Establishment of Reference Intervals of Thyrotropin and Free Thyroid Hormones during The First Week of Life

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Abstract. Serum concentrations of TSH, freeT4 (f-T4) and freeT3 (f-T3) were determined simultaneously in 199 fullterm newborn babies from 12 to 168 hours after birth. In addition, cord sera from 29 fullterm neonates were analyzed similarly. Cord blood concentrations of TSH were higher, and of f-T3 were lower than seen in adults and f-T4 was similar to the adult range. Serum concentrations of TSH showed a marked increase during the first day of life followed by a decline to the adult range on day four. The free thyroid hormone values increased to the maximum levels within 24 to 48 hours followed by a decrease, but remained higher than in adults even at 168 hours. The fT3/fT4 ratio was found to be lower than in adults except between 12 hours and 24 hours. These data allow us more detailed discussion with regard to hormonal dynamics in the newborn. Furthermore, our study is the first to successfully establish the reference intervals for TSH, f-T4 and f-T3 during the first seven days of life utilizing the most widely used kits and sufficient numbers of samples. These references will be of great assistance for prompt and proper evaluation of neonates at risk for thyroid dysfunction.

Key words: TSH, freeT4 (f-T4), freeT3 (f-T3), reference interval, neonates

Introduction

Thyroid hormone, which is indispensable for cerebral and skeletal development, is fully supplied during the third trimester by fetuses’ own thyroid glands(1, 2). Although the neonatal diagnosis of congenital hypothyroidism can be made easily in many cases typically with a marked increase in TSH, precise evaluation of thyroid function during the neonatal period is often difficult because of the lack of the proper reference intervals. Since subtle derangement may have been overlooked. It is not appropriate to compare neonatal thyroid function with the adult standard range, as evidenced by many reports referring to the changes in thyroid function in the early neonatal period (1–11). Postnatal hypersecretion of TSH in the newborn is already well known (1, 4, 6, 7, 12–15). The subsequent increase in serum concentrations of thyroid hormones, T4 and T3, is also described by many authors (1–8, 11, 13, 14, 16–18).

Nevertheless, simultaneous measurements of serum TSH and free thyroid hormones (f-T4 and f-T3) have not been reported. The purpose of this study is to investigate thyroid gland function changing with time after birth, and to define the
Subjects

Two hundred and twenty-eight Japanese newborn infants (111 males and 117 females) born between January 1998 and January 1999 in Toranomon Hospital were enrolled when (1) gestational age was between 37 and 42 weeks, (2) height, weight and head circumference were within the 5th to 95th percentile range for their gestational age, (3) the APGAR score was equal to or more than eight at both 1 and 5 minutes, and (4) the regional neonatal screening program gave normal results. They were all in good condition apart from occasionally having idiopathic hyperbilirubinemia. Ten neonates required phototherapy. Three were delivered with the aid of forceps, eight by caesarean section and the remainder were delivered vaginally. The maternal history provided no evidence of thyroid disease or drug abuse. Iodine disinfectant was not used for disinfection of the umbilicus or of the maternal side before and after delivery. Written consent for the study was obtained from the parents of the subjects. All the samples were used for the measurement of TSH, f-T4 and f-T3, and, ten randomly selected samples of cord blood and 30 samples of neonatal blood in sufficient amounts were also analyzed for total T4 (t-T4), total T3 (t-T3) and TBG.

Methods

The cord was clamped one to two minutes after delivery of the baby and twenty-nine cord samples were obtained from umbilical veins by needle aspiration. All samples taken from the newborns were collected by venipuncture before feeding at hunger. Neonates were fed eight times a day commencing at eight hours after birth. Venous blood was drawn from five infants at 12 hours, 24 infants at 24 hours, 33 infants at 48 hours, 46 infants at 72 hours, 31 infants at 96 hours, 32 infants at 120 hours, 23 infants at 144 hours, and 5 infants at 168 hours after birth. Variations of the timing of blood sampling were within three hours before and after the respective time points. The sampling for the study was limited to once from a single subject and to the timing when blood sampling was needed for clinical purposes: for example, premature rupture of the membrane, group B streptococcus detected in the mother's vagina, puerperal fever, suspected idiopathic hyperbilirubinemia, and the neonatal screening program. All blood samples were separated by centrifugation at room temperature and sera were stored at –20°C until assayed. All measurements were run in duplicate.

