

# Normal ranges of T4 screening values in low birthweight infants

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**SUMMARY** Thyroxine (T4) screening values in infants of low birthweight in relation to birthweight and gestational age are reported. There were 86 healthy infants of low birthweight (group 1), and 29 preterm infants with respiratory distress syndrome (group 2). All the group 2 infants and 36 % of those in group 1 had a T4 screening value below the cut-off point ( $-2.1$  SD). In group 1 there was a significant increase in T4 with birthweight at a given gestational age, as well as with gestational age at a given birthweight. In group 2 there was also a significant increase in T4 values in relation to birthweight and gestational age, but it could not be ascertained whether this increase existed at a given gestational age or birthweight. A statistical model giving normal ranges of T4 for both groups of infants is presented, which, if applied to low birthweight infants, makes it possible to estimate the effect of low birthweight on T4 screening values, provided the birthweight and gestational age are known. In this manner the sensitivity of screening for congenital hypothyroidism is enhanced and the recall rate reduced.

Screening for congenital hypothyroidism started in The Netherlands at the beginning of 1981. Blood is obtained by heel prick between the 6th and 8th day of life, spotted on to filter paper, and the thyroxine (T4) concentration determined. Mean and standard deviation (SD) values are calculated for each day. Thyroid-stimulating hormone (TSH) levels are measured in the 20% of blood samples with the lowest T4 values, and a patient is investigated further if his T4 is lower than  $-2.1$  SD of the daily mean (the cut-off point for T4), or if the TSH is higher than 20 mU/l. T4 measurements, supplemented by TSH measurements, are used in Canada,<sup>1</sup> and in several parts of the USA.<sup>2-3</sup> In a trial undertaken in Rotterdam<sup>4</sup> the incidence of congenital hypothyroidism was found to be 1:2600.

A low birthweight infant is often found to have a low T4 concentration, particularly on day 7, and this is generally present without any increase in TSH.<sup>5-7</sup> Occasionally there is transient hypothyroidism.<sup>8</sup>

Bernard *et al.*,<sup>9</sup> using cord serum from healthy infants of 31-46 weeks' gestation, reported a significant increase in T4 levels with gestational age and birthweight, and with birthweight at a constant gestational age. Fisher *et al.*<sup>10</sup> showed a progressive increase in cord T4 concentration between 22 weeks' gestational age and term.

Particularly low serum T4 levels at birth and in the first days of life were found in preterm infants with idiopathic respiratory distress syndrome (RDS); cord T4 levels in preterm infants differed significantly between those with RDS and those without.<sup>11,12</sup> Such a difference could not be confirmed by Klein *et al.*<sup>13</sup>

We describe the correlation between T4 serum concentration and T4 screening values, show the effects that birthweight and gestational age have on T4 screening values in low birthweight infants with and without RDS, and give normal ranges of T4 screening values for such infants. In addition, the T4 concentration in cord blood is compared with that in sera obtained on the 7th day of life in order to evaluate the suggestion of Walfish *et al.*<sup>14</sup> that cord blood should be used and that the optimal screening time is at birth.

## Patients and methods

Venous blood samples were taken on day 7 of life from 115 infants of low birthweight. If possible samples were also taken at birth from the cord. T4 screening values were obtained from heel pricks performed on days 6-8 post partum. The study was approved by the Committee of Ethics of the Academic Hospital, University of Amsterdam.

Two groups were formed. Group 1 comprised 86 infants who were healthy and either appropriate (AGA) or small (SGA) for gestational age. Infants with birthweights below the 10th centile for gestational age (Kloosterman's growth chart)<sup>15</sup> were considered as SGA. Birthweight varied from 700 to 2920 g, and gestational age from 26 to 39 weeks. Group 2 comprised 29 AGA infants suffering from RDS. Birthweight varied from 800 to 2360 g and gestational age from 26 to 34 weeks. The diagnosis of RDS was made using the criteria given by Usher.<sup>16</sup>

Because of premature labour 19 infants in group 1 had received prenatal treatment with betamethasone (a maternal dose of 12 mg) to prevent RDS; all these infants were born before 34 weeks' gestational age. In group 2, 11 infants had received betamethasone prenatally.

Infants with congenital malformations or sepsis, or infants born of mothers with diabetes mellitus were excluded from the study.

Gestational age was calculated from the mother's last menstrual period. In cases of doubt, gestational age was estimated from the Dubowitz score.<sup>17</sup> Gestational age for both groups is shown in Fig. 1.

