Low Dietary Iodine Intake and the Functional Activity of the Rat Thyroid[1,2] (34837)

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Low iodine diets in some experimental animals and humans have been reported to result in goiter when iodine deficiencies occur. Since iodine is the essential raw material for synthesis of thyroid hormones, chronic iodine deficiency would tend to decrease and ultimately cause reduction in hormone synthesis leading to iodine deficiency symptoms such as goiter production. Remington and his associates (1–4) produced a diet from grains grown in iodine-deficient soil. It contained 0.015–0.080 ppm of iodine. This level of iodine in the diet has been found to be variably effective in producing goiter (5–13). Although the experimental conditions differed only slightly and the iodine content of the diet varied little in these experiments, the results were that goiter in the rat was produced in 2 weeks in some reports while in other cases thyroid enlargement did not occur for many weeks.

The object of this study was to determine the functional activity of the thyroid in the albino rat at intervals for 3 months after feeding a low iodine diet. While low iodine diets have been studied for goiter formation and iodine metabolism, little study has been reported on the thyroid hormone-secretion rate (TSR) in the rat after iodine restriction. Besides TSR, other parameters of thyroid activity, such as PBI, thyroidal-^{131}I uptake and release, and the DNA content of the thyroid, were also studied to supplement TSR observations.

Materials and Methods. A low iodine diet was prepared in the Agricultural Chemistry Department, University of Missouri, Columbia (Ag. Chem.-LID). This low iodine diet contained corn starch, 61%; vitamin-free casein, 18%; brewer's yeast, 10%; B_{12} (3 g cyancobalamine, Merck, per kg of corn starch), 1%; salt mixture No. 6112, 5%; cottonseed oil, 4%; vitamine D, E, and K in cottonseed oil, 1%; and carotene, 0.001%. The composition of salt mixture No. 6112 was as follows: $CaCO_3$, 21.4%; $CaHPO_4 \cdot 2H_2O$, 33.3; $MgCO_3$, 2.0; $MgSO_4$, 1.2; NaCl, 10.2; KCl, 1.7; $FePO_4$, 3.2; KH_2PO_4, 25.2; $MnSO_4 \cdot H_2O$, 1.5; $CuSO_4 \cdot 5H_2O$, 0.13; $AlK(SO_4)_2 \cdot 12H_2O$, 0.02; $CoCl_2 \cdot H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.04; NaF, 0.08; Total, 100%. The gross energy value of this diet was 4.36 kcal per gram. The iodine content of this diet was analyzed by Wisconsin Alumni Research Foundation, Madison, and was reported to contain 0.05 ppm iodine.

The control diet used in this study was Purina Lab Chow. It was reported to have a gross energy value of 4.25 kcal per gram, a protein content of 23.4%, and an iodine content of 1.7 ppm.

Young female rats of the Sprague–Dawley–Rolfsmeyer strain were used in all the experiments. They were housed in wire cages in groups of five and the cages were frequently cleaned of fecal material to reduce coprophagy. The rats were housed in a large animal room at 25.5° with 14 hr of artificial light daily.

TSR. The individual TSR of a group of 30-day-old rats was estimated by the method

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previously described (14). A goitrogen, methimazole-tapazole, was also injected sc at the level of 0.4 mg/100 g body wt/day during the entire period of TSR estimation to block the recycling of metabolized ^{131}I. The rats were then divided into two groups on equal TSR basis. One group of rats received the low iodine diet and the other group received Purina Lab Chow. Since tap water in this locality contained little iodine (0.004 ppm), the rats in both the groups were given tap water. The Ag. Chem.-LID was fed to the experimental rats for a total period of 3 months, and TSR was estimated at intervals of 1 month. For TSR determinations thyroidal-^{131}I neck counts were made on a Nuclear Chicago Model DS-5 scintillation probe containing a 2 × 2-in. sodium iodide crystal attached to a Picker Ratemeter, Model 5866. The rat was slightly anesthetized with ether and the neck-thyroid region was pressed slightly against the surface of the scintillation probe for a period of 20–30 sec until the pointer was at rest. The highest thyroidal-^{131}I count obtainable at the time of counting was recorded.

