

ORIGINAL ARTICLES

Circulating lipids and glycaemic control in insulin dependent diabetic children

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Abstract

The prevalence of dyslipidaemia in children with insulin dependent diabetes mellitus (IDDM) and its relation to glycaemic control was studied in a group of 51 diabetic children and a control population of 132 schoolchildren. The prevalence of dyslipidaemia in the fasting state was increased in the diabetic group (39%) compared with control subjects (17%). Serum cholesterol concentration alone was raised in 25% of diabetic subjects while serum cholesterol and triglycerides were raised in 14%, compared with 16% and 0.7% respectively in control subjects. Serum total cholesterol (5.1 v 4.5 mmol/l), low density lipoprotein cholesterol (3.2 v 2.6 mmol/l), non-esterified fatty acids (0.91 v 0.50 mmol/l), and triglycerides (0.94 v 0.76 mmol/l) were higher in diabetic children. Serum total cholesterol, triglycerides, and apolipoprotein (apo)B concentrations increased with worsening control, while serum high density lipoprotein cholesterol and apoA-I concentrations were unaltered. There were also positive correlations between glycated haemoglobin and total cholesterol, triglycerides, and apoB in diabetic children. Thus, abnormalities in circulating lipids are common in young subjects with IDDM but largely disappear if blood glucose concentrations are reasonably controlled.

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Insulin dependent diabetes mellitus (IDDM) is the most common metabolic disorder of childhood. Although treatment with injected insulin has revolutionised the outlook, patients with IDDM show increased premature morbidity and mortality from cardiovascular disease.¹ Dyslipidaemia is a major risk factor for cardiovascular disease and is common in non-insulin dependent diabetes (NIDDM) where it undoubtedly plays a part in the susceptibility of these patients to macrovascular disease.² The situation is less certain in IDDM where more normal lipid profiles have been reported in adults.³ Less is known of lipid abnormalities in diabetic children, although some have shown normal values two years after diagnosis⁴ while others

have shown raised high density lipoprotein (HDL) cholesterol⁵ or conversely low HDL cholesterol,⁶ the latter being associated with poor glycaemic control.

The present study was conducted to establish whether lipid profiles were indeed abnormal in children and adolescents with established IDDM and whether any such abnormalities were related to glycaemic control.

Subjects and methods

SUBJECTS

Fifty one diabetic children and adolescents attending the special diabetic clinics at the Royal Victoria Infirmary and Freeman Hospital, Newcastle upon Tyne, were studied. Subjects were included if, at the time of screening, diabetes had been diagnosed for at least one year, they were not acutely ill and did not have any coexisting disease associated with hyperlipidaemia, particularly hypothyroidism, Down's syndrome, liver disease, or renal disease.

In order to obtain control data, 128 children from two local schools participated. As the school population consisted of children 11 years and over, a small number (15) of younger children admitted for minor surgery who were otherwise fit were also investigated.

CLINICAL PROCEDURES

The project was approved by the local ethical committee. Informed consent was obtained from parents and where appropriate from the child. All patients were seen by a paediatrician with a special interest in diabetes or by the main investigator, who filled in a diabetic follow up flow sheet and carried out a physical examination. Subjects were investigated post-prandially in the outpatient department between two to five hours after breakfast. In order to obtain fasting samples an early morning visit to the patient's home was made and blood drawn with the patient having fasted overnight and before the morning injection of insulin was given.

The schoolchildren were approached initially by the headteacher who outlined the need for the procedure. In addition, letters were sent out before the sampling to parents, explaining briefly the project and the procedure it

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Table 1 Fasting circulating glucose and lipid levels in diabetic and control subjects; values are mean (95% confidence interval)

