FATTY LIVER IN PROTEIN-CALORIE MALNUTRITION*

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In the fatty liver of protein-calorie malnutrition, i.e. kwashiorkor and marasmus, the lipid that accumulates is predominantly triglyceride[1,2] as in other types of fatty liver.[3]

In general there are 4 possible mechanisms which may result in fatty liver:[4]

1. Increased mobilization of free fatty acids from adipose tissue.
2. Increased fatty-acid synthesis in the liver.
3. Decreased oxidation of fatty acids.
4. Decreased release of fats from the liver to plasma in lipoproteins.

It has been suggested that the fatty livers produced in experimental animals by puromycin and ethionine result from reduced hepatic synthesis of the protein part of plasma lipoproteins.[5,6] The fatty liver of kwashiorkor might have a similar pathogenesis. Plasma concentrations of lipids are low,[7,8] and there is indirect evidence of reduced hepatic synthesis of another protein, plasma albumin,[9] in this state of human protein-calorie malnutrition.

Secondly, the question has not yet been fully answered whether deficiency of lipotropic factors plays a part in causing the fatty liver of protein-calorie malnutrition. If fatty liver ever results from dietary deficiency of methionine and choline in man, it might be expected in this type of malnutrition.

The studies reported here were planned to give some answers to these 2 hypotheses. Serum was collected from children with protein-calorie malnutrition before and during treatment. Various lipid fractions were measured and compared with the degree of fatty liver shown by needle biopsy soon after admission.

CLINICAL AND LABORATORY METHODS

Children with untreated kwashiorkor or marasmus were admitted to the metabolic ward at the Red Cross Children's Hospital, Cape Town. They were rehydrated and given vitamin K_1. Biopsy specimens of the liver were obtained percutaneously with the Menghini needle as soon as parental consent, prothrombin values and the child's condition permitted—usually one or two days after admission.

The patients were treated with high protein, very low fat diets, vitamins (but no lipotropic factors), minerals, penicillin and sulphadiazine. Other treatment was given as necessary. Blood was taken after an overnight fast the day after admission and every fourth day thereafter for 3 weeks. All the children recovered.

The liver biopsy specimens were stained with haematoxylin and eosin and graded independently by a pathologist (0-4+) according to the degree of fatty change present histologically.

Serum lipoproteins were separated by horizontal paper electrophoresis[10] with 1% human albumin included in the buffer. Alpha- and β-lipoprotein bands were cut out and extracted, and cholesterol concentration was measured by the Abell method.[10]

Lipoproteins were also separated by the newer vertical electrophoretic method of Lees and Hatch.[11] Strips were stained with oil red O (Gurr) and examined for pre-beta-lipoprotein bands.

For phospholipid determinations, serum was extracted with chloroform-methanol, 2 : 1. Phospholipids were fractionated by thin-layer chromatography on silica gel G in the solvent system chloroform-methanol-acetic acid-water (80 : 30 : 8 : 4). They were visualized with iodine vapour. Lecithin and sphingomyelin bands were scraped off. Phosphorus was determined in these and in the original lipid extract of serum by the method of Parker and Petersen.[12]

Free fatty acids were determined by the method of Trout *et al.*,[13] except that Nile blue A indicator and a Beckman Spinco micro-titrator were used. Serum albumin was measured by biuret after salting out the globulins with 27% sodium sulphate—a standard method used in our laboratory for many years.[14,15]

RESULTS

Kwashiorkor

In the first part of this work we studied 19 children with kwashiorkor. Some preliminary results have already been reported.[16,17] Half had 3+ or 4+ fatty liver and half had lesser grades of liver fat. It was therefore possible to divide the patients into 2 groups. Those with severe fatty liver had lower initial serum β-lipoprotein cholesterol, triglycerides and albumin, but α-lipoprotein and phosphatidyl choline (lecithin) were not reduced.

During treatment serum lipids rose above normal levels (triglyceride before cholesterol) and pre-beta-lipoprotein bands appeared (Fig. 1).

