

# The Expression of the Sodium/Iodide Symporter Is Up-Regulated in the Thyroid of Fetuses of Iodine-Deficient Rats

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**Is the fetal thyroid already capable to increase its iodide uptake in response to iodine deficiency? To answer this question, we analyzed the expression of the  $\text{Na}^+/\text{I}^-$  symporter and several other genes in the thyroid of rat fetuses at 21 d of gestation from control mothers presenting a mild or more severe iodine deficiency. Female rats were placed on a low iodine diet, not supplemented, or supplemented with iodide or perchlorate for 3 months. The maternal and fetal thyroidal iodide uptake was measured 24 h after injection of 10  $\mu\text{Ci}$   $\text{Na}^{125}\text{I}$  into the dams. The absolute iodide uptake of the maternal thyroid was unchanged in a low iodine diet, not supplemented, compared with one supplemented with iodide. In contrast, the fetal thyroid absolute iodide uptake of a low iodine diet, not supplemented, and one supplemented with perchlorate was decreased by 70% and 95% compared with that supplemented with iodide.  $\text{Na}^+/\text{I}^-$  symporter mRNA was detected in the fetal thyroid of supplemented with iodide and increased about 2- and 4- fold in the thyroid of fetuses from a low iodine diet, not supplemented, and one supplemented with perchlorate, re-**

**spectively.  $\text{Na}^+/\text{I}^-$  symporter expression was induced in the fetal side of the placenta in both a low iodine diet, not supplemented, and one supplemented with perchlorate; in contrast,  $\text{Na}^+/\text{I}^-$  symporter mRNA was never detected in the maternal side of the placenta. Fetal thyroid thyroglobulin and type I deiodinase mRNA contents were only significantly increased with a diet supplemented with perchlorate. Glucose transporter 4 mRNA was decreased in the fetal thyroid of both a low iodine diet, not supplemented, and one supplemented with perchlorate compared with one supplemented with iodide.**

**In conclusion, although the up-regulation of  $\text{Na}^+/\text{I}^-$  symporter expression in fetal thyroid and placenta in the low iodine diet, not supplemented group did not lead to restoration of a normal absolute iodide uptake, our data show that all adaptive and/or defending mechanisms against iodine deficiency are already present in the fetus. (Endocrinology 142: 3736–3741, 2001)**

**T**HE UPTAKE OF iodide is an essential step in thyroid hormone synthesis. This transport process is mediated by the  $\text{Na}^+/\text{I}^-$  symporter (NIS). Before the identification of NIS, the iodide transport process had already been characterized, being energy, temperature, sodium, and, above all, TSH dependent (1). The rat NIS cDNA has been cloned by Dai *et al.* (2), followed in the same year by the cloning of the human NIS (3). Antibodies directed against the C-terminal part of the protein had been used to characterize the molecular forms of rat NIS and to analyze the distribution of the protein in the rat thyroid and in FRTL-5 cells (4, 5). NIS mRNA and protein levels of FRTL-5 cells increase in response to TSH, whereas TGF- $\beta$ 1, TNF- $\alpha$  suppress the TSH-induced increase of NIS expression (6, 7). Alterations of the structure and/or expression of the NIS gene are implicated in various thyroid disorders: congenital hypothyroidism (8, 9), autoimmunity (10, 11), treatment of cancer (12, 13), and iodine deficiency (14). In a recent report, it has been suggested that a decrease in NIS expression might play a role in the escape from the acute Wolff-Chaikoff effect (15).

Abbreviations: AIN, American Institute of Nutrition; AIU, absolute iodide uptake; BAT, brown adipose tissue; D1, deiodinase type 1; MID, mild iodine diet; NIS,  $\text{Na}^+/\text{I}^-$  symporter; PII, plasma inorganic iodide; Tg, thyroglobulin.

