Screening for familial hyper- β -lipoproteinaemia in children in hospital

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Leonard, J. V., Fosbrooke, A. S., Lloyd, J. K., and Wolff, O. H. (1976). *Archives of Disease in Childhood*, **51**, 842. **Screening for familial hyper- β -lipoproteinaemia in children in hospital.** 1510 plasma cholesterol estimations were made in 1391 children admitted to hospital as part of a biochemical profile. Babies under 1 year and children known to have familial hyperlipoproteinaemia were excluded. The mean concentration was $4.28 \text{ mmol/l} \pm 1.04$ (1 SD) ($165.3 \text{ mg/100 ml} \pm 38.6$), and levels exceeded 5.93 mmol/l (229 mg/100 ml) in 68 children. Repeat estimations on 55 of these children showed 34 still to have values $> 5.93 \text{ mmol/l}$ and family studies were performed in 19 of these. In 8 children hypercholesterolaemia was secondary and no familial lipoprotein disorder was present. Familial hyper- β -lipoproteinaemia (FH) was diagnosed in 3 children and in 2 of the families there was a history of early ischaemic heart disease. In 2 children the diagnosis was in doubt. In the remaining 6 children FH and secondary hyperlipoproteinaemia were excluded so the hypercholesterolaemia was presumably environmentally induced, possibly in association with polygenic inheritance. In the present state of knowledge screening of the childhood population for FH by means of plasma cholesterol determinations cannot be recommended. Studies of lipoproteins should, however, be made in children from families known to have FH or early coronary heart disease.

Familial hyper- β -lipoproteinaemia (FH) is one of the most common inherited metabolic disorders in childhood. Its importance lies in the high risk of coronary heart disease in affected individuals, especially males, in early adult life (Slack, 1969; Stone *et al.*, 1974). Population screening for the disorder during childhood is not recommended (Lloyd, 1975) partly because long-term results of treatment are not known, and partly because there is little information about the precision with which the diagnosis can be made in children on the basis of estimation of total plasma cholesterol concentration as the screening method. As part of a study to assess the value of a biochemical profile on children admitted to hospital (Leonard, Clayton, and Colley, 1975) we measured plasma cholesterol and report our findings.

Patients and methods

The details of two controlled trials carried out on children admitted to the Hospital for Sick Children in

1973 to assess the value of a routine biochemical profile have already been described (Leonard *et al.*, 1975). All the children admitted were eligible to be included except for day cases and those admitted to two of the surgical wards. A capillary or venous blood sample was collected soon after admission and the children were not necessarily fasting.

In the present study children under one year of age, and those known to have a primary disorder of serum lipoproteins at the time of admission were excluded. The decision to exclude babies was made because the type of milk at this age is known to have a marked influence on serum cholesterol concentrations thus making interpretation of results difficult (Darmady, Fosbrooke, and Lloyd, 1972).

For the preliminary identification of children likely to have FH, it was decided to apply an arbitrary cut-off value for plasma cholesterol above which children would be further investigated. When the plasma cholesterol concentration of 200 consecutive patients had been determined, 5% were found to have values $> 5.93 \text{ mmol/l}$ (229 mg/100 ml) and this value was then chosen as the cut-off point. Children with values exceeding 5.93 mmol/l in the initial (profile) study had another specimen of blood collected, after a period of fasting lasting at least 8 hours, for the estimation of serum cholesterol and

Received 4 March 1976.

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triglyceride, and for ultracentrifugation to determine lipoprotein concentrations. In many cases this specimen could not be obtained while the child was still in hospital because of the design of the controlled trials of the biochemical profiles (Leonard *et al.*, 1975), and also because it was decided to allow at least 6 weeks to elapse after an operation or serious illness before collecting the second sample. At the same time appropriate investigations were undertaken to identify conditions likely to cause secondary hypercholesterolaemia.

If the value of the repeat cholesterol estimation was <5.93 mmol/l no further studies were done. If the value exceeded 5.93 mmol/l fasting blood specimens were obtained from the parents, and if possible from sibs. The purpose of the study was explained to the parents, and their permission and that of the family practitioner was obtained. The study was approved by the Ethical Committee of the Institute of Child Health and the Hospital for Sick Children.

