mean cholesterol levels were 241 ± 19 and 158 ± 7 mg/dl, and mean triglyceride levels were 282 ± 61 and 75 ± 4 mg/dl (±SEM) (9–11). None of the diabetics had received any specific therapy for their hyperglycemia. Other characteristics of the diabetic and control groups were similar to those described (3). Blood was collected after an overnight fast into 0.05 vol of ice-cooled 0.2 M sodium citrate (pH 7.0). Plasma was obtained by centrifugation at 2000 × *g* for 30 min at 0–2°C and used immediately for the assays described below.

Determination of the Rate of Cholesterol Esterification in Plasma. Plasma was brought to 0.01 M with Tris·HCl buffer (pH 7.4) and to 1 mM with Na_2EDTA. Pentuplicate samples taken at zero time were extracted with chloroform/methanol (12), and free cholesterol concentration was determined fluorimetrically (13). Cholesterol esterification rate was calculated as the rate of decrease in plasma free cholesterol when plasma was incubated at 37°C (3, 7). This decrease was inhibited (>97%) by inclusion of 1.5 mM 5,5′-dithiobis(2-nitrobenzoic acid) (Nbs_2), an inhibitor of LCATase (14), in the incubation medium. This demonstrates that LCATase was the major or only source of *de novo* synthesis of cholesteryl esters in plasma under these conditions.

Determination of Cholesteryl Ester Transfer. Plasma was brought to 0.01 M Tris·HCl (pH 7.4) and 1 mM Na_2EDTA and then mixed with 0.1 vol of 15 mM Nbs_2 in 0.1 M phosphate buffer (pH 7.4) to inhibit LCATase (14). An initial sample was mixed with 0.05 vol of 2 M $MgCl_2$ and 0.05 vol of dextran sulfate (20 mg/ml; Pharmacia, Uppsala, Sweden) to precipitate total VLDL and LDL. After standing in ice for 30 min, the precipitate was removed by centrifugation (2000 × *g*, 30 min, at 0–2°C) and pentuplicate samples of the supernatant solution were extracted and taken for analysis of free and esterified cholesterol (13). The remainder of the plasma + Nbs_2 was then incubated at 37°C for 60 min and samples were taken as before for precipitation, centrifugation, and analysis. We have previously shown (15) that while the generation of cholesteryl esters by LCATase is inhibited by Nbs_2, the transfer of preformed cholesteryl esters from HDL to VLDL + LDL is unchanged and linear with time under these conditions. Accordingly, the decrease in supernatant (HDL) cholesteryl ester as a function of time represents the rate of accumulation of cholesteryl ester in the precipitated

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LCATase, lecithin:cholesterol acyltransferase; NIDDM, non-insulin-dependent diabetes mellitus; Nbs_2, 5,5′-dithiobis(2-nitrobenzoic acid).

VLDL and LDL. Any change in supernatant free cholesterol under the same conditions of incubation represents unlinked (LCATase-independent) net transfer of free cholesterol between HDL and VLDL + LDL.

Heparin-Agarose Column Chromatography. Chromatography was carried out at 4°C by using columns (1 × 40 cm) of heparin-agarose (Pharmacia) equilibrated with 0.15 M NaCl/1 mM Na_2EDTA, pH 7.4 (saline/EDTA). Plasma (3–4 ml) was passed through at 2–4°C at a flow rate of 10–15 ml/hr, and fractions of nonadsorbed material containing detectable protein (16) or cholesterol (13) were pooled (2–3 plasma vol). After the column had been washed with a further 10 column vol of saline/EDTA, the adsorbed lipoproteins were eluted with 3 M NaCl/1 mM EDTA in ≈2 plasma vol and dialyzed against saline/EDTA. These lipoproteins were either reincorporated with the nonadsorbed fraction of plasma for studies of cholesterol metabolic activities or were fractionated by ultracentrifugation (35,000 rpm, 18 hr, 2–4°C) at ρ 1.019 g/ml for analysis of lipoprotein lipid and protein composition. The lipoproteins were brought to the indicated density with KBr solution and centrifuged in a Beckman L3-40 or L3-50 preparative ultracentrifuge. In some studies the infranatant (LDL) fraction was recentrifuged at ρ 1.063 g/ml under the same conditions. The nonadsorbed fraction from heparin-agarose affinity chromatography was also centrifuged at ρ 1.019 g/ml, and floating lipoprotein was recovered for analysis.

