

## REVIEW

# Thyroid hormone and central nervous system development

S Chan and M D Kilby<sup>1</sup>

Department of Obstetrics and Gynaecology, Birmingham Women's Hospital, Edgbaston, Birmingham B15 2TG, UK

<sup>1</sup>Department of Fetal Medicine, Division of Reproductive and Child Health, University of Birmingham, Edgbaston, Birmingham B15 2TG, UK

(Requests for offprints should be addressed to M Kilby; Email: m.d.kilby@bham.ac.uk)

### Introduction

It is well established that the thyroid status of neonates and children has a significant long-term impact on their behaviour, locomotor ability, speech, hearing and cognition (Legrand 1986). Delay in restoring normal thyroid status in the neonate can lead to irreversible damage. Prompt thyroid supplementation following the diagnosis of neonatal hypothyroidism can restore neurodevelopment to within the normal range (Fisher 1979). Even so, there are still subtle abnormalities of language, visuo-spatial impairments and lower mean IQs later in childhood compared with euthyroid controls. This implies that brain development may, in part, be thyroid hormone sensitive not only in the neonatal period but also prior to birth (New England Congenital Collaborative 1990, Heyerdahl 1991). Development of different areas of central nervous system has been associated with the timing and duration of thyroid hormone deficiency, suggesting that there are critical periods during which various parts of the brain are sensitive to thyroid hormone supply (Rovet *et al.* 1992).

### Central nervous system histology and the effects of thyroid status

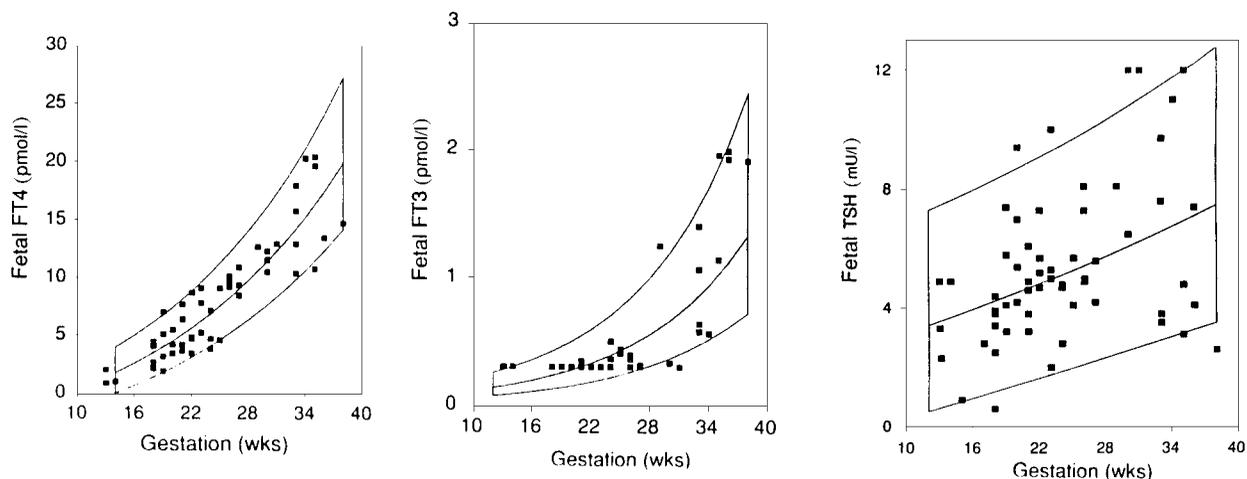
The clinical signs of neurodevelopmental delay can be explained by the accompanying histological and biochemical changes, mainly observed in animal models. Thyroid hormones regulate the processes of terminal brain differentiation such as dendritic and axonal growth, synaptogenesis, neuronal migration and myelination (Eayrs & Taylor 1951, Eayrs & Horne 1955, Eayrs 1955). In hypothyroid rats there is retarded development of the neuropil in the cerebral cortex and the cerebellar Purkinje cells. Neuronal bodies are smaller and more densely packed, there is diminished dendritic branching and elongation, as well as altered distribution of dendritic spines and delayed cell proliferation and migration (Nicholson & Altman 1972). Deficiencies of myelination have been observed in the cerebral cortex, visual and

