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2.1 THYROID HORMONE PRODUCTION AND METABOLISM

The thyroid gland secretes two main hormones; thyroxine (T4) and tri-iodothyronine (T3) (Figure 2.2). Thyroxine is produced in greater quantity than T3 (at a rate 10:1), but T3 is the major biologically active thyroid hormone and is mostly derived from T4 in the peripheral tissues. The thyroid gland utilizes and conserves iodine to produce thyroid hormones. Iodine is obtained from the diet, converted to iodide, actively transported to the thyroid, and incorporated into thyroglobulin (TG) by way of the enzyme thyroid peroxidase (TPO). This leads to production of mono-iodotyrosine and di-iodotyrosine, which are then coupled to form T4, T3 and reverse T3 (rT3). Reverse T3 has no biological activity. The thyroid hormones are part of the TG stored in the colloid of thyroid follicles until excreted into the circulation.

Thyroid hormone production is regulated by the pituitary through the action of thyrotropin (thyroid-stimulating hormone, TSH). TSH comprises two subunits and it has one alpha-subunit in common with luteinizing hormone, follicle stimulating hormone and human chorionic gonadotropin (hCG), and one specific beta-subunit. TSH shows circadian and pulsatory secretion – its secretion peaks at around midnight and declines during the day. The function of the pituitary is controlled by the hypothalamus, which excretes thyrotropin-releasing hormone (TRH). It accelerates the production of TSH, whereas dopamine and somatostatin hinder it. The thyroid hormones have a negative feedback effect on the pituitary and hypothalamus (Figure 2.1), which is modified by the T4 concentration in the serum and the conversion of T4 to T3 locally in the brain. Therefore, if T4 concentration in the serum drops, the inhibitory stimulus is decreased due to a diminished local effect of T3 in the pituitary and TSH levels rise to stimulate the thyroid gland (Ganong, 2005; Hadley and Levine, 2007).

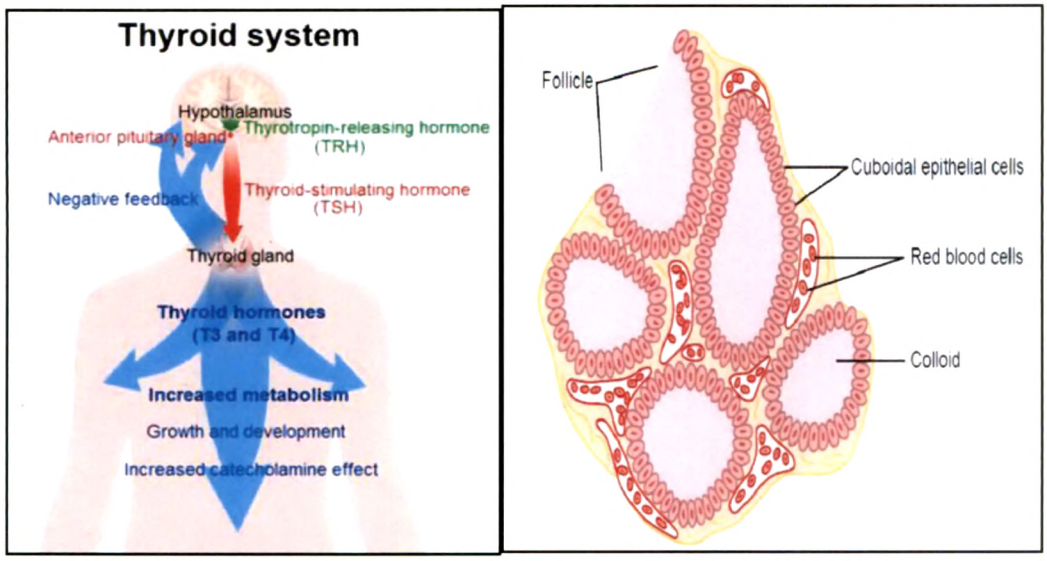


Figure 2.1: Thyroid gland and follicle cell

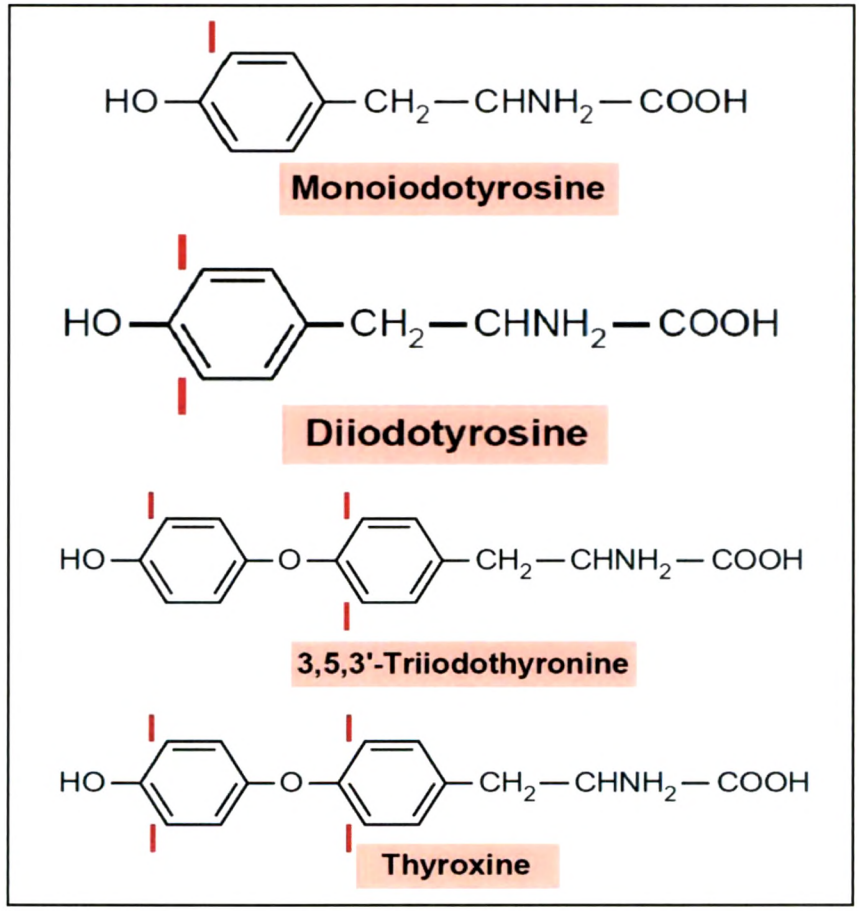
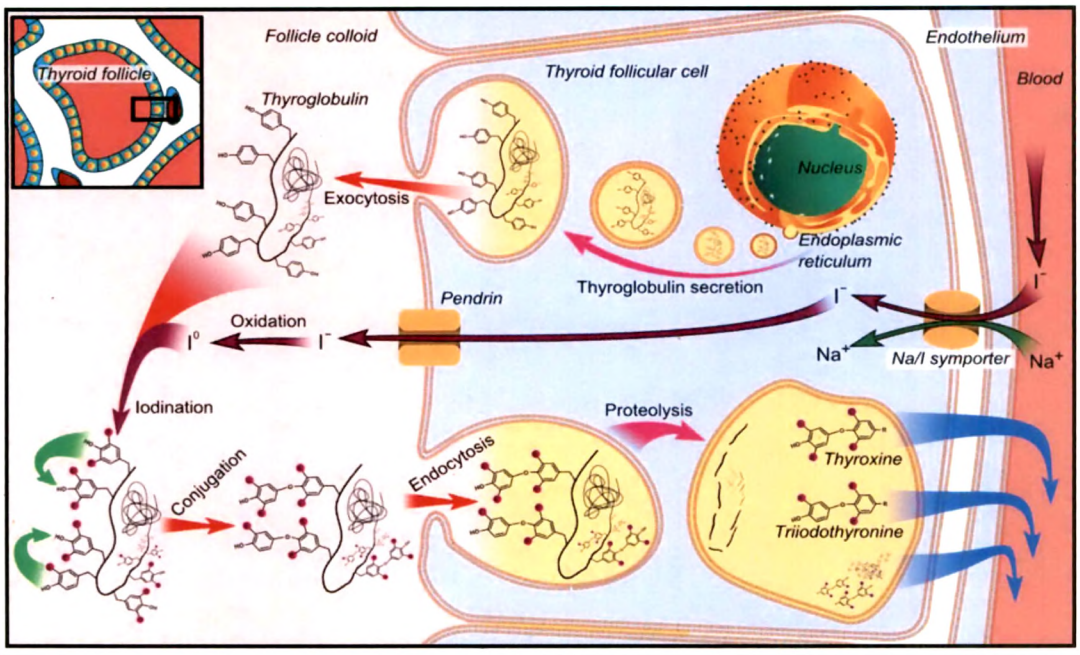


Figure 2.2: Structure of thyroid hormones

Figure 2.3: Synthesis of the thyroid hormones in thyroid follicular cell

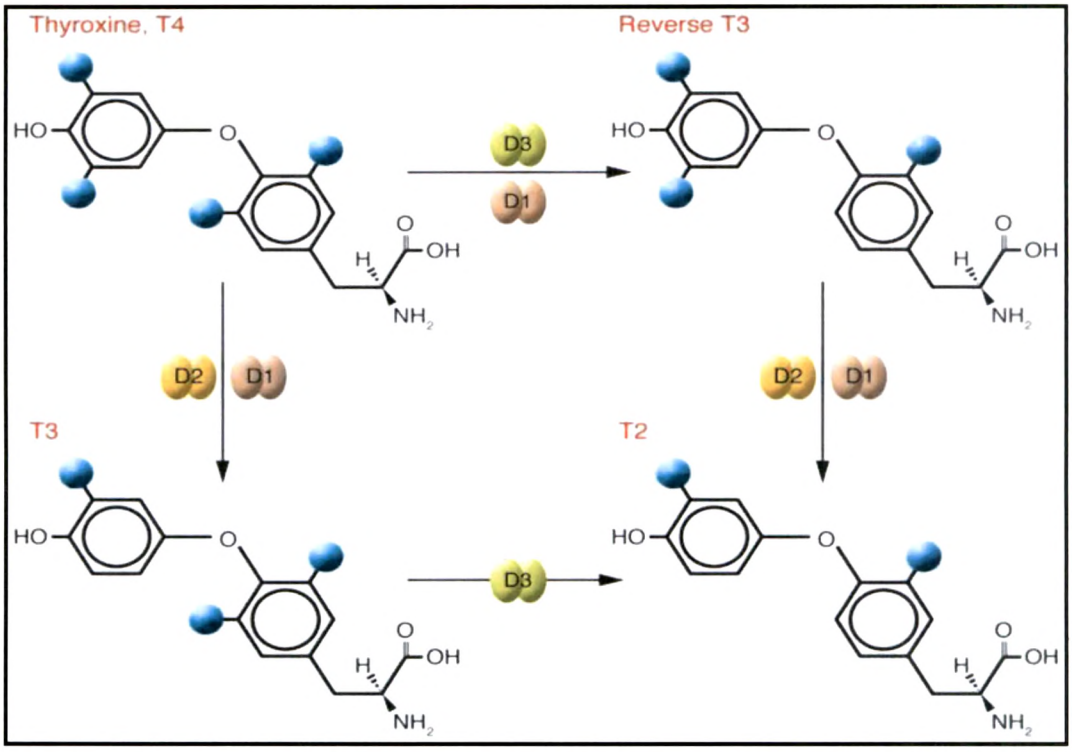


- Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis.
- Meanwhile, a sodium-iodide (Na/I) symporter pumps iodide (I) actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms.
- This iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in purportedly passive manner.
- In the colloid, iodide (I-) is oxidized to iodine (I) by an enzyme called thyroid peroxidase. Iodine (I) is very reactive and iodinates the thyroglobulin at tyrosyl residues in its protein chain (in total containing approximately 120 tyrosyl residues).
- In conjugation, adjacent tyrosyl residues are paired together. The entire complex re-enters the follicular cell by endocytosis.
- Proteolysis by various proteases liberates thyroxine and triiodothyronine molecules, which enter the blood by largely unknown mechanisms.

Source: Peterson, 2011

The thyroid hormones are protein-bound in the serum, and only 0.02% of T4 and 0.2% of T3 are free, biologically active hormones. 45-70% of thyroid hormones are bound to thyroxine-binding globulin (TBG), and the rest to transthyretin and albumin.

Figure 2.4: The basic deiodinase reactions



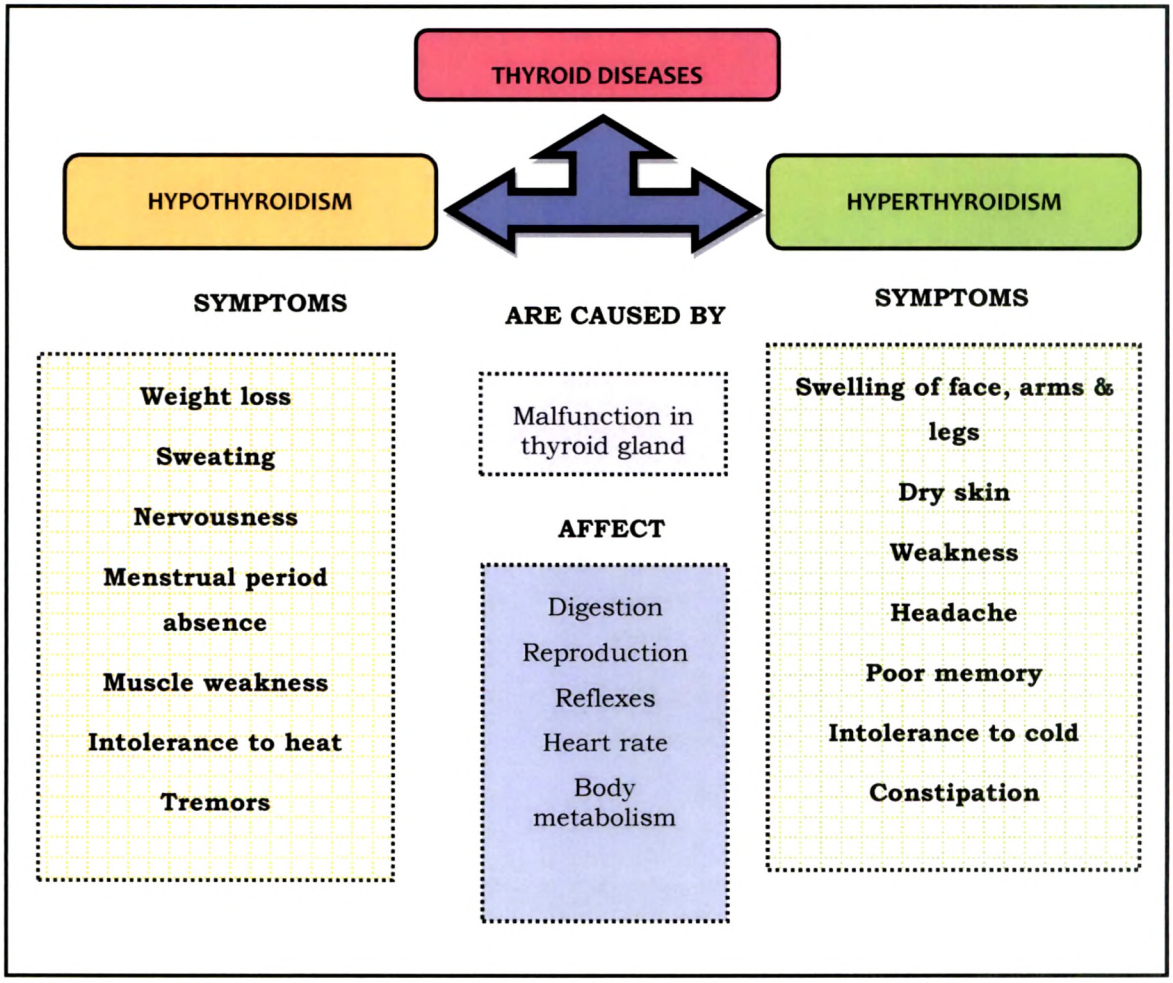
Source: Bianco and Kim, 2006

Free T4 (fT4) is metabolized in the tissues to the active form free T3 (fT3) by three deiodinase enzymes. The tissues have different rates of fT3 production and uptake according to the presence of the deiodinase enzymes. Type I deiodinase (D1) is located in the liver, kidneys, thyroid and pituitary and is primarily responsible for fT3 formation. Its activity is low in the fetus. Type II deiodinase (D2) is located in the central nervous system and pituitary and it produces a supply of fT3 to the brain. Type III deiodinase (D3) is located in the brain and in reproductive tissues and it inactivates both fT4 and fT3, maintaining an equilibrium in the fT3 concentration. Only D2 and D3 have been detected in human placental tissue, the former providing the placenta with a supply of fT3, and the latter maintaining its equilibrium (Ganong, 2005).

Effects of thyroid hormones

The thyroid hormones stimulate oxygen consumption and increase the metabolic rate. They have an effect on the heart and connective tissues and affect growth and development. Thyroid hormones have a marked effect on brain development, especially on the cerebral cortex and the basal ganglia. Lack of thyroid hormones during development due to iodine deficiency leads to cretinism- a condition which can be fully prevented with iodine prophylaxis. In addition, congenital hypothyroidism, if unnoticed and untreated, leads to intellectual deficiency, which fortunately can be prevented by screening programmes and thyroxine treatment started in infancy (Hadley and Levine, 2007).

Figure 2.5: Thyroid diseases and their symptoms



High concentrations of thyroid hormones can elevate the body temperature as they increase the metabolic rate. A rise in body temperature activates the cardiovascular system to dissipate heat. Also, peripheral vascular resistance decreases due to vasodilatation, and blood volume and cardiac output increases through the direct actions of thyroid hormones. Thyroid hormones also lead to protein catabolism from muscles and increase carbohydrate absorption. Therefore they have an effect on glucose metabolism. They also lower circulating cholesterol levels by increasing the hepatic removal of cholesterol (Ganong, 2005).

2.2 THYROID DISEASES

Hypothyroidism

Hypothyroidism is a deficiency of T4. It is present in approximately 2% of women and 0.1–0.2% of men (Tunbridge et al, 1977; Vanderpump et al, 1995). The prevalence of congenital hypothyroidism is 1/4000. Hypothyroidism can present with different symptoms such as fatigue, dry and coarse skin, puffiness, weight gain, diminished perspiration and poor cold endurance, as well as menstrual abnormalities and infertility in women (Figure 2.5). Hypothyroidism is often caused by factors associated with the thyroid itself (primary hypothyroidism). Only 5% of hypothyroidism is the result of central hypothyroidism, i.e. due to lack of TSH or its effects. The most common causes of hypothyroidism are autoimmune thyroiditis, radioiodine therapy and thyroid surgery, but also iodine, medicines or rare genetic disorders may cause hypothyroidism.

Overt hypothyroidism

Overt hypothyroidism is diagnosed by laboratory testing and is presented as low concentrations of circulating thyroid hormones with raised concentrations of TSH. Overt hypothyroidism is often detected and treated before the onset of pregnancy, since it causes infertility and recurrent miscarriages (Krassas et al, 1999). However, 0.2–0.5%

of all pregnant women have overt hypothyroidism (Allan et al, 2000; Casey et al, 2005), representing either new undiagnosed cases or inadequate treatment of previously detected disease.

Subclinical hypothyroidism

Subclinical hypothyroidism is present when TSH concentrations are raised but thyroid hormone concentrations are still normal. The presence of thyroid antibodies reveals if hypothyroidism is caused by chronic autoimmune thyroiditis. Subclinical hypothyroidism has a prevalence of approximately 2.2–2.5% in pregnant women (Allan et al, 2000; Casey et al, 2005). Hypothyroidism is treated with thyroxine (Valimaki and Schalin-Jantti, 2009). Nearly all levothyroxine treated mothers need an approximately 30–50% increase in their dosage to maintain euthyroidism during pregnancy (Mandel et al, 1993; Alexander et al, 2004). The dosage should be raised to 2.0–2.4 μg/kg when trying to conceive or at least when pregnancy tests are positive. It is noteworthy that women without residual thyroid function (after radioiodine treatment or thyroidectomy) require a greater increase in their daily dose of levothyroxine than women with chronic autoimmune thyroiditis.

Hypothyroid mothers should be followed by using thyroid function tests (serum TSH and fT4) throughout pregnancy (4 weeks apart if test results are abnormal and 6–8 weeks apart if results are within reference intervals) and their thyroxine dosage altered when necessary. This can be based on the degree of TSH elevation: for women with serum TSH levels of 5–10 mIU/L the increment in thyroxine is 25–50 μg/day; for those with serum TSH levels of 10–20 mIU/L the increment is 50–75μg/day and for those with serum TSH levels of > 20 mIU/L the increment is 75–100 μg/day.

A newly diagnosed case of hypothyroidism during pregnancy should be vigorously treated. Thyroxine treatment should be initiated with a dose of 100–150 μg thyroxine/day or a dose calculated according to body weight. In severe hypothyroidism, therapy may be initiated with

a double dose of the estimated final daily dose for the first few days. The treatment of hypothyroidism during pregnancy should be adjusted so that TSH and fT4 levels remain within the established reference ranges for pregnant women (Abalovich et al, 2007).

Hypothyroxinemia

Hypothyroxinemia is defined as a low serum fT4 concentration with normal TSH concentrations. Its prevalence during pregnancy is approximately 1.3–2.1% (Casey et al, 2007; Cleary-Goldman et al, 2008). The clinical significance of hypothyroxinemia is still largely unknown. It is noteworthy that central hypothyroidism shows similar features. Hypothyroxinemia has been particularly associated with iodine deficiency and it has a high prevalence in iodine-deficient populations. Due to lack of raw materials (iodine), the thyroid produces more T3 instead of T4, thus leading to hypothyroxinemia (Glinoer, 1997).

Chronic autoimmune thyroiditis

Chronic autoimmune thyroiditis is the most common cause of primary hypothyroidism. A high affinity marker of the disease is thyroid peroxidase antibody (TPO-Ab), and high concentrations of TPO-Ab can be measured in most cases of autoimmune thyroiditis. A similar marker of autoimmune thyroiditis is thyroglobulin antibody (TG-Ab). Autoimmune thyroiditis is a silent disease process in which the thyroid may be enlarged or atrophied and the production of thyroid hormones may decrease resulting in hypothyroidism. The prevalence of positive thyroid auto antibodies increases with age, with the highest frequency observed in women aged 40–60 years (Vanderpump et al, 1995; Hollowell et al, 2002).

Positive TPO-Ab and/or TG-Ab test results are found in approximately 5% of euthyroid pregnant women. However, a thyroid autoantibody prevalence of up to 15% has been found in pregnant populations (Cleary-Goldman et al, 2008). At parturition, 56% of thyroid antibody-

positive mothers have been reported to have high TSH values. During gestation, a significant amount of TPO-Ab and/or TG-Ab positive women are at risk of developing hypothyroidism, since they have lower thyroid function reserve (Glinoer et al, 1994). Chronic autoimmune thyroiditis is the most important cause of hypothyroidism in pregnant women, and up to 90% of women with hypothyroidism during pregnancy test positive for thyroid antibodies (Klein et al, 1991).

Hyperthyroidism

Hyperthyroidism, overproduction of thyroid hormone, has a prevalence of approximately 1% in the population. Thyrotoxicosis refers to increased amounts of thyroid hormones in the circulation, the cause of which can be also other than thyroid hormone overproduction. The symptoms of hyperthyroidism include anxiousness, tachycardia or even atrial fibrillation, increased heat sensitivity, perspiration, skin changes, loss of weight, insulin resistance, fatigue and shivering. Overt hyperthyroidism complicates approximately 0.05–0.2% of pregnancies (Burrow, 1993). Hyperthyroidism is associated with ovulatory dysfunction, miscarriages and difficulties conceiving (Krassas et al, 1999; Anselmo et al, 2004) unless treated. Subclinical hyperthyroidism has a prevalence of approximately 1.7% in pregnant women (Casey et al, 2006).

