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Thyroid hormones and their placental deiodination in normal and pre-eclamptic pregnancy

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ABSTRACT

Pre-eclampsia is associated with lower serum selenium concentrations and glutathione peroxidase expression/activity; total thyroid hormones are also lower.

Objectives, study design and main outcome measures: We hypothesised that the placental selenoprotein deiodinase (D3) will be protected in pre-eclampsia due to the hierarchy of selenoprotein biosynthesis in selenium deficiency. Venous blood and tissue from three standardised placental sites were obtained at delivery from 27 normotensive and 23 pre-eclamptic women. mRNA expression and enzyme activity were assessed for both deiodinases (D2 and D3); protein expression/localisation was also measured for D3. FT_4, FT_3 and TSH concentrations were measured in maternal and umbilical cord blood.

Results: No significant differences in D3 mRNA or protein expression between normotensive and pre-eclamptic pregnancies. There was a significant effect of sampling site on placental D3 activity only in pre-eclamptic women ($P = 0.034$; highest activity nearest the cord). A strong correlation between D3 mRNA expression and enzyme activity existed only in the pre-eclamptic group; further strengthened when controlling for maternal selenium ($P < 0.002$). No significant differences were observed between groups for any of the maternal thyroid hormones; umbilical TSH concentrations were significantly higher in the pre-eclamptic samples ($P < 0.001$).

Conclusions: D3 mRNA and protein expression appear to be independent of selenium status. Nevertheless, the positive correlation between D3 mRNA expression and activity evident only in pre-eclampsia, suggests that in normotensive controls, where selenium is higher, translation is not affected, but in pre-eclampsia, where selenium is low, enzyme regulation may be altered. The raised umbilical TSH concentrations in pre-eclampsia may be an adaptive fetal response to maximise iodide uptake.

1. Introduction

The availability and integration of the trace element selenium into the selenocysteine amino acid (Sec) is crucial to the enzymatic function of deiodinases (D1, D2 and D3). The regulation of selenoprotein synthesis is highly selenium-dependent; it has been shown that a hierarchy exists for the synthesis of different selenoproteins, both via differential mRNA translation and sensitivity to nonsense-mediated decay with D3 being prioritised [1]. The placenta is a key site for the activity of many selenoproteins such as the antioxidant glutathione peroxidase (GPx), iodothyronine deiodinase, and redox signalling thioredoxin reductase families [2]. Many of these roles appear to be particularly relevant to the aetiology of the pregnancy-specific condition of pre-eclampsia, a hypertensive disorder of pregnancy that occurs in ~3% of all pregnancies (*de novo* proteinuric hypertension), a leading cause of maternal and perinatal mortality and morbidity worldwide [3]. Placental and maternal systemic oxidative stress are components of the syndrome [4] and contribute to a generalised maternal systemic inflammatory activation [5]. Placental ischaemia-reperfusion injury has been implicated in excessive production of reactive oxygen species, which could cause release of placental factors that mediate the inflammatory responses [6]. We have recently shown increased maternal and fetal plasma thiobarbituric acid reactive substances (TBARS) concentrations which were measured as a global marker of oxidative stress in pre-eclampsia [4].

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There are three iodothyronine deiodinases, which all utilise Sec at their active site. Deiodinase types 1 and 2 (D1 and D2) primarily catalyse the removal of an iodine from the outer (phenolic) ring and in so doing convert inactive T_4 to T_3. Deiodinase type 3 (D3) catalyses the deiodination of the inner (tyrosyl) ring of both T_4 and T_3 to produce the inactive products reverse T_3 (rT_3) and 3, 3′-diiodothyronine (T_2), respectively [7,8]. D2 and D3 mRNA and activity have both been identified in homogenates of human placenta from near the cord insertion site [9–12]; their activity decreases with gestational age from the end of the first trimester [10,11]. D2 is an integral membrane protein found mainly in the endoplasmic reticulum [13], while D3 is localised in the plasma membrane of the intra-placental cells; the highest levels of D3 are found in the placenta [12]. In the human feto-placental unit, D3 metabolises T_4 to rT_3 throughout pregnancy [14]; only later in pregnancy there is an increase in T_4 to T3 conversion by D1 and D2. Fetal thyroxine-binding globulin (TBG) concentrations rise to non-pregnant levels by the late 3rd trimester, although remaining lower than maternal [15]; the fetal T_4:TBG ratio is, however, higher at term.