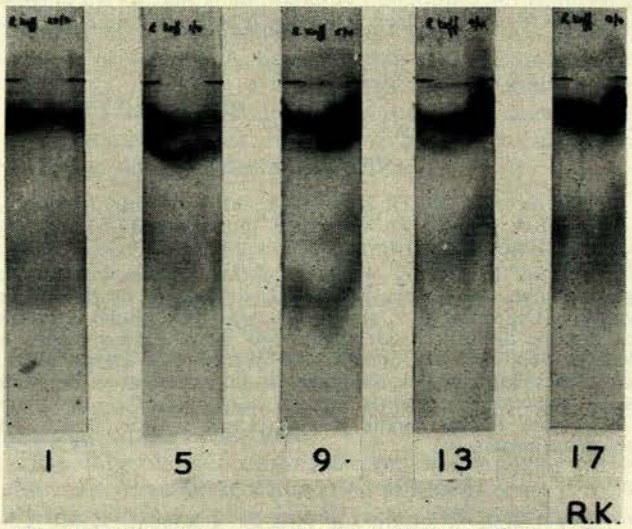

Fig. 1. Paper electrophoretic strips of serum lipoproteins in a typical patient with kwashiorkor, Lees and Hatch method,[11] stained with oil red O. The ink marks indicate the line of application. Note the double β-lipoprotein bands which appear on day 5. The lower of these double bands is pre-β-lipoprotein. α-lipoproteins are more faint and appear about half-way between the β-lipoproteins and the bottom of the strips. Numbers under the strips indicate days after admission. Blood was taken on day 1 before protein feeding commenced.

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To find out how quickly serum triglycerides respond to treatment, blood was taken every day for the first 5 days in a further 7 kwashiorkor patients. Liver biopsy was omitted. Triglycerides rose very fast, reaching normal levels after one day's treatment and their highest concentrations after 3 days. Pre-beta-lipoproteins usually appeared after 1 or 2 days on treatment.

Marasmus

We then studied 18 cases of marasmus. They had higher mean serum lipids on admission. In some of them triglycerides were above normal and pre-beta-lipoprotein was present before treatment (Fig. 2). Biopsy of specimens obtained by needle puncture showed no fat in the livers of 12 cases. The other 6 had some fatty liver—up to 3+.

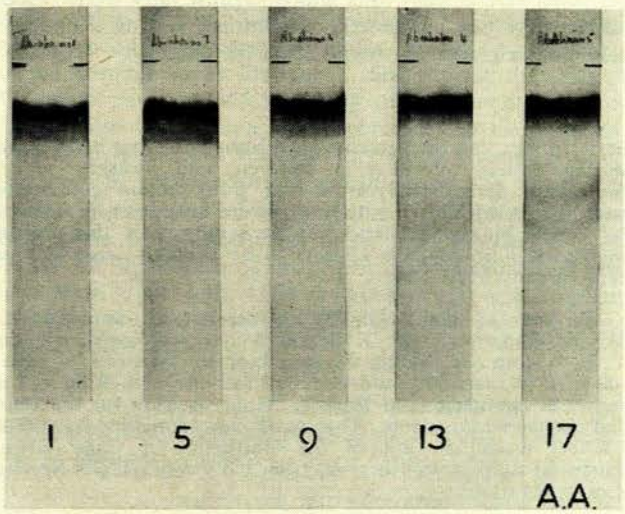

Fig. 2. Paper electrophoresis strips of serum lipoproteins in a typical patient with marasmus. Note that there is a pre-β-lipoprotein band before treatment, on day 1. Methods as for Fig. 1.

Serum β-lipoprotein cholesterol and triglycerides were lower in those with fatty liver; total phospholipids and α-lipoprotein cholesterol were a little lower. Plasma free-fatty-acid concentrations were variable, but usually moderately increased before treatment and rather higher in those without fatty liver. Serum lipids did not reach such high peaks during treatment as in kwashiorkor.

