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Iodothyronine Deiodinase Activities in Fetal Rat Tissues at Several Levels of Iodine Deficiency: A Role for the Skin in 3,5,3′-Triiodothyronine Economy?*

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ABSTRACT

Iodothyronine deiodinases, types I, II, and III (D1, D2, and D3) activities were measured in tissues of fetal rats, at 18 and 21 days of gestation, at several levels of iodine deficiency (ID): mild ID diet (MID) and moderately severe ID, MID + 0.005% perchlorate (MID+P). D2 was present in fetal skin, increased between days 18 and 21, and also in MID and MID+P. In skin, D3 increased during ID at day 18, whereas there was a decrease at day 21. Skin T_4 decreased in MID and MID+P, showing an inverse relationship with D2. Skin T_3 decreased at day 18 in MID and MID+P but increased at day 21, probably because of the increased D2 and decreased D3, maintaining T_3 concentrations. No effect of ID was observed on hepatic D1. D2 increased in brain and brown adipose tissue at day 21 in MID+P. No changes were found in maternal placental D2 and D3, but D2 and D3 increased in the fetal placenta at day 18 in MID+P.

A higher level of D2 is present in fetal skin than in the brain. As the activity is increased, in even mild ID (and already at 18 days) it can be concluded that skin D2 is likely to be of considerable physiological importance, at least for fetal thyroid hormone economy, by contributing to the intracellular T_3 content of the skin and, possibly, to the plasma T_3. (*Endocrinology* **139:** 2229–2234, 1998)

T_4 IS BIOACTIVATED into T_3 by enzymatic deiodination. T_3 homeostasis in tissues is maintained by the iodothyronine deiodinase isoenzymes, types I, II, and III (D1, D2, and D3). Their activity is dependent on the thyroid (hormone) and nutritional status and is regulated by glucocorticoids and growth factors (1, 2). The presence and activities of these enzymes are tissue specific. D1 is found in the liver, kidney, pituitary, and thyroid; D2 is found in the central nervous system, pituitary, brown adipose tissue (BAT), placenta, (human) thyroid, and muscle; D3 is present in the brain, skin, placenta, and several fetal tissues (1–5). The ontogenic appearance of D1 in the rat fetal liver and lung, of D2 in the fetal brain and BAT (6–8), and of D3 in the cerebral cortex (9) and in fetal tissues (2) are well described. Changes in tissue deiodinase activity are of great importance in those organs, which are dependent on locally produced T_3 (1–8).

During pregnancy, thyroid hormones of maternal origin are found in fetal rat tissues before onset of fetal thyroid function (10–13), as well as in humans (14, 15), and are, at present, believed to play a role in early development (16–18) and to have a protective role when fetal thyroid function is impaired (19), although this role is sometimes contested (20). Until onset of its own thyroid function, the fetus is mainly dependent on the maternal supply of T_4 (10–13). During pregnancy, T_4 and T_3 levels in maternal plasma and tissues in the rat are decreased (21).

One of the situations in which thyroid function is impaired is iodine deficiency (ID). ID is characterized by a decrease in plasma and tissue T_4 concentrations, normal or even higher than normal plasma T_3, and an increased plasma TSH. The thyroid is enlarged (6, 22–32). There is a preferential synthesis and secretion of T_3 by the thyroid (23, 24), which is also stimulated by the TSH-dependent increase in thyroid D1 activity (29, 33). This results in higher T_3/T_4 ratios in plasma and tissues. In this situation, less T_4 from the maternal circulation is available for the fetus (6, 12, 13, 18, 27). Fetal plasma and tissue T_4 and T_3 levels are lowered and D2 activity is increased in fetal brain and BAT, whereas D1 activity in liver decreased (6). Most of these studies were performed with severely iodine-deficient rats, whose fetuses had lowered plasma T_3 levels.

Even in adult rats that were only marginally ID, with slightly lowered plasma T_4 levels, D2 activity increased in the brain (34).

Our aim was to obtain more information about changes in iodothyronine deiodinase activities in fetal rat tissues (brain, BAT, liver, placenta, and skin) at degrees of ID that are more comparable with the mild and moderate ID that affects inhabitants in large parts of the world. We considered it of interest to assess the capacity of the different iodothyronine

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deiodinases of the developing fetus, to respond to these situations, and their contribution to the maintenance of T_3 levels.

