Lipoprotein Patterns in Diet, Sulphonylurea, and Insulin Treated Diabetics

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Summary. In order to study the lipoprotein pattern in diabetes mellitus, plasma lipoproteins were isolated by rate zonal centrifugation in 12 control subjects (median fasting blood glucose level: 80 mg/dl (range: 74–86)), 14 diabetic patients treated by diet alone 104 mg/dl (76–153), 27 patients treated by diet plus insulin (180 mg/dl (106–404)), and 32 patients treated by diet plus sulphonylurea [178 mg/dl (103–361)]. No significant differences of median relative body weight existed between the four groups. Neither the diabetic group on diet alone nor the insulin-treated group differed significantly from control subjects with respect to lipid and lipoprotein concentrations. Diabetics treated with diet plus sulphonylurea, however, differed significantly from the control group with regard to the following parameters (median and range); plasma triglycerides (210 [75–620) mg/dl; $p<0.01$)] and intermediate density lipoproteins (65 (10–338) mg/dl; $p<0.05$)) were higher; low density lipoproteins (236 (82–418) mg/dl; $p<0.05$)) and high density lipoproteins$_2$ (HDL$_2$) [51 (12–121) mg/dl; $p<0.01$)] concentrations were lower. When data from all 85 studied individuals were analysed together, significant positive correlations were observed between fasting blood glucose and plasma triglyceride concentration ($r = 0.28$, $p<0.01$), and between fasting blood glucose and plasma very low density lipoproteins (VLDL) ($r = 0.23$, $p<0.05$). A negative correlation was found between blood glucose and plasma HDL$_2$ ($r = -0.29$, $p<0.01$). In addition, VLDL correlated negatively with HDL$_2$ ($r = -0.89$, $p<0.001$) but not with plasma HDL$_3$ concentration. It is concluded that the deranged lipoprotein metabolism in diabetes mellitus may be better controlled by insulin than by sulphonylureas.

Key words: Non-insulin-dependent diabetes, insulin treatment, sulphonylurea treatment, triglycerides, VLDL, IDL, LDL, HDL fractions

Diabetes has been reported to be a major contributor to cardiovascular morbidity [1] and mortality with a twofold to threefold increase of clinical atherosclerosis [2]. The incidence of macroangiopathy does not, however, correlate well with diabetic control, and atherosclerosis in the general population is more closely linked to abnormalities of plasma lipids than to hyperglycaemia. It is well-known that abnormal lipid metabolism often coexists with hyperglycaemia in the diabetic patient. Thus, in most surveys, the incidence of fasting hyperlipoproteinaemia in diabetes is 30 to 40 percent [3, 4]. Most commonly, fasting plasma triglyceride levels are elevated, but cholesterol concentration may also be increased in the diabetic population [3, 4, 5, 6]. The absence of fasting hyperlipidaemia does not, however, assure either normal lipid homeostasis or freedom from vascular disease.

Recently, attention has been paid to the relationship of HDL (high density lipoproteins) and diabetes. In contrast to VLDL (very low density lipoproteins) and LDL (low density lipoproteins), HDL have been suggested to protect against atherosclerosis [7, 8]. In most studies, HDL cholesterol was used as a measure of HDL concentration in plasma, in spite of the fact that Gofman et al. [9] demonstrated quite clearly that only the HDL$_2$ fraction is lowered in patients with atherosclerosis.

Many recent studies have dealt with the investigation of HDL plasma concentration in patients with diabetes mellitus [10–15], and significant correlations between control of diabetes and HDL concentration have been reported by some [10, 12, 13] although not by others [9, 14]. The interpretation of the results obtained so far has been difficult, because: a) diabetics form a highly heterogeneous group; many other factors such as alcohol intake, body weight, sex, and smoking habits influence the concentrations of plasma HDL; b) HDL consist of at least two different subgroups, HDL$_2$ and HDL$_3$, which differ from each other with regard to their

Table 1. Clinical and biochemical characteristics of the patients

	Controls	Diabetics treated by diet alone	Diabetics treated by insulin	Diabetics treated by sulphonylureas
Male/Female	4/8	5/9	10/17	12/20
Age	66 (46–74)	66 (47–77)	65 (40–83)	70 (50–86)
Duration of diabetes (years)		4.4 (1–12)	5.7 (1–20)	4.7 (1–17)
Relative body weight	1.03 (0.86–1.20)	0.97 (0.87–1.18)	0.97 (0.63–1.22)	1.04 (0.69–1.36)
Fasting blood glucose, mg/dl	80 (74–86)	104 (76–153)[c]	180 (106–405)[c]	178 (103–361)[c]
K_g, %/min	1.80 (1.56–2.38)	0.79 (0.34–1.32)[c]	0.40 (0.14–1.01)[c] a	0.49 (0.10–1.14)[c]
Fasting plasma insulin, mu/l	21 (12–34)	19 (8–58)	13 (0–48)[c] b	24 (8–85)[c]
Early insulin response[a]	2.35 (1.41–3.82)	1.77 (0.45–3.35)	0.99 (0–2.00)[c]	1.02 (0.29–1.82)[c]

