Ontogenesis of Thyroid Function and Interactions with Maternal Function

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Abstract

Fetal and neonatal development of thyroid function involves the embryogenesis, differentiation and maturation of the thyroid gland, of the hypothalamic-pituitary-thyroid axis and of the systems controlling thyroid hormone metabolism. We focus here on aspects related to neurodevelopment. Throughout gestation, thyroxine (T4) transferred from the mother, present in embryonic fluids by 4 weeks, protects the fetal brain. Free T4 (FT4) in fetal fluids increases rapidly, approaching adult levels by midgestation, in concentrations that are determined by the maternal serum T4. T3 remains very low throughout pregnancy. In the cerebral cortex T3, generated from T4, reaches adult values by midgestation and is partly bound to specific nuclear receptor isoforms. The iodothyronine deiodinases are important for the spatial and temporal presence of T3 in different fetal brain areas. After onset of fetal thyroid secretion at midgestation, maternal transfer of T4 continues to contribute importantly to fetal serum T4, protecting neurodevelopment until birth. In rats, even a transient period of maternal hypothyroxinemia disrupts neurodevelopment irreversibly, supporting epidemiological evidence for its negative role in human neurodevelopment. The prompt treatment of maternal hypothyroidism or hypothyroxinemia should mitigate negative effects on neurodevelopment. Neurodevelopmental deficits of preterm infants might also result from an untimely interruption of the maternal transfer of T4 [Morreale de Escobar et al: J Clin Endocrinol Metab 2000;85:3975–3987; Best Pract Res Clin Endocrinol Metab 2004;18:225–248; Eur J Endocrinol 2004;151(suppl 3):U25–U37].

Ontogenesis of Thyroid Function

The thyroid gland develops mostly during fetal and early postnatal life. The thyrocytes forming the functional unit of the gland – the thyroid follicle – derive from the embryonic endoderm in the floor of the primitive pharynx, forming a
bud already visible at embryonic day 16 (E16) in humans. After proliferation of the cells and migration at E24–E32 in humans, the thyroid reaches its final position at E40–E50 [1]. An error during morphogenesis results in thyroid abnormalities, such as agenesis or ectopies, as shown in null mice with targeted deletions of thyroid transcription factors, fully described in the chapter by De Felice and Di Lauro [pp. 1–14].

The histological differentiation of the thyrocytes and formation of the follicle are accompanied by the progressive appearance of specific proteins: thyroglobulin, thyroid peroxidase, sodium/iodine symporter (NIS) and TSH receptor, all necessary for the synthesis and secretion of T4 and T3. The main physiological regulator of thyroid gland activity is TSH, but TSH-independent autoregulatory mechanisms also play an important role in the postnatal adaptation to fluctuations in iodine availability. Thyroglobulin has been detected in human thyroid as early as the 5th week of gestation, before the gland reaches its final position. Under the artificial conditions of organ culture, iodine uptake starts at 12–13 weeks after conception, coinciding with the closure of follicles. In vivo, however, significant uptake of iodine, a prerequisite for the synthesis and secretion of fetal thyroid hormones, is minimal until midgestation (18–20 weeks) coinciding with full development of the pituitary-portal vascular system.

For obvious reasons, studies in human fetuses are scarce and much of our knowledge of the role of thyroid hormones during fetal life comes from experimental work performed in rats [table 3 in 2; 3–5], where thyroid hormone secretion starts at E17.5–E18. Fetal serum T4 concentrations increase 10-fold between E18 and the end of gestation (E22), a developmental period comparable to that of a human fetus at the beginning of the third trimester. T3, however, is very low throughout gestation.

