

# Thyroid hormone and carrier protein interrelationships in children recovering from kwashiorkor<sup>1-3</sup>

W John Kalk, MRCP, Karen J Hofman, MD, Aletta M Smit, BSc,  
Maryna van Drimmelen, MSc, L Andre van der Walt, PhD, and Roland E Moore, MD

**ABSTRACT** We have studied 15 infants with severe protein energy malnutrition (PEM) as a model of nutritional nonthyroidal illness. Changes in circulating thyroid hormones, binding proteins, and their interrelationships were assessed before and during recovery. Serum concentrations of total thyroxine and triiodothyronine and of thyroxine-binding proteins were extremely reduced, and increased progressively during 3 wk of refeeding. The  $T_4$ :TBG molar ratio was initially  $0.180 \pm 0.020$ , and increased progressively, parallel to the increases in  $TT_4$ , to  $0.344 \pm 0.038$  after 21 days ( $p < 0.025$ ). The changes in free  $T_4$  estimates varied according to the methods used—FTI and analogue  $FT_4$  increased, dialysis  $FT_4$  fraction decreased. Serum TSH levels increased transiently during recovery.

It is concluded 1) there is reduced binding of  $T_4$  and  $T_3$  to TBG in untreated PEM which takes 2–3 wk to recover; 2) there are methodological differences in evaluating free  $T_4$  levels in PEM; 3) increased TSH secretion appears to be an integral part of the recovery from PEM. *Am J Clin Nutr* 1986;43:406–413.

**KEY WORDS** Protein energy malnutrition, thyroxine, triiodothyronine, reverse triiodothyronine, thyroxine-binding globulin, prealbumen, thyrotropin

## Introduction

A variety of changes in circulating thyroid hormones may occur in nonthyroidal illnesses (NTI) (1–3). Low serum levels of triiodothyronine ( $T_3$ ) are found most often (1, 2), and are attributed to the reduction of peripheral deiodination of thyroxine ( $T_4$ ) to  $T_3$ , often accompanied by an accumulation of reverse  $T_3$  ( $rT_3$ ) with resultant relative or absolute elevations of this metabolite (2). The serum-free fraction of  $T_4$ , which is in equilibrium with the protein-bound hormone, is characteristically elevated in NTI (2, 3). Free  $T_4$  levels ( $FT_4$ ) have been said to be influenced by alterations in concentrations of thyroxine-binding proteins—albumen (4), thyroxine-binding prealbumen (TBPA) (5, 6), and thyroxine-binding globulin (TBG) (7), in addition to inhibitors of  $T_4$  binding to carrier proteins. Albumen and especially TBPA (8) are sensitive to nutritional status.

Most studies of NTI have involved groups of patients with established diseases and have

not evaluated serial changes in thyroid function tests as the patients' condition improves. In infantile protein energy malnutrition (PEM) concentrations of all three thyroid hormone-binding proteins are extremely low (8–11) and recover rapidly with refeeding. This condition thus provides a nutritional model of NTI in which thyroid hormones, carrier proteins, and their interactions can be studied at various protein concentrations as recovery supervenes. We have therefore investigated prospectively

<sup>1</sup> From the Department of Medicine, Endocrinology and Metabolism Unit, Department of Paediatrics, and Department of Chemical Pathology (SAIMR), University of the Witwatersrand Medical School, and Department of Physiology, Trinity College, Dublin, Ireland.

<sup>2</sup> Supported by a grant from the South African Medical Research Council. REM was a recipient of the Wellcome-Lowenstein Research Travel Grant.

<sup>3</sup> Address reprint requests to: Dr WJ Kalk, Department of Medicine, Medical School, York Road, Parktown, Johannesburg, 2193, South Africa.

Received May 28, 1985.

Accepted for publication September 24, 1985.

changes in circulating thyroid hormone levels and the relation of T<sub>4</sub> and T<sub>3</sub> to carrier protein concentrations in children with PEM, before and during progressive recovery to normality. Various methods for expressing free T<sub>4</sub>, direct and derived, have also been evaluated.

### Patients and methods

Fifteen children with PEM, ages 7 to 27 mo (mean, 12.5 mo) were studied. All presented with edema and the typical clinical evidence of severe kwashiorkor (Wellcome Working Party classification) (12). All but one child presented with a history of vomiting associated with diarrhea in most, with respiratory infections in three and impetigo in three others. All were edematous. Only three did not require initial intravenous therapy, which generally lasted 3 to 4 days, especially if parenteral antibiotics were used. All children were given antibiotics (ampicillin, or penicillin and gentamycin) and vitamin supplements. Intercurrent infections and enteritis were usually better within the first week. Only enteral refeeding was used, starting within the first 24 h after admission, and was continued until full clinical recovery had been achieved, after 14 to 21 days. One patient received a blood transfusion. Tests of renal and liver function are shown in Table 1. Serum urea and creatinine levels were initially low; urea rose with refeeding. Liver function tests were normal.

