High-density-lipoprotein-cholesterol in protein–energy malnutrition

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1. Plasma total cholesterol, high-density-lipoprotein (HDL)-cholesterol total protein, albumin and globulin levels were estimated in blood samples from thirty children with kwashiorkor, thirty-five with marasmus, twenty-eight with marasmic-kwashiorkor and twenty-seven control children.

2. HDL-cholesterol was estimated after the very-low-density and low-density lipoproteins were precipitated from the plasma with heparin and manganese chloride.

3. The distribution of HDL-cholesterol in the control and in the children with PEM was skewed and the range of values was wide. The values were independent of age and sex.

4. After logarithmic transformation of the HDL-cholesterol and HDL-cholesterol:total cholesterol values, the geometric mean values for the three groups of children with PEM were significantly decreased when compared with values for the control children.

5. The decrease in the mean HDL-cholesterol value for the children with kwashiorkor was more than for children with marasmus and marasmic-kwashiorkor.

Most studies on lipoprotein changes in protein-energy malnutrition (PEM) have been consistent in their finding of a reduced level of B-lipoprotein in kwashiorkor (Chatterjee & Chaudhuri, 1961; Coward & Whitehead, 1972; Onitiri & Boyo, 1975). However, there are divergent views about the changes in the α-lipoproteins. Some have reported a reduced level of α-lipoprotein in kwashiorkor (Chatterjee & Chaudhuri, 1961; Coward & Whitehead, 1972; Cravioto *et al.* 1959), while others (Truswell *et al.* 1969; Devi *et al.* 1976) did not find any alterations in the relative amounts of lipoproteins. These divergent views could be due to a number of factors, such as the differences in ages and sexes of the malnourished children studied, as well as the differences in the techniques employed.

The electrophoretic method of lipoprotein separation was used by the earlier workers on lipoproteins in PEM (Chatterjee & Chaudhuri, 1961; Cravioto *et al.* 1959; Truswell *et al.* 1969). Flores *et al.* (1970) used the preparative ultracentrifugation method, Coward & Whitehead (1972) immunological technique and Onitiri & Boyo (1975) employed a turbidimetric method. It is known that the most accurate method for lipoprotein estimation is the preparative ultracentrifugation method, but this has its limitations. A fast, simple and accurate precipitation method for estimating cholesterol concentration of the high-density lipoprotein (HDL) is now commonly used.

This method gives values which are comparable with those of the preparative ultracentrifugation method. The present study employed the precipitation method using heparin and manganese chloride. Because the method is simple and fast, it was possible to make a comparative study of HDL-cholesterol in a large sample of patients with kwashiorkor, marasmus and marasmic-kwashiorkor.

METHOD

Patients

Using the International classification recommended by a Wellcome sponsored working group (Wellcome Foundation Classification, 1970) thirty children with kwashiorkor, thirty-five with marasmus and twenty-eight with marasmic-kwashiorkor who were

attending the Paediatric Gastroenterology/Nutrition clinic at the University College Hospital, Ibadan, Nigeria, were studied. Twenty-seven control children who were attending the hospital for minor surgical elective procedure such as herniorrhaphy were also studied. The ages, sexes, weights and heights of these children were recorded.

Blood collection and biochemical tests

Blood (10 ml) was collected from each of the children with PEM and control children without fasting. The blood samples were mixed with Na_2 EDTA (1 mg/ml) and placed on ice. The cells were removed by centrifugation at 4° within 2 h of collection. The precipitation of the very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) from the plasma was usually carried out immediately. The precipitation method described by Burstein & Samaille (1960) was used. Heparin (0·12 ml; 5000 units/ml) and 1 M-manganese chloride (0·15 ml) were added separately to 3 ml plasma and mixed by using a Vortex mixer and allowed to stand for 30 min at 4°.

The precipitate was then centrifuged at 1500 ***g*** for 30 min. The clear supernatant fraction which contained the HDL was carefully removed using a pasteur pipette. The remaining plasma and the HDL fraction were stored at −20° until their cholesterol concentrations were determined according to a modified method described by Searcy & Berquist (1960). This method involves the extraction of the lipids with chloroform–methanol (2:1, v/v) and the use of acetic acid saturated with ferrous sulphate plus sulphuric acid as the colour development reagent. In calculating the cholesterol concentration of the HDL fraction a factor of 1·09 was used to correct for the dilution due to the addition of heparin and manganese chloride. Wellcomtrol (Wellcome Reagent Ltd) and a pooled sera were always included as control sera for each batch of cholesterol determination. Plasma total protein, albumin and globulin were determined using the Biuret method (Gornall *et al.* 1949).

Statistical tests

The means, logarithm of means, medians and standard deviation were calculated using standard methods. The Student's *t* test was used to determine the level of significance of paired data.

