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## Plasma transport proteins in rehabilitated protein-energy malnourished children in Nigeria

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### Summary

Blood levels of prealbumin, retinol-binding protein, ceruloplasmin and transferrin were monitored during the rehabilitation of thirty protein-energy malnourished children. The children aged between six and fifty-four months were rehabilitated on an out-patient basis and fed locally prepared good quality food while the mothers were given nutrition education coupled with health education. The initial mean anthropometric values and plasma transport protein levels were significantly lower in these children when compared with values obtained from 10 apparently healthy children presenting with no signs or symptoms suggestive of any form of malnutrition. At the end of the rehabilitation period, there was no significant difference in plasma transport protein levels between the previously malnourished children and the normal controls although the malnourished children still demonstrated significant weight deficit.

**Key words:** protein-energy malnutrition, rehabilitation; prealbumin, retinol-binding protein, ceruloplasmin, transferrin.

### Introduction

One of the consequences of dietary protein-energy inadequacy in young children is weight deficit although the functional significance of such observed weight deficit in these malnourished children has not been clearly defined. Recently, there has been renewed interest in other indicators of protein-energy malnutrition (PEM) especially those which allow early detection of PEM and with some functional significances. Thus, special attention has been given to

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serum transport proteins of hepatic origin (prealbumin, retinol-binding protein, transferrin and ceruloplasmin), since the liver is one of the organs first affected by PEM. In this respect, transferrin and prealbumin have been proposed as indices of the severity of PEM as well as indices for the field assessment of nutritional status (McFarlene et al., 1969; Reeds and Laditan, 1976; Ingebleek et al., 1972). These transport proteins are highly sensitive for evaluating changes in nutritional status compared with other nutritional indicators. In a recent study in Nigeria low levels of prealbumin were found in apparently normal or near normal nutritional status thus allowing for identification of all pre-kwashiorkor stages and early stages of marasmus, even when other parameters lie within normal limits (Ogunshina and Hussain, 1980).

In the present study, transport proteins were monitored in the plasma of thirty protein-energy malnourished children for a period of twelve weeks after treatment with an improved diet in a rehabilitation centre. The Nutrition Rehabilitation Centre provides service for preschool children suffering from malnutrition. An important objective of the centre is nutrition education of the mother. Laditan and Ayeni (1977) had earlier suggested that improved nutrition as well as education of the adult population would probably go a long way to curtail improper feeding of these children. Thus, during the rehabilitation period, the mothers of these malnourished children were given nutrition education coupled with health education.

### **Subjects, Materials and Methods**

#### *Subjects*

The subjects for the study were children selected from a group attending the general out-patient department of the University College Hospital. They were aged between six and fifty-four months presenting for the first time with various forms of protein-energy malnutrition (PEM). Such children were examined for clinical signs of malnutrition before they were referred to the Institute of Child Health where the Nutrition Rehabilitation Centre is located.

At the Rehabilitation Centre, clinical examination, anthropometric measurements of mid-arm circumference (= MAC), body weight and height of the children were made according to methods described by Gurney and Omololu (1971) and Gurney et al. (1972). Ten children with an apparently normal and healthy physique and presenting with no clinical or anthropometric signs or symptoms suggestive of any form of malnutrition were used as the control group. This group of children were brought for weighing and blood sampling at stipulated intervals. During the first visit to the Centre, the mothers gave information on the feeding habits, qualitative and quantitative food intake of the children and also on the educational and socio-economic background of the parents. The mothers were then instructed to bring their children to the Rehabilitation Centre which operates once a week. Clinical examination and anthropometric measurements were carried out during weekly visits to the centre. The importance of the Centre and its activities were explained to the mothers. The parasitologic result from previous dietary studies showed widespread hookworm infestation in this age group linked with anaemia (Republic of Nigeria, 1965). Apart from anti-malaria drugs, no other form of medication was provided.

