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Developmental Trends in Cord and Postpartum Serum Thyroid Hormones in Preterm Infants

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The purpose of this study was first to clarify postnatal trends in sera T_4, free T_4 (FT_4), T_4-binding globulin, TSH, T_3, rT_3, and T_4 sulfate levels in cord and at 7, 14, and 28 d in groups of preterm infants at 23–27 wk (n = 101), 28–30 wk (n = 196), and 31–34 (n = 253) wk gestation, and second to compare these trends to those of term infants and also with cord sera levels of equivalent gestational ages (n = 812; 23–42 wk gestation). In all preterm groups, TSH and rT_3 decrease to below, T_4-binding globulin increases to within, and T_3 and T_4 sulfate increase to above cord levels of equivalent gestational age. Term infants are hyperthyroxinemic relative to cord and nonpregnant adult levels of T_4. Postnatal T_4 increases are attenuated in 31- to 34-wk infants, absent in 28- to 30-wk infants (although levels are equivalent to gestational age), and crucially reversed in 23- to 27-wk infants. This immature group is hypothyroxinemic relative to other groups and to cord levels of equivalent gestational age. Compared with term infants, postnatal FT_4 increases are lower in 31- to 34-wk infants, attenuated in 28- to 30-wk infants, and absent in 23- to 27-wk infants. The 23- to 27-wk group is distinctive; they are hypothyroxinemic on T_4 levels, yet FT_4 levels are within the cord levels of equivalent gestational age. (*J Clin Endocrinol Metab* 89: 5314–5320, 2004)

THYROID HORMONE IS essential for normal development of the human brain. Numerous factors control levels of receptor-active thyroid hormone (T_3), and disruption of any of these at critical phases of human development can lead to severe and persistent cognitive and motor deficits. Transient hypothyroxinemia in preterm infants is common, characterized by low levels of plasma T_4 and T_3 but normal levels of TSH (1). This condition was thought to be without long-term sequelae or a requirement for thyroid hormone replacement (2). However, more recent studies that measured only T_4 have linked low T_4 levels in preterm infants with later neurodevelopmental deficits in motor and cognitive function (3–5) and low plasma T_3 with reductions in IQ at 8 yr of age (6). These associations persist after adjustment for perinatal illness (5, 6).

The comparison of preterm infant cord and postnatal thyroid hormone sera levels with term infant and/or adult values has established that these groups have important differences in sera levels of iodothyronines and related parameters (1, 7–10). The accepted definition of preterm birth is one at less than 37 wk completed gestation, but the lower limit of viability extends to around 23 wk gestation. It is therefore not surprising that thyroid hormone levels differ in preterm infants grouped by gestational age (11, 12). This makes it essential for observational studies, or comparative studies of treatment regimens, to analyze and interpret data from groups based on relatively narrow gestational age ranges (13–15). To date, longitudinal studies of postnatal thyroid hormone sera levels in gestationally grouped preterm infants are limited because they are restricted in number and also vary in the range and nature of the serum parameters analyzed (14, 16–18). Other studies are limited to the early postnatal period (12) or to measurement of a single iodothyronine (19). Indeed, the three seminal studies that established the statistical relationship between neurodevelopmental outcome and hypothyroxinemia were based on a single measurement of postnatal T_4 level (3–5). The pathophysiological processes of transient hypothyroxinemia in preterm infants are complex, and to inform further understanding this requires a longitudinal postnatal study of sufficient power and with measurement of a comprehensive range of thyroid hormones in gestationally restricted age groups.

The definition of optimal ranges for serum thyroid hormones in preterm infants related to gestation and postnatal age has not been resolved. Preterm infant reference ranges should be derived from populations of gestationally restricted age groups (12, 20). But are these reference ranges optimal for the maturation of critical organs such as brain? Ideally, and in the longer term, measures of physiological function, such as long-term neurodevelopmental outcome,

Abbreviations: FT_4, Free T_4; TBG, T_4-binding globulin; T_4S, T_4 sulfate.

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related to postnatal thyroid hormone levels should be used to determine the optimal postnatal serum thyroid hormone levels. Such studies have begun (18). In the interim, other means of informing the definition of optimal levels need to be explored. We propose that the relationship of postnatal to cord levels of iodothyronines, T_4-binding globulin (TBG), and TSH may be assessed only once the cord levels have been corrected to equivalent gestational age had the fetus remained *in utero*.

Subjects and Methods

Data were collected between January 1998 and September 2001. The study encompassed two distinct groups. A cohort of mothers and infants delivered at 23–34 wk gestation and who were part of a multicenter study of transient hypothyroxinemia in 11 level III Scottish neonatal intensive care units. And a consecutive sample of mothers and infants delivered at 35–42 wk gestation and recruited in a single center at Ninewells Hospital and Medical School, Dundee. In addition, we increased our term data set with data obtained from Rotunda Hospital, Dublin, and from several different hospitals in The Netherlands. Data on mothers have been described elsewhere (21) and will not be discussed further in this paper. Gestational age of infants was calculated from menstrual history and, in most instances, was confirmed by ultrasound examination in the first trimester. Exclusion criteria from the study were known viral hepatitis or HIV positivity (or at high risk), major congenital abnormality, or inability of mothers to provide informed consent.

All infants had intensive care support as required, including intermittent positive ventilation and, where appropriate, correction of fluid, electrolyte, blood glucose, and acid-base abnormalities. Blood pressure was supported with inotropes, plasma, or other blood products as required. Infants with significant persistence of the ductus arteriosus were treated with diuretics and indomethacin, or surgical ligation if appropriate. Parenteral nutrition regimens, if required, were based on a solution of electrolytes, 10% dextrose, amino acids (Vaminolact, Fresenius Kabi, Cheshire, UK), a phosphate supplement (Addiphos, Fresenius Kabi), water-soluble vitamins (Solvito N, Fresenius Kabi), and trace elements (Peditrace, Fresenius Kabi) to levels recommended by the manufacturer. In tandem, a fat emulsion solution (Intralipid 20%, Fresenius Kabi) with added fat-soluble vitamins (Vitlipid, Fresenius Kabi) was used. Enteral feeds were started when the condition of the infant was stable. Thereafter, enteral feed volumes were gradually increased as determined by the infants' clinical condition, with reciprocal reductions in the volume of parenteral nutrition infused.