auditory cortex, hippocampus and cerebellum, areas which relate to the observed neurodevelopmental delay (Balazs *et al.* 1969, 1971, Rosman *et al.* 1972). These findings, together with a marked decrease in the number and delayed maturation of microtubules within these cells, indicate potentially profound changes in the central nervous system compared with the euthyroid state (Legrand 1967, Faivre *et al.* 1984). All these effects in rats can be reversed by thyroid supplementation but only if supplementation is started before the end of the second week of extrauterine life. The greater the delay in thyroid replacement the less the chance of recovery (Eayrs 1971, Legrand 1986). Rat brain development is such that postnatal maturation corresponds to changes in the human brain which occur at the end of intrauterine life.

### Maternal and fetal thyroid status in the antenatal period

Both maternal and fetal thyroid status *in utero* may be critical in brain development. In a reported case of Pit-1 deficiency, an inherited condition which results in severe hypothyroidism in both the mother and fetus secondary to failure of thyrotroph development, there was severe impairment in the neonate's neurological development together with delay in cardiopulmonary function and bone maturation (de Zegher *et al.* 1995). Observational studies performed in iodine-deficient parts of the world have shown that iodine supplementation before pregnancy and in the first and second trimesters reduces the incidence of cretinism but supplementation beginning later in pregnancy does not improve the neurodevelopmental status of the offspring (Pharoah *et al.* 1971, Cao *et al.* 1994). Even children of marginally iodine-deficient mothers show psychomotor and cognitive impairment (Pharoah *et al.* 1984). Such data indicates the sensitivity of the developing CNS to maternal thyroid metabolism *in utero*.

It has been clearly demonstrated that maternal thyroid hormones cross the placenta into the fetal circulation. In athyroid fetuses at term Vulsma *et al.* (1989) reported



**Figure 1** The ontogeny of fetal thyroid hormone metabolism. Concentrations of fetal TSH, T4 and T3 during gestation. Data from fetal blood samples obtained by cordocentesis (Thorpe-Beston *et al.* 1991b).

concentrations of circulating thyroid hormones around 25–50% those of normal infants. These concentrations appear adequate in preventing phenotypic expression of congenital hypothyroidism at birth and confer some protection of fetal brain development (Illig 1979). This may explain why in cases of human congenital hypothyroidism in which the mother is euthyroid neonatal thyroxine supplementation can result in neurodevelopment within the normal range (Fisher 1991).

In contrast, maternal hypothyroidism, not necessarily due to iodine deficiency, has been associated with poorer neuropsychological outcome in offspring. In the Netherlands, 220 mothers with a low serum free thyroxine (T4) concentration at 12 weeks gestation gave birth to babies who at 10 months of age had scores at or below the tenth percentile on the psychomotor developmental index of the Bayley scales of infant development (Pop *et al.* 1999). In the United States, mothers who had a low serum butanol-extractable iodine (a measure of circulating thyroid hormones employed in the 1960s) before 24 weeks gestation and who were not adequately treated, had infants with lower Bayley Scores. These children were later shown to have lower IQ scores at 4 and 7 years of age (Man *et al.* 1991). Recently, another study was performed in the United States on the offspring of 62 women with serum thyrotrophin (TSH) concentrations above the 98th percentile during pregnancy comparing them with 124 matched controls. None of the neonates had hypothyroidism at birth. The children were assessed between the ages of 7 and 9 years. Those with hypothyroid mothers performed less well on all 15 of the neuropsychological tests and had on average a 4-point lower IQ score on the Wechsler intelligence scale for children than the controls. Forty-eight of the 62 biochemically hypothyroid women were not treated during pregnancy as they were clinically

