Fetal sex and the circulating renin-angiotensin system in early gestation in women who later develop preeclampsia or gestational hypertension

Short Title: Hypertension in pregnancy and angiotensin peptides

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Abstract

There are fetal sex-specific differences in the balance between angiotensin (Ang) II and Ang-(1-7) in the maternal circulation during pregnancy. To determine whether at 15 weeks' gestation plasma levels of Ang II and Ang-(1-7) as well as prorenin and angiotensin-converting enzyme (ACE) predicted the development of gestational hypertension (GH) or preeclampsia (PreE) and were associated with estimates of fetal and maternal health, women who later developed GH (*n*=50) or PreE (*n*= 50) were compared with BMI-matched controls (*n*=100). Women who subsequently developed PreE or GH had increased Ang-(1-7) levels at 15 weeks' gestation compared to women with normal pregnancies. When separated by fetal sex, this difference was only seen in women carrying a female fetus. Prorenin and ACE concentrations were not useful biomarkers for prediction of either PreE or GH at 15 weeks' gestation. Women with a male fetus who developed PreE and women who subsequently developed GH had increased blood pressures (BPs) at 15 weeks' gestation compared to women with normal pregnancies, suggesting that these women were on an early trajectory for development of hypertension. We propose that measurement of Ang-(1-7) in early gestation could be useful in predicting which women will go on to develop new-onset hypertension in pregnancy.

Key words: Angiotensin II, Angiotensin-(1-7), preeclampsia, gestational hypertension, prorenin, angiotensin-converting enzyme

Summary Table

What is known about topic

- The RAAS is significantly altered during normal pregnancy and is implicated in the pathogenesis of hypertensive diseases of pregnancy including preeclampsia and gestational hypertension.
- Maternal circulating angiotensin II and angiotensin-(1-7) are both decreased at term in women who have preeclampsia.

What this study adds

- Maternal circulating angiotensin-(1-7) levels measured at 15 weeks' gestation are increased in women who subsequently develop either preeclampsia or gestational hypertension compared to women with normal pregnancy outcomes, well before clinical diagnosis of disease. Angiotensin-(1-7) may be a useful biomarker for new-onset hypertension during pregnancy.
- Fetal sex alters the maternal circulating RAS in women who subsequently develop new-onset hypertension.
- Measurement of maternal circulating concentrations of angiotensin II, prorenin and ACE were not able to differentiate between normal or hypertensive pregnancies.

Trial Registry Name: Screening nulliparous women to identify the combinations of clinical risk factors and/or biomarkers required to predict preeclampsia, SGA babies and spontaneous preterm birth.

URL: http://www.anzctr.org.au.

Registration number: ACTRN12607000551493

Introduction

Hypertensive diseases of pregnancy are the leading cause of maternal mortality in Latin America and the Caribbean and the second highest cause of maternal mortality in developed countries, accounting for 25.7% and 16.1%, respectively.[1] In a clinical setting it is often difficult to determine early in gestation which women will develop GH or PreE. This is of concern as untreated PreE can develop into eclampsia which has a very high mortality rate.[2]

Adult BP exhibits a sexually dimorphic pattern, being higher in men.[3,4] The renin-angiotensin-aldosterone system (RAAS), which responds to sex steroids,[3-6] is implicated as a contributor to this dimorphism since it is a major regulator of BP. We have previously shown that at 15 weeks' gestation women who had normal pregnancy outcomes and carrying a female fetus had higher Ang II levels and Ang-(1-7) to Ang II ratios were lower than in women carrying male fetuses.[7]

PreE is associated with poor placentation[8] and since Ang peptides have been implicated in placental development[9] we thought the RAAS may be involved. The uteroplacental unit might contribute prorenin to the maternal circulation. We postulated that the impaired placentation characteristic of PreE would lead to a decrease in prorenin and subsequent Ang II levels in the maternal circulation early in pregnancy. We further proposed that women with GH, who are unlikely to have poor placental development, would have a different circulating RAAS profile to women who develop PreE. Therefore we assessed levels of RAAS components in the maternal circulation of women who go on to develop hypertensive diseases of pregnancy to determine if any of these components might be useful biomarkers. Other clinical and laboratory estimates

of maternal health were also assessed to determine if they were associated with any differences in the measured circulating RAAS components. The effect of fetal sex was considered in all analyses.