Blood was withdrawn on the day of admission to hospital and weekly thereafter for 2 (all subjects) or 3 wk (six subjects). The separated serum was stored at -20°C for up to 6 mo before all assays were completed. Sera from the first 10 subjects studied were assayed for albumen, TBG (13), total T<sub>4</sub> (TT<sub>4</sub>), T<sub>3</sub>, rT<sub>3</sub>, the T<sub>3</sub> resin uptake test (T<sub>3</sub>RU), free T<sub>4</sub> by a direct analogue method (AFT<sub>4</sub>) (Amerlex FT<sub>4</sub>, Amersham, UK), and thyrotropin (TSH) (14); the free thyroxine index (FTI) was calculated T<sub>4</sub> × T<sub>3</sub>RU ÷ 100. In six subjects FT<sub>4</sub> was measured by equilibrium dialysis (DFT<sub>4</sub>) (15) and TBPA was assayed by immunoelectrophoresis (rocket electrophoresis) (16) in addition to the T<sub>4</sub> measurements. All sera from each subject were assayed together to avoid interassay variability.

**Electrophoretic studies.** 50 ul of serum was incubated with 50 ul <sup>125</sup>I-T<sub>4</sub> in phosphate buffered saline (20,000 cpm) for 30 min at 37°C prior to cellulose acetate electrophoresis in 0.05 M barbitone buffer, pH 8.6, with sodium azide as preservative. After autoradiography the TBG, albumen, and TBPA areas were cut out and counted. Sera from six patients, from before and after recovery were evaluated in this manner; each pair was studied in duplicate

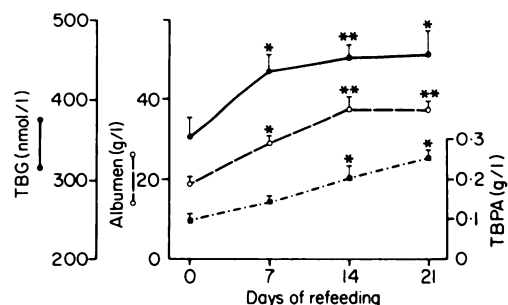


FIG 1. Changes during refeeding of mean (±SEM) serum concentrations of thyroxine-binding globulin (TBG), albumen, and thyroxine-binding prealbumin (TBPA) in children with protein energy malnutrition. \* = compared to day 0, *p* < 0.05. \*\* = compared to day 0, *p* < 0.01.

in the same electrophoretic run. (One subject was studied during and after recovery.)

The committee for Research on Human Subjects of the University of the Witwatersrand approved the study.

**Statistical analyses.** The paired *t* test was used to evaluate changes with time. Correlations were assessed by linear regression analysis, and the slopes and intercepts of regression lines were compared by the analysis of covariance. Data are expressed as the mean ± SEM.

### Results

On admission to the hospital the levels of albumen, TBPA, and TBG were subnormal in every subject but had risen to within the normal ranges by the day 14 (Fig 1) (normal ranges: albumen, 34–46 g/l; TBPA, 0.24–0.40 g/l; TBG, 344–718 nmol/l).

The alterations in TT<sub>4</sub>, T<sub>3</sub>, rT<sub>3</sub>, and TSH after refeeding are shown in Figure 2 (normal ranges: TT<sub>4</sub>, 60–150 nmol/l; T<sub>3</sub>, 1.5–3.0 nmol/l; rT<sub>3</sub>, 0.10–0.37 nmol/l; TSH, <8 μU/ml). At presentation T<sub>3</sub> levels were subnormal in every case, and TT<sub>4</sub> levels in 10 of the 15 children; by day 14, levels were normal in every patient. Reverse T<sub>3</sub> levels were elevated in

TABLE 1

Tests of kidney and liver function in PEM children before and 21 days after refeeding

	Initial	Final	Reference range
Urea (mmol/l)	1.8 ± 0.3	4.0 ± 0.5	<6.0
Creatinine (μmol/l)	27.3 ± 3.3	29.9 ± 2.0	30–60
Alkaline Phosphase (μ/l)	84.7 ± 5.6	101.1 ± 12.2	150–380
ALT (μ/l)	11.9 ± 0.6	17.3 ± 2.9	<35
AST (μ/l)	7.3 ± 1.1	14.3 ± 2.9	<40

(*p* < 0.01)