	Control subjects (n=143)	Diabetic subjects (n=51)	p Value*
Total cholesterol (mmol/l)	4.5 (4.3 to 4.6)	5.1 (4.8 to 5.5)	0.001
Triglycerides (mmol/l)	0.76 (0.72 to 0.80)	0.94 (0.83 to 1.08)	0.007
HDL cholesterol (mmol/l)	1.5 (1.4 to 1.6)	1.5 (1.4 to 1.6)	0.56
LDL cholesterol (mmol/l)	2.6 (2.5 to 2.7)	3.2 (2.9 to 3.4)	<0.001
ApoA-I (g/l)	1.5 (1.45 to 1.55)	1.6 (1.49 to 1.61)	0.21
ApoB (g/l)	1.0 (0.99 to 1.11)	1.0 (0.92 to 1.08)	0.54
HDL cholesterol/LDL cholesterol	0.34 (0.32 to 0.36)	0.30 (0.27 to 0.32)	0.005
LDL cholesterol/apoB	5.2 (5.0 to 5.3)	4.5 (4.3 to 4.6)	<0.001
Glucose (mmol/l)	4.0 (4.0 to 4.1)	12.6 (11.1 to 14.1)	<0.001
NEFA (mmol/l)	0.50 (0.46 to 0.53)	0.91 (0.78 to 1.05)	<0.001
Glycerol (mmol/l)	0.08 (0.02 to 0.15)	0.11 (0.004 to 0.22)	0.001

*Unpaired Student's *t* test.

would entail. Signed consent was obtained. Children fasted overnight before investigation; postprandial samples were collected between one and a half hours and five hours of their normal breakfast. A questionnaire was filled out by a doctor or qualified nurse to exclude subjects with a family history of major illnesses, particularly diabetes, cardiovascular disease, or hyperlipidaemia; subjects receiving medication or with a chronic illness were also excluded.

A conventional venepuncture with minimal venous stasis was used to withdraw 20 ml of blood which was allowed to clot at room temperature, a specimen for glycerol measurement being taken into preweighed tubes containing 5% v/v perchloric acid. Serum was separated by centrifugation at 800 *g* and apolipoprotein (apo)B-containing lipoproteins were precipitated from samples for HDL cholesterol determination within four hours. Samples for lipid analysis were stored at 4°C before analysis, which was undertaken within three days. Samples for apolipoproteins were stored at -20°C before analysis.

LABORATORY METHODS

Cholesterol was analysed by a cholesterol oxidase based procedure using kits supplied by BCL (Lewes) and a Cobas Bio Analyser (Roche, Welwyn Garden City); triglycerides were measured by a lipase-glycerol kinase method without correction for free glycerol using a Cobas Bio centrifugal analyser and

kits supplied by Roche. The between batch coefficients of variation for cholesterol were 2.2% at 3.9 mmol/l and 1.9% at 7.8 mmol/l and for triglyceride were 3.2% at 2.31 mmol/l and 3.0% at 3.47 mmol/l. HDL cholesterol was estimated using the modification of Warnick and Albers,⁷ in which apo apoB-containing lipoproteins were precipitated by heparin manganese. Cholesterol was measured in the supernatant, the between batch coefficient of variation being 3.6% at 0.97 mmol/l. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula⁸:

LDL cholesterol =

$$[\text{total cholesterol}] - [\text{HDL cholesterol}] - [\text{total triglycerides}/2.2]$$

Apolipoproteins were quantified by immunonephelometry using an automated Behring Laser Nephelometer (Hoechst UK Ltd, Hounslow). Behring standards and antisera were used and between batch coefficients of variation were 5.6% at a concentration of 0.92 g/l for apoA-I and 4.9% at 0.88 g/l for apoB.

Blood glucose concentration was measured using a Technicon AA-II autoanalyser (Technicon, Basingstoke) with a glucose oxidase based enzymatic colorimetric method. Glycated haemoglobin (HbA1) was measured by electroendosmosis after removal of the labile adduct with semicarbazide, using plates and apparatus supplied by Corning (Halstead). Plasma non-esterified fatty acids (NEFA) were determined by an automated enzymatic method.⁹ C peptide was measured by radioimmunoassay using a commercial radioimmunoassay kit (Novo, Bagsvaerd, Denmark).¹⁰ Glycerol was determined by an automated enzymatic technique.¹¹

