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

H.Al Kadi[1], H.Nasrat[2] and F.Broughton Pipkin[3]

[1]Department of Physiology, [2]Department of Obstetrics and Gynaecology, Faculty of Medicine, King Abdulaziz University Hospital, King Abdulaziz University, Jeddah, Saudi Arabia and [3]Department of Obstetrics and Gynaecology, Queen's Medical Centre, Nottingham NG7 2UH, UK

[4]To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, D Floor, East Block, Queen's Medical Centre, Nottingham NG7 2 UH, UK. E-mail: Fiona.broughton-pipkin@nottingham.ac.uk

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 (Krakoff, 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 PRA 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 $F_{1\alpha}$ (PG 6-keto $F_{1\alpha}$), the stable metabolite of prostacyclin and thromboxane B_2 (Tx B_2), the stable metabolite of thromboxane A_2 in normal, nulliparous women in the follicular phase of a menstrual cycle and on at least four occasions during the first trimester of pregnancy.

Materials and methods

Subjects and sampling

The study was approved by the Ethical committee of the King Abdulaziz University Hospital, Jeddah, Saudi Arabia. Twelve volunteers were initially recruited by word of mouth from a cohort of women, with no known personal history of renal, cardiovascular or metabolic disease, who were either hoping to conceive naturally within the near future ($n = 10$), or who were already in very early pregnancy at the time of recruitment ($n = 2$). None was using any form of medication. All women were nulliparous. All gave written informed consent to the study.

Blood samples were obtained during the follicular phase (days 5–7) in 10 women. Once pregnancy was confirmed (biochemical, followed by dating ultrasound scan in later gestation), a first pregnancy sample was obtained from all women between 4 and 8 weeks gestation (median 6 weeks). Three to five (median 4.5) further blood samples were obtained from each woman, up to 13 completed weeks of pregnancy (median 12 weeks). At each visit, a 10 ml venous blood sample

was taken from an antecubital vein, with the woman comfortably seated with her arm supported. The blood sample was placed immediately in a chilled tube containing 0.5 ml of a mixture of 0-phenanthroline (0.025 mol/l) and EDTA (0.125 mol/l; 1:1 v/v), transported on ice to the laboratory and centrifuged at 1900 *g* for 15 min at 4°C. Plasma was decanted and stored at –20°C until assay.

Eleven of the women also provided midstream urine samples immediately before each blood sample. These were centrifuged at 1900 *g* for 15 min at 4°C and stored at –20°C before transfer to a –80°C freezer.

Pregnancy outcome was recorded for each woman. The corrected birthweight centile of the baby was calculated, using data from women of Middle Eastern origin (Gardosi and Francis, 2003). This takes into account maternal body mass index (BMI) and parity, gestation age at delivery, baby sex and weight.

Assays

Plasma samples were air-freighted on dry ice to Nottingham for assay of plasma renin and angiotensinogen concentrations (PRC; Aogen), using established radioimmunoassays (Tetlow and Broughton Pipkin, 1983; Broughton Pipkin *et al.*, 1984). Samples were assayed in duplicate, and all samples from individual patients were run in the same assay. The within-assay coefficient of variation (CV) was 8.4% for PRC and 6.3% for Aogen.

