Lipid and lipoprotein aberrations in Indian patients with non-insulin-dependent diabetes in the young

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Summary
The present study was undertaken to examine the lipid and lipoprotein status of 85 Indian patients with non-insulin-dependent diabetes in the young (NIDDY) and 85 matched Indian controls. There were no significant differences between patients and controls with regard to total serum cholesterol, low-density lipoprotein (LDL) cholesterol or apoprotein A-I (apo A-I) levels. However, serum triglyceride and apoprotein B (apo B) levels (females only) were significantly higher and serum high-density lipoprotein (HDL) cholesterol levels significantly lower in the NIDDY patients than in the controls. Serum triglyceride values correlated significantly with glycosylated haemoglobin levels ($r = 0.23$) and apo B levels ($r = 0.42$). The hypertriglyceridaemia and increased apo B levels appeared to emanate from the very-low-density lipoprotein class. Since HDL cholesterol levels were decreased and apo A-I levels were normal, these findings could be interpreted as reflecting an abnormal HDL composition. Obesity did not appear to have a significant influence on the lipid and lipoprotein abnormalities manifested by the patients in this study.

Patients and methods

Patients
Criteria for the diagnosis of NIDDDY included the following: age of onset less than 35 years; patients symptomatic but not ketonuric at presentation; and prevention of ketonuria and satisfactory control of symptoms with diet and oral hypoglycaemic agents for at least 1 year. In addition to satisfying all of the above criteria, the patients had to fulfill the criteria of the World Health Organization for the diagnosis of diabetes. Details of the patients' clinical presentation and biochemical features have been reported previously. Ideal body weight (IBW) was calculated using tables of the Metropolitan Life Insurance Company, and patients weighing 120% of their IBW or more were considered obese. The mean age of the patients at the time of the study was 32.2 years (range 14 - 49 years), while the mean weight was 122% of the IBW (range 86 - 171%).

A full clinical examination was performed to exclude other causes of hyperlipoproteinaemia, and patients with evidence of hepatic, renal or thyroid disease, as determined by standard laboratory techniques, were excluded.

The 85 Indian patients with NIDDY were matched for age, sex and weight with 85 healthy Indian volunteers whose response to a 75 g oral glucose load indicated that their glucose tolerance was normal. Informed consent was obtained from all participants in the study. All patients and controls were specifically asked about their smoking habits and alcohol consumption and were encouraged to adhere to their normal diet for at least 2 weeks before sampling; patients were requested to omit the overnight dose of the oral hypoglycaemic agent they were taking on the day before blood samples were withdrawn. None of the patients or controls was on any other medication known to affect lipid metabolism and all were ambulant at the time of sampling. Furthermore, none of the participants had had any major or minor illnesses within 6 months and 2 weeks respectively of sampling. None of the patients or controls was actively involved in any exercise programme.

Blood samples were obtained with minimal venostasis after an overnight fast of 12 - 14 hours. After allowing samples for lipoprotein quantitation to clot at room temperature, they were centrifuged within 2 hours. The sera obtained were stored at 4°C and assays for determination of total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were performed within 3 days from the time of sampling. However, the sera for apoprotein A-I (apo A-I) and apoprotein B (apo B) assays were stored at −20°C and assays were only performed when samples had been obtained from all participants. Samples were also taken for glycosylated haemoglobin (Hb AI) estimation, which was performed on the day of sampling.

As only 9 of the 85 patients (11%) admitted to smoking or taking alcohol, the effect of cigarette smoking and alcohol consumption on lipid and lipoprotein profiles was not analysed statistically because of the small sample size.
The refrigerator test (as described by Lewis) was performed on the sera of 9 patients with a serum total triglyceride concentration of over 5 mmol/l to determine the relative contribution of very-low-density lipoprotein (VLDL) and chylomicrons to the hypertriglyceridaemia.

Analytical methods

Serum cholesterol and triglyceride levels were quantitated by enzymatic procedures (Boehringer Mannheim); HDL cholesterol was measured by a polyanion precipitation method using phosphotungstate and magnesium chloride. The LDL cholesterol concentration was obtained by subtracting the HDL cholesterol from the HDL + LDL level determined by a precipitation method using 10% sodium dodecyl sulphate. Serum apo A-I and apo B levels were quantitated by radioimmunoassay using the double-antibody technique, as described previously. The assays were similar to those described by previous workers, with minor modifications. Briefly, the apoproteins were isolated by preparative ultracentrifugation, delipidation and chromatography; antibody was raised in rabbits and the apoproteins were labelled by the indirect conjugation labelling method of Bolton and Hunter. Hb A1c was determined by cation exchange chromatography (Helena Laboratories, Houston, Texas, USA). The intra- and inter-assay coefficients of variation for the methods used were as follows: cholesterol, 2.0%, 2.3%; triglycerides, 2.4%, 4.8%; HDL cholesterol, 3.5%, 7.9%; HDL + LDL cholesterol, 2.5%, 2.7%; apo A-I, 3.8%, 9.8%; apo B, 4.4%, 5.5%; and Hb A1c, 3.9%, 7.2%. Composite standard curves for both the apo A-I and apo B assays are shown in Fig. 1.

