

Neonatal Hypothyroxinemia: Effects of Iodine Intake and Premature Birth*

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ABSTRACT

We have investigated the effects of iodine (I) intake on urinary I excretion in preterm (PT) babies up to 2 months after birth and its effect on serum T_4 , free T_4 (FT_4), T_3 , TSH, and thyroglobulin (Tg) levels compared to those in term (T) newborns.

Very premature and very sick infants were in negative I balance for the first weeks after birth. Later, these same infants, as well as the other PT and T newborns, were in positive balance; 75–80% of the ingested I was not accounted for in the urine. The urinary I levels of PT and T neonates cannot be equated to their I intakes.

T_4 , FT_4 , and T_3 levels in PT and T neonates increased with post-

menstrual age, whereas Tg decreased and TSH did not change. Serum FT_4 , T_3 , Tg, and TSH levels in PT neonates were affected negatively, independently from age, by a low I intake. PT birth also affected T_4 , FT_4 , and Tg negatively, independently from I intake and postmenstrual age, for at least 6–8 weeks after birth.

Care should be taken to avoid I deficiency in PT neonates. However, even when I intake is adequate, PT newborns are hypothyroxinemic compared to T babies during an important period of brain development. This suggests the possible convenience of interventions that might mimic the intrauterine hormone environment and accelerate maturation. (*J Clin Endocrinol Metab* 82: 1704–1712, 1997)

DURING THE first half of gestation, thyroid hormone available to the fetus is predominantly of maternal origin (1). Once fetal thyroid secretion starts, fetal supplies are of mixed fetal and maternal origin. Although fetal thyroidal secretion is believed to constitute an increasing proportion of the hormone available to the developing fetus, maternal transfer of T_4 may still contribute significantly to fetal needs (20–50% of normal values) up until term (2), mitigating the consequences of an inadequate fetal thyroid function (3). Although the capacity to synthesize thyroglobulin (Tg), to concentrate iodine (I), and to synthesize T_4 and T_3 may start earlier, secretion of iodinated hormones by the fetal thyroidal is believed to start at midgestation, when hypothalamic-pituitary circulation matures, and TSH is secreted into the fetal blood (4, 5). As shown in fetal serum obtained *in utero* by cordocentesis (6, 7), T_4 and free T_4 (FT_4) do not clearly increase until 20–24 weeks gestation, and free T_3 (FT_3) is mostly undetectable until after 32 weeks. Thus, the earlier in pregnancy, the more immature the fetal gland and the more important the proportion of fetal requirements that are met by the maternal contribution of hormone. Fetal thyroidal secretion has barely started in

premature neonates of 24 weeks gestational age (GA) and has been contributing to fetal requirements for only a few weeks in those born at 27 weeks GA, compared to 3 months in term (T) babies.

Preterm (PT) babies have low circulating levels of T_4 and FT_4 (8); the degree of hypothyroxinemia is greater in the very premature neonates (9). For years their alterations in circulating parameters of thyroid function have been considered an expression of their physiological hypophyseal-pituitary-thyroid immaturity or a manifestation of nonthyroidal illness, but 2 recent follow-up studies of 640 PT children up to 5 and 9 yr of age (10) and of 400 premature infants up to 2 yr of age (11) have confirmed that severe hypothyroxinemia plays an important causative role in their frequently impaired mental development and disabling cerebral palsy.

Worldwide, the most frequent cause of hypothyroxinemia is I deficiency, which is at present recognized as the major cause of preventable mental retardation throughout the world and a major cause of disabling cerebral palsy. The severity of the damage is not only related to the degree of the deficiency, but also to the developmental phase during which it is suffered, the most severe being the consequence of I deficiency during the first two trimesters of pregnancy (12, 13). The I intake of the mother ought to ensure that her thyroid synthesizes enough hormone for her needs and those of the developing fetus as well as an adequate supply of I for fetal and neonatal thyroid function, both *in utero* and during lactation. An inadequate I supply might be especially dangerous in the case of PT babies, who have been prematurely deprived not only of the maternal supply of hormone, but

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also of I, before their own glands have been able to accumulate as much I as in T newborns.

This lead us to investigate the I content of formula preparations used for premature infants and for T neonates (14). The present study investigates the role of I intake on the thyroid function of PT infants. It reports the relationship between I intake and urinary I excretion in PT neonates at different postmenstrual ages (PMA)¹ and compares the results with those of T neonates. It also investigates whether their circulating thyroid hormone levels would reach the same values as those of T neonates once their I intakes become comparable. As will be seen, although a higher I intake results in an amelioration of several circulating parameters of thyroid function, a certain degree of hypothyroxinemia persists for months, even if I intake is comparable to that of newborn neonates.

Subjects and Methods

Patients

As part of a study on thyroid function of premature babies, we determined the I intake and I excretion of 115 premature and 28 healthy T infants born in the Neonatology Unit of the Hospital La Paz (Madrid, Spain). The premature infants, born between 27–36 weeks GA (body weights ranging from 900–4200 g), were studied during the first week of life and every 15 days for up to 2–4 months after birth. The T infants, born between 37–42 weeks GA, were followed for a shorter period, up to 1–1.5 months. During the study period I-containing topical antiseptics were banned from the Neonatology Unit. Infants exposed to external sources of I as a result of radiological or surgical examinations were excluded from the study as were those receiving parenteral feeding, because an I-containing contrast medium had been used to position the catheters (15). The study was approved by the ethics committee of the Hospital La Paz.

