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# Urinary angiotensinogen excretion in Australian Indigenous and non-Indigenous pregnant women

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ABSTRACT

The intrarenal renin-angiotensin system (iRAS) is implicated in the pathogenesis of hypertension, chronic kidney disease and diabetic nephropathy. Urinary angiotensinogen (uAGT) levels reflect the activity of the iRAS and are altered in women with preeclampsia. Since Indigenous Australians suffer high rates and early onset of renal disease, we hypothesised that Indigenous Australian pregnant women, like non-Indigenous women with pregnancy complications, would have altered uAGT levels. The excretion of RAS proteins was measured in non-Indigenous and Indigenous Australian women with uncomplicated or complicated pregnancies (preeclampsia, diabetes/gestational diabetes, proteinuria/albuminuria, hypertension, small/large for gestational age, preterm birth), and in non-pregnant non-Indigenous women.

Non-Indigenous pregnant women with uncomplicated pregnancies, had higher uAGT/creatinine levels than non-Indigenous non-pregnant women ($P < 0.01$), and levels increased as pregnancy progressed ($P < 0.001$). In non-Indigenous pregnant women with pregnancy complications, uAGT/creatinine was suppressed in the third trimester ($P < 0.01$). In Indigenous pregnant women with uncomplicated pregnancies, there was no change in uAGT/creatinine with gestational age and uAGT/creatinine was lower in the 2nd and 3rd trimesters than in non-Indigenous pregnant women with uncomplicated pregnancies ($P < 0.03$, $P < 0.007$, respectively). The uAGT/creatinine ratios of Indigenous women with uncomplicated or complicated pregnancies were the same.

A decrease in uAGT/creatinine with advancing gestational age was associated with increased urinary albumin/creatinine, as is seen in preeclampsia, but it was not specific for this disorder. The reduced uAGT/creatinine in Indigenous pregnant women may reflect subclinical renal dysfunction which limits the ability of the kidney to maintain sodium balance and could indicate an increased risk of pregnancy complications and/or future renal disease.

## 1. Introduction

There are known alterations in the circulating renin-angiotensin aldosterone system (RAAS) in complications of pregnancy including gestational hypertension, preeclampsia [1,2], and gestational diabetes [3,4]. However, the underlying pathogenic mechanisms of these disorders remain uncertain.

Normal pregnancy is associated with activation of the circulating renin-angiotensin aldosterone system (RAAS). This is partly through oestrogen-induced increases in hepatic angiotensinogen (AGT) synthesis and secretion [5,6]. In response to the substantial fall in systemic vascular resistance that threatens to lower blood pressure there is also an increase in the release of both renin and prorenin [5–8]. These changes occur very early in pregnancy and the circulating RAAS plays a critical role in stimulating salt and water reabsorption, which is necessary to expand maternal blood volume and to offset the diuretic

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effects of an increased glomerular filtration rate (GFR) and high progesterone levels [9]. Angiotensin (Ang) II, the major end product of the interaction between renin and angiotensinogen, causes vasoconstriction and stimulates renal sodium reabsorption directly and by releasing aldosterone from the adrenal cortex [9]. Preeclampsia, in particular when associated with delivery of small for gestational age (SGA) infants, and normotensive SGA pregnancies, are known to be associated with impaired plasma volume expansion [10] and a reduction in the activity of the circulating RAS [11].

There is also an intrarenal renin-angiotensin system (iRAS), which is likely to contribute, in part, to the increased retention of sodium in pregnancy. Key components of the RAS are synthesised in the renal tubules. AGT is synthesised in the proximal convoluted tubule. Its synthesis is stimulated by Ang II/$AT_1$R-mediated activation of the MAPK/NFκB pathway [12]. Prorenin and renin are not only synthesised in the juxtaglomerular apparatus but also in the distal nephron [13]. Their secretion is stimulated by Ang II formed in the proximal tubule acting via the $AT_1$R [13]. Renin can act on intratubular AGT spilling over into the distal nephron from the proximal tubule [14]. ACE is found throughout the entire nephron [15]. While uAGT excretion is not increased in response to a low sodium diet, medullary renin is increased [16]. As well, tubular flow rate, i.e GFR, is associated with increased expression and excretion of AGT into urine [17]. Therefore both the high circulating Ang II levels present in pregnancy and the hyperfiltration of pregnancy could stimulate the iRAS. Furthermore, both the circulating and intrarenal RASs could play a role in regulating sodium homeostasis in pregnancy.

Intrarenal RAS activity, as measured by uAGT/creatinine is increased in individuals with hypertension [18] and chronic kidney disease [19], and in children with type 1 diabetes [20]. It is also elevated in normotensive patients with Type II diabetes mellitus, prior to the onset of albuminuria, suggesting that it may be an early marker of renal disease [21]. Despite these findings, there have been very few reports on the activity of the iRAS in human pregnancy. Three reports found that uAGT levels were depressed in women with preeclampsia and one found it increased [22–25].

