A prospective, longitudinal study of the renin–angiotensin system, prostacyclin and thromboxane in the first trimester of normal human pregnancy: association with birthweight

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BACKGROUND: Very early human pregnancy is a state of cardiovascular underfilling. The renin–angiotensin system (RAS) is directly concerned with sodium and water homeostasis. Angiotensinogen is known to be the rate-limiting component in the generation of angiotensin I, and hence angiotensin II, in pregnancy. The usual measurement of ‘renin activity’ does not differentiate between enzyme and substrate. We hypothesized that the RAS is activated from the start of pregnancy; plasma renin concentration (PRC) and angiotensinogen will show differential regulation and might stimulate the rise in prostacyclin.

METHODS: A prospective study of 12 nulliparous normal women. PRC and angiotensinogen and excretion of prostacyclin and thromboxane metabolites were measured pre-pregnancy and four to six times after conception to 13 weeks. RESULTS: By 6 weeks gestation, mean PRC was markedly raised and remained stable to 13 weeks. The initial angiotensinogen response varied, but rose consistently after 6–8 weeks. Regression analysis showed angiotensinogen in the first trimester to be strongly associated with corrected birthweight centile ($P < 0.001$). Excretion of eicosanoid metabolites was very variable, but rose significantly from 6 weeks; the ratio between prostacyclin and thromboxane excretion did not alter over this time. There was no correlation between the various hormones measured. CONCLUSION: Angiotensinogen is known to be rate-limiting in pregnancy. Its association with birthweight may be through effects on early plasma volume expansion and may have implications for intrauterine growth restriction and pre-eclampsia.

Key words: angiotensinogen/birthweight/human pregnancy/prostacyclin/renin

Introduction

By the time a woman is in the sixth week of her pregnancy, her cardiac output has risen by ~20% (Robson et al., 1989; Chapman et al., 1998; Spaanderman et al., 2000b), her calculated total peripheral resistance has fallen by slightly more and her plasma osmolality is in the process of falling by 10 mOsm/kg to a new level (Davison et al., 1981) which will be maintained to the end of her pregnancy. These changes are proactive, in the sense that the fetus will, as yet, be putting minimal extra demands on the maternal organism, and have been suggested to be triggered by a primary fall in systemic vascular tone (Duvekot et al., 1993) which is initiated in the luteal phase (Chapman et al., 1998). These haemodynamic changes are similar to those accompanying the vasodilatation of other sodium-retaining conditions. Their successful implementation is considered to be central to normal pregnancy outcome. In particular, poor plasma volume expansion has been linked to low birthweight (Campbell and MacGillivray, 1972; Gibson, 1973; Pirani et al., 1973; Salas et al., 1993), the development of hypertension and pre-eclampsia (Gallery et al., 1979; Brown et al., 1989; Zamudio et al., 1993), and to intrauterine fetal death in women with chronic hypertension (Sibai et al., 1982).

The fall in systemic vascular tone in the first trimester is accompanied by a shift away from sympathetic towards vagal modulation of tone (Kuo et al., 2000), and, at least by the late first trimester, activation of the renin–angiotensin system (RAS) (see below), decreased pressor responsiveness to angiotensin II (ANG II) (Gant et al., 1973; Baker et al., 1992) and an increase in synthesis of such vasodilators as prostacyclin (Fitzgerald et al., 1987). During this period, a first wave of remodelling of the spiral arteries occurs, which will finally lead to their conversion into floppy, thin-walled conduits, optimizing blood flow to the fetal side of the placenta (Meekins et al., 1997). mRNA for the enzyme renin, its substrate angiotensinogen (Aogen), angiotensin converting enzyme and the angiotensin type AT1 receptor (AT1R) have been demonstrated in close proximity to the spiral arteries in first trimester human pregnancy (Morgan et al., 1998) and it has been
suggested that the uterine RAS is concerned in the vascular remodelling.