There were 15 PT infants of 27–30 weeks GA, but only the data obtained for 10 of them before the onset of parenteral feeding are included in the present study as well as all data from the 5 infants who were always fed orally. Of the 31–36 weeks GA PT babies included in the study, 52 were healthy; there were 9 small for GA (SGA) infants, and 35 were considered sick PTs with different pathologies, mostly hyaline membrane disease, transitory distress syndrome, severe infection, cardiovascular abnormalities, anemia, persistent ductus arteriosus, intracranial hemorrhage, and perinatal hypoxia.

On each occasion, samples of the different formulas or maternal milk given to each baby were kept for the direct determination of I content, and the ingested volume over a 24-h period was recorded in each case, as described previously in more detail (14). The 24-h urine was collected at each study period for the determination of I and creatinine concentrations. Venous blood samples were withdrawn for the determination of TSH, total T₄, FT₄, T₃, and Tg. When the blood volume obtained was too small for all of the determinations, only FT₄, T₃, and Tg were measured.

I determinations

The concentration of I in formula and milk samples was determined by a modification of the method described by Benotti and Benotti (16) for serum, with duplicate 40- μ L aliquots of each preparation being digested in 2 mL chloric acid. The inter- and intraassay coefficients of variation were $14.7 \pm 6.7\%$ (\pm sd) and $12.5 \pm 1.8\%$, respectively. The concentration of I in the urine was determined using 0.5-mL aliquots and 3 mL chloric acid. This method determines the total I content of milk and urine regardless of its initial chemical form.

Thyroid function tests

Serum TSH (Dynotest TSH from Henning Berlin, Berlin, Germany), T₄ and T₃ (RIA kit from Clinical Assays, Baxter, Cambridge, MA), and

FT₄ (Two Step Gammacoat Free T₄ RIA kit from Clinical Assays) concentrations were measured in duplicate by specific RIAs. Tg was measured by immunoradiometric assay (Dynotest Tg from Henning Berlin).

Statistical analysis

Mean values (\pm SEM) are shown. Data were subjected to one-way ANOVA after testing for homogeneity of variance. The significance of the difference between groups was identified by the Newman-Keuls test for multiple comparisons. All calculations were performed as described by Snedecor and Cochran (17). Multiple regression and partial correlation analyses were performed using the SPSS program (SPSS Inc., Chicago, IL).

Whenever it is stated that a certain variable was higher or lower for different groups or that correlations existed between the variables, it is implicit that the differences or correlation coefficients were statistically significant, at $P < 0.05$ or less.

Results

I intake

The I intake is calculated from the I concentration of the formula or maternal milk, which we have previously reported in detail (14), and the volume ingested.

The volume of milk ingested during a 24-h period by all the PT and T babies at different PMA, corrected for differences in body weight, is shown in Fig. 1. The very small premature babies did not ingest the 150–200 mL/kg BW-day recommended for T babies (18) until they were approximately 1 month old and weighed 2 kg or more.

Figure 2 shows the I intake of all PT and T infants at different PMA. As a consequence of the small volume of milk ingested, the I intake of the 27–30 weeks GA infants and that of all sick and SGA infants were lower during the first week of life ($7.5 \pm 1.8 \mu\text{g I/day}$) than that of the healthy PT babies ($16.5 \pm 1.6 \mu\text{g I/day}$). The corresponding values at 1 month of age were, respectively, $13.1 \pm 2.2 \mu\text{g I/day}$ (27–30 weeks GA and sick neonates), $19.3 \pm 2.5 \mu\text{g I/day}$ (SGA neonates), and $25.1 \pm 2.8 \mu\text{g I/day}$ (T neonates). The differences disappeared at later ages.

I excretion into urine

Urinary I excretion was calculated from the volume and the I concentration of the 24-h urine sample. We obtained data for urinary I concentration in 182 samples, but reliable 24-h urine collection was not always successful. This reduced

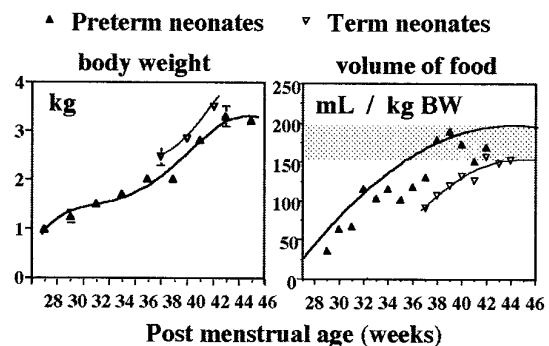


FIG. 1. The left panel shows the mean (\pm SEM) body weights of PT and T neonates in the present study at different PMA. The right panel shows the volumes of daily ingested formula or milk in reference to body weight. The shaded area corresponds to the volume of 150–200 mL/kg BW usually recommended as normal for neonates (18).

¹ PMA is the GA plus the postnatal age, in weeks.

the daily I excretion data to 86 values, from 69 PT and 28 T infants. The volume of urine was correlated to both the body weight of the PT baby and the PMA, and the data were fitted ($P < 0.02$) by the following equation: volume of urine (mL/day) = $162 - 3.6 \times \text{PMA} + 29.7 \times \text{BW}$, where the PMA is given in weeks and the body weight in kilograms.

