# Apolipoprotein B Levels and Altered Lipoprotein Composition in Diabetes

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SUMMARY

**Hyperlipoproteinemia is found in only 25 to 50 per cent of persons with diabetes. However, carbohydrate, lipid and lipoprotein metabolism are so closely related that one would expect some alterations in lipoprotein metabolism in nearly all diabetic subjects. We wondered whether the metabolism of lipoproteins in normolipemic diabetic patients was normal and whether the metabolism of lipoproteins in subjects with hyperlipoproteinemia and diabetes differed from those without diabetes. As a first approach to these questions, the levels of lipoprotein lipids and apolipoproteins were determined in ninety-seven diabetics and seventy-two nondiabetics, and lipoprotein compositions were computed. Among those with diabetes, seventy-one were normolipemic—twelve were type IIa and fourteen were type IV. In each of the diabetic groups, low density and high density lipoproteins were enriched in triglyceride ($p < 0.01$). These alterations in lipoprotein composition were not accounted for by differences in treatment modality, sex, race, age, or ponderal index. Thus the presence of diabetes itself appears to result in altered low and high density lipoprotein compositions. The mechanism(s) for the changes in the high density lipoproteins is (are) unknown, but in light of present knowledge the presence of triglyceride-enriched LDL could mean that a less dense moiety of low density lipoproteins may be accumulating in diabetic plasma, and this could result from altered rates of turnover of ApoB containing lipoproteins. Of course appropriate studies are needed to confirm this formulation.** DIABETES *23*:827-34, October, 1974.

A close examination of lipoprotein metabolism in diabetes mellitus is important because of the relationship of lipoproteins to atherosclerosis and the high incidence of early atherosclerotic complications and death among persons with diabetes.[1] Nikkila has recently reviewed the great number of studies which link the metabolism of carbohydrates and insulin to tissue and blood lipids and lipoproteins in normal[2] and diabetic[3] man and animals. It is clear that the insulin lack of juvenile diabetic patients[4,5] and the obesity and hyperinsulinemia of adult-onset diabetic patients[4] may each affect the factors regulating hepatic input of lipoproteins into plasma, i.e. hepatic lipogenesis, triglyceride and protein synthesis, and very low density lipoprotein secretion. Factors contributing to the catabolism or outflow of lipoproteins from plasma, as lipoprotein lipase,[6] may also be affected.[7] Glucagon too may regulate lipoprotein production.[8,9] Thus, there are a number of mechanisms whereby abnormalities in lipoprotein metabolism could be produced in diabetics. Yet, according to prevalence studies of hyperlipoproteinemia,[10,11] 60 to 75 per cent of persons with diabetes have what are generally accepted as normal lipid levels and/or lipoprotein electropherograms. We wondered whether this implied that the metabolism of lipoproteins was normal in normolipemic diabetics. We also wondered whether the metabolism of lipoproteins in subjects with hyperlipoproteinemia and diabetes differed from those without diabetes. This study represents initial attempts to answer these questions. Plasma levels and compositions of lipoproteins and of apolipoproteins were examined in outpatient diabetic subjects and in groups of control subjects. Differences were found in the compositions of the low density and high density lipoproteins.

## PATIENTS, MATERIALS AND METHODS

The diabetic group consisted of patients who visited the diabetes outpatient clinic of the St. Louis City Hospital over a four-week period. Each of the patients had fasting plasma glucose levels > 135 mg./dl. and glycosuria on several occasions. None of the ninety-seven patients were acutely ill and although the de-

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grees of control in terms of the blood sugar level were highly variable, none of the patients were ketotic. All had reasonably stable body weights (less than 1.5 kilogram change over the preceding month). These subjects represented about 35 per cent of the total outpatient diabetic population and were thought to be representative of the whole. All of the normolipemic and five of the hyperlipoproteinemic nondiabetic subjects were volunteers who were recruited from the general outpatient medical clinic of the City Hospital. None had any previously known endocrine metabolic, renal or hepatic diseases. Most were occasional visitors to the clinic who came with a variety of minor musculoskeletal or gastrointestinal complaints. None were acutely ill and all had relatively stable body weights. The majority of the nondiabetic patients with hyperlipoproteinemia were patients of the Washington University Lipid Research Center. In these people secondary causes of hyperlipemia were excluded by appropriate clinical tests. Many had one or more relatives with the same lipoprotein phenotype; all were closely followed as outpatients. Type IIa had total TG $<$ 200 mg./dl. and LDL-C $>$ 200 mg./dl. Type IV had total TG $>$ 200 mg./dl. and LDL-C $<$ 200 mg./dl. and no chylomicrons or floating beta (for abbreviations see below).

