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The Impact of Iodine Excess on Thyroid Hormone Biosynthesis and Metabolism in Rats

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Abstract Thyroid function ultimately depends on appropriate iodine supply to the gland. There is a complex series of checks and balances that the thyroid uses to control the orderly utilization of iodine for hormone synthesis. The aim of our study is to evaluate the mechanism underlying the effect of iodine excess on thyroid hormone metabolism. Based on the successful establishment of animal models of normal-iodine (NI) and different degrees of high-iodine (HI) intake in Wistar rats, the content of monoiodotyrosine (MIT), diiodotyrosine (DIT), T_4, and T_3 in thyroid tissues, the activity of thyroidal type 1 deiodinase (D1) and its (D*io1*) mRNA expression level were measured. Results showed that, in the case of iodine excess, the biosynthesis of both MIT and DIT, especially DIT, was increased. There was an obvious tendency of decreasing in MIT/DIT ratio with increased doses of iodine intake. In addition, iodine excess greatly inhibited thyroidal D1 activity and mRNA expression. T_3 was greatly lower in the HI group, while there was no significant difference of T_4 compared with NI group. The T_3/T_4 ratio was decreased in HI groups, antiparalleled with increased doses of iodine intakes. In conclusion, the increased biosyntheses of DIT relative to MIT and the inhibition of thyroidal D*io1* mRNA expression and D1 activity may be taken as an effective way to protect an organism from impairment caused by too much T_3. These observations provide new insights into the cellular regulation mechanism of thyroid hormones under physiological and pathological conditions.

Keywords Thyroid hormone · Type 1 deiodinase · Monoiodotyrosine · Diiodotyrosine · Iodine intakes

Introduction

The thyroid's only clearly established function is to make its hormones, T_4 (3,5,3′,5′-tetraiodo-L-thyronine, thyroxine) and T_3 (3,5,3′-triiodo-L-thyronine). Orderly formation of

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thyroid hormone depends on the proper amount of iodine available. Both iodine deficiency and iodine excess will lead to thyroid dysfunction. Up to now, significant progress has been accomplished in understanding the relationship between iodine deficiency and thyroid disorders [1, 2]. However, the mechanism underlying the effect of iodine excess on thyroid function is poorly defined. Therefore, it is of great necessity to establish animal models and make a thorough evaluation of the impact of excessive iodine intakes on thyroid function to ascertain the nature and significance of this influence.

Recent studies have shown that there is a complex series of checks and balances that the thyroid uses to control the orderly utilization of iodine for hormone synthesis [3]. Monoiodotyrosine (MIT) and diiodotyrosine (DIT), the precursors of thyroid hormones, represent the most abundant iodoamino acids in thyroid gland. Therefore, effects of different iodine intake levels will be reflected by a change in the relative amount of these iodotyrosines, which will further lead to the fluctuations of thyroid hormones.

In addition, in order for thyroid hormones to exert their effects at the nuclear level, the prohormone thyroxine (T_4) must be transformed intracellularly into its most active form of metabolites (T_3) after deiodination of its outer (5′) ring [4, 5]. The deiodinases function at a prereceptor level in tissues to modulate the concentrations and, thus, the actions of thyroid hormones [6]. In many different tissues, T_4 is converted into T_3 catalyzed by one or both of two isoenzymes called type 1 (D1) and type 2 (D2) deiodinases. D1 activity is found in many tissues, including liver, kidney, and thyroid, etc., while D2 is mainly expressed in the central nervous system and pituitary [4, 7]. Studies indicate that a great proportion of T_4 is deiodinated in the thyroid, generating approximately 40–50% of circulating T_3 in rats [4, 8]. Therefore, D1 plays a very important role in thyroid hormone metabolism [9].

In this study, based on the successful establishment of animal models of normal-iodine (NI) and different degrees of excess-iodine intake in Wistar rats, the content of MIT, DIT, T_4, and T_3 in thyroid tissues, the thyroid D1 activity and its mRNA expression level were measured. The aim of the present study was to investigate the possible regulation mechanism by which iodine exerts its effect on thyroid function.

Materials and Methods

Animals

Wistar rats, weaning 1 month and weighting 120–140 g, half males and half females, were randomly divided into five groups according to body weight and sex (for each group, n= 60): (1) NI, (2) fivefold high iodine (5HI), (3) tenfold iodine (10HI), (4) 50-fold iodine (50HI), and (5) 100-fold iodine (100HI). They were fed with normal feedstuff (average iodine content is 300–400 μg/kg). Rats in the NI group drank tap water (average iodine content is 5 μg/L), whereas rats in HI groups drank tap water containing different concentrations of potassium iodate, and the iodine content in water was 820, 1,845, 10,045, and 20,295 μg/L, respectively. Then, 3, 6, and 12 months after administration, they were killed and thyroid glands were excised.