The mean volume of urine was 46.7 ± 2.4 mL/kg BW·day for PT neonates and 33.5 ± 4.6 mL/kg BW·day for T infants. These values are within the normal ranges given by others for both PT and T infants (18). The volume of urine (in milliliters per kg BW) decreased with PMA in the PT infants, whereas no age-related change was observed in T neonates.

The pattern of 24-h urinary I excretion is shown for PT and T babies in Fig. 2. During the first weeks of life (see the *inset* of Fig. 2), I excretion was higher in the majority of the 27–30 weeks GA babies than in the same babies at later postnatal ages. On the contrary, I excretion in PT infants of 31–36 weeks GA increased with PMA and, to a lesser degree, with I intake. When the results were grouped according to PMA and/or the presence of illness, it was observed that the 27–33 weeks PMA sick infants excreted approximately 1.5 times the amount of I into the urine as healthy T babies despite their lower I intake.

I excretion vs. intake

Figure 2 compares the urinary I excretion with the intake for both PT and T infants. Initially (Fig. 2, *inset*), the mean I excretion was usually higher than the intake, but starting at about 31–32 weeks PMA, the mean urinary I excretion was lower than the intake. The differences increased markedly with age.

When urinary I excretion was plotted against the daily intake (not shown), it was observed that the majority of PT babies excreted much less I than they ingested. The 24-h I intake and urinary excretion were positively correlated, but with a regression coefficient ($n = 74$; $r = 0.35$) that, although

statistically significant, was relatively poor for estimation of I intake from the 24-h urinary excretion data. When the more numerous data of urinary I concentration ($n = 182$) were considered instead of the 24-h excretion, and PMA and body weight were also taken into account, the data fitted the following equation ($P < 0.001$): I intake ($\mu\text{g}/\text{day}$) = $-74.9 + 2.02 \times \text{PMA} + 8.2 \times \text{BW} + 63.7 \times \text{urinary I}$ ($\mu\text{g}/\text{mL}$).

To test how far the I intakes might be assessed from the above equation when the 24-h collection was incomplete and only the urinary I concentration was known, we performed a paired *t* test comparing the actual measured intakes (21.1 ± 1.7 μg I/day) with the calculated ones (21.1 ± 1.1 μg I/day); no difference was found. When this equation was applied to the T newborns, up to 1.5 months of age there was no difference between the measured intakes (38.0 ± 4.2 $\mu\text{g}/\text{day}$) and the calculated intake values (37.8 ± 2.2 $\mu\text{g}/\text{day}$), suggesting that during this neonatal period the above equation might also be used to estimate the I intake. This equation should be considered as merely tentative, as we do not know whether it would be valid for PT infants receiving a much higher or lower I intake than those reported in the present study, in which the maximum I intake was 90 $\mu\text{g}/\text{day}$.

Urinary creatinine

The mean urinary creatinine concentration of PT infants was lower (7.2 ± 0.4 mg/dL) than that of T newborns (13.0 ± 3.6 mg/dL; $P < 0.001$). No reliable correlation was found between the urinary creatinine concentration and PMA, body weight, or body length in either PT or T babies. When the creatinine concentrations were used to calculate the I to creatinine ratios, the previous correlations between urinary I concentration and I intake, PMA, and body weight were not improved, but were actually weakened.

I balance

The daily I balance was calculated as the difference between the I intake and the I excretion. As evident from the data shown in Fig. 2, the I balance of very premature infants appeared to be negative, whereas at about 31–32 weeks of PMA and later, the I balance was clearly positive. For this

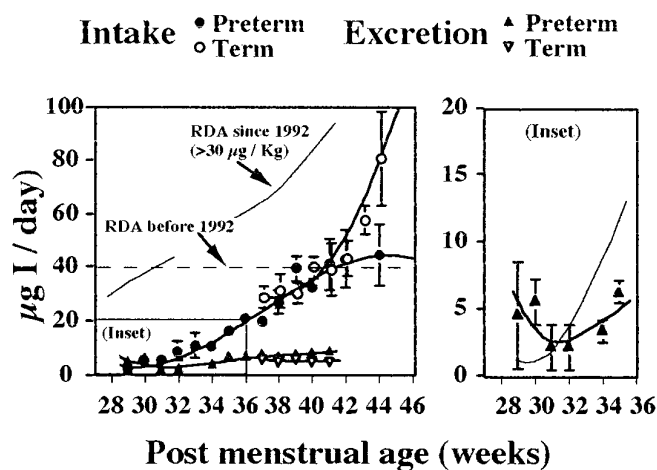


FIG. 2. The mean (\pm SEM) daily I intake and excretion of PT and T neonates are shown as a function of PMA. The dashed horizontal line indicates the recommended daily allowance defined before 1992; the more recent recommendation (19) of more than $30 \mu\text{g}/\text{kg}\cdot\text{day}$ is also shown as a thin curve. The *inset* amplifies the urinary I excretion data between 29–35 weeks PMA. The corresponding I intake is shown as a thin curve.