Estimation of cholesterol and serum triglyceride. In the initial examination (profile study) total cholesterol was estimated on unextracted plasma by the Liebermann-Burchard reaction using the Technicon SMA 12/micro multichannel analyser (Leonard, 1975). In the repeat examination and for the family studies the analyses were performed by the method of Fosbrooke and Pringle (1970) on extracted serum using a semi-automated ferric chloride procedure. The two methods did not give identical results, those obtained by the ferric chloride method being consistently higher. Comparisons on 32 paired sera showed that the regression of the Liebermann-Burchard values (y) on the ferric chloride results (x) was $y = 0.86x + 0.33$ (correlation coefficient = 0.99, standard error of the slope 0.02, standard error of the intercept 0.16). Thus a value of 5.93 mmol/l (229 mg/100 ml) in the profile study corresponds to 6.53 mmol/l (252 mg/100 ml) in the repeat analysis. Though all the repeat estimations were made by the ferric chloride method the same numerical cut-off point as used with the Liebermann-Burchard method (5.93 mmol/l) was used to decide whether or not to proceed with a family study. This value corresponds to a value by the Liebermann-Burchard method of about 5.43 mmol/l (210 mg/100 ml), and thus it is unlikely that children with FH will have been missed.

Serum triglyceride was measured either by a gas liquid chromatographic technique (Fosbrooke and Tamir, 1968) or by an automated fluorometric method (Claude, Corre, and Levallois, 1968). Preparative ultracentrifugation was carried out by the method of Cornwell *et al.* (1961).

Reference values and criteria for diagnosis of FH. Reference values for children over the age of one year were those obtained on healthy children whose serum was analysed in the same laboratory performing the repeat analyses and using identical methods (Table). Reference values for adults (Table) were taken from the data of Fredrickson, Levy, and Lees (1967). The criteria used to establish the diagnosis of FH were serum

cholesterol concentrations exceeding the upper reference value with increased β -lipoprotein cholesterol on ultracentrifugation in the index patient and at least one first-degree relative.

Results

In the profile study 1510 plasma cholesterol estimations were performed on 1391 children; the mean concentration was 4.28 mmol/l ± 1.04 (1 SD) (165.3 mg/100 ml ± 38.6). In 69 patients values exceeded 5.93 mmol/l (229 mg/100 ml) (Fig. 1), and Fig. 2 illustrates the further investigation on these children. Repeat estimations were made on 55 children within 15 months of the profile study. The reasons for not obtaining blood from the remaining 13 patients are given in Fig. 2. Of the 8 children in whom it might have been possible to obtain a further specimen, 2 had left the country and in one other, who had severe brain damage following encephalitis, investigation would have been inappropriate. 2 of the 8 children had the nephrotic syndrome (cholesterol values 6.03 and >12.9 ; 233 and >498 mg/100 ml), a condition known to be associated with secondary hypercholesterolaemia, and the other 6

FIG. 1.—Plasma cholesterol in 1391 children in biochemical profile study. The shaded area represents the 68 children whose values exceeded 5.93 mmol/l.

Case no.	Propositus			Sibs		
	Age (yr), sex, diagnosis	Serum cholesterol (LDL cholesterol) (mmol/l)	Triglyceride (mmol/l)	Age (yr), sex	Serum cholesterol (LDL cholesterol) (mmol/l)	Triglyceride (mmol/l)
1	1.0 M Truncal ataxia	8.60(6.27)	0.82	2.5 M	8.37(6.53)	0.93
2	5.2 M Epilepsy	7.20(*)	0.73	5.6 M 8.3 F 11.1 F	5.08(3.24) 6.73(4.87) 5.57(*)	0.45 — —
3	14.4 M Mental retardation	6.99(4.53)	—	None	—	—
4	3.1 M Acrodermatitis enteropathica	6.01(3.19)	1.02	7.1 F 9.8 F 10.9 F 12.1 M	7.02(4.22) 4.87(2.18) 6.16(2.99) 5.96(3.06)	0.65 0.71 0.78 0.78
5	12.1 M Inguinal herniotomy	7.43(4.4)	0.78	None	—	—
6	9.3 M Perthes's disease	7.10(4.77)	—	None	—	—
7	1.4 F Mental retardation after encephalitis	6.34(4.20)	1.01	None	—	—
8	11.4 F Short stature	10.80(4.74)	0.64	None	—	—
9	12.6 M Cystinuria	7.51(5.98)	0.73	2 sisters not tested		
10	8.7 F Osteosclerotic lesion of spine	6.53(3.65)	0.67	2 sisters and 2 brothers not tested		
11	5.6 M Urinary diversion for urethral stricture	6.11(2.93)	1.05	None	—	—