All patients were following their usual diets. There is disagreement at the present time as to the optimum composition of diabetic diets, but everyone agrees on the desirability of maintaining ideal body weight. Our patients were therefore instructed on diets which contained 40 to 45 per cent carbohydrates, 35 to 40 per cent fat and 15 per cent protein. Sufficient calories were prescribed to maintain ideal body weight; in insulin-treated patients stress was laid on the timing of meals to match the physical activity of the subjects and the actions of the various preparations of insulin. Patients had not received any medications known to affect plasma lipids and lipoproteins, except for oral hypoglycemic agents and insulin, for the two months before the blood sampling procedure. Venous blood samples were drawn into EDTA tubes (1 mg. per milliliter) after twelve to sixteen hour fasts. Plasmas were overlaid with 0.16 M NaCl, 1 mM EDTA, pH 8.6, d = 1.006 (EDTA-saline), and the very low density lipoproteins (VLDL) were floated to the tops of the tubes through the EDTA-saline by ultracentrifugation for 1 x $10^8$ g minutes in a 40.3 rotor in a Model L265B Beckman ultracentrifuge.[12] Flotation through EDTA-saline was performed to minimize contamination of the VLDL by other density fractions or proteins. Nevertheless, some contamination was found (see below). Since the plasmas contained no detectable chylomicrons by inspection or by lipoprotein electrophoresis,[13] no attempt was made to isolate any chylomicron fractions and the "compositions" reported for VLDL (see below) are probably representative of VLDL alone "uncontaminated" by chylomicrons. The low density lipoprotein (LDL) was precipitated from the infranates of the above ultracentrifugations by heparin-$MnCl_2$,[14] leaving the high density lipoproteins (HDL) in solution. Triglycerides (TG) and cholesterol (Chol) were assayed by AutoAnalyzer II (Technicon) in the Core Laboratory of the Washington University Lipid Research Center.[15] Total-TG and Total-Chol represent results obtained on isopropanol extracts of whole plasma; HDL-TG and HDL-Chol were obtained on extracts of the heparin-$MnCl_2$ supernates. LDL-TG = ultracentrifugal infranate-TG (containing HDL and LDL) - HDL-TG; LDL-Chol = infranate-Chol - HDL-Chol. VLDL were analyzed directly. Results are reported in milligrams per deciliter plasma. The coefficients of variation of the chemical procedures ranged from 3 to 5 per cent. The sums of the lipoprotein lipids represented 80 to 90 per cent of the total plasma lipids. Results have not been corrected for the losses of 10 to 20 per cent of the lipids.

VLDL-protein was determined by the method of Lowry,[16] VLDL-ApoB* was measured by a previously described radioimmunoassay (RIA).[17] VLDL-ApoC = VLDL-protein - VLDL-ApoB. The determinations of VLDL-protein and VLDL-ApoC are accurate only if the VLDL as isolated contained no contaminating plasma proteins. To check on this point, twelve VLDL preparations were analyzed by immunodiffusion.[18] Albumin and IgG were found. These VLDL were then "washed" by floating them up through EDTA-saline in a second ultracentrifugation performed as above. No contaminating proteins were detected immunologically in these "washed" VLDL. VLDL and "washed" VLDL were analyzed for TG, Chol, ApoB and protein (table 1). The levels of the first three were altered variably but always by less than 10 per cent, whereas the levels of VLDL-protein decreased by 25 $\pm$ 3 per cent and the decreases were proportional to the initial concentration (correlation of difference in pro-

*The major classes of apolipoproteins are ApoA, ApoB and ApoC. Chylomicrons, very low density lipoproteins (VLDL), and low density lipoproteins (LDL) each contain ApoB and ApoC in varying proportions. The high density lipoproteins (HDL) contain ApoA and ApoC.

TABLE 1

Concentrations of lipids and apoproteins in VLDL and "washed" VLDL

| | TG | Chol | ApoB | Protein |
|---|---|---|---|---|
| | | mg./dl. plasma | | |
| VLDL | 103.3 ±105.5 | 25.6 ±22.8 | 4.6 ±3.9 | 25.6 ±17.3 |
| Washed VLDL | 98.4 ±101.7 | 23.3 ±21.0 | 4.2 ±3.7 | 19.1 ±13.7 |

VLDL were isolated with one ultracentrifugation at d 1.006; "washed" VLDL consisted of aliquots of the above VLDL which were recentrifuged at d 1.006. Losses of TG, Chol and ApoB were < 10 per cent. Twenty-five per cent of protein was lost.