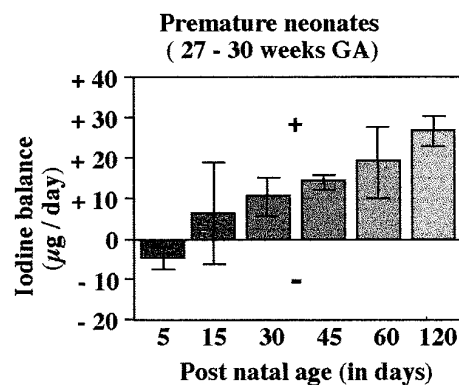


FIG. 3. Mean (\pm SEM) I balance of 27–30 weeks GA neonates at different postnatal ages. The mean I balance was negative (–) in practically all neonates for the first week after birth, but became positive (+) in practically all of them by 1 month of age.

TABLE 1. Percentage of preterm and term neonates in negative balance

Postnatal age (days)	Group by GA (weeks)					Term
	27–30	31–33		34–36		
		Sick	Healthy	Sick ^a	Healthy	
5	75	28	11	21	11	7
15	33	14	0	0	0	0
30	0	0	0	0	0	0

Percentages were calculated using as 100% the number of infants in each group with complete 24-h urine excretion data.

^a The SGA infants are included in this subgroup.

reason, the results of these different groups are described separately.

Negative I balance

As indicated above, the very immature, 27–30 weeks PMA PTs showed a different pattern of I balance than the 31–36 weeks PMA healthy PTs; most of the former group were in negative balance during the first week of life. This negative balance was greater the more immature the PT baby. As illustrated in Fig. 3, this condition was only transient, with the balance changing from negative to positive with increasing PMA.

Many of the 31–36 weeks GA PT infants who were sick and many of the SGA neonates also excreted more I into the urine for the first weeks of life than healthy babies of the same PMA, as summarized in Table 1, despite the fact that their I intake was not higher.

When the data from all 17 PT babies in negative balance were considered as a whole, it was found that a negative balance was more frequent for the first days of postnatal life (9.0 ± 1.6 days) when their mean GA was 32.5 ± 0.7 weeks and their mean weight was less than 2.0 kg (1.9 ± 0.2 kg). The negative I balance of these babies decreased with their PMA and body weight. The urinary I excretion of the PT neonates who were in negative balance increased with their I intake. This finding suggests that the premature infants in negative balance are unable to retain all the I they are ingesting, and that an increased intake would not correct their negative I balance.

When the volumes of urine of all PTs in negative balance were compared to those of PMA-matched PT babies in positive I balance, it was observed that the volume of urine excreted by the former (102 ± 8 mL/day) was higher than that in the PT neonates in positive balance (73 ± 9 mL/day). The negative I balance of these PT babies was not related to corticosteroid treatment of the mothers at risk of premature delivery.

Positive I balance

Most of the 31–36 weeks GA healthy PT infants and T babies were in positive I balance (Table 1), which increased with I intake and, to a lesser degree, with PMA. As I intake was also correlated to PMA, multiple regression analysis was performed to assess the relative contributions of both variables to I balance. I intake proved to be the major factor influencing I balance regardless of the stage of maturation, as measured by PMA.

The urinary I excretion was positively correlated to the I

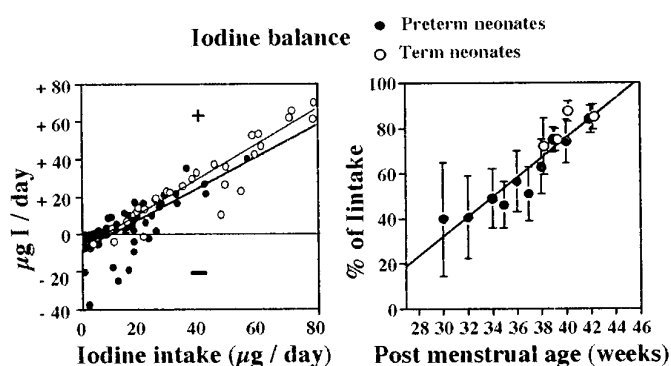


FIG. 4. *Left panel*, Individual values for I balance in both PT and T neonates are plotted against their I intakes. When, regardless of PMA and body weight, the I intake was the same, there was no statistically significant difference between the I balances of PT and T neonates. *Right panel*, The positive I balance after 30 weeks PMA is expressed as a percentage (mean \pm SEM) of the I intake and shown as a function of PMA for both PT and T neonates.

intake, as indicated for immature infants in negative balance, but the slope of the linear function was much lower than that obtained for premature infants in negative balance.

Comparisons between PT and T babies

I balance. Figure 2 not only shows the I intake and urinary I excretion of PT babies but also those of T infants during their first 6 weeks after birth. To correct for differences in PMA between the PT and T neonates, I balance was plotted against I intake (Fig. 4, *left panel*). No differences were found between the linear regressions for PT vs. T infants. This indicates that when I intake is the same, the amount of I that is retained by the PT infant is the same as that retained by T infants. The *right panel* of Fig. 4 shows the positive I balances of the premature and full-term neonates as a percentage of their I intakes and as a function of PMA. As may be seen, the capacity to retain the ingested I was not constant, but increased with PMA. There was also a great individual variability around the mean value for a given PMA in the capacity to retain the ingested I, which was greater the younger the neonate.

