3,3′-Diiodothyronine sulfate cross-reactive material (compound W) in human newborns

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BACKGROUND: Thyrosulfoconjugation appears to facilitate fetal-to-maternal transfer of 3,3′-diiodothyronine-sulfate (T_2S). Elevated maternal levels of T_2S cross-reactive material (compound W) are found in humans, with higher levels found in venous cord blood than in arterial samples. These findings are consistent with the postulate that the placenta plays an essential role in compound W production.

METHODS: Serum compound W levels were measured by a T_2S-specific radioimmunoassay in 60 serum samples from newborns with hyperbilirubinemia, age 1–30 d. In addition, 59 maternal serum samples, from day 1 to day 7 after uneventful deliveries, were studied.

RESULTS: As compared with day 1, at day 5, the mean (±SE) compound W level fell to 43.5 ± 6.8% (decay half-life ($t_{1/2}$) = 4.12 d) and to 33.7 ± 4.6% (decay $t_{1/2}$ = 2.82 d) in the newborn and maternal groups, respectively. In the mothers, the level continued to decline along the same slope through day 7. In the newborns, however, the mean compound W level entered a slower phase of decay after the fifth day with a decay $t_{1/2}$ = 10.9 d.

CONCLUSION: Compound W is cleared at similar rates in newborn and postpartum maternal sera. This is consistent with the postulate that compound W is produced in the placenta.

Iodothyronine sulfoconjugation is one of the major pathways of thyroid hormone metabolism in developing mammals (1,2). High serum concentrations of sulfated iodothyronine analogs have been shown in ovine and human fetal and newborn infant sera. These include thyroxine sulfate (T_4S), 3,3′,5-triiodothyronine sulfate (T_3S), reverse 3,3′,5′-triiodothyronine sulfate (rT_3S), and 3,3′-diiodothyronine sulfate (T_2S). A kinetic study using the steady-state constant infusion method in sheep showed that the major pathways of thyroid hormone metabolism in the fetus convert T_4 to the inactive metabolites rT_3, T_4S, rT_3S, and T_3S via sulfotransferase and type 3 deiodinase enzyme systems in late gestation (2,3). In sheep, thyrosulfoconjugation appears to facilitate fetal-to-maternal transfer of 3,3′-T_2S, a metabolite from monodeiodination of both rT_3S and T_3S. In humans, a presumably similar process results in high maternal levels of T_2S cross-reactive material (compound W) that disappears rapidly from maternal circulation after delivery (4,5). The measurement of compound W in maternal serum or urine during pregnancy may reflect iodothyronine levels in the fetus and therefore be a marker of fetal thyroid function (5,6). Compound W is not authentic T_2S and, unlike T2S, is not hydrolyzed by hot acid. Hydrolyzable T_2S constitutes a minor fraction of total T_2S cross-reactivity in serum from mothers at term and in cord serum (4,5). A higher level of compound W has been found in venous cord blood than in arterial samples. These findings are consistent with the postulate that the placenta plays an essential role in compound W production and preferential transfer to the maternal compartment (ref. 4 and Figure 1). This study was conducted to answer the question: What are the relative rates of clearance of compound W from newborn and maternal postpartum sera?

RESULTS

The age distributions and percentage reduction of compound W levels of the 60 newborn samples were shown as solid lines in Figure 2. The percentage reduction of compound W levels in the newborns is presented as the average reduction on each postnatal day. The number of samples collected on each day was day 1, 10; day 2, 5; day 3, 5; day 4, 7; day 5, 4; days 7–10, 9; days 11–15, 8; days 18–22, 6; and days 24–32, 6. Fifty-nine postpartum maternal serum samples, from day 1 to day 7 after uneventful deliveries, were also studied. Maternal samples were collected as follows: day 1, 15; day 3, 22; day 5, 14; and day 7, 8.

As shown in Figure 2, at day 5 after delivery, the mean (±SE) compound W level was reduced to 43.5 ± 6.8% (decay half-life ($t_{1/2}$) = 4.12 d) and to 33.7 ± 4.6% (decay $t_{1/2}$ = 2.82 d) in newborn and maternal sera, respectively. In the postpartum mothers, the compound W level continued to decline along the same slope to 23.1 ± 2.2% at day 7. In the newborns, however, the mean compound W level declined

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Received 9 March 2012; accepted 8 June 2012; advance online publication 12 September 2012. doi:10.1038/pr.2012.116