Materials and Methods

Population studied

The current study is a nested case-control study within the Adelaide SCOPE (Screening for Pregnancy Endpoints) cohort, an international prospective cohort study recruiting patients in Australia, New Zealand, UK and Ireland, that aims to predict and prevent the major complications of late pregnancy.[10] Nulliparous women with a singleton pregnancy were recruited into the Adelaide SCOPE cohort after providing written informed consent during antenatal visits at the Lyell McEwin Hospital (Elizabeth Vale, South Australia). Women who were less than 15 weeks' gestation and who had less than three previous terminations of pregnancy or miscarriages were included. Women had a normal pregnancy outcome if they remained normotensive (<140 and/or <90 mmHg prior to labour), showed no proteinuria, delivered a live born baby after 37 weeks who was not small for gestational age and had no other sign of pregnancy complications. GH (n=50) was determined from two BP measurements at least 4 hours apart after 20 weeks' gestation and before the onset of labour, in which systolic BP (SBP) ≥140 mmHg and/or diastolic BP (DBP) ≥90 mmHg (Korotkoff V). These women did not develop proteinuria. PreE (n=50) was defined as GH with proteinuria (urinary protein ≥300 mg/24 h or spot urine protein:creatinine ratio ≥30 mg/mmol creatinine or urine dipstick protein ≥++).[10] Our PreE and GH cohorts were classified as having mild disease severity since we excluded women that delivered pre-term (< 37 weeks), had

small for gestational age infants, or who presented with other complications of pregnancy such as gestational diabetes. Women with either PreE or GH were matched according to BMI with two women who had a normal pregnancy outcome (n=100). Ethics approval for this work was given by the Ethics of Human Research Committee Central Northern Adelaide Health Service (REC 1712/5/2008) and informed written consent was obtained from all participants.

Doppler sonography

At 20 weeks' gestation, Doppler sonography was conducted to measure resistance in the umbilical and uterine arteries, respectively. Data are shown as the resistance index (RI), calculated by measuring blood flow velocity at peak systole and dividing this by the sum of peak blood flow at diastole combined with systole. This produces a ratio that has a maximum value of 1, which represents the absence of end diastolic flow.

Sample collection

Blood and urine were sampled by a research midwife at 15 weeks' gestation from all women who were recruited and before any diagnosis of PreE or GH, which occurred after 20 weeks' gestation. Non-fasting blood samples were collected by venepuncture into EDTA tubes and placed on ice, before centrifugation at 2400 g for 10 min at 4˚C; plasma was stored in 250 µL aliquots at -80˚C. Patients were either sitting or supine for 10 min prior to blood collection. Mid-stream urine samples were collected into 50 mL pots and placed on ice. Urine was stored in 900 µL aliquots at -80˚C. All samples were placed on ice before processing and storage at -80˚C within 30 min of collection. Other clinical measurements such as BP, height and weight were also recorded at the 15 week

antenatal visit. Fetal sex as well as birth and placental weights were recorded at delivery.

Measurement of Ang II and Ang-(1-7)

Ang II and Ang-(1-7) peptide levels were measured by ProSearch International Australia Pty. Ltd. (Malvern, Victoria, Australia) using a direct radioimmunoassay as described previously.[7] The Ang-(1-7) to Ang II ratio was derived from the plasma levels of these peptides. Combined production of Ang peptides was calculated by adding Ang II and Ang-(1-7) levels.

Measurement of ACE and prorenin levels

Maternal plasma levels of ACE (Duoset, R&D Systems, MN, USA) and prorenin (Molecular Innovations, MI, USA) were measured using commercially available enzyme-linked immunosorbent assay kits and conducted according to manufacturer's instructions.

Measurement of other biological markers

All other biological analytes were measured by SA Pathology at the Institute of Medical and Veterinary Science (IMVS, South Australia, Australia). Plasma and urinary electrolytes were measured using ion-selective electrodes, while creatinine was measured using the Jaffé method, and C-reactive protein determined with an immuno-turbidimetric assay. An Olympus AU5400 Chemistry-Immuno Analyzer was used for reading all assays performed.

Data analyses

161 The rate pressure product (an index of cardiac work) was calculated as the product of
162 heart rate and SBP.

163 The urinary protein to creatinine (UPr:Cr, g/mmol), urinary albumin to creatinine
164 (UAlb:Cr, mg/mmol) and urinary sodium to potassium (UNa:K) ratios were all
165 determined and the fractional excretion of sodium (FENa) was calculated from the
166 formula;

167 FENa (%) = (UNa x [PCr/1000]) / (PNa x UCr)*100

168 Where UNa and PNa are urinary and plasma sodium levels (mmol/L), respectively and
169 UCr and PCr are urinary (mmol/L) and plasma creatinine levels (μmol/L), respectively.

170 Mann-Whitney U Tests were used to determine significant differences between cases
171 and controls. Fetal sex- differences were analysed using Mann-Whitney U Tests in all
172 four groups; PreE cases (male=27, female=23), PreE controls (male=48, female=52),
173 GH cases (male=29, female=21), GH controls (male=51, female=49). Data were
174 analysed using Stata/IC 11.0 (StataCorp LP, Texas, USA) and figures produced using
175 GraphPad Prism 5.0. Differences were considered statistically significant if $P \leq 0.05$.

176 **Results**

177 *Women who developed PreE*

178 In women who developed PreE we found sex-specific differences in the components of
179 the RAAS, compared to women who had normal pregnancy outcomes (Fig. 1). Ang II
180 peptide levels were not affected by pregnancy outcome or fetal sex but Ang-(1-7) levels
181 (P<0.003), Ang-(1-7) to Ang II ratios (P<0.03) and the sum of the two peptides

182 (P=0.05) were higher in women who had female fetuses and developed PreE compared
183 to those women with female fetuses who had normal pregnancies.