Iodine deficiency induces goiter formation and several thyroidal changes that contribute to increase the efficiency of the synthesis of thyroid hormones (16, 17). Despite adaptive mechanisms, there are changes in circulating thyroid hormone levels. Mild iodine deficiency in rats is characterized by a lowered plasma  $\text{T}_4$  concentration, a normal  $\text{T}_3$  concentration, and normal to slightly elevated TSH levels. In moderately severe iodine deficiency, very low  $\text{T}_4$ , normal or lowered  $\text{T}_3$ , and elevated TSH levels are found (16–24) and thyroids are enlarged. During mild iodine deficiency, the absolute iodide uptake (AIU) of the thyroid of pregnant rats is not changed, but that of the fetal thyroid is decreased (25).

Thyroid hormones are essential during fetal development, especially for the brain (26–28). Fetal brain development is especially dependent on the maternal supply of  $\text{T}_4$  before its own thyroid starts to function (at d 17.5) (29, 30). After the onset of the fetal thyroid function, a large part of circulating  $\text{T}_4$  is also coming from the mother (31, 32). Not much  $\text{T}_3$  is transferred from the mother to the fetus (31). When iodine intake is low, less thyroid hormones are synthesized by the maternal thyroid, and, as a consequence, during pregnancy there will be less  $\text{T}_4$  available from the mother for the fetus (24, 28, 30).

This study was undertaken to determine whether NIS

expression is modified in the fetal thyroid, when the mother is exposed to iodine deficiency. To study this, the effect of different levels of iodine deficiency of the mother on the expression of the NIS and other genes, in fetal tissues of the rat, was investigated. The fetuses were obtained from pregnant rats at 21 d of gestation. We analyzed in the fetal thyroids NIS, glucose transporters (Glut1 and Glut4), iodothyronine deiodinase type 1 (D1), and thyroglobulin (Tg) mRNA expression. Maternal and fetal thyroïdal iodide uptake was measured 24 h after administration of 10  $\mu$ Ci Na<sup>125</sup>I to the dams. We describe clear changes in the rat fetal thyroids in response to mild to moderate iodine deficiency conditions of the mother.

## Materials and Methods

### Animals

The experiments were approved by the Local Committee on Animal Care. Three groups of rats (CPB/WU, Iffa Credo, Brussels, Belgium) were used (body weight, 210  $\pm$  5 g). The rats (n = 6 per group) were housed at 22 C, with alternating 14-h light and 10-h dark periods. They were fed the American Institute of Nutrition (AIN) diet (33), without iodide [mild iodine diet (MID)]. In one group potassium perchlorate (0.005%) was added to this diet (MID+P), and in the third group potassium iodide (1.5  $\mu$ g per day) was added to the diet (MID+I). This last group served as controls (24). After 3 months rats were mated, and the morning that sperm was present was taken as day zero of pregnancy. Rats were housed in metabolic cages for 5 d. This allowed us to collect urine over 24-h periods of time and to measure the iodine excretion according to Sandell and Kolthoff (34). At d 21 of pregnancy, rats were bled and perfused under light ether anesthesia. Fetal tissues (brown adipose tissue, brain, liver, skin, thyroid, fetal and maternal placenta, skin) were taken, and kept frozen at -70 C until used. The skin was taken from the rump, excluding that of the head, legs, and tail. The fetal and maternal (basal) sides of the placenta were isolated by separation of the two distinct layers from each other with blunt forceps on ice (24). At d 21 of pregnancy fetal and maternal iodine uptake was measured as described previously (25). Dams received an ip injection of 10  $\mu$ Ci Na<sup>125</sup>I at day 20, and were placed in metabolic cages for collection of urine. After 24 h rats were bled and perfused under light ether anesthesia. The maternal and fetal thyroids were dissected, counted, and the measured radioactivities were expressed as percentages of the injected dose. Plasma inorganic iodide (PII) and AIU were calculated from the values of the thyroid radioiodide content (percentage of the dose) and the specific activity of the maternal urinary iodide (25).