The study was approved, as appropriate, by the Multicenter Research Ethics Committee (Edinburgh, UK) and the Tayside Committee on Medical Research Ethics; in all cases, written informed consent was obtained.

TABLE 1. Postnatal levels of iodothyronine, TSH, and TBG in cord and at d 7, 14, and 28

Gestation (wk)	Mean concentration ± SD (n)			
	Cord	d 7	d 14	d 28
T_4 (nmol/liter)				
23–27	70 ± 26 (57)	52 ± 23 (100)[a,b]	61 ± 33 (101)[b]	79 ± 30 (97)
28–30	81 ± 26 (123)	81 ± 27 (196)	85 ± 29 (191)	96 ± 30 (156)[a,b]
31–34	98 ± 29 (253)[b]	121 ± 44 (252)[a,b]	117 ± 46 (198)[a]	115 ± 38 (104)[a]
≥37	118 ± 25 (255)[b]	163 ± 37 (14)[a]	138 ± 18 (6)[a]	125 ± 28 (9)
FT_4 (pmol/liter)				
23–27	16.5 ± 5.3 (56)	18.9 ± 7.2 (93)[a]	18.6 ± 6.5 (96)[a]	19.3 ± 5.5 (92)[a]
28–30	18.6 ± 5.5 (122)	23.4 ± 8.5 (187)[a,b]	21.2 ± 5.6 (186)[a,b]	22.0 ± 5.5 (151)[a,b]
31–34	19.2 ± 4.3 (251)	27.6 ± 7.4 (246)[a,b]	25.2 ± 5.5 (193)[a]	24.2 ± 5.9 (105)[a,b]
≥37	18.1 ± 5.0 (253)	34.7 ± 7.3 (14)[a,b]	26.1 ± 3.6 (6)[a,b]	21.2 ± 4.4 (8)
TBG (mg/liter)				
23–27	18.8 ± 5.7 (54)	17.1 ± 4.0 (80)	18.9 ± 5.2 (79)	22.8 ± 6.0 (85)[a,b]
28–30	19.6 ± 5.1 (118)	20.2 ± 4.9 (146)	21.1 ± 5.2 (156)[a]	22.0 ± 6.1 (137)[a,b]
31–34	24.0 ± 8.2 (249)	24.4 ± 7.9 (210)	23.4 ± 7.9 (169)	23.1 ± 7.6 (95)
≥37	29.2 ± 5.6 (242)	33.5 ± 10.7 (14)	27.5 ± 3.8 (5)	27.3 ± 67 (8)
TSH (mU/liter)				
23–27	6.8 [6.4] ± 2.9 (55)	3.5 [2.9] ± 2.6 (78)[a,b]	3.9 [3.2] ± 2.7 (83)[a,b]	3.8 [2.8] ± 4.7 (82)[a,b]
28–30	7.0 [6.4] ± 3.7 (120)	3.6 [3.1] ± 2.5 (155)[a,b]	4.9 [3.3] ± 11.2 (162)[a,b]	3.6 [2.8] ± 2.5 (137)[a,b]
31–34	7.9 [7.2] ± 5.2 (248)	3.6 [2.6] ± 4.8 (212)[a,b]	3.8 [2.5] ± 9.3 (170)[a]	3.5 [2.4] ± 3.4 (97)[a,b]
≥37	6.7 [5.4] ± 4.8 (247)	2.6 [1.9] ± 1.8 (14)[a,b]	2.5 [1.8] ± 2.0 (6)[a,b]	1.8 [1.7] ± 0.9 (8)[a]
T_3 (nmol/liter)				
23–27	0.30 ± 0.23 (57)	0.50 ± 0.31 (98)[a,b]	0.63 ± 0.38 (100)[a]	0.97 ± 0.42 (96)[a,b]
28–30	0.44 ± 0.32 (123)	0.86 ± 0.37 (194)[a,b]	1.11 ± 0.43 (187)[a,b]	1.34 ± 0.48 (154)[a,b]
31–34	0.54 ± 0.36 (252)	1.41 ± 0.55 (250)[a,b]	1.68 ± 0.63 (197)[a,b]	1.84 ± 0.62 (105)[a,b]
≥37	0.92 ± 0.53 (255)	2.27 ± 0.77 (14)[a,b]	2.57 ± 0.48 (6)[a,b]	2.70 ± 0.49 (8)[a]
rT_3 (nmol/liter)				
23–27	5.8 ± 2.6 (57)	1.3 ± 0.6 (92)[a,b]	1.2 ± 0.5 (93)[a,b]	1.1 ± 0.4 (89)[a,b]
28–30	4.9 ± 2.1 (122)	1.6 ± 0.7 (179)[a,b]	1.2 ± 0.4 (179)[a,b]	1.1 ± 0.5 (148)[a,b]
31–34	4.6 ± 1.9 (250)	1.7 ± 0.8 (231)[a,b]	1.3 ± 0.5 (187)[a,b]	1.1 ± 0.5 (102)[a,b]
≥37	3.4 ± 1.1 (255)	1.7 ± 0.7 (14)[a,b]	1.2 ± 0.5 (5)[a,b]	0.9 ± 0.4 (6)[a]
T_4S (pmol/liter)				
23–27	1165 ± 370 (52)	2364 ± 810 (62)[a,b]	1774 ± 1322 (61)[a,b]	1645 ± 1709 (64)[a,b]
28–30	1124 ± 449 (111)	2022 ± 906 (113)[a,b]	1073 ± 699 (133)	833 ± 698 (119)[a,b]
31–34	1068 ± 407 (240)	1716 ± 783 (163)[a,b]	807.0 ± 378 (146)[a]	560 ± 386 (84)[a]
≥37	711 ± 265 (67)	907 ± 577 (9)	395 ± 286 (5)	155 ± 53 (5)[a]

Data in *brackets* are medians. Conversion factors are as follows: T_3 and rT_3, multiply by 65.1 for ng/dl; T_4, multiply by 0.0777 for μg/dl; FT_4, multiply by 0.0777 for ng/dl; T_4S, multiply by 0.085 for ng/dl.

