The Effects of an Acute Load of Thyroxine on the Transport and Peripheral Metabolism of Triiodothyronine in Man

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ABSTRACT In order to examine the question of whether thyroxine-binding globulin (TBG) influences significantly the peripheral metabolism of 3,3',5-triiodo-L-thyronine (T_3) in vivo, paired studies of the effects of a large intravenous load of L-thyroxine (T_4) on the kinetics of ^{131}I-labeled T_3 metabolism were carried out in five normal subjects. After the T_4 load, both the early distributive loss of labeled T_3 from serum and the volume of T_3 distribution, observed after distribution equilibrium had been attained, were greatly increased. These alterations were consistent with those to be expected from displacement of T_3 from its extracellular binding sites. After the T_4 load, however, the fractional rate of T_3 turnover was decreased. This finding is ascribed either to competition between T_3 and T_4 for common intracellular pathways of degradation or excretion or to displacement of T_3 from sites of more rapid to sites of less rapid metabolism. These effects of alterations in the binding activity of TBG on the peripheral metabolism of T_3, together with those previously reported by others, are consistent with the interpretation that T_3 is significantly bound by TBG in vivo. However, it is suggested that the effects of alterations in the T_3-TBG binding interaction on the metabolism of T_3 are obscured by alterations in the extracellular-cellular partitioning of T_4 that would result from concurrent alterations in T_4-binding by TBG.

INTRODUCTION

On the basis of in vitro studies, it is clear that 3,3',5-triiodo-L-thyronine (T_3) is strongly bound by serum proteins since normally no more than a fraction of 1% of the total T_3 in serum exists in the free or unbound state (1). Electrophoretic studies have indicated that thyroxine-binding globulin (TBG) is the serum protein to which T_3, like L-thyroxine (T_4), is predominantly bound (2, 3). Despite this, it has been reported that clinical states associated with alterations in the binding activity of TBG are not accompanied by alterations in the kinetics of peripheral T_3 metabolism in vivo similar to those which occur in the case of T_4 under the same conditions (4–7). This has been taken to indicate that T_3 in contrast to T_4 is not bound by TBG to a significant extent in vivo. To investigate this problem further, we have studied the kinetics of peripheral T_3 metabolism in normal subjects before and after a single large intravenous dose of T_4. It was anticipated that this maneuver would limit the access of T_3 to binding sites shared by both hormones and therefore would permit an assessment of the influence of such binding sites on the peripheral metabolism of T_3.

METHODS

The effects of T_4 loading on the transport and peripheral metabolism of ^{131}I-labeled T_3 were assessed in paired studies conducted in five normal subjects.[1]

Assessment of the kinetics of peripheral T_3 metabolism. This was carried out as described in the companion report (8), except that in four of the five subjects additional blood samples were collected at 20 and 50 min after injection of the ^{131}I-labeled T_3.[2] 1 wk after the injection of ^{131}I-labeled T_3 for the control study, a blood sample was collected for measurement of the residual trichloroacetic acid (TCA)–precipitable ^{131}I. Immediately thereafter, an intravenous load of 4 mg of T_4 in a 1% (w/v) solution of human serum

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[1] The five normal subjects studied were among the seven presented in the companion report (8). They included two of the authors, one physician attached to the medical unit, and two fully-informed volunteers. The latter two subjects were hospitalized for the studies.

[2] Obtained from The Radiochemical Centre, Amersham, England.