STATISTICAL METHODS

Before analysis the distribution of all variables were tested separately in diabetic and control groups using the Kolmogorov-Smirnov goodness of fit procedure. The distributions of triglycerides were not Gaussian, although logarithmic transformation converted these to normal. Results are expressed as mean (95% confidence intervals of the mean), antilogarithms being calculated for triglycerides for clarity of presentation. Differences between diabetic and control groups were assessed using the unpaired Student's *t* test and differences between fasting and postprandial values were examined using the paired *t* test in each group. Diabetic subjects were further classified as having good (HbA1 up to 8.5%), fair (HbA1 8.6-10.0%), and poor control (HbA1 >10.1%). Differences between variables in these groups were assessed using one way analysis of variance (ANOVA) with Duncan's procedure to test for significance. Correlations between variables were assessed by calculating Pearson's correlation coefficients. Differences in the number of subjects with hyperlipidaemia in diabetic and control groups were investigated using the χ^2 with Yates's correction.

Table 2 Comparison of fasting and postprandial lipids in normal and diabetic subjects; values are mean (95% confidence interval)

Variable	Fasting	Postprandial	p Value*
Total cholesterol (mmol/l)			
Control	4.3 (4.1 to 5.4)	4.2 (4.0 to 4.4)	0.94
Diabetic	5.1 (4.7 to 5.4)	4.9 (4.6 to 5.2)	0.57
Triglycerides (mmol/l)			
Normal	0.74 (0.69 to 0.81)	0.93 (0.85 to 1.23)	<0.001
Diabetic	0.95 (0.83 to 1.10)	1.04 (0.91 to 1.20)	0.09
HDL cholesterol (mmol/l)			
Normal	1.4 (1.3 to 1.5)	1.3 (1.2 to 1.4)	0.001
Diabetic	1.5 (1.4 to 1.6)	1.5 (1.4 to 1.6)	0.66
LDL cholesterol (mmol/l)			
Normal	2.5 (2.3 to 2.7)	2.4 (2.3 to 2.6)	0.06
Diabetic	3.2 (2.9 to 3.4)	2.9 (2.7 to 3.1)	0.001
ApoA-I (g/l)			
Normal	1.45 (1.38 to 1.52)	1.56 (1.46 to 1.64)	<0.001
Diabetic	1.53 (1.46 to 1.59)	1.50 (1.42 to 1.56)	0.226
ApoB (g/l)			
Normal	0.96 (0.90 to 1.02)	0.87 (0.82 to 0.92)	<0.001
Diabetic	1.00 (0.90 to 1.08)	1.07 (0.96 to 1.16)	0.117

*Paired *t* test.

Table 3 Blood glucose and lipid concentrations in relation to glycaemic control; values are mean (95% confidence interval)

	Glycaemic control		
	Good	Fair	Poor
HbA1 (%)	5.0-8.5	8.6-10	>10
Number	11	13	27
Glucose (mmol/l)	10.3 (6.6 to 13.9)	11.1 (8.4 to 13.7)	14.4 (12.3 to 16.5)*
Total cholesterol (mmol/l)	4.6 (4.2 to 5.0)	4.7 (4.4 to 5.1)	5.5 (4.9 to 6.1)*
Triglycerides (mmol/l)	0.67 (0.56 to 0.80)	0.84 (0.76 to 0.93)	1.1 (0.91 to 1.44)*
HDL cholesterol (mmol/l)	1.6 (1.4 to 1.9)	1.5 (1.2 to 1.8)	1.4 (1.3 to 1.6)
LDL cholesterol (mmol/l)	2.6 (2.3 to 3.0)	2.9 (2.6 to 3.3)	3.5 (3.0 to 3.9)*
ApoA-I (g/l)	1.6 (1.4 to 1.7)	1.5 (1.4 to 1.6)	1.6 (1.5 to 1.7)
ApoB (g/l)	0.8 (0.7 to 0.9)	0.9 (0.8 to 1.0)	1.1 (1.0 to 1.2)*
HDL cholesterol/LDL cholesterol	0.35 (0.30 to 0.41)	0.30 (0.26 to 0.36)	0.27 (0.23 to 0.31)*
LDL cholesterol/apoB	5.5 (5.1 to 5.9)	5.3 (4.9 to 5.6)	5.0 (4.8 to 5.2)*

*Different from good control group ($p < 0.05$, ANOVA, Duncan's statistic).