*No result for technical reasons.
LDL, low density lipoprotein.

Reference values:

Conversion: SI to traditional units—Cholesterol : 1 mmol/l $\approx$ 39 mg/100 ml.

Cholesterol (mmol/l)

	Age	Mean	90% confidence limits
Children	2-13	4.92	3.86-5.98
Adults	20-29	4.66	3.11-6.22
	30-39	5.31	3.63-6.99
	40-49	5.83	3.88-8.03
	50-59	6.34	4.41-8.55

had cholesterol values between 6.09 and 6.24 mmol/l (235 and 241 mg/100 ml).

Of the 55 children in whom repeat estimations were made, 34 had cholesterol values greater than 5.93 mmol/l and 21 had values below this level. 5 of the latter patients had conditions known to be

associated with secondary hypercholesterolaemia (diabetes mellitus 1, nephrotic syndrome 2, haemolytic-uraemic syndrome 1, hypopituitarism 1); because they had all improved with treatment the fall in serum cholesterol between the two examinations is not surprising.

family studies

Father			Mother			Other details
Age (yr)	Serum cholesterol (LDL cholesterol) (mmol/l)	Triglyceride (mmol/l)	Age (yr)	Serum cholesterol (LDL cholesterol) (mmol/l)	Triglyceride (mmol/l)	
26	4.69(2.69)	—	23	10.20(7.77)	0.84	Mother—corneal arcus; Maternal grandfather—died of myocardial infarct aged 43
32	8.96(5.70)	1.72	29	5.65(3.11)	0.72	Paternal grandfather died of myocardial infarct aged 50
—	—	—	38	8.13(5.0)	0.77	Parents divorced—father not traceable
35	5.72(2.46)	—	37	7.95(4.48)	1.16	
—	—	—	40	7.46(*)	1.02	Father died of myocardial infarct aged 65
43	7.33(3.86)	—	51	6.37(3.31)	—	
45	7.67(4.32)	0.65	40	6.34(*)	0.86	
54	6.63(4.12)	2.49	52	6.66(2.56)	1.31	Propositus: no cause for short stature found after extensive investigation
49	6.66(4.20)	0.81	42	5.21(*)	—	
40	5.80(2.74)	—	38	5.28(2.41)	—	
37	4.35(2.36)	0.83	33	6.40(3.29)	0.96	

Triglyceride: 1 mmol/l $\approx$ 88 mg/100 ml.

LDL cholesterol (mmol/l)	
Mean	90% confidence limits
2.43	1.06–3.81
2.90	1.55–4.40
3.26	1.88–4.92
3.47	2.07–4.92
4.17	2.07–5.44

Of the 34 children with serum cholesterol concentrations greater than 5.93 mmol/l, family studies were completed in 19; the reasons for not obtaining a family study in the other 15 are given in Fig. 2. Hypercholesterolaemia in 12 of these was probably secondary (diabetes mellitus 2, nephrotic syndrome

5, chronic renal disease 3, hypothyroidism 1, hypopituitarism 1). One of the remaining children had heavy proteinuria after penicillamine therapy for cystinosis and the other 2 had cholesterol values of 6.29 and 5.98 mmol/l (243 and 231 mg).

