Transferrin Kinetics Are Altered in Children with Severe Protein-Energy Malnutrition

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ABSTRACT This study was undertaken to determine the following: 1) the kinetic changes responsible for the depletion and repletion of plasma transferrin (Tr) concentration in children with protein-energy malnutrition (PEM); 2) the role of infection in mediating these changes; and 3) whether plasma Tr concentration is related to body protein status. We measured plasma Tr concentration, and fractional (FSR) and absolute (ASR) Tr synthesis rates with the use of a constant intragastric infusion of $^{2}H_{3}$-leucine in 14 children with PEM, at 2 d postadmission (study 1), 8 d postadmission when infections were under control (study 2), and at recovery (study 3). In studies 1 and 2, the children synthesized less Tr and had lower Tr concentrations compared with values at recovery. When infections were controlled, plasma Tr concentration rose, but Tr synthesis was unchanged. There were only fair correlations ($P < 0.05$) between plasma Tr concentrations and indices of wasting. Concerning malnourished children, we reached the following conclusions: 1) changes in the Tr pool size are achieved mainly through changes in synthesis rate; 2) infections play a minor role in reducing the Tr pool through either changes in the rate of catabolism or loss from the intravascular space; and 3) Tr concentration is not a very good indicator of protein nutritional status. J. Nutr. 127: 1469–1474, 1997.

KEY WORDS: • transferrin concentration • transferrin synthesis • protein-energy malnutrition • children

Severe protein-energy malnutrition (PEM) remains a major health problem worldwide. The underlying derangements of protein metabolism in PEM, which are thought to be pivotal in the pathogenesis of the disease, have not been clearly elucidated. Although whole-body protein turnover is reduced in children with PEM (Golden et al. 1977), the rates of synthesis of the individual plasma proteins are unknown. PEM is marked by lower plasma protein concentrations (Coward and Lunn 1981, Ingenbleek et al. 1975, Reeds and Laditan 1976) including transferrin (Tr), the major iron-binding plasma protein that transports iron to the erythropoietic tissues, liver and muscle (De Jong et al. 1990). Transferrin also serves an important bacteriostatic function because its iron-binding property deprives bacteria of iron. Furthermore, the reduction in plasma Tr concentration has important clinical implications in children with PEM because it is associated with a greater mortality rate (Ingenbleek et al. 1975, McFarlane et al. 1970, Ramdath and Golden 1989, Reeds and Laditan 1976).

In normal health, only 30% of available Tr is involved in the binding and transport of iron; hence there is a substantial excess of iron-binding capacity (Ramdath and Golden 1989). However, because of the decreased availability of Tr in children with PEM, plasma transferrin becomes more saturated with iron, thereby markedly reducing the iron-binding capacity of malnourished children. This has been offered as an explanation for the fact that plasma iron concentration is not lower in severe malnutrition (Golden and Ramdath 1987). It has been suggested that the overwhelming systemic infection associated with the high mortality rate of malnourished children with very low (<0.33 g/L) plasma Tr concentrations may be due to the increased availability of iron for bacterial growth (McFarlane et al. 1970).

In children with PEM, the depletion of the Tr pool may be the direct result of a reduced rate of synthesis of the protein mediated either by changes in iron status or by dietary protein deficiency. However, plasma iron concentration is not different in severely malnourished children (Golden and Ramdath 1987). Hence, it has been proposed that a reduction in Tr synthesis rate secondary to chronic protein deficiency is the...
cause of Tr depletion in malnutrition. Direct evidence for this proposal was obtained from rat studies in which the hepatic Tr mRNA was reduced after 48 h of food deprivation (De Jong et al. 1988) and after rats had consumed a diet providing 60% of protein requirements for 14 d (Moullac et al. 1992). However, a direct measure of the actual Tr synthesis rate has not been made in infants with PEM.

A potential mediator of the changes in Tr kinetics in PEM is infection, but its precise role is not known. Because concurrent infection is a common feature of severe PEM (Christie et al. 1985) and the plasma Tr concentration is reduced in inflammatory and infective states (Fleck 1989), it is likely that the lower plasma Tr concentration is due to a higher rate of catabolism, possibly mediated by infection. This may impede recovery because Tr has an additional important role in host defense against infection (Ramdath and Golden 1989).