Results are means ± 1 S.D.

tein (VLDL - "washed" VLDL) vs. VLDL-protein = 0.93). The reported VLDL-protein values were corrected for this difference. Corrected VLDL-protein values were also used in the other calculations (e.g. VLDL-ApoC, see also tables 4 to 6).

Since LDL-protein consists primarily of ApoB,[19] LDL-ApoB was taken to represent LDL-protein. The former was determined by RIA on the infranates of the ultracentrifugations at d 1.006. We have shown previously that ApoB is not detectable in any of the plasma fractions of density greater than that of LDL. The ApoB contained in the d 1.006 infranates is, therefore, associated only with LDL. The apoprotein values too are reported in milligrams per deciliter plasma.

Using the apoprotein and lipid data, "compositions" for VLDL and LDL are computed as follows: per cent VLDL-TG = VLDL-TG x 100 ÷ (VLDL-TG + VLDL-Chol + VLDL-protein) (i.e. "Total" VLDL); per cent VLDL-Chol = VLDL-Chol x 100 ÷ "Total" VLDL, etc. Phospholipid measurements were not obtained. The inclusion of these measurements would have required isolating the LDL fractions from each of the plasmas—a sizeable undertaking. It was felt that the TG, Chol and protein data were sufficiently indicative of VLDL and LDL compositions for the purposes of this study. For similar methodologic reasons the protein and phospholipid data were not obtained on HDL. This is a significiant omission particularly for HDL. Therefore, it should be understood that in comparing the various groups under study, the HDL-Chol/HDL-TG ratio is used merely as an index of HDL composition.

## STATISTICAL METHODS

The information obtained from each subject was keypunched and entered into an SPSS data base[20] for statistical analysis. Comparisons of various plasma concentrations and "compositions" were made between a diabetic group and its comparable lipoprotein phenotype control group, e.g. normolipemic diabetic group against normolipemic nondiabetic group. These tests were based on the standard Student's *t* test.

Because some of the groups were not directly comparable in terms of racial compositions, age, sex and degree of obesity (see below), the relevant variables were used to construct an analysis of variance model to verify that the differences in lipoproteins were in fact due to the presence of the diabetes. The model consisted of four main factors: (a) diagnosis (DX, diabetic vs. nondiabetic); (b) lipoprotein phenotype (TYPE);

TABLE 2

Comparative data on the study populations

| | Diabetic | | | Nondiabetic | | |
|---|---|---|---|---|---|---|
| | Lipids Normal (71) | Type IIA (12) | Type IV (14) | Lipids Normal (41) | Type IIA (19) | Type IV (12) |
| Age (yr.)* | 56 ± 13 | 53 ± 12 | 50 ± 15 | 54 ± 17 | 44 ± 18† | 47 ± 10 |
| Height (in.)* | 65 ± 4 | 63 ± 2 | 65 ± 8 | 65 ± 3 | 64 ± 4 | 69 ± 3 |
| Weight (lbs)* | 175 ± 40 | 175 ± 23 | 182 ± 49 | 162 ± 39 | 148 ± 38† | 175 ± 23 |
| Race (W/B) | 24/47 | 2/10 | 10/4 | 21/20 | 15/4 | 10/2 |
| Sex (M/F) | 20/51 | 3/9 | 7/7 | 12/29 | 6/13 | 10/2 |
| FBS (mg./dl.)* | 204 ± 83 | 198 ± 106 | 302 ± 147 | 110 ± 15† | 94 ± 6† | 98 ± 7† |

*Data are presented as means ± 1 S.D.

†Significantly different from their diabetic counterparts ($p \leq 0.05$).

Type IIa = low density lipoprotein cholesterol (LDL-C) > 200 mg. per deciliter; total triglyceride (Total-TG) < 200 mg. per deciliter.

Type IV = Total TG > 200 mg. per deciliter, LDL-C < 200 mg. per deciliter.

TABLE 3

Diabetic population

| | FBS* | 2 hr. PC* | Phenotype† Normolipemic | Type IIA | Type IV |
|---|---|---|---|---|---|
| Treatment Modality | | | | | |
| Insulin (35) | 241 | 335 | 22 (63) | 9 (26) | 4 (11) |
| Oral Hypoglycemics (49) | 208 | 259 | 40 (82) | 2 (4) | 7 (14) |
| Diet (13) | 195 | 230 | 9 (69) | 1 (8) | 3 (23) |

*Mean fasting and two hour post cibum blood sugar levels in milligrams per deciliter.