Experimental Protocols

Experiment 1: Reverse-Phase High-Performance Liquid Chromatography of Iodotyrosines Rat thyroid tissue was firstly homogenized in phosphate-buffered saline at 1:20 [weight/volume (W/V)] dilution and then digested with Pronase E (10% W/V) (type XIV, bacterial from

Streptomyces griseus, Sigma, St. Louis, MO, USA) at 37°C for 24 h. The hydrolysate was centrifuged at 12,000 rpm for 10 min. Extraction solution (methanol/aqua ammonium, 99/1 by volume) was added to the supernatant and then recentrifuged at 12,000 rpm for 10 min. The supernatant was kept for high-performance liquid chromatography (HPLC) analysis.

The HPLC apparatus consisted of a Model LC-6A solvent metering pump (Shimadzu, Kyeto, Japan), Model 7125 syringe loading sample injector with a 50 μl loop (Rheodyne, Oak Harbor, WA, USA), a SPD-6AV analytical ultraviolet detector, and a Anastar recorder–integrator was used to control the chromatographic system and collect data.

Stock 1-mg/ml solutions of MIT and DIT (Sigma) were prepared by dissolving these iodotyrosines in distilled water. Standard working solutions for chromatography were obtained by mixing MIT and DIT stock solutions and then further diluted to give concentrations of 1, 2, 3, 4, 5, 6, 10, 20, and 40 μg/ml, respectively.

Standard working solutions and thyroid extractions were transferred onto the reversed-phase Kromasil C_{18} column (5 μm, 250×4.6 mm inner diameter, Bohus, Sweden) and successively eluted with methanol/water (30/70 by volume, contained 0.05 M NaH_2PO_4, pH=2) solvent system. Chromatography was continued isocratically for 30 min at room temperature. The flow rate was 1.0 ml/min. Detection was monitored in a 1-cm flow-through cell at 225 nm. All the chemicals and solvents used for HPLC analysis were of HPLC grade.

Experiment 2: Absolute Quantitative Real-Time Reverse Transcriptase-Polymerase Chain Reaction for Dio1 Transcript Total RNA was extracted from thyroid tissue using the chloroform–isopropyl-alcohol method with Trizol. The quality of RNA samples was assessed by electrophoresis through denaturing agarose gels and staining with ethidium bromide, and the 18S and 28S RNA bands were visualized under ultraviolet illumination. The extraction yield was quantified spectrophotometrically. Three micrograms of total RNA for each sample was reverse transcribed using Oligo $(dT)_{18}$ primer and M-MuLV reverse transcriptase (Fermentas, Burlington, Canada).

The cDNAs were then subjected to polymerase chain reaction (PCR) using the following thermal profile: 95°C for 10 min followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. All the PCR primers were designed to be intron spanning, using Primer Express Software version 1.0 (Applied Biosystems, Foster City, CA, USA) and were purchased from Invitrogen (Carlsbad, CA, USA). The sequence of the primers, spanning 101 bp for D*io1* and 107 bp for G*apdh*, was D*io1* sense primer: 5′-TCGTAGATGACTTTGCCTCCAC-3′, antisense primer: 5′-CCGGATGTCCACGTTGTTC-3′; G*apdh* sense primer: 5′-CATGGCCTT CCGTGTTCCTA-3′, antisense primer: 5′-ATGCCTGCTTCACCACCTTCT-3′.

After purification, amplicones were inserted to the pGEM plasmid vector using pGEM®-T Easy Vector System I (Promega, Madison, WI, USA) and cloned into chemically competent *Escherichia coli.* Positive blue colonies were isolated by LB agar plates added with X-Gal solution. The resulting recombinant plasmid was extracted and purified from transformed *E. coli* cell cultures using QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA, USA). Then, plasmid DNA was linearized by *Pv*uII (Toyobo, Tokyo, Japan) digestion and sequenced to test the orientation of D*io1* and G*apdh* amplicones. To generate the standard curve, plasmid DNA was quantified by spectrophotometric absorbance readings at 260 nm (A260) and was serially diluted in nuclease-free water to produce standard template ranging from 0 to 10^{10} copies/μl. Plasmid preparations are advantageous because these preparations generate high-quality, pure, and concentrated standards that can be independently quantified and converted to a number of copies of target DNA [10].

Real-time quantitative PCR was achieved using SYBR® Premix Ex Taq™ kit (TaKaRa, Tokyo, Japan) according to the manufacturer's instructions and was developed on the ABI

Prism 7500 Sequence Detection System (Perkin-Elmer Applied Biosystems) The reactions were carried out in 25-μL mixtures containing SYBR Premix Ex Taq™, 10-μM concentration of each forward and reverse primer, and ROX™ as a passive reference dye. Real-time reactions were processed through 40 cycles of two-step PCR, including 10 s of denaturation at 95°C and 34 s of annealing–elongation at 60°C. At the end, a dissociation stage was added. In each 96-well plate, the dilution series of the plasmid standard was run along with the unknown samples. Each sample was assayed in triplicate, in the presence of no template controls, and the intra-assay coefficient of variation was less than 1%. All reactions were repeated at least three times independently to ensure the reproducibility of the results.