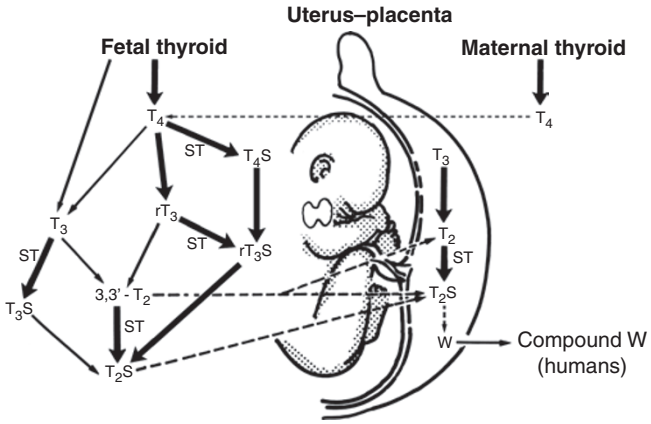

Figure 1. Postulated metabolic pathways for human fetal thyroid hormones. Heavy solid lines indicate pathways that are more active in fetuses than in adults, whereas thin solid lines show pathways that are less active in fetuses. The upper horizontal light dotted line depicts T_4 of maternal origin moving to the fetal compartment in the first trimester, before the fetal thyroid begins functioning. Other broken lines represent unconfirmed pathways. rT_3S, reverse 3,3′,5′-triiodothyronine sulfate; ST, iodothyronine sulfotransferases; T_2S, 3,3′-diiodothyronine sulfate; T_3S, 3,3′,5-triiodothyronine sulfate; T_4S, thyroxine sulfate.

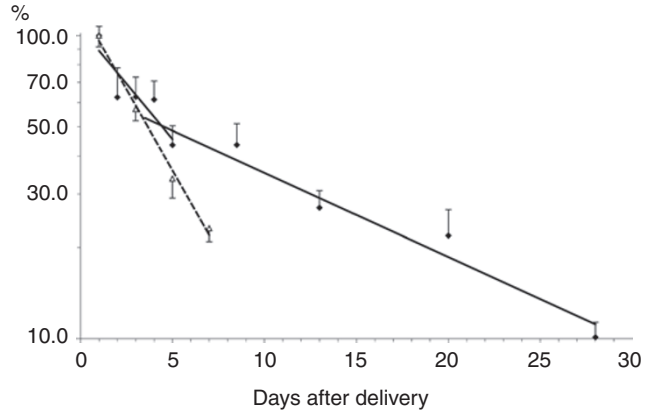

Figure 2. The percentage reduction of compound W levels in newborn and maternal groups in semi-log plot. Solid lines represent newborns; dashed line represents postpartum mothers. Vertical bars indicate 1 SE. The percentage reduction of compound W levels in the newborns is presented as the average reduction on each postnatal day. The number of samples collected on each day was day 1, 10; day 2, 5; day 3, 5; day 4, 7; day 5, 4; days 7–10, 9; days 11–15, 8; days 18–22, 6; and days 24–32, 6; total number of samples = 60. Maternal samples were collected as follows: day 1, 15; day 3, 22; day 5, 14; and day 7, 8; total number of samples = 59.

along a slower slope after the fifth day to a level of 10.1 ± 1.2% at 24–30 d ($t_{1/2}$ = 10.9 d).

As shown in Table 1, comparing liver function tests in newborns at days 2–5 (fast phase) and days 7–32 (slow phase), there are significant elevations of serum direct bilirubin and alkaline phosphatase levels in the slow phase. The total bilirubin was 257 ± 15 μmol/l at 2–5 d as compared with 293 ± 24 μmol/l at 7–32 d (not significant). The direct bilirubin was 3.5 ± 1.1 μmol/l at 2–5 d as compared with 9.3 ± 1.5 μmol/l at 7–32 d ($P < 0.01$). The alkaline phosphatase was 129 ± 8 IU/l at 2–5 d as compared with 213 ± 14 IU/l at 7–32 d ($P < 0.001$).

DISCUSSION

In humans, we have shown high levels of compound W in maternal serum (4,5) and urine (6). Levels increased with the progression of pregnancy and peaked before parturition. At delivery, a 20-fold increase in serum compound W was found as compared with levels in nonpregnant women, and levels returned to nonpregnant values by 7–10 d. The compound W in maternal serum did not co-chromatograph with synthetic T_2S by high-performance liquid chromatography (4) and, in contrast to authentic T_2S, was not hydrolyzed by hot-acid digestion. Using hot-acid digestion, the recovery of compound W was 77.1% in fetal and 87.4% in maternal serum (5).

