Regional physiological adaptation of the central nervous system deiodinases to iodine deficiency

ROBIN PEETERS,1 CSABA FEKETE,2 CARLA GONCALVES,1 GABOR LEGRADI,2 HELEN M. TU,1 JOHN W. HARNEY,1 ANTONIO C. BIANCO,1 RONALD M. LECHAN,2 AND P. REED LARSEN1

1Thyroid Division, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston 02115; and 2Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine, Department of Medicine, New England Medical Center-Tufts University School of Medicine, Boston, Massachusetts 02111

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Regional physiological adaptation of the central nervous system deiodinases to iodine deficiency. Am J Physiol Endocrinol Metab 281: E54–E61, 2001.—The goal of the present investigation was to analyze the types 2 (D2) and 3 (D3) iodothyronine deiodinases in various structures within the central nervous system (CNS) in response to iodine deficiency. After 5–6 wk of low-iodine diet (LID) or LID + 2 μg potassium iodide/ml (LID+KI; control), rats’ brains were processed for in situ hybridization histochemistry for D2 and D3 mRNA or dissected, frozen in liquid nitrogen, and processed for D2 and D3 activities. LID did not affect weight gain or serum triiodothyronine, but plasma thyroxine (T4) was undetectable. In the LID+KI animals, D3 activities were highest in the cerebrum cortex (CO) and hippocampus (HI), followed by the olfactory bulb and was lowest in cerebellum (CE). Iodine deficiency decreased D3 mRNA expression in all CNS regions, and these changes were accompanied by three- to eightfold decreases in D3 activity. In control animals, D2 activity in the medial basal hypothalamus (MBH) was similar to that in pituitary gland. Of the CNS D2-expressing regions analyzed, the two most responsive to iodine deficiency were the CO and HI, in which an ~20-fold increase in D2 activity occurred. Other regions, i.e., CE, lateral hypothalamus, MBH, and pituitary gland, showed smaller increases. The distribution of and changes in D2 mRNA were similar to those of D2 activity. Our results indicate that decreases in the expression of D3 and increases in D2 are an integral peripheral component of the physiological response of the CNS to iodine deficiency.

thyroid; selenium; goiter; nutrition; trace element; development; growth

IODE IS AN ESSENTIAL COMPONENT of thyroid hormones. The thyroglobulin-derived iodothyronine molecules contain three or four iodine atoms that are covalently bound during iodide organification. This process, also known as iodination, is catalyzed by thyroid peroxidase and requires that the thyroid cell concentrate iodide from plasma (19). Iodine is readily available from the ocean, and salt-water vertebrates, the first life forms to develop a thyroid gland, are not at risk for iodine deficiency. However, in terrestrial vertebrates, including humans, iodine availability can be rate limiting depending on the proximity to the ocean and the iodine content of the soil. Fortunately, a multiplicity of physiological mechanisms has evolved to mitigate the consequences of iodine deficiency on thyroid hormone synthesis. In fact, these mechanisms can be so efficient that a recent survey indicates that nearly 2.3 billion people can live in geographical areas with low-iodine soil content (8). In areas of extreme deficiency, however, iodine intake is so low that the compensation mechanisms are inadequate. The consequence of this is the iodine deficiency disorders, including hypothyroidism, irreversible mental retardation, goiter, reproductive failure, increased infant mortality, and socioeconomically hardships.

When rats are placed on a low-iodine diet (LID), the hypothalamic-pituitary-thyroid axis undergoes a series of rapid physiological adaptations to maintain the delivery of thyroid hormones to tissues. The changes in thyroidal iodothyronines have been well described and are designed to maintain plasma triiodothyronine (T3) in the normal range (1, 12, 26, 27). This assures that most target tissues are only mildly affected during moderate iodine deficiency. As an example, no differences in O2 consumption or thermal homeostasis were detected in iodine-deficient rats (33). In addition, growth and relative weight of various organs are not affected in rats with moderate iodine deficiency despite an ~10-fold higher thyroid-stimulating hormone (TSH) and nearly undetectable plasma thyroxine (T4) (25). Not surprisingly, however, if iodine deficiency is severe and prolonged, signs of hypothyroidism will develop, i.e., reduced O2 consumption (24) and liver α-glycerophosphate dehydrogenase (24) and malic en-
zyme activities (23, 31), indicating a finite capacity of adaptive physiological mechanisms.