Parameters of thyroid function. As evident from the *upper panels* of Fig. 5, the age-related changes in circulating parameters of thyroid function were different in PT and T infants. The circulating T₃, T₄, and FT₄ levels in the PT infants increased, whereas Tg levels decreased, as functions of PMA. On the contrary, in the T neonates described here we found that circulating T₃ hardly changed with increasing PMA, whereas

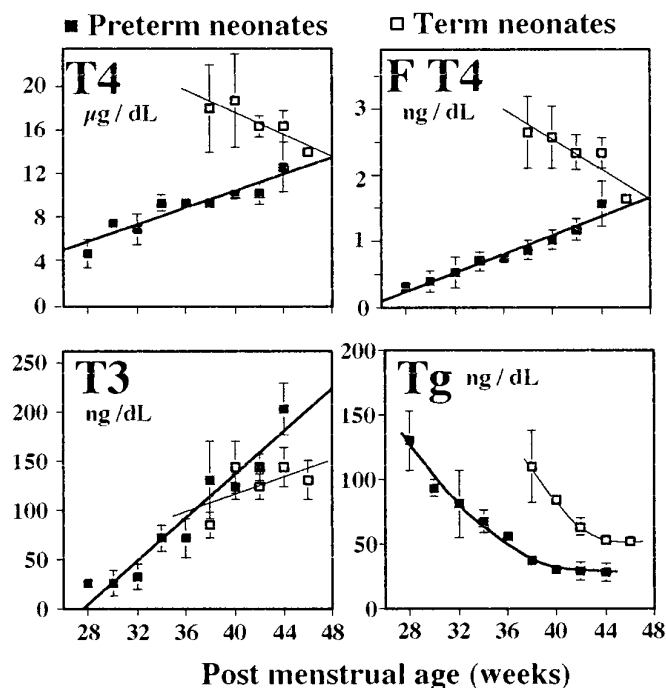


FIG. 5. Mean (\pm SEM) circulating concentrations of T_4 , FT_4 , T_3 , and Tg are plotted against PMA for both PT and T neonates.

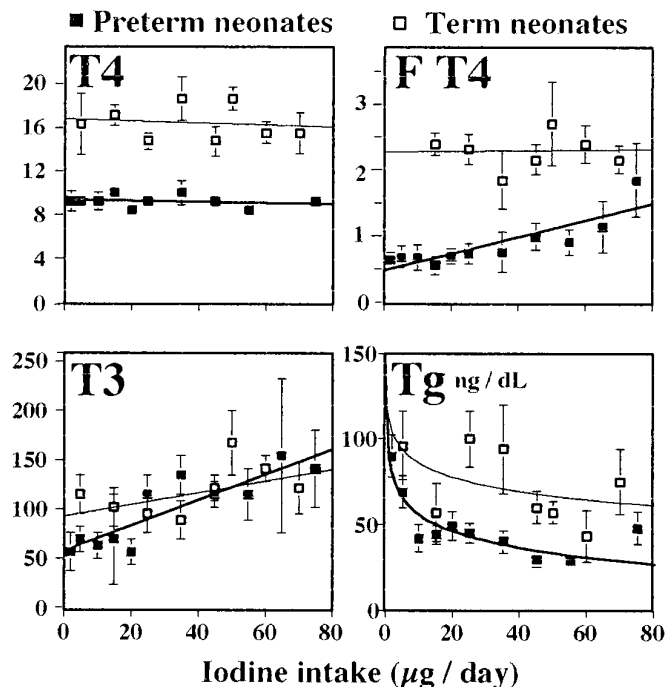


FIG. 6. Mean (\pm SEM) circulating concentrations of T_4 , FT_4 , T_3 , and Tg are plotted against the daily I intake for both PT and T neonates.

T_4 , FT_4 , and Tg decreased. Serum TSH (not shown) decreased during the first week after birth in both PT and T neonates, but did not later correlate with age.

Figure 6 shows the possible relationship between circulating parameters of thyroid function and I intake. T_4 was not related to I intake in either PT or T infants. TSH (not shown),

FT_4 , and T_3 were correlated with I intake in PT, but not T, neonates. Circulating Tg decreased with I intake in both groups. Results were quite similar when these variables were plotted against the calculated I balance.

It, therefore, appeared of interest to assess whether circulating parameters of thyroid function in premature neonates would be comparable to those in T infants when their I intakes (and balance) were the same.

As I intake was also affected by PMA (Fig. 2), it appeared important to exclude the influence of PMA on the relationships illustrated in Fig. 6. Partial correlation analysis was performed using only the data from those cases without any missing variable; all cases where one or more variables were missing were omitted from the study. This avoided uneven weights of variables due to differences in the number of data. We also omitted the data from very sick infants to avoid hormone alterations that might be a consequence of the neonatal pathology. This stringency reduced the study to 90 cases. As summarized in Table 2, this partial correlation analysis indicated that some of the circulating parameters of thyroid function were correlated to I intake independently from PMA; results were different for PT and T neonates (Table 2). In PT babies, the circulating levels of T_3 , FT_4 , Tg, and TSH were indeed related to I intake, independently of PMA. On the contrary, the only circulating parameter of thyroid function that appeared to be related to the I intake in T babies was the serum concentration of Tg, which decreased with increasing intake.

Figure 6 also shows that differences persisted between PT and T infants in the circulating levels of T_4 , FT_4 , and Tg, even when I intakes were the same. To evaluate the influence of PT vs. T birth, a multiple regression analysis was performed. The variables were I intake and circulating T_4 , FT_4 , T_3 , Tg, and TSH; PT or T birth was the qualitative condition separating the data into two groups. When PMA was also entered among the variables, the conclusions were comparable. The results are summarized in Table 3 and show that being born prematurely is the main factor responsible for the lower circulating levels of T_4 , FT_4 , and Tg and persists even when I intakes are comparable.