Outside pregnancy, the rate of reaction between plasma renin and Aogen, which results in the generation of angiotensin I, is driven by the plasma renin concentration (a first order reaction). This is regarded as the rate-limiting step in the generation of ANG II (Poulsen, 1973). Plasma renin activity (PRA), the most frequently-reported measure of activity of the RAS, is measured as the amount of angiotensin I generated by a plasma sample under physiological conditions of temperature and pH per unit time. It is therefore affected by both enzyme (renin) and substrate (Aogen) concentrations, but in non-pregnant subjects it is driven by the renin concentration, with which it is strongly correlated. PRA is therefore used as a surrogate measure of plasma renin concentration (PRC) outside pregnancy. Plasma concentrations of Aogen are nevertheless close to those which would affect the rate of reaction in the non-pregnant state, and, in hyper-estrogenic states (use of oral contraception; pregnancy), the raised concentration of Aogen becomes at least as important in regulating the rate of angiotensin I generation (Kraakoff, 1973; Skinner et al., 1972). It is therefore desirable, in pregnancy studies, to measure both PRC and Aogen individually, to understand factors which may be influencing either or both.

Serial measurements of PRC during the first trimester showed a significant rise by 6 weeks gestation (Chapman et al., 1998), which was more marked than that of aldosterone. This did not identify whether increases in the enzyme renin or its substrate Aogen were driving the rise in PRA. There also appears to be no information about very early changes in prostacyclin and thromboxane in human pregnancy. Since changes in any or all of these parameters might be implicated in the very early cardiovascular response to pregnancy, we have made serial measurements of PRC and Aogen concentration, and urinary 6-keto prostaglandin F1α (PG 6-keto F1α), the stable metabolite of prostacyclin and thromboxane B2 (Tx B2), the stable metabolite of thromboxane A2, concentrations were measured by enzyme immunoassay (Amersham Biosciences UK Ltd, Little Chalfont, Buckinghamshire, UK) after extraction and purification on Amprep C18 minicolumns (recovery CV 8.1 and 10% respectively; Brown et al., 1992). The eicosanoid concentration was expressed in relation to urinary creatinine (Brown et al., 1992). Urinary creatinine concentrations were measured on an AutoAnalyser (Hitachi), with a within-assay CV of 7%. All samples from a patient were run in the same assay.

Statistical methods
Data were tested for normality of distribution and normalized using log10 as necessary. Central tendency is expressed as mean ± SD or median (interquartile range) as appropriate; group comparisons were made using the Mann–Whitney test. Regression analysis was performed on normalized data.

Results
The women were aged between 19 and 36 (mean 25.0 ± 4.4) years and had pre-pregnancy BMI between 17.9 and 29.7 (mean = 22.0 ± 3.6) kg/m². All women remained normotensive throughout their pregnancies and delivered at or after 37 weeks gestation (mean 38.9 ± 1.0 weeks). The median birthweight centile of their babies was 58.5% (12.5–89.5) and all were healthy.

At a median of 6 weeks gestation, PRC had risen sharply, from 2.6 (1.7–3.8) during the follicular phase to 7.1 (5.8–12.0) ng/ml/h (P < 0.001), while plasma Aogen was unchanged [0.54 (0.37–0.91) compared with 0.58 (0.50–0.91)]. As Figure 1a shows, although PRC remained high until the end of the first trimester, there was no further significant rise in concentration after 6 weeks.

In contrast, overall plasma Aogen concentration rose steadily to 13 weeks (Figure 1b; r = 0.333; P = 0.004). However, inspection of the individual data sets where both follicular and
early pregnancy data were available \((n = 10; \text{Figure 2})\) showed that in five of the women, plasma Aogen concentration initially fell before beginning the rise. Those women in whom an early fall in Aogen concentration was seen had significantly lower average Aogen concentration over the entire first trimester \((\text{median } 0.57 \mu g/ml \text{ compared with } 1.28 \mu g/ml; P < 0.001, \text{Mann–Whitney})\); their babies were also of lower birthweight centile \((\text{median } 45\% \text{ compared with } 77\%; P = 0.152)\). Regression analysis identified a highly-significant positive association between the birthweight centile and the log10 plasma Aogen concentration measured in the first trimester \((\text{Figure 3}; r = 0.575; F = 26.18; P < 0.0001)\). The association was stronger for women whose [Aogen] did not show an early fall \((\text{solid symbols}: r = 0.619, F = 22.39, P < 0.0001)\), but was also present in those in whom the earliest \((\text{Aogen})\) fell slightly, just failing to reach statistical significance \((\text{open symbols}: r = 0.3250, F = 4.026, P = 0.053)\).