The family studies showed no evidence in support

FIG. 2.—Investigations of children with plasma cholesterol greater than 5.93 mmol/l in the profile study.

of a diagnosis of FH in 7 children, all of whom had a disease known to cause hypercholesterolaemia (diabetes mellitus 2, nephrotic syndrome 2, chronic renal disease 2, glycogen storage disease 1). In one child with anorexia nervosa the initial serum cholesterol concentration was 8.86 mmol/l (342 mg) but after one year of treatment during which time she had improved but not completely recovered, the level had fallen to 6.11 mmol/l (236 mg). Her father had a serum cholesterol of 9.04 mmol/l (349 mg) with low density lipoprotein (LDL) cholesterol of 5.57 mmol/l (215 mg) (both raised), and his brother had had a myocardial infarction at the age of 47 years. Although FH is probably present in this family, it is not possible to state categorically whether the patient is affected as anorexia nervosa is known to cause hypercholesterolaemia which reverts to normal with treatment (Blendis and Crisp, 1968).

Details of the family studies in the remaining 11 patients are given in the Table. In 3 families (Cases 1, 2, and 3) FH was identified in the patient according to our criteria, and in 2 of these families there was also a history of early ischaemic heart disease. In 4 families (Cases 6, 7, 8, and 9) the patients, none of whom had diseases known to be associated with hypercholesterolaemia, had raised levels of total cholesterol and LDL cholesterol but neither parent had increased β -lipoprotein cholesterol and thus a diagnosis of FH was unlikely. In 2 families (Cases 4 and 5) a firm conclusion could not be reached. In family 4, one sib had raised total and LDL cholesterol, another sib and the mother had raised total cholesterol but normal LDL cholesterol; in the patient himself the normal LDL cholesterol could be the result of malabsorption caused by acrodermatitis enteropathica. In Case 5 the father had died from a myocardial infarct at the age of 65 and details of his serum lipids were not available. Cases 10 and 11 had raised total cholesterol but normal LDL cholesterol, and both parents were normal.

Thus FH was diagnosed with a high degree of probability in 3 of the 11 children, the diagnosis was in doubt in 2, and was excluded in 6.

Discussion

Diagnosis of heterozygous FH is usually based on raised total plasma cholesterol concentration and/or raised LDL cholesterol concentration in the proband and at least one first-degree relative. There is no precise cut-off point for either of these cholesterol determinations above which the diagnosis can be made with certainty (Fredrickson, 1971). Different cut-off points have been used by Fredrickson *et al.* (1967) of 90% confidence limits, and Slack and Nevin (1968) of two standard deviations above the mean. The values are arbitrary and depend on the population studied and the method of lipid analysis.

Our study illustrates the diagnostic difficulties which are likely to arise in the interpretation of plasma cholesterol concentrations in children. In 5 of our patients family studies excluded FH, and no other disease known to cause raised lipid levels was present. In these children hypercholesterolaemia may be environmentally induced and polygenic inheritance may play a part. The risk of coronary heart disease in adult life for such children is not known but is likely to be less than for those with FH (Slack, 1974).

The incomplete follow-up in our study was due to the constraints imposed by the larger biochemical profile study of which our investigations formed a part, and because many of the families lived a long

way from the hospital. Nevertheless we do not consider that our overall conclusions have been affected. We are not able to give a precise prevalence rate for FH but the figure cannot be less than 0.22%, which is of the same order as that derived by others (Carter, Slack, and Myant, 1971; Darmady *et al.*, 1972; Goldstein *et al.*, 1973; Tsang, Fallat, and Glueck, 1974). Precise diagnosis and accurate estimation of the prevalence will only be possible when the basic defect is known. The work of Goldstein and Brown (1975) suggests that this may be failure of surface binding of LDL and its degradation. Further work is needed before such knowledge can be applied to the diagnosis of the disorder.

In our opinion screening of the childhood population for FH by means of plasma cholesterol determination cannot be recommended at this time. In children from families known to have FH and/or a history of early coronary disease, however, studies of plasma lipoproteins should be undertaken (Chase, O'Quin, and O'Brien, 1974; Lloyd, 1975). Such a selective procedure would in fact have detected 2 out of 3 of the affected children in our study.

We are grateful to Professor B. E. Clayton for the initial estimations in the profile study, to the Consultant Staff of The Hospital for Sick Children, Great Ormond Street for permission to study their patients, to Dr. J. Slack for helpful criticisms; and to the British Heart Foundation for financial support.

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