

Role of thyroid hormone during early brain development

Gabriella Morreale de Escobar, María Jesús Obregón and Francisco Escobar del Rey

Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas (CSIC) y Universidad Autónoma de Madrid (UAM), Arturo Duperier, 4, 28029-Madrid, Spain

(Correspondence should be addressed to G Morreale de Escobar; Email: gmorreale@iib.uam.es)

Abstract

The present comments are restricted to the role of maternal thyroid hormone on early brain development, and are based mostly on information presently available for the human fetal brain. It emphasizes that maternal hypothyroxinemia – defined as thyroxine (T4) concentrations that are low for the stage of pregnancy – is potentially damaging for neurodevelopment of the fetus throughout pregnancy, but especially so before midgestation, as the mother is then the only source of T4 for the developing brain. Despite a highly efficient uterine–placental ‘barrier’ to their transfer, very small amounts of T4 and triiodothyronine (T3) of maternal origin are present in the fetal compartment by 4 weeks after conception, with T4 increasing steadily thereafter. A major proportion of T4 in fetal fluids is not protein-bound: the ‘free’ T4 (FT4) available to fetal tissues is determined by the maternal serum T4, and reaches concentrations known to be of biological significance in adults. Despite very low T3 and ‘free’ T3 (FT3) in fetal fluids, the T3 generated locally from T4 in the cerebral cortex reaches adult concentrations by midgestation, and is partly bound to its nuclear receptor. Experimental results in the rat strongly support the conclusion that thyroid hormone is already required for normal corticogenesis very early in pregnancy.

The first trimester surge of maternal FT4 is proposed as a biologically relevant event controlled by the conceptus to ensure its developing cerebral cortex is provided with the necessary amounts of substrate for the local generation of adequate amounts of T3 for binding to its nuclear receptor. Women unable to increase their production of T4 early in pregnancy would constitute a population at risk for neurological disabilities in their children. As mild–moderate iodine deficiency is still the most widespread cause of maternal hypothyroxinemia in Western societies, the birth of many children with learning disabilities may already be preventable by advising women to take iodine supplements as soon as pregnancy starts, or earlier if possible.

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Introduction

The association between alterations of thyroid function early after birth and neurodevelopmental disorders has been recognized for more than a century. For many years of the 20th century there has been, however, less consensus regarding the stage during fetal life when thyroid hormone becomes necessary for normal brain development (more extensively reviewed by us in (1–4)). This has mostly been due to opposing views regarding the importance of maternal thyroid hormones for the fetus. On the one hand, those who had personal field experience of iodine-deficiency disorders (IDDs) were convinced of its importance, because the severity of the central nervous system (CNS) damage of the progeny was related to the degree of maternal thyroxine (T4) deficiency and could only be prevented when the latter was corrected before midgestation. On the other hand, Western-trained physicians usually adhered to the idea that maternal thyroid hormones did not play a role in early neurodevelopment, an idea apparently supported by the good results obtained with prompt treatment of congenital

hypothyroidism (CH). This success was interpreted as proof that the developing fetal brain did not need thyroid hormone until after birth. The idea that the maternal thyroid hormones were of little relevance for the early fetal brain was reinforced by the increasing evidence of the existence of an efficient utero–placental ‘barrier’ that prevented the transfer of maternal thyroid hormones into the fetal compartment in amounts that could be physiologically relevant. The importance of an adequate provision of thyroid hormones for brain development during later phases of pregnancy was, however, increasingly accepted when the transfer of maternal T4 up to birth was shown in man (5) and its possible protective role in cases of CH was recognized (6).

Despite the increasing awareness that thyroid hormone is already required for normal brain development during fetal life, the general consensus as late as 1999 was summarized by Utiger (7): ‘Thyroid deficiency during the latter two thirds of gestation and the first months after delivery can result in mental retardation and sometimes neurological deficits. Whether thyroid hormone is needed during the first trimester is less

certain. If it is, it must be supplied by the mother, because none is secreted by the fetus until the middle trimester'. The same editorial drew attention to the many severe neurological defects found in children born to iodine-deficient mothers that require adequate intervention before midgestation for their prevention, and that do not occur in untreated CH infants. In the present contribution we will try to summarize what is known, and what is still unknown, regarding early fetal thyroid hormone physiology and its dependence on the production of T4 by the mother.

Findings from experimental rat models

Much of our present knowledge regarding transfer of thyroid hormones from the mother to the fetus and its possible role in fetal brain development has been prompted by previous findings in experimental animal models, especially in rats. There is an important similarity between man and rat with respect to placentation, which is hemochorial in both species. There are, however, major differences between human and rat brain development if we take birth as the point of reference, because the rat is born at a less mature stage, the newborn pup being comparable to a human fetus nearing the third trimester. Conversely, the human newborn might be compared with a 2- to 3-week-old rat pup, but with a very important difference that is often forgotten when postnatal findings in hypothyroid rat models are extrapolated to the third trimester human CH fetus. In man development of the fetal brain may still be protected by the transfer of maternal T4 during a period of development when the rat is deprived of this potential benefit, as rat milk does not contain thyroid hormone in relevant amounts.

Valid comparisons may, however, be made by using the onset of active fetal thyroid function (FTF) as the milestone for comparisons. This coincides in both species with full maturation of the pituitary portal vessels, and occurs at E17.5-18 in the rat (with E0 being the day of conception and E21-22 the day of birth), and at 18-20 weeks of postmenstrual age (PMA) in man (at 16-20 weeks postconception), with birth at 36-40 weeks PMA. Thus, most of the comparisons between both species will be restricted to development and maternal-fetal interrelations before FTF, unless stated otherwise. Findings relevant to the present topic are very briefly summarized here. For pertinent detailed references, see Table 3 in a previous review (1). References are mostly restricted to more recent studies.

T4 and triiodothyronine (T3) of maternal origin are present, albeit at very low concentrations, in very early rat embryonic and fetal tissues, brain included, before onset of FTF, their concentrations being directly influenced by those in the maternal circulation, especially those of T4. Thyroid hormone receptor (TR) isoforms are already present in the brain at neural

tube closure and are likely to mediate biological effects of the T3 that has been locally generated from T4 transferred from the mother. Therefore, if the mother is hypothyroxinemic, the brain of a hypothyroid rat fetus is T3-deficient, even if maternal and fetal T3 are normal, because during early development, serum-derived T3 hardly contributes to cerebral T3 (6). Important phases of the development of the neocortex are altered by a period of maternal hypothyroxinemia preceding onset of FTF (8, 9), showing directly that thyroid hormone of maternal origin is important for neurodevelopment.