[a] Significantly different ($P < 0.05$) from appropriate gestational cord level.

[b] Significantly different ($P < 0.05$) from cord level of equivalent gestational age. The mean gestational age at birth was determined for each of the following groups: 26 wk for the 23- to 27-wk group, 29 wk for the 28- to 30-wk group, 33 wk for the 31- to 34-wk group, and 39 wk for term infants.

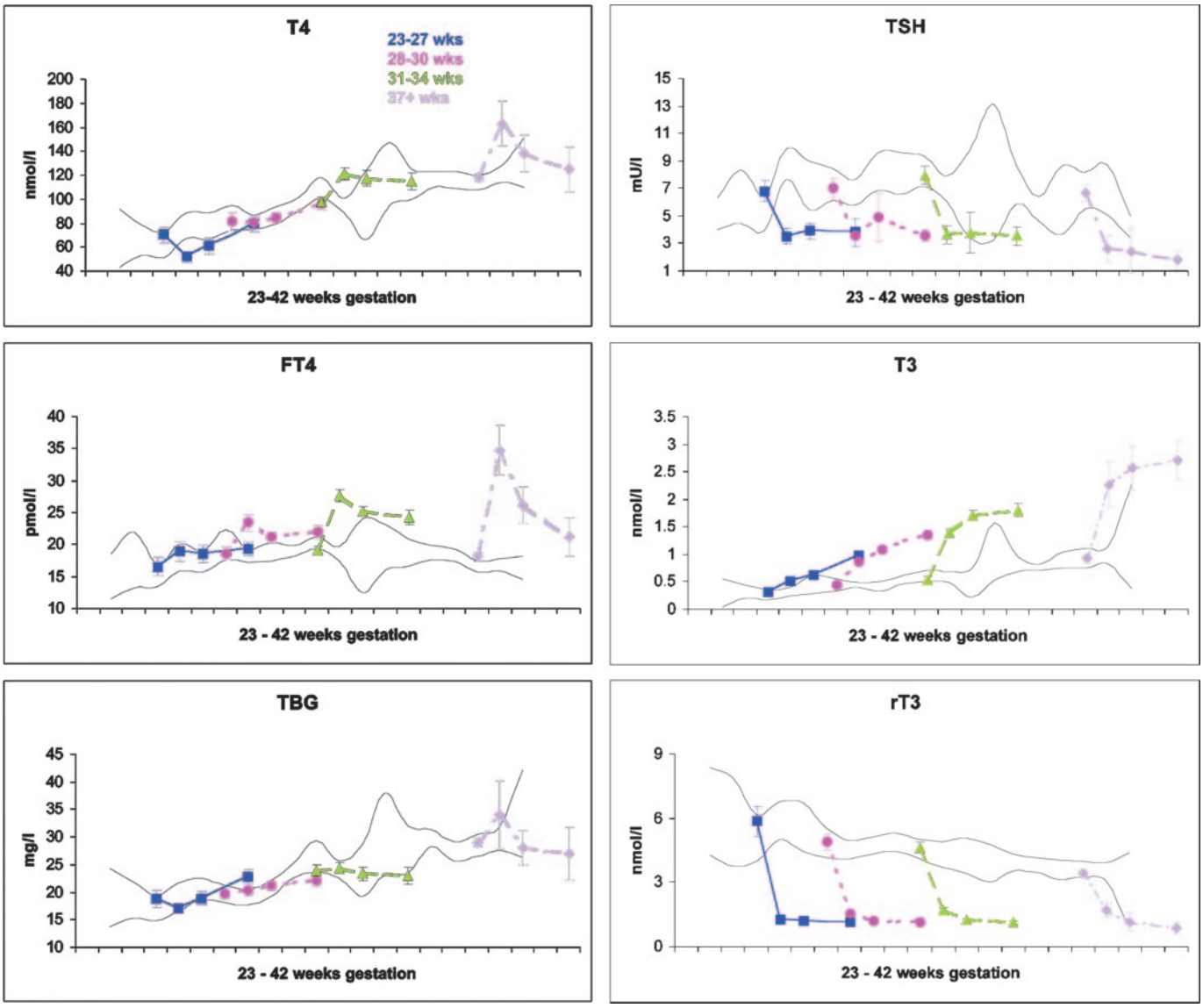

FIG. 1. Sera levels of iodothyronines, TBG, and TSH in cord and at d 7, 14, and 23 postpartum. The *error bars* for T_3 and rT_3 are small and have been obscured by the symbol depicting the mean levels of these iodothyronines.

Cord blood (n = 695) was collected into a tube without anticoagulant as soon as possible after delivery of the live-born infants (23–42 wk gestation) from fetal vessels running over the placental surface. Blood was also collected at postnatal d 7, 14, and 28. The blood samples were allowed to clot for at least 15 min and then centrifuged at 4000 rpm for 5 min. If collected outside of normal laboratory hours, the blood was stored at 4 C (maximum 12 h) before processing. The serum was removed, stored, and transported at a maximum of –20 C for assays in one laboratory (T.J.V.).