Lipid and Lipoprotein Analysis. Immunoassays of lipoprotein proteins B and E were performed by the method of Mancini *et al.* (17), as described (18, 19).

Agarose strip gel electrophoresis of lipoproteins was carried out with 0.5% agarose in barbital buffer (pH 7.4) on polyester film (Gelbond, Bioproducts, Rockland, ME), essentially as described by Noble (20). After development the strips were fixed in ethanol/acetic acid/water (75:5:20), air dried, stained in sudan black in 60% (vol/vol) ethanol/water, and then destained in ethanol/water.

The lipid composition of isolated plasma lipoproteins was determined after extraction into chloroform/methanol (12). Portions of chloroform phase were taken for fluorimetric determination of free and esterified cholesterol (13). Triglyceride and phospholipid were separated by thin-layer chromatography on layers of silicic acid on glass plates (Merck, Darmstadt, F.R.G.) and developed in hexane/diethyl ether/acetic acid, 83:16:1 (vol/vol/vol). Triglyceride glycerol was determined after periodate oxidation with chromotropic acid (21). Lipid phosphorus was determined with ammonium molybdate (22). Lipoprotein cholesteryl esters, separated by thin-layer chromatography as described above, were hydrolyzed with methanol/sulfuric acid (23). Fatty acid methyl esters were analyzed by gas/liquid chromatography (model 2920B with M-1 computerized integrator; Perkin–Elmer, Norwalk, CT), using a column of 5% DEGS-PS on 100/200 Supelcoport (Supelco, Bellefonte, PA).

RESULTS

When plasma from normoglycemic donors was incubated at 37°C, there was a mean increase in the amount of cholesteryl ester present in VLDL + LDL (15.6 ± 4.8 μg/ml of plasma per hr). Since LCATase activity was inhibited by Nbs_2, this represents the amount of cholesteryl ester transferred from HDL to VLDL and LDL. This transfer rate was significantly greater ($P < 0.005$) than the value of 3.1 ± 2.5 μg/ml of plasma per hr from patients with NIDDM. These results are similar to our previous observations (3), with subjects showing lower triglyceride levels, that cholesteryl ester transfer to VLDL + LDL in these diabetics is markedly reduced. Plasma from which VLDL and LDL had been removed by heparin-agarose affinity chromatography showed no detectable cholesteryl ester transfer in the precipitation assay (see *Materials and Methods*), indicating that only lipoproteins retained by heparin-agarose were of significance in cholesteryl ester transfer. In contrast to the finding with cholesteryl ester transfer, cholesteryl ester synthesis—i.e., the total increase in plasma cholesteryl ester—was similar in normal and diabetic subjects (28.6 ± 12.1 vs. 27.6 ± 7.3 μg of cholesterol esterified per ml of plasma per hr; $P > 0.1$), and in neither group was this rate reduced significantly by the removal of VLDL + LDL from the plasma. These findings indicated that the defect of cholesterol metabolism in diabetic plasma lay not in the generation of cholesteryl esters but in their transfer to HDL, rather than to VLDL and LDL.

One explanation of this defect in transfer would be that the acceptor lipoproteins (VLDL and LDL) present in diabetic plasma were themselves abnormal. To test this hypothesis, the nonadsorbed fraction of diabetic plasma from heparin-agarose was recombined with the VLDL + LDL fraction from normoglycemic plasma, prepared by the same fractionation technique. As shown in Fig. 1, the cholesteryl ester transfer rate, which was low in native diabetic plasma, was markedly stimulated ($P < 0.02$). Conversely, addition of VLDL + LDL from the plasma of NIDDM patients to the nonadsorbed fraction of control plasma markedly decreased ($P < 0.05$) the rate of cholesteryl ester transfer observed (Fig. 1). These experimental results indicate that the abnormality in plasma from diabetic subjects that inhibits cholesteryl ester transfer lies in the VLDL and LDL retained by heparin-agarose.

