Selective deficiency of hepatic triglyceride lipase and hypertriglyceridaemia in kwashiorkor

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1. Serum postheparin lipolytic activities (PHLA), triglyceride and free fatty acid concentrations were determined in children with kwashiorkor before and after treatment and also in normal control children.
2. Using the range (571–1650 μmol/l) of serum triglyceride of the control children as normal, five (20 %) of the twenty-five children with kwashiorkor had low (less than 570 μmol/l), thirteen (52 %) had normal (571–1650 μmol/l) and seven (28 %) had high (more than 1650 μmol/l) serum triglyceride levels.
3. The serum PHLA did not show any definite correlation with the level of circulating triglycerides, although the lowest levels of PHLA were found in the malnourished children with highest triglyceride level.
4. While the hepatic PHLA in the malnourished children was significantly less than control value, the extrahepatic PHLA did not differ significantly.
5. After treatment, serum PHLA rose significantly and the mean levels were within normal range.
6. Our findings suggest that a defect in catabolism of very-low-density lipoprotein caused by a low hepatic PHLA may cause hypertriglyceridaemia in children with kwashiorkor.

An elevated serum free fatty acid (FFA) occurs commonly in kwashiorkor, and this has usually been one of the explanations for the fatty liver often found in this condition (Lewis *et al.* 1964; Fletcher, 1966). Both serum cholesterol and phospholipids are low (Schwartz & Dean, 1957; Macdonald *et al.* 1963; Rao & Prasad, 1966) while the serum triglyceride may be normal or reduced (Lewis *et al.* 1964; Truswell *et al.* 1969; Flores *et al.* 1970).

An important mechanism regulating the concentration of circulating blood lipids is believed to involve a complex system in which the enzyme lipoprotein lipase (LPL) plays an important role. LPL is involved in the hydrolysis of lipoprotein-bound triglyceride in the serum and its activity may modify triglyceride transport and distribution to the tissues (Robinson, 1970). LPL is present in tissues such as the heart, adipose tissue, aorta and liver (Desnuelle, 1972; Fredrickson & Levy, 1972), and the injection of heparin or similar polyionic substances causes its displacement into circulation from the binding sites (Korn, 1959). Serum postheparin lipolytic activity (PHLA) apparently includes at least two major enzymes originating from liver and extrahepatic tissues such as adipose tissue (Krauss *et al.* 1973), and both enzymes are equally capable of hydrolysing triglyceride-rich lipoproteins (Klose *et al.* 1977; Mordasini *et al.* 1977).

The purpose of this study was to determine serum PHLA, triglyceride and FFA in children with kwashiorkor before and after treatment in an attempt to further determine the role of LPL activity in the deranged lipid metabolism which occurs in kwashiorkor.

METHODS

Patients

Twenty-five children with kwashiorkor, ranging in age from 1 to 5 years, and ten normal control children within the same age-range were studied. All malnourished patients had typical features of kwashiorkor such as growth failure, hypoalbuminaemia, oedema and characteristic skin and hair changes. The control children were children admitted to the

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hospital for minor surgical elective procedures such as herniorrhaphy. The study protocol was approved by the Ethical Committee of the Department of Paediatrics, Faculty of Medicine, University of Ibadan, Nigeria, and informed consent was obtained from the parents of the children before the commencement of the investigations.

The initial biochemical studies in the malnourished children were carried out as soon as possible after hospital admission but before being started on nutritional rehabilitation. Malnourished children with severe complications like diarrhoea, hypothermia and significant hypoglycaemia were excluded from the study.

After the intial studies, the patients were placed on a diet of skimmed milk with multivitamin and haematinic supplements until they improved enough to take a normal ward (balanced) diet. They were then discharged and cared for as out-patients. At 3–4 months after complete nutritional rehabilitation as evidenced by loss of oedema and satisfactory weight gain, the patients who had not defaulted from the clinic were re-admitted as day cases for repeat studies.

Blood collection and processing of serum

After an overnight fast 3–5 ml venous blood samples were obtained before and at 10 min after rapid intravenous injection of 10 IU heparin (Evans Medical Ltd, Poole, England)/kg body-weight. The blood was collected into a universal bottle in each instance, placed in an ice-bath and allowed to clot. Serum was separated by centrifugation at 4° and stored at −20° until assays of lipolytic activities and other biochemical tests were carried out.

