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Thyroid Function and Deiodinase Activities in Rats with Marginal Iodine Deficiency

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ABSTRACT

The hypothesis tested was whether marginal iodine deficiency for a period of 6 wk affects iodothyronine deiodinase activities in liver and brain of rats. Male rats were fed purified diets either deficient or sufficient in iodine; the diets were fed on a restricted basis (60% of *ad libitum* intake). Body weight gain of the two groups was comparable. Iodine deficiency was evidenced by increased thyroid weight (26%), reduced urinary iodine excretion (80%), and reduced plasma T_4 concentrations (22%). Activities of liver type I and brain type III deiodinase were unchanged, but the activity of type II deiodinase in brain was increased (28%) in the iodine-deficient rats. Food restriction *per se* significantly lowered T_3 (30%) and T_4 (22%) concentrations in plasma and decreased type III deiodinase activity in brain (30%). These results indicate that in marginal iodine deficiency the activities of hepatic type I deiodinase and brain type III deiodinase are unchanged, whereas that of brain type II deiodinase is increased.

Index Entries: Rats; iodine deficiency; food restriction; thyroid; deiodination; liver; brain.

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Abbreviations: DTT = dithiotreithol; Hepes = *N*-[2-hydroxyethyl]piperazine-*N*'-[2-ethanesulfonic acid]; PTU = propylthiouracil; I = iodine; rT_3 = reverse T_3 = 3,3',5'-triiodothyronine; 3,3'-T_2 = 3,3'-diiodothyronine; T_3 = 3,3',5-triiodothyronine; T_4 = 3,3',5,5'-tetra-iodothyronine; TSH = thyroid stimulating hormone; IRD = inner ring deiodination; ORD = outer ring deiodination.

INTRODUCTION

Cretinism and goiter are often caused by iodine deficiency (*1–3*). Iodine deficiency is associated with slightly elevated TSH, decreased T_4, and normal or slightly elevated T_3 levels in plasma (*4*). Hypothyroidism is characterized by high TSH, low T_4, and low T_3 levels in plasma (*5,6*).

In rats with hypothyroidism there is a marked decrease in type I iodothyronine deiodinase activity in liver (*7,8*). A decrease in type III deiodination has also been found in brains of hypothyroid rats (*9,10*). However, the activity of brain type II deiodinase is enhanced in hypothyroidism (*8–10*).

The type I enzyme, mainly present in liver, kidney, and thyroid, catalyzes the deiodination of the outer and/or inner ring of different iodothyronines and is very important for the production of plasma T_3; it is most efficient in outer ring deiodination (ORD) of rT_3. Type II deiodinase only catalyzes ORD of T_4 to T_3 and rT_3 to 3,3'-T_2 and is important for the local production of T_3 in tissues such as brain. Type III deiodinase inactivates T_4 and T_3 by inner ring deiodination (IRD) and probably is important for the clearance of plasma T_3 (*7*).

The abovementioned studies with rats with severe hypothyroidism may not be relevant for the situation in humans in areas where moderately reduced intake is a problem. Therefore, we have induced in rats a marginal iodine deficiency that is not associated with growth retardation or other clinical signs. Using this animal model we determined the activities of type I deiodinase in liver homogenates and those of type II and III deiodinases in brain homogenates.

MATERIALS AND METHODS

The experimental protocol was approved and its execution supervised by the animal welfare officer of the Wageningen Agricultural University.

Animals and Housing

Male, 30-d-old Wistar rats (Cpb/Hsd; Harlan Inc, Zeist, The Netherlands) were used. During the pre-experimental period of 2 wk (d-14–0), the rats were housed in groups of six animals in stainless-steel cages (60 × 42 × 19 cm) with wire mesh bases. During the experimental period of

6 wk (d 0–42) they were kept individually in metabolic cages (314 cm^2 × 15 cm). The cages were placed in a room with controlled temperature (20–22°C), relative humidity (45–65%), and a 12-h light-dark cycle (lights on: 7 AM to 7 PM).

