The placenta as modulator of fetal prosperity
Buimer, M.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 5:

Transient hypothyroxinemia in severe hypertensive disorders of pregnancy

M. Buimer, A.G. van Wassenaer, W. Ganzevoort, H. Wolf, O.P. Bleker, J.H. Kok

Obstetrics and Gynecology 2005; 106: 973-979
Chapter 5

Abstract

Objective: Assess whether and to what extent thyroid function is affected in pregnant women with early and severe hypertensive disorders and their newborns.

Methods: Patients were 80 women with preeclampsia, hemolysis, elevated liver enzymes and low platelet count syndrome or gestational hypertension combined with fetal growth restriction in the 24th to 34th week of singleton pregnancies. Maternal thyroid hormone levels and thyroid peroxidase antibodies (TPOab) were determined at admission and three months post term. Neonatal levels were determined from cord blood at delivery. Maternal hypothyroxinemia was defined as fT\textsubscript{4} value below 9 pmol/l.

Results: At admission 26 (33%) women in the Study Group had fT\textsubscript{4} levels below 9 pmol/l, with spontaneous normalization during pregnancy. There were, however, no statistically significant differences between thyroid hormone values in women in the study group compared to 10 normotensive pregnant women in their third trimester. Three months post term 97.5% of patients had thyroid hormone levels in the normal range. TPOab were elevated in 10% of women post partum. Their infants, born at a median gestational age of 30\textsubscript{6}/\textsubscript{7} weeks, had lower cord blood fT\textsubscript{4} and TSH values compared to preterm infants of the comparison group, appropriate for gestational age. Cord blood fT\textsubscript{4} had no correlation with gestational age or maternal fT\textsubscript{4}, but there was a significant correlation of cord blood fT\textsubscript{4} with umbilical artery pH.

Conclusion: Women with severe hypertensive disorders of pregnancy may have transiently lower fT\textsubscript{4} levels, without evidence of a thyroid disorder. Their neonates have lower fT\textsubscript{4} levels at birth unrelated to maternal fT\textsubscript{4}, but related to prenatal acidosis.
Sufficient provision of thyroid hormone in the first trimester of pregnancy is essential for normal fetal brain development. There is growing evidence, however, suggesting that maternal thyroid hormone levels remain important until term. In the debate on benefits of screening for hypothyroidism in pregnancy, the question on the optimal free T₄ (fT₄) level remains unanswered, as reports on thyroid function in normal pregnancy are scarce.

Preeclamptic patients are at particular risk. Several reports describe an association between preeclampsia and maternal thyroid dysfunction, and low birth weight, some authors even suggested maternal thyroid function abnormalities to be a causal factor. Free T₄ is generally found to be lower in umbilical cord samples from neonates born from preeclamptic pregnancies than in infants from normotensive pregnancies, but reports remain inconclusive. A low fT₄ can be a consequence of lower maternal thyroid hormone levels, however, it can also be caused by impaired transfer of T₄ due to placental insufficiency or fetal disease due to fetal growth restriction (FGR) and fetal acidosis.

The aim of the present study was to assess whether and to what extent thyroid function is impaired in women with severe and early hypertensive disorders in pregnancy, whether autoimmunity is involved, and to what extent neonatal thyroid function is affected.

Patients and Methods

This prospective cohort study was performed between April 1st 2001 and June 1st 2003, in a subset of women with severe hypertensive disorders of pregnancy, participating in the Preeclampsia Eclampsia TRial of Amsterdam. This randomized clinical intervention trial in women with severe hypertensive disorders of pregnancy was carried out in two tertiary care perinatal centers in Amsterdam from September 1st 2000 to June 1st 2003. In our study of thyroid function only the subset of women and neonates admitted in one of the centers (Academic Medical Center) were included. As blood collection, for thyroid hormone parameters at admission, preceded the study intervention data from both treatment arms were joined for our further analyses.

The study protocol was approved by the institutional review board of the Academic Medical Center. After informed consent, women were included upon admission if they were in the 24th to 34th week of a singleton pregnancy with either pregnancy induced hypertension in combination with fetal growth restriction (diastolic blood pressure >90 mmHg and estimated fetal weight < 5th centile or abdominal circumference <10th percentile) or severe preeclampsia (diastolic blood pressure > 110 mmHg and proteinuria > 0.3 g/L) or HELLP-syndrome (lactate dehydrogenase (LDH) > 600 U/L, aspartate aminotransferase > 70 U/L, Platelet Count < 100 x 10⁹/L). After informed consent, patients were randomly allocated to a temporizing management...
strategy with or without plasma volume expansion. Randomization was preformed by use of a designated palmtop computer with random number generation software. In all patients, obstetric management aimed to improve fetal prognosis through increasing gestational age at birth. Pregnancy was prolonged until deterioration of fetal or maternal condition necessitated delivery. In the absence of normal reference values for thyroid hormone in pregnancy, apart from comparing thyroid function results with our local reference ranges for the specific assays, they were also compared with earlier described results in a prospectively followed group of 10 healthy women who became pregnant by artificial insemination because of male infertility. Baseline, non-pregnant thyroid function was also known in these women. None of these pregnant women showed any sign of hypertensive disorders.

