Decreased Serum Triiodothyronine in Starving Rats is Due Primarily to Diminished Thyroidal Secretion of Thyroxine

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Abstract

Although thyroxine (T_4) 5'-deiodinase activity is diminished in liver homogenates of starved rats, no information is available regarding the effect of starvation on net T_4 to triiodothyronine (T_3) conversion in the intact rat. It appeared important to clarify this relationship since rat liver homogenates are widely used as a model for the study of the factors responsible for reduced circulating T_3 in chronically ill and calorically deprived patients. In contrast to the expected selective decrease in circulating T_3 levels in calorically restricted humans due to diminished T_4 to T_3 conversion, 5 d of starvation of two groups of male Sprague-Dawley rats resulted, paradoxically, in a greater decrease in serum T_4 than in serum T_3 levels. Kinetic studies show that starvation is associated with no change in the metabolic clearance rate (MCR) of T_3, a 20% increase in the MCR of T_4, a 67% reduction in turnover rate of T_4, but only a 58% reduction in the turnover rate of T_3. Moreover, in the first group of rats studied, direct chromatographic analysis of the isotopic composition of total body homogenates after the injection of ^{125}I-T_4 showed that 21.8% of T_4 is converted to T_3 in control rats and 28.8% in starved rats, suggesting that virtually all extrathyroidal T_3 in starved and control rats is derived from the peripheral conversion of T_4, and that there is little or no direct thyroidal secretion of T_3. Our findings strongly point to a reduced thyroidal secretion of T_4 as the primary cause of the observed reduction in circulating T_3. Since the mechanisms leading to reduced levels of plasma T_3 differ in humans and rats, it may be important to reexamine the use of liver homogenate preparations as models for study of the pathogenesis of the "low T_3 syndrome" in humans.

Introduction

In man, the low concentration of serum triiodothyronine (T_3)[1] associated with starvation and catabolic disease results from diminished peripheral conversion of thyroxine (T_4) to T_3. This conclusion is based primarily on the finding that, in starvation, the turnover of T_3 is more markedly reduced than the turnover of T_4, and that the bulk of circulating T_3 is derived from the monodeiodination of T_4 rather than direct thyroidal secretion (1, 2).

Experimental efforts to elucidate the biochemical basis and possible functional significance of the "low T_3 syndrome" have prompted the widespread use of animal models. The starving rat is most commonly used because in this model both the circulating level of T_3 and the in vitro conversion of T_4 to T_3 in liver homogenates are reduced (3–6). Several reports indicate, however, that the relative reduction of the plasma T_4 observed in the starved rat exceeds that of T_3 (3, 7), precisely the opposite of the expected consequence of diminished fractional conversion. Moreover, the low thyroid-stimulating hormone (TSH) levels reported in this model (8, 9) further suggest that reduced thyroidal secretion of T_4, rather than impaired monodeiodination, could fully account for the diminished circulating T_3. Nevertheless, we were unaware of any studies that attempt to quantitate the effects of starvation on the kinetics of iodothyronine metabolism in vivo. We therefore determined, in this study, the effects of starvation on T_4 and T_3 plasma turnover rates and assessed T_4 to T_3 conversion in vivo from an analysis of the isotopic composition of whole body homogenates.

Methods

Male Sprague-Dawley rats weighing 175–200 g were obtained from Bio-Lab, Inc. (White Bear Lake, MN) and housed individually. Control rats had free access to food (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL). The experimental groups in all cases were used for study after 96 h of complete starvation. Water was always freely available to both groups. ^{125}I-labeled T_4 (^{125}I-T_4) and T_3 (^{125}I-T_3) were obtained from Abbott Laboratories, North Chicago, IL. ^{131}I-labeled T_3 (^{131}I-T_3) was prepared in the laboratory as previously described (10). All isotopic preparations were checked for purity by paper chromatography using the system of Bellabarba et al. (t-amyl alcohol/hexane/ammonia, 5:1:6, vol/vol) (11). Contaminating ^{125}I-T_3 in ^{125}I-T_4 preparations was found to be <0.8% in all cases. Less than 1% ^{125}I-T_4 was observed in the radiolabeled T_3.