Preparation of triolein emulsion (substrate)

The triolein emulsion used as substrate in PHLA assays was prepared under strictly standardized conditions. The following components: 3 ml bovine serum albumin (300 mg), glycerol (2-^{3}H) trioleate (The Radiochemical Centre, Amersham, Bucks.), triolein (270 mg), lysolecithin (16·2 mg), 1·6 ml 2 M-Tris-HCl buffer (pH 8·0) were made up to a total of 6 ml with water, in a beaker. The microtip of a Branson (MSE, London) sonicator was placed approximately 5 mm below the surface of the solution and the mixture was sonicated in an ice-bath for a total of 8 min alternating 60 s sonication with a 60 s pause. The substrate was activated by incubating with 12 ml heated fasting human serum for 30 min at 37° in a water-bath with a shaker. (The fasting human serum was first heated for 10 min at 62° in a water-bath to inactivate any endogenous LPL that might have been present in the body.) Freshly prepared substrate was used for each test.

Standard assay for serum PHLA

The selective measurement of LPL activity was based on inactivation extrahepatic activity by protamine sulphate as originally described by Krauss *et al.* (1973). In the standard assay, postheparin serum was incubated in a total volume of 0·4 ml Tris-HCl buffer (pH 8·0) for 10 min at 37°. After this incubation, 0·6 ml substrate medium was added and further incubation was carried out for 45 min at 37°. The reactions were terminated by adding 1 ml trichloroacetic acid (100 g/l TCA). In the assay of hepatic lipase, the samples were also incubated in the same buffer containing protamine sulphate (3 mg/0·1 ml serum) for 10 min at 37°. The assay of LPL was then carried out as described previously. In each instance, the (^{3}H) glycerol extracted into the TCA supernatant was counted in Instagel using a Packard Tricarb liquid-scintillation spectrometer. Total release of fatty acid was calculated and the enzyme activity expressed as nmol fatty acid/ml per min. The extraction of glycerol was 100% efficient and there was no need for any correction factor in the calculation. The extrahepatic enzyme activity was the difference between total PHLA and hepatic lipase activity.

Table 1. *Mean body-weight, serum free fatty acid, triglycerides and postheparin lipolytic activities (PHLA) in children with kwashiorkor and control children**

(Mean values with their standard errors; no. of children in parentheses)

Group . . .	1		2		3		Control	
Serum triglyceride (μmol/l)	0–570 (5)		571–1650 (13)		> 1650 (7)		571–1650 (10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt (kg)	6·5	0·6	7·5	0·3	8·3	0·3	10·0	1·0
Triglyceride (μmol/l)	377	97	1020	80	2135	190	990	63
Free fatty acid (μmol/l)	1154	343	1814	226	1388	191	780	59
PHLA								
Total (nmol fatty acid/ml per min)	121	15	154	30	82	16	394	70
Hepatic (nmol fatty acid/ml per min)	44	4	53	15	25	5	213	47
Extrahepatic (nmol fatty acid/ml per min)	77	14	101	23	57	14	181	41

* For details, see p. 352.

Other biochemical tests

The concentration of triglyceride was measured by the method of Gottfried & Rosenberg (1973) using triolein (Sigma Co., St Louis, Mo., USA) as standard. Serum FFA was estimated by Dole's titrimetric method as modified by Trout *et al.* (1960). Thymol blue (0·01% in water) was the indicator and palmitic acid (Sigma Co.) was the standard acid used.

Statistical tests

The mean, standard error of mean and χ^2 were calculated using standard methods. The test of significance was carried out using Student's t test; $P > 0{\cdot}05$ was regarded as not significant.

RESULTS

The range of serum triglyceride (μmol/l) was 154–4213 in the patients with kwashiorkor and 571–1650 in the control group. Relative to control values, the malnourished children could be classified into three distinct groups based on serum triglyceride levels (μmol/l): group 1 with low (0–570) serum triglyceride; group 2 with normal level (571–1650) and group 3 with high serum triglyceride level (more than 1650). The percentage distribution of the patients studied in each of these groups was 20·0, 52·0 and 28·0 respectively.

