Lipoprotein Lipase Activity in Marasmic Type of Protein-Calorie Malnutrition*

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Kwashiorkor and marasmus constitute the two major syndromes of protein-calorie malnutrition encountered in infants and young children in many parts of the world.

The clinical and metabolic characteristics of these syndromes differ significantly from one another, one difference being the fat metabolism. The increase of free fatty acids (FFA) in plasma has been accepted as the key factor causing fatty liver in kwashiorkor (Korn, 1955; Feigelson *et al.*, 1961; Lewis *et al.*, 1964; Fletcher, 1966; Bar-on and Stein, 1968). The role of lipoprotein lipase (LPL) in the mobilization of FFA is well known.

In this paper, plasma FFA and LPL levels, as well as the effect of heparin on the activation of this enzyme and its kinetics in plasma in various stages of marasmus will be presented.

Material and Methods

Fourteen cases of marasmus have been included in the study. The mean age of the series was 14.3 months (range 8 to 25 months). The mean weight loss (as a percentage of normal weight for age) was 48.0% (range 29.4% to 57.9%). Retardation in height (as a percentage of normal height for age) was 12.4% (range 2.3% to 21.5%). On clinical examination, hepatomegaly was not present in any of the cases. There were no other noteworthy clinical findings.

The first group of tests was carried out within 11 days of admission to the hospital, the second group of tests 6 weeks later, and the final group of tests 8 weeks after the second one.

Five normal controls were selected. Instead of the marasmic infants' age-group, children of 8 months to 12 years old were chosen to facilitate the study technically. It has already been established that the post-heparin LPL activity found in the newborn and in children up to 17 years of age showed no appreciable difference (Högstedt and Lindquist, 1963).

Heparin tests. After a fasting period of 12 hours, 12 U/kg. of heparin was administered intravenously. Blood was collected before and at the 15th, 45th, 60th, and 90th minutes after the administration of heparin. Plasma LPL activity, FFA, and glucose levels were determined within one hour of the collection.

FFA in plasma were determined according to Dole's method as modified by Burns (Dole and Meinertz, 1960; Burns *et al.*, 1963). Kern's method was used in the determination of LPL activity, details of which were given previously (Kern, Steinmann, and Sanders, 1961; Gürsön and Etili, 1968). LPL activity was expressed as the amount of FFA in $\mu\text{Eq/ml. per 60 min.}$ produced from the substrate.

A glucose oxidase method was used to determine blood glucose (Marks, 1959).

Pilocarpine iontophoresis was carried out on all cases in this series.

The statistical validity of the results was investigated by using the Student test. Half-life, zero time, extrapolated activity, and K slope constants were calculated in accordance with Boberg and Carlson's formulae (Boberg and Carlson, 1964).

Results

The mean LPL activity in normal controls was found to be $0.298 \pm 0.167 \mu\text{Eq/ml. per 60 min.}$ The mean values after heparin at 15, 45, 60, and 90 minutes were 4.013 ± 0.894 , 1.289 ± 0.491 , 0.544 ± 0.343 , and $0.399 \pm 0.319 \mu\text{Eq/ml. per 60 min.,}$ respectively.

In cases of marasmus, the results of the first tests were as follows: the mean initial value was $0.404 \pm 0.299 \mu\text{Eq/ml. per 60 min.}$ The values after heparin were 2.125 ± 0.916 , 0.678 ± 0.432 , 0.469 ± 0.263 , and $0.325 \pm 0.247 \mu\text{Eq/ml. per 60 min.}$ at the 15th, 45th, 60th, and 90th minutes.

When the tests were repeated on 9 of the cases 6 weeks later, the mean initial value for LPL activity was found to be $0.294 \pm 0.128 \mu\text{Eq/ml. per 60 min.}$ and the values after heparin at the same time intervals as above were 2.221 ± 0.964 , 0.874 ± 0.470 , 0.473 ± 0.208 , and $0.270 \pm 0.129 \mu\text{Eq/ml. per 60 min.,}$ respectively.