Results are given as median and range

[a] $\dfrac{\text{Plasma insulin, 10 minutes after glucose}}{\text{Fasting plasma insulin}}$

Difference from control group: [c] p<0.01
Differences between insulin group and sulphonylurea group: a: p<0.05; b: p<0.01

Table 2. Plasma lipid and lipoprotein concentrations of the patients

mg/dl	Controls	Diabetics treated with diet alone	Diabetics treated by insulin		Diabetics treated by sulphonylureas
Cholesterol	254 (183–290)	232 (112–325)	247 (165–334)		237 (138–380)
Triglycerides	122 (73–189)	129 (64–369)	174 (74–880)	a	210 (75–620)[b]
Phospholipids	219 (183–252)	206 (149–282)	238 (137–394)		234 (125–394)
HDL-cholesterol	66 (41–97)	64 (46–89)	62 (35–105)	b	52 (26–92)[b]
VLDL	65 (23–208)	63 (20–407)	103 (19–750)		149 (40–407)
IDL	27 (10–114)	39 (15–144)	57 (10–273)		65 (10–338)[a]
LDL	323 (210–400)	279 (154–410)	267 (120–492)		236 (82–418)[a]
HDL_2	89 (63–116)	81 (37–152)	72 (18–232)	b	51 (12–121)[b]
HDL_3	233 (186–279)	227 (174–528)	206 (113–343)		232 (135–380)

Results are given as median and range
Lipoprotein densities VLDL: d<1.006; d<1.006–1.020; LDL: d<1.020–1.063; HDL_2: d<1.063–1.125; HDL_3: d<1.125–1.21 g/ml
Difference from control group: [a] p<0.05; [b] p<0.01
Differences between insulin group and sulphonylurea group: a: p<0.05; b: p<0.01

plasma concentration, chemical composition, and their metabolic function.

The aim of this study was to investigate the lipo-protein patterns of patients with diabetes mellitus treated by diet alone, as well as with diabetes treated by insulin or sulphonylureas.

Patients and Methods

Eighty-five individuals were studied (see Table 1 for clinical and biochemical characteristics). Twelve age and sex matched persons with a normal glucose disappearance rate after intravenous glucose ($K_g > 1.5$) served as a control group. These individuals were obtained from the population who had attended the diabetic out-patient clinic for routine exclusion of diabetes mellitus.

The 73 diabetic patients had been seen regularly at our diabetic outpatient clinic for at least the last 3 years. The diet-treated diabetics had been seen at 3 monthly intervals, the insulin-treated patients and the sulphonylurea-treated patients at least every 2 months. Each patient had received the same treatment for at least the last year. The insulin-treated group received a long-acting insulin preparation (Insulin Novo Lente) in a single dose daily in the morning. The average dose in the 27 insulin-treated patients was 40 U/day (range 24–60 U). On the day of investigation, the patients received no insulin. The sulphonylurea group took either tolbutamide, 1 g twice daily (16 patients), or glibenclamide, 5 mg twice daily (16 patients). The evening dose was ommitted on the day before the study. All individuals had ceased appreciable nicotine and alcohol consumption for at least 1 year before the investigation. Relative body weight of the individuals was calculated by division of their actual body weight by their average body weight [16]. All patients gave informed consent for the study.

Methods

An IV glucose tolerance test was performed at 0800 h in the recumbent position by rapid injection of glucose 0.33 g/kg body weight. The glucose disappearance rate after glucose (K_g) was calculated from the semi-logarithmic expression of the fall in blood glucose levels. Immediately before the test, approximately 100 ml venous blood were drawn using heparin as an anticoagulant. Plasma concentrations were determined for free and esterified cholesterol using the Cholesterol Enzymatic Color Test (Boehringer Mannheim GmbH) [17, 18], triglycerides [19], phospholipids [20], insulin (Phadebas), and HDL cholesterol. The coefficients of variation of our methods were for total cholesterol 4.7%, triglycerides 4.7%, phospholipids 5.3%, HDL cholesterol 3.6%, and insulin 9.4%. Insulin was determined after pretreatment of plasma with polyethyleneglycol in order to remove antibodies which may be present as a result of insulin treatment [21]. Ten minutes after glucose injection, insulin was again determined to assess the early insulin response. HDL cholesterol was determined after precipitation of VLDL and LDL by sodium phosphotungstate and magnesium chloride [22]. Glucose concentration was determined in whole blood by an automated hexokinase method.