euthyroid, and the children of this subgroup of women performed less well, with an average 7-point lower IQ score than controls. Nineteen per cent of these scored 85 or less. Interestingly, the serum total T4 and free T4 concentrations in the treated and the non-treated undiagnosed women were similar during pregnancy. This study demonstrates that subclinical hypothyroidism in women can result in neuropsychological deficits in their offspring, and thyroxine supplementation can improve the outcome even when supplementation is inadequate (Haddow *et al.* 1999). A further study in Canada suggested subclinical autoimmune hypothyroidism in the mother could be associated with a higher incidence of transient congenital hypothyroidism, probably through disruption of the fetal thyroid gland by the transplacental passage of maternal anti-thyroid antibodies. Unfortunately, the long-term neuropsychological outcomes in these children were not reported (Dussault & Fisher 1999). Certainly in the rat, maternal hypothyroxinaemia has been shown to result in long-term biochemical and behavioural dysfunction in the progeny (Pickard *et al.* 1997).

### Circulating thyroid hormone concentrations in human pregnancy

There is a rise in fetal circulating concentrations of total T4, free T4, free triiodothyronine (T3) and thyroxine binding globulin (TBG) with gestation (Fisher 1992, Burrow *et al.* 1994, Kilby *et al.* 1998) (Fig. 1). In the first and second trimesters there is a much higher concentration of free T4 in the maternal circulation compared with the fetal circulation. The difference decreases towards term as fetal thyroid function matures. However, even at term maternal serum free T3 concentrations are two- to

threefold more than those in the fetus and approximately 30% of thyroid hormones measured in cord blood are still derived from the mother (Thorpe-Beeston *et al.* 1992, Delbert & Fisher 1997b).

### Thyroid hormone concentrations in fetal brains

In humans, both T3 and T4 can be detected in the first trimester brain before the fetal thyroid gland becomes active, possibly indicating that thyroid hormones transferred from the mother play an important role (Bernal & Pekonen 1984, Sinha *et al.* 1997). T3 is not detectable in other fetal tissues apart from the brain at this stage, lending support to the theory that there is a specific role for thyroid hormones in very early brain development. T4 is detected in the brain at 11–14 weeks, the level increasing 2.5 times by 15–18 weeks. Even after the fetus begins to produce its own thyroid hormones in the second trimester, maternal thyroid hormones make a significant contribution towards the supply to the fetal brain. This is indicated by positive correlations between maternal serum T4 concentrations, fetal cerebro-cortical T4 and maternal urinary iodine excretion at this stage (Sinha *et al.* 1997).

Concentrations of circulating thyroid hormones determine cellular supply to the brain and, in turn, effects at the cellular level are influenced by 5-monodeiodinase activity. T4 is converted to the active ligand T3 by type II 5-monodeiodinases locally in brain tissue. The rise in circulating thyroid hormones is accompanied by evidence of increasing 5-monodeiodinase activity in the brain up to 19–22 weeks gestation but the activity declines thereafter (Sinha *et al.* 1997). The need to increase local T3 levels lends further support to the theory that thyroid hormones play an important role in brain development in the late first trimester and early second trimester.