Table 1 shows that the body-weight tended to increase with increasing level of circulating serum triglycerides and the mean body-weight in group 3 was significantly higher than the value for group 1 ($P < 0{\cdot}01$). These values were significantly less than the control value except in group 3, where the reduction was not significant. Also there were no significant differences in serum FFA values in the three groups of malnourished children. These values were significantly higher than the control value except in group 1 where the increase was not significant (Table 2).

The mean serum total, hepatic and extrahepatic PHLA in the three malnourished groups were not significantly different except that the hepatic PHLA in group 3 was significantly less than the value in group 1 ($P < 0{\cdot}01$). The total and hepatic PHLA in these three groups were significantly less, but the reductions in extrahepatic PHLA were not significant when compared with corresponding control values.

In four malnourished children who died during the course of treatment, the mean level

Table 2. *Statistical comparison of body-weights, free fatty acid, postheparin lipolytic activities (PHLA) within and between the groups of children with kwashiorkor (groups 1–3)* and control (C) children†*

Groups compared	1 *v.* 2	1 *v.* 3	2 *v.* 3	C *v.* 1	C *v.* 2	C *v.* 3
Body-wt	NS	0·01	NS	0·05	0·02	NS
Free fatty acid	NS	0·01	NS	NS	0·001	0·002
PHLA: Total	NS	NS	NS	0·02	0·002	0·002
Hepatic	NS	0·01	NS	0·05	0·002	0·01
Extrahepatic	NS	NS	NS	NS	NS	NS

NS, not significant ($P > 0·05$).

* Grouped according to serum triglyceride levels (μmol/l) group 1 0–570, group 2 571–1650; group 3 > 1650.

† For details, see Table 1.

Table 3. *Postheparin serum lipolytic activities in children with kwashiorkor before and after treatment*

(Control values are shown in parentheses)

	PHLA values (μmol fatty acid/ml per min)					
	Before treatment			After treatment		
Patient	Total	Hepatic	Extrahepatic	Total (394±70)	Hepatic (213±47)	Extrahepatic (181±41)
L.F.	154	43	111	474	284	190
M.S.	51	32	19	174	79	95
A.G.	82	14	68	104	87	17
L.M.	101	54	47	299	85	214
O.A.	320	146	174	380	180	200
S.S.	90	37	53	120	57	63
F.M.	97	12	85	290	100	190
M.G.	193	48	125	436	222	214

of hepatic PHLA was less than the mean level in those who survived. However, there was no significant correlation (χ^2_1 3·20) between the number of deaths and reduced hepatic PHLA.

Table 3 shows that in seven of the eight children with kwashiorkor who were studied after full clinical recovery, the total, hepatic and extrahepatic PHLA increased to within normal levels.

DISCUSSION

This study has shown that in accord with previous reports (Lewis *et al.* 1964; Fletcher, 1966; Rao & Prasad, 1966; Flores *et al.* 1970; Taylor, 1971), the serum FFA is significantly elevated in patients with kwashiorkor. The serum triglyceride concentrations showed wide variations but the over-all mean level was not significantly different from the value in healthy controls. Taking the range of serum triglyceride values in the control children as normal, the malnourished children could be divided into three distinct groups as having low, normal or high serum triglycerides, as previously reported by Flores *et al.* (1973).

The exact cause of the wide variations in the levels of serum triglycerides among protein-malnourished children is unknown, but many hypotheses have been suggested. Arroyave *et al.* (1961) were of the opinion that the concentration of serum triglyceride may be influenced by the extent of fatty infiltration of the liver, the concentration being lowest in patients with the greatest extent of fatty liver. This view is supported by Truswell (1975). The importance of fatty liver in kwashiorkor is that prognosis in this disorder correlates

with the extent of fatty infiltration of the liver (Waterlow *et al.* 1960) and the variation in the levels of serum triglyceride may therefore reflect the severity of the disease. In the present study, liver enlargement was observed in the majority of the patients. Although hepatomegaly in kwashiorkor is believed to be due to fatty infiltration of the liver, liver palpation is a poor measure of the extent of fatty liver (Trowell *et al.* 1954). The relationship between the level of serum triglycerides and severity of protein-malnutrition needs further investigation, although Taylor (1971) did not observe any definite association between the severity of kwashiorkor and serum cholesterol levels.