Because it is known that there are developmental changes of D1, D2, and/or D3 activities during the last stage of gestation, two time-points were chosen, *i.e.* day 18 of gestation (just as the fetal thyroid starts to function) and day 21 (1 day before birth) (6–9).

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 (BW, 210 ± 5 g). The rats were housed at 22 C, with alternating 14-h light, 10-h dark periods. They were fed the American Institute of Nutrition (AIN) diet (35), without iodine (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 μg/day) was added to the diet (MID+I). This last group served as controls. After 3 months, rats were mated, and the day that sperm was present was taken as day zero of pregnancy. During 5 days, the rats were housed in metabolic cages. This allowed us to collect 24-h urine and to measure the iodine excretion according to Sandell and Kolthoff (36). At 18 and 21 days of pregnancy, three rats were bled and perfused under light ether anesthesia. Fetal tissues were taken and were kept frozen at −70 C until used. The skin was taken, 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 (37).

Chemicals

T_4, T_3, dithiothreitol (DTT), and propylthiouracil (PTU) were from Sigma Chemical Co. (St. Louis, MO). ^{125}I and ^{131}I were from New England Nuclear-Dupont (Dreieich, Germany), inner-ring labeled T_3 was purchased from Formula (Berlin, Germany). Anion exchange resins Dowex AG 1X2 and 50WX2 were obtained from Bio-Rad (Richmond, CA).

Determinations of plasma TSH, T_3, and T_4 in plasma and skin

In plasma, the concentrations of T_4 and T_3 were assayed by rat-specific RIA (38). Plasma TSH was measured by the specific RIA developed for the rat by the NIDDK (NIH, Bethesda, MD). Reference preparation-2 was used as a standard.

In skin, thyroid hormones were determined as previously described in detail for rat tissues (39). The fetal skin was frozen in liquid nitrogen and crushed to a fine powder while frozen. The powder was taken up quantitatively and homogenized in methanol, after which the procedure of extraction and purification used for other tissues was followed.

Determination of 5′-D1, D2, and D3 activities

For determination of D1, D2, and D3 activity the release of [^{125}Iodide] from [^{125}I]-labeled substrates was measured using homogenates.

Before use, the labeled substrates were purified by paper electrophoresis, to separate them from the contaminating iodide. The contribution of the mass of radioactive substrates added was taken into account for calculation of the total substrate concentrations. After the reaction, the [^{125}I] iodide released was separated, by ion exchange chromatography, on Dowex 50W-X2 columns, as previously described (6–8, 40). The protein content was measured by the method of Lowry *et al.* (41).

The conditions were as set forth below.

For D1. [^{125}I]rT_3 (60.000 cpm/tube) was used in the presence of 200 nM rT_3 and 2 mM DTT, after 15 min incubation at 37 C, using 20–50 μg protein/100 μl.

For D2. [^{125}I]T_4 (60,000 cpm/tube) was used in the presence of 2 nM T_4, 1 μM T_3, 20 mM DTT, and 1 mM PTU, after 1 h incubation at 37 C; 150–200 μg protein/100 μl was used (42). For skin, we checked with paper chromatography and found that equal molar amounts of iodide and T_3 were released. Endogenous T_4 from the skin contributed less than 2% to the total substrate concentration.

For D3. [^{125}I, 3]T_3 (60,000 cpm/tube) was used in the presence of 20 nM T_3, 20 mM DTT, and 1 mM PTU, after 1 h incubation at 37 C; 30–50 μg protein/100 μl was used.

Statistics

Mean values ± SE are given. Statistical analysis was performed by ANOVA (43), and differences between mean values were considered statistically significant at $P \leq 0.05$.

The program SPSS 6.1 (Real Stats, Real Easy, Chicago, IL) was used for comparison of the slopes of the linear regression.