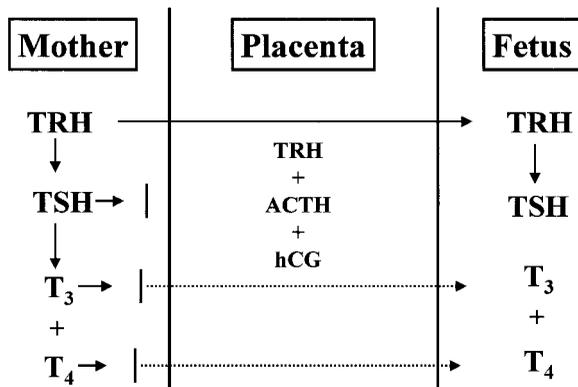
Studies in the rat have shown that maternal T4 transported across the placenta can provide normal concentrations of T3 in hypothyroid fetal brains (Calvo *et al.* 1990). Also, brains of human fetuses carried by mildly iodine-deficient mothers with biochemical hypothyroidism have been reported to have T4 levels similar to those of normal fetuses in both the first and second trimesters of pregnancy. The T3 levels in the cerebral cortex of human fetuses normally peaks at 15–18 weeks gestation, coinciding with the onset of fetal thyroid hormone production. In mild iodine deficiency, however, T3 is maintained at a high level for longer, until 22 weeks gestation, but the absolute amount is still significantly less than in the iodine-sufficient group (Karmarkar *et al.* 1993). Activity of the type II 5-monodeiodinase enzyme is significantly higher in iodine deficiency and when the circulating T4 concentration is low (Leonard *et al.* 1984, Karmarkar *et al.* 1993, Sinha *et al.* 1994). All this evidence suggests that the preferential uptake of T4 by the thyroid hormone-deficient brain, together with the up-regulation

of the enzyme occur as part of a compensatory homeostatic mechanism to improve thyroid hormone supply to the developing brain in iodine deficiency (Sinha *et al.* 1997). However, it is unlikely that in more severe maternal hypothyroidism this mechanism of compensation is capable of maintaining adequate thyroid hormone levels in fetal brains.

As mentioned earlier, mildly iodine-deficient and hypothyroid mothers do produce neurodevelopmentally compromised offspring and fetuses in such circumstances are known to have normal T4 concentrations but reduced T3 concentrations in the brain. It is possible that there are other factors, apart from thyroid hormone levels *per se* in the fetal brain, that mediate the thyroid hormone-related pathogenesis of impaired neurodevelopment. Our understanding so far does not help us to ascertain whether the effects of maternal hypothyroidism on fetal brain development are completely mediated through a direct effect on the fetal thyroid status. In pregnancy these effects may be secondary to the impaired supply of iodide and T4 to the fetus, or through materno-fetal transfer of anti-thyroid antibodies. Alternatively, they may perhaps be mediated indirectly by maternal metabolic impairment and detrimental effects on placental function. It has been documented that T3 can stimulate the production of 17 $\beta$ -oestradiol and epidermal growth factor in human placenta, and may have a role in placental development (Maruo *et al.* 1991, Kilby *et al.* 1998).

### The placental transport of hormones and fetal pituitary function

Circulating thyroid hormones in the human fetus are of both maternal and fetal origin, and their presence are dependent on a functioning placenta for T4 transport and supply of iodide substrate (Fig. 2). The placenta rapidly breaks down much of the T4 presented to it but significant amounts of T4 are still transferred (Delbert & Fisher 1997b). The placenta is freely permeable to iodide and thyrotrophin-releasing hormone (TRH) but impermeable to TSH. Maternal TRH may have a role in controlling fetal thyroid function (Polk *et al.* 1991) before the maturation of the hypothalamic-pituitary-thyroid axis that occurs near term. TRH can be detected in the fetal hypothalamus by the end of the first trimester, at the same time as the thyroid begins to concentrate iodine. TSH can be found in the pituitary at 10–12 weeks gestation, with serum levels rising towards term to values exceeding those of the adult (Fisher *et al.* 1977, Thorpe-Beeston *et al.* 1991a). Our data has indicated that the human placenta expresses all thyroid hormone receptor (TR) isoforms (both protein and mRNA) and that this expression increases with gestational age (Kilby *et al.* 1998). The role of these receptors in placental tissue is unknown at present.