The kinetics of T_3 and T_4 metabolism were studied in two separate experiments in which rats received intravenous injections of tracer doses (<10 ng/100 g body wt) of ^{125}I-T_3 or ^{125}I-T_4. Blood was sampled from the tail veins 10 min before injection and 5 min, 3, 6, 15, and 24 h after injection. Residual hormone in plasma was separated from ^{125}I-iodide by precipitation with TCA. We have shown that TCA precipitation yields values for T_3 that are not consistently different from those resulting from chromatographic techniques (12). Metabolic clearance rate (MCR), fractional removal rates, and volume of distribution (V_d) were calculated using noncompartmental methods. The interval from $t = 0$ to $t = 24$ h was integrated with graphic displays of the data. The interval from $t = 24$ h to $t = \infty$ was calculated by use of equations previously defined (13). Plasma samples taken before injection of isotope were used for measurement of immunoassayable T_4, using a Quantitope kit (Kallestad Laboratories, Inc., Austin, TX). Unextracted plasma or serum was used for this assay, so we tested for possible changing specificity due to alterations in serum components resulting from experimental manipulation. Recovery of added T_4 (2.5–22 μg/dl) was measured in pools of serum of control and starved rats.

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1. *Abbreviations used in this paper:* CR, conversion ratio; ^{125}I-T_3, ^{125}I-labeled T_3; ^{125}I-T_4, ^{125}I-labeled T_4; ^{131}I-T_3, ^{131}I-labeled T_3; MCR, metabolic clearance rate; PR, production rate; PTU, propylthiouracil; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone; V_d, volume of distribution.

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Recovery was 103.8±6.0% and 101.4±2.9% in control and starved pools, respectively. T_3 was measured by the method of Surks et al. (14). Hormone production rates (PR) were estimated from the product of the MCR and the plasma hormone concentration.

The rate of conversion of T_4 to T_3 in the whole rat was measured as previously described (15) in rats from the group used for the first turnover studies. Doses of ^{125}I-T_4 (100 μCi/rat) were injected via the tail vein. 2 mg of iodide were simultaneously injected intraperitoneally to prevent recycling of radioactive iodide. Rats were killed 48 h later. To correct for spurious deiodination during the analytical procedure other rats were similarly injected and killed within 2 min. Intestinal contents and the urine in the bladder were removed. The entire carcass was then homogenized with added ice-cold water containing 10^{-4} M propylthiouracil (PTU). Two 5-g aliquots of homogenate were extracted with absolute ethanol containing 10^{-4} M PTU until 80–90% of the counts were removed. ^{131}I-T_3 was added to the extract to serve as a marker for T_3 during chromatographic isolation of the ^{125}I-T_3 derived from the injected ^{125}I-T_4 and to allow corrections for losses during the isolation procedure. Further, a constant ^{125}I-T_3 to ^{131}I-T_3 ratio was used as a strict criterion of purity of the isolated ^{125}I-T_3. The extracts were concentrated *in vacuo* and applied to 3MM paper (Whatman Inc., Clifton, NJ) together with unlabeled T_3 and T_4. The products were separated by chromatography in t-amyl alcohol/hexane/ammonia. The T_3 area was then visualized under ultraviolet light, eluted with methanol/ammonia (95:5), and rechromatographed. This procedure was repeated for a total of three runs. The final chromatogram was stained with diazotized sulfanilic acid (16) and the T_3 area cut into 0.5-cm pieces. The ratio of ^{125}I-T_3 (from ^{125}I-T_4) to ^{131}I-T_3 was determined, with appropriate corrections for spillover from the ^{131}I channel to the ^{125}I channel as well as for recovery of the ^{131}I-T_3. Carcass content of ^{125}I-iodide was estimated from chromatography of a small aliquot of the original extract. The percent of total ^{125}I counts present in the carcass as T_3 was calculated from the equation (15):

$$(^{125}I/^{131}I) \times {}^{131}I\text{-}T_3 \times 100/{}^{125}I,$$

where ^{125}I/^{131}I is the corrected ratio of isotopes in the T_3 area of the chromatogram; ^{131}I-T_3, the known amount of counts added to the original extract; and ^{125}I, the total counts of this isotope present in the carcass. The percent of ^{125}I-T_4 remaining in the carcass was taken as the difference between the total ^{125}I radioactivity and the fraction of ^{125}I-iodide and ^{125}I-T_3. The fractional turnover of T_4 was estimated from the percent of T_4 and the residual radioactivity in a weighed aliquot of the carcass homogenate. The fractional turnover of T_3 was similarly measured in four separate rats killed 18 h after the intravenous injection of ^{125}I-T_3. The conversion ratio (CR) was calculated as follows (14):

$$CR = 200(T_3^*/T_4^*)/([\lambda_3/\lambda_4] - 1),$$

where the factor 200 is used to account for random deiodination in the 3′ and 5′ positions (15) and expression of the result as a percent; T_3^*/T_4^* is the isotopic ratio in the carcass; and λ_3 and λ_4 are the fractional removal rates of the hormones.