If such experimental findings were relevant for man, they would explain why in most cases of CH there is no permanent severe CNS damage when T4 is supplied starting soon after birth. Most fetuses with CH have a normal mother, supplying enough T4 to the developing brain throughout gestation to preferentially avoid cerebral T3 deficiency. As a result, the fetal brain has not been severely damaged before birth, and its normal development can still be achieved by prompt postnatal treatment with T4. They 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. The more severe damage would be expected to occur when both the mother and fetus are hypothyroxinemic throughout pregnancy, as occurs in iodine-deficient environments, and as increasingly confirmed by case reports (1-4, 10).

Thyroid hormones and their nuclear receptors in the human fetal brain

As already indicated, during most of the second half of the 20th century the prevailing idea was that the early embryo actually developed in the absence of thyroid hormones. Supporting this conclusion was the evidence of a placental 'barrier' system that drastically limited their transfer from the mother. Recent information has confirmed the widespread distribution, mostly of deiodinases D2 and D3, in the utero-placental unit and the expression of D3 in fetal epithelia (11-14).

As in experimental animals, the existence of an active barrier, however, does not necessarily exclude that some iodothyronines of maternal origin actually do reach fetal tissues, and we shall briefly summarize the existing evidence, mostly as pertaining to the brain. Most of the experimental findings in the rat models, are being confirmed - and actually extended - in humans.

From conception to midgestation

Attempts to measure the very low concentrations of iodothyronines in fetal tissues had to await the development of adequate extraction methods that permitted their purification and determination by sensitive and specific RIAs. Most commercially available methods

that have been developed for human sera are not adequate for the very low concentrations found up to midgestation in human fetal serum. Commercial kits for the estimation of 'free' T4 (FT4) are even less adequate, because of the great qualitative and quantitative differences in the composition of T4-binding proteins between fetal fluids and adult serum.

During the 1980s, T4 and T3 were measured in tissue extracts from cerebral cortex, liver and lung of 8–18 week PMA human fetuses, using improved specific and highly sensitive RIAs (15–17). In liver, lung and heart, only T4 was found during the second trimester, although T3 could be demonstrated at somewhat earlier ages in lung nuclear extracts. T3 was quantified in purified extracts from human fetal brain, however, as early as 9–10 weeks PMA, and increased steadily up to 18 weeks PMA, despite the fact that during this period plasma T3 was undetectable and <10% of adult values. By midgestation the concentration of T3 in the fetal brain reached 34% of adult values, much higher than would have been inferred from their very low circulating T3.

Another important methodological improvement was the development of transvaginal ultrasound-guided puncture of the embryonic cavities (Fig. 1) to obtain

samples from the fetal compartment without severing vascular connections with the mother. This procedure, combined with specific and sensitive RIAs, disclosed important new information regarding fetal thyroid hormones early in pregnancy (18). T4, T3 and reverse T3 (rT3) were found in the first trimester coelomic and amniotic fluids from 5.8–11 weeks PMA (3.8–9 weeks postconceptional age) (Fig. 2). The T4 concentrations in the coelomic fluid were positively correlated with the maternal circulating concentrations, but were <1% of the maternal values. T3 was at least 10-fold lower than T4, with rT3 being clearly higher than T4, findings that confirmed the high D3 activities of the placental 'barrier' and fetal epithelia. Concentrations in the coelomic fluid were higher than in the amniotic compartment. Because of the minute amounts of the iodothyronines found in these fluids, their possible biological significance was often questioned.

The results were essentially confirmed and extended in a second study (19) where transvaginal ultrasound-guided puncture of these cavities and of fetal blood was performed up to 17 weeks PMA. A specific methodology was developed for the determination of FT4. We confirmed the previous observation (18) that T4 in fetal fluids is more than 100-fold lower than in

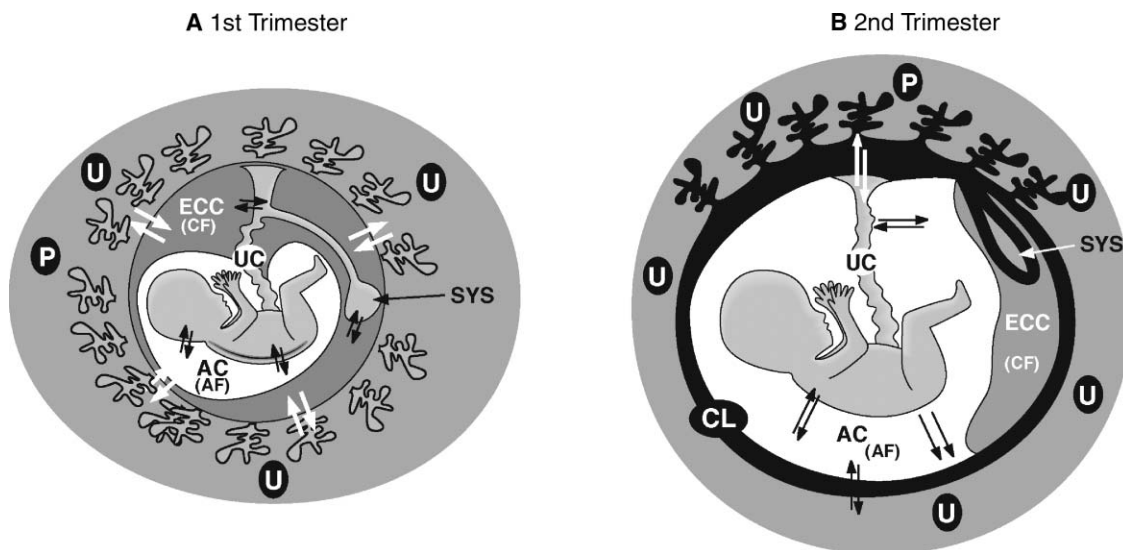


Figure 1 Schematic representation of the maternal–fetal unit during the 1st (A) and 2nd (B) trimesters of pregnancy. (A) The human fetus is surrounded by two distinct fluid cavities separated from each other by a thin membrane: the inner, or amniotic cavity (AC) contains the fetus and the outer, or exocoelomic cavity (ECC), separates the amniotic cavity from the placenta and contains the secondary yolk sac (SYS). The latter is directly connected to the fetal digestive tract and circulation. The ECC is the site of important molecular exchanges between the mother and the fetus and contains the coelomic fluid (CF) that results from an ultrafiltrate of maternal serum with the addition of specific placental and SYS bioproducts. The ECC is a physiological liquid extension of the early placenta (P) and acts as a reservoir for nutrients needed by the developing fetus. There is no direct vascular connection between the mother and the umbilical cord of the fetus. (B) A second mode of transfer starts at the end of the 1st trimester. The SYS and two-thirds of the placental mass degenerate and the ECC is progressively obliterated by the growing AC containing the amniotic fluid (AF) surrounding the fetus. These major anatomical transformations modify considerably the spatial relationships between maternal tissue and developing fetus, and, consequently, the maternal–fetal exchange pathways. From 11–12 weeks onwards maternal nutrients, including thyroid hormone, are transferred from the placenta directly into the fetal circulation. The AF contains fetal urine and waste products. U, uterus; UC, umbilical cord; CL, chorion laeve (membranes in development).