### Determinations of plasma TSH, T<sub>3</sub>, and T<sub>4</sub> in plasma

Plasma T<sub>4</sub> and T<sub>3</sub> concentrations were assayed by rat-specific RIAs (35). Plasma TSH was measured using the RIA developed for rat TSH by the NIDDK (NIH, Bethesda, MD). Reference preparation-2 was used as a standard.

### Northern blot analysis of NIS, Tg, D1, Glut1, and Glut4 mRNA levels

Total RNA was extracted with guanidine-HCl as described previously (36), using ethanol precipitation. From each experimental group three dams were used for the fetal tissues. Four to five fetal thyroids were pooled. Total recovery of RNA was 50–150  $\mu$ g. Total RNA (20–25  $\mu$ g) was denatured and subjected to electrophoresis on a 2.2-M formaldehyde 1% agarose gel in 1 $\times$  MOPS buffer and transferred to nylon membranes (Nytran, NY13N) (36). A NIS cDNA fragment (5': 561/3': 1333) of 773 bp obtained by RT-PCR from FRTL-5 mRNA, Glut 1, and 4 cDNA (provided by Dr. G. Bell, Howard Hughes Medical Institute and Department of Biochemistry and Molecular Biology and Medical University of Chicago, Chicago, IL), Tg cDNA (provided by Dr. R. Di Lauro, Stazione Zoologica, Anton DOHRN, Naples, Italy), and rat D1 cDNA (provided by Dr. P. R. Larsen, Brigham & Women's Hospital, Boston, MA) were used as probes. The cDNA probes were labeled with

[ $\alpha$ -<sup>32</sup>P]deoxy-CTP using random primers. The filters were hybridized overnight at 42 C, and autoradiographs were made. They were scanned with an Instant Imager (Packard, Downers, Grove, IL) and quantified using the software packet Instant Quant (Packard). The 18S ribosomal RNA or cyclophilin mRNA were used to correct for differences of RNA loading.

### Statistics

Values are given as the mean  $\pm$  SEM. Statistical analysis was performed by ANOVA (37), and differences between mean values were considered significant at *P* less than 0.05.

## Results

The experimental set-up chosen provided three distinct levels of iodine intake: a normal iodide intake (MID+I), intermediate (MID), and low iodide intake (MID+P). The daily urinary iodide excretion was: 1.62  $\pm$  0.08  $\mu$ g (MID+I); 0.42  $\pm$  0.04  $\mu$ g (MID); 0.60  $\pm$  0.05  $\mu$ g (MID+P). The excretion of iodide in MID+P was higher than that in MID due to the higher PII, because the uptake of I, not only from the diet but also from thyroid hormone metabolism, was blocked by P, whereas in MID rats part of this iodide can be reused for thyroid hormone synthesis. The maternal body weight (at d 21, 321  $\pm$  6 g), the number and weight of fetuses were not altered in MID and MID+P (data not shown). The maternal thyroids were significantly enlarged in MID+P (58.0  $\pm$  5.5 mg) compared with those of MID+I (21.0  $\pm$  4.2 mg) and MID (27.2  $\pm$  2.4 mg).

T<sub>4</sub>, T<sub>3</sub>, and TSH concentrations in maternal and fetal plasma are reported in Fig. 1. Maternal plasma T<sub>4</sub> was decreased by 40% in the MID group and was close to the detection limit (<1.5 nmol/liter) in the MID+P group. Plasma T<sub>3</sub> concentrations did not change. Plasma TSH concentration was slightly (however, not significantly) elevated in MID and increased 10 times in MID+P. Changes in the fetal plasma were comparable with those found for the dams: a decrease in T<sub>4</sub> concentration, unchanged plasma T<sub>3</sub> concentration, and a marked increase in plasma TSH in MID+P.