**Figure 2** Classically the placenta has been considered as a 'barrier' to transfer of free T<sub>3</sub> and T<sub>4</sub> to the fetus. However, recent epidemiological data has rekindled interest as to the possibility of transplacental transfer of thyroid hormones to the fetus from early gestation. (TRH, thyroid releasing hormone; TSH, thyrotrophin; T<sub>3</sub>, triiodothyronine; T<sub>4</sub>, thyroxine; ACTH, adrenocorticotrophin; hCG, human chorionic gonadotrophin.)

### Thyroid hormone receptor expression and function in the human fetus

The T<sub>3</sub> binding capacity and TR concentration in the human fetal brain also rises steadily with gestation. A tenfold increase in the maximal binding capacity for T<sub>3</sub> in human fetal brains between 10 and 16 weeks has been described (Bernal & Pekonen 1984). However, the mere presence of TRs at any time does not necessarily imply a concurrent thyroid-dependent action as demonstrated by studies of the development of the cochlea in rats. Although TRs are present in the primitive cochlea from the twelfth day of gestation, the critical period for its thyroid hormone-dependent development is from the eighteenth day of gestation till the fifth postnatal day (Uziel 1986).

Four important TR isoforms have been characterised in humans. The *erbA $\alpha$*  locus on chromosome 17 encodes TR $\alpha$ 1 and TR $\alpha$ 2 proteins, whilst the *erbA $\beta$*  locus on chromosome 3 expresses TR $\beta$ 1 and TR $\beta$ 2 (Oppenheimer & Schwartz 1997, Sinha *et al.* 1997). A greater than threefold rise in TR $\beta$ 1 transcript levels between 11 and 22 weeks in human fetal brains has been described.

TR $\alpha$ 1, TR $\beta$ 1 and TR $\beta$ 2 bind T<sub>3</sub> with similar affinity and binding kinetics. The  $\alpha$ 2 protein does not bind T<sub>3</sub> and a modulatory role mediated by competitive binding of protein to thyroid hormone response elements on DNA has been postulated (Koenig *et al.* 1989, Lazar *et al.* 1989). All TR isoform-mRNAs were detectable in fetal cerebral cortex using RT-PCR as early as 9 weeks. Weak immunostaining for TR $\alpha$ 1 and TR $\alpha$ 2 proteins within cortical pyramidal cells and cerebellar Purkinje cells in the first trimester has been described. By the second trimester all of the isoforms were expressed in the same areas. A rise

of at least fourfold was observed for each isoform between the first and second trimester. By the third trimester every neuron in these same areas expressed all of the TR isoforms (Verhaeg *et al.* 1999).

The existence of various TR isoforms implies the presence of specific roles for each of them. For example, patients with homozygous deletion of the TR $\beta$  gene suffer with deaf-mutism but demonstrate normal cognitive function (Takeda *et al.* 1992). This implies a specific role for the TR $\beta$  protein in the development of the auditory apparatus and cortex.

