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EPIDEMIOLOGY OF THYROID HORMONE ABNORMALITIES IN EXTREMELY PREMATURE INFANTS

By

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ABSTRACT

EPIDEMIOLOGY OF THYROID HORMONE ABNORMALITIES IN EXTREMELY PREMATURE INFANTS

By

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Transient hypothyroxinemia of prematurity (THOP) is manifest by very low levels of serum thyroxine (T4) and tri-iodothyronine (T3), but normal thyroid stimulating hormone (TSH) levels from shortly after birth until up to 6 weeks after birth. THOP is commonly seen among extremely premature infants born before 28 weeks of gestational age (GA). Studies have shown that THOP could increase risk of cerebral palsy (CP), a major neurodevelopmental disability outcome among children born prematurely, and may also cause other neurodevelopmental disabilities at later ages. Recent evidence suggests thyroid hormone (TH) supplementation may prevent some cases of CP and improve cognitive outcomes among premature infants. Little has been written about the correlates of baseline TH levels or postnatal TH levels in infants at risk for THOP.

From April 2005 to August 2007, we conducted a randomized clinical trial of TH supplementation in preterm infants born between 24 and 28 weeks of GA. Neonatal free T4 (FT4), total T4 (TT4), and TSH values were measured within 24 hours after birth but prior to treatment (as baseline), and then on day 3, 7, 14, 21, 42, 56, and at discharge. Maternal FT4, TT4, and TSH were measured shortly after delivery. FT4 was assessed by direct equilibrium dialysis and TT4 and TSH by chemiimmunoluminescent assay. Maternal and labor exposures were abstracted through medical records. Neonatal information was kept in daily charts. Discharge information was recorded in study discharge form.

This dissertation reviewed the characteristics and the correlates of neonatal baseline and postnatal TH levels, and analyzed correlates of neonatal baseline and postnatal TH levels (log-scale) among very premature infants using the THOP trial data. Linear regression was used for analysis on correlates of baseline TH levels and mixed model for correlates of postnatal TH levels.

Adjusted analyses showed that neonatal baseline FT4 levels related to GA (percentage change; p-value: +17%/wk; <0.01), fetal growth ratio (-6%/0.1; <0.01), race (Black vs. Hispanic +30%; <0.01), multiple birth (-14%; 0.06), and intrauterine growth restriction (-18%; 0.06), while TT4 levels related to GA (+16%/wk; p<0.01), antenatal steroid use (+22%; 0.03), magnesium use (+14%; 0.06), and prolonged rupture of membrane (PROM) (+32%; <0.01). Postnatal FT4 levels were not significantly associated with any factor examined. Postnatal TT4 levels on day 3-21 were all significantly lower than on day 56, ranging from 33-46% (p<0.01). Postnatal TT4 levels also were associated with GA (+12%/wk; <0.01), duration of ventilator use (-5.6%/10day; <0.01), and necrotizing enterocolitis (NEC) (-53%; 0.01).

With the exception of GA, different groups of factors were correlated with FT4 and TT4 levels in extremely premature infants. Baseline FT4 was more closely related to fixed attributes such as multiple birth, race, and fetal growth, while baseline TT4 was more closely related to pregnancy conditions and treatments, such as antenatal steroid and magnesium use, and PROM. Postnatal FT4 levels were not clearly affected by any prenatal or postnatal exposures examined. Postnatal TT4 levels increased significantly over postnatal age and were significantly associated with GA, duration of ventilator use, and NEC.

To my beloved parents, Donghai Hong and Tianlan Ma,
and husband, Hong Chen

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Chapter 1 Background

In the past three decades, the development of laboratory techniques has improved our understanding of perinatal thyroid physiology. However, as the survival of premature infants increases, we are facing the new challenge of transient hypothyroxinemia of prematurity, which is manifest by relatively low serum free thyroxine (T4), normal serum thyroid stimulating hormone (TSH). Literature on premature thyroid is sparse, especially on the correlates of transient hypothyroxinemia in prematurity newborns and how premature thyroid reacts to postnatal life. Thus this dissertation aims to answer some of these questions using data collected in a randomized clinical trial.

In this chapter, brief overview of thyroid hormones is provided, covering the physiology of thyroid hormones, the role of iodine in thyroid physiology, and the ontogenesis of the hypothalamic-pituitary-thyroid system. Then a brief description of thyroid function changes in healthy term infants is given, followed by a review of the changing patterns of thyroid hormones and prenatal/postnatal correlates of transient hypothyroxinemia in premature infants. At the end, a summary discusses associations of transient hypothyroxinemia with neurodevelopmental outcomes in premature infants, and the rationale for attempting to discover whether there are beneficial effects of thyroxine supplements in this population.

1.1 Physiology of thyroid hormone (Figure 1.1)

The thyroid gland produces a variety of thyroid hormones, also called iodothyronine, to provide the daily needs of the human body. Iodothyronine molecules

differ from each other by the number of iodine atoms and positions on the benzene-ring. The major iodothyronines are:

1) 3, 5, 3', 5'-L-tetraiodothyronine (T4),

2) 3, 5, 3'-triiodo-L-thyronine (T3),

3) 3, 3', 5'-triiodothyronine (reverse T3 (rT3)),

4) 3, 3'-diiodothyronine (T2). Conversion among iodothyronines is catalyzed by human iodothyronine selenodeiodinases.

Among all iodothyronines, T4 has the highest concentration in plasma and is the only thyroid hormone exclusively secreted by the thyroid gland. T3 is also secreted by the thyroid gland, but 80% of T3 (and almost all rT3) is produced by conversion from T4 in the liver, kidney, brain, skeletal muscle, and adipose tissue (1-4). In the circulation, there are two forms of thyroid hormone: free and bound. Because free thyroid hormones degrade rapidly, most T4 and T3 molecules are transported to target organs bound to thyroid binding globulin (TBG). However, since only free thyroid hormone can be taken up into the cell, bound T4 and T3 serve as a thyroid hormone reservoir to maintain thyroid hormone homeostasis (1).

Transporting free thyroid hormone molecules into cells is an energy-dependent process, mediated by the Na+/taurocholate cotransporting polypeptide and L-type amino acid transporters. Free T3 (FT3) molecules enter cells and bind to specific nuclear DNA-bound thyroid hormone receptors (TR) to regulate gene expression (5). With a 15-fold higher binding affinity to TR's than T4, T3 is the major biologically active thyroid hormone. Intracellular FT3 can either enter the nucleus and bind to the TRs, or be degraded to T2 by type-3 selenodeiodinase (D3) (1). The intracellular free T4 (FT4)

molecule becomes active T3 by having a single 5'-iodine atom detached by type 2 (D2) human iodothyronine selenodeiodinase. FT4 can also be converted to inactive rT3 by removing a single 5-iodine atom, a reaction catalyzed by D3. The activation and degradation pathways of iodothyronines are illustrated in Figure 1.1.

1.2 Role of Iodine in thyroid physiology

Iodine is an essential element in thyroid hormone synthesis and metabolism and, in humans, is completely dependent on dietary intake. Iodine deficiency is often seen in mountain area and lowland regions far from the oceans, such as central Africa, northern China, Himalayas area, India, many countries in Europe, and South America, etc. Those who consume only locally produced foods in these areas are at great risk for Iodine deficiency.

When iodine supply is reduced, the human body takes compensatory reactions to maximally conserve iodine and improve the efficiency of iodine use. These adjustments occur at the hypothalamic, pituitary, thyroid, and peripheral tissue levels. For instance, during iodine deficiency, a plasma T4 decrease may increase D2 expression in the central nervous system (CNS), hypothalamus, and pituitary gland, which promotes the conversion of T4 to T3. D3 expression in the CNS may decrease to allow T3 to stay active for relatively a longer period in the face of iodine deficiency (6-8). This preference for T3 during iodine deficiency is physiologically more efficient as T3 consumes one iodine atom less than T4, but has a three-fold higher biological potency (1).

In human adults, when total iodine intake is less than 75 μg/day, the above compensatory adjustments in thyroid function will be activated. Similar physiological adjustments have been found in disorders in which there is difficulty utilizing iodide,

such as Hashimoto's disease and Grave's disease during antithyroid drug treatment treatment (9, 10). Despite the compensatory mechanisms to help the human body to preserve iodine and increase the efficacy of iodine utilization, prolonged and severe iodine deficiency will eventually lead to clinical hypothyroidism.

Since insufficient iodine can cause hypothyroidism, one might assume that excessive iodine would induce hyperthyroidism. However, the contrary is true. Excessive iodine exposure inhibits thyroid hormone production and may cause transient hypothyroxinemia. Prolonged excessive exposure to iodine places the thyroid gland at risk of goiter or hypothyroidism. With iodide overload, the thyroid gland reacts first by increasing, and then by decreasing organification of iodide, ultimately producing a reduction in iodothyronine synthesis (11). This decrease intrathyroidal organification of iodide is known as the *Wolff-Chaikoff Effect.* The decreased iodide organification is thought to result from the overly high concentration of inorganic iodide within the thyroid gland, which possibly interferes with the effect of thyroid peroxidase (TPO). After experiencing the *Wolff-Chaikoff Effect* for a short time period, the normal thyroid gland partially regains the ability to uptake iodide into the cell for iodothyronine production. This process lasts until the iodide level falls below the threshold for maintaining the *Wolff-Chaikoff Effect.* This *adaptation* or *escape* phenomenon prevents the development of hypothyroidism and iodide goiter. However, if excessive iodine intake continues and iodine levels stay higher than the threshold, goiter or hypothyroidism will eventually develop. Although this *escape* mechanism exists in fetus or premature infants is not confirmed, cases of infants born with goiters large enough to cause respiratory distress have been reported as a result of maternal iodide ingestion (12-

14). Potassium iodide, once commonly used for cough treatment, was thought to be one of the most frequent culprits (15). Thus prolonged overload iodine intake during pregnancy or infancy (for preterm infants) may cause hypothyroidism or goiter and should be avoided as much as possible (11, 16, 17).

1.3 Ontogenesis of hypothalamic-pituitary-thyroid system

The development of the fetal hypothalamic-pituitary-thyroid (HPT) system includes three phases: I) embryogenesis, II) hypothalamic maturation, and III) maturation of hypothalamic-pituitary-thyroid system function (18, 19). Although the time windows overlap with each other, distinct differences exist in anatomy, histology, biochemistry, and biological function across the three phases.

Phase I: embryogenesis of the pituitary and thyroid gland occurs during the first trimester. The thyroid embryo anlage becomes recognizable as early as one month after conception and the embryogenesis of thyroid gland is the mostly completed by 10 to 12 weeks of gestational age (GA) (1, 20). Toward the end of this period, tubule-like structures are seen in the fetal thyroid, soon after which the follicular structure appears and then the follicles are filled with colloid. While the thyroid structure develops, the fetal thyroid develops the ability to concentrate iodide and to synthesize iodothyronines (1, 18, 19, 21-22). Meanwhile, the fetal pituitary undergoes histological differentiation and starts synthesizing TSH by 14 weeks' gestation. Fetal serum T4, T3, and TSH become measurable by the end of this phase (23-26), but concentrations remain low until mid-gestation.

Phase II: hypothalamic maturation overlaps with Phase I. The time window, from 4-5 weeks of gestation to around 30-35 weeks of gestation, is when continuing

development of the hypothalamus and pituitary glands proceeds. Anatomically, hypothalamic nuclei and fibers of the supraoptic tract become visible by 12 to 14 weeks. Biochemically, TRH, gonadotropin-releasing hormone, and somatostatin reach measurable levels in hypothalamic tissue by 12 weeks, indicating that the hypothalamus can synthesize hormones by then. From the 10-12th to the 35th week, hypothalamic maturation continues as the pituitary-portal vascular system matures (18, 19).

Phase III: the elevation of fetal serum T4 concentrations marks the beginning of the maturation of the hypothalamic-pituitary-thyroid system. Around 18-20 weeks' gestation, pituitary and serum TSH begin to rise. Serum TSH concentrations peak early in the third trimester and decline slowly from about 15 μU/ml at 30 weeks to 10 μU/ml at term (27-29). Fetal serum T4 and FT4 levels gradually climb up in the third trimesters until term.

More importantly, the hypothalamic-pituitary-thyroid negative feedback system starts regulating thyroid hormone production during this period. Infants born after 26 to 28 weeks of GA have been found to respond to exogenous TRH injections (40 μg) with serum TSH elevations (30, 31). Also, increased cord and serum T4 levels after intra-amniotic injection of T4 (700 μg) greatly suppressed both cord TSH levels and neonatal TSH surges in term infants (32). These up and down-regulations of thyroid hormone levels are mediated through hypothalamic stimulation of pituitary TSH secretion and subsequent stimulation of the thyroid gland (19). The hypothalamic-pituitary-thyroid feedback matures with gestational age and HPT development is complete at term (28).

1.4 Maternal-Fetal thyroid hormone interaction

The fetal thyroid hormone supply relies a great deal on maternal sources throughout pregnancy, especially the first two trimesters. It is well known that transplacental passage of TSH from mother to fetus is negligible, but maternal T4 can cross the placental barrier and freely enter the fetal circulation. T4 has thus been detected in coelomic and amniotic fluids prior to the onset of fetal thyroid function (22). Moreover, measurable cord T4 levels are found in newborns with congenital hypothyroidism (33). With increasing tissue needs resulting from fetal growth, maternal thyroid hormone production rises as pregnancy proceeds. In the late second or early third trimester, the source of fetal thyroid hormone supply gradually shifts, however, from complete maternal dependence to partial maternal dependence, because of maturation of fetal thyroid function and of hypothalamic-pituitary-thyroid regulation.

Can the fetus suffer hyperthyroxinemia when the mother transfers too much thyroid hormone to the fetus? Most likely no, because large amount of type 3 monodeiodinase existed in placenta tissue, possibly in uterus as well, which prevents the fetus from developing hyperthyroxinemia by increasing the degradation of T4 to rT3 or T3 to T2. Nonetheless, there have been reports on neonatal thyrotoxicosis secondary to maternal hyperthyroidism (34, 35). This could be due to placental transfer of immunoglobulin, or possibly abnormality of neonatal thyroid.

1.5 Postnatal thyroid hormone surge

In term neonates, serum TSH levels surge immediately after birth and usually peak at 30 minutes of postnatal life. A sharp decrease in serum TSH appears immediately after the peak in the first few hours, followed by a much slower decline until

TSH drops significantly below cord level at about 48-72 hours after birth (36). Serum T4 levels rise to respond to the TSH elevation, reaching an apex at 24 hours after birth, and then slowly decreasing to a plateau over the first week of life (37-41). Serum T3 increases in a similar fashion to T4, except that its rise is not a direct resulted of the TSH boost, but rather is produced by 1) reduced T3 degradation due to sudden loss of D3 from placenta tissue, 2) decreased D3 expression in newborn liver and brain tissues, and 3) increased peripheral conversion of T4 to T3 shortly after birth (19, 37, 41).

The acute TSH surge after birth is thought to be stimulated by the cold exposure in the extrauterine environment, and the subsequent thyroid hormone elevations are known to be responsible for non-shivering thermoregulation in newborns. The thyroid hormones influence the brown adipose tissue (BAT) in heat production through many physiologic pathways, such as by increasing uncoupling protein activity in BAT, controlling lipid composition and the size of brown adipocyte, and increasing expression of proteins involved in brown adipocyte metabolism (oxidation). All of these mechanisms promote the production of high energy phosphate bonds that are ultimately converted to heat in BAT (42). Results from many animal experiments support the role of thyroid hormone in non-shivering thermoregulation in neonates. In addition to the thyroid hormone surge triggered by cold exposure, it has been found that thyroid hormone levels are positively related to rectal temperature, and that higher body temperature are observed in euthyroid animals compared to animals with hypothyroidism (43-46).

However, premature infants, especially extremely low gestational age infants (GA < 28 completed weeks), are expected to have immature hypothalamic-pituitary-thyroid

system and less fat tissue. Immaturity affects the thyroid hormone surge owing to the lack of capacity to synthesize hormone and/or the lack of effectiveness of HPT hormone regulation. The latter deficit affects the thyroid hormone surge by reducing intracellular conversion of T4 to T3 in BAT, which is rich in D2. As a result, the brown adipocyte is smaller, has an altered lipid composition. There is a lower circulating free fatty acid concentration, and eventually less heat production (18, 47-49).

1.6 Transient hypothyroxinemia of prematurity (Table 1.1)

Transient hypothyroxinemia of premature (THOP) infants is manifest by relatively low free T4, normal serum TSH, and normal responses of TSH and T4 to TRH (19). In general, THOP is a laboratory based diagnosis and is mostly asymptomatic. However, signs of hypothyroidism, such as hypoactivity, lethargy, poor feeding, constipation, jaundice, edema, and hoarse cry, have been observed in preterm infants with THOP (50). Although these signs are not specific to hypothyroidism, the clinical response to thyroxine replacement therapy, exclusion of other possible causes in addition to the infants' low serum T4 levels and normal TSH levels, have strongly suggested that hypothyroxinemia was likely the cause. With increasing postnatal age, serum thyroid hormone levels of preterm infants increase and catch up with term infants by 3-8 wks postnatal age. The temporary presence of low thyroid hormone levels is the reason for use of the term transient hypothyroxinemia.

Frequencies of THOP reported in different preterm cohorts have varied because of differences in 1) the gestational age groups studied, 2) the postnatal ages when tested, 3) the definition of transient hypothyroxinemia applied, and 4) the sample sizes. Table 1 summarizes published frequencies of THOP (50-59). Although variations exist across

studies, the trend is clear in showing that the THOP rate decreases dramatically as gestational age increases. Within the same gestation group, the THOP rate declines over postnatal age, although the effect of postnatal growth on thyroid hormone levels is not as strong as that of gestational maturation.

1.6.1 The different patterns of postnatal thyroid hormone levels in preterm infants

In the past three decades, advances in laboratory techniques have improved our knowledge of the physiology of the thyroid hormones. With the broad use of surfactant, prenatal steroids, and the development in neonatal intensive care, more and more very premature newborns survive. The physiology and pathophysiology of these premature thyroid glands and HPT regulation systems have been studied, but are still not well understood. This section provides a brief summary of the changing patterns of postnatal thyroid hormones and TSH concentrations in preterm infants, especially extremely premature infants, and the differences from term infants.

1.6.1.1 Cord blood thyroid hormone levels in preterm infants (Table 1.3-1.5; Figure 1.2-1.4)

At birth, preterm infants have low cord serum thyroid hormone levels. Many studies have reported significantly lower cord FT4, TT4, and TSH levels in preterm infants than in term infants (60-63). The baseline hormone level differences between preterm and term infants diminish as gestational age increases (64). Tables 1.3-1.5 summarize the cord FT4, TT4, and TSH levels reported in different populations by different gestational age groups (2, 37, 54, 59, 65, 66). FT4 levels had slight increase over gestational age with the highest values appearing at 36 weeks. TT4 levels increase steadily along with gestational age. TSH levels remain nearly flat over gestational age.

One Japanese study reported TSH levels being highest around 30-34 weeks, but the same trend was not seen among other studies (66).

Williams et al. presented the cord thyroid hormone and TSH levels of 620 infants in a cross-sectional fashion. Study infants were born between 23 and 42 weeks of GA and enrolled from 11 Scottish neonatal intensive care units, as part of a cohort study of transient hypothyroxinemia (63). Cord thyroid hormone levels of every single gestational week were reported and results were difficult to be included in table 1.3-1.5 because other studies reported thyroid hormone levels by clustered gestational age groups. To visualize the changing pattern, we have converted the thyroid hormone levels reported by Williams into graphs. Figure 1.2-1.4 illustrate the approximate changing pattern of cord FT4, TT4, and TSH with gestational age. FT4 reached the highest value around week 33 and then decreased thereafter. The turning point in this graph appeared much earlier than what is observed in table 1.3, and the degree of decrement is much greater in William's population. These differences were probably caused by three factors. First, clustered gestational age groups (table 1.3) can not show trends as clearly as individual gestational weeks (figure 1.2). Second, the sample sizes in gestational weeks 35, 36, and 37 were 4, 9, and 10 respectively in Williams' report. Small sample sizes might introduce variation and might not accurately reflect the hormone levels of infants born within these gestational weeks. Third, infants in Williams' report were all admitted to neonatal intensive care units, implying that they had different levels of illness. Their illness levels might be related to their thyroid hormone levels and the relation is likely to be stronger in term than in preterm infants. Patterns seen in both TT4 and TSH, in the Williams' data, are in agreement with those observed in table 1.4.

1.6.1.2 Postnatal thyroid hormone changes in preterm infants

After birth, as aforementioned, the TSH levels of term infants rise quickly and reach an apex value within 30 minutes. Serum iodothyronine levels rise to respond to the TSH stimulation and climax at around 24 hours after birth. Both TSH and thyroid hormone levels drop after the peak levels but remain much higher than in adults.

The thyroid function of preterm infants behaves differently from term infants. According to the maturity level, preterm infants can be categorized into two groups, moderately preterm group (29-36 completed weeks of GA) and extremely premature group (less than 28 completed weeks of GA). We will address these two groups separately.

1.6.1.2.1 Postnatal thyroid hormone changes in moderately preterm infants (Table 1.6-1.8) (2, 54, 58, 64-71)

The moderately preterm group refers to infants born between 29-36 completed weeks of gestational age. As these neonates are closer to term, their thyroid glands and HPT regulation systems have gained a certain level of capacity to adapt to the extra-uterine environment, but the potency of these system is not as great as those of term infants. Baseline (at birth) thyroid hormones and TSH levels of this group are lower than those of term infants and are positively associated with gestational age. The TSH surge is seen in this population and rising T4 and T3 levels are observed in response to the TSH surge. The surges of TSH and thyroid hormones in moderately preterm infants are qualitatively similar to those of term infants, but lesser in degree (19, 72).

1.6.1.2.2 Postnatal thyroid hormone changes in extremely premature infants (Table 1.9-1.11) (2, 54, 57, 61, 64)

The extremely premature group consists of neonates born prior to 28 completed weeks of gestational age. This population is delivered at the end of the second trimester or the beginning of the third trimester. This time window coincides with the third phase of the ontogenesis of the HPT system, during which the fetal thyroid gland and the HPT regulation system begin to mature and function (see section 1.3) (18, 19). This process is interrupted by parturition. The coordination between the fetal thyroid gland and the HPT regulation system is not yet developed at birth. Clinically, TSH levels at baseline are extremely low in this population and they increase with gestational age. The TSH surge, is greatly suppressed or even absent among extremely premature infants (2, 61, 73). The reasons for this absence could include a lack of TRH response to declining thyroid hormone levels, or an immature HPT system that has limited TRH secretion. Alternatively, the central nervous system may be too immature to respond to cold stimulation after delivery (74).

Among extremely premature infants, FT4 concentrations start at a much lower level compared to moderately preterm or term infants and the FT4 increase is transient and minimal. In a randomized clinical trial, in which thyroxine supplement was provided to preterm infants less than 30 weeks of gestational age, Van Wassenaer et al. reported a small, insignificant increase (0.3 ng/dl) of the mean serum FT4 levels on day 1, followed by a significant decrease with a nadir appearing on day 7 among 100 preterm newborns in the placebo group (75). In another large randomized clinical trial which examined the effect of T3 and hydrocortisone on lung disease in very premature infants (23-29 weeks),

no thyroid hormone surge was seen in 128 subjects randomized to the placebo group (61). In addition, the thyroid hormone concentrations barely changed over the first two weeks of life in this study cohort. A study of 22 infants born between 24 and 27 weeks of gestational age, whose thyroid hormone levels were measured in cord blood and at 1, 7, and 24 hours after birth, found small and transient increases consistent with other studies (2).

The total T4 increase is also greatly attenuated in these very low gestational age newborns. Murphy et al. reported severely reduced TT4 elevation after birth in neonates born between 24 and 27 gestational weeks (2). Hadeed et al. also found infants less than 1000 grams (GA < 30 wks) had small degrees of TT4 elevation. Table 1.7 summarizes the previously reported serum TT4 values in the first 3 weeks of life in different gestational age groups. Cross-sectionally, TT4 levels increase with gestational age; longitudinally, TT4 increases slightly by postnatal age, but with great variation across different studies.

Interestingly enough, baseline rT3 levels (the inactive form of thyroid hormone) remain much higher in extremely premature infants than in both moderately preterm and term infants (2, 37, 68, 71). Cord rT3 levels decrease with increasing gestational age. After birth, the serum rT3 levels gradually decrease to the same levels as term infants. rT3 is also known as the major iodothyronine form in the fetus. This similarity between extremely premature infants and fetuses strongly suggests that thyroid function and HPT regulation are not functioning, only minimal functioning, in this population.

In summary, both baseline and postnatal thyroid hormone levels are low in preterm infants. Hormone changes regularly seen in term infants, especially the hormone

surges after birth, are suppressed in preterm infants. The degree of attenuation is the greatest in extremely premature newborns, and sometimes the surges even disappear. Transient hypothyroxinemia of prematurity reflects a delay in maturation of both thyroid gland function and hypothalamic-pituitary-thyroid regulation system. The maturity of newborn, namely gestational age, plays the most important role in this process.

1.6.2 Prenatal factors that affect thyroid hormone levels in preterm infants at birth

Besides prematurity, there are other prenatal factors that may affect the thyroid function of preterm infants at birth, such as intrauterine growth restriction, chorioamnionitis, placenta insufficiency, antenatal steroid use, etc. In this section we briefly summarize most of the prenatal factors previously discussed in the literature.

1.6.2.1 Intrauterine growth restriction and THOP (Table 1.12) (58, 60, 76, 77)

Intrauterine growth restriction (IUGR) is associated with transient hypothyroxinemia in preterm infants. Many studies have compared the thyroid hormone levels of small for gestational age (SGA) infants with those of appropriate for gestational age (AGA) infants at birth or during neonatal period. Consistently low thyroid hormone levels have been found in SGA infants independent of gestational age (41, 58, 71, 78). However, in light of our limited knowledge of fetal thyroid development, and our restriction to observational data from previous reports, it is hard to determine the causal relationship between IUGR and THOP. Three mechanisms may explain the association between IUGR and THOP. First, thyroid hormone is known to be critical in fetal growth. In athyrosis cases or when fetal thyroid development was interrupted, intrauterine hypothyroidism has been known to cause fetal growth restriction. Second, in fetus with growth restriction from intrauterine insults, the thyroid gland could be one of the affected

organs and hypothyroxinemia might develop. Last, a common risk factor may affect both fetal growth and thyroid gland development or function.

1.6.2.2 Histological chorioamnionitis and THOP (Table 1.12-1.13) (58, 60, 76, 77)

It is worth mentioning an interesting finding about the association of histological chorioamnionitis (HCA) and low thyroid hormone levels in preterm infants. De Felice et al. examined placentas of 155 preterm newborns (GA 29.36 ± 2.39 wks) and measured the thyroid hormone levels of these infants on postnatal day 4 and day 40 (76). They found that after adjustment for birthweight and respiratory distress syndrome, having HCA increased the risk of having first week TT4 (day 4) more than 1 standard deviation below the mean by 32-fold (95% CI 8.9-115.6). Although this is the only study, to our knowledge, designed for testing the relationship between asymptomatic HCA and hypothyroxinemia, many other groups repeatedly have established the significant impact of intrauterine infection on elevated risks of preterm delivery and other neonatal morbidities of the preterm infants (79-81). De Felice's finding suggests that THOP may be one of many consequences of a fetal inflammatory response to intrauterine infection.

However, in another study whose primary aim was to examine the association between antenatal glucocorticoids and THOP, the opposite relationship was discovered between chorioamnionitis (CA) and THOP. The unadjusted analyses showed that the median TT4 level of 200 infants with CA was significantly higher than that of 100 infants without CA (TT4 6.6 vs. 6.2 μg/dl, p=0.02). Although whether this relationship remained significant in an adjusted model was not indicated in this report, it draws attention to the unsettled effect of CA on THOP.

Except for comparable prevalence rates of CA (63.2% (98/155) vs. 66.7% (200/300)), we found several features in study designs and populations that differed and which may explain their contradictory results (table 1.13). The most important difference is that De Felice et al. included IUGR newborns, but Martin et al. did not. As noted above, SGA infants have lower thyroid hormone levels than do AGA infants. CA is known to be a risk factor for IUGR (81). Excluding IUGR subjects could artificially elevated TT4 levels in the chorioamnionitis population and attenuate the differences. In addition, De Felice et al. studied moderately preterm infants, but Martin et al. studied extremely premature infants. The latter are known to have very low thyroid hormone levels and a minimal thyroidal response to the extra-uterine environment. Population difference could thus be a second reason for the different results. Moreover, Martin's group used the thyroid hormone results assessed by the State newborn screening program and excluded all subjects with no or delayed first week thyroid hormone values, while De Felice et al. obtained day 4 thyroid hormone values on every study subject. We know that overall severity of illness tends to interfere with newborn screening and cause delays in blood sample collection. According to Martin, the subjects with missing hormone values in their study were less mature and had a higher mortality rate. We also know that sickness severity is tightly bound to low thyroid hormone levels and CA may also contribute to the sickness. In the Martin et al study, there might have been more subjects with CA encountering missing hormone value problems than subjects without CA. This might also raise the apparent hormone levels in chorioamnionitic infants. Finally, De Felice et al. studied only infants with asymptomatic CA, while Martin's group did not differentiate

asymptomatic from symptomatic. It might be that asymptomatic and symptomatic CA might affect fetuses through different mechanisms.

Since the study conducted by De Felice's group was designed to study the association between HCA and THOP among premature infants, their findings provided us evidence to believe that HCA may be associated with low thyroid hormone levels in preterm infants. However, histological chorioamnionitis is a complicated condition and may affect fetal growth in various ways. For further understanding of this association, more studies with careful definition of histological chorioamnionitis need to be conducted for this specific hypothesis.

1.6.2.3 Placenta insufficiency and THOP (Table 1.12) (58, 60, 76, 77)

Two small studies (n=62 and n=28) compared thyroid hormone levels of moderately preterm infants born to preeclamptic mothers with "placenta deficiency" (PD) assessed through ultrasonography and mothers without (60, 62). Both studies found low cord thyroid hormone levels in infants born to preeclamptic mothers with placenta insufficiency (PI). However, most infants born to preeclamptic mothers with PI in these two studies had IUGR. The association between PI and THOP thus might possibly have been mediated by IUGR. Alternatively, THOP could also result from a condition other than IUGR, primarily induced by PI. For instance, Buimer et al. found that low cord FT4 levels were not related to maternal FT4 levels, but to prenatal acidosis, which was an outcome of PI (82).

1.6.2.4 Maternal thyroid hormone levels and THOP

It is well accepted that fetal thyroid hormone levels are strongly influenced by maternal thyroid function. Will this also be true after delivery? Studies that examined

both cord and maternal serum thyroid hormone levels are needed to answer this question. In a cross-sectional study, Hume et al. measured thyroid hormone levels (FT4, T4, T3, & TSH) of 428 mother-infant pairs at different gestational age (GA 15-42 wk) (37). The correlation analyses revealed an overall lack of association between maternal thyroid hormones and TSH levels at delivery with neonatal cord levels. However, in another study engaged in newborn thyroid hormone changes in the first 24 hours of life, the same research team found a strong correlation between maternal TSH and neonatal TSH in 72 mother-infant pairs with gestational age between 24 and 34 weeks (2). The relationship between maternal and neonatal thyroid hormone levels at birth thus remains unclear.

1.6.2.5 Antenatal steroid treatment and THOP

Controversies exist in literature with respect to the association between antenatal steroid exposure and early neonatal thyroid hormone levels among premature infants. Franklin et al. failed to find any effect of antenatal steroids use on neonatal thyroid function at birth or during the postnatal period (n=97) (83). But a recent study, in a much larger preterm population (n=556; GA 23-28 wk), found a complete dose of antenatal glucocorticoids increased first-week mean T4 level by 0.81 μg/dl (p=0.03) (77). Except for the difference in the number of subjects received antenatal steroids treatment (20 vs. 135), which gave more power in detecting association, the latter study also found much less illness frequency among newborns receiving antenatal glucocorticoids treatment, including lower rates of PDA, IVH, etc. These findings suggested that antenatal steroid treatment could possibly promote the maturation of multiple organs, including the thyroid gland, and reduce overall illness severity, both of which are known to be related to improved serum thyroid hormone levels.

1.6.2.6 Other factors and THOP

A number of studies looked at other prenatal risk factors for THOP. One large study (n=365) found that low thyroxine levels at newborn screening were related to older postnatal age at test (OR=1.6/day, 95% CI 1.3-2.1), and maternal education higher than 12 years was protective (OR=0.4, 95% CI 0.2-1.0) (84). Although not tested in other studies, we have already learned that illness severity is related to both delay in newborn screening sample collection and low thyroid hormone levels. This might explain the relation between older postnatal age at test and lower thyroxine levels. However, the role of maternal education, a representation of social economic status, is unclear in relation to neonatal thyroid hormone levels.

Other studies also examined the effects of the following factors on thyroid hormone levels in preterm infants, such as gender, race, multiple birth, route of delivery, birthweight < 1500 g, premature rupture of membrane, placenta infarction, gestational diabetes, prenatal antibiotics/beta-2-sympathicomimetics therapy within 24 hours prior to delivery, etc. No significant association has been detected consistently (65, 68, 78).

1.6.3 Factors that affect postnatal thyroid hormone levels in preterm infants

After birth, postnatal exposures, in addition to residual effects from intrauterine and labor exposures, add more complexity in revealing the association between postnatal factors and THOP. As in adults, thyroid hormone levels in preterm infants decrease dramatically as illness or nutritional deprivation becomes severe. Our limited knowledge on the pathophysiology of how the neonatal thyroid gland responds to the extra-uterine environment makes it difficult to reveal the causal relations between neonatal illnesses and THOP.

1.6.3.1 Postnatal illness and THOP (Table 1.14 -1.15) (54, 58)

Impaired lung function is the most commonly seen condition in preterm infants with THOP. Compared to healthy preterm controls, significantly lower cord and postnatal serum T4 levels have been found in infants who subsequently developed respiratory distress syndrome (RDS) and in those who suffered RDS, respectively (54, 58, 84). The T4 difference between preterm infants with and without RDS decreases as gestational age increases, and no T4 difference is found between term infants with and without RDS.

The association between RDS and THOP is intriguing. Some have suggested that THOP could be secondary to RDS. However, the finding of suppressed cord thyroxine levels in preterm infants who developed RDS later, as well as the lack of a T4 difference between term infants with and without RDS did not support this hypothesis. The etiology of this association is more likely to be intrauterine for premature infants. A few mechanisms may explain the relationship.

First, intrauterine thyroid hormone deficiency may lead to a shortage of cortisol, a key stimulator of pulmonary surfactant synthesis. Lack of cortisol would delay lung maturation (85), produce surfactant deficiency and impose a greater risk of having RDS in the premature population (58). Second, it could be that the overall immaturity in premature infants leads to both RDS and THOP. Third, thyroid hormone dependent pulmonary maturation could be hindered directly by postnatal hypothyroxinemia. The severity of pulmonary illness could be worsened and its duration prolonged (84). Fourth, once RDS occurs, it could possibly affect neonatal thyroid function and deteriorate

hypothyroxinemia, which in turn could slow down the thyroid hormone dependent pulmonary maturation and RDS may become worse.

Interestingly enough, although T4 levels decrease in infants with RDS, TSH showed very little difference between infants with and without RDS within the same gestation groups(58). This finding suggested that the HPT system was immature for hormone regulation in preterm infants. It could be that either the hypothalamus does not respond to serum low thyroxine levels, or the pituitary reduces responding to TRH stimulation.