To explore the possible origin of compound W, the serum concentrations of sulfated iodothyronines from cord arterial and venous samples were compared. There were no significant differences between the mean T_3S, T_4S, or rT_3S concentrations of arterial and venous serum samples. However, the mean compound W concentration in paired arterial/venous cord sera was significantly higher in venous than in arterial samples (5). In addition, the maternal serum concentrations were significantly lower than those of the paired cord serum. These findings support the postulate that compound W is produced in the placenta. The rapid disappearance from both maternal and newborn sera immediately after delivery in this study supports this hypothesis.

In the newborns, there was a slower phase of compound W decay after the fifth day through days 24–30 with a decay $t_{1/2}$ = 10.9 d. Possible liver dysfunction related to the elevated direct bilirubin and alkaline phosphatase (Table 1) may have played a role in the slower metabolism of compound W.

Prior studies suggest strongly that compound W is a metabolite of fetal thyroid hormone capable of transplacental fetal-to-maternal transfer (3–5,7). Both maternal and fetal compound W levels increased progressively during gestation with significant direct correlation (in both mothers and fetuses). In addition, in paired cord and maternal sera obtained at delivery, highly significant positive correlations were observed between respective compound W levels and fetal free thyroxine (FT_4) (5). A significant positive correlation was also observed between maternal and fetal compound W whereas no correlation was observed between maternal serum compound W and maternal serum FT_4 in euthyroid or hyperthyroid women. These data suggest that fetal FT_4 contributes significantly to placental production of compound W. Therefore, the concentration of compound W in maternal serum reflects fetal thyroid function. However, the presence of significant compound W levels in maternal serum during the first trimester contradicts this conclusion because fetal thyroid hormones are supplied by the mother only before the onset of hormonal synthesis in fetal thyroid gland at midgestation. Our current postulate is that compound W is produced in the placenta from substrate T_2S in the fetal circulation. In turn, the amount of T_2S is determined by the T_4 and T_3 levels, whether from mother or fetus, present in the fetal circulation.

Studies indicate that abnormalities in intelligence quotient and other neuropsychological tests may be found in children of women with subclinical hypothyroidism during pregnancy

Table 1. Comparison of liver function in newborns between days 2–5 and days 7–32

Metabolite or enzyme	W[a]	TB (μmol/l)	DB (μmol/l)	ALB (g/l)	ALT (IU/l)	AST (IU/l)	GGT (IU/l)	ALK (IU/l)
Reference range	—	1.7–20	0.5–1.0	35–55	4–50	4–50	2–30	30–114
Days 2–5								
(*n*)	(21)	(21)	(21)	(18)	(18)	(18)	(18)	(18)
Mean ± SE	58.6 ± 5.2	257 ± 15	3.5 ± 1.1	34.0 ± 0.4	11.2 ± 1.1	48.8 ± 9.9	127 ± 20	129 ± 8
Days 7–32								
(*n*)	(29)	(26)	(26)	(26)	(26)	(26)	(26)	(26)
Mean ± SE	27.6 ± 3.5	293 ± 24	9.3 ± 1.5	36.8 ± 0.5	16.9 ± 2.9	44.3 ± 4.6	152 ± 14	213 ± 14
	$P < 0.001$	NS	$P < 0.01$	$P < 0.002$	NS	NS	NS	$P < 0.001$

ALB, albumin; ALK, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; GGT, γ-glutamyl transpeptidase; NS, nonsignificant; TB, total bilirubin; W, compound W (3,3′-diiodothyronine sulfate cross-reactive material).

[a]Mean ± SE compound W value as a percentage of the mean of day 1 level.

(8–10). However, in a recent report (11), women who had severe hypothyroidism in early pregnancy, but who were restored to normal later in pregnancy, had offspring who developed normally. The authors postulate that iodine deficiency may be the factor that leads to abnormal development. In any case, we have no data on maternal levels of compound W in this group of mothers. In a prior study, Cortelazzi *et al.* (7) found no significant correlation between serum levels of maternal compound W and maternal FT_4. However, these serum samples were collected from pregnant women of different gestational ages. It is possible that any significant correlation between maternal FT_4 and maternal compound W levels before the onset of fetal function may be negated by fetal T_4 secretion in the later trimesters. Nevertheless, whether maternal hypothyroidism may result in lower compound W values in the first trimester is yet to be studied.