The TR proteins are characterised by a DNA binding domain containing two zinc fingers, a carboxy terminal that contains the ligand binding domain and the trans-activation site, and finally an amino-terminal domain with a poorly defined function. The T<sub>3</sub>-TR complex binds to specific DNA sequences on thyroid hormone response elements (TRE) found in the regulatory regions of target genes (Oppenheimer *et al.* 1996, Onate *et al.* 1995). All the following research data concerning thyroid hormone-regulated gene expression have been gathered from animal models. Examples of such genes include myelin basic protein (MBP), myelin-associated glycoprotein (MAG) and proteolipid protein (PLP) whose mRNAs expressed in oligodendrocytes are reduced by at least 50% in hypothyroid fetal rats (Oppenheimer & Schwartz 1997). The lack of these proteins may be implicated in the delayed myelination observed. Effective TREs have been found in the promoters of mouse MBP and PLP genes, suggesting direct regulation by the T<sub>3</sub>-TR complex (Bogazzi *et al.* 1994, Farsetti *et al.* 1991, 1992). The capacity of cultured cells to enhance MBP expression in response to T<sub>3</sub> coincided with the appearance of TR $\beta$ 1 mRNA (Strait *et al.* 1997). The cerebellar Purkinje cells express genes including calbindin, myo-inositol-triphosphate (IP<sub>3</sub>) receptor, and Purkinje cell protein-2 (PCP-2), all of which show significantly delayed mRNA expression in neonatal hypothyroid rats. Interestingly despite ultimate normalisation of these mRNA levels, the pups demonstrated a marked deficit in Purkinje cell maturation, confirming the importance of timing in thyroid hormone-dependent neurodevelopment (Strait *et al.* 1992). It has been postulated that there are suppressive factors that control the predetermined times at which thyroid hormone augmentation of specific genes should occur. It has been shown that injecting pregnant rats with large doses of T<sub>4</sub> resulting in supraphysiological levels of T<sub>3</sub> in fetal brains failed to augment the expression of PCP-2 and MBP prematurely (Schwartz *et al.* 1997). Potential suppressors, in the form of DNA binding nuclear proteins, have been found to bind to the promoter regions of the PCP-2 and MBP genes, and interestingly their disappearance coincides with the time the gene expression becomes thyroid hormone responsive (Anderson *et al.* 1997).

### Extranuclear mechanisms of thyroid hormone action

Only a limited number of specific genes in the CNS have been found to be directly regulated by thyroid hormones through ligand–receptor complexes binding to DNA response elements and the difficulty in identifying more has been surprising (Oppenheimer & Schwartz 1997). In contrast to the examples above, the neurogranin (RC3) and calbindin mRNAs expressed in neurons are markedly depressed in hypothyroid rats but a functional TRE has not been identified upstream of these genes (Iniguez *et al.* 1993, 1994). This suggests the possibility of an indirect regulatory mechanism in action. It is possible that this mechanism could still involve the binding of T<sub>3</sub>–receptor complexes to DNA, for example through controlling the expression of genes encoding nucleoproteins that in turn regulate RC3 expression (Oppenheimer & Schwartz 1997).

*In vivo* studies using animal models have raised the possibility of extranuclear thyroid hormone action but definitive evidence is lacking to date. The production of microtubule-associated proteins (MAP) and tau are altered by thyroid status but this difference is imperceptible at the mRNA level (Silva & Rudas 1990, Nunez *et al.* 1991, 1992). It has been demonstrated that thyroid hormones can regulate the timing of the splicing mechanism involved in the replacement of juvenile tau mRNA with adult versions, so thyroid hormones may control post-transcriptional activity (Aniello *et al.* 1991).

Granular immunostaining of TRs has been reported within neuronal cell cytoplasm where they may have a role. In rats T<sub>3</sub> binding sites have also been found on cell membranes, synaptosomes and in mitochondria (Sterling *et al.* 1977, Dratman *et al.* 1989). In the presence of thyroid hormones amino acid and glucose uptake by brain tissue is rapidly enhanced possibly through the direct regulation of membrane porosity (Buchanan & Tapley 1966, Sinha *et al.* 1997, Pickard *et al.* 1987, 1991, 1997).

It is known that T<sub>4</sub> can rapidly down-regulate the action of type II monodeiodinase, an activity which is affected by cytochalasin B but not cycloheximidine or actinomycin D. This suggests an action not mediated through gene expression and that requires an intact actin cytoskeleton (Leonard *et al.* 1990). It has been suggested that type II monodeiodinase binds actin F in the presence of T<sub>4</sub> triggering the internalisation of the enzyme into endosomal pools by the movements of microfilamentous cytoskeletons (Farwell *et al.* 1993). This ability of thyroid hormones to influence cytoskeletal activity in this way raises the possibility that similar mechanisms involving different cytoplasmic proteins could be responsible for neuronal migration, dendritic and axonal elongation and neuronal differentiation (Lauffenburger & Horwitz 1996, Mitchison & Cramer 1996).