In addition to the thyroidal changes, in some tissues including brain, pituitary gland, and brown adipose tissue (BAT), intracellular T₃ may be regulated in a more complex manner, since the intracellular production of T₂ from T₄ catalyzed by type 2 iodothyronine deiodinase (D2) occurs in these tissues. The activity of this enzyme is increased during iodine deficiency or hypothyroidism (5, 13, 20, 22), thus increasing the fractional conversion of T₄ to T₃. We (40) and others (14, 28) have demonstrated that D2 activity and mRNA are heterogeneous within the rat central nervous system (CNS) and that D2 is especially concentrated in the hypothalamus, particularly in the tanyocytes and the arcuate nucleus–median eminence region. Recently, D2 mRNA was also shown to peak dramatically around postnatal day 7 in the mouse cochlea, a structure known to be exquisitely sensitive to thyroid hormones (4).

All of these results point to the potential for region-specific regulation of intracellular T₃ production in the CNS.

The CNS has yet another potential physiological mechanism for adaptation to iodine deficiency. A second deiodinase, type 3 (D3), which inactivates T₄ and T₃, is also expressed in this tissue. D3 is a T₃-dependent gene (37, 41) and is positively correlated with thyroid status (16). Despite its critical role, it was only recently demonstrated that its distribution in the CNS, like that of D2, is heterogeneous with high focal expression in the hippocampal pyramidal neurons, granule cells of the dentate nucleus, and layers II-VI of the cerebral cortex (9, 41). These data imply that there may also be region-specific regulation of D3.

Much of our understanding of adaptive regulation of selenodeiodinases is derived from studies in animals with altered thyroid status. During hypothyroidism, there is an increase in D2 activity in various regions of the CNS (28), and the opposite is observed for D3 mRNA levels (41). However, hypothyroidism is observed only in moderate-to-severe iodine deficiency (31). In addition, given the exquisite sensitivity of D2 to reductions in plasma T₄, it is likely that an increase would occur long before the onset of hypothyroidism (21). From a teleological point of view, primary hypothyroidism due to autoimmune disease is extremely rare compared with iodine deficiency and is unlikely to be the condition for which these peripheral physiological- adaptive mechanisms evolved.

The goal of the present investigation was to document changes in D2 and D3 mRNA levels and activities within specific structures of the CNS of iodine-deficient rats. Our results show marked regional alterations in the expression of both enzymes, illustrating that increases in D2 combined with compensatory decreases in D3 are an integral peripheral component of the tissue physiological response of the vertebrate to iodine deficiency.

### MATERIAL AND METHODS

**Animals and diets.** Unless specified otherwise, all drugs and reagents were purchased from Sigma Chemical (St. Louis, MO). Experiments were performed on male Sprague-Dawley rats weighing 100–125 g obtained from Harlan (Madison, WI). Studies were conducted in accordance with the highest standards of humane animal care under a protocol approved by the Standing Committee on Animal Research. Animals were maintained on a 12:12-h light-dark cycle at 21°C and had free access to diet and water. Some animals were placed on a Remington low-iodine diet (LID; Harlan) during 5–6 wk with supplemental 0.5% sodium perchlorate in the drinking water during the 1st wk to induce rapid depletion of thyroidal iodine. The control group (LID supplemented with potassium iodide (LID+KI)) received the same diet with supplemental 2 µg KI/ml in the drinking water.

**In situ hybridization histochemistry.** All animals (n = 7 in each group) were deeply anesthetized with pentobarbital sodium (50 mg/kg) and perfused through the heart first with saline followed by 4% paraformaldehyde in PBS for 20 min. The brains were removed and immersed in 20% sucrose in PBS overnight. Blocks of the forebrain were frozen on dry ice and sectioned in the coronal plane at 18 µm on a cryostat. Sections were mounted onto Superfrost Plus glass slides (Fisher) and stored at −80°C until subjected to hybridization. The hybridization protocols were based on methods previously reported from our laboratories (40, 41). For D2 mRNA, tissue sections were hybridized with a single stranded [35S]UTP-labeled cRNA probe generated from pH7-1575. The rD2 fragment contains a portion of 5′ flanking region (FR) and the entire 798 nt of coding region (kindly provided by Drs. D. St. Germain and V. A. Galton, Dartmouth Medical School, Lebanon, NH). As a control, the same plasmid was used to generate sense RNA with T₃ RNA polymerase. Hybridization was performed under plastic coverslips in buffer containing 50% formamide, a twofold concentration of standard sodium citrate (2× SSC), 10% dextran sulfate, 0.25% BSA, 0.25% Ficoll 400, 0.25% polyvinylpyrrolidone 360, 250 mM Tris (pH 7.5), 0.5% sodium pyrophosphate, 0.5% sodium dodecyl sulfate, 250 µg/ml denatured salmon sperm DNA, and 6 × 10⁶ cpm of the radiolabeled probe for 16 h at 55°C. Slides were dipped into Kodak NTB2 autoradiography emulsion (Eastman Kodak, Rochester, NY), and the autoradiograms were developed after 7 days of exposure at 4°C.