TABLE I

The Effects of L-Thyroxine (T_4) Loading on the Early Phase of the Peripheral Metabolism of ^{131}I-Labeled 3,3',5-Triiodo-L-Thyronine (T_3)

Subject	Age, Sex	Body weight	20 min volume of T_3 distribution		50 min volume of T_3 distribution		50 min TCA-precipitable ^{131}I / 20 min TCA-precipitable ^{131}I	
	yr	*kg*	*liters*		*liters*		*%*	
			Control	*T_4 load*	*Control*	*T_4 load*	*Control*	*T_4 load*
1	31, M	68	9.8	14.9	13.5	18.0	73	83
2	30, M	75	9.5	14.4	13.0	19.2	74	75
3	58. M	53	7.0	10.8	9.1	15.2	77	71
4	39, M	57	9.7	11.6	12.6	16.5	76	70
Mean			9.0	12.9	12.0	17.2	75	75
SE			0.7	1.0	1.0	0.9	1	3
Mean difference			3.9		5.2		1	
SEM difference			0.7		0.6		5	
*P**			<0.02		<0.01		NS	

* Analysis by the paired *t* test.

albumin was administered, followed immediately by a second injection of 50 μCi of ^{131}I-labeled T_3. In the studies performed after the T_4 load, corrections were made for the concentration in serum of TCA-precipitable ^{131}I remaining from the first injection. The volumes of distribution of T_3 at 20 and 50 min were calculated as the quotient of the amount of injected ^{131}I and the concentrations in serum of TCA-precipitable ^{131}I at the corresponding times. This calculation assumed that significant degradation of T_3 had not yet occurred, a supposition that was borne out by measurement of urinary ^{131}I at these times.[3] The kinetics of peripheral T_3 metabolism after attainment of distribution equilibrium were assessed as described in the companion report (8).

Assessment of the per cent of T_3 in serum bound by TBG. 0.5-ml aliquots of serum samples obtained from four of the five subjects 20 min after injection of the ^{131}I-labeled T_3 were subjected to conventional electrophoresis in filter paper sheets in glycine (0.2 M)-acetate (0.13 M) buffer at pH 8.6. In the remaining subject, serum obtained 8 hr after injection of the ^{131}I-labeled T_3 was enriched with a very small quantity of labeled T_3 before electrophoresis. Serum enriched with ^{131}I-labeled T_4[4] (2 μCi/ml) was subjected to electrophoresis in another segment of the same filter paper sheet to serve as a radioactive marker. After electrophoresis, the filter paper sheets were cut into 1 cm strips and then were counted. The counts were plotted on graph paper and the ^{131}I in the strips corresponding to the TBG peak in the labeled T_4 marker was expressed as a per cent of the total ^{131}I in the sample.

Assessment of the per cent of free T_3 in serum. The general equilibrium dialysis method of Oppenheimer, Squef, Surks, and Hauer was employed (10). Aliquots (100 μl) of serum samples obtained at least 24 hr after each injection of ^{131}I-labeled T_3 were diluted with 2.40 ml of Krebs-Ringer phosphate buffer (KRP) at pH 7.4 and enriched with 20 μl of a solution which yielded the equivalent of 0.17 μg of ^{131}I-labeled T_3 added per 100 ml of undiluted serum (final dilution of the serum, 1:25.2). 1 ml of the diluted sample was placed in a sac made from dialysis tubing (Union Carbide Corp., New York, size 20) and dialyzed against 5 ml of KRP in a 25 ml Erlenmeyer flask for 20 hr at 37°C. After dialysis, aliquots were taken from inside and outside the dialysis sac. To these were added equal volumes of serum containing carrier iodide and a few milligrams of propylthiouracil. The protein was precipitated with cold 20% TCA. The precipitates were washed twice with cold 5% TCA, dissolved in 2 N sodium hydroxide, and made up to a standard volume for counting. Sufficient counts were obtained to reduce the probable counting error to a maximum of 3%. The amount of TCA-precipitable ^{131}I per milliliter of dialysate was expressed as a fraction of the amount of TCA-precipitable ^{131}I in the 1 ml of dilute serum within the dialysis sac. To obtain a value for the per cent of free T_3, this fraction was multiplied by 100 and divided by the dilution factor of the serum within the sac (1:25.2). Serum samples obtained from a given subject during the control study and after the T_4 load were always analyzed concurrently and in duplicate.