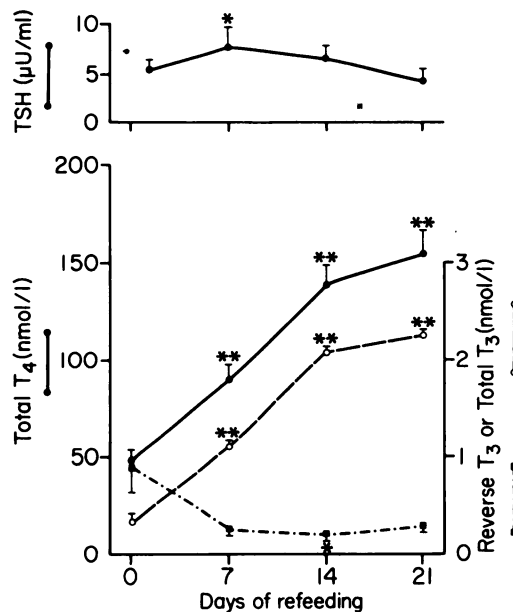


FIG 2. Alterations in mean ( $\pm$ SEM) serum concentrations of thyrotropin (TSH), total thyroxine (TT<sub>4</sub>), total triiodothyronine (TT<sub>3</sub>), and reverse T<sub>3</sub> (rT<sub>3</sub>) in children with protein energy malnutrition, before and during 21 days refeeding. \* = compared to day 0,  $p < 0.05$ . \*\* = compared to day 0,  $p < 0.01$ . ○ = compared to TSH level on day 7,  $p < 0.05$ .

11 children and declined rapidly within 7 days of refeeding and remained fairly constant thereafter. In patients with normal rT<sub>3</sub> levels on admission initial TT<sub>4</sub> concentrations were extremely low (12–29 nmol/l). Mean serum TSH levels increased from  $5.1 \pm 0.9$  μU/ml (range, 2.2–11.9 μU/ml) to peak levels on either day 7 or day 14 (two subjects). The mean value of the peak levels was  $8.2 \pm 1.8$  μU/ml (range, 2.2–18.4 μU/ml;  $p < 0.05$ ) (increments ranged from  $-0.5$  to  $+12.3$  μU/ml); and then declined in every case by day 21, to  $3.0 \pm 1.5$  ml/ml (vs peak levels  $p < 0.05$ ); at this stage TSH levels were below initial values in four of the six children studied.

The differences in the rates of relative changes (percent of recovery values) of TT<sub>4</sub>, TBG, and the T<sub>4</sub>:TBG molar ratio are shown in Figure 3. Only after 7 days did the T<sub>4</sub>:TBG ratio increase, parallel with the rise in T<sub>4</sub>. TBG levels were very close to normal during this period.

The various assessments of Free Thyroxine are shown in Table 2. Mean values of the

T<sub>3</sub>RU test declined progressively from baseline until day 14. Despite this fall FTI values increased progressively from day 1 to day 21. The T<sub>4</sub>:TBG molar ratio was very low on day 1 and did not change significantly in the first week. Thereafter values rose progressively, and significantly by days 14 and 21. Levels of FT<sub>4</sub> measured by the analogue method, (AFT<sub>4</sub>) also rose progressively with recovery. In contrast the free fraction of thyroxine, assessed by equilibrium dialysis, declined progressively. However, the mean-derived dialysis FT<sub>4</sub> (DFT<sub>4</sub>) remained virtually constant for the 21 days of the study.

Serum T<sub>3</sub> levels were undetectable ( $<0.1$  nmol/l) in half the children on admission to hospital, but measurable levels were found 1 wk later in all. The T<sub>3</sub>:TBG molar ratio increased progressively with refeeding from  $0.0027 \pm 0.0006$  on day 7 to  $0.0045 \pm 0.0005$  on day 14 ( $p < 0.025$ ) to  $0.0053 \pm 0.0006$  on day 21 ( $p < 0.05$ ). The molar T<sub>3</sub>:T<sub>4</sub> ratio was extremely low on day 1, and increased substantially (200% of basal) by day 7 and remained constant thereafter (Fig 4). There was a reciprocal fall in the rT<sub>3</sub>:T<sub>4</sub> ratio between days 1 and 7, values remaining almost constant over the subsequent 2 wk.

**Correlations.** Total T<sub>4</sub> was significantly cor-

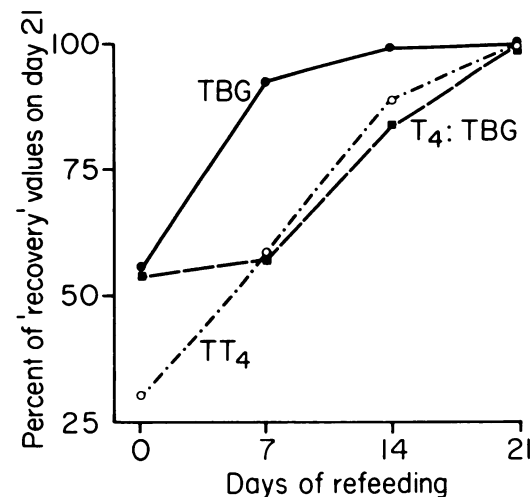


FIG 3. Relative changes in thyroxine-binding globulin (TBG), and total thyroxine (TT<sub>4</sub>) and the T<sub>4</sub>:TBG molar ratio in children with protein calorie malnutrition, before and during refeeding. Recovery values on day 21 were designated 100%.