The standard curve for the D*io1* gene was constructed by plotting the cycle threshold (Ct) values, with 95% confidence intervals, against the logarithm of the initial copy numbers. Target DNA copy number and Ct values are inversely related, i.e., a sample containing a high number of copies of the target DNA will cross the threshold at an earlier cycle than a sample with a lower number of copies of the same target [11, 12]. Therefore, the absolute levels of D*io1 in the experimental samples were determined by extrapolating the Ct values from the linear regression of that standard curve.*

Reverse transcriptase-PCR (RT-PCR)-specific errors in the quantification of mRNA transcripts are easily compounded by any variation in the amount of starting material between samples [13, 14]. To normalize for differences in the amount of total RNA added to the reaction, amplification of G*apdh* RNA was performed as an endogenous control.

Experiment 3: Thyroid D1 Activity Assay This method was first reported by Hotz in 1996 [15], and we only made a few modifications. In brief, thyroid was homogenized in cold homogenization solution [10 mM pH 7.0 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid buffer, 1 mM dithiothreitol (DTT), 320 mM sucrose] at 1:20 (W/V) dilution. Homogenates were centrifuged at 4°C and 20 μl supernatant was added to 100 μl 37°C preincubated ^{125}I-rT_3 incubation solution (0.005 μM ^{125}I-rT_3, 0.495 μM 5′-L-rT_3, 2 mM DTT, 100 mM pH 7.0 potassium phosphate buffer, 1 mM EDTA). Total reaction time was 11 min then stopped by adding 200 μl of cold T_4/PTU stop solution (10 μM T_4, 10 μM PTU). Reactions for each sample began and stopped at 30-s intervals to make sure that the reaction time for each sample is accurate. Centrifuge filter columns, packed with Dowex-50w ion exchange resin (Nankai University, Tianjin, China) and fitted with receiver tubes, were loaded with 256 μl of the reaction mixture and centrifuged (1,500 g) for 10 min. Columns were eluted with 0.5 ml 10% (V/V) acetic acid, then recentrifuged and re-eluted as above. The receiver tubes containing the total filtrate were removed and the released $^{125}I^-$ was counted in a gamma counter. Protein content was determined by biuret reaction method. D1 activity was expressed as picomole I^- released/milligram protein per minute.

Experiment 4: Determination of Total T4 and T3 Content of the Thyroid Gland The level of thyroid tissue hormones (T_3 and T_4) was analyzed by the specific RIA kit (Northern Biological Reagent Institute, Beijing, China). The entire procedure was carried out following the fabricant recommendations.

Statistical Analysis

SPSS was used to analyze the collected data. Data were expressed as means ± SD. Difference among groups were examined by one-way analysis of variance followed by a

Fisher test. LSD or Dunnett's T3 post hoc test was used when it was appropriate. A *P* value less than 0.05 was considered statistically significant.

Results

Reverse-Phase HPLC of Iodotyrosines

Separation of Standard Iodotyrosines Figure 1 shows the separation of standard MIT and DIT within 25 min on isocratic elution with methanol/water (30/70 by volume, contained 0.05 M NaH_2PO_4, pH=2). MIT and DIT were separated according to their degree of iodination by reverse-phase HPLC. With a standard mixture, the retention time was 7.70± 0.13 min for MIT and 19.45±0.15 min for DIT.

Standard Curves Figure 2 depicts the calibration curves (peak areas plotted vs concentration) for the quantitative detection of MIT and DIT. Each linear calibration curve is composed of nine points, and each point represents the average of five determinations. Both MIT and DIT gave good linear response, and very low quantities of these compounds were detected. The regression coefficients (r^2) were 0.9960 for MIT and 0.9936 for DIT.

The analytical sensitivity determined as the minimum detectable amount was 16 ng for MIT and 40 ng for DIT with the recorder set at a full-scale deflection of 0.02 A. At maximum sensitivity (0.005 A full-scale deflection), quantitation of the peaks was less reliable because the baseline became more irregular.

Intra-assay variation from six experiments and interassay variations from one analysis per day for 6 days were calculated for 2, 10, 40 μg/ml standard working solutions. The intra- and interassay coefficients of variation ranged from 2.78% to 4.6% for MIT and 1.43% to 4.72% for DIT and from 2.36% to 3.61% for MIT and 3.56% to 5.40% for DIT, respectively. These data were reproducible if careful adherence to constant HPLC chemicals and elution conditions were maintained.

HPLC Separation of Rat Thyroid Tissue Extract Using the same conditions as for the standard curve, we injected the rat thyroid tissue extract onto the column. As Fig. 3 shows,

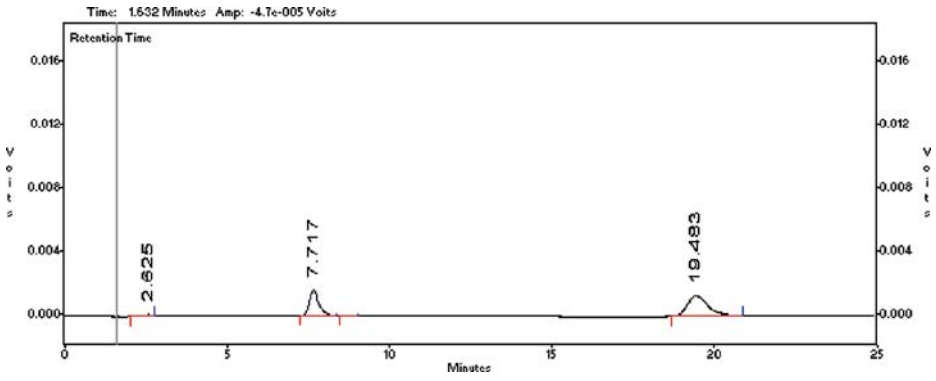

Fig. 1 Separation by HPLC of iodotyrosine standards. The column was injected with 50 μl standard working solutions. The chromatographic conditions were described in the Materials and Methods