Serum butanol-extractable iodine (BEI) This was measured by the method of Benotti and Pino (11).[5]

RESULTS

The intravenous T_4 load was well tolerated. Three subjects complained of mild lassitude, but no other untoward effects were encountered.[6] The mean value for the serum BEI was 22.4 μg/100 ml (range, 20.0–26.0)

[3] Absence of significant deiodination of the administered labeled T_3 during this period was confirmed by the concurrent administration of inorganic ^{125}I according to a modification of the method of Anbar, Guttman, Rodan, and Stein (9).

[4] Obtained from Abbott Laboratories, North Chicago, Ill.

[5] Performed by the Boston Medical Laboratory, Boston, Mass.

[6] In the one subject in whom it was measured throughout the study, the urinary excretion of creatine increased from approximately 40 mg/g of creatinine during the control period to 120 mg/g of creatinine during the period from 48 to 72 hr after the T_4 load.

TABLE II

The Effects of L-Thyroxine (T_4) Loading on the Peripheral Metabolism of ^{131}I-Labeled 3,3',5-Triiodo-L-Thyronine (T_3) after Attainment of Distribution Equilibrium

Subject	Age, Sex	Body weight	Volume of T_3 distribution		Fractional T_3 turnover rate		T_3 clearance rate		Urinary maximum	
	yr	*kg*	*liters*		*%/24 hr*		*liters/24 hr*		*% dose*	
			Control	*T_4 load*	*Control*	*T_4 load*	*Control*	*T_4 load*	*Control*	*T_4 load*
1	31, M	68	43	54	67	57	28.8	30.8	83	86
2	30, M	75	46	61	60	44	27.6	26.8	81	93
3	58, M	53	34	64	45	35	15.3	22.4	54	71
4	39, M	57	41	47	68	56	27.9	26.3	70	73
5	38, M	74	43	64	40	34	17.2	21.8	91	93
Mean	–		41	58	56	45	23.4	25.6	76	83
SE			2	3	6	5	2.9	1.6	6	5
Mean difference			17		11		2.3		7	
SEM difference			4		2		1.6		3	
P*			< 0.02		< 0.01		NS		NS	

* Analysis by the paired t test.

20 min after the T_4 load, 16.0 μg/100 ml (range, 14.8–17.4) at 24 hr, and 13.7 μg/100 ml (range, 12.2–15.0) at 72 hr.

Table I presents the values for the volumes of distribution of ^{131}I-labeled T_3 at 20 and 50 min after injection and the effects thereon of T_4 loading. The mean control value for the volume of distribution of T_3 at 20 min was 9.0 ±0.7 liters (mean ±SE), and at 50 min, 12.0 ±1.0 liters. T_4 loading was consistently followed by major increases in the volumes of distribution of T_3 at both times; and for the group as a whole, these increases were significant statistically. The fractional rate of disappearance of T_3 from 20 to 50 min, as judged from the ratio of the concentrations of TCA-precipitable ^{131}I in serum at the two times, did not change after the T_4 load.

The volume of distribution and fractional rate of turnover of T_3 after attainment of distribution equilibrium and the effects thereon of T_4 loading were calculated from the data obtained from 24 to 72 hr after administration of the ^{131}I labeled T_3. Verification that distribution equilibrium of the labeled T_3 had been attained by 24 hr was obtained by the method described in the companion report (8). In the control study, the values for the pooled 24-hr fractional rates of disappearance were 54 ±2% (mean ±SE) during the period from 24 to 48 hr and 55 ±1% during the period from 24 to 72 hr. After the T_4 load, the corresponding values were both 44 ±1%. The excellent agreement of the 24–48 and 24–72 hr values indicated a single exponential rate of disappearance during this time and, therefore, that distribution equilibrium of the residual labeled T_3 had been attained. Values for the volume of distribution and fractional rate of turnover of T_3 derived from the data obtained in each subject from 24 to 72 hr after each injection of T_3 are presented in Table II. T_4 loading was consistently followed by a major increase in the volume of T_3 distribution and by a decrease in the fractional rate of T_3 turnover. For the group as a whole, both these changes were significant statistically. The calculated rate of T_3 clearance did not change significantly after the T_4 load. T_4 loading was followed by a slight but consistent increase in the calculated value for the proportion of the injected ^{131}I ultimately appearing in the urine (urinary maximum), suggesting that the proportion of ^{131}I ultimately appearing in the feces (fecal maximum) might have decreased. These changes, however, were not significant statistically.