This does not mean that parameters of thyroid function are not ameliorated by increasing the I intake of PT babies, as their circulating T_3 , FT_4 , Tg, and TSH levels are improved with higher I intakes independently of PMA, as apparent from Table 2 and in agreement with our previous report (14) in which I intake of PT neonates above or below 40 μ g I/day was used as the qualitative condition separating the data for covariance analysis.

Comparisons between premature babies and fetuses in utero. We have compared the present findings to those obtained *in utero* by cordocentesis (7). The result for FT_4 is shown in Fig. 7. As may be seen, the FT_4 of premature infants was clearly below the 95% confidence interval of the values given for fetuses of the same age *in utero*. It is unlikely that this is related to methodological differences between the two different laboratories, as the FT_4 levels of the T neonates in the present study were not lower than those reported by Thorpe-Beeston *et al.* (7). Although not shown here, we also found that the circulating TSH levels of both the PT and T neonates were

TABLE 2. Partial correlation analysis: regression coefficients (r) and significance (P)

	T ₃	T ₄	Free T ₄	Tg	TSH
Preterm babies					
Postmenstrual age vs.	r = 0.28 P < 0.01	r = 0.17 P < 0.05	r = 0.8 P < 0.01	r = -0.96 P < 0.001	NS
Iodine intake vs.	r = 0.3 P < 0.001	NS ^a	r = 0.8 P < 0.001	r = -0.3 P < 0.001	r = -0.3 P < 0.05
Term babies					
Postmenstrual age vs.	r = 0.58 P < 0.05	r = -0.9 P < 0.01	r = -0.75 P < 0.01	r = -0.87 P < 0.01	NS
Iodine intake vs.	NS	NS	NS	r = -0.34 P < 0.05	NS

^a NS = not statistically significant.

TABLE 3. Multiple linear regression analysis: effect of premature or term birth on circulating T₄, FT₄, and Tg concentrations

Variable	Effect of prematurity	95% Confidence interval	P
T ₄	80.1 nmol/L lower	60–100	<0.001
FT ₄	20.9 nmol/L lower	18–26	<0.001
Tg ^a	46.0 ng/dL lower	28–64	<0.001

^a Linearity was obtained after logarithmic transformation.

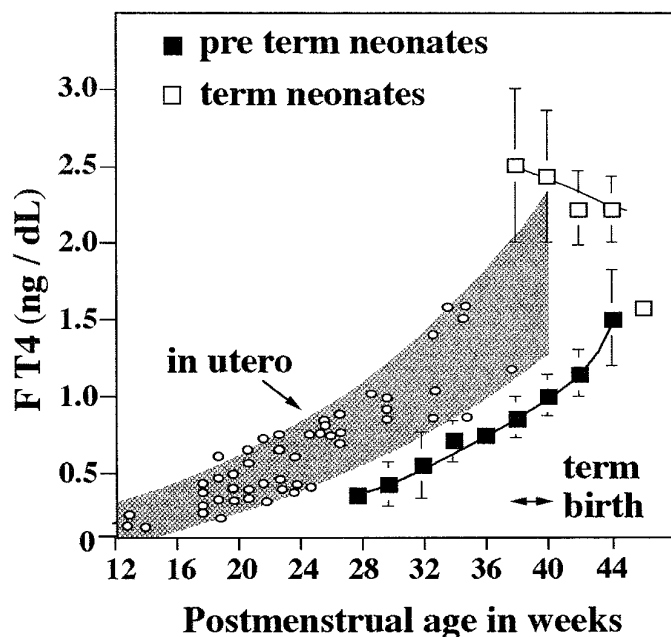


FIG. 7. The mean (\pm SEM) concentrations of FT₄ corresponding to PT and T neonates in Fig. 5 as a function of PMA are superimposed on data published for fetuses *in utero* by Thorpe-Beeston *et al.* (7). The shaded area corresponds to the 95% confidence intervals for the fetal FT₄ data.

below the confidence intervals for the fetal TSH levels, which are clearly higher than those found in T neonates, their mothers, and nonpregnant adults.

Discussion

I intake

In a previous study (14) we determined the I content of breast milk, 32 formulas from different brands used in Spain, and 127 formulas from 14 other countries. Breast milk contained more I ($10 \pm 1 \mu\text{g I/dL}$) than the majority of formulas,

especially those for premature infants. The I content ranged from 1.5–11 $\mu\text{g I/dL}$ for the formulas used in Spain and from 2–17 $\mu\text{g I/dL}$ for those used in the other countries.

Taking into account the amount of formula and/or breast milk actually ingested by the premature babies, their daily I intake was far below the recommended daily allowance of more than 30 $\mu\text{g I/kg}$ accepted since 1992 (19) and hardly reached the previous recommendations of 40 $\mu\text{g I/day}$. The I intake of the T infants was also clearly lower than the more recently recommended allowance; only a few babies reached a value of 30 $\mu\text{g I/kg}$ when they were 4–6 weeks old. Present I intakes are similar to those described for 29 4-week-old PT babies born in Belgium (20, 21) and for T neonates born in France (22).