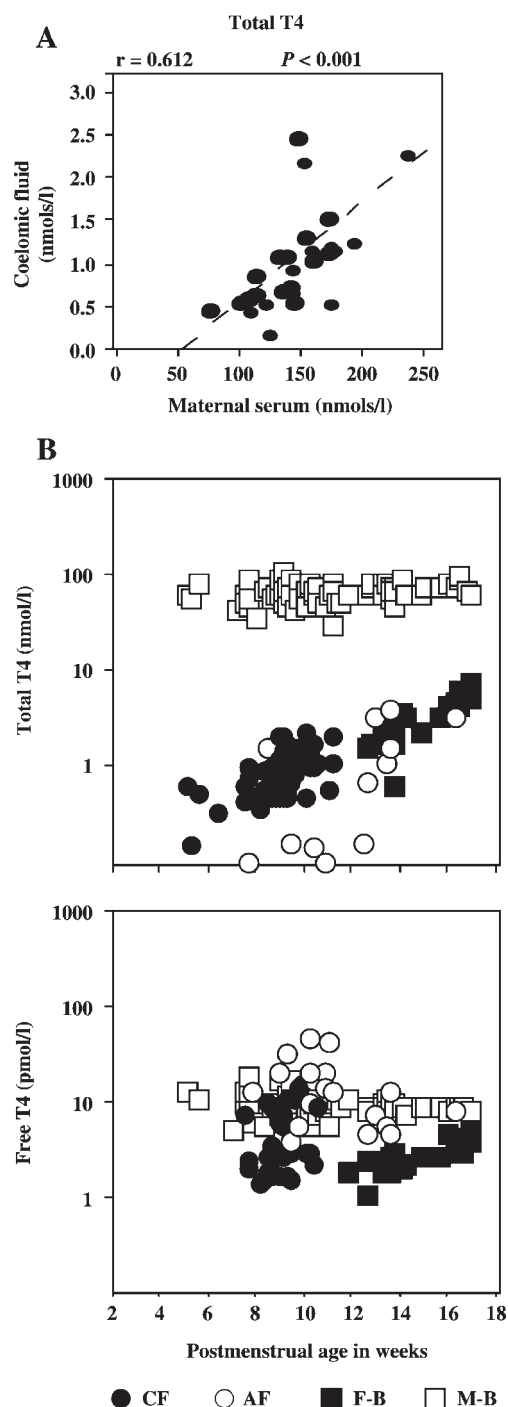


Figure 2 (A) Changes in concentrations of total T4 and FT4 in fetal fluids up to midgestation, as a function of maternal serum T4 values, which were within the normal range (18). (B) Concentrations of total and FT4, as a function of postmenstrual age, in fetal coelomic fluid (CF), amniotic fluid (AF), fetal blood (F-B) and maternal blood (M-B). The ordinates in both panels are on a logarithmic scale, in order to better visualize the similarity of the FT4 concentrations in fetal fluids to those in the corresponding mother, whereas there is a greater than 100-fold difference in the T4 concentrations (data from (19)). For both (A) and (B) the fetal fluids were obtained without severing maternal to fetal vascular connections.

maternal serum, with T3 being even lower. The new finding was that this great difference between the maternal and fetal concentrations of total T4 might be misleading with respect to their potential biological significance, because the proportion of T4 that is not bound to proteins is so much higher than in adult sera that the concentrations of T4 actually 'available' to developing tissues, namely those of FT4, reach values which are comparable to those known to be biologically active in their mothers (Fig. 2B).

The FT4 levels in the fetal fluids are defined by the concentrations of T4-binding proteins and the concentrations of maternal T4 or FT4 that have escaped the placental 'barrier'. The T4-binding capacity of the proteins in fetal fluids is determined ontogenically, is independent of the maternal thyroid status, and is far in excess of the amounts of total T4 that reach the fetal fluids. Thus, the availability of FT4 for embryonic and fetal tissues is ultimately determined by the maternal circulating T4 or FT4 and would decrease in hypothyroxinemic women, even if they are clinically euthyroid. The results explained why an efficient 'barrier' to maternal thyroid hormone transfer is actually necessary. If total T4 and T3 reached the same concentrations in fetal fluids as those in the maternal serum, the developing tissues would be exposed to inappropriately high, and possibly toxic, concentrations of FT4 and 'free' T3 (FT3). An inordinately high FT4 and/or FT3 could result in adverse effects on the timely sequence of thyroid hormone-sensitive developmental events in the human fetus, as recently confirmed (20).

That thyroid hormone-sensitive developmental events may already occur before midgestation and onset of FTF is supported by the early presence of nuclear TRs in the human fetal brain. These were detected in the earliest samples of the cerebral cortex (9 weeks PMA) studied by Bernal & Pekonen (15) with their concentration increasing at least 10-fold by 18 weeks. Despite the very low fetal serum concentrations of T3, the occupation of the TRs by this iodothyronine was 25–30% throughout the study period (15–17), strongly suggesting that biological effects of the hormone might already be occurring in the cerebral cortex during the first trimester of human pregnancy. A recent study (21) has confirmed the early expression of TR gene isoforms and related splice variants in the whole fetal brain studied between 8.1 and 13.9 weeks PMA. Expression of the TR β 1, TR α 1 and c-erbA α 2 isoforms was detected in the 8.1 week brain sample, with TR α 1 being the predominant form in early development, increasing steadily up to 13.9 weeks; so did the c-erbA α 2 isoform. TR β 1 expression appeared to present a more complex ontogenic pattern. The authors moreover point out that the repressor activity of un-liganded Tr α 1 on basal gene transcription may also become relevant when the maternal supply of hormone decreases: a decrease in the amount of T3 available for receptor binding

would increase the proportion of the un-liganded isoform and its repressor activity, and further interfere with thyroid hormone-sensitive biological effects.