The 24-h AIU of the maternal thyroid was similar in MID and MID+I (Fig. 2), but was very low in MID+P. In contrast,

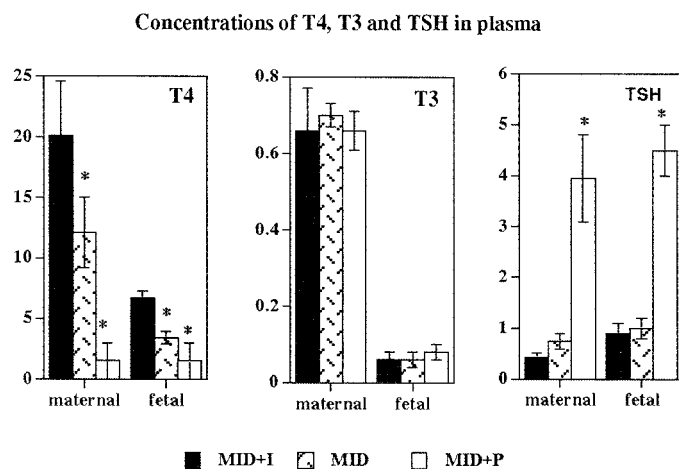


FIG. 1. Changes in T<sub>4</sub> (nmol/liter), T<sub>3</sub> (nmol/liter), and TSH (ng/ml) concentrations in maternal and fetal plasma at 21 d of gestation in MID+I (n = 6), MID (n = 6), and MID+P (n = 6). Plasma of the fetuses was pooled per dam. Values are mean  $\pm$  SEM. \*, *P* < 0.05 vs. MID+I.

the fetal AIU (that is the amount of iodide derived from the maternal circulation) decreased by about 65% (from 70 to 24 ng/24 h) in MID and decreased even more in MID+P (about 93%). The PII concentration in the dams and fetuses was only decreased in MID.

Figure 3 shows that NIS mRNA was detected in the fetal thyroid and in the fetal side of the placenta but was neither found in fetal brain, brown adipose tissue (BAT), liver, heart, and skin nor in the maternal side of the placenta during MID+I. This was also the case during MID and MID+P (data not shown). Figure 4 shows the changes in NIS expression in response to iodine deficiency. In the fetal thyroid, NIS mRNA level was already increased about 2-fold in MID, and increased 3- to 4-fold in MID+P (Fig. 4). NIS mRNA was hardly detected in the fetal side of the placenta in MID+I, but was strongly induced in MID and MID+P (Fig. 5). Noteworthy, these changes were not observed in the maternal side of the placenta (basal layer).

We analyzed the expression of several other genes in the fetal thyroid (Fig. 6). Tg mRNA content was similar in MID+I

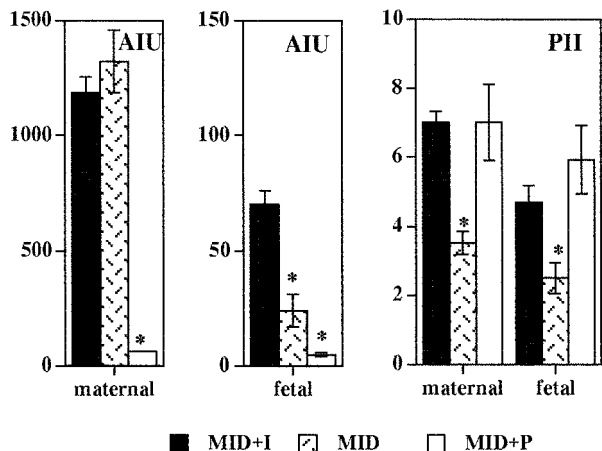


FIG. 2. AIU (ng/24 h) of maternal and fetal thyroids and maternal and fetal PII concentration (ng/ml). Fetal thyroids and plasma of the fetuses were pooled per dam. Values are mean  $\pm$  SEM (n = 6/group). \*,  $P < 0.05$  vs. MID+I.