†Number of patients (for definition of lipoprotein phenotypes see table 1).

( )Per cent.

(c) race; and (d) sex. In addition, the interactions of DX and TYPE and of DX and SEX were included. Since these factors are not independent, and the cell frequencies are not proportional, the model required a nonorthogonal solution. The SAS[21] procedure REGR was utilized for the calculations. The plasma concentrations were converted to logarithms as their distributions were then closer to normal. The "partial F value" was used to assess statistical significance, i.e. the significance of a given factor was evaluated after the effects of all other factors had been removed. Age and the ponderal index were also introduced as covariates into the model to eliminate any potential contamination by these factors.

## RESULTS

### *Study Populations*

Clinical data of the study groups are presented in tables 2 and 3. A total of 169 subjects, ninety-seven of whom were diabetic and seventy-two nondiabetic, were studied. The diagnoses of diabetes in this group were well established. They were made on the basis of clinical symptoms and compatible blood and urine sugar levels. Diabetic control was variable but all subjects were outpatients, and none were ketotic. The exclusion of latent and chemical diabetes is more difficult requiring rigorously controlled glucose and cortisone-glucose tolerance testing.[22] In this study such procedures were not carried out. Those classified as nondiabetic subjects were free of symptoms, had fasting blood sugar levels of 120 mg. per deciliter or less, and had no glucosuria on repeated testing. Therefore, it is conceivable that our nondiabetic patients included some with "chemical diabetes." The diagnostic criteria for hyperlipoproteinemia were similar to those used by others.[23] Of the diabetic subjects, 73 per cent were normolipemic, 12.5 per cent had Type IIa and 14.5 per cent had Type IV hyperlipoproteinemia. Of those who had hyperlipoproteinemia, 37 per cent were taking insulin, 18 per cent were on oral drugs and 31 per cent were on diet alone (table 3). Of the Type IIa patients, 75 per cent were taking insulin, and of Type IV, 72 per cent were not taking insulin. The difference in the distribution of phenotypes between insulin-requiring and other diabetics was significant by $X^2$ analysis at $p < 0.05$ ($X^2 = 9.023$, d.f. = 2). These prevalence figures agree with those of Wilson et al.[11] and other researchers[10,24] and confirm the increased prevalence of hyperlipoproteinemia in diabetic patients over normal subjects.[25,26]

The diabetic and nondiabetic groups of patients with normal lipid and lipoprotein values were similar in age, height and weight and contained similar pro-

TABLE 4

Lipid and lipoprotein levels in diabetes*

| | Total TG | VLDL-TG | LDL-TG | HDL-TG | Total-Chol | VLDL-Chol | LDL-Chol | HDL-Chol |
|---|---|---|---|---|---|---|---|---|
| Diabetic | | | | | | | | |
| Normolipemic (71) | 113 ± 41 | 62 ± 33 | 33 ± 7 | 13 ± 6 | 202 ± 35 | 11 ± 7 | 132 ± 32 | 48 ± 13 |
| Type IIA (12) | 124 ± 33 | 61 ± 24 | 46 ± 11 | 12 ± 2 | 299 ± 32 | 12 ± 6 | 239 ± 34 | 48 ± 10 |
| Type IV (14) | 416 ± 421 | 337 ± 398 | 49 ± 14 | 16 ± 6 | 234 ± 73 | 61 ± 65 | 128 ± 35 | 29 ± 7 |
| Nondiabetic | | | | | | | | |
| Normolipemic (41) | 97 ± 41 | 50 ± 35 | 26 ± 11 | 13 ± 7 | 199 ± 38 | 10 ± 7 | 126 ± 35 | 59 ± 23 |
| Type IIa (19) | 107 ± 34 | 56 ± 27 | 38 ± 9 | 9 ± 4 | 356 ± 85 | 11 ± 6 | 295 ± 99 | 44 ± 15 |
| Type IV (12) | 401 ± 229 | 356 ± 234 | 25 ± 9 | 13 ± 4 | 237 ± 35 | 58 ± 26 | 134 ± 42 | 34 ± 13 |

*Results expressed in milligrams per deciliter ± 1 S.D. Underlined results are significantly different from nondiabetic counterparts ($p \leq 0.05$).