Maternal Blood Samples at Admission
At admission, maternal blood samples were collected for determination of T₄, T₃, Thyroid Stimulating Hormone (TSH), freeT₄ (fT₄), Thyroxin Binding Globulin (TBG), reverse-T₃ (rT₃), and Thyroid Peroxidase antibodies (TPOab). Normal values of fT₄ in pregnancies are not known. Therefore, we defined low maternal thyroxin levels by the lower limit of our laboratory (i.e. nonpregnant) reference range: fT₄ of <9 pmol/l. A second sample was taken if fT₄ was < 9 pmol/l or TSH was < 0.4 or > 4 mU/l. If fT₄ or TSH in the second sample were also outside these limits, the patient was referred to an endocrinologist for further evaluation.

Neonatal Blood Samples
At birth, an umbilical cord blood sample for determination of T₄, T₃, TSH, fT₄, TBG and rT₃ was collected. If the amount of cord blood after determination of arterial pH was limited and thyroid hormone analysis was incomplete, preference was given to determining fT₄, TSH, T₄ and T₃. We compared results with data previously collected in our hospital using the same assays, in a group of 114 premature infants below 30 weeks gestational age (mean gestational age 28 1/7 weeks ± 8 days) of whom 90% were appropriate for gestational age.

Maternal Blood Sample Post Partum
Three months after term date, at a scheduled visit, a maternal post partum blood sample was obtained for determination of T₄, T₃, TSH, fT₄, TBG, rT₃ and TPO-antibodies. Values were compared with reference values of the laboratory. If one of these determinations was outside the reference range, the patient was further evaluated by an endocrinologist for thyroid disorders.

Assays
Blood was centrifuged immediately and stored at -20°C until assay. T₄, T₃ and rT₃ were measured by in-house RIA methods. Detection limits were 5.0, 0.3 and 0.03 nmol/l, respectively; intra-assay coefficients of variation...
were 2-4%, 3-4% and 4-5%, respectively, and interassay coefficients of variation were 3-6%, 7-8% and 5-9%, respectively. FreeT\textsubscript{4} was measured by time-resolved fluoroimmunoassay (Delfia fT\textsubscript{4}, Wallac Oy, Turku, Finland). Detection limit was 2 pmol/l, intra-assay coefficient of variation was 4-6% and inter-assay coefficient of variation was 5-8%. TSH was also measured by time-resolved fluoroimmunoassay (Delfia hTSH Ultra, Wallac Oy, Turku, Finland). Detection limit was 0.01 mU/l, intra-assay coefficient of variation was 1-2% and inter-assay coefficient of variation was 3-4%.

Anti-TPO antibodies were measured by chemiluminescence immunoassay (LUMI-test anti-TPO, BRAHMS, Berlin, Germany). Detection limit was 30kU/l, intra- and inter-assay coefficient of variation were 3-7% and 8-12%, respectively. Thyroxin Binding Globulin was determined by commercial radioimmunoassay (Eiken Chemical Co, Tokyo, Japan). Detection limit was 30 nmol/l, intra- and interassay coefficient of variation were 2-4% and 4-6% respectively.

**Placental Insufficiency Parameters**

We used three measures of severity of placental insufficiency. Ultrasound Doppler examination of a free loop of umbilical artery was performed twice weekly, and the most recent Pulsatility Index, calculated from the flow velocity profile, was recorded. Secondly, we used Birth Weight Ratio as a measure for dysmaturity. The Birth Weight Ratio is the observed birth weight divided by the expected weight at the corresponding gestational age according to the customized antenatal growth chart.\textsuperscript{33} By definition, an infant with the appropriate weight for gestational age is to have a BWR of 1, whereas a birth weight ratio less than 0.86 corresponds to a birth weight less than the 10\textsuperscript{th} percentile (SGA). The net weight of the placenta was measured after birth after removal of cord and membranes; centile values were calculated by means of Dutch reference curves, stratified for parity and gender of the neonate.\textsuperscript{34}