Finally, it has been proposed that plasma Tr concentration is a good indicator of protein intake and protein nutritional status (Reeds and Laditan 1976, Shetty et al. 1979) because plasma Tr concentrations have been shown to be lower in children with PEM (Ingenbleek et al. 1975, Reeds and Laditan 1976), to be reduced by protein deprivation in rats (Moullac et al. 1992) and humans (Shetty et al. 1979) and to increase on refeeding (Shetty et al. 1979). However, the validity of this assertion has been questioned because the plasma Tr concentration can also be affected by infection and inflammatory states (Golden 1982). Thus, another goal of this study was to determine whether a relationship exists between plasma Tr concentration and body protein status.

The present study, therefore, was undertaken to determine the following: 1) the kinetic changes responsible for the depletion and repletion of plasma Tr concentration in PEM; 2) the role of concurrent infection, apart from that of an inadequate diet, in mediating these kinetic changes; and 3) the possible relationship between plasma Tr concentration and body protein status.

SUBJECTS AND METHODS

Subjects. This study was approved by the Medical Ethics Committee of the University Hospital of the West Indies and the Baylor Affiliates Review Board for Human Subject Research of Baylor College of Medicine. Fourteen Jamaican children (10 boys, 4 girls) were enrolled in the study after informed consent was received from their parents. The physical and clinical characteristics of the children have been described in detail previously (Morlese et al. 1996). The main criterion for selection into the study was a deficit in body weight of ≥20%. The weight of the children was measured using a beam balance (Sartorius GMBH, Gottingen, Germany) and height was measured on a horizontally mounted stadiometer (Holtain, Crymych, United Kingdom).

The children were admitted to the metabolic ward of the Tropical Metabolism Research Unit and managed according to a standard treatment protocol (Jackson and Golden 1988). This involved correction of fluid and electrolyte imbalances and administration of broad-spectrum antibiotics. The course of antibiotics consisted of parenteral penicillin and gentamycin, and oral metronidazole. After admission, the children immediately received a maintenance milk-based diet which provided 417 kJ of energy and 1.2 g/(kg·d) of protein (Table 1) with additional supplements of vitamins and trace elements until appetite was restored (postadmission d 8). During the catch-up growth phase, the patients were fed a milk-based formula (made energy dense by the addition of an oil) that provided 625–750 kJ of energy and ~3 g protein/(kg·d).

The children were fed the maintenance diet at each study. The first isotope infusion (study 1) was performed immediately after fluid resuscitation when the children were stable as indicated by blood pressure, heart rate and respiration rate and had been receiving the maintenance diet for 2 d. Study 2 was undertaken 8 d postadmission while the children were still receiving the maintenance diet. They had lost edema, their affect and appetite had recovered, and their infections were under control as determined by normalization of temperature, respiratory and pulse rate, resolution of clinical features of the infective episode, e.g., cessation of diarrhea and absence of chest crepitations. Study 3 was performed at ~59 d postadmission when full recovery had occurred, that is, after the catch-up growth rate had started to reach a plateau and weight-for-height was at least 90%. At this point, the child again received the maintenance diet for 3 d before the isotope infusion.

Isotope infusion. A sterile solution of 2H3-leucine (Cambridge Isotope Laboratories, Woburn, MA) prepared in 9 g/L NaCl was infused for 8 h to measure the rate of synthesis of transferrin (Tr) as previously described (Morlese et al. 1996). Briefly, ~40% of the subject’s daily food intake was given by constant intragastric infusion starting 2 h before the isotope infusion commenced and continuing throughout the isotope infusion. After a 2-mL venous blood sample was drawn, the 2H3-leucine was infused nasogastrically at a rate of 26 μmol/(kg·h) for a period of 8 h. Additional 2-mL blood samples were drawn at 2-h intervals throughout the infusion. The same infusion and blood sampling protocol was repeated in the second and third studies.