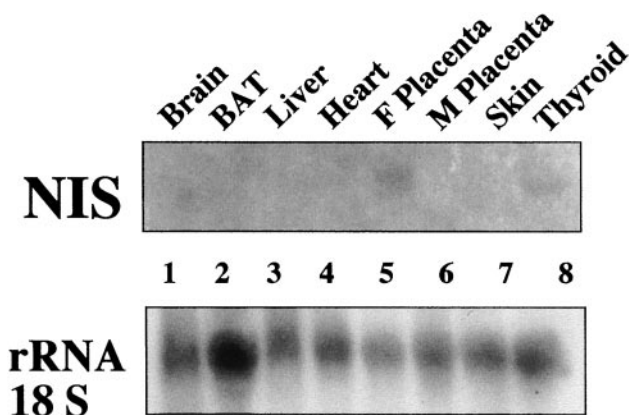


FIG. 3. Northern blot analysis of NIS mRNA content of fetal organs. Ribosomal 18S RNA is shown to correct for differences in total RNA input, as cyclophilin expression is variable in the different tissues examined. F, Fetal placenta; M, maternal or basal layer of the placenta.

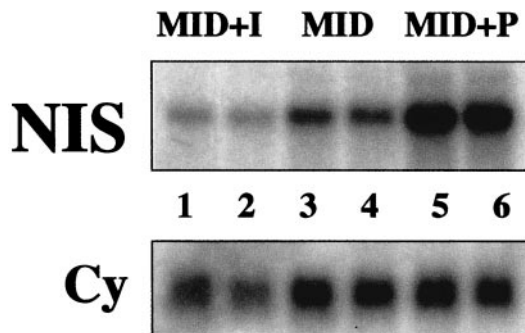


FIG. 4. NIS mRNA content of fetal thyroids at 21 d of gestation from iodine-sufficient (MID+I) and iodine-deficient (MID and MID+P) dams. Hybridization with cyclophilin (Cy) is shown to correct for differences in total RNA input.

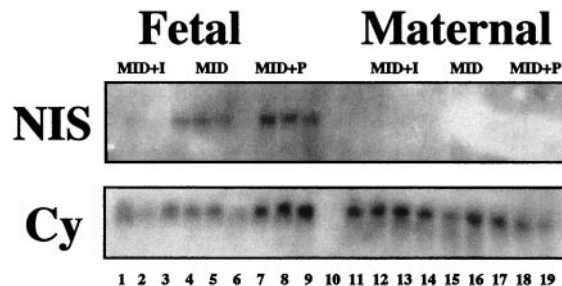


FIG. 5. Evidence for an induction of NIS gene expression in the fetal side of the placenta at 21 d of gestation of MID+I, MID, and MID+P rats. Northern blot analyses were performed on 15  $\mu$ g total RNA extracted from the fetal and maternal sides of the placenta. Hybridization with cyclophilin (Cy) is shown to correct for differences in RNA input.

and MID and increased 2-fold in MID+P. D1 mRNA did not change in MID but increased about 2-fold in MID+P (Fig. 6). On the contrary, Glut4 mRNA decreased in both MID and MID+P, whereas Glut 1 mRNA content was similar in the three groups.

**Discussion**

Plasma  $T_4$ ,  $T_3$ , and TSH levels of the control rats (MID+I) are comparable with those of rats on commercial pellet diet and with those of rats receiving potassium iodide (10 mg/liter) in their drinking water (24). Using the AIN diet without adding extra I, we induced a mild iodine deficiency with lowered  $T_4$  and normal  $T_3$  and TSH levels. It can be deduced from urinary iodide excretion measurements in MID, that rats received some I, probably from casein present in the AIN diet (17), but also from the metabolism of their own thyroid hormones. This degree of iodine deficiency can be compared with that occurring in large parts of the world, where goiter exists due to the low iodide intake, often aggravated by the interference of other nutritional and/or environmental factors acting on the iodide uptake, organification, and/or thyroid hormone secretion processes (38).