The measurement of hormones was done by the following methods. Serum TSH was measured by an immunoradiometric assay (IRMA) (19) using a TSH RIA Beads kit (Dainabott, Tokyo, Japan). Serum concentrations of f-T4 and f-T3 were determined with Amerlex MAB freeT4 and freeT3 kits (Ortho-Clinical Diagnostics Inc., Bucks, UK) (20). Serum TBG was measured by RIA with an RIAgnost TBG kit (CIS Diagnostic K.K, Chiba, Japan). Serum t-T4 and t-T3 were measured by RIA with a T4 RIA Beads kit and a T3 RIA Beads kit (Dainabott, Tokyo, Japan). Utilizing these assays we had previously established reference intervals in 345 healthy adults; the mean TSH concentration was 1.69 µU/ml with a reference interval of 0.5 to 5.0 µU/ml (defined as means ± 2 SD of the logarithmic values), the mean f-T4 concentration was 1.31 ng/dl with a reference interval of 0.8 to 1.8 ng/dl (± 2 SD of the mean), and the mean f-T3 concentration was 3.07 pg/ml with a reference interval of 2.3 to 3.8 pg/ml. Similarly, we established reference intervals in 76 healthy adults; the mean concentrations of t-T4, t-T3, and TBG were 8.45 ± 1.32 µg/dl, 1.07 ± 0.24 ng/ml, and 19.1 ± 3.4 µg/ml, respectively.

Statistical analysis

All measurements except TSH showed normal distribution. TSH values after logarithmic
Conversion were handled similarly to normally distributed data. Results were expressed as the mean ± SD.

We used Multiple Comparison test of Dunnet’s test and Tukey-Kramer HSD, and also Student’s t-test, where appropriate.

## Results

Reference intervals for the three hormones are summarized in Table 1. They were defined as the mean ± 2 SD of the values after exclusion of the figures outside the initial calculation of the mean ± 2 SD. The reference interval for TSH was determined in the same way after the logarithmic conversion of the raw data.

### Cord blood

Although the mean fT4 concentration was not different from the adult reference range, fT3 was significantly lower (p<0.001), and TSH was higher (p<0.001) than the adult values.

Only ten samples were used for the measurement of tT4, tT3, and TBG. The concentration of tT4 (9.8 ± 1.1 µg/dl) was overlapped with the adult reference interval although the mean was higher than in adults (p<0.01). The mean tT3 value (0.64 ± 0.10 ng/ml) was lower than in the adults (p<0.001) as is the case for fT3. Although the TBG value (29.5 ± 3.4 µg/ml) was higher (p<0.001) than the adult, it was lower than in the pregnant women (48.1 ± 7.1 µg/ml, p<0.001).

### Changes with time during the first week of life

**TSH:** Age-related changes were clearly seen in the serum concentrations of TSH (Fig. 1). The serum TSH concentration was higher at 12 hours of age than at any other time and became lower until 72 hours. TSH concentrations were significantly different between 0 and 12 hours, 12 and 24 hours, 24 and 48 hours, and 48 and 72 hours (p<0.001, respectively). The mean TSH concentration reached its bottom level from 72 hours through 168 hours of age, and did not differ significantly from the adult mean.

**fT4:** Figure 2 shows the fT4 values during the first seven days of life. The mean concentration of fT4 was significantly higher at 24 hours after birth (p<0.001). There was no difference between 24 hours and 48 hours. The concentration of fT4 was very low from 72 hours (p<0.001), and continued declining toward 144 hours. But values were still higher than the adult mean even at 144 hours after birth (p<0.001).

