Thyroxine administration to infants of less than 30 weeks gestational age decreases plasma tri-iodothyronine concentrations

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Abstract

Objective: To investigate the effect on thyroid hormone metabolism of the administration of thyroxine to very preterm infants.

Design and methods: Two hundred infants of less than 30 weeks gestation were enrolled into a randomized, double-blind, placebo-controlled trial. Thyroxine (T₄) (at a fixed daily dose of 8 μg/kg birthweight) or placebo was started 12–24 h after birth and discontinued 6 weeks later. Plasma concentrations of T₄, tri-iodothyronine (T₃), reverse T₃ (rT₃), TSH, and thyroxine-binding globulin were measured weekly during trial medication and 2 weeks thereafter.

Results: The T₄ and the placebo group each comprised 100 infants. Antenatal, perinatal, and postnatal clinical characteristics were comparable in both groups. T₄ and rT₃ were significantly increased in the T₄ group. TSH concentrations were depressed in the T₄ group and T₃ was significantly decreased, probably as a result of TSH depression. The T₄/T₃ and T₄/rT₃ ratios differed significantly between the two study groups.

Conclusions: Daily T₄ administration during the first 6 weeks after birth to infants of less than 30 weeks gestation prevents hypothyroxinemia, but decreases plasma T₃ concentrations. Our finding possibly implies that very preterm infants should receive supplements of both T₄ and T₃.

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Introduction

Transient hypothyroxinemia is common in premature and low birthweight infants (1–10). Apart from transient low serum concentrations of thyroxine (T₄) and free thyroxine (FT₄), low concentrations of tri-iodothyronine (T₃) are also found, whereas thyroid-stimulating hormone (TSH) concentrations are in the normal range (1). The reaction of TSH to stimulation by thyrotropin-releasing hormone (TRH) is described as normal (1, 9). The degree of hypothyroxinemia is related to gestational age, birthweight and the severity of neonatal disease (1–10).

Transient hypothyroxinemia has been ascribed to immaturity of the hypothalamo–pituitary–thyroid axis (6), tertiary hypothyroidism (2, 7), non-thyroidal illness (2–4, 8), insufficient iodine intake (10) or premature withdrawal of a maternal contribution to fetal thyroid hormone concentrations (4, 11, 12). The last of these presumes transplacental passage of T₄ from the mother to the fetus (13).

An association has been described between low neonatal T₄ values and an increased risk of impaired developmental outcome at the age of 24 months (14) and 9 years (15). Low T₄ values are also associated with a greater morbidity and mortality (3, 7, 8). There is no proof, however, that these associations are causal. Thyroid hormone is required for normal brain development (16), and therefore administration of thyroid hormones could possibly improve the neurodevelopmental outcome of preterm infants. We have recently described, however, that administration of T₄ to infants of less than 30 weeks gestational age did not affect neurodevelopmental outcome at the age of 24 months. A subgroup of infants of less than 27 weeks gestation might benefit (12). Mortality and morbidity were comparable in the treated and untreated study groups.

Thyroid hormone metabolism is still immature in very preterm infants, because of high concentrations of type III deiodinase and low concentrations of hepatic type I deiodinase (17). In our preparatory, non-randomized study (11), administration of T₄ did not result in an increase in plasma T₃, but plasma concentrations of reverse T₃ (rT₃) were increased. Two other studies, using different treatment protocols, had similar results (18, 19). In the present study, we investigated changes in thyroid hormone concentrations caused by daily administration of T₄ to 100 infants.
of less than 30 weeks gestation during the first 6 weeks after birth, compared with the concentrations in 100 placebo-treated infants who had the same gestational age and also did not differ regarding the extent of clinical disease (12). Apart from plasma thyroid hormone concentrations, \( T_4/T_3 \) and \( T_3/rT_3 \) ratios, indicators of thyroid hormone metabolism, were calculated. As will be seen, plasma \( T_3 \) concentrations decreased as a result of \( T_4 \) supplementation.

**Methods**

**Eligibility and randomization of infants**

The trial was carried out in infants who met the following criteria: a gestational age less than 30 weeks, absence of severe congenital malformations, no maternal endocrine disease, and no maternal illicit drug use. After informed consent had been obtained from at least one parent, the infants were randomly and blindly assigned to receive either \( T_4 \) or placebo. Randomization was performed in blocks of 10 infants, using a computer program.

This study was approved by the Committee of Medical Ethics of the Academic Medical Center in Amsterdam.