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In four of the cases, the heparin test was repeated 14 weeks after admission. The initial LPL activity at this time was found to be 0.354 ± 0.160 $\mu\text{Eq/ml. per 60 min.}$; and the values after heparin 3.670 ± 1.405 , 1.790 ± 1.374 , 1.079 ± 0.564 , and 0.777 ± 0.475 $\mu\text{Eq/ml. per 60 min.}$ (Fig.).

An analysis of the results showed that no significant differences existed between plasma LPL activity before heparin in normal controls and in cases of marasmus in severe as well as recovery stages. On the other hand, the LPL activities after heparin at 15 minutes in the severe stage of marasmus were significantly lower than in normal controls ($p < 0.01$).

Table I shows the mean half-life for LPL activity, the mean zero time, the mean LPL activity extrapolated to zero time, and the mean K slope constant in normal controls as well as in different stages of marasmus.

Table II presents the initial mean plasma FFA level in normal controls and the values after heparin in the case of marasmus.

The results of pilocarpine iontophoresis showed the sodium values to be 23.2 ± 13.9 mEq/l.; those for chloride were 23.7 ± 15.5 mEq/l., and those for potassium 14.1 ± 3.9 mEq/l.

Comments

In order to clarify the causes of fatty liver in kwashiorkor, a study of material taken from biopsies and necropsies enables us to make certain

hypotheses in connexion with the origin of fat accumulation in the liver.

From an analysis of the composition of fat found in the liver and in other fat tissues in the body, it was apparent that the lipid accumulating

FIG.—Plasma lipoprotein lipase activity in marasmic cases and in normal controls after heparin administration at different times and stages (PCM: Protein-calorie malnutrition).

TABLE I

Kinetics of LPL Activity in Plasma of Marasmic Cases after Single Dose of Heparin at Different Stages of Disease

	Elimination Rate of Lipoprotein Lipase $t_{1/2}$ (min.)	Zero Time ($\mu\text{Eq/ml. per 60 min.}$)	K slope constant (% min.)	LPL Activity Extrapolated to Zero Time ($\mu\text{Eq/ml. per min.}$)
Controls (5)*	24 ± 2	6.10 ± 1.3	2.90 ± 0.25	0.0712 ± 0.0094
Marasmic cases				
Upon admission (14)	28.2 ± 5.9	3.2 ± 1.4	2.54 ± 0.48	0.0397 ± 0.0180
6 weeks later (9)	28.2 ± 4.4	3.3 ± 1.7	2.50 ± 0.36	0.0403 ± 0.0198
14 weeks later (4)	26.5 ± 4.2	5.3 ± 1.8	2.66 ± 0.43	0.0589 ± 0.0067

* Figures in parentheses represent the number of cases.

	Controls vs. Marasmus (upon admission)	Marasmus (upon admission) vs. 14 weeks later
Half-life time	Not significant	Not significant
Zero time	$p < 0.01$	$p < 0.05$
K slope constant	Not significant	Not significant
Extrapolated activity	$p < 0.01$	Not significant

TABLE II

Free Fatty Acids (mEq/l.) in Plasma of Marasmic Cases after Heparin Administration at Different Times and Stages

	Initial	15 min.	45 min.	60 min.	90 min.
Controls (5)*	0.450 ± 0.199	0.650 ± 0.229	0.585 ± 0.256	0.485 ± 0.283	0.505 ± 0.250
Upon admission (14) ..	0.493 ± 0.226	0.663 ± 0.315	0.615 ± 0.272	0.569 ± 0.236	0.595 ± 0.305
6 weeks later (9)	0.418 ± 0.122	0.574 ± 0.165	0.520 ± 0.175	0.532 ± 0.135	0.528 ± 0.143
14 weeks later (4)	0.538 ± 0.119	0.577 ± 0.075	0.527 ± 0.033	0.516 ± 0.104	0.462 ± 0.065

* Figures in parentheses represent the number of cases.

No significant differences existed in the FFA values between normal controls and cases of marasmus in the various stages.

in the liver was not derived from the adipose tissue. The fatty acid composition of the fat suggests that it might be synthesized in the liver from dietary carbohydrates (MacDonald, Hansen, and Bronte-Stewart, 1963; Fletcher, 1966). Contrary to this theory, it has been postulated that adipose tissue may be the chief source of the lipid accumulating in the liver during the late stage of kwashiorkor, being transported to that organ in the form of FFA (Lewis *et al.*, 1964). Levels of FFA in plasma were found to be high in kwashiorkor. Hadden and Rutishauser (1967) showed that FFA in plasma in marasmus, where a grossly fatty liver is not characteristic, were not increased as in kwashiorkor. The present study supports these findings.