Sepsis is another illness linked to THOP. It shares the same attributes with RDS in relation to THOP, in that premature newborns with sepsis have lower serum thyroxine levels. The association of sepsis with THOP appears to be independent of gestational age and TSH is not affected by sepsis (54, 58). THOP has also been found to be related to intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), chronic lung disease (CLD), and other neonatal disorders (4, 55, 84, 86-88).

Not many studies have investigated prenatal or postnatal risk factors of THOP, and the consistency of published findings needs to be confirmed by more observational studies with larger populations and broader risk factors of interest.

1.6.3.2 Medical procedure and THOP

As aforementioned, the thyroid glands of premature infants do not have the ability to escape from *Wolff-Chaikoff Effect* when exposed to excessive iodine exposure. Thyroid hormone production will be suppressed and prolonged iodine exposure could induce permanent hypothyroidism in this population. Topical iodine antiseptics for skin (before or after surgery), umbilical cord disinfection, and iodine containing contrast

media are widely used in neonatal intensive care units. They are the major sources of excessive iodine exposure for preterm infants. Khashu et al. reported severe hypothyroxinemia in a premature infant after prolonged use of iodinated skin disinfectants for a complex skin lesion (16). Weber et al. studied 23 infants exposed to excessive iodine (89), among which eleven were through maternal iodine exposure (iodine containing antiseptics used for vaginal application before or after delivery (n=4), or caesarean section (n=5), or skin disinfectant applied in the second trimester of pregnancy (n=2)) and twelve through neonatal exposure (topical iodine antiseptics used for surgical intervention or umbilical cord disinfection (n=7), or contrast media used for venous catheterization (n=4) or urography (n=1)). They have found that more than 50% of premature infants who had THOP were exposed to iodinated skin disinfectants or iodine-containing contrast media. The exposed neonates also had increased TSH levels and urinary iodine excretion.

These findings suggest that iodine containing disinfectants, drugs, or contrast agents should be used with great caution in premature infants, and that TSH monitoring is advisable for detecting thyroid hormone suppression.

1.7 How do thyroid hormones affect brain development?

Thyroid hormones affect both fetal brain development and long term neurodevelopment. The former outcome is more related to maternal thyroid hormone levels during pregnancy since maternal-fetal thyroid hormone transfer is the major source of fetal thyroid hormone supply. The latter outcome is more associated with neonatal thyroid function after birth.

Three major molecular and cellular level findings support that thyroid hormone is essential to fetal brain development. First, thyroid hormone receptors in the brain appear early in fetal life (around 10 weeks after conception), along with detectable levels of T3 in the brain. Moreover, a large increase in the number of thyroid hormone receptors has been found in parallel with neuroblast proliferation during the 2nd trimester. All three discoveries strongly suggested that the fetal brain is a target organ of thyroid hormone from early onset of pregnancy (90, 91).

Second, in first-trimester moderate/transient hypothyroxinemia rat models, neocortical genesis and neuron migration were found to be interrupted and altered (92). The observed changes could be avoided by timely T4 infusion, and became irreversible with delayed or absent T4 treatment. Absence of benefit with delayed thyroxine treatment implies that a critical window exists in brain development for the effects of thyroid hormones. Similar findings from other authors reinforce the view that fetal thyroid hormone deficiency can result in blurred layering in the neocortex, abnormal distribution of callosal connections, decreased myelin deposition, and altered neuron differentiation and axon arborization in fetal brain (93-97). Lastly, but not the least, free T4 is the only iodothyronine that can cross the blood-brain barrier, undergo intracellular conversion of T4 to T3, and modulate gene expression and protein production in brain tissue (98). Thus T4 is critical in fetal brain development.

1.8 THOP and adverse neurodevelopmental outcomes (Table 1.16) (51, 53, 87, 99, 100)

THOP has repeatedly been shown to be related to adverse neurodevelopmental outcomes. Three large observational studies linked first week thyroid function of

premature infants and their later neurodevelopmental outcomes. All three studies followed large cohorts of premature infants over a long period of time and examined these children with widely accepted reliable neurological instruments. Each study found different degrees of cognitive delay in early childhood (18-24 months) with THOP during neonatal period, all were statistically significant. The average reduction in the Bayley mental development index (MDI) among children experiencing neonatal THOP varied from 6.8 to 8.3 (87, 99). The cognitive delay persisted until later ages (age 6, 8, and 9), at which time IQ reductions were found to vary from 6 to 8 points, and more school failures were observed in these children than controls (51, 87, 99, 100). Neonatal THOP was also found to increase the risk of cerebral palsy by 3.6 to 4.4 fold at age 2, and the risk of motor development delay, especially walking disability, by 4.9 fold at age 6 (87). Their findings were consistent with each other and strongly suggested that THOP during the neonatal period is associated with increased risk of adverse neurodevelopmental outcomes. However, the question of whether premature infants with THOP should be treated with thyroxine can only be answered by a randomized clinical trial with valid long term neurodevelopmental follow-up.

1.9 Neurodevelopmental outcomes from randomized clinical trials of supplemental thyroxine for THOP (Table 1.17-1.18) (75, 101-105)

Since the late seventies, six randomized clinical trials (RCT) have been done to evaluate the effect of thyroid hormone supplement, among which three early studies focused on neonatal mortality and morbidity, and three later studies also included neurodevelopmental outcomes at later ages. One study detected a significant protective effect of thyroid hormone (RR=0.23, 95% CI 0.07 - 0.74) on neonatal mortality in

moderately preterm infants (104), but other studies, although they detected similar trends, failed to find statistical significance (Chowdry et al. RR=0.46, 95% CI 0.05 - 4.38; Van Wanssnaer et al. RR=0.67, 95% CI 0.36-1.24) (75, 103, 104). These negative studies were underpowered to detect moderate effect of supplements on cognitive development.

In a small RCT (n=44, 22 per group) supplementing preterm infants (GA < 32 wks) with two doses of 25 μg T3 for the first two days of life, Amato et al. reported a 20% mean decrement of the fraction of inspired oxygen (FIO2) in T3 treated neonates (peak FIO2 0.58 ± 0.12 (T3 treated) vs. 0.78 ± 0.09 (control) liters, $p<0.05$), however, similar effects were not seen in other trials, including an earlier trial conducted by the same team using T4 as the treatment (75, 101, 102, 105). The inconsistency could be due first to a different biological effect of T3 and T4 treatment. Second, Amato's group studied 44 premature infants with RDS, while others focused on general preterm populations. By and large, thyroid hormone supplement had no beneficial effect on infant growth, duration of mechanical ventilation/CPAP, neonatal morbidities, such as sepsis, patent ductus arteriosus, necrotizing enterocolitis, brain lesion, etc.

Among the three trials which studied neurodevelopmental outcomes at later ages, Chowdry et al. found 11.4 points of MDI reduction (not statistically significant) in thyroxine treated group at 12 months of age and no MDI difference at 24 months. However, their conclusion could be misleading because their sample size was extremely small, 5 in treatment group and 3 in placebo group at 12-month follow up, and 2 in each group at 24-month follow up (103). Vanhole's group also examined Bayley MDI and psychomotor development index (PDI), but at an age (7 months) too young for a reliable CP diagnosis to be made (105).

The most important contribution was from Van Wassenaer's group, because it was by far the largest RCT of thyroid hormone supplementation in premature infants. 200 premature infants less than 30 weeks of gestational age were enrolled, with half receiving 8 μg T4 per kilogram of body weight per day for 6 weeks and another half receiving placebo. FT4, TT4, T3, rT3, TSH, and TBG were repeatedly measured at postnatal day 0, 3, 7, 14, 21, 28, 35, 42, and 56. Standard Bayley examinations were performed at 6, 12, and 24 months of corrected postnatal age. No difference was found in neurodevelopment between two study groups. However, in subgroup analyses, treated subjects who were born less than 27 weeks of gestation had an 18-point increment in mean MDI ($p=0.01$). Among subjects born at or after 27 weeks, a 10-point decrease in mean MDI was seen in the treatment group ($p=0.03$). These findings suggested that thyroxine treatment affects newborns born before and after 27 weeks differently, although non-pre-hypothesized interactions in a trial should be treated with caution, infants below 27 weeks, i.e. extremely preterm infants, were indeed short of thyroxine. Thyroxine treatment appeared to benefit this population and improve their neurodevelopmental outcomes. Nonetheless, the sample size for the subgroup analysis in infants < 27 weeks was relatively small, 13 in the thyroxine group and 18 in the placebo group. To confirm that thyroxine supplement is beneficial to extremely premature infants, a larger RCT is needed.

From April 2005 to March 2007, we conducted a RCT of thyroxine supplement among infants born between 24 and 28 completed weeks of gestational age. This study aimed to find the optimal dosing schedule and route for thyroxine supplementation in extremely premature infants. The methodology is described in detail in chapter 2. In

stead of the treatment effects, this dissertation will focus on the epidemiology of the thyroid hormone abnormalities in very premature infants.

SUMMARY OF CHAPTER ONE

In summary, compared to term infants, the thyroid hormone levels of preterm infants are much lower and are positively correlated with gestational age. The postnatal hormone surges are in general attenuated, characterized by being qualitatively similar to term infants but quantitatively less in moderately preterm infants (28-36 wks), and being broadly suppressed in extremely preterm infants ($\leqq$28 wks). Independent of gestational age, intrauterine growth restriction (-), histological chorioamnionitis (-), placenta deficiency (-), and antenatal steroid use (+) were found possibly related to thyroid hormone levels at birth, although the databases were modest for drawing conclusions. Respiratory distress syndrome, sepsis, chronic lung disease, necrotizing enterocolitis, and intraventricular hemorrhage were all found to be associated with low thyroid hormone levels during the postnatal period, but the causal effect directions were uncertain.

Previous observational studies have suggested that neonatal transient hypothyroxinemia of prematurity might impose greater risks of adverse neurodevelopmental outcomes, such as delayed cognitive or motor development, school failure, and cerebral palsy. Follow-up results from randomized clinical trials have suggested that thyroxine supplement might improve neurodevelopmental outcomes in preterm infants with neonatal transient hypothyroxinemia. As yet, however, the benefits of such treatment in terms of the survival, respiratory maturation, and decreased morbidities/illnesses remain uncertain.

Table 1.1 Percentages of transient hypothyroxinemia of prematurity.

Author, year	Definition of THOP	Time of testing	N	Gestational age (wk)	Percentage (%)
Diamond, 1979	Serum T4 < 4.8 μg/dl	Day 3-5	64	< 37	44
Hadeed, 1981	Cord T4 < 6.5 μg/dl	Cord blood	40	28-30	52
			59	31-33	33
			92	34-36	12
			215	28-36	25
Uhrmann, 1981	Serum T4 < 3 μg/dl	Cord blood	18	28-36	11
		Day 1	32		3
		Day 2	39		5
		Day 3	31		16
		Day 7	50		18
		Day 14	46		11
		Day 21	44		2
Romagnoli, 1982	Serum T4 < 6 μg/dl	Day 3-60	36	≦ 33	69.4
			64	34-36	40.6
			110	≧ 37	17.2
Mercado, 1987	Serum T4 < 5 μg/dl	Week 1	292	-	6.5
Mercado, 1988	Serum T4 < 5.1 μg/dl	Day 0	108	23-31	58
		Day 1			67
		Day 3			83
		Week 1			84
		Week 3			63
		Week 4			49
		Week 5			48
		Week 6			36
Den Ouden, 1995	Serum T4 < 4.7 ng/dl (3SD below mean)	Week 1	717	<26	83.3
				26-27	70.8
				28-29	44.1
				30-31	24.1
				≧32	15.3
				Total	32
Lucas, 1996	Serum T3 < 19.5 ng/dl	Week 1	280	30.2 ± 0.2	21.8
Reuss, 1997	Serum T4 < 4 μg/dl	Week 1	711	23	40
				24	31
				25	31
				26	25
				27	15
				28	10.2
Paul, 2000	Serum T4 < 10th p of babies screened on that day & TSH < 25 μIU/ml	Week 1	247	< 1500 g	91
		Week 2-4			59
Rabin, 2004	Serum FT4 < 0.8 ng/dl with normal TSH level	Day 7-14	114	≦ 27	16
				28-30	4
				31-33	0
				Total	7.9

Table 1.2 Summary of studies reviewed in tables 1.3-1.11.

Author, year	Time when blood specimens collected	N	Gestational age (wk)	Hormones tested	Technique if FT4 tested
Adams, 1995	Cord blood or Week (W) 1	174	25-36	FT4	Direct equilibrium dialysis
Belet, 2003	Cord blood; Day (D) 1, 3, 7, 21	62	29-37	FT4, T4, FT3, T3, TSH, TBG	Enzyme Immunoassay (EIA)
Biswas, 2002	< 5 Hour (Hr); D 1, 2, 3, 7, 10, 14	128	23-29	FT4, TT4, FT3, TT3, TSH	Radioimmunoassay (RIA)
Bongers-Schokking, 1984	Cord blood; Hr 3-4, 24-30; D 6-9, 13-20	107	29-43	T4, T3, TSH, FT3I	-
Carrascosa, 2004	Cord blood; Hr 1, 24; D 7, 21; Month (M) 2, 4, 6, 12	75	30-35	FT4, T4, T3, rT3, TSH	Immunochemo-luminescence
Chen, 1994	D 1, 5	80	27-41	FT4, T4, T3, TSH	EIA
Cuestas, 1979	D 30, 60, 90	48	30-35	T4, T3, FT4I	-
De Felice, 2005	D 4, 40	155	29 ± 2.4	T4 & TSH	-
Dembinski, 2001	D 14-21, 35-49	92	23-36	FT4 & TSH	Microparticle EIA
Eggermont, 1984	D 0, 10, 20, 30, 40; M 4, 7, 12	38	< 31	FT4, T4, FT3, T3, rT3, TSH, TBG, TG	FT4 immunophase technique
Etling, 1983	D 1, 5	29	26-41	T4, T3, TSH, rT3, TBG	-
Fetter, 1998	Cord blood; D 1, 3, 5, 7, 14, 21	28	28-33	FT4, T3, rT3, TSH	RIA
Fuse, 1990	D 4-5; weekly until postnatal age 40 wk	26	23-39	FT4, FT3, TSH	RIA
Hadeed, 1981	Cord blood; D 5; W 2, 4, 24, 52	54	8-35	FT4, TT4, TSH	RIA
Harkavy, 1991	W 1, 2, 3, 4, > 4	75	25-42	FT4, T4, T3	RIA
Hashimoto, 1991	TRH test between D 10-20	26	30-40	FT4, T4, T3, TSH	RIA
Hirano, 1985	Cord blood; D 2; W 1, 2	36	27-41	FT4, T4, TSH, TBG	RIA
Huang, 2002	D 9-22	54	24-37	FT4, T4, TSH	Solid phase RIA
Hume, 2004	Cord blood	428	15-20[1] 23-42	FT4, T4, T3, rT3, T4 sulfate (T4S), TSH, TBG	Vitros ECi technology
Ishaik, 2000	W 2, 40	15	30-35	FT4, TT4, TSH	Immuno 1 Bayer method
Jacobsen, 1979	D 7-13, 14-29, 30-49, 50-79, 80-119, 120-240	306	25-42	T4, T3, TSH, TBG	-
John, 1987	D 1-3 or 4-10	104	28-41	FT4, FT3	Amerlex analogue method
Martin, 2001	First week (newborn screening)	719	23-30	TT4	-
Martin, 2005	First week (newborn screening)	521	23-28	TT4, TSH	-

Table 1.2 Summary of studies reviewed in tables 1.3-1.11. (cont'd)

Author, year	Blood specimens	N	Gestational age (wk)	Hormone tested	Technique if FT4 tested
Mercado, 1988	At birth; D 1, 3, 7, 21, 28, 35, 42	108	23-31	T4, T3, TSH, TBG	-
Murphy, 2004	Cord blood; Hr 1, 7, & 24	72	24-34	FT4, T4, T3, rT3, T4S, TSH, TBG	Vitros ECi technology
Pavelka, 1997	Cord blood; D 1, 3, 7, & 14	61	22-32	T4, T3, rT3, TSH	-
Paul, 2000	D 5, 14-28	247	23-35	TT4, TSH	-
Rabin, 2004	Cord blood or D 7-14	114	< 33	FT4, TSH	Direct equilibrium dialysis
Reuss, 1997	First week (newborn screening)	365	< 32	TT4, TSH	-
Reuss, 1997	First week (newborn screening)	919	< 29	TT4, TSH	-
Romagnoli, 1982	Cord blood; D 3, 10, 20, 40, 60	352	30-41	T4, TSH	-
Rooman, 1996	D 1 & 14	263	26-41	FT4, TSH	RIA
Simpson, 2005	Cord blood; D 1, 7, 14, 28	441	23-34	FT4, T4, T3, rT3, T4S, TSH, TBG	Vitros ECi technology
Uhrmann, 1978	Cord blood; D 1, 2, 3, 7, 14, 21	35	31-34	T4, T3, TSH, rT3, TBG	-
Uhrmann, 1981	Cord blood; D 1, 2, 3, 7, 14, 21	54	29-36	T4, TSH	-
Van Wassenaer, 1997	Cord blood; D 1, 3; W 1, 2, 3, 4, 5, 6, 8	100	< 30	FT4, T4, T3, rT3, TSH, TBG	RIA
Williams, 2005	Cord blood;	620	23-42	FT4, T4, T3, rT3, TSH, T4S, TBG	Vitros ECi technology

1 Samples obtained from induced abortion subjects.

Table 1.3 Cord FT4 (pmol/l) levels (mean ± SD) by gestational age groups reported by different studies.

Author, year	Gestational age (wk)				
	23-27	28-30	31-34	35-36	37-42
Carrascosa, 2004	-	-	13.9 ± 3.7 [3]	-	17.8 ± 4.5 [6]
Hirano, 1985	-	23.4 ± 7.9 [2]	21.1 ± 5.7 [4]	28.7 ± 7.4 [5]	23.4 ± 5.9 [7]
Hume, 2004	16.5 ± 5.3	18.6 ± 5.5	19.3 ± 4.3	19.2 ± 5.2	17.6 ± 3.0
Murphy, 2004	15.9 ± 3.3 [1]	17.9 ± 4.8	18.6 ± 2.1	-	-

Table 1.4 Cord TT4 (nmol/l) levels (mean ± SD) by gestational age groups reported by different studies.

Author, year	Gestational age (wk)				
	23-27	28-30	31-34	35-36	37-42
Carrascosa, 2004	-	-	127.7 ± 39.9 [3]	-	139 ± 27 [6]
Hirano, 1985	-	85.8 ± 32.5 [2]	81.9 ± 23.4 [4]	124.8 ± 27.8 [5]	127.4 ± 26.0 [7]
Hume, 2004	69.6 ± 25.5	81.2 ± 26.4	97.0 ± 29.0	112.8 ± 37.5	116.5 ± 20.6
Mercado, 1988 [8]	49 ± 19 [9]	66 ± 22 [10]	-	-	-
Murphy, 2004	65 ± 22 [1]	70 ± 13	98 ± 22	-	-
Uhrmann, 1981	-	-	79.8 [11] (range 20.6-126.1)	-	-

Table 1.5 Cord TSH (mU/l) levels (mean ± SD) by gestational age groups reported by different studies.

Author, year	Gestational age (wk)				
	23-27	28-30	31-34	35-36	37-42
Carrascosa, 2004	-	-	6.63 ± 3.76 [3]	-	-
Hirano, 1985	-	10.9 ± 1.2 [2]	14.8 ± 8.5 [4]	6.4 ± 3.0 [5]	9.6 ± 2.8 [7]
Hume, 2004	6.8 ± 2.9	7.0 ± 3.73	8.0 ± 5.12	7.6 ± 5.88	6.4 ± 4.85
Mercado, 1988 [8]	16 ± 8 [9]	16 ± 7 [10]	-	-	-
Murphy, 2004	4.2 ± 1.9 [1]	4.8 ± 1.9	5.8 ± 3.1	-	-

1 GA 24-27 wk; 2 GA 29.6 ± 1.2 wk; 3 GA 30-35 wk; 4 GA 31.6 ± 1.2 wk; 5 GA 33.7 ± 1.4 wk; 6 GA term infants; 7 GA 39.2 ± 1.0 wk.
8 Hormones measured at birth, not cord blood; 9 GA 23-28 wk; 10 GA 28.1-31; 11 GA 32.6 ± 3.2 wk;

Table 1.6 Postnatal serum FT4 (nmol/l) levels (mean ± SD) over postnatal age by gestational weeks in moderately premature infants (29-36 completed weeks of GA).

Postnatal age at testing	Author, year	Gestational age (wk)			
		28-30	31-34	35-36	> 36
Hour 1	Carrascosa, 2004	-	18.1 ± 5.9 [3]	-	-
	Murphy, 2004	21.6 ± 5.9	26.8 ± 8.9	-	-
Hour 7	Murphy, 2004	25.8 ± 9.6	27.1 ± 7.8	-	-
Day 1	Carrascosa, 2004	-	24.2 ± 5.9 [3]	-	-
	Chen, 1994	16.2 ± 4.9 [1]	-	16.9 ± 5.3 [6]	16 ± 3.9 [11]
	Murphy, 2004	26.9 ± 7.8	29.1 ± 4.2	-	-
	John, 1987	-	-	15.9 ± 4.4 [7]	27.4 ± 7.0 [12]
Day 5	Chen, 1994	11.8 ± 2.6 [1]	-	15.4 ± 4.0 [6]	16 ± 3.3 [11]
Week 1	Carrascosa, 2004	-	17.9 ± 4.0 [3]	-	-
	Adams, 1995	25.8 ± 9.0	30.9 ± 9.0 [4]	36 ± 10.3 [8]	46.9 ± 10.6 [13]
	Harkavy, 1991	-	-	21.5 ± 7.2 [9]	25.9 ± 7.9 [14]
	Hirano, 1985	13.9 ± 4.0 [2]	19.4 ± 5.6 [5]	22.5 ± 5.9 [10]	31.6 ± 3.3 [15]
	John, 1987	-	-	17.8 ± 8.1 [7]	21.7 ± 4.3 [12]
Week 2	Harkavy, 1991	-	-	22.3 ± 5.6 [9]	25.9 ± 5.7 [14]
	Hirano, 1985	13.5 ± 4.5 [2]	24.2 ± 10.0 [5]	26.8 ± 4.8 [10]	38.8 ± 3.8 [15]
Week 3	Carrascosa, 2004	-	16.3 ± 3.3 [3]	-	-

1 GA 27-31; 2 GA 29.6±1.2; 3 GA 30-35; 4 GA 31-33 5 GA 31.6±1.2; 6 GA 32-36; 7 GA 29-36; 8 GA 34-36; 9 25-37, 38-42; 10 GA 33.7±1.4; 11 GA 38-41; 12 GA 37-41; 13 GA 37-42; 14 38-42; 15 GA 39.2±1.0.

Table 1.7 Postnatal serum T4 (nmol/l) levels (mean ± SD) over postnatal age by gestational weeks in moderately premature infants (29-36 completed weeks of GA).

Postnatal age at testing	Author, year	Gestational age (wk)			
		28-30	31-34	35-36	> 36
Hour 1	Carrascosa, 2004	-	145.8 ± 42.5 [4]	-	-
	Murphy, 2004	73 ± 21	98 ± 23	-	-
Hour 7	Murphy, 2004	83 ± 23	125 ± 39	-	-
Day 1	Etling, 1983 [1]	-	74.6 ± 20.6	114.5 ± 10.3	141.6 ± 14.1 [12]
	Mercado, 1988	63 ± 25	-	-	-
	Chen, 1994	106.8 ± 27 [2]	-	112 ± 29.6 [8]	130 ± 24.5 [13]
	Murphy, 2004	84 ± 24	125 ± 38	-	-
	Carrascosa, 2004	-	193.6 ± 49.7 [4]	-	-
	Uhrmann, 1978 [1]	-	124.8 ± 14.1 [5]	-	-
Day 3	Romagnoli, 1982 [1]	-	104.2 ± 7.7 [6]	121 ± 5.1 [9]	149.3 ± 5.1 [14]
	Mercado, 1988	57 ± 26	-	-	-
Day 5	Etling, 1983 [1]	-	75.9 ± 16.7	109.4 ± 9	133.8 ± 10.3 [12]
	Chen, 1994	72.1 ± 16.7 [2]	-	103 ± 32.2 [8]	126.1 ± 38.6 [13]
Week 1	Mercado, 1988	53 ± 26	-	-	-
	Carrascosa, 2004	-	162.4 ± 42.2 [4]	-	-
	Harkavy, 1991	-	-	102 ± 52 [10]	163 ± 62 [15]
	Hirano, 1985	33.8 ± 14.3 [3]	71.5 ± 22.1 [7]	96.2 ± 35.1 [11]	159.9 ± 10.4 [16]
	Uhrmann, 1978 [1]	-	114.5 ± 12.9 [5]	-	-
Day 10	Romagnoli, 1982 [1]	-	87.5 ± 6.4 [6]	106.8 ± 5.1 [9]	127.4 ± 3.9 [14]

1 mean ± sem; 2 GA 27-31; 3 GA 29.6±1.2; 4 30-35 wk; 5 GA 30-34; 6 GA ≦33; 7 GA 31.6 ± 1.2; 8 GA 32-36; 9 GA 34-36; 10 GA 25-37; 11 GA 33.7 ± 1.4; 12 Small for gestational age term infants; 13 GA 38-41; 14 GA≧37; 15 GA 38-42; 16 GA 39.2 ± 1.0.

Table 1.7 Postnatal serum T4 (nmol/l) levels (mean ± SD) over postnatal age by gestational weeks in moderately premature infants (29-36 completed weeks of GA). (cont'd)

Postnatal age at testing	Author, year	Gestational age (wk)			
		28-30	31-34	35-36	> 36
Week 2	Uhrmann, 1978 [1]	-	121 ± 11.6 [5]	-	-
	Harkavy, 1991	-	-	98 ± 39 [10]	151 ± 65 [15]
	Hirano, 1985	29.9 ± 2.6 [3]	88.4 ± 18.2 [7]	98.8 ± 23.4 [11]	182.0 ± 23.4 [16]
Week 3	Carrascosa, 2004	-	134.1 ± 27.8 [4]	-	-
	Mercado, 1988	70 ± 24	-	-	-
	Uhrmann, 1978 [1]	-	121 ± 7.7 [5]	-	-

1 mean ± sem; 2 GA 27-31; 3 GA 29.6±1.2; 4 30-35 wk; 5 GA 30-34; 6 GA ≤33; 7 GA 31.6 ± 1.2; 8 GA 32-36; 9 GA 34-36; 10 GA 25-37; 11 GA 33.7 ± 1.4; 12 Small for gestational age term infants; 13 GA 38-41; 14 GA≧37; 15 GA 38-42; 16 GA 39.2 ± 1.0.

Table 1.8 Postnatal serum TSH (mU/l) levels (mean ± SD) over postnatal age by gestational weeks in moderately premature infants (29-36 completed weeks of GA).

Postnatal age at testing	Author, year	Gestational age (wk)			
		28-30	31-34	35-36	> 36
Hour 1	Carrascosa, 2004	-	40.49 ± 19.93 [4]	-	-
	Murphy, 2004	19.9 ± 15.1	22.7 ± 9.2	-	-
Hour 7	Murphy, 2004	7.4 ± 5.3	9.8 ± 6.8	-	-
Day 1	Etling, 1983 [1]	-	12.2 ± 2.8	11.8 ± 1.5	20.0 ± 5.6 [13]
	Mercado, 1988	13 ± 5	-	-	-
	Chen, 1994	9.8 ± 6.5 [2]	-	18.4 ± 11.0 [9]	22.6 ± 14.1 [14]
	Murphy, 2004	4.5 ± 2.6	7.3 ± 4.8	-	-
	Carrascosa, 2004	-	12.38 ± 6.13 [4]	-	-
	Uhrmann, 1978 [1]	-	10.5 ± 3.1 [5]	-	-
Day 3	Romagnoli, 1982 [1]	-	14.8 ± 2.1 [6]	14.7 ± 1.8 [10]	16.6 ± 1.4 [15]
	Mercado, 1988	10 ± 6	-	-	-
Day 5	Chen, 1994	8.6 ± 8.0 [2]	-	7.4 ± 4.4 [9]	10.7 ± 5.0 [14]
	Etling, 1983 [1]	-	9.1 ± 2.4	5.8 ± 1.2	5.4 ± 0.9 [13]
Week 1	Carrascosa, 2004		4.56 ± 2.41 [4]	-	-
	Mercado, 1988	12 ± 6	-	-	-
	Adams, 1995	10.4 ± 5.1	14.3 ± 6.8 [7]	11.4 ± 5.1 [11]	20 ± 9.5 [16]
	Hirano, 1985	6.4 ± 2.9 [3]	8.7 ± 5.8 [8]	3.5 ± 3.5 [12]	6.6 ± 4.9 [17]
	Uhrmann, 1978 [1]	-	3.6 ± 0.3 [5]	-	-
Day 10	Romagnoli, 1982 [1]	-	15.1 ± 2.2 [6]	12.8 ± 1.5 [10]	11.5 ± 1.2 [15]

1 mean ± sem; 2 GA 27-31; 3 GA 29.6 ± 1.2; 4 GA 30-35; 5 GA 30-34; 6 GA ≦33; 7 GA 31-33; 8 GA 31.6 ± 1.2; 9 GA 32-36; 10 GA 34-36; 11 GA 34-36; 12 GA 33.7 ± 1.4; 13 Small for gestational age term infants; 14 GA 38-41; 15 GA≧37; 16 GA 37-42; 17 GA 39.2 ± 1.0

Table 1.8 Postnatal serum TSH (mU/l) levels (mean ± SD) over postnatal age by gestational weeks in moderately premature infants (29-36 completed weeks of GA). (cont'd)

Postnatal age at testing	Author, year	Gestational age (wk)			
		28-30	31-34	35-36	> 36
Week 2	Hirano, 1985	10.4 ± 5.9 [3]	9.6 ± 3.9 [8]	2.5 ± 1.3 [12]	6.0 ± 0.9 [17]
	Uhrmann, 1978 [1]	-	3.4 ± 0.2 [5]	-	-
Week 3	Mercado, 1988	10 ± 4	-	-	-
	Carrascosa, 2004	-	3.39 ± 1.73 [4]	-	-
	Uhrmann, 1978 [1]	-	3.3 ± 0.2 [5]	-	-

1 mean ± sem; 2 GA 27-31; 3 GA 29.6 ± 1.2; 4 GA 30-35; 5 GA 30-34; 6 GA ≦33; 7 GA 31-33; 8 GA 31.6 ± 1.2; 9 GA 32-36; 10 GA 34-36; 11 GA 34-36; 12 GA 33.7 ± 1.4; 13 Small for gestational age term infants; 14 GA 38-41; 15 GA≧37; 16 GA 37-42; 17 GA 39.2 ± 1.0

Table 1.9 Postnatal serum FT4 (pmol/l) levels (mean ± SD) over postnatal age by gestational weeks in extremely premature infants (≦ 28 completed weeks of GA).

Postnatal age at testing	Author, year	Gestational age (wk)
		24 - 27
Hour 1	Murphy, 2004	21.8 ± 4.5
Hour 7	Murphy, 2004	20.1 ±3.8
Day 1	Murphy, 2004	19.1 ± 7.1
	Biswas, 2002 [1]	12.6 ± 0.4 [2]
Week 1	Adams, 1995	18 ± 5.2 [3]
	Biswas, 2002 [1]	9.7 ± 0.5 [2]
Week 2	Biswas, 2002 [1]	11.0 ± 0.4 [2]

1 mean ± sem; 2 GA 23-29 wk; 3 GA 25-27 wk.

Table 1.10 Postnatal serum T4 (nmol/l) levels (mean ± SD) over postnatal age by gestational weeks in extremely premature infants (≦ 28 completed weeks of GA).

Postnatal age at testing	Author, year	Gestational age (wk)					
		≦ 23		24 - 27			~ 28
Hour 1	Murphy, 2004			67 ± 27			
Hour 7	Murphy, 2004			64 ± 24			
	Biswas, 2002 [1]			83.5 ± 4.9 [2]			
Day 1	Mercado, 1988			40 ± 21			
	Murphy, 2004			59 ± 27			
Day 3	Mercado, 1988			35 ± 19			
Week 1	Biswas, 2002 [1]			68.2 ± 4.1 [2]			
	Mercado, 1988			29 ± 16			
Week 2	Biswas, 2002 [1]			74.8 ± 4.2 [2]			
Week 3	Mercado, 1988			50 ± 23			
		≦ 23	**~ 24**	**~ 25**	**~ 26**	**~ 27**	**~ 28**
Week 1	Reuss, 1997	65.6 ± 32.2	75.9 ± 36.0	75.9 ± 42.5	79.8 ± 37.3	92.7 ± 38.6	100.4 ± 38.6

1 mean ± sem; 2 GA 23-29 wk;

Table 1.11 Postnatal serum TSH (mU/l) levels (mean ± SD) over postnatal age by gestational weeks in extremely premature infants (≦ 28 completed weeks of GA).

Postnatal age at testing	Author, year	Gestational age 24-27 weeks
Hour 1	Murphy, 2004	8.3 ± 7.2
Hour 7	Murphy, 2004	3.6 ± 1.3
	Biswas, 2002 [1]	2.6 ± 0.3 [2]
Day 1	Mercado, 1988	16 ± 6
	Murphy, 2004	2.3 ± 1.5
Day 3	Mercado, 1988	11 ± 5
	Adams, 1995	15.3 ± 7.5 [3]
Week 1	Biswas, 2002 [1]	4.3 ± 0.6 [2]
	Mercado, 1988	12 ± 7
Week 2	Biswas, 2002 [1]	3.3 ± 0.4 [2]
Week 3	Mercado, 1988	11 ± 4

1 mean ± sem; 2 GA 23-29 wk; 3 GA 25-27 wk.

Table 1.12 Thyroid hormone levels (mean ± sem) by prenatal factors that are significantly correlated with newborn thyroid hormone levels at birth.

Author, year	Postnatal age at testing / Hormone	Gestational age (wk)	Prenatal exposure variable		
			IUGR +	IUGR -	p-value
Romagnoli, 1982	Day 3 / T4 µg/dl	≦ 33 wk	4.6 ± 0.5	8.3 ± 0.7	p < 0.05
		34-36 wk	8.9 ± 1.1	9.6 ± 0.5	n.s.
		≧ 37 wk	11.9 ± 0.4	10.8 ± 0.7	n.s.
			Placenta deficiency +	**Placenta deficiency -**	p-value
Belet, 2003	Cord / FT4 ng/dl		0.9 ± 0.2	1.2 ± 0.2	p < 0.05
	Cord / T4 µg/dl		8.7 ± 0.4	9.7 ± 0.5	n.s.
	Cord / TSH µIU/ml		8.4 ± 0.6	5.7 ± 0.5	p < 0.05
			Histological chorioamnionitis +	**Histological chorioamnionitis -**	OR, 95% CI
De Felice, 2005	Day 4 / TT4 µg/dl		2.91 ± 1.25	6.58 ± 2.29	32, 8.9-115.6
Martin, 2005	Day 4 / TT4 µg/dl		6.6 (median)	6.2 (median)	p = 0.02 [1]

Table 1.13 Comparison between two studies on the association between histological chorioamnionitis and THOP.