In most fetal tissues, T4 increases in parallel to the increases in fetal plasma T4 while T3 concentrations are highly variable in different tissues: in liver, as in plasma, T3 is low throughout gestation, but in other tissues, such as brain or brown adipose tissue (BAT), T3 almost reaches adult levels. This suggests that T3 is required for the development and maturation of these tissues, as confirmed for certain brain areas or in BAT, which is being prepared for the transition, at birth, to lower environmental temperatures. The higher T3 observed in some tissues is due to ontogenic increases in 5'-deiodinase activities: D2 in fetal brain and BAT and D1 in lung, aimed at the production of T3 when and where T3 is required [3, 5]. The presence of 5-deiodinase (D3) activity is high during most of the fetal life. D3 deiodinates T3 and T4 in the inner ring, leading to inactive compounds, and is a key enzyme during the fetal period. It is activated in proliferating states, and is present in high amounts in placenta, uterus and fetal membranes [6, 7]. The role of D3 is to act as a ‘barrier’, which prevents
excessive amounts of maternal thyroid hormones from reaching the conceptus and keeps $T_3$ low throughout fetal development.

Thyroid hormones are important for normal development and the role of the deiodinases during development is to achieve the timely and tissue-specific appropriate $T_3$ levels. For example, D2 peaks in the mouse cochlea before the onset of hearing; D2 is thought to be involved in the maturation of the auditory function. In the rat brain, D2 activity peaks near birth at E21 and at day 15 after birth, dates that coincide with important periods of neuronal and glial maturation [4]. High D2 and $T_3$ concentrations are found in fetal BAT during the active recruitment of the tissue occurring before birth. Many other tissues such as the skin, the retina or the bone are also being studied. The role of D2 and D3 during fetal development is being studied in mice with targeted deletion of D2 and D3 [6]. The D2 knockout mice have pituitary resistance to $T_4$, impaired auditory and visual functions, become hypothermic in the cold and have reduced anxiety levels. The D3 knockout mice are hypothyroid, have growth retardation and impaired fertility associated with D3 being an imprinted gene.

In the present contribution we try to summarize what is known, and what is still unknown, regarding early and late fetal thyroid hormone physiology, its importance in neurodevelopment, its dependence on the production of $T_4$ by the mother, and the likely consequence of the untimely interruption caused by premature birth.

**The Influence of Maternal Thyroid Hormones until Midgestation**

Irreversible brain damage is found when thyroid hormone deficiency occurs during brain development. Epidemiological findings in areas of neurological cretinism clearly indicate an early involvement of the maternal thyroid hormones in fetal CNS development [2, 8, 9]. The severity of the CNS damage found is related to the degree and timing of maternal $T_4$ deficiency and is only prevented when the maternal hypothyroxinemia is corrected before midgestation [tables 1 and 2 in 2]. These ideas were difficult to reconcile with the success of the prompt postnatal treatment with $T_4$ of children with congenital hypothyroidism (CH). This success was considered proof that the developing fetal brain did not need thyroid hormone until after birth, because no major CNS damage was observed if the athyrotic newborn was promptly treated with $T_4$. However, new insights into maternal transfer of thyroid hormones throughout gestation and its likely role in fetal neurodevelopment have reconciled the findings in CH and in neurological cretinism caused by iodine deficiency.
**Findings from Experimental Rat Models**

Before onset of fetal thyroid function (FTF), T₄ and T₃ of maternal origin are present at very low concentrations in rat embryonic and fetal tissues, including the brain, that are directly influenced by the maternal serum T₄ [10]. The thyroid hormone receptor isoforms are already present long before the onset of FTF, at neural tube closure, and are likely to mediate biological effects of the T₃, locally generated from T₄ [11–13].

After onset of FTF, maternal transfer of thyroid hormones continues until term and represents an important proportion of thyroid hormones available to the fetus. In case of fetal thyroid failure, the amount of maternal T₄ reaching the fetal brain is enough to selectively prevent the cerebral T₃ deficiency of a hypothyroid rat [14]. Maternal T₄ and T₃, however, are not equivalent for the fetal brain, because during fetal and early postnatal development, cerebral T₃ depends on its local generation from T₄ – through D2 activity – and is not affected by circulating T₃ levels. Therefore, if the mother is hypothyroxinemic, the brain of a hypothyroid fetus is T₃-deficient, even if maternal and fetal circulating T₃ is normal or actually increased. Fetal brain T₃ is also protected from an excess of maternal T₄. Such results suggest that overtreatment of the mother with T₄ is less damaging for the fetal brain than maternal hypothyroxinemia. In contrast, there is almost no protection of the fetal brain from an excess of circulating T₃ [15].