Figure 2.6: Normal, over-active and under-active thyroid gland

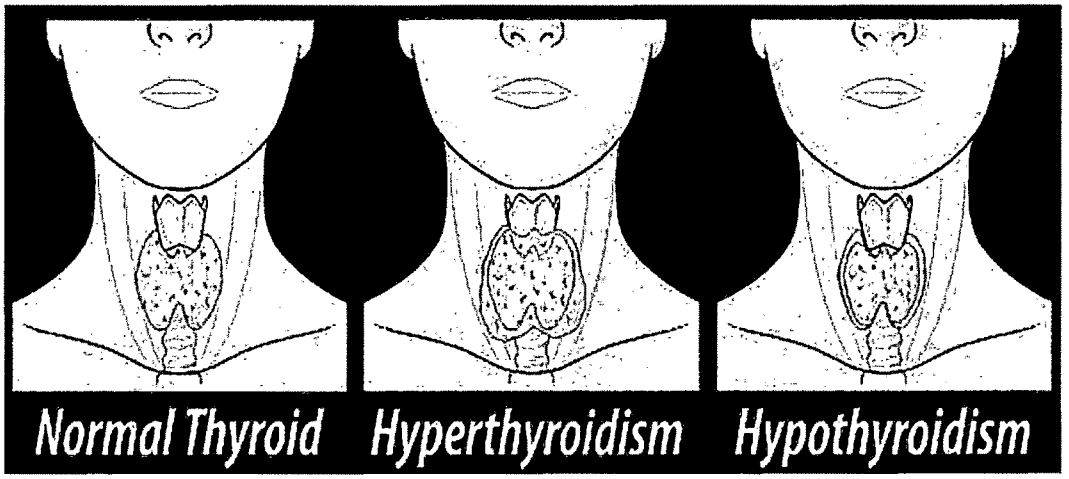


Table 2.1: Etiology of hypothyroidism in pregnancy

Hashimoto disease
Post-thyroid ablation/removal
Iodine deficiency
Primary atrophic hypothyroidism
TSH-dependent hypothyroidism

Source: Mestman et al, 1995

Table 2.2: Etiology of hyperthyroidism in pregnancy

Graves's disease (85–90% of all cases)
Sub-acute thyroiditis
Toxic multi-nodular goiter
Toxic adenoma
TSH-dependent thyrotoxicosis
Exogenous T3 or T4
Iodine-induced hyperthyroidism
Pregnancy-specific associations
Hyperemesis gravidarum

Source: Browne-Martin and Emerson, 1997

Table 2.3: Etiology of postpartum thyroid dysfunction

Hyperthyroidism
Primary
Postpartum thyroiditis
Postpartum Graves's disease
Secondary
None*
Hypothyroidism
Primary
Postpartum thyroiditis
Secondary
Lymphocytic hypophysitis
Postpartum pituitary necrosis

Source: Mulder, 1998

*TSH-producing pituitary tumors and thyroid hormone resistance are secondary causes of hyperthyroidism, but they do not have a predilection for the postpartum period.

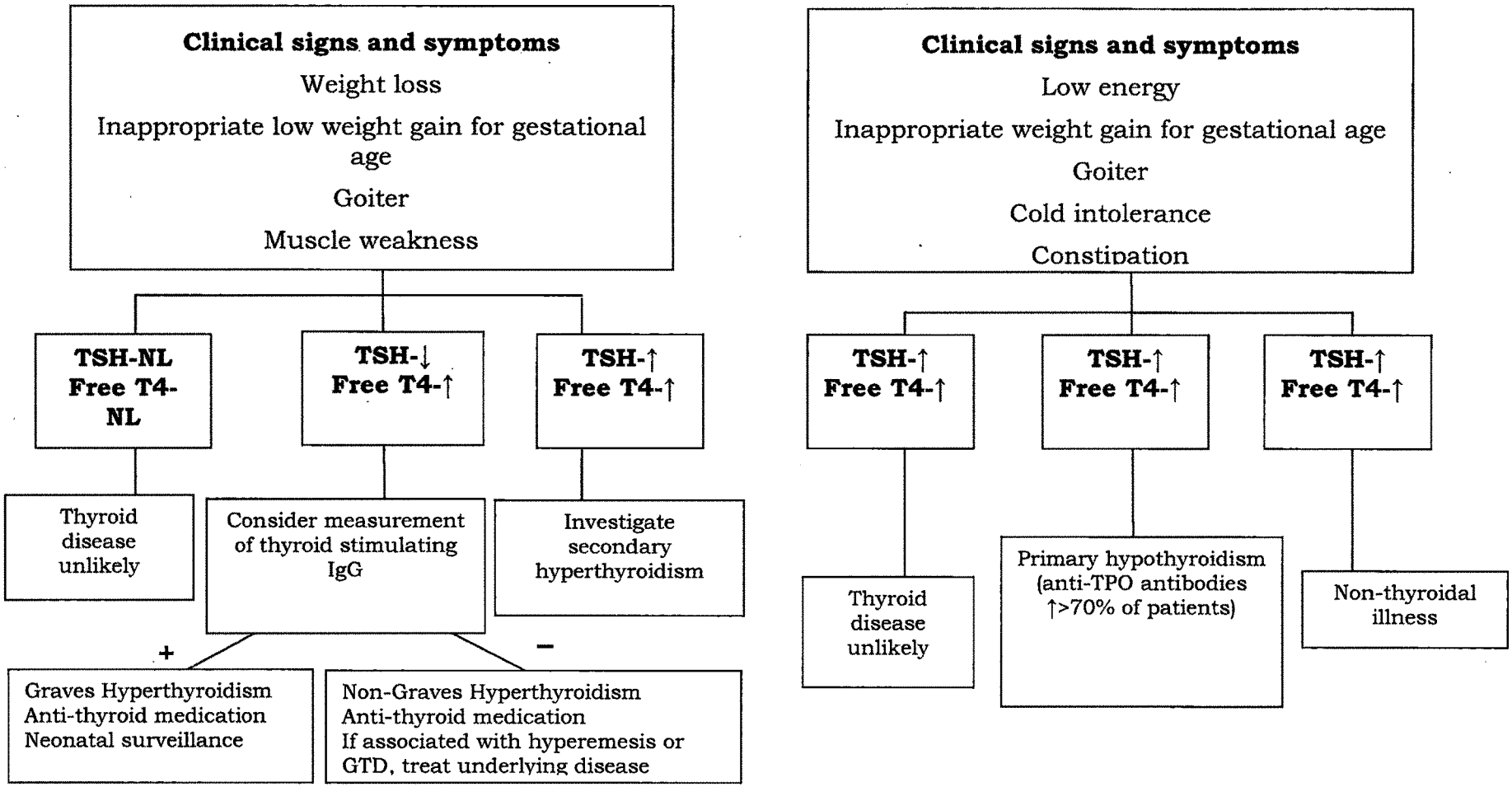


Figure 2.7: Algorithm for the evaluation of hyperthyroidism [left] and hypothyroidism [right] during pregnancy

Source: Fantz et al, 1999

2.3 THYROID FUNCTION DURING PREGNANCY

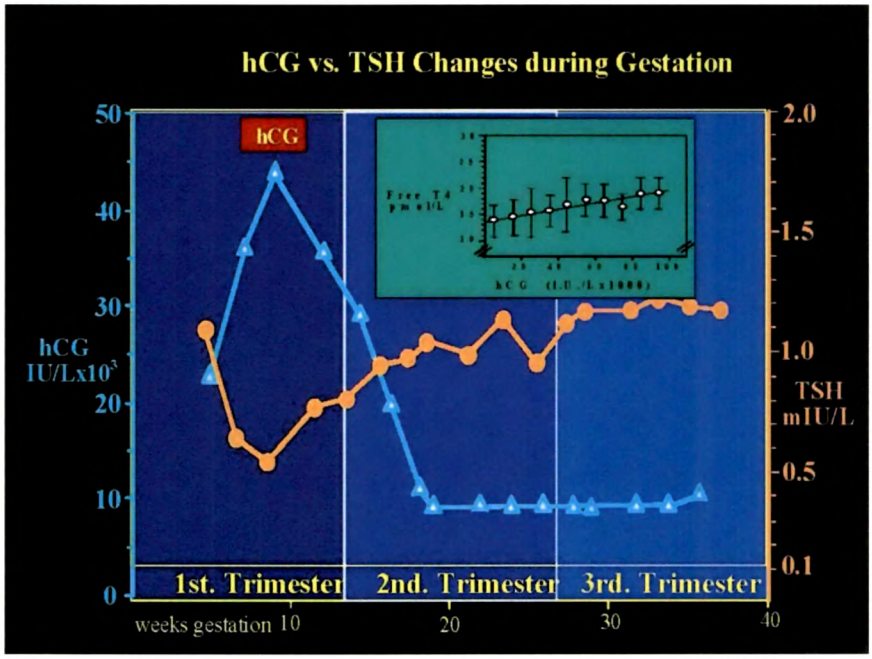
During normal pregnancy, the maternal thyroid produces up to 50% more thyroid hormones. This rise in thyroid hormones results from physiological changes in pregnancy.

Factors affecting thyroid physiology during normal pregnancy

1. Thyroid stimulation by hCG

hCG has mild thyrotropic activity (Yoshimura, 1995; Goodwin, 1997). During the first trimester of pregnancy, when hCG is at its greatest concentration, serum TSH concentrations drop, creating the inverse image of hCG (Figure 2.8). In most pregnancies, this decrease in TSH remains within the health-related reference interval (Glinoer, 1990). Under pathological conditions in which hCG concentrations are markedly increased for extended periods, significant hCG-induced thyroid stimulation can occur, decreasing TSH and increasing free hormone concentrations.

Figure 2.8: Changes in TSH and hCG during gestation



Source: Glioner, 1990

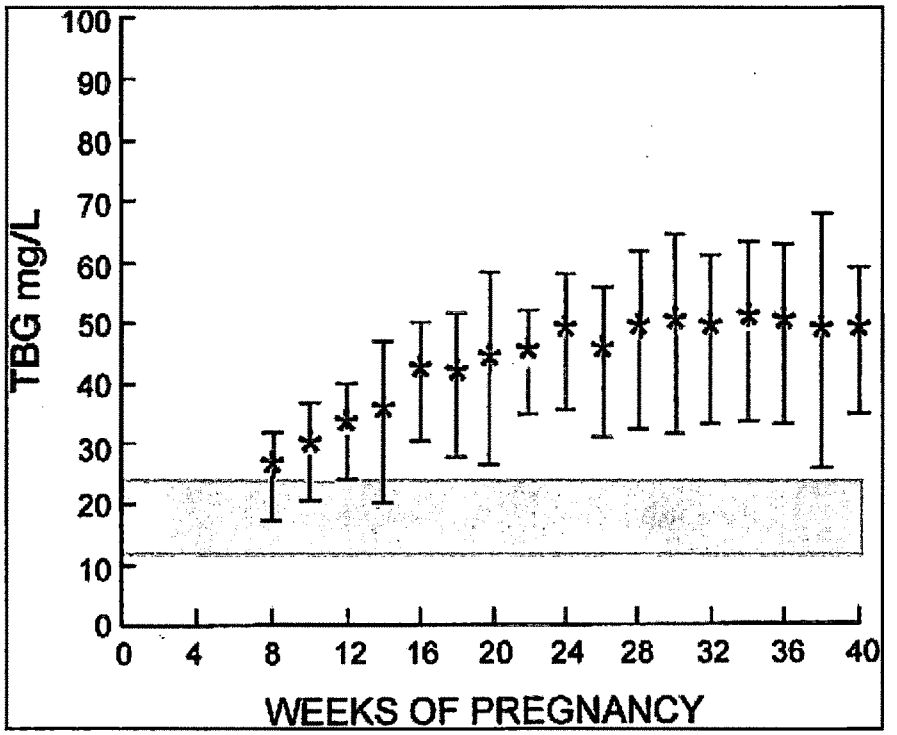
2. Increase in thyroid binding globulins

Thyroid hormones are transported in serum bound to three proteins: thyroxine binding globulin (TBG), transthyretin, and albumin. Although TBG is present in low abundance in serum, it has a high

affinity for thyroid hormones and is responsible for the transport of the majority of T4-68% and T3-80% (Larsen, 1998). During pregnancy, the affinities of the three binding proteins for T4 and T3 are not significantly altered, but the circulating concentration of TBG increases two to threefold, whereas the concentrations of albumin and transthyretin remain unchanged (Ain et al, 1987; Glinoer, 1997 and Skjoldebrand et al, 1982). Serum TBG increases a few weeks after conception and reaches a plateau during mid-gestation (Figure 2.9) (Skjoldebrand et al, 1982). The mechanism for this increase in TBG involves both an increase in hepatic synthesis of TBG and an estrogen-induced increase in sialylation, which increases the half-life of TBG [from 15 min to 3 days for fully sialylated TBG (Ain et al, 1987; Glinoer, 1997 and Brent, 1997)].

Figure 2.9: TBG during normal pregnancy (mean±2sd) in 2-week intervals

[Shaded area-reference interval for non-pregnant fertile women]



Source: Fantz et al, 1999

3. Increase in total T4 and T3

Plasma concentrations of total T4 and T3 are also increased during pregnancy, often outside the health-related reference interval. Total T4

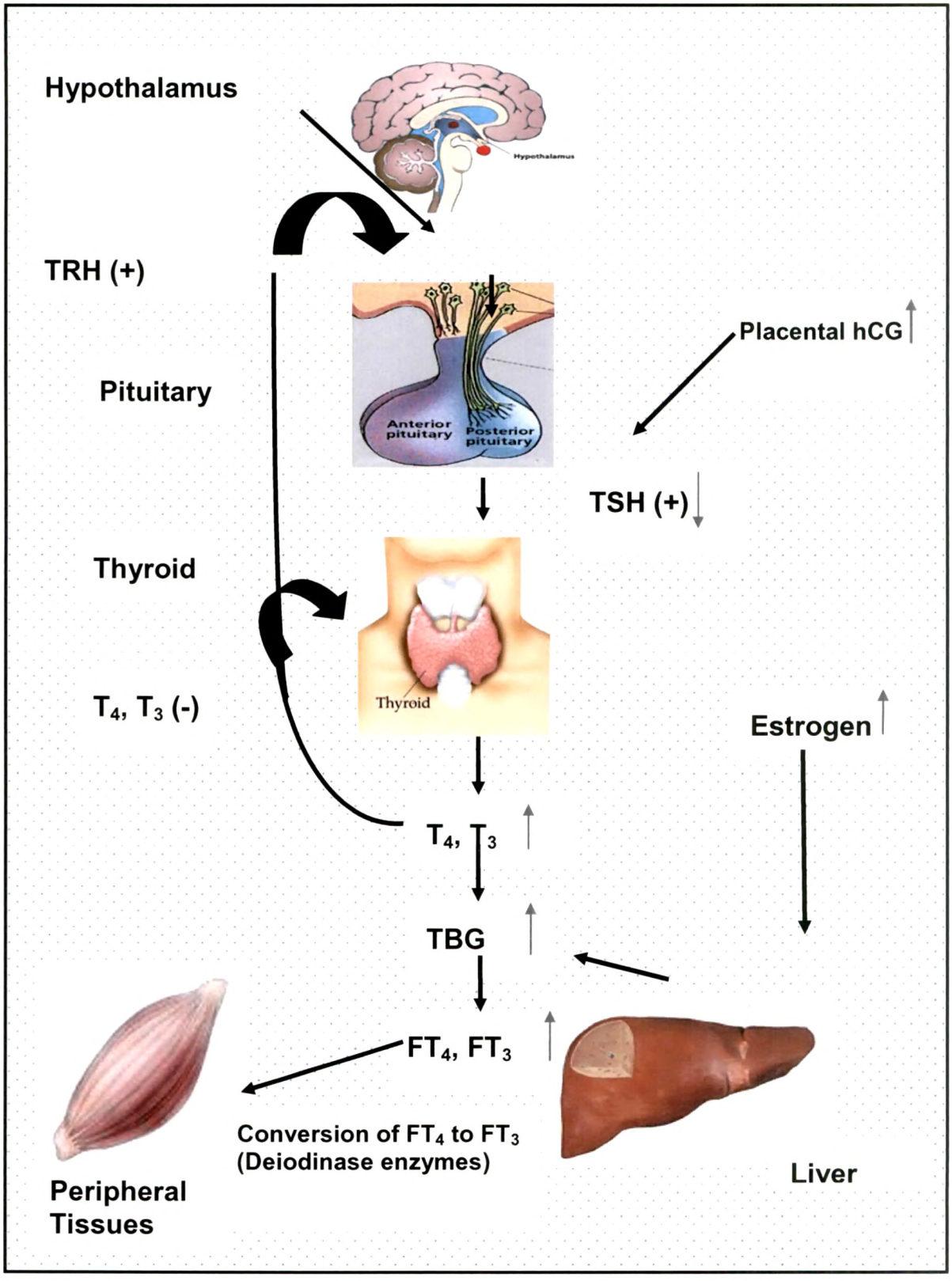
and total T3 concentrations increase sharply in early pregnancy and plateau early in the second trimester at concentrations 30–100% greater than pre pregnancy values (Skjoldebrand et al, 1982; Guillaume et al, 1985). The etiology of this increase in total circulating thyroid hormones involves, primarily, increased concentrations of plasma TBG (Glinoer, 1997; Skjoldebrand et al, 1982 and Guillaume et al, 1985). Another proposed mechanism for this increase in total thyroid hormone concentrations is production of type III deiodinase from the placenta (Glinoer, 1997). This enzyme, which converts T4 to reverse T3, and T3 to diiodotyrosine (T2), has extremely high activity during fetal life (Burrow et al and Fisher et al, 1994). Increased demand for T4 and T3 has been suggested to increase production of these hormones with, ultimately, increased concentrations in the circulation (Glinoer, 1997). Changes in free T4 and T3 concentrations during pregnancy have been controversial.

Table 2.4: Thyroid function during pregnancy

Physiologic change	Resulting change in thyroid activity
↑ Serum estrogens	↑ Serum TBG
↑ Serum TBG	↑ Demand for T4 and T3 ↑ In total T4 and T3
↑ hCG	↓ TSH (in reference range unless hCG >50,000 IU/L) ↑ FT4 (in reference range unless hCG >50,000 IU/L)
↑ Iodine clearance	↑ In dietary requirement for iodine ↓ In hormone production in iodine deficient areas ↑ Goitre in iodine deficient areas
↑ Type III deiodinase	↑ T4 and T3 degradation ↑ Demand for T4 and T3
↑ Demand for T4 and T3	↑ Serum thyroglobulin ↑ Thyroid volume ↑ Goitre in iodine deficient areas

Source: Fantz et al, 1999

Figure 2.10: Changes in the hypothalamus-pituitary-thyroid axis during pregnancy



4. Increase in serum thyroglobulin

Thyroglobulin is frequently increased during pregnancy, reflecting the increased activity of the thyroid gland during pregnancy (Glinoer, 1997). The increase in thyroglobulin can be seen as early as the first trimester, but it is more pronounced in the latter part of pregnancy (Glinoer, 1997). Increased serum thyroglobulin concentrations are also associated with an increase in thyroid volume.

5. Increase in renal iodide clearance

In pregnancy, the renal clearance of iodide increases substantially because of an increased glomerular filtration rate (Glinoer, 1997). The iodide loss lowers the circulating concentrations of iodide and produces a compensatory increase in thyroidal iodide clearance. In areas of the world where iodine intake is sufficient, such as the US, the iodide losses in the urine are not clinically important. In other areas of the world, however, iodine deficiency during pregnancy can lead to hypothyroidism and goiter and poses a serious public health issue. Approximately 500 million people live in areas of overt iodine deficiency (Glinoer, 1997). In the non pregnant condition, adequate iodine intake is estimated to be 100–150 mg/day.

2.4 DEVELOPMENT OF THE THYROID GLAND AND FETAL THYROID HORMONE SUPPLY

Thyroid hormones and neurodevelopment

Thyroid hormones have no influence on very early developmental events, such as neural induction and establishment of polarity, but regulate later processes, including neurogenesis, myelination, dendrite proliferation and synapse formation (Bernal et al, 2003; Zoller and Rovet, 2007) (Figure 2.11). Numerous thyroid hormone responsive genes have been identified (Bernal et al, 2003) and the timing of the onset of thyroid hormone action in the developing brain is crucial (Zoller and Rovet, 2007; de Escobar et al, 2004; Obregon et al, 2007).

For example, endemic neurological cretinism is due to maternal iodine deficiency and the resulting maternal hypothyroxinemia, which is defined as thyroxine (T4) concentrations that are low for the stage of pregnancy. Low maternal T4 levels cause neurological hypothyroidism in the foetus, which results in profound mental retardation, cerebral spastic diplegia, deaf-mutism and squint in the absence of general signs of hypothyroidism (Porterfield and Hendrich, 1993). Although endemic cretinism can be prevented by public health measures, such as iodine supplementation, that prevent or correct first trimester maternal hypothyroxinemia, iodine deficiency remains the commonest endocrine disorder worldwide and the most frequent cause of preventable mental retardation (de Escobar et al, 2000). By contrast, neurological features in neonatal hypothyroidism are less severe, dependent on the severity of hypothyroidism and largely preventable by immediate thyroid hormone replacement, although deficits in memory and IQ may persist (Zoller and Rovet, 2007). Untreated neonates exhibit growth retardation and general features of hypothyroidism with mental retardation, tremor, spasticity and speech and language deficits (Zoller and Rovet, 2007; Porterfield and Hendrich, 1993). Differences between endemic cretinism and congenital hypothyroidism illustrate that the timing of thyroid hormone action is fundamental for neurodevelopment.

Timing of thyroid hormone action in the brain

Three stages of thyroid hormone dependent neurological development can be recognised (Figure 2.11).

First stage: The first occurs before the onset of foetal thyroid hormone synthesis, which occurs at 16–20 weeks post-conception in humans or by embryonic day E17.5–18 in the rat. During this period, thyroid hormone exposure comes only from maternally synthesised hormone (de Escobar et al, 2004; Obregon et al, 2007) and influences

neuronal proliferation and migration of neurones in the cerebral cortex, hippocampus and medial ganglionic eminence (Narayanan, 1985; Lucio et al, 1997; Cuevas et al, 2005; Auso et al, 2004).

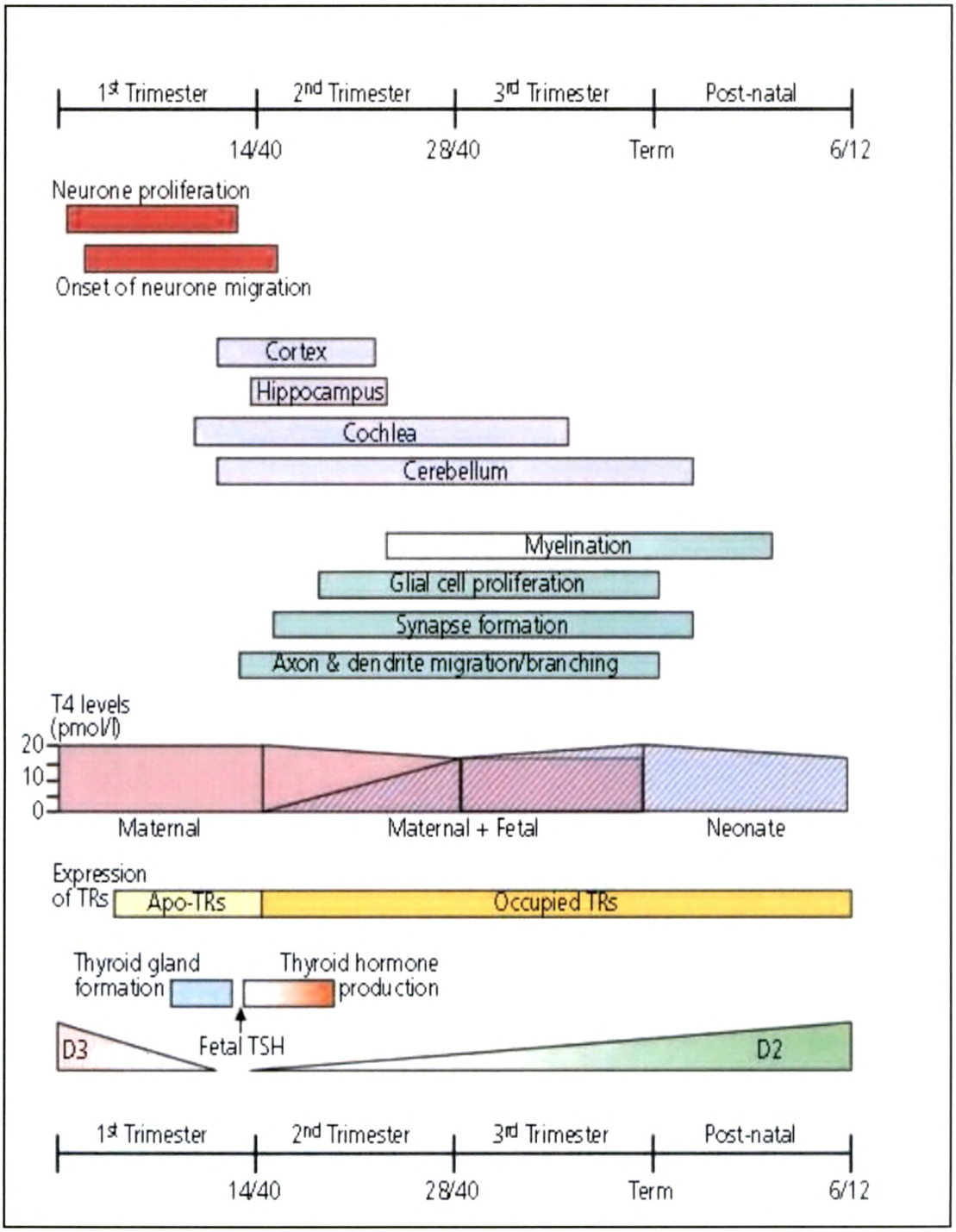
Second stage: The second stage occurs during the remainder of pregnancy after the onset of foetal thyroid function when the developing brain derives its supply of thyroid hormones from both the foetus and the mother (de Escobar et al, 2004; Obregon et al, 2007). During this period, thyroid hormone dependent processes include neurogenesis, neurone migration, axonal outgrowth, dendritic branching and synaptogenesis, together with the initiation of glial cell differentiation and migration and the onset of myelination (Bernal, et al, 2003; Porterfield and Hendrich, 1993; de Escobar et al, 2000).