**Administration of \( T_4 \)/placebo**

For each infant who entered the study, a numbered set of 50 ampuls, containing 25 \( \mu g/ml \) \( T_4 \) or placebo, was prepared by the hospital pharmacy. \( T_4 \) or placebo was started 12–24 h after birth in a fixed dose of 8 \( \mu g/kg \) birthweight per day, once daily. Trial medication was administered intravenously during the period of parenteral nutrition and orally thereafter.

The treatment period lasted 6 weeks. This treatment schedule was chosen on the basis of results of a preparatory study, in which we showed that \( T_4 \) 8 \( \mu g/kg \) per day during the first 6 postnatal weeks prevents the nadir of \( FT_4 \) that is seen in untreated very preterm infants, whereas \( FT_3 \) concentrations remain below the upper reference value for term infants (11). The investigators, medical and nursing staff, and the parents were unaware of the treatment given.

**Assays**

A blood sample of 1 ml (or less if the clinical condition did not allow for this quantity) was drawn before \( T_4 \)/placebo was started, on day 3, weekly during trial medication, and 2 weeks after discontinuation of the treatment. Cord blood was taken if available. Measurements of \( T_4 \), \( T_3 \), \( rT_3 \), TSH and thyroxine-binding globulin (TBG) were carried out in each of these specimens. The following assays were used: for \( T_4 \), in-house RIA (detection limit 5 nmol/l; intra-assay variation 3.0%; interassay variation 5.1%); for \( T_3 \), in-house RIA (detection limit 0.3 nmol/l, intra-assay variation 4.0%, interassay variation 6.3%); for \( rT_3 \), in-house RIA (detection limit 0.03 nmol/l, intra-assay variation 4.6%, interassay variation 4.6%); for TSH, immunochemiluminescent metric assay (Behring, Amsterdam, The Netherlands) after tenfold dilution (detection limit 0.01 mU/l, intra-assay variation 4.0%, interassay variation 6.0%); and for TBG, RIA (Eiken Chemical Co., Tokyo, Japan: detection limit 20 nmol/l, intra-assay variation 6.0%, interassay variation 7.0%).

**Statistical analysis**

Data are presented in the figures as mean values±SEM. Differences between the hormone concentrations in the \( T_4 \) and the placebo group were tested by analysis of variance for repeated measurements (BMDP 5V), using the model in which hormone concentration was examined as a function of time, \( T_4 \) supplementation, and an interaction term between time and \( T_4 \).

**Table 1 Baseline and clinical characteristics of the infants in the two study groups.**

<table>
<thead>
<tr>
<th></th>
<th>( T_4 ) (( n = 100 ))</th>
<th>Placebo (( n = 100 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>1078 ± 218</td>
<td>1077 ± 239</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>197 ± 8</td>
<td>196 ± 9</td>
</tr>
<tr>
<td>APGAR score at 5 min</td>
<td>8.3 ± 1.8</td>
<td>8.1 ± 1.8</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)</td>
<td>54</td>
<td>53</td>
</tr>
<tr>
<td>Surfactant therapy (n)</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Time on respirator (days)†</td>
<td>4.3 ± 5.5</td>
<td>4.9 ± 7.2</td>
</tr>
<tr>
<td>Time on parenteral nutrition (days)†</td>
<td>13.7 ± 7.8</td>
<td>14.8 ± 8.0</td>
</tr>
<tr>
<td>Sepsis (n)‡</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (n)</td>
<td>37</td>
<td>34</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (n)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Deaths</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups.

† Analysis of surviving infants only.

‡ Confirmed by positive bacterial culture.
supplementation. A significant interaction term was considered as a treatment effect. The distribution of the residuals was checked for skewness. A log transformation was performed when necessary. The resulting $P$ values are given in the text. When a significant interaction term was found, the difference between the values of the two study groups at corresponding timepoints was determined, using the $P$-value of the difference of the estimated mean values of each timepoint. The results of these tests are given in the figures. Student’s $t$-test was used to calculate the difference in mean hormone concentrations before trial medication was started — that is, on days 0 and 1.

**Results**

**Patient population**

Two hundred infants were enrolled (12). The T4 group and the placebo group each comprised 100 infants. During the period of trial medication, a total of seven infants were withdrawn from the study, because of severe congenital malformations diagnosed after study entry ($n = 4$: septum pellucidum agenesis, congenital hypothyroidism, Crouzon syndrome and severe heart malformation) and because of parental discomfort with the study ($n = 3$). In the T4 group, 96 infants remained; in the placebo group there were 97 infants. Statistical analysis of the hormone concentrations was carried out, in accordance with the intention-to-treat principle, including the initial 200 infants.