The ontogenic patterns of the concentrations of T4, T3, rT3, and of D1, D2 and D3 activities have now been studied (22) in nine different cerebral areas from fetuses of 13–20 weeks PMA, when fetal serum T4 increased significantly from about 3 to 15 pmol/ml, whereas T3 did not correlate with PMA and remained at about 0.5 pmol/ml throughout the same developmental period (19). The ontogenic profiles of the concentrations of the iodothyronines in the different areas of the brain, and of their D2 and D3 activities, showed both spatial and temporal specificity, but with divergence in the cerebral cortex as compared with other brain areas (Fig. 3). In the cortex the concentration of T4 was increasing with PMA, as expected from the increase in fetal serum T4. But, in contrast with the very low and practically constant circulating levels, T3 increased significantly with PMA in the cortex between 13 and 20 weeks PMA to levels comparable to those reported in adults (2.5 pmol/g). These findings show that in the human cerebral cortex T3 is also generated locally from T4, and is hardly influenced by circulating T3. Considerable D2 activity was indeed found in the human cerebral cortex, whereas D3 activity was very low. In contrast, cerebellar D3 activities were very high until midgestation, and T3 was very low, only increasing after midgestation, when D3 activity was decreasing. Other regions with high D3 activities (midbrain, basal ganglia, brain stem, spinal cord,

hippocampus) also had low T3 concentrations up to mid-gestation. The study (22) supports the hypothesis that T3 is indeed required by the human cerebral cortex before midgestation, when the mother is the only source of the FT4 that is available to the fetal tissues. It confirms the important roles of D2 and D3 in the local bioavailability of cerebral T3 during fetal life; D2 generates T3 from T4 and D3 protects different brain regions from an untimely, or excessive, T3 until this hormone is required for differentiation.

We do not, however, have precise information on the stage of human brain development when down-regulation of D2 expression and/or activity might contribute to T3 bioavailability in conditions of maternal or fetal hypothyroxinemia; if down-regulation is delayed with respect to the onset of its expression, a decrease in maternal T4 would ultimately result in a lower intracellular concentration of T3 (4).

Between midgestation and birth

There is a dearth of information on the ontogenic patterns of thyroid hormone concentrations and iodothyronine deiodinase expression and/or activities in different fetal tissues during the second half of pregnancy. Studies performed so far have relied on autopsy material of babies who died of different causes and at variable intervals after a premature birth, and results are subject to many confounding factors other than their PMA. The most important ones are likely to be the premature interruption of the

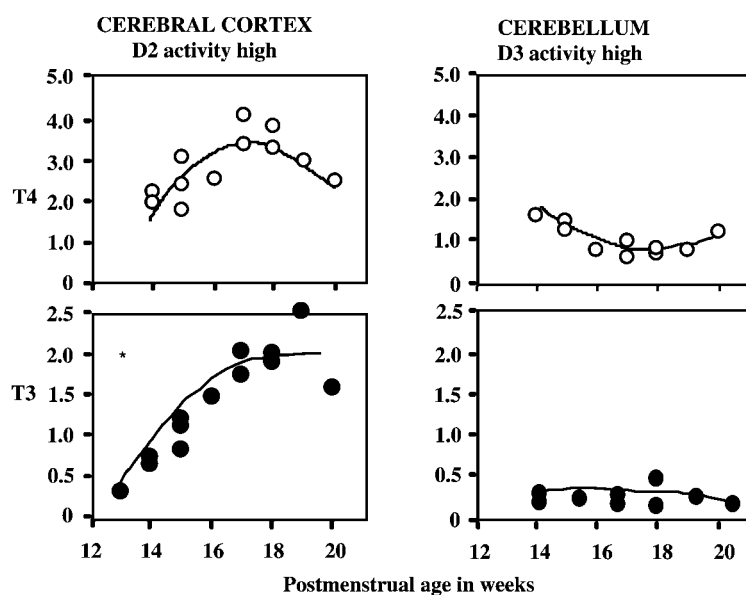


Figure 3 Ontogenic changes in the concentrations of T4 (upper panels), and T3 (lower panels) in the human cerebral cortex (left-hand panels) and cerebellum (right-hand panels) up to midgestation when the mother is the only source of thyroid hormone for the developing fetus (data from (22)). During this period T4 in the fetal serum increases about 5-fold, from 3 to 15 pmol/ml, whereas circulating T3 remains very low, about 0.5 pmol/ml (equivalent to 0.5 pmol/g), and does not increase with PMA (19). D3 activities are very high in the cerebellum throughout this period.

maternal supply of T4 and the sudden major decrease in circulating thyrotropin (TSH). For this reason such studies will not be included here. We will only summarize what is known regarding thyroid hormones and related indices of fetal thyroid physiology that have been obtained *in utero* and *in vivo*, restricting our knowledge to data obtained from serum samples.

For many years most diagrams (23) describing ontogenic patterns of changes in fetal circulating levels of T4, T3, rT3, TSH, T4-binding globulin (TBG) etc. had been derived from premature babies, after interruption of maternal connections. Early in the 1990s, however, ultrasound-guided blood sampling from the umbilical cord and the heart was used to obtain fetal samples between 12 and 37 weeks PMA (24, 25) (Fig. 4). Some of the patterns previously described (23) for different parameters of FTF were confirmed: T3 and FT3 were very low throughout fetal life, as compared with those in both the maternal serum and in the adult population. In striking contrast, however, both T4 and FT4 increased steadily with fetal age, and reached maternal and adult concentrations by the beginning of the third trimester (25). Contrasting with previous reports of a negative feed-back between the fetal thyroid and the hypothalamic-pituitary system during the third trimester, Thorpe-Beeston *et al.* (25) found that FT4 and TSH were both increasing until birth.

An even greater discrepancy concerned the intrauterine fetal levels of TSH that were much higher than maternal and adult values throughout the study period, a finding confirmed in our later study (19). This was in conceptual agreement with reports that in both normal and anencephalic fetuses, TSH bioactivity is greatly increased with respect to that circulating in the mothers, confirming that fetal serum TSH is neither of maternal origin, under hypothalamic neuroendocrine control, nor under negative feed-back control by thyroid hormones, whether of maternal or fetal origin (26). Fetal TSH levels are already high well before full maturation of hypothalamic-pituitary connections at midgestation. This poses unanswered questions regarding its origin; synthesis of TSH by the rat and monkey brain have been reported (27). More recently, a TSH receptor has been found in early human fetal brain and human astrocytes in primary culture (28). This receptor mediates extrathyroidal cAMP-independent biological effects of TSH, among which is, quite interestingly, the stimulation of D2 in astroglial cells. The possible cAMP-independent 'extrathyroidal' actions of TSH throughout human fetal development are most intriguing, especially if acting in brain development as a growth factor. Equally intriguing is why the high intrauterine TSH concentrations plummet with birth. Sudden severance from the placenta might be playing a role, as the placenta produces high amounts of thyrotropin-releasing hormone-like peptides that might be stimulating extrapituitary synthesis of TSH or TSH-like proteins.