Agarose gel electrophoresis indicated that the nonadsorbed fraction from heparin-agarose gel chromatography of plasma from either normal or diabetic subjects contained not only HDL but also a pre-β-migrating fraction recovered in the supernatant fraction by centrifugation at a medium density of ρ 1.019 g/ml. The adsorbed lipoproteins contained the β-migrating LDL and a second pre-β band recovered by flotation at ρ 1.019 g/ml. These electrophoretic patterns were unchanged following a second fractionation of the same material on heparin-agarose. Therefore, plasma from both normal subjects and patients with NIDDM contained two fractions of VLDL, separable by heparin-agarose chromatography. However, since cholesteryl ester transfer was not detectable in the nonadsorbed fraction of plasma from either normal or diabetic individuals, the nonadsorbed fraction of VLDL did not play a major role in transfer in the plasma.

The nonadsorbed and adsorbed fractions of VLDL and LDL were purified by flotation and their compositions were determined. As shown in Table 1, the nonadsorbed fraction

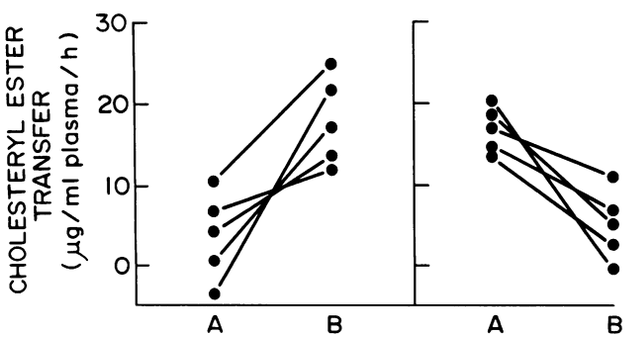

FIG. 1. Effects of the reconstitution of VLDL + LDL isolated from normal plasma with plasma from diabetic patients (left) or VLDL + LDL isolated from diabetic subjects with plasma from normal individuals (right) on cholesteryl ester transfer activity. A, Whole native plasma; B, reconstituted plasma. Plasma was passed through heparin-agarose and the nonadsorbed fraction was mixed in the original proportions of plasma with adsorbed lipoproteins eluted with 3 M NaCl and then dialyzed vs. 0.15 M NaCl/1 mM EDTA, pH 7.4.

Table 1. Lipid and protein composition of VLDL and LDL from plasma of diabetic and normal subjects

Lipoprotein	Composition, weight %						
	Protein	Free cholesterol	Phospholipid	Triglyceride	Cholesteryl ester	FC/PL	CE/TG
Unadsorbed VLDL							
Diabetic	5.8 ± 1.1	5.3 ± 0.3	9.6 ± 0.2	75.0 ± 2.0	4.5 ± 0.5	0.54 ± 0.02	0.06 ± 0.01
Normoglycemic	6.0 ± 1.6	4.6 ± 0.5	12.8 ± 2.4	71.6 ± 3.3	6.4 ± 1.8	0.37 ± 0.04	0.08 ± 0.01
Adsorbed VLDL							
Diabetic	11.5 ± 2.1	6.9 ± 2.0	13.8 ± 4.3	54.1 ± 10.5	13.7 ± 4.2	0.50 ± 0.04	0.27 ± 0.13
Normoglycemic	10.4 ± 1.3	5.8 ± 1.4	15.2 ± 4.1	53.4 ± 6.2	13.9 ± 3.6	0.39 ± 0.04	0.25 ± 0.09
LDL							
Diabetic	25.0 ± 1.7	10.8 ± 1.5	21.5 ± 3.7	7.7 ± 4.9	35.0 ± 3.3	0.50 ± 0.03	6.3 ± 3.3
Normoglycemic	25.0 ± 1.7	8.6 ± 0.7	20.9 ± 1.6	3.5 ± 1.1	41.9 ± 1.8	0.41 ± 0.02	13.6 ± 6.5