At a median of 6 weeks gestation, both PG 6-keto F1α:Cr and TxB2:Cr were unchanged from follicular values \([95.9 \text{ (53.7–132.2) compared with } 95.9 \text{ (68.1–138.4) pmol/mmol and } 89.3 \text{ (38.5–105.5) compared with } 84.6 \text{ (66.7–163.2) pmol/mmol}].\) Both rose thereafter \((\text{Figure 4a and b}; \text{PG } 6\text{-keto } F_{1\alpha}:\text{Cr}: r = 0.389; P = 0.001; \text{TxB2}:\text{Cr}: r = 0.307; P = 0.013)\). There was an overall correlation between urinary output of the two metabolites during the first trimester when gestation age was controlled for \((r = 0.3451; P = 0.011)\). The median ratio
but were apparently symptom-free 5 months later had a considera-
ble rise in both plasma ANG II and plasma volume in the
first trimester, but a live fetus at the time of sampling, the 31
women who subsequently aborted had mean Aogen concentration only
two-thirds that of the 26 who had continuing pregnancies.

The strong association between plasma (Aogen) in the first tri-
mester and corrected birthweight centile 6 months later (Figure 3)
may reflect an impact of plasma (Aogen) on the plasma volume expansion needed for normal adaptation to preg-
nancy. The association between plasma volume expansion and
birthweight has been documented for >30 years (Campbell and
MacGillivray, 1972; Salas et al., 1993). A low plasma volume has
been reported in association with both hypertensive and non-
hypertensive intrauterine growth restriction (IUGR), an associa-
tion first noted in 1978 (Croall et al., 1979; Brown et al., 1989; Zamudio et al., 1993). If Aogen is
rate-limiting in the generation of ANG II in pregnancy, then, other
things being equal, lower Aogen will be associated with lower
ANG II concentrations, lesser stimulus to aldosterone synthesis
and release and less sodium and water retention. Although the
evidence for this is indirect, women who had had pre-eclampsia
but were apparently symptom-free 5 months later had a considera-
bly smaller rise in both plasma ANG II and plasma volume in the
luteal phase than did control women (Spaanderman et al., 2000a).
Such women also showed a smaller increase in plasma (aldoster-
one) in response to infused ANG II (Spaanderman et al., 2004).
Their plasma Aogen concentration was not measured. ANG II
also directly stimulates drinking (Fitzsimons, 1998).

Discussion
The acquisition of sequential samples before pregnancy and
during the first trimester of normal, continuing pregnancy is
extremely difficult. We were fortunate that the cultural back-
ground of our volunteers is such that all were hoping to con-
ceive immediately after their marriage. Nineteen women were
initially recruited, but seven found the study too demanding of
their time, and withdrew after the first one or two samples had
been obtained. However, although the final study group of 12
is relatively small, we were able to obtain sequential samples
from before conception and as early as 4 weeks gestation
through to the end of the first trimester.

There is a rise in PRA and (ANG II) during the luteal phase of
ovulatory menstrual cycles (Chapman et al., 1997; Spaanderman
et al., 2000a). This prospective study has shown that the rise in
PRA previously reported by 6 weeks gestation (Chapman
et al., 1998), and which seems likely simply to be an amplifica-
tion of the luteal rise, is due to an initial rise in PRC (Figure 1a)
which then remains relatively stable until the end of the first
trimester. Others have previously shown that there is a further,
less marked, increase between the end of the first and second
trimesters (Baker et al., 1992). The initial sharp rise in PRC is
presumably a response to the renal sodium loss (Persson, 2003)
which would otherwise be evoked by the increasing progester-
one concentrations of early pregnancy, which act as competi-
tive inhibitors to aldosterone. In addition to its effects on the
adrenal cortex (Persson, 2003), ANG II also directly stimulates
proximal tubular sodium reabsorption (Cogan, 1990).