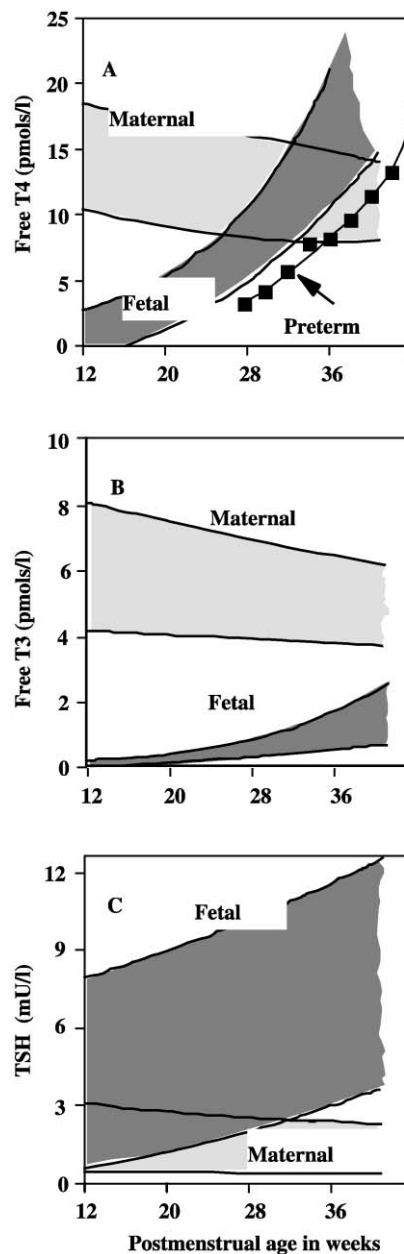


Figure 4 (A–C) The ontogenic changes in different parameters of thyroid hormone status from 12 weeks PMA until birth, obtained *in vivo* by cordocentesis, without interfering with the normal connections between mother and conceptus. The shaded areas enclose the values reported by Thorpe-Beeston *et al.* (25). (A and B) Show that fetal serum FT4 values reach maternal concentrations shortly after midgestation, whereas those of FT3 are low throughout pregnancy. (C) Draws attention to the very high levels of fetal TSH, most of which were higher than those of the mother. (A) Also shows the FT4 levels found in sera from premature babies (■, preterm) (30, 31) as compared with those *in utero* (25).

Fetal circulating T4 and FT4 are already increasing steadily *in utero* before the fetal thyroid is likely to be able to maintain such concentrations when deprived

of the maternal contribution; the degree of iodination of thyroglobulin (Tg) and its T4 and T3 contents are very poor before 42 weeks PMA (29). This lack of full maturation of the fetal thyroid would contribute significantly to the neonatal hypothyroxinemia of premature infants, a possibility that is strongly supported when serum FT4 concentrations of such infants are plotted as a function of PMA and superimposed on the pattern found for age-paired living fetuses that are still *in utero* (Fig. 4) (30, 31). Such observations imply that the mother continues to contribute significantly to fetal circulating T4 and FT4 until birth, as described for experimental animals.

That the transfer of maternal T4 to the fetus continues until the umbilical cord is severed was conclusively shown in 1989 by Vulsma *et al.* (5) who found concentrations of T4 in cord blood of seven neonates with complete organification defect, namely, a complete inability to iodinate proteins and, therefore, to synthesize the iodinated hormones. These concentrations varied between 35 and 70 nmol/l, values that are about 30–60% of the mean concentrations reached by the normal fetus at term, 109 nmol/l (25). In hypothyroid rat fetuses, serum T4 concentrations ranging between 30 and 60% of normal, together with the compensatory increase of D2 activity in the brain, would be enough to preferentially avoid cerebral T3 deficiency (6). Extrapolation of the latter findings to the human CH fetus suggests that after midgestation the down-regulation of cerebral D2 is also operative in the human brain, and has contributed to protect it from major CNS damage until birth.

Summarizing

Results so far confirm for the human developing brain the same principles that appear to modulate T3 bioavailability in different developing structures in many species, in a temporally and spatially specific sequence of events, namely by the ontogenetically programmed expression of the iodothyronine deiodinase isoenzymes, mainly D2 and D3. We have less information regarding mechanisms other than those involving the iodothyronine deiodinase isoforms that might also play important roles in tailoring T3 bioavailability to changing needs of developing human brain structures.

Thus, both T4 and T3 are present in human embryonic and fetal fluids, with the FT4 reaching concentrations that are known to be biologically relevant in adults. The FT4 concentrations in these fluids are, moreover, directly dependent on the maternal T4 supply, and so is the FT4 available to fetal tissues, including the brain. It appears plausible to conclude that the lower the maternal T4 early in pregnancy, the lower the FT4 available to the fetal cortex and, presumably, the lower the amounts of T3 available for binding to cerebral TRs exerting biological effects. It is important to realize that this could already

occur with maternal circulating levels that are still within the normal reference range for adults (Fig. 2).

Although valuable new insights have been obtained regarding the ontogenic patterns of change 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. As summarized recently in somewhat more detail (4), little is known regarding the roles, in determining the availability of circulating T4 to the fetal brain, of the activities of the deiodinating enzyme isoforms in other fetal tissues, as well as those of the sulfotransferases, glucuronidases and sulfatases, and of recently identified specific iodothyronine plasma membrane transporters into, and out of, the fetal brain. We have already remarked (4) 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. 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. Mainly for this reason, effective procedures that might improve their neurodevelopment have not yet been fully established (32).

Direct evidence of a role of maternal T4 in neurogenesis

Present findings regarding regulatory mechanisms involved in the bioavailability of T3 in the human fetal cortex early in development, as well as the early expression of nuclear TRs, already occupied by T3, strongly support the hypothesis that an adequate supply of maternal T4 is already needed by the cerebral cortex early in pregnancy. In man, such an hypothesis cannot be directly verified, or negated, for obvious ethical constraints.