Combined Kwashiorkor and Marasmus (Protein-Calorie Malnutrition)

The data for kwashiorkor and marasmus patients were combined to obtain a large series of 37 cases of protein-calorie malnutrition, which were subdivided according to the liver biopsy findings into 3 groups: those with severe fatty liver (3 or 4+), mild fatty liver (2+ down to less than 1+), or no fat at all in their livers. The mean serum lipoproteins, lipids and albumin before treatment are presented in Table I. Except for α-lipoprotein, they showed a striking stepwise inverse relationship with the degree of fatty liver. Beta-lipoprotein cholesterol had the strongest negative association, followed by triglyceride and albumin. The effect was weaker for phospholipids and not shown by α-lipoprotein.

TABLE II. MEAN SERUM TRIGLYCERIDES (MG./100 ML.) DURING TREATMENT IN 3 FATTY LIVER GROUPS IN COMBINED SERIES OF PROTEIN-CALORIE MALNUTRITION (KWASHIORKOR + MARASMUS)

Liver fat grade	*No. of cases*	*Day 1*	*Day 5*	*Day 9*	*Day 14*	*Day 18*
3 and 4 +	11	78	217	187	147	125
1 and 2 +	6	105	250	203	163	156
0	15	125	192	189	125	121

The changes of serum triglycerides during treatment in the 3 fatty-liver groups are shown in Table II. Although triglycerides started lower in the groups with fatty liver, the peaks during treatment were higher and lasted longer than in children with no liver fat. Triglycerides were maximal by day 5, cholesterols usually on day 9.

DISCUSSION

Free-Fatty-Acid Mobilization

Lewis *et al.*,[8] in our unit, first reported increased fasting plasma free fatty acids in kwashiorkor. Other workers have confirmed this.[18-20] Lewis went on to show, with ^{14}C-palmitate, that the flux of free fatty acids through plasma was increased in infants with protein-calorie malnutrition.[21]

There are several reasons why this cannot be the only mechanism producing fatty liver in protein-calorie malnutrition. The increased free-fatty-acid concentration can be restored to normal by feeding starch and sucrose but no protein for a few days,[8] which suggests that it is caused by the anorexia and undernutrition that occur in the late stage of kwashiorkor. The liver is not usually fatty in marasmus, yet Lewis *et al.*[8] found increased plasma free fatty acids in this form of protein-calorie malnutrition as well, although Hadden[20] was unable to confirm this in Uganda. If an increased flux of free fatty acids were the only mechanism producing fatty liver, serum lipids would not be reduced. They would be normal or elevated.[22]

The present observations confirmed that plasma free fatty acids are usually increased in marasmic patients in Cape Town before treatment. However, free fatty acids

TABLE I. MEAN SERUM LIPOPROTEINS, LIPIDS AND ALBUMIN IN 3 FATTY-LIVER GROUPS IN COMBINED SERIES OF PROTEIN-CALORIE MALNUTRITION (KWASHIORKOR + MARASMUS) BEFORE TREATMENT

	Liver fat grade	*No. of cases*	*Pre-beta-lipoprotein*	*Triglyceride (mg./100 ml.)*	*Total cholesterol (mg./100 ml.)*	*Beta-lipoprotein cholesterol (mg./100 ml.)*	*Alpha-lipoprotein cholesterol (mg./100 ml.)*	*Total phospholipid (mg./100 ml.)*	*Albumin (G/100 ml.)*
(A)	3 and 4 +	13	Never	78	84	50	34	128	1·72
(B)	1 and 2 +	9	Never	105	97	63	33	141	2·35
(C)	0	15	5/15	125	131	91	32	160	2·79
	$\frac{C-A}{\frac{1}{2}(C+A)}$			46%	44%	59%	−5%	22%	47%

were actually lower in the cases with fatty liver. These observations are therefore further evidence that the increased free-fatty-acid concentration is not the main determinant of fatty liver in protein-calorie malnutrition.