Sample analyses. Blood was drawn in prechilled tubes (containing Na2EDTA and a cocktail of sodium azide, merthiolate and soybean trypsin inhibitor) and immediately centrifuged at 1000 × g for 15 min at 4°C. The plasma was removed and stored at −70°C for later analysis.

Plasma Tr concentration was measured by radial immunodiffusion using Human Transferrin NL RID kits (The Binding Site, Birmingham, England) as previously described (Jahoor et al. 1996). The immunoprecipitate was subjected to sodium dodecyl polyacrylamide gel electrophoresis to separate Tr from its degradation products. The dried protein precipitates and gel bands were hydrolyzed in 6 mol/L HCl at 110°C for 12 h. The amino acids released by the children immediately received a maintenance milk-based diet

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Composition of the milk-based maintenance diet1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maintenance diet</td>
</tr>
<tr>
<td></td>
<td>g/100g</td>
</tr>
<tr>
<td>Protein</td>
<td>7.6</td>
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<tr>
<td>Carbohydrate</td>
<td>28.4</td>
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<tr>
<td>Fat</td>
<td>53.2</td>
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<tr>
<td>Linoleate</td>
<td>2.8</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.5</td>
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</table>

1 Vitamin supplement: a compound vitamin preparation (Tropivite, Federation Pharmaceuticals, Kingston, Jamaica) was given daily at a dose of 2 mL/kg, providing (per kg): retinol, 21 μmol; cholecalciferol, 104 nmol; thiamine, 6 nmol; riboflavin, 8.5 nmol; niacin, 84 nmol; vitamin B-6, 19.5 nmol; vitamin C, 681 μmol. Folate was given at 10.6 nmol/d.

2 Trace element supplement: a solution of mineral mix given daily provided (per kg): potassium, 4 mmol; magnesium, 1 mmol; copper acetate, 1.65 μmol; zinc acetate, 16 μmol.
infections were under control as indicated by the absence of all signs and symptoms of infection. All of the children were severely protein-energy malnourished with a mean weight-for-age of 55 ± 2.2% and a mean weight-for-length of 73 ± 1.4% of expected. These indices of nutritional status were unchanged from study 1 to study 2 (Table 2). Although there was only a fair degree of correlation between plasma Tr concentration and weight-for-age or weight-for-length (whether or not the infected-malnourished were included or excluded in the analysis), these correlations were all significant (Table 3).

The tracer/tracee ratio of leucine bound in VLDL apoB-100 reached a steady state after 4 h of isotope infusion in all three studies, and there was a linear increase in the amount of labeled leucine incorporated into plasma transferrin during this period of time (Fig. 1). Hence, the FSR of transferrin was calculated from the rate of incorporation of labeled leucine into the protein during the last 4 h of the isotope infusion.

In study 1, the plasma Tr concentration was significantly lower ($P < 0.01$) compared with the recovered value in study 3 (Fig. 2). Compared with the study 1 value, the plasma Tr concentration increased significantly ($P < 0.05$) by study 2 (when infections were under control but the children were still malnourished) but remained significantly lower than recovered values ($P < 0.05$). The fractional synthesis rate (FSR) of Tr was faster at studies 1 and 2 compared with the recovered value ($P < 0.05$). The intravascular absolute synthesis rate (ASR) of Tr was significantly lower in study 1 ($P < 0.05$) and study 2 ($P < 0.05$) compared with the rate at recovery (Fig. 2).

In both edematous and non-edematous groups, plasma Tr concentrations were significantly lower in study 1 compared with recovered values ($P < 0.05$). In study 2, plasma Tr concentration of the edematous group remained significantly lower ($P < 0.05$) compared with the recovered value, but in the non-edematous group of children, there was no difference between the study 2 and recovered values. There was no difference in the plasma Tr concentration of edematous and non-edematous children in any of the studies (Fig. 3).