Features	De Felice, 2005	Martin, 2005
Gestational age (wk)	29.36 ± 2.39	23-28
Birthweight (g)	1072 ± 256	500-1,000
Sample size (HCA +, %)	155 (98, 63.2%)	300 (200, 66.7%)
Definition of HCA	≧10 polymorphonuclear leukocytes per field in 10 non-adjacent 400-power fields in membranes and/or placental chorionic plate.	Polymorphonuclear cells in the subchorion, chorion, or amnion.
Exclusion criteria		
Death prior to the first week of life	Yes	Yes
Congenital hypothyroidism	Yes	No
Symptomatic chorioamnionitis	Yes	No
IUGR	No	Yes
Maternal thyroid disease	No	Yes
No sample for the first week of life	n/a	Yes

Table 1.14 Association between neonatal TSH (mU/l) levels (mean ± sem) and postnatal exposures in preterm infants.

Author, year	Postnatal age at testing	Exposure variable		p-value
		Hyaline membrane disease +	**Hyaline membrane disease -**	
Mercado, 1998	At birth	19 ± 17	17 ± 8	n.s.
	Day 1	15 ± 6	13 ± 5	n.s.
	Day 3	11 ± 5	10 ± 6	n.s.
	Week 1	12 ± 7	12 ± 6	n.s.
	Week 3	11 ± 4	10 ± 5	n.s.
		Respiratory distress syndrome +	**Respiratory distress syndrome -**	
Romagnoli, 1982	Day 3	18.8 ± 2.79	16.41 ± 2.15	n.s.
	Day 10	16.46 ± 2.15	13.30 ± 2.11	n.s.
		Sepsis +	**Sepsis -**	
	Day 3	20.85 ± 4.31	16.15 ± 3.12	n.s.
	Day 10	18.1 ± 4.14	15.67 ± 3.43	n.s.

Table 1.15 Association between neonatal T4 (nmol/l) levels (mean ± sem) and postnatal exposures in preterm infants.

Author, year	Postnatal age at testing	Gestational age (wk)	Exposure variable		p-value
			Hyaline membrane disease +	**Hyaline membrane disease -**	
Mercado, 1998	At birth		33 ± 18	74 ± 23	p < 0.002
	Day 1		48 ± 19	75 ± 28	p < 0.001
	Day 3		40 ± 22	65 ± 30	p < 0.002
	Week 1		37 ± 23	52 ± 28	p < 0.001
	Week 3		59 ± 22	70 ± 29	p < 0.05
			Respiratory distress syndrome +	**Respiratory distress syndrome -**	
Romagnoli, 1982	Day 3 / T4 µg/dl	≦33	5.03 ± 0.48	9.38 ± 1.06	P < 0.001
		34-36	6.13 ± 0.74	10.64 ± 0.76	P < 0.001
		≧37	12.72 ± 1.43	13.70 ± 1.97	n.s.
		Total	6.95 ± 0.65	10.71 ± 0.71	P < 0.001
	Day 10 / T4 µg/dl	≦33	5.70 ± 0.77	7.17 ± 0.78	P < 0.05
		34-36	6.49 ± 0.69	8.47 ± 0.70	P < 0.05
		≧37	9.94 ± 0.25	11.62 ± 1.33	n.s.
		Total	6.84 ± 0.52	8.53 ± 0.56	P < 0.05
			Sepsis +	**Sepsis -**	
	Day 3 / T4 µg/dl	≦33	5.59 ± 2.2	8.65 ± 3.28	P < 0.02
		34-36	9.45 ± 3.76	12.46 ± 3.67	P < 0.01
		≧37	11.09 ± 3.03	11.97 ± 1.92	n.s.
		Total	8.24 ± 0.97	11.69 ± 1.05	P < 0.05
	Day 10 / T4 µg/dl	≦33	4.89 ± 2.83	7.06 ± 1.13	P < 0.05
		34-36	5.69 ± 2.87	8.15 ± 1.44	P < 0.01
		≧37	8.88 ± 1.63	8.68 ± 0.94	n.s.
		Total	5.81 ± 0.78	7.83 ± 0.36	P < 0.05

Table 1.16 Adverse neurodevelopmental outcomes of children with THOP.

Author, year	Sample size	GA (wk) or BW (g)	THOP Definition
Lucas 1988, 1996	236	GA 30.2 ± 0.2 or BW<1,850	T3 < 0.3 nmol/l
Meijer, 1992 and Den Ouden, 1996	563 (age 2); 640 (age 5); 552 (age 9)	GA<32 & BW<1,500	T4 < 3 SD below mean
Reuss, 1996	466	BW < 2,000	T4 < 2.6 SD below mean

Table 1.16 Adverse neurodevelopmental outcomes of children with THOP. (cont'd)

Author, year	THOP cognitive outcomes (early age)	THOP IQ (older age)	Other THOP neurological abnormalities
Lucas 1988, 1996	8.3-point MDI [4] ↓ at 18m.	WISC [5] 6.6-point ↓ at age 7.5-8.	PDI [8] 7.4-point ↓ at 18 m.
Meijer, 1992 and Den Ouden, 1996 [2]	2.97-fold risk of failing national screening test.	School failure (OR=1.3, 95% CI 1.1-1.6) at age 9.	Neurologic dysfunction (OR=1.3, 95% CI 1.1-1.6) at age 5.
Reuss, 1996 [3]	6.8-point MDI ↓ at 24m.	8.6-point S-B [6] ↓ and 7.1-point TVPS [7] ↓ at age 6.	3.6-4.4 fold risk ↑ of CP at age 2; 4.9-fold risk of unable to walk at age 6.

1 adjusted for multiple potential confounders, including several measures of illness severity; 2 adjusted for gestational age and perinatal factors;
3 adjusted for 21 potential confounders in their analyses; 4 MDI: Bayley Scales of Infant Development - mental development index;
5 WISC: Wechsler Intelligence Scales for Children; 6 S-B: Standford-Binet, 4th edition;
7 TVPS: Test of Visual Perceptual Skills; 8 PDI: Bayley Scales of Infant Development - psychomotor development index.

Table 1.17 Study designs of six randomized clinical trials of thyroxine supplement treatment.

Author, year	N (treatment/ placebo)	Gestational age (wk)	Dosage	Treatment duration
Amato, 1988	18/18	29 - 34	50 μg T4	2 doses at 1 and 24 hr
Amato, 1989	22/22	< 32	25 μg T3	Twice a day × 2 days
Chowdry, 1984	12/11	25 - 28	10 μg/kg T4 [3]	6-7 weeks
Schonberger, 1981	45/55 [1]	< 37 or < 2,200 g	25 μg T4 + 5 μg T3	Daily dose for entire neonatal stay
VanHole, 1997	20/20 [2]	25 - 30	20 μg/kg T4	2 weeks
Van Wassenaer, 1997	100/100	< 30	8 μg/kg T4	6 weeks

1 Five subjects who were assigned to receive treatment was inadvertently treated with placebo and resulted in 45/55 ratio;
2 Each group had 3 patients died within the first week;
3 Thyroxine dose was increased to 15 μg/kg when T4 did not rise after the first week of treatment.

Table 1.18 Findings of six randomized clinical trials treating THOP infants with thyroxine supplement.

Author, year	Bayley exam (effect of treatment)	Other effects of treatment
Amato, 1988	-	No effect on peak FIO2
Amato, 1989	-	Peak FIO2 ↓ 11.4% ($p<0.05$)
Chowdry, 1984	n.s. (12 & 24 months)	No effect on growth
Schonberger, 1981	-	Length of mechanical ventilation/CPAP ↓ ($p<0.05$)
VanHole, 1997	n.s. (7 months)	No effect on heart rate, FIO2, and weight gain.
Van Wassenaer, 1997	n.s. (24 months); < 27 wk group: MDI ↑ 18 points ($p = 0.01$) ≧27 wk group: MDI ↓ 10 points ($p = 0.03$)	No effect on sepsis, FIO2, PDA or brain lesions.

MDI: mental development index.

Figure 1.1 The metabolism of human iodothyronines.

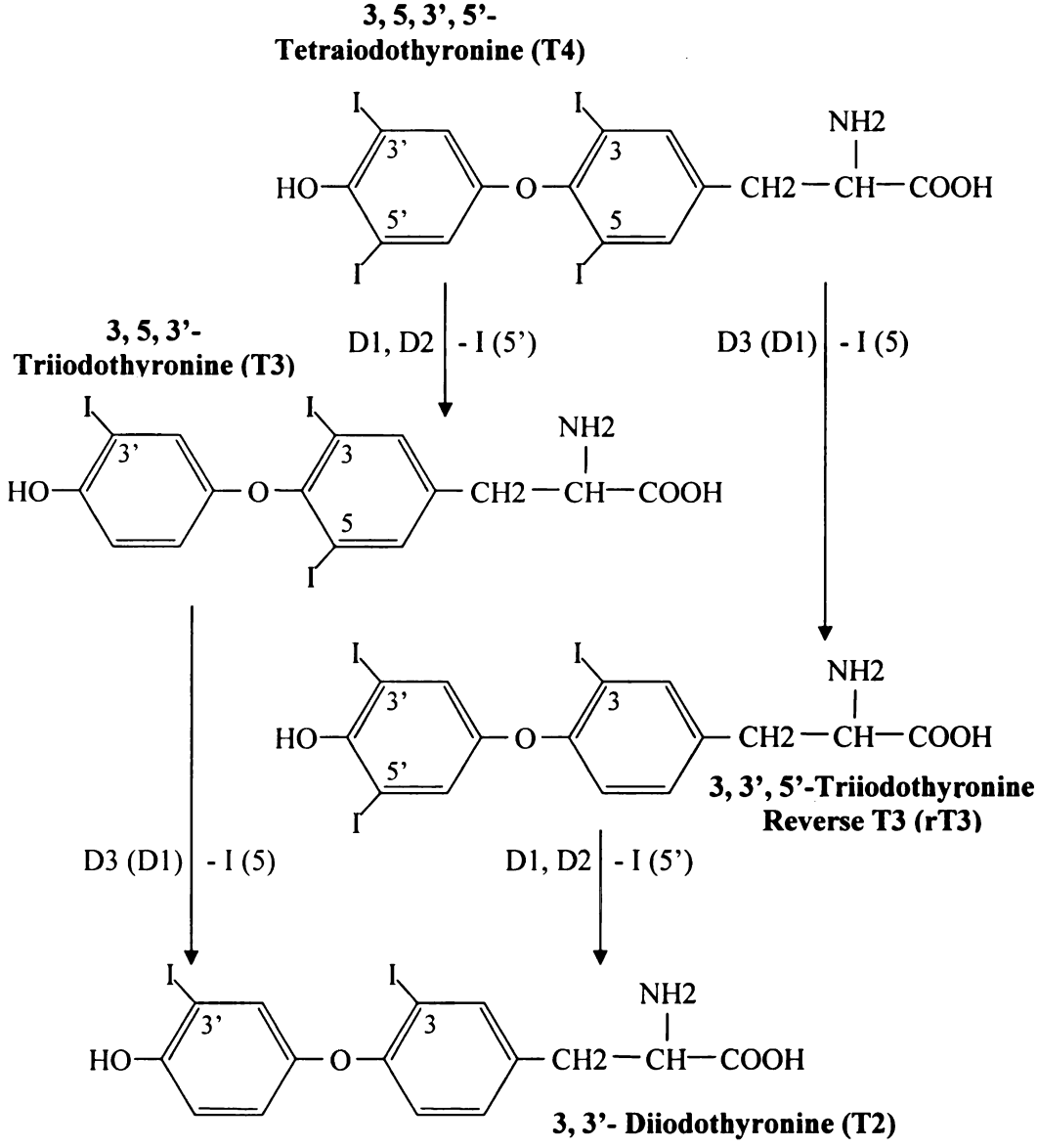


Figure 1.2 Cord serum FT4 levels by gestational age (plot based on data cited from Williams et al. 2005 (63)). (1 ng/dl = 12.87 pmol/l)

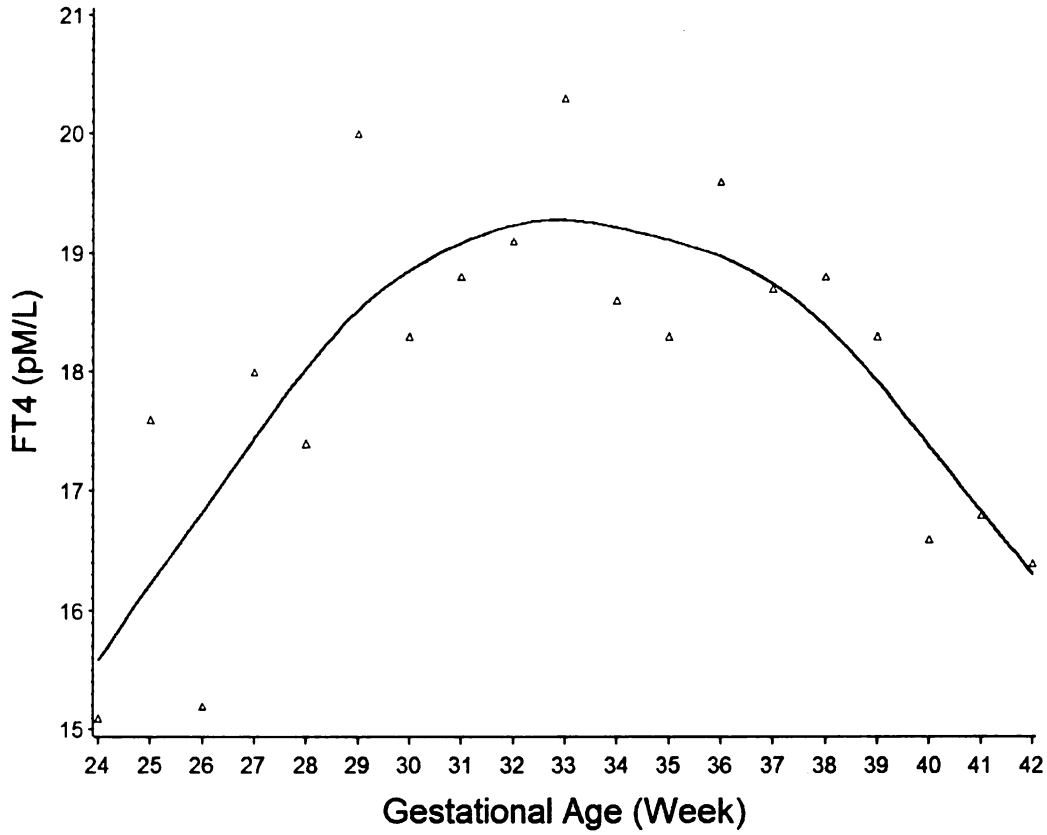


Figure 1.3 Cord serum T4 levels by gestational age (plot based on data cited from Williams et al. 2005 (63)). (1 μg/dl = 12.87 nmol/l)

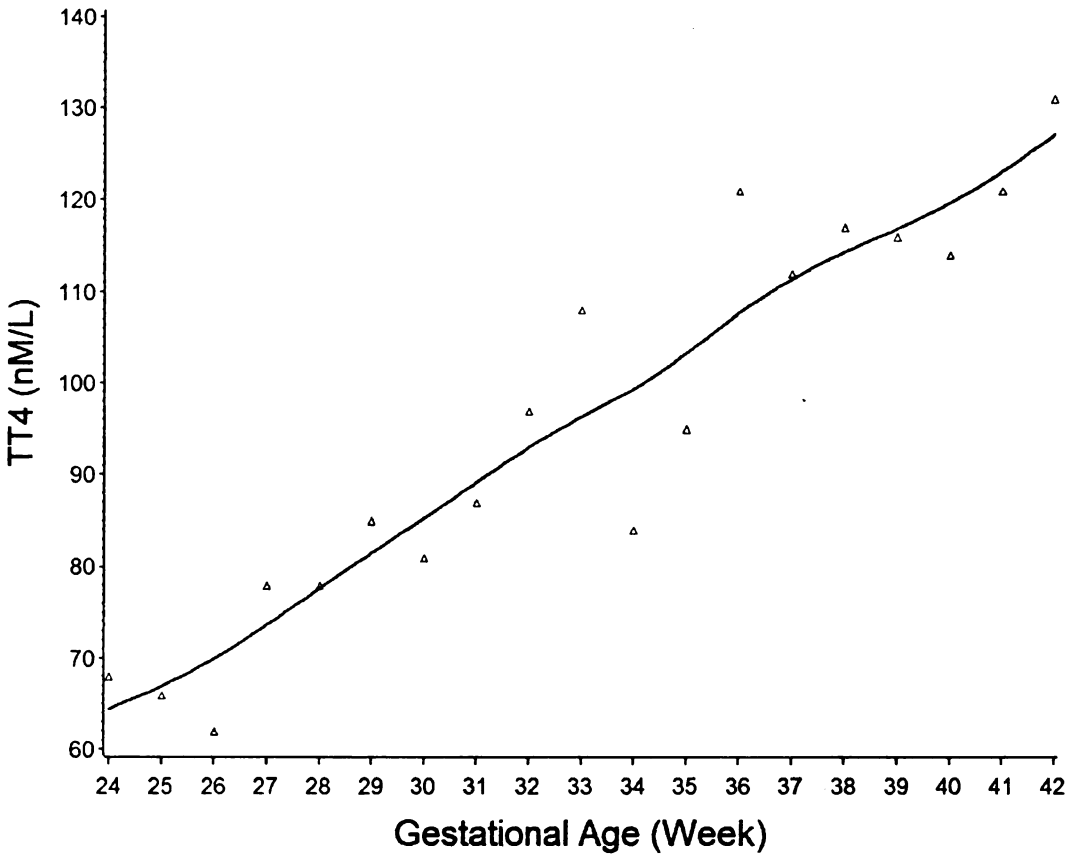
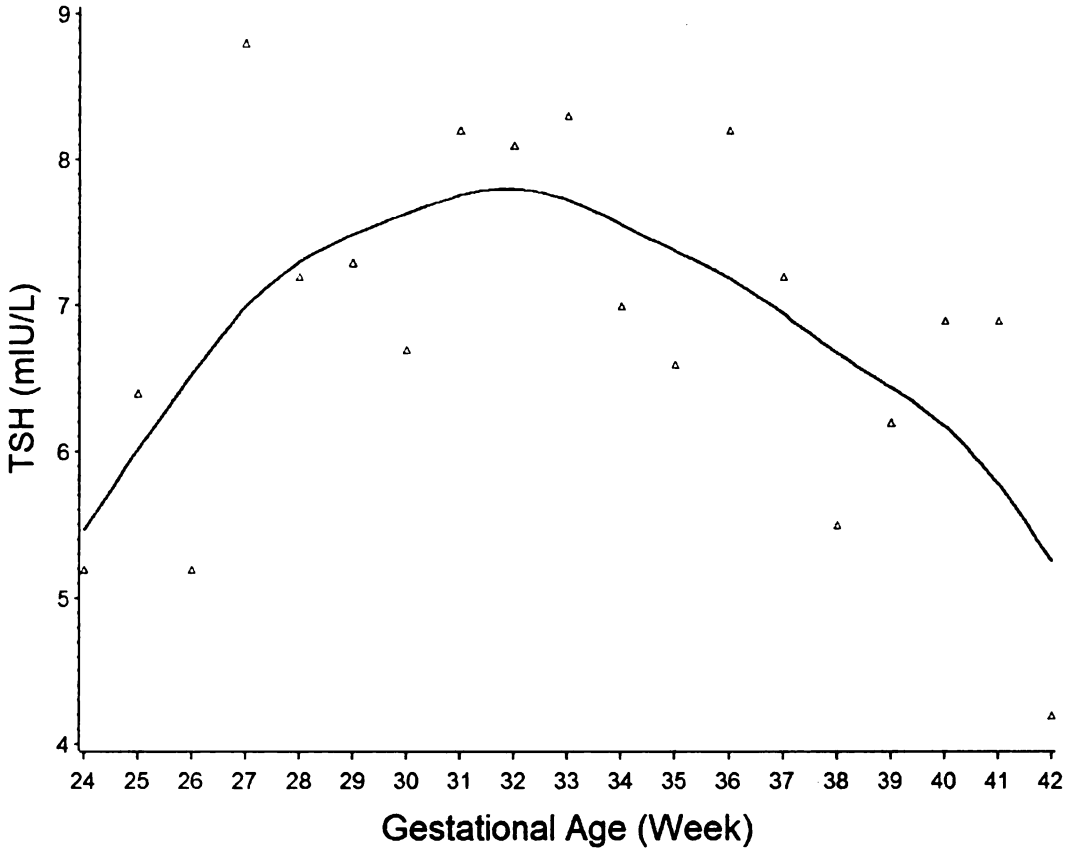


Figure 1.4 Cord serum TSH levels by gestational age (plot based on data cited from Williams et al. 2005 (63)).



BIBLIOGRAPHY

1. Larsen PR, Davies TF, Schlumberger M, Hay ID. Williams Textbook of Endocrinology. Chapter 10 THYROID physiology and Diagnostic Evaluation of Patients with Thyroid Disorders., 2002.

2. Murphy N, Hume R, van Toor H, et al. The hypothalamic-pituitary-thyroid axis in preterm infants; changes in the first 24 hours of postnatal life. J Clin Endocrinol Metab 2004;89:2824-31.

3. Pavelka S, Kopecky P, Bendlova B, et al. Tissue metabolism and plasma levels of thyroid hormones in critically ill very premature infants. Pediatr Res 1997;42:812-8.

4. Simpson J, Williams FL, Delahunty C, et al. Serum thyroid hormones in preterm infants and relationships to indices of severity of intercurrent illness. J Clin Endocrinol Metab 2005;90:1271-9.

5. Lazar MA. Williams Textbook of Endocrinology. Chapter 4 Mechanism of Action of Hormones That Act on Nuclear Receptors., 2002.

6. Campos-Barros A, Meinhold H, Walzog B, Behne D. Effects of selenium and iodine deficiency on thyroid hormone concentrations in the central nervous system of the rat. Eur J Endocrinol 1997;136:316-23.

7. Meinhold H, Campos-Barros A, Walzog B, Kohler R, Muller F, Behne D. Effects of selenium and iodine deficiency on type I, type II and type III iodothyronine deiodinases and circulating thyroid hormones in the rat. Exp Clin Endocrinol 1993;101:87-93.

8. Peeters R, Fekete C, Goncalves C, et al. Regional physiological adaptation of the central nervous system deiodinases to iodine deficiency. Am J Physiol Endocrinol Metab 2001;281:E54-61.

9. Izumi M, Larsen PR. Triiodothyronine, thyroxine, and iodine in purified thyroglobulin from patients with Graves' disease. J Clin Invest 1977;59:1105-12.

10. Larsen PR. Thyroidal triiodothyronine and thyroxine in Graves' disease: correlation with presurgical treatment, thyroid status, and iodine content. J Clin Endocrinol Metab 1975;41:1098-104.

11. Theodoropoulou A, Vagenakis AG, Makri M, Markou KB. Thyroid hormone synthesis and secretion in humans after 80 milligrams of iodine for 15 days and subsequent withdrawal. J Clin Endocrinol Metab 2007;92:212-4.

12. Ayromlooi J. Congenital goiter due to maternal ingestion of iodides. Obstet Gynecol 1972;39:818-22.

13. Carswell F, Kerr MM, Hutchison JH. Congenital goitre and hypothyroidism produced by maternal ingestion of iodides. Lancet 1970;1:1241-3.

14. Iancu T, Boyanower Y, Laurian N. Congenital goiter due to maternal ingestion of iodide. Am J Dis Child 1974;128:528-30.

15. Bostanci I, Sarioglu A, Ergin H, Aksit A, Cinbis M, Akalin N. Neonatal goiter caused by expectorant usage. J Pediatr Endocrinol Metab 2001;14:1161-2.

16. Khashu M, Chessex P, Chanoine JP. Iodine overload and severe hypothyroidism in a premature neonate. J Pediatr Surg 2005;40:E1-4.

17. Markou K, Georgopoulos N, Kyriazopoulou V, Vagenakis AG. Iodine-Induced hypothyroidism. Thyroid 2001;11:501-10.

18. Fisher DA, Dussault JH, Sack J, Chopra IJ. Ontogenesis of hypothalamic--pituitary--thyroid function and metabolism in man, sheep, and rat. Recent Prog Horm Res 1976;33:59-116.

19. Fisher DA, Klein AH. Thyroid development and disorders of thyroid function in the newborn. N Engl J Med 1981;304:702-12.

20. Brown R, Larsen PR. Thyroid Disease Manager©. Chapter 15. Thyroid Gland Development and Disease in Infancy and Childhood.: Endocrine Education, Inc., 2005.

21. Ballabio M, Nicolini U, Jowett T, Ruiz de Elvira MC, Ekins RP, Rodeck CH. Maturation of thyroid function in normal human foetuses. Clin Endocrinol (Oxf) 1989;31:565-71.

22. Contempre B, Jauniaux E, Calvo R, Jurkovic D, Campbell S, de Escobar GM. Detection of thyroid hormones in human embryonic cavities during the first trimester of pregnancy. J Clin Endocrinol Metab 1993;77:1719-22.

23. Costa A, Arisio R, Benedetto C, et al. Thyroid hormones in tissues from human embryos and fetuses. J Endocrinol Invest 1991;14:559-68.

24. Thorpe-Beeston JG, Nicolaides KH. Fetal thyroid function. Fetal Diagn Ther 1993;8:60-72.

25. Thorpe-Beeston JG, Nicolaides KH, Felton CV, Butler J, McGregor AM. Maturation of the secretion of thyroid hormone and thyroid-stimulating hormone in the fetus. N Engl J Med 1991;324:532-6.

26. Thorpe-Beeston JG, Nicolaides KH, McGregor AM. Fetal thyroid function. Thyroid 1992;2:207-17.

27. Fisher DA, Hobel CJ, Garza R, Pierce CA. Thyroid function in the preterm fetus. Pediatrics 1970;46:208-16.

28. Hashimoto H, Sato T, Horita S, Kubo M, Ohki T. Maturation of the pituitary-thyroid axis during the perinatal period. Endocrinol Jpn 1991;38:151-7.

29. Oddie TH, Fisher DA, Bernard B, Lam RW. Thyroid function at birth in infants of 30 to 45 weeks' gestation. J Pediatr 1977;90:803-6.

30. Jacobsen BB, Andersen H, Dige-Petersen H, Hummer L. Thyrotropin response to thyrotropin-releasing hormone in fullterm, euthyroid and hypothyroid newborns. Acta Paediatr Scand 1976;65:433-8.

31. Jacobsen BB, Andersen H, Dige-Petersen H, Hummer L. Pituitary-thyroid responsiveness to thyrotropin-releasing hormone in preterm and small-for-gestational age newborns. Acta Paediatr Scand 1977;66:541-8.

32. Klein AH, Hobel CJ, Sack J, Fisher DA. Effect of intraamniotic fluid thyroxine injection on fetal serum and amniotic fluid iodothyronine concentrations. J Clin Endocrinol Metab 1978;47:1034-7.

33. Vulsma T, Gons MH, de Vijlder JJ. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. N Engl J Med 1989;321:13-6.

34. Markham LA, Stevens DL. A case report of neonatal thyrotoxicosis due to maternal autoimmune hyperthyroidism. Adv Neonatal Care 2003;3:272-82; quiz 283-5.

35. Walker P, Dussault JH, Hart IR, Langelier P, Szots F. Thyrotoxicosis detected in a mass-screening program for neonatal hypothyroidism: demonstration of placental transfer of an immunoglobulin with marked lipolytic activity. J Pediatr 1977;91:400-3.

36. Fisher DA. Thyroid function in premature infants. The hypothyroxinemia of prematurity. Clin Perinatol 1998;25:999-1014, viii.

37. Hume R, Simpson J, Delahunty C, et al. Human fetal and cord serum thyroid hormones: developmental trends and interrelationships. J Clin Endocrinol Metab 2004;89:4097-103.

38. Abuid J, Klein AH, Foley TP, Jr., Larsen PR. Total and free triiodothyronine and thyroxine in early infancy. J Clin Endocrinol Metab 1974;39:263-8.

39. Abuid J, Stinson DA, Larsen PR. Serum triiodothyronine and thyroxine in the neonate and the acute increases in these hormones following delivery. J Clin Invest 1973;52:1195-9.

40. Erenberg A, Phelps DL, Lam R, Fisher DA. Total and free thyroid hormone concentrations in the neonatal period. Pediatrics 1974;53:211-6.

41. Jacobsen BB, Hummer L. Changes in serum concentrations of thyroid hormones and thyroid hormone-binding proteins during early infancy. Studies in healthy fullterm, small-for-gestational age and preterm infants aged 7 to 240 days. Acta Paediatr Scand 1979;68:411-8.

42. Polk DH. Thyroid hormone effects on neonatal thermogenesis. Semin Perinatol 1988;12:151-6.

43. Bird JA, Clarke L, Symonds ME. Influence of thyrotrophin-releasing hormone on thermoregulation in newborn lambs. Biol Neonate 1998;73:52-9.

44. Cabello G. Endocrine reactivity (T3, T4, cortisol) during cold exposure in preterm and full-term lambs. Biol Neonate 1983;44:224-33.

45. Heasman L, Clarke L, Symonds ME. Influence of thyrotropin-releasing hormone administration at birth on thermoregulation in lambs delivered by cesarean. Am J Obstet Gynecol 2000;183:1257-62.

46. Polk DH, Callegari CC, Newnham J, et al. Effect of fetal thyroidectomy on newborn thermogenesis in lambs. Pediatr Res 1987;21:453-7.

47. Gluckman PD, Sizonenko SV, Bassett NS. The transition from fetus to neonate--an endocrine perspective. Acta Paediatr Suppl 1999;88:7-11.

48. Liggins GC. The role of cortisol in preparing the fetus for birth. Reprod Fertil Dev 1994;6:141-50.

49. Nathanielsz PW. Thyroid function in the fetus and newborn mammal. Br Med Bull 1975;31:51-6.

50. Mercado M, Szymonowicz W, Yu VY, Gold H. Symptomatic hypothyroxinemia with normal TSH levels in preterm infants. Clin Pediatr (Phila) 1987;26:343-6.

51. Den Ouden AL, Kok JH, Verkerk PH, Brand R, Verloove-Vanhorick SP. The relation between neonatal thyroxine levels and neurodevelopmental outcome at age 5 and 9 years in a national cohort of very preterm and/or very low birth weight infants. Pediatr Res 1996;39:142-5.

52. Hadeed AJ, Asay LD, Klein AH, Fisher DA. Significance of transient postnatal hypothyroxinemia in premature infants with and without respiratory distress syndrome. Pediatrics 1981;68:494-8.

53. Lucas A, Morley R, Fewtrell MS. Low triiodothyronine concentration in preterm infants and subsequent intelligence quotient (IQ) at 8 year follow up. Bmj 1996;312:1132-3; discussion 1133-4.

54. Mercado M, Yu VY, Francis I, Szymonowicz W, Gold H. Thyroid function in very preterm infants. Early Hum Dev 1988;16:131-41.

55. Paul DA, Leef KH, Stefano JL, Bartoshesky L. Thyroid function in very-low-birth-weight infants with intraventricular hemorrhage. Clin Pediatr (Phila) 2000;39:651-6.

56. Rabin CW, Hopper AO, Job L, et al. Incidence of low free T4 values in premature infants as determined by direct equilibrium dialysis. J Perinatol 2004;24:640-4.

57. Reuss ML, Leviton A, Paneth N, Susser M. Thyroxine values from newborn screening of 919 infants born before 29 weeks' gestation. Am J Public Health 1997;87:1693-7.

58. Romagnoli C, Curro V, Luciano R, et al. Serial blood T4 and TSH determinations in low birth weight infants. Influence of gestational age, birth weight and neonatal pathology on thyroid function. Helv Paediatr Acta 1982;37:331-44.

59. Uhrmann S, Marks KH, Maisels MJ, Kulin HE, Kaplan M, Utiger R. Frequency of transient hypothyroxinaemia in low birthweight infants. Potential pitfall for neonatal screening programmes. Arch Dis Child 1981;56:214-7.

60. Belet N, Imdat H, Yanik F, Kucukoduk S. Thyroid function tests in preterm infants born to preeclamptic mothers with placental insufficiency. J Pediatr Endocrinol Metab 2003;16:1131-5.

61. Biswas S, Buffery J, Enoch H, Bland JM, Walters D, Markiewicz M. A longitudinal assessment of thyroid hormone concentrations in preterm infants younger than 30 weeks' gestation during the first 2 weeks of life and their relationship to outcome. Pediatrics 2002;109:222-7.

62. Fetter WP, Waals-Van de Wal CM, Van Eyck J, Samson G, Bongers-Schokking JJ. Thyroid hormone concentrations in preterm infants born to pre-eclamptic women with placental insufficiency. Acta Paediatr 1998;87:186-90.

63. Williams FL, Mires GJ, Barnett C, et al. Transient hypothyroxinemia in preterm infants: the role of cord sera thyroid hormone levels adjusted for prenatal and intrapartum factors. J Clin Endocrinol Metab 2005;90:4599-606.

64. Adams LM, Emery JR, Clark SJ, Carlton EI, Nelson JC. Reference ranges for newer thyroid function tests in premature infants. J Pediatr 1995;126:122-7.

65. Carrascosa A, Ruiz-Cuevas P, Potau N, et al. Thyroid function in seventy-five healthy preterm infants thirty to thirty-five weeks of gestational age: a prospective and longitudinal study during the first year of life. Thyroid 2004;14:435-42.

66. Hirano T, Singh J, Srinivasan G, Pildes R. Post-natal thyroid function in low birth weight infants; a longitudinal assessment of free thyroxine and thyroid hormone binding globulin. Acta Endocrinol (Copenh) 1985;110:56-60.

67. Chen JY. Thyroid function in healthy and sick neonates. Zhonghua Yi Xue Za Zhi (Taipei) 1994;54:51-6.

68. Etling N, Padovani E, Gehin-Fouque F, Tato L. Serum and urine thyroid hormone levels in healthy preterm and small for date infants on the first and fifth day of life. Helv Paediatr Acta 1984;39:223-30.

69. Harkavy KL, Enecio CE. Free thyroxine levels in hospitalized newborns: depressed levels in critical, nonthyroidal illness. J Perinatol 1991;11:117-21.

70. John R, Bamforth FJ. Serum free thyroxine and free triiodothyronine concentrations in healthy fullterm, preterm and sick preterm neonates. Ann Clin Biochem 1987;24 (Pt 5):461-5.

71. Uhrmann S, Marks KH, Maisels MJ, et al. Thyroid function in the preterm infant: a longitudinal assessment. J Pediatr 1978;92:968-73.

72. Diamond FB, Jr., Root AW. Thyroid function in the preterm infant. Adv Exp Med Biol 1991;299:227-41.

73. Fuse Y, Shimizu M, Uga N, Fujii T, Irie M. Maturation of feedback control of thyrotropin in premature infants. J Dev Physiol 1990;14:17-22.

74. Rooman RP, Du Caju MV, De Beeck LO, Docx M, Van Reempts P, Van Acker KJ. Low thyroxinaemia occurs in the majority of very preterm newborns. Eur J Pediatr 1996;155:211-5.

75. van Wassenaer AG, Kok JH, de Vijlder JJ, et al. Effects of thyroxine supplementation on neurologic development in infants born at less than 30 weeks' gestation. N Engl J Med 1997;336:21-6.

76. De Felice C, Bagnoli F, Toti P, et al. Transient hypothyroxinemia of prematurity and histological chorioamnionitis. J Perinat Med 2005;33:514-8.

77. Martin CR, Van Marter LJ, Allred EN, Leviton A. Antenatal glucocorticoids increase early total thyroxine levels in premature infants. Biol Neonate 2005;87:273-80.

78. Martin CR, Van Marter LJ, Allred EN, Leviton A. Growth-restricted premature infants are at increased risk for low thyroxine. Early Hum Dev 2001;64:119-28.

79. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res 1997;42:1-8.

80. Dammann O, Leviton A. Inflammation, brain damage and visual dysfunction in preterm infants. Semin Fetal Neonatal Med 2006;11:363-8.