### Intrauterine growth restriction and prematurity in human pregnancy

Fetal development and growth depend on several endocrine, paracrine and autocrine events within the fetoplacental unit (Hill 1988). Malfunction of this unit can result in intrauterine growth restricted (IUGR) fetuses, with brain weight usually maintained relative to body weight while other organs like the liver are significantly smaller. IUGR babies contribute significantly to perinatal and neonatal mortality and morbidity. Ten per cent of low birth weight babies suffer some degree of physical handicap (Gaffney 1994) and 5% show neurodevelopmental delay at age 9 (Kok *et al.* 1998). *In utero* fetal blood sampling has shown that fetuses with severe IUGR have significantly lower levels of circulating free T<sub>4</sub>, free T<sub>3</sub> and a slight elevation in TSH (Thorpe-Beeston *et al.* 1991b, Kilby *et al.* 1998). Very low birth weight (VLBW) infants also have lower serum total T<sub>4</sub> and T<sub>3</sub> concentrations (Klein *et al.* 1997). This is accompanied by a reduction in the expression of all TR isoforms in the cerebellum and cerebral cortices of the IUGR fetus (Verhaeg *et al.* 1999). Histological abnormalities, which are similar to those described in thyroid deficiency, have also been noted in the cerebral and cerebellar cortices of growth restricted animal models (Mallard *et al.* 1998). Therefore thyroid status has been postulated to play a role in the pathogenesis of neurodevelopmental impairment in IUGR and VLBW infants.

Premature infants of less than 30 weeks gestational age (not necessarily with IUGR) also experience a transient period of hypothyroxinaemia with a fall in serum free T<sub>4</sub> concentrations without TSH elevation. These neonates have a problem with autoregulation of thyroidal iodine uptake as well as an immature hypothalamic–pituitary–thyroid axis resulting in an inability to compensate for the loss of maternal T<sub>4</sub> supply in the short term (Rooman *et al.* 1996, Ares *et al.* 1997, Delbert & Fisher 1997a, Van Wassenaer *et al.* 1997). Comparing free T<sub>4</sub> concentrations of premature infants with intrauterine fetuses of similar gestational age revealed a 50% difference initially, a phenomenon not observed in term babies (Ares *et al.* 1997). This difference is regarded by many neonatologists to represent a physiological response because thyroid hormone supplementation in premature infants has not demonstrated any clear benefit to the long-term neurodevelopment of these infants (Chowdry *et al.* 1984). However analysis of a subgroup of neonates of 25–26 weeks gestational age showed a higher IQ in the treated group compared with placebo treated controls. Conversely, oversupplementation in neonates older than 27 weeks gestational age has been associated with a reduction in mean IQ relative to controls (Van Wassenaer *et al.* 1997). However there are only a few studies of thyroid supplementation in premature infants, with small numbers of patients, and these initial results need further verification.

## Conclusions

There is strong evidence pointing towards a crucial role for thyroid hormones in fetal brain development. This is supported by evidence at histological, biochemical and molecular levels mainly in animal models. There needs to be caution when extrapolating these findings to humans. Initial research on human fetuses has shown many parallel findings especially regarding the thyroid hormone levels and receptors in fetal brains. The precise timing for thyroid hormone-dependent central nervous system development in the human fetus is, however, unclear, and may be phasic and different for specific areas of the brain. The relative contribution of maternal and fetal thyroid hormones to brain development is also unclear. We know that some degree of compensation occurs if one or other is lacking, but differences in the neuropsychological development are still demonstrable in either case compared with euthyroid controls. There is suggestion that reduction in circulating thyroid hormone concentrations is one of the factors mediating impaired neurological development in IUGR, VLBW and premature babies. More research is required to resolve these questions and ultimately show if thyroid hormone or iodide supplementation in hypothyroid mothers in the antenatal period and in premature neonates can reduce the prevalence of neurodevelopmental delay.

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