In 77 infants (62 of group 1, and 15 of group 2) cord serum T4 levels were determined. T4 and TSH levels were determined from all serum samples taken on day 7. T4 concentrations from serum or filterpaper eluates were determined by radioimmunoassay as described by Chopra.<sup>18</sup> The intra-assay coefficient of variation in serum was between 3.4 and 6.3%, the inter-assay coefficient of variation between 3.4 and 11.8%.<sup>19</sup> In eluates these figures were 8% and 10%

respectively. Serum TSH concentrations were determined using the method described by Odell *et al.*<sup>20</sup> T4 screening values were expressed in terms of pg/punch (the diameter of a punch being 3.2 mm) and, after mean and SD of the daily population had been established, converted to SD of the daily mean. In the laboratory that screened for congenital hypothyroidism in north west Netherlands in 1981 the daily mean of 29 349 T4 determinations was 115 pg/punch with an SD of 29.6 pg/punch.

Statistical analysis was performed using multiple linear regression, analysis of variance, multiple correlation coefficients, and Pearson's correlation coefficients.

**Results**

Fig. 2 shows the relation between the T4 screening values of all 115 infants, and serum T4 concentrations on day 7 (venous puncture). A highly significant correlation between both can be seen ( $r=0.88$ ).

In group 1 (healthy AGA and SGA infants) 36% of the infants had a screening value below the cut-off point ( $-2.1$  SD). In group 2 (RDS infants) all infants had screening values below  $-2.1$  SD. The Table shows the distribution of screening values for both groups. All 115 infants had TSH concentrations below 20 mU/l on day 7.

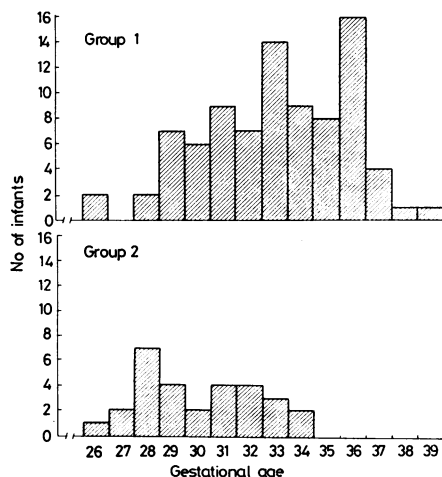


Fig. 1 Distribution of number of infants per gestational age in healthy AGA and SGA infants (group 1), and in AGA infants with RDS (group 2).

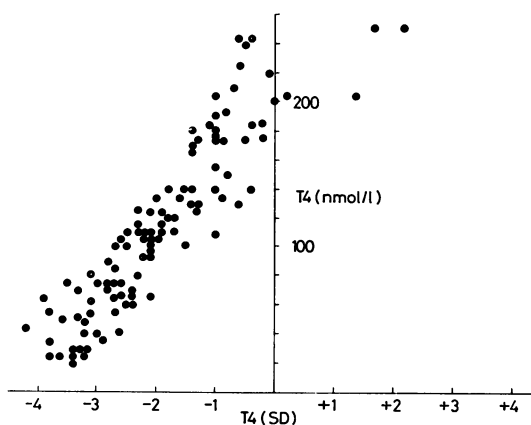


Fig. 2 Correlation between T4 screening values (in SD from the daily mean) and T4 serum concentrations on day 7 (in nmol/l) ( $r=0.88$ ).

Table T4 screening values in SD (mean, minimum, and maximum).

Group	Mean	Minimum	Maximum
1 (healthy)	-1.6	-3.9	+2.2
2 (with RDS)	-3.0	-4.2	-2.1

Statistically the screening values show a normal distribution. Multiple linear regression was used to calculate the dependency of T4 in SD values on gestational age (GA in weeks) and birthweight (BW in g) for each group. In group 1 the regression equation was found to be:  $T4 (SD) = -9.87 + (0.2155 \pm 0.043) \times GA + (0.00066 \pm 0.00024) \times BW \pm 0.79$ . The adequacy of this model was tested in two ways. Firstly, it was tested to see if the regressions on birthweight at different gestational ages were parallel. This hypothesis could be accepted (F-test:  $P=0.67$ ). Secondly, given parallelism, it was tested to see if the intercept increased linearly with gestational age (as implied by the model above). This hypothesis could also be accepted (F-test:  $P=0.38$ ).

Fig. 3 shows the estimated mean of T4 in SD values for different gestational ages as a function of birthweight. The lower 95% confidence limits are shown for corresponding gestational ages. T4 values increase significantly with birthweight ( $P<0.01$ ), with gestational age ( $P<0.001$ ), and with the combination of both these ( $P<0.00001$ ).