Results

SUBJECTS

The diabetic children were free of retinopathy on fundoscopy and no patient had test strip positive albuminuria. Patients were taking a sugar free, carbohydrate controlled diet and all subjects were on twice daily insulin regimens except three who received once daily injections. One girl was taking a low dose contraceptive pill and another girl was on treatment with sodium valproate for epilepsy. Patients were receiving a mean dosage of 0.9 (0.85-0.95) units of insulin per kg of body weight. Mean duration of diabetes mellitus was 5.3 (4.5-6.1) years; 76.6% of the diabetic children were C peptide negative (fasting concentration < 0.02 mmol/l). The rest had low but detectable concentrations (up to 0.1 mmol/l). Both fasting and postprandial specimens were obtained from 48 subjects; there were 33 boys and 18 girls. The control group comprised 68 boys and 64 girls and postprandial specimens were obtained from 55 subjects. The mean age of the diabetic patients was 12.6 (11.5-13.7) years, younger than the control subjects (15.3 (13.5-17.2) years; $p = 0.02$).

BIOCHEMICAL VARIABLES

Fasting results

The mean values of the variables measured in the fasted state are shown in table 1. As expected, HbA1 and glucose concentrations were higher in the diabetic than in the control subjects. Serum total cholesterol, triglycerides, LDL cholesterol, and plasma NEFA concentrations were higher while LDL cholesterol/apoB ratios and HDL cholesterol/LDL cholesterol ratios were lower in the diabetic group. The difference in triglyceride concentrations

between the diabetic and control subjects (0.18 mmol/l) was greater than differences in free glycerol concentrations (0.03 mmol/l).

Proportion of subjects with dyslipidaemia

Out of 51 diabetic subjects examined in the fasted state, 20 (39%) had a serum total cholesterol above the cut off point of 5.2 mmol/l; 13 children (25%) had raised cholesterol alone; seven had a serum triglyceride concentration exceeding 1.7 mmol/l (14%), the children with a raised triglyceride concentration also had a raised cholesterol. None of the children had hypertriglyceridaemia alone. By contrast only 24 out of 132 healthy controls (17%) had a raised serum cholesterol and only one (0.7%) had high triglycerides. This latter subject also had a high cholesterol value. The proportions with hypercholesterolaemia ($\chi^2 = 11.0$; $p < 0.001$) and hypertriglyceridaemia were significantly higher in the diabetic subjects ($\chi^2 = 13.5$, $p < 0.001$).

Postprandial results

As expected, within subject increases of triglycerides postprandially were seen in the normal group, although the increase failed to achieve significance in the diabetic patients (table 2). HDL cholesterol and apoB decreased while apoA-I increased in the normal but not in diabetic subjects. LDL cholesterol decreased in diabetic patients and a similar trend was seen in control subjects, although this just failed to achieve significance ($p = 0.06$).

Relationship of lipid variables with glycaemic control

Diabetic patients in poor control had higher fasting total cholesterol, triglyceride, LDL cholesterol, and apoB concentrations when compared with patients in good control (table 3). In addition, HDL cholesterol/LDL cholesterol and LDL cholesterol/apoB ratios were lower in patients with poor control than in those with good control. These relationships appeared to be continuous in the diabetic patients rather than occurring at a particular threshold of HbA1 as intermediate values were seen in patients with fair control

Table 4 Relationship between lipid variables and HbA1 concentrations

Variable	Diabetic patients	Control subjects
Total cholesterol	0.42**	0.03
Triglycerides	0.58**	0.08
HDL cholesterol	-0.25	0.17
LDL cholesterol	0.36**	-0.09
ApoA-I	-0.17	0.15
ApoB	0.54**	0.08
HDL cholesterol/LDL cholesterol	-0.37*	0.21*
LDL cholesterol/apoB	-0.34	-0.06
ApoA-I/apoB	-0.37*	0.09

Values are Pearson's correlation coefficients: ** $p < 0.001$, * $p < 0.01$.

and there were significant correlations between these variables and HbA1 concentrations (table 4). However, HbA1 concentrations did not appear to be related to these variables in control subjects. There was no relationship between apoA-I or HDL cholesterol concentrations and HbA1 in any of the study groups.