This study has also shown that the heavier patients had the highest concentration of serum triglycerides, a finding again similar to that of Flores *et al.* (1973). This tendency to hypertriglyceridaemia in some patients with kwashiorkor may be similar to the lipemia observed in obesity (Gofman, 1954) as some patients with kwashiorkor have more adipose mass than healthy controls (Garrow, 1966).

Dietary habit greatly influences serum lipid patterns in man (Scott *et al.* 1964) and differences in the type of food consumed by these children may account for the wide variation in serum triglyceride concentrations. However, the commonest weaning diet among the people of low socio-economic group in our environment is maize starch, and therefore the effect of the type of diet may be minimal in the present study.

The role of LPL in triglyceride metabolism in kwashiorkor has hitherto received scant attention. Probably the most remarkable result of this study is that the reduced serum total PHLA in the different groups of kwashiorkor is mostly due to diminished hepatic triglyceride lipase activity.

Furthermore, the serum hepatic triglyceride lipase was lowest in the group of malnourished patients with the highest triglyceride concentrations. The significance of this finding is not immediately clear, as the specific role of hepatic triglyceride lipase in triglyceride metabolism is not definitely known. However, in a number of conditions a secondary hypertriglyceridaemia usually accompanies reduced hepatic triglyceride lipase activity (Klose *et al.* 1977; Mordasini *et al.* 1977). In the present study, the majority of the patients had normal or higher than normal levels of serum triglyceride. This has been confirmed in a study involving a large number of malnourished patients (Agbedana & Taylor, unpublished observation). It is tempting, therefore, to speculate that accumulation of triglyceride-rich lipoproteins in some of the children is a consequence of the demonstrated low hepatic triglyceride lipase activity. This hypothesis does not necessarily imply that the conversion of intermediate lipoprotein to LDL occurs in the liver, but the demonstration of insignificant reduction in extrahepatic triglyceride lipase in all the children with kwashiorkor is strongly in favour of this hypothesis.

In explaining the fatty liver in kwashiorkor many workers have proposed that there is a defective release of very-low-density lipoprotein (VLDL; triglyceride-rich) probably as a consequence of decreased apoprotein synthesis by the liver (Truswell & Hansen, 1969; Truswell *et al.* 1969; Coward & Whitehead, 1972). Thus there are two major opposing factors affecting the levels of circulating triglycerides in kwashiorkor; a decreased release of VLDL causing hypotriglyceridaemia and reduced serum hepatic triglyceride lipase causing hypertriglyceridaemia. Protein-malnourished children with varying extents of triglyceridaemia may be considered as representing different stages of the equilibrium between the rates of release and catabolism of triglycerides. This probably explains why dietary therapy which has been shown to cause release of liver lipids into circulation (Arroyave *et al.* 1961) leads to a pronounced hypertriglyceridaemia within 2–10 d of dietary rehabilitation in treatment of kwashiorkor followed by a return to normal level at full recovery (Rao & Prasad, 1966; Taylor, 1971). Our study has shown that the postheparin serum hepatic triglyceride lipase activity rises rapidly to normal level after recovery in the patients tested.

In the few fatal cases the initial hepatic triglyceride lipase activity tended to be lower than the activity in the patients who survived. The paucity of the number of such patients precluded meaningful comparison of the results in the fatal and non-fatal cases. It has been suggested that liver failure may be an important cause of sudden death among children with severe kwashiorkor (McLean, 1962). It is probable that the changes in the activity of the enzyme may reflect the extent of liver damage, and its measurement may be a very sensitive and useful prognostic test.

In conclusion, our results suggest that in kwashiorkor a defect in catatabolism of VLDL caused by a low serum hepatic triglyceride lipase leads to accumulation of triglyceride-rich lipoproteins. However, in some patients an associated defective release of VLDL probably due to excessive fatty infiltration of the liver prevents the occurrence of hypertriglyceridaemia.

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