In the rat, changes in maternal thyroid hormone availability during early stages of development – equivalent to the end of the first, and beginning of the second, trimester in man – affect neurogenesis irreversibly. Two models have been studied so far. One involved iodine-deficient rat dams (8). The other involved rat dams treated for only 3 days with a goitrogen (methyl-mercapto-imidazole (MMI)), a protocol that resulted in a transient and very mild degree of maternal thyroid hormone deficiency (3dMMI model) (9). In both models the dams were hypothyroxinemic between E14 and E16, a period of very active neurogenesis and of migrations of radial neurons into the developing cerebral cortex and hippocampus, the mother being the only source of thyroid hormone available to the developing fetus. The final location of

the cells generated during this period was aberrant, with neurons appearing in layers of the somatosensory cortex and hippocampus where they are never found in pups from normal dams. The cytoarchitectures of the barrel cortex and hippocampus were also affected. The short transient period of moderate maternal thyroid hormone deficiency between E12 and E15 (3dMMI model) (9) was sufficient to derange successive radial waves of neuronal migrations and to result in cytoarchitectural abnormalities that could only be prevented by the timely infusion of T4. This was of no benefit when delayed beyond the critical period of corticogenesis. An increased susceptibility to acoustic stimulation was also observed in a high proportion of the pups born to 3dMMI dams.

Such findings clearly support the importance of an adequate early supply of maternal thyroid hormone for neurodevelopment. Extrapolation to man would define the period in human gestation when the fetal cerebral cortex is especially sensitive to changes in the availability of maternal thyroid hormone within the first half of pregnancy. In man, the two main waves of radial migrations of neurons into the cortex peak at 11 and 14 weeks PMA. The first one coincides approximately with the human chorionic gonadotropin (hCG)-driven maternal FT4 surge.

Thyroid function of the mother

It has been known for decades that important changes occur in thyroid hormone physiology during normal pregnancy. When it was initially observed that maternal circulating T4 was higher than in non-pregnant women, it was believed that this was a direct consequence of the estrogen-stimulated increase of circulating TBG and of TBG moieties that are more highly sialylated and have longer biological half-lives. The increase in circulating T4 was deemed necessary in order to keep circulating FT4 within the normal range, but the expected transient decrease in FT4, followed by a rise in TSH, necessary to attain the new equilibrium was not detected.

The transient initial surge of maternal circulating FT4

We now know that the increases in T4 and TBG do not occur simultaneously, and FT4 is actually significantly increased for several weeks before TBG concentrations plateau at midgestation (33). The increased concentrations of hCG in the maternal and fetal compartments are essential for the maintenance of the pregnancy and are imposed by the presence of the conceptus. During this period the woman's thyroid is under the control of the high concentrations of hCG and hCG-related molecules that have TSH-like activity. During early pregnancy, when these are highest, secretion of both T4 and T3 is stimulated to the point that maternal circulating

TSH is suppressed. Plotting mean maternal serum hCG levels as a function of gestational age shows peak concentrations at the end of the first trimester, with TSH levels falling in a mirror image (Fig. 5).

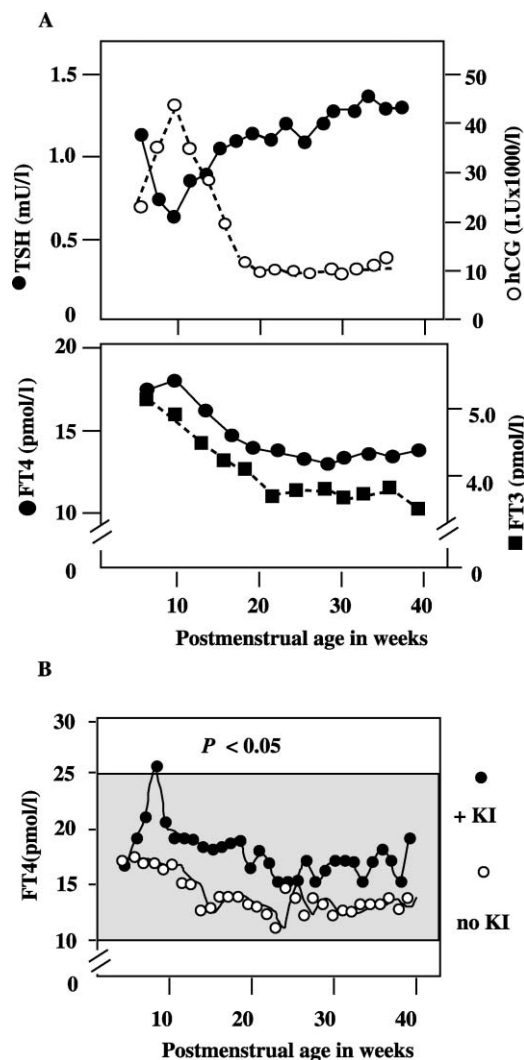


Figure 5 (A) shows that during the first trimester there is an increase in circulating hCG (A, upper panel) that results in a first trimester surge of maternal FT4 and FT3 (A, lower panel), and a suppression of circulating TSH (A, upper panel). These results support the present hypothesis that the surge in maternal FT4 before midgestation is controlled by the conceptus, and may have a biologically relevant role, and that an inadequate surge might not be detected using as the indicator an increase of serum TSH above the normal population range. Drawn using mean values reported by Glinoe (33). (B) An example of the effects of the iodine intake on the first trimester FT4 surge and on FT4 values throughout pregnancy, based on data from a study (46) of pregnant women with a median urinary iodine of 90–95 μg l/l throughout gestation, and of those in the same area advised to take potassium iodide (KI) supplements (approximately 250 μg l/day) from early pregnancy, and a mean urinary iodine excretion double that of the non-supplemented women. First trimester median FT4 values for non-supplemented and supplemented women were, respectively, 16.9 and 19.9 pmol/l.

This phenomenon has even been observed in a case of thyroid hormone resistance (34); the already high serum T4 and T3 increased further at the onset of pregnancy and reached peak values when circulating hCG was highest, at 10–12 weeks of gestation, with a mirror image in serum TSH that was temporarily suppressed.

This initial adaptation of maternal thyroid physiology to the presence of the fetus may well be one more example of the control exerted by the conceptus on the maternal endocrine system. One possible interpretation is that it is essential for the conceptus to ensure, for its own benefit, high maternal FT4 concentrations that are relevant for early neurodevelopment. To achieve this, it would be necessary to transiently override the control of thyroid function through the negative hypothalamic–pituitary–thyroid feed-back mechanism.