![Fig. 1. Composite standard curves for apo A-I and apo B assays generated from 5 consecutive assays.](image)

Results

Serum cholesterol, LDL cholesterol and apo A-I levels were not significantly different between the patients and the controls, irrespective of sex (Tables I and II). However, total serum triglyceride values were significantly higher and HDL cholesterol values lower in the patients than in the controls; these aberrations were manifested by both sexes (Tables I and II). While apo B levels in the female patients were significantly higher than those in the female controls, there were no significant differences between the apo B levels of the male patients and the controls.

Table IV shows the correlation between deranged variables. There was a significant positive correlation between Hb A1c and serum total triglyceride values above 5 mmol/l revealed diffuse lactescence (increased VLDL) in 8 cases, while the sample from the remaining patient displayed a diffuse lactescence with a creamy layer on top (increased VLDL and chylomicrons).

To examine the effect of obesity on lipid and lipoprotein levels in NIDDY, the patients were divided into obese and non-obese subgroups and the lipid and lipoprotein levels were compared. As shown in Table III, there were no significant differences between the two subgroups.

Table IV shows the correlation between deranged variables. There was a significant positive correlation between Hb A1c and serum total triglyceride values (r = 0.23; P < 0.05); correlations between Hb A1c and HDL cholesterol values (r = -0.02; P > 0.05) and Hb A1c and apo B levels (r = 0.19; P > 0.05) were not significant. Serum triglyceride values correlated significantly with the HDL cholesterol levels (r = -0.37; P < 0.001) but not with apo A-I levels (r = 0.13; P > 0.05). Serum apo B levels correlated significantly with both LDL cholesterol (r = 0.52; P < 0.001) and triglyceride values (r = 0.42; P < 0.001). The correlations between % IBW and triglyceride values (r = 0.17; P > 0.05) and between % IBW and HDL cholesterol levels (r = -0.01; P > 0.05) were not significant.
Table III. Comparison of Lipid and Lipoprotein Levels in Non-obese and Obese Patients with NIDDM

<table>
<thead>
<tr>
<th>Non-obese</th>
<th>Obese</th>
</tr>
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<tbody>
<tr>
<td>(10 m, 33 l)</td>
<td>(7 m, 35 l)</td>
</tr>
<tr>
<td>% IBW</td>
<td>104.5 (range 86 - 119)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.71 ± 0.40</td>
</tr>
<tr>
<td>Total triglycerides (mmol/l)</td>
<td>2.71 ± 0.71</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.88 ± 0.03</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.69 ± 0.20</td>
</tr>
<tr>
<td>Apo A-I (g/l)</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.19 ± 0.05</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>12.85 ± 0.45</td>
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</table>

*P < 0.05
**P < 0.001
*No. of subjects in parentheses.

Table IV. Interrelationship between Lipids, Lipoproteins and Glycosylated Haemoglobin Levels

<table>
<thead>
<tr>
<th>N</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A, and triglycerides</td>
<td>80</td>
<td>0.23 &lt; 0.05</td>
</tr>
<tr>
<td>Hb A, and apo B</td>
<td>80</td>
<td>0.19 &gt; 0.05</td>
</tr>
<tr>
<td>Hb A, and HDL cholesterol</td>
<td>80</td>
<td>-0.02 &gt; 0.05</td>
</tr>
<tr>
<td>Triglycerides and HDL cholesterol</td>
<td>85</td>
<td>-0.37 &lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides and apo A-I</td>
<td>76</td>
<td>0.13 &lt; 0.05</td>
</tr>
<tr>
<td>Apo B and HDL cholesterol</td>
<td>77</td>
<td>0.52 &lt; 0.001</td>
</tr>
<tr>
<td>Apo B and triglycerides</td>
<td>77</td>
<td>0.42 &lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides and % IBW</td>
<td>85</td>
<td>0.17 &gt; 0.05</td>
</tr>
<tr>
<td>HDL cholesterol and % IBW</td>
<td>85</td>
<td>-0.01 &gt; 0.05</td>
</tr>
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Discussion

The present study clearly identifies hypertriglyceridaemia and decreased HDL cholesterol levels as the major lipid abnormalities in Indian patients with NIDDM. These aberrations together with the normal serum total and LDL cholesterol levels are in accord with the findings in many studies of NIDDM. It is generally believed that hypertriglyceridaemia in non-insulin-dependent diabetics is the result of enhanced de novo hepatic synthesis of VLDL from carbohydrate. This finding is substantiated in the present study, since there was a significant positive correlation between plasma triglyceride and glycosylated haemoglobin levels.

The finding of lower serum HDL cholesterol levels in NIDDM patients than in controls accords with the experience of most previous investigators who have reported on HDL cholesterol levels in non-insulin-dependent diabetes mellitus (NIDDM). Although lower HDL cholesterol levels have been recorded in obese individuals than in non-obese controls the lower levels in the present study cannot be explained on this basis, for there was no significant difference between the HDL cholesterol levels of the obese and non-obese subgroups, nor was the correlation between percentage of IBW and serum HDL cholesterol significant. This is in agreement with the results of many previous workers who failed to demonstrate any relationship between HDL cholesterol and obesity in patients with NIDDM. Also in accordance with the findings of most previous workers reporting on NIDDM, the present study failed to demonstrate any significant correlation between HDL cholesterol and Hb A levels, suggesting that glycaemia status does not influence HDL cholesterol levels directly.