Half-life of thyroidal-^{131}I. Two separate groups of rats on equal TSR basis were used to determine the $t_{½}$ of thyroidal-^{131}I at 1-month intervals. Rats fed Purina Lab Chow were injected ip with 10 μCi ^{131}I. The LID-fed rats were injected ip with 2–3 μCi ^{131}I since a higher thyroidal uptake of the radioiodine was expected. The first thyroidal neck count was taken after 48 hr. At this time 0.4 mg/100 g body wt tapazole was injected sc to each rat in both groups and thereafter once each day until five thyroidal-^{131}I counts were obtained at 2-day intervals. The half-life ($t_{½}$) of thyroidal-^{131}I was calculated by the equation 0.693/m, where m was the slope of the regression line (percentage dose against time) (15).

PBI. At the end of the 3-month experimental period, blood samples were collected by heart puncture, and serum was obtained for PBI determinations (16). This was based on dry ashing and consisted of use of anionic exchange resins and elution of the iodine with the arsenious reagent. The arsenious ion developed a color reaction with ceric ion and the optical density was measured in a DU-Spectrophotometer at 420 mμ. The reagents and glassware were obtained from Hycel, Inc. (Houston, Texas).

Wet gland weights and DNA. The rats were killed in ether, and the thyroid, adrenal, and pituitary glands were collected on ice, cleaned, and weighed on a torsion balance, accurate to 0.2 mg. The thyroid and adrenal glands were stored at −20° in closed glass vials for later estimation of DNA. To obtain dry fat-free tissue (DFFT) the thyroids and adrenals of six rats were pooled and extracted in 95% hot ethyl alcohol for 8 hr, then reextracted with fresh alcohol for another 8 hr, and finally with ether for 8 hr. The DFFT thus obtained was weighted and finely ground in a Wiley mill. A known quantity of the powdered DFFT was then assayed for DNA (17). The total DNA gave an estimate of the cell population and the DNA per mg of DFFT gave an estimate of the cell size.

Results. The effects of feeding the LID to rats for 3 months on thyroidal ^{131}I uptake, TSR, and biological half-life of thyroidal-^{131}I are shown in Table I. A decline in the TSR of the control rats was observed due to advancing age (18, 19). The high uptake by the thyroid of the low iodine diet fed rats suggested a gradual depletion in thyroidal and extrathyroidal iodine pool and that the "iodine trap" was greatly stimulated. The significant decrease in biological $t_{½}$ of thyroidal-^{131}I in the low iodine diet-fed group indicated a faster turnover of thyroidal iodine. Since the TSR measured the net daily secretion of thyroid hormones, the results suggested that the rats fed low iodine diet were able to alleviate any deficiency of dietary iodine by increasing "iodide trap" mechanisms and by a faster turnover of thyroid hormones.

Food intake measurements (Table II) in the low iodine diet-fed group provided a basis for calculating the daily iodine intake. Since rats obtained very little iodine from tap water, the total daily iodine intake from feed (12–15 g per day) was 0.60–0.75 μg based on the iodine content of the diet (0.05 ppm). The mean wet thyroid, adrenal, and pituitary weights are shown in Table III. Thyroids of

TABLE I. Effect of Low Dietary Iodine Intake on TSR, Thyroidal-^{131}I Uptake, and $T_{½}$ of Thyroidal-^{131}I Release in Female Rats.

Group	Diet	Age (days)	Body wt (g)[a]	Thyroidal-^{131}I uptake (%)	TSR μg L Thyroxine /100 g body wt	$T_{½}$ of thyroidal-^{131}I release (hr)
I	Lab Chow (21)[b]	30	79.0 ± 1.9	7.9 ± 0.6	1.53 ± 0.06	
		60	178.3 ± 4.7	8.5 ± 0.7	1.18 ± 0.07	84.7 ± 5.7 (8)
		90	203.3 ± 5.2	11.3 ± 0.9	1.08 ± 0.05	67.8 ± 5.5 (8)
		120	248.3 ± 6.7	8.3 ± 0.6	0.90 ± 0.05	57.9 ± 5.9 (8)
II	LID (21)	30	75.7 ± 1.8	8.8 ± 0.6	1.51 ± 0.07	
		60	159.8 ± 5.6	51.3 ± 2.6	1.17 ± 0.07	54.5 ± 4.4 (8)
		90	224.5 ± 5.0	43.0 ± 1.9	1.32 ± 0.05	40.5 ± 2.5 (8)
		120	227.4 ± 4.2	42.9 ± 2.4	1.27 ± 0.03	29.8 ± 2.2 (8)
Probability Student's t test p		30	NS	NS	NS	
		60	.02	.001	NS	.001
		90	.01	.001	.01	.001
		120	.02	.001	.001	.001