For D3 mRNA, tissue sections were hybridized with a single-stranded [35S]UTP-labeled cRNA probe generated from a linearized 0.84-kb rD3 Cys no. 4 cDNA in pHT-1600. The rD3 fragment contains a mutation in its coding region resulting in a cysteine codon in place of the TGA selenocysteine codon. This fragment also contains no 5′ FR, 833 bp of coding region, and 5 bp of 3′ FR including the stop codon. The original rD3 cDNA was the kind gift of Drs. D. St. Germain and V. Galton (37). The sense probe was made by transcribing with T₃ RNA polymerase and the antisense probe by transcribing with T₄ RNA polymerase. The rat D3 antisense probe contained the entire 833-nt coding region and 5 nt of 3′ untranslated region. Hybridization was performed as above, and the autoradiograms were developed after 7 days of exposure at 4°C. All sections were cleared in graded solutions of ethanol and Histosol, and the sections were coverslipped in Histomount. The autoradiograms were visualized and photographed under dark-field illumination with a Spot digital camera attached to a Zeiss Axioskop 2 binocular microscope.

**Analytical procedures.** At the end of the experimental period, rats were killed by decapitation, the brains were...
rapidly removed, and specific regions were dissected and frozen in liquid nitrogen, as previously described (28). D2 activity was measured as previously described (29) using two different protein concentrations (4-fold difference for each sample), depending on the cerebral structure, i.e., 2–30 mg for medial basal hypothalamus (MBH), 90–500 mg for lateral hypothalamus, 60–400 mg for hippocampus, 60–350 mg for cerebral cortex, 75–370 mg for cerebellum, and 2–10 mg for pituitary gland. For D3 activity, 80–100 mg of protein was assayed during 2 h at 37°C as described (30). The reaction was stopped by the addition of 200 ml of normal horse serum and 100 ml of TCA, and the supernatant was used for determination of the [125I]T2 and [125I]T1 produced. This was done after the supernatant was loaded onto a 2-ml LH-20 column and washed subsequently with water and 20% [125I]-T1 and 50% [125I]-T2 ethanol. Nonspecific deiodination was estimated similarly as for D2 and was always <1.5%.

Blood was collected, and serum concentrations of T4 and T3 were measured by RIA by the Clinical Research Center at the Brigham and Women’s Hospital.

Statistical analysis. Results are expressed as means ± SD throughout the text, table, and figures. Comparisons were done by Student’s t-test.

RESULTS

Animals from both groups, LID and LID+KI, gained ~100 g body wt during the 5- to 6-wk experimental period, with body weights that were not significantly different (305 ± 13 vs. 286 ± 25 g; P > 0.05) at the completion of the study. On the other hand, rats on LID without supplemental KI increased their thyroid gland weight about threefold (Table 1). Iodine deficiency reduced serum T4 to undetectable levels, but the 30% decrease in serum T3 was not significant (Table 1; P > 0.05). In both groups, D2 and D3 activities were quantified in cerebral cortex, hippocampus, cerebellum, and pituitary gland. In addition, D3 activity was measured in olfactory bulb, and D2 activity was quantified in the MBH and lateral hypothalamus. Other animals from the same groups were also studied for changes in D2 and D3 mRNA by in situ hybridization histochemistry to allow qualitative estimates of the changes in the mRNAs encoding these proteins.

