Free Triiodothyronine Measured in Dried Blood Spots from Normal, Low-Birth-Weight, and Hypothyroid Neonates

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Free triiodothyronine (FT₃) has rarely been studied in neonates, primarily because of obvious problems in obtaining serum samples from neonates for research purposes. We adapted an analog radioimmunoassay for measuring FT₃ in dried blood samples and used it to assay 361 samples selected from those collected for the screening for neonatal hypothyroidism. The mean FT₃ concentration in healthy neonates on the fourth postpartum day is 2.55 pmol per liter of whole blood (SD 0.78 pmol/L, n = 145), the same as in healthy adults. Low-birth-weight infants have a decreased concentration of FT₃; and this decrease is much greater in premature neonates than in infants at low weight for gestational age. In six hypothyroid newborns, the FT₃ concentration was normal or increased, clearly indicating that FT₃ assay is not a diagnostic tool for hypothyroidism. FT₃ was barely detectable in one case of congenital galactosemia.

Concentrations of thyroid hormones in the serum of neonates are less well documented than in adults, primarily because of difficulties in obtaining large blood samples for research purposes.

Assaying dried blood samples allows measurement of thyrotropin (TSH), FT₄, and FT₃ in less than 100 μL of blood. Dried blood samples from a great number of neonates are easily available from a laboratory that performs screening for neonatal hypothyroidism and phenylketonuria.

TSH has been extensively studied and is now considered as the "gold standard" in laboratory diagnosis of neonatal hypothyroidism. We have already studied FT₄ and FT₃ in dried blood collected from 10 000 neonates (I) at the fourth postnatal day. Preterm babies have low concentrations of FT₄ and the values are related to birth weight when it is less than 2500 g. We have shown that FT₄ measurement could be a specific screening test for neonatal hypothyroidism. Currently, we use FT₄ measurement as a confirmatory test of hypothyroidism when the TSH concentration in blood exceeds 25 milli-int. units/L. Thus the diagnosis is not delayed by the need to collect and assay a second sample.

FT₃ has been studied little in neonates. Its concentration is lower in infants at low weight for gestational age and in premature infants than in full-term, healthy infants (2). The concentration of FT₃ is low in cord blood, increasing to a maximum at 12 h postpartum (3). However, few cases have been studied. Precise physiological studies are needed to evaluate the usefulness of FT₃ measurement for the diagnosis of neonatal hypothyroidism and for the follow-up of hypothyroid children. If normal values for FT₃ were well documented, its measurement could be useful for the diagnosis of neonatal hyperthyroidism and for the assessment of thyroid function in the neonate born to a hyperthyroid or hypothyroid mother.

We have adapted an analog radioimmunoassay kit for FT₃ for use with dried blood samples (4) and used it to test samples that we obtained at day 4 postpartum to screen for neonatal hypothyroidism. Besides determining the normal values for FT₃ at day 4, we also assessed the influence of birth weight and gestational age on FT₃ concentration.

Additional Keyphrases: thyroid status · radioimmunoassay · dried blood as sample · screening · galactosemia · reference interval · birth weight as related to gestational age.

Materials and Methods

Samples: We selected 361 samples from all those collected for the screening for neonatal hypothyroidism in Normandy, France. The selection was based on the quality of the blood spots and criteria of birth weight and gestational age. One blood sample (100 μL) was collected per infant, on or after day 4 but no later than day 11. The blood was spotted on filter paper (Macherey-Nagel no. 818; OSI, Paris, France). The hypothyroid newborns were detected out of approximately 30 000 neonates, i.e., a one-year screening. The 5th and 90th percentiles of birth weight were determined from data on 24 000 neonates (twins excluded) born between 1981 and 1987 in the same region as those from the present study. The 97 adult subjects tested to assess the correlation between serum and dried blood assays were euthyroid (n = 83), hypothyroid (n = 4), or hyperthyroid (n = 10) patients.