**Statistical Analysis**

Data were analyzed with the statistical program SPSS 10.0.7 for Windows (SPSS Inc., Chicago, Illinois, USA). Patient characteristics as well as hormone values were checked for normal distribution as indicated by one sample Kolmogorov-Smirnov test. All analyses were done for the whole group and within subgroups according to low fT\textsubscript{4} or admission diagnosis. Groups were compared using the Student t test and the Chi square test. One way ANOVA was used to compare groups according to admission diagnosis. Linear regression analysis and Pearson correlation were used in correlations of continuous variables. Multivariable regression analysis was performed by the enter model, with the significant factors of the univariate analyses, combined with gestational age and maternal fT\textsubscript{4}. The statistical power to detect a clinically significant difference of 1.5 pmol/l in maternal fT\textsubscript{4} level between our study group and the comparison group was calculated as 80% (effect size = 0.84; N=10 compared with N=80; α=0.05).
Chapter 5

Results

The mothers
In the study period, 80 women were included. Table 1 shows the demographic and obstetric data at admission. There were 69 live-born babies and 11 stillbirths. Median gestational age at birth was 306/7 weeks (range 261/7 - 366/7 weeks), birth weights ranged 525–2310 grams (median 1100) and BWR ranged 0.36 -0.88 (median 0.63): all babies but one were SGA. Median umbilical cord artery pH was 7.20, ranging 6.94 – 7.44. Table 2 shows mean maternal thyroid hormone levels at admission. There were no statistically significant differences between the study group and the comparison group, although TT4, fT4, T3 and TBG were lower than in the comparison group, in combination with a higher TSH.

<table>
<thead>
<tr>
<th>Study Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
</tr>
<tr>
<td>Maternal Age</td>
<td>years</td>
</tr>
<tr>
<td>Nulliparity</td>
<td></td>
</tr>
<tr>
<td>Maternal Weight</td>
<td>kg</td>
</tr>
<tr>
<td>Gestational Age at admission</td>
<td>weeks</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>mmHg</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>mmHg</td>
</tr>
<tr>
<td>Admission Diagnosis*</td>
<td></td>
</tr>
<tr>
<td>- HELLP</td>
<td>18    (23)</td>
</tr>
<tr>
<td>- Severe preeclampsia</td>
<td>23    (29)</td>
</tr>
<tr>
<td>- Gestational hypertension and FGR</td>
<td>39   (49)</td>
</tr>
<tr>
<td>Interval admission-birth</td>
<td>days</td>
</tr>
<tr>
<td>Lowest Recorded Platelet Count</td>
<td>109/l</td>
</tr>
<tr>
<td>Highest Recorded Proteinuria</td>
<td>g/24 hrs</td>
</tr>
</tbody>
</table>

Table 1: Maternal characteristics at admission and during observation. Values are mean (+/- standard deviation) median (range) or n(%), as appropriate. *During clinical observation, in the Severe Preeclampsia Group 9 patients developed HELLP Syndrome whereas in the FGR Group 11 patients developed HELLP Syndrome. At birth 38 patients (48%) had HELLP, 15 (19%) had Severe Preeclampsia, and 27 (34%) had FGR.

However, in 26 patients (33%) fT4 was <9 pmol/l, their mean fT4 was 8.0 (±0.66) pmol/l, ranging 6.8–8.9 pmol/l. In the Comparison Group, 2 patients (20%) had fT4 levels < 9 pmol/l. On reassessment one to three weeks later a mean fT4 of 10.5 (±2.8) and a TSH of 3.9 (±2.8) were found. Only one patient developed a specific thyroid disorder (see post partum section). When
Preeclampsia and Thyroid Hormone Levels

We compared clinical characteristics on admission (systolic and diastolic blood pressure, admission diagnosis, ultrasound Doppler PI of the umbilical artery, highest level of proteinuria, lowest platelet count during observation, gestational age at delivery, interval admission-delivery, birth weight, BWR, Apgar score at 5`, placenta weight centile) of women with low fT4 to women with normal fT4 values, no statistically significant differences were found. TPO antibodies were elevated in 7 patients at admission, but there was no significant relationship between the presence of TPO antibodies and fT4 below 9 pmol/l (p = 0.22, χ² = 1.5, df=1). In univariate regression analyses, T₃ and T₄ levels had significant positive correlations with TBG levels, which is expected, as T₃ and T₄ are predominantly bound to TBG. Concentrations of this binding protein, subsequently, were significantly lower in women with a higher quantity of proteinuria (Pearson R = -0.33, p = .006), suggesting that lower T₄ and T₃ concentrations are due to loss of binding protein.