Urinary 6-keto prostaglandin $F_{1\alpha}$ (PG 6-keto $F_{1\alpha}$), the stable metabolite of prostacyclin, and thromboxane B_2 (Tx B_2), the stable metabolite of thromboxane A_2, 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 log_{10} 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
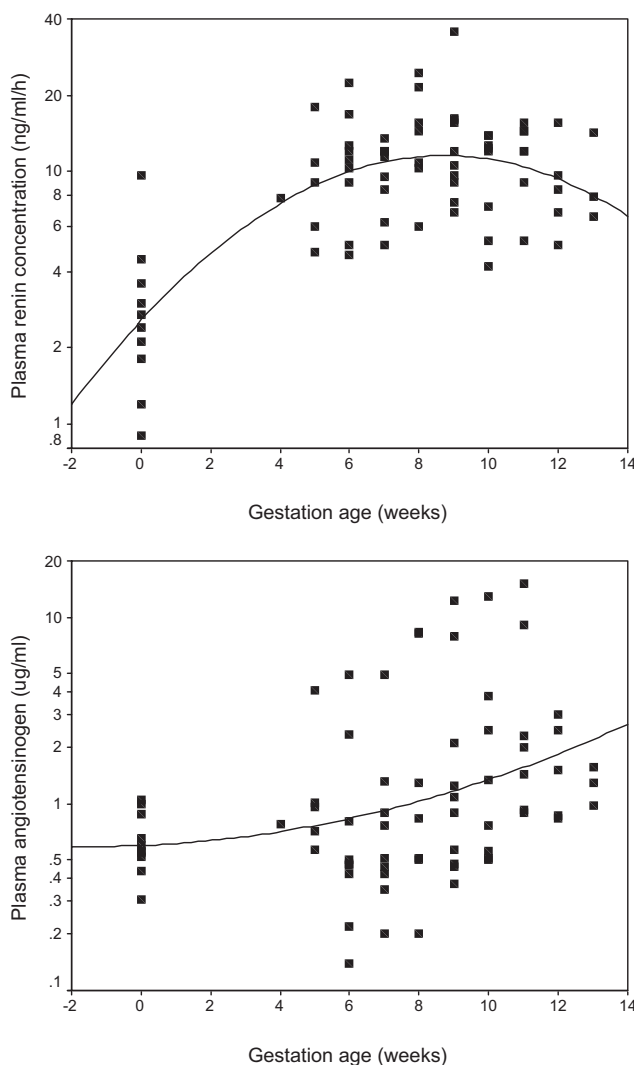
Figure 1. The upper panel shows plasma renin concentration (PRC) and the lower panel plasma angiotensinogen concentration ([Aogen]) before pregnancy and during the first trimester in 10 normal, nulliparous women. PRC had risen sharply by the time of the first pregnancy measurement (4–6 weeks gestation; $P < 0.001$) and did not rise further during the first trimester. Aogen had not risen significantly at the time of the first measurement, but rose steadily thereafter ($P = 0.004$).
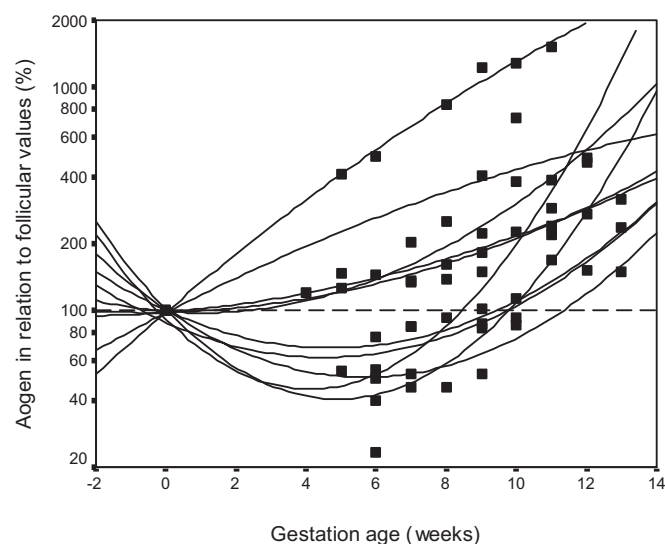