Provided sufficient serum was available, T_4, free T_4 (FT_4), TSH, T_3, rT_3, T_4 sulfate (T_4S), and TBG levels were determined. Serum T_4, T_3, and rT_3 were measured by in-house RIA; FT_4 by Vitros ECI technology (Ortho-Clinical Diagnostics, Amersham, UK); TSH by Dynotest immunoradiometric assay; and TBG by Dynotest RIA (Brahms, Berlin, Germany). T_4S was prepared by the method of Eelkman Rooda *et al.* (22). The measurements of T_4S in serum were done by a specific antibody, as described previously (23). Within-assay coefficients of variation were calculated as 2–8% for T_4, 3–7% for FT_4, 2–6% for T_3, 3–4% for rT_3, 6–17% for T_4S, 2–5% for TSH, and 2–4% for TBG. Between-assay coefficients of variation were 5–10% for T_4, 5–10% for FT_4, 8% for T_3, 9–14% for rT_3, 4–19% for T_4S, 2–14% for TSH, and 2–3% for TBG.

Because ratios tend not to follow a Gaussian distribution, all ratios were log transformed, so that the data approximated a normal distribution; this allowed the determination of group means and SE of the means. The median values are given in parentheses for TSH because it has a skewed distribution; however, the data are presented untransformed so that they can be readily compared with other published studies. The data were subdivided into four gestational age groups: 23–27, 28–30, 31–34, and 37 or more weeks. Infants born at or under 30 wk gestation constitute the extreme preterm infant.

All the graphs were constructed as follows. For individual gestational weeks between 23 and 42 wk, the mean and twice the SEM of cord blood levels was plotted for each iodothyronine, TSH, and TBG. This baseline graph shows the joined and smoothed lines of plus twice the SEMs and minus twice the SEMs. The data were grouped by gestational age at delivery into 23–27 (n = 101), 28–30 (n = 196), and 31–34 (n = 253) wk. The mean and ± twice the SEM were calculated for each iodothyronine, TSH, and TBG for cord and for postnatal d 7, 14, and 28. When the SE bars do not overlap, the difference between (any) two means may be assumed to be statistically different ($P < 0.05$).

The mean gestational age at birth was determined for each of the groups: 25.8 wk for the 23- to 27-wk group, 29.2 wk for 28- to 30-wk group, and 32.5 wk for the 31- to 34-wk group. The postnatal data at d 7, 14, and 28 were plotted onto the baseline graph starting at the ap-

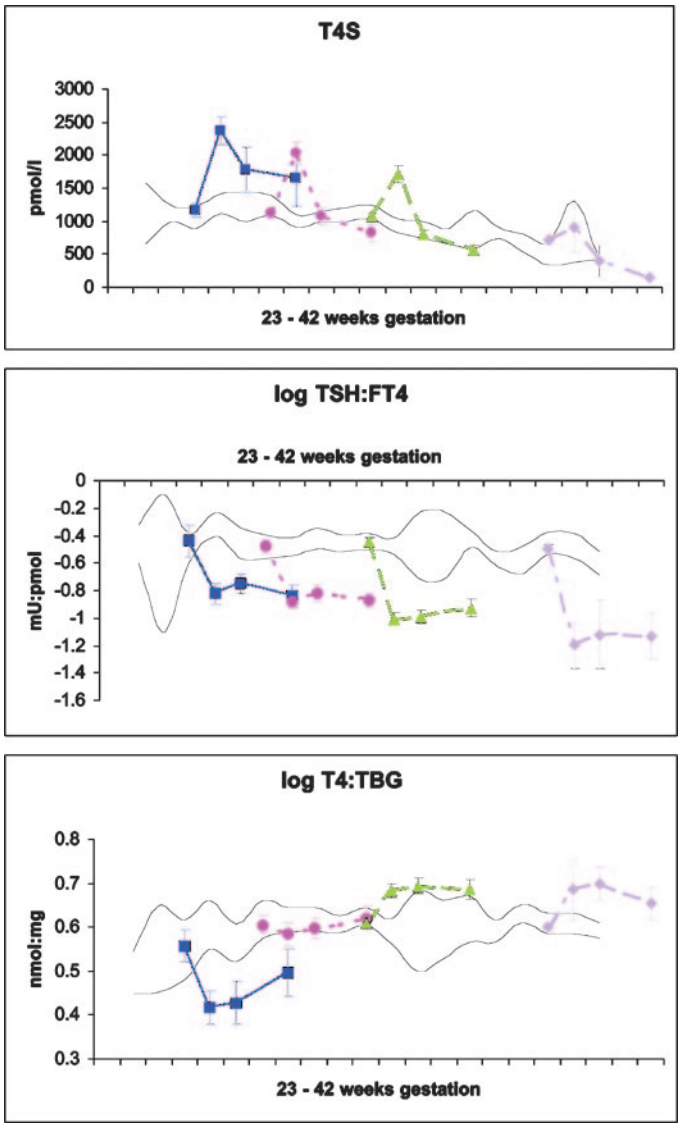

FIG. 1. *Continued*

propriate mean birth gestation for each group. For example, the most immature group started at 26 wk gestation and d 7 was plotted against 27 wk, d 14 against 28 wk, and d 28 against 30 wk. Thus, data for any given particular postnatal age (*i.e.* 7, 14, or 28 d) was plotted and described in relation to the equivalent gestational age had the fetus remained *in utero* (referred to hereafter as equivalent gestational age). The assumption we make is that the cord levels of iodothyronines, TBG, and TSH reflect those of the normal fetus *in utero* of equivalent gestational age and who progress to a term delivery.

Results

The infants were subdivided into gestational age groups for data analysis: 23–27 wk, 28–30 wk, 31–34 wk, and term (Table 1). The retention of these subdivisions is important in our analysis of this data set, because both Murphy *et al.* (24) and van Wassenaer *et al.* (15, 18) have described discontinuities in thyroid hormone responsiveness and metabolism with extreme prematurity.

The first month of life in term infants is characterized typically by a period of hyperthyroxinemia (T_4 and FT_4) relative to cord values and levels for nonpregnant women (Table 1 and Fig, 1) (21). The postnatal elevation in serum T_4 is attenuated in 31- to 34-wk infants, absent in 28- to 30-wk infants (although levels are similar to cord values of equivalent gestational age), and reversed in 23- to 27-wk infants at d 7 and 14 with hypothyroxinemia relative to other preterm and term groups and to cord values of equivalent gestational age (Table 1 and Fig. 1).