FC, free unesterified cholesterol; PL, phospholipid; CE, cholesteryl ester; TG, triglyceride. Values are means ± SD for five experiments. Adsorbed VLDL, VLDL retained with LDL on heparin-agarose in 0.15 M NaCl; unadsorbed VLDL, VLDL eluted from heparin-agarose in 0.15 M NaCl. Cholesteryl ester mass was calculated as ester cholesterol × 1.72.

of VLDL from both normal and diabetic donors showed a higher concentration of triglyceride and a lower concentration of cholesteryl ester (both $P < 0.001$) than the adsorbed VLDL. The major cholesteryl ester fatty acid in both VLDL fractions and LDL from both diabetic and normal plasma was linoleate ($C_{18:2}$) (data not shown), suggesting the origin of most of the cholesteryl ester in each lipoprotein from the LCATase reaction (24). Also evident was a doubling of the triglyceride content of LDL isolated from diabetic plasma, a finding in agreement with earlier data (1). However, such a difference was not present when the VLDL of normal and diabetic plasma were compared. There was no difference between diabetic and normal plasma in the apoprotein composition of the equivalent lipoprotein fractions (data not shown). The nonadsorbed VLDL fraction of both diabetic and control plasma contained no detectable apolipoprotein E, while the apolipoprotein E/apolipoprotein B mass ratios of adsorbed VLDL, determined by specific immunoassay, were 0.55 ± 0.17 and 0.55 ± 0.14, respectively. The only observed consistent difference between each of the lipoproteins isolated from diabetic plasma and the corresponding lipoprotein from normal plasma lay in the consistently higher ratio ($P < 0.005$) of free cholesterol to phospholipid present in the lipoproteins from patients with NIDDM. If this difference were important in the inhibition of cholesteryl ester transfer in diabetic plasma, transfer would be expected to decrease as the free cholesterol/phospholipid weight ratio became greater. This relationship is observed in the data in Fig. 2.

If the free cholesterol content of VLDL and LDL from diabetic patients were increased and if this increase were important in directing cholesteryl esters away from VLDL and LDL, then the existence of an effective chemical potential gradient for cholesterol is implied between these acceptor lipoproteins and HDL. This was investigated directly by measuring whether there was a net transfer of free cholesterol from VLDL + LDL to HDL during incubation of plasma when LCATase activity (which could independently drive such transfer to supply substrate for cholesterol esterification) was inhibited by Nbs_2. The results of these determinations are shown in Fig. 3, which also includes unpublished measurements from a similar group of diabetics whose plasma cholesterol metabolism was studied earlier (3). These results clearly document the fact that there was little or no transfer of free cholesterol from VLDL and LDL to HDL (0.5 ± 1.2 μg/ml of plasma per hr) when LCATase was inhibited in the plasma of normal subjects. This finding indicates that the chemical potential of cholesterol in each of the lipoprotein fractions of normal plasma is equivalent. It can also be seen that this lack of free cholesterol transfer from VLDL and LDL to HDL in normal plasma is associated with substantial rates of cholesteryl ester transfer to VLDL and LDL. Conversely, the chemical potential of cholesterol in diabetic VLDL and LDL was much greater than in diabetic HDL, as was shown by the much greater rate of spontaneous transfer of free cholesterol from these lipoproteins when LCATase was inhibited (6.0 ± 1.7 μg/ml of plasma per hr) ($P < 0.005$) and this was associated with a very low rate of cholesteryl ester transfer. These findings support the concept that increased acceptor free cholesterol content (rather than a decreased phospholipid content) is an important factor in the inhibition of cholesteryl ester transfer in diabetes.