As is known, the LPL enzyme brings about the hydrolysis of triglyceride, which produces the FFA. Animal experiments and observations on man have shown that the enzyme may be formed in the heart, liver, kidney, and fatty tissues (Korn, 1955; Robinson and French, 1960; Boberg, Carlson, and Normell, 1964; Condon, Tobias, and Datta, 1965; Datta, 1966).

A number of diseases where LPL activities have been reported to be low include cystic fibrosis of pancreas, coeliac disease (Slack *et al.*, 1962; Jakovic and Hsia, 1963) and biliary cirrhosis (Datta, 1963; Fabian *et al.*, 1967).

This study has shown that LPL activity after heparin in the severe stages of marasmus was lower than that in normal controls. Studies carried out in cases of cystic fibrosis of pancreas would favour the explanation that the decrease of LPL after heparin in patients with this disease is the result of a lack of stimulation of the enzymes due to malabsorption of triglycerides. This conclusion was supported by the observation that a fall in plasma LPL activity was seen after two weeks on a low-fat isocaloric diet and a rise to the initial levels after two weeks of the reintroduction of fat to the diet.

In these marasmic cases, low LPL activity after heparin may not be due to fat malabsorption because comparison of LPL activities after heparin in the initial severe stage of marasmus and those observed 6 weeks later revealed no significant difference (except in two cases with values of 3.425 and 4.090), in spite of the fact that the children showed no signs of intolerance to fat in the diet and that a clinical improvement was evidenced by a gain in weight (33.5 g. daily). LPL activities after heparin determined in 4 cases 14 weeks after starting treatment showed little difference from values found in the severe stage. Therefore it may be accepted that the low LPL activity after heparin in marasmus continues for a long time and is not dependent on fat malabsorption.

The low LPL activity reported in cases of biliary cirrhosis of the liver is believed to be due to inability of damaged liver cells to carry out the release of LPL (Datta, 1966; Bosadjiewa *et al.*, 1967; Röding and Liese, 1967). The low LPL activity after heparin in cirrhotic subjects may be due to several causes. SGOT shows a significant negative relation with LPL levels after heparin, from which it may be determined that decreased production appears to be involved.

GOT, GPT, and ICD activities in serum are increased in approximately 50% of cases of kwashiorkor. The excess enzymes are derived principally from skeletal muscle but also from the liver as a result of cell damage or increased cell permeability (Edozien, 1961; Waterlow, 1963). Some investigators found no increases in GOT and GPT in children with either marasmus or kwashiorkor (Smith, 1962).

For this reason the low LPL activity cannot be attributed solely to liver damage. A kinetic study of LPL activity may constitute a helpful criterion in evaluating the significance of enzyme levels (Boberg and Carlson, 1964; Geday, 1966). Disappearance rates from plasma and half-life

calculation revealed no significant differences in this group of cases from values obtained in normal controls.

It, therefore, appears that the low level of LPL activities after heparin in marasmus cannot be explained entirely by the above hypotheses. It is quite possible that the defective synthesis of LPL in cases of marasmus has a common aetiology with other enzyme defects reported in protein-calorie malnutrition. It can also be stated that this functional defect is not corrected in the early stages of recovery.

Summary

The effect of heparin on LPL activity, the kinetics in the plasma of this enzyme, and the FFA levels in 14 cases of marasmus were investigated in the severe and recovery stages of the disease.

The free fatty acid levels were found to be comparable to normal control values. This finding was in agreement with the absence of fatty liver enlargement in this type of protein-calorie malnutrition.

LPL activity in the plasma after the administration of heparin did not rise as high as observed in the normal controls. This is probably the expression of a defect in the synthesis of this enzyme similar to defects in other enzyme systems observed in states of malnutrition. The defect in synthesis of the enzyme persisted for up to 14 weeks.

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