In control animals supplemented with KI, D3 activities (Fig. 1A) were highest in the cerebral cortex and hippocampus, followed by the olfactory bulb and cerebellum. No D3 activity was detected in the pituitary gland. Iodine deficiency caused a marked decrease in the expression of D3 in all CNS regions analyzed. As

![Fig. 1. Type 3 iodothyronine deiodinase (D3) activity in various regions of the central nervous system (CNS) of rats fed a low-iodine diet (LID) or LID supplemented with KI (LID+KI). Immediately after the animals were killed, the CNS was carefully dissected, and various regions, i.e., cerebral cortex (CO), hippocampus (Hi), cerebellum (CE), and olfactory bulb (OLF) were frozen in liquid N2 and later processed for D3 activity. A: absolute D3 activity; B: ratio of D3 activity LID to LID+KI. *P < 0.05 vs. LID+KI by Student’s t-test.](http://ajpendo.physiology.org/)

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### Table 1. Thyroid weight and thyroid hormone serum concentration of rats fed LID or LID + KI

<table>
<thead>
<tr>
<th></th>
<th>LID + KI</th>
<th>LID</th>
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<tbody>
<tr>
<td>Thyroid weight, mg</td>
<td>25 ± 11(7)</td>
<td>67 ± 21(7)</td>
</tr>
<tr>
<td>mg/100 g body wt</td>
<td>8.2 ± 2.9(7)</td>
<td>23 ± 6.9(7)</td>
</tr>
<tr>
<td>Serum T4, ng/ml</td>
<td>27.2 ± 9.5(4)</td>
<td>&lt;5 (5)</td>
</tr>
<tr>
<td>Serum T3, ng/ml</td>
<td>0.94 ± 0.09(4)</td>
<td>0.68 ± 0.36(5)</td>
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</table>

Values are means ± SD. The number of animals in each group is shown in parenthesis. LID, low-iodine diet; KI, potassium iodide. T4, thyroxine; T3, triiodothyronine. *P < 0.05 vs. LID + KI.
shown in Fig. 1B, D3 activity in the iodine-deficient animals was reduced five- to eightfold relative to that in LID+KI animals, except in the olfactory bulb, where it was decreased to ~40% of control.

The pattern of distribution of D3 mRNA, as detected by in situ hybridization, paralleled the expression of D3 activity. D3 mRNA was distributed in a diffuse pattern over the forebrain in control animals but was

![Image](image1)

**Fig. 2.** In situ hybridization autoradiograms of D3 mRNA in rats fed LID+KI (A and C) or iodine-deficient LID (B and D) rats. Note the distribution of silver grains with particularly high concentrations in Hi, dentate (A) and CO (B). Iodine deficiency decreases D3 mRNA in all regions. Magnification ×50. More anatomical details and landmarks can be found elsewhere (41).

![Image](image2)

**Fig. 3.** Type 2 iodothyronine deiodinase (D2) activity in various regions of the CNS of rats fed LID or LID+KI. Samples were obtained and processed as in the legend to Fig. 1. The regions also included pituitary gland (Pit), medial basal hypothalamus (MBH), and lateral hypothalamus (LH). A: absolute D2 activity; B: ratio of D2 activity LID/LID+KI. *P < 0.05 vs. LID+KI by Student's t-test.
particularly apparent in the hippocampal pyramidal cells, granule cells of the dentate (Fig. 2A), and layer II of the pyriform cortex (Fig. 2C). In the iodine-deficient rats, the D3 mRNA hybridization signal was reduced throughout (Fig. 2, B and D). However, the regional distribution was maintained.

D2 activity was ~10-fold higher in MBH than in any other CNS region analyzed, including the immediately adjacent lateral hypothalamus (Fig. 3A), due to the presence of the tanycytes in the region (40). D2 activity in MBH was not different from that in the pituitary gland. Of the brain regions examined for D2 activity, the two most responsive D2-expressing structures to iodine deficiency were the cerebral cortex and hippocampus, in which an ~20-fold increase in D2 activity occurred. Five- to tenfold increases in D2 activity were detected in cerebellum, lateral hypothalamus, MBH, and pituitary gland of the animals with iodine deficiency (Fig. 3B).