Diets and Feeding

The diets were in powdered form and stored at 4°C until feeding. During the pre-experimental period, all rats were fed a purified control diet and twice-distilled water *ad libitum*. The control diet was iodine-sufficient (1.2 μmol added iodine/kg) and its composition was as follows: ovalbumin, 151 g; corn oil, 25 g; coconut fat, 25 g; glucose, 709.4 g; cellulose, 30 g; $CaCO_3$, 12.4 g; $NaH_2PO_4 \cdot 2H_2O$, 15.1 g; $MgCO_3$, 1.4 g; KCl, 1.0 g; $KHCO_3$, 7.7 g; KIO_3, 0.25 mg; iodine-free mineral premix, 10 g; vitamin premix, 12 g. The mineral premix consisted of (mg): MnO_2, 79; NaF, 2; NH_4VO_3, 0.2; $FeSO_4 \cdot 7H_2O$, 174; $NiSO_4 \cdot 6H_2O$, 13; $ZnSO_4 \cdot H_2O$, 33; $CuSO_4 \cdot 5H_2O$, 15.7; $SnCl_2 \cdot 2H_2O$, 1.9; $Na_2SeO_3 \cdot 5H_2O$, 0.3; $CrCl_3 \cdot 6H_2O$, 1.5; corn meal, 9679.4. The vitamin premix consisted of (mg): thiamin, 4; riboflavin, 3; pyridoxine, 6; niacinamide, 20; D,L-calcium pantothenate, 17.8; folic acid, 1; biotin, 2; cyanocobalamine, 50; choline chloride, 2000; D,L-α-tocopheryl acetate, 60; menadione, 0.05; retinyl acetate and retinyl palmitate, 8 (1200 retinol equivalents); cholecalciferol, 0.025; corn meal, 9828.125.

At the end of the pre-experimental period (d 0), the rats were divided into three groups, which were stratified for body weight. One group of 12 rats and one group of 6 rats remained on the control diet. The other group of 12 rats received an iodine-deficient diet. To prepare the iodine-deficient diet, KIO_3 was omitted from the control diet. The group of 6 rats was fed *ad libitum*. The other two groups received 60% of *ad libitum* intake; with this feeding regimen any difference in feed intake and growth rate between these groups would be precluded. Food was administered daily. Twice distilled water was freely available.

Experimental Procedures

Body weight was measured once a week. Food intake was measured daily. During the last 3 d of the experiment (d 39–42) urine was collected quantitatively from each rat and stored at −20°C until analysis. At the end of the experiment (d 42), the rats were anesthetized with diethyl ether and blood samples were collected in heparinized tubes by aorta puncture. The anesthetized rats were killed subsequently by CO_2 inhalation. Organs were removed and weights determined immediately. Plasma was stored at −20°C until analysis. Brains and livers were collected in liquid nitrogen and stored at −80°C until analysis.

Urinary iodine and creatinine were determined as described by Kolthoff and Sandell (*11*) and by Folin (*12*), respectively. Plasma T_3 and T_4 were analyzed by radioimmunoassays without prior extraction accord-

ing to the method of Larsen (*13*), with minor modifications as described by Van Hardeveld and Kassenaar (*14*).

To prepare liver homogenates, a portion of the liver was weighed and homogenized in 10 volumes of ice-cold buffer A (10 m*M* Hepes, 0.25*M* sucrose and 10 m*M* DTT, pH 7). Brains (cerebral cortex, striatum, and midbrain) were homogenized in 5 vol of ice-cold buffer A. Homogenates were stored at −70°C until analysis. Protein content of tissue homogenates were measured with the Bio-Rad protein assay (Pierce Europe, Oud Beijerland, The Netherlands) using bovine serum albumin as standard.

All deiodinase assays were performed in the presence of DTT by analysis of radioiodide production from ^{125}I-labeled substrates, similarly to the method of Mol et al. (*15*). All deiodinase reactions were stopped by adding 100 μL pooled human serum and 500 μL ice-cold 10% TCA. Released $^{125}I^-$ was separated from protein-bound iodothyronines by centrifugation. The data were corrected for nonenzymatic deiodination as determined in the absence of tissue homogenate. For calculation of deiodinase activities, random labeling of 3′ and 5′ positions of [^{125}I]rT_3 and [^{125}I]T_4 and of the 3 and 5 positions of [^{125}I]T_3 was taken into account.

Hepatic type I deiodinase activity was determined by incubation of the appropriate amount of homogenate protein (final concentration 0.05 mg/mL) for 20 min at 37°C with 75 nCi [3′,5′-^{125}I]rT_3 and 1 μ*M* rT_3 in the presence of 10 m*M* DTT in buffer B (final concentrations: 0.1*M* sodium-phosphate, 2 m*M* EDTA, pH 7.2; final volume: 200 μL).