TABLE 2

Changes in total T<sub>4</sub> levels, T<sub>3</sub>RU values and the various methods of expressing Free Thyroxine before and during refeeding in children with PEM (only six children were studied on day 21). (AFT<sub>4</sub> = FT<sub>4</sub> by analogue method; DFT<sub>4</sub> = FT<sub>4</sub> by equilibrium dialysis)

Days of refeeding	0	7	14	21	Normal range
TT <sub>4</sub> (nmol/l)	46.9 ± 5.3	89.0 ± 8.0**	138.8 ± 10.5**	154.7 ± 10.8**	60–150
T <sub>3</sub> RU (%)	40.4 ± 2.5	32.8 ± 1.6**	28.5 ± 3.2**	29.8 ± 1.9*	30–40
FTI	15.5 ± 2.5	29.7 ± 2.7*	38.7 ± 2.5**	44.2 ± 1.9*	25–60
AFT <sub>4</sub> (pmol/l)	7.6 ± 1.4	10.9 ± 1.2	14.2 ± 3.8*	22.7 ± 4.1*	12–25
DFT <sub>4</sub> (%)	0.037 ± 0.007	0.024 ± 0.007	0.020 ± 0.005	0.017 ± 0.002*	0.018–0.040
DFT <sub>4</sub> (pmol/l)	19.5 ± 3.9	19.1 ± 4.2	18.1 ± 4.9	20.8 ± 4.6	16.9–38.4
T <sub>4</sub> :TBG	0.180 ± 0.020	0.192 ± 0.018	0.281 ± 0.025**	0.344 ± 0.038*	—

\* vs day 0,  $p < 0.05$ .

\*\* vs day 0,  $p < 0.01$ .

related with TBG levels on days 0, 14, and 21 of the study ( $p < 0.05$ – $p < 0.001$ ) (Figure 5). The slopes of the regression lines were all comparable, but the y intercept on day 1 was significantly ( $p < 0.01$ ) lower than on day 21. There were no correlations between T<sub>4</sub> or T<sub>3</sub> and albumen or TBPA, and T<sub>3</sub> levels did not correlate with TBG.

Analogue FT<sub>4</sub> values did not correlate with any of the carrier protein concentrations, nor with the T<sub>4</sub>:TBG molar ratio or the FTI on each day of the study. FTI values correlated with T<sub>4</sub>:TBG molar ratios on day 7 ( $r = 0.87$ ,  $p < 0.02$ ) and on day 14 ( $r = 0.65$ ) ( $p < 0.05$ ). Dialysis FT<sub>4</sub> fraction correlated negatively with the T<sub>4</sub>:TBG molar ratio ( $r = -0.71$ ,

$p < 0.01$ ), but not with TBG, TBPA, or albumen levels.

Changes in T<sub>3</sub> or T<sub>4</sub> levels and TSH did not correlate.

*Electrophoretic studies (Table 3).* In this small group of children the changes in levels of TT<sub>4</sub>- and T<sub>4</sub>-binding proteins before and after recovery were similar to the group as a whole. The bound T<sub>4</sub>:TBG molar ratio increased by 36% ( $p < 0.05$ ) during recovery from PEM, reflecting the changes in the serum T<sub>4</sub>:TBG ratio. In contrast, the bound T<sub>4</sub>:TBPA ratio was 55% greater before refeeding compared to recovery values ( $p < 0.05$ ). The changes in albumen-bound T<sub>4</sub> were inconsistent.

## Discussion

The circulating concentrations of thyroid hormones and their binding proteins in our children with PEM were similar to previously published values (8, 10, 11). With refeeding and recovery all levels normalized rapidly. Serum TBG concentrations after recovery were comparable to those seen in West African children and were higher than reported in Caucasian children (10, 11). During refeeding TBG levels recovered most rapidly, while the recovery of albumen concentrations appeared to be faster than those of TBPA.

Most circulating T<sub>4</sub> is normally bound to TBG (17). However, in untreated children with PEM the very low serum concentrations of T<sub>4</sub> cannot be explained only by the reduced levels of TBG and the other T<sub>4</sub>-binding proteins. After 3 wk of refeeding the mean TBG concentration had increased by about 65% of

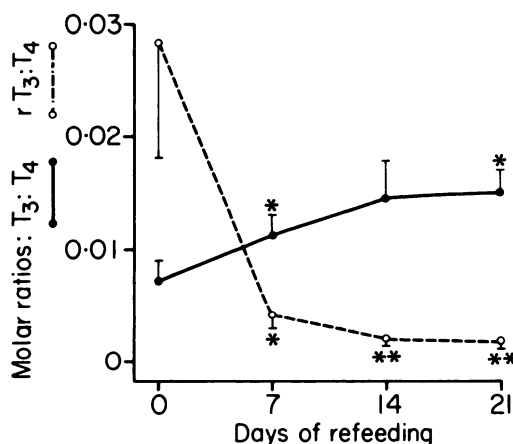


FIG 4. The molar ratios of total triiodothyronine:thyroxine and reverse triiodothyronine:thyroxine in children with protein energy malnutrition before and during refeeding. \* = compared to day 0,  $p < 0.05$ . \*\* = compared to day 0,  $p < 0.01$ .