The FSR of Tr was faster in both the edematous and non-edematous children at study 1 when compared with the respective rates at recovery (Fig. 3). There was no significant difference between the two groups of children. In study 2, when infection was under control, the edematous children still had a faster FSR compared with the rate at recovery, but the non-edematous group had an FSR that was not different from the rate at recovery. That is, FSR of the non-edematous group was significantly slower ($P < 0.03$) at study 2 than at study 1.

### RESULTS

At admission, 13 of the children had signs and symptoms of infection based on the presence of one or more of the following: leucocyte count $> 11 \times 10^9$ cells/L, temperature at admission $> 37^\circ C$ or $< 35.5^\circ C$, positive blood, urine or stool culture (see Table 3 of Morlese et al. 1996). All of the children were anemic with hemoglobin concentrations ranging from 4.71 to 6.45 mmol/L (mean 5.4 ± 0.17). At study 2, the

### TABLE 2

<table>
<thead>
<tr>
<th>Physical characteristic</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mo</td>
<td>12.3 ± 1.5</td>
<td>12.5 ± 1.5</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>5.3 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>65 ± 0.7</td>
<td>65 ± 0.8</td>
<td>67 ± 0.8</td>
</tr>
<tr>
<td>Weight-for-age, %</td>
<td>55 ± 2.2</td>
<td>54 ± 2</td>
<td>72 ± 2</td>
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<tr>
<td>Weight-for-length, %</td>
<td>73 ± 1.4</td>
<td>72 ± 2</td>
<td>94 ± 1.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, $n = 14$

2 Percentage of the expected reference value derived from the Harvard Standards (Stuart and Stevenson, 1959).

$$FSR \, (\%/d) = \frac{PE_t - PE_f}{E_t} \times \frac{2400}{t_f - t_i}$$

where $PE_t - PE_f$ is the increase in enrichment of Tr-bound leucine over the time period, 4 to 8 h ($t_f - t_i$) of the infusion, and $E_t$ is the plateau enrichment of apoB-100–bound leucine. In this calculation, the plateau enrichment of apoB-100–bound leucine in plasma is assumed to represent the enrichment of the intrahepatic leucine pool from which the Tr is synthesized (Jahoor et al. 1994). A steady-state tracer/tracee ratio was obtained by finding the average of the individual tracer/tracee ratio values after the tracer/tracee ratio-time curve reached a plateau. Plateau was defined as previously described (Jahoor et al. 1994).

The intravascular absolute synthesis rate (iv ASR) of Tr was estimated as the product of the Tr FSR and the intravascular (iv) Tr mass as follows:

$$iv \, ASR \, [mg/(kg \cdot d)] = iv \, transferrin \, mass \times FSR$$

where the intravascular Tr mass is the product of the plasma volume and the plasma concentration of Tr. The plasma volume of the acutely ill child was assumed to be 42 mL/kg edema-free body weight (Viart 1976); the value of 50 mL/kg was used for the recovered children (James and Hay 1968, Viart 1976).

The sample size of seven subjects per group is sufficient to detect a 1.5 SD difference between groups and a change of 1.2 SD within groups. This assumes a correlation between studies of 0.5, a type 1 error of 0.05 and power equal to 0.80. All results are presented as mean values ± SEM. The data were analyzed using ANOVA with repeated measures utilizing the SPSS statistical package (SPSS, Chicago, IL). Time was the within-subject factor. At the first stage of the analysis, group (i.e, edematous and non-edematous) was entered as the between-subject factor. When the interaction between time and group was not significant, the analysis was repeated with both groups combined. Potentially confounding variables were included in the analysis as covariates, and the results remained unchanged. When there were significant differences over time, individual time points were compared with univariate post-ANOVA contrasts. Correlation analysis was also performed to determine the relationship between anthropometric variables and plasma transferrin concentrations. Significance of difference was assumed at $P < 0.05$.

### TABLE 3

<table>
<thead>
<tr>
<th>Anthropometric variable</th>
<th>Correlation coefficient</th>
<th>Probability, $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-for-age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All values (studies 1 &amp; 2)$^1$</td>
<td>0.340</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Study 2 values$^2$</td>
<td>0.387</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Weight-for-length</td>
<td></td>
<td></td>
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<tr>
<td>All values (studies 1 &amp; 2)$^1$</td>
<td>0.481</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Study 2 values$^2$</td>
<td>0.470</td>
<td>&lt;0.05</td>
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</tbody>
</table>

1 $n = 28$; 2 $n = 14$.  