Third stage: The third stage occurs in the neonatal and post-natal period when thyroid hormone supplies to the brain are entirely derived from the child and critical for continuing maturation. During this period, migration of granule cells in the hippocampal dentate gyrus and cerebellum, pyramidal cells in the cortex and Purkinje cells in the cerebellum are sensitive to thyroid hormones and thyroid hormone-dependent gliogenesis and myelination continues (Bernal, et al, 2003; Porterfield and Hendrich; 1993; de Escobar et al, 2004).

Figure 2.11: Relationship between thyroid hormone action and development of the brain

1. In the first trimester of pregnancy early neuronal proliferation and migration is dependent on maternal thyroxine (T4). In fetal tissues, inactivating type 3 deiodinase (D3) enzyme expression falls and development of the thyroid gland commences.
2. By the end of the first trimester, development of the hypothalamic-pituitary axis has occurred and a surge in thyroid-stimulating hormone (TSH) secretion results in the onset of fetal thyroid hormone production, expression of the activating type 2 iodothyronine deiodinase enzyme (D2) and increasing occupation of thyroid hormone receptors (TRs) by 3,5,3'-L-triiodothyronine (T3).

3. Continuing development of the brain in the second and third trimesters relies increasingly on T4 produced by both the fetus and mother. Continued post-natal development is entirely dependent on neonatal thyroid hormone production. Apo-TR, unliganded unoccupied thyroid hormone receptor.

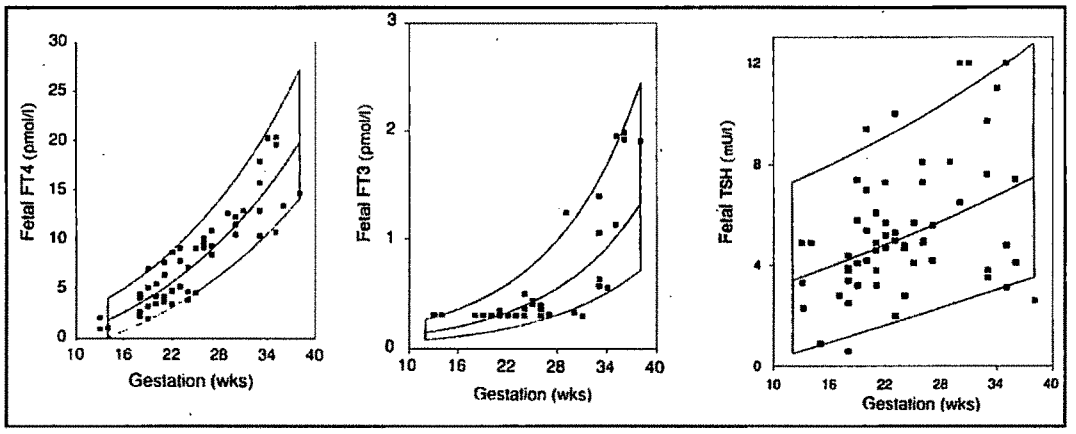


Source: Williams, 2008

Circulating thyroid hormone concentration 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) (Figure 2.12).

Figure 2.12: The ontogeny of fetal thyroid hormone metabolism and concentrations of fetal TSH, T4 and T3 during gestation



Source: Chan and Kibly, 2000

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 and Fisher 1997b).

Thyroid hormone concentrations in fetal brain

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 and 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).

Utero–placental transfer of thyroid hormones

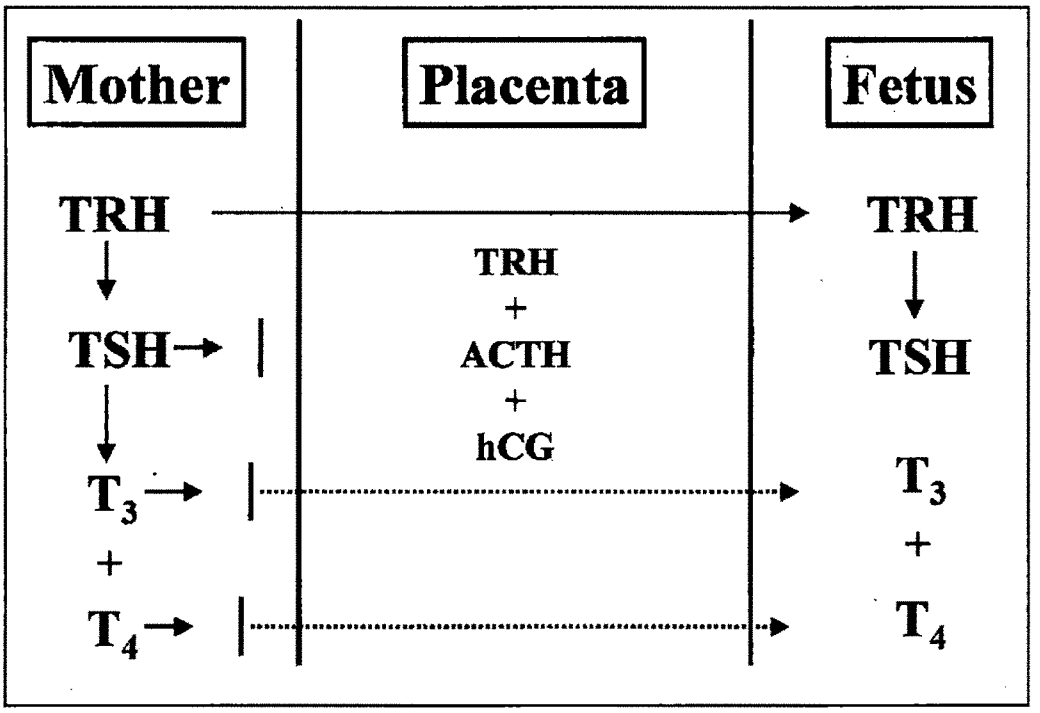
Circulating thyroid hormones in the human fetus are of both maternal and fetal origin and their presence is dependent on a functioning placenta for T4 transport and supply of iodide substrate (Figure 2.13). The placenta rapidly breaks down much of the T4 presented to it but significant amounts of T4 are still transferred (Delbert and 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, 1991). Chan and Kibly (2000) have 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.

By analogy, if the total thyroid hormone levels in the mother and fetus were similar, fetal tissues would be exposed to elevated levels of free T4 and free T3 (de Escobar et al, 2004) that are also detrimental to the critical temporal sequence of thyroid hormone responses during fetal development (Anselmo et al, 2004). These considerations underlie the

requirement for an efficient but incomplete utero-placental barrier to maternal thyroid hormone transfer that limits supplies and ensures that 'euthyroid' free hormone concentrations are maintained in fetal fluids and tissues. Following the onset of fetal thyroid hormone production, levels of total and free T3 remain very low in the fetus compared to the mother, whereas total and free T4 concentrations reach adult levels by the beginning of the third trimester (Thorpe-Beeston et al, 1991).

Figure 2.13: Placental transfer of thyroid hormones



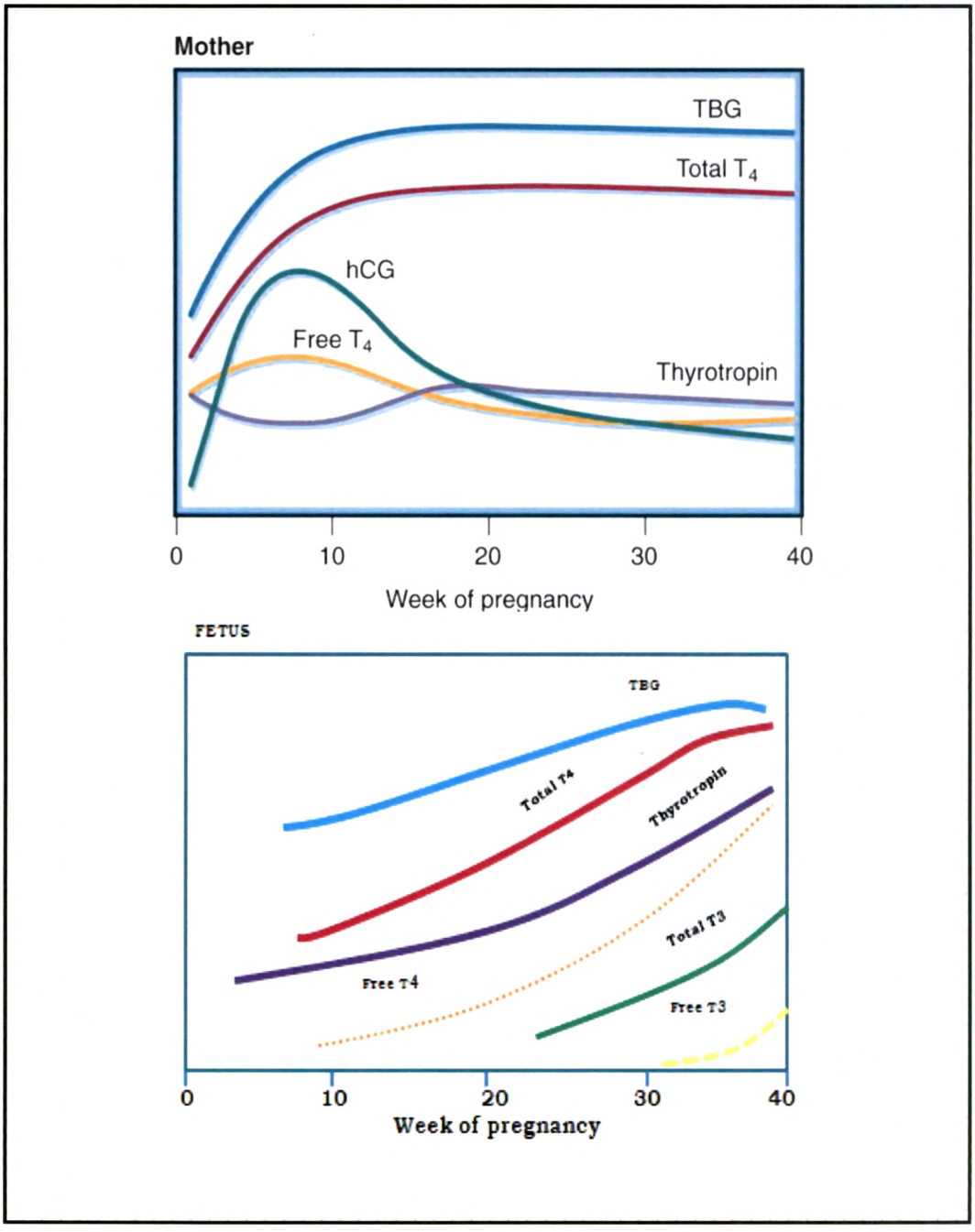
(TRH, thyroid releasing hormone; TSH, thyrotrophin; T3, triiodothyronine; T4, thyroxine; ACTH, adrenocorticotrophin; hCG, human chorionic gonadotrophin)

Source: Chan and Kilby, 2000

Despite the increasing concentrations of T4 in the fetus as gestation progresses, the fetal thyroid reserve remains low and the gland does not mature fully until birth (van den Hove and Vulsma et al, 1989). Thus, maternal thyroid hormones continue to contribute to fetal T4 levels until birth, as demonstrated in neonates who cannot synthesize their own thyroid hormones because of complete organification defects (Vulsma et al, 1989). Likewise, hypothyroxinemia in premature babies

results from a complete absence of maternal T4 and may account in part for their increased risk of cerebral palsy and neurological deficit (Zoller and Rovet, 2004; de Escobar et al, 2004; Lafranchi, 1999).

Figure 2.14: Relative changes in maternal and fetal thyroid function during pregnancy



Source: Burrow et al, 1994

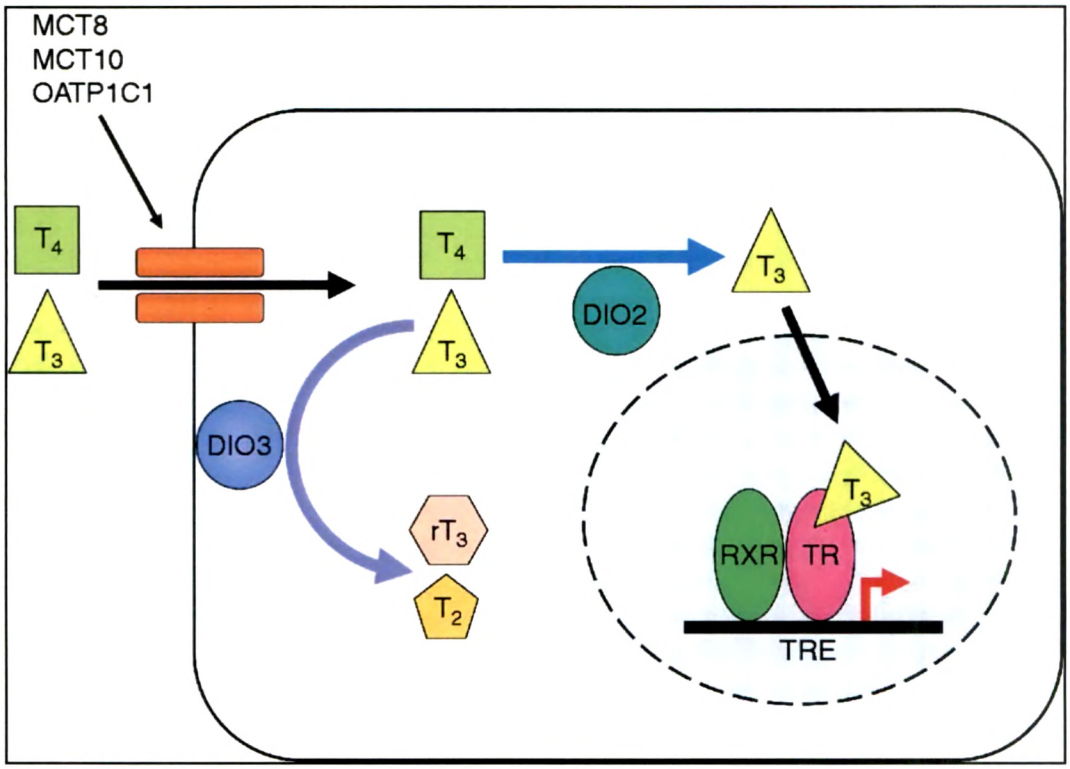
Delivery of thyroid hormones to target tissues

Thyroid hormones are lipophilic and poorly soluble in water. They bind reversibly to plasma transport proteins of varying affinity, which include thyroxine-binding globulin (TBG), transthyretin (previously called thyroxine-binding prealbumin), albumin and various lipoproteins. The free-fractions of circulating thyroid hormones are dependent on the concentrations and saturations of binding proteins and in normal serum free T4 represents 0.02% of the total T4 concentration, whereas free T3 is 0.3% of total T3 because of its lower affinity for TBG. As a result, total circulating concentrations of T4 are 50–60 folds higher than total T3, whereas free T4 levels are only approximately four-fold higher than free T3.

Circulating T4 is derived solely from synthesis and secretion by the thyroid gland, whereas 80% of circulating T3 is produced in peripheral tissues by enzymatic removal of an outer ring 5'-iodine atom from T4. Both T4 and T3 are transported into target tissues with equal efficiency and do not compete for uptake. Until recently, the mechanism of cellular entry of free thyroid hormones was not clear but was presumed to occur by passive diffusion because of their lipophilic nature (Friesema et al, 2005). In fact, the hormones enter target cells and cross the placenta via an energy-dependent, ATP-requiring, stereo-specific and saturable transport mechanism that is mediated by the mono-carboxylate transporter-8 (MCT8) (Friesema et al, 2003) and other transporter proteins such as OATP1c1, a member of the Na+- independent organic anion transporter protein (OATP) family (Jansen et al, 2005; Heuer, 2007).

Figure 2.15: Regulation of intracellular supplies of T3 to the nucleus of T3 target cells

MCT8 and MCT10, monocarboxylate transporters 8 and 10; OATP1C1, organic acid transporterprotein-1C1; DIO2 and DIO3, type 2 and 3 deiodinase enzymes; TR, thyroid hormonereceptor, RXR, retinoid X receptor; T4, thyroxine; T3, 3,5,3'-L-triiodothyronine; rT3,3,3',5'-triiodothyronine; T2, 3,3'-diiodothyronine.



Source: Williams and Bassett, 2011

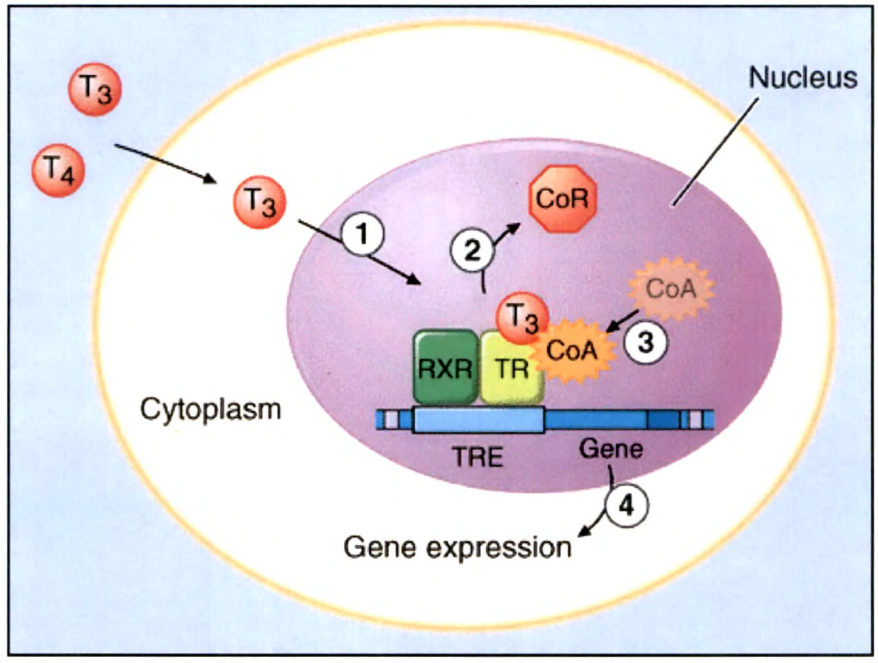
Thyroid hormone action

Thyroid hormone receptors (TRs) bind T3 with high affinity and function as ligand-inducible transcription factors that regulate expression of T3-responsive target genes. TRα and TRβ are members of the steroid / thyroid hormone receptor super family (Sap et al, 1986; Weinberger et al, 1986). The TRα gene encodes three C-terminal variants in mammals: TRα1 binds T3 and DNA and is a functional receptor, whereas TRα2 and TRα3 do not bind T3 (Harvey and Willliams, 2002). The TRβ gene encodes two N-terminal variants in all vertebrates TRβ1 and TRβ2, both of which are functional receptors.

Unlike most nuclear receptors, unliganded apo-TRs compete with liganded TRs for DNA response elements and act as potent repressors that exert important physiological roles during the development of specific tissues including the brain (Hashimoto et al, 2001; Chassande, 2003; Venero et al, 2005; Wallis et al, 2008). Apo-TRs interact with co-repressor proteins, which recruit histone deacetylases and maintain a non-permissive chromatin structure to inhibit gene transcription. By contrast, liganded TRs bind to co-activators in a T3-

dependent manner. Co-activators possess intrinsic histone acetyl transferase activity, which facilitates formation of permissive nucleosomes and activation of gene expression. Thus, the opposing effects of unoccupied and occupied TRs result in greatly increased amplitude of transcriptional response to T3 (Harvey and Williams, 2002). Although circulating levels of free T4 are four-fold higher than free T3, the TR has at least a 15-fold greater affinity for T3 (Lin et al, 1990), indicating that T4 is a pro-hormone that must be converted to T3 prior to the onset of thyroid hormone action (Bianco et al, 2006).

Figure 2.16: Mechanism of thyroid hormone receptor action



Source: Longo et al, accessible at www.accessmedicine.com

The thyroid hormone receptor (TR) and retinoid X receptor (RXR) form heterodimers that bind specifically to thyroid hormone response elements (TRE) in the promoter regions of target genes. In the absence of hormone, TR binds co-repressor (CoR) proteins that silence gene expression.

The numbers refer to a series of ordered reactions that occur in response to thyroid hormone: (1) T4 or T_3 enters the nucleus; (2) T_3 binding dissociates CoR from TR; (3) Coactivators (CoA) are recruited to the T_3-bound receptor; (4) gene expression is altered.

Thyroid hormone metabolism (iodothyronine)

Placenta

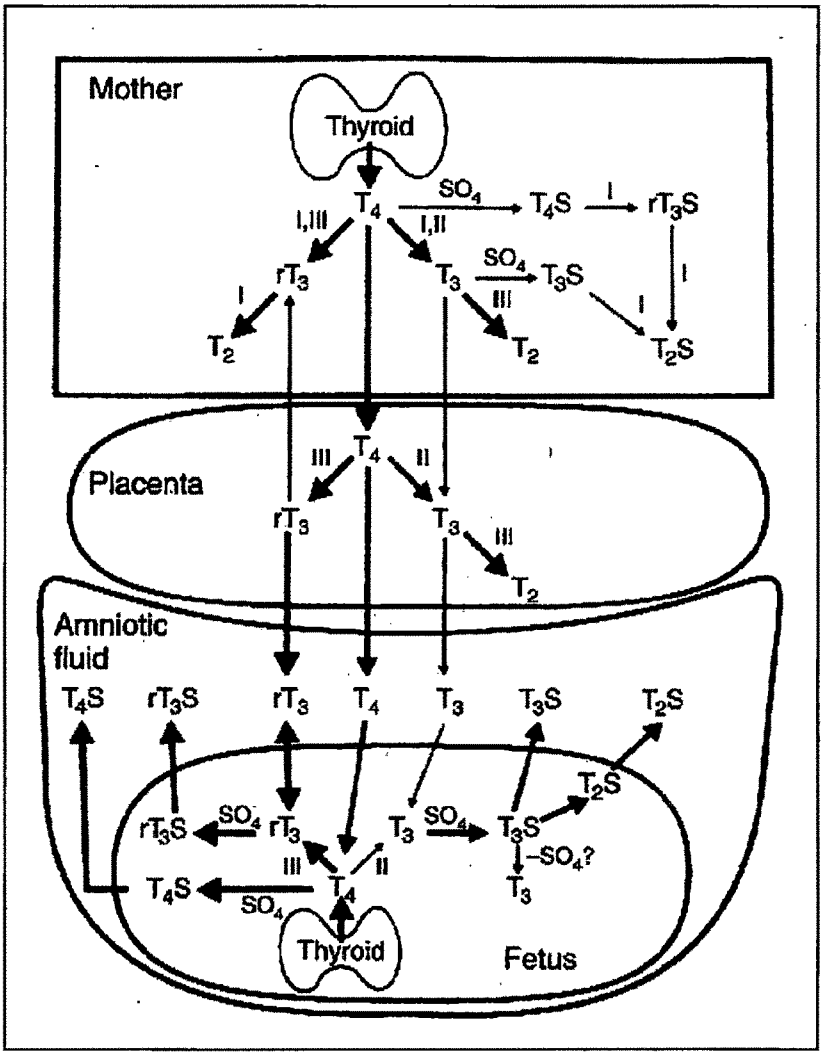
Three enzymes catalyze the deiodination of iodothyronine in human tissues (Larsen et al, 1981). Type I deiodinase, which catalyzes the deiodination of both the outer and inner rings, is a seleno-protein expressed in adults in liver, kidney, thyroid, and pituitary gland (Mandel et al, 1992). This enzyme is responsible for most of the T3 in serum. It also catalyzes the 5′ deiodination of 3,3′,5′-triiodothyronine (reverse T3), which is the rate limiting step in the clearance of reverse T3, as well as inner ring deiodination, particularly of T3 sulfate and T4 sulfate (Figure 2.17) (Otten et al, 1983; Mol and Visser, 1985).