Method: We used the "Amerlex" FT₃ kit (Amersham International, Les Ulis, France) reagents, diluted in phosphate-buffered saline (PBS; NaCl 0.15 mol/L, phosphate 50 mmol/L, pH 7.4). The magnetizable antiserum suspension was diluted eightfold, the [¹²⁵I]T₃ analog fourfold. We punched from the dried-blood-soaked filter paper a disc 4.25 mm in diameter (corresponding to 5 μL of blood) and incubated this at room temperature for 2 h with 500 μL of antiserum. We then added 500 μL of [¹²⁵I]T₃ analog and incubated for another 3 h at 37 °C. After adding 2 mL of PBS to each sample tube, we centrifuged them for 15 min (4 °C, 1000 × g). We aspirated the supernatant liquid, washed the disc a second time with PBS, centrifuged, and re-aspirated the supernate. The radioactivity of the bound fraction remaining with the paper disc in the tubes was counted for 2 min in a Multigamma counter (LKB, Paris, France). We assayed all samples in duplicate and expressed the results as picomoles of FT₃ per liter of whole blood. To measure nonspecific binding, we incubated one disc of zero standard with 500 μL of the kit buffer that had been diluted eightfold in PBS, then followed the procedure detailed above.

Statistical analysis: We used BMDP software (BMDP, Los Angeles, CA) to analyze results for 361 neonates. The relationship between FT₃, the dependent variable, and an independent variable (birth weight or gestational age) was

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* Nonstandard abbreviations: FT₃, free triiodothyronine; FT₄, thyroxin; FT₃, free triiodothyronine; FT₄, thyroxin; TSH, thyrotropin; and PBS, phosphate-buffered saline.

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* J. F. Viel and G. Muller, personal communication.
quantiﬁed by use of a polynomial regression, the highest
degree of the polynomial being assessed by a goodness-of-ﬁt
test. Because birth weight and gestational age are correle-
ated, we used a multiple linear regression to analyze simulta-
neously the FT3 dependence on birth weight, gestational
age, sex, and age at sampling. We did not use a multiple
polynomial regression for this because the clinical inter-
pretation was much simpler with a multiple regression of
degree one. Values falling at or below the detection limit (n
= 5) were included in the statistical analyses because their
binding ratios were inferior to the binding capacity, allow-
ing extrapolation on the standard curve.

Results

Figure 1 illustrates the standard curve obtained for 11
analytical runs (mean ± 2 SD). The mean ± 2 SD for the
binding capacity is 35.2% ± 6.4%, and for the nonspeciﬁc
binding 4.7% ± 2%. The assay detection limit, determined
from the standard curve with 95% assurance, is 0.48 pmol/L.

To assess analytical recovery, we added a series of discs
of blood containing 3 pmol ft per liter to one disc of blood
standards with FT3 concentrations ranging from 0 to 20
pmol/L, and assayed. The slope for the curve that compared
the measured concentration and the expected concentration
was 0.96 (r = 0.99, least-squares analysis), indicating a 96%:
recovery.

The correlation between the results (in picomoles per
liter) obtained for serum and dried blood from 97 adult
subjects is expressed by the following equation: blood FT3 =
0.58 serum FT3 − 1.28 pmol/L (P < 10−4, r = 0.931, least-
squares analysis). The slope, 0.58, corresponds to a mean
packed-cell volume of 42%, which is approximately the
usual hematocrit for adults.
The within-assay CV was 13.6% (n = 30) and the
between-assay CV 16% (n = 9) for a sample containing 2.6
pmol of FT3 per liter.

Samples could be stored at 4 °C for two months without
signiﬁcant changes in the measured concentration.

To determine the normal reference intervals, we assayed
two hundred postpartum day 4 from 145 neonates
weighing 3000 to 3700 g, whose gestational age ranged from
39 to 41 weeks. Figure 2 is a histogram of the FT3 values
obtained. The mean and SD mirror those of adults.