Type II deiodinase activity was determined by incubation with 0.5 n*M* [3′,5′-^{125}I]T_4 for 2 h at 37°C with brain homogenate (4 times diluted) in the presence of 1 μ*M* T_3, 1 m*M* PTU, and 25 m*M* DTT in buffer B. PTU and T_3 were added to prevent possible interference by type I and type III deiodinase. This was verified by HPLC analysis of parallel incubations (n = 12). Therefore the deiodinase reaction was stopped by adding 200 μL methanol to the incubation mixture. Iodothyronine metabolites were analyzed using HPLC (*16*). Furthermore, the correlation coefficient between [^{125}I]T_3 production measured with HPLC and $^{125}I^-$ production measured according to Mol et al. (*15*) was 0.9067 (p = 0.001).

Type III deiodinase activity in brain homogenates was determined by incubating 5 n*M* [3,5-^{125}I]T_3 (courtesy of H. Rokos, Henning, Berlin, FRG) for 20 min at 37°C with diluted brain homogenate (final protein concentration 0.5 mg/mL) in the presence of 1 μ*M* rT_3, 0.1 m*M* PTU and 50 m*M* DTT in 200 μL buffer B. PTU and rT_3 were added to block possible type I deiodination of the labeled T_3.

Statistics

Data were analyzed using the Statistical Package for Social Sciences (SPSS) (*17*). The level of significance was preset at $p < 0.05$. Data were checked for normality. For normally distributed data, group means were

compared using a two-tailed, unpaired Student's *t*-test. To take into account the increasing risk of a type I error caused by multiple comparisons, Bonferroni's adaptation of the *p* value was applied, leading to a significance level of $p < 0.025$. Mann-Whitney U test was used to evaluate differences between group means of not normally distributed data (*18*).

RESULTS

Body Weight and Organ Weights

Initial (d 0) body weight of the rats was 138.1 ± 11.8 g (mean ± SD, $n = 30$). Average feed intake during the experiment by the group fed *ad libitum* was 21.8 ± 1.6 g/d (mean ± SD, $n = 6$). Growth rates, final (d 42) body and organ weights of the experimental groups are shown in Table 1. Restricting feed intake to 60% of *ad libitum* intake reduced body weight gain by about 57% (Table 1). The rats fed the restricted amount of the control diet also had lower absolute organ weights. However, when expressed relative to body weight, the rats fed on a restricted basis had higher brain and pituitary weights, but lower liver weights. This can be explained by a lower degree of adiposity in the animals fed the restricted amount of feed. Restricted feeding of the iodine-deficient diet did not affect body weight, when compared with restricted feeding of the control diet (Table 1).

Iodine Status

Iodine deficiency significantly increased mean thyroid weight by 26% (Table 2). Relative thyroid weight was also significantly increased by iodine deficiency. Restricted feeding *per se* decreased thyroid weight by on average 33%, but relative thyroid weight was unchanged. Restricted feeding did not alter the iodine:creatinine ratio in urine. The ratio was significantly lowered in iodine deficiency.

Thyroid Hormones

Restricted feed intake lowered T_4 levels in plasma (Table 2). Reduced plasma T_4 concentrations were also observed in the iodine-deficient rats. Rats fed restricted amounts of the control diet had significantly lower mean T_3 concentrations than their counterparts fed *ad libitum*. T_3 concentration was not affected by iodine deficiency.

Iodothyronine Deiodinase Activities

No differences between groups in protein concentration of liver and brain homogenates were found (Table 3). In rats fed *ad libitum*, specific activities of hepatic type I deiodinase and brain type II deiodinase were

Table 1
Effects of Iodine Deficiency and/or Food Restriction on Growth, Body Weight, and Organ Weights

Feeding regimen:	Restricted				*Ad libitum*	
Diet:	Control, $n = 12$		I deficient, $n = 12$		Control, $n = 6$	
	Mean	SD	Mean	SD	Mean	SD
Growth, g/d	2.3[a]	0.3	2.2	0.2	5.3	0.3
Body weight, g	234.2[a]	8.8	229.0	10.3	364.0	9.0
Liver weight, g	6.01[a]	0.56	5.81	0.57	14.28	1.09
Liver weight, g/100 g BW	2.57[a]	0.24	2.53	0.20	3.92	0.27
Brain weight, g	1.71[b]	0.04	1.67	0.08	1.80	0.07
Brain weight, g/100 g BW	0.73[a]	0.02	0.73	0.05	0.50	0.03
Weight kidneys, g	1.57[a]	0.10	1.57	0.10	2.31	0.19
Weight kidneys, g/100 g BW	0.67	0.05	0.68	0.04	0.63	0.05
Spleen weight, g	0.41[a]	0.03	0.40	0.03	0.60	0.07
Spleen weight, g/100 g BW	0.17	0.01	0.17	0.02	0.17	0.02
Pituitary weight, mg	7.1[a]	0.5	7.0	0.7	9.8	0.9
Pituitary weight, mg/100 g BW	3.0[b]	0.2	3.0	0.3	2.7	0.2