**Table 1** The reference intervals of TSH, fT4, and fT3 during the first week of life

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>TSH (µU/ml)</th>
<th>fT4 (ng/dl)</th>
<th>fT3 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± ref. interval</td>
<td>mean ± SD</td>
<td>ref. interval</td>
</tr>
<tr>
<td>Cord</td>
<td>29</td>
<td>8.11 ± 4.54–14.50</td>
<td>1.33 ± 1.05–1.61</td>
<td>1.81 ± 1.43–2.19</td>
</tr>
<tr>
<td>24 hour</td>
<td>24</td>
<td>8.96 ± 3.61–22.20</td>
<td>3.15 ± 1.91–4.39</td>
<td>5.99 ± 3.25–8.73</td>
</tr>
<tr>
<td>48 hour</td>
<td>33</td>
<td>5.90 ± 2.76–12.63</td>
<td>3.46 ± 2.40–4.52</td>
<td>5.96 ± 3.58–8.34</td>
</tr>
<tr>
<td>72 hour</td>
<td>46</td>
<td>2.18 ± 0.86–5.51</td>
<td>2.70 ± 1.82–3.58</td>
<td>4.13 ± 2.71–5.55</td>
</tr>
<tr>
<td>96 hour</td>
<td>31</td>
<td>1.91 ± 0.65–5.57</td>
<td>2.55 ± 1.81–3.29</td>
<td>3.91 ± 2.65–5.17</td>
</tr>
<tr>
<td>120 hour</td>
<td>32</td>
<td>1.57 ± 0.43–5.65</td>
<td>2.41 ± 1.69–3.13</td>
<td>3.64 ± 2.24–5.04</td>
</tr>
<tr>
<td>144 hour</td>
<td>23</td>
<td>1.53 ± 0.51–4.57</td>
<td>2.37 ± 1.83–2.91</td>
<td>3.61 ± 2.65–4.57</td>
</tr>
<tr>
<td>168 hour</td>
<td>5</td>
<td>1.90 ± 1.21–3.00</td>
<td>2.44 ± 1.46–3.42</td>
<td>3.65 ± 2.43–4.87</td>
</tr>
<tr>
<td>Adult</td>
<td>345</td>
<td>1.69 ± 0.50–5.00</td>
<td>1.31 ± 0.80–1.80</td>
<td>3.07 ± 2.30–3.80</td>
</tr>
</tbody>
</table>

Data are mean ± SD for fT4 and fT3, and geometric mean for TSH.
f-T3: Serum f-T3 values are shown in Fig. 3. The value at 24 hours was significantly higher than in cord blood (P<0.001). No difference was seen between 24 hours and 48 hours. Concentrations of f-T3 at 72 hours were significantly lower than at 48 hours after birth (P<0.001), and tended to decline with time from 72 hours until 144 hours after birth. Higher values than the adult reference interval lasted until 144 hours after birth (p<0.01).

f-T3/f-T4 ratio: As shown in Fig. 4, the f-T3/f-T4 weight ratio was obviously low in the cord blood (p<0.001 vs. adult mean), and it was significantly lower than those at 12 and 24 hours (p<0.001). Slight differences were seen between 24 and 48 hours and between 48 and 72 hours (p<0.05 and p<0.001, respectively). There was no significant difference from 72 hours to 168 hours after birth. When all the values in this period were combined, the average was lower than in adults (p<0.001).

Discussion

Drastic changes in the serum concentrations of TSH and thyroid hormones have been reported during the first week of life (1–11). Many studies have shown that the TSH concentration is higher in cord blood than in adults, increases to the peak level at 30 min, is still high at 12 hours, starts decreasing approximately at 24 hours and reaches the adult level on day 5 (1, 4, 6, 7, 12–15). Concentrations of both thyroid hormones are reported to increase after birth to the peak at 24 through 48 hour followed by a gradual decline (1–8, 11, 13, 14, 16–18). In some papers, an early rise of T3 at about three hours after birth is described (3, 5, 13) and in another a delayed increase in T3 after T4 on days 1 and 2 is reported (4, 11).

In most previous studies, however, time-dependent changes in individual hormones have been mainly addressed. Our attempt is also to measure TSH and thyroid hormones in each
sample simultaneously and analyze the mutual relationships among the hormones. Our present study also allows the establishment of reference intervals for f-T4, f-T3 and TSH concentrations on a daily basis, which have not previously been reported in spite of their clinical importance.

To clarify the time-dependent changes in the hormones, a longitudinal study that consists of repeated sampling from each subject is preferred, but such a study would not be permitted considering the total volume of blood to be drawn off and the painful procedures on consecutive days soon after birth. Filter paper methods, which may partly solve these problems, give wider SDs (21) and less reproducible results and, furthermore, direct comparison with the figures from other laboratories will not be justified. We therefore selected measurement of the serum concentrations from the venous blood which was drawn off only once from a single neonate.

We first examined several factors which may influence neonatal thyroid function. Sex difference was not found in any hormones at any observation point. Regarding the mode of birth, because there were no significant differences between vaginal delivery and Caesarean section, we decided to combine all the values irrespective of sex and mode of delivery for further analyses.

Our study does not include neonates who have received iodine-containing disinfectant directly, i.e. application to the neonates’ skin, or indirectly, i.e. to the mothers’ genitalia. As excessive iodine is known to cause neonatal hypothyroidism in variable degrees (7, 22–24), application of iodine-containing disinfectant even in a “usual” dose conceivably affects neonatal thyroid function. This setting of our study is therefore essential to investigate and define normal thyroid function in a condition free from excessive iodine. It should also be noted that our reference intervals can be

![Figure 2](image-url)

**Fig. 2** F-T4 concentrations during the first 168 hours after birth. Open circles and vertical bars indicate the means and reference intervals.
**Fig. 3**  F-T3 concentrations during the first 168 hours after birth. Open circles and vertical bars indicate the means and reference intervals.