Type II deiodinase acts only on the outer ring and prefers T4 and reverse T3. It is expressed in brain, pituitary gland, brown adipose tissue, keratinocytes, and placenta (Hidal and Kaplan, 1985; Kaplan et al, 1988; Houstek et al, 1993). Type III deiodinase, present in high amounts in placental tissue, brain, and epidermis, catalyzes the conversion of T4 to reverse T3 and T3 to 3, 3′ –di-iodothyronines (T2) (Roti et al, 1981). The activity of type II deiodinase is higher in the chorionic and decidual membranes of the placenta than in the amniotic membranes, whereas type III deiodinase is found mostly in trophoblasts (Hidal and Kaplan, 1985). The mixture of type II and type III deiodinases in the placenta provides for the conversion of T4 to T3 and of T4 and T3 to reverse T3 and T2, respectively (Figure 2.17).

As in other tissues, the activity of type II deiodinase increases when the availability of T4 decreases (Hidal and Kaplan, 1985). This suggests that deiodinase activity represents a homeostatic mechanism for maintaining T3 production in the placenta when maternal serum T4 concentrations are reduced (e.g., during hypothyroidism or iodine deficiency). Most of the beneficial effects of T3 generated by the action of maternal type II deiodinase are probably restricted to the placental cells because of the highly active placental type III deiodinase. In fact, placental type III deiodinase seems designed to maintain low serum T3

concentrations in the fetus while protecting decidual cells from hypothyroidism. The high rate of placental blood flow explains the absence of measurable difference in serum T4 and T3 concentrations between the umbilical artery and vein that might otherwise be anticipated given the high level of type III deiodinase activity in placenta (Abuid et al, 1973).

Figure 2.17: Interaction of maternal, placental and fetal thyroid metabolism



Source: Burrow et al, 1994

Fetus

The ontogeny of the three deiodinase that catalyze the progressive deiodination of T4 differs in the developing fetus. Type II and type III deiodinases appeared at mid-gestation, whereas type I deiodinase is

not evident until later (Fisher and Polk, 1989) (Figure 2.17). Accordingly fetal serum T3 concentrations are low (<15 ng/dl) before 30 weeks gestation; they increase gradually to about 50 ng/dl at term (Table 2.5) (Ballabio et al, 1989). By contrast maternal serum values are approximately 200 ng/dl. The preterm increase in fetal serum T3 concentration is due to an increase in type I deiodinase activity. Serum concentration of reverse T3, T4 sulfate, T3 sulfate, and reverse T3 sulfate in the umbilical cord at this time are high (Wu et al, 1993). The sulfated metabolites accumulate in fetal serum as a result of the very low type I deiodinase activity in fetal tissues and because the sulfated iodo-thyronines are not substrates for placental type III deiodinase (Santini et al, 1992*). Although T3 sulfates does not bind to nuclear T3 receptors and therefore has not biological activity, its parenteral administration in rats after thyroidectomy increases serum T3 concentrations and biological responses (Santini et al, 1993). Thus, T3 sulfate can be desulftaed in the gut. The liver, kidney, and the brain of adult rats have sulfatase activity, and desulfation of T3 sulfate to T3 occurs in the liver and brain of fetal rats (Kung et al, 1988; Santini et al, 1992). Thus, T3 sulfate could serve as a local source of T3 in fetal tissues containing sulfatase.

The importance of iodothyronines in amniotic fluid

In addition to being linked by the umbilical cord, the mother and fetus are linked by the amniotic cavity and amniotic fluid, providing a second pathway for fluid and molecular exchange. The amniotic fluid volume is the net balance of inflow, consisting of fetal urine and lung fluid, and outflow, consisting of fetal-maternal trans-amniotic fluid exchange and fetal swallowing (Tomoda et al, 1985). The pattern of iodothyronines in amniotic fluid reflects the effects of type III deiodinase activity in placental and fetal tissues, and the iodothyronine concentrations in amniotic fluid thus reflect both maternal and fetal thyroid hormone metabolism (Figure 2.17).

Table 2.5: Iodothyronine concentrations in maternal and fetal serum and amniotic fluid

Iodothyronine	Maternal serum	Amniotic fluid (ng/dl)		Fetal serum (ng/dl)	
	(mid-gestation)	20 week	Term	20 week	Term
T4	12,000	250	570	3,100	11,000
T3	200	8.6	6.6	13	49
T2	2.2	5.8	6.2	-	11
rT3	24	130	69	250	270
T4 sulfate	1.8	28	-	-	21
T3 sulfate	2.9	6.6	-	6.7	12
rT3 sulfate	3.8	8.6	-	-	50

Source: Burrow et al, 1994

Tissue thyroid status

D3 is expressed in fetal tissues and the placenta where it initially prevents maternal thyroid hormone access to the developing fetus (Wasco et al, 2003). At this time, unoccupied TRs are critical factors that generally maintain cell proliferation and prevent differentiation (Flamant et al, 2002). Increased pituitary D2 expression correlates with maturation of the hypothalamic-pituitary-thyroid (HPT) axis whereas its expression in T3-target tissues, concomitant with reduced expression of D3, results in conversion of unoccupied TRs into occupied TRs and the initiation of cell differentiation (Campos-Barros et al, 2000). Thus, the TR acts as a deiodinase-dependent developmental switch that regulates maturation of T3-dependent tissues. Expression of D2 is increased in hypothyroidism whereas D3 expression is increased in thyrotoxicosis, ensuring that the balance between D2 and D3 activities acts as a critical homeostatic regulator of T3 availability to the cell nucleus even at extremes of thyroid function. In the brain, D2 activity is markedly up-regulated in the

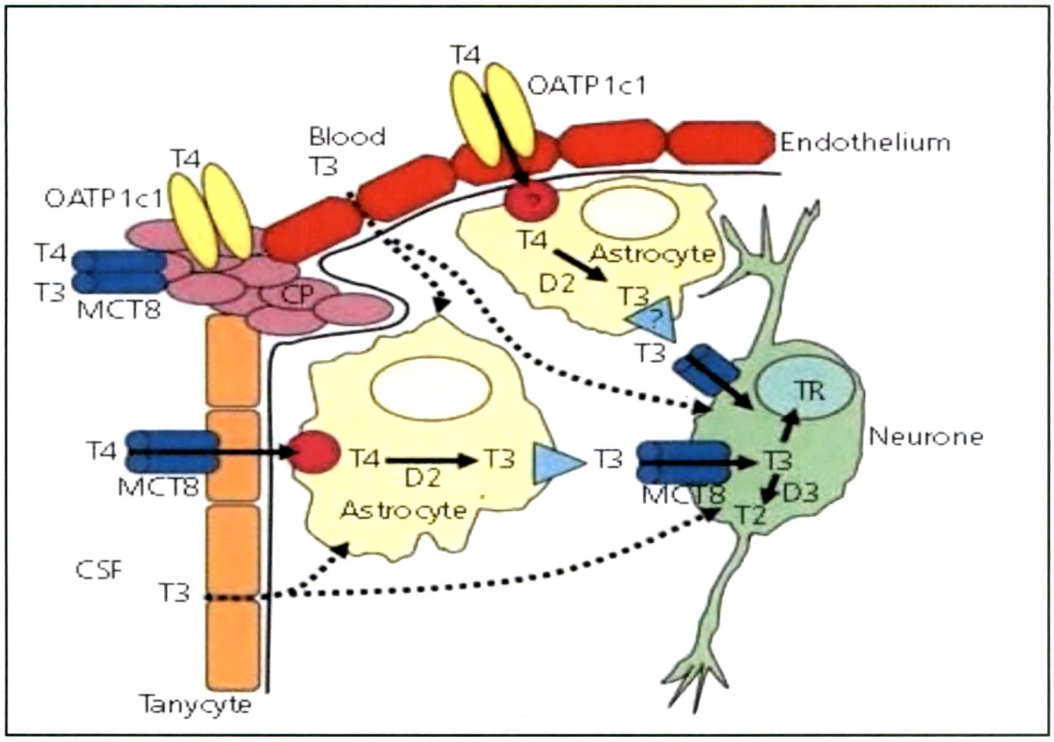
presence of low thyroid hormone levels (Burmeister et al, 1997), whereas D3 activity is strongly decreased (Friedrichsen et al, 2003).

This adaptation is thought to protect the susceptible brain from normal fluctuations in circulating thyroid hormone levels as well as to some extent protecting from the detrimental effects of hypothyroidism or hyperthyroidism, particularly during development (Guadano-Ferraz et al, 1999). Thus, local regulation of thyroid status in specific regions of the brain is achieved by the coordinated regional expression of the D2 and D3 enzymes (Kester et al, 2004) and T3 homeostasis results from compensatory reciprocal changes in activities of the D2 and D3 enzymes (Tu et al, 1997).

Control of T3 availability and action in the brain

The main cellular site of T3 action in the brain is the neuron, but T3 must gain access to neurons by a circuitous route that is subject to local regulation (Fliers et al, 2006) (Figure 2.18). The highly specific iodothyronine transporters OATP1c1 and MCT8 are both expressed in the central nervous system (CNS) and, in the last 5 years, our understanding of thyroid hormone uptake into the brain has progressed remarkably. Most likely, control of local T3 levels in brain tissue takes place in functional units of astrocytes and neurons (Friesema et al, 2006). T4 (and T3) must first cross the blood–brain barrier, and the T4-specific transporter OATP1c1 is ideally placed to achieve this as it is expressed at high levels in capillary endothelium throughout the CNS (Tohyama et al, 2004). Some T3 transport across the blood–brain barrier may also be mediated by MCT8 (Heuer et al, 2005). Thyroid hormones also enter via the choroid plexus–cerebrospinal fluid (CSF) barrier. OATP-1 and MCT-8 are expressed in choroid plexus and likely to mediate transport of T4 (Heuer et al, 2005), whilst MCT8 and D2 are co-expressed in tanycytes lining the third ventricle and facilitate access of thyroid hormones to hypothalamic nuclei and thyrotrophin-releasing hormone (TRH) neurons (Alkemade et al, 2005).

Figure 2.18: Delivery of thyroid hormones to neurons



Source: Williams, 2008

Circulating thyroid hormones enter the cerebrospinal fluid via the choroid plexus, which expresses both monocarboxylate transporter-8 (MCT8) and Na+-independent organic anion transporter protein 1c1 (OATP1c1) thyroid hormone-specific transporter proteins. The pro-hormone, T4 is transported across the blood–brain barrier via OATP1c1 in endothelial cells or MCT8 in tanycytes lining the third ventricle. Thyroxine (T4) enters glial cells including astrocytes via an unknown mechanism and is activated to 3,5,3'-L-triiodothyronine (T3) via the activating type 2 iodothyronine deiodinase (D2) enzyme. T3 is exported from glial cells by an unknown transporter to facilitate MCT8-dependent entry into neurons. T3 may also enter neurons directly from blood or cerebrospinal fluid (CSF) by a poorly defined route. T3 acts via thyroid hormone receptors (TRs) expressed in neurons or is metabolized by the inactivating type 3 deiodinase (D3) enzyme to inactive 3,3'-diiodothyronine (T2).

CP, choroid plexus, unknown transporter protein

Having entered the CNS, T4 is taken up by astrocytes via an unidentified transporter, where it is converted to T3 via local activity of D2 (Alkemade et al, 2005). T3 generated in astrocytes is then transported out of the cell via an unknown transporter before active uptake into neurons is mediated via MCT8 (Alkemade et al, 2005). T3 then exerts its major actions directly in neurons by regulating expression of T3-target genes (Bradley et al, 1992). T3 is finally metabolized and degraded by D3 in neurons (Alkemade et al, 2005).

Thyroid hormone transport in the brain

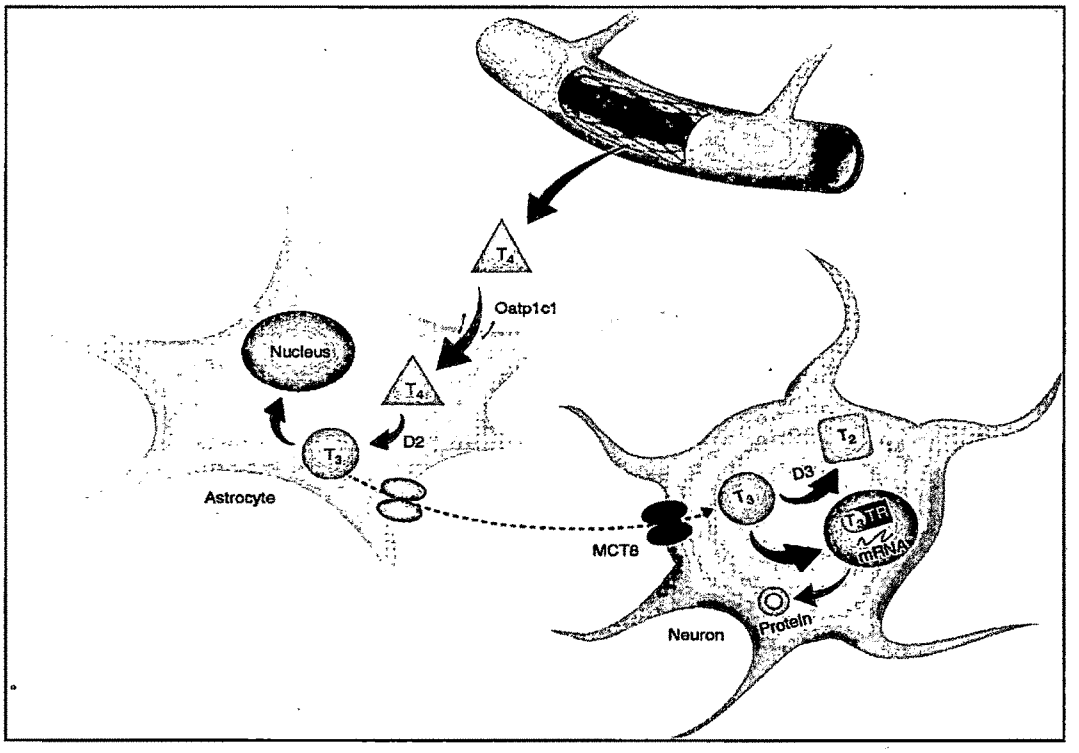
In situ hybridization, studies have shown MCT8 mRNA is expressed at high levels in choroid plexus, olfactory bulb, cerebral cortex, hippocampus and amygdala, at moderate levels in striatum and cerebellum and at low levels in some neuroendocrine nuclei. Colocalisation studies revealed that MCT8 is predominantly expressed in neurons. Together with a spatiotemporal pattern of MCT8 expression during the perinatal period, these data indicate that MCT8 plays an important role in CNS development by transporting thyroid hormones into neurons (Heuer et al, 2005). MCT8 is also expressed in pituitary folliculo-stellate cells (Alkemade et al, 2006); the same cells that express TSH receptor and may be involved in ultra-short feedback control of TSH secretion (Brokken et al, 2005). In support of this view, MCT8 protein is expressed in human hypothalamic paraventricular, supraoptic and infundibular nuclei and in the lining of ependymal cells of the third ventricle, which are all locations involved in negative feedback of TRH (Alkemade et al, 2005).

MCT8 mutations in humans

A key physiological role for thyroid hormone transport was confirmed in patients with mutations in SLC16A2 (previously MCT8) located on chromosome Xq13.2 (Friesema et al, 2004). There is global developmental delay including poor communication skills, no speech development, poor head control, mental retardation and varying degrees of truncal hypotonia, athetoid movements and motor

deficiency, which may include spastic quadriplegia in severe cases (Refetoff and Dumitrescu, 2007).

Figure 2.19: Thyroid hormone transporters in the brain



Source: Patel et al, 2011

T4 is transported to the brain thyroid hormone-binding proteins such as transthyretin (TTR), where T4 then passes out through endothelial cells lining the blood vessels. T4 is rapidly transported through the cell membrane transporter, Oatp1c1, located on the surface of astrocytes. T4 is then metabolized intra cellularly by D2 to T3, where it can then be transported out from astrocytes by an as yet unidentified cell membrane transporter. Within the brain parenchyma, T3 is then promptly uptaken by neurons and oligodendrocytes via the MCT8 cell membrane transporter. Within the cell, T3 can either translocate and bind to thyroid hormone receptors (TRs), resulting in thyroid hormone action, or be metabolized via D3 to biologically inactive T2.

Thyroid hormone metabolism in the brain

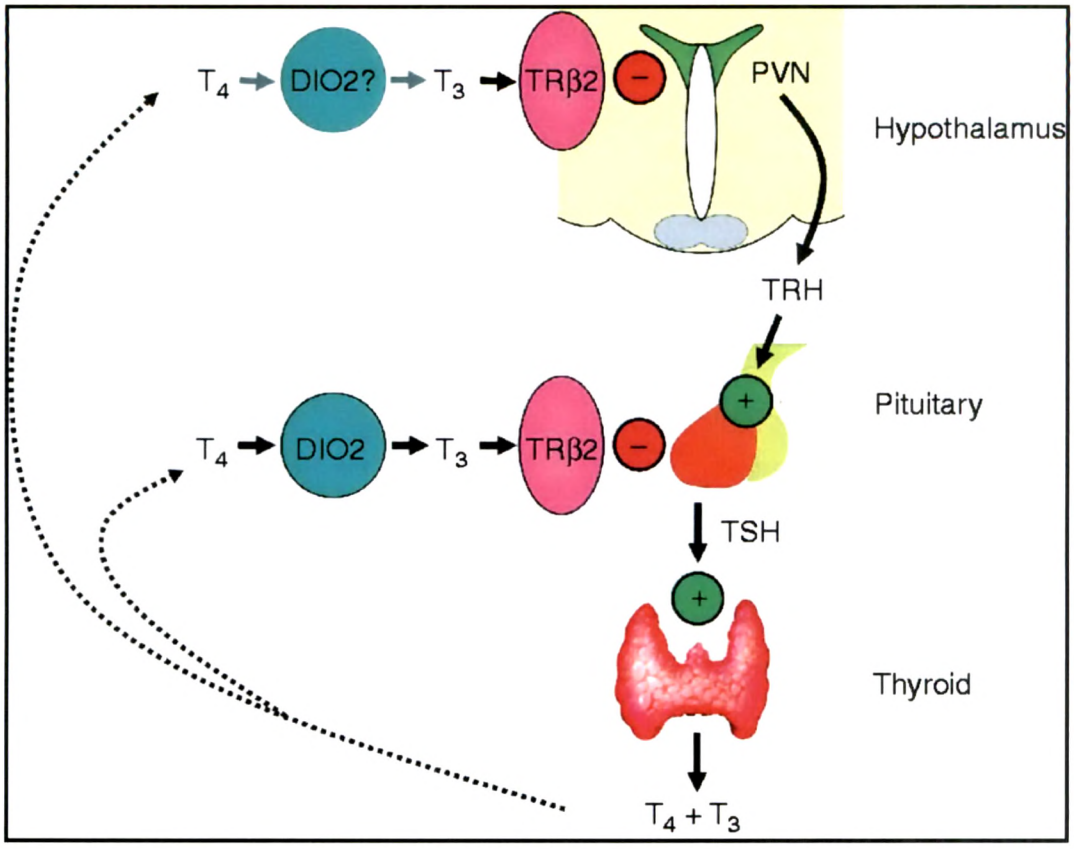
Type 2 deiodinase

The activating D2 enzyme is expressed in glial cells, third ventricle tanycytes, astrocytes and some sensory neurons including nuclei within the trigeminal, auditory and visual pathways (Guadano-Ferraz et al, 1999). Understanding of key neurodevelopment roles for D2 has come from a series of elegant studies in mice. In the cochlea, D2 is expressed in periosteal connective tissue surrounding the internal sensory tissues, with enzyme activity peaking before the onset of hearing. TR expression, however, is localized to the cochlea sensory epithelium, suggesting that periosteal D2 provides a spatiotemporally regulated supply of T3 to the sensory epithelium that is necessary for correct timing of the development and maturation of the cochlea (Campos-Barros et al, 2000). This hypothesis was supported by the finding that D2-deficient mice exhibit delayed cochlea development and defective auditory function despite circulating levels of thyroid hormones that normally are permissive for development of hearing. Thus, D2-dependent local generation of T3 to the cochlea is essential for auditory function (Ng et al, 2004). In this case, the activating D2-enzyme functions as a local amplifier of T3 action to regulate sensory development.

By contrast, the inactivating D3 enzyme can influence spatiotemporal development of sensory pathways by inhibiting T3 action locally. For example, D3 regulates localized asymmetrical growth of the dorsal retina during development of the eye in Xenopus by reducing local T3 concentrations to inhibit T3-dependent proliferation of lateral projecting ganglion cells (Marsh-Armstrong et al, 1999).

D2-deficient mice also have elevated circulating T4 and TSH levels but normal T3 concentrations and display an impaired negative feedback TSH response to T4 but not T3, demonstrating that D2 is required for local T3 generation in the pituitary and essential for normal control of the HPT axis (Schnieder et al, 2001).

Figure 2.20: Negative feedback regulation of the hypothalamic–pituitary–thyroid axis



Source: Williams and Bassett, 2011

The role of DIO2 in negative feedback control of the HPT axis occurs predominantly in thyrotrophs of the anterior pituitary gland. PVN, para-ventricular nucleus; TRH, thyrotropin-releasing hormone; DIO2, type 2 deiodinase enzyme; TRb2, thyroid hormone receptor b2; T4, thyroxine; T3, 3,5,3'-Ltriiodothyronine

Type 3 deiodinase

The inactivating D3 enzyme is highly expressed in the developing rat brain (Bates et al, 1999) and is also present in neurons throughout the adult rat brain especially in pyramidal cells of the hippocampus, granule cells in the dentate and in cerebral cortex (Tu at el, 1999). In early studies, heterogeneous levels of D3 enzyme activity were detected throughout the brain (Kaplan et al, 1981). Studies in D3-deficient mice have also revealed a critical role for this enzyme in

maturation and activity of the HPT axis at the levels of the hypothalamus, pituitary and thyroid gland.