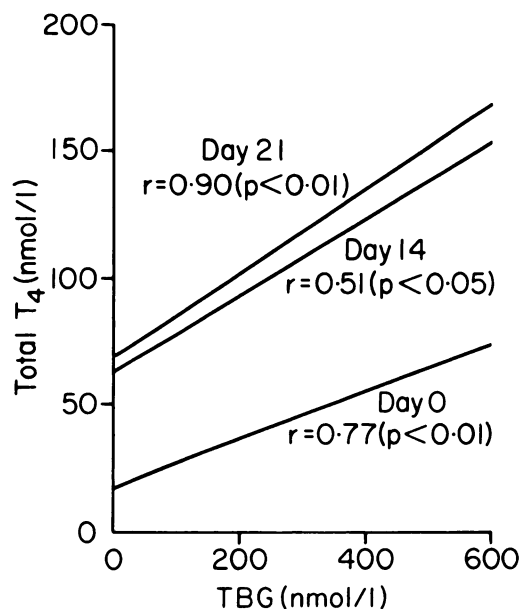


FIG 5. The linear regression lines of total thyroxine ( $TT_4$ ) against thyroxine-binding globulin (TBG) concentrations in children with PEM before therapy and on days 14 and 21. The y intercept ( $TT_4$ ) was significantly greater on day 21 compared to day 0 ( $p < 0.01$ ). Day 0:  $y = 0.095x + 17.7$ ; day 21:  $y = 0.17x + 69.1$ .

pretreatment values, but  $T_4$  levels rose by more than 200%. Moreover, in each individual the  $T_4$ :TBG molar ratios were initially extremely low and increased substantially in every case only after 7 days of treatment. Thus the degree of saturation of TBG with  $T_4$  remained virtually unchanged during the first week of re-

feeding, and then increased progressively from day 7 to full recovery on day 14 or 21, in parallel with the increases in  $TT_4$ . The parallelism of the  $T_4$ :TBG correlations on days 0 and 21, with an increase in the  $T_4$  intercept also demonstrates the overall increase in TBG saturation after recovery.

In the untreated patients reduced thyroid hormone production may have contributed to the extremely low level of TBG saturation with  $T_4$ : the  $T_4$ -degradation rate, and by implication its production rate, is reduced in the steady state of untreated PEM (11). After 7 days of optimal refeeding  $T_4$  kinetics have largely normalized (12), yet in our patients TBG saturation remained unchanged, despite increases in both  $T_4$  and TBG. These findings suggest that there is interference with  $T_4$  binding to TBG in PEM. This concept is also supported by the significant rise in the  $T_4$  intercept of the  $T_4$ :TBG correlations between days 0 and 21, and by the electrophoretic studies. This pattern of decreased  $T_4$  binding has been observed in patients with NTI, with either normal or elevated TBG concentrations (13). Thus the effects of NTI contribute substantially to the very low serum concentrations of  $T_4$  found in PEM. The change from high to low normal values of the  $T_3$ RU test, which reflects the number of available thyroid hormone-binding sites on carrier proteins (18), also supports this concept.

A similar pattern of recovery in the  $T_3$ :TBG molar ratio was also evident from days 7 to 21 of refeeding. Most circulating  $T_3$  is derived

TABLE 3

Concentrations of  $TT_4$ , binding proteins, and percentage 125I- $T_4$  bound to each protein after electrophoresis of serum, before and during refeeding in six PEM children

	Serum $TT_4$	TBG			TBPA			Albumen		
		Concentrations	$T_4$ -bound	$T_4$ -bound/TBG	Concentrations	$T_4$ -bound	$T_4$ -bound/TBPA	Concentrations	$T_4$ bound	$T_4$ -bound/albumen
	nmol/l	nmol/l	%		nmol/l	%		g/l	%	
Before treatment	62.2 (8.9)*	524.7 (110.6)	63.2 (3.5)	0.095 (0.022)	1186.7 (210.3)	16.8 (2.4)	0.0070 (0.0029)	24.1 (1.9)	20.0 (1.8)	0.57 (0.06)
During recovery	112.3 (15.2)	682.9 (56.9)	69.9 (2.1)	0.130 (0.021)	3277.1 (574.7)	13.3 (1.7)	0.0045 (0.0026)	38.6 (1.5)	16.8 (1.5)	0.53 (0.08)
Significance	$p < 0.025$	$p < 0.025$	$p < 0.05$	$p < 0.025$	$p < 0.05$	NS†	$p < 0.05$	$p < 0.01$	$p < 0.1$	NS

The bound  $T_4$ :protein ratio is a measure of the degree of saturation of the protein with  $T_4$ .

\* SEM in parenthesis.