The study groups were comparable regarding gestational age, birthweight and all other baseline and clinical characteristics (Table 1) (12).

**Hormone concentrations**

Figure 1 shows the changes in plasma T4 concentrations. In the placebo group, plasma T4 concentrations decreased after an initial increase on the first postnatal day, reaching a minimum on day 7, and thereafter showed a steady increase; after day 28, the plasma T4 concentrations stabilized. T4 administration increased plasma T4 concentrations significantly ($P < 0.0001$; interaction term). After discontinuation of T4 administration, the plasma T4 concentrations in both study groups were comparable. The time course of FT4 concentrations in both study groups was comparable to the T4 time course (14).

In contrast to T4 and FT4 concentrations, those of T3 were significantly decreased ($P < 0.0001$; interaction term) by T4 supplementation from day 7 onwards, until the last day (56) of the protocol (Fig. 1b). In both the T4 and the placebo group, a steady increase in plasma T3 concentrations was found. However, between days 3 and 14, the T3 increase in the T4 group was slower, resulting in constantly lower T3 concentrations in the T4 group compared with those in the placebo group until the last day of the study. Figure le shows plasma concentrations of rT3. In the placebo group, a fast decrease in rT3 concentrations occurred from birth until day 7, and a minimal decrease thereafter. T4 supplementation increased rT3 concentrations from day 1 onwards, until termination of T4 treatment ($P < 0.0001$; interaction term). Figure ld shows the changes in TSH concentrations. In the placebo group, an increase in TSH was found between days 7 and 28. In the T4 group, TSH concentrations stayed significantly lower during the T4 administration period ($P < 0.0001$, calculated after log-transformation; interaction term), despite a gradual increase from day 7 onwards. This TSH increase continued after termination of T4 administration. On day 56, TSH concentrations were the same in both groups. As depicted in Fig. lc, T4 administration did not change plasma TBG values. Both study groups had a parallel time course of TBG concentrations, showing a zig-zag pattern between birth and day 7. After day 7, the increase in TBG was steady, reaching a plateau on day 21.

The changes in T4/T3 ratio are shown in Fig. 2a. In the placebo group, the ratio decreased about twofold in the first week after birth and subsequently showed a very gradual decrease in the following 5 weeks. In the T4 group, however, the T4/T3 ratio increased immediately after the start of T4 supplementation and remained significantly increased throughout the period of T4 supplementation ($P < 0.0001$; interaction term). After termination of T4 supplementation, the ratio was the same in both groups.

Figure 2b shows changes in the T4/rT3 ratio. During the first week after birth, the T4/rT3 ratio was the same in both groups. After day 7, the ratio was significantly greater in the placebo group ($P = 0.0015$; interaction term) until the end of the T4 supplementation period. In both groups, the T4/rT3 ratio increased about sevenfold between birth and day 56.

All analyses were also carried out with only the surviving infants included, and without results from the seven infants who were withdrawn from the trial. The results of these analyses were not different from those described above.

**Discussion**

This study describes the effects of T4 administration to infants of less than 30 weeks’ gestational age on thyroid hormone metabolism. It was carried out in an iodine-replete area (20).

The study groups were comparable regarding birthweight, gestational age, and severity of neonatal disease, therefore the picture of how T4 supplementation influences the thyroid hormone metabolism in very preterm infants was not distorted by known confounders. As expected (11, 18, 19), the dosage scheme of 8 μg/kg birthweight per day resulted in
Figure 1 Mean ± S.E.M. plasma concentrations of T₄ (a), T₃ (b), rT₃ (c), TSH (d), and TBG (e) in the two study groups during the first 8 weeks after birth. ○, T₄ group; □, placebo group. *P < 0.05; **P < 0.01, ***P < 0.005 compared with placebo. All S.E.M. values for mean T₃ and rT₃ and some for mean TSH are smaller than the size of the symbols.
As I signficantly increase in plasma T₄ and FT₄ during the 6-week duration of the T₄ treatment, preventing the profound hypothyroxinemia usually found in the first 3 weeks of life. TSH secretion was depressed during T₄ supplementation. After discontinuation of supplemental T₄, there was a twofold increase in plasma TSH concentrations, showing an appropriate response of the pituitary to the withdrawal of the external supply of hormone. In the placebo group, an increase in TSH concentrations was found between days 7 and 14. This TSH increase corresponds with the T₄ nadir on day 7, again showing an intact response mechanism of the pituitary. T₄ administration increased plasma rT₃ concentrations. However, there was a remarkable decrease in plasma T₃ concentrations in the T₄ group. This decrease was 0.5 nmol/l at most, or 20–30% of the T₃ concentration in the placebo group, and may have been caused by a lower endogenous thyroid hormone production as a result of TSH suppression. In addition, the intrathyroidal T₄ to T₃ conversion could have been influenced by the decreased TSH, as both thyroid type I and type II deiodinase may have been depressed (21, 22). Formation of thyroglobulin with a lower T₃ content (23), or a slower liberation of T₃ from the thyroid (24) as a result of lower TSH concentrations are also possible explanations. Finally, type III deiodinase activity, stimulated by the increase in plasma T₄ (17), might have resulted in a faster degradation of T₃.

