Lipoprotein measurements—a necessity for precise assessment of risk in children from high-risk families

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SUMMARY Serum lipids and lipoproteins were measured in 67 high-risk children, aged between 2 and 17 years, who were the offspring of 33 fathers with hypercholesterolaemia. 18 of the 33 fathers had had a myocardial infarction (MI) before 44 years of age. In 15 of the fathers there was no history of accelerated coronary heart disease (CHD). No difference in the concentrations of the serum lipids and lipoproteins was found between the children whose fathers had a history of MI ($n = 31$) and the children with no family history of CHD ($n = 36$). Serum lipids and lipoproteins were also measured in a control group of 19 children, aged between 2 and 17, whose parents were normolipaemic and had no history of atherosclerosis. Serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDLc) concentrations were significantly higher— 5.47 ± 1.36 mmol/l (211 ± 52 mg/100 ml) and 3.55 ± 1.16 mmol/l (137 ± 45 mg/100 ml) respectively—in the 67 'high-risk' children than in the control group—TC, 4.46 ± 0.64 mmol/l (172 ± 25 mg/100 ml); LDLc, 2.56 ± 0.64 mmol/l (99 ± 25 mg/100 ml). No statistically significant difference was found in serum triglyceride (TG) or high-density lipoprotein-cholesterol (HDLc) concentrations between these two groups.

In 7 of the high-risk children, raised levels of serum TC >5.74 mmol/l (>221 mg/100 ml) were due to raised HDLc >1.95 mmol/l (>75 mg/100 ml) and normal LDLc <3.84 mmol/l (<148 mg/100 ml). In spite of their increased TC levels these children are probably at a lower risk for CHD as a result of the protective effect of HDL. In 3 children, raised concentrations of LDLc (>3.84 mmol/l) were accompanied by low levels of HDLc (<1.19 mmol/l) and normal concentrations of TC (<5.74 mmol/l). These children are probably at the highest risk for CHD.

The correct diagnosis would have been missed in 10 (15%) of the children had only TC been measured. It is therefore imperative to measure lipoprotein levels in addition to TC in all children from 'high-risk' families, regardless of their TC concentrations.

During the last two decades some risk factors associated with coronary heart disease (CHD) in young adults have been identified. Ways have been found to reduce these risks as a possible means to prevent, or at least delay, CHD (Berenson *et al.*, 1978; Glueck *et al.*, 1978a, b). As many of these risk factors can be identified in childhood, attempts to change relevant behaviour—for example, diet, physical activity, and smoking—may usefully be made at this age.

In adults there is a positive association between

CHD and serum total cholesterol (TC) and low-density lipoprotein (LDL) (Kannel *et al.*, 1971; Tyroler *et al.*, 1975; Castelli *et al.*, 1977). More recently, an inverse association between high-density lipoprotein (HDL) and CHD has been established (Tyroler *et al.*, 1975; Glueck *et al.*, 1976; Castelli *et al.*, 1977).

Of the known risk factors for CHD, plasma lipids and lipoproteins are particularly relevant to children because (1) hypercholesterolaemia can be detected at an early age, and (2) dietary modification, combined in severe cases with cholestyramine, can lower the levels of serum cholesterol in children (Segall *et al.*, 1970; West and Lloyd, 1973; Glueck *et al.*, 1978b).

Although screening large groups of children to detect familial hypercholesterolaemia (FH) is unlikely to be profitable (Leonard *et al.*, 1976; Lloyd,

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1977), efforts should be made to screen children from high-risk families (Leonard *et al.*, 1976). Many children from families with accelerated CHD and hypercholesterolaemia will also have raised plasma lipids (Tamir *et al.*, 1972).

However, recent evidence has suggested that measurement of lipids only may be insufficient to assess the true risk for CHD. For example, Glueck *et al.* (1978a) reported that about 16% of school-children with raised plasma TC levels had normal concentrations of LDL and the increase in TC was due solely to raised plasma HDL. In view of the protective effect of HDL, these children were putatively at a lower risk for CHD, which obviated the need for intervention.

We report on the distribution of serum lipids and lipoproteins in 67 children from 33 high-risk families, and reiterate the need to measure lipoproteins, in addition to TC and triglyceride (TG), for the correct diagnosis of abnormalities in such children.