I excretion and I balance

The amounts of I excreted by the full-term babies of the present study are similar to those reported by Etlin *et al.* (22). In the present PT neonates, I excretion changed with PMA, initially decreasing between 27–30 weeks PMA and then increasing steadily. PT and full-term infants of similar PMA excreted the same amounts of I.

Delange *et al.* (20, 21) reported that 40% of 29 healthy PT babies studied at 34 weeks PMA were in negative I balance. A negative balance was also observed in PT babies of the present study, but was mostly limited to very immature, sick, or SGA infants. The mean weight of the babies studied by the above researchers was $1.75 \pm 0.37 \text{ kg}$, and most of them appeared to have been born at less than 30 weeks GA. Thus, our present findings would confirm this report for such neonates, but not for most of the less immature and healthy PT neonates. Moreover, the present results show that the negative I balance is transient and becomes positive as the PT neonate matures and/or illness is controlled.

Although in most premature neonates both I intake and I excretion increased with PMA, they did so at different rates; the proportion of the ingested I that was not excreted into the urine was not a constant fraction of the intake, with a high degree of individual variability, especially in the younger PT neonates, and increased with age. Considering that excretion of I into the feces represents a minor proportion of excreted I in both PT and T infants (20, 21), we have assumed that the positive I balance represents the amount of I that is retained by the neonate. The present results indicate that this capacity appears to be the more limited and variable the younger the

PT neonate, but that it becomes the same as that in T infants of comparable I intake and PMA.

The facts that PT and T babies were either in negative or clearly positive I balance and that the proportion of ingested I that is retained is not constant clearly show that their urinary I excretion cannot be equated to their I intake, as usually done in field studies of I nutrition in schoolchildren and adults.

It is very likely that most of the ingested I that is retained (not excreted into urine or feces) is being taken up by the neonate's thyroid gland. It is known that during fetal life the amount of I accumulating in the gland increases steadily as a result of a marked increase in both the weight of the gland and the I uptake (23, 24). Thus, a positive I balance would be expected during fetal and early postnatal life and would continue thereafter, as adult glands contain about 10–20 mg I/gland compared to less than 100 μg at T. We do not know at which age the thyroidal I content stabilizes, and intake and excretion become comparable, so that intake may be assessed from urinary I.

It has been suggested (22) that one of the factors leading to the negative I balance of PT babies is the immaturity of their kidneys. Renal immaturity alone, however, does not offer a convincing explanation; unlike other electrolytes, tubular reabsorption of iodide is negligible, and glomerular filtration depends entirely on its circulating concentration (25). Other factors are likely to be involved. Mobilization of previously accumulated I (or thyroid hormone) pools is likely to occur, considering that the loss of I into the urine is greater than its intake. In full term infants neonatal requirements for T_4 are quite high: the substitution dose for congenitally hypothyroid neonates is 8–10 $\mu\text{g } T_4/\text{kg BW}\cdot\text{day}$ compared to approximately 2 $\mu\text{g}/\text{kg BW}\cdot\text{day}$ for hypothyroid adults. The dose chosen for T_4 treatment of premature babies without congenital hypothyroidism to correct for their hypothyroxinemia is 8 $\mu\text{g } T_4/\text{kg BW}\cdot\text{day}$ (26). Deiodination of T_4 would represent the release of 12 $\mu\text{g I}/\text{day}$ for a PT neonate weighing 2 kg, an amount clearly higher than the I intake of the very immature neonates. Therefore, the negative balance would indicate that the neonates are meeting their hormonal needs by depleting their prenatal intrathyroidal stores. Moreover, the net loss of I into the urine was greater in PTs receiving the higher I intakes, and this suggests that the capacity of the thyroid to accumulate iodide and synthesize T_4 is limited, as indicated above.

Parameters of thyroid hormone function

As prematurity is not a normal physiological situation, circulating levels of T_4 , FT_4 , T_3 , and TSH are usually compared to those of healthy T newborns. Our results are in general good agreement with those reported in other studies for both PT and T infants. T_4 , FT_4 , and T_3 levels are lower in PT neonates compared to T newborns, increasing progressively with PMA, whereas TSH levels are the same (27–31). To our knowledge, the possibly independent effects of I intake *vs.* PT birth on these parameters of thyroid function have not been assessed by others. As indicated previously (14), I intake does affect the circulating levels of FT_4 , T_3 , Tg, and TSH in PT infants independently of their PMA. We also

show here that premature birth by itself affects T_4 , FT_4 , and Tg independently of I intake and PMA. Circulating levels are lower in PT than in T neonates of comparable PMA and I intake (or balance), at least up to 44 weeks PMA and an intake of 80 $\mu\text{g}/\text{day}$. These results also indicate that the thyroid of PT infants is unable to accumulate I or synthesize and/or secrete the same amounts of T_4 as that of T neonates despite equal I intake. Indeed, Van den Hove *et al.* (32) (personal communication) found that the Tg content and degree of Tg iodination in the thyroid of PT babies are low compared to those in T neonates, and the percentage of intrathyroidal I found as T_4 plus T_3 is also reduced until they reach a PMA corresponding to normal T. These findings might also explain the lower circulating Tg levels of the PT neonates despite the same degree of thyroid stimulation by comparable levels of circulating TSH.