Abbreviations: TG = triglyceride; Chol = cholesterol; VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.

TABLE 5

Apolipoprotein levels in diabetes*

| | Total ApoB | VLDL-Protein | VLDL-ApoB† | VLDL-ApoC | LDL-ApoB |
|---|---|---|---|---|---|
| Diabetic | | | | | |
| Normolipemic (71) | 110 ± 36 | 15 ± 7 | 4 ± 2 | 11 ± 5 | 95 ± 28 |
| Type IIa (12) | 194 ± 69 | 16 ± 7 | 4 ± 2 | 12 ± 5 | 145 ± 20 |
| Type IV (14) | 146 ± 48 | 46 ± 32 | 16 ± 13 | 29 ± 20 | 119 ± 32 |
| Nondiabetic | | | | | |
| Normolipemic (41) | 104 ± 45 | 14 ± 8 | 4 ± 4 | 9 ± 8 | 92 ± 32 |
| Type IIA (19) | 184 ± 59 | 14 ± 5 | 4 ± 2 | 10 ± 5 | 167 ± 51 |
| Type IV (12) | 107 ± 36 | 36 ± 15 | 12 ± 5 | 24 ± 16 | 86 ± 35 |

*Results expressed in milligrams per deciliter, mean ± 1 S.D. Underlined results are significantly different from nondiabetic counterparts ($p \leq 0.05$).

VLDL-ApoC = (VLDL-Protein) - (VLDL-ApoB).

ApoB = apolipoprotein B

ApoC = apolipoprotein C

For other abbreviations see table 2.

portions of male and female subjects. However there were disproportionately more blacks among the diabetic patients (W/B = 24/47 vs. 21/20). The diabetic and nondiabetic Type IV groups were also comparable by the above criteria except for the relatively larger number of women in the diabetic group (M/F = 7/7 vs. 10/2). The diabetic Type IIa subjects were older and weighed more than their nondiabetic counterparts. The former group also contained relatively more black people (W/B = 2/10 vs. 15/4).

*Lipids and Lipoproteins*

In the diabetic and nondiabetic normolipemic subjects, the mean TG and Chol *levels* were similar for most fractions. The exceptions were LDL-TG and HDL-Chol (table 4). Mean apolipoprotein levels were also similar (table 5). Nor did diabetic and nondiabetic VLDL "*compositions*" differ significantly (table 6). However, the LDL of the diabetic subjects contained more TG (per cent LDL-TG = 13 vs. 11) than did the LDL of nondiabetic subjects (table 6).

By definition,[23] total-Chol and LDL-Chol *levels* of Type IIa subjects were higher than those of the normolipemics (tables 4 and 5). Total ApoB and LDL-ApoB were also high. However, mean TG and apolipoprotein levels of diabetic and nondiabetic Type IIa patients were comparable. In Type IIa also, the VLDL "*compositions*" of the diabetic and nondiabetic groups were similar, while the LDL of the diabetic

TABLE 6

Lipoprotein "composition" in diabetes

| | VLDL* | | | LDL* | | | HDL |
|---|---|---|---|---|---|---|---|
| | TG | CHOL per cent | PROT | TG | CHOL per cent | ApoB | TG/CHOL |
| Diabetic | | | | | | | |
| Normolipemic (71) | 70 ± 6 | 12 ± 4 | 19 ± 5 | 13 ± 4 | 51 ± 6 | 36 ± 5 | 0.29 ± 0.12 |
| Type IIA (12) | 69 ± 6 | 13 ± 3 | 18 ± 4 | 11 ± 2 | 56 ± 4 | 34 ± 3 | 0.28 ± 0.13 |
| Type IV (14) | 73 ± 6 | 14 ± 3 | 13 ± 4 | 17 ± 4 | 43 ± 5 | 40 ± 4 | 0.57 ± 0.22 |
| Nondiabetic | | | | | | | |
| Normolipemic (41) | 66 ± 8 | 12 ± 5 | 21 ± 5 | 11 ± 5 | 52 ± 9 | 37 ± 7 | 0.23 ± 0.12 |
| Type IIa (19) | 68 ± 5 | 13 ± 4 | 19 ± 4 | 8 ± 2 | 58 ± 6 | 33 ± 6 | 0.21 ± 0.09 |
| Type IV (12) | 76 ± 7 | 14 ± 5 | 10 ± 3 | 11 ± 5 | 53 ± 7 | 36 ± 6 | 0.43 ± 0.25 |

*Results are proportions of single components relative to the whole (e.g. $\frac{TG}{TG + CHOL + PRO}$ x 100, concentrations in milligrams per deciliter). Since phospholipids were not quantified, the results do not represent the true compositions of the lipoproteins.