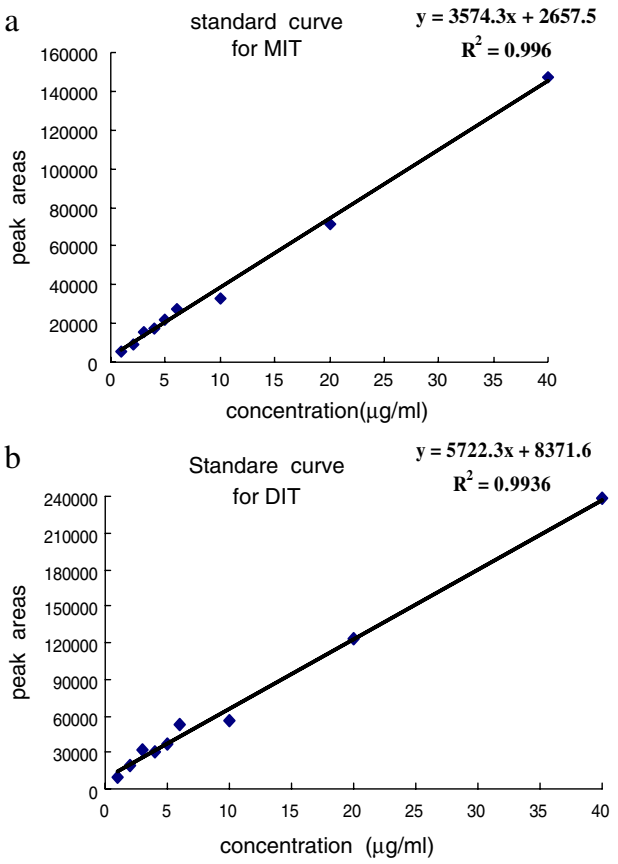

Fig. 2 Standard curve for MIT (**a**) and DIT (**b**). Standard graphs obtained by plotting peak area against concentrations of MIT and DIT. The iodotyrosine amount was 1, 2, 3, 4, 5, 6, 10, 20, and 40 μg/ml, respectively, and each point represents the average of five determinations

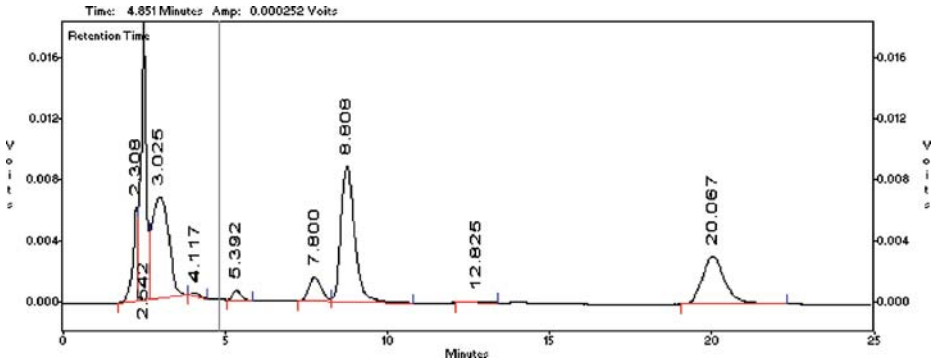

Fig. 3 Separation by HPLC of rat thyroid tissue samples. Using the same conditions as for the standard curve, MIT and DIT in rat thyroid extract were detected with retention time of 7.800 and 20.067 min. Tyrosine and tryptophan were eluted at 5.392 and 8.808 min, respectively. Therefore, they did not interfere with the iodotyrosine assay. The several peaks appeared between 1 and 4 min partly reflected the solvent front

MIT and DIT display regular and well-defined peaks. They were detected with retention times similar to the standards (7.800 min for MIT and 20.067 min for DIT). Although tyrosine and tryptophan were eluted along with MIT from the resin, these aromatic amino acids did not interfere with the iodotyrosine assay.

There were also several unidentified peaks that migrated faster (between 1 and 4 min) than tyrosine, which may partly reflect the solvent front. The thyroid extract obviously contains some contaminants, but they all come off at the solvent and do not elute near the iodotyrosines. These unknown peaks may have been the artifacts of the digestion procedure or protein-resistant peptides and glycopeptides that contained tyrosyl and tryptophanyl residues [16].

Retention time of MIT and DIT was reproducible and consistent with an array of sequential experiments of biological samples carried out on the same or different days. Differences between retention times were always lower than 10 s, permitting a precise correlation between MIT or DIT peak and its retention. By using internal standards, the percentage of recovery was determined from 90% to 101% for MIT and from 86% to 106% for DIT. This demonstrates the reliability of this procedure.