† NS = not significant.

from the peripheral conversion of T<sub>4</sub>, by 5'deiodinase (19), an enzyme system which also rapidly metabolizes rT<sub>3</sub> (20). Thus the relatively elevated serum levels of rT<sub>3</sub>, and the very low levels of T<sub>3</sub> reflect inhibition of the 5'deiodinase systems, characteristic of NTI (21). In PEM, the rapid decline of rT<sub>3</sub> to very low levels suggests that the 5'deiodinase system recovers within a week of refeeding. The reciprocal rise in T<sub>3</sub> levels, however, takes longer to normalize. The progressive increase in T<sub>3</sub>:TBG ratio suggests that binding of T<sub>3</sub> to TBG is also initially inhibited in PEM, and takes time to recover. Additionally, the unchanged T<sub>3</sub>:T<sub>4</sub> molar ratio from day 7 to 21 suggests that the availability of T<sub>4</sub> might be a limiting factor in the full recovery of serum T<sub>3</sub> levels. Lack of T<sub>4</sub> may also have contributed to the relatively low levels of rT<sub>3</sub> seen in a few subjects. This concept is supported by the studies of isolated perfused rat livers in which hepatic T<sub>3</sub> production is dependent on the T<sub>4</sub> concentrations in the perfusate (22). Moreover, in NTI the T<sub>4</sub>-protein binding inhibitory factors may also reduce the accumulation of cellular T<sub>4</sub> (23).

The initial mean serum TSH level was somewhat raised compared to the mean recovery value (24), and concentrations increased significantly with early refeeding, and then fell to lower levels with complete recovery. Prolonged fasting in normal subjects lowers basal and TRH-stimulated TSH levels (25); thus the rise in TSH with refeeding may represent recovery from the effects of starvation. Recent improvement in nutritional status may account for the occasional elevated TSH level seen in patients with NTI. An alternative explanation, that the pituitary sensitivity to ambient thyroid hormone levels may normalize with improved nutritional status, leading to initial increases in TSH secretion in the presence of subnormal T<sub>4</sub> concentrations, appears unlikely. The pituitary retains sensitivity to rising T<sub>3</sub> levels and to TRH in kwashiorkor (24). Moreover, the thyroidal radioactive iodine uptake and clearance are normal in kwashiorkor (26); and thirdly the thyroid remains sensitive to TSH even after prolonged periods of severe malnutrition (27). Furthermore the absence of correlations between changes in TSH and T<sub>3</sub> or T<sub>4</sub> levels in the present and a previous report (28) also argue

against this hypothesis. Our data from patients with moderately severe infective illnesses and severe malnutrition, do not support the concept that TSH levels are universally low in severe NTI (29) although the TSH assay was not ultrasensitive. After 21 days of refeeding TSH concentrations were lower than initial values in four of six cases. Nevertheless, the rise in TSH accompanying refeeding is likely to accelerate the recovery of circulating T<sub>4</sub> and T<sub>3</sub>.

The free or unbound fraction of T<sub>4</sub> is generally considered to be the biologically available and therefore the active form of the hormone. For this reason we evaluated a variety of in vitro indices of free thyroxine during recovery from PEM. The FTI, T<sub>4</sub>:TBG ratio, and analogue FT<sub>4</sub> measurements were all extremely low at presentation and recovered progressively during refeeding. In contrast, the FT<sub>4</sub> fraction, assessed by equilibrium dialysis, progressively declined with refeeding; this fall was fully compensated for by the reciprocal rise in TT<sub>4</sub>, resulting in unchanged levels of DFT<sub>4</sub>, confirming an earlier report (11). The normal DFT<sub>4</sub> in childhood PEM contrasts with the elevated levels observed in adult malnutrition and other NTIs, where both the free fraction and absolute FT<sub>4</sub> are often elevated (30). The reasons for this discrepancy are not clear.

Very reduced concentrations of FT<sub>4</sub> measured by the direct analogue method have recently been reported in PEM (31) comparable to levels we observed using a similar method. Subnormal FT<sub>4</sub> values assayed by these methods can be caused by reduced levels of albumen (4) or TBPA (6) and by elevated levels of free fatty acids (32). We could not confirm an association with either of these proteins, nor with TBG, before, during, or after recovery from PEM, in individual children or in the group as a whole. Nor did AFT<sub>4</sub> correlate with DFT<sub>4</sub> or FTI, nor with the T<sub>4</sub>:TBG ratio, although these measurements correlate well in normal subjects (33). Precisely what AFT<sub>4</sub> levels reflect in patients with NTI is unclear (34, 35). On the other hand the dialysis FT<sub>4</sub> fraction, but not the absolute level, did correlate, inversely, with the T<sub>4</sub>:TBG ratio.

The reasons for these differences in apparent free T<sub>4</sub> levels may be explained by the principles of the assay methods. The radio-labeled T<sub>4</sub> analogue used in the assay system we used

binds to serum proteins (6). Any substance which displaces  $T_4$  from proteins should also reduce the proportion of protein-bound analogue, resulting in an increased proportion of unbound analogue. Thus the assay antibody will take up more label giving a spuriously low  $FT_4$  estimation. In contrast, reduced  $T_4$  binding is measured directly as an increase in the free  $T_4$  fraction in the equilibrium dialysis system, and is reflected by a reduced  $T_4$ :TBG ratio.