Proposed interpretation of present results

Birth severs vascular connections between mother, placenta, and fetus, with sudden interruption of transplacental passage or placental production of nutrients and hormones. The persistent hypothyroxinemia of the PT neonates could be at least partly the result of the premature interruption of the maternal supply of I, thyroid hormone, and placental TRH occurring at a stage of development when the hypothalamic-pituitary-thyroid system is not fully mature, and the fetal thyroid is not ready to meet postnatal requirements.

The hypothesis underlying this proposal is that the prolongation of the maternal transfer of thyroid hormone during the second and third trimesters is a physiological event that meets part of the fetal thyroid hormone requirements, permitting the fetal thyroid to mature fully, so as to accumulate I and iodinated Tg, and to produce enough hormone to meet future needs once the maternal supply is interrupted. Premature deprivation of the maternal source of I and thyroid hormone would unduly strain the immature gland of the neonate, and depletion of previous thyroid stores would be a likely consequence. Experimental data obtained in rat fetuses deprived of the maternal supply of thyroid hormone support this possibility; the fetuses manage to meet their extrathyroidal hormone requirements, but do so at the expense of the intrathyroidal stores (33).

Tg, T_4 , and T_3 are clearly lower in the thyroids of premature infants who survived for a few days or weeks after birth at a mean GA of 35 weeks compared to those of younger aborted fetuses at a mean GA of 22 weeks (34). Such findings may be affected by the pathological process that lead to their death, but the FT_4 levels of the present PT infants, many of whom were healthy, were also clearly lower than those reported for fetuses *in utero* (7), in conceptual agreement with the above findings (34), and the hypothesis proposed here.

TSH is necessary for full maturation of the fetal thyroid, as shown by the atrophy of the gland in cyclocephaly, where the anterior and posterior lobes of the pituitary are absent (35). TSH is positively correlated with FT_4 in the fetus, independently of GA, and continues to increase up to term, even when adult FT_4 levels have been reached (7). TSH levels in the fetus are much higher than those in T neonates and adults. As evident when we compared the present results

with intrauterine TSH levels (7), the high fetal levels drop abruptly with premature birth, and the maturational effects of high serum TSH on the thyroid are thus lost. As shown by the relatively normal pituitary and thyroid development in anencephaly, where hypothalamus and posterior pituitary are absent (35), intrauterine TSH secretion is under the control of extrahypothalamic sources of TRH. High levels of TRH are found in amniotic fluid, when hypothalamic TRH is still low and the hypothalamic-pituitary portal system is not yet fully developed, and in newborns' cord blood (36, 37). An important source of TRH that is interrupted at birth is the placenta (38). The sudden decrease in TRH might also have effects other than a decrease in TSH secretion, as neuro-modulator functions have been attributed to extrahypothalamic TRH.

The persistent hypothyroxinemia of the premature neonate might also further delay attainment of full thyroid gland maturation by several mechanisms that are not mutually exclusive. In many species thyroid hormones are needed to attain normal concentrations of TRH in the median eminence (39), for differentiation of the preoptic nuclei and hypothalamic-hypophyseal tract (40), and for appearance of monoaminergic axon terminals and penetration of capillaries into the median eminence (41). Such effects of thyroid hormone on the hypothalamic-pituitary portal system might also influence functions other than TSH secretion, including regulation of GH and glucocorticosteroid economy, which would also be affected by premature birth.

Consequences of the proposed interpretation of the present results

The immediate obvious conclusion regarding interruption of the maternal supply of I is that care should be taken to provide the recommended minimum I intake, so that the external I supply would not be a rate-limiting factor for the synthesis of thyroid hormone once the gland is mature enough to concentrate iodide and incorporate it into thyroid hormone. To assess the I intake, the actual volume of food ingested should be taken into account, not only the I content of the formula preparation. The addition of supplements poses no risk, as there is a wide margin between the recommended I intake and a thyroid-blocking I overload.

Even if the I supply is adequate, it may not be sufficient to avoid the neonatal hypothyroxinemia of premature neonates. Mimicking intrauterine conditions by transient postnatal treatment with T₄ might improve some of the thyroid-related events, such as maturation of thyroidal T₄ and T₃ synthesis. Van den Hove *et al.* (personal communication) observed that treatment of the very premature (24–32 weeks GA) PT neonates for 3 days or more with 10 µg T₄/kg BW·day resulted in increases in the degree of iodination of Tg and the proportion of I found as T₄ and T₃, to values similar to those in T neonates.

Several groups have already performed trials to improve postnatal development of PT neonates with transient postnatal T₄ treatment, obtaining either inconclusive or conflicting results. A recent randomized double blind study involving 100 PT neonates of less than 30 weeks GA receiving 8 µg T₄/kg·day for 6 weeks and an equal number receiving pla-

cebo has shown that this treatment may improve the developmental outcome measured at 2 yr of age, although the latter beneficial effect is restricted to PT infants of less than 27 weeks GA (26).

As discussed above, postnatal substitution with T₄ would compensate for the sudden decrease in FT₄ of the premature neonate compared to the FT₄ level to which fetal tissues were exposed *in utero* and may well represent an improvement. However, it would not mimic the intrauterine environment in other aspects, such as the increased serum TRH and TSH levels, which might also contribute to maturation of the hypothalamic-pituitary-thyroid system and other systems involved in brain development. Obviously, more information is needed before such possibilities are considered for clinical trials.

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