Discussion

The current definitions of hypercholesterolaemia for adult subjects specify total serum cholesterol values <5.2 mmol/l as desirable and >6.5 mmol/l as requiring active intervention in some groups of patients.^{12 13} However, serum cholesterol concentrations rise with age and lower cut off values in children have been proposed, 4.1 mmol/l by the World Health Organisation¹⁴ and 4.3 mmol/l by the National Cholesterol Education Program in the United States,¹⁵ which also defined 4.4–5.2 mmol/l as borderline and >5.2 mmol/l as raised. The principle finding of the present study was that the mean serum total cholesterol concentration approached this definition in IDDM and the prevalence of hypercholesterolaemia was higher in IDDM patients than in control subjects. Definitions of hypertriglyceridaemia have received less attention but using the definition of good control proposed by the European Non-Insulin-Dependent Diabetes Policy Group (<1.7 mmol/l),¹⁶ the prevalence of hypertriglyceridaemia and the mean blood triglyceride concentrations were increased in IDDM patients. A potential confounding factor is free glycerol concentrations as these are measured in the triglyceride assay and are higher in diabetic subjects. Although free glycerol concentrations were raised in the diabetic subjects in the present study these were not great enough to explain the raised triglyceride concentrations.

The present data are in agreement with some previous reports,^{6 17–20} although others have reported total serum cholesterol to be similar in IDDM and control groups.^{4 5 21–23} Possible reasons for differences between reports include case selection criteria, the nature of the control population, the diet of the general population, the duration and severity of diabetes, the degree of glycaemic control, and variations in laboratory methods. One possible confounding factor in the present study is that the control group was older than the diabetic patients. However, we have shown previously that age has no effect on cholesterol and triglyceride concentrations in children within the age range studied.²⁴ The present data confirm suggestions that glycaemic control is a major factor affecting serum lipid concentrations in juvenile diabetes^{6 18 20} as strong correlations were found between total lipid values and HbA1 concentrations and results from patients with good control were similar to those in control subjects.

HDL cholesterol concentrations, which reflect antiatherogenic lipoproteins, are low in untreated adult IDDM but increase with good

glycaemic control, often exceeding those in control subjects.³ Some previously published data in children have been similar to results from adults, with low HDL cholesterol values found in patients with poor control^{6 18} and raised concentrations in well controlled subjects.²⁵ In common with the findings of others,^{4 20 21} HDL cholesterol values did not differ from control values in the present study and there was no relationship with glycaemic control. We determined HDL cholesterol values using a precipitation technique that was similar to the procedure used by others who have reported conflicting data.²⁵ Thus, methodological differences do not appear to explain discrepant results. The main difference in studies that have reported changes in HDL cholesterol in juvenile diabetes is that they have included subjects up to the age of 20 years.^{6 25} HDL cholesterol concentrations in non-diabetic children fall after puberty.²⁶