Patients and methods

67 children (38 boys and 29 girls) aged between 2 and 16 years (mean 8.8) were investigated. They were the offspring of 33 hypercholesterolaemic (that is—serum TC >95th centile) fathers and normolipidaemic mothers. Serum TG concentrations in all the fathers were normal. The fathers of 31 children had a history of CHD verified from hospital records (pathognomonic ECG changes and increased serum enzymes), whereas in the fathers of 36 children there was no history of atherosclerosis. All the fathers were less than 44 years old at the time of examination.

For comparative purposes, we also measured serum lipids and lipoproteins in 19 children (mean age 11.6; range 2–17 years) whose parents were normolipidaemic and had no history of atherosclerosis.

Blood samples were obtained after an overnight fast (at least 12 hours). Children under 5 years were allowed water until 2 hours before examination. Serum TC was estimated by the method of Rappaport and Eichhorn (1960) and TG by the method of Shafir and Khassis (1969). Because TG concentrations were <3.25 mmol/l (<298 mg/100 ml) in all samples, HDL-cholesterol (HDLc) was assessed by precipitation of the very low density lipoprotein (VLDL) and LDL with a heparin-manganese chloride solution and measurement of the cholesterol content of the supernatant. VLDL-cholesterol was assumed to be equal to serum TG/2, and LDL-cholesterol (LDLc) was calculated as the quantity: TC-HDLc-VLDLc.

Results

The concentrations of serum lipids and lipoproteins in the three groups of children are shown in Tables 1 and 2. For all the parameters studied, no statistically significant differences were found between the children of fathers without CHD (group 1) and the children of fathers with CHD (group 2). Therefore, these two groups were combined for subsequent analyses. The children from the high-risk families (groups 1 and 2) had significantly higher levels of TC ($P < 0.01$), and LDLc ($P < 0.001$), than the children in the control group. No significant differences were detected in the concentration of TG or HDLc.

The Figure shows that in 30 (40%) of the high-risk children, TC concentrations exceeded the mean level of the control group by >2 SDs. In three of these, serum TG levels were also raised. One child had normal TC but an increased TG level. Of the 30 children in whom hypercholesterolaemia was present, only 23 had high TC levels resulting from raised LDLc. In 7 children, the TC levels were high but LDLc concentrations were normal. In these 7 children HDLc exceeded the mean of the control

Table 1 Mean ($\pm$ SD) serum lipid and lipoprotein levels in children with hypercholesterolaemic and normolipidaemic parents

Group	Age (years) (mean, range)	TC (mmol/l)	TG (mmol/l)	LDLc (mmol/l)	HDLc (mmol/l)
1 (n = 36)	8.3 (2–16)	5.58 $\pm$ 1.62	0.61 $\pm$ 0.29	3.67 $\pm$ 1.38	1.68 $\pm$ 0.38
2 (n = 31)	9.5 (3–16)	5.34 $\pm$ 1.00	0.57 $\pm$ 0.30	3.41 $\pm$ 0.90	1.64 $\pm$ 0.40
P		NS	NS	NS	NS
1 and 2 (n = 67)	8.9 (2–16)	5.47 $\pm$ 1.36	0.58 $\pm$ 0.29	3.55 $\pm$ 1.16	1.66 $\pm$ 0.38
Control (n = 19)	11.6 (2–17)	4.46 $\pm$ 0.64	0.69 $\pm$ 0.29	2.56 $\pm$ 0.64	1.57 $\pm$ 0.38
P		<0.02	NS	<0.001	NS

Group 1 were children with normolipidaemic mothers and hypercholesterolaemic fathers who had a history of MI. Group 2 were children with normolipidaemic mothers and hypercholesterolaemic fathers who had no history of MI.

TC = Total cholesterol, TG = triglyceride, LDLc = low-density lipoprotein cholesterol, HDLc = high-density lipoprotein cholesterol.

Conversion: SI to traditional units—cholesterol: 1 mmol $\approx$ 38.6 mg/100 ml, triglyceride: 1 mmol $\approx$ 88 mg/100 ml.