81. Williams MC, O'Brien WF, Nelson RN, Spellacy WN. Histologic chorioamnionitis is associated with fetal growth restriction in term and preterm infants. Am J Obstet Gynecol 2000;183:1094-9.

82. Buimer M, van Wassenaer AG, Ganzevoort W, Wolf H, Bleker OP, Kok JH. Transient hypothyroxinemia in severe hypertensive disorders of pregnancy. Obstet Gynecol 2005;106:973-9.

83. Franklin RC, Purdie GL, O'Grady CM. Neonatal thyroid function: prematurity, prenatal steroids, and respiratory distress syndrome. Arch Dis Child 1986;61:589-92.

84. Reuss ML, Paneth N, Lorenz JM, Susser M. Correlates of low thyroxine values at newborn screening among infants born before 32 weeks gestation. Early Hum Dev 1997;47:223-33.

85. Cuestas RA, Engel RR. Thyroid function in preterm infants with respiratory distress syndrome. J Pediatr 1979;94:643-6.

86. Huang CB, Chen FS, Chung MY. Transient hypothyroxinemia of prematurity is associated with abnormal cranial ultrasound and illness severity. Am J Perinatol 2002;19:139-47.

87. Reuss ML, Paneth N, Pinto-Martin JA, Lorenz JM, Susser M. The relation of transient hypothyroxinemia in preterm infants to neurologic development at two years of age. N Engl J Med 1996;334:821-7.

88. Williams FL, Ogston SA, van Toor H, Visser TJ, Hume R. Serum thyroid hormones in preterm infants: associations with postnatal illnesses and drug usage. J Clin Endocrinol Metab 2005;90:5954-63.

89. Weber G, Vigone MC, Rapa A, Bona G, Chiumello G. Neonatal transient hypothyroidism: aetiological study. Italian Collaborative Study on Transient Hypothyroidism. Arch Dis Child Fetal Neonatal Ed 1998;79:F70-2.

90. Bernal J, Pekonen F. Ontogenesis of the nuclear 3,5,3'-triiodothyronine receptor in the human fetal brain. Endocrinology 1984;114:677-9.

91. Dobbing J, Sands J. Timing of neuroblast multiplication in developing human brain. Nature 1970;226:639-40.

92. Auso E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 2004;145:4037-47.

93. Berbel P, Guadano-Ferraz A, Martinez M, Quiles JA, Balboa R, Innocenti GM. Organization of auditory callosal connections in hypothyroid adult rats. Eur J Neurosci 1993;5:1465-78.

94. Gravel C, Hawkes R. Maturation of the corpus callosum of the rat: I. Influence of thyroid hormones on the topography of callosal projections. J Comp Neurol 1990;291:128-46.

95. Gravel C, Sasseville R, Hawkes R. Maturation of the corpus callosum of the rat: II. Influence of thyroid hormones on the number and maturation of axons. J Comp Neurol 1990;291:147-61.

96. Lavado-Autric R, Auso E, Garcia-Velasco JV, et al. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 2003;111:1073-82.

97. Lucio RA, Garcia JV, Ramon Cerezo J, Pacheco P, Innocenti GM, Berbel P. The development of auditory callosal connections in normal and hypothyroid rats. Cereb Cortex 1997;7:303-16.

98. Morreale de Escobar G. The role of thyroid hormone in fetal neurodevelopment. J Pediatr Endocrinol Metab 2001;14 Suppl 6:1453-62.

99. Lucas A, Rennie J, Baker BA, Morley R. Low plasma triiodothyronine concentrations and outcome in preterm infants. Arch Dis Child 1988;63:1201-6.

100. Meijer WJ, Verloove-Vanhorick SP, Brand R, van den Brande JL. Transient hypothyroxinaemia associated with developmental delay in very preterm infants. Arch Dis Child 1992;67:944-7.

101. Amato M, Guggisberg C, Schneider H. Postnatal triiodothyronine replacement and respiratory distress syndrome of the preterm infant. Horm Res 1989;32:213-7.

102. Amato M, Pasquier S, Carasso A, Von Muralt G. Postnatal thyroxine administration for idiopathic respiratory distress syndrome in preterm infants. Horm Res 1988;29:27-30.

103. Chowdhry P, Scanlon JW, Auerbach R, Abbassi V. Results of controlled double-blind study of thyroid replacement in very low-birth-weight premature infants with hypothyroxinemia. Pediatrics 1984;73:301-5.

104. Schonberger W, Grimm W, Emmrich P, Gempp W. Reduction of mortality rate in premature infants by substitution of thyroid hormones. Eur J Pediatr 1981;135:245-53.

105. Vanhole C, Aerssens P, Naulaers G, et al. L-thyroxine treatment of preterm newborns: clinical and endocrine effects. Pediatr Res 1997;42:87-92.

Chapter 2 Methodology

This chapter includes four major aspects of the Phase 1 Clinical Trial of Thyroid Hormone in Extremely Premature Infants (THOP): 1) study design; 2) study population; 3) exposures of interest and outcomes of interest; 4) statistical analysis. In the last section, two research questions of this dissertation are framed, followed by their analytical samples statistical methods used.

2.1 Study Design (Table 2.1)

The Thyroid Hormone in Extremely Premature Infants study, a multi-center randomized clinical trial, is designed to establish the optimal dosing schedule for very preterm infants to increase plasma T4 and T3 levels to the normal range without increasing risks of morbidity or mortality, and without suppressing TSH. This trial was conducted at the neonatology intensive care units of three hospitals: Maria Fareri Children's Hospital at Westchester Medical Center, Valhalla, New York; Emma Children's Hospital, Amsterdam, Netherlands; and University Hospital La Paz, Madrid, Spain. The data management team at Michigan State University, East Lansing, Michigan, was responsible for data management and monitoring.

The THOP trial was designed to have six study arms. Four treatment groups received 4 or 8 μg/kg/day of T4 by bolus or by continuous infusion in the 6 weeks of life, and also received 1 μg/kg/day of T3 by continuous infusion in the first 2 weeks of life. A fifth group received 30 μg/kg/day of potassium iodide (KI), in the form of SSKI drops, via enteral route, for 6 weeks. The last group was a control group, subjects of which received Dextrose 5% in Water (D5W) by continuous infusion for 6 weeks. Treatment

was required to begin within 24 hours after birth. The six study treatment groups were coded as 1, 2, 3, 4, 5, and 6. The contents of group 1-6 were only revealed to study pharmacists and the data management team. Patients, clinicians, and study coordinators were blinded to the treatment throughout the study period, except the group 6, which revealed itself by the enteral route.

From April 2005 to March 2007, 168 preterm infants born between 24 and 28 completed weeks of gestational age were enrolled. The randomization procedure was first stratified by study centers to ensure the enrollment ratio is 2:1:1 in New York, Amsterdam, and Madrid respectively. Gestational age (below or above 26 weeks) and gender (male or female) were also used in stratification to minimize imbalance in gestational age and gender across six study groups at enrollment.

Subjects who expired or who withdrew from the THOP study prior to 7 days after birth were considered as non-completers. As the study was designed to monitor hormone changes over time (from birth to discharge), non-completers would not provide sufficient information on hormone levels, we replaced them with the next eligible subject. Information of non-completers was kept in the database for analysis. A note was provided to identify the reason for not being able to complete the study treatment.

2.2 Inclusion and exclusion criteria (Table 2.2)

We screened all preterm infants who were born in or transferred to the three study centers during the enrollment period and whose gestational age was between 24 and 28 completed weeks. Gestational age estimation was based on the last menstrual period and was also confirmed by obstetric ultrasound scans.

Infants born to women less than 18 years of age were excluded because of the complexity of getting parental consent for an underaged mother. Infants with congenital thyroid disease or major/lethal anomalies, such as trisomy 13 and 18, CNS structural anomaly, and so on, were excluded. Infants born to mothers who had thyroid disease, who planned to breastfeed meanwhile taking anti-thyroid medications/supplemental iodine in therapeutic doses, or who had substance abuse history during the current pregnancy were all excluded. We also avoided extremely sick infants, for instance, liver failure, sepsis, severe cardiopulmonary disease, etc. Neonates who were enrolled in another clinical trial, or have no parental consent or assent from treating neonatologist were also excluded.

2.3 Adverse events

As in all clinical trials, adverse events were closely monitored throughout the study period and were generally reported within 24 to 48 hours of detection. In this trial, we classified adverse events into two categories: 1) severe adverse events, which include death and brain parenchymal lesion on ultrasound; 2) mild/moderate adverse events, which include changes in thyroid gland function, pulmonary cardiovascular status, metabolic status, gastrointestinal system status, central nervous system status, and skin system status. Information of each adverse event was recorded in detail, including the starting and ending time points, symptoms of the adverse event, concomitant medications used for treating the adverse event, and the outcome of the adverse event. Whether and when the study treatment was suspended, stopped, or restarted were also documented.

The attending neonatologist was responsible for filling the study adverse event form online. An instant email notification was sent to three parties: the data management team at MSU, which would update the mortality report if it was a death; the Data Safety Monitoring Board (DSMB), formed by clinicians, statisticians, and project officers

appointed by the funding agency of this study---the National Institute of Health to oversee the THOP trial; and the Independent Medical Monitor (IMM), who, a neonatologist in another institute blinded from study treatment, was responsible for making an independent judegement of whether the adverse event was related to the study treatment based on the information provided in the study adverse event form.

2.4 Study Population (Figure 2.1)

During the two-year enrollment period, 239 preterm infants born between 24 and 28 completed weeks of gestational age were screened. 188 (188/239, 78.7%) newborns were eligible for enrollment. 168 (89.4%) neonates, born to 151 women, were consented and randomized. A total of 32 study subjects expired before hospital discharge, among which 7 expired before day 7 of life. Six subjects withdrew from the study, four of whom withdrew before day 7 of life and 2 withdrew at day 12 and day 41 of life respectively. The total number of non-completers is 11.

2.4.1 Early termination of group 2 (Table 2.3 and Figure 1)

As of July 21, 2006, 113 subjects were enrolled in the THOP trial. 19 subjects expired, 4 of which died prior to day 7 of life. Table 2.3 lists the deaths and mortality rates of each group at that time. At the time 20 subjects were enrolled in group 2. No death occurred before 7 days of life and the mortality rate of this group was 30%, which was higher than any other group, although not statistically significant. This high mortality rate raised concern among the DSMB members, which believed that the group 2 dosing level put patients in this group at an unacceptable risk. On July 21, 2006, the DSMB notified the THOP study team to stop recruiting study subjects into Group 2. The decision was immediately carried out in three study sites. The randomization program

was subsequently modified to adapt to the change and to maintain the balance across the rest five study groups.

2.4.2 Unexpected dosing and coding errors in Madrid site (Table 2.4)

On October 24, 2006, a double dosing error was reported by the study PI from the Madrid site. In the THOP trial, study drug was shipped directly from the pharmaceutical company to each study site. The local pharmacist of each study site is responsible for diluting the study drug into two different doses used in the THOP trial. In each package, a standard manufacture dilution instruction was included, however it was different from the THOP dilution protocol written in the manual of procedure. The hospital pharmacist in Madrid site followed the manufacture dilution instruction instead of the study manual of procedure, which omitted one dilution step and led to double dosing error in all treatment groups. The same dilution procedure had been applied in Madrid since the beginning of the THOP trial in Madrid, until the error was detected.

While investigating the dosing error, a second error was found in Madrid. Unbeknownst to the data center, the Madrid site, at the randomization stage, used a group coding system different from the one assigned to all study sites. In itself, this violation would not have violated the principles of randomization, since all groups were assigned randomly from a central minimization/randomization program. However, part of the study subjects enrolled in Madrid site were classified into wrong groups. Table 2.4 compared the difference of the group coding between Madrid site and the THOP study.

As a result of the double-dosing error, we lost all subjects in two 4 μg/kg/day T4 groups in Madrid and accidentally created two new groups, 16 μg/kg/day T4 by continuous or bolus infusion.

2.4.2.1 Impact of double dosing error and coding error in Madrid on study group assignment (Table 2.5)

While one might have expected all six Madrid groups to differ from the protocol because of the Madrid coding error, as seen in table 2.5, only three actually did. This is because 1) group 6 was the KI group in all three sites, and 2) Madrid coded group 2 as B4 group and group 3 as C4 group, whereas they were supposed to be B8 and C8 groups respectively. The double dosing error restored these two groups to their original assignment.

2.4.2.2 Regrouping procedure

After the errors were identified, we regrouped the study participants in Madrid and put the mis-grouped subjects into the correct dosing groups based on the true treatment they received. The regrouping procedure followed the rules below:

Subjects assigned to group **1** in Madrid were regrouped to group **4 of THOP**;
Subjects assigned to group **2** in Madrid remained in group **2 of THOP**;
Subjects assigned to group **3** in Madrid remained in group **3 of THOP**;
Subjects assigned to group **4** in Madrid were regrouped to group **7 of THOP**;
Subjects assigned to group **5** in Madrid were regrouped to group **8 of THOP**;
Subjects assigned to group **6** in Madrid remained in group **6 of THOP**.

Two subjects were enrolled in Madrid after the dosing error was corrected. One subject was randomized to group 6, and therefore remained in group **6 of THOP**. The other subject was randomized to Madrid group 3, which was group **1 of THOP** after regrouping.

2.4.2.3 Termination of enrollment and treatment in Madrid

On October 25, 2006, The DSMB decided to close Madrid site for both enrollment and treatment. This decision turned the two last-enrolled subjects, who

received correct treatment dose from the beginning, into non-completers by ending the treatment before 7 days of life and also withdrew the study treatment from another one subject, who had been enrolled for 12 days at that time.

2.4.2.4 Impact of the errors on study sample size

After regrouping based on the true treatment each subject received, Madrid had subjects in groups 1, 2, 3, 4, and 6 of THOP. No subject in Madrid received 4 μg/kg/day T4 treatment except the last enrolled subject, who was enrolled after the dosing error was corrected.

The total loss of study sample size was 17, of which 15 received incorrect treatment dose and 2 became non-completers by withdrawing from treatment prior to day 7 of life. The effective sample size of the THOP trial went down from 139 to 124. The New York and Amsterdam sites continued enrollment until the end of the trial.

2.5 Collection of biological specimens

2.5.1 Blood specimens

A series of 2-milliliter (ml) neonatal blood sample were collected for every study subject at enrollment (day 0), on day 3, 7, 14, 21, 42, 56, and at discharge. Serum Free T4 (FT4), Total T4 (TT4), Total T3 (TT3), TSH, thyroid binding globulin (TBG), and cortisol levels were measured in neonatal blood specimens. Neonatal serum hormone results were available for 164 study subjects. Four subjects did not have hormone results because of either early withdrawal from the study or insufficient specimen volume for laboratory testing.

A 3-ml maternal venous blood was collected at enrollment. FT4, TT4, and TSH were measured in maternal blood samples. Blood samples were not obtained for 25

women because some mothers were discharged home early or project manager missed maternal visits. Hormone testing results of one woman was excluded in the analysis because her blood sample was taken 18 days after delivery, which was too far away from delivery and may not reflect the thyroid function at delivery. Maternal serum hormone results were available for 125 mothers.

2.5.2 Urine and breast milk specimens

A series of 2 to 5-ml urine samples were collected for each study subject at enrollment (day 0), on day 7, 21, 42, 56, and at discharge. A single 2 to 5-ml urine sample was taken from participating mothers at enrollment. A series of 2-ml breast milk samples were collected from mothers whose breast milk was available on day 3, 7, 14, 21, 42, and 56. Iodine levels were measured in all urine and breast-milk samples.

2.5.3 Storage, transportation, and assessment of biological specimens

All specimens were stored at -20 degrees Celsius (°C). Blood specimens were transported in dry ice by over-night FedEx to the Quest Diagnostics, at Chantilly, Virginia, where all hormone assessments were completed. Direct equilibrium dialysis (DED) was used to assess serum FT4 levels and chemiimmunoluminescent assay was used for other hormone assessments. In a situation when sample volume was insufficient to complete the tests of all six biomarkers, we prioritized FT4 testing and then TSH, TT4, TT3, TBG, and cortisol.

Urine and breast-milk specimens were transported in dry ice to the Molecular Endocrinology Lab at the Institute of Biomedical Research "Alberto Sols", Autonomous University of Madrid, in Madrid, Spain. Ammonium Persulfate method and Chloric Acid Digestion method were used for urine and breast milk iodine assessment respectively.

2.6 Exposure of interest

We are interested in three groups of exposures in this clinical trial. First, intra-uterine exposures. At enrollment, a concise questionnaire was used to collect maternal information on health history/behavior (e.g. gravidity, parity, addiction, etc.) and current pregnancy (e.g. preeclampsia, fever, HELLP syndrome, etc.). Medication prescribed to mothers prior to delivery was also recorded (e.g. antibiotics, magnesium, steroids, etc.). Second, effects of breast-feeding. Iodine, immunoglobulins, and very low levels of thyroid hormones would be transported to the neonates through breast-milk (1, 2). We measured the iodine levels in breast-milk, maternal urine, and neonatal urine and adjusted the iodine levels in thyroid hormone analysis. Third, neonatal exposures. Neonatal birth characteristics, such as birthweight, gestational age, multiple birth status, and Apgar score at 1 and 5 minutes, were collected at enrollment for each study subject. Daily records (such as vital signs, blood gases, calorie intake, growth, respiratory support, etc.) and illness episodes during neonatal period were also recorded in detail.

2.7 Outcomes of interest

As the ultimate goal of this study was to look for the optimal dosing schedule of thyroid hormone treatment for very preterm infants to increase plasma T4 and T3 levels to normal range, without increasing risks of morbidity or mortality, and without suppressing TSH, the primary outcomes were the neonatal thyroid hormone levels (FT4, TT4, TT3, and TSH) at different time points, death, severe morbidity such as brain lesion.

Most studies measured serum FT4 concentrations by analog immunoassay or FT4 index methods, both of which could be biased by the serum protein levels and/or protein-bound T4 concentrations (3). Hence low FT4 values of preterm infants could be partially

attributed to reduced T4 binding to proteins. DED method is by far the least biased method by changes in serum proteins or protein binding of T4 and we believe it can most accurately reflect the serum FT4 levels in extremely premature infants (4).

Each death was reported to the study PI, the data management team, the DSMB, and the IMM within 24 hours of occurrence. The DSMB received appropriate mortality analyses update from the data management team within 1-2 days.

Brain lesions (such as parenchymal lesion, hemorrhagic lesions, periventricular leukomalacia (PVL), hydrocephalus, etc.) were diagnosed through cranial ultrasound scans. All subjects received three cranial ultrasound scans at postnatal day 1-3, day 7-10, and > 4 weeks. All scans were sent to one central ultrasound reader, who was blinded from treatment assignment throughout the study period.

Secondary outcomes include neonatal morbidities, such as chronic lung disease, necrotizing enterocolitis, retinopathy of prematurity, etc., which were recorded in the study discharge/death form.

2.8 Statistical analysis

In this dissertation, two research questions will be studied. First, what are the correlates of thyroid hormone levels at birth among extremely premature infants ($\leqq$ 28 wks)? Linear regression (with log-transformation) will be used for both unadjusted and adjusted analyses.

Second, what are the correlates of postnatal thyroid hormone levels among extremely premature infants ($\leqq$ 28 wks)? Repeatedly measured neonatal thyroid hormone levels will be treated as an outcome in a whole. Proc Mixed procedure will be used for analysis to account the between and within subjects variations. Backward model

selection approach will be applied. All analyses will be carried out using SAS software 9.1.3.

Table 2.1 The six THOP arms.

Study arm	Treatment	Group abbreviation
1	Continuous Infusion T4(4μg/kg/d) × 42days + Continuous Infusion T3 (1μg/kg/d) × 14days	C4
2	Bolus Infusion T4 (8μg/kg/d) × 42 days + Continuous Infusion T3 (1ug/kg/d) × 14 days	B8
3	Continuous Infusion T4 (8μg/kg/d) × 42 days + Continuous Infusion T3(1μg/kg/d) × 14days	C8
4	Placebo (D5W infusion)	Placebo
5	Bolus Infusion T4 (4μg/kg/d) × 42 days + Continuous Infusion T3 (1μg/kg/d) × 14 days	B4
6	Iodine Supplementation alone (SSKI) (30μg/kg/d) × 42 days	Iodine

Table 2.2 Inclusion and exclusion criteria of the THOP trial.

Inclusion criterion:

Infants born between 24 and 28 complete weeks of gestational age (GA, confirmed by early obstetric ultrasound scan).

Exclusion criteria:

1. Mother of newborn is less than 18 years of age.
2. Maternal or congenital thyroid disease (e.g. propylthiouracil, PTU).
3. Women who plan to breast feed and who take anti-thyroid medications or supplemental iodine in therapeutic doses.
4. Substance abuser (heroin, methadone, alcohol > 1oz per day, or other).
5. Known disorders of fatty acid metabolism or inherited metabolic diseases.
6. Liver Failure (e.g. enzymes in the thousands).
7. Newborns with known major or lethal anomalies such as:
   1) *Known chromosomal anomalies Trisomy 13 & 18.*
   2) *CNS structural anomaly (e.g. hydrocephalus, migration disorders, etc.).*
   3) *Major congenital malformations requiring surgery.*
   4) *Structural congenital heart disease (e.g. cyanotic heart defects).*
   5) *Anomalies of the gastrointestinal tract (e.g. gastroschesis).*
8. Newborn with arrhythmia.
9. Proven sepsis at birth (clinical signs, positive body fluid culture, and antibiotic treatment > 5 contiguous days).
10. Death expected within 48 hours of birth due to severity of initial cardiopulmonary disease.
11. A concurrent clinical trial with another randomized drug.
12. Other concern by treating physician that either mandates or prohibits study treatment.
13. Absence of treating neonatal physician's assent.
14. Absence of parental written consent.

Table 2.3 Deaths (total, < 7 days, and ≧7 days) and mortality rates (total, < 7 days, and ≧7 days) by group assignment in the THOP trial as of July 21, 2006.

Study group	1	2	3	4	5	6	p-value	Total
Number of subjects	19	20	20	19	19	16		113
All deaths	2	6	2	4	2	3	0.50 [3]	19
(Mortality %)	(10.5)	(30)	(10)	(21.1)	(10.5)	(18.8)		(16.8)
Deaths < 7 days	1	0	2	1	0	0	-	4
(Mortality %) [1]	(5.3)	(0)	(10)	(5.3)	(0)	(0)		(3.5)
Deaths ≧7 days	1	6	0	3	2	3	0.11 [3]	15
(Mortality %) [2]	(5.6)	(30)	(0)	(16.7)	(10.5)	(18.8)		(13.8)

1 The total number of subjects enrolled in each group was used as the denominator;
2 The total number of subjects survived ≧ 7 days in each group was used as the denominator.
3 p-value by exact analysis (Pearson test).

Table 2.4 The study group coding difference between Madrid site and the THOP trial.

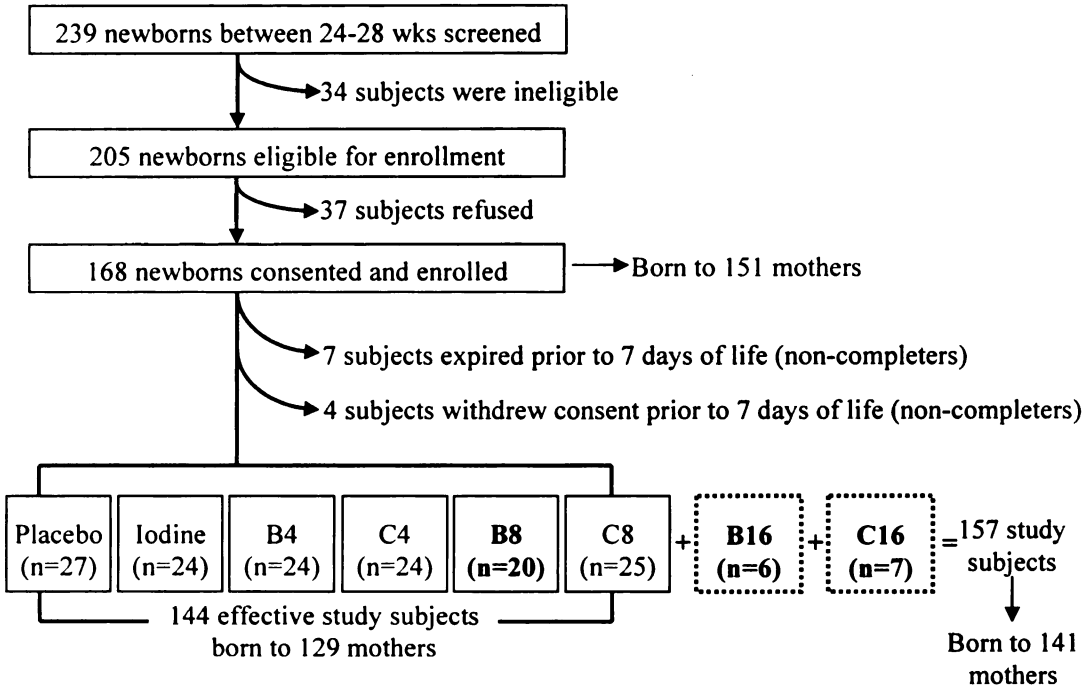
Study Group	Group coding	
	THOP	**Madrid**
1	C4	Placebo
2	B8	B4
3	C8	C4
4	Placebo	C8
5	B4	B8
6	Iodine	Iodine

Table 2.5 Impact of Madrid double dosing and coding error on treatment groups.

Madrid group coding		Impact of Madrid double dosing error on the groups			Impact of Madrid double dosing error and coding error in comparison with the THOP protocol grouping standard
1	Placebo	**1**	Placebo	**4**	Placebo
2	B4	**2**	B8	**2**	B8
3	C4	**3**	C8	**3**	C8
4	C8	**4**	C16		No corresponding group – named as group **7** [1]
5	B8	**5**	B16		No corresponding group – named as group **8** [2]
6	Iodine	**6**	Iodine	**6**	Iodine

1 **Group 7**: Continuous infusion T4 (16 μg/kg/d) × 42 days + continuous infusion T3 (1 μg/kg/d) × 14 days;
2 **Group 8**: Bolus infusion T4 (16 μg/kg/d) × 42 days + continuous infusion T3 (1 μg/kg/d) × 14 days;

Figure 2.1 Flow chart of the THOP enrollment.



BIBLIOGRAPHY

1. Smith VC, Svoren BM, Wolfsdorf JI. Hypothyroidism in a breast-fed preterm infant resulting from maternal topical iodine exposure. J Pediatr 2006;149:566-7.

2. van Wassenaer AG, Stulp MR, Valianpour F, et al. The quantity of thyroid hormone in human milk is too low to influence plasma thyroid hormone levels in the very preterm infant. Clin Endocrinol (Oxf) 2002;56:621-7.

3. Nelson JC, Weiss RM, Wilcox RB. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. J Clin Endocrinol Metab 1994;79:76-9.

4. Rabin CW, Hopper AO, Job L, et al. Incidence of low free T4 values in premature infants as determined by direct equilibrium dialysis. J Perinatol 2004;24:640-4.

Chapter 3 Results: Population characteristics

This chapter describes the general characteristics of the THOP cohort. Information covers: 1) attributes of 239 potential subjects screened for eligibility in the two-year enrollment period; 2) maternal characteristics, such as demographic information (e.g. maternal age, ethnicity) and health history during current pregnancy; and 3) neonatal characteristics, such as demographic and birth attributes.

3.1 Recruitment (Table 3.1)

Among 239 potential subjects we screened, 168 (70%) were successfully enrolled in this clinical trial. The major reasons for declining participation is that parents of 36 (15%) potential subjects were either not interested in research, afraid of research, too stressed to decide, or wanted incentives to participate. 2 (0.9%) refused because of religious beliefs.

33 potential subjects were ineligible for enrollment, among which 3 (1.3%) were contacted after the study eligibility time frame (24 hours after birth), 10 (4.2%) were very sick at birth and death was expected within 48 hours, 8 (3.3%) had mothers younger than 18 years old, and another 8 (3.3%) had mothers with diseases listed in the exclusion criteria. Ineligible for enrollment due to other reasons, such as maternal addiction (1, 0.4%), mother too sick to approach (1, 0.4%), and iodine exposure prior to delivery (2, 0.9%) were all under 1%.

3.2 Comparison of consented and refused subjects (Table 3.2)

We compared study site, race, gender, birthweight, and gestational age of subjects who were enrolled in the THOP study versus those who were not. Subjects of Hispanic

origin were less like to be enrolled in the THOP study, with a relative risk of 0.4 (95% CI 0.17-0.83) compared to whites, 0.3 (95% CI 0.1-0.7) compared to blacks, and 0.2 (95% CI 0.02-1.5) compared to other race category. Except for race, other factors did not seem to influence parental decision making in trial participation.

3.3 Study cohort (Table 3.3-3.4)

During the two-year enrollment period, we recruited a total of 168 subjects. 77 (45.8%) were enrolled in New York, 52 (31%) in Amsterdam, and 39 (23.2%) in Madrid. Parents of four subjects withdrew their consent within 7 days after birth and another two withdrew after 7 days of life. Seven subjects (4.3%) expired prior to day 7 of life and 25 (15.9%) more died after 7 days. About 63% of the study population is white, 24% is black, 9% is Hispanic, and other ethnicities account for about 4%.

Birthweight of all study subjects (n=168) ranged between 440 to 1550 grams with a mean of 851 grams and a standard deviation of 190.7 (se=14.7). Gestational age ranged between 24 to 27.9 completed weeks with a mean of 26.2 weeks and a standard deviation of 1.1 (se=0.1).

Among the 168 enrolled subjects, we consider 144 of them as effective study subjects, since 9 subjects either expired or withdrew from the study prior to day 7 of life and 15 subjects received 16 μg/kg/day thyroxine due to an unexpected dilution error as aforementioned in chapter 2. With the early termination of group 2 (Bolus 8 μg group) at group size of 20, we enrolled 4 more subjects to complete the projected sample size of 144. These four subjects were randomized to the placebo group (n=3) and the continuous 8 μg group (n=1).

3.4 Maternal characteristic of the THOP cohort

The 168 subjects were born to 151 women. Maternal information is complete for 150 women. 73 (48.3%) were enrolled from New York site, 42 (27.8%) were enrolled from Amsterdam site, and 36 (23.8%) were enrolled from Madrid site. 62% of mothers were white, 25% were black, and 8% were Hispanic.

During the current pregnancies of the 150 mothers, 4 (2.7%) had HELLP, 20 (13.4%) had preeclampsia, 16 (10.7%) had fever ($\geq$ 100.4 F) during pregnancy, 40 (28.8%) had preterm premature rupture of membrane (ROM) for more than 24 hours prior to onset of labor, and 69 (46%), 124 (82.7%), and 42 (28%) were treated with antibiotics, antenatal steroids, and magnesium sulfate respectively prior to delivery. These mothers had an average age of 29.5 years, ranging between 18 and 47 years old.

Except for HELLP syndrome, we found significant differences across three study sites on all maternal characteristics we studied. This suggests that population or clinical management difference may exist among three sites. NY tended to have younger mothers than other sites ($p=0.0034$). Both black and Hispanic enrollees were mostly recruited from New York site and the major race group in both Amsterdam and Madrid is white.

NY also had the highest rates of preeclampsia, fever during pregnancy, and ROM greater than 24 hours. The large difference in preeclampsia (PE) rates between NY and Amsterdam in this study, although not significant, could have several explanations. First, the urinary excretion of 300 mg of protein or higher in a 24-hour urine collection is required for PE diagnosis in Amsterdam, but not in the US. Given the potential difficulty of obtaining a 24-hour urine sample, this criterion may have restricted women from

getting a preeclampsia diagnosis. On the other hand, less restrictive criteria may have led to an overestimation of preeclampsia in NY. Second, the racial composition is quite different. A large proportion of NY subjects were blacks and Hispanics, while Amsterdam had very few blacks and no Hispanics, both of whom are known to have higher risk of preeclampsia. Third, clinical practice in Amsterdam allows fetal demise, which possibly could have caused fewer preeclamptic women to carry the fetus to delivery. Potentially this difference in practice has introduced selection bias in our sampling and an under-representation of preeclamptic pregnancy.

More than half the women in NY received antibiotics (68%) and magnesium sulfate (56%) treatment prior to delivery, while only 20-30% of women received antibiotics in the other two sites and magnesium sulfate treatment was rare in Amsterdam (0%) and Madrid (5.6%). Both treatments are related to management of premature ROM, preeclampsia, and preterm labor, therefore differences were partially related to the low rates of preeclampsia in Amsterdam, and low rates of prolonged premature ROM in Amsterdam and Madrid. In addition, clinical practice may have contributed to the difference, for instance, Magnesium sulfate may not be a standard treatment for preeclampsia and preterm labor in Amsterdam (need to clarify with Amsterdam).

3.5 Neonatal characteristic of the THOP cohort (Table 3.6)

Significant birthweight and gestational age differences existed across study sites. The average birthweight of NY subjects was 150 grams less than that of Amsterdam subjects. The mean gestational age was 3.5 days younger in NY and Madrid than in Amsterdam. With bigger babies, Amsterdam also had the highest FGR level among three sites.

The characteristics of the 168 study subjects across three study sites are given in table 3.6. No gender difference was found in the THOP study across three study sites. The racial composition differed significantly by study site. Madrid had the highest number of subjects in the whites group and NY had the highest number of subjects in both black and Hispanic groups. The difference is not surprising as it reflects the racial composition of the three cities where study sites are located.

In both Amsterdam and Madrid, more than 80% of the subjects had Apgar score at 1 minute (APGAR1) higher than 3, but in NY only 65.7% did. The difference in APGAR1 across study sites was significant, possibly because Amsterdam had bigger or more mature babies who might perform well at birth. After resuscitation, Apgar score at 5 minute (APGAR5) of all subjects in Amsterdam and Madrid went up to 4 and above, while APGAR5 of five subjects in NY remained under 3. The Apgar score difference by sites was no longer significant at five minutes.

Findings through comparisons among study sites suggest that there are differences in both population and clinical practice across sites. We will adjust for the effect of study sites in later analyses.

Table 3.1 Reasons for declining or exclusion.

	Number of potential subjects	Percentage
Reasons for declining		
Not interested, or afraid of research, or wants payment, or too stressed to decide	36	15.1%
Religious beliefs	2	0.9%
Reasons for exclusion		
Eligible time frame expired (< 24 hr after birth)	3	1.3%
Death expected within 48 hours	10	4.2%
Mother < 18 years old	8	3.3%
Maternal disease during pregnancy	8	3.3%
Maternal substance abuse	1	0.4%
Mother is too ill after delivery	1	0.4%
Iodine exposure prior to delivery	2	0.9%
Total number of enrolled subjects	168	70%
Total number of screened subjects	239	100%

Table 3.2 Characteristics of included and excluded subjects.

	Included (n=168)	Excluded (n=71)	p-value
	N (%)	N (%)	
Study site			
New York	77 (65.6)	42 (35.3)	
Amsterdam	52 (77.6)	15 (22.4)	0.1516 [1]
Madrid	39 (73.6)	14 (26.4)	
Race/ethnicity			
White	107 (71.3)	43 (28.7)	
Black	40 (78.4)	11 (21.6)	**0.0221** [1]
Hispanic	15 (48.4)	16 (51.6)	
Other	6 (85.7)	1 (14.3)	
Gender			
Male	93 (72.1)	36 (27.9)	0.5096 [1]
Female	75 (68.2)	35 (31.8)	
	Mean (Se)	Mean (Se)	
Birthweight (g)	852.2 (14.7)	853.9 (25.3)	0.9526 [2]
Gestational age (wk)	26.2 (0.1)	26 (0.1)	0.2708 [2]

1 P-value by Chi-square test;
2 P-value based on t-test.

Table 3.3 Characteristics of the THOP cohort.