Thyroid hormone receptors in the brain

TR expression

In the brain, TRs are expressed prior to the onset of fetal thyroid hormone production (Forrest et al, 1991). TRα1 is the major isoform expressed during fetal life (Forrest et al, 1990) but, prior to birth, there is increased expression of TRβ1, which is distributed widely as development proceeds (Strait et al, 1990). Nevertheless, TRα has been estimated to account for 70–80% of total TR expression in the brain (Schwartz et al, 1992). TRα1 and TRβ1 also exhibit differential spatiotemporal expression in neurons throughout the post-natal and adult brain (Mellstrom et al, 1991), suggesting discrete roles for the two isoforms during development and in the mature CNS. For example, in cerebellum, TRα1 is expressed in granular cells whereas both TRα1 and TRβ1 are present in Purkinje cells. Accordingly, T3 acts via TRα1 to regulate granular cell migration and via both TRα1 and TRβ1 to control Purkinje cell differentiation (Morte et al, 2002). Similarly, differences in levels of TRα1 and TRβ1 expression in GABAergic interneurons in cerebral cortex and hippocampus correlate with behavioral phenotypes characterized in TR mutant mice (Guadano-Ferraz et al, 2003).

Furthermore TRα1, but not TRβ, has been shown to regulate the onset of oligodendrocyte precursor cell differentiation and control the timing of oligodendrocyte maturation and migration in the optic nerve (Bilion et al, 2002). The actions of TRα1 and TRβ1 in the brain, however, are not necessarily discrete; recent studies have revealed cooperative interactions between the two isoforms during astrocyte maturation (Morte et al, 2004). Expression of the TRβ2 isoform, by contrast, is localized and restricted to the hypothalamus (Cook et al, 1992), anterior pituitary (Wood et al, 1991; Hodin et al, 1989), developing cochlea (Bradley et al, 1994) and neural retina (Sjoberg et al, 1992).

Regulation of the HPT axis by TRs

In the hypothalamus and pituitary, TRβ controls the HPT axis. Mice lacking all TRβ isoforms or harboring a dominant negative knocking mutation of Thrβ display the biochemical features of resistance to thyroid hormone (RTH) seen in patients with mutations in THRB (Weiss and Refetoff, 2000). TRβ knockout mice or mice expressing dominant-negative TRβ proteins display defective HPT axis regulation in both hypothalamus and pituitary resulting in elevated T3 and T4 concentrations and inappropriately normal or elevated TSH (Abel et al, 2003).

2.5 SCREENING FOR THYROID DISORDERS ASSOCIATED WITH PREGNANCY

There are no recommendations for universal screening for thyroid dysfunction in women before or during pregnancy. As the overall benefits of screening for thyroid dysfunction (primarily hypothyroidism) have not yet been universally justified by current evidence-based medicine, recent international guidelines have recommended 'aggressive' case finding among the following groups of women who are at high risk, preferably already prior to pregnancy or during early gestation (Abalovich et al, 2007).

High risk women for whom screening is recommended:

- Women with a history of hyperthyroid or hypothyroid disease, postpartum thryoiditis, thyroid lobectomy, and women who already take thyroxine prior to conception.
- Women with a family history of thyroid disease.
- Women with a goiter.
- Women with thyroid antibodies (when known).
- Women with symptoms or clinical signs suggestive of thyroid under-function or over-function, including anemia, elevated cholesterol, and hyponatremia

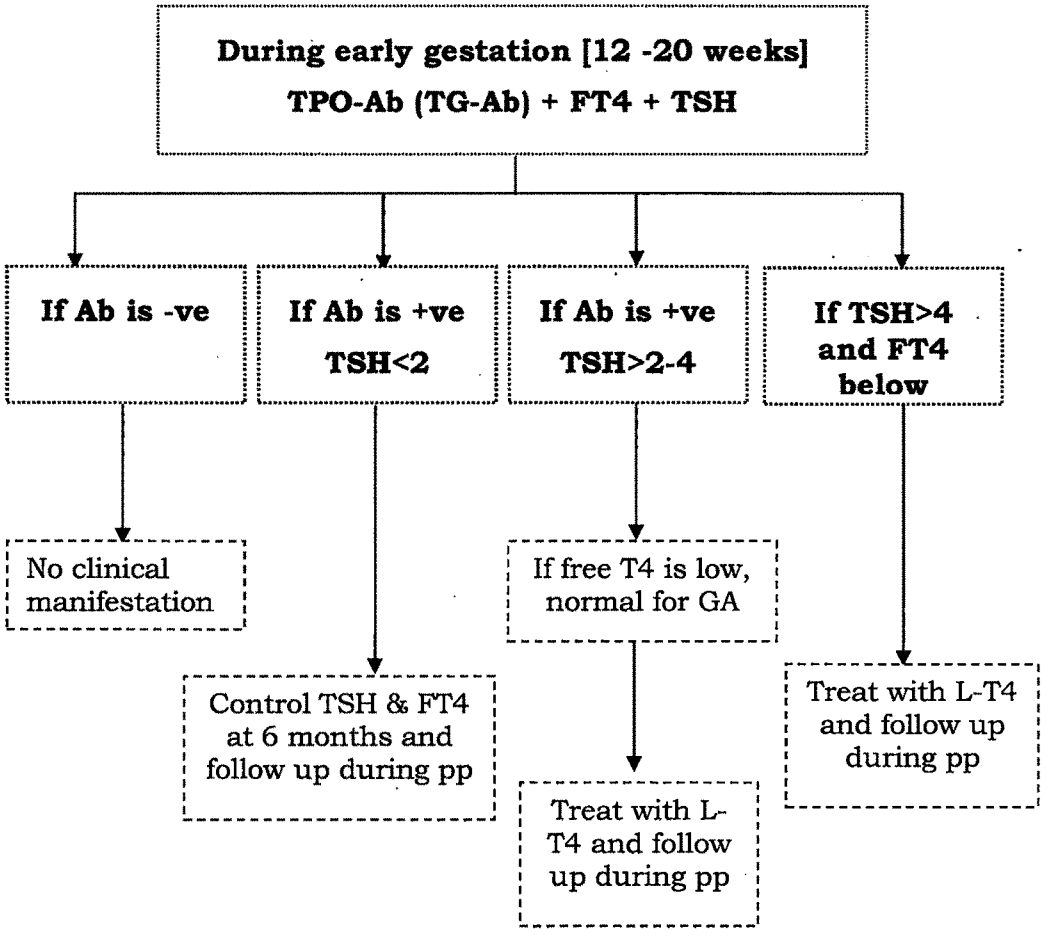
- Women with type I diabetes
- Women with other autoimmune disorders
- Women with a prior history of head and neck irradiation
- Women with infertility should have screening with TSH as part of their infertility work-up
- Women with a prior history of miscarriage and preterm delivery

Among possible screening algorithms, the following scheme has been proposed (Figure 2.21). The first step in the algorithm is to measure serum TSH and thyroid antibodies in early gestation. Because isolated hypothyroxinemia may occur in some women (without concomitant rise in serum TSH), it is reasonable to include systematically a free T4 determination. Ideally, both TG-Ab and TPO-Ab should be determined; however, if for economic reasons only one antibody can be measured, then it is preferable to measure TPO-Ab because it yields the best diagnostic score. When serum TSH is elevated or free T4 clearly below normal, and irrespective of the presence (or absence) of thyroid autoimmunity (TAI), women should be considered to have thyroid under-function and treated with thyroxine.

The next step concerns those women with TAI and normal thyroid function. When serum TSH is <2.5 mIU/L (most frequently associated with low antibodies titers and normal free T4 levels), thyroxine treatment is not systematically warranted, and serum TSH and free T4 should be monitored later during gestation. For women with TAI and a serum TSH that lies within the normal range in early gestation, but is already slightly shifted to higher 'normal' values, i.e. between 2.5-4.0 mIU/L (most frequently associated with higher antibody titers and low-normal free T4 levels), obstetric care providers should consider thyroxine treatment.

Figure 2.21: An algorithm for systematic screening of thyroid autoimmunity and hypothyroidism during pregnancy based on the determination of thyroid antibodies (Ab)

[serum TSH and free T4 concentrations during the first half of gestation, GA = gestational age; NL = normal limits; PP = postpartum]



Source: Glinoer, 1998

It is important to keep in mind that serum TSH is down-regulated under the influence of peak hCG values in the 1st half of gestation, and also that the thyroid deficit tends to deteriorate as gestation progresses in TAI-positive women. Because the potential deleterious effects, for both mother and progeny, are not due to high serum TSH per se but to low free T4 concentrations, clinical judgment should be based on serum free T4. If low or low-normal for gestational age, thyroxine treatment is probably justified. In daily practice, when such a scheme is systematically applied, most-if not all-of the pregnancies followed are successful and uneventful (Abalovich et al, 2002).

Even though more prospective studies are needed to assess the final clinical relevance of the proposed scheme, the recent study of Negro et al, provided strong arguments in favor of early thyroxine administration in women with AITD and normal thyroid function during early gestation (Negro et al, 2006).

Systematic screening for AITD during early pregnancy also allows to delineating women who are prone to developing thyroid dysfunction after parturition. Thus, even when no specific treatment is warranted during gestation, systematic screening is useful to clinicians for organizing the monitoring of potential postpartum thyroid dysfunction (Kamijo et al, 1990).

Guidelines for treatment of thyroid disorders (Banerjee, 2011)

Hypothyroidism during pregnancy

1. Both maternal and fetal hypothyroidism exerts serious adverse effects on the fetus, so maternal hypothyroidism should be avoided by early diagnosis at the first prenatal visit or at diagnosis of pregnancy.
2. In cases of hypothyroidism diagnosed before pregnancy, adjust the preconception T4 dose to reach a TSH level not higher than 2.5 μIU/ml before pregnancy.
3. By 4-6 weeks of gestation, the T4 dosage needs to be increased by about 30-50%.
4. If overt hypothyroidism is diagnosed during pregnancy, thyroid function should be normalized as rapidly as possible. The target is to achieve and maintain TSH concentrations below 2.5 μIU/ml in the first trimester (or 3 μIU/ml in the second and third trimesters) or to trimester specific normal TSH ranges. This can be achieved by rapidly titrating the T4 dosage to reach and maintain the target TSH levels. A re-assessment of the thyroid function should be carried out within 30 to 40 days.
5. Women who have thyroid antibodies in the early stages of their pregnancy but are otherwise euthyroid should be monitored for

elevations of TSH above the normal range because they are risk of developing hypothyroidism.

6. Sub clinical hypothyroidism: Recommend T4 replacement as T4 treatment has been shown to improve obstetrical outcome, though do not modify long-term neurological development in the offspring.
7. After delivery, dose of T4 need to be decreased in most hypothyroid women.

Autoimmune and thyroid disease and miscarriage

Universal screening for anti-thyroid antibodies and possible treatment cannot be recommended as there are very few reports regarding positive association between the presence of thyroid antibodies and pregnancy loss.

Iodine nutrition during pregnancy

Increase daily iodine intake to 250 µg on an average during pregnancy and breastfeeding by encouraging the use of iodized salt.

Postpartum thyroiditis (PPT)

1. TSH estimation at 3 and 6 months in women known to be thyroid peroxidase antibody positive, for women with type 1 diabetes mellitus (PPT 3-fold greater).
2. Women with a history of PPT have a markedly heightened risk of developing permanent primary hypothyroidism within 5 to 10 years, should undergo annual TSH assessments.
3. Asymptomatic women with PPT who have a TSH above the reference range but less than 10 µIU/ml and who are not planning a subsequent pregnancy do not necessarily require intervention but should, if untreated, be re-monitored in 4–8 weeks. Symptomatic women and women with a TSH above normal and who are attempting pregnancy should be treated with levothyroxine.
4. Women with postpartum depression should be screened for hypothyroidism and appropriately treated.

2.6 SCREENING OF NEONATES

Neonatal screening programs for detection of CH in neonatal period are widespread in the developed countries for the last three decades (Dussault, 1999) and are fast gaining momentum in the developing world as well (Wu et al, 1999). In most screening programs blood samples are collected at 5-6 days age, but with large number of babies being discharged early, cord blood samples are being used as well (Ordookhani et al, 2003).

In our country, it is very difficult to call back babies once discharged. Also, an effective social system whereby babies could be reached at home is practically non-existent. Thus cord blood remains a very practical alternative for screening purposes, and thus is the practice in some Asian countries (Ordookhani et al, 2003). The Indian Academy of Pediatrics recommends the use of cord blood samples for screening for CH.

2.7 GESTATIONAL AGE-SPECIFIC REFERENCE INTERVALS

Laboratory reference intervals for thyroid function tests have traditionally been derived from non-pregnant subjects who are free from thyroid disease. Their validity in pregnant women is debatable as pregnancy produces profound physiological changes in the mother, which in turn complicate the interpretation of maternal thyroid function tests. Therefore, a local reference range for thyroid hormones in pregnant women is needed (Price et al, 2001) along with international reference ranges.

Table 2.6 and 2.7 presents trimester specific reference intervals for thyroid hormones, developed worldwide using percentile method and mean/median method respectively. For India we have trimester specific reference intervals (thyroid hormone) given by Marwah et al (2008) using percentile method (table 2.6) and Kumar et al (2003) using mean/median method (table 2.7). However, in these two studies, trimester specific reference intervals for urinary iodine were not exercised.

Table 2.6: Worldwide trimester specific reference intervals [percentiles- 5th & 95th, 2.5th & 97.5th]

Reference intervals based on INDIAN population *Source: Marwah et al, 2008*			
Thyroid Hormone	**Trimester**	**5th Percentile**	**95th Percentile**
TSH [mIU/L]	First	0.6	5.0
	Second	0.435	5.78
	Third	0.74	5.7
FT4 [pM/L]	First	12	19.45
	Second	9.48	19.58
	Third	11.3	17.71
FT3 [pM/L]	First	1.92	5.86
	Second	3.2	5.7
	Third	3.3	5.18
Reference intervals based on UAE and ASIAN population *Source: Dhatt et al, 2006*			
Thyroid Hormone	**Trimester**	**2.5thPercentile**	**97.5thPercentile**
		UAE	
TSH [mIU/L]	First	0.06	8.3
	Second	0.17	5.9
	Third	0.21	6.9
FT4 [pM/L]	First	8.9	24.6
	Second	8.4	19.3
	Third	8.0	18.0
		ASIAN	
TSH [mIU/L]	First	0.12	7.4
	Second	0.3	5.5
	Third	0.3	4.85
FT4 [pM/L]	First	11.3	21.9
	Second	9.7	18.5
	Third	8.9	16.6
Reference intervals based on CHINESE population *Source: Yu et al, 2010*			

Thyroid Hormone	Trimester	2.5thPercentile	97.5thPercentile
TSH [mIU/L]	First	0.02	3.65
	Second	0.36	3.46
	Third	0.44	5.04
FT4 [pM/L]	First	11.85	21.51
	Second	9.45	16.25
	Third	9.30	17.14
TPO-Ab [IU/mL]	First	5	19.69
	Second	5	19.62
	Third	5	21.96

Reference intervals based on CHINESE population

Source: Panesar et al, 2001

Thyroid Hormone	Pregnancy Status (wk)	2.5thPercentile	97.5thPercentile
TSH [mIU/L]	7	0.05	2.3
	11	0.03	2.3
	15	0.03	2.8
	19	0.03	3.1
	23	0.03	3.7
	27	0.08	3.5
	31	0.46	3.8
	35	0.13	3.4
	39	0.03	3.56
FT4 [pM/L]	7	11.8	20.8
	11	11.1	22.9
	15	10.1	17
	19	9.5	15.4
	23	8.1	16.7
	27	8.7	15.1
	31	7.8	13.7
	35	8.5	14.4
	39	9.1	15.6

Thyroid Hormone	Pregnancy Status (wk)	2.5thPercentile	97.5thPercentile
FT3 [pM/L]	7	3	5.7
	11	3	5.7
	15	2.8	4.9
	19	2.5	4.9
	23	2.8	4.2
	27	2.5	4.1
	31	2.3	3.9
	35	2.4	4.1
	39	2.4	4.2

Reference intervals based on AUSTRALIAN population

Source: Gilbert et al, 2008

Thyroid Hormone	Pregnancy Status (wk)	2.5thPercentile	97.5thPercentile
TSH [mIU/L]	9	0.05	2.20
	10	0.02	2.13
	11	0.02	2.16
	12	0.06	2.00
	13	0.06	2.54
FT4 [pM/L]	9	11.0	17.4
	10	10.6	17.6
	11	10.4	18.6
	12	10.3	17.9
	13	9.7	16.7
FT3 [pM/L]	9	3.3	5.5
	10	3.3	5.6
	11	3.3	5.6
	12	3.3	5.9
	13	3.2	6.0

Reference intervals based on SPANISH population

Source: Bocos-Terraz et al, 2009

Thyroid Hormone	Pregnancy Status (wk)	2.5th Percentile	97.5thPercentile
TSH [mIU/L]	<11	0.10	2.65
	11-20	0.03	2.57
	21-30	0.12	2.64
	31-36	0.23	3.56
	>36	0.36	-
FT4 [ng/dl]	<11	0.83	1.38
	11-20	0.77	1.34
	21-30	0.70	1.14
	31-36	0.66	1.17
	>36	0.17	-
FT3 [pg/mL]	<11	2.34	4.34
	11-20	2.24	4.43
	21-30	2.47	4.18
	31-36	2.25	4.16
	>36	2.59	-
TPO-AB [UI/mL]	<11	0.0	0.68
	11-20	0.0	1.04
	21-30	0.0	0.89
	31-36	0.0	1.27
	>36	0.0	-
TG-Ab [UI/mL]	<11	0.41	3.11
	11-20	0.34	3.31
	21-30	0.37	2.60
	31-36	0.38	2.45
	>36	0.46	-

Table 2.7: Worldwide trimester specific reference intervals [mean/median]

Country (year)	Analyte	Trimester			PP
		I	II	III	
UK Asians (2001)	TSH (mIU/L)	0.9	1.3	-	1.3
	FT4 (pmol/mL)	12.6	11.5	-	13.1
	UI (µg/L)	125	170	-	-

Country (year)	Analyte	Trimester			PP
		I	II	III	
UK whites (2001)	TSH (mIU/L)	0.9	1.3		1.7
	FT4 (pmol/mL)	12.4	11.5		13.2
	UI (µg/L)	125	170	147	-
Belgium (1990)	TSH (mIU/L)	0.75	1.05	1.29	-
	T4 (nmol/L)	138	148	148	-
	FT4 (pmol/mL)	17.9	14.5	13.4	-
	UI (µg/L)	58	58	53	-
Italy (2002)	TSH (mIU/L)	1.1	-	-	-
	Tg (ng/mL)	25	-	-	-
	FT4 (pmol/mL)	10.4	-	-	-
	UI (µg/L)	116	-	-	-
India (2003)	***TSH (mIU/L)***	***1.20***	***2.12***	***3.3***	-
	T4 (nmol/L)	***164***	***165***	***159***	-
	T3 (nmol/L)	***1.85***	***2.47***	***1.82***	-
Nigeria (2005)	TSH (mIU/L)	2.7	2.12	2.29	3.1
	T4 (nmol/L)	129	157	173	139
	T3 (nmol/L)	2.6	7.9	8.2	7.4
	UI (µg/L)	-	-	-	79
Japan (2005)	TSH (mIU/L)	1.05	1.51	1.23	2.96
	FT3 (pg/mL)	3.60	3.39	3.17	3.57
	FT4 (ng/dL)	1.43	1.11	1.02	1.08
UAE (2006)	TSH (mIU/L)	0.71	1.04	1.20	1.32
	FT4 (pmol/L)	14.6	12.7	12.0	13.7
Other Arabs (2006)	TSH (mIU/L)	0.63	1.1	1.30	-
	FT4 (pmol/L)	14.9	13.3	12.4	-
Asian (Ind) (2006)	TSH (mIU/L)	0.95	1.30	1.1	-
	FT4 (pmol/L)	15.7	13.4	12.1	-
Sweden (2004)	TSH (mIU/L)	0.89	1.17	1.16	1.06
	FT4 (pmol/L)	12.3	10.5	10.5	13.7
	Tg (ng/mL)	15.48	14.92	18.55	13.95
	UI (µg/L)	180	170	145	-
Singapore (2001)	TSH (mIU/L)	0.65	1.2	-	-
	UI (µg/L)	107	116	124	105
Sudan (2000)	TSH (mIU/L)	1.1	1.2	1.0	-
	FT4 (pmol/L)	11.4	9.6	10.2	-

Source: Soldin et al, 2007 [PP- postpartum]

2.8 Repercussions of hypothyroidism on pregnancy outcome

Despite the known association between decreased fertility and hypothyroidism, the latter condition does not preclude the possibility to conceive. This is probably the main reason why, until a few years ago, hypothyroidism had been considered wrongly to be relatively rare during pregnancy (Krassas, 2000). In a study by Abalovich and team, 34% of hypothyroid women became pregnant without thyroxine treatment, 11% of them had OH and 89% SCH (Abalovich et al, 2002). When hypothyroid women become pregnant and maintain the pregnancy, they carry an increased risk for early and late obstetrical complications (Table 2.8 and Table 2.9).

Table 2.8: Pregnancy outcome associated with hypothyroidism: maternal aspects

MOTHER	Frequency	%	Type of Hypo†	Reference
Anemia	Increased	31 %	OH **	Davis, 1988
PP hemorrhage	Increased	4 %	SCH **	Leung, 1993
PP hemorrhage	Increased	19 %	OH	Davis, 1988
Cardiac dysfunction	Increased	n. a.	OH	Davis, 1988
Pre-eclampsia	Increased	15 %	SCH	Leung, 1993
Pre-eclampsia	Increased	22 %	OH	Leung, 1993
Pre-eclampsia	Increased	44 %	OH	Davis, 1988
Pre-eclampsia	Increased	n. a.	OH	Mizgala, 1991
Placenta abruption	Increased	19 %	OH	Davis, 1988

† Hypothyroidism; ** SCH: subclinical hypothyroidism; ** OH: overt hypothyroidism, PP: postpartum

Source: Glioner, 2008

Table 2.8 and 2.9 clearly shows that both obstetrical and fetal complications occur with an increased frequency in pregnant women with hypothyroidism. As expected, these complications are both more frequent and more severe in women with OH than SCH. Most

importantly, adequate treatment with thyroid hormone greatly reduces risks of a poorer obstetrical outcome (Tan et al, 2006).

Table 2.9: Pregnancy outcome associated with hypothyroidism: fetal and neonatal aspects

FETUS-NEWBORN	Frequency	%	Type of Hypo†	Reference
Fetal distress in labour	increased	14 %	OH **	Wasserstrum, 1995
Prematurity	increased	31 %	OH	Davis, 1988
/LBW*	increased	9 %	SCH **	Leung, 1993
	increased	22 %	OH	Leung, 1993
	increased	13 %	OH	Abalovich 2002
	increased	R.R.: 1.8 ***	SCH	Casey, 2005
	increased	O.R.: 3.6 ***	OH	Idris, 2005
Breech presentation	increased	O.R.: 4.7 ***	Early hypo-T4	Pop, 2004
Cesarean section	increased	29 %	OH	Idris, 2005
Impaired intra-uterine growth	increased	n. a.	OH	Blazer, 2003
Congenital malformations	increased	4 %	OH	Leung, 1993
	increased	6 %	OH	Abalovich, 2002
Fetal death	increased	4 %	OH	Leung, 1993
	increased	12 %	OH	Davis, 1988
	increased	3 %	OH	Abalovich 2002
	increased	8 %	OH	Allan, 2000
Perinatal death	increased	9-20 %	OH	Montoro, 1981
	increased	3 %	OH	Allan, 2000

† Hypothyroidism; * LBW: low birth weight; ** SCH: subclinical hypothyroidism; ** OH: overt hypothyroidism; *** O.R.: Odds Ratio; *** R.R.: Relative Risk

Source: Glioner, 2008

2.9 IODINE PROPHYLAXIS

Salt is the most widely used food vehicle for iodine fortification. USI, that is iodization of all salt for human (food industry and household) and livestock consumption, is the strategy recommended by WHO for the control of iodine deficiency (WHO, 2008). Adequate iodization of all salt will deliver iodine in required quantities to the population on a continuous and self-sustaining basis. In 1994, a special session of the WHO and UNICEF joint committee on health policy recommended USI as a safe, cost effective and sustainable strategy to ensure sufficient intake of iodine in all individuals (UNICEF/2005).

In nearly all countries where iodine deficiency occurs, it is now well recognized that the most effective way to achieve the virtual elimination of IDD is through USI. Salt iodization programmes are currently implemented in over 70 countries around the world where IDD is a public health problem (WHO, 2008). The additional cost of iodine fortification in the process of salt production should eventually be borne by the consumer, but is negligible. This will greatly assist sustainability.