#### *Nutrition rehabilitation*

All the malnourished children were rehabilitated on an out-patient basis. The nutrition rehabilitation session normally lasted about 4–5 h. The primary objective of the Centre is to educate

Table 1. Mean values of anthropometric measurements of normal children (N = 10) and the rehabilitated malnourished children (N = 30)

| Parameter                  | Week 0 |              | Week 4 |              | Week 8 |              | Week 12 |              |
|----------------------------|--------|--------------|--------|--------------|--------|--------------|---------|--------------|
|                            | Normal | Malnourished | Normal | Malnourished | Normal | Malnourished | Normal  | Malnourished |
| <b>Weight (%)*</b>         |        |              |        |              |        |              |         |              |
| Mean ....                  | 96.84  | 59.41        | 96.64  | 61.97        | 96.94  | 70.08        | 97.54   | 74.77        |
| SD .....                   | 8.26   | 17.04        | 4.46   | 12.80        | 4.84   | 8.16         | 4.24    | 5.54         |
| <b>Height (%)*</b>         |        |              |        |              |        |              |         |              |
| Mean ....                  | 95.62  | 85.77        | 95.94  | 88.83        | 96.08  | 87.28        | 96.42   | 89.45        |
| SD .....                   | 3.58   | 5.05         | 3.24   | 2.92         | 3.48   | 2.05         | 2.88    | 3.15         |
| Mid arm circumference (cm) |        |              |        |              |        |              |         |              |
| Mean ....                  | 12.71  | 9.46         | 12.80  | 9.68         | 12.91  | 10.18        | 12.90   | 10.48        |
| SD .....                   | 1.16   | 0.85         | 1.08   | 0.76         | 1.04   | 0.81         | 0.92    | 0.62         |

SD = Standard deviation

\* Percentage of the local standard

the mothers on the dietary needs of their children. Usually, on the previous day to the nutrition rehabilitation sessions, one of the mothers was provided with money to purchase locally available foodstuffs, from a nearby local market. The mothers were taught to prepare the food for their children with minimum nutrient losses occurring. They were also taught the various categories of foodstuffs and their functions in the body through the use of visual aids. The need for clean water and hygienic conditions were highly emphasized. They were also made to realise the effect of insanitary condition on the health of their children. The mothers were taught how to mix various kinds of foodstuffs available in their locality to make excellent weaning diets for their children and how to take care of themselves before, during and after pregnancy. Emphasis was laid on breastfeeding and its advantages. The mothers prepared the food under the supervision of the Institute's trained staff. They also shared the food (between 150–250 g per child) and fed the children after which they washed the plates and cleaned up the kitchen. Samples of foods prepared were collected for laboratory analysis.

#### *Dietary study*

A 24-h dietary intake of the children was recalled by their mothers. Questionnaires were administered. The quantity of food was obtained in terms of descriptive measures. Models of some household utensils were used during the recall interview to aid the mothers. Recall interviews were carried out twice on each malnourished child during the study at the first visit to the Rehabilitation Centre and towards the end of the study in their homes during home visits. Each interview lasted about 15 min. The mother was first asked to describe the different foods eaten by the child during the previous day, starting with the first food of the day and continuing in chronological sequence. She was then asked to give the cost of such food consumed by the child on each occasion since most of the foods were bought from the vendors. Information on vendors' recipes was collected and the amount of nutrients contained in the diets were calculated on a dry weight basis using the appropriate food composition tables (Food and Agricultural organization, 1968). Intake from breastmilk was specifically excluded when calculating the nutrient intake of the subjects.