Table 1 shows the relation between birth weight and FT3
concentration. The value is lower in low-birth-weight in-
fants. A maximum is observed for birth weights of 3 to 3.5
kg. The data ﬁt best into a quadratic regression (see
Table 2).

Table 1 also shows the relation between gestational age
and FT3 concentration. FT3 decreases when gestational age
is low. The decrease seems more remarkable before the 37th
gestational week. A maximum is observed at 40 weeks. The
most adequate model is a cubic regression (Table 2).

Because birth weight and gestational age are correlated
we have grouped the data into four categories. Figure 3
shows the mean FT3 concentration and the standard devia-
tion for each category. The slopes indicate the 5th and 90th
percentiles of birth weight as a function of gestational age.
Hypotrophy is associated with low FT3 concentration. The
decrease in FT3 concentration is most remarkable in prema-
ature infants before the 36th gestational week; the values are
<1.5 pmol/L, even when birth weight is normal for age.

We have performed a linear multivariate regression anal-
ysis to test the relation between FT3 concentration and birth
weight, gestational age, sex, and age at sampling (Table 3).
Three variables are signiﬁcantly and positively related to
FT3 concentration: birth weight (P = 0.03), gestational age
(P < 10−4), and sex—girls having higher FT3 values than
boys (P = 0.03). On the other hand, age at sampling between
four and 11 days postpartum is not signiﬁcantly linked to
the FT3 concentration.

We have tested six hypothyroid newborns by this method
before replacement therapy (Table 4). All of them had high
values for TSH and low values for FT4. In contrast, the
value for FT3 was normal in ﬁve cases and high in one case
(dyshormonogenesis).

One infant with normal birth weight and normal gesta-
tional age, who was selected as a subject for the determina-
tion of normal values, had a very low concentration of FT3:
0.45 pmol/L. FT4 was low: 7 pmol/L (normal mean – 2 SD =

Fig. 1. Standard curve (mean ± 2 SD) for FT3 concentration in dried
blood for 11 analytical runs performed in duplicate
FT3, pmol per liter of whole blood; B/B0, binding ratio; normal range for FT3
concentration in neonates, 1–4 pmol/L.

Fig. 2. Frequency distribution of FT3 concentrations in blood spots from
145 euthyroid neonates at the fourth postpartum day
Gestational age, 39–41 weeks; birth weight, 3000–3700 g
Table 1. Relation between Birth Weight or Gestational Age and FT₃ Concentration

<table>
<thead>
<tr>
<th>Birth weight, kg</th>
<th>FT₃, pmo/L</th>
<th>Gestational age, weeks</th>
<th>FT₄, pmo/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n*</td>
</tr>
<tr>
<td>1</td>
<td>0.5-1.0</td>
<td>0.60</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1.0-1.5</td>
<td>0.86</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>1.5-2.0</td>
<td>0.86</td>
<td>6</td>
</tr>
<tr>
<td>33</td>
<td>2.0-2.5</td>
<td>1.55</td>
<td>2</td>
</tr>
<tr>
<td>56</td>
<td>2.5-3.0</td>
<td>2.17</td>
<td>10</td>
</tr>
<tr>
<td>137</td>
<td>3.0-3.5</td>
<td>2.52</td>
<td>8</td>
</tr>
<tr>
<td>68</td>
<td>3.5-4.0</td>
<td>2.48</td>
<td>12</td>
</tr>
<tr>
<td>25</td>
<td>4.0-4.5</td>
<td>2.38</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>4.5-5.0</td>
<td>2.02</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>5.0-5.5</td>
<td>2.10</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>

*a n is the number of cases.