The diet supplemented with 0.005% perchlorate (MID+P) resulted in moderately severe iodine deficiency, with very low plasma  $T_4$ , high TSH, but still normal  $T_3$  levels not only in rat dams, but also in their fetuses. The normal  $T_3$  levels in moderately iodine-deficient adult rats has long been known (16-24). How can the fetus maintain normal  $T_3$  levels in mild

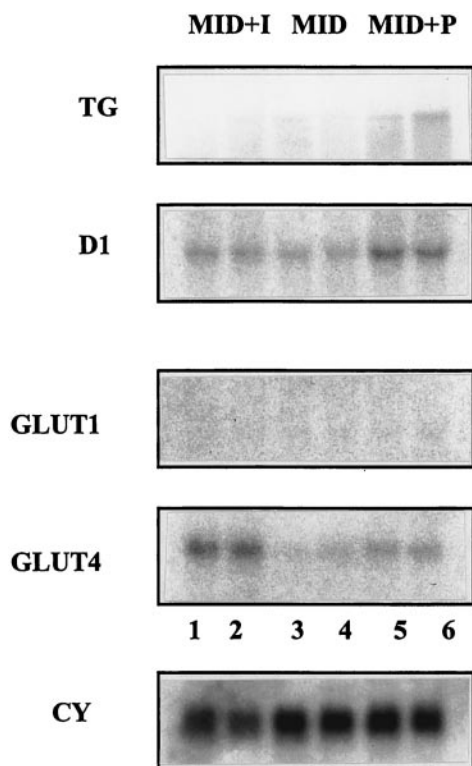


FIG. 6. Northern blot analyses of the Tg, D1, and glucose transporters (Glut 1 and 4) mRNA contents of the fetal thyroids at 21 d of gestation. Each lane was loaded with 15  $\mu$ g total RNA extracted from the thyroids of fetuses obtained from control (MID+I) and iodine-deficient (MID and MID+P) dams. Hybridization with cyclophilin is shown to correct for differences in RNA input.

and moderately severe iodine-deficient conditions, considering that the maternal supply of  $T_4$  is so much lower than in the normal situation, and that the maternal to fetal transfer of  $T_3$  is expected to be insufficient to ensure normal fetal plasma  $T_3$  (31)? This is not related to an increased flux of iodide from the mother to the fetus because the PII of both dams and fetuses are decreased in MID. Apparently, the increase in NIS mRNA expression in the fetal placenta is not sufficient to increase or normalize the fetal PII. In the maternal placenta, NIS mRNA seems not to be present, not even in the iodine-deficient situation; it is still possible that the maternal placenta is rate-limiting for iodide transport, or that in the maternal placenta another transport system is present (*i.e.* ion channels, which are not increased during iodine deficiency). But also an adequate supply of  $T_4$  to the maternal placenta might provide a means of transporting maternal iodine stores to the fetus for fetal thyroid hormone synthesis due to a difference in PII of the mother compared with that of the fetus (39). The AIU of the fetal thyroid is only the amount of iodide derived from the inorganic iodide pool of the maternal circulation. The amount of iodide produced by type II and type III deiodinase (D2 and D3) activity in the placenta that partly is taken up by the fetal thyroid cannot be measured. Thus, the measured fetal AIU is an underestimation of the real total iodide uptake. By using the specific activity of the iodide in the amniotic fluid, an AIU of 404 ng/24 h for MID+I and 154 ng/24 h for MID was obtained,

indeed, much higher than the fetal AIU in Fig. 2. This seems unlikely, due to the fact that the total iodide content of the fetal thyroid at d 21 is about 60–80 ng per total gland (23). However, the decrease in MID compared with MID+I is similar (as a percentage) in both calculations.