National salt iodization programmes are now well implemented worldwide and have followed a common pattern of evolution, which includes the following phases:

- **Decision phase:** This phase involves making the decision for USI supported by industry, backed by standards and regulation, and supported by an implementation plan.
- **Implementation Phase:** This phase ensures the infrastructure for iodization and packaging of all human and livestock salt, and supports that infrastructure with quality assurance measures, communication and demand creation, regulation and enforcement.
- **Consolidation Phase:** Once the goal of USI is achieved, it needs to be sustained and assessed through on-going process and impact monitoring, as well as periodic evaluations, the latter may include international multidisciplinary teams.

A successful salt iodization programme depends upon the implementation of a set of activities at the national level by various sectors:

- Government ministries (legislative and justice, health, industry, agriculture, education, communication and finance.
- Salt producers, salt importers and distributors, food manufacturers.
- Concerned civic groups, including consumer associations.
- Nutrition, food and material scientists, and other key opinion makers.

USI has been remarkably successful in many countries. Over 30 countries have achieved the goal of USI (>90 % of households using iodized salt), and many others are on track (WHO/UNICEF/ICCIDD, 2007).

The choice of salt as the preferred vehicle for the delivery of iodine is based on the following factors:

1. Salt is one of the few commodities consumed by everyone
2. Consumption is fairly stable throughout the year
3. Salt production is usually limited to a few geographical areas, which facilitates its quality control
4. Salt iodization technology is easy to implement and available at a reasonable cost throughout the developing world
5. The addition of iodine to salt does not affect its colour, taste or odour
6. The quality of iodized salt can be monitored at the production, retail and household levels (Allen L et al, 2006).

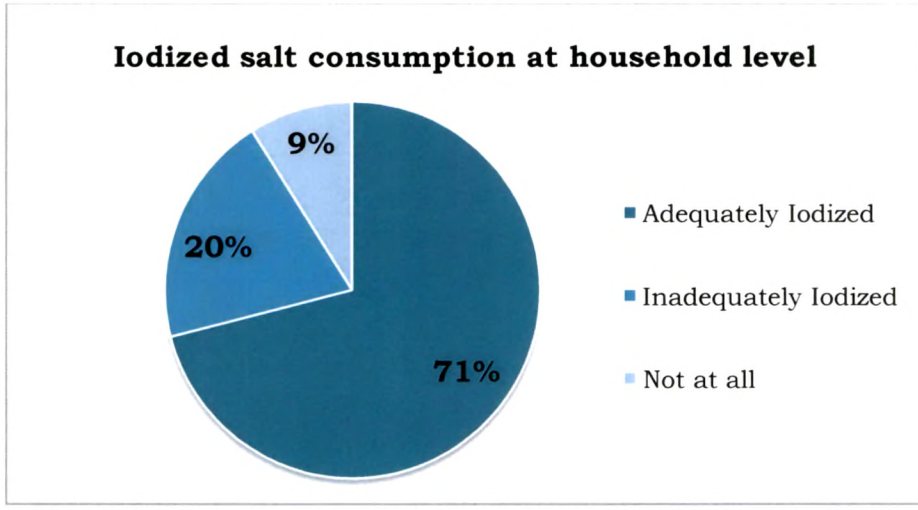
Some obstacles to the implementation of USI are:

1. Difficulties in enforcing legislation on iodized salt.
2. Problems caused by having a high number of small-scale salt producers and the absence of an effective operational monitoring system.
3. The lack of monitoring of the salt quality may result in variations in the iodine content of salt, inadequate supply of fortificants, inadequate supply and use of iodized versus non-iodized salt, and population levels of salt intake (measured versus estimated).

Status of iodized salt in INDIA

A lift on the ban of sale of non-iodized edible salt between 2000 and 2005 left iodized salt use suspended at 51% of households as per the NFHS-3 (National Family Health Survey). The ban's 2005 re-instatement combined with heightened consumer awareness, effective monitoring, and improvements in iodization practices and packaging helped to boost the use of iodized salt from 51% of households in 2006, to 71% by 2009 (Figure 2.24). Amidst this impressive increase in coverage, equitable distribution remains a challenge: consumption of iodized salt is much higher in urban areas (83%) than in rural areas (66%) leaving the most vulnerable population at a greater risk (Figure 2.23).

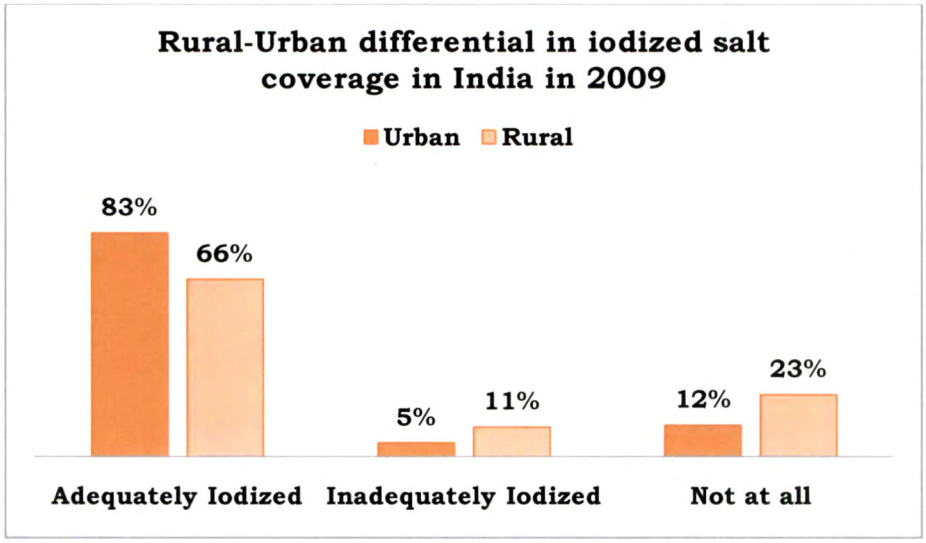
Figure 2.22: Iodized salt consumption at household level in INDIA



Source: IDD Newsletter, May 2011

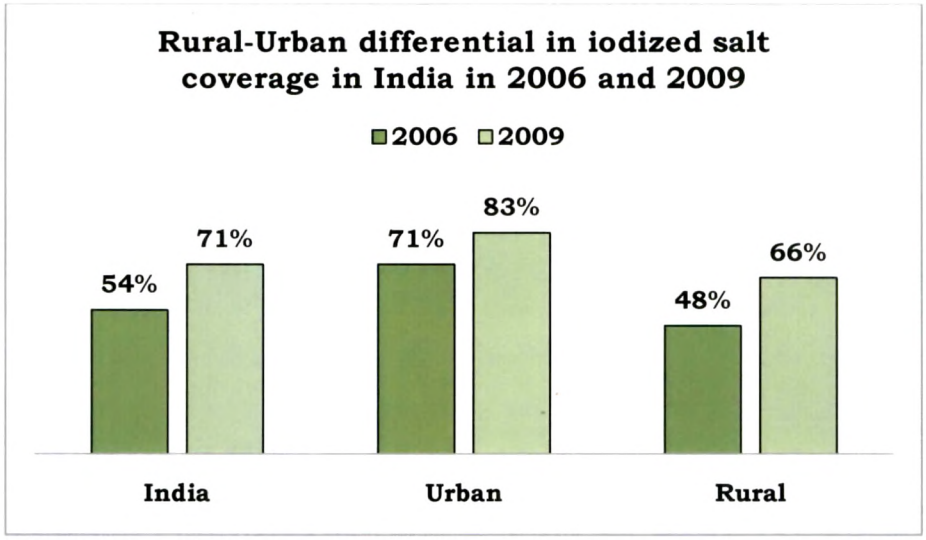
As per UNICEF CES (2009), overall 71% of the households were using cooking salt which was iodized at the recommended level of 15 ppm or more (Figure 2.22). Only 9% of the households used salt that was not iodized at all and 20% used salt that was iodized inadequately (<15 ppm). The rural-urban differential in salt iodization was found to be pronounced (Figure 2.23). Around 83% of households in urban areas used salt with 15 ppm or more iodine content compared with 66% of households in rural areas.

Figure 2.23: Difference in iodized salt coverage



Source: IDD Newsletter, May 2011

Figure 2.24: Comparison of difference in IS coverage [2006-09]



Source: IDD Newsletter, May 2011

The proportion of households using non-iodized salt was greater in rural areas (12%) than in urban areas (5%). The use of iodized salt was high in northeastern States and in States of Delhi, Goa, Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab and all UTs, ranging from 80% to 94% (Figure 2.24). In the States of Karnataka, Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, Orissa, and Jharkhand use of iodized salt was low as compared to other States (Figure 2.25).

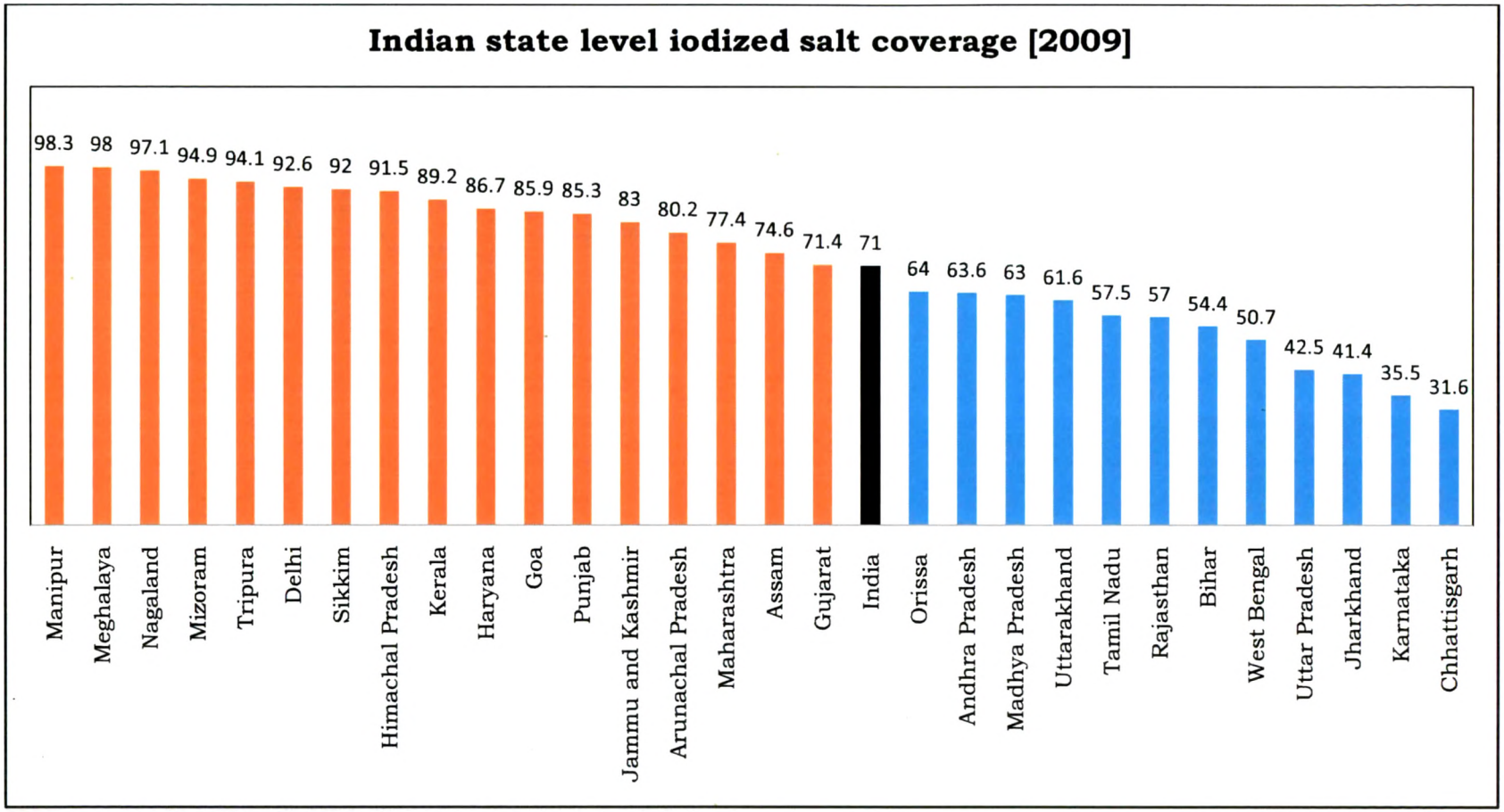


Figure 2.25: State level iodized salt coverage in Indian in 2009

Source: IDD Newsletter, May 2011

Harvesting of salt

Figure 2.26: Glimpses of production of iodized salt in India

The results of the UNICEF CES 2009 shows that tremendous progress was made towards achieving USI in India in recent years. In the last national level survey conducted in 2005-06 (National Family Health Survey 3) the consumption of adequately iodized salt at household level was only 51%. No increase in iodized salt coverage was seen between the two national level surveys in 1998-99 (NFHS 2) and 2005-2006 (NFHS 3). The stagnation in the household level coverage of adequately iodized salt between 1998 and 2006 was primarily because of the lifting of the ban on the sale of non-iodized salt in India in year 2000.

The remarkable progress made in iodized salt coverage in the country was driven by a multitude of factors and by bringing together all stakeholders of USI at National and State level.

The results of the UNICEF CES (2009) survey are extremely encouraging and with further acceleration of the efforts to eliminate IDD in India, the country may achieve Universal Salt Iodization (USI) soon. Keys to achieving USI in India is better understanding of changing consumer preferences, and the trend towards an increasing share of refined/branded/packaged edible salt in India.

The Indian salt industry has made rapid progress over last decade with an increase in both quantity and quality of iodized salt produced in the country. In year 2008-2009, for the first time, the iodized salt production (5.37 million MT in year 2008-09) was greater than the national requirement of edible salt (5.2 million MT) (IDD Newsletter, May 2011). There has been a trend in recent years towards an increasing share of refined salt and packaged in edible salt category. The share of refined edible salt in total salt production in India is increasing by approximately10% annually (currently approximately 50% of salt production in India consists of refined salt).

2.10 IODINE DEFICIENCY

Iodine deficiency (ID) used to be a major public health problem. It is the leading cause of mental retardation during childhood. Concerted

international action taken since 1990 has aimed at the sustainable elimination of IDDs using salt iodization as main strategy (WHO/ UNICEF/ICCIDDD, 1996).

GLOBAL progress

A worldwide effort has dramatically raised the proportion of people consuming iodized salt from less than 20% in 1990 to about 70% by 2000. Thirty-four countries have achieved the elimination of iodine deficiency through universal salt iodization. By 2006, more than 120 countries were implementing salt iodization programmes, an increase of one third in just six years compared to the 90 countries with such programmes in 2000 (UNICEF, 2008).

Sustainable elimination of iodine deficiency

- Countries that have met the goal-34
- Countries on track -38
- Countries declining or lagging-24
- Countries with low coverage (<20%) and no progress-12

NATIONAL progress

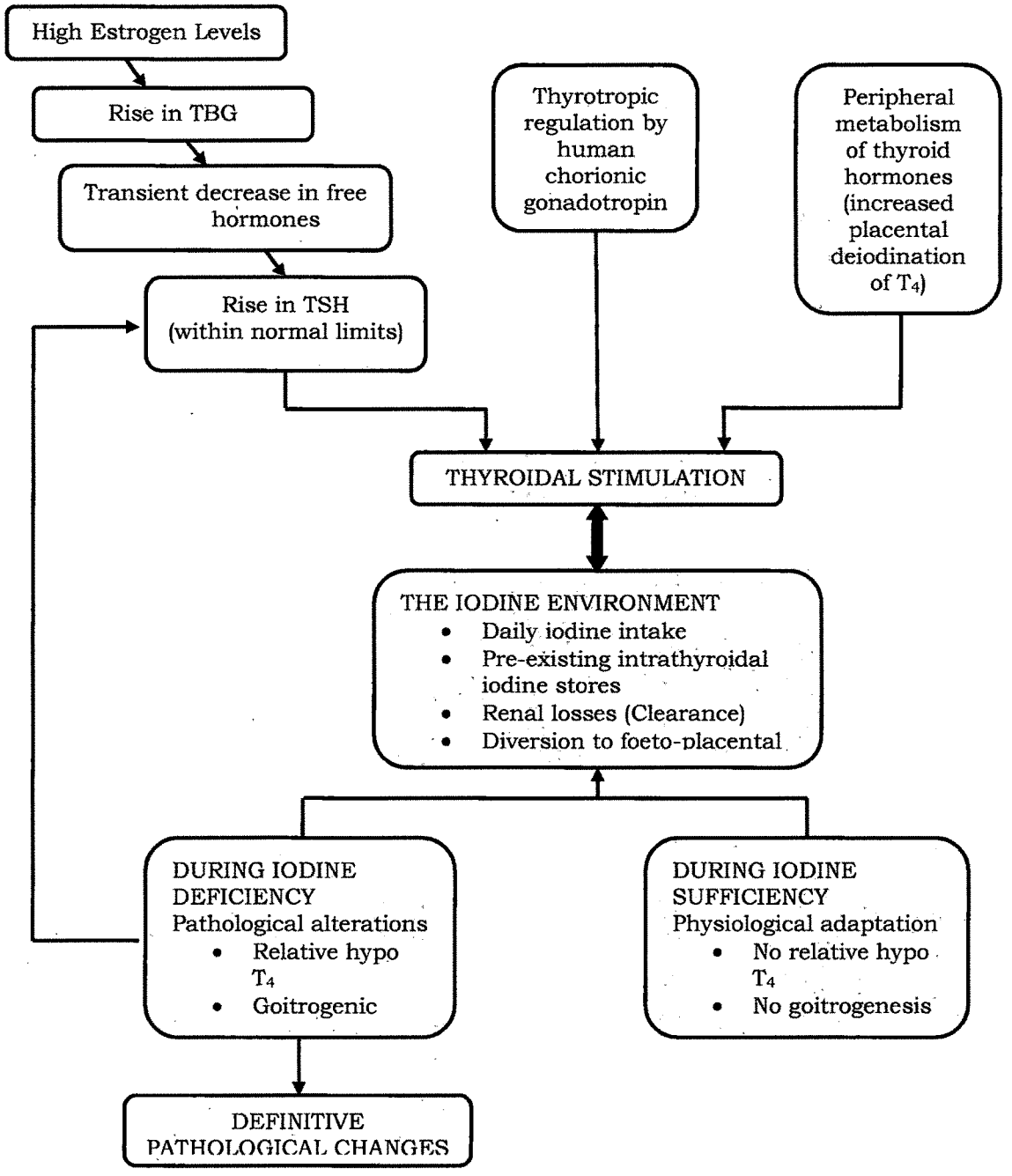
Since the United Nations Special Session on Children in 2002, many countries have reported continued progress towards universal salt iodization. However, some countries have made little tangible progress. In 2006, UNICEF identified 16 'make or break' countries that need additional support to accelerate their efforts. These are major salt-producing countries with high numbers of unprotected newborns; low levels of salt iodization, and a need for special advocacy and technical support to renew national IDD elimination programmes. If these 16 countries achieve universal salt iodization, the global average of households consuming adequately iodized salt will be about 85%. These countries are India, Pakistan, China, Russian Federation, Ethiopia, Indonesia, Ukraine, Philippines, Sudan, Bangladesh, Afghanistan, Egypt, Ghana, Angola, Niger and Senegal (UNICEF, 2008).

2.11 PREGNANCY AND IODINE DEFICIENCY

Physiologic adaptation of the thyroidal economy associated with normal pregnancy is replaced by pathologic changes when pregnancy

takes place in conditions with iodine deficiency or even only mild iodine restriction.

Figure 2.27: From physiological adaptation to pathological alterations of the thyroidal economy during pregnancy



Source: Glinoer, 1997*

Globally, the changes in maternal thyroid function that occur during gestation can be viewed as a mathematical fraction, with hormone requirements in the numerator and the availability of iodine in the denominator. When availability of iodine becomes deficient during gestation, at a time when thyroid hormone requirements are increased, this situation presents an additional challenge to the maternal thyroid (Glinoer, 1997*). Figure 2.27 illustrates the steps through which pregnancy induces a specific challenge for the thyroid gland and the profound difference between glandular adaptation in conditions with iodine sufficiency or deficiency.

Thus during pregnancy, the physiologic changes that take place in maternal thyroid economy lead to an increase in thyroid hormone production of ~50% above preconception baseline hormone production. In order to achieve the necessary increment in hormone production, the iodine intake needs to be increased during early pregnancy.

Metabolism of iodine during normal pregnancy

After reduction to iodide, dietary iodine is rapidly absorbed from the gut. Then, iodide of dietary origin mixes rapidly with iodide resulting from the peripheral catabolism of thyroid hormones and iodothyronines by deiodination, and together they constitute the extra-thyroidal pool of inorganic iodide (PII). This pool is in a dynamic equilibrium with two main organs, the thyroid gland and the kidneys.

A normal adult utilizes ~80 μg of iodide to produce thyroid hormones (TH) and the system is balanced to fulfill these daily needs. When the iodine intake is adequate (150 μg/day) in non-pregnant conditions, a kinetic balance is achieved with a 35% uptake of the available iodine by the thyroid (Panel A Figure 2.28). From the 80 μg of hormonal iodide produced each day by TH catabolism, 15 μg of iodide is lost in feces, leaving 65 μg to be redistributed between the thyroid

compartment (hence, providing 25 µg for daily TH production) and irreversible urinary losses. In such conditions, the metabolic balance is in equilibrium, with 150 µg of iodide 'in' and the same amount 'out', and 80 µg available for daily hormone production. Thus, with an iodine intake level of 150 µg/day (or above) in non-pregnant healthy adults, the system is able to maintain plentiful intra-thyroidal stores, in the order of 15-20 mg of iodine.

In contrast, when the iodine intake level is restricted to only 70 µg/day, the system must up-regulate the glandular iodine trapping mechanisms and increase the relative iodine intake to 50% (Panel B Figure 2.28). The higher uptake allows to recover 35 µg of iodine from dietary intake and 33 µg from TH catabolism but, in these conditions in a non-pregnant healthy adult, this is no longer strictly sufficient to sustain requirements for the production of TH, since 80 µg of iodide is still required daily. To compensate for the missing amount (i.e. ~10-20 µg), the system must use the iodine that is stored in the gland, which therefore becomes progressively depleted to lower levels (~2-5 mg of stable iodine). Over time, if the nutritional situation remains unchanged and despite some adaptation of urinary iodine losses, the metabolic balance becomes negative. The thyroid gland tries to adapt by an increased uptake, glandular hypertrophy, and a higher setting of the pituitary thyrostat.

During pregnancy, two fundamental changes take place. There is a significant increase in the renal iodide clearance (by ~1.3 to ~1.5 fold) and, concomitantly, a sustained increment in TH production requirements (by ~1.5 fold), corresponding to increased iodine requirements, from 80 to 120 µg iodide/day. Since the renal iodide clearance already increases in the first weeks of gestation and persists thereafter, this constitutes a non-avoidable urinary iodine loss, which tends to lower circulating PII levels and, in turn, induce a

compensatory increase in the thyroidal clearance of iodide. These mechanisms underline the increased physiologic thyroidal activity during pregnancy. Panel C in Figure 2.28 indicates that when the daily iodine intake is only 70 μg during pregnancy, despite an increase in glandular uptake to 60%, the equilibrium becomes more or less rapidly unbalanced, since the iodide entry resulting from both uptake and recycling is insufficient to fulfill the increased requirements for TH production.

Calculations show that in such conditions, ~20 μg of iodine are missing daily and, in order to sustain TH production, the glandular machinery must draw from already depleted intra-thyroidal iodine stores. Thus in about one trimester after conception, the already low intra-thyroidal iodine stores become even more depleted and, when iodine deprivation prevails during the first half, it tends to become more severe with the progression of gestation to its final stages.

A second mechanism of iodine deprivation for the mother occurs later in gestation, from the passage of a part of the available iodine from maternal circulation to the fetal-placental unit. The extent of iodine passage has not yet been precisely established. At mid-gestation, the fetal thyroid gland has already started to produce TH, indispensable for the adequate development of the fetus.