The physical characteristics of the children when severely malnourished (studies 1 and 2) and when fully recovered (study 3)*
of the Tr pool of children with PEM. When infections were controlled, there was a modest but significant improvement in plasma Tr concentration that was not associated with any change in the amount of intravascular Tr synthesized. This finding suggests that concurrent infections were playing a minor, though significant role in mediating the depletion of the Tr pool during malnutrition. Furthermore, the effect of infections on the Tr pool size was mediated through either changes in the rate of catabolism or the loss of Tr from the intravascular space. Finally, because there was only a fair degree of correlation between plasma Tr concentration and indices of wasting, plasma Tr concentration is not a good indicator of protein nutritional status.

In agreement with the earlier findings of Ingenbleek and Reeds (Ingenbleek et al. 1975, Reeds and Laditan 1976), in the present study, the plasma Tr concentration of the acutely malnourished children was markedly lower compared with values at recovery and values from normal children (2.6–4.0 g/L). In normal health, the pool size of a plasma protein is determined by the balance between its rates of synthesis and catabolism or loss. Thus, the pool size of a plasma protein could be reduced by one of two potential kinetic mechanisms, i.e., either a decrease in synthesis rate unbalanced by a change in the rate of catabolism, or an increase in the catabolic rate relative to the synthesis rate. In severely malnourished children with concurrent infection(s), increased transfer of a protein from the intravascular to the extravascular space is another potential factor that may alter the pool size of the plasma protein. In injury and infection, there are marked losses of

**DISCUSSION**

The results of this study show that the lower plasma Tr concentration of severely malnourished children was associated with a decrease in the amount of Tr synthesized per unit of time both in the presence of concurrent infections and after the infections were treated. Conversely, normalization of the plasma Tr pool during nutritional rehabilitation was associated with an increase in the amount of Tr synthesized per unit of time. These findings suggest that changes in the amount of Tr synthesized play a major role in the depletion and repletion of the Tr pool of children with PEM.
plasma proteins, including Tr, from the intravascular space as a result of increased transcapillary escape or excretion in the urine (Davies et al. 1962, Fleck 1989, Fleck et al. 1985). This also includes loss through the gut in those children (four in the present study) who have gastrointestinal infections (Sarker et al. 1986). In the present study, the malnourished children were synthesizing less Tr per unit of time and had smaller Tr pools whether the infections were active or controlled, suggesting that the major factor responsible for the depletion of the Tr pool of children with PEM was a reduction in the amount of Tr synthesized. Interestingly, in studies 1 and 2, the fractional rate of synthesis of Tr, that is, the fraction of the Tr pool catabolized and resynthesized per unit of time, was always faster when the size of the Tr pool was smaller. This can be seen even more clearly in study 2 when the subjects were divided into edematous and non-edematous groups. The edematous group, which still had a reduced Tr pool in study 2, also had a faster fractional rate of synthesis of Tr compared with the value at recovery.

On the other hand, there was a modest but significant increase in plasma Tr concentration without a parallel change in absolute intravascular synthesis rate just 8 d postadmission when the children were still severely malnourished (Table 2) but their infections were under control. This finding suggests that concurrent infections also were involved in mediating the reduction of the Tr pool through either changes in the rate of Tr catabolism or loss from the intravascular space. The evidence suggests that, when the malnourished child has a concurrent infection, the Tr pool size is regulated by changes in both the rates of synthesis and catabolism during the course of illness and during rehabilitation.