<table>
<thead>
<tr>
<th></th>
<th>Study Group</th>
<th>Comparison Group*¹⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>TT₄ nmol/l</td>
<td>147 (33)</td>
<td>158 (26)</td>
</tr>
<tr>
<td>TT₃ nmol/l</td>
<td>2.7 (0.8)</td>
<td>2.88 (0.5)</td>
</tr>
<tr>
<td>TSH mU/l</td>
<td>3.1 (3.9)</td>
<td>1.4 (0.6)</td>
</tr>
<tr>
<td>TBG nmol/l</td>
<td>733 (140)</td>
<td>805 (72)</td>
</tr>
<tr>
<td>fT₄ pmol/l</td>
<td>9.8 (1.7)</td>
<td>10.2 (1.6)</td>
</tr>
<tr>
<td>rT₃ nmol/l</td>
<td>0.38 (0.13)</td>
<td>0.32 (0.08)</td>
</tr>
<tr>
<td>TPOab low/undetectable</td>
<td>69 (86)</td>
<td>7</td>
</tr>
<tr>
<td>elevated 70 – 2240</td>
<td>7 (9)</td>
<td></td>
</tr>
<tr>
<td>missing</td>
<td>4 (5)</td>
<td></td>
</tr>
<tr>
<td>Ratio T₃/rT₃</td>
<td>7.9 (3.2)</td>
<td>9.5 (3.0)</td>
</tr>
</tbody>
</table>

Table 2: Maternal Hormone Levels at admission
Values are mean (sd) or n (%), as appropriate. *Ten women in the Comparison Group¹⁰ had a mean age of 33.2(±3.5) years; 2(20%) were nulliparous; had a mean body weight of 79(±11) kg; median gestational age at testing was 35⁷/₇ weeks, ranging 29²/₇–41¹/₇.

Postpartum maternal blood samples, taken 14 to 27 (mean 22) weeks after delivery were obtained in all patients but 1, who was lost to follow-up. Table 3 lists the results of maternal thyroid hormone levels at three months post term date. TPO antibodies were elevated in 8 patients (10%). There were two patients (2.5%) with abnormal thyroid hormone parameters. One patient, with a low fT4 level during hospitalization, was diagnosed with Graves’ Hyperthyroidism. The second patient had suppressed TSH (0.08 mU/l), elevated TT₄ (2.75 nmol/l) and normal T₃ (150 nmol/l) and fT₄ (15.7 pmol/l), suggesting T₃ hyperthyroidism. All other patients with a prior low fT₄ now
Chapter 5

had normal thyroid function. Umbilical cord blood thyroid hormones were assessed in of 46 (67%) of live-born neonates. Results are shown in Table 4. As gestational age in the study group was 3 weeks higher than in the comparison group, higher concentrations of TT₄, fT₄, T₃, and TBG anticipated in the study group. Free T₄, however, was significantly lower in this group than in cord blood of the comparison group. There was no correlation between maternal and cord blood fT₄, as shown in Figure 1 (Pearson R = 0.17, p= 0.27).

<table>
<thead>
<tr>
<th>N</th>
<th>Study Group</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT₄ nmol/l</td>
<td>117 (27)</td>
<td>70 -150</td>
</tr>
<tr>
<td>T₃ nmol/l</td>
<td>2.0 (0.7)</td>
<td>1.3 – 2.7</td>
</tr>
<tr>
<td>TSH mU/l</td>
<td>1.8 (1.2)</td>
<td>0.4 – 4</td>
</tr>
<tr>
<td>TBG nmol/l</td>
<td>405 (125)</td>
<td>200 – 650</td>
</tr>
<tr>
<td>rT₃ pmol/l</td>
<td>13.7 (3.9)</td>
<td>10 – 22</td>
</tr>
<tr>
<td>Ratio T₄/T₃</td>
<td>8.5 (2.7)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Maternal hormone levels, 3 months after term date
Values are mean (sd) or n (%), as appropriate. Reference levels are normal lab values. One patient was lost to follow up.