Underlined figures are significantly different from nondiabetic counterparts ($p \leq 0.01$).

group contained slightly more TG (per cent LDL-TG = 11 vs. 8) (table 6). It is worth noting that the LDL of both Type IIa groups contained less TG than did their respective normolipemic and Type IV counterparts.

Mean lipid and ApoB *levels* in Type IV diabetic and nondiabetic subjects were the same. The exceptions were LDL-TG, total ApoB and LDL-ApoB, which were higher in diabetic patients (tables 4 and 5). Again, the VLDL of diabetic and nondiabetic patients did not differ in "*composition*", while the "diabetic LDL" contained more than the expected amounts of triglyceride. Thus, significant differences in LDL "*composition*" (at $p < 0.01$) were found in each of the diabetic groups.

HDL compositions behaved as did LDL; i.e. they differed according to phenotype, and they were also affected by the diabetes (tables 6 and 7).

Since the diabetic subjects consisted of three treatment groups (table 3), it was important to ascertain whether any of the above differences in lipoproteins could be ascribed to therapy. Accordingly, the data were grouped by treatment modality and the same parameters presented in tables 4 to 6 were recalculated. Mean lipid and apoprotein levels of the insulin, oral hypoglycemic, and diet groups did not differ significantly from each other or from the nondiabetic groups. There was one exception, the LDL-C, which was higher in the insulin group than in any of the others (162 vs. 125-140 mg. per deciliter). However, the VLDL and LDL compositions of the groups did not differ significantly (e.g. mean values for the three treatment categories ranged from 12 to 13 per cent for per cent LDL-TG, and 64 to 67 per cent for per cent VLDL-TG), nor was there a relationship between fasting blood sugar and lipid or lipoprotein levels.

The diabetic and nondiabetic groups differed from each other in a number of attributes such as race (normolipemics), sex (Type IV), age and degree of obesity (Type IIa) (table 2). The possible confounding effects of these variables on lipoprotein compositions were evaluated by the analysis of variance model described under Methods (table 7). Clearly the compositions of VLDL, LDL, and HDL were related to the phenotype. However LDL and HDL compositions were also affected very significantly ($p < 0.01$) by diabetes itself and not by a number of other factors including the ponderal index. Thus, the diabetic state appears to have an effect on lipoprotein compositions.

TABLE 7

Significant statistical relationships by analysis of variance

| | Composition (per cent) | | | | | | |
|---|---|---|---|---|---|---|---|
| | VLDL | | | LDL | | | HDL |
| | TG | Chol | Prot | TG | Chol | ApoB | TG/Chol |
| Factor | | | | | | | |
| Diabetes | | | | * | * | | * |
| Phenotype | † | | * | * | * | | * |
| Sex | | | | | | | |
| Race | | † | | | | | |
| Age | | | | | † | | |
| Ponderal Index | | † | | | | | |
| Diabetes x Phenotype | | | | * | † | | |
| Diabetes x Sex | | † | | | | | |

Statistical relationships between factors and compositions were calculated by an analysis of variance model (see method).

* = Significance at $p \leq 0.01$, † = $p \leq 0.05$.

Ponderal index = height (in.) $/\sqrt[3]{\text{weight (lbs.)}}$

## DISCUSSION

Atherosclerotic cardiovascular disease is an important cause of morbidity and the leading cause of mortality among human diabetic patients.[1,27] One reason for this may be the increased prevalence of hyperlipoproteinemia.[10,11] In addition the major risk factors for the development of atherosclerotic disease—hypertension, smoking and hypercholesterolemia—seem to be more potent in combination with diabetes mellitus than without it, so that hypercholesterolemia in the presence of diabetes represents a greater risk than does hypercholesterolemia alone. Thus, diabetics may have an increased "sensitivity" to the risk factors compared with nondiabetics. This could follow from alterations of the metabolism of the arterial walls[28] or of the metabolism of lipoproteins, or of both. Therefore, it seemed important to us to study the lipoproteins in diabetes in some detail. This study represents initial efforts in that direction. Significant differences were found in the compositions of LDL and HDL in diabetic patients by univariate analysis. However, before any conclusions as to the influence of the diabetes per se could be drawn, the specificity of the effect of diabetes had to be demonstrated. This is a difficult problem because many factors influence the metabolism of lipoproteins and it is impossible to match control and experimental groups for all of them. Therefore a number of statistical procedures were carried out to determine whether the effects of diabetes were confounded by some of the most obvious factors, such as treatment modality, blood sugar, age, sex, race, ponderal index[29] and some combinations of the above. The analyses sug-

gested that the relative enrichment of LDL and HDL by TG in diabetes was related to several factors, but that a most important relationship was to the diabetes itself.