RESULTS

The mean values for age, height, weight, total protein, albumin, globular, total cholesterol and HDL cholesterol for the control group and those with kwashiorkor, marasmus, marasmic-kwashiorkor and all PEM children are presented in Table 1. The differences between any pair of the four groups were examined and the results are shown in Table 2.

Although there were no significant differences between the mean ages of the control group and those of each of the three PEM groups, the mean weight for the control group was significantly higher than that of the PEM groups. There was no significant difference between the mean height of the group with kwashiorkor and that of the control group, but the control group had a significantly higher mean height than those of the groups with marasmus and marasmic-kwashiorkor. Plasma total protein levels in the control group were significantly higher than the levels in the PEM groups. The mean plasma albumin level was significantly higher in the control subjects than in children with kwashiorkor and marasmic-kwashiorkor but not higher than the one for children with marasmus. The mean plasma albumin level in children with marasmus was also significantly higher than the mean values for children with kwashiorkor and marasmic-kwashiorkor. The mean plasma globulin level in the control children was significantly higher than those for children with kwashiorkor and marasmic-kwashiorkor but not higher than that for children with marasmus. There was no significant difference in the mean level of plasma globulin in the three groups of children with PEM.

Table 1. *Mean values for plasma proteins, total cholesterol and high-density-lipoprotein (HDL)-cholesterol in control and malnourished children*

(Mean values and standard deviations, no. of subjects given in parentheses)

	Age (months)		Weight (kg)		Height (m)		Total protein (g/l)		Albumin (g/l)		Globulin (g/l)		Total cholesterol (mg/l)		HDL-cholesterol (mg/l)		HDL-cholestrol / Total cholesterol	
Subjects	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean*	SD	Mean*	SD
Control (27)	22·8	17·6	10·31	3·06	0·80	0·12	71	5	40	6	32	6	1420	410	314	16	0·24	0·02
Kwashiorkor (30)	26·3	11·7	8·60	2·15	0·80	0·08	50	10	23	2	28	7	1070	400	211	18	0·22	0·02
Marasmus (35)	20·0	12·4	7·87	2·19	0·73	0·08	65	11	37	10	28	8	1100	330	243	17	0·24	0·02
Marasmic-kwashiorkor (28)	20·3	11·0	6·48	1·47	0·73	0·08	53	13	27	9	26	8	1130	340	248	18	0·23	0·02
All PEM children (93)	22·1	11·8	7·69	1·99	0·76	0·08	57	11	29	8	27	8	1100	360	234	18	0·23	0·02

* Geometric mean.

Table 2. *Level of significance for the comparison of pairs of the four groups of control (group 1) and PEM children (groups 2–4) for values of variables given in Table 1*

Groups compared	Age	Weight	Height	Total protein	Albumin	Globulin	Total cholesterol	HDL-cholesterol	HDL-cholesterol / Total cholesterol
1 *v.* 2	0·9459	2·4020*	0·0727	10·2470***	13·2417***	2·3250*	3·2553***	20·2721***	3·7698***
1 *v.* 3	0·7122	3·5080***	2·3547*	2·8604***	1·3779	2·2502*	3·3115***	15·6257***	0·0000
1 *v.* 4	0·6351	5·8827***	2·3310*	6·8525***	5·8415***	3·1535***	2·8500***	14·0071***	1·8538
2 *v.* 3	2·1053*	1·3534	3·4530***	5·7522***	8·0964***	0·0000	0·3314	6·4345***	4·0216***
2 *v.* 4	2·0130*	4·4090***	3·3835***	0·9931	2·3017*	1·3541	0·6173	6·8505***	1·9029
3 *v.* 4	0·1003	3·0041***	0·0000	3·8933***	4·1703***	0·9860	0·3532	0·7893	1·9732

HDL high-density-lipoprotein.
* $P < 0·05$, *** $P < 0·001$.
Group 2, children with kwashiorkor; group 3, children with marasmus; group 4, children with marasmic-kwashiorkor.

Table 3. *Median, range and number at different levels of plasma high-density-lipoprotein-cholesterol in the control and malnourished children*

HDL-cholesterol (mg/l)	100–200			200–300			> 300		
	Median	Range	*n*	Median	Range	*n*	Median	Range	*n*
Control (27)	160	120–190	3	230	210–300	7	410	330–750	17
Kwashiorkor (30)	120	60–200	15	240	220–270	6	420	340–590	9
Marasmus (35)	120	90–180	10	270	220–300	10	350	320–560	15
Marasmic-kwashiorkor (28)	120	70–180	9	270	220–300	10	440	310–640	9