Results

The experimental model chosen provided three levels of iodine intake: normal iodine intake (MID+I), moderate (MID), and low iodine intake (MID+P). The daily urinary iodine excretion was: 1.62 ± 0.08 μg (MID+I); 0.42 ± 0.04 μg (MID); and 0.60 ± 0.05 μg (MID+P). The excretion of iodine in MID+P is higher than that in MID because the uptake of iodide, not only from the diet but also from thyroid hormone metabolism, is completely blocked by perchlorate, whereas in MID, part of this iodide can be reused for thyroid hormone synthesis. The maternal BW (at day 18: 296 ± 8 g; at day 21: 321 ± 6 g) and the number and weight of fetuses were not influenced by MID and MID+P (data not shown). The maternal thyroids were enlarged in MID+P (58.0 ± 5.5 mg), compared with those of controls (21.0 ± 4.2 mg) and MID (27.2 ± 2.4 mg).

Table 1 shows the T_4, T_3, and TSH values in maternal and fetal plasma. In MID, maternal plasma T_4 was decreased to 60% of MID+I and decreased below detection limit (<1.5 nM) in the MID+P group at 21 days of gestation. Plasma TSH increased 10× in MID+P. The plasma T_3 concentrations did not change. Similar changes were observed at days 18 and 21 of gestation.

In the fetal plasma, we found changes comparable with those described for the mothers: a decrease in T_4 concentration in MID, with T_4 being below detection limit in MID+P,

TABLE 1. Concentrations of T_4, T_3, and TSH in maternal and fetal plasma

	MID+I	MID	MID+P
At 21 days of gestation			
Maternal			
T_4 nM	20.1 ± 4.5	12.1 ± 2.9[a]	<1.5[a]
T_3 nM	0.66 ± 0.11	0.70 ± 0.03	0.66 ± 0.05
TSH ng/ml	0.43 ± 0.08	0.75 ± 0.15	3.95 ± 0.86[a]
Fetal			
T_4 nM	6.7 ± 0.6	3.4 ± 0.5[a]	<1.5[a]
T_3 nM	0.06 ± 0.02	0.06 ± 0.02	0.08 ± 0.02
TSH ng/ml	0.9 ± 0.2	1.0 ± 0.2	4.5 ± 0.5[a]
At 18 days of gestation			
Concentrations of T_4, T_3 and TSH in maternal plasma			
T_4 nM	24.3 ± 3.2	17.7 ± 2.9[a]	4.8 ± 2.5[a]
T_3 nM	0.75 ± 0.06	0.68 ± 0.05	0.72 ± 0.03
TSH ng/ml	0.44 ± 0.08	1.03 ± 0.18[a]	4.95 ± 0.70[a]

[a] At least $P < 0.05$ *vs.* MID+I.

together with an unchanged plasma T_3; an increase in plasma TSH was only observed in MID+P.

Deiodinase activities

Liver. The developmental pattern, an increase in activity from day 18 to day 21, was present in all three groups. Hepatic D1 activity was not affected by MID and MID+P either on day 18 or on day 21 (Fig. 1).

BAT. No D2 activity could be measured at day 18, whereas it was clearly detected on day 21. The mean value of D2 activity was slightly higher at day 21 in MID, compared with MID+I, but the difference was not statistically significant. A marked increase in D2 activity was present in MID+P at day 21 (Fig. 2).

Brain. An increase in D2 activity was seen during development, from day 18 to day 21. At day 18, no statistically significant increases in D2 activity were found in MID and MID+P. At day 21, D2 activity clearly was increased in MID+P (but not in MID) fetuses.

In brain, the D3 activity at day 18 decreased in MID, and further in MID+P fetuses, to 30% of MID+I values. On the contrary, at day 21, the D3 activity increased 2-fold, both in MID and MID+P (Fig. 3).

Fetal side of the placenta. D3 activity was lower at day 21 than at day 18 in all three groups (Fig. 4).

D2 activity increased marginally (but significantly) in MID+P at day 18. This increase in D2 activity was not found at day 21. The same increase in D3 activity was observed in MID+P at day 18.

Maternal placenta. There were no changes in D2 and D3 activities in the maternal basal side of the placenta caused by either gestational age or by MID and MID+P (Fig. 5).

D2 activity in the maternal side of the placenta was found to be about 10 times higher than in the fetal side of the placenta, whereas D3 activities were comparable.