Determination of Iodotyrosines in Rat Thyroid Tissue The MIT and DIT content in biological samples were calculated based on the standard linear calibration curves. As Fig. 4 shows, compared with NI groups, the biosynthesis of both MIT and DIT was increased in the case of iodine excess. The amount of MIT reached its maximum in 50HI at 3 months, 10HI at 6 months, and 5HI at 12 months. On the other hand, DIT formation was relatively favored over MIT formation in HI groups, presented as the tendency of decreasing in MIT/DIT ratio with increased does of iodine intakes.

Determination of Dio1 mRNA Level Using Real-Time RT-PCR

Figure 5 shows the real-time PCR standard curve for the D*io1* gene. A linear relationship was observed between the input copy number of the template and the Ct values. The regression coefficients (r^2) were 0.9997.

The specificities of the products amplified by SYBR Green PCR were monitored by analyzing the amplification profiles and the corresponding dissociation curves of each amplicon. Figure 6 provides examples of amplification profile and the corresponding dissociation curve of the D*io1* gene product. As expected, a dissociation curve with a single peak at the melting temperature of the amplicon was obtained.

As Fig. 7 shows, D*io1* mRNA expression was down-regulated in all HI groups compared with NI groups. There was a tendency of decreasing in D*io1* mRNA expression with increased doses of iodine intake. A significant difference was found in 50HI and 100HI at 3 months, 100HI at 6 months, and all HI groups at 12 months.

Thyroidal D1 Activity Assay

Compared with NI groups, thyroidal D1 activity was decreased in HI groups at three different months. There was an obvious tendency of decreasing in D1 activity with increased doses of iodine intakes. When fed with excess iodine for 6 and 12 months, rats of 50HI and 100HI have markedly lower D1 activity levels. However, there was no significant difference between HI groups and NI groups at 3 months (Fig. 8).

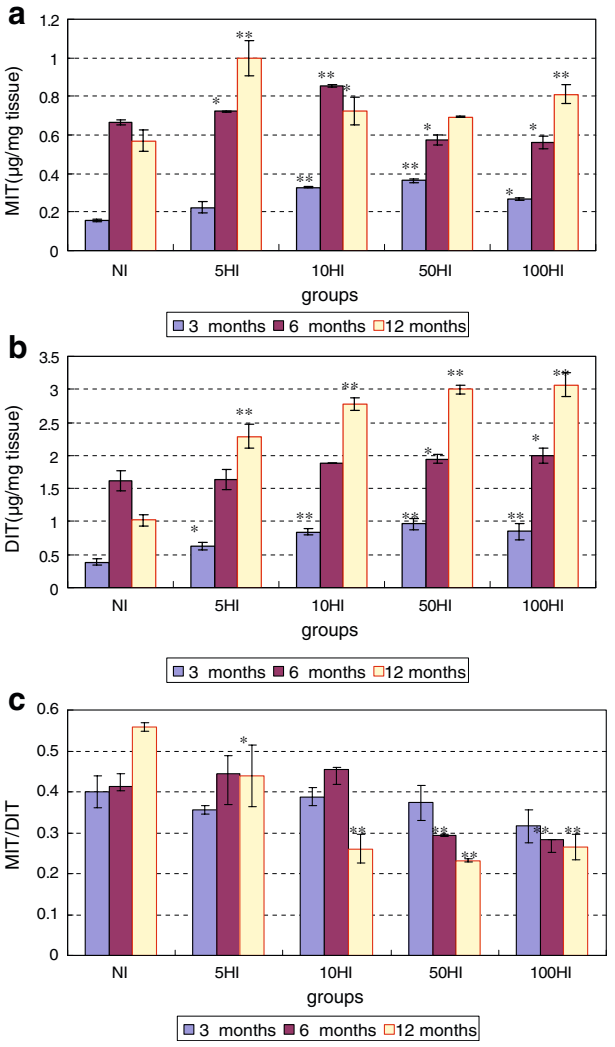

Fig. 4 The content of MIT (**a**), DIT (**b**), and MIT/DIT (**c**) in rat thyroid with different iodine intakes. Data are shown as mean ± SD. $n=5$ rats/group. P value indicates difference between groups by analysis of variance. *$P<0.05$, **$P<0.01$, vs NI groups

Determination of Total T4 and T3 Content of the Thyroid Gland

In all three different intervention time groups, T_3 was decreased in all HI groups compared with the NI groups. Significant difference was found in 10HI, 50HI, and 100HI at 3 months; 5HI and 100HI at 6 months; and 50HI and 100HI at 12 months. However, there was no difference of T_4 in thyroid tissue between HI groups and NI group, except for 100HI at 6 and 12 months. T_3/T_4 ratio was significantly decreased at 3 and 6 months (Fig. 9).