Table 2 Total cholesterol and lipoprotein cholesterol (means) in children from 'high-risk' families with raised* TC but normal LDLc levels

Case	Age (years)	Sex	TC (mmol/l)	LDLc (mmol/l)	HDLc (mmol/l)	VLDLc (mmol/l)
1	13	F	5.77	2.86	2.26	0.65
2	8	M	5.87	3.67	2.00	0.23
3	11	F	6.16	3.27	2.65	0.23
4	14	M	5.95	3.27	2.31	0.36
5	8	F	5.79	3.35	2.26	0.18
6	7	M	5.85	3.22	2.31	0.31
7	11	M	6.34	3.64	2.31	0.39

*Exceeding the mean for control group by > 2 SDs.

Figure Total number of children at high risk.

group by 1 SD, and thus was the main contributor to the raised TC levels. In 3 children, raised levels of LDLc (>3.84 mmol/l) were accompanied by low HDLc (<1.95 mmol/l), resulting in normal TC (<5.74 mmol/l).

Discussion

The importance of detecting risk factors for CHD as early as possible in childhood is now well accepted (Glueck *et al.*, 1974; Levy and Rifkind, 1974; Lauer *et al.*, 1975). It is assumed, but not yet confirmed, that instituting measures to correct these risk factors early in childhood will prevent, or at least delay, the onset of atherosclerosis and its main manifestation—CHD.

Raised plasma or serum lipids, mainly TC, are among the main factors that are especially associated with increased risk for CHD in young adults (Kannel *et al.*, 1971; Blackburn, 1974; Keys, 1975; *Lancet*, 1977). Moreover, the risk for developing early atherosclerosis is particularly high in patients with FH, especially in young men (Slack, 1969; Stone *et al.*, 1974). Hence, screening children to detect FH is recommended by some but not all paediatricians (Lloyd, 1975). However, it is generally agreed that children from high-risk families—that is families with a history of accelerated atherosclerosis or hyperlipoproteinaemia—should be examined for detection of

FH (Tamir *et al.*, 1972; Glueck *et al.*, 1974; Leonard *et al.*, 1976; Lloyd, 1977).

Diagnosis of FH is usually based on a raised level of TC or LDLc in the proband and in at least one first-degree relative. However, because about 70% of plasma TC is carried in the LDL, it has been generally accepted that in most instances measurement of TC is sufficient for appropriate diagnosis.

The importance of the inverse relationship of HDLc and CHD has been reported (Tyroler *et al.*, 1975; Glueck *et al.*, 1976; Castelli *et al.*, 1977) and HDL is now considered a 'negative risk factor' for CHD. Measurement of lipoproteins is thus assuming greater importance for proper diagnosis and treatment. In addition to the study by Glueck *et al.* (1978a), in which up to 16% of a group of schoolchildren with raised TC levels had normal LDLc but increased HDLc, it has also been shown that about one-third of children referred because of hypercholesterolaemia had increased concentrations of HDLc with normal LDLc (Neill *et al.*, 1977). Curiously, these children are putatively at a lower risk for CHD, in spite of their raised TC levels.

In 10 (15%) of the 67 children reported here, the correct diagnosis would have been missed if TC and TG alone had been measured. In 7, the increased TC levels were caused by increased concentrations of HDLc (with normal concentrations of LDLc). These children probably have a lower than normal

risk for developing CHD; therefore, any measures aimed at reducing their TC are probably not warranted. At the same time, however, the normal TC levels in 3 children were the result of increased LDLc but low HDLc. These children, who are probably at the highest risk for CHD (Kwiterovich, 1977), would also have been missed had only TC been estimated.

Our results confirm earlier reports that approximately 40% of the children born to matings of familial hypercholesterolaemic and normolipaeic parents will have FH (Tamir *et al.*, 1972; Kwiterovich *et al.*, 1974). The need for accurate diagnosis is especially important in the children from these high-risk families; it is likely that they carry the genetic form of hypercholesterolaemia, which has been shown to be associated with a very high risk for CHD.

We advocate measurement of lipoproteins in these children—although it is more complex and requires special skill and equipment—as it clearly enhances diagnostic precision and improves the assessment of their risk. Furthermore, it facilitates appropriate treatment and prognostication. This is important not only for the fortunate children whose raised TC levels result from raised HDLc, who are thus putatively at lower risk for CHD, but it is of vital importance for the children whose normal TC levels (but low HDLc) do not accurately reflect their high-risk status.

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