At day 18, D3 activities in the fetal and maternal placenta were similar, but they differed at day 21, when the activity in the maternal placenta was much higher than in the fetal side.

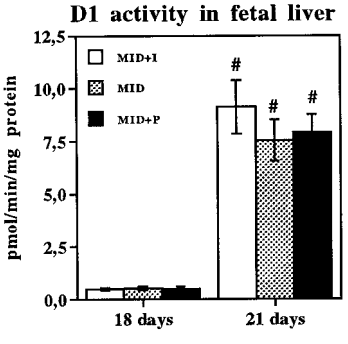

FIG. 1. Hepatic D1 activities in fetuses at day 18 and day 21 in MID+I, MID, and MID+P. Values are the means ± SE. n = 8 fetal livers. For this and the following figures: *, statistically significant difference at a given age, with respect to MID+I; &, statistically significant difference between MID and MID+P; #, statistically significant difference for a given group between 18 and 21 days.

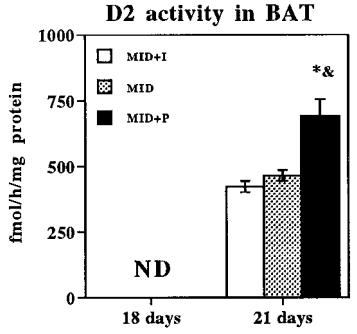

FIG. 2. D2 activity in fetal BAT at day 21. ND, BAT of 18 days was not present. Values are the means ± SE. *, At least $P < 0.05$ (n = 8 fetal BAT); *, statistically significant difference, at a given age, with respect to MID+I; &, statistically significant difference between MID and MID+P.

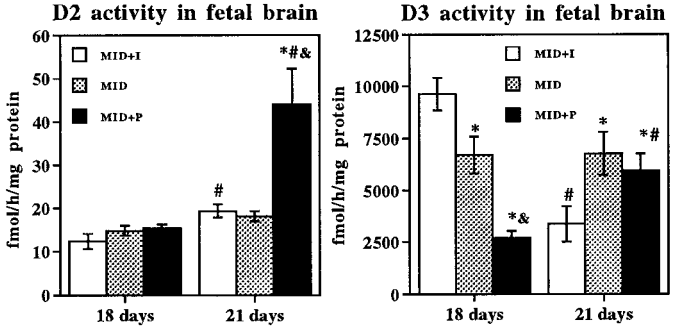

FIG. 3. D2 activity (*left panel*) and D3 activity (*right panel*) in brain from 18- and 21-day-old fetuses. Values are the means ± SE. n = 8 fetal brains. *, #, and &, as explained in Fig. 1 legend.

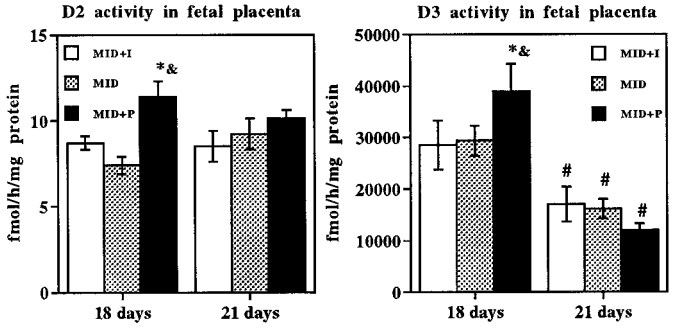

FIG. 4. D2 activity (*left panel*) and D3 activity (*right panel*) in the fetal side of the placentas from 18- and 21-day-old fetuses. Values are the means ± SE. n = 8 fetal placentas. *, #, and &, as explained in Fig. 1 legend.

Skin. D1 activity was not present in measurable amounts in fetal skin. The changes in D2 and D3 activity in fetal skin are presented in Fig. 6. The developmental increase in D2 activity, from day 18 to day 21, was present in all three groups. Not only is D2 activity present in the fetal skin, but it increased in MID fetuses and further increased 3- to 4-fold in MID+P, both at day 18 and at day 21.