	N (%)
Study site	
New York	77 (45.8)
Amsterdam	52 (31)
Madrid	39 (23.2)
Withdrawal	
Prior to 7 days [1]	4 (2.4)
After 7 days [2]	2 (1.2)
Total	6 (3.6)
Race/ethnicity	
White	107 (63.7)
Black	40 (23.8)
Hispanic	15 (8.9)
Other	6 (3.6)
Gender	
Male	93 (55.4)
Female	75 (44.6)
Birthweight (g)	
Mean (se)	851 (14.7)
Median (range)	827 (440, 1550)
Gestational age (week)	
Mean (se)	26.2 (0.1)
Median (range)	26.2 (24, 27.9)
Total	168

1 The denominator is the number of subjects who did not withdraw within the first 7 days of life;
2 The denominator is the number of subjects who survived for the first 7 days of life;

Table 3.4 Treatment group assignment of the THOP cohort.

Treatment	Number enrolled	Noncompleter [1]	Number of effective subject
Study groups	N (%)		N (%)
Placebo	28 (18.3)	1	27 (18.7)
Iodine	26 (17.0)	2	24 (16.7)
Bolus 4 μg	25 (16.3)	1	24 (16.7)
Continuous 4 μg	27 (17.7)	3	24 (16.7)
Bolus 8 μg	20 (13.0)	0	20 (13.9)
Continuous 8 μg	27 (17.7)	2	25 (17.3)
Total	153	9	144
Accidental groups			
Bolus 16 μg	7 (46.7)	1	6 (46.2)
Continuous 16 μg	8 (53.3)	1	7 (53.8)
Total	15	2	13
Total	168	11	157

1 Noncompleter refers to subjects who expired or withdrew consent prior to day 7 of life.

Table 3.5 Maternal characteristics of the THOP cohort.

	New York (n=72)	**Amsterdam (n=42)**	**Madrid (n=36)**	**p-value**	**Total (n=150)**
	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)
Maternal age (yr)	28 (5.9)	31.6 (4.9)	30.2 (5.9)	**0.0034**[1]	29.5 (5.8)
	N (%)	N (%)	N (%)		N (%)
Maternal race					
White	29 (39.7)	32 (76.2)	33 (91.7)	**<0.0001**[2]	94 (62.2)
Black	28 (38.4)	9 (21.4)	1 (2.8)		38 (25.2)
Hispanic	11 (15.1)	0 (0)	2 (5.6)		13 (8.6)
Other	5 (6.8)	1 (2.4)	0 (0)		6 (4)
HELLP	4 (5.6)	0 (0)	0 (0)	0.1606[2]	4 (2.7)
Preeclampsia	14 (19.4)	1 (2.4)	5 (14.3)	**0.0355**[3]	20 (13.4)
Fever (≥ 100.4 F) during pregnancy	12 (16.7)	4 (9.5)	0 (0)	**0.0303**[2]	16 (10.7)
Antibiotics administration	49 (68.1)	13 (31)	7 (19.4)	**<0.0001**[2]	69 (46)
Antenatal steroids administration	61 (84.7)	38 (90.5)	25 (69.4)	**0.0409**[3]	124 (82.7)
Magnesium administration	40 (55.6)	0 (0)	2 (5.6)	**<0.0001**[3]	42 (28)
Rupture of membrane ≥ 24 hr	25 (40.3)	9 (21.9)	6 (16.7)	**0.0230**[3]	40 (28.8)

1 P-value based on F-test;
2 P-value by exact analysis (Pearson test);
3 P-value by Chi-square test.

Table 3.6 Neonatal characteristics of the THOP cohort.

	New York (n=77)	Amsterdam (n=52)	Madrid (n=39)	p-value	Total (n=168)
	N (%)	N (%)	N (%)		N (%)
Gender (male)	35 (45.5)	34 (65.4)	24 (61.5)	0.0557[1]	93 (55.4)
Race					
White	30 (39)	41 (78.9)	36 (92.3)	**< 0.0001**[2]	107 (63.7)
Black	29 (37.7)	10 (19.3)	1 (2.6)		40 (23.8)
Hispanic	13 (16.9)	0 (0%)	2 (5.1)		15 (8.9)
Other	5 (6.5)	1 (1.9%)	0 (0)		6 (3.6)
C-section	48 (63.2)	7 (13.5)	22 (56.4)	**< 0.0001**[1]	77 (46.1)
Multiple birth					
Singleton	66 (86.8)	32 (61.5)	32 (82.1)	**< 0.0001**[2]	130 (77.8)
Twins	10 (13.2)	14 (26.9)	7 (17.9)		31 (18.6)
Triplets	0 (0)	6 (11.5)	0 (0)		6 (3.6)
Intrauterine growth restriction	13 (16.9)	3 (5.8)	4 (10.3)	0.1505	20 (11.9)
Apgar score at 1 min					
≤ 3	25 (34.3)	9 (18.8)	6 (16.7)	**< 0.0001**[1]	40 (25.5)
4 - 6	29 (39.7)	12 (25)	24 (66.6)		65 (41.4)
Apgar score at 5 min					
≤ 3	5 (6.7)	0 (0)	0 (0)	0.1362[2]	5 (3)
4 - 6	13 (17.6)	9 (17.3)	5 (3.2)		27 (16.5)
Apgar score change from 1 min to 5 min					
≤ 3 to (4 - 6)	7 (9.6)	7 (4.6)	2 (5.6)	**0.0016**[2]	16 (10.2)
(4 - 6) to ≥ 7	23 (31.5)	10 (20.8)	21 (58.3)		54 (34.4)
≤ 3 to ≥ 7	13 (17.8)	2 (4.2)	4 (11.1)		19 (12.1)
	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)
Birthweight	796.9 (177.1)	948.4 (202.3)	830.9 (151.4)	**< 0.0001**[3]	852 (190.8)
Gestational age	26 (1.1)	26.5 (0.8)	26 (1.2)	**0.0352**[3]	26.2 (1.1)
Fetal growth ratio	0.82 (0.2)	0.93 (0.2)	0.85 (0.1)	**0.0001**[3]	0.86 (0.2)

1 P-value by exact analysis (Pearson test);
2 P-value by Chi-square test;
3 P-value based on F-test.

Chapter 4 Correlates of thyroid hormone levels at birth among extremely premature infants

Transient hypothyroxinemia of prematurity (THOP) is manifest by very low levels of serum thyroxine (T4) and tri-iodothyronine (T3), but normal thyroid stimulating hormone (TSH) levels from shortly after birth until up to 6 weeks after birth. The disorder is seen with increasing frequency in infants born before 32 weeks of gestation, and especially below 28 weeks (1-5). The major cause of THOP is thought to be the immaturity of the thyroid gland and the hypothalamic-pituitary-thyroid axis in preterm infants. Despite variations in study populations and THOP definitions, studies have consistently shown clear decreases in THOP as gestational age increases.

Many studies have suggested that hypothyroxinemia during the neonatal period in premature infants may be associated with neurodevelopmental disabilities (1, 6-9). Preventing and treating THOP in extremely premature newborns ($\leqq$ 28 wks) has the potential to decrease the risk of adverse neurodevelopmental outcomes. It seems reasonable to suppose that premature infants vary in their thyroid hormone levels at birth, but little is known about the correlates of such variation. In this chapter, we use data from the Phase 1 Study of Thyroid Hormone in Extremely Premature Infants to investigate associations between prenatal exposures and newborn thyroid hormone levels at birth (baseline). The examination of correlates of thyroid hormones can lead to a better understanding of THOP.

4.1 Analytical sample (Figure 4.1)

To reduce the effect of the correlation between infants born to the same mother, we only included the first born of any multiple birth set if more than one infant in this set was enrolled. Among the 168 enrolled subjects, 131 were singleton and 37 were multiple births. The multiple births included 13 pairs of twins (the second-born excluded), five single twin enrolled from five different twin pairs (zero excluded), and two births from two triplet sets (four excluded). After the exclusion of 17 multiple births, seven more subjects were removed from this analysis because no baseline thyroid hormone levels were available. The final analytical sample thus excluded 24 of the 168 enrolled subjects, leaving 144 infants born between 24 and 28 completed weeks of gestational age. Neonatal baseline FT4 levels were available for 144 subjects, TT4 levels were available for 128 subjects, and TSH levels were available for 135 subjects. Missing data were due to insufficient sample volume, because of which multiple hormone testing could not be completed.

4.2 Exposures and outcomes

4.2.1 Maternal exposures

Four types of maternal information were obtained from maternal interview, medical records abstraction, and laboratory tests: 1) maternal demographic information (race, age); 2) previous pregnancy history (gravida, parity); 3) current pregnancy and delivery history (route of delivery, preeclampsia, prolonged premature rupture of membrane, and antenatal antibiotic/steroids/magnesium use prior to delivery); and 4) maternal thyroid function tests (FT4, TSH, and TT4).

Maternal venous blood (3 ml) was collected at enrollment for thyroid function tests. Among 144 mothers, blood samples were not obtained for 22 women due to early discharge. FT4, TT4, and TSH were measured in maternal blood samples. Three women were missing TT4 and two were missing TSH results. The time of collection varied from 0-18 days after delivery. Half of the specimens (52%) were drawn within 24 hours after delivery, 31% were drawn within 3 days of delivery, and 16% within a week. Test results for one woman whose blood sample was taken 18 days after delivery were excluded from the analysis, because such a sample may not reflect thyroid function at delivery.

4.2.2 Neonatal exposures

Neonatal birth characteristics consisted of exposures with two different attributes: 1) fixed exposures, e.g. birthweight (BW), gestational age (GA), gender, race, fetal growth ratio (FGR), intrauterine growth restriction (IUGR), multiple birth status, and Apgar score at 1 minute (APGAR1) and 5 minute (APGAR5); and 2) changing exposures, e.g. the change of Apgar score from 1 minute to 5 minute.

FGR was defined as the ratio of birthweight to the median birthweight of infants of the same gestational week. IUGR was defined as birthweight below 10th percentile of the same gestational week. Both FGR and IUGR were generated using an external gestational-age and plurality-specific birthweight standard, derived by Alexander et al. from the 1991-1995 US Natality Data (10).

At 1 minute and 5 minutes after birth, neonatal Apgar scores were evaluated for each subject on a 0 to 10 scale, with 0 being the worst and 10 being the best. We divided Apgar scores into three groups: ≤ 3, 4 - 6, and ≥ 7. To understand how neonates changed

from 1 minute to 5 minute, we created a new variable, Apgar score change from 1' to 5', based on Apgar scores at these two time points. Subjects were categorized into four groups: from ≤ 3 to 4 - 6, from ≤ 3 to ≥ 7, from 4 - 6 to ≥ 7, and no change.

4.2.3 Log transformation of thyroid hormone levels

Thyroid hormone levels of subjects at birth serve as the only outcome variable in this analysis. We tested the normality of the distribution of both neonatal and maternal FT4, TT4, and TSH levels. Results suggested that all five hormones followed the lognormal distribution. Therefore, a logarithmic transformation (base *e*) was applied to all hormone results and the report below is based on log-transformed hormone values.

4.3 Model selection approach

Unadjusted analyses were carried out to study the associations between each maternal/neonatal exposure and neonatal FT4/TT4 levels, as continuous variables. Linear regression was used to test relationships involving continuous exposures. One-way ANOVA was used to test associations involving categorical exposures. Unadjusted associations between maternal/neonatal exposures and baseline FT4/TT4 levels are reported first, before adjusted associations. .

Multivariable model selection involved four major steps. First, we included all variables that were related to the neonatal baseline thyroid hormone levels in *unadjusted* analyses at a significance level of 0.1. Second, we eliminated variables with *adjusted* p-values less than 0.05 from the full model, one at a time, beginning with the least significant variable. Third, we added each one of the remaining variables, which were excluded at the first step and dropped at the second step, back to the model one at a time starting with the most significant variable in univariable analyses. If any variable showed

a significant association with the outcomes after adjusting for other variables, it would be added into the model and the third step would be repeated one more time with the new model. Finally, when every variable that was significantly (α=0.05) related to the outcomes was included, we increased the significance level to 0.1 and included any variable that was biologically meaningful but not statistically significant (α=0.05).

4.4 Results

4.4.1 Neonatal and maternal characteristics (Table 4.1)

Birthweight of the 144 subjects ranged from 440 to 1550 grams with a mean of 855 grams and a standard deviation of 198.3 grams. Gestational ages ranged from 24 to 27.9 completed weeks with a mean of 26.2 weeks and a standard deviation of 1.1 weeks. Fetal growth ratio varied from 0.43 to 1.33 with a mean of 0.86 and a standard deviation of 0.2. FGR was defined as the ratio of birthweight to the median birthweight of infants of the same gestational week and was generated using an external gestational-age and plurality-specific birthweight standard, derived by Alexander et al. from the 1991-1995 US Natality Data (10).

55% of the enrolled subjects were male. The majority of this population was White (62.5%). 45.8% of subjects were born by C-section, 14% were one of a multiple-birth set, and 13% had intrauterine growth restriction. About 27% of subjects had Apgar scores at 1 minute below 4 points. At five minutes, only 3% remained under 4. Apgar scores from 1 minute to 5 minutes were improved in 57% of the subjects. The rest remained in the same Apgar score range (e.g., 0-3, 4-6, 7-10). About 12% of subjects had the most improvement, changing from less than 4 points at 1 minute to greater than 7 points at 5 minutes.

The 144 subjects were born to 144 mothers, whose age ranged from 18 to 47 years with a mean of 29.5 years and a standard deviation of 5.8. 14% of mothers had preeclampsia and 10% reported fever during the pregnancy. Prior to delivery, 46%, 83%, and 30% of mothers were treated with antibiotics, antenatal steroids and magnesium sulfate, respectively. About 30% of women had rupture of membranes for longer than 24 hours (ROM > 24 hours) prior to onset of labor.

4.4.2 Neonatal and maternal hormone levels (Table 4.2 - 4.5)

Neonatal and maternal serum FT4 levels were similar and both averaged around 1.5 μg/dl. The mean maternal TT4 level was close to two times higher than that of neonates, while the mean maternal TSH level was only half the neonatal level.

We studied hormone levels across the three study sites. Results in NY and Amsterdam were comparable. Madrid had the lowest neonatal but the highest maternal thyroid hormone levels, with significant differences in neonatal FT4 and maternal FT4 and TT4. Lower iodine levels in Spain may explain the low neonatal thyroid hormone levels, but do not explain the higher maternal thyroid hormone levels. Study site will be included in the multivariable analyses to account for site differences.

Both neonatal and maternal thyroid hormone levels were right-skewed and followed lognormal distribution. We reported thyroid hormone using the original scales in Table 4.2-4.5, and log transformed hormone levels were used in linear regression analyses (Table 4.6-4.9).

4.4.3 Unadjusted association between neonatal exposures and neonatal baseline thyroid hormone levels (Table 4.6)

Baseline FT4: Gestational age was significantly correlated with neonatal baseline FT4 levels ($p< 0.0001$), but birthweight was not. FGR was inversely associated with FT4 ($p=0.0117$). Subjects enrolled in the NY site had the highest FT4 values and subjects from Madrid the lowest. Female subjects tended to have higher baseline FT4 levels than males. Black infants had the highest baseline hormone levels, while Hispanics had the lowest ($p=0.032$). Multiple birth status also was related to neonatal baseline FT4 levels, but at the border of significance ($p=0.0649$). In subgroup analysis, singleton births had significantly higher baseline thyroid hormone levels than did twin births ($p=0.0432$). APGAR1 was directly, but not significantly associated with FT4 ($p=0.0804$), but APGAR5 was not. With respect to Apgar score change from 1 to 5 minutes, stable subjects whose Apgar scores remained in the same category had the highest FT4 levels ($p=0.0810$). The lowest FT4 levels were seen in subjects who had the most dramatic Apgar score improvement, i.e., from $\leqq 3$ to $\geqq 7$.

Baseline TT4: Compared to neonatal baseline FT4, fewer variables were correlated with baseline TT4 levels. Birthweight and gestational age were both significantly related to TT4 levels at birth. APGAR1 ($p=0.0098$) and Apgar score change from 1 minute to 5 minute ($p=0.0445$) were significantly related to TT4 levels in the same fashion as to FT4 levels. Interestingly, IUGR, a widely accepted risk factor for low thyroid hormone levels in neonates (5, 11-13), was not related to either FT4 or TT4 levels.

4.4.4 Unadjusted association between maternal exposures and neonatal baseline thyroid hormone levels (Table 4.7)

Log-transformed maternal TT4 levels were inversely correlated with neonatal baseline FT4 levels (p=0.0218). No other maternal exposures were related to neonatal baseline FT4 levels at significance level of 0.1.

However, several maternal exposures were significantly correlated with neonatal baseline TT4 levels in unadjusted analyses. Infants born to women who received antibiotics (p=0.0648), antenatal steroids (p=0.0112), and magnesium sulfate (p=0.0809) treatment prior to delivery had higher baseline TT4 levels. Women who had ROM > 24 hours (p=0.0005) prior to onset of labor also had higher TT4 levels at birth.

4.4.5 Correlates of neonatal FT4 level at birth (Table 4.8, Figure 4.2-4.3)

Previously, we found that subjects enrolled in the three centers had different attributes reflecting variations in population and clinical management across centers. We therefore studied the association between study sites and neonatal baseline thyroid hormone levels. No significant results were found. We also adjusted for study centers in the final linear regression model and center was insignificant (p>0.1). As study center has no effect on the outcomes of interest, we did not include center in the final model.

Gestational age, fetal growth ratio, race, multiple birth status, and intrauterine growth restriction were significantly correlated with neonatal baseline FT4 levels. Newborn baseline FT4 levels rose as gestational age increased. Every one week increase in gestational age increased FT4 by 17% (p < 0.0001). FGR was found inversely correlated with baseline FT4 levels. Every 10% increase in fetal growth ratio resulted in a 6% reduction in baseline FT4 levels. IUGR was not significantly related to neonatal

FT4 level in the unadjusted model, but became marginally significant after adjusting for gestational age and other factors. Subjects with growth restriction had an 18% reduction in FT4 level compared to subjects without.

On average, FT4 levels of Blacks were 14% higher than Whites (p=0.0570) and 30% higher than Hispanics (p=0.0016, data not shown). Subjects born as one of a multiple-birth set had on average a 14% reduction in FT4 levels compared to singleton births (p=0.0642).

It is interesting to see that although both FGR and IUGR represent fetal growth, they were related with FT4 levels in different directions. To further understand why FGR was negatively, while IUGR was positively, correlated with FT4 levels, we plotted log-transformed FT4 levels over FGR values by IUGR status. Figure 4.2-4.3 suggests that for subjects who had IUGR, there was no linear relationship between FGR and neonatal baseline FT4 levels (regression p=0.7684, r2=0.0056); for subjects who did not have IUGR, the higher the FGR, or birthweight, the lower the FT4 levels (regression p=0.0034, r2=0.0069).

4.4.6 Correlates of neonatal TT4 level at birth (Table 4.9)

As with baseline FT4 levels, gestational age remained directly significantly correlated with neonatal TT4 levels at birth adjusted for other factors. For every one week increase in gestational age, TT4 increased about 16%.

Neonatal baseline TT4 levels were associated with antenatal steroid use, magnesium use prior to delivery, and ROM > 24 hours prior to onset of labor with adjusting for gestational age. Infants born to women with prolonged ROM had a 32% increase in TT4 levels ($p < 0.0001$). Infants who received antenatal steroids treatment

had a 22% increase in TT4 levels (p=0.0272). Infants born to women who received magnesium sulfate prior to delivery had an average increase of 14% in TT4 levels (p=0.0266). Although both antenatal steroid use (p=0.006) and rupture of membrane for greater than 24 hours (p=0.034) are significantly correlated with magnesium sulfate use prior to delivery, these three factors remain significant in the model after adjusting for each other, which suggests that these three factors were all independently related to neonatal baseline TT4 levels.

4.5 Discussion

In the literature, correlates of neonatal FT4 at birth have seldom been studied, mainly because most studies used thyroid hormone levels obtained from various local newborn screening programs, which normally do not test FT4 values. In our study, we had the opportunity to study both neonatal FT4 and TT4 levels at birth. Our results suggest that neonatal baseline FT4 and TT4 levels were associated with distinct groups of factors: 1) FT4 levels were associated with fixed attributes, such as fetal growth ratio, multiple birth status, race, and IUGR; 2) TT4 levels were associated with pregnancy conditions, such as antenatal steroids/magnesium use and ROM > 24 hours prior to onset of labor.

Gestational age was positively associated with both baseline FT4 and TT4 levels adjusting for other factors. This finding is consistent with previous reports that have shown this association not only in serum samples at birth, but also in cord blood (13-18).

4.5.1 Correlates of neonatal baseline FT4 in premature infants between 24-28 weeks of gestational age (Figure 4.4-4.5)

An interesting discovery is the inverse relationship between fetal growth ratio and neonatal baseline FT4 levels. FGR is an index representing intrauterine growth by comparing birthweight with the gestational week specific median birthweight. A slight decreasing trend in FT4 levels over FGR is shown in figure 4.4. Infants with lower FGR's have higher FT4 levels, and vice versa.

More interestingly, both FGR and IUGR represent fetal growth, one would assume these two variables should relate to baseline FT4 levels similarly. However, in this analysis, presence of IUGR, defined as the lowest 10th percentile of birthweight for gestational age, had an opposite effect. In them, FT4 levels were lower rather than higher.

To further explore the opposite association between FGR and IUGR in relation to baseline FT4 levels, we plotted log FT4 levels over FGR within IUGR stratum (figure 4.2-4.3) and found a significant inverse relationship between log FT4 levels and FGR only among infants who did not have IUGR ($p=0.0034$). Figure 4.5 (mean of log-transformed FT4 by every 10th percentile of FGR) revealed a quadratic shape between FT4 levels and FGR percentiles. FT4 levels were low in the lowest 10th percentile, gradually increased in the second 10th percentile, then decreased until the highest percentile. This illustrates the opposite direction of effect of FGR on FT4 within IUGR and within non-IUGR babies. In effect, infants with the lowest and highest FGR had low FT4 levels, while infants in the middle had higher FT4 levels.

Few studies have examined race and multiple birth status in relation to thyroid hormone levels at birth. In our study, race ($p=0.0167$) and multiple birth status

(p=0.0642, marginally significant) were both correlated with baseline FT4 levels after adjusting for gestational age and other factors. Black infants had the highest FT4 levels at birth compared to Whites (p=0.0570) and Hispanics (p=0.0016, not shown in Table). Singleton births had higher FT4 levels than multiple births. The mean FT4 level difference between singletons and twin births was especially significant (p=0.0489, not shown in Table). To our best knowledge, no one has ever reported any significant associations between race/ethnicity and multiple birth status in relation to baseline thyroid hormone levels. We do not know whether our findings are a true reflection of biological relationships or were due to chance because we do not have a large sample in testing FT4 level differences across race/ethnicity group (power = 71%) or multiple birth status (power = 56%). Future research with a larger sample size stratified by social demographic factors and race profile /multiple birth status is needed.

4.5.2 Correlates of neonatal baseline TT4 in premature infants between 24-28 weeks of gestational age

Martin et al. reported that newborns with a complete dose of antenatal steroid treatment had a mean increase of 0.81 μg/dl in serum TT4 levels (19). Our results support this finding. Infants who received antenatal steroid treatment prior to delivery had a 22% increase in neonatal TT4 levels. Both results, although not comparable, agreed on the directionality of the association between antenatal steroids and newborn baseline TT4 levels.

We also found that ROM > 24 hours prior to onset of labor was related to higher (32%) TT4 levels. Magnesium use prior to delivery, although marginally significant, was also associated with higher TT4 levels. Although a significant correlation existed

between ROM > 24 hours and magnesium use, both factors remained significant after adjusting for each other and additional variables, which suggests that their effects on neonatal baseline TT4 levels were independent.

Other factors, such as gender, route of delivery, Apgar score at 1 minute, and antenatal antibiotic use, have been reported in the literature to be associated with neonatal FT4 and TT4 levels. In our unadjusted analyses, we found FT4 levels, at the significance level of 0.1, were associated with gender and Apgar score at 1 minute, and TT4 levels were associated with Apgar score at 1 minute and antibiotic use prior to delivery. All these associations disappeared after adjusting for gestational age and other factors.

4.5.3 Relationship between neonatal baseline thyroid hormone levels and maternal thyroid hormone levels (Figure 4.6 - 4.8)

Hume et al. reported an overall lack of association between neonatal cord and maternal thyroid hormone levels (16). We looked at the relationship between neonatal baseline and maternal thyroid hormone levels. Maternal TT4 and neonatal FT4 levels were correlated, but the significance disappeared after adjusting for other factors. No associations in FT4 or TT4 levels were observed between maternal and neonatal serum samples within different gestational week groups (figure 4.6-4.8).

Murphy et al. found a strong relationship between maternal and neonatal TSH levels (18). Significant associations between maternal and neonatal TSH within each gestational week group were also seen in our study (figure 4.8).

4.6 Strength and limitation (Table 4.10)

Our study collected information on 168 neonates born between 24 and 28 completed weeks of gestational age and is the largest study for this unique premature population. We also are the first group to study a broad range of prenatal exposures and baseline thyroid hormone levels at birth.

Unlike most previous studies, we measured serum FT4 levels by the direct equilibrium dialysis (DED) method. Studies have shown that analog immunoassay or FT4 index methods, commonly used by earlier studies, could be biased by the concentrations of serum proteins and protein-bound T4 (3, 20). The DED method can maximally remove bias from serum protein levels, thus provides us the most accurate FT4 levels.

Baseline FT4 levels were measured between birth and 24 hours of life. One may question whether the time of collection matters in this population. The literature has shown that in term infants, thyroid hormone surges soon after birth. TSH surges within half an hour after birth and decreases soon after its maximum level. Serum T4 and T3 levels increase quickly following TSH surge and peak at 24 hours of life. Various time points of sample collection may result in capturing FT4 and TT4 levels in different stages of the increasing phase, which may not reflect the baseline thyroid hormone levels of the study subjects.

Several studies have shown that among extremely premature infants, the hypothalamic-pituitary-thyroid system may not respond to the extra-uterine environment in the same way or at the same level of magnitude as in the term or moderately preterm infant. Hormone surges were found to be attenuated, and some even disappeared, due to

immaturity of the thyroid gland and the hypothalamic-pituitary-thyroid system. To test whether time of sample collection affected our results, we calculated the mean and standard deviation of neonatal FT4 and TT4 levels on day 0 for all study participants and on other days among the subjects who did not receive thyroid hormone treatment (Table 4.10). We found that FT4 levels hardly changed between day 0 and day 3, which suggested that time of collection on day 0 would not affect the associations we observed. TT4 levels began to decline for about 10-20% by day 3, which implied that most of the relationships we found in the current analyses would be at most mildly affected by time of collection.

4.7 Conclusion

In conclusion, we found that except for gestational age, different groups of factors were associated with baseline FT4 and TT4 levels among premature infants born between 24-28 weeks of gestational age. Baseline FT4 levels were associated with fixed attributes, such as race, fetal growth ratio, intrauterine growth restriction, and multiple birth status; while baseline TT4 levels were associated with conditions such as antenatal steroids and magnesium treatment, and ROM > 24 hours prior to onset of labor.

Neonatal baseline FT4/TT4 levels were not related to maternal FT4/TT4 levels, however, TSH levels of neonates and mothers were positively correlated with each other.

Figure 4.1 Flow chart of analytical sample selection.

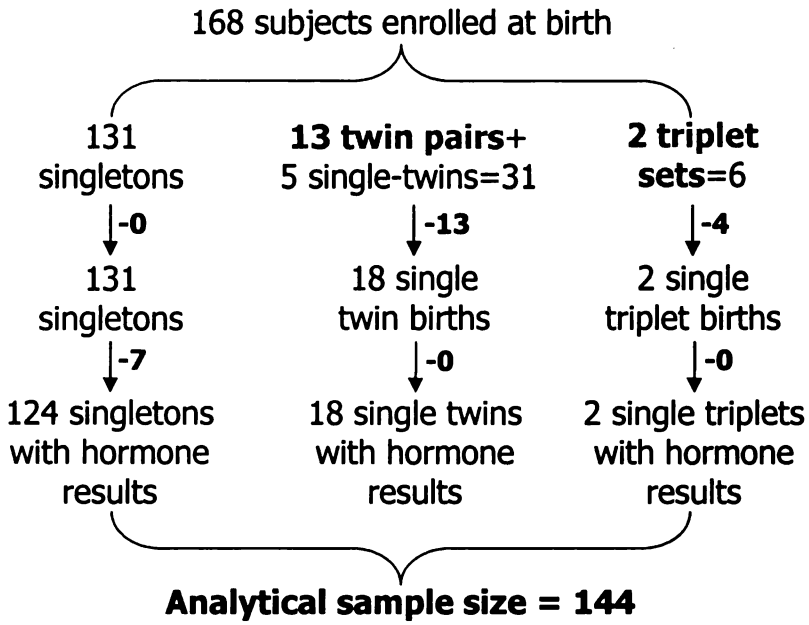


Figure 4.2 Correlation between log-transformed FT4 and FGR in infants with IUGR.

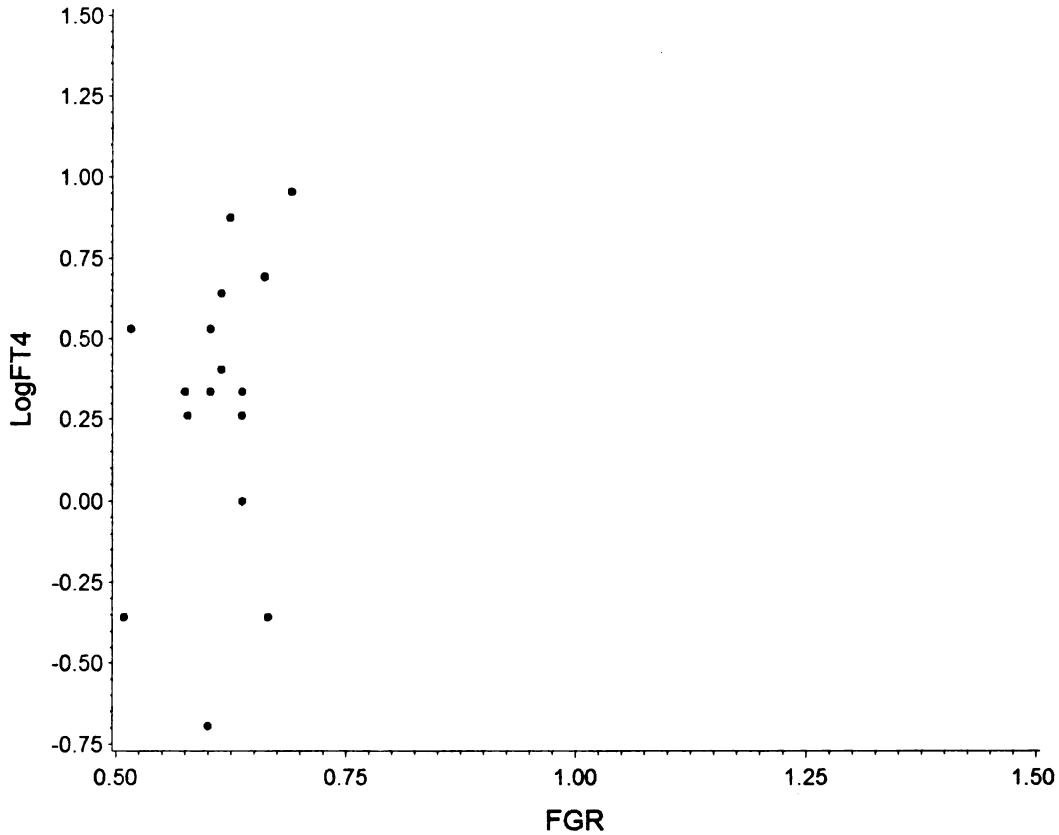


Figure 4.3 Correlation between log-transformed FT4 and FGR in infants without IUGR.

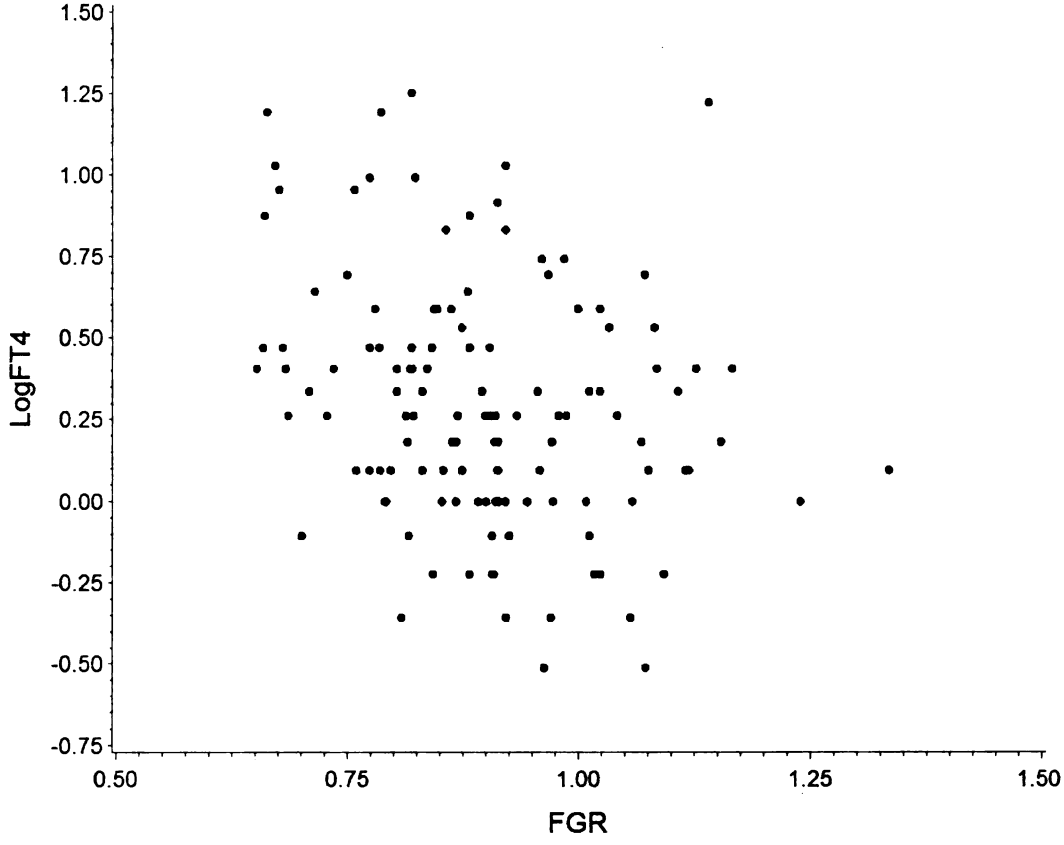
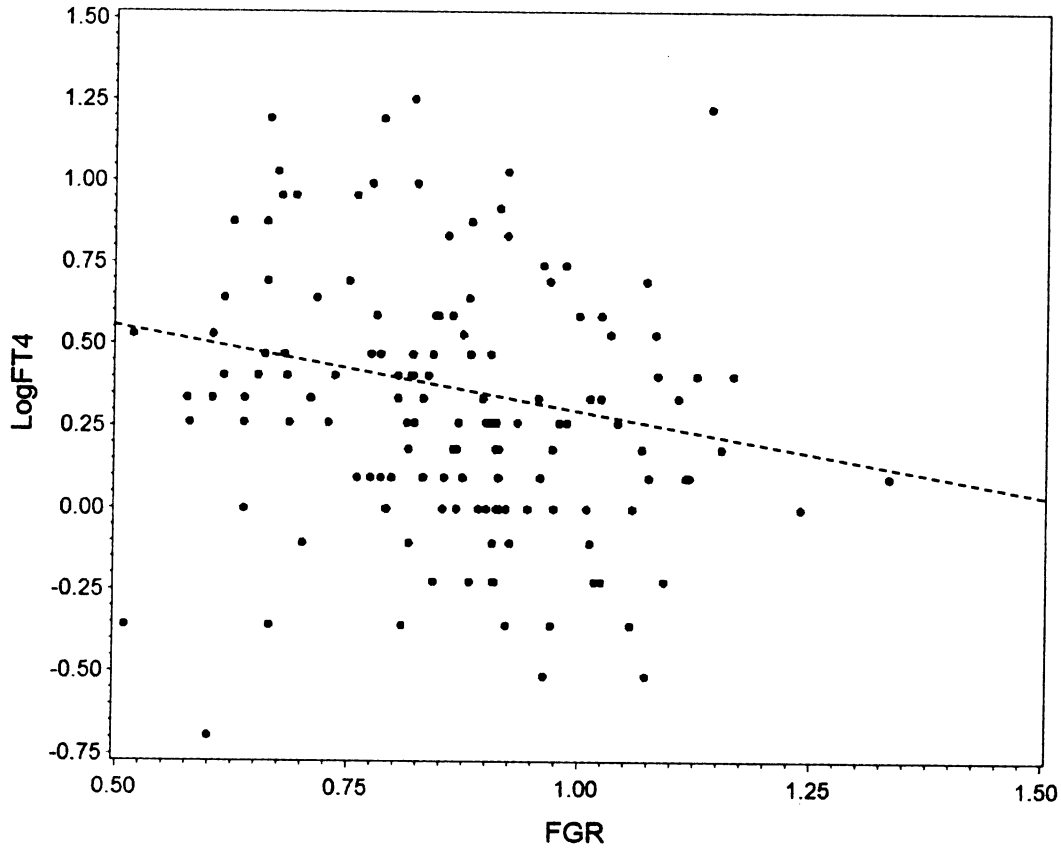


Figure 4.4 Log-transformed FT4 distribution over FGR.