Placental D3 enzyme activity is 100–400 fold greater than D2 activity and the D3/D2 mRNA ratio varies from 0.5 to 50 [10]. Placental D2 mRNA concentrations correlate with neither protein nor activity rates [10]. Placental D3 activity is unaffected by plasma T_4 concentrations [9,16] and is controlled by post-transcriptional and post-translational regulation [17] such as the TGF-β via Smad-dependent pathway [18].

Total T_3 and T_4, as well as TBG concentrations in women with pre-eclampsia have been reported to be lower compared to normotensive pregnant women but TSH concentrations are higher [19–21]; these changes have also been observed in fetal samples from pre-eclamptic pregnancies [22]. We have also shown maternal and umbilical venous serum selenium concentrations to be decreased in pregnancy and to be further reduced in pre-eclamptic pregnancy [4]. A strong positive relationship exists between GPx activity and serum selenium concentrations in both maternal plasma and placental tissue and we have reported significant reductions in maternal and fetal GPx protein expression and activity in both plasma and placental tissue [4,23]. The hierarchal control of selenoproteins appears to exist in selenium deficient conditions and ranks deiodinases higher than GPxs [24]. Systematic investigation of the placental deiodinases in relation to pre-eclampsia appears not to have been undertaken.

We hypothesised that D3 would be preserved in placentae from pre-eclamptic women despite their lower serum selenium. We also hypothesised that decreased selenium would be associated with increased TSH due to the role of deiodinases in extrathyroidal production of T_3, to maintain FT_3 and FT_4 concentrations.

2. Methods

Subjects: The investigations were approved by the Nottingham Hospital Ethics Committee; written, informed consent was obtained from each participant. Pre-eclampsia was defined as a systolic blood pressure of 140 mm Hg or more and diastolic pressure (Korotkoff V) of 90 mm Hg or more on 2 occasions after 20 weeks gestation in a previously normotensive woman together with proteinuria ≥300 mg/L, ≥500 mg/day or ≥2+ on dipstick analysis of midstream urine (MSU) if 24-h collection result was not available [25]. The study population consisted of White European women who had either a normotensive ($n = 27$) or pre-eclamptic ($n = 23$) pregnancy (Table 1) [4]. Umbilical venous blood samples were obtained from babies from 24 of the normotensive and 14 pre-eclamptic women. Medical and obstetric histories, including delivery data, were obtained for each woman. The birthweight centile for each baby was computed, correcting for gestation age, sex, maternal parity and body mass index (BMI) [26].

Sample collection: Venous blood samples were taken from mothers before delivery; where possible, umbilical venous samples were also taken, immediately after placental delivery. Samples were taken into chilled tubes with no anticoagulant and the serum fraction stored at −80 °C until required. Two full depth placental tissue samples were collected from three standardised locations between the cord insertion and placental periphery (1 cm from the cord insertion (Near), 1 cm from the periphery (Outer), and midway between the two (Middle)), avoiding placental infarcts. The placental samples were taken within 10 min of delivery, membranes removed and the tissue washed in ice cold 1× PBS to remove maternal blood contamination. One set of samples was snap frozen in liquid nitrogen and stored at −80 °C for measurement of deiodinase activity and RNA assessment; the other was formalin fixed and wax-embedded for immunohistochemical analysis.

Table 1
Demographic and pregnancy data of Subject Groups.[a] Data are presented as mean ± SD or median [IQR] as appropriate, except for proteinuria: (Median (min, max) and parity and Caesarean sections (no. (%)). $^*P < 0.05$ between normal and pre-eclamptic pregnancies; $^{**}P < 0.001$ between normal and pre-eclamptic pregnancies.