Lipoprotein fractions were not assessed directly in the present study so that the exact pattern of hyperlipidaemia could not be determined. LDL cholesterol values were calculated, this method having the drawback that it includes intermediate density lipoprotein (IDL), which accumulates in diabetes, in the calculation²⁷ and the assumption that true triglyceride concentrations have been determined. The small differences in free glycerol concentrations found in the present study were not great enough for the effect of these on triglyceride concentrations to invalidate the calculation of LDL and our finding that raised LDL cholesterol is related to glycaemic control in children with IDDM is in agreement with a study in which LDL cholesterol concentrations were determined by ultracentrifugation, a procedure that avoids the interference of IDL.¹⁸ Correlations between glycosylated proteins and LDL cholesterol concentrations in juvenile diabetes have been found by others, even though LDL cholesterol concentrations were not raised.²² As LDL cholesterol was increased and HDL cholesterol unchanged, the HDL cholesterol/LDL cholesterol ratio was increased in the diabetic subjects. This ratio has been shown to be an index of the atherogenicity of serum lipoproteins, particularly in the presence of hypertriglyceridaemia.²⁸

In common with other reports, the present investigation has demonstrated no differences in apolipoprotein concentrations between study groups in treated patients,²² but increased apoB concentrations in diabetic children with worsening glycaemic control.^{6 18} ApoA-I is the major protein component of HDL while apoB is found mainly in LDL. Thus these results confirm the lipid data and suggest further that the latter are not confounded by methodological inaccuracies. The measurement of apolipoproteins is limited currently by the lack of uniform standardisation and calibration procedures,²⁹ but if these problems can be overcome apolipoproteins may prove to be more valuable as predictors of future atherosclerotic disease than lipid measurements.^{30 31}

Although it is the major proatherogenic lipoprotein, LDL is not homogenous and two distinct subclasses that have different sizes and densities are recognised. Austin *et al* have described two distinct LDL phenotypes, type A which is characterised by a predominance of large buoyant particles with high cholesterol/apoB ratios, and type B in which the particles are smaller, denser, triglyceride enriched and have low cholesterol/apoB ratios.³² The LDL subclass pattern associated with small dense particles (pattern B) is associated with a threefold greater risk of myocardial infarction than the type A pattern, independently of LDL concentration.³³ In the present study the LDL/apoB ratio was raised in the diabetic as compared with control children, suggesting a predominance of small, dense LDL, this abnormality being related to the degree of glycaemic control. This finding is consistent with data from adult IDDM patients,³⁴ in addition to NIDDM patients in which increased particle density and reduced particle size occur have been shown to be directly related to reduced LDL cholesterol/apoB ratios, these abnormalities occurring even when lipid concentrations were not raised significantly.³⁵ To our knowledge, the present report is the first describing small dense LDL in juvenile diabetes.

We have demonstrated that NEFA and glycerol concentrations are significantly raised in diabetic subjects. This suggests increased adipose tissue lipolysis, one of the basic biochemical disturbances of insulin deficiency,³⁶ provides fuel for hepatic very low density lipoprotein (VLDL) triglyceride synthesis. The mechanism of increased LDL in poorly controlled diabetes is not clearly understood although overproduction of VLDL, the precursor of LDL, would partly explain the increase. In addition, it is possible that compositional abnormalities of LDL,³⁵ non-enzymatic glycation of LDL apoB,³⁷ or direct effects of insulin on the LDL receptor slow high affinity receptor mediated clearance of LDL from the circulation. Support for this hypothesis is provided by a report that describes reduced clearance of LDL apoB in NIDDM, this being increased by insulin treatment.³⁸

Investigation of postprandial lipids in our study groups was of interest for two reasons. First, although serum triglyceride concentrations increase in adults after a meal it has been suggested that this phenomenon is age dependent.³⁹ Investigation protocols would be simplified if postprandial lipaemia did not occur to a significant extent in children as it might not be necessary to take fasting samples for lipid analysis. Second, the extent of postprandial lipaemia has been proposed a marker of the atherogenicity of triglyceride-rich lipoproteins.⁴⁰ Postprandial increases in triglycerides and other lipoproteins were found in control subjects although significant changes did not occur in diabetic subjects, apart from a fall in LDL cholesterol. We conclude that any postprandial changes in serum lipids are small in diabetic children.

In summary we have shown that various circulating lipid variables are raised in young patients with IDDM, these being related to glycaemic control. The important long term implication, therefore, is that lipid concentrations need not be worse than in the non-diabetic population, providing that good glycaemic control is achieved.

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