R-square=0.0439
Adjusted R-square=0.0371

Figure 4.5 Mean FT4 (log-transformed) by FGR 10th percentile.

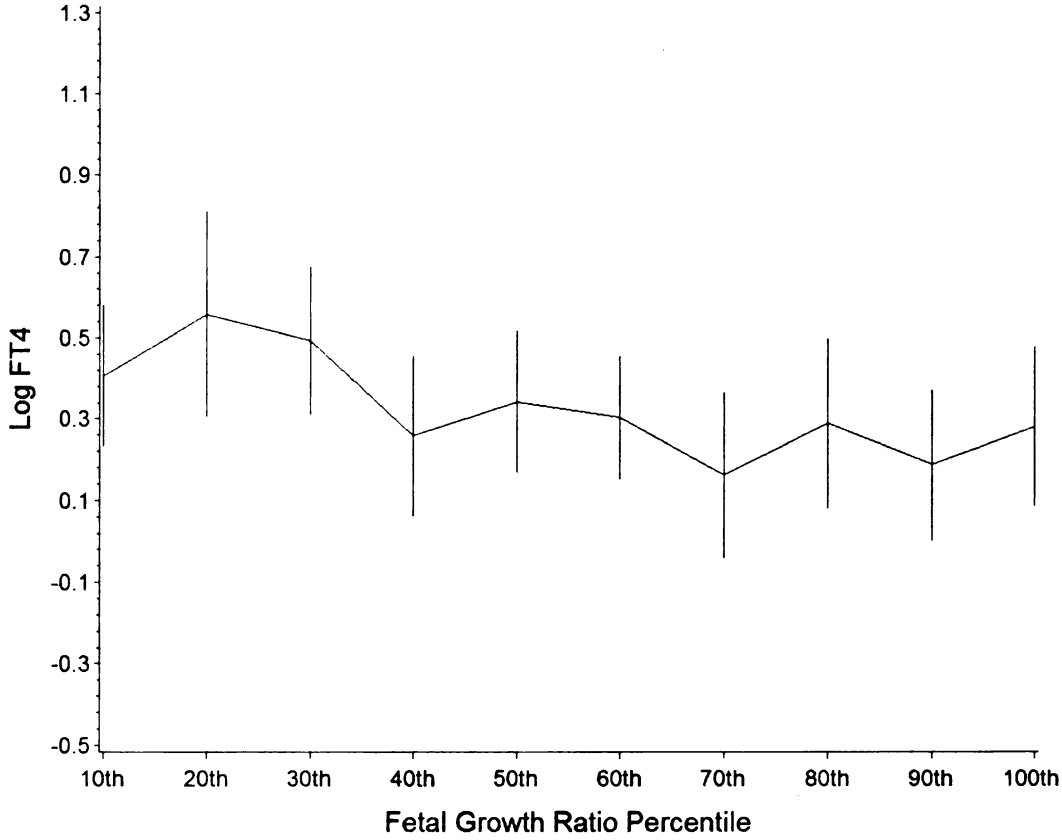


Figure 4.6 Correlation between maternal and neonatal FT4 (log-transformed) by gestational week among 144 subjects.

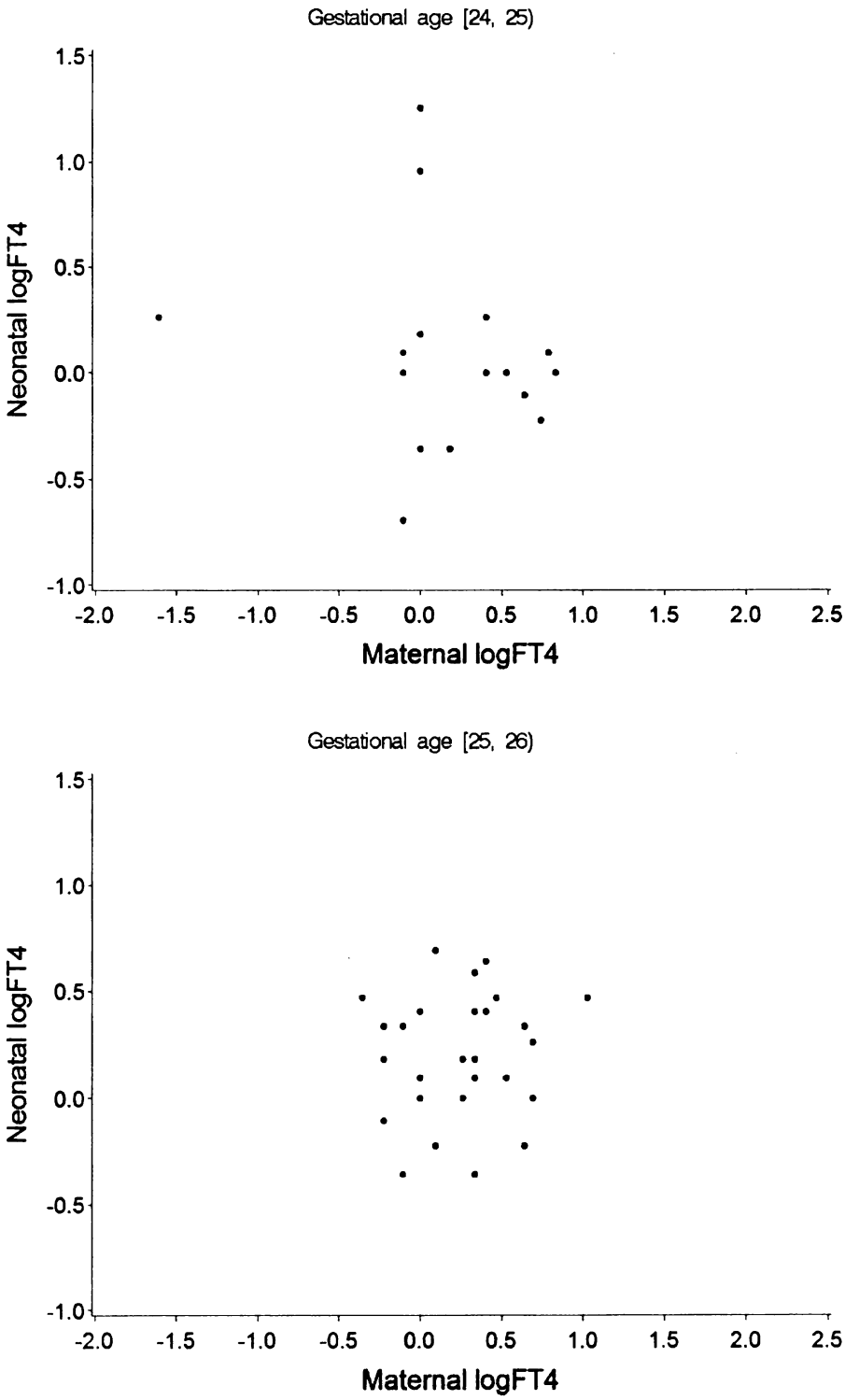
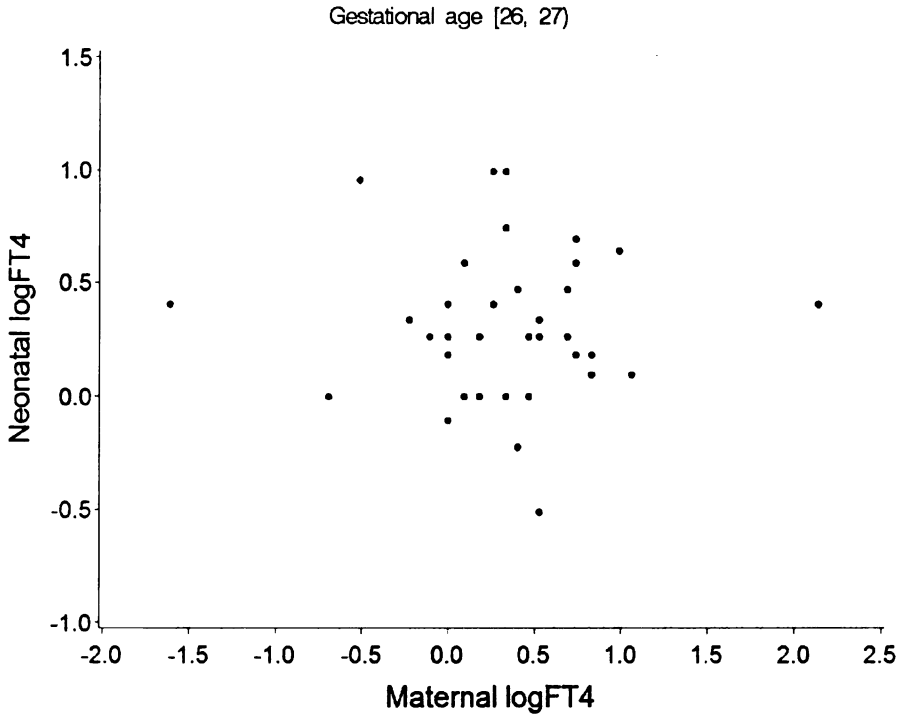


Figure 4.6 Correlation between maternal and neonatal FT4 (log-transformed) by gestational week among 144 subjects. (cont'd)



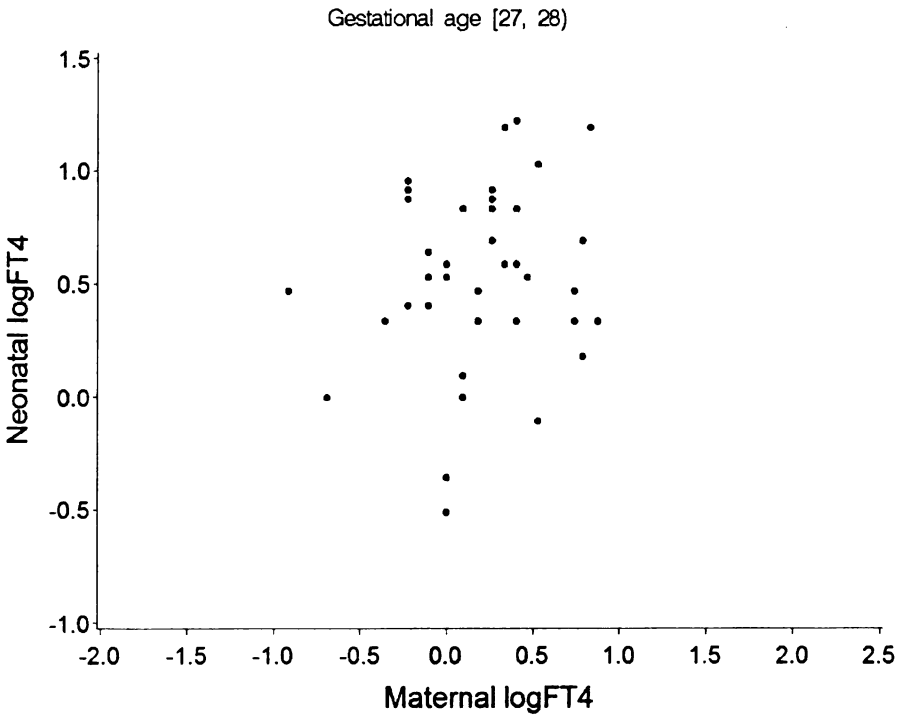


Figure 4.6 Correlation between maternal and neonatal FT4 (log-transformed) by gestational week among 144 subjects. (cont'd)

GA (week)	β coefficient	P-value
24-25	-0.1365	0.5321
25-26	0.0528	0.7212
26-27	-0.0118	0.9025
27-28	0.1698	0.3032

Figure 4.7 Correlation between maternal and neonatal TT4 (log-transformed) by gestational week among 144 subjects.

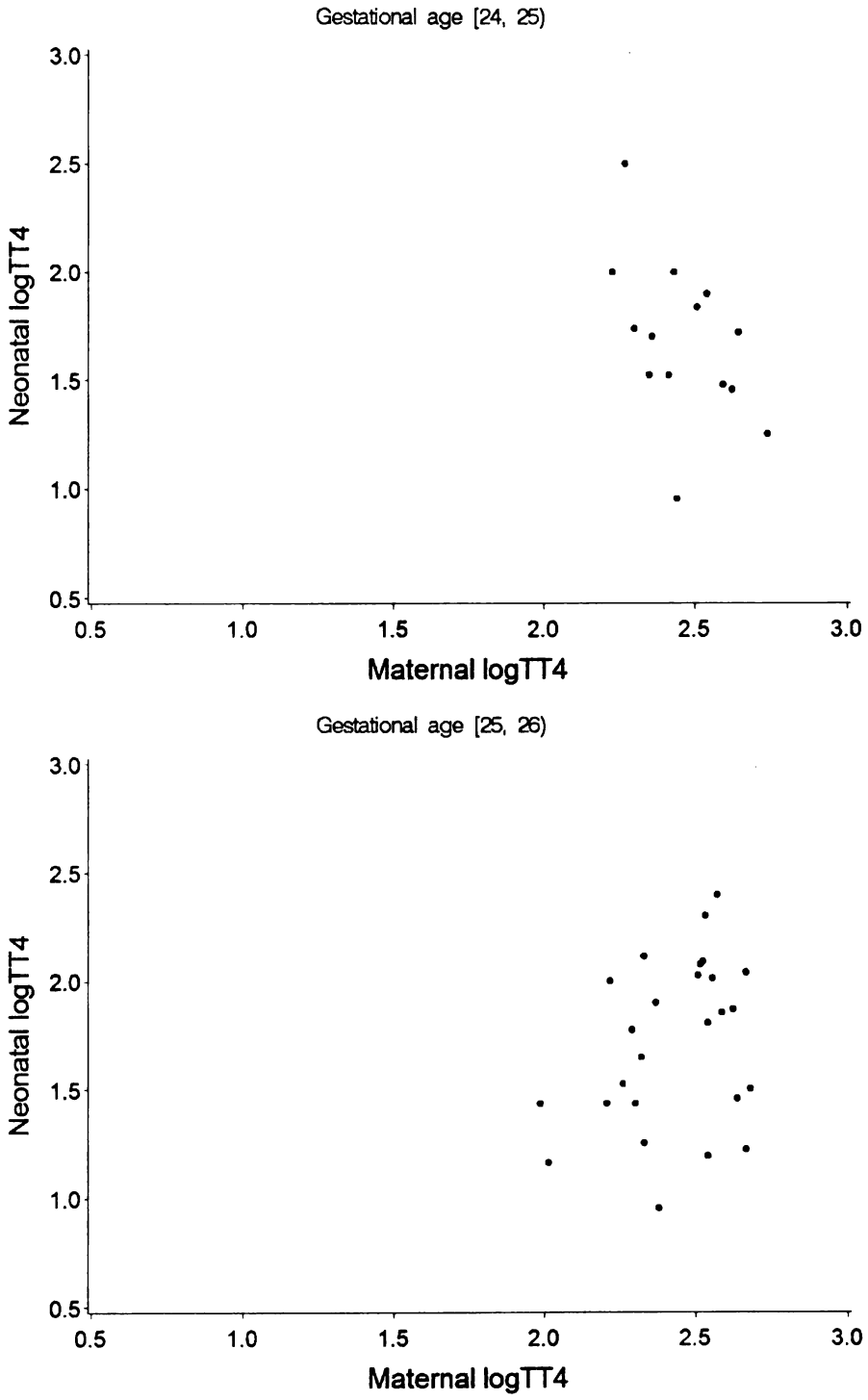


Figure 4.7 Correlation between maternal and neonatal TT4 (log-transformed) by gestational week among 144 subjects. (cont'd)

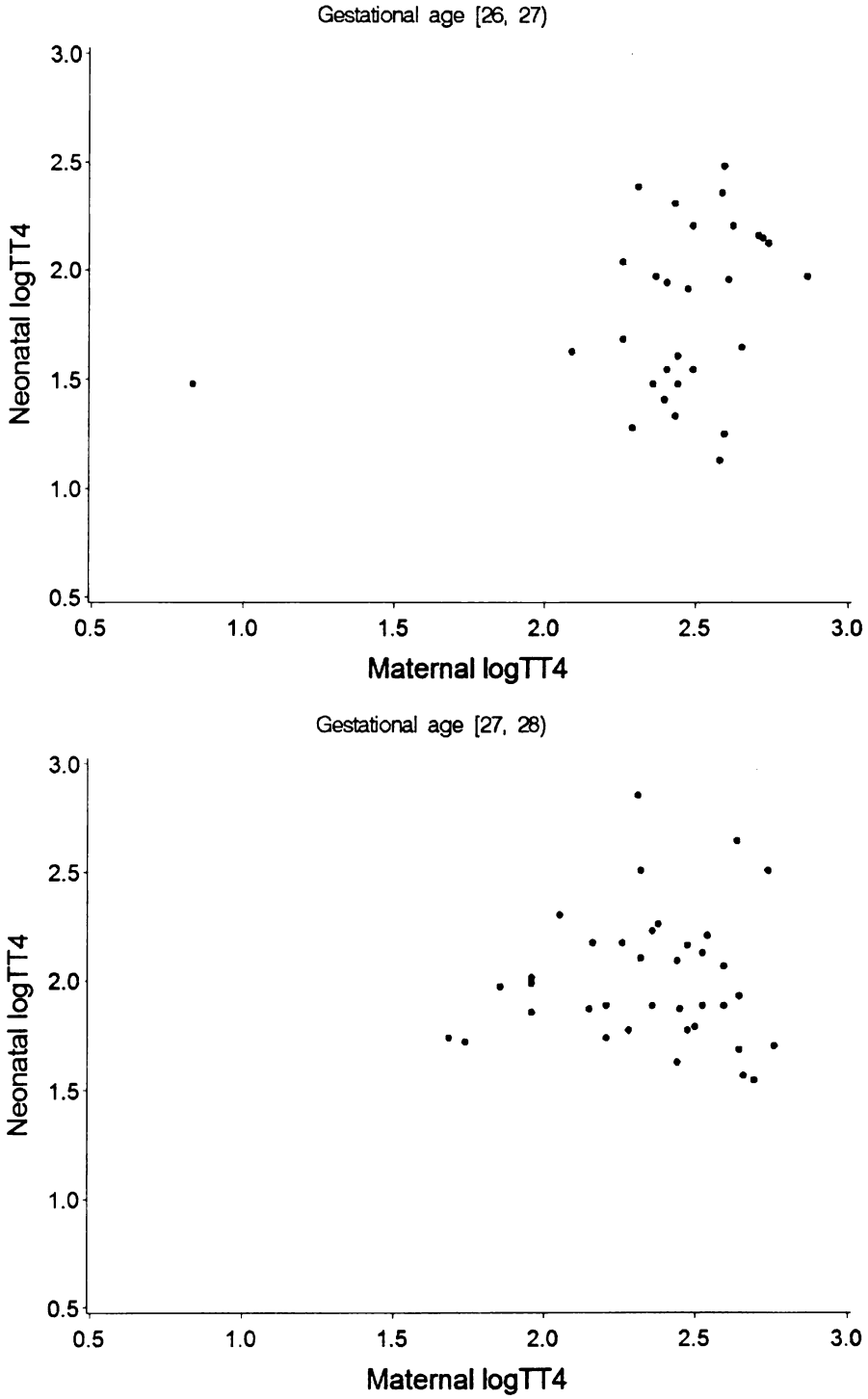


Figure 4.7 Correlation between maternal and neonatal TT4 (log-transformed) by gestational week among 144 subjects. (cont'd)

GA (week)	β coefficient	P-value
24-25	-0.3051	0.0697
25-26	-0.1601	0.4757
26-27	0.2113	0.0538
27-28	-0.0029	0.9817

Figure 4.8 Correlation between maternal and neonatal TSH (log-transformed) by gestational week among 144 subjects.

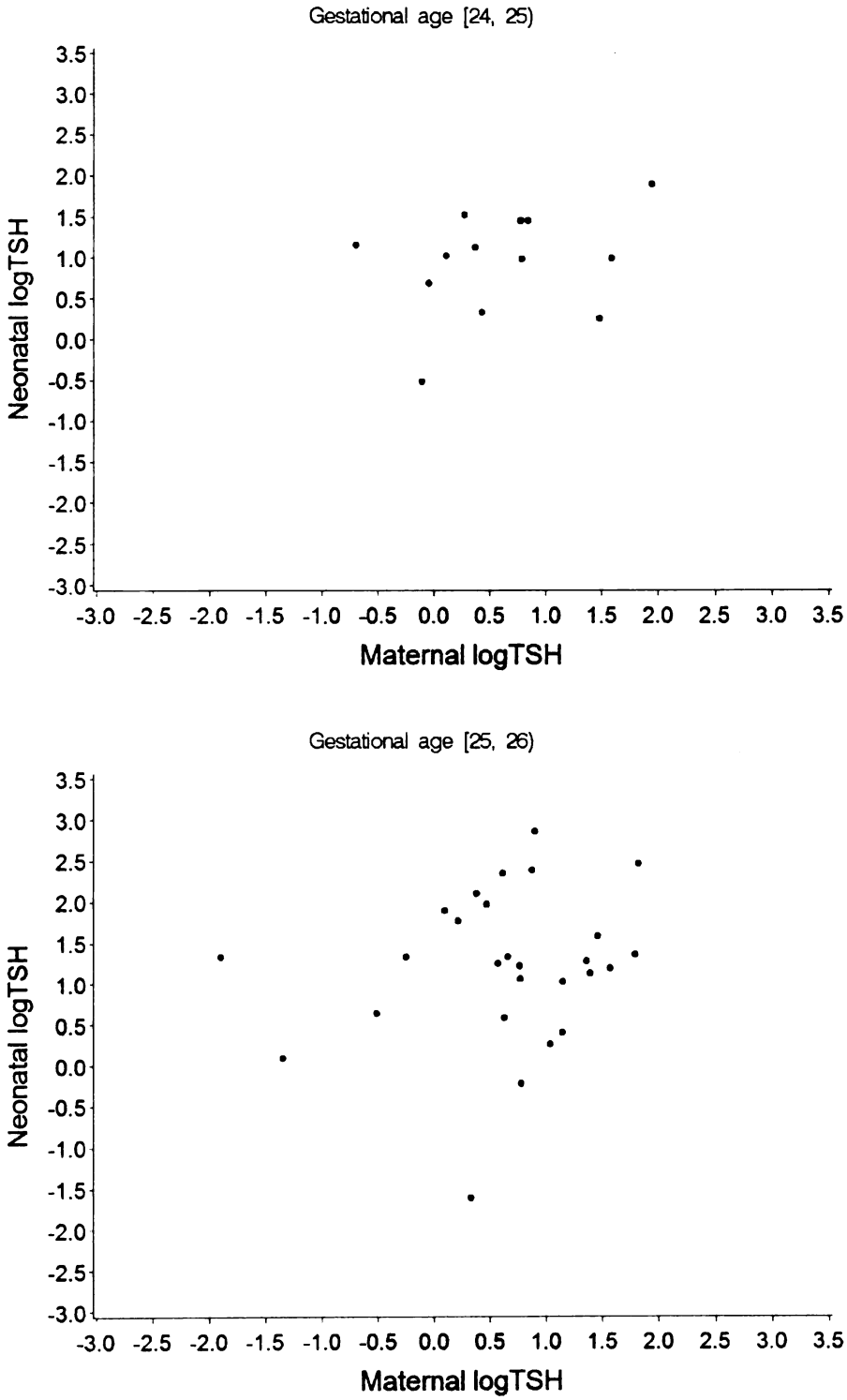


Figure 4.8 Correlation between maternal and neonatal TSH (log-transformed) by gestational week among 144 subjects. (cont'd)

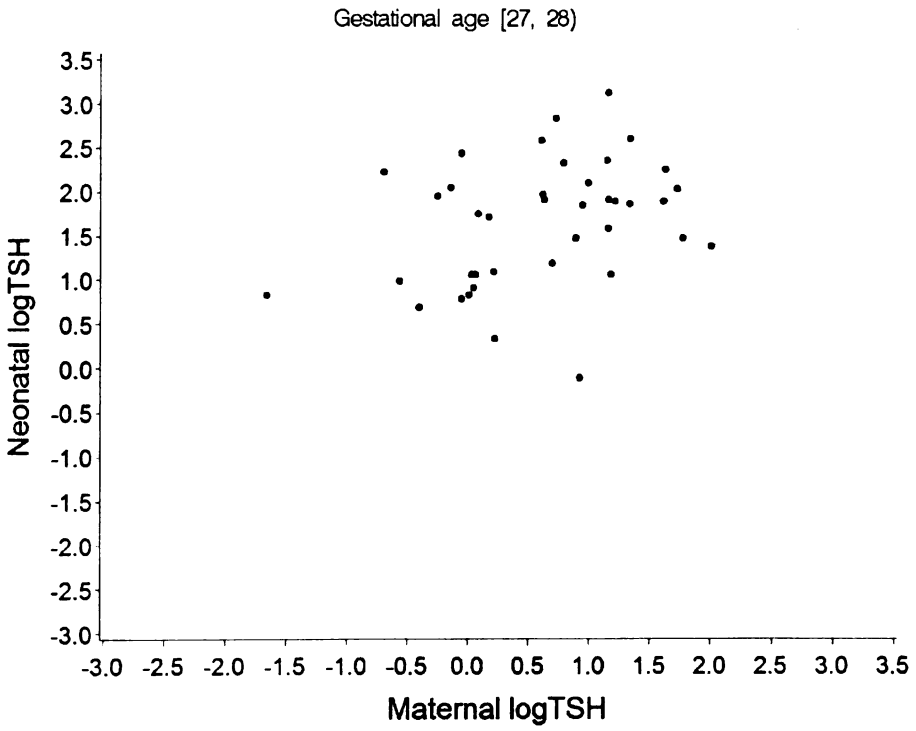


Figure 4.8 Correlation between maternal and neonatal TSH (log-transformed) by gestational week among 144 subjects. (cont'd)

GA (week)	β coefficient	P-value
24-25	0.2591	0.3767
25-26	-0.1410	0.8089
26-27	0.0551	0.7522
27-28	-0.0670	0.8232

Table 4.1 Neonatal and maternal characteristics of enrolled subjects.

Neonatal characteristics		**N=144**
Birthweight (g)	Mean (SD)	855 (198.3)
	Median (Range)	830 (440, 1550)
Gestational age (wk)	Mean (SD)	26.2 (1.1)
	Median (Range)	26.3 (24, 27.9)
Fetal growth ratio	Mean (SD)	0.86 (0.2)
	Median (Range)	0.87 (0.43, 1.33)
		N (%)
Gender	Male	79 (54.9)
Race	White	90 (62.5)
	Black	35 (24.3)
	Hispanic	13 (9)
Multiple birth (twins and triplets)		20 (13.9)
C-section		66 (45.8)
Intrauterine growth restriction (IUGR)		18 (12.5)
Apgar score at 1 min	≤ 3	36 (26.5)
	4 - 6	56 (41.2)
	7-10	44 (32.3)
Apgar score at 5 min	≤ 3	4 (2.8)
	4 - 6	26 (18.3)
	7-10	112 (78.9)
Apgar score change from 1 min to 5 min	≤ 3 to (4 - 6)	16 (11.8)
	(4 - 6) to ≥ 7	46 (33.8)
	≤ 3 to ≥ 7	16 (11.8)
Maternal characteristics		**N=144**
Maternal age (yr)	Mean (SD)	29.5 (5.8)
	Median (range)	29 (18, 47)
		N (%)
HELLP syndrome		4 (2.8)
Preeclampsia		20 (14)
Fever during pregnancy		15 (10.4)
Antibiotics administration		66 (45.8)
Antenatal steroids administration		119 (82.6)
Magnesium administration		42 (29.2)
ROM > 24 hr		40 (30.1)

Table 4.2 Neonatal and maternal thyroid hormone levels at birth.

Hormone	**N**	**Mean (SD)**	**Median**	**Range**	**Percentile**			
					10th	**25th**	**75th**	**90th**
Neonatal								
FT4 (μg/dl)	144	1.5 (0.6)	1.3	(0.5, 3.5)	0.8	1.1	1.7	2.4
TT4 (ng/dl)	128	6.8 (2.7)	6.5	(2.5, 17.4)	3.9	4.7	8.2	10.6
TSH (mIU/ml)	135	5.2 (3.8)	4.3	(0, 22.8)	1.5	2.7	6.7	10.6
Maternal								
FT4 (μg/dl)	122	1.4 (0.8)	1.3	(0.2, 8.5)	0.8	1.0	1.7	2.1
TT4 (ng/dl)	119	11.5 (2.5)	11.6	(2.3, 17.6)	8.1	10.1	13.4	14.4
TSH (mIU/ml)	120	2.3 (1.6)	1.9	(0.1, 7.4)	0.7	1.1	3.1	4.9

Table 4.3 Neonatal thyroid hormone levels at birth by study site.

Hormone/Site	N	Mean (SD)	Median	Range	Percentile				p-value
					10th	25th	75th	90th	
FT4 (μg/dl)									
New York	70	1.6 (0.7)	1.4	(0.5, 3.5)	0.8	1.0	2.0	2.6	
Amsterdam	40	1.5 (0.5)	1.3	(0.8, 2.8)	1.0	1.1	1.8	2.2	**0.0206**
Madrid	34	1.2 (0.3)	1.3	(0.6, 1.9)	0.8	1.0	1.4	1.6	
TT4 (ng/dl)									
New York	69	7.1 (3.1)	6.6	(2.5, 17.4)	3.9	4.6	8.3	12.3	
Amsterdam	34	6.7 (2.2)	6.5	(3.4, 12.0)	4.2	4.8	8.4	10.1	0.2804
Madrid	25	6.0 (2.1)	6.0	(2.6, 10.6)	3.3	4.4	7.4	8.7	
TSH (mIU/ml)									
New York	67	5.6 (3.9)	4.8	(0.2, 17.5)	1.4	2.8	7.2	11.8	
Amsterdam	38	4.9 (4.1)	3.7	(0.8, 22.8)	1.8	2.3	5.8	10.6	0.3711
Madrid	30	4.5 (2.9)	4.3	(0, 14.5)	1.3	2.5	5.8	7.6	

Table 4.4 Maternal thyroid hormone levels by study site.

Hormone/Site	N	Mean (Se)	Median	Range	Percentile				p-value
					10th	25th	75th	90th	
FT4 (μg/dl)									
New York	63	1.2 (0.5)	1.2	(0.2, 2.3)	0.8	0.9	1.5	2.0	
Amsterdam	32	1.5 (1.3)	1.3	(0.2, 8.5)	0.8	1.1	1.6	2.0	**0.0043**
Madrid	27	1.9 (0.5)	1.9	(0.9, 2.9)	1.0	1.5	2.1	2.7	
TT4 (ng/dl)									
New York	61	11.1 (2.5)	11.0	(2.3, 15.5)	8.1	9.8	12.9	14.0	
Amsterdam	33	11.2 (2.5)	11.5	(5.7, 15.2)	7.1	10.2	12.6	14.1	**0.0026**
Madrid	25	13.0 (2.0)	12.7	(9.1, 17.6)	10.6	11.5	14.1	15.5	
TSH (mIU/ml)									
New York	61	2.2 (1.6)	1.8	(0.1, 6.6)	0.6	1.0	2.5	4.4	
Amsterdam	33	2.3 (1.4)	2.2	(0.2, 5.9)	0.8	1.1	3.2	4.0	0.4977
Madrid	26	2.6 (1.9)	1.9	(0.2, 7.4)	1.1	1.3	3.4	5.6	

Table 4.5 P-values of pair-wise comparison between sites on thyroid hormone levels.

Comparison	Neonatal			Maternal		
	FT4	TT4	TSH	FT4	TT4	TSH
NY vs. Amsterdam	0.4342	0.5794	0.3348	0.1245	0.8079	0.6039
NY vs. Madrid	**0.0055**	0.1120	0.1978	**0.0011**	**0.0009**	0.2421
Amsterdam vs. Madrid	0.0651	0.3309	0.7199	0.1006	**0.0049**	0.5355

Table 4.6 Unadjusted associations between neonatal exposures and baseline serum Free T4 and Total T4 levels (log-transformed).

	Log FT4 (n=144)	P value	Log TT4 (n=128)	P value
Fixed attributes				
	β (se)	P value	β (se)	P value
Birthweight (100 g)	0.01 (0.02)	0.4156	0.04 (0.02)	**0.0237**
Gestational age (wk)	0.14 (0.03)	**< 0.0001**	0.12 (0.03)	**0.0001**
Fetal growth ratio	-0.51 (0.2)	**0.0117**	-0.08 (0.2)	0.7206
	mean (se)		mean (se)	
Study site				
New York	0.36 (0.05)	**0.0555**	1.87 (0.05)	0.3240
Amsterdam	0.35 (0.05)		1.86 (0.06)	
Madrid	0.17 (0.05)		1.74 (0.08)	
Gender				
Male	0.26 (0.04)	**0.0783**	1.79 (0.05)	0.1527
Female	0.37 (0.05)		1.89 (0.05)	
Race				
White	0.29 (0.03)	**0.0320**	1.77 (0.04)	0.1042
Black	0.44 (0.07)		1.92 (0.07)	
Hispanic	0.08 (0.14)		1.93 (0.14)	
Other	0.26 (0.27)		2.04 (0.17)	
Multiple birth				
Singleton	0.33 (0.04)	**0.0649**	1.86 (0.04)	0.2372
Twins or triplets	0.16 (0.07)		1.74 (0.08)	
Route of delivery				
Cesarean section	0.36 (0.04)	0.1677	1.83 (0.05)	0.8084
Vaginal delivery	0.27 (0.05)		1.85 (0.05)	
Intrauterine growth restriction				
Yes	0.35 (0.11)	0.6461	1.84 (0.09)	0.9477
No	0.30 (0.03)		1.84 (0.04)	
Changing attributes				
Apgar score at 1'				
≤ 3	0.21 (0.06)	**0.0804**	1.71 (0.05)	**0.0098**
4 - 6	0.30 (0.05)		1.82 (0.06)	
≥ 7	0.41 (0.06)		1.98 (0.06)	
Apgar score at 5'				
≤ 3	0.36 (0.17)	0.9418	1.82 (0.10)	0.2824
4 - 6	0.29 (0.09)		1.74 (0.06)	
≥ 7	0.32 (0.03)		1.87 (0.04)	
Apgar score change from 1 min to 5 min				
≤ 3 to 4-6	0.22 (0.11)	**0.0810**	1.68 (0.08)	**0.0445**
≤ 3 to ≥ 7	0.17 (0.09)		1.70 (0.08)	
4-6 to ≥ 7	0.28 (0.05)		1.81 (0.07)	
No change	0.41 (0.06)		1.94 (0.05)	

Table 4.7 Unadjusted associations between maternal exposures and baseline serum Free T4 and Total T4 levels (log-transformed).