Increased production of T4 by the maternal thyroid

Maternal thyroid hormone production during the first half of human pregnancy obviously has to increase very soon after its onset, in order to ensure the early surge in circulating FT4, considering, among other factors, that the plasma volume increases rapidly. There is also an increased degradation of the iodothyronines by the very high activity of D3 in the uterine–placental unit, possibly also a consequence of the increased estrogen levels (12). The increase in the size of the maternal T4 pool early in pregnancy has not yet been defined precisely, and may differ in different pregnancies of the same woman. Available information suggests that it imposes a considerable burden on the maternal thyroid. It has been known for years that hypothyroid women very often have to increase their T4 dose during pregnancy (35) to 'normalize their TSH', the standard goal of treatment for non-pregnant patients. A very recent study (36) has drawn attention to the need for increasing the levothyroxine dose already by the fifth week of gestation by about 50%, in order to keep TSH within the normal range. This early and significant increase of the T4 dose, however, failed to reproduce the first trimester FT4 peak and TSH nadir found in normal pregnant women. An even greater increase in the dose might have been required if the aim had been attainment of the physiological trimester-specific FT4 and TSH values (37). This possibility is in conceptual agreement with the marked increase in iodine requirements (38, 39), which almost double from the onset of pregnancy, and is not entirely explained by the increased renal iodide clearance.

Conditions required for the maternal thyroid to meet the demands imposed by the conceptus

There are two principal requisites for the maternal thyroid to be able to meet the burden imposed by the conceptus, namely (i) that thyroid tissue is not

functionally impaired, and (ii) that the supply of iodine for the synthesis of sufficient T4 is almost double. It is becoming increasingly evident that the frequency with which women from Western industrialized countries might not be able to respond adequately to the greatly increased demands of T4 placed by the presence of the conceptus, is a 100-fold greater, or more, than that of CH babies, whose detection and early treatment by mass screening programs have proved so successful.

Impaired thyroid function In a recent summary by Glinoe & Smallridge (39) on 'The impact of Maternal Thyroid Disease on the Developing Fetus: Implications for Diagnosis, Treatment and Screening', four different conditions were discussed from a practical point of view: (i) clinical hypothyroidism, with low serum FT4 and high serum TSH; (ii) subclinical hypothyroidism, with normal FT4 and high TSH; (iii) thyroid autoimmunity features, with normal FT4, normal TSH with thyroid antibodies; and hypothyroxinemia with low FT4 and normal TSH, and (iv) hypothyroxinemia with low FT4 and normal TSH, and clinical euthyroidism.

From North-American and Western European evaluations, up to 0.5% of pregnant women (1 in 200) may have overt hypothyroidism and up to 2.5% of them (1 in 40) subclinical hypothyroidism, undetected before pregnancy. With respect to the third condition, between 6 and 12% of women of child-bearing age (1 in 16 to 1 in 8) may have thyroid antibodies, with strictly normal FT4 and TSH. Most of these reports relied on an upper limit for 'normal' TSH of approximately 5 mU/l. The number of women diagnosed as clinically or subclinically hypothyroid would probably increase further if the newer upper limit of 2.5 mU TSH/l were used, and even more if first trimester-specific ranges of serum FT4 and TSH values were available (37). Our present drawback is that we still lack reliable basic information regarding appropriate trimester-specific reference ranges for FT4, T4 and TSH obtained with samples from normal pregnancies in euthyroid women with a confirmed appropriate iodine intake (250 µg I/day) and without autoimmune disease (2, 35, 37).

Maternal hypothyroxinemia Of the four conditions indicated above, maternal hypothyroxinemia is the most frequent, even in industrialized Western societies. In The Netherlands, a population considered as iodine-sufficient, neurodevelopmental deficits have been reported (40) in one out of every two offspring from women with first trimester FT4 below the 10th percentile (1 in 20 births). The etiology of the maternal hypothyroxinemia reported in these studies has not been clarified.

The maternal hypothyroxinemia that is caused by an iodine intake that fails to meet the increased needs imposed by the conceptus, is likely to be much more frequent than primary thyroid failure and thyroid autoimmunity. It has been amply documented that iodine

deficiency is, worldwide, the most frequent cause of reproductive failure, decreased mental and motor functions, and cerebral palsy (41–43). Neurodevelopmental deficits not only occur in areas of severe iodine deficiency, more recent findings show that even mild degrees of iodine deficiency are potentially adverse for the outcome of pregnancy (38, 42, 44). A very recent 10-year follow up (45) of the progeny of women with mild iodine deficiency has shown an unusually high proportion (70%) of children with attention deficit hyperactivity disorders among those born to mothers who had been hypothyroxinemic during the first half of pregnancy. This, superimposed on the decrease, albeit moderate, of their intelligence quotients, constitutes an important handicap in our increasingly competitive societies. A recently published study (4) reveals that as many as 25% of pregnant women in the United States have iodine intakes that are less than half those recommended during pregnancy. Higher frequencies of this pregnancy-related iodine insufficiency are being reported from Western European populations where schoolchildren and non-pregnant women have an adequate iodine intake (3, 10, 39, 46, 47).

Remarks regarding maternal hypothyroxinemia caused by iodine deficiency

Why is this condition, potentially the most frequent preventable cause of learning disabilities in our industrialized societies, receiving so little attention in medical practice? We should like to point out a few of the possible reasons.

Unchanged serum TSH

Regulation of thyroid function through the hypothalamic–pituitary–thyroid negative feed-back is so ingrained in our thinking, that a low T4 is automatically associated with a high TSH. Thus, the definition of hypothyroxinemia itself – a decreased T4 without an increase of TSH above normal – is instinctively rejected. Very efficient mechanisms controlling thyroid function – other than the negative feed-back – are overlooked by, or are even unknown to, most physicians. More than half a century ago it was shown that the immediate response of the gland to decreased circulating iodide triggers very efficient autoregulatory mechanisms that result, among others, in an increase in thyroid vascularity, iodine uptake, acinar cell height, hyperplasia and serum T3/T4 ratios. All these changes are independent from TSH, and occur even when hypophysectomized rats, or hypophysectomized animals on TSH substitution, are fed a diet low in iodine (48–50). Their autonomy from TSH in man has recently been confirmed (51). Among the many changes that occur, one directly related to the present topic regarding maternal hypothyroxinemia is that the synthesis and

secretion of thyroid hormones is switched towards a preferential use of the decreasing iodine supply in favor of T3 over T4 (50, 52). As a consequence, circulating T4 decreases, but T3 does not, and may actually increase, preventing both an increase in serum TSH and clinical manifestations of hypothyroidism (53–55). Indeed, increased serum TSH is rarely found in goitrous individuals from areas with iodine deficiency alone, with increased Tg concentrations being a much more frequent finding in mild to moderate iodine deficiency. In the seminal studies by Glinoe and colleagues (10, 33, 56) on thyroid function in pregnant women from a population with moderate iodine deficiency, increased TSH levels were not found, even among the women with the lowest first trimester FT4 levels, whereas increased T3/T4 molar ratios and serum Tg, were already observed from the onset. Serum TSH tended to increase by the third trimester, but mostly remained within the normal range.