184 In line with previous work,[7] Ang-(1-7) to Ang II ratios were lower in women with
185 normal pregnancies who had carried a female fetus compared to women carrying a male
186 fetus (P<0.02).

187 At 15 weeks' gestation, women who carried a male fetus and subsequently developed
188 PreE had higher BPs (SBP and DBP: P<0.02 and P<0.01, respectively) than women
189 who had normal pregnancies and carried a male fetus (Table 1). Women who had male
190 fetuses and subsequently developed PreE also had higher DBP (P<0.04) than women
191 with female fetuses who developed PreE.

192 Women with a female fetus who developed PreE had greater uterine artery resistance
193 indices than women who had normal pregnancies and a female fetus (P=0.05). Overall
194 the umbilical artery resistance indices at 20 weeks' gestation were greater if the fetus
195 was female irrespective of pregnancy outcome (control: P<0.05, PreE: P<0.06),
196 although this didn't quite reach significance in PreE women. Newborn females of
197 women who developed PreE were smaller than newborn females from normal
198 pregnancies (P<0.05).

199 *Women who developed GH*

200 Like PreE, Ang-(1-7) levels and Ang-(1-7) to Ang II ratios were higher in women with
201 female fetuses if the women later developed GH (P<0.03, P<0.02) compared with
202 women who had normal pregnancies and carried females.

BMI-matched controls for the GH study also had lower Ang-(1-7) to Ang II ratios if the fetus was female, compared with women carrying a male fetus ($P<0.02$).

Prorenin levels were significantly lower in GH women carrying a female fetus compared with levels in those GH women carrying a male fetus ($P<0.02$). Interestingly, prorenin levels were also lower in women with female fetuses who developed GH compared to women who subsequently developed PreE and carried female fetuses ($P<0.03$).

Independent of the fetal sex, women who later developed GH had higher BPs (male: SBP and DBP, $P<0.02$, female: SBP and DBP, $P<0.004$) and rate pressure products (male: $P=0.05$, female: $P<0.03$) compared to women with normal pregnancy outcomes (Table 2).

Women who developed GH gave birth to smaller infants if the newborn was female ($P<0.04$). These female newborns were also smaller than females born to women who had normal pregnancies ($P<0.02$). Female placental weights were also less in women who developed GH compared to male GH placentae ($P<0.05$).

Women who developed either PreE or GH

Data from women who developed either PreE or GH were assessed as a single cohort (as were the previously separated control groups) to determine if the measured components of the RAAS could be markers for new-onset hypertension in pregnancy irrespective of fetal sex. Ang-(1-7) was higher in women who developed hypertension in pregnancy (median: 74.5 pg/mL, interquartile range: 48.9-120.2) compared to women

224 who had normal pregnancies (55.1 pg/mL, 42.0-82.8; *P*=0.003) and was the only RAS
225 component that was altered between these two populations.

226 **Discussion**

227 The aim of this study was to see if components of the RAAS, namely prorenin, ACE,
228 Ang II and Ang-(1-7) measured in a clinical setting early in gestation would be useful in
229 predicting the probability of the later development of PreE and GH. We have
230 demonstrated that Ang-(1-7) levels are significantly higher at 15 weeks' gestation in
231 women who go on to develop new-onset hypertension in pregnancy.

232 Ang II concentrations have been studied extensively during pregnancy but most often in
233 women after 30 weeks' gestation.[11,12] Baker *et al*. have reported Ang II concentrations
234 in the first, second and third trimesters and those values are lower than the values
235 reported here.[13] Women with PreE have lower Ang II and Ang 1-7 levels at term,
236 compared to women with normal pregnancy outcomes. [14-17]

237 Our reports of Ang-(1-7) in early gestation in women who subsequently develop PreE
238 or GH are novel. The concentrations we report here for women who develop PreE are
239 nearly 10 times higher than those levels reported at term in women with PreE.[15,17] These
240 discrepancies could be due to the gestational age at sampling, greater sample numbers
241 used in the current study, ethnicity and sample handling methodology. However another
242 plausible explanation is that ACE2 changes with gestation. Our group and others have
243 shown that placental *ACE2* expression is greatest in early gestation and ACE2 protein is
244 localised to the syncytiotrophoblasts and villous stroma.[18-20] This may contribute to the
245 10-fold difference seen in Ang-(1-7) at 15 weeks gestation and levels found later in
246 gestation by others.[15,17] Since PreE is a disease marked by placental dysfunction,[21-23]

247 perhaps *ACE2* expression in late gestation is further decreased beyond that seen in
248 normal pregnancy resulting in very low Ang-(1-7) levels in PreE at term, as reported by
249 Merrill *et al*. as well as Velloso and colleagues.[15,17] Although no difference has been
250 reported for placental *ACE2* mRNA abundance at term between women who had
251 normal pregnancies and those who had preeclampsia,[20] placental ACE2 activity may be
252 altered. Furthermore, autoantibodies to the AT_1R (AT_1R-AA) are increased in PreE
253 women at term.[24-26] This increase in AT_1R-AAs in women with PreE could result in
254 feedback suppression of renal renin release via AT_1R activation, causing the observed
255 low levels of Ang peptides in PreE women at term.