In summary, augmentation of iodine trapping is the fundamental mechanism by which the thyroid adapts to changes in the iodine supply, and such mechanism is the key to understanding thyroidal adaptation to iodine deficiency. During pregnancy, increased hormone requirements and iodine losses alter the preconception steady-state. When the iodine supply is restricted (or more severely deficient), pregnancy triggers a vicious circle that leads to excessive glandular stimulation (Glinoer, 1999).

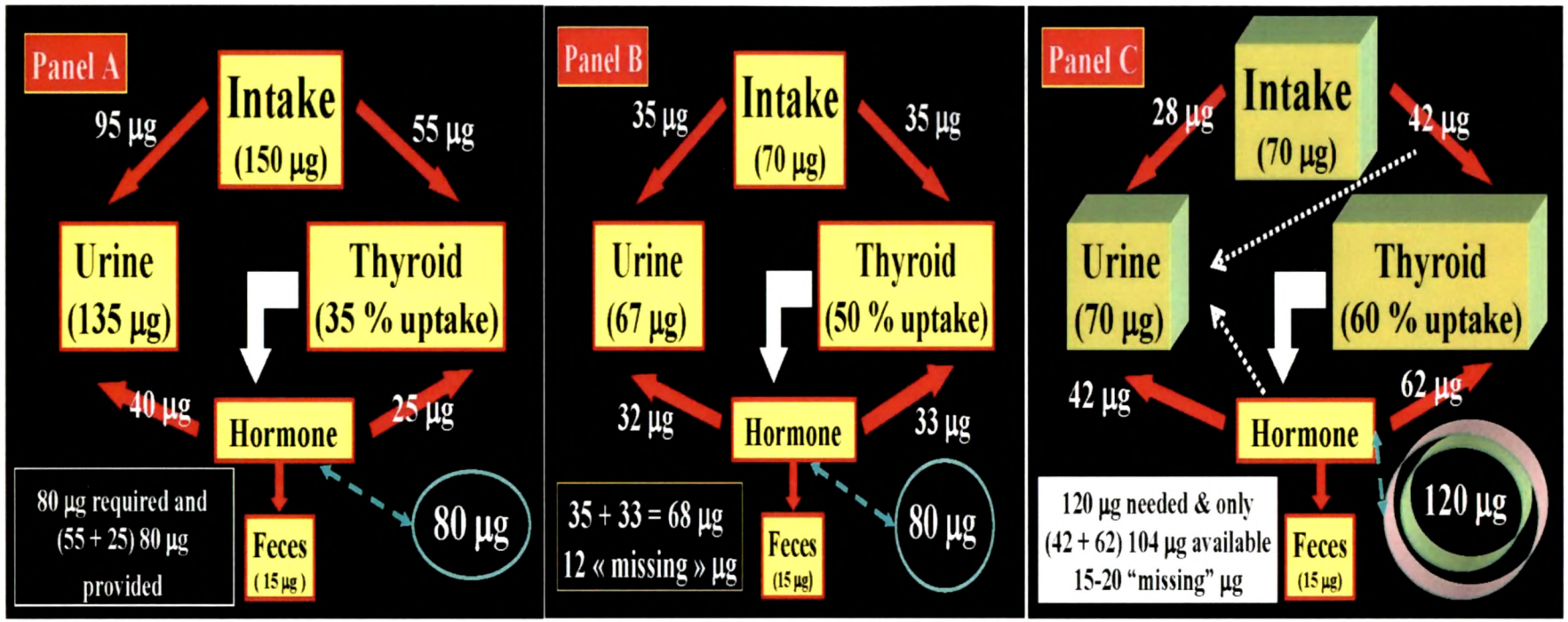


Figure 2.28: Schematic representation of the kinetics of iodide in healthy non-pregnant and pregnant adults

Panel A: non-pregnant adult with an adequate iodine intake of 150 µg/day. Panel B: non-pregnant adult with a restricted iodine intake, corresponding to 70 µg/day. Panel C: the latter condition is compared with an identically restricted level of iodine intake (i.e. 70 µg/day) in a pregnant woman. Daily TH production was set at 80 µg of iodine/day (in non-pregnant) and increased by 1.5-fold to 120 µg/day during pregnancy

Source: Glinoer, 1999

Iodine nutrition of pregnant women in India

Very limited data is available regarding the iodine nutritional status of pregnant women in India. Review (systematic review) of published literature assessing the iodine nutrition status of pregnant women in India clearly shows a significant iodine deficiency in pregnant women (Yadav et al, 2012).. There is a need to conduct national level representative survey to better quantify the iodine nutrition of pregnant women in India.

Even in countries that have achieved iodine sufficiency, the status of iodine nutrition in pregnant and lactating women may still be inadequate. For example, in the United States of America, where the status of iodine nutrition is adequate in the general population, with a median UI concentration of 145 µg/L, 6.7% of pregnant women are nevertheless affected by moderate to severe ID and have a UI concentration below 50 µg/L. This is probably largely due to the fact that women are recommended to limit their intake of salt during pregnancy, which includes iodized salt, but also because of the metabolic changes that occur during pregnancy and lactation that result in an increased requirement for iodine (Beckers and Reinwein, 1991). Yet pregnant women are the most sensitive group in the population to the effects of ID, maternal hypothyroxinemia due to ID occurring early during gestation, even before the onset of fetal thyroid function, is the cause of irreversible brain damage in the fetus resulting in mental deficiency in the offspring (Zoller, 2003).

Hence, iodine status of the pregnant women needs to be addressed on priority.

Iodine requirements during pregnancy, lactation and Infancy: new recommendations

The thyroid economy undergoes a series of metabolic changes during pregnancy and lactation (Glinoer, 1997*). One of the factors involved in these changes is the increased requirement of iodine in the mother due to the transfer of thyroxine (T4) and of iodide from mother to fetus during pregnancy and to the loss of iodide in breast milk during

lactation. These two processes are required in order to ensure normal brain development and prevention of mental retardation in the offspring (de Escobar et al, 2000; IOM, 2001). Because of these factors, the recommended dietary intake of iodine during pregnancy is higher than the value of 150 µg/day recommended for non-pregnant adults and adolescents (WHO/UNICEF/ICCIDD, 2001; IOM, 2001) (Table 2.10). Below this critical threshold of 150µg/day, the iodine balance is negative during pregnancy (Dworkin et al, 1966).

Table 2.10: Iodine requirements during pregnancy, lactation and neonatal period

Age or Population group	IOM EAR	AI or RDA	Age or Population group	WHO RNI
Infant 0-12 m	-	110-130	Children 0-5 y	90
Children 1-8 y	65	90	Children 6-12 y	120
Children 9-13 y	73	120		
Adults > 14 y	95	150	Adults >12 y	150
Pregnancy	160	220	Pregnancy	250
Lactation	200	290	Lactation	250

Source: Marwah and Gopalkrishnan, 2011

2.12 METHODS TO ASSESS IODINE STATUS

Four methods are generally recommended for assessment of iodine nutrition in populations: urinary iodine concentration (UI), the goitre rate, serum TSH, and serum Tg. These indicators are complementary, in that UI is a sensitive indicator of recent iodine intake (days) and Tg shows an intermediate response (weeks to months), whereas changes in the goitre rate reflect long-term iodine nutrition (months to years).

Thyroid size

Two methods are available for measuring goitre:

1. Neck inspection and palpation
2. Thyroid ultrasonography

By palpation, a thyroid is considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the subject being examined.

In the classification system of WHO (2007):

- Grade 0 is defined as a thyroid that is not palpable or visible
- Grade 1 is a goitre that is palpable but not visible when the neck is in the normal position (i.e., the thyroid is not visibly enlarged)
- Grade 2 goitre is a thyroid that is clearly visible when the neck is in a normal position.

Urinary iodine concentration

More than 90% of dietary iodine eventually appears in the urine therefore urinary iodine (UI) is an excellent indicator of recent iodine intake. UI can be expressed as a concentration (mg/l), in relationship to creatinine excretion (mg iodine/g creatinine), or as 24-h excretion (mg /day).

For populations, because it is impractical to collect 24-h samples in field studies, UI can be measured in spot urine specimens from a representative sample of the target group and expressed as the median, in mg/L. Although the median UI does not provide direct information on thyroid function, a low value suggests that a population is at higher risk of developing thyroid disorders.

There is no information about iodine nutrition for pregnant and lactating women in the WHO assessment table, and the upper limits of the median UI for lactating women and children less than two years of age were not specified (Table 2.11).

1. The term excessive means in excess of the amount needed to prevent and control iodine deficiency.
2. In lactating women, the numbers for median UI are lower than the iodine requirements because of the iodine excreted in breast milk.

However, the median UI is often misinterpreted. Individual iodine intakes and, therefore, spot UIs are highly variable from day to day and a common mistake is to assume that all subjects with a spot UI less than 100 µg/L are iodine deficient. Daily iodine intake for

population estimates can be extrapolated from UI, using estimates of mean 24-h urine volume and assuming an average iodine bioavailability of 92% using the formula: urinary iodine (µg/L) x 0.0235 x body weight (kg) = daily iodine intake. Using this formula, a median UI of 100µg/l corresponds roughly to an average daily intake of 150 µg.

Table 2.11: Epidemiological criteria from the WHO for assessment of iodine nutrition in a population based on median or range of UI

UI	Iodine intake	Iodine Nutrition
School-aged children		
<20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Optimum
200-299	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible group
>300	Excessive	Risk of adverse health consequences

Pregnant women		Lactating women		Children <2 yr of age	
<150	Insufficient	<100	Insufficient	<100	Insufficient
150-249	Adequate	≥100	Adequate	≥100	Adequate
250-499	More than adequate				
≥500	Excessive				

Source: WHO/UNICEF/ICCIDD, 2007

Thyroid stimulating hormone

Serum TSH is determined mainly by the level of circulating thyroid hormone, which in turn reflects iodine intake, TSH can be used as an indicator of iodine nutrition. However, in older children and adults, although serum TSH may be slightly increased by iodine deficiency, values often remain within the normal range. TSH is therefore a relatively insensitive indicator of iodine nutrition in adults. In contrast, TSH is a sensitive indicator of iodine status in the newborn period.

Thyroglobulin

Tg is synthesized only in the thyroid and is the most abundant intra thyroidal protein. In iodine sufficiency, small amounts of Tg are secreted into the circulation, and serum Tg is normally less than 10 µg/L. In areas of endemic goitre, serum Tg increases due to greater thyroid cell mass and TSH stimulation. Serum Tg is well correlated with the severity of iodine deficiency as measured by UI. Commercially available assays measure serum Tg, which requires vein puncture, centrifugation, and frozen sample transport, which may be difficult in remote areas.

Thyroid hormone concentrations

In contrast, thyroid hormone concentrations are poor indicators of iodine status. In iodine-deficient populations, serum T3 increases or remains unchanged, and serum T4 usually decreases. However, these changes are often within the normal range, and the overlap with iodine-sufficient population is large enough to make thyroid hormone levels an insensitive measure of iodine nutrition

Assessment during pregnancy

According to the WHO/ICCIDD/UNICEF for evaluating the status of iodine nutrition in pregnant women, evaluating the median urinary iodine concentration (UI) is recommended (WHO/UNICEF/ICCIDD, 2007). The recommended daily iodine intake can be used to extrapolate the expected UI in µg/L. This assumes the median 24-h urine volume for girls in the age group of 7-15 years to be 0.9 ml/h/kg; for adult women to be 1.5 L; and the mean iodine bioavailability to be 92%. Using this model, the UI during pregnancy is derived to be approximately 135-150 µg/L, corresponding to the recommended daily iodine intake of 220-250 µg for the period of pregnancy. It may also be necessary to take into account the variations in age of pregnant women, particularly in developing countries, where adolescent pregnancy in not uncommon. For

example, for a15-yr-old weighing 50 kg, the UI value comes about to be approximately 185-215µg/L, considering the recommended daily intake of 220-250 µg. However, given the physiological alterations during pregnancy, an increase in the glomerular filtration rate and possibly renal iodine clearance (RIC), this estimation of UI may stand less valid. These uncertainties were reflected in the recent WHO expert group report, which recommended an adequate iodine intake in pregnancy to be 150-249 µg, and suggested that the UI be extrapolated from this value.

Assessing status during lactation

Since the mammary gland is able to concentrate iodine, iodine supply to the newborn via the breast milk may be maintained even in the face of maternal iodine deficiency. Iodine supply to the infant may be maintained even in cases of maternal iodine deficiency because of the ability of the mammary glands to concentrate iodine. This may explain the higher values of BMICs than expected (based on UI) that are observed in lactating mothers in areas of iodine deficiency. The full-term infant's iodine requirement is approximately 7µg/kg body weight.

Assuming that the iodine in breast milk is 95% absorbed, and the mean breast milk excretion of iodine is 0.78L in the first six months post-childbirth, the minimum BMIC required to fulfill the infant's iodine requirement (of 50µg/day) is 80µg/L until introduction of food. Although maternal iodine requirement during lactation is high (200-290 µg/day), the median UI that indicates adequate iodine nutrition status in a lactating woman is the same as that of a non-pregnant, non-lactating woman, keeping in mind the amount of iodine lost in breast milk (WHO/UNICEF/ICCIDD, 2007).

Assessing status during infancy

WHO recommendations state, a median UI of atleast 100µg/L in infants is sufficient. At the same time, they recommend an iodine intake of 90µg/d during infancy and suggest extrapolating from this

to a median UI assuming a urine volume of 300–500 ml/d, but this would produce a higher cut-off of at least 180 μg/L (Zimmerman, 2009*).

2.13 IODINE DEFICIENCY, THYROID DYSFUNCTION AND INFANT DEVELOPMENT

In areas of iodine sufficiency, healthy women maintain iodine stores of 15–20 mg in the thyroid. During pregnancy, to help meet the approximately 50% increase in maternal iodine requirements; women may draw on this significant iodine store (Zimmerman, 2009*). In areas of chronic iodine deficiency, women enter pregnancy with already depleted iodine stores. With little thyroidal iodine to draw on to meet the increased maternal iodine requirement, pathological changes goiter and hypothyroidism may occur that can adversely affect maternal and fetal health.

Whether mild-to-moderate maternal iodine deficiency causes more subtle impairment of cognitive and/or neurological function in the offspring is uncertain. Two case-control studies in iodine-sufficient women with mild thyroid hypofunction have reported developmental impairment in their offspring. In the United States (Haddow et al, 1999), the IQ scores of 7- to 9-yr-old children of mothers with subclinical hypothyroidism during pregnancy (an increased TSH in the second trimester) were 7 points lower compared with children from mothers with normal thyroid function during pregnancy. In The Netherlands (Pop et al, 1999), infant development to 2 yr was impaired in children of women with a free T_4 (FT4) below the 10th percentile at 12 wk gestation. These studies suggest that cognitive deficits may occur in the offspring even if maternal hypothyroidism is mild and asymptomatic. However, the maternal thyroid dysfunction in these studies was presumably not due to iodine deficiency because they were done in iodine-sufficient populations. Table 2.12 gives psycho-neurological outcome in the progeny associated with maternal hypothyroidism due to iodine deficiency.

Table 2.12: Neuropsychiatric and intellectual deficits in infants and schoolchildren born to mothers residing in conditions with mild to moderate iodine deficiency

REGION	TESTS	MAIN FINDINGS	AUTHOR
Spain	Locally adapted: - BAYLEY - McCARTHY - CATTELL	Lower psychomotor and mental development	Bleichrodt (1989)
Italy (Sicily)	BENDER-GESTALT	Low perceptual integrative motor ability & neuromuscular and neuro-sensorial abnormalities	Vermiglio (1990)
Italy (Tuscany)	WECHSLER RAVEN	Low verbal IQ, perception, motor and attentive functions	Fenzi (1990)
Italy (Tuscany)	WISC Reaction time	Lower velocity of motor response to visual stimuli	Vitti (1992) Aghini Lombardi (1995)
India	**Verbal, pictorial learning tests, Tests of motivation**	**Lower learning capacity**	**Tiwari (1996)**
Iran	BENDER-GESTALT RAVEN	Retardation in psychomotor development	Azizi (1993)

Source: Glinoer and Delange, 2000

Infant development testing scales (Spreen and Strauss, 1998)

The Cattell Infant Intelligence Scale- The Cattell was designed to assess infants and toddlers from 2 through 30 months old.

The Standard Raven Progressive Matrices- Drawing on Spearman's theory of general ability consists of 60 matrix problems, which are

separated into five sets of 12 designs each. Within each set of 12, the problems become increasingly difficult. Each individual design has a missing piece. The participant's task is to select the correct piece to complete the design from among six to eight alternatives. Correct responses are based on various organizing principles, such as increasing size, reduced or increased complexity, and number of elements. The SPM uses nonverbal stimuli, and it is assumed that it does not require a specific knowledge base.

The Wechsler Intelligence Scale for Children- This test is an individually administered clinical instrument for assessing the intellectual abilities of children aged 6 years through 16 years, 11 months. The instrument consists of three main composite scores: Verbal IQ (comprising Information, Similarities, Arithmetic, Vocabulary, Comprehension, and Digit Span subtests), Performance IQ (comprising Picture Completion, Coding, Picture Arrangement, Block Design, Object Assembly, Symbol Search, and Mazessubtests), and Full Scale IQ.

The Denver Developmental Screening Test- The Denver Developmental Screening Test is one of the most popular developmental screening tests (with an age range of 1 month to 6 years). The driving factor of the Denver is its brevity-it takes 15-20 minutes to administer. The content of the test includes personal-social, motor, language, and adaptive domains. The scoring is based on parent reports, direct child assessment, and observation. The assessment results expressed by a single score assigning the child to one of the four descriptive categories: Pass, Questionable, Abnormal, or Un-testable.

The McCarthy Scales of Children's Abilities- The McCarthy Scales of Children's Abilities form a well standardized and psychometrically sound measure of the cognitive abilities of young children (ages 2 1/2 to 8 1/2 years). The test is individually administered and takes about 45 to 60 minutes to administer, depending on the age of the child. The McCarthy Scales have some unique features valuable for the assessment of young children with learning problems or other

exceptionalities. The test produces a general measure of intellectual functioning called the General Cognitive Index (GCI), as well as a profile of abilities that includes measures of verbal ability, nonverbal reasoning ability, number aptitude, short-term memory, coordination, and hand dominance.

Bayley Scales of Infant Development- This scale is arguably the most widely used measure of the development of infants and toddlers. In addition, the BSID has an extensive psychometric history and a very respectable track record. The BSID-II is applicable to children from 1 through 42 months of age. The administration takes about 25 to 35 minutes for infants under 15 months of age and up to 60 minutes for children over 15 months.

Baroda Development Screening Tests for Infants (BDSTI) (Pathak and Khurana, 1991)

A screening test for the assessment of the motor-mental development of infants was developed by selecting items from the Bayley Scales of Infant Development (BSID). Many items of the BSID use standardized equipment (cubes, pegboard, form-board, etc.) and standardized techniques (certain performances timed by stop-watch). The age-placements cannot be presumed to be the same when non-standardized tools and techniques are used. For instance, it was reported that the size, weight and surface of the cubes affects the performance of the child in building a tower of cubes and using any cubes or boxes is wrong. The BSID is a detailed test and has many items for testing the development of one skill-some of them very close in the developmental sequence. For instance, there are ten items related to the skill of sitting. Items like: sits with support, effort to sit, sits alone momentarily; sits alone 30 sec or more and sits down require some experience and good judgment on the part of the health worker. For instance one must be sure that the child sat down and did not slump down.

Table 2.13: Screening test items (BDSTI)

Age group	Sr. No.	Items
1	1	Arms and legs thirst in play *
	2	Momentary regard
	3	Lateral head movement (prone) *
2	4	Responds to sound
	5	Follows moving person
	6	Free inspection of surroundings
3	7	Social smile/vocalizes
	8	Eye co-ordination
	9	Head erect and steady*
4	10	Holds head steady*
	11	Recognizes mother
	12	Elevates on arms*
5	13	Play with rattle/hand play
	14	Reaches for dangling ring
	15	Sits with slight support*
6	16	Turns head to sound
	17	Turns from back to side*
	18	Exploitive paper play
7	19	Discriminates strangers
	20	Pulls to sit*
8	21	Bangs to play
	22	Sits alone steadily*
9	23	Retails two things in two hands
	24	Pulls to stand*
	25	Playful response to mirror images
	26	Sits with good coordination*
10	27	Pulls string-secures toy
	28	Co-operates in play
	29	Crawling (pre-walking) *
11	30	Rings bell purposefully
	31	Fine prehension*
	32	Raises to sit*
	33	Stands by furniture*
12	34	Adjusts to words
	35	Says da-da

13-15	36	Inhibits on command
	37	Midline skills *
	38	Walks with help*
	39	Turns pages
16-18	40	Imitates words
	41	Stands alone*
	42	Spontaneous scribble
	43	Throws ball*
	44	Aufstein I*
	45	Walks alone*
	46	Gestures for wants*
19-24	47	Shows shoes, etc.
	48	Two words
	49	Walks up and down stairs with* help
	50	Words for wants
25-30	51	Two word sentences
	52	Name three objects
	53	Stands on one foot*
	54	Walks up and down stairs without help*

Source: Pathak and Khurana, 1991 *Motor scale items

Only those items which were simple and easy to administer and to assess and not requiring any special training, experience and equipment were selected. Further duplication of items with similar age-placement was avoided. While some items of similar nature like eye co-ordination were grouped together. In this way a total of 54 items 22 motor and 32 mental were selected for the screening test (Table 2.13).

2.14 IRON DEFICIENCY DURING PREGNANCY

The overall iron requirement during pregnancy is significantly greater than that in the non-pregnant state despite the temporary iron losses incurred during menstruation.

Iron requirements during pregnancy

If the demand for iron were spread evenly throughout gestation, iron requirements could be met more easily by a sustained rise in the rate

of iron absorption. The need for iron, however, varies markedly during each trimester of pregnancy.

First trimester: Iron requirements decrease during the first trimester because menstruation stops, which represents a median saving of 0.56mg Fe/d (160 mg/pregnancy) (Hallberg and Rossander-Hulten, 1991). The only iron losses that must be met during this period are the obligatory ones from the body via the gut, skin, and urine, which accounts to ~0.8 mg/d in a 55-kg woman (14g/kg/d or 230 mg/pregnancy) (Green et al, 1968). Early hemodynamic changes include generalized vasodilation, some increase in the plasma volume, and an increase in red blood cell 2, 3- diphosphoglycerate concentrations (Hallberg and Hulten, 1996). There is also some evidence that erythropoietic activity may be reduced during this period, with a slight reduction in red blood cell mass (Taylor and Lind, 1979), a reduction in the number of reticulocytes, and a rise in the serum ferritin concentration (Kaufer and Casanueva, 1990).

Second Trimester: During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder of pregnancy. The increase in oxygen consumption by both mother and fetus is associated with major hematologic changes.

Most studies in women supplemented with iron show a change in total blood volume of ~45%, with an increase in plasma volume of ~50% and an increase in red blood cell mass of ~35%. The rise in hemoglobin mass is similar at ~30% (de Leeuw et al, 1966). There has been some difficulty in establishing the normal hemoglobin concentration in pregnancy because of both the disproportionate increases in the plasma volume and the frequent occurrence of iron deficiency anemia..