For the determination of concentration and composition of the main lipoprotein density classes, namely VLDL, IDL (intermediate density lipoproteins), LDL, HDL_2, and HDL_3, we used rate zonal ultracentrifugation [23]. The lipoprotein concentrations were calculated as the sum of total cholesterol, triglyceride, phospholipid, and protein [24] in each lipoprotein fraction. Because of dilution of lipoprotein fractions during separation by high salt concentrations, the ultracentrifugal fractions were concentrated by membrane pressure filtration (Collodion-Bags SM 13200 Sartorius-Membranfilter GmbH, Göttingen) to about one fifteenth of their original volume, then diluted with water to plasma density, and finally concentrated again. Triglyceride concentration was thus determined in a 15-fold concentrated sample.

Since many of the measured paramenters had a lognormal distribution (fasting blood glucose, K_g, plasma concentration of insulin, triglycerides, HDL cholesterol, VLDL, IDL, HDL_2, HDL_3), median and range of all parameters were indicated in the tables. Statistical calculations were performed using the non-parametric Wilcoxon test for comparison of unpaired measurements. Correlation analyses were performed by the method of the least squares. In the case of log-normally distributed variables, correlations were calculated out after logarithmic transformation of the data.

Results

Table 1 shows the clinical and biochemical characteristics of the 4 groups of patients. There were no significant differences between the groups with regard to duration of diabetes, sex, age, or relative body weight. Fasting blood glucose was lowest in the control group, slightly elevated in the diet-treated group, and highest in the insulin and sulphonylurea groups. Correspondingly, K_g and early insulin response were low in the insulin and sulphonylurea treated groups. In spite of the fact that no significant differences of fasting blood glucose were found between the insulin and sulphonylurea groups, K_g was significantly higher in the sulphonylurea group.

Table 2 shows the lipid and lipoprotein concentrations of the four groups. The cholesterol and phos-
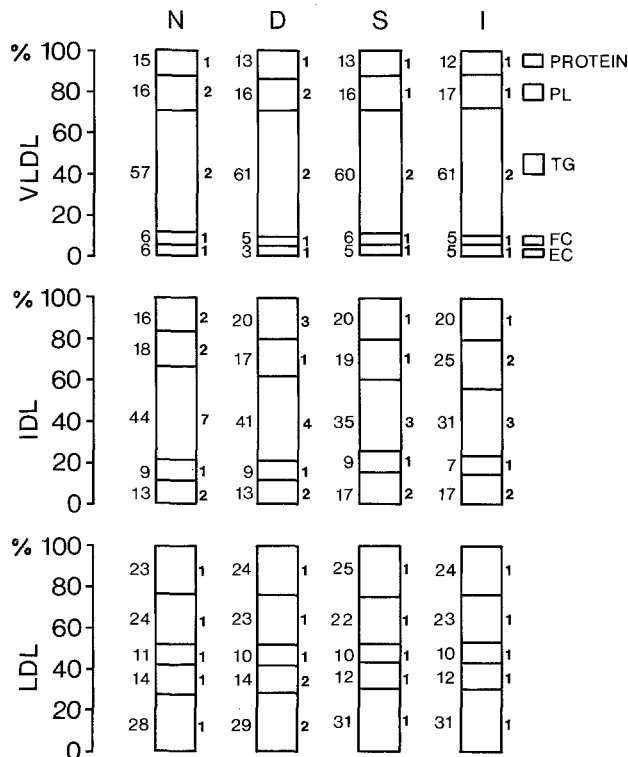
COMPOSITION OF LIPOPROTEINS I

Fig. 1. Average percentage chemical composition of the lipoprotein fractions VLDL, IDL, and LDL of 12 normal individuals (N), 14 diet-treated diabetics (D), 32 sulphonylurea-treated diabetics (S), and 27 insulin-treated diabetics (I). EC: Esterified cholesterol; FC: Free cholesterol; TG: Triglycerides; PL: Phospholipids. The differences in the percentage content of protein, PL, TG, FC, and EC between the four groups of individuals were not significant for all three investigated lipoprotein fractions. The numbers on the left side of columns signify the mean values, whereas the SEM are indicated on the right side of the columns