256 Ang II and Ang-(1-7) have well described opposing effects on the maternal vasculature
257 with Ang II causing vasoconstriction through both peripheral[27] and central[28] actions
258 while Ang-(1-7) is reported as a vasodilator acting through the Mas receptor.[29] The
259 increase in Ang-(1-7) levels in PreE and GH women was largely due to values obtained
260 in women who had female fetuses. Even so, we propose that measurement of Ang-(1-7)
261 in early gestation could be useful in predicting which women will go on to develop
262 new-onset hypertension in pregnancy. It may reflect the overall increase in production
263 of Ang peptides at this stage of gestation as Ang II is rapidly hydrolysed to Ang-(1-7),[30]
264 possibly at the placental interface by the high levels of ACE2 found in the
265 syncytiotrophoblast.[18-20] If pregnant women were routinely screened for Ang peptides in
266 early gestation, any woman with high Ang-(1-7) levels could be monitored more
267 closely, particularly if the fetus was female. While soluble endoglin and the soluble
268 VEGF receptor (sFlt) have both been shown to be important in the pathogenesis of PreE
269 and biomarkers of the syndrome, significant differences in these proteins aren't seen
270 between PreE and women with normal pregnancies until 28 weeks or later.[31,32]

Therefore, we think that Ang-(1-7) could be one of the earliest detectable predictors of PreE. The validity of this claim relies on more practical methodologies for detecting Ang-(1-7) in a clinical laboratory rather than using radioimmunoassay. One suggested method is the recent development of the "RAS-fingerprint", using liquid chromatography tandem mass spectrometry (Attoquant Diagnostics GmbH, Vienna, Austria), which may become available for clinical use.

If maintenance of maternal vascular tone is related to the balance between the two peptides, we would have anticipated that the effects of a high Ang-(1-7) to Ang II ratio would be vasodilation and hypotension.

The increased production of Ang peptides in PreE produces higher than normal Ang-(1-7) levels and results in an imbalance in the vasodilator/vasopressor ratio in women carrying female fetuses. This might explain the lack of an early rise in BP in women with female fetuses who later develop PreE compared to their PreE predisposed counterparts who carried male fetuses. It may also have compounded the effects of the high uterine resistance indices seen at 20 weeks' gestation resulting in the observed reduction in the birth weights of these female neonates. Supporting this hypothesis is the fact that males born of PreE women had normal birth weights, their uterine artery resistance indices were normal and maternal BPs were higher.

On the other hand, at 15 weeks gestation women who later developed GH and were carrying female fetuses already had higher BPs than their BMI-matched controls, possibly because their Ang-(1-7) levels were not as high as found in women carrying female fetuses who developed PreE. They also had no increase in uterine resistance indices. Even so, their female neonates were also smaller at birth compared with

294 controls. Therefore, other factors are involved in determination of the birth weights of
295 females whose mothers subsequently develop hypertension.

296 Both women who later developed GH and those who carried a male fetus and developed
297 PreE had higher BPs at 15 weeks' gestation compared with women who had normal
298 pregnancy outcomes. While these women did not at this stage of gestation have BPs that
299 were classified as 'hypertensive', our findings suggest that they were already on a
300 trajectory that would result in clinical hypertension later in gestation. Interestingly,
301 women who later developed GH or PreE and carried a male fetus also had increased
302 CRP levels. We postulate that this may be associated with the higher blood pressures
303 since several recent studies have shown a link between CRP and hypertension outside of
304 pregnancy.[33]

305 Women in this cohort who developed PreE did not have severe PreE, i.e. women with
306 HELLP or multisystem disorders of PreE, or those delivering preterm were not studied.
307 While we did see a negative influence on female birth weight in both PreE and GH
308 women, no women delivered a small for gestational age infant. Women who develop
309 severe PreE may have a sharper rise in BP at 15 weeks' gestation than those PreE
310 women analysed in the current study, as previously reported for women with severe
311 PreE after 21 weeks' gestation.[34]

312 To see if there were other changes in components of the circulating RAAS apart from
313 those described above, we looked at two upstream components of the RAAS; prorenin
314 and ACE. Prorenin levels weren't different in either group (PreE and GH) from their
315 respective controls but women carrying female fetuses who developed GH had lower
316 prorenin levels than women carrying males; their prorenin levels were also less than

levels in women who developed PreE who were carrying a female fetus (Figures 1 and 2). The clinical usefulness of this difference is limited because it was confined to 'female' pregnancies and was not evident when fetal sex wasn't taken into account.