Table 2. Mean values of plasma transport proteins of normal children (N = 10) and rehabilitated malnourished children (N = 30)

| Transport protein*             | Week 0 |              | Week 4 |              | Week 8 |              | Week 12 |              |
|--------------------------------|--------|--------------|--------|--------------|--------|--------------|---------|--------------|
|                                | Normal | Malnourished | Normal | Malnourished | Normal | Malnourished | Normal  | Malnourished |
| <b>Prealbumin</b>              |        |              |        |              |        |              |         |              |
| Mean . . . .                   | 13.56  | 7.35         | 14.53  | 13.0         | 16.70  | 14.11        | 16.90   | 14.54        |
| SD . . . .                     | 2.71   | 3.43         | 3.70   | 2.86         | 2.83   | 3.83         | 2.72    | 2.81         |
| <b>Retinol-binding protein</b> |        |              |        |              |        |              |         |              |
| Mean . . . .                   | 3.40   | 1.38         | 3.80   | 2.70         | 4.24   | 3.28         | 4.42    | 3.82         |
| SD . . . .                     | 1.01   | 1.10         | 0.81   | 0.92         | 0.76   | 0.88         | 0.91    | 0.84         |
| <b>Transferrin</b>             |        |              |        |              |        |              |         |              |
| Mean . . . .                   | 398.94 | 162.43       | 410.24 | 296.84       | 443.67 | 339.80       | 464.72  | 387.24       |
| SD . . . .                     | 72.23  | 56.85        | 66.78  | 88.45        | 57.16  | 87.35        | 84.56   | 90.37        |
| <b>Ceruloplasmin</b>           |        |              |        |              |        |              |         |              |
| Mean . . . .                   | 32.40  | 19.44        | 37.36  | 26.63        | 44.98  | 40.07        | 47.34   | 46.50        |
| SD . . . .                     | 9.33   | 8.27         | 8.79   | 6.03         | 6.12   | 7.42         | 9.75    | 6.25         |

SD = Standard deviation

\* in  $\mu\text{g}/\text{dl}$

#### Blood sampling and analysis

Venous blood drawn from the femoral vein was collected into heparinized tubes and centrifuged at 3000 rpm for 20 min to ensure a good separation of plasma from the blood cells. The plasma was carefully decanted into clean bottles and stored at  $-20^\circ\text{C}$  until required analysis. Blood samples were collected at the beginning of the study and at every four week interval till the 12th week.

Plasma prealbumin, ceruloplasmin and transferrin were determined by using M-Partigen radial immunodiffusion plates, retinol-binding protein using LC-Partigen plates with standards obtained from Calbiochem-Behring Corporation, La Jolla, California and from Behring Diagnostics, Somerville, New Jersey, USA.

#### Statistical analysis

Student t-test was used to test if there was any significant difference in the parameters determined for the two groups (Snedecor and Cochran, 1967). T-values were calculated and significance was denoted where they were more than t-tabulated at 0.05.

## Results

Table 1 shows the results of the anthropometric measurements expressed as percentage of the local standard (Janes, 1973). Mean weight for age of the malnourished children was below 60% of the local standard at the beginning of

Table 3. Calorie und protein intake of malnourished subjects during the first and second dietary studies (intake expressed as percentage of FAO/WHO 1973 recommended allowance)

|             | First<br>dietary study | Second<br>dietary study |
|-------------|------------------------|-------------------------|
| Calorie (%) |                        |                         |
| Mean ....   | 44.14                  | 94.04                   |
| SD .....    | 20.7                   | 27.53                   |
| Protein (%) |                        |                         |
| Mean ....   | 60.35                  | 96.68                   |
| SD .....    | 22.62                  | 19.82                   |

Table 4. Calorie and protein content of some of the food items prepared and served at the Nutrition Rehabilitation Centre

| Food item                  | Calorie<br>(Kcal/100 g) | Protein<br>(Crude-g/100 g) |
|----------------------------|-------------------------|----------------------------|
| Rice and beans .....       | 408.2                   | 30.02                      |
| Melon stew with fish ....  | 342.37                  | 65.31                      |
| Plain stew with fish ..... | 277.93                  | 58.53                      |
| Yam flour (Amala) .....    | 304.6                   | 4.42                       |

the study. By week 12, the mean weight of this group had reached 75% levels of the local standard. This increase was significant at  $P < 0.05$ . Mean height for age of the malnourished children was also significantly lower than that of the control children at the start of study ( $P < 0.05$ ), but this difference was not observed by week 12. Similarly, the mid-arm circumference (MAC) was significantly reduced in the malnourished children at the start of the study ( $P < 0.05$ ) and this reduction persisted till week 12.