If such experimental findings were relevant for man, they would explain why in most cases of promptly treated CH there is no permanent severe CNS damage. Most CH fetuses have a normal mother, supplying enough T₄ to the developing brain throughout gestation to avoid cerebral T₃ deficiency. As a result, the fetal brain has not been severely damaged before birth, and its normal development is thereafter achieved with T₄ treatment. These observations would also explain the irreversible damage caused by an insufficient supply of T₄ during early development, when the mother is the only source of hormone to the brain. Indeed, in the rat important phases of the development of the neocortex are altered by a period of maternal hypothyroxinemia preceding the onset of FTF [16, 17], showing directly that T₄ of maternal origin is important for early neurodevelopment. The most severe damage would be expected to occur when both the mother and fetus are hypothyroxinemic throughout pregnancy, as actually confirmed in humans [18].

**Findings in the Human Fetus**

During the last decades, our knowledge regarding thyroid hormone economy of the human fetus has increased both quantitatively and qualitatively and contributed to important new insights.

Major technical advances have made this possible, among them a) the development of highly sensitive methods to estimate very low concentrations of
iodothyronines in fetal fluids and tissues (for which commercial kits are inadequate) and b) the development of transvaginal, ultrasound-guided puncture of the embryonic cavities to obtain samples from the fetal compartment without severing vascular connections with the mother [8, 9]. The latter option has changed some previously held concepts, which had been based on findings from aborted fetuses. We will artificially divide the information into two periods, namely the first half of gestation, when the mother is the major source of thyroid hormones available to the fetus, and the second half, when active FTF starts and the maternal contribution is still quite important. Most of the information is focused on the developing brain.

**From Conception to Midgestation**

Thyroxine, T₃ and reverse 3’,5’,3-triiodothyronine (rT₃) have been found in coelomic and amniotic fluids [19] from 5.8–11 weeks’ postmenstrual age (PMA), which is 3.8–9 weeks’ postconceptional age. The concentration of T₄ in the coelomic fluid is positively correlated with the concentration of the hormone in the mother’s circulation, but values are extremely low compared with those of the mother [19]. The concentration of T₃ is at least 10-fold lower than that of T₄, whereas that of rT₃ is clearly higher. Concentrations in the coelomic fluid were higher than in the amniotic compartment. These low concentrations of T₄ and T₃ confirmed the efficiency of the placental ‘barrier’. Because of the minute amounts of iodothyronines found in these fluids, their possible biological significance was often questioned, and therefore a second study included serum samples up to 17 weeks’ PMA [19]. It confirmed that the concentration of T₄ in fetal fluids, including serum, is more than 100-fold lower than in maternal serum, and the concentration of T₃ is even lower.

Free T₄ (FT₄), however, was found to reach concentrations that are biologically active in their mothers (see fig. 2 in [9]): FT₄ in the fetal fluids is determined by the very low concentration of the T₄-binding proteins and the maternal T₄ that has escaped through the placental ‘barrier’. The T₄-binding capacity of these proteins is determined ontogenically, is independent from maternal thyroid status, and is far in excess of the amounts of total T₄ that reach the fetal fluids. Thus, the availability of FT₄ to embryonic and fetal tissues is ultimately determined by the maternal circulating T₄ or FT₄, and will decrease in hypothyroxinemic women, even if clinically euthyroid [19]. The results obtained in these studies also explain why an efficient ‘barrier’ to maternal thyroid hormone transfer is actually necessary: without it the developing tissues would be exposed to inappropriately high, and possibly toxic, concentrations of free iodothyronines. Both a decrease and an inordinately high increase in the availability of FT₄ and/or FT₃ could result in adverse effects on the timely sequence of thyroid hormone-sensitive developmental events in the human brain.
It was also observed [19] that the concentration of TSH circulating in the fetal serum, obtained before severing maternal-fetal vascular connections, was very high, ranging from 2.9 to 7.2 mIU/l, and remained higher than in the maternal serum throughout pregnancy, confirming those values reported by Thorpe-Beeston et al. [20] using samples collected by cordocentesis, without disturbing maternal-fetal connections.