The reduction in plasma Tr concentration is of clinical importance in children with PEM because it is associated with a greater mortality rate (Ingenbleek et al. 1975, McFarlane et al. 1974, 1975, Ramdath and Golden 1989, Reeds and Laditan 1976). The severely malnourished child has a lower iron-binding capacity because of the decreased availability of transferrin. Golden and Ramdath (Golden and Ramdath 1987, Ramdath and Golden 1989) have suggested that this is the reason why severely malnourished children have normal plasma free iron and high tissue iron concentrations. Because plasma free iron facilitates bacterial growth (Weinberg 1975), McFarlane and Smith (McFarlane et al. 1970, Smith et al. 1989) have suggested that the systemic infection associated with the death of malnourished children during the early postadmission period may be due to the increased availability of iron for bacterial growth. This has been proposed as an explanation for the increased mortality in malnourished children treated with ferrous sulfate in the early postadmission period (McFarlane et al. 1970, Ramdath and Golden 1989, Smith et al. 1989).

The results of the present study suggest that a shortage of amino acid precursors secondary to a chronic reduction in dietary protein intake may be responsible for the decrease in Tr synthesis by children with PEM. This is in agreement with the conclusion of Moullac et al. (1992) who reported reduced hepatic Tr mRNA after rats had consumed a diet providing only 60% of protein requirements for 14 d. Although other factors such as deficiencies in micronutrients, including retinol, copper and iron, can affect Tr synthesis (Golden 1982), there was no change in the ASR of Tr from study 1 to study 2 after the children had received 6 d of supplementation with vitamins and trace elements. In the case of iron, although it is true that the availability of iron is a major regulator of transferrin synthesis, it is unlikely that iron deficiency is involved in mediating the suppressed Tr synthesis in these malnourished children. First, although plasma iron concentration was not measured in the present study, it is unlikely that these children were iron deficient despite having low hemoglobin concentrations. For example, previous work by our colleagues and others (Ramdath and Golden 1989, Smith et al. 1989) have demonstrated that children with both PEM and anemia, in Jamaica and Africa, are not iron deficient because they have normal plasma free iron and high tissue iron concentrations. Furthermore, iron deficiency would cause a stimulation and not a suppression of Tr synthesis (Huebers and Finch 1987).

The marked reduction in plasma Tr concentration in severe PEM and the rapid increase during nutritional rehabilitation led to its proposed use as an indicator of dietary protein intake and protein nutritional status (De Jong et al. 1988, Ingenbleek et al. 1975, Pressac et al. 1990, Shetty et al. 1979, Young and Hill 1978). However, this use has been questioned because the plasma Tr concentration is also affected by other factors such as the presence of infection or deficiencies of micronutrients, including retinol, copper and iron (Golden 1982). In agreement with the previous finding of Smith et al. (1989), in the present study there was only a fair correlation between plasma Tr concentration and indices of wasting (weight-for-age and weight-for-length). For example, in study 1, although all of the children had severe malnutrition, four of them had plasma Tr concentrations (2.03–2.37 g/L) near the normal range (2.6–4.0 g/L).

Our results do not corroborate the findings of previous studies that non-edematous malnourished (marasmic) children have higher plasma Tr concentrations than edematous children (Ramdath and Golden 1989, Reeds and Laditan 1976).
In our study, there was no difference in the plasma concentrations or absolute rates of synthesis of Tr between the two groups of children at 2 d postadmission. This may be due to the fact that most of the children studied had evidence of infection at admission, which we have shown to have an effect on Tr homeostasis. Interestingly, in study 2, when the infections were under control but the children were still malnourished, the mean plasma Tr concentration of the marasmic children was 75% higher than that of the edematous group of children. However, because of the wide intersubject variability, a finding also reported by Smith et al. (1989), the difference was not significant ($P = 0.08$).

In conclusion, these findings suggest that the depletion and repletion of the Tr pool in children with PEM is achieved primarily through changes in the synthesis rate of Tr. The presence of concurrent infections also plays a minor role in mediating the reduction of the Tr pool through either changes in the rate of Tr catabolism or loss from the intravascular space. Our results also suggest that Tr concentration is not a very good indicator of protein nutritional status.

ACKNOWLEDGMENTS

We are grateful to the nursing staff of the TMRU for their care of the children and to Leslie Loddeke for editorial assistance.

LITERATURE CITED