Our findings confirm that the hepatic type I deiodinase activity is still low in this period of life (25), probably because of low amounts of enzyme protein (26); an increase in plasma T₄ did not result in an increase in plasma T₃. Both the low amount of hepatic type I deiodinase and high concentrations of type III deiodinase (17) can explain the increase in rT₃ concentrations in the T₄ group. To give more insight into changes in thyroid hormone metabolism, T₄/T₃ and T₄/rT₃ ratios were calculated. The increased T₄ and decreased T₃ concentrations explain the increased T₄/R
T3 ratio in the T4 group. The decreased T4/rT3 ratio in the T4 group only became apparent from day 7 onwards. Before this time, type III deiodinase converted relatively equal amounts of T4 to rT3 in both groups. After day 7, the rT3 concentration in the placebo group stabilized at a low concentration; the production of rT3 and its conversion to di-iodothyronine by type I deiodinase had reached a steady state. Probably, rT3 concentrations in the T4 group were too high to be converted completely by the hepatic type I deiodinase available at that time. Alternatively, higher plasma T3 concentrations in the placebo group could have enhanced type I deiodinase in the liver (27), resulting in a greater rate of deiodination of rT3 in this group.

T4 administration did not alter TBG concentrations. In both groups, TBG concentrations increased during the first 6 weeks, as has been described earlier in these very preterm infants (7). However, to our knowledge, the zig-zag pattern between day 0 and 7 has not previously been found. Acute liver insufficiency, increased concentrations of interleukin-6 during the first days after birth as a result of respiratory distress syndrome, and relative undernutrition all might play a part in these variable TBG concentrations (28–30).

Interestingly, the only other randomized study describing the effect on thyroid hormone concentrations of 20 mg/kg T4 during the first 2 weeks after birth did not find a significant decrease in T3 (21). This could reflect both the lower numbers in that study (40 compared with the 200 infants in our study) and the shorter period of T4 administration (2 weeks compared with 6 weeks). Vanhole et al. (18) described a transient increase in T3 in response to TRH administration. This also points towards a thyroid origin of the plasma T3, mediated by TSH. Thus T4 treatment alone results in an increase in plasma T4 and rT3, depression of TSH secretion and either no change in plasma T3 or a decreased plasma T3, depending on the duration of T4 treatment; however, T4 treatment alone does not change clinical outcome (12, 21). In fullterm neonates,
T_{3} shows an eightfold increase on the first day after birth (31); this T_{3} surge is believed to be responsible for metabolic and functional changes that occur in the transition from fetal to extraterrestrial life (17). Very preterm infants, both without and with T_{4} supplementation, lack this T_{3} surge. We therefore suggest that future studies on clinical effects of thyroid hormone treatment in very preterm infants also include a combined T_{4} plus T_{3} regimen. Such a regimen might improve or normalize intracellular T_{3} concentrations in all tissues (32). It is known that lung maturation is T_{3}-dependent (33). A combined T_{4}/T_{3} treatment could stimulate maturation in both central nervous tissue and peripheral organs such as the lungs. In addition, T_{3} enhances expression of the type I deiodinase gene (27), and T_{3} treatment thus could increase endogenous T_{3} production. It may, however, be difficult to find an appropriate T_{4}/T_{3} regimen, because a middle course must be found between a T_{3} dose by which maturation is enhanced and morbidity is decreased (33, 34), and a T_{3} dose that does not increase protein catabolism (35). Treatment with T_{3} alone is not advisable, as it will result in lower plasma T_{4} concentrations by negative feedback (19), and thus will increase the risk of depletion of cerebral thyroid hormone supply.

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