Maternal compound W levels also seem to reflect the effects of drugs on fetal thyroid function. In women on propylthiouracil, maternal serum compound W levels were in the low normal range and did not show the usual increase with progression of gestation (7). The lack of progression in maternal compound W levels was confirmed in a recent study of 22 pregnant women treated with antithyroid medication (12). A significant increase in maternal compound W was observed when the propylthiouracil dose was decreased or discontinued.

Cortelazzi, *et al.* (7) suggest that fetal hypothyroidism is predictable by the absence of the normal rise of compound W during gestation. Serial measurements of compound W in maternal serum can be considered a safe and practical test for the assessment of fetal thyroid function, particularly in hyperthyroid women treated with antithyroid drugs (12).

At present, there is no practical and noninvasive marker for fetal thyroid function available to specialists managing high-risk pregnancies. Amniotic fluid levels of T_3, T_4, and thyroid-stimulating hormone do not consistently reflect fetal plasma concentrations, and direct assessment via percutaneous umbilical cord blood sampling carries a 1% risk of fetal mortality (13). Serial ultrasonographic examinations are relatively cumbersome (14). The possibility that the transferred compound W may serve as a convenient and noninvasive marker of fetal thyroid function deserves further exploration.

In conclusion, our result, showing that T_2S cross-reactive material (compound W) is cleared from newborn and postpartum maternal sera at similar rates is consistent with the postulate that compound W is produced in the placenta. The rate of decay in the newborn may be affected by coexisting hyperbilirubinemia, which may partially explain the slower phase of decay in infants returning after 5 d of follow-up. Additional data on decay of compound W in normal healthy newborns would provide useful information, and such studies are planned.

METHODS

Serum compound W levels were measured by radioimmunoassay using a polyclonal antibody against T_2S. Labeled T_2 (^{125}I-3,3′-T_2) was prepared by radioiodination with ^{125}I (NEZ033L) from NEN-PerkinElmer (Boston, MA) using the method described previously (15,16). The anti-L-T_2S antibody (W0213) was obtained from rabbits immunized with a 3,3′-T_2S-bovine serum albumin conjugate (4). T_2 was purchased from Henning–Berlin (Berlin, Germany). The lower limit of detection of the assay was 3.3 fmol (2 pg), or 33.1 pmol/l in a 300 μl ethanol extract of serum. Intra-assay variations were 1.9–9.1% and interassay variations were 6.0–19.5%, depending on the measured concentrations.

Sixty serum samples, from newborns aged 1–30 d (38 males), were studied. These are banked sera collected for clinical management of neonatal hyperbilirubinemia (total bilirubin is 95–364 μmol/l; normal range, 1.7–20 μmol/l). The gestational age of the newborns averaged 38.7 wk (range 36.0–41.9). No neonatal hypothyroidism (thyroid-stimulating hormone >20 mU/l) was found in this group of newborns (17). Samples from newborns with gestation period <36 wk were excluded. All blood-sampling protocols were approved by the institutional review board of the Wuxi Hospital for Maternal and Child Health Care. Because anonymous banked serum samples were used in the study, the institutional review board determined that no informed consent was needed. Banked serum samples were stored at −20 °C in the clinical laboratory and studied within 12 mo. Preliminary studies have shown that frozen samples at the same temperature are stable for 1 y in repeat measurement of compound W, 101.2 ± 2.5% (mean ± SE, percentage of initial values of compound W, $n = 14$) (S.Y.W., unpublished data).

In addition, 59 maternal (unrelated to newborns studied) serum samples, from day 1 to day 7 after uneventful deliveries, were also studied. Compound W levels, expressed as ng/dl of T_2S-equivalent in

both neonatal and maternal groups, were converted to percentage of the mean level of day 1 for each respective group to eliminate variations in assays performed in different laboratories (newborn samples were measured in China, and maternal samples in the United States). Serum bilirubin, alanine aminotransferase, aspartate aminotransferase, γ glutamyl transpeptidase, and alkaline phosphatase were measured by a Hitachi 7170A Full-automated Biochemical Analyzer (Hitachi, Tokyo, Japan).

Statistical Analysis

Student's unpaired *t*-test was used to assess between-group differences. ANOVA was used to test multigroup comparisons (18). Significance was defined as $P < 0.05$. Results are reported as the mean ± 1 SE. In addition, semi-log linear regression analysis of the serum compound W concentrations was used to assess and compare the decay half-life in postpartum women and the newborn samples (19). Half-life decay values were calculated from the PK Functions software package for Microsoft Excel.

STATEMENT OF FINANCIAL SUPPORT

This work was supported by grants from the Department of Veterans Affairs and the Southern California Institute for Research and Education.

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