The regression equation for group 2 is calculated as:  $T4 (SD) = +5.59 + (0.0698 \pm 0.071) \times GA + (0.00033 \pm 0.00040) \times BW \pm 0.47$ . Tests on parallelism and linearity gave P values of 0.79 and 0.082.

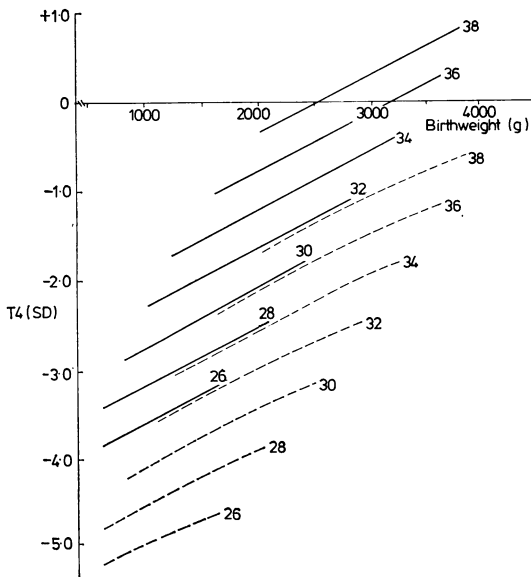


Fig. 3 Regression lines for T4 screening values (T4 SD) on birthweight for seven different gestational ages in 86 healthy AGA and SGA infants (solid lines). Lower 95% confidence limits are shown for each gestational age (broken lines).

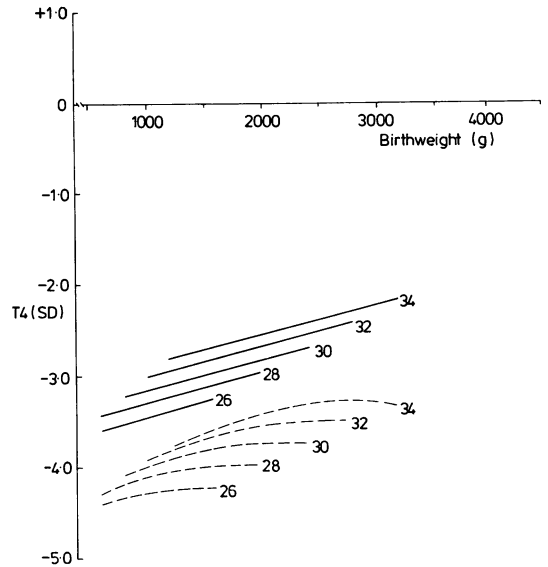


Fig. 4 Regression lines for T4 screening values (T4 SD) on birthweight for five different gestational ages in 29 infants with RDS (solid lines). Lower 95% confidence limits are shown for each gestational age (broken lines).

Fig. 4 shows the estimated mean of T4 in SD values for different gestational ages in correlation with birthweight for group 2, with the lower 95% confidence limits for the corresponding gestational ages. As can be seen from the slopes and the associated SDs, either of the two factors (gestational age or birthweight) can be omitted from the model without altering the fit significantly. However, both factors cannot be left out simultaneously ( $P=0.014$ ). The regression equation of group 2 differs significantly from that of group 1 ( $P<0.00001$ ).

The multiple correlation coefficient of T4, birthweight, and gestational age in samples of cord blood from healthy infants is lower than that on day 7 ( $r = 0.30$  and  $r = 0.76$ , respectively). In the RDS infants there is no difference in the multiple correlation coefficients between cord blood and samples on day 7 ( $r = 0.63$  and  $r = 0.61$ , respectively).

### Discussion

Since T4 screening values are expressed in terms of SDs of the daily mean, which is a relative value, factors which influence this daily mean might reduce the reliability of the screening values. Such a factor could be the number of low birthweight infants concerned in the daily screening population, since their T4 values could influence the daily mean

negatively. One could argue, that this factor might be negligible because the relative number of low birthweight infants is small (1:120). However the total number of all daily screened infants in north west Netherlands is small too (about 150 infants a day). Moreover the number of low birthweight infants in this population appears to vary daily. Therefore the influence of low T4 values of low birthweight infants on the daily mean and the outcome of the screening is unpredictable. We estimated it by correlating T4 screening values in SD with T4 serum concentrations in day 7 samples (Fig. 2). Since this correlation appears to be strong, it can be concluded that T4 screening values expressed in SD are sufficiently reliable.