[a] Mean ± standard error.

[b] Numbers in parentheses indicate number of rats.

rats on LID were significantly larger than their corresponding controls on per unit body weight basis and appeared hyperemic on visual examination. Unfortunately, histological examinations of these thyroids were not made, but the total DNA/thyroid indicated hyperplasia. Since DNA/mg DFFT is an index of cell size assuming constant DNA per epithelial cell, the data indicated that thyroids were also hypertrophied (Table IV) (20). The observation that adrenals of rats fed low iodine diet had significantly higher DNA/mg DFFT cannot be explained at this stage and requires further investigation.

The mean PBI in the low iodine diet group of rats was determined to be 4.6 μg/100 ml, as compared to 5.3 μg/100 ml in Lab Chow-fed rats, which were not significantly different. It may be pointed out that the method used for PBI determination may not be sensitive enough or the PBI levels in these two groups of rats were actual values within limits of experimental error.

Discussion. The effect of the level of marginal and submarginal iodine intake in experimental animals has not been settled. It is recognized by various workers that rat thyroids have the capacity to maintain an euthyroid state for a considerable period of time on low dietary iodine intake. Low iodine diets available commercially in 1953–1955 contained 0.08 ppm and produced goiter in rats very slowly while another diet containing 0.015 ppm iodine and no longer available produced goiter in 6 weeks (13). A low iodine diet (0.08 ppm) was reported to cause thyroid enlargement in the rat after 4 months or longer (13) whereas another low iodine diet (0.03 ppm) caused goiter in Sprague–Dawley–Rolfsmeyer strain male adult rats

TABLE II. Effect of a Low Iodine Diet on Food Consumption in Rats.

Age (days)	Feeding period (months)	No. of rats	Purina Lab Chow			Ag. Chem. LID		
			Mean body weight (g)	Food intake		Mean body weight (g)	Food intake	
				g/day	g/100 g body wt[a]		g/day	g/100 g body wt
100	2	12	238.5	16.5	6.9 ± 0.2	207.1	12.4	5.9 ± 0.2
130	3	12	258.7	18.7	7.2 ± 0.2	238.7	15.3	6.4 ± 0.3

[a] Mean ± standard error.

TABLE III. Effect of a Low Iodine Diet for 3 Months on the Thyroid, Adrenal, and Pituitary Wet Weights and on PBI.

Diet	No. of rats	Body wt (g)[a]	Thyroid		Adrenal		Pituitary		PBI (μg/100 ml)
			Total (mg)	/100 g body wt (mg)	Total (mg)	/100 g body wt (mg)	Total (mg)	/100 g body wt (mg)	
Lab Chow	21	266.8 ± 8.8	16.8 ± 0.8	6.3 ± 0.3	59.9 ± 1.9	22.6 ± 0.8	11.0 ± 0.5	4.1 ± 0.2	5.3 ± 0.5
LID	21	233.7 ± 4.2	19.6 ± 0.8	8.4 ± 0.2	59.3 ± 1.5	25.5 ± 0.7	11.7 ± 0.2	5.0 ± 0.4	4.6 ± 0.5
Probability Student's t test p		.001	.02	.001	—	.02	—	.001	—

[a] Mean ± standard error.

by Day 7 and it continued to increase linearly (11). More recently it was reported that a low iodine diet (0.02 ppm) fed to rats for 10 months enlarged the thyroid size 5–8 times the normal size (21).