	Log FT4 (n=144)	**P value**	**Log TT4 (n=128)**	**P value**
	mean (se)		mean (se)	
Primigravida				
Yes	0.36 (0.06)		1.89 (0.06)	
No	0.29 (0.04)	0.3216	1.81 (0.04)	0.2893
Preeclampsia				
Yes	0.42 (0.08)		1.91 (0.60)	
No	0.29 (0.04)	0.1797	1.83 (0.04)	0.4170
Fever				
Yes	0.36 (0.11)		1.99 (0.11)	
No	0.30 (0.03)	0.6037	1.82 (0.04)	0.1160
Antibiotics use during pregnancy				
Yes	0.29 (0.05)		1.90 (0.05)	
No	0.33 (0.04)	0.5038	1.78 (0.04)	**0.0648**
Antenatal steroids use				
Yes	0.31 (0.03)	0.8763	1.88 (0.04)	**0.0112**
No	0.30 (0.09)		1.64 (0.08)	
Magnesium use				
Yes	0.33 (0.07)		1.93 (0.06)	
No	0.30 (0.04)	0.7579	1.80 (0.04)	**0.0809**
Rupture of membrane ≥ 24 hr				
Yes	0.32 (0.07)		2.03 (0.07)	
No	0.29 (0.04)	0.6812	1.76 (0.04)	**0.0005**
	β (se)		β (se)	
Maternal age (yr)	2e-3 (6e-3)	0.7259	5e-3(6e-3)	0.4197
Maternal gravida	0.01 (0.01)	0.4784	-5e-3(0.02)	0.7472
Maternal log Free T4	-2.4e-4 (0.07)	0.9974	0.01 (0.07)	0.9036
Maternal log Total T4	-0.31 (0.13)	**0.0207**	0.11 (0.13)	0.4065
Maternal log TSH	-0.02 (0.05)	0.5832	0.06 (0.05)	0.1827

Table 4.8 Adjusted association between maternal/neonatal exposures and baseline serum Free T4 levels (log-transformed).

	Estimate (n=144)[1]	% of change	SE	t Value	p-value[2]	Overall p-value[3]
Intercept	-3.57	-	0.73	-4.91	**<.0001**	**<.0001**
Gestational age (wk)	0.16	**17% ↑**	0.03	5.89	**<.0001**	**<.0001**
Fetal growth ratio	-0.063	**6% ↓**	0.023	-2.76	**0.0067**	**0.0067**
Race/ethnicity						
Black[4]	0.13	14% ↑	0.07	1.92	0.0570	**0.0167**
Hispanic	-0.23	**20% ↓**	0.10	-2.28	**0.0240**	
Other	0.04	4% ↑	0.14	0.27	0.7851	
White	ref	-	-	-	-	
Multiple birth						
Multiple birth	-0.15	14% ↓	0.08	-1.87	0.0642	0.0642
Singleton	ref	-	-	-	-	
Intrauterine growth restriction						
Yes	-0.20	18% ↓	0.11	-1.88	0.0627	0.0627
No	ref	-	-	-	-	

1 Adjusted for everything else in the table.
2 P-value by Wald-test.
3 P-value by F-test.
4 The % of change in comparison of Black vs. Hispanic is 30% (p=0.0016).

Table 4.9 Adjusted association between maternal/neonatal exposures and baseline serum total T4 levels (log-transformed).

	Estimate (n=144)[1]	% of change	SE	t Value	p-value[2]	Overall p-value[3]
Intercept	-2.17	-	0.77	-2.81	**0.0058**	**0.0058**
Gestational age (wk)	0.15	**16% ↑**	0.03	5.16	**<.0001**	**<.0001**
Antenatal steroids						
Yes	0.20	**22% ↑**	0.09	2.24	**0.0272**	**0.0272**
No	ref	-	-	-	-	
ROM > 24 hours						
Yes	0.28	**32% ↑**	0.07	4.22	**<.0001**	**<.0001**
No	ref	-	-	-	-	
Magnesium sulfate						
Yes	0.13	14% ↑	0.07	1.88	0.0625	0.0625
No	ref	-	-	-	-	

1 Adjusted for everything else in the table.
2 P-value by Wald-test.
3 P-value by F-test.

Table 4.10 Possible variations of FT4 and TT4 introduced by various time of collection.

		FT4		TT4	
	Postnatalday	N	Mean (SD)	N	Mean (SD)
Complete cohort	0	160	1.45 (0.59)	144	6.63 (2.72)
	0	50	1.51 (0.64)	47	6.42 (2.80)
	3	51	1.64 (0.96)	41	5.32 (2.43)
	7	51	1.84 (1.34)	45	4.44 (1.90)
Placebo + Iodine group	14	49	1.52 (0.81)	44	4.45 (2.16)
	21	48	1.50 (0.77)	42	5.29 (2.42)
	42	48	1.78 (0.96)	46	6.98 (2.35)
	56	43	1.64 (0.96)	39	7.26 (2.13)
	0	25	1.45 (0.5)	25	6.52 (2.59)
	3	27	1.35 (0.44)	22	5.12 (2.21)
	7	27	1.70 (1.20)	23	4.77 (2.00)
Placebo group only	14	27	1.46 (0.99)	26	4.32 (1.97)
	21	24	1.48 (0.73)	21	5.44 (2.34)
	42	26	1.43 (0.88)	25	6.49 (1.56)
	56	25	1.47 (0.59)	22	6.89 (2.27)

BIBLIOGRAPHY

1. Den Ouden AL, Kok JH, Verkerk PH, Brand R, Verloove-Vanhorick SP. The relation between neonatal thyroxine levels and neurodevelopmental outcome at age 5 and 9 years in a national cohort of very preterm and/or very low birth weight infants. Pediatr Res 1996;39:142-5.

2. Hadeed AJ, Asay LD, Klein AH, Fisher DA. Significance of transient postnatal hypothyroxinemia in premature infants with and without respiratory distress syndrome. Pediatrics 1981;68:494-8.

3. Rabin CW, Hopper AO, Job L, et al. Incidence of low free T4 values in premature infants as determined by direct equilibrium dialysis. J Perinatol 2004;24:640-4.

4. Reuss ML, Paneth N, Lorenz JM, Susser M. Correlates of low thyroxine values at newborn screening among infants born before 32 weeks gestation. Early Hum Dev 1997;47:223-33.

5. Romagnoli C, Curro V, Luciano R, et al. Serial blood T4 and TSH determinations in low birth weight infants. Influence of gestational age, birth weight and neonatal pathology on thyroid function. Helv Paediatr Acta 1982;37:331-44.

6. Lucas A, Morley R, Fewtrell MS. Low triiodothyronine concentration in preterm infants and subsequent intelligence quotient (IQ) at 8 year follow up. Bmj 1996;312:1132-3; discussion 1133-4.

7. Lucas A, Rennie J, Baker BA, Morley R. Low plasma triiodothyronine concentrations and outcome in preterm infants. Arch Dis Child 1988;63:1201-6.

8. Meijer WJ, Verloove-Vanhorick SP, Brand R, van den Brande JL. Transient hypothyroxinaemia associated with developmental delay in very preterm infants. Arch Dis Child 1992;67:944-7.

9. Reuss ML, Paneth N, Pinto-Martin JA, Lorenz JM, Susser M. The relation of transient hypothyroxinemia in preterm infants to neurologic development at two years of age. N Engl J Med 1996;334:821-7.

10. Alexander GR, Kogan M, Martin J, Papiernik E. What are the fetal growth patterns of singletons, twins, and triplets in the United States? Clin Obstet Gynecol 1998;41:114-25.

11. Jacobsen BB, Hummer L. Changes in serum concentrations of thyroid hormones and thyroid hormone-binding proteins during early infancy. Studies in healthy fullterm, small-for-gestational age and preterm infants aged 7 to 240 days. Acta Paediatr Scand 1979;68:411-8.

12. Martin CR, Van Marter LJ, Allred EN, Leviton A. Growth-restricted premature infants are at increased risk for low thyroxine. Early Hum Dev 2001;64:119-28.

13. Uhrmann S, Marks KH, Maisels MJ, et al. Thyroid function in the preterm infant: a longitudinal assessment. J Pediatr 1978;92:968-73.

14. Carrascosa A, Ruiz-Cuevas P, Potau N, et al. Thyroid function in seventy-five healthy preterm infants thirty to thirty-five weeks of gestational age: a prospective and longitudinal study during the first year of life. Thyroid 2004;14:435-42.

15. Hirano T, Singh J, Srinivasan G, Pildes R. Post-natal thyroid function in low birth weight infants; a longitudinal assessment of free thyroxine and thyroid hormone binding globulin. Acta Endocrinol (Copenh) 1985;110:56-60.

16. Hume R, Simpson J, Delahunty C, et al. Human fetal and cord serum thyroid hormones: developmental trends and interrelationships. J Clin Endocrinol Metab 2004;89:4097-103.

17. Mercado M, Yu VY, Francis I, Szymonowicz W, Gold H. Thyroid function in very preterm infants. Early Hum Dev 1988;16:131-41.

18. Murphy N, Hume R, van Toor H, et al. The hypothalamic-pituitary-thyroid axis in preterm infants; changes in the first 24 hours of postnatal life. J Clin Endocrinol Metab 2004;89:2824-31.

19. Martin CR, Van Marter LJ, Allred EN, Leviton A. Antenatal glucocorticoids increase early total thyroxine levels in premature infants. Biol Neonate 2005;87:273-80.

20. Nelson JC, Weiss RM, Wilcox RB. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. J Clin Endocrinol Metab 1994;79:76-9.

Chapter 5 Correlates of postnatal thyroid hormone levels among extremely premature infants born between 24-28 weeks of gestational age

Studies have shown that differences in postnatal thyroid hormone levels exist between extremely preterm infants (< 28 completed weeks of gestational age) and both term and moderately preterm infants (29-36 completed weeks of gestational age). At birth, the TSH levels of term infants peak within 30 minutes, followed by a sharp decrease (1-3). Serum T4 and T3 levels increase quickly following the TSH surge and peak at 24 hours after delivery. The thyroid hormone surges of moderately preterm infants are similar to term infants, but on a much smaller scale (1, 4). For extremely preterm infants, however, the thyroid hormone surges have been found greatly attenuated, or even absent (5, 6).

It is not well known, however, among extremely preterm infants, whether prenatal and postnatal exposures are associated with the postnatal thyroid hormone levels. Though previous findings have suggested that postnatal thyroid hormone levels are associated with gestational age, postnatal age, respiratory distress syndrome, and sepsis, they have not taken into account the correlation among hormone measurements within each subject in their analyses (7-10). In addition, very little is known about the association between necrotizing enterocolitis (NEC) and decreased postnatal thyroid hormone levels.

In this chapter, we use data collected from the Phase 1 Study of Thyroid Hormone in Extremely Premature Infants 1) to observe the postnatal thyroid hormone level changes over time in premature infants born between 24 and 28 weeks of gestational weeks; and 2)

to study the correlates of thyroid hormone levels of premature infants during the neonatal period, adjusting for correlation among hormone measurements within each subject. The results of this analysis may help identify infants at risk for THOP and possibly reduce the risk of adverse neurodevelopmental outcomes through timely supplementary treatment.

5.1 Analytical sample (Figure 5.1)

Among the 168 study subjects, we excluded 121 participants in this analysis for the following reasons: 1) received thyroxine supplementary treatment (i.e. subjects in the four thyroxine supplementary treatment groups), 2) expired or withdrew from the study prior to day 7 of life, 3) were not the firstborn of any multiple birth set if more than one infant in this set was enrolled, and 4) data were missing on postnatal thyroid hormone results, maternal history, obstetric information, or postnatal exposures.

Among the 54 study subjects who did not receive thyroxine treatment, 28 were randomized to the placebo group and 26 to the potassium iodine (KI) group. One subject in the placebo group was excluded from the analysis because of death before 7 days of life. Two subjects in the KI group were excluded because parents withdrew consent before 7 days of life. Among the remaining 51 subjects, 40 were singletons and 11 were multiple births. The multiple births included one pair of twins (the second-born excluded), 4 single twin enrolled from four different twin pairs (zero excluded), and five births from two triplet sets (three excluded). We excluded a total of seven infants from multiple births. The final analytical sample included 47 subjects.

5.2 Exposures

The findings from chapter 4 suggest that baseline FT4 levels were associated with gestational age, race, fetal growth ratio, intrauterine growth restriction, and multiple birth

status. Baseline TT4 levels also were associated with antenatal steroid and magnesium treatment, and rupture of membrane for greater than 24 hours (ROM > 24 hours) prior to the onset of labor.

In this analysis, we would like to explore whether these factors will continue to have similar associations with postnatal thyroid hormone levels over time. In addition, previous studies have shown that postnatal illnesses, such as respiratory distress syndrome, chronic lung disease, and sepsis, are associated with low serum thyroxine levels in preterm infants. Hence, we will study these factors as well in relation to postnatal thyroid hormone levels.

Three types of exposure variables were studied: 1) prenatal and birth exposures (e.g., antenatal medication, gestational age, fetal growth ratio); 2) postnatal characteristics during hospitalization (e.g., death, postnatal age, length of hospital stay, duration of ventilator/oxygen use, chronic lung disease, NEC, receiving antibiotic treatment for greater than 5 consecutive days (antibiotic > 5 days)); and 3) neonatal thyroid hormone levels at birth. Most exposures were fixed attributes (e.g., gestational age, fetal growth ratio). NEC and antibiotic > 5 days were time dependent variables and were both binary exposures, which were absent at birth and became present when the disease occurred.

Prenatal and birth information were collected at birth through maternal interview. Neonatal baseline thyroid hormone levels were tested within 24 hours after birth but before the study treatment was administered. Postnatal information was collected on a daily basis and summarized and recorded in discharge/death form at discharge or death.

5.3 Outcome

Postnatal thyroid hormone levels collected on day 3, 7, 14, 21, 42, and 56 were the outcome variables in this analysis. We tested the normality of neonatal FT4, TT4, and TSH distributions and found that all hormones followed the lognormal distribution. Therefore, a log transformation was applied to all hormone results and the report below is based on log transformed hormone values.

Missing postnatal thyroid hormone results. Because blood specimens were collected for each study subject on postnatal day 3, 7, 14, 21, 42, and 56, the 47 subjects in our study would potentially have a total of 282 measurements in hormone levels. However, results of 12 subjects were partially incomplete because of death (11 samples of 5 subjects) and missing records (7 samples of 7 subjects). Most samples (85%, n=224) had all three hormone results (FT4, TT4, and TSH) available, 11% (n=30) of the samples had two test results available, and 4% (n=10) had only one test result available for analysis. Missing data were due to insufficient sample volume, because of which multiple hormone testing could not be completed. Missingness was considered as random.

5.4 Statistical analyses

As the outcome variables of this analysis are a series of repeated hormone levels on different postnatal days, we used analysis for repeated measurements to study the associations between exposures and outcomes. A repeated measurement analysis allows us to account for the correlation among hormone levels collected at different time points within each study subject. The Mixed procedure in SAS software (ver. 9.1.3) was used in all analyses.

5.5 Results

5.5.1 Prenatal and birth infant characteristics of 47 enrolled subjects (Table 5.1)

Birthweight of the 47 subjects ranged from 440 to 1325 grams (mean ± standard deviation (SD): 864 g ±194). Gestational age ranged from 24 to 27.9 completed weeks (mean ± SD: 26.2 weeks ± 1.2). Fetal growth ratio varied from 0.51 to 1.24 (mean ± SD: 0.87 ± 0.15). More than half (60%) of the analytical sample was male. The majority of the population was White (62%). 15% of the subjects were one of a multiple-birth set, 36% were born through C-section, and 9% had intrauterine growth restriction. About 25% of subjects had an Apgar score at 1 minute below 4 points. By five minutes, the Apgar scores of most infants (56%) had improved and only 2% remained under 4. About 16% of the subjects had a large improvement in Apgar scores, changing from less than 4 points at 1 minute to greater than 7 points at 5 minutes.

5.5.2 Maternal characteristics of 47 enrolled subjects (Table 5.1)

The 47 subjects were born to 47 mothers, whose ages ranged from 19 to 40 years (mean ± SD: 29.5 years ± 5.7). During this current pregnancy, 89% of the mothers received antenatal steroid and 26% received magnesium treatment prior to delivery. About 24% of women had ROM > 24 hours prior to onset of labor. Both neonatal and maternal characteristics of the 47 study subjects were similar to those attributes in the 144 subjects studied in previous chapter.

5.5.3 Postnatal characteristics of 47 study subjects (Table 5.2)

The 47 subjects were followed until discharge or death. The weight at the end of follow-up ranged from 750 to 6335 grams (mean ± SD: 2797g ±1045). On average, subjects stayed in the neonatal intensive care unit for 92 days before discharge or death.

Except for one outlier case hospitalized for 343 days, most subjects (72%) stayed in the hospital for under 100 days and 26% stayed for more than 100 and less than 200 days. Duration of ventilator use varied from 0 to 300 days (mean ± SD: 28 d ± 53). About 30% of the subjects were on a ventilator for less than 28 days and only three (7%) were on a ventilator for above 60 days. The outlier case mentioned above was on a ventilator for 300 days. Compared to ventilator use, subjects were on CPAP for a shorter time. The mean duration on CPAP was 21 days, varying from 0 to 100 days.

During the study treatment (up to 42 days postnatal) and follow-up period (up to discharge or death), eight (17%) subjects expired, 35 (75%) were still on oxygen supplement past postnatal age 28 days, 17 (36%) were still oxygen dependent at postnatal age 36 weeks, 22 (47%) received one or more antibiotic treatments for five or more consecutive days, and three (6%) developed NEC.

5.5.4 Neonatal baseline thyroid hormone levels of 47 study subjects (Table 5.3-5.4)

Average baseline FT4, TT4, and TSH levels of the 47 subjects were similar to those of the 144 subjects discussed in chapter 4, except that no hormone level differences existed across three study centers. Hormone levels also were compared between placebo and KI groups. The FT4 levels of the KI group tended to be slightly higher and TSH levels slightly lower than that of placebo group, but no significant difference was found in any of the three hormones between two study groups. In the adjusted analysis, we included baseline thyroid hormone levels to test any association between baseline and postnatal thyroid hormone levels.

5.5.5 Postnatal thyroid hormone levels of 47 study subjects (Figure 5.2-5.7)

In general, mean FT4 levels of the 47 study subjects appeared reasonably stable over the first 56 days of life. The mean FT4 levels increased approximately 0.5 ng/dl over the first week, decreased back to the baseline level, and then remained flat. Compared to the placebo group, subjects of the KI group had slightly higher FT4 levels at all time points except on day 21. The FT4 level differences between two groups were not statistically significant.

Mean TT4 levels of the 47 subjects decreased about 2 μg/dl in the first week, stayed at the lowest level (4 μg/dl) until the end of the second week (day 7-14), and gradually rose after the end of the second week (day 14). The mean TT4 level at postnatal day 56 was slightly above the baseline level. Similar to FT4, TT4 levels of the KI group were slightly higher than those of the placebo group except on day 7. The TT4 level differences between two groups were not statistically significant either.

Mean TSH levels of the 47 subjects initially decreased about 3.5 mU/L in the first three days, then increased until around day 21, but still below the baseline mean value, and plateaued thereafter. TSH levels of the KI group were lower than that of the placebo group at most time points except on day 14 and 21. No significant differences between TSH levels of two groups were detected. Regardless of the lack of a statistically significant difference between the placebo and KI group for each hormone studied, we included treatment assignment in the adjusted analysis.

5.5.6 Correlates of postnatal FT4 levels in 47 study subjects (Table 5.5)

We studied the correlates of postnatal FT4 levels with adjustment for correlation among measurements within each subject and other factors. Although the mean FT4

level on day 56 was higher than those on other days (n.s.), the mean FT4 levels had little variation over postnatal growth. We also observed that the FT4 levels of the KI group were slightly higher than that of the placebo group at most of the time points, however, the difference was not statistically significant. Among the rest of the variables we tested, no variable was correlated with FT4 levels over 56-day postnatal period.

5.5.7 Correlates of postnatal TT4 levels in 47 study subjects (Table 5.6)

TT4 levels were positively and significantly associated with postnatal age. Figure 5.4 showed that the TT4 levels decreased in the first week and increased steadily since the end of the second week. The TT4 difference between the two study groups also was tested but no significant association was found.

Among the prenatal exposures we studied, gestational age remained positively and significantly correlated with TT4 levels over time after adjusting for correlation among hormone levels within each study subject and other factors. This association was not seen in FT4 levels. Baseline TT4 levels were not found to be associated with postnatal TT4 levels.

Among the postnatal exposures, duration of ventilator use was negatively and significantly correlated with TT4 levels. On average, every 10 days of ventilator use was associated with a 5.6% decrease in TT4 levels ($p < 0.0001$). No association was found for either CPAP use or oxygen use. NEC also was significantly associated with TT4 levels. Subjects who developed NEC had a 53% reduction in TT4 levels ($p=0.0167$). No other postnatal exposure variables were significantly associated with postnatal TT4 levels.

5.6 Discussion

It has consistently been reported that thyroid hormone surges in extremely premature infants (< 28 weeks of gestation) are greatly attenuated, or even absent (2, 5). In our study, no postnatal thyroid hormone surge was observed in either FT4 or TT4. However, we only have data at birth and day 3 of life. Due to the limited time points of sample collection, it is possible that we missed the known 24-hour thyroid hormone surge seen in term infants.

In a thyroxine supplement trial conducted in Amsterdam, Van Wassenaer et al. found that the mean plasma FT4 concentrations of 100 preterm infants (25-30 wk) in their placebo arm increased slightly (about 0.4 ng/dl) from birth on day 1 followed by a nadir on day 7, and then increased again (6). Our results differed from their findings in FT4 changing pattern. We found the mean FT4 level on day 7 was not the lowest, but in fact the highest among FT4 measurements.

We further explored the hormone changes over time by three study sites (Figure 5.8-5.9). We found that the FT4 levels of the 12 Amsterdam subjects followed a pattern similar to that described by Van Wassenaer's group. On the other hand, the mean FT4 levels of subjects from both New York (n=25) and Madrid (n=10) were highest on day 7; New York was lowest on day 21, and Madrid lowest on day 14. The variation by site could be due to population differences, or due to a slight difference in gestational age between the 12 Amsterdam subjects and those of New York and Madrid (25-28 vs. 24-28 weeks of GA). This also could explain why our finding among Amsterdam subjects is consistent with that of Van Wassenaer and colleagues. We next examined the FT4 levels over time by treatment group in the Amsterdam subjects and found no effect of treatment.

This was expected given that our Amsterdam FT4 trend was similar to the placebo group studied by Van Wassenaer et al. Due to the differences by site, our data suggest that for infants under 28 weeks of gestation, there is hardly any pattern in FT4 changes in the early neonatal period, but modest variation exists during the first three weeks of life.

In chapter 4, baseline FT4 levels of preterm infants were found to be associated with a group of attributes (e.g., gestational age, race, fetal growth ratio), but postnatal FT4 levels were not associated with any of these factors. In addition, postnatal exposures (e.g., ventilator or oxygen use, postnatal illnesses) did not seem to affect serum FT4 levels either. Postnatal FT4 has been rarely studied and we do not have a clear understanding of the role of FT4 in postnatal life. As thyroid hormone levels could be affected by many factors, this lack of association may be due to the limited variables we examined in this analysis. It also is possible that since the times of sample collection were fixed, while the measurement of exposures was not, we may have missed the etiologically relevant time window for certain exposures studied (11).

After a decrease in the first week of neonatal life, TT4 tended to rise gradually with increasing postnatal age. We hypothesize that this increase was related to the increasing production of both thyroxine and serum thyroid binding globulin (TBG) concentrations in preterm infants. Each TBG molecule has one iodothyronine binding site, hence, the T4 binding capacity of TBG in normal human serum is equivalent to its blood concentration (12). If our hypothesis is correct, we would expect increasing production of TBG as neonates grow. If the increasing rate of TT4 and TBG were equal, we would observe flat TT4/TBG ratio over time; however if TT4 production was greater than that of TBG, we would see increasing TT4/TBG over time. In Figure 5.10, the mean

TBG level of the 47 subjects increased steadily from 15 to 21 μg/ml. The TT4/TBG ratio, which paralleled the change of TT4, increased as well after an initial decrease. These findings suggest a decrease in thyroxine synthesis in the first week of life, but not in TBG synthesis. In addition, both TT4 and TBG production increased in postnatal life and the increase of TT4 was greater than that of TBG.

In our study, TT4 levels were negatively associated with duration of ventilator use. Ventilator use reflects pulmonary function. In the literature, respiratory distress syndrome (RDS) has repeatedly and consistently been reported to be associated with low TT4 levels in preterm infants. Impaired lung function has been seen to be the most common condition in preterm infants with hypothyroxinemia. Many groups have reported that significant reductions in serum T4 levels were found in preterm infants who had RDS or who subsequently developed RDS (8-10). In our analysis, we used duration of ventilator use, duration of CPAP use, and oxygen dependence at 36 weeks postnatal to reflect neonatal lung function. After adjustment, a 5.6% reduction in TT4 levels was associated with each 10-day increase of ventilator use.

We have three possible mechanisms to explain the relationship between impaired lung function and neonatal hypothyroxinemia. During early fetal life, thyroxine has been known to be a key factor to fetal growth. Intrauterine thyroid hormone deficiency may lead to a shortage of cortisol, a key stimulator of pulmonary surfactant synthesis. Lack of cortisol would delay lung maturation (7), produce surfactant deficiency, and impose a greater risk of having lung problems in premature infants (10). Although intrauterine thyroxine deficiency could impede fetal lung development, one thing to keep in mind is that the condition itself is very likely a result of poor pregnancy conditions but not a

cause. Second, prematurity could lead to both lung underdevelopment and hypothyroxinemia. Third, thyroid hormone-dependent pulmonary maturation could be hindered directly by postnatal hypothyroxinemia. The severity of pulmonary illness could be worsened and its duration prolonged (9). Unfortunately, given the restriction of the data, the time order of the relationship between impaired lung function and hypothyroxinemia cannot be resolved.

Simpson et al. classified preterm infants by severity level of postnatal illnesses (prematurity, chronic lung disease, severe respiratory disease, cerebral pathology, patent ductus arteriosus, and NEC) and found a statistically significant reduction in postnatal TT4 levels among newborns with maximal intensive care (13). In our study, subjects with NEC had a 52% reduction in TT4, compared to those without. Although our findings are similar to Simpson's, results are not directly comparable because Simpson et al. analyzed the effect of postnatal illnesses as a group, while we specifically looked at NEC. Our results were limited because the power to detect the association between NEC and TT4 levels was restricted by our study objectives, which aimed to establish the optimal dosing schedule for very preterm infants. Only three out of 47 subjects developed NEC during the 56-day follow-up period. Such a small sample size can only suggest a possible association between NEC and reduced postnatal TT4 levels. Future study with larger sample size is needed to confirm this finding.

5.7 Strengths and limitations

The present analyses have several strengths that must be considered. Studies have measured postnatal thyroid hormone levels longitudinally, however, unlike our study, very few have accounted for the correlation among measurements within each subject in

their analyses of hormone trends. Even fewer studies have examined the correlates of postnatal thyroid hormone levels using analysis for repeated measurements. In an improvement over previous studies, we assessed the serum FT4 concentrations using the Direct Equilibrium Dialysis method, which maximally avoided possible biases from changes in serum proteins or protein binding of T4, and accurately reflected the serum FT4 levels in extremely premature infants. An additional advantage of our analyses is that we had very few subjects with missing outcome data and all missingness was random, and hence should not bias our results. Finally, the trends of postnatal thyroid hormone observed in this study can be generalized to extremely premature infants.

We analyzed data from this clinical trial as a prospective cohort study. Restricted by the original study design, we can only examine hormone results on 47 subjects from the two non-treatment groups. This limited the power to detect associations between exposures of interest and postnatal thyroid hormones levels in our study. Further, even if power had been sufficient for our analyses, it is possible that we may have missed the etiologically relevant time window for certain exposures studied. In addition, as expected, we had some lost to follow-up due to deaths (5, 10.6%), however, the number was minimal and is unlikely to affect our results.

5.8 Conclusion

In summary, among preterm infants born between 24 and 28 weeks of gestational age: 1) a postnatal FT4 surge did not exist in this study population and hardly any pattern in FT4 levels variations over time was present; 2) a postnatal TT4 surge was not observed and TT4 levels remained lowest in the first week before increasing; 3) postnatal thyroid hormone levels increased over postnatal age and only the elevation in serum TT4 levels

was statistically significant; 4) potassium iodine treatment did not affect postnatal thyroid hormone levels compared to placebo; 5) postnatal FT4 levels were not clearly affected by any prenatal or postnatal exposures examined; and 6) postnatal TT4 levels were significantly associated with gestational age, postnatal age, duration of ventilator use, and NEC diagnosis.

Due to the limited knowledge in this field, though this study contributes to the understanding of postnatal thyroid hormone level changes and correlates, prospective cohort studies with larger sample sizes are needed to more rigorously evaluate these associations.

Figure 5.1 Flow chart of analytical sample selection.

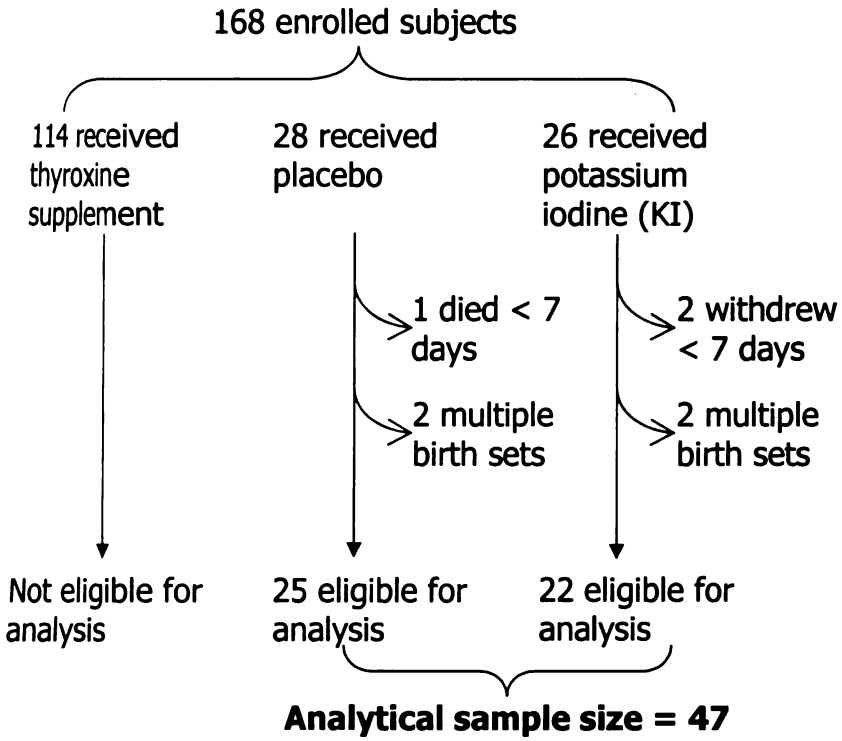


Figure 5.2 Mean structure of FT4 levels over postnatal age in 47 study subjects.

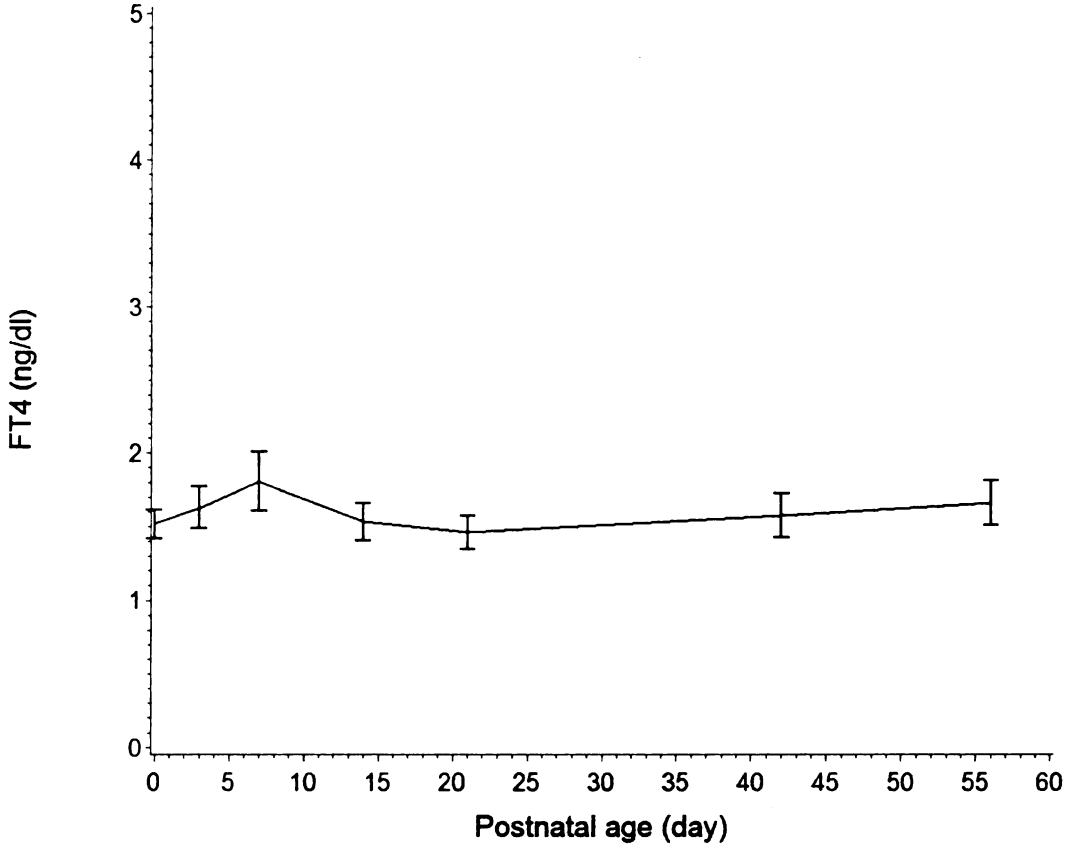


Figure 5.3 Mean structure of FT4 levels over postnatal age in 47 study subjects by treatment groups.