A compositional change of this sort implies that the mean density of LDL in diabetes is decreased. On analytic ultracentrifugation, this is usually found to correlate with increases in the less dense SF 12-20 subclass of LDL relative to the more dense SF 0-12 subclass.[30] For example, Gofman et al.[31] have studied lipoproteins in the analytic ultracentrifuge and reported increases in the SF 12-20 to SF 0-12 ratio in pregnancy; we find increases in per cent LDL-TG (Hillman, L., Schonfeld, G., et al., submitted for publication). Fisher[32] found "light" LDL fractions in Type IV hyperlipoproteinemia, where we find elevated per cent LDL-TG. Therefore, the alterations in LDL composition in diabetes are probably accompanied by increases in the SF 12-20 fraction as well. Of course this surmise needs experimental verification. (It is interesting to note that the alterations of lipoproteins in pregnancy, a "diabetogenic" state,[33] resemble those seen in clinical diabetes itself.)

Current concepts of lipoprotein metabolism hold that LDL is a metabolic product of intravascular VLDL (and chylomicron) catabolism.[34] If this is so, the accumulation of TG-enriched LDL implies that there may be alterations in the catabolic pathway in diabetes[3,4,7]—even in those ambulatory diabetic patients with normal lipoprotein levels. More studies of lipoprotein turnover in diabetic patients, particularly those with normal lipoprotein levels, are needed to test the validity of this formulation. HDL-TG levels are directly related to total TG, [30] and some alterations in HDL compositions occur during the absorption of dietary fat[35] and in pregnancy (Hillman and Schonfeld, see above). These fluctuations in HDL are unexplained.

The greater prevalence of Type IIa in insulin-requiring than in other diabetic subjects has been reported by others.[11] Differences in diet do not account for this finding, nor are the elevations in LDL-C secondary to other diseases, e.g. disorders of liver, kidney, thyroid, cancer, dysproteinemia. Secondary causes for Type IV have also been similarly ruled out. Thus the hyperlipoproteinemia appears to be "primary." More studies are needed to determine whether these cases are familial and, if so, whether or not the diabetes and hyperlipoproteinemia are inherited together or separately.

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REFERENCES

[1]National Heart and Lung Institute Task Force on Arteriosclerosis: Arteriosclerosis. Washington, D.C., U.S. Government Printing Office, 1971, vol. 2, p. 100.

[2]Nikkila, E. A.: Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin. Adv. Lipid Res. 7:63-132, 1969.

[3]Nikkila, E. A.: Triglyceride metabolism in diabetes mellitus. *In* Progress in Biochemical Pharmacology, vol. 8, Macdonald, I., Ed. New York, S. Karger, 1973, p. 271.

[4]Nikkila, E. A., and Kekki, M.: Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism *22*:1, 1973.

[5]Bagdade, J. D., Porte, D., Jr., and Bierman, E. L.: Acute insulin withdrawal and the regulation of plasma triglyceride removal in diabetic subjects. Diabetes *17*:127-32, 1968.

[6]Bagdade, J. D., Porte, D., Jr., and Bierman, E. L.: Diabetic lipemia—A form of acquired fat-induced lipemia. N. Engl. J. Med. *276*:427-38, 1967.

[7]Lewis, B., Moncini, M., Mallock, M., Chait, A., and Fraser, T. R.: Plasma triglyceride and fatty acid metabolism in diabetes mellitus. Eur. J. Clin. Invest. *2*:445-53, 1972.

[8]Heimberg, M., Weinstein, I., and Kohout, M.: The effects of glucagon, dibutyryl-3′, 5′-adenosine monophosphate, and concentration of free fatty acid on hepatic lipid metabolism. J. Biol. Chem. *244*:5131-39, 1969.

[9]Eaton, R. P., and Schade, D. S.: Glucagon resistance as a hormonal basis for endogenous hyperlipaemia. Lancet *1*:973-74, 1973.

[10]Bergquist, N.: Serum lipids in an ambulatory diabetic clientele. Acta Med. Scand. *187*:213-18, 1970.