The electrophoretic studies confirm a reduction of  $T_4$  binding by TBG in untreated compared to recovering PEM. Moreover, there appeared to be a reciprocal *increase* in relative  $T_4$  binding to TBPA, but not to albumen, in untreated cases, suggesting that TBPA, although reduced in concentration, buffers some of the excess unbound  $T_4$  displaced from TBG. A similar observation has been made in uremic animals (14).

The nature of the interference with the  $T_4$ -TBG interaction and  $T_4$  to  $T_3$  deiodination in NTI has not been fully elucidated. Recent evidence, however, strongly suggests that elevated plasma levels of free fatty acids (FFA) may displace  $T_4$  from TBG and increase the dialysable fraction (36), and reduce measured levels of  $AFT_4$  (32). Moreover, *in vitro* studies have shown that fatty acids interfere with the production of  $T_3$  from  $T_4$  and may also be active *in vivo* (37). Plasma concentrations of FFA are considerably elevated in untreated Kwashiorkor and fall rapidly during refeeding (38), perhaps contributing to the changes in  $T_4$  binding and to the low serum  $T_3$  levels.

Thus it is apparent that the changes in bound and free thyroid hormones in recovering PEM did not reflect only changes in binding protein concentrations. Only changes in TBG, and not those of albumen or TBPA, were reflected by the  $FT_4$  fraction measured by equilibrium dialysis. There are also qualitative changes in thyroid hormone binding—a decrease in TBG binding with relative increases in TBPA binding, the former predominating and contributing substantially to the very low levels of  $T_4$  and  $T_3$  found in untreated PEM. An increase in TSH secretion appears to be characteristic of recovery from PEM and may accelerate the normalization of circulating thyroid hormone concentrations. ■

We thank Dr E Sochett for allowing us to study some of his patients.

## References

1. Vermaak WJH, Kalk WJ, Zakolski WJ. Frequency of euthyroid sick syndrome as assessed by free thyroxine index and a direct Free Thyroxine assay. *Lancet* 1983;1:1373-5.
2. Chopra IJ, Hershman JM, Pardridge WM, Nicoloff JT. Thyroid function in nonthyroidal illnesses. *Ann Intern Med* 1983;98:946-57.
3. Kaptein EM, Robinson WJ, Grieb DA, Nicoloff JT. Peripheral serum thyroxine, triiodothyronine and reverse triiodothyronine kinetics in the low thyroxine state of acute nonthyroidal illnesses. *J Clin Invest* 1982;69:526-33.
4. Amino N, Nishi K, Nakatani K, et al. Effect of albumen concentration on the assay for serum free thyroxine by equilibrium radioimmunoassay with labelled thyroxine analog (Amerlex Free  $T_4$ ). *Clinical Chem* 1983;29:321-5.
5. Oppenheimer JH, Squef R, Surks M, Hauer H. Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. *J Clin Invest* 1963;42:1769-82.
6. Stockigt JR, de Garis M, Csicsman T, Barlow JW, White EL, Hurley DM. Limitations of a new free thyroxine assay (Amerlex Free  $T_4$ ). *Clin Endocrinol* 1981;15:313-8.
7. Woeber KA, Madax BA. Thyroid hormone binding in nonthyroidal illness. *Metabolism* 1981;30:412-6.
8. Ingenbleek Y, de Visscher M, de Nayer P. Measurement of prealbumin as index of protein calorie malnutrition. *Lancet* 1972;2:106.
9. Graham GG, Baertl JM, Claeysen G, et al. Thyroid hormonal studies in normal and severely malnourished infants and small children. *J Pediatr* 1973;83:321-31.
10. Ingenbleek Y, de Nayer P, de Visscher M. Thyroxine binding globulin in infant protein calorie malnutrition. *J Clin Endocrinol Metab* 1974;39:178-80.
11. Ingenbleek Y, Malvaux P. Peripheral turnover of thyroxine and related parameters in infant protein-calorie malnutrition. *Am J Clin Nutr* 1980;33:609-16.
12. Classification of infantile malnutrition. *Lancet* 1970;II:302-3.
13. Kalk WJ, Kew MC, Danilewitz MD, Jacks F, van der Walt LA, Levin J. Thyroxine binding globulin and thyroid function test in patients with hepatocellular carcinoma. *Hepatology* 1982;2:72-6.
14. Kalk WJ, van Drimmelen M, Fitzpatrick M, Myburgh JA, Smit JA, van der Walt LA. Circulating thyroid hormones in progressive renal failure in the baboon (*Papio ursinus*). *J Endocrinol Invest* 1984;8:299-306.
15. Wilson F, Rankel S, Linke EG, Henry JB. Free thyroxine—an abbreviated practical assay. *Am J Clin Pathol* 1974;62:383-97.
16. Laurel CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analytical Chemistry* 1966;15:45-52.
17. Oppenheimer JH. Role of plasma proteins in the