The presence of thyroid hormone receptors early in the development of the human fetal brain supports the hypothesis that thyroid hormone-sensitive developmental events might already occur before midgestation. These receptors were detected in the earliest samples of the cerebral cortex studied by Bernal et al. [11] at 9 weeks’ PMA, with their concentrations increasing at least 10-fold by 18 weeks. The occupation of the thyroid hormone receptors by T₃ was 25–30% throughout the study period, despite the very low serum concentrations of this iodothyronine. This very important finding supports the hypothesis that the biological effects of the hormone might already occur in the cerebral cortex during the first trimester of human pregnancy. This possibility is supported by the more recent study by Iskaros et al. [13], which confirmed the early expression of TR genes in the whole fetal brain studied between 8.1 and 13.9 weeks PMA [11–13, 21].

The ontogenic patterns of the concentrations of T₄, T₃, rT₃, and the activity of the iodothyronine deiodinases D1, D2 and D3 have now been studied in nine different areas of the brain between 13 and 20 weeks’ PMA. The developmental patterns of the iodothyronines, and of the activity of D2 and D3, showed both spatial and temporal specificity, but with divergence in the cerebral cortex and other brain areas, especially the cerebellum [22] (fig. 1). T₃ increased in the cerebral cortex between 13 and 20 weeks’ PMA reaching concentrations similar to those in adult cortex, despite the very low concentration of circulating T₃. The data support the idea that T₃ in the human cerebral cortex is also locally generated from T₄, with considerable D2 activity being indeed found together with very low D3. In contrast, T₃ in cerebellum was very low, and increased only after midgestation, probably because cerebellar D3 activities were the highest of those found in the brain areas studied, and decreased only after midgestation. These findings support the hypothesis that T₃ is indeed required by the human cerebral cortex before midgestation, when the mother is the only source of the FT₄ available to fetal tissues, and confirm the important opposite roles of D2 and D3 in the local and timely bioavailability of cerebral T₃ during fetal life. It is important to realize that during the first trimester there is a maternal FT₄ peak [see the chapter by Glinoer, pp. 62–85] that appears imposed by the conceptus to ensure enough T₄ for generation of T₃ in the cerebral cortex up to midgestation [8, 9].
Availability of Thyroid Hormones to the Fetus from Midgestation to Birth

Fluids of the Fetal Compartment and Fetal Brain

Most diagrams summarizing changes in circulating levels of T₄, T₃, rT₃, and TSH of the human fetus had for many years been based on serum samples from premature babies who had died for different reasons at variable intervals after birth and at different stages of gestation, the results being thus affected by many factors other than developmental age. As already indicated, ultrasound-guided blood sampling without interruption of the maternal to fetal vascular connections, finally assessed the fetal thyroid hormone situation in vivo [19, 20], confirming only some of the patterns previously described. Thorpe-Beeston et al. [20] confirmed that T₃ and FT₃ serum concentrations were very low throughout fetal life. In striking contrast to previous reports, however, T₄ and FT₄ were found to reach maternal and adult concentrations already at the beginning of the third trimester, and increased steadily with fetal age until birth (fig. 2). Fetal

*Fig. 1.* Changes in the concentrations of T₄ and T₃ in the cerebral cortex and cerebellum of human fetuses before onset of FTF. T₄ in fetal serum increases 5-fold, from 3 to 15 pmol/ml; T₃ in fetal serum is very low throughout, approximately 0.5 pmol/ml (drawn using information from Kester et al. [22]). The asterisk indicates that the increase is significant.
serum TSH and FT₄ concentrations were found positively correlated \( r = 0.896, p < 0.001 \) until birth and not negatively, as previously thought. Moreover, fetal serum TSH concentrations throughout pregnancy were well above the maternal TSH levels, reaching up to 12 mIU/l near term [20] (fig. 2).