Although ACE is a key enzyme in angiotensin peptide production,[35] there was no evidence that ACE levels had any predictive value for GH or PreE in either sex, nor could it predict hypertension in pregnancy in the new onset hypertensive cohort.

In conclusion, we have shown that women who subsequently develop PreE or GH have increased Ang-(1-7) levels at 15 weeks' gestation, prior to the clinical diagnosis of disease. Prorenin and ACE concentrations were not biomarkers for the later onset of either PreE or GH. Women who subsequently develop GH and those women with a male fetus who develop PreE have increased BPs at 15 weeks' gestation compared to women who have normal pregnancy outcomes, although they are not clinically hypertensive, suggesting that they are already on a trajectory for hypertension. Our findings suggest that in women who develop either PreE or GH there are changes in circulating Ang peptides, which are different from those seen once the condition has manifested. We propose that measurement of Ang-(1-7) levels at 15 weeks' gestation may be useful as a biomarker for predicting women that will develop new-onset hypertensive diseases of pregnancy, however further research in a larger cohort is necessary to confirm this.

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Conflict of Interest

The authors declare that there are no conflicts of interest

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494
495 **Tables**

496 **Table 1**: Maternal health characteristics measured in women who had normal
497 pregnancies who later developed PreE and split by fetal sex.

	Women with male fetus		**Women with female fetus**	
Maternal Health Parameters	Normal *n*=48	PreE *n*=27	Normal *n*=52	PreE *n*=23
Age	22 (19-27)	22 (19-26)	23 (20-27)	23 (20-25)
Cigarettes/Day	0 (0-15)	0 (0-0) *	0 (0-7)	0 (0-5)
BMI	26 (21-33)	28 (23-34)	26 (23-34)	25 (23-33)
Height (cm)	165 (160-168)	165 (160-171)	166 (160-169)	161 (157-166)
Weight (kg)	69 (59-84)	71 (59-99)	73 (61-89)	68 (62-84)
SBP (mmHg)	107 (100-112)	110 (106-118) *	108 (104-115)	108 (102-116)
DBP (mmHg)	60 (60-68)	70 (60-74) * #	64 (59-70)	64 (58-70)
Heart Rate (bpm)	84 (78-90)	84 (78-90)	82 (75-88)	86 (78-93)
Rate Pressure Product	8 747 (7 800-9 744)	9 450 (8 850-10 560)	8 768 (8 015-9 856)	9 504 (8 200-9 870)
C-reactive Protein (mg/L)	4.5 (2.6-8.5)	7.1 (4.5-13.0) *	6.9 (3.2-11.0)	3.8 (2.8-12.0)
FENa	0.45 (0.27-0.62)	0.39 (0.23-0.54)	0.50 (0.34-0.71)	0.40 (0.34-0.54)
UNa:K	2.0 (1.3-2.8)	1.8 (1.1-2.3)	2.3 (1.5-3.1)	2.1 (1.6-2.4)
UAlb:Cr	0.5 (0.4-0.8)	0.7 (0.3-0.8)	0.5 (0.4-0.6)	0.5 (0.4-0.7)
UPr:Cr	0.010 (0.008-0.015)	0.012 (0.008-0.015)	0.010 (0.008-0.014)	0.009 (0.006-0.013)

Uterine Artery RI	0.60 (0.54-0.62)	0.59 (0.53-0.64)	0.58 (0.52-0.61)	0.62 (0.55-0.68) *
Umbilical Artery RI	0.73 (0.71-0.78)	0.74 (0.71-0.78)	0.76 (0.73-0.79) #	0.78 (0.74-0.81) ^
Placental Weight (g)	596 (500-700)	620 (500-720)	600 (500-700)	520 (465-665)
Birth Weight (g)	3 540 (3 285-3 783)	3 510 (3 180-3 690)	3 470 (3 310-3 840)	3 270 (3 000-3 725) *

All data were collected at 15 weeks' gestation except the Doppler sonography (20 weeks' gestation) and obstetric data (recorded at birth). Data are presented as median (interquartile range). BMI=body mass index, BP=blood pressure, FENa (%)=fractional excretion of sodium, UNa:K=urinary sodium to potassium ratio, UAlb:Cr (mg/mmoL)=urinary albumin to creatinine ratio, UPr:Cr (g/mmoL)=urinary protein to creatinine ratio, RI=resistance index. Mann-Whitney U tests used to identify significant differences. *$P<0.05$ differences between pregnancy outcome, $^{\#}P\leq0.05$ differences between fetal sex.

513 **Table 2**: Measurements of maternal health analysed by fetal sex, in women who had
514 normal pregnancies or who subsequently developed GH.