Fatty Acid Synthesis and Oxidation

One would not expect increased fatty acid synthesis in the liver of children with protein-calorie malnutrition because they are underweight for age and must have been in negative calorie balance for some time. The 10% of linoleic acid in liver triglycerides which has been found in kwashiorkor[8] would not occur if the fat were synthesized in the liver, because linoleic acid is an essential fatty acid, ultimately derived from the diet. Lastly, Fletcher[18] has measured fatty acid synthesis from ^{14}C acetate *in vitro* in liver biopsy samples from children with protein-calorie malnutrition. The synthesis rate was reduced.

Using very small doses of ^{14}C-palmitate, Lewis *et al.*[23] found that plasma free fatty acids were oxidized to respiratory CO_2 more rapidly than normal in kwashiorkor and marasmus. This is the opposite of what would be expected if the fatty liver resulted from decreased oxidation of fatty acids.

Release of Fats from the Liver

There are at least 2 ways in which fatty liver in protein-calorie malnutrition might result from decreased lipoprotein secretion into the plasma: by reduced synthesis of the protein moiety of plasma lipoproteins or of the component phospholipids.

The principal phospholipid in plasma is lecithin (phosphatidyl choline). If fatty liver was caused by deficiency of lipotropic factors—choline and methionine—cases with gross fatty liver would be expected to have low serum phosphatidyl choline with total phospholipids reduced more than other lipid classes. We did not find this, so that these results are fresh evidence against lipotropic factor deficiency.

Previous reports on serum lipids in protein-calorie malnutrition have not agreed whether triglycerides were reduced.[8,19,24] It is important to be sure about this, because triglyceride is the principal lipid that accumulates in the liver. Our results seem to be quite clear. Fasting serum triglycerides were reduced in malnourished children who had fatty livers. When the livers were not fatty, triglycerides were normal or increased. As little as one day's protein feeding can restore reduced triglycerides to normal. As to serum lipoproteins, the only two previous reports both described great reduction of α-lipoprotein, with[25] or without[26] reduced β-lipoproteins. The lipoprotein we found related to fatty liver was β-lipoprotein. Beta-lipoprotein cholesterol showed a stronger degree of inverse relationship than any other lipid or serum albumin. On the other hand, serum α-lipoprotein cholesterol was not related to the degree of fatty liver.

The present results support the hypothesis of decreased hepatic synthesis of the protein moiety of plasma lipoproteins as a major mechanism for fatty liver in protein-calorie malnutrition. But β-lipoproteins appear to be much more sensitive to dietary protein deficiency than α-lipoproteins. This is somewhat unexpected, because α-lipoproteins contain more protein than β-lipoproteins.[27]

The rise of serum triglycerides during treatment and the appearance of pre-beta-lipoprotein presumably show fat being mobilized out of the liver because our patients were eating diets very low in fat. Serum triglycerides were higher for a longer time in cases with fatty liver (Table II). The recovery from protein-calorie malnutrition is, therefore, another cause of secondary type IV hyperlipoproteinaemia (pre-beta-lipoproteinaemia).[27,28]

Our results might help the clinician to diagnose fatty liver in children with protein-calorie malnutrition. Fatty liver is more likely when the clinical picture is kwashiorkor than in marasmus,[29] but we did encounter marasmus patients with 3+ fatty liver and kwashiorkor with no liver fat. Palpation of the liver is not very reliable in our experience. Low serum cholesterol and low albumin are the standard biochemical tests which suggest fatty liver.[30] And, where they can be done, pre-treatment fasting β-lipoprotein and triglyceride should increase diagnostic accuracy.

SUMMARY

Serum lipids and lipoproteins have been measured before and during protein refeeding in children with kwashiorkor or marasmus. Serum triglyceride and β-lipoprotein cholesterol were lowest in children with most severe fatty liver as assessed by needle biopsy. Reduced hepatic synthesis of the protein moiety of β-lipoproteins appears to be a major cause of this type of fatty liver.

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A NEW PERIDOL DERIVATIVE IN THE TREATMENT OF