Finally, if an increase in free cholesterol content of VLDL + LDL from diabetic subjects plays a role in the block of cholesteryl ester transfer, recombination experiments similar to those outlined in Fig. 1, which modified cholesteryl ester transfer, should lead to predictable changes in free cholesterol transfer from VLDL + LDL to HDL. The results of such experiments, seen in Fig. 4, support this concept. The rate of cholesteryl ester transfer from VLDL + LDL to HDL that occurs in plasma of normal subjects is seen in column A and confirms the fact that there is essentially no potential difference of free cholesterol between lipoproteins in the plasma of normal subjects. On the other hand, when VLDL + LDL from patients with diabetes are exchanged for

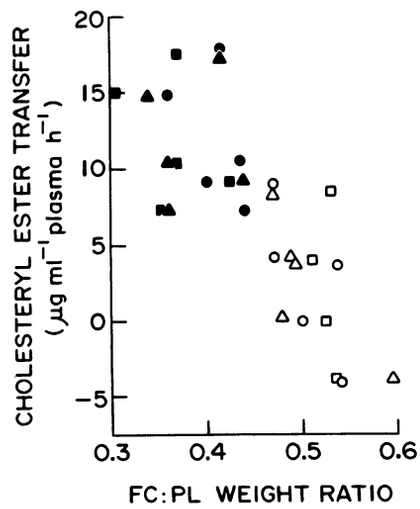

FIG. 2. Relationship between VLDL or LDL free cholesterol/phospholipid (FC/PL) weight ratio and the ability of the same lipoproteins to support cholesteryl ester transfer ($r = -0.82$). Closed symbols, lipoproteins from normal plasma; open symbols, lipoproteins from diabetic plasma. ●, ○, LDL; ▲, △, the adsorbed fraction of VLDL; ■, □, the nonadsorbed fraction of VLDL.

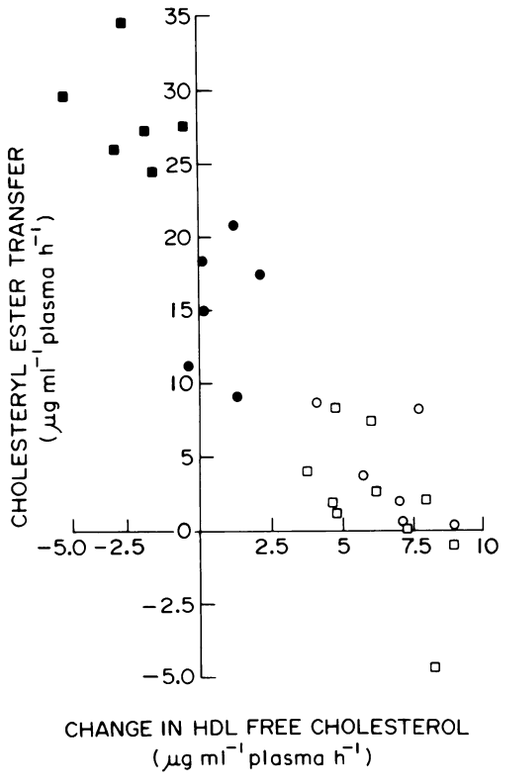

FIG. 3. Relationship between the spontaneous net transfer of cholesterol (in the absence of LCATase-mediated cholesterol esterification) and cholesteryl ester transfer activity ($r = -0.76$). ○, Diabetic plasma; ●, normal plasma. Also shown are the unpublished free cholesterol transfer data from an earlier study (ref. 3): □, diabetic plasma; ■, normal plasma.

VLDL + LDL present in plasma of normal subjects (column B), a significant amount of free cholesterol moves from VLDL + LDL to HDL ($P < 0.02$). Similar changes, but opposite in direction, are seen in columns D and C of Fig. 4. Thus, column D reconfirms the presence of the chemical potential difference in free cholesterol that exists between lipoproteins in native plasma from diabetics, resulting in a substantial transfer of free cholesterol from VLDL + LDL to