Discussion

Iodine is an essential component for the synthesis of thyroid hormones. Thyroid function ultimately depends on appropriate iodine supply to the gland. Thyroid concentrates iodide

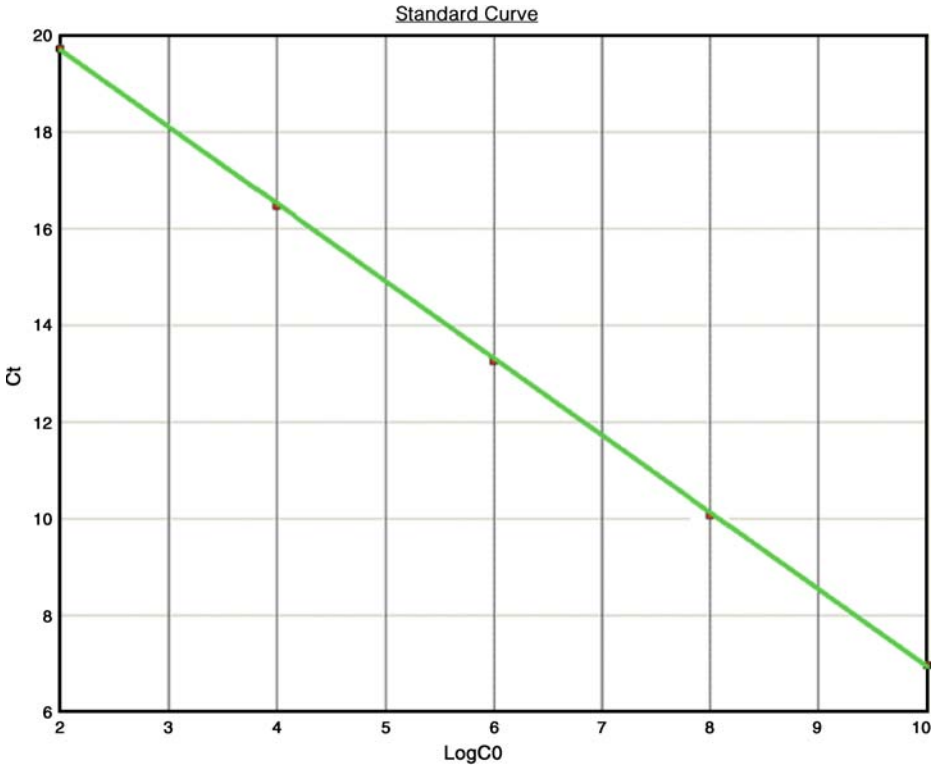

Fig. 5 Calibration curve for Dio1 mRNA using the SYBR green PCR analysis. Standard curve plotting the log of the input amount vs the threshold cycle (Ct) was determined as described in the Materials and Methods. The threshold cycle represents the PCR cycle at which an increase in reporter fluorescence above a baseline signal was first detected

from the serum and oxidizes it at the apical membrane, attaching it to tyrosyl residues within thyroglobulin (Tg) to make MIT and DIT. Then, two residues of DIT couple to make T_4, or one DIT and one MIT to make T_3, all still within the Tg molecule [17]. Though MIT and DIT are the inactive precursors of thyroid hormones, they contain about two-third of Tg's iodine. Under conditions of NI supply and thyroid activity, a Tg molecule contains about 2.5 residues of T_4, 0.7 residues of T_3, 4.5 residues of DIT, and five residues of MIT [3]. Thus, MIT and DIT represent the most abundant iodoamino acids. Therefore, effects of different iodine intake levels on the thyroid will be reflected by a change in the relative amount of these iodotyrosines. A convenient and reliable method is thus required to quantitate them in thyroid in order to further investigate the effects of iodine intake on thyroid function and how it possibly works.

However, the quantitation of iodotyrosines, especially from biological samples, has been a complicated study in the thyroid research field. In the last few years, iodotyrosines have been analyzed by numerous chromatographic, spectrophotometric, and competitive radioassay procedures [18–20]. Among them, HPLC has been more frequently used and gives better results than any other method due to its high efficiency and reproducibility. Under various conditions, purified standards of iodotyrosines and iodothyronines have been separated by HPLC [21–25]. Although these methods may have useful chemical and biomedical applications, they suffer from a lack of sensitivity and require a relatively large