In comparison with term infants, the postnatal increase in FT_4 levels is lower in 31- to 34-wk infants, further attenuated in 28- to 30-wk infants (nevertheless still higher than cord values of equivalent gestational age), and still further attenuated in 23- to 27-wk infants with FT_4 levels remaining within cord values equivalent to gestational age (Table 1 and Fig. 1).

The trend in postnatal TBG levels in all preterm infant groups is to increase and generally to follow, with minor variations, the equivalent gestational age limits (Fig 1). In term infants, postnatal TBG levels are similar to cord values at birth (Table 1 and Fig. 1).

The postnatal log T_4:TBG ratios (Fig. 1) show similar trends, within the gestational age groups, to the pattern of postnatal T_4 levels. In term infants, postnatal log T_4:TBG ratios increase above cord values of equivalent gestational age. The postnatal elevation in serum log T_4:TBG is similar in 31- to 34-wk infants, attenuated in 28- to 30-wk infants (although levels are similar to cord values of equivalent gestational age), and reversed in 23- to 27-wk infants (Fig. 1).

The TSH values were similar in all gestational groups in cord and at all ages postnatally; the values were highest in cord, fell by d 7, and remained constant thereafter (Fig. 1). TSH levels were lower (with two exceptions) at all ages postnatally than cord values of equivalent gestational age.

Cord log TSH:FT_4 ratios are similar in all groups including term infants. At d 7 and 14, the 23- to 27- and 28- to 30-wk gestational groups have higher ratios than the 31- to 34-wk and term infants. The log TSH:FT_4 ratios in nonpregnant women [mean, –1.13, and twice SEM, 0.04 (21)] are identical to the postnatal values at d 7, 14, and 28 in term infants (Fig. 1).

In all preterm infants, mean serum T_3 levels rise appreciably from similar cord values in all gestational groups, but the magnitude of the increments are related to gestational and postnatal age. Mean serum T_3 values show a remarkable continuity of apparent linear increments in postnatal levels extending through the gestational groups to term. In effect, all preterm infant groups from d 7 are exposed to increasingly higher serum levels of T_3 than cord levels of equivalent gestational ages (except d 14 in the 23- to 27-wk group) (Table 1 and Fig. 1).

The rT_3 values at all postnatal ages and in all groups are substantially below the cord values of the equivalent gestational ages (Table 1 and Fig. 1).

In all our preterm groups, postnatal T_4S levels increase, with maximal values measured in d 7 sera. The 23- to 27-wk gestation group are distinctive because they not only have the highest d 7 mean serum level, but in contrast to the more mature preterm groups (28–30 and 31–34 wk gestation), mean T_4S levels at postnatal d 14 and 28 remain generally elevated above cord levels of equivalent gestational ages.

Discussion

This series represents the largest data set investigating postnatal iodothyronine, TBG, and TSH serum levels in preterm infants less than or at 34 wk gestation. Cord serum samples were collected from these infants, and from a cohort of infants more than 34 wk gestation, which allowed us to compare changes in serum levels at fixed postnatal times relative to cord levels of equivalent gestational ages. This approach is the best approximation we have to compare postnatal changes in preterm infants to the *in utero* condition; it has been exploited only to a limited extent (17). This assumes that prenatal and intrapartum factors do not have a significant impact on cord levels; preliminary analysis of our data supports this view (unpublished observations). Clearly cordocentesis levels in normal pregnancies are ideal, but the small sample sizes in the series available limit interpretation of developmental trends (25, 26).

Thyroid hormones are essential for adaptation to extrauterine life, including lung development and fluid control (27), adaptive thermogenesis (28), and a diverse range of metabolic processes in liver including gluconeogenesis (29). In thyroidectomized rats, only combined treatment with T_4 and T_3 ensures euthyroidism in all tissues (30). An adequacy of T_3 and T_4 may similarly be important for the maturation of essential functions in human infants.

In term infants, serum T_3 levels increase approximately 3-fold from cord values by 3–4 wk postnatal age (11, 31) to values of 2.5–3.0 nmol/liter (31). Preterm infants of 32–33 wk gestation also increase postnatal T_3 levels by approximately the same magnitude albeit from lower cord levels (7, 8, 19). In preterm infants less than 32 wk gestation, T_3 changes are more variable; some series record no postnatal changes over 2 wk (32) or even 6 wk (16); in other studies, T_3 levels increased postnatally (13–15). Serum T_3 levels rise appreciably from similar cord values in all our preterm groups. Importantly, in our 23- to 27-wk group, postnatal increments in T_3 occur at a time when mean T_4 (but not FT_4 levels) are decreased below cord levels of equivalent gestational age. T_3 levels can be increased in preterm infants of less than 30 wk gestation by T_3 supplementation, but this decreases FT_4 levels (33). Even a single administration of T_3 to infants of less than 28 wk gestation, although increasing T_3, results in a transient decrease in FT_4 (34). Maintaining serum FT_4 levels in extreme preterm infants may be a priority for sustaining postnatal brain development and neurodevelopmental outcome (35) because sufficient circulatory FT_4, rather than total T_4, is necessary for T_3 generation within brain cells (36).

Both T_4 and TBG in cord serum increase over the range 15–42 wk gestation (21). Log T_4:TBG ratios are low at early gestations but increase until late second trimester, as temporal increments in T_4 are greater than those of TBG (21). This is in agreement with the data shown by Greenberg *et al.* (37). In the third trimester, cord T_4 and TBG levels both increase, but the log T_4:TBG ratio is now constant (21). In our 23- to 27-wk group, postnatal reductions in log T_4:TBG ratios, to levels equivalent to earlier gestations, are the result of a larger decrease in T_4 than TBG levels. In contrast, the 31- to 34-wk group, where T_4 and TBG levels increase postnatally, the log T_4:TBG ratios also increase substantially, a result of increased T_4 levels to values above those equivalent to the corrected gestational ages.