- binding, distribution and metabolism of the thyroid hormones. *N Engl J Med* 1968;278:1153-62.
18. Ingbar SH, Woeber WA. The thyroid gland. In: Williams RH, ed. Textbook of endocrinology. London: WB Saunders Co, 1981:117-247.
19. Surks MI, Schadow AR, Stock JM, Oppenheimer RH. Determination of iodothyronine absorption and conversion of L-thyroxine (T<sub>4</sub>) to L-triiodothyronine (T<sub>3</sub>) using turnover rate techniques. *J Clin Invest* 1973;52:805-11.
20. Geola FL, Chopra LJ, Gefner DL. Patterns of 3-3'-5'-triiodothyronine monodeiodination in hypothyroidism and nonthyroidal illnesses. *J Clin Endocrinol Metab* 1980;50:336-40.
21. Chopra LJ, Chopra U, Smith SR, Reza M, Solomon DH. Reciprocal changes in serum concentrations of 3,3',5'-triiodothyronine (reverse T<sub>3</sub>) and 3,3',5'-triiodothyronine (T<sub>3</sub>) in systemic illnesses. *J Clin Endocrinol Metab* 1975;41:1043-8.
22. Jennings DS, Ferguson DC, Utiger RD. Regulation of conversion of thyroxine to triiodothyronine in the perfused rat liver. *J Clin Invest* 1979;64:1614-23.
23. Oppenheimer JH, Schwartz HL, Marias CN, Kaiser E. Evidence for a factor in the sera of patients with nonthyroidal illness which inhibits iodothyronine binding in solid matrixes, serum proteins and rat hepatocytes. *J Clin Endocrinol Metab* 1982;54:757-66.
24. Pimstone BL, Becker D, Hendricks S. TSH response to synthetic thyrotropin-releasing hormone in human protein calorie malnutrition. *J Clin Endocrinol Metab* 1973;36:779-83.
25. Vinik AI, Kalk WJ, McLaren H, Hendricks S, Pimstone BL. Fasting blunts the TSH response to synthetic thyrotropin-releasing hormone. *J Clin Endocrinol Metab* 1975;40:509-11.
26. Ingenbleek Y, Bechers C. Thyroidal iodide clearance and radioiodide uptake in protein calorie malnutrition. *Am J Clin Nutr* 1978;31:408-15.
27. Beas F, Monckeberg F, Horwitz I, Figuera M. The response of the thyroid gland to thyroid stimulating hormone in infants with malnutrition. *Paediatrics* 1966;38:1003-8.
28. Ingenbleek Y, Beckers C. Triiodothyronine and thyroid stimulating hormone in protein-calorie malnutrition in infants. *Lancet* 1975;2:845-8.
29. Wehman RE, Gregerman RI, Burns WH, Saral R, Santos GW. Suppression of thyrotropin in the low-thyroxine state of severe nonthyroidal illness. *N Engl J Med* 1985;312:546-52.
30. Chopra LJ, Smith SR. Circulating thyroid hormones and thyrotropin in adult patients with protein energy malnutrition. *J Clin Endocrinol Metab* 1975;40:221-7.
31. Hatemi N, Khaktan M, Genca E, Cuma T. Thyroid function in protein energy malnutrition. *Turkish J Paed* 1982;24:29-34.
32. Vermaak WJH, Kalk WJ, Kuyl JM, Smith AM. Spectrum of methodological artefacts and true thyroid hormone changes during episodes of artificially raised NEFA in healthy volunteers. *Annales de Endocrinologie* 1984;45:48(abSTRACT).
33. Franklyn JA, Sheppard MC, Ramsen DB, Wilkinson R, Hoffenberg R. Measurement of free thyroxine and free triiodothyronine in thyrotoxicosis and hypothyroidism. *Clin Endocrinol* 1984;20:107-10.
34. Stockigt JR, White EL, Barlow JW. What do radioimmunoassay methods for free thyroxine using "unbound analogues" actually measure? *Lancet* 1982;2:712.
35. Ekins RP, Jackson T, Edwards P, Salter C, Ogier I. Euthyroid sick syndrome and free thyroxine assays. *Lancet* 1983;2:402.
36. Chopra LJ, Huang TS, Hurd RE, Beredo A, Solomon DH. A competitive ligand binding assay for the measurement of thyroid hormone-binding inhibitor in serum and tissues. *J Clin Endocrinol Metab* 1984;58:619-28.
37. Chopra LJ, Huang T-S, Beredo A, Solomon DH, Chua Teco GN, Mead JF. Evidence for an inhibitor of extrathyroidal conversion of thyroxine to 3,5,3'-triiodothyronine in sera of patients with non thyroidal illnesses. *J Clin Endocrinol Metab* 1985;60:666-72.
38. Lewis B, Hansen JDL, Wittman W, Krut LH, Stewart F. Plasma free fatty acids in Kwashiorkor and the pathogenesis of the fatty liver. *Am J Clin Nutr* 1964;15:161-8.