The pattern was different for D3 activity in fetal skin. At day 18, the D3 activity increased almost 2-fold in MID and in MID+P fetuses, whereas at day 21, a sharp decrease in D3 activity was found: in MID fetuses, the activity was 50% of the MID+I value; and in MID+P fetuses, it was 30% of the MID+I value.

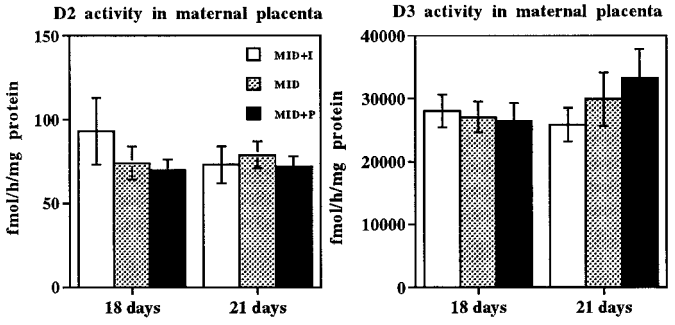

FIG. 5. D2 activity (*left panel*) and D3 activity (*right panel*) in the maternal side of placentas from 18- and 21-day-old fetuses. Values are the means ± SE. n = 8 maternal placentas.

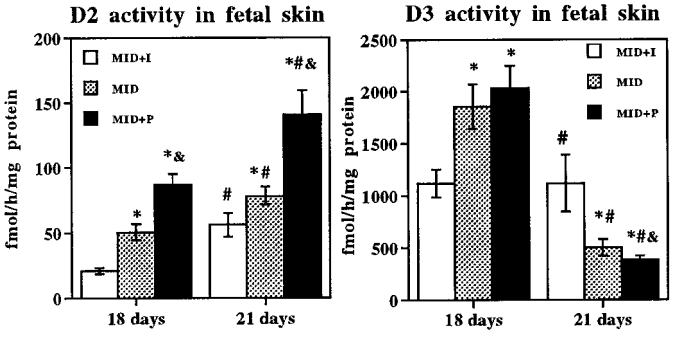

FIG. 6. D2 activity (*left panel*) and D3 activity (*right panel*) in fetal skin from 18- and 21-day-old fetuses. Values are the means ± SE. n = 8 fetal skins. *, #, and &, as explained in Fig. 1 legend.

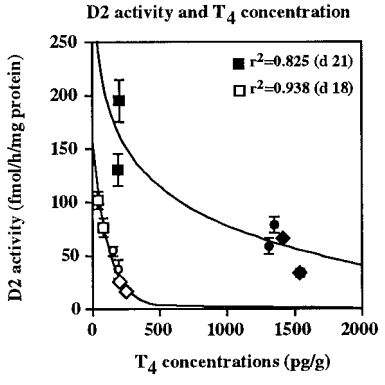

FIG. 7. An inverse relationship was present between D2 activity and the T_4 concentration in the skin. As T_4 content decreased, D2 activity increased, both at day 18 (*open symbols*) and day 21 (*filled symbols*). Each correlation is obtained using the mean values obtained from fetal samples from each dam of the three experimental groups (the total number is 24 for each correlation) and the SE for the mean of the fetal values from each dam. MID+I, *diamonds*; MID, *circles*; MID+*P*, *squares*. The slope of the linear regression at day 18 is significantly different from that at day 21 ($P < 0.0001$, $t = 9.7$, df = 8). Values for the slopes are −0.419 ± 0.026 at day 18; −0.086 ± 0.018 at day 21.

An inverse logarithmic relationship was found between the D2 activity and the T_4 concentration in skin, both at day 18 and at day 21 (Fig. 7). A different sensitivity of D2 activity to changes in the concentration of T_4 is observed at both gestational days. This suggests that the activity of the enzyme is suppressed by smaller amounts of T_4 at day 18. T_4 concentration in the fetal skin increased from day 18 to day 21, and it decreased in the MID fetuses and was more pronounced in the MID+P group (Fig. 8). The T_3 concentration responded in an opposite manner to MID and MID+P, with a decrease at day 18 and an increase at day 21.