Values for both the proportion of T_3 bound by TBG and the per cent of free T_3 in serum are depicted in Fig. 1. T_4 loading was consistently followed by a decrease in the proportion of T_3 bound by TBG and by an increase in the per cent of free T_3 in serum; for the group as a whole, both these changes were significant statistically.

DISCUSSION

It has generally been accepted that phenomena related to the binding of thyroid hormones in vitro provide a qualitatively accurate reflection of the thyroid hormone-protein binding interactions that pertain in vivo. Within the framework of the concept that the extracellular binding of hormone limits the access of hormone to the tissues, the many circumstances in which alterations in T_4 binding, as assessed in vitro, are associated with predictable reciprocal alterations in the rate of clearance of T_4 in vivo have tended to support the applicability of in vitro binding phenomena. A large body of data

obtained in vitro suggests that TBG is the major binding protein for T_3 (2, 3). Nevertheless, several recent observations indicate that changes in the binding activity of TBG do not produce alterations in the in vivo metabolism of T_3 similar to those which occur in the case of T_4 (4–7). Consequently, it has been suggested that T_3 is not bound to TBG in vivo to a significant extent. This conclusion challenges the relevance of in vitro binding phenomena to the in vivo situation not only in the case of T_3 but also with respect to T_4.

Two lines of evidence derived from kinetic data have been taken to indicate that T_3 is not bound to a significant extent by TBG in vivo. First, Zaninovich, Farach, Ezrin, and Volpé have reported that alterations in the binding activity of TBG do not influence the rate of disappearance of ^{131}I-labeled T_3 from serum during the period from 20 to 50 min after its intravenous administration (4). On the other hand, similarly induced alterations in the binding activity of TBG did produce the expected alterations in the rate of disappearance of T_4 during the same period. In order to examine this interesting observation further, we have attempted to disrupt the extracellular T_3-TBG binding interaction by saturating the binding sites on TBG through the administration of a large T_4 load. In common with the findings cited above, the rate of disappearance of T_3 from serum during the period from 20 to 50 min was unaltered. On the other hand, calculated volumes of distribution were greatly increased by the T_4 load. The interpretation of such findings is exceedingly complex.

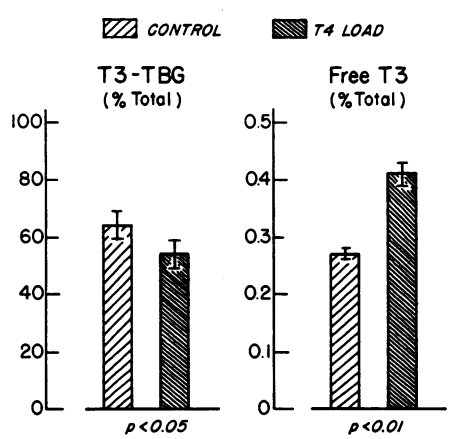

FIGURE 1 The effects of an intravenous load of L-thyroxine (T_4) on the in vitro binding of 3,3',5-triiodo-L-thyronine (T_3) in serum. T_3-TBG refers to the per cent of labeled T_3 bound by T_4-binding globulin (TBG), as assessed by conventional filter paper electrophoresis of serum obtained after an intravenous tracer dose of ^{131}I-labeled T_3. In vitro enrichment with a very small quantity of ^{131}I-labeled T_3 was required in only one instance before electrophoresis. The mean ±SE of the values obtained in the five subjects are shown.