Plasma Aogen rises more slowly during the first trimester
(Figure 1b), but then continues to rise to ≥36 weeks (Baker et al.,
1992). Both exogenous and endogenous estrogens are potent stim-
uli to hepatic Aogen synthesis and release (see Tewksbury, 1983)
and the pregnancy-induced rise in Aogen is assumed to be prima-
arily due to rising concentrations of estradiol. The data reported
here show marked variability in Aogen response in early preg-
nancy. Serial measurements of estradiol concentrations in early
human pregnancy are also very variable (Lenton et al., 1982), and
the two observations are presumably causally linked. Interest-
ingly, Aogen concentration measured before 14 weeks gestation
has been identified as being at least equivalent to plasma (estradi-
ol) in identifying pregnancies at risk of spontaneous abortion
(Siimes et al., 1983). Among women who had uterine bleeding in
the first trimester, but a live fetus at the time of sampling, the 31
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between the two metabolites was 1.15 (1.04–1.68) during
the follicular phase, and did not change over the period of
study (r = 0.036; P > 0.75).

Figure 4. The excretion of 6-keto prostaglandin F$_{1\alpha}$ in relation to
creatinine (upper panel) and of thromboxane B$_2$ in relation to creatinine
(lower panel). Mean values were not significantly altered by the first
pregnancy measurement, and although excretion of both metabolites
clearly rises during the first trimester (r = 0.036; P = 0.013), there is
substantial overlap with non-pregnant values.
Very early pregnancy shares many of the features of a low sodium state, presumably because of the raised circulating prostaglandin concentrations. There are increased plasma renin and ANG II concentrations and decreased AT1R (Baker et al., 1992) and a blunting of pressor responsiveness (Gant et al., 1973). Angiotensinogen-null mice have significantly greater early embryonic waste, much reduced 21 day survival rate and much slower post-natal growth among the survivors (Templer et al., 2000). Intriguingly, a significant association has been reported between the T allele of the Aogen M235T polymorphism and a low plasma volume in nulligravid women (Bernstein et al., 1998) and the same allele has been reported in significant excess in association with IUGR (Zhang et al., 2003). A suitably activated RAS may be a primary requirement to respond to the demands of pregnancy.

It has been reported that the early rise in prostacyclin metabolite excretion seen in normal pregnancy is blunted by the end of the first trimester in women who go on to develop pre-eclampsia (Broughton Pipkin et al., 1984). The effect of prostaglandin E2 upon the biochemical response to infused angiotensin II in human pregnancy. Clin Sci (Lond) 66,399–406.

Very early pregnancy shares many of the features of a low sodium state, presumably because of the raised circulating prostaglandin concentrations. There are increased plasma renin and ANG II concentrations and decreased AT1R (Baker et al., 1992) and a blunting of pressor responsiveness (Gant et al., 1973). Angiotensinogen-null mice have significantly greater early embryonic waste, much reduced 21 day survival rate and much slower post-natal growth among the survivors (Templer et al., 2000). Intriguingly, a significant association has been reported between the T allele of the Aogen M235T polymorphism and a low plasma volume in nulligravid women (Bernstein et al., 1998) and the same allele has been reported in significant excess in association with IUGR (Zhang et al., 2003). A suitably activated RAS may be a primary requirement to respond to the demands of pregnancy.

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In conclusion, our data confirm very early activation of the RAS in normal human pregnancy, initially through a rise in PRC and secondarily through increased Aogen. The association between first trimester Aogen and corrected birthweight centile has not been reported previously, but fits with other early pregnancy data. It is suggested that an appropriate response of the renin–angiotensin system, especially of the rate-limiting Aogen, is necessary for the proper expansion of plasma volume in very early pregnancy. Thus inadequate Aogen synthesis might contribute to the pathogenesis of IUGR and pre-eclampsia.

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References


RAS and eicosanoids in the first trimester

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