Low iodine diets have been reported to cause increased synthesis of L-triiodothyronine (L-T_3) in the thyroid of rats. Chromatograms of thyroid hydrolyzates show increased MIT/DIT and L-T_3/L-T_4 ratios (11, 22–25). Increased L-T_3 content of the thyroid gland suggests increased synthesis and is assumed to reflect increased release into circulation. Previous studies have indicated that in rats, L-T_3 is over twice as active biologically as L-T_4 on an equimolar basis (26, 27). Increased synthesis and release of L-T_3 by the thyroid in the case of low dietary iodine intake would indicate an endogenous compensatory mechanism to meet the metabolic demands at the tissue level (23). Studies have indicated that on low iodine diet the thyroidal and body pool of iodine is greatly reduced. There is high uptake of radioiodine (28), but it is equally rapidly discharged by the thyroid. The tremendous increase in turnover rate of the thyroidal iodine (hormones) approached total compensation in moderate dietary iodine deficiency.

The data in the present study indicated that feeding a low iodine diet (with casein) containing 0.05 ppm iodine for 3 months to Sprague–Dawley–Rolfsmeyer strain young female rats did not decrease the thyroid hormone secretion rate on a daily basis, and thus the rats were able to maintain near normal levels of thyroid hormones in the blood stream. Since the young rats have been reported to require larger amounts of thyroid hormones for growth and reproduction than the adults per unit weight basis, it was interesting to find that 0.05 ppm iodine in the diet was not low enough to depress the net daily hormone secretion in young female rats for up to 3 months. On the contrary, it was observed that the TSR in the experimental group of rats fed the low iodine diet for 2 and 3 months was significantly higher than the corresponding controls fed Lab Chow.

Based on the present study under the existing experimental conditions, the authors be-

TABLE IV. Effect of a Low Iodine Diet on DNA Content of Thyroid and Adrenal.

Group	Diet	No. of rats	Mean body wt (g)[a]	Thyroid				Adrenal			
				Wet wt (mg)	DFFT (mg)	DNA/mg DFFT (μg)	Total DNA (μg)	Wet wt (mg)	DFFT (mg)	DNA/mg DFFT (μg)	Total DNA (μg)
1	Lab Chow	21	266.8 ± 8.8	16.8 ± 0.8	3.01 ± 0.15	20.19 ± 0.91	60.33 ± 0.76	59.3 ± 1.5	7.46 ± 0.42	22.37 ± 2.66	167.19 ± 22.75
2	LID	21	233.7 ± 4.2	19.6 ± 0.8	2.58 ± 0.21	28.39 ± 2.82	71.78 ± 3.35	59.9 ± 1.9	6.84 ± 0.26	30.48 ± 1.83	207.45 ± 4.64
Probability Student's t test p			.001	.02	—	.05	.01	—	—	.05	—

[a] Mean ± standard error.

lieve that unless there is chronic deficiency of dietary iodine coupled with other alterations in body adaptations, such as secretion of thyrotropin, presence of goitrogens in the feed, reabsorption of thyroid hormones in the gut, and utilization at cellular level, the thyroid of the young adult female rat has tremendous capacity to increase its activity to alleviate any moderate degree of iodine deficiency for up to 3 months.

Summary. The effects of low dietary iodine intake on the functional activity of the thyroid were studied in young female albino rats. The low iodine diet contained 0.05 ppm iodine, with casein as the primary protein source. Feeding this diet to 30-day-old rats for periods of up to 3 months increased the thyroid hormone secretion rate (TSR). The PBI was within the normal range but thyroid size was slightly increased as compared to the corresponding control rats of the same age fed Purina Lab Chow. On the low iodine diet, the percentage uptake of thyroidal-^{131}I was greatly increased, and the biological half-life of thyroidal-^{131}I was significantly reduced, indicating a faster turnover of thyroidal-iodine (hormones). The DNA content of the thyroids indicated hyperplasia and hypertrophy suggesting stimulation of the gland. It was concluded that under these experimental conditions the rat thyroid has the capacity to increase its activity to compensate for any moderate low dietary iodine for at least 3 months by augmenting recycling mechanisms and thyroidal turnover of hormones.

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