The mean plasma total cholesterol level in the control group was significantly higher than those for the PEM groups. The geometric mean (antilogarithm of the mean logarithm) of HDL-cholesterol in the control group was significantly higher than that for the PEM groups. The geometric mean HDL-cholesterol level for the children with kwashiorkor was significantly less than those for children with marasmus and marasmic-kwashiorkor. The geometric mean values for HDL-cholesterol:total cholesterol in the children with kwashiorkor was significantly less than that for the control group and children with marasmus, but not significantly different from that for the children with marasmic-kwashiorkor. When the variables in Table 1 were categorized according to sex, there were no significant differences within each of the four groups. There was also no correlation between the total and HDL-cholesterol and the ages of the subjects in the four groups. Table 3 shows the range of values, the number of subjects and median for specified levels of HDL-cholesterol in the four groups. Most of the children with kwashiorkor (50%) had values for HDL-cholesterol which were below 200 mg/l, while only 11% of the control children had similar values.

DISCUSSION

Previous studies on lipoprotein metabolism in malnutrition have been concerned with the changes in low-density lipoprotein (LDL) on hospitalized malnourished children (Cravioto *et al.* 1959; Truswell *et al.* 1969). Because it is expensive to keep these children on admission for a long period, such studies have usually involved only a few patients. An obvious advantage in this type of study is that the children can serve as their own controls. However, it is difficult to carry out such studies in many hospitals where the patient turnover and the consequent demand for bed-space are very high. It was one of the objectives of the present study to investigate a large sample of PEM children at the outpatient clinic without the expense of admitting them.

The observed reduced weight, plasma total protein, albumin and total cholesterol values in the children with PEM when compared with values for the control children are known biochemical changes in this condition. This study has shown that the HDL-cholesterol was reduced in all the children with PEM, with the change in the children with kwashiorkor being more marked than those in the children with marasmus or marasmic-kwashiorkor. Similarly, the HDL-cholesterol:total cholesterol value in the children with kwashiorkor was significantly reduced when compared with the control and children with marasmus. The observed valued of HDL-cholesterol in the control children was not significantly different from that for children 1–10 years old from a rural community (Oladunni Taylor, unpublished results). The HDL-cholesterol levels in the control and in children with PEM were independent of age and sex.

Previous studies on HDL changes in PEM have been inconsistent. Truswell *et al.* (1969) did not find any change in the level of HDL-cholesterol before and after treatment of malnourished children. Devi *et al.* (1976) did not find any alteration in the relative amounts of plasma HDL in children with kwashiorkor but the HDL band after electrophoresis on agarose was found to be faint in four of the eighteen children with kwashiorkor. It would appear that the differences in the previous studies might not depend mainly on the accuracy of the techniques used for estimating HDL but on the pattern of distribution of HDL values in both the control and in children with PEM. In the present study, it was observed that the pattern of HDL-cholesterol in the control and in the children with PEM was skewed and the range of values was very wide. Statistical analysis became more meaningful only when logarithmic transformation of the data was carried out. It would appear that because previous studies were carried out on fewer subjects, the results obtained could make statistical interpretation difficult and this could partly account for the previous conflicting reports.

The study by Laditan & Reeds (1976) has emphasized the importance of inadequate diet and infection in the aetiology of kwashiorkor and marasmus. It is therefore difficult to separate the effects of these two factors on the observed levels of HDL-cholesterol in children with PEM. Schonfeld *et al.* (1976) observed a decrease in the level of HDL-cholesterol by feeding a formula diet which had 80% carbohydrate and 20% protein. Sacks *et al.* (1975) also showed that vegetarians who consumed a macrobiotic diet which consisted mainly of whole grains, beans and fresh vegetables for an average of 3 years had plasma HDL-cholesterol of 420 mg/l compared with 490 mg/l for the control subjects. Similarly, a 9% decrease in HDL-cholesterol level was reported in subjects with mild upper respiratory infection compared with those who were free of such infection in the community (Miller *et al.* 1979).

Our study of children with malaria and other infections showed a reduction in HDL-cholesterol when compared with values for healthy children (Oladunni Taylor, unpublished results). It could therefore be suggested that the observed changes in the HDL-cholesterol in the children with PEM could be due to the combined effects of inadequate nutrition and infections such as malaria, measles and bronchitis.

It is however pertinent that the present study has shown that some control and malnourished children had a high proportion of HDL-cholesterol to total cholesterol. The relevant characteristics of this group of children should be further investigated in an attempt to determine the factors which could increase the proportion of HDL-cholesterol in the blood. The study of Schaefer *et al.* (1978) suggested a relation between the metabolism of triglyceride-rich lipoprotein and HDL. Our earlier report (Agbedana *et al.* 1979) showed the relationship between triglycerides and hepatic lipase in malnourished children. The relationship between plasma HDL-cholesterol, triglyceride and hepatic lipase activity in PEM should therefore be of much interest.

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