The generalized, but inaccurate idea that iodine deficiency not only lowers T4 production, but results necessarily in increased circulating TSH is mostly derived from studies in iodine-deficient areas where additional factors (i.e. goitrogens, selenium deficiency, etc.) result in loss of functional thyroid tissue, and even in glandular atrophy (57, 58), curtailing adaptation through autoregulatory mechanisms.

Maintenance of euthyroidism

Iodine deficiency is also inaccurately associated with clinical manifestations of hypothyroidism; individuals are frequently referred to as hypothyroid, even in recent reviews (i.e. see (59) and keywords in (33)). This statement is correct in individuals from the same iodine-deficiency goiter endemias with myxoedema indicated above where TSH is increased. This widespread assumption has been inadvertently compounded by the inclusion of 'hypothyroidism' in the long list of IDD's that summarized findings from areas with endemic goiter (60). No distinction was made between IDD's reported from areas of iodine deficiency alone from those reported from areas where additional factors result in loss of functional thyroid tissue. Individuals from areas where the autoregulatory mechanism permit their adaptation to the inadequate iodine supply, are clinically euthyroid, even in situations of severe iodine deficiency (53) because of their normal, or increased, circulating T3.

To further complicate the issue, tissues (such as the brain) that depend mostly on T4 for their availability of T3 may, however, be T3-deficient and selectively hypothyroid (60) without clinical manifestation of hypothyroidism of the individual as a whole. They are often described as 'dull', with whole populations appearing to 'wake up' when the iodine deficiency – and the hypothyroxinemia – are corrected (61).

Maternal vs fetal adaptation to iodine deficiency

The fetus constitutes an exception with respect to our previous comments regarding the unchanged serum TSH and the clinical euthyroidism of inhabitants of areas with iodine deficiency alone, because autoregulatory mechanisms are not yet fully operative until after birth (62); there would be no preferential secretion of T3 preventing an increase in serum TSH and hypothyroidism. This is in conceptual agreement with the observation that the proportion of newborns at screening with whole blood TSH above 5 mU/l is increased above 3% in populations with iodine deficiency (51). For the same reason, they would not be protected from hypothyroidism. Statements pertaining to the thyroid status of the mother should be dissociated from those regarding the fetus, as they are not necessarily the same.

Final comments

Presently available information that has been summarized here supports the hypothesis that an inappropriate first trimester surge in maternal FT4, whatever the circulating TSH, would interfere with the development of the cerebral cortex, even if maternal euthyroidism is maintained by normal circulating T3 (Fig. 6).

There is at present increasing consensus that maternal hypothyroidism, both clinical and subclinical, requires early detection and prompt treatment, because of its important negative effects for the woman, the pregnancy and the child (i.e. see (1, 2, 4, 10, 35, 39, 59)). Their early detection, and that of women with thyroid autoimmunity, by mass screening programs poses considerable logistic problems in large countries such as the United States, but ought to be implemented in European countries already providing special health care for pregnant women, without further controlled prospective trials regarding the efficacy of treatment, which might no longer be ethically acceptable. With respect to mass screening for maternal hypothyroxinemia, the most frequent cause of preventable neurodevelopmental handicaps, there are still uncertainties regarding the cut-off points of laboratory data for its definition. This is the bad news. But the good news is that most cases of maternal hypothyroxinemia are related to a relative iodine deficiency during pregnancy that can be so easily prevented, with minimal expense, without risk (63) and with worldwide success (38, 41). It follows that we can already prevent a very frequent cause of learning disabilities of new generations by promoting: (i) the use of iodized salt throughout life, possibly by universal salt iodization (41); and (ii) the use of iodine supplements, both as vitamin–mineral mixtures that contain potassium iodide, or as potassium iodide tablets, where available, from the onset of pregnancy – or earlier if pregnancy is planned – just as folate supplements are

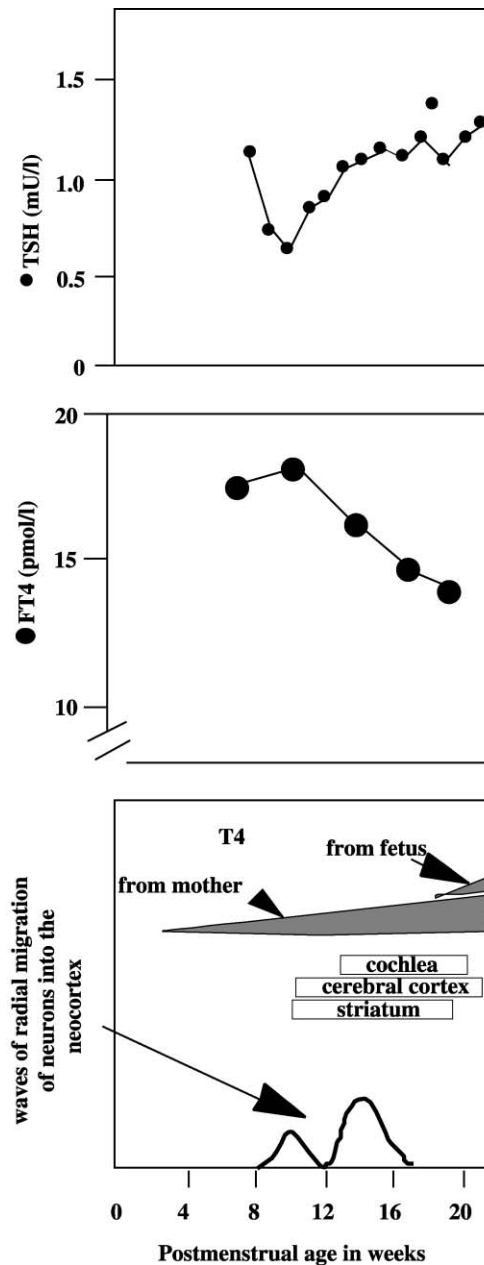


Figure 6 Possible timing in man of cerebral developmental events that are sensitive to maternal thyroid hormones in the rat (9) (lower panel), with respect to timing of first trimester events in maternal thyroid physiology controlled by the conceptus, such as the surge in maternal FT4 (middle panel), despite the suppression of circulating TSH (upper panel).

extensively promoted, whether or not folate deficiency is confirmed.

In the early 1920s, David Marine, a precursor of present programs for the worldwide elimination of IDD, expressed his views that ‘simple goiter is the easiest to prevent of all known diseases. It can be excluded from the list of human diseases as soon as society decides to undertake the necessary effort’

(quoted by Langer (64)). The same may still be said, more than 80 years later, about most cases of learning handicaps related to maternal hypothyroxinemia.

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