Because low T4 values are found in low birthweight infants, the recall rate in these infants is consequently high (in 1981 in the north west Netherlands 30 326 T4 determinations were carried out. Six hundred and thirty-seven infants had T4 values below the cut-off point, which means a recall rate of 2.1%, and 41% of these recalls were of low birthweight infants). Since all low birthweight infants reported here had TSH levels below 20 mU/l, no case of primary hypothyroidism was included in our study. In healthy low birthweight infants we found that the early gestational ages in particular (<30 weeks) had T4 screening values below the cut-off point of  $-2.1$  SD.

In a study of preterm infants with and without RDS, Hadeed *et al.*<sup>7</sup> have shown a normal degree of response of pituitary and thyroid glands by stimulation with thyrotrophin releasing hormone (TRH) at ages 4 to 14 days. According to them the low T4 levels are caused by a relative delay in maturation of the hypothalamic-pituitary-thyroid control system. We did not study the TRH response, but in follow-up studies of the recalled infants TSH levels remained low, while T4 levels increased spontaneously in about 3 weeks to levels found in term neonates. It is possible that the infants had suffered from transient tertiary hypothyroidism, but another possibility may be that present in these infants were circulating substances inhibiting the binding of T4 to plasma proteins, as described by Chopra *et al.*<sup>21</sup>

During this study period we saw 3 infants with high TSH levels, who for that reason were excluded. These infants were suffering from transient hypothyroidism as described by Delange *et al.*<sup>8</sup> All 3 infants were severely ill, 2 of them suffering from RDS while the third had been asphyxiated at birth. T4 screening values of the 2 infants with RDS were  $-3.7$  and  $-3.6$  SD, birthweight and gestational age were 1150 g 29 weeks, and 2520 g 34 weeks respectively. The asphyxiated infant (1630 g and 31 weeks) had a T4 screening value of  $-3.6$  SD.

There was a spontaneous recovery of T4 and TSH levels within one month after birth.

Fig. 3 shows that SGA infants have T4 screening values in concordance with gestational age and not with birthweight. For example, a T4 screening value of  $-1.75$  SD is normal for an infant of 1200 g and 34 weeks, but for an infant of 1200 g and 28 weeks a value of  $-3.05$  SD would be normal.

In the RDS infants it was notable that all screening values were below  $-2.1$  SD. Unlike others,<sup>7,11</sup> we did not observe RDS in any infant with a gestational age greater than 34 weeks. It could not be ascertained whether birthweight and gestational age influenced T4 independently of each other in such infants. This is probably due to the high correlation between gestational age and birthweight ( $r = 0.84$ ). However, the influence of gestational age on T4 seems to be weaker than in healthy low birthweight infants, as can be seen from the difference between the regression lines (Figs 3 and 4). It seems that the influence of RDS on T4 levels is an important factor that should not be overlooked.

There are conflicting data<sup>11-13</sup> about the presence of significantly low T4 levels in cord blood of RDS infants compared with healthy low birthweight infants. We found a marginal difference statistically between mean T4 serum concentrations in cord blood in both groups ( $P=0.0505$ ). As long as convincing evidence concerning a possible difference in cord T4 levels between infants with RDS and those without is lacking, it is still arguable whether RDS infants ought to be treated with thyroxine supplementation or not. Despite the fact that Schönberger *et al.*<sup>22</sup> have found a lower mortality rate in T4-treated RDS infants, Klein *et al.*<sup>13</sup> do not recommend T4 treatment. We wish to stress that T4 treatment may increase oxygen consumption. For this reason an already existing tendency towards hypoxaemia may be enhanced, and an even more unfavourable condition in infants with RDS may be created. If one decides not to treat low birthweight infants with low T4 screening values, it will be necessary to take repeated measurements of T4 and TSH serum concentrations during the next weeks.

In conclusion, low birthweight is associated with low T4 levels, which generally are not correlated with primary hypothyroidism, since TSH values are normal. Therefore in interpreting T4 screening values in low birthweight infants, birthweight and gestational age must be taken into account. It is more reliable to correct T4 screening values for birthweight and gestational age in samples taken on day 7 rather than in cord blood, since the multiple correlation coefficient in healthy infants is considerably higher in 7-day samples than in cord blood samples. Day 7 will consequently be about the best time to screen

such infants. It is possible to find out if the T4 screening value of a low birthweight infant is in the normal range (Figs 3 and 4). In this way we expect that the recall rate can be considerably reduced. As long as information about T4 screening values of low birthweight infants with primary hypothyroidism is lacking, it will be necessary to determine TSH concentrations using the same criteria as before, in order not to overlook primary hypothyroidism.

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