**Fig. 4**  F-T3/f-T4 ratios during the first 168 hours after birth. Open circles and vertical bars indicate the mean ± SD.
adequately used for all the neonates, with or without exposure to exogenous iodine, for the precise evaluation of thyroid function.

Our results were in agreement with the previously described hypersecretion of TSH in the early neonatal period, but we found no significant difference in the serum TSH concentrations between 72 to 168 hours and adulthood. Most neonatal screening programs in our country use the TSH value to detect congenital hypothyroidism and the majority of the blood spot samples are taken at 5 days after birth. Our results support the conclusion that it is reasonable to use the samples taken on day 5 and even on day 4 in order to avoid over-detection and false positive results because of the narrow range of variation at these postnatal days.

We confirmed the finding in the literature indicating that f-T4 has a peak between 24 and 48 hour and a gradual decline toward day 5 (1–8, 11, 13, 14, 16–18). Our observation that f-T4 still remains above the normal adult range at 144 hours is noteworthy. This implies that even if neonates on day 5 have f-T4 values within the adult standard range, they may be hypothyroid.

Whereas the mean serum f-T4 levels increased within the first 24 to 48 hours of extra-uterine life, the increase in the mean serum f-T3 concentrations also occurred during the same period. Although a delayed increase in T3 after T4 on days 1 and 2 has been reported in some papers (4, 11), it is more conceivable that the mean serum f-T3 levels change in parallel with f-T4, judging from our simultaneous measurement of both f-T4 and f-T3. The present results probably indicate quick and enhanced conversion of T4 to T3 in the peripheral tissues at this early stage of life (on days 1 and 2) as discussed below.

The rapid increase in serum f-T3 concentrations in the newborns could be due to augmented thyroid hormone secretion caused by TSH stimulation, mobilization of TBG-bound hormone and increased deiodination of T4 to T3 in the peripheral tissues. Nevertheless, because serum T3 is fundamentally attributable to peripheral conversion from T4 by deiodinases, we considered the T3/T4 ratio to indicate the conversion activity. The mean serum t-T3/t-T4 ratio derived from 16 cases from 72 hours to 144 hours after birth in our study was $7.8 \times 10^{-3}$, whereas its adult mean was $12.2 \times 10^{-3}$ (p<0.001). Moreover, as shown in Fig. 4, the f-T3/f-T4 ratio during the first 6 days was almost always lower than the adult level with the exception of the short period between 12 and 24 hours. As the age-depended pattern of the fT3/fT4 ratio resembled that of TSH and, in addition, TSH is known to stimulate deiodinase I activity (25), the correlation between the TSH and f-T3/f-T4 ratio was investigated. We found a weak but significant correlation at 12 and 24 hours after birth (r=0.41, p<0.05). These findings suggest low conversion activity during the early days of life and transient recovery in response to the TSH surge.

We also evaluated the free/total ratio of both T4 and T3 in the randomly selected neonates in whom total hormones were also measured. The data are summarized in Table 2. The mean f-T4/t-T4 values and the f-T3/t-T3 ratio in neonates from 72 to 144 hours are $0.18 \times 10^{-3}$ and $3.60 \times 10^{-3}$, respectively, whereas those in adults are $0.16 \times 10^{-3}$ and $2.96 \times 10^{-3}$ (p<0.001, respectively). Such a high proportion of free hormones in neonates in spite of abundant TBG may be partly explained by a low serum concentration of albumin. Nevertheless, if free/total ratios in the cord blood are also taken into consideration, the explanation becomes more complex. Serum thyroid hormone binding also may be altered in the neonates by other factors than TBG and albumin. Questions may also arise as to whether or not the present measurements of free hormones are appropriate in newborn sera in which the constituents are changing daily.

In conclusion, our findings clearly demonstrated a definite preponderance of f-T4 over f-T3 in comparison with the adult status and probable low conversion of T4 into T3, which is
transiently recovered by high TSH. In the more clinical setting, accurate evaluation of thyroid status is mandatory in neonates whose thyroid function may be impaired. The best examples are neonates born to mothers with autoimmune thyroid disorders with or without antithyroid drug treatment. Our study is of great clinical importance, because it successfully settled the reference intervals for TSH, f-T4 and f-T3 utilizing the most widely used kits.

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References

2. LaFranchi S. Thyroid function in the preterm infant. Thyroid 1999; 9: 71–8.