Figure 2. Individual lines of best fit are shown for plasma Aogen concentration during the first trimester, as a percentage of the follicular concentration measured pre-pregnancy. In five women, concentrations were somewhat lower than follicular in the earliest part of pregnancy. There was clear tracking of values in individual women. The dotted line gives the follicular reference (100%).
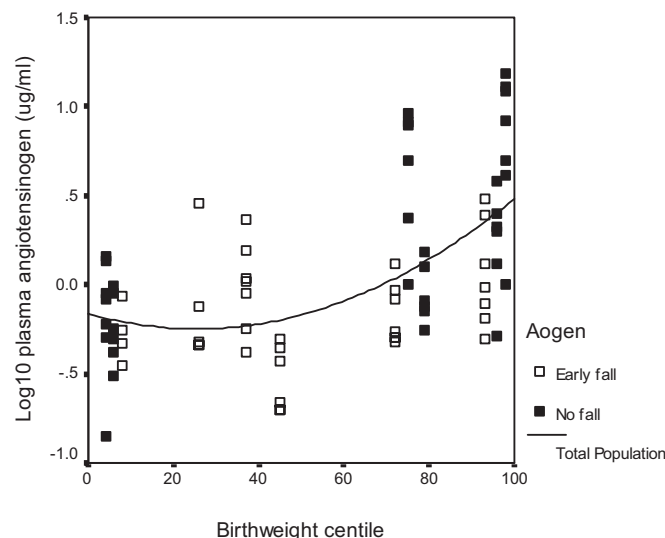

Figure 3. Overall, $\log_{10}$ plasma Aogen concentration ([Aogen]) measured during the first trimester of normal pregnancy correlated strongly with the birthweight centile ($r = 0.575$; $F = 26.18$; $P < 0.0001$). The association was stronger for women whose [Aogen] did not show an early fall (solid symbols: $r = 0.619$, $F = 22.39$, $P < 0.0001$), but was also present in those in whom the earliest (Aogen) fell slightly, just failing to reach statistical significance (open symbols: $r = 0.3250$, $F = 4.026$, $P = 0.053$).

early pregnancy data were available ($n = 10$; 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 (median 0.57 μg/ml compared with 1.28 μg/ml; $P < 0.001$, Mann–Whitney); their babies were also of lower birthweight centile (median 45% compared with 77%; $P = 0.152$). Regression analysis identified a highly-significant positive association between the birthweight centile and the $\log_{10}$ plasma Aogen concentration measured in the first trimester (Figure 3; $r = 0.575$; $F = 26.18$; $P < 0.0001$). In this model, a contribution from $\log_{10}$ urinary PG 6-keto $F_{1\alpha}$:Cr also approached statistical significance ($P = 0.051$).

At a median of 6 weeks gestation, both PG 6-keto $F_{1\alpha}$:Cr and TxB$_2$:Cr were unchanged from follicular values [95.9 (53.7–132.2) compared with 95.9 (68.1–138.4) pmol/mmol and 89.3 (38.5–105.5) compared with 84.6 (66.7–163.2) pmol/mmol]. Both rose thereafter (Figure 4a and b; PG 6-keto $F_{1\alpha}$:Cr: $r = 0.389$; $P = 0.001$; TxB$_2$: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
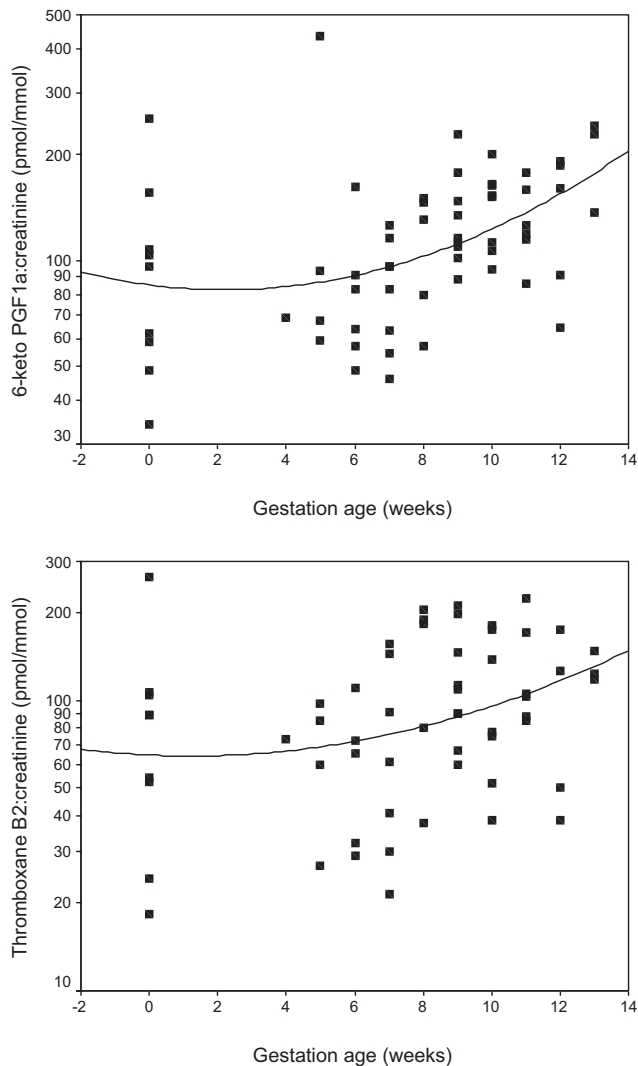
Figure 4. The excretion of 6-keto prostaglandin $F_{1\alpha}$ in relation to creatinine (upper panel) and of thromboxane B_2 in relation to creatinine (lower panel). Mean values were not significantly altered by the first pregnancy measurement, and although excretion of both metabolites clearly rises during the first trimester ($P < 0.001$, $P = 0.013$), there is substantial overlap with non-pregnant values.