Until the late second trimester, the developmental pattern of log T_4:TBG ratios shows a remarkable similarity to that of FT_4; both increase with gestational age. Thereafter, log T_4:TBG ratios are constant, but FT_4 levels peak at 31–34 wk and decrease to term (21). In our postnatal preterm infants, this relationship of log T_4:TBG ratios to FT_4 levels shows additional inconsistencies. The reasons for this may be multiple and vary in different gestational age groups and postnatal ages. In our term and 31- to 34-wk infants, T_4 levels increase postnatally with almost constant TBG levels. As a consequence, in this group, log T_4:TBG ratios increase above gestational age-related limits to levels greater than maternal and nonpregnant women (21), and as a result, FT_4 levels also increase. In contrast, in the 23- to 27-wk group, T_4 levels decrease postnatally against a background of postnatal TBG levels that are maintained within limits corrected for gestational age. As a result, postnatal log T_4:TBG ratios decrease below gestational age-related limits. In these circumstances, it was expected that FT_4 levels would also decrease as a feature of transient hypothyroxinemia (1, 15, 16). In fact FT_4 levels in our 23- to 27-wk gestation group remain within cord values of equivalent gestational age, levels apparently adequate to allow normal brain development *in utero* assuming these cord iodothyronine levels reflect those of the normal fetus *in utero* of equivalent gestational age and progressing to a term delivery.

The depression of T_4 levels and maintenance of FT_4 levels at 2 wk postnatal age shown in our 23- to 27-wk group has been previously described in a group of very low birth weight infants (<1500 g), changes that were exaggerated by the most severely ill subgroup of five infants (38). FT_4 levels in that study, as determined by the two-stage method of AutoDelfia, were 67% of those of equilibrium dialysis FT_4 levels (38). The measurement of sera FT_4 by commercial kits is problematic, overestimating the FT_4 levels at high serum binding protein concentrations and underestimating FT_4 at low protein concentrations (39), and there are differences in FT_4 estimation between the kits (40). It is possible that FT_4 levels in our study were also similarly underestimated, although the Vitros ECI FT_4 method has one of the best concordances with the dialysis method (40) (van Toor, H., and T. J. Visser, unpublished data). This underestimation will also apply to other studies in preterm infants where postnatal FT_4 levels have been measured by commercial kits (1, 15, 17). Calculation of the FT_4 index using T_4:TBG ratios is also problematic; TBG binding capacity can be altered independent of changes in concentrations of TBG protein (39) in agreement with our data.

Displacement of T_4 from TBG by drugs, metabolites, and free fatty acids (41, 42) is a possible explanation for the increases in FT_4 relative to the T_4:TBG ratio evident in our most immature group. Elevated levels of free fatty acids, particularly when serum albumin is low, as is common in preterm infants (43), can displace protein-bound T_4 and elevate FT_4 (44). In our study, parenterally fed newborns are infused with Intralipid at levels previously shown to generate average ratios of free fatty acids to albumin of 12 or more; the ratio needed to inhibit protein binding *in vitro* is

approximately 3 (44, 45). The patency of arterial and venous catheters is maintained by heparin infusions; this activates lipoprotein lipase, which can generate free fatty acids in postvenesection serum and falsely augment FT_4 levels *in vitro* (46). Extreme preterm infants are more likely to have conditions necessary for displacement of protein-bound T_4, such as parenteral nutrition, lower serum albumin levels, heparin infusions, and other drug usage. In preterm infants, serum FT_4 levels increase relative to those of T_4 in the first hour of life and before exposure to heparin or iv triglyceride emulsions (24); this suggests endogenous factors influence FT_4 levels. An additional explanation for increases in FT_4 levels may be the result of polymorphonuclear leukocyte elastase cleavage of TBG during sepsis and inflammation liberating bound T_4 (47). Polymorphonuclear leukocyte elastase activity is 8-fold higher in cord blood than maternal sera (48). Cleaved TBG is present in cord blood and in the absence of sepsis may be part of the physiological inflammatory response in the newborn (49).

Cord log TSH:FT_4 ratios are similar in our preterm gestational groups and term infants. This is in contrast to the work of Fisher *et al.* (50), who used combined data from multiple individual studies and where cord values of very low birth weight infants were higher than those of term infants. Our postnatal log TSH:FT_4 ratios in the 23- to 27- and 28- to 30-wk groups are significantly higher at 7 and 14 d compared with the 31- to 34-wk groups and term infants. This pattern is in contrast to the data interpreted by Fisher *et al.* (50), which is relatively constant in the first postnatal week in groups of infants from 25–42 wk gestation. The mean postnatal log TSH:FT_4 of term infants is similar to that of our nonpregnant women (21), which is very different from the work of Fisher *et al.* (50), who suggest that TSH:FT_4 does not reach adult levels until the early 20s. However, as with all ratios, changes in each parameter, provided they happen together because of a change in the set point, will not be reflected in a change in the ratio.

Cord T_4S levels increase through the second trimester to reach a peak in late second to early third trimester before the last-trimester decline in T_4S levels (21). In this study, T_4S levels transiently increase postnatally before declining; the explanation for these changes is not obvious. For example, the contribution of different sulfotransferase isoforms and tissues to T_4S serum levels is not known, or even whether the contribution changes through fetal life, or postnatally (51, 52). T_4S is exclusively metabolized to inactive rT_3S; elevation in serum T_4S levels is associated with reduced type 1 iodothyronine deiodinase activity (53), but there is no supportive evidence for this scenario in studies of human hepatic deiodinase development (54).

We have previously shown that the late second/early third trimester is a critical transition period in fetal thyroid hormone metabolism, which may be interrupted by preterm birth and contribute to postnatal thyroid dysfunction (21, 24). In this present study, we provide more evidence that the 23- to 27-wk group of infants is particularly distinctive from other preterm groups; they are hypothyroxinemic on T_4 levels, yet FT_4 levels are equivalent to gestational age values, and T_3 increases to levels above equivalent gestational age values.