By in situ hybridization histochemistry, silver grains denoting the location of D2 mRNA were distributed widely but in low abundance throughout the forebrain and were only marginally greater than background. In the tuberal region of the hypothalamus, however, hybridization was intense and primarily concentrated between the rostral pole of the hypothalamic median eminence and the infundibular recess. Three different rostrocaudal sections of the hypothalamus are shown in Fig. 4. Hybridization of the D2 mRNA is seen in the most rostral region at the external zone of the median eminence (Fig. 4A), whereas a midsection shows D2 mRNA hybridization in the floor of the third ventricle (Fig. 4C). In the most caudal region, silver grains accumulated over cells lining the infralateral wall and floor of the third ventricle and extended into the stalk median eminence, closely associated with portal vessels (Fig. 4E). Interestingly, hybridization was also found in
DISCUSSION

This is the first comparative analysis of the effects of moderately severe iodine deficiency on the physiological response of the iodothyronine deiodinases in discrete subregions of the CNS. The expression of D2 and D3 in brain is well documented as are the changes induced by alterations in thyroid status in this organ as a whole (15, 16). The results indicate that the physiological response to iodine deprivation in the cerebral cortex and hippocampus is a combination of 8- to 20-fold increases in D2 activity and 3- to 8-fold decreases in that of D3 (Figs. 1–4). Parallel, but less dramatic, changes occur in cerebellum, and qualitatively similar effects are observed in the mRNAs encoding these two enzymes by in situ hybridization. These changes are in the same direction but much greater than the approximately threefold alterations observed in the whole brain (22).

The regional specificity of the changes indicates that deiodinase regulation is a specific physiological adaptation of the CNS to this challenge. Previous data obtained by in situ hybridization show that D3 mRNA is expressed at its highest levels in the cerebral cortex and hippocampus. In fact, of the brain regions examined, these are the regions where the highest D3 activities were found in the iodine-deficient rats, indicating that the D3 message is being translated into active protein. Furthermore, it is known that D3 mRNA and activities are regulated by thyroid status being markedly increased in hyperthyroid rats and decreased relative to euthyroid levels in hypothyroid rats (16, 41). In addition, iodine deficiency has been shown to modulate D3 activity in the CNS. Both total fetal (32) and adult (22) rat brains respond to iodine deficiency by decreasing D3 activity, but only modest (2-fold) reductions were observed. By focusing on specific subregions of the CNS, we found that D3 activity is decreased by 80–90% in the cerebral cortex, hippocampus, and cerebellum, changes of a much higher magnitude than occur in the brain in general. The consequences of the marked fall in D3 activity are twofold. First, there will be an increase in the residence time of T3 within the tissue, because the rate of T3 degradation via inner-ring deiodination will be reduced. Second, because T4 is also a substrate for this enzyme, relatively more of this prohormone will remain within the tissue for conversion to T3 by D2. Particularly in tissues such as brain, where the exchange of T3 with plasma is slow and where most of the T3 is generated in situ, it is likely that fluctuations in the rate of T3 degradation will have a greater influence on tissue levels of T3 than will occur in tissues that are in rapid equilibration with plasma, such as liver and kidney (7). This prediction has been borne out using dual-labeling in vivo techniques in which the disappearance of tracer T3 from cerebral cortex and cerebellum was found to be significantly slower in hypothyroid rats, a situation where CNS D3 is also decreased (36).

As can be appreciated from the in situ hybridization results (Fig. 4), there was an increase in D2 mRNA in iodine-deficient animals, especially within those subregions of the brain expressing high D2 activity. An increase in D2 mRNA would be expected from the negative regulation of transcription of D2 by T3 (17). However, the increases in D2 activity of 20-fold in the cerebral cortex and hippocampus appear to be much greater than those in D2 mRNA (Fig. 3 vs. Fig. 4). This discrepancy is analogous to results recently reported in hypothyroid rat cerebral cortex in which a 1.7-fold increase in D2 mRNA was associated with a 4.6-fold increase in D2 activity (3). The much greater increase in D2 activity than in D2 message can be explained by the hypothryoxinemia of iodine deficiency per se acting at a posttranslational level. The mechanism by which T4, the preferred substrate for D2, reduces D2 protein levels is a consequence of a substrate-induced increase in the rate of D2 ubiquitination and subsequent proteasomal degradation (11, 38, 39). When plasma T4 falls, D2 half-life is prolonged, leading to an increase in the D2 protein-to-mRNA ratio. During modest reductions in iodine intake, the increased D2 will increase the fractional rate of T4 to T3 conversion, thus maintaining T3 production despite a decrease in T4.