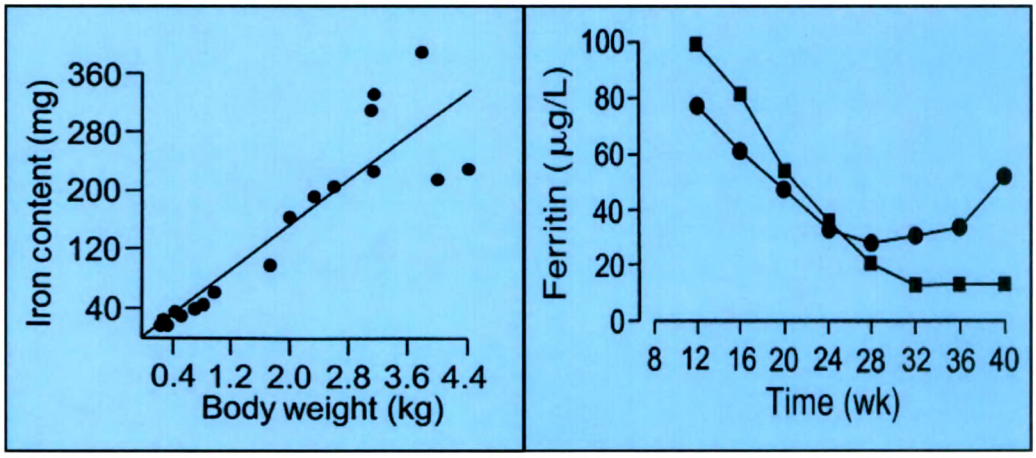
In studies in which iron deficiency was prevented, however, the average decrease in hemoglobin concentration appeared to be <10g/L (Bonner and Goldberg, 1969). Translated into iron requirements, the increase in red blood cell mass that occurs in a normal pregnancy

amounts to ~450 mg Fe in a 55-kg woman (de Leeuw et al, 1966). Whereas this represents a significant drain during the later part of the pregnancy, it does not affect long-term iron balance because the iron is returned to the body's stores at the end of pregnancy, when the red blood cell volume gradually reverts to normal.

Third Trimester: As pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most of the iron accumulating during the third trimester (Figure 2.29a). The average iron content of a fetus weighing >3kg is ~270mg (Widdowson and Spray, 1951).

In determining iron requirements during pregnancy, the losses incurred during parturition must also be added. These include an average maternal blood loss equivalent to 150 mg Fe and a further 90 mg present in the placenta and umbilical cord.

Figure 2.29: Left (a)- Relation between body weight and body iron content in the fetus and newborn child (de Leeuw et al, 1966) & Right (b)- Effects of iron supplementation on serum ferritin concentration in pregnancy (Fenton et al, 1977)



[Supplemented group- ● , un-supplemented group- ■]

Period after delivery: In the period after delivery, there is a small additional iron loss of ~0.3mg/d through lactation (Fransson and Lonnerdal, 1980), but this is offset by the absence of menstruation, except when breast-feeding is continued long after the return of menstruation.

To summaries, the total iron requirement during pregnancy for a 55-kg woman is ~1,040 mg (Table 2.14). At delivery, there is a further loss of maternal blood, which raises the total cost of pregnancy to ~1,190 mg iron. The net cost, however, is only 580 mg because the iron used to increase the red blood cell mass is returned to stores and overall losses are further offset by the absence of menstruation during pregnancy.

Table 2.14: Iron requirements in pregnancy

Total cost of pregnancy	**Amount (mg)**
Fetus	270
Placenta	90
Expansion of red blood cell mass	450
Obligatory basal losses	230
Sum	1040
Maternal blood loss at delivery	150
Total cost	1190
Net cost of pregnancy	
Contraction of maternal red blood cell mass	-450
Absence of menstruation during pregnancy	-160
Subtotal	-610
Net cost	580

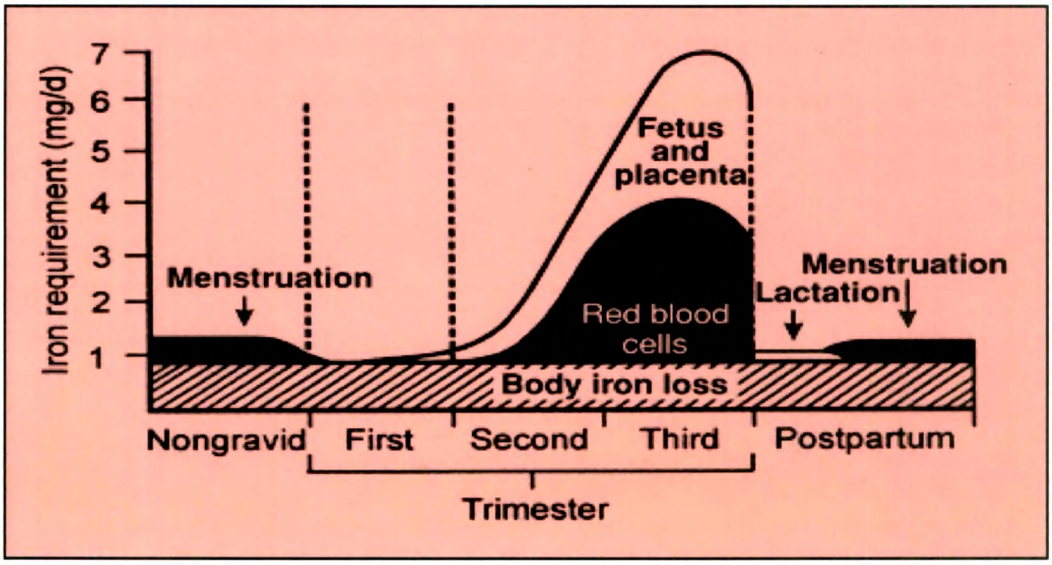
Source: Bothwell, 2000

Iron balance in pregnancy

When total iron requirements during pregnancy are translated into increased daily needs, it is apparent that there is an unequal distribution over time (Figure 2.30). Although reduced during the first trimester, iron requirements rise to between 4 and 6 mg in the second and third trimesters, respectively (FAO, 1988). Because major changes in the red blood cell mass start occurring only in the middle of the second trimester (Lund and Donovan, 1967), iron requirements may reach as much as 10 mg/d during the last 6–8 wk of pregnancy (Hallberg, 1992).

Irrespective of the exact value, it is apparent that daily iron requirements cannot be met from dietary absorption alone in the latter part of pregnancy, even from the most optimal diet. In diets containing large quantities of bio-available iron diets in which there are generous quantities of meat, poultry, fish, and foods containing high amounts of ascorbic acid, overall iron absorption is usually 3–4 mg/d and, at most, 5 mg/d. The amount of iron absorbed is much lower when the diet contains only small amounts of bio-available iron (FAO, 1988), as is often the case in many developing countries where the staple food is cereal and the intake of meat and ascorbic acid is limited.

Figure 2.30: Estimated daily iron requirements during pregnancy in a 55-kg woman



Source: Bothwell, 2000

Importance of iron stores

Iron balance can be maintained in pregnancy only when there are adequate iron stores at the start of pregnancy. If a woman routinely eats a diet high in bio-available iron, a pre-pregnancy iron store of 300 mg is probably sufficient to carry her through pregnancy, although a higher amount of stored iron is needed when the diet is less than optimal.

Assessment of iron status in pregnancy

Assessing iron status during pregnancy is filled with difficulties because the profound hemodynamic changes associated with pregnancy affect several indices of iron status. During pregnancy, hemo-dilution leads to a reduced hemoglobin concentration, whereas both serum iron and ferritin concentrations decrease and total iron-binding capacity increase (Fenton et al, 1977).

The relative contributions of pregnancy per se and a pregnancy induced negative iron balance in bringing about these changes can be assessed by measuring the changes in hemoglobin, serum iron, serum ferritin, and total iron-binding capacity that occur during pregnancy in women rendered iron replete after adequate iron supplementation during pregnancy (Fenton et al, 1977). The disproportionate increase in plasma volume during pregnancy leads to a drop in the hemoglobin concentration of <10g/L. Although hemoglobin concentrations <110 g/L have been occasionally reported in iron-replete women, this concentration has proved to be a useful cutoff for defining anemia in pregnancy (FAO, 1988). There is a moderate drop in the concentration of serum iron that stabilizes in the middle of pregnancy (Fenton et al, 1977). More striking than the changes in either hemoglobin or serum iron concentrations is the steady rise in total iron-binding capacity during pregnancy to <50% above normal, which reflects an increase in the concentration of transferrin in plasma (Fenton et al, 1977). As a result, there is a drop in transferrin saturation. As discussed previously, there is some evidence that serum ferritin rises modestly early in pregnancy, presumably because of reduced erythropoietic activity; thus, iron is diverted to stores (Kaufer and Casanueva, 1990). Thereafter, however, the serum ferritin concentration drops steadily to <50% of normal at midterm (Figure 2.29b) (Milman et al, 1991). These changes reflect hemo-dilution and the mobilization of iron from stores to meet the increased demands of pregnancy. It is, therefore, apparent

that all the indexes associated with iron deficiency including hemoglobin, transferrin saturation, and serum ferritin concentrations are reduced during pregnancy even in iron-replete women. In contrast, the concentrations of circulating transferrin receptor have been found to be normal in pregnancy, only being raised if iron deficiency is present. This suggests that serum transferrin receptor concentrations may prove to be a useful tool for diagnosing iron deficiency in pregnancy.

Strategies to combat iron deficiency in pregnancy

Daily supplementation

Iron supplementation regimens in pregnancy vary depending on the characteristics of the population. In developed countries most women enter pregnancy with normal hemoglobin concentrations and variable amounts of stored iron. In contrast, large numbers of women in developing countries are anemic at the onset of pregnancy (WHO, 1992). Prenatal iron supplementation is not compulsory in many industrialized countries and the recommended dose is often small (30 mg ferrous iron daily), but has been as high as 240 mg/d in developing countries. In India (Sood et al, 1975) the World Health Organization (WHO) recommended universal supplementation of all pregnant women with 60 mg ferrous iron twice daily in populations where gestational anemia is common and once daily in populations where overall iron nutrition is better (de Maeyer et al, 1989). This recommendation was subsequently modified to a single daily dose of 60 mg Fe for 6 month in pregnancy or 120 mg Fe if 6 month duration cannot be achieved (Stoltzfus and Dreyfus, 1998). Keeping the dose as low as is compatible with unimpaired effectiveness is an important principle because the side effects of iron therapy, which can seriously limit compliance, are dose-dependent phenomena (Solvell, 1970).

Two strategies that merit consideration are programs to modify dietary habits and iron fortification of foods (Bothwell and MacPhail, 1992). The second has the advantage that it can be applied to large population groups at low cost and the identification and cooperation of deficient or potentially deficient individuals is not a prerequisite, as it is with supplementation.

2.15 DOUBLE FORTIFIED SALT (DFS)

Salt has been the vehicle for the world's most successful food fortification initiative to date - Universal Salt Iodization. The fortification of salt with iodine has been hailed as one of the world's great public health advancements. Now breakthrough technology that allows salt to be double fortified with iron as well as iodine has created an exciting new opportunity to reach the world with supplemental iron easily and inexpensively, without having to change people's habits.

Iodine deficiency is the world's leading cause of preventable intellectual disability

Iron deficiency anemia is the most common and wide-spread nutritional disorder in the world

Iodine and Iron

Previously incompatible in food fortification, they can now be combined through new technology in Double Fortified Salt.

Billions of people are affected by the hidden hunger of micronutrient deficiencies. Double Fortified Salt is an innovative new fortified food product - delivering crucial amounts of iodine and iron to human beings through their diet. Double Fortified Salt presents one of the most cost-effective opportunities to deliver two of the most critical micronutrients for mental capacity, maternal and infant survival and human productivity.

Fortification of common salt with iron has been developed by the National Institute of Nutrition (NIN) as a public health strategy for the

control of IDA on the lines of iodization of salt for the effective control of IDD (Working group report, 1982). However, with the advent of universal iodization of edible salt as a National policy in 1988, NIN undertook research studies aimed at development and testing of double fortified salt (DFS) containing iodine and iron for reducing the deficiencies of both these micronutrients (Narasinga Rao, 1994).

In view of their antagonistic chemical properties, the incorporation of iron and iodine in salt requires a stabilizer. NIN developed a DFS formulation using sodium hexa-metaphosphate (SHMP) as a stabilizer. SHMP is intended to protect iodine and prevent the interaction between the iron and iodine and also with the other constituents of the salt. The stability, bioavailability and acceptability of DFS were determined and found to be good (Narasinga Rao, 1994).

The Micronutrient Initiative (MI) in Canada (MI report, 1999) and a company in Chennai with the trade name of the salt "Nutrisalt" (Rajagopalan and Malvika, 2000) have developed two other formulations of DFS, in which physical separation of iodine was achieved by barrier methods. Parallel to these developments, studies were continued at NIN to explore other formulations. On the suggestion and support from the International Life Sciences Institute (ILSI, Washinton D.C.), NIN also tested other DFS formulations containing sodium ferric EDTA as a source of iron along with Iodine and also SHMP and EDTA as promoters of iron absorption (NIN, 2002). Some formulations were identified as promising second-generation preparations. DFS containing encapsulated iron salt was found to have good stability characteristics and iron bioavailability, even with powdered common crystal salt. Working with powdered common salt reduces the cost of salt but increases the cost of iron incorporation, so that at the final product level there may not be any major difference in cost between these different approaches.

Table 2.15: Comparative Physico-Chemical Features of different DFS Formulations

Characteristics	NIN formulation	MI formulation	Nutrisalt
Clinical constituents	30-40 ppm I, KIO3 or KI Ferrous sulphate SHMP Stabilizer and promoter	50 ppm I, KI–Ferrous fumarate Encapsulation of iodine by dextrin	30 ppm I, KIO3, Iron salt Barrier
Stability	Stable up to 9 months	Stable for 12 months.	Report claims good stability
Acceptability	Full-fledged acceptability described	Not acceptable with some foods	Report claims good acceptability and stability during cooking
Bioavailability of iron and iodine	Demonstrated (Iron absorption 6.1%) Urinary iodine increased like within IS	Demonstrated (Iron absorption variable 13.5%) Urinary iodine equal to IS	Not reported
Pilot scale/ plant scale production tested	Plant scale production	Not tested	Not known

Source: Shivkumar, 2004

Table 2.16: Comparative bio-impact features of different DFS formula (Source: Shivkumar, 2004)

Characteristics	**NIN formulation**	**MI formulation**	**Nutrisalt**
Study Populations	Tribal villages in (AP) single blind, placebo Residential school children, Hyd. Double blind, placebo	Mothers and children in Ghana, double blind, placebo	Tea estate labourers, Valpari, South India Double blind, placebo
De-worming treatment	No treatment	No treatment	Simultaneously de-wormed
Stability of iodine at the Location	Done	Done	Data not given
Impact on iron and iodine Status	Goitre prevalence decreased and urinary iodine improved. Iron status benefited only in some groups of the tribals. Benefited residential school children both in iron status and iodine status. The impact on iron status was mainly on control of anemia.	Maintain good iodine levels in children and mothers. Children showed small reduction in prevalence of anemia with DFS while the prevalence of anemia increased in controls. Mothers too showed improvement with DFS, though the anemia prevalence at baseline was not comparable to control.	Benefited only in females. De-wormingis an important requisite for response.
Cost	About Rs. 4.50/kg	Worked out	Worked out (Rs. 4.50/kg)
Productivity	Not worked out	Not Worked out	Measured Plucking of tea leaves
Safety issues, if any, due to components	Safety of SHMP evaluated in rats as well as in children	Perhaps issues are not involved	Not known, if any safety issues are involved

The NIN also carried out extensive studies on the safety and impact of its DFS (both experimental and community situations) on both iron and iodine statuses (Sivakumar and Nair, 2002). Trials of large-scale production, operational feasibility of distribution and acceptability in the community have been repeatedly demonstrated with the NIN DFS (Table 2.15).

Limited studies dealing with the acceptability and impact were reported on the other two formulations. The MI formulation, though stable, developed an unacceptable colour with some recipes (MI report, 1999). No detailed studies are available on the organoleptic properties of Nutrisalt.

Both the NIN and MI formulations demonstrated a limited, but significant impact on both iron and iodine status of different population groups. Nutrisalt was found to have an impact on iron status (as measured by an increase in blood hemoglobin) at a lower probability than the other formulations of DFS (Rajagopalan and Malavika, 2000) (Table 2.16).

It has to be understood that the impact of DFS on hemoglobin increase will not be very striking since providing iron through DFS is a preventive measure and not a therapeutic one. However, it is a sustainable method of doubling iron intake of one billion plus population for several decades.

Iron fortified Iodized Salt to be promoted to battle malnutrition in the country (www.pmindia.nic.in)

Public health experts from various part of our country have different opinion regarding promotion of double fortified salt (table 2.17). However, our Prime Minister has agreed to promote DFS in ICDS and MDM programmes.

A meeting was held in the Prime Minister's Office on the promotion of consumption of Iron fortified Iodized Salt as a measure to deal with malnutrition in the country. The meeting on 18.4.2011 was chaired by

the Principal Secretary to the Prime Minister and was attended by the officers of Ministries of Health, Women and Child Development, Department of Industrial Policy and Promotion, Director of National Institute of Nutrition and a Member of the Prime Minister's National Council on India's Nutrition Challenges.

Actions on the following lines were agreed upon:

- To begin with, Ministries dealing with food and nutrition programmes like ICDS and Mid-day Meal Programme will make the use of iron fortified iodized salt (double fortified salt) mandatory in those programmes, in an appropriate manner;
- The Dept. of Food and Public Distribution will examine the possibility of supplying DFS through the PDS;
- A major mass media campaign will be taken up to promote the use of iron fortified iodized salt (DFS) by the Ministries of Women and Child Development and Health and Family Welfare;
- The communication campaign and the decision to use DFS in Government programmes needs to be associated with efforts to increase the supply of DFS in the country. The Department of Industrial Policy and Promotion will work with the private industry and cooperatives to promote manufacture of iron fortified iodized salt (DFS). The Department will also explore the possibility of taking up a scheme to promote capital investment and technology up-gradation so that the installed capacity for producing iron fortified iodized salt (DFS) in the country is substantially stepped up.
- The Departments of Health and Family Welfare and Health Research under the Ministry of Health and Family Welfare will take necessary measures for promoting the use of DFS in the country, including advising the Ministries of Women and Child Development, Human Resource Development and Consumer Affairs, Food and Public Distribution on use of DFS in Government programmes.

Table 2.17: Responses from various Public health experts/ scientists towards double fortification of salt

Public Health Expert	Response on use of DFS
***Umesh Kapil (AIIMS, New Delhi)**	DFS developed by NIN needs to be tested under public health conditions to demonstrate its effectiveness both on iodine and iron status. The results of limited controlled field studies undertaken in residential school children do show the effectiveness of the DFS. However, before DFS is introduced as a National Programme or in to a National Programme like ICDS, we need to have effectiveness studies of DFS under public health conditions.
***R. K. Bakshi (GMC, Baroda)**	Experience of even single fortification - Iodized salt utilization has been far from satisfactory. Apparently, there is no reason for its low consumption. Gujarat has followed up the history of low utilization in face of very significant production of the edible salt. A lot of organized effort is on to improve. It has been the priority of the state as well as many international agencies.
***Prakash Kotecha (GMC, Baroda)**	In a culturally diverse country like India, across the country a common food that can be fortified and would work is salt. It is with this background that we are trying to reach out to explore double fortified salt. ICMR has been in to systemic review currently with committees working on this and data are favourable though not satisfactory to absolute scientific standards. We need to generate more data on this and to do this at various levels we will have to try out rather than wait for a green signal from high bodies to start use of DFS at national level. Studies have not very convincingly shown the efficacy but have shown its acceptability and overall recommendation is favourable for its use. More trial to study both efficacy and effectiveness

	and simultaneously acceptability are required. If we need to control anemia effectively supplementation alone will be main approach but by itself would not be enough as global experience documents. Fortified salt is a big hope and let us hope it becomes a reality in near future.
***Subadra Seshadri (MSU, Baroda)**	There are two ways of getting more and better bio available iron through diets. One is dietary diversification, improving access to foods that are good sources of iron and ascorbic acid, for a predominantly vegetarian population. The other is to fortify foods with iron. In India, since salt is universally consumed, salt has been chosen as a vehicle for fortification. As pointed out by others, quantity of iron added to salt is small, 1mg per g. Given the low bioavailability when consumed with typical vegetarian meals of India, we cannot expect the fortified salt to produce any dramatic reduction in anemia prevalence even over a period of a year or two. However, what the iron-fortified salt will do is to add small but useful amounts of iron to our diets daily (because we consume salt every day) and possibly prevent a decline in HB that seems to occur under many conditions in our day-to-day lives. There is some good evidence to support this from the NIN studies. So fortified salt should be considered as one of the several approaches for adding some iron to our diets. This will have to be combined with diet diversification, nutrition awareness promotion and other approaches, for a more lasting solution to the problem of anemia.
***H S Sharma (Consultant, Gurgaon)**	We are discussing only fringe subject like DFS while the malady lies somewhere else. The DFS would be of use when the people get adequate amount of nutrition in terms of carbohydrate and protein. We are going the wrong way why should poverty line be pegged at 1700 K cal in India while it is 3.000 K cal in China?

Public Health Expert	Response on use of DFS
****S Nair & K Joshi (MSU, Baroda)**	Our experiences with fortification are suggestive that, it is highly beneficial in urban as well as rural scenario. Major success remains when we are able to convince the consumers who are using it, especially it is their right to know what deficiency do they have, thus they need to consume the fortified items. We have used Double fortified salt as a replacement from the daily salt though minor, the changes are playing a contributory role when it is used in long term strategies and programmes mainly because of sustained release of Iron and Iodine. Using it in a pregnant mother's diet did not bring any significant change in iron, but contributory roles in Iodine levels were observed. In children, along with dietary counseling and improved food intake, mothers opined that they have become more active and fatigue is not seen. This is suggestive that we need to promote consumer education before introducing the fortificant. It would add more meaning to our contributions.
****B Sesikaran (NIN, Hyderabad)**	Double-fortified salt (DFS) which is fortified with both iron and iodine is identified as a potential public health tool for delivering nutritional iron to population at large. National Institute of Nutrition (NIN), Hyderabad has carried out extensive studies on stability, bioavailability, acceptability, safety and impact (including in community) of DFS. Feasibility both at factory level production and community level implementation have been worked out. It has been tried and distributed through a state school feeding program in the state of Tamil Nadu.
****Deepika Anand (RA, Chhattisgarh)**	Around 50 percent of women in Chhattisgarh were anemic. Iron Fortified Salt Distribution (IFSD) Scheme was launched wherein iron fortified salt was distributed to the beneficiaries through ICDS programme. The results indicated that this salt was beneficial in reducing the level

	of anemia among pregnant women, lactating mothers and under-6 years children. Thus, the programme should be continued regularly without any interruption.
****Mehtab B Bamji (Dangoria Charitable Trust, Hyderabad)**	It is a pity that a promising technology to address a major nutrition problem i.e. iron deficiency, is allowed to languish for some reasons. No one is questioning the need for dietary diversification. However, with the price of vegetables, pulses and even millets, besides animal foods, which are rich sources of iron, so high, our field experience shows that even the farmers who grow vegetables prefer to sell them to earn money, this despite heavy dose of nutrition education. It is not the case of either or, but both or all strategies that would help to increase body iron stores. Hemoglobin increase is the end result. Even improvement in body store has impact on functions of iron like brain function. DFS is meant to build iron stores and not treat anemia. In a country where the burden of anemia is high, treatment strategies have to accompany food- based strategies. DFS is also afoot based strategy.
****Prema Ramachandran, (Nutrition Foundation of India, New Delhi)**	All the studies on dietary intake of iron in Indians have shown that the current intake is very low. Any and every method of increasing iron intake is therefore needed. Dietary diversification is one method. Use of iron and iodine fortified salt is another. These would improve iron intake and overtime improve the iron status, iron stores and Hb levels across the population groups. Relationship between high salt consumption and hypertension are well documented and nutrition education to reduce salt consumption is underway in all countries of the world. Added iron or iodine in the salt does not in any way increase the risk of hypertension and therefore the worry that DFS may increase risk of hypertension is not correct. Currently majority of Indians are anemic; DFS

	intake and dietary diversification alone cannot correct anemia. Therefore detection and treatment of anemia will have to continue to receive attention.
****Sheila C. Vir (Public Health Nutrition and Development Centre, New Delhi)**	I have been following the DFS responses and totally agree that we have the technology and supportive research studies which indicate benefits of DFS and establish the stability, acceptability, bioavailability and safety of DFS. There is no doubt that we need to consider DFS as one of the strategies for addressing the problem of iron deficiency and anemia. However, we need to be extremely cautious prior to rolling out the programme in the country. We need to accept the fact that public health strategy should be evidence based in terms of effectiveness as well as capacity for sustained quality production of DFS.

Source: Solution Exchange, 2006*; Solution Exchange, 2012**