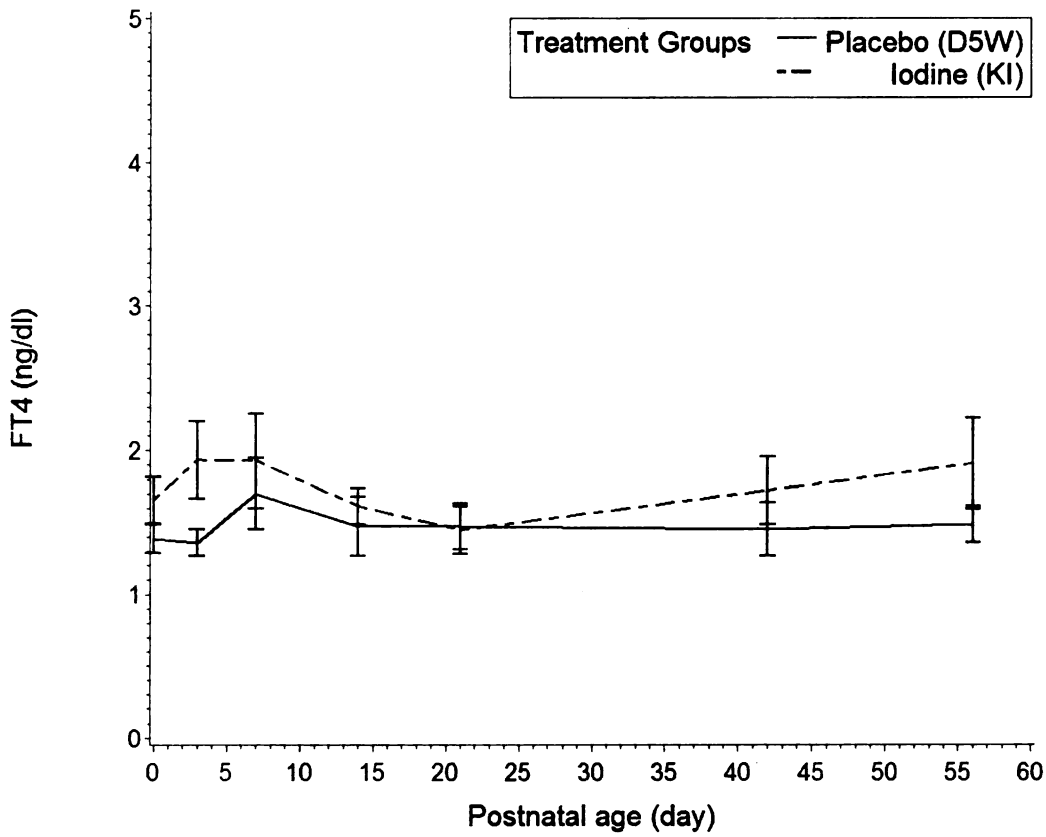


Figure 5.4 Mean structure of TT4 levels over postnatal age in 47 study subjects.

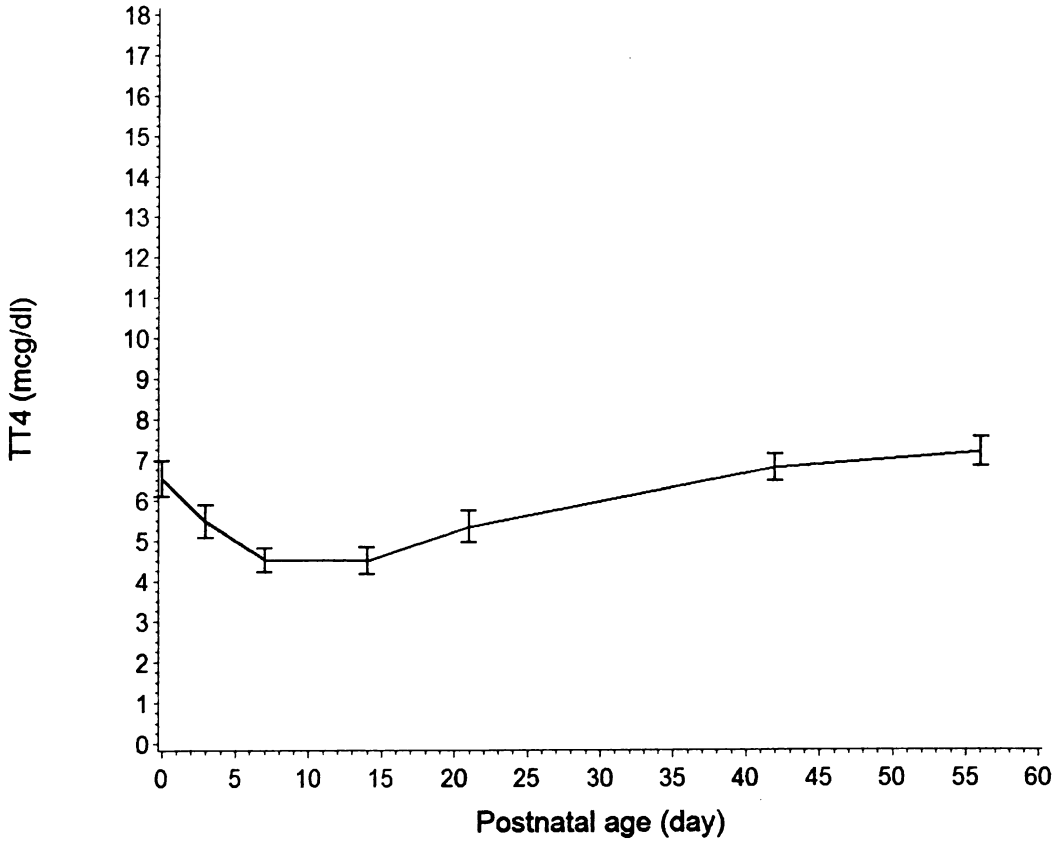


Figure 5.5 Mean structure of TT4 levels over postnatal age in 47 study subjects by treatment groups.

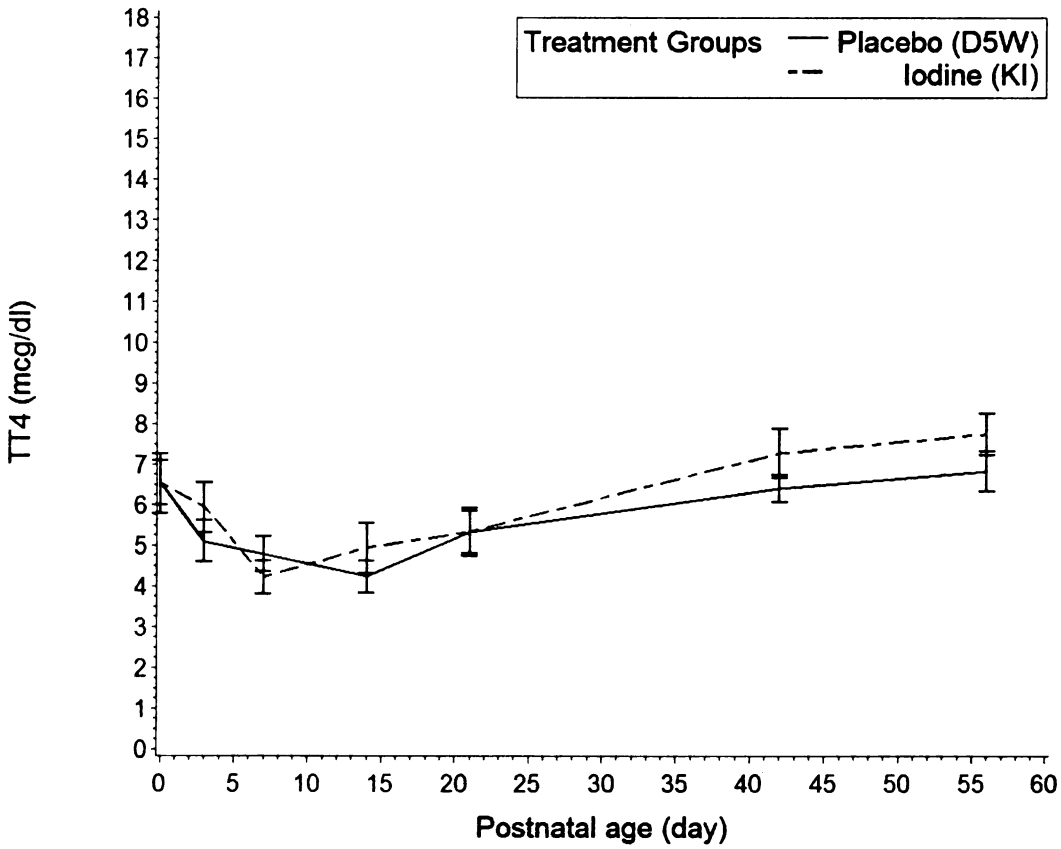


Figure 5.6 Mean structure of TSH levels over postnatal age in 47 study subjects.

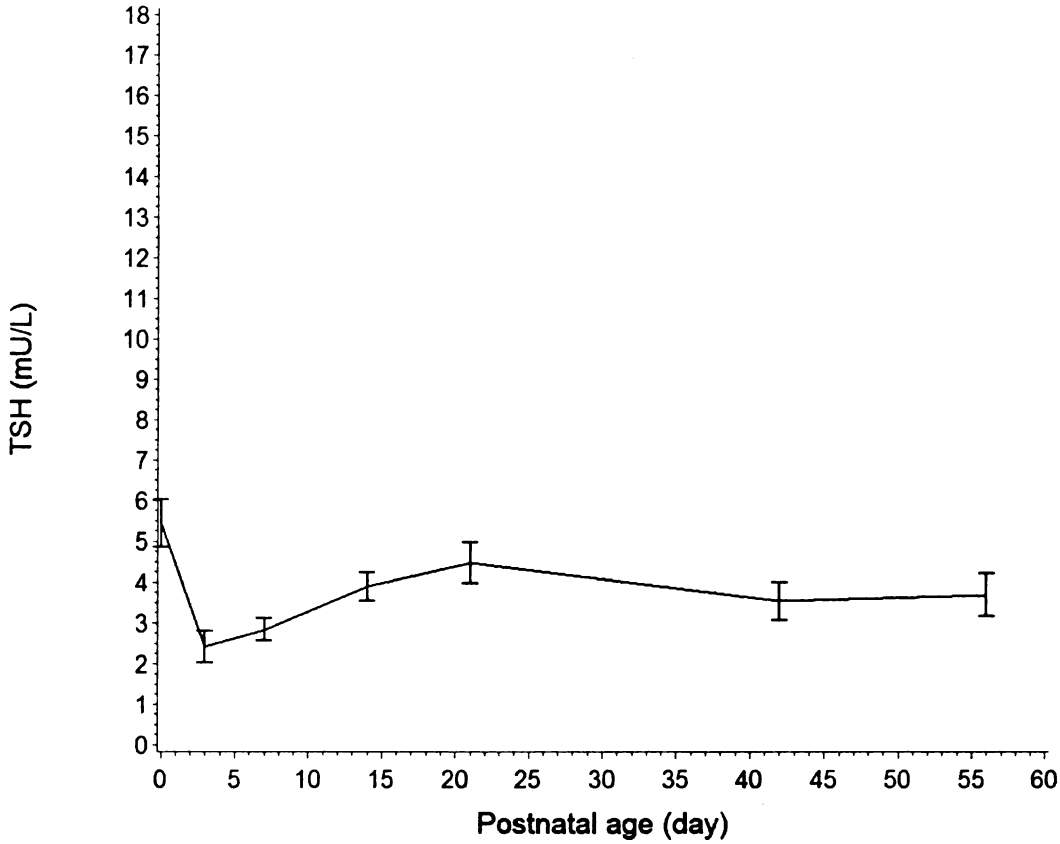


Figure 5.7 Mean structure of TSH levels over postnatal age in 47 study subjects by treatment groups.

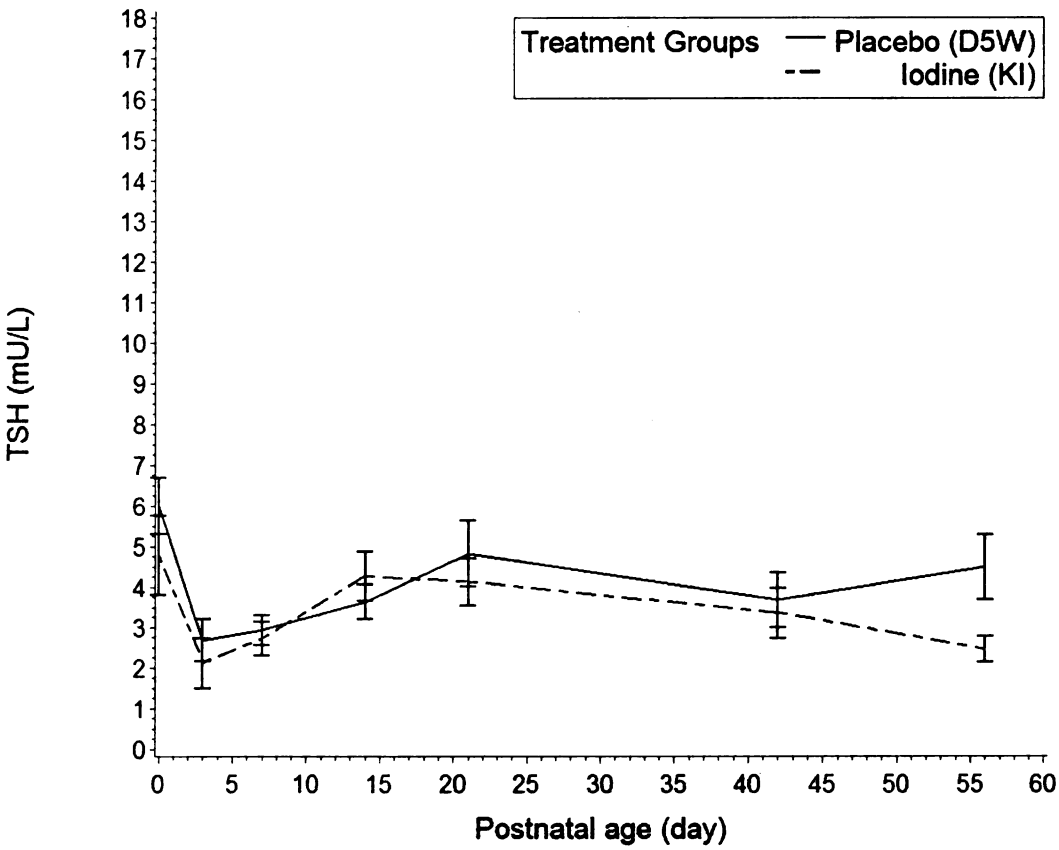


Figure 5.8 Mean structure of FT4 levels over postnatal age in 12 study subjects enrolled from Amsterdam site.

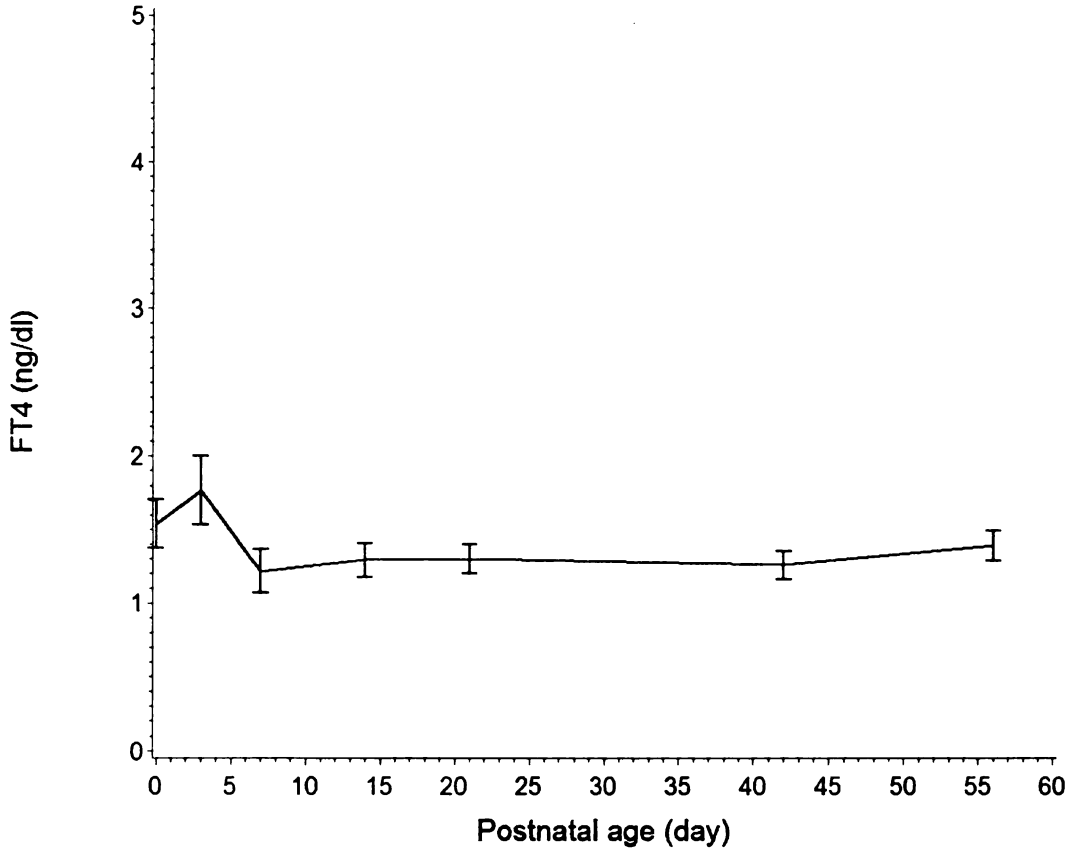


Figure 5.9 Mean structure of FT4 levels over postnatal age in 12 study subjects enrolled from Amsterdam site by treatment groups.

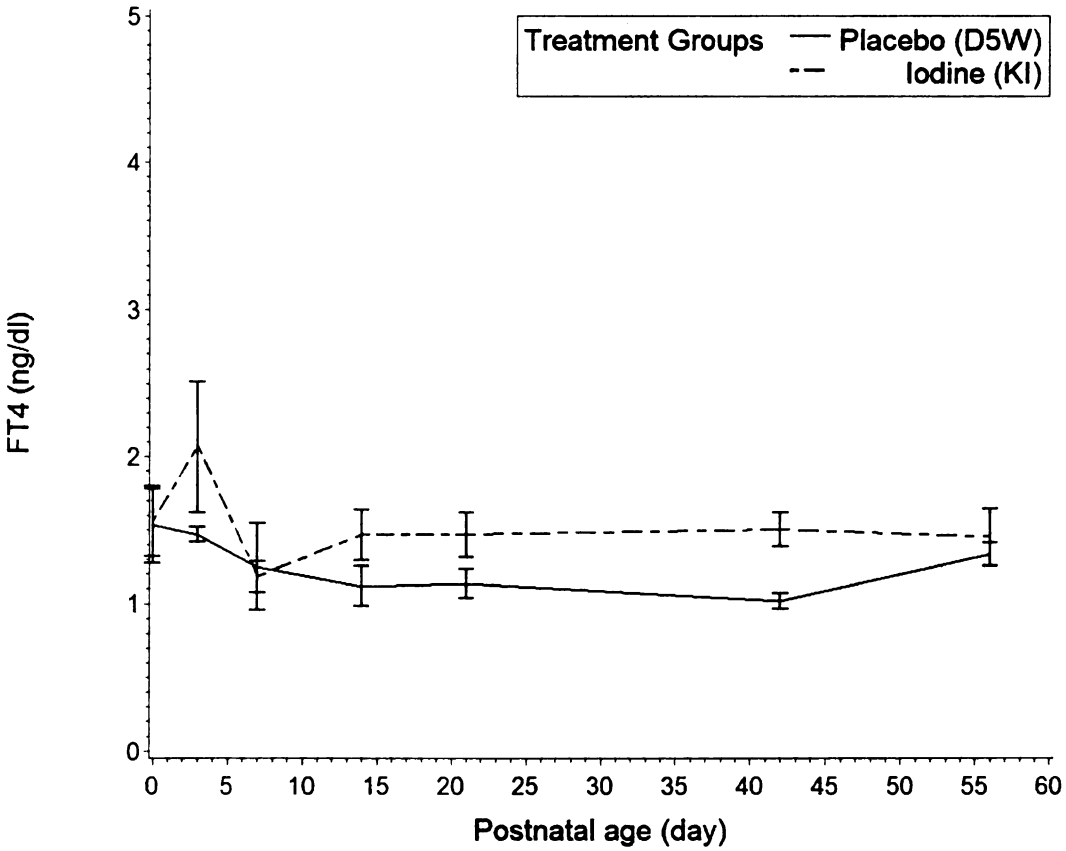


Figure 5.10 Mean structure of TT4/TBG and TBG levels over postnatal age in 47 study subjects.

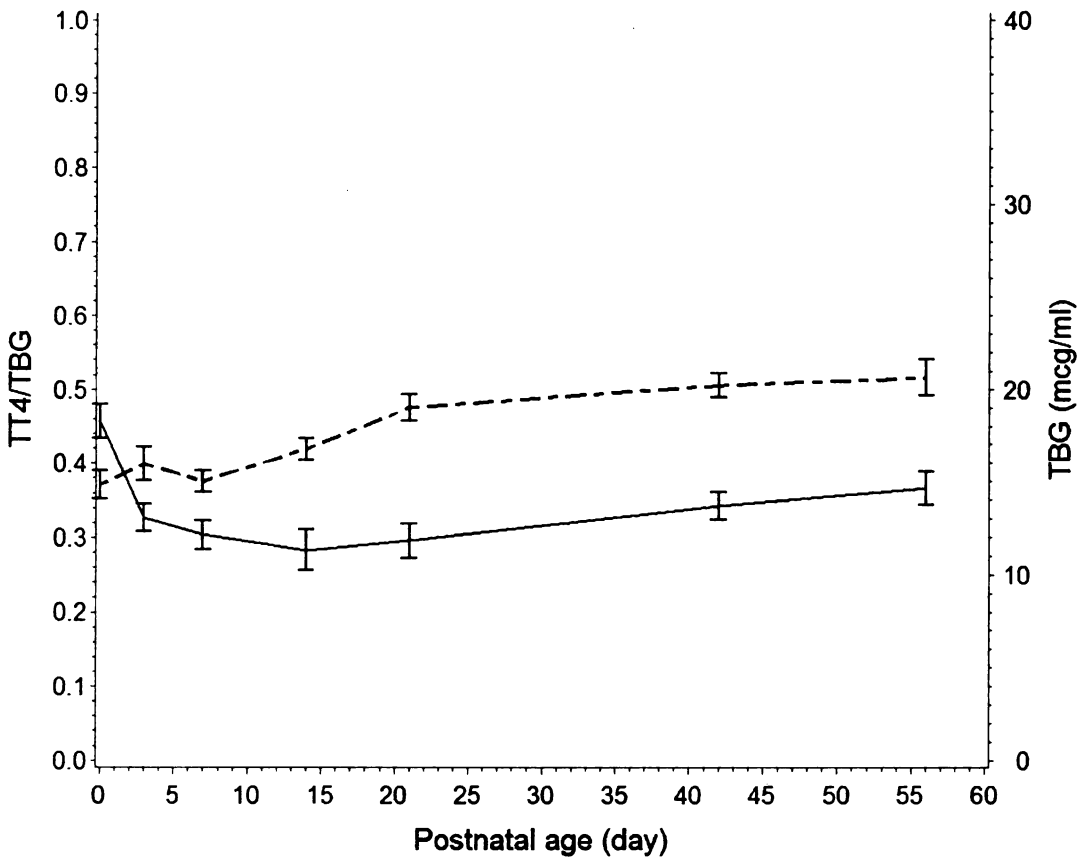


Table 5.1 Neonatal and maternal characteristics of 47 study subjects.

Neonatal characteristics		**N=47**
Birthweight	Mean (SD)	864 (194)
	Median (Range)	860 (440, 1325)
Gestational age	Mean (SD)	26.2 (1.2)
	Median (Range)	26.4 (24, 27.9)
Fetal growth ratio	Mean (SD)	0.87 (0.15)
	Median (Range)	0.87 (0.51, 1.24)
		N (%)
Gender	Male	28 (60)
Race	White	29 (62)
	Black	13 (28)
	Hispanic	4 (9)
Multiple birth (twins and triplets)		7 (15)
C-section		17 (36)
Intrauterine growth restriction (IUGR)		4 (9)
Apgar score at 1 min	≤ 3	11 (25)
	4 - 6	17 (39)
Apgar score at 5 min	≤ 3	1 (2)
	4 - 6	5 (11)
Apgar score change from 1 min to 5 min	≤ 3 to (4 - 6)	3 (7)
	(4 - 6) to ≥ 7	15 (34)
	≤ 3 to ≥ 7	7 (16)

Maternal characteristics		**N=47**
Maternal age (yr)	Mean (SD)	29.5 (5.7)
	Median (range)	29 (19, 40)
		N (%)
Antenatal steroids administration		42 (89)
Magnesium administration		12 (26)
ROM > 24 hr		11 (24)

Table 5.2 Postnatal characteristics of 47 study subjects.

Postnatal characteristics		**N=47**
Discharge weight (gram)	Mean (SD)	2797 (1045)
	Median (range)	2614 (750, 6335)
Duration of hospital stay (day)	Mean (SD)	92 (53)
	Median (range)	85 (15, 343)
Duration of ventilator use (day)	Mean (SD)	28 (53)
	Median (range)	9 (0, 300)
Duration of CPAP use (day)	Mean (SD)	21 (19)
	Median (range)	19 (0, 100)
		N (%)
Death		8 (17)
Oxygen dependent > 28 days		35 (75)
Oxygen dependent at postnatal 36 weeks		17 (36)
Necrotizing enterocolitis		3 (6)
Germinal matrix/intraventricular hemorrhage		15 (33)
White matter damage		5 (11)
Treating with antibiotics > 5 consecutive days		22 (47)

Table 5.3 Neonatal baseline thyroid hormone levels by study centers.

Hormone/Site	N	Mean (SD)	Median	Range	Percentile				p-value
					10th	25th	75th	90th	
Baseline FT4 (μg/dl)									
New York	24	1.6 (0.8)	1.4	(0.7, 3.5)	0.7	1.0	1.9	2.4	
Amsterdam	12	1.5 (0.6)	1.4	(1.0, 2.8)	1.1	1.1	1.9	2.3	0.7390
Madrid	9	1.4 (0.4)	1.3	(0.8, 1.9)	0.8	1.1	1.6	1.9	
Total	45	1.5 (0.6)	1.4	(0.7, 3.5)	0.8	1.1	1.8	2.4	
Baseline TT4 (ng/dl)									
New York	24	6.5 (3.2)	5.9	(3.1, 17.4)	3.5	4.3	7.2	9.1	
Amsterdam	10	6.4 (2.5)	5.8	(3.4, 10.2)	3.8	4.3	8.4	10.2	0.9474
Madrid	8	6.8 (2.5)	7.3	(3.5, 10.9)	3.5	4.5	8.4	10.9	
Total	42	6.5 (2.9)	5.9	(3.1, 17.4)	3.5	4.3	7.5	10.1	
Baseline TSH (mIU/ml)									
New York	23	6.1 (4.5)	4.5	(1.3, 17.1)	1.4	2.5	10.3	11.8	
Amsterdam	12	4.1 (2.8)	3.8	(0.8, 12.0)	1.8	2.5	4.7	5.4	0.3544
Madrid	8	5.5 (2.7)	5.6	(1.3, 10.4)	1.3	4.0	6.6	10.4	
Total	43	5.4 (3.8)	4.4	(0.8, 17.1)	1.4	2.8	7.1	11.5	

Table 5.4 Neonatal baseline thyroid hormone levels by treatment groups.

Hormone/ Study group	N	Mean (SD)	Median	Range	Percentile				p-value
					10th	25th	75th	90th	
Baseline FT4 (μg/dl)									
Placebo	23	1.4 (0.5)	1.3	(0.7, 2.8)	1.0	1.0	1.6	1.9	0.166
KI	22	1.7 (0.8)	1.6	(0.7, 3.5)	0.8	1.1	2.0	2.4	
Total	45	1.5 (0.6)	1.4	(0.7, 3.5)	0.8	1.1	1.8	2.4	
Baseline TT4 (ng/dl)									
Placebo	23	6.5 (2.6)	5.9	(3.1, 13.0)	3.5	4.3	7.5	10.2	0.976
KI	19	6.5 (3.2)	5.9	(3.4, 17.4)	3.5	4.3	8.0	9.1	
Total	42	6.5 (2.9)	5.9	(3.1, 17.4)	3.5	4.3	7.5	10.1	
Baseline TSH (mIU/ml)									
Placebo	23	6.0 (3.3)	5.4	(1.3, 13.6)	2.2	3.9	7.1	11.8	0.303
KI	20	4.8 (4.3)	3.1	(0.8, 17.1)	1.3	2.2	5.8	11.2	
Total	43	5.4 (3.8)	4.4	(0.8, 17.1)	1.4	2.8	7.1	11.5	

Table 5.5 Correlates of postnatal FT4 levels in 47 preterm infants born between 24 and 28 completed weeks of gestational age.

	Estimate (n=47)	SE	t Value	p-value	Overall p-value
Intercept	2.22	1.57	1.41	0.1665	0.1665
Postnatal age (day)					
Day 3	-0.14	0.12	-1.14	0.2540	
Day 7	-0.06	0.12	-0.48	0.6285	
Day 14	-0.08	0.12	-0.65	0.5196	0.8500
Day 21	-0.15	0.12	-1.17	0.2430	
Day 42	-0.07	0.12	-0.55	0.5828	
Day 56	ref	-	-	-	
Study group					
Potassium iodine	0.03	0.11	0.31	0.7564	0.7564
Placebo	ref	-	-	-	
Prenatal exposures					
Gestational age (wk)	-0.04	0.06	-0.63	0.5295	0.5295
Fetal growth ratio	-0.60	0.41	-1.48	0.1411	0.1411
Race					
Black	-0.24	0.13	-1.82	0.0710	0.2190
Hispanic	0.20	0.19	1.02	0.3089	
Other	-0.19	0.37	-0.52	0.6021	
White	ref	-	-	-	
Baseline thyroid hormone levels					
Log (Baseline FT_4)	0.22	0.17	1.35	0.1775	0.1775
Postnatal exposures					
Duration of hospital stay (day)	2.2 e-3	2.4 e-3	0.90	0.3670	0.3670
Duration of ventilator use (day)	-2.2 e-3	2.0 e-3	-1.11	0.2679	0.2679
Duration of CPAP use (day)	-3.3 e-3	4.2 e-3	-0.78	0.4339	0.4339
Oxygen dependent at postnatal 36 weeks					
Yes	0.01	0.14	0.10	0.9237	0.9237
No	ref	-	-	-	
Receiving antibiotic treatment > 5 consecutive day					
Yes	0.12	0.12	1.02	0.3075	0.3075
No	ref	-	-	-	
Necrotizing enterocolitis					
Yes	0.32	0.31	1.04	0.2995	0.2995
No	ref	-	-	-	

* adjusted for the rest of the variables.

Table 5.6 Correlates of postnatal TT4 levels in 47 preterm infants born between 24 and 28 completed weeks of gestational age.

	Estimate (n=47)	SE	t value	p-value	Overall p-value
Intercept	-2.29	1.10	-2.08	**0.0467**	**0.0467**
Postnatal age (day)					
Day 3	-0.45	0.10	-4.62	**< 0.0001**	
Day 7	-0.62	0.09	-6.75	**< 0.0001**	
Day 14	-0.55	0.10	-5.74	**< 0.0001**	**< 0.0001**
Day 21	-0.40	0.10	-4.17	**< 0.0001**	
Day 42	-0.07	0.09	-0.82	0.4126	
Day 56	Ref	-	-	-	
Study group					
Potassium iodine	0.04	0.08	0.49	0.6243	0.6243
Placebo	Ref	-	-	-	
Prenatal exposures					
Gestational age (wk)	0.14	0.04	3.22	**0.0016**	**0.0016**
Antenatal steroids use prior to delivery					
Yes	-0.01	0.13	-0.08	0.9336	0.9336
No	Ref	-	-	-	
Magnesium sulfate use prior to delivery					
Yes	0.06	0.09	0.66	0.5111	0.5111
No	Ref	-	-	-	
Rupture of membranes > 24 hours prior to delivery					
Yes	0.11	0.10	1.09	0.2778	0.2778
No	Ref	-	-	-	
Baseline thyroid hormone levels					
Log (Baseline TT_4)	0.07	0.13	0.55	0.5854	0.5854
Postnatal exposures					
Duration of hospital stay (day)	0.8 e-3	1.7 e-3	0.49	0.6253	0.6253
Duration of ventilator use (day)	-5.8 e-3	1.4 e-3	-4.18	**< 0.0001**	**< 0.0001**
Duration of CPAP use (day)	1.3 e-3	3.3 e-3	0.41	0.6860	0.6860
Oxygen dependent at postnatal 36 weeks					
Yes	0.11	0.10	1.05	0.2956	0.2956
No	Ref	-	-	-	
Receiving antibiotic treatment > 5 consecutive day					
Yes	0.06	0.10	0.57	0.5680	0.5680
No	Ref	-	-	-	
Necrotizing enterocolitis					
Yes	-0.76	0.30	-2.55	**0.0117**	**0.0117**
No	Ref	-	-	-	

* adjusted for the rest of the variables.

BIBLIOGRAPHY

1. Fisher DA, Klein AH. Thyroid development and disorders of thyroid function in the newborn. N Engl J Med 1981;304:702-12.

2. Hume R, Simpson J, Delahunty C, et al. Human fetal and cord serum thyroid hormones: developmental trends and interrelationships. J Clin Endocrinol Metab 2004;89:4097-103.

3. Jacobsen BB, Hummer L. Changes in serum concentrations of thyroid hormones and thyroid hormone-binding proteins during early infancy. Studies in healthy fullterm, small-for-gestational age and preterm infants aged 7 to 240 days. Acta Paediatr Scand 1979;68:411-8.

4. Diamond FB, Jr., Root AW. Thyroid function in the preterm infant. Adv Exp Med Biol 1991;299:227-41.

5. Murphy N, Hume R, van Toor H, et al. The hypothalamic-pituitary-thyroid axis in preterm infants; changes in the first 24 hours of postnatal life. J Clin Endocrinol Metab 2004;89:2824-31.

6. van Wassenaer AG, Kok JH, de Vijlder JJ, et al. Effects of thyroxine supplementation on neurologic development in infants born at less than 30 weeks' gestation. N Engl J Med 1997;336:21-6.

7. Cuestas RA, Engel RR. Thyroid function in preterm infants with respiratory distress syndrome. J Pediatr 1979;94:643-6.

8. Mercado M, Yu VY, Francis I, Szymonowicz W, Gold H. Thyroid function in very preterm infants. Early Hum Dev 1988;16:131-41.

9. Reuss ML, Paneth N, Lorenz JM, Susser M. Correlates of low thyroxine values at newborn screening among infants born before 32 weeks gestation. Early Hum Dev 1997;47:223-33.

10. Romagnoli C, Curro V, Luciano R, et al. Serial blood T4 and TSH determinations in low birth weight infants. Influence of gestational age, birth weight and neonatal pathology on thyroid function. Helv Paediatr Acta 1982;37:331-44.

11. White E, Hunt JR, Casso D. Exposure measurement in cohort studies: the challenges of prospective data collection. Epidemiol Rev 1998;20:43-56.

12. Larsen PR, Davies TF, Schlumberger M, Hay ID. Williams Textbook of Endocrinology. Chapter 10 THYROID physiology and Diagnostic Evaluation of Patients with Thyroid Disorders., 2002.

13. Report of working group of the British Association of Perinatal Medicine and Neonatal Nurses Association on categories of babies requiring neonatal care. Arch Dis Child 1992;67:868-9.