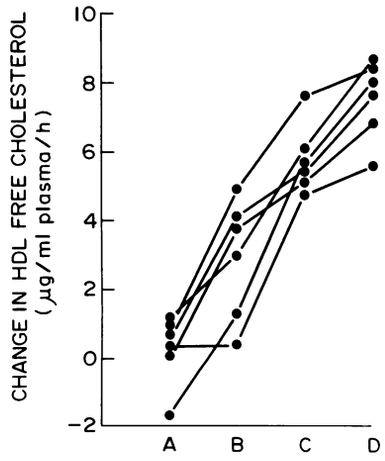

FIG. 4. Effects of added exogenous VLDL + LDL on spontaneous free cholesterol transfer. A, Unfractionated normal plasma; B, the nonadsorbed fraction of normal plasma reconstituted with VLDL + LDL from diabetic plasma; C, the nonadsorbed fraction of diabetic plasma reconstituted with VLDL + LDL from normal plasma; D, unfractionated diabetic plasma. The lines connect the individual values obtained with the same donor pair in each set of experiments.

HDL (dextran sulfate/$MgCl_2$ supernatant fraction). However, the results in column C indicate that the free cholesterol transfer rate in plasma from diabetics is significantly reduced ($P < 0.01$) when the VLDL + LDL from normal patients are exchanged for native VLDL + LDL. These findings provide additional support for the concept that a free cholesterol gradient exists between VLDL + LDL and HDL in the plasma of patients with NIDDM and that the increased free cholesterol content of VLDL + LDL in NIDDM plays a major role in the inhibition of cholesteryl ester transfer to these lipoproteins.

DISCUSSION

The defect of cholesteryl ester transfer in diabetic plasma was largely reversed by the substitution of normal for diabetic VLDL + LDL in diabetic plasma. Conversely, the exchange of diabetic for normal VLDL + LDL in plasma from normal subjects led to a marked decrease in the cholesteryl ester transfer rate seen in the plasma of normal individuals. These data provide strong evidence that the inhibition of cholesteryl ester transfer seen in the plasma of patients with NIDDM is due to an abnormality in the acceptor lipoproteins, VLDL and LDL. The absence of detectable transfer to nonadsorbed VLDL may indicate a role for apolipoprotein E in transfer to VLDL; however, LDL, in the adsorbed fraction, appears in any case to be the major acceptor of cholesteryl ester in plasma (15). Fractions with similar composition to nonadsorbed and adsorbed VLDL, separated by electrophoresis (25) or heparin-Sepharose chromatography (26), have been identified; the former may represent nascent VLDL.

The evidence obtained suggests that it is the increased concentration of free cholesterol, found in both VLDL and LDL isolated from patients with NIDDM, that is responsible for the block of cholesteryl ester transfer. First, the level of free cholesterol relative to phospholipid was increased in both VLDL and LDL isolated from the plasma of patients with NIDDM as compared to normal subjects, and the block in cholesteryl ester transfer was inversely correlated with the increase in free cholesterol/phospholipid ratio of VLDL + LDL. In addition, net transfer of free cholesterol from VLDL + LDL to HDL could be documented in plasma of patients with NIDDM, indicative of the presence of a chemical potential gradient of free cholesterol between plasma lipoproteins in this situation. In contrast, essentially no transfer of free cholesterol from VLDL + LDL to HDL was seen in plasma from normals. Furthermore, the magnitude of the free cholesterol gradient between VLDL + LDL and HDL was inversely correlated with cholesteryl ester transfer rate. Finally, the degree of the free cholesterol gradient was increased in plasma from normal subjects when their VLDL + LDL were substituted with VLDL + LDL from diabetic subjects and decreased in plasma from patients with NIDDM when the corresponding substitution was made. Taken together, these data strongly support the concept that the free cholesterol content of VLDL + LDL is a determinant of cholesteryl ester transfer rate and that the increased free cholesterol/phospholipid ratio of these acceptor lipoproteins in NIDDM is a major factor in the reduced cholesteryl ester transfer rates that are a characteristic of this disease. The finding that cholesteryl ester transfer in diabetics was completely normalized by control VLDL + LDL (Fig. 1) under conditions in which the free cholesterol gradient was only partially reduced probably reflects an additional abnormality in the composition of the acceptor (HDL) fraction of diabetics, although this remains to be determined.