Although low HDL cholesterol levels in diabetics treated with sulphonylurea drugs have been reported by some investigators, Paisey et al., have reported a significant increase in HDL cholesterol levels after 1 year of sulphonylurea therapy. We have previously shown in a prospective study that there was no significant difference between HDL cholesterol levels before sulphonylurea therapy and those after treatment for 1 year, which suggested that sulphonylurea drugs are not primarily involved in the lowering of HDL cholesterol levels. This accords with the experience of most previous workers who failed to show any adverse effect of sulphonylurea drugs on HDL cholesterol in NIDDM.

What is of particular interest is the significant negative correlation between HDL cholesterol and serum triglyceride levels, a finding that has been confirmed by numerous studies. The physical basis for this correlation is apparently enrichment of HDL with triglycerides, and this has been demonstrated in diabetics. Recently Biesbroeck et al. have suggested that hypertriglyceridaemia induces a change in HDL composition so that there is a greater amount of triglycerides and a smaller amount of cholesterol associated with each particle, and that the total number of HDL particles are not affected. The argument was based in part on the inverse relationship between serum triglyceride and HDL cholesterol concentrations. It is also of interest that the HDL cholesterol levels of the obese and non-obese patients no significant differences were found, and furthermore the correlation between the serum triglyceride values and percentage of IBW was not significant. It is therefore decided that the hypertriglyceridaemia associated with the non-obese patients is a coincidental primary hyperlipoproteinaemia. Lewis et al. suggested that if the fasting plasma glucose level exceeds 6.6 mmol/l and the patient has a positive family history of diabetes it is usual to diagnose hypertriglyceridaemia secondary to diabetes rather than primary hyperlipoproteinaemia. If these proposals are adopted in the present study, it can be inferred that the hypertriglyceridaemia is secondary to the diabetic state, since 84% of the patients had a positive family history of diabetes and all had a fasting plasma glucose level above 6.6 mmol/l. In addition, the finding of a significant positive correlation between the serum triglyceride and Hb A levels suggests that the increased triglyceride level is related to the diabetic state. Inasmuch as hypertriglyceridaemia is considered important in the development of vascular disease in diabetics, this finding emphasizes the importance of adequate control in this group of young patients.

The genesis of the hypertriglyceridaemia is undetermined. Increased triglyceride levels have been reported in the obese, and as a group the patients in the present study were certainly obese. However, on comparing the triglyceride levels of the obese and the non-obese patients no significant differences were found, and furthermore the correlation between the serum triglyceride values and percentage of IBW was not significant. It is therefore decided that the obesity caused the hypertriglyceridaemia in the present study. It is generally believed that hypertriglyceridaemia in non-insulin-dependent diabetics is the result of enhanced de novo hepatic synthesis of VLDLs from carbohydrate. This finding is substantiated in the present study, since there was a significant positive correlation between plasma triglyceride and glycosylated haemoglobin levels.
correlation between serum triglycerides and HDL cholesterol, normal apo A-I levels, absence of correlation between serum triglycerides and apo A-I levels, and a significant positive correlation between serum triglycerides and HDL triglycerides. The present study confirms that apo A-I levels are not changed in the patients and that they are not related to serum triglyceride levels, thus supporting the proposal of an abnormal composition of HDL.

However, it should be borne in mind that low HDL cholesterol levels are only partly related to hypertriglyceridaemia and that low HDL cholesterol levels were encountered in patients with normal triglyceride levels. These findings have also previously been recorded in patients with NIDD.

The finding of normal serum apo A-I levels in the present study is similar to the experience of most previous workers reporting on NIDD, although decreased levels of apo A-I have been reported in patients with NIDD in one study.

Serum apo B levels were significantly increased in female patients with NIDD, and although apo B levels were higher in the male patients than in controls, this did not attain significance at the 5% level. This could be attributed to the small number of male patients (15) studied. The obvious question which arises is where the increased level of apo B emanates from, because both LDL and VLDL account for the majority of circulating apo B in fasting man. In the present study, apo B levels correlated significantly with both LDL cholesterol (r = 0.52) and serum triglyceride (r = 0.42) levels. Since LDL cholesterol levels were not raised and serum triglyceride levels were, it would not be unreasonable to suppose that the increase in apo B came predominantly from the VLDL class. Increased apo B levels have been reported in diabetics who have both increased cholesterol and triglyceride levels, and in addition increased VLDL apo B levels have been reported in NIDD.

In conclusion, the present study clearly demonstrates aberrations in lipid metabolism in Indian patients with NIDD which manifest as hypertriglyceridaemia, low levels of HDL cholesterol and increased apo B levels (in female patients only). There therefore appears to be no difference between patients with NIDD and those with NIDD with onset at a later age or between Indian and white diabetics with regard to lipid and lipoprotein abnormalities.

REFERENCES