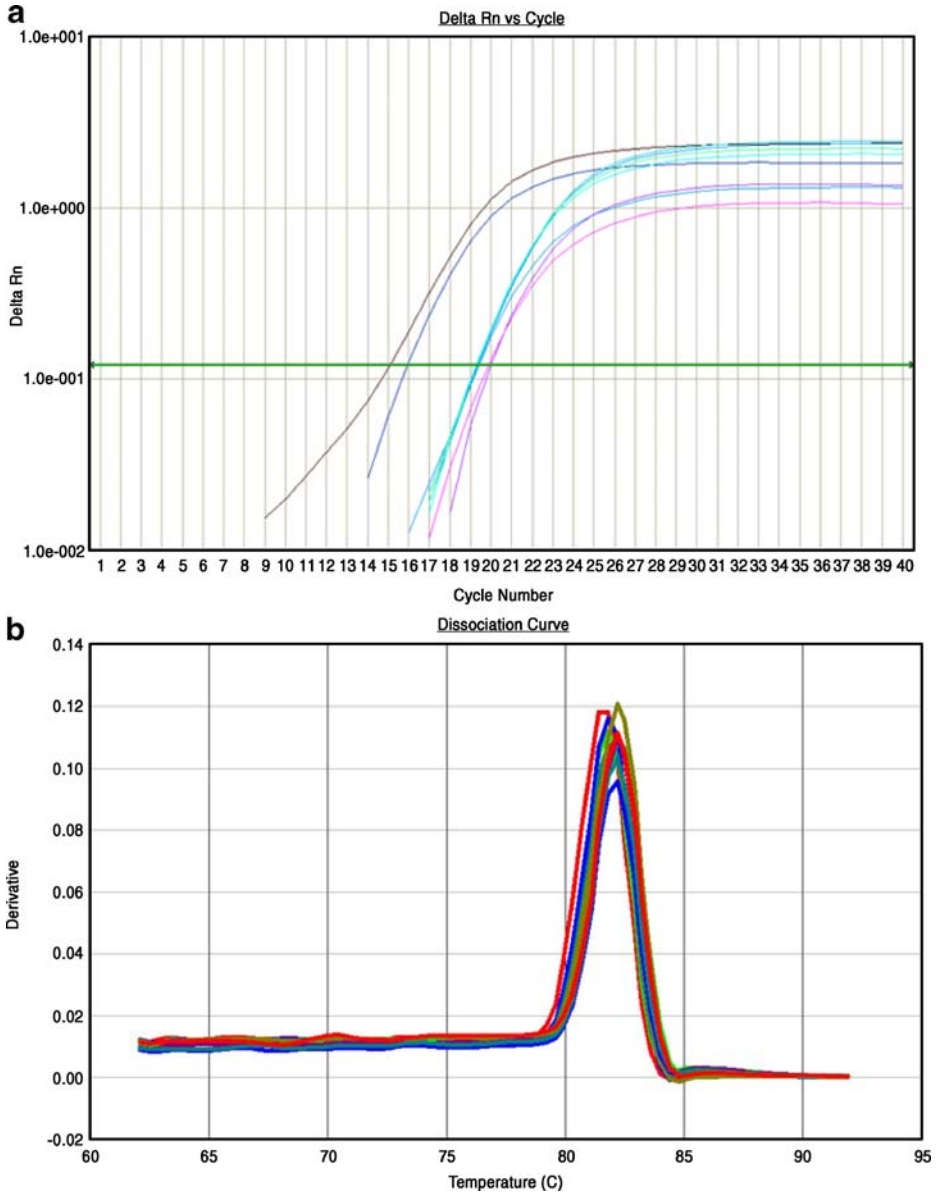

Fig. 6 Amplification profiles (**a**) and dissociation curves (**b**) of Dio1 gene. For every SYBR green PCR amplification described in this paper, samples were routinely analyzed by their amplification profile and the dissociation curve to ensure the specificity of the intended product

amount of substrate for reliable detection. The more sensitive methods require a previous treatment, such as derivatization and transformation of the samples [26]. This is time-consuming and makes it impossible to recover active iodotyrosines for future studies of biological samples in clinical assays. Therefore, these reported methods were found to be inappropriate for our needs.

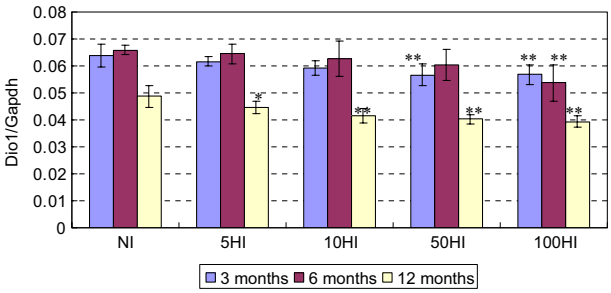

Fig. 7 The expression of Dio1 mRNA in thyroid in rats with different iodine intakes. The initial copy numbers of D*io1* with that of G*apdh* was compared to determine the expression levels of D*io1* mRNA. Data are shown as mean ± SD. n=6 rats/group. P value indicates difference between groups by analysis of variance. *P<0.05, **P<0.01 vs NI groups

The procedure we describe here seems particularly convenient because of its high sensitivity, reproducibility, and rapidity. Moreover, it needs a very small amount of thyroid tissue (5 μg) and avoids the use of radio-labeled iodine. To our knowledge, it is the first time that the tissue concentrations of MIT and DIT were directly measured in rat thyroid. In this way, this method greatly expands the thyroid researchers' potential for determining the biosynthesis of thyroid hormones from iodinated precursors.

On the other hand, MIT and DIT are not the only factors that determine T_4 and T_3 levels in the thyroid. Once T_4 and T_3 are removed from the Tg molecule by proteolysis, T_4, the main secretary product of the thyroid gland, is partly converted to its biologically active metabolite, T_3, by thyroidal D1 before leaving the cell [4, 5]. In fact, the intrathyroid conversion of T_4 to T_3 may account for much more of the T_3 released by thyroid rather than its de novo synthesis [27–29]. Therefore, it can be ascertained that thyroidal D1 plays a critical role in regulating the balance of different thyroid hormone species inside the thyroid gland, although it is still doubtful whether the skeletal muscle D2 activity could be responsible for a major part of the peripheral T_4 to T_3 deiodination in the rats [6, 30, 31].