It is clear from the data in Table 1 that cholesteryl esters in fact are present in both the VLDL and LDL isolated from the plasma of these patients. In addition, as stated earlier,

the cholesteryl ester fatty acid spectrum of VLDL and LDL from diabetic subjects is like that of the corresponding normal lipoproteins, rich in linoleate ($C_{18:2}$), as would be expected for esters derived from LCATase activity (24). Nevertheless, direct assay of diabetic plasma indicates a greatly reduced rate of transfer of LCATase-derived cholesteryl esters into VLDL and LDL. The most likely explanation of this apparent paradox is that the VLDL and LDL of diabetics represents the "end-product" of cholesteryl ester transfer to these lipoproteins (15), beyond which further transfer is inhibited. This suggestion is strongly supported by the phase diagram for free cholesterol, phospholipids, and cholesteryl ester + triglyceride (27), which indicates that the composition of VLDL and LDL from patients with NIDDM places them almost exactly on the phase boundary at which crystalline cholesterol hydrate would be expected to separate out at the lipoprotein surface (Fig. 5). This result indicates that major changes in the properties of surface lipids of these lipoproteins are to be expected as the result of a relatively small increase in free cholesterol content, and it provides a physical explanation of the major metabolic changes associated with increased free cholesterol concentration on plasma cholesterol metabolism.

Finally, the relevance of the present findings with NIDDM plasma to those obtained with plasma from other groups at risk for coronary artery disease (7) deserves to be considered. It is now a consistent finding in dysbetalipoproteinemics, hyperbetalipoproteinemics, and diabetics that a block in cholesteryl ester transfer is associated with an uptake of free cholesterol from such plasma by cell membranes. In each of these conditions, the free cholesterol/phospholipid ratio of LDL is significantly increased above that of normal LDL (refs. 28 and 29; Table 1). On the basis of the earlier data (3, 7) and those contained in the present study, we suggest that it may be a common metabolic defect in the plasma of those at risk for atherosclerosis, including diabetics, that an increased acceptor lipoprotein free cholesterol content inhibits the normal pathway of cholesteryl ester transfer, by means of which in normal metabolism cholesterol of peripheral origin is returned to the liver for catabolism.

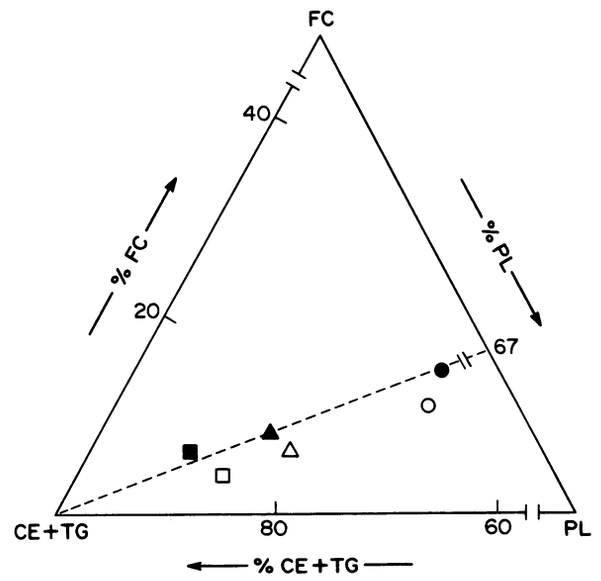

FIG. 5. Phase diagram for the lipoprotein lipid moiety (after ref. 27). FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid. The dashed line represents the boundary between two- and three-phase regions, beyond which cholesterol monohydrate separates as a separate phase. ●, ○, LDL; ▲, △, the adsorbed fraction of VLDL; ■, □, the nonadsorbed fraction of VLDL. Open symbols, lipoproteins from normoglycemic plasma; closed symbols, lipoproteins from diabetic plasma. Values are the means derived from five experiments.

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