Fetal T₄ and FT₄ are already increasing steadily in utero before the fetal thyroid itself is able to maintain such serum concentrations: the degree of iodination of thyroglobulin and its T₄ and T₃ contents are very low before 42 weeks’ PMA [23]. The fetal contribution to its serum T₄ and FT₄ would be smaller, the earlier the gestational age. This may well be an important factor in the neonatal hypothyroxinemia of premature infants that will be discussed further on.

Due to obvious ethical constraints there is very little information regarding thyroid hormone concentrations and iodothyrone deiodinase expression and/or activities in different fetal tissues during the second half of pregnancy. Studies performed so far, including our own [22], have relied on autopsy material of premature babies affected by many factors other than development age. For these reasons, we will not review information obtained so far, as it is not possible to define the ontogenic developments per se, free from confounding factors.

**The Role of the Mother from Midgestation to Birth**

The transfer of maternal T₄ to the fetus continues until the umbilical cord is severed, as conclusively shown in 1989 by Vulsma et al. [24] who found concentrations of T₄ in cord blood of 7 neonates with complete organification defect that represented about 30–60% of the mean values reached by the normal fetus at term. In hypothyroid rat fetuses, serum T₄ concentrations that are

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**Fig. 2.** Changes in the concentrations of FT₄, FT₃ and TSH throughout gestation, in maternal (M) and fetal (F) serum, before and after onset of FTF. The shaded areas enclose the values reported by Thorpe-Beeston et al. [20], obtained by cordocentesis without interruption of maternal-fetal vascular connections. Black squares and circles correspond to serum values found in premature infants, as reported by Morreale de Escobar and Ares [33].
30–60% of those of normal fetuses, together with the response of D2 activity in the brain, are enough to preferentially avoid cerebral T3 deficiency until birth [14]. Extrapolation of the latter findings to the human fetus suggests that after midgestation a normal maternal supply of T4, together with the increase in cerebral D2 activity that occurs when the fetal thyroid does not secrete enough hormone, is sufficient to protect the brain from T3 deficiency, and the accompanying CNS damage, until birth, explaining the good results of prompt postnatal treatment of CH infants.

Although valuable new insights have been obtained regarding the ontogenic patterns of cerebral thyroid hormone concentrations, their nuclear receptors, and the roles of the deiodinating isoenzymes in tailoring the bioavailability of T3 to the developmental requirements of different cerebral structures, it is likely that we are still quite far from understanding all the mechanisms that may be involved, and their interrelationships. Very little is known regarding the role, in determining the availability of circulating T4 to the fetal brain [for a review, see 9], of the activities of the deiodinating enzyme isoforms in other fetal tissues, as well as those of the sulfotransferases, glucoronidases, and sulfatases. Even less is known regarding the possible role of the recently identified specific iodothyronine plasma membrane transporters into, and out of, the fetal brain. We have already remarked upon our ignorance with respect to a possible developmental role of the high levels of TSH throughout gestation, as well as the cause for their rapid decrease after premature birth [9]. We still have insufficient information regarding the capacity of the fetal thyroid to meet the needs of the newborn preterm infant faced with the untimely interruption of the maternal supply of hormone, or how to improve it.

The Brain of the Fetus from Midgestation to Birth

In the rat, so often successfully used as experimental models for the study of the influence of thyroid hormones on brain development, the maturation of the brain is severely and irreversibly impaired when the postnatal thyroid function of the pup is inadequate. During the first few weeks of the suckling period brain development comprises phases that occur during the second half of gestation in humans. Figure 3a shows the human cerebral cortex at different stages of fetal development, specifically the development of layer V pyramidal neurons [25]. It clearly illustrates that major phases of corticogenesis still have to develop in the brain of the premature neonates as compared to those of the children born at term. Figure 3b and c also shows the complexity of changes between birth and weaning. Taking into consideration that an inadequate supply of thyroid hormones, especially of T4, during the first half of human gestation, and that a delay in postnatal treatment with T4 of congenitally hypothyroid newborns both result in central nervous system damage, it seems fair to conclude that neurodevelopment during the second half of pregnancy also requires an adequate supply of maternal...
thyroid hormones. This conclusion receives direct experimental support in rats, where maternal hypothyroxinemia late in pregnancy, during a developmental period comparable to that occurring in the human brain during the second half of pregnancy, results in important irreversible alterations of cerebral cortex and hippocampal structures, and of behavior [16, 17].