We bring evidence for a clear increase in NIS expression in MID, and this increase occurs without significant increase in TSH at that moment. We think that the order of events is: when the intake of iodide is lowered, the PII is lower, resulting in a decreased AIU. It takes some time before  $T_4$  is lowered, but when that happens TSH increases transiently and, thus, NIS will increase; AIU will increase, leading to an increase in mainly  $T_3$  synthesis, but still a lowered  $T_4$ . However, from this and earlier studies from our group, the up-regulation of NIS expression does not seem to be sufficient by itself to increase the AIU in the fetal thyroid, the values are even lower (25). An increase in NIS mRNA does not only reflect the change in the AIU. The AIU can also be influenced by changes in pendrin and thyroid peroxidase. It is possible that when pendrin and thyroid peroxidase (not measured) would also increase, the organification will increase, and thus there will be an increased retention of thyroidal iodide. This can be the case for the mother in MID but not for the fetus, because there the AIU is decreased.

Data from another experiment (similar iodide intake/level and same plasma  $T_4$ ,  $T_3$ , and TSH concentrations) showed that NIS expression in the maternal thyroid increased about 3-fold in MID and about 5-fold in MID+P. The fetal thyroid has to compete with the maternal thyroid and loses this competition. Due to a higher increase in NIS expression in the maternal thyroid, together with the higher amount of tissue of the maternal thyroid compared with that of the fetuses, this leads to an unchanged AIU and a lowered fetal AIU. As in the adult, Glut4 is present in the fetal thyroid (40). A putative thyroid hormone response element within the rat Glut4 promoter was defined in skeletal muscle (41). Glut4 in heart and BAT are under control of thyroid hormone (42). We can hypothesize that Glut4 also in the thyroid is regulated by thyroid hormone like D1 expression, which is stimulated by  $T_3$  mediated by a thyroid hormone response element in the D1 promoter. Yet, D1 expression is increased in hypothyroidism and decreased in hyperthyroidism because of the overriding stimulatory effect of TSH. This may also apply to genes such as Glut4. Thus, D1 and Glut4 are decreased in MID (compared with MID+I) and increased in MID+P (compared with MID). Because the process of the AIU is energy dependent it can be suggested that the basal intracellular energy (glucose) availability is diminished as indicated by a decreased Glut4 mRNA expression.

As the adult thyroid, the fetal thyroid can preferentially synthesize  $T_3$  (16–23). This was demonstrated in fetuses from 18–21 d of gestation, obtained from dams receiving low iodine diets (23). The  $T_3/T_4$  ratio in Tg extracted from the thyroid of fetuses obtained from iodine-deficient dams was 3- to 4-fold higher than that measured in the fetal thyroid obtained from iodine-sufficient mothers. A preferential  $T_3$  secretion by the fetal thyroid could also participate to normal plasma  $T_3$  levels in the fetuses. As in the thyroid of adult rats (17, 19) the free  $T_3/T_4$  ratio was found elevated (5- to 8-fold) in the thyroid of rat neonates (23). A possible preferential  $T_3$

secretion is supported by the observed increased D1 expression, induced by increased TSH that could lead to an increased T<sub>4</sub> to T<sub>3</sub> conversion as already reported in the adult rat (43).

An increase in T<sub>3</sub> production by peripheral D2 in extra-thyroidal tissues might also contribute to normal T<sub>3</sub> concentrations in the fetus. In tissues known for local T<sub>3</sub> production, there is an increase in D2 activity [brain (23), BAT (36), placenta (24, 44–46)]. In the fetus, the skin is very active in converting T<sub>4</sub> to T<sub>3</sub> and has high D2 activity (24).

In conclusion, all adaptive and/or defending mechanisms against effects of iodine deficiency are already present in the fetus. This means that each of the important steps in the thyroid hormone economy, iodide uptake through an increased expression of NIS, iodide organification, thyroid hormone synthesis, secretion and peripheral bioactivation is activated to work toward the delivery of sufficient amounts of T<sub>3</sub> to fetal tissues when iodide supply is limited.

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