	Women with male fetus		**Women with female fetus**	
Maternal Health Parameters	Normal *n*=51	GH *n*=29	Normal *n*=49	GH *n*=21
Age	23 (19-28)	24 (19-26)	23 (20-27)	23 (22-29)
Cigarettes/Day	0 (0-15)	0 (0-4)	0 (0-7)	0 (0-15)
BMI	27 (24-36)	25 (24-35)	27 (24-36)	29 (26-34)
Height (cm)	165 (161-168)	166 (163-169)	166 (160-169)	165 (162-171)
Weight (kg)	74 (63-96)	76 (65-99)	73 (66-96)	82 (76-93)
SBP (mmHg)	108 (100-112)	116 (110-120) *	108 (104-116)	118 (110-124) *
DBP (mmHg)	62 (60-69)	68 (60-72) *	64 (60-70)	70 (66-80) *
Heart Rate (bpm)	84 (78-89)	86 (81-92)	82 (75-88)	84 (80-89)
Rate Pressure Product	8 960 (8 148-9 744)	9 968 (8 680-10 506) *	8 820 (8 000-9 912)	10 080 (8 874-11 040) *
C-reactive Protein (mg/L)	5.0 (2.8-8.9)	9.0 (4.4-18.5) *	7.1 (3.2-11.0)	6.7 (3.3-11.0)
FENa (%)	0.37 (0.24-0.54)	0.43 (0.28-0.52)	0.50(0.34-0.68)	0.45 (0.37-0.49)
UNa:K	1.7 (1.2-2.7)	1.5 (1.2-1.8)	2.4 (1.5-3.1)	1.6 (1.3-2.3)
UAlb:Cr	0.5 (0.4-0.7)	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.6)
UPr:Cr	0.01 (0.008-0.015)	0.01 (0.007-0.013)	0.01 (0.008-0.014)	0.01 (0.009-0.012)
Uterine Artery RI	0.60 (0.53-0.63)	0.58 (0.53-0.63)	0.58 (0.52-0.62)	0.62 (0.55-0.66)
Umbilical Artery	0.73 (0.71-0.78)	0.75 (0.72-0.78)	0.76 (0.73-0.79)	0.75 (0.73-0.79)

RI				
Placental Weight (g)	600 (520-695)	600 (520-670)	600 (500-700)	525 (410-630) #
Birth Weight (g)	3 560 (3 310-3 785)	3 575 (3 290-3 928)	3 550 (3 320-3 846)	3 350 (3 160-3 510) * #

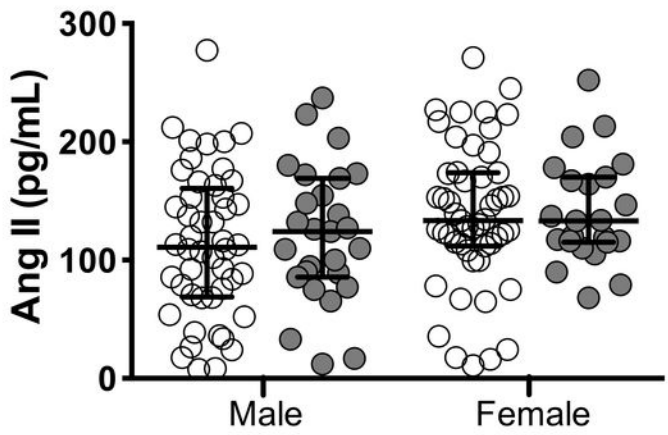
All data were collected at 15 weeks' gestation except the Doppler sonography (20 weeks' gestation) and obstetric data (recorded at birth). Data are presented as median (interquartile range). BMI=body mass index, BP=blood pressure, FENa (%)=fractional excretion of sodium, UNa:K=urinary sodium to potassium ratio, UAlb:Cr (mg/mmoL)=urinary albumin to creatinine ratio, UPr:Cr (g/mmoL)=urinary protein to creatinine ratio, RI=resistance index. Mann-Whitney U tests used to identify significant differences. *$P<0.05$ differences between pregnancy outcome, #$P\leq0.05$ differences between fetal sex.