Table 2. Polynomial Regression of FT₃ Concentration (pmol/L) on Birth Weight or Gestational Age

<table>
<thead>
<tr>
<th>Degree</th>
<th>Birth weight, kg</th>
<th>Regression coefficient</th>
<th>P-value</th>
<th>Gestational age, weeks</th>
<th>Regression coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>-2.248</td>
<td>&lt;10⁻⁸</td>
<td>40.920</td>
<td>&lt;10⁻⁸</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2.665</td>
<td>&lt;10⁻⁹</td>
<td>-36.320</td>
<td>&lt;10⁻⁹</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>-0.356</td>
<td>&lt;10⁻⁹</td>
<td>1.013</td>
<td>&lt;10⁻⁷</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>—</td>
<td></td>
<td>-0.009</td>
<td>&lt;10⁻⁷</td>
<td></td>
</tr>
</tbody>
</table>

8 pmol/L; the value for TSH was normal: <20 milli-int. units/L. This child had congenital galactosemia. The hormonal status was normalized by a lactose-free diet.

Discussion

The adaptation of radioimmunoassays for use with dried blood samples allows simple, non-invasive sampling in the neonate. TSH and FT₄ determinations in dried blood spots were already possible, so FT₃ measurement, as reported here, completes the assessment of thyroid function in newborns. The mean FT₃ concentration at day 4 postpartum is the same as for adults. Prematurity induces a twofold decrease of FT₃ concentration. This decrease is observed only when gestational age is less than 37 weeks. It is much more important than the decrease observed for FT₄ concentration: the mean blood FT₄ value is 17 pmol per liter of blood at the 39th to 40th gestational week (SD 4.5 pmol/L), 14.3 pmol/L (SD 4.1 pmol/L) at the 35th to 36th week, and 10.3 pmol/L (SD 3 pmol/L) at the 29th to 30th week (1). This suggests an immaturity of the peripheral conversion from T₄ to T₃ before the 37th gestational week. Severe hypothyrophy of the neonate induces a decrease of FT₃ concentration, which is far less important than that due to prematurity.

FT₃ concentrations are within normal limits in hypothyroid infants, so FT₃ measurement should not be used as a diagnostic test for neonatal hypothyroidism. The normal FT₃ value reported in the case of thyroid agenesis suggests that thyroid tissue is not completely absent, even if not seen at the scintiscan. FT₃ concentration at diagnosis could reflect the degree to which the tissues lack thyroid hormones before replacement therapy and, in this respect, be a prognostic tool.

In the case of galactosemia that we detected, severe hepatic insufficiency and low protein concentration could

Table 3. Multiple Linear Regression with FT₃ as the Dependent Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, kg</td>
<td>0.1821</td>
<td>0.03</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>0.1453</td>
<td>&lt;10⁻⁷</td>
</tr>
<tr>
<td>Sex</td>
<td>0.1966</td>
<td>0.03</td>
</tr>
<tr>
<td>Age (days) at sampling</td>
<td>0.0314</td>
<td>0.39</td>
</tr>
<tr>
<td>Intercept</td>
<td>-4.5144</td>
<td></td>
</tr>
</tbody>
</table>

Multiple r² = 0.2079.

Table 4. Concentrations of Thyroid Hormones in Dried Blood from Six Hypothyroid Infants

<table>
<thead>
<tr>
<th>Pathology</th>
<th>TSH, milli-int. units/L</th>
<th>FT₃, pmol/L</th>
<th>FT₄, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agenesis</td>
<td>150</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Ectopic gland</td>
<td>170</td>
<td>2.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Dyshormonogenesis</td>
<td>35</td>
<td>3.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Transient hypothyroidism</td>
<td>180</td>
<td>3.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Normal values</td>
<td>&lt;25</td>
<td>&gt;8</td>
<td>1-4</td>
</tr>
</tbody>
</table>
explain the low values for FT3 and FT4, as they do with analogs assays of serum (5).

The assay of FT3 in dried blood allows physiological studies in the neonatal period. The low values reported for low-birth-weight infants are more remarkable in premature infants than in infants at low weight for gestational age. Hypothyroidism cannot be detected by measurement of FT3.

References