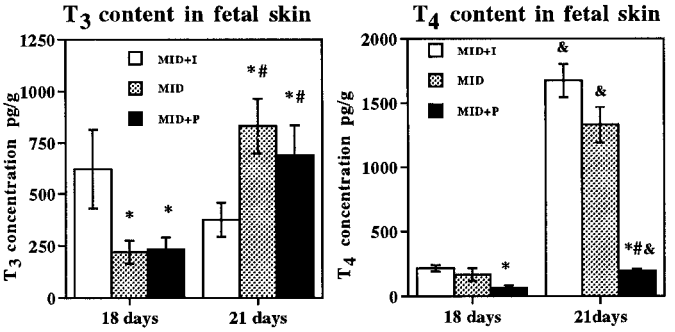

FIG. 8. In the *left panel*, fetal skin T_3 concentrations are shown at day 18 and at day 21 in MID+I, MID, and MID+P. In the *right panel*, skin T_4 concentrations are presented at day 18 and at day 21 in MID+I, MID, and MID+P. For both panels, values are the means ± SE. *, at least $P < 0.05$; n = 6–8 fetal skins. *, #, and &, as explained in Fig. 1 legend.

Discussion

The plasma T_4, T_3, and TSH levels of the MID+I rats are comparable with the plasma levels of rats on a commercial pellet diet and with those of rats in kinetic experiments receiving potassium iodide (10 mg/liter) in their drinking water to prevent reuse of labeled iodide by the thyroid (44).

The degree of ID in this study was not as marked as that reported in previous studies (6, 12, 13, 24–27). Using the AIN diet, without adding extra iodine, we induced a marginal ID. As was seen from the urinary iodine excretion in MID, the rats received some iodine, probably from the casein in the AIN diet (23). This degree of ID can be compared with that occurring in large parts of the world, where endemic goitre exists because of the low intake of iodine, often aggravated by the interference of other nutritional or environmental factors with the iodine-uptake, organification, and thyroid hormone secretion (45).

The diet, together with 0.005% perchlorate (200 times less than the amount that is goitrogenic), resulted in the moderately severe ID, with undetectable plasma T_4, high TSH, but still normal T_3 levels in rat mothers and their fetuses. This degree of ID was less than reported in previous studies (6, 12, 13, 24–27) using a Remington-type diet, and in which fetal plasma T_3 decreased.

The normal T_3 levels in ID in adult rats has long been known (12, 22–26, 29–32). How can the normal T_3 levels in the mild and moderately severe ID fetus be explained, considering that the maternal supply of T_4 is so much lower than in the normal situation, and that maternal-to-fetal transfer of T_3 is not sufficient to ensure normal fetal plasma T_3 (19)? One explanation can be the increase in fetal thyroidal T_3 secretion. In the adult rat, TSH-stimulated D1 activity in the thyroid (33); D1 activity was increased in the thyroid during ID (29). But it is not known whether this is the case in the fetal thyroid. The finding that the fetal thyroid is not stimulated to increase iodine uptake gives the impression that the fetal thyroid is not yet fully responsive to TSH (46). Another

possibility is that extrathyroidal responses of the iodothyronine deiodinases contribute to this phenomenon.

Until now, D2 activity had been found in the central nervous system, pituitary gland, BAT, pineal gland, placenta, and muscle. Although Kaplan *et al.* (42) showed the presence of D2 in human cultured epidermal keratinocytes from the neonate and adult, no activity was found in homogenates of fresh fetal or cadaveric epidermis. But because the skin is a large part of the body mass in the fetus (15%), it thus seemed an interesting target for our study.

This is the first time that the presence of D2 activity in the fetal rat skin is reported. It seems to be higher than D2 activities in fetal brain, and moreover, it responds to degrees of ID that do not affect brain or BAT D2, and it does so already at day 18. Because D2 is of crucial importance for both local production of T_3 in particular tissues and systemic thyroid hormone homeostasis, and because BAT D2 is considered responsible for a large proportion of the circulating T_3 under hypothyroid conditions (47), the role of the skin in producing T_3 has to be taken into account. Because of the total mass of the skin, the D2 activity might contribute, in an important way, not only to the skin T_3 content but eventually also to the plasma T_3, especially in situations in which the maternal supply of T_4 is lowered. As can be calculated, the amount of T_3 in skin is 11.6% of the total amount of T_3 in the extrathyroidal fetal pool, as measured by RIA (unpublished data), much more than brain (5.8%), or even BAT (1.8%).