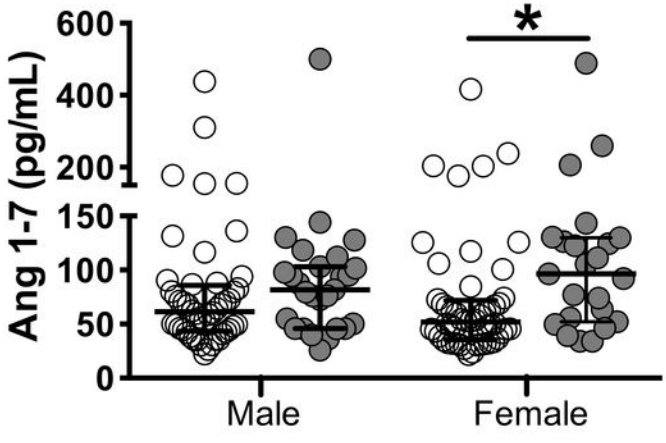
535 **Figures**

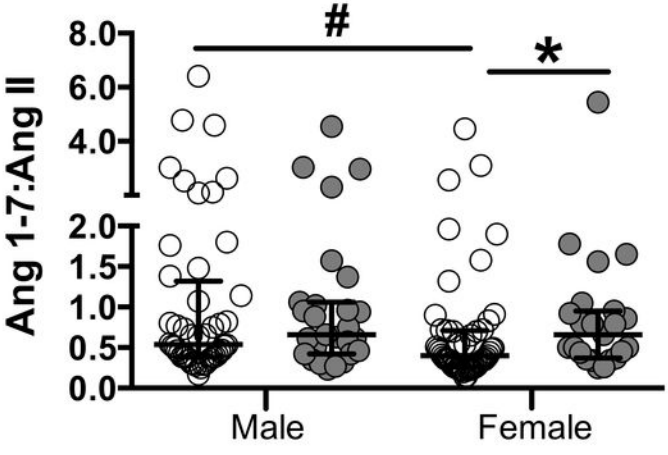
536 **Figure 1**: The fetal-sex associated differences in the maternal RAAS profile at 15
537 weeks' gestation in women who had normal pregnancies (○, M: *n*=48, F: *n*=52) or who
538 were later diagnosed with PreE (●, M: *n*=27, F: *n*=23). Data are presented as scatter
539 plots with median and interquartile range shown by the coloured lines. **P*≤0.05 and
540 $^{\#}P$≤0.05 using Mann-Whitney U tests to identify differences between outcomes within
541 each subpopulation of fetal sex and differences between fetal sexes within in each
542 subpopulation of outcome, respectively.

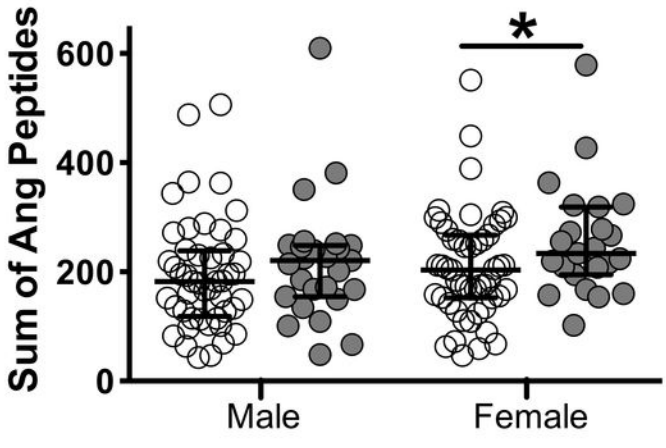
543

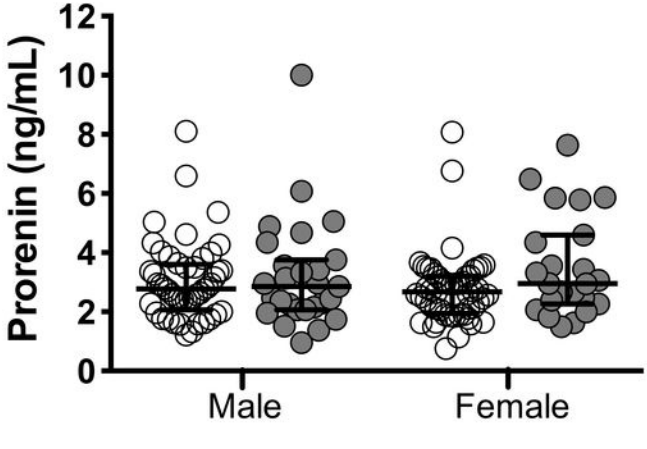
544 **Figure 2**: The maternal RAAS profile at 15 weeks' gestation and the influence of fetal
545 sex in women who had normal pregnancies (○, M: *n*=51, F: *n*=49) or who
546 subsequently developed GH (●, M: *n*=29, F: *n*=21). Data are presented as scatter plots
547 with median and interquartile range shown by the coloured lines. **P*≤0.05 and $^{\#}P$≤0.05
548 using Mann-Whitney U tests to identify differences between outcomes within each
549 subpopulation of fetal sex and differences between fetal sexes within in each
550 subpopulation of outcome, respectively.