[a]Restricted control vs *ad libitum* control diet, $p < 0.001$.
[b]Restricted control vs *ad libitum* control diet, $p < 0.025$.

similar to those in rats fed on a restricted basis. Type I deiodinase activity in whole liver had decreased by restricted feeding. Restricted feeding significantly lowered type III deiodinase activity in brain. Iodine deficiency did not affect the specific activity of type I deiodinase in liver, but increased group mean type II deiodinase activity in brain. The stimulatory effect of iodine deficiency on brain type II deiodinase activity did not reach statistical significance ($p = 0.049$). In a parallel experiment (unpublished), we used diets that were identical to those in the present study, but without selenium ($Na_2SeO{\cdot}5H_2O$) added to the mineral premix. Selenium contents of the diets were determined using two different methods (*19,20*). Analysis showed that the control diet of the present study contained 0.19 (*19*) or 0.15 (*20*) mg Se/kg. The analyzed selenium concentrations of the diets used in the present and parallel study were as follows: iodine sufficient diet without added selenium, 0.09 (*19*) or 0.07 (*20*) mg Se/kg; iodine deficient diet with added selenium, 0.10 mg Se/kg; iodine deficient diet without added selenium, 0.09 mg Se/kg. The latter two diets were analyzed using the method of Koh and Benson (*19*) only.

Table 2
Effect of Iodine Deficiency or Food Restriction on Iodine Status

Feeding regimen:	Restricted				*Ad libitum*	
Diet:	Control, n = 12		I deficient, n = 12		Control, n = 6	
	Mean	SD	Mean	SD	Mean	SD
Urinary iodine, µgI/d	3.21	1.11	0.66[c]	0.20	3.90	0.97
Urinary creatinine, m*M*	6.79[a]	2.52	6.79	3.31	12.03	2.97
Urinary iodine, µgI/g creatinine	290.7	111.4	58.3[c]	17.5	230.6	70.9
Thyroid weight, mg	17.8[a]	2.0	22.4[c]	3.9	26.5	5.8
Thyroid weight, mg/100 g BW	7.6	0.9	9.8[d]	1.9	7.3	1.6
Plasma T_4, n*M*	63[b]	10	49[c]	8	81	9
Plasma T_3, n*M*	0.35[a]	0.09	0.38	0.15	0.50	0.09

[a]Restricted control vs *ad libitum* control diet, $p < 0.001$.
[b]Restricted control vs *ad libitum* control diet, $p < 0.025$.
[c]Restricted iodine deficient vs restricted control diet, $p < 0.001$.
[d]Restricted iodine deficient vs restricted control diet, $p < 0.025$.

Table 3
Effect of Iodine Deficiency or Food Restriction on Deiodinase Activities

Feeding regimen:	Restricted				*Ad libitum*	
Diet:	Control, n = 12		I deficient, n = 12		Control, n = 6	
	Mean	SD	Mean	SD	Mean	SD
Hepatic deiodinase-I, pmol/mg/min	264	51	252	42	296	82
Brain deiodinase-II, fmol/mg/h	4.74	1.02	6.00	1.82	3.98	0.90
Brain deiodinase-III pmol/mg/h	3.39[a]	0.50	3.43	0.32	4.82	0.69
Protein content of liver homogenates, mg/mL	16.5	1.1	16.3	0.9	14.7	2.1
Protein content of brain homogenates, mg/mL	14.8	0.8	15.6	1.0	14.4	1.3

[a]Restricted control vs *ad libitum* control diet, $p < 0.001$.

The diets without added selenium used in the parallel study were also fed to rats at a level of 60% of *ad libitum* intake for a period of 42 d. Iodine deficiency as induced with the diets without added selenium was found to significantly ($p = 0.001$) increase brain type II deiodinase activity, the activities in deficient and sufficient animals being 7.3 ± 2.2 and 4.6 ± 0.8 fmol/h per mg protein (means ± SD, $n = 12$). In that experiment type III deiodinase activity in brain homogenates was not affected by iodine deficiency.