Recently, several methods have been used to detect tissue D1 activity [8, 32–36]. The method described here has been proved to be reliable, reproducible, and sensitive. Sizes as small as 20 μl of tissue homogenate were used for assay, making this procedure especially useful for the analysis of small tissues such as thyroid [15].

Results showed in this study indicate that, in the case of iodine excess, the biosynthesis of both MIT and DIT, especially DIT, was increased. There was an obvious tendency of decreasing in MIT/DIT ratio with increased doses of iodine intake. In addition, iodine excess greatly inhibited thyroidal D1 activity and D*io1* mRNA expression. Therefore, as revealed by thyroid tissue hormone assay, T_3 was greatly lower in the HI group, while there was no significant difference of T_4 compared with the NI group. The T_3/T_4 ratio was decreased in HI groups, antiparalleled with increased doses of iodine intake. As we know,

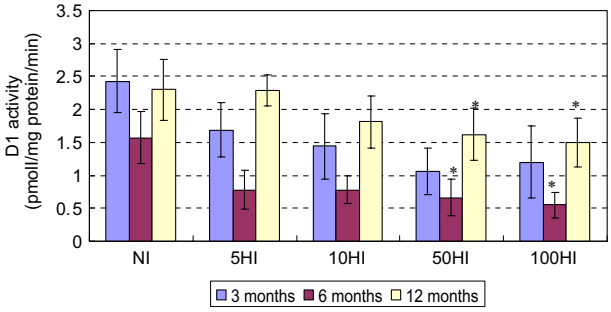

Fig. 8 Thyroidal D1 activity in rat with different iodine intakes. Data are shown as mean ± SD. n=6 rats/group. D1 activity was determined using optimal assay conditions (see Materials and Methods). P value indicates difference between groups by analysis of variance. *P<0.05 vs NI groups

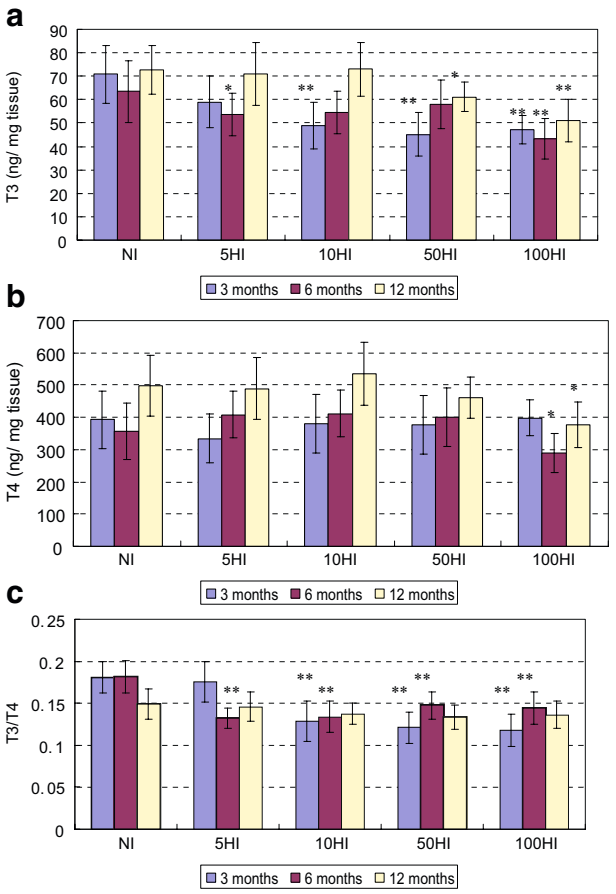

Fig. 9 Thyroid tissue hormone (**a** T_3, **b** T_4, **c** T_3/T_4) in rats with different iodine intakes. Data are shown as mean ± SD. $n=10$ rats/group. P value indicates difference between groups by analysis of variance. $^*P<0.05$, $^{**}P<0.01$, vs NI groups

the classical effects of thyroid hormones are exerted by binging T_3 to its specific nuclear receptors, thereby influencing the expression of target genes. Therefore, the increased biosyntheses of DIT relative to MIT and the inhibition of thyroidal D*io1* mRNA expression and D1 activity may be taken as an effective way to protect organisms from impairment caused by too much T_3 [37, 38].

Physiologically, thyroid function is controlled by two pathways, i.e., the hypothalamic–pituitary–thyroid axis regulation and thyroid autoregulation [39]. Our data show that the fluctuation of iodine intake will first result in the activation of thyroid autoregulation. The control of biosynthesis of thyroid hormone precursors and conversion of T_4 to T_3 is an effective way to maintain normal thyroid function. Wistar rats exhibit strong tolerance to iodine excess through adaptation mechanism [40].

In summary, different iodine intakes will impact on MIT and DIT biosynthesis, thyroidal D1 activity, and D*io1* mRNA expression to different degrees. The experiment needs to be extended, with a constant eye on how the results translate to the normal physiology of the intact thyroid. The convergence of biochemical, molecular biological, and nutritional studies on iodine and thyroid hormone metabolism has yielded considerable advancements in our understanding of the thyroid function regulation mechanism. We hope our study can provide new insights into the cellular regulation mechanism of thyroid hormones under

physiological and pathological conditions, with implications ranging from the basic to clinical research.

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