Results

Starvation resulted in the reduction of serum T_4 in group I and group II rats to 21 and 32% of the basal levels, respectively (Table I). In both cases the decreases in circulating T_3 were proportionally smaller, to 42 and 48% of the respective fed values. The MCR of T_4 was increased in both groups by 20%, whereas that of T_3 appeared unaffected. Despite the small increase in the MCR of T_4, the turnover of this hormone was markedly reduced, to 28 and 32% of control in the two starved groups. In each experiment, the reduction in T_4 turnover was proportionally greater than that for T_3. The latter was reduced only to 40 and 46% of control values in groups I and II, respectively. The disproportionate reduction of T_4 turnover rate is the opposite of the reduction expected from major impairment of fractional T_4 to T_3 conversion. Rather, the magnitude of the decrease in plasma T_4 levels (68 and 79%) in the face of only a 20% increase in the MCR of T_4 strongly suggested that the major cause of the hypothyroxinemia is reduced thyroidal secretion. Though it seemed unlikely, we could not entirely exclude the theoretical possibility that diminished fractional conversion of T_4 to T_3 was obscured by a relative increase in T_3 secretion.

To address this issue directly, we quantitated conversion of ^{125}I-T_4 to ^{125}I-T_3 in a group of rats studied simultaneously with the first group of rats subjected to turnover studies (Table II). Normal rats exhibited a conversion ratio of 21.8±5.1% (mean±SD) that did not differ statistically from the ratio in starved animals of 28.8±14.3%.

Discussion

Our finding that starvation in the rat results in a marked reduction in turnover of T_4 is consistent with previous reports of lowered levels of T_4 and TSH in starved rats (8, 9). Since the turnover rate of T_4 actually decreased more sharply in

Table I. Plasma Thyroid Hormone Kinetics in Two Groups of Starved Rats

	Group I		Group II	
	Control	Starved	Control	Starved
	n = 4	*n = 5*	*n = 6*	*n = 6*
Plasma T_4 (*ng/ml*)	47±2*	10±6‡	62±5	20±5‡
MCR T_4 (*ml/100 g body wt/d*)	30±2	36±4‡	34.3±3.1	41.0±8.2
PR T_4 (*ng/100 g body wt/d*)	1,400±11	390±250‡	2120±215	804±164‡
λ4 (*h*)	0.048±0.002	0.050±0.009	0.059±0.010	0.074±0.023
$(V_d)_{T_4}$ (*ml/100 g body wt*)	25.8±1.3	27.6±1.6	24.7±4.1	23.7±2.5
Plasma T_3 (*ng/ml*)	0.73±0.09	0.31±0.08‡	0.54±0.05	0.26±0.05‡
MCR T_3 (*ml/100 g body wt/d*)	315±78	314±89	366±53	335±41
PR T_3 (*ng/100 body wt/d*)	233±88	93±20‡	196±34	89±25‡
λ3 (*h*)	0.085±0.041	0.104±0.018	0.102±0.077	0.093±0.028
$(V_d)_{T_3}$ (*ml/100 g body wt*)	150.1±107.2	133.1±50.5	194.4±68.8	162.6±38.4

λ3 and λ4 indicate the fractional removal rates of T_3 and T_4 as determined by noncompartmental analysis. * Mean±SD. ‡ Different from control; $P < 0.05$ by t test.

Table II. T_4 to T_3 Conversion in Whole Rats

	Control	Starved
	n = 4	
CR (%)	21.8±5.1*	28.8±14.3 (*n* = 4)
λ3 (/*h*)	0.096±0.014	0.110±0.016 (*n* = 5)
λ4 (/*h*)	0.046±0.003	0.045±0.008 (*n* = 4)

λ3 and λ4 are the fractional removal rates of T_3 and T_4 determined from the residual radioactivity in the carcass 18 h and 48 h, respectively, after intravenous injection of ^{125}I-T_3 or ^{125}I-T_4.
* Mean±SD.

both groups of rats studied than did the turnover rate of T_3, there appears to be no need to postulate diminished overall conversion of T_4 to T_3 to account for the lowered levels of T_3. The possibility that diminished conversion of T_4 to T_3 by peripheral tissues could be offset by a concomitant increase in the relative secretion of T_3 is highly unlikely since increased rather than decreased levels of TSH are generally believed to preferentially stimulate thyroidal T_3 secretion (17). Nevertheless, in conjunction with the study of the first group of rats, we attempted to measure the fractional conversion of isotopic T_4 to T_3 by chromatographic analysis of total body homogenates, as previously described from our laboratory (15). The results of these experiments provided no evidence of a decrease in the conversion ratio.