[11]Wilson, D. E., Schreibman, P. H., and Arky, R.: Hyperlipidemia in an adult diabetic population. J. Chronic Dis. *23*:501-06, 1970.

[12]Dole, V. P., and Hamlin, J. T.: Particulate fat in lymph and blood. Physiol. Rev. *42*:674-701, 1962.

[13]Noble, R. P.: Electrophoretic separation of plasma lipoproteins in agarose gel. J. Lipid Res. *9*:693-700, 1968.

[14]Burstein, M., Scholnick, H. R., and Morfin, R.: Rapid method for the isolation of lipoproteins from human plasma by precipitation with polyanions. J. Lipid Res. *11*:583-95, 1970.

[15]Manual of Laboratory Methods. Lipid Research Clinics Program. Chapel Hill, University of North Carolina, 1972.

[16]Lowry, O. H. Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*:265-75, 1951.

[17]Schonfeld, G., Lees, R. S., George, P. K., and Pfleger, B.: Assay of total plasma apolipoprotein B concentration in human subjects. J. Clin. Invest. *53*:1458-67, 1974.

[18]Ouchterlony, O.: Handbook of immunodiffusion and immunoelectrophoresis. Ann Arbor, Ann Arbor-Humphrey Science Publishers, 1968.

[19]Scanu, A. M., and Wisdom, C.: Serum lipoproteins structure and function. Ann. Rev. Biochem. *41*:703-30, 1972.

[20]Nie, N. H., Bent, D. H., and Hull, C. H.: Statistical Package for the Social Sciences. St. Louis, McGraw Hill, 1970.

[21]Service, J.: A User's Guide to the Statistical Analysis System, North Carolina State University, Raleigh, N. C., Student Supply Stores, 1972.

[22]Fajans, S. S., and Conn, J. W.: An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. Diabetes *3*:296-304, 1954.

[23]Fredrickson, D. S., and Levy, R. I.: Familial hyperlipoproteinemia. *In* Metabolic Basis of Inherited Diseases, 3rd ed., Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds. New York, McGraw Hill, 1972, p. 545.

[24]Santen, R. J., Willis, P. W., and Fajans, S. S.: Atherosclerosis in diabetes mellitus. Arch. Intern. Med. *130*:833-43, 1972.

[25]Wood, P. D. S., Stern, M. P., Silvers, A., Reaven, G. M., and Von Der Groeben, J.: Prevalence of plasma lipoprotein abnormalities in a free-living population of the Central Valley, California. Circulation *45*:114-26, 1972.

[26]Leren, P., and Haabrekke, O.: Blood lipids in normals. Acta Med. Scand. *189*:501-04, 1971.

[27]Bradley, R. F., and Partamanian, J. O.: Coronary heart disease in the diabetic patient. Med. Clin. N. Amer. *49*:1093-1104, 1965.

[28]Clements, R. S., Morrison, A. D., and Winegrad, A. I.: Polyol pathway in aorta: regulation by hormones. Science *166*:1007-08, 1969.

[29]Bierman, E. L., and Porte, D.: Carbohydrate intolerance and lipemia. Ann. Intern. Med. *68*:926-33, 1968.

[30]Ewing, A. M., Freeman, N. K., and Lindgren, F. T.: The analysis of human serum lipoprotein distributions. Adv. Lipid Res. *3*:25-63, 1965.

[31]Gofman, J. W., DeLalla, O., Glazier, F., Freeman, N. K., Lindgren, F. T., Nichols, A. V., Stisower, B., and Tamplin, A. R.: The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease. Plasma *2*:413-84, 1954.

[32]Fisher, W. R.: The characterization and occurrence of an Sf 20 serum lipoprotein. J. Biol. Chem. *245*:877-84, 1970.

[33]Freinkel, N., Herrera, E., Knopp, R. H., and Ruder, H. J.: Metabolic realignments in late pregnancy: a clue to diabetogenesis. *In* Advances in Metabolic Diabetes, Camerini-Davalos, R. A., and Cole, H. S., Eds. New York, Acad. Press, Suppl. 1, 1970, p. 205.

[34]Levy, R. I., Bilheimer, D. W., and Eisenberg, S.: The structure and metabolism of chylomicrons and very low density lipoproteins. *In* Plasma Lipoproteins, Smellie, R. M. S., Ed. New York, Acad. Press, 1971, p. 3.

[35]Havel, R. J., Kane, J. P., and Kaseyap, M. L.: Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. J. Clin. Invest. *52*:32-38, 1973.