**Prematurity**

Survival of premature infants has increased drastically with the improvements that have been introduced over the last decades, such as antepartum steroids, surfactant replacement, minimized volutrauma, optimized fluid maintenance, transfusion and nutritional intake, reduction in infections, and neonatal management techniques that emphasize stress reduction. This is also true for the survival of infants born at 23–27 completed weeks of gestation, with <1,000 g at birth, who we refer to here as ‘great prematures’. But their improved survival rate has carried considerable costs [26], both emotional and economical, for the children themselves, their families and society: a large proportion of these children suffer from long-term disabilities, including disabling cerebral palsy; the only clearly associated factor is male sex. The results obtained in many other similar studies were also rather discouraging, and ‘defining the limits of hope’ [27].
Several observations, however, have pointed to the identification of causal factors that might be amenable to interventions ameliorating the developmental outcome of premature infants. During the last two decades several studies have been published that strongly support a causal connection between low circulating T₃ or T₄ [28, 29] during neonatal development of the premature infant and permanent cognitive and/or neurological abnormalities, including disabling cerebral palsy. The conclusions from these studies, was that the transient hypothyroxinemia or hypotiroiddothyroninemia frequently accompanying prematurity should not be considered either ‘physiological’ or ‘harmless’.

Although for years the low levels of circulating T₄ or T₃, without increased serum TSH, had been considered a ‘physiological’ consequence of fetal hypothalamic-pituitary-thyroid immaturity, results such as those illustrated in figure 2, however, strongly suggest an important role of the sudden interruption of the maternal supply of thyroid hormone, at phases of development when the thyroid hormone requirements of the neonate cannot be adequately met because of the neonate’s hypothalamic-pituitary-thyroid immaturity. The ‘physiological’ situation of the premature infant, that is, to continue developing in utero, would have been quite different. Circulating T₄ and FT₄ are significantly higher for the fetus in utero than for age-matched prematurely born neonates. Moreover, the fetus is exposed to very high TSH levels, which drop abruptly with interruption of the maternal-fetal vascular connections. More recent studies performed on 620 premature infants [30] have confirmed that at 7 days after birth many of them had serum T₄ concentrations that are below those of term babies, and that 41% of neonates of the 23- to 27-week group had T₄ values below –1 SD of the cord levels adjusted for gestational age, TSH being also lower. Such results confirm that the intrauterine availability of both T₄ and T₃ is higher than that provided by the immature fetal thyroid, and that their postnatal hypothyroxinemia is not ‘physiological’, leading to a different outlook, in which the premature interruption of maternal transfer of thyroid hormones acquires an important causative role in the postnatal hypothyroxinemia of ‘great’ prematures.

It also became increasingly evident that this condition should not be considered ‘harmless’, because of the possibility that it is causally related to developmental deficits [28, 29]. The possibility that postnatal substitution therapy with thyroid hormones might ameliorate the outcome was explored. Among these studies, the randomized, placebo-controlled, double-blind trial of thyroxine supplementation in 200 infants born at less than 30 weeks’ gestation, performed by van Wassenaer et al. [31] merit special attention. Half of the cohort was treated for 6 weeks after birth with T₄, the other 100 with placebo. Initial results at 24 months of age showed an important benefit of treatment in the few premature babies born at <27 weeks’ gestation, with an 18 points higher developmental score, that reached normal values. In contrast, postnatal treatment of
older prematures suggested negative effects. Developmental evaluation at 10 years of age confirmed the initial results, and supported the need for new double-blind treatment trials, especially in infants born at 27 weeks or less. A trial enabling project is at present being carried out to define doses and modes of postnatal treatments of such infants, in order to mimic the circulating levels of thyroid hormones that they would have if they were still developing in utero [32]. It is hoped that such attempts might change the present pessimistic ‘definitions of the limits of hope’ [27] for such infants.

References


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