Parameter	Normotensive ($n = 27$)	Pre-eclampsia ($n = 23$)
Maternal age, y	29 ± 6.8	32 ± 5.8
Primipara, *n* (%)	18 (63)	19 (68)
Booking body mass index, kg/m^2	26.9 ± 5.9	26.9 ± 5.5
Max. systolic blood pressure outside labour, mm Hg	116.6 ± 4.1	158 ± 12.2**
Max. diastolic blood pressure outside labour, mm Hg	76.4 ± 3.0	98.6 ± 5.9**
Proteinuria, g/L		1.0 (0.3, 11.5)
Gestation age at delivery, wks	39.9 ± 1.1	36.4 ± 3.6*
Caesarean section, *n* (%)	4 (15)	12 (43)*
Birthweight, kg	3.46 [3.24, 3.82]	2.92 [2.14, 3.49]*
Birthweight centile	45 [23, 67]	32 [3, 81]
Placental weight (g)	684.8 ± 118.9	592.6 ± 157*
Placental/birthweight ratio	1.95 ± 0.31	2.1 ± 0.8*

[a] The demographic data from these women have already been published as part of our study of GPx in normal and pre-eclamptic pregnancy [4].

Quantitative real-time PCR: Total RNA was extracted from a known amount of placental tissue (100 mg) using QIAzol lysis reagent (Qiagen, Crawley, UK). RNA concentration and quality were verified spectrophotometrically, using the Nanodrop ND-1000 (Nanodrop Technologies, Labtech, Ringmer, UK); all samples had an A_{260}/A_{280} ratio greater than 1.96 and were stored at −80 °C. RNA (1 μg) was then reverse transcribed using the QuantiTect Reverse Transcription Kit containing a mix of random primers and Oligo dT (Qiagen, Crawley, UK) in a Primus 96 advanced gradient thermocycler (Peqlab Ltd, Fareham, UK). Quantitative real time PCR (7500 FAST thermocycler; Applied Biosystems) was used to examine the expression of D2 and D3 relative to stably expressed beta -2-microglobulin (B2M) [27,28]. Reactions set up in duplicate were carried out in total volume of 20 μl comprising 10 μl FAST SYBR Green Master Mix (Applied Biosystems), 500 nM forward primer, 500 nM reverse primer, nuclease-free water and 1 μl cDNA. The PCR programme ran at 95 °C (20 s) followed by 40 cycles of 95 °C (3 s), 60 °C (30 s). Melt-curve analysis was performed at 95 °C − 60 °C to confirm the presence of one single product. Two negative controls were included with each set of samples: (1) no RNA template; (2) RNA provided but no reverse transcription. The crossing point (CP) values were used for analysis, using a mathematical model for relative quantification developed by Pfaffl [29]. The relative expression ratio (*R*) of the target gene is calculated based on efficiency (*E*) and the *CP* deviation of an unknown sample versus a calibrator, and is expressed in comparison to a housekeeping gene [29,30]. Primer sequences for D2 and D3 and for the housekeeping genes were as previously reported [31].

Immunohistochemistry: Immunohistochemical analysis was performed using the Dako Envision™ visualisation system (Dako, Ely, UK) as previously described [23,32]. D3 antibody (Abcam) was used at 0.5 μg/ml respectively, after determination of optimal dilutions (data not shown). Rabbit IgG was used in place of the specific antibodies as a negative control. Cerebral cortex was used as the positive control for the D3 antibody to verify specificity. A specific antibody for D2 in placentae could not be found and therefore not assayed.

D2 and D3 activity assays: The activities of specific deiodinase subtypes were estimated using methods previously described [33]. Briefly, the placental samples were homogenised in 10 vol 0.1 M phosphate (pH 7.2), 2 mM EDTA and 10 mM dithiothreitol (P100E2D1 buffer). Protein concentrations were estimated using the Bradford method [34]. D2 activity was determined by HPLC analysis of the production of radioactive iodide and T3 outer ring-labelled T_4, and D3 activity by HPLC analysis of the formation of radioactive T_2 and 3′-iodothyronine from outer ring-labelled T_3. Deiodination in the presence of placental homogenate (1 mg protein/ml) was corrected for non-enzymatic deiodination in the absence of homogenate.

D2 activity assay: Incubations were carried out for 120 min at 37 °C with 1 nM (10^5 cpm) [3′,5′-^{125}I]T_4 in the presence of 1 μM T_3 to block D3 and in the absence or presence of 100 nM T_4 to saturate D2, in 0.1 ml P100E2D10 buffer. Deiodinase activity was ascribed to D2 if inhibited by excess unlabelled T_4.