DISCUSSION

At the end of the experiment, the control and iodine-deficient groups fed restricted amounts of feed had similar body and organ weights, except for the thyroid gland. As would be expected (*4*), iodine deficiency as only variable raised thyroid weight and lowered plasma T_4 concentrations. These results indicate that the iodine-deficient rats had a mild form of hypothyroidism, but growth rate was not affected.

The control groups fed either *ad libitum* or on a restricted basis had similar daily excretions of iodine. Thus, the control rats fed restricted amounts of feed can be considered iodine sufficient; their iodine intake was on average 0.015 μmol/d. Iodine intake of the rats fed the control diet *ad libitum* was on average 0.025 μmol/d. However, this difference in iodine intake owing to *ad libitum* vs restricted feeding, did not significantly affect daily iodine excretion. Furthermore, the decrease in daily iodine excretion owing to restricted feeding, was not proportional to the decrease in iodine intake. It can be hypothesized that iodine excretion in the form of T_4 and its metabolites in feces is decreased, leading to a higher proportion of iodine in urine (*21,22*).

Urinary iodine:creatinine ratios were determined to monitor the iodine status of the rats. After six weeks, the ratio was lowered by about 80% in the rats fed the diet without added iodine when compared with the controls who were fed on a restricted basis. Restricted feeding lowered urinary creatinine levels. A lowering effect of malnutrition on urinary creatinine concentration has also been reported for humans (*23*). Thus, the iodine:creatinine ratio as such may be an unreliable index to compare iodine status of undernourished people with that of well-nourished people. Twenty-four-hour-urinary iodine excretion is a better index of the iodine status in areas where malnutrition occurs.

Iodine deficiency *per se* did not influence hepatic T_4 to T_3 monodeiodination as based on type I deiodinase activity. Possibly, type I monodeiodinase activity is decreased only when circulating thyroid hormones are markedly reduced. Evidence for this notion has been presented earlier (*7,8*).

Iodine-deficient rats had an increased activity of type II deiodinase in brain. The activity that we found is rather low when compared with

other studies (*8,9*). This can be explained by the fact that we used homogenates of brain tissue consisting of cerebral cortex, striatum, and midbrain. Unlike others (*9,10*) we did not include cerebellum, which has a relatively high activity of type II deiodinase (*9,10*). Moreover, we used homogenates instead of microsomes. Our results do show that a mild reduction in serum T_4 is associated with an increase in apparent type II deiodinase activity. Interference of type II deiodinase measurement by endogenous T_4 cannot be excluded. However, it is unlikely that a change in endogenous T_4 in the homogenates accounts for the observed increase in type II deiodinase activity in iodine-deficient rats. The T_4 concentration in the incubation mixture (endogenous plus tracer) as estimated according to Van Doorn et al. (*24*) was far below the apparent K_m (1 n*M*) of the type II deiodinase (*7*). Furthermore, the contribution of endogenous T_4 probably is low compared to the added tracer T_4. Thus, variations in brain T_4 concentrations may not have measurably affected deiodinase type II activity.

Marginal iodine deficiency did not affect brain type III deiodinase activity. Studies using thyroidectomized rats with severely reduced T_4 levels have shown decreased type III deiodinase activities (*9,10*).

Food restriction lowered plasma T_4 and T_3 concentrations. The decreased T_3 levels in plasma may limit energy expenditure during a period of restricted feed intake. Schröder-van der Elst and Van der Heide (*21*) described that restricted feeding of rats lowers peripheral T_3 production. The present results show that specific hepatic type I deiodinase was not reduced by food restriction. However, because of the decrease in liver weight, type I deiodinase activity in whole liver was decreased. The lowering effect of food restriction on T_3 to T_2 monodeiodination in brain can be considered as a mechanism, protecting the brain against shortage of T_3, even in situations when T_4 levels are decreased.

In sum, under the present experimental conditions marginal iodine deficiency had predictable effects on thyroid function. Food restriction alone also influenced thyroid hormone metabolism as evidenced by a fall of plasma T_4 and T_3 levels and a significant decrease in type III deiodinase activity in brain. In addition, in rats with mild hypothyroidism brain type II deiodinase activity was increased. Type II deiodinase in brain seems more sensitive to minor changes in plasma levels of T_4 than type I and III deiodinases. This can be seen as a compensatory mechanism to maintain a normal tissue T_3 concentration in spite of a fall in circulating T_4 level.

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