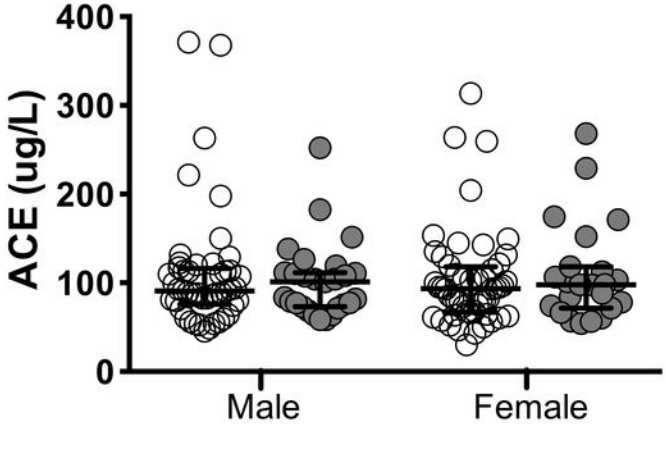

Ang II (pg/mL)
300
200
100
0
Male
Female

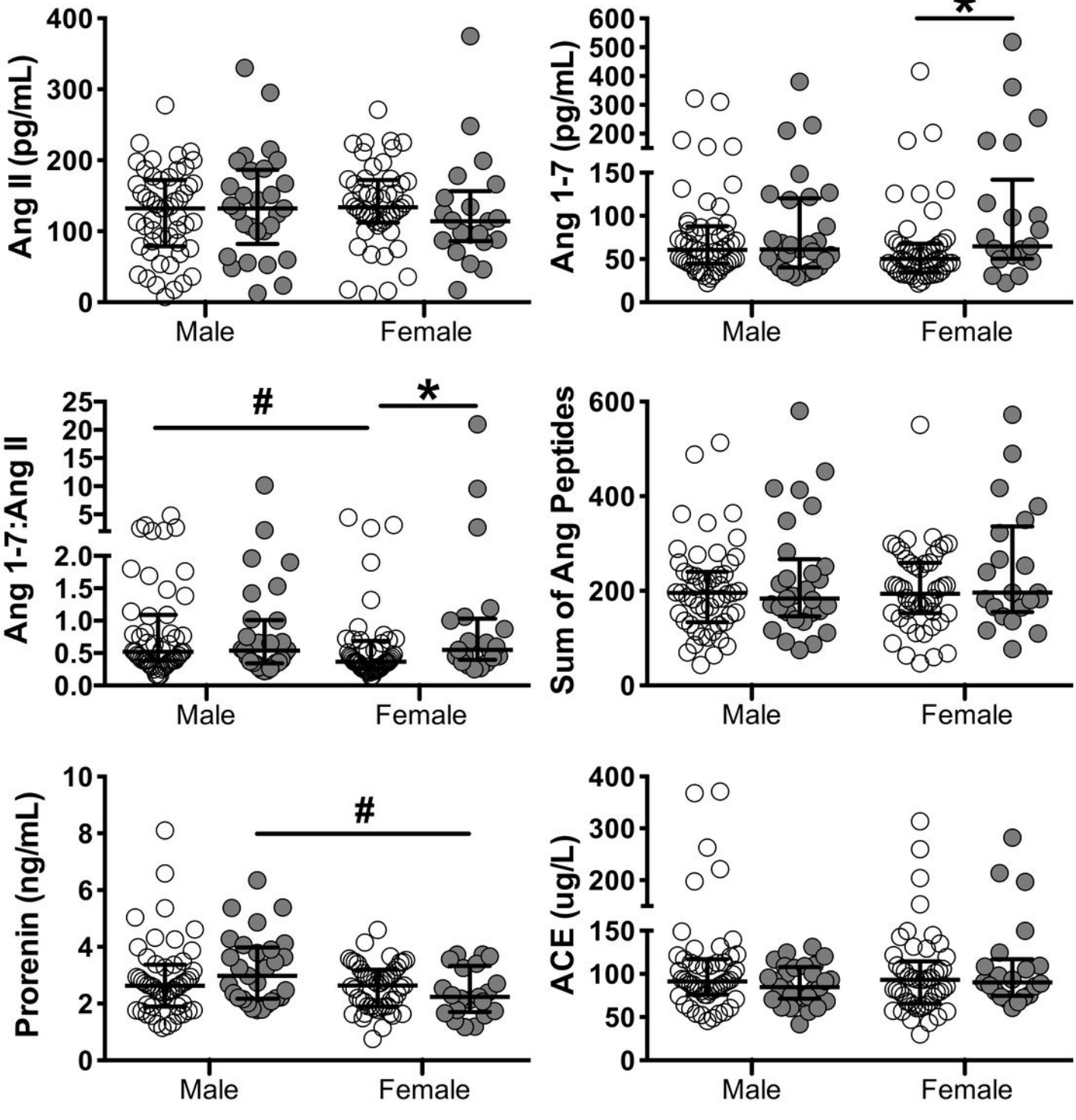

Ang 1-7 (pg/mL)
600
400
200
150
100
50
0
*
Male
Female


Ang 1-7:Ang II
8.0
6.0
4.0
2.0
1.5
1.0
0.5
0.0
#
*
Male
Female


Sum of Ang Peptides
600
400
200
0
*
Male
Female


Prorenin (ng/mL)
12
10
8
6
4
2
0
Male
Female


ACE (ug/L)
400
300
200
100
0
Male
Female


Ang II (pg/mL)
400
300
200
100
0
Male
Female
Ang 1-7 (pg/mL)
600
500
400
300
200
150
100
50
0
*
Male
Female
Ang 1-7:Ang II
25
20
15
10
5
2.0
1.5
1.0
0.5
0.0
#
*
Male
Female
Sum of Ang Peptides
600
400
200
0
Male
Female
Prorenin (ng/mL)
10
8
6
4
2
0
#
Male
Female
ACE (ug/L)
400
300
200
150
100
50
0
Male
Female