In addition to the availability of T_4 in fetal tissues during gestation and the level of ID, D3 activity also plays an important role in fetal skin in regulating T_3 levels by inactivating the biological active thyroid hormone. There is no significant difference between the T_3 concentration in skin between day 18 and day 21 in MID+I, whereas there is a clear and significant increase in MID and MID+P from day 18 to day 21. Based only on D2 and D3 activity, we cannot find a cause for this discrepancy.

At day 18, D3 increases in ID, which explains the lowering of the T_3 levels in the skin. But at day 21, a sharp decrease in D3 activity is found in MID and MID+P, resulting in higher than normal levels of T_3 in this tissue; and so, the skin might contribute to systemic T_3. Skin T_4 levels decreased in accordance with the decrease in T_4 in other tissues (plasma, brain, and liver) during ID (6, 12, 13, 25). The inverse relationship between T_4 and D2 was found at both 18 and 21 days. This had been previously shown on day 21 in BAT (8) and brain (7) in hypo and hyperthyroidism. Interesting is the fact that in skin, the changes in D2 and D3 activity are already pronounced in MID on day 18, whereas such an effect was not found in brain, suggesting an earlier maturation of the skin.

D1 activity in liver did not change, indicating a diminished T_3 production caused by the lowered availability in substrate (T_4), as was described in the adult and fetal rat (6, 29). D2 activity in brain and BAT increased in MID+P, in agreement with previous findings, at day 21, under conditions of severe ID (6, 12–13, 18, 27). Present data show that MID is not enough to elicit a D2 response in the fetal brain and BAT, even at day 21. But even MID is enough to affect D3 activity at both day 18 and day 21. The decrease in cerebral D3 activity at day 18 is as expected in situations where lower T_3 levels occur. But at day 21, there is an unexpected increase in D3 activity in MID and MID+P, counteracting the increase of D2, at least in MID+P. No information is available about the response of D3 in MID and severe ID. An essential aspect can be that, at a certain time-point, one tissue will be more mature than the other. This may cause differences in behavior and regulation of activity levels of D3 in tissues such as skin and brain. Moreover, changes in growth factors might influence the developmental patterns in tissues (48). The induction of D3 activity by growth factors is stronger than that caused by thyroid status. So, a minor change in growth factor (increase in D3) at a given age can have a more important impact on D3 activity than thyroid status (decrease in D3) (49).

D2 activity increased in the the fetal side of the placenta at day 18. A developmental pattern of D2 was not observed in the placenta, in contrast to D3. The developmental decrease in D3 activity was found in the fetal side of the placenta, in accordance with McCann *et al.* (2). In our study, there is no clear role for D2 and D3 in the placenta, for the regulation of plasma T_3 levels. The absence of an effect in maternal placenta might indicate that D2 and D3 activities in this tissue are regulated by plasma T_3. This was also shown in human placenta during hypothyroidism (50).

Summary and conclusions

Mild and moderate ID caused a decrease in T_4 concentrations, although the developmental increase from day 18 to day 21 was still observed. In spite of the lowered amount of substrate, the marked increased D2 and decreased D3 activities at day 21 resulted in higher than normal intracellular T_3 concentrations. T_3 can remain entirely in the skin itself, but there might be a net outflux of T_3 into the circulation.

The presence of D2 in a tissue is considered an indication that this tissue needs local production of T_3 (as is the case of brain and BAT). These results show that high D2 activity is present in fetal skin, even higher than in fetal brain. Because the activity is increased (as early as day 18) during both mild and moderately severe ID, it can be concluded that skin D2 is likely to be of considerable physiological importance, at least for fetal thyroid hormone economy, by contributing to the intracellular T_3 content of the skin, for its own development and possibly by contributing to the plasma T_3. The latter would be a benefit for those tissues dependent on plasma T_3, but not for the brain, because this organ is mainly dependent on T_4 and its own D2 for its intracellular T_3 concentration.

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