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References

1. **Rooman RP, DuCaju MVL, DeBeeck LO, Docx M, VanReempts P, VanAcker KJ** 1996 Low thyroxinaemia occurs in the majority of very preterm newborns. Eur J Pediatr 155:211–215
2. **Fisher DA** 1990 Euthyroid low thyroxine (T_4) and triiodothyronine (T_3) states in prematures and sick neonates. Pediatr Clin North Am 37:1297–1312
3. **Meijer WJ, Verloove-Vanhorick SP, Brand R, van den Brande JL** 1992 Transient hypothyroxinemia associated with developmental delay in very preterm infants. Arch Dis Child 67:944–947
4. **den Ouden AL, Kok JH, Verkerk PH, Brand R, Verloove-Vanhorick SP** 1996 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 39:142–145
5. **Reuss ML, Paneth N, Pinto-Martin JA, Lorenz JM, Susser M** 1996 The relation of transient hypothyroxinemia in preterm infants to neurologic development at two years of age. N Engl J Med 334:821–827
6. **Lucas A, Morley R, Fewtrell MS** 1996 Low triiodothyronine concentration in preterm infants and subsequent intelligence quotient (IQ) at 8 year follow up. BMJ 312:1132–1133
7. **Abassi V, Merchant K, Abramson D** 1977 Postnatal triiodothyronine concentrations in healthy preterm infants and in infants with respiratory distress syndrome. Pediatr Res 11:802–804
8. **Uhrmann S, Marks KH, Maisels MJ, Friedman Z, Murray F, Kulin HE, Kaplan M, Utiger R** 1978 Thyroid function in the preterm infant: a longitudinal assessment. J Pediatr 92:968–973
9. **Cuestas RA** 1978 Thyroid function in healthy premature infants. J Pediatr 92:963–967
10. **Jacobsen BB, Andersen HJ, Peitersen B, Dige-Petersen H, Hummer L** 1997 Serum levels of thyrotropin, thyroxine and triiodothyronine in full term, small-for-gestational age and preterm newborn babies. Acta Paediatr Scand 66:681–687
11. **Fisher DA, Klein AH** 1981 Thyroid development and disorders of thyroid function in the newborn. N Engl J Med 304:702–712
12. **Adams LM, Emery JR, Clark SJ, Carlton EI, Nelson JC** 1995 Reference ranges for newer thyroid function tests in premature infants. J Pediatr 126:122–127
13. **Ballard PL, Ballard RA, Ning Y, Cnann A, Boardman C, Pinto-Martin J, Polk D, Phibbs RH, Davis DJ, Mannino FL, Hart M** 1998 Plasma thyroid hormones in premature infants: effect of gestational age and antenatal thyrotropin-releasing hormone treatment. Pediatr Res 44:642–649
14. **Biswas S, Buffery J, Enoch H, Bland JM, Walters D, Markiewicz M** 2002 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 109:222–227
15. **van Wassenaer AG, Kok JH, Dekker FW, de Vijlder JJM** 1997 Thyroid function in very preterm infants: influences of gestational age and disease. Pediatr Res 42:604–609