The results of the changes in mean plasma transport proteins of the malnourished children before and after dietary treatment are given in Table 2. The very low initial levels of the plasma transport proteins of the malnourished children confirm that all the children in this group are severely depleted of proteins. Mean prealbumin level in the malnourished group was less than 60% of the control group but rose significantly ( $P < 0.01$ ) by week 4 and this increase was maintained till week 12. Changes in retinol-binding protein were similar in trend to those observed for prealbumin. The steady increase observed among the control subjects was found to be non-significant. By week 4 of the study, the mean retinol-binding protein level had increased 2-fold in the malnourished group. Mean transferrin and ceruloplasmin levels were significantly lower ( $P < 0.05$ ) in the malnourished group at the beginning of the study but these also significantly rose to values very close to those of the control subjects. Thus,

there was no significant difference in the plasma transport proteins between the two groups by week 8 of the study.

Tables 3 and 4 show the results of the dietary studies conducted at the start and end of the project and the chemical analysis of some of the food items served during the rehabilitation, respectively. In Table 3, calorie and protein intake of the malnourished group were computed at 44% and 60% of the FAO/WHO 1973 recommended allowance, respectively, at the beginning of the study. By week 12 both calorie and protein intake had significantly ( $P < 0.01$ ) increased to 94% and 96%, respectively.

## Discussion

Changes in body weight still remain a very useful indicator of a change in overall nutritional status, though in short term studies, such changes may be unreliable because of changes in fluid balance. Indeed, in the present study the malnourished children still demonstrated significant weight deficit after 3 months of rehabilitation. The height is also affected by malnutrition but much less than the body weight. Well-nourished Nigerian children living in good conditions have been shown to have growth pattern similar to that of their well reared caucasian counterparts (Collis et al., 1962; Janes, 1973). Arm muscle circumference is also a useful indicator of nutritional status in young children since the muscle is the largest protein containing organ in the body. There is evidence that the myoglobin protein reserves of the musculature are particularly depleted in PEM in early childhood (Jelliffe and Jelliffe, 1969). The mid-arm circumference of the malnourished children slightly increased from 75% of the control at the beginning of the study to a little over 80% by the end of the study period (week 12). Laditan and Ayeni (1977) showed that after 12 months of rehabilitation the children with kwashiorkor had mid-arm muscle circumference appropriate for their ages while children with marasmus were not completely repleted in muscle mass as estimated by mid-arm muscle circumference. It is probable that a study period of 12 weeks may be insufficient for adequate catch-up in muscle growth.

The transport proteins represent highly sensitive parameters for changes in the nutritional status as regards protein (Gofferje, 1978). In studies where the nutrition rehabilitation involved the use of high-protein diets (Gofferje, 1978; Shetty et al., 1979), prealbumin and retinol-binding protein levels dropped significantly during low protein diet or during total fasting and rose again when the protein-diets were given. In the present study, prealbumin and retinol-binding protein levels which were initially low in the malnourished subjects increased by 2-fold within four weeks of rehabilitation. Low levels of the metal-transporting proteins, transferrin and ceruloplasmin have also been observed in protein-energy malnutrition (Reeds and Laditan, 1976) and Ones et al. (1980) demonstrated a constant rise in serum ceruloplasmin following treatment. A

similar trend was observed in this study. The functional significance of the increase in plasma transport proteins inspite of the glaring weight deficit demonstrated by the malnourished children is still not clear. The results show that the rehabilitation both in terms of diet supplementation (Table 4) and nutrition education resulting in improved calorie and protein intake (Table 3) has contributed to the linear rise in the plasma levels of the transport proteins while the growth retardation remains an adaptation to the previous protein-energy status of the malnourished children. There is a need to monitor these changes on a long term basis to determine the extent to which this increase in the transport protein levels is sustained.

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