pholipid concentrations did not differ significantly between the groups, whereas the plasma triglyceride concentration in the sulphonylurea group was significantly higher than in the control group. There were no striking differences of HDL cholesterol between control group, diet group, and insulin group. In contrast, there was a significant decrease of HDL cholesterol in the sulphonylurea group. Diabetics treated with sulphonylureas had, unlike the insulin-treated group, significantly higher triglyceride, VLDL, and IDL levels and significantly lower LDL levels than the control group. A highly significant decrease of HDL_2 was observed in the sulphonylurea group in comparison with the three other groups. There were no significant differences in HDL_3 concentrations between the four groups. A direct comparison between the insulin and sulphonylurea groups revealed a significantly higher concentration of plasma triglycerides and significantly lower concen-
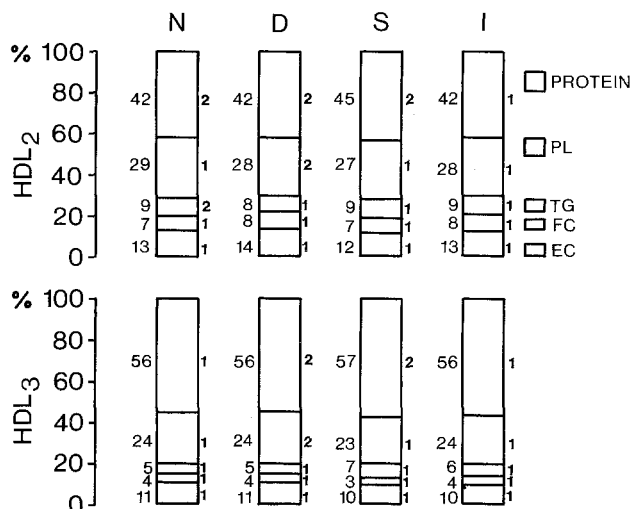
COMPOSITION OF LIPOPROTEINS II

Fig. 2. Average percentage chemical compositon of the lipoprotein fractions HDL_2 and HDL_3 of 12 normal individuals, 14 diet-treated diabetics, 32 sulphonylurea-treated diabetics, and 27 insulin-treated diabetics. The differences in the percentage content of protein, PL, TG, FC, and EC between the four groups of individuals were not significant for the two HDL fractions. The numbers on the left side of the columns signify the mean values, whereas the SEM are indicated on the right side of the columns. For abbreviations see the legend to Figure 1

trations of HDL cholesterol and HDL_2 in the sulphonylurea group.

There were no significant differences in the chemical composition of VLDL, IDL, LDL, HDL_2, and HDL_3 fractions between the control group and the three other diabetic groups.

If all studied individuals were analysed together, significant positive correlations were observed between the logarithm of fasting blood glucose on the one hand and the logarithm of plasma triglycerides ($r = +0.28$, $p<0.01$) and the logarithm of plasma VLDL ($r = +0.23$, $p<0.05$) on the other hand. The logarithm of blood glucose correlated negatively with the logarithm of plasma HDL_2 concentration ($r = -0.29$, $p<0.01$). Furthermore, the logarithm of plasma VLDL concentration could be correlated negatively with the logarithm of HDL_2 ($r = -0.89$, $p<0.001$) and positively with the logarithm of IDL ($r = +0.33$, $p<0.01$).

Discussion

The presented data demonstrate the well-known disturbance of lipid metabolism in patients with diabetes mellitus. The metabolic derangement was slight in patients who required only dietary treatment and

more severe in insulin-treated and sulphonylurea-treated individuals. As compared with a control group, however, no difference was found in lipid and lipoprotein metabolism in the diet-treated group or in the insulin-treated group. In contrast, the study revealed significant differences in the sulphonylurea group in comparison with the control group with regard to the plasma concentrations of triglycerides, VLDL, and IDL (higher) and the concentrations of HDL cholesterol, LDL, and HDL_2 (lower). These data suggest impaired catabolism of triglyceride rich lipoproteins in tablet treated diabetics. It is known that lipoprotein lipase is under hormonal regulation by insulin [25, 26] and the activity of the enzyme in adipose tissue and muscle has been shown to be reduced in uncontrolled diabetes [27]. As there is a relationship between HDL cholesterol and lipoprotein lipase activity, both in post-heparin plasma and adipose tissue [28, 29], it is suggested that HDL_2 are an end product of catabolism of triglyceride rich lipoproteins [30].

Because of the increases of triglyceride, VLDL, and IDL and the decrease of HDL_2 concentration in the plasma of tablet treated diabetics as compared with the insulin-treated group, it appears that insulin, unlike sulphonylurea treatment, may at least partially overcome the defect in lipoprotein catabolism seen in diabetes. In view of the assumptions that HDL protect against atherosclerosis [7, 8], of the observation that HDL_2 are lowered in patients with atherosclerosis [9], and of a negative association between vascular disease and HDL cholesterol in diabetics [15], it is possible that the atherogenic potential of diabetes mellitus may be reduced more effectively by insulin than by tablet treatment.

The correlation analyses on all our subjects favour the assumption that control of blood glucose has a key relationship to the regulation of lipoprotein homeostasis. Even though fasting blood glucose was nearly identical in the insulin and sulphonylurea groups, it can be assumed that the patients treated with long-acting insulin show better blood glucose values through the day. Further longitudinal studies on the effects of insulin and sulphonylureas on the lipoprotein pattern in diabetes mellitus are warranted to elucidate this problem.

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