Before the attainment of distribution equilibrium, the conceptual differentiation between volume of distribution and fractional rate of disappearance is probably spurious. It is apparent that if T_4 loading increased the volume of distribution of T_3 at 20 min, then it must also have increased the rate of disappearance of T_3 from serum before that time. It is clear that the degradation of T_3 during this early period is negligible. Hence, the disappearance of T_3 from serum is totally a reflection of distribution. Whether viewed as an increase in the volume of distribution at 20 min or as an increased rate of disappearance before 20 min, the present data indicate a greater early distributive loss of T_3 from serum after the T_4 load. This is consistent with the classical binding concept since the T_4 load would be expected to increase the proportion of free T_3 (as was actually observed in vitro), and it is generally held that the free hormone is more readily accessible to the tissues. Thus, the present data suggest that T_3 is significantly bound in vivo and that such binding can be disrupted by an increase in the concentration of T_4; they do not, however, bear upon the question as to whether T_3 is significantly bound by TBG per se. Evidence bearing upon this question is discussed below.

The second line of evidence from which it has been concluded that T_3 is not significantly bound by TBG in vivo derives from studies of the effects of alterations in the binding activity of TBG on the kinetics of peripheral T_3 metabolism after distribution equilibrium has been attained (5–7). Thus, several studies have demonstrated that when the binding activity of TBG is increased the fractional rate of T_3 turnover is enhanced and that when the binding activity of TBG is decreased the fractional rate of T_3 turnover is diminished. These alterations are the converse of those observed in the case of T_4 (see review, reference 12). In the present study, T_4 loading was associated with an increase in the ultimate volume of distribution of T_3, a change again consistent with that to be expected from a disruption of the extracellular binding of T_3. However, the fractional rate of turnover of T_3 was decreased after the T_4 load. This observation is concordant with the previously observed effects on T_3 turnover produced by primary alterations in the binding activity of TBG cited immediately above. Although these alterations in the fractional rate of T_3 turnover appear superficially inconsistent with the expected effects of a T_3-TBG binding interaction, they do not constitute evidence that T_3 is not significantly bound by TBG in vivo. If T_3 were indeed not bound by TBG, one would expect that alterations in the binding activity of TBG would have no effect on the metabolism of T_3; such is obviously not the case. Hence, it must be concluded that regardless of

whether T_3 is bound by TBG, alterations in the binding activity of TBG lead to some other change which in turn is reflected in the observed alterations in T_3 metabolism.

The most likely possibility is that the changes in T_3 metabolism are owing to a redistribution of T_4, probably into the liver. The few data available would suggest that when there is a primary decrease in the binding activity of TBG T_4 is shifted to cellular sites, particularly in liver, so that the cellular pool of hormone comprises a greater than normal fraction of the total extrathyroidal pool (13–15). Furthermore, in experiments comparable to those presented here, acute intravenous loads of T_4 have been shown to produce acute displacement of labeled (and hence stable) T_4 into extravascular sites, at least partly in the liver (16). Increased localization of T_4 within the liver resulting from either primary decreases in TBG or from T_4 loading could produce the observed alterations in T_3 metabolism in one of two ways. First, it could inhibit hepatic uptake of T_3, while permitting enhanced uptake at other sites where T_3 degradation might occur more slowly. Alternatively, enhanced hepatic uptake of T_3 owing to decreased extracellular binding may occur, but accumulated T_4 may compete with accumulated T_3 for degradative or excretory pathways. Either explanation would be consistent with the slight decrease in the calculated fecal maximum that might have occurred after the T_4 load since this is a reflection of T_3 metabolism by the liver.

In conclusion, if it is granted that the data demonstrate that alterations in the binding activity of TBG do influence the peripheral metabolism of T_3, but that their effects on T_3 metabolism are secondary to some other change consequent to the alteration in the binding activity of TBG, then it is no longer necessary to postulate that T_3 is not significantly bound by TBG in vivo. One need only postulate that the secondary effects on T_3 metabolism preponderate over the primary effects of alterations in the T_3-TBG binding interaction. The latter conclusion would tend to preserve intellectual order and would be in accord with the great likelihood that in vitro binding interactions of T_3 do qualitatively reflect those that occur in vivo.

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