The mean conversion ratio in normal rats, 21.8%, is in general agreement with our previously published value of 17% (15) and the 22% value recently reported by Silva et al. (18), and it lies within the theoretical range of 14–24%, predicted by DiStefano et al. (19). Higher values (33 and 27%), however, have been reported by Boonnamsiri et al. (20) and Zimmerman et al. (21), respectively. If the value 21.8% is applied to the measured T_4 turnover rate in the group I rats, the corresponding PR of T_3 yields a value 110% resulting from direct measurements. Similarly, if the value 28% is applied to the PR in the starved rats of group I, the PR of T_3 from T_4 is 101% the total T_3 turnover. These findings suggest, therefore, that essentially all of the extrathyroidal T_3 in the rat is produced by peripheral conversion from thyroxine, a conclusion differing markedly from our 1971 estimate (15) that only 20% of T_3 in the rat is produced by extrathyroidal conversion. However, our earlier calculations were based on borrowed literature values for T_3 and T_4 obtained by isotopic equilibration studies performed in the preradioimmunoassay era as well as on an MCR of T_4 that was considerably less than those measured in the present experiments. When, however, the conversion ratios obtained with group I rats were used in conjunction with the turnover ratios of group II rats, the calculated T_3 PR was approximately twice the PR observed by direct measurement. An examination of the data in Table I indicates that the higher PR of T_4 in the second group of rats was largely due to a higher circulating T_4. We have been impressed by the substantial variation in the level of T_4 reported in normal rats, with recent reports as low as 29 ng/ml (19) and as high as 70 ng/ml (22). To verify the validity of our methods, we carefully checked recovery of increasing concentrations of T_4 added to pooled sera samples from fed and starved euthyroid rats and fed hypothyroid rats, and with each group, we obtained nearly 100% recovery, a finding that minimizes the possibility of a systematic analytic error. Moreover, we measured plasma T_4 in groups of rats from different suppliers. Individual groups ranged from 44±8 (mean±SD, 6/group) to 61±5 ng/ml, measurements which approximate those of our two groups of experimental rats. We therefore believe that the variation in T_4 levels is not spurious but represents the effect of as yet undefined genetic, environmental, or dietary factors. Consequently, we believe that it is likely that the conversion ratio of group II rats was lower than the value determined for group I rats. Thus, if one assumes no thyroidal T_3 secretion in these rats, one can calculate a maximal conversion ratio of 11% in the control and 13.2% in the starved group II rats, suggesting the operation of an autoregulatory system similar to that proposed by Lum et al. in humans (23). With increased concentrations of circulating T_4, decreased fractional conversion of T_4 to T_3 would occur. We did not make any effort to test this hypothesis because of the difficulty in determining the fractional conversion of T_4 to T_3: multiple steps in the procedure, each with an inherent experimental error, lead to relatively imprecise net results. An inordinate number of rats exhibiting different basal T_4 levels would be required to establish valid distinctions in conversion rates.

Regardless of these considerations, it is clear that in the rat, in contrast to the results in humans, starvation consistently produced a greater reduction in T_4 than in T_3 turnover. Direct measurement of the conversion ratio by the isotopic technique provided no evidence for net reduction in peripheral T_4 to T_3 conversion. These results point to a reduction in thyroidal T_4 secretion as the major cause of the low T_3 levels in starved rats.

Our failure to infer an overall decrease in T_4 monodeiodination contrasts with many reports of a 40–50% reduction in the rate of T_4 to T_3 conversion in liver homogenates from starving rats (3–6). Unfortunately, there are no firm data that allow a quantitative estimate of the contribution of hepatic deiodination to the circulating pool of T_3 either in the fed or starved rat. DiStefano et al. (19) have estimated that ~50% of the T_3 generated in the normal rat is derived from tissues that equilibrate rapidly with T_4, namely liver and kidney, whereas the remaining T_3 appears to be derived from more slowly equilibrating tissues such as muscle and brain. It is possible that the contribution of the liver to total T_4 monodeiodination is sufficiently small to obscure reduction in overall hepatic monodeiodination in the intact rat. Hypothetically, reduction in the rate of hepatic monodeiodination could be offset by augmented deiodination in other tissues. Larsen et al. (24) have shown that monodeiodination may proceed by separate enzymatic mechanisms in individual tissues and may respond in a differential manner to agents such as PTU. Moreover, Kaplan and Yaskoski (25) have shown that starvation causes a 60% increase in the rate of deiodination of hypothalamic tissue. It is possible, therefore, to postulate that increased deiodination occurs in nonhepatic tissues in starved rats. Alternatively, monodeiodination, as measured under in vitro conditions, may fail to accurately reflect the changes that occur in vivo. Under any circumstances it may be premature to assume that the biochemical mechanisms responsible for diminished monodeiodination in liver homogenates from starved rats can serve as a valid model for the pathogenesis of the low T_3 syndrome in humans.

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