<table>
<thead>
<tr>
<th>N</th>
<th>Study Group</th>
<th>Comparison Group n =114</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT₄ nmol/l</td>
<td>96 (37)</td>
<td>87 (30)</td>
</tr>
<tr>
<td>TT₃ nmol/l</td>
<td>0.87 (0.48) †</td>
<td>0.6 (0.3)</td>
</tr>
<tr>
<td>TSH mU/l</td>
<td>7.9 (3.0) †</td>
<td>10.4 (11)</td>
</tr>
<tr>
<td>TBG nmol/l</td>
<td>389 (114)</td>
<td>353 (96)</td>
</tr>
<tr>
<td>fT₄ pmol/l</td>
<td>11 (3.7) †</td>
<td>14.7 (4.5)</td>
</tr>
<tr>
<td>rT₃ nmol/l</td>
<td>2.9 (0.9)</td>
<td>3.1 (1.3)</td>
</tr>
</tbody>
</table>

Table 4: Cord blood hormone levels of live-born neonates
Values are mean (sd). †p < 0.001 vs. cord blood of reference group, Student t test. As gestational age in the study group was 3 weeks higher than in the comparison group, higher concentrations of TT₄, fT₄, T₃, and TBG anticipated in the study group.
Cord blood freeT₄ values for 46 infants, measured at birth, by maternal blood fT₄ values, measured at admission. Pearson R = 0.17, p= 0.27

Contrary to normal pregnancies, in these growth restricted neonates, there was no relation between fT₄ levels and gestational age (Figure 2: Pearson R = 0.18, p= 0.22). On further univariate testing of perinatal factors, only umbilical artery pH (Pearson R = 0.48, p= 0.001) and gender (Pearson R = -0.33, p= 0.03) were significant determinants and gestational age, maternal fT₄, Doppler PI, BWR, placenta weight centile and treatment by plasma volume expansion were not. Multivariate linear regression analysis showed that umbilical cord fT₄ was significantly dependent on umbilical artery pH and gender, and was only slightly influenced by gestational age and maternal fT₄.
Figure 2: Cord blood fT₄ in relation to gestational age

Cord blood freeT₄ values for 46 infants by gestational age at birth. Pearson R = 0.18, p= 0.22
**Discussion**

This observational study of maternal and neonatal thyroid function was carried out in women referred to a tertiary care center because of early and severe hypertensive disorders of pregnancy. Although we did not find statistically significant differences with thyroid hormone levels of a group of 10 healthy women, TT4, fT4, and T3 were somewhat lower, consistent with literature. Moreover, 33% of patients had fT4 concentrations below the lower limit of our local reference range of 9 pmol/l. These women had no identifiable maternal disorder or specific clinical course. In contrast to some authors,14-16;23 we found total T4 and T3 to be of limited clinical value in assessing thyroid function in preeclampsia, as they reflect low TBG due to proteinuria. The observed fT4 levels spontaneously changed to normal at reassessment during pregnancy and more so three months after term, at the scheduled post partum visit.

In the light of the present discussion on the necessity for screening thyroid function in pregnancy, it is therefore pivotal that the normal lower limit of fT4 levels is identified, especially since no pathophysiological pathway has been determined to explain the observed fT4 values in our study group. Notably, two subjects (20%) in the comparison group of healthy women had a third trimester fT4 level below 9 pmol/l.

We anticipated a high prevalence of thyroid disorders and thyroid autoimmunity in the women in our study. However, three months post term specific thyroid abnormalities were diagnosed in only 2 women (2.5%), which is the normal prevalence of post partum thyroid disease in the non-preeclamptic population.35

In this study, umbilical fT4 levels in the neonates were lower than in the comparison group, a finding in concordance with literature.13;14;17;22;26 This low umbilical fT4 was not related with maternal fT4, therefore it is not likely to result from decreased maternal supply of fT4 or impaired transfer. According to our data, these low fT4 levels are due to prenatal acidosis as a result of utero placental insufficiency.

In the present study we were not able to investigate the duration of low fT4 levels in utero. In a cordocentesis study, high fetal TSH and low fT4 levels were found to be correlated to PO2 levels in FGR fetuses without signs of fetal distress, suggesting slowly advancing chronic hypothyroxinemia.36 The present study confirms the general concern about adequate fT4 supply in FGR fetuses. It raises the question whether low fT4 is just a derivative of intrauterine malnutrition. It could well be an independent cause of impaired brain development and the observed impairment of neuropsychological development in infants who were born growth restricted.37,38 These data stress the importance of an adequate follow up of growth restricted preterm infants, as they are at risk of hypothyroxinemia, and follow-up of preterm neonates shows a high prevalence of developmental disorders, especially after low postnatal thyroid hormone levels.39
In summary, we have demonstrated that transient hypothyroxinemia is common in women with severe hypertensive disorders, but is not associated with an increased incidence of thyroid disorders. The neonates show low fT₄ and TSH levels, unrelated to maternal fT₄ levels. These lower fT₄ levels are most likely caused by fetal acidosis. Follow-up is in progress and will reveal whether developmental outcome is associated with low fT₄ levels of mother, low fT₄ levels of the neonate, or merely with the deleterious consequences of fetal growth restriction itself.
Preeclampsia and Thyroid Hormone Levels

Reference List