between the two metabolites was 1.15 (1.04–1.68) during the follicular phase, and did not change over the period of study ($r = 0.036$; $P > 0.75$).

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 background of our volunteers is such that all were hoping to conceive 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 amplification 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 progesterone concentrations of early pregnancy, which act as competitive inhibitors to aldosterone. In addition to its effects on the adrenal cortex (Persson, 2003), ANG II also directly stimulates proximal tubular sodium reabsorbtion (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 stimuli to hepatic Aogen synthesis and release (see Tewksbury, 1983) and the pregnancy-induced rise in Aogen is assumed to be primarily due to rising concentrations of estradiol. The data reported here show marked variability in Aogen response in early pregnancy. 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. Interestingly, Aogen concentration measured before 14 weeks gestation has been identified as being at least equivalent to plasma (estradiol) 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 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 trimester and corrected birthweight centile 6 months later (Figure 3) may reflect an impact of plasma (Aogen), via ANG II, on the plasma volume expansion needed for normal adaptation to pregnancy. 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 nonhypertensive intrauterine growth restriction (IUGR), an association first noted in 1978 (Croall *et al.*, 1978; Rosso *et al.*, 1993). Furthermore, poor plasma volume expansion has been linked to the development of hypertension and pre-eclampsia (Gallery *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 considerably 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 (aldosterone) 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).

Very early pregnancy shares many of the features of a low sodium state, presumably because of the raised circulating progesterone 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 postnatal growth among the survivors (Tempfer *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 (Fitzgerald *et al.*, 1987). We were thus interested to investigate whether this might be a primary or secondary effect (i.e. an early rise, which was then suppressed by some other factor). All our subjects remained normotensive throughout pregnancy. Since their output of PG 6-keto $F_{1\alpha}$:Cr was not higher than that in the follicular phase of the menstrual cycle until the end of the study period, it seems likely that lower levels in future hypertensive women may be primary. There are numerous interactions between the RAS and the eicosanoids, which can be very complex (Nasjletti, 1998). Although direct stimulatory interactions between ANG II and prostacyclin have been recorded *in vitro*, evidence *ex vivo* in man tends to suggest an inverse association, at least in low sodium states (Watson *et al.*, 1984). In our study, there was no statistical evidence for an association between either PRC or Aogen and PG 6-keto $F_{1\alpha}$:Cr or TxB_2:Cr although Aogen and the excretion of both eicosanoid metabolites showed a similar pattern of rise with gestation age (Figures 1 and 4).

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.

Acknowledgements

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