The differences in the magnitude of the changes in D2 and D3 activities in different subregions (Figs. 1–4) suggest that the responses to iodine deficiency within the CNS are region specific. Previous studies in iodine-deficient rats have examined only the global changes in these activities in the entire brain, which may mask large changes in small regions. For example, we confirmed that the MBH has the highest level of D2 activity of any CNS structure analyzed, comparable to that in pituitary (28). However, the increases in D2 activity during iodine deficiency in both of these tissues are of far less magnitude than are those in cerebral cortex and hippocampus (Fig. 3B). This suggests either differential sensitivity of these regions to the same stimulus or regional differences in the supply of T4 or T3 within the CNS. Teleologically, one might argue that, because the hypothalamus and pituitary are critical for the control of TSH secretion, which must be increased as part of the central physiological response to iodine deficiency, the lower magnitude of the D2 response is appropriate. In addition, Koenig et al. (18) have shown that D2 activity in populations of pituitary cells relatively enriched in thyrotrophs is the least responsive to the absence of T3.

The increased fractional production of T3 from T4 by D2 combined with the prolonged residence time of T3 will mitigate the effects of iodine deficiency, as has
been demonstrated in mild-to-moderate hypothyroidism by tracer studies (34). These predictions were confirmed directly by Campos-Barros et al. (5), who measured thyroid hormone concentrations in various regions of the CNS in iodine-deficient rats. As expected, tissue T₄ was markedly decreased, whereas tissue T₃ concentrations were reduced by only 50%. This illustrates the effectiveness of these compensatory mechanisms.

The CNS is an unusual tissue with respect to thyroid function, since the occupancy of the nuclear T₃ receptors in these tissues is ~80–90% (7). Similar high T₃ receptor saturation provided by the action of D₂ is seen in pituitary and also in BAT during cold stress (6, 35). However, nuclear T₃ receptors in the remainder of peripheral tissues are only 50% saturated, since plasma T₃ is the primary source of receptor-bound T₃ in these tissues. The decrease of ~50% in CNS T₃ in the aforementioned iodine-deficient animals would reduce nuclear T₃ receptor occupancy to ~45%. Thus, despite all of the compensatory mechanisms that occur, the CNS is modestly hypothyroid. This is the cause of the decrease in D₃ mRNA and activity. However, this reduction in D₃ can also be viewed as a second line of defense for an amelioration of the hypothyroidism. The requirement for high T₃ receptor saturation for normal D₃ synthesis in brain can explain this increase. It suggests that, at least for this T₃-dependent parameter, the partial desaturation of the receptors decreases D₃ gene transcription. Interestingly, a similar phenomenon occurs for the uncoupling protein 1 gene in BAT, for which high T₃ receptor occupancy must be achieved for optimal gene expression (2). The analysis of T₃-dependent enzymes in the cerebral cortex of neonatal rats mentioned earlier (33) suggests that certain T₃-dependent events in the cerebral cortex can be preserved even with a reduction of nuclear T₃ receptor saturation to only 40%.

In iodine deficiency, the onset of generalized hypothyroidism will depend on the severity of the challenge and the success of compensation, which may vary from tissue to tissue. The present results indicate that, even within the CNS, the adaptive mechanisms for maintaining T₃ concentrations are not generalized. Although an increase in D₂ and a decrease in D₃ activities occur throughout the CNS, there is a regional specificity in the magnitude of this response that has not been previously recognized. The increase in D₂ activity arises, in part, from an increase in D₂ mRNA but, predominantly, by an increase in D₂ protein half-life. The decrease in D₃, however, appears to parallel the fall in D₃ mRNA. These changes will mitigate the effects of modest iodine deficiency on intracellular T₃ concentrations in these tissues. Although it is difficult to examine the long-term consequences of this in experimental animals, the syndrome of endemic cretinism in geographical regions with severe iodine deficiency suggests that the residual nuclear T₃ saturation in the brain of severely iodine-deficient infants and children is not adequate to permit normal intellectual development. Thus stringent efforts must continue to increase the level of iodine intake to amounts that can provide sufficient T₄ for D₂-catalyzed T₃ production. This would allow individuals in these regions to benefit from the intrinsic physiological compensatory mechanisms in this critical tissue.

Serum concentrations of thyroid hormones were measured by the Clinical Research Center at the Brigham and Women’s Hospital.

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