16. **Mercado M, Yu VYH, Francis I, Szymonowicz W, Gold. H** 1988 Thyroid function in very preterm infants. Early Hum Dev 16:131–141
17. **Ares S, Escobar-Morreale HF, Quero J, Durán S, Presas MJ, Herruzo R, Morreale de Escobar G** 1997 Neonatal hypothyroxinemia: effects of iodine intake and premature birth. J Clin Endocrinol Metab 82:1704–1712
18. **van Wassenaer AG, Kok JH, de Vijlder JJM, Briët JM, Smit BJ, Tamminga P, van Baar A, Dekker FW, Vulsma T** 1997 Effects of thyroxine supplementation on neurologic development in infants born at less than 30 weeks' gestation. N Engl J Med 336:21–26
19. **Lucas A, Rennie J, Baker BA, Morley R** 1988 Low plasma triiodothyronine concentrations and outcome in preterm infants. Arch Dis Child 63:1201–1206
20. **Clark SJ, Demming DD, Emery JR, Adams LM, Carlton EI, Nelson JC** 2001 Reference ranges for thyroid function tests in premature infants beyond the first week of life. J Perinatol 21:531–536
21. **Hume R, Simpson J, Delahunty C, van Toor H, Wu S-Y, Williams FLR, Visser TJ** Human fetal and cord serum thyroid hormones: developmental trends and interrelationships. J Clin Endocrinol Metab 89:4097–4103
22. **Eelkman Rooda SJ, Kaptein E, van Loom MAC, Visser TJ** 1988 Development of a radioimmunoassay for triiodothyronine sulfate. J Immunoassay 9:125–134
23. **Wu S-Y, Huang W-S, Polk D, Florsheim WH, Green WL, Fisher DA** 1992 Identification of thyroxine-sulfate (T_4S) in human serum and amniotic fluid by a novel T_4S radioimmunoassay. Thyroid 2:101–105
24. **Murphy N, Hume R, van Toor H, Matthews TG, Ogston SA, Wu S-Y, Visser TJ, Williams FLR** 2004 The hypothalamic-pituitary-thyroid axis in preterm infants; responsiveness to birth over the first 24 hours of postnatal life. J Clin Endocrinol Metab 89: 2824–2831
25. **Thorpe-Beeston JG, Nicolaides KH, McGregor AM** 1992 Fetal thyroid function. Thyroid 2:207–217
26. **Ballabio M, Nicolini U, Jowett T, Ruiz de Elvira MC, Ekins RP, Rodeck CH** 1989 Maturation of thyroid function in normal human foetuses. Clin Endocrinol (Oxf) 31:565–571
27. **Hume R** 2002 Thyroid hormones and lung development. In: Hennemann G, Visser TJ, eds. Hot thyroidology, November, No. 3. www.hotthyroidology-.com
28. **Lowell BB, Spiegelman BM** 2000 Towards a molecular understanding of adaptive thermogenesis. Nature 404:652–660
29. **Feng X, Jiang Y, Meltzer P, Yen PM** 2000 Thyroid hormone regulation of hepatic genes *in vivo* detected by complementary DNA microarray. Mol Endocrinol 14:947–955
30. **Escobar-Morreale HF, del Rey FE, Obregon MJ, de Escobar GM** 1996 Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. Endocrinology 137:2490–2502
31. **Santini F, Chiovato L, Ghirri P, Lapi P, Mammoli C, Montanelli L, Scartabelli G, Ceccarini G, Coccoli L, Chopra IJ, Boldrini A, Pinchera A** 1999 Serum iodothyronines in the human fetus and the newborn: evidence for an important role of placenta in fetal thyroid hormone homeostasis. J Clin Endocrinol Metab 84:493–498
32. **Pavelka S, Kopeck P, Bendlová P, Stolba P, Vítková L, Vobruba V, Plavka R, Houstek J, Kopeck J** 1977 Tissue metabolism and plasma levels of thyroid hormones in critically ill very premature infants. Pediatr Res 42:812–818
33. **Biswas S, Buffery J, Enoch H, Bland M, Markiewicz M, Walters D** 2003 Pulmonary effects of triiodothyronine (T_3) and hydrocortisone supplementation in preterm infants less than 30 weeks gestation: results of the THORN trial–thyroid hormone replacement in neonates. Pediatr Res 53:48–56
34. **Cools F, van Wassenaer AG, Kok JH, de Vijlder JJM** 2000 Changes in thyroid hormone levels after a single dose of triiodothyronine in premature infants of less than 30 weeks gestational age. Eur J Endocrinol 143:733–740
35. **van Wassenaer AG, Briet JM, van Baar A, Smit BJ, Tamminga P, de Vijlder JJM, Kok JH** 2002 Free thyroxine levels during the first weeks of life and neurodevelopmental outcome until the age of five years in very preterm infants. Pediatrics 109:532–539
36. **Morreale de Escobar G, Obregon MJ, Escobar Del Rey F** 2000 Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin Endocrinol Metab 85:3975–3987
37. **Greenberg AH, Czernichow P, Reba RC, Tyson J, Blizzard RM** 1970 Observations on the maturation of thyroid function in early fetal life. J Clin Invest 49:1790–1803
38. **Klein RZ, Carlton EL, Faix JD, Frank JE, Hermos RJ, Mullaney D, Nelson JC, Rojas DA, Mitchell ML** 1997 Thyroid function in very low birth weight infants. Clin Endocrinol (Oxf) 47:411–417
39. **Spencer C** 2003 Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13:23–24
40. **Sapin R, Schlienger JL, Gasser F, Noel E, Lioure B, Grunenberger F, Goichot B, Grucker D** 2000 Intermethod discordant free thyroxine measurements in bone marrow-transplanted patients. Clin Chem 46:418–422
41. **De Groot LJ** 1999 Dangerous dogmas in medicine: the nonthyroidal illness syndrome. J Clin Endocrinol Metab 84:151–164
42. **Chopra IJ** 1997 Euthyroid sick syndrome: is it a misnomer? J Clin Endocrinol Metab 82:329–334
43. **Fryer AA, Jones P, Strange R, Hume R, Bell JE** l993 Plasma protein levels in normal human fetuses 13–41 weeks gestation. Br J Obstet Gynaecol 100:850–855
44. **Lim C-F, Docter R, Visser TJ, Krenning EP, Bernard B, Van Toor H, De Jong M, Hennemann G** 1993 Inhibition of thyroxine transport into cultured rat hepatocytes by serum of nonuremic critically ill patients: effects of bilirubin and nonesterified fatty acids. J Clin Endocrinol Metab 76:1165–1172
45. **Kao LC, Cheng MH, Warburton D** 1984 Triglycerides, free fatty-acids albumin molar ratio, and cholesterol levels in serum of neonates receiving long-term lipid infusions: controlled trial of continuous and intermittent regimens. J Pediatr 104:429–443
46. **Jaume JC, Mendel CM, Frost PH, Greenspan FS, Laughton CW** 1996 Extremely low doses of heparin release lipase activity into the plasma and can thereby cause artifactual elevations in the serum-free thyroxine concentration as measured by equilibrium dialysis. Thyroid 6:79–83
47. **Pemberton PA, Stein PE, Pepys MB, Potter JM, Carrell R** 1988 Hormone binding globulins undergo serpin conformational change in inflammation. Nature 336:257–258
48. **Adeyemi E, Abdulle AS** 1998 Comparison of maternal and cord blood polymorphonuclear leukocyte elastase levels. J Perinat Med 26:89–93
49. **Khan NS, Schussler GC, Holden JB** 2002 Thyroxine-binding globulin cleavage in cord blood. J Clin Endocrinol Metab 87:3321–3323
50. **Fisher DA, Nelson JC, Carlton EI, Wilcox RB** 2000 Maturation of human hypothalamic-pituitary-thyroid function and control. Thyroid 10:229–234
51. **Kester MHA, Kaptein E, Roest T J, van Dijk CH, Tibboel D, Meinl W, Glatt H, Coughtrie MWH, Visser T J** 1999 Characterization of human iodothyronine sulfotransferases. J Clin Endocrinol Metab 84:1357–1364
52. **Kester MHA, van Dijk CH, Tibboel D, Hood AM, Rose NJM, Meinl W, Pabel U, Glatt H, Falany CN, Coughtrie MWH, Visser TJ** 1999 Sulfation of thyroid hormone by estrogen sulfotransferase. J Clin Endocrinol Metab 84:2577–2580
53. **Visser TJ** 1996 Role of sulfate in thyroid hormone sulfation. Eur J Endocrinol 134:12–14
54. **Richard K, Hume R, Kaptein E, Sanders JP, de Herder WW, den Hollander JC, Visser T** 1998 Ontogeny of type I and type III iodothyronine deiodinases in human liver. J Clin Endocrinol Metab 83:2868–2874

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