Indigenous Australian women are more likely to develop preeclampsia [26], and gestational diabetes [27], have a significant incidence of microalbuminuria, and have a 10 times greater incidence of chronic kidney disease [28] compared with non-Indigenous women. Therefore, we set out to measure and compare levels of urinary RAS components in Indigenous and non-Indigenous Australian women in pregnancy. We considered both uncomplicated and complicated pregnancy outcomes to determine if there were significant changes in excretion of RAS proteins, and if there was any association between uAGT/creatinine and poor pregnancy outcome in these populations. In particular, we aimed to determine if uAGT/creatinine and other measured RAS proteins were altered when there was evidence of renal dysfunction as the iRAS has been shown to be activated and implicated in the pathogenesis of chronic kidney disease [19].

## 2. Methods

### 2.1. Study design

This study used samples and data from several different cohort studies. Women self-identified as being of Aboriginal or Torres Strait Islander descent or not, and on this basis, were classified as Indigenous or Non-Indigenous, respectively.

Non-Indigenous non-pregnant women (n = 10) were recruited in Newcastle, NSW as previously reported [29]. Non-Indigenous pregnant women were recruited from the Adelaide SCreening fOr Pregnancy Endpoints (SCOPE) cohort study (n = 216), the Birth, Estrogen, Estradiol and Progesterone (BEEP) study (n = 16) and from the Gomeroi Gaaynngal Study (Tamworth and Walgett, NSW) (n = 9, [30]). Women in the SCOPE cohort were selected based on their pregnancy outcome (uncomplicated: n = 95, preeclampsia: n = 71, GDM: n = 38, proteinuria: n = 15). Indigenous pregnant women were enrolled in the Gomeroi Gaaynngal Study (n = 116) recruited from Tamworth, Walgett and Newcastle, NSW. All participants gave written, informed consent.

The Gomeroi gaaynngal study is a prospective, longitudinal cohort study involving Indigenous and non-Indigenous pregnant women. Pregnant women who identified as Indigenous Australians, or pregnant non-Indigenous women with Indigenous partners were eligible to participate and could enrol at any stage in their pregnancy. Participants were recruited by Indigenous research assistants at antenatal clinic locations at all sites, including Indigenous antenatal birth centres and an Aboriginal Community Controlled service [31]. The research team aimed to see participants once per trimester during pregnancy, with sample collections and physical measures occurring at times that suited participants (range: 6–40 weeks). The Adelaide SCOPE cohort aimed to predict the major complications of late pregnancy. Nulliparous women with a singleton pregnancy were recruited into the cohort during antenatal visits at the Lyell McEwin Hospital (Elizabeth Vale, South Australia). Healthy women who were less than 15 weeks' gestation and who had less than three previous terminations of pregnancy or miscarriages were eligible.

Non-Indigenous term pregnant women who delivered between 41 and 42 weeks' gestation were recruited from the John Hunter Hospital in Newcastle in late gestation as part of the BEEP study.

Data from the pregnancy cohorts were pooled to form 4 groups in order to compare Indigenous and non-Indigenous uncomplicated and complicated pregnancies. The patient outcomes are outlined in Table 1. Non-Indigenous and Indigenous women had an uncomplicated pregnancy if they remained normotensive (SP < 140 and/or DP < 90 mmHg prior to labour), showed no proteinuria or albuminuria, delivered a live born baby after 37 weeks who was not small or large for gestational age and had no other sign of pregnancy complications. Since pregnancy complications often occur together (e.g. preeclampsia with preterm delivery or SGA) it can be very difficult to define each pregnancy. Therefore, for the purposes of this study we chose to define adverse pregnancy outcomes according to whether they were primary or secondary outcomes. For example, women with preeclampsia who delivered prematurely were defined as having preeclampsia (the primary outcome), since the preterm delivery was likely a result of the preeclampsia. For these reasons, preeclampsia, hypertension and diabetes were defined as primary outcomes and may include women that delivered preterm or an SGA baby. Conversely, women who were classified as being in the preterm, LGA, SGA, or Preterm + SGA groups had no evidence of any other maternal disease. Non-Indigenous women with pregnancy complications (n = 135) included 70 women with preeclampsia (PreE), 38 with diabetes in pregnancy (37 with GDM, 1 with type 2 diabetes), and 3 with concurrent hypertension and diabetes in pregnancy. Indigenous women with pregnancy complications (n = 69) included 23 who had proteinuria and or albuminuria in pregnancy without any evidence of hypertension, 8 who delivered

**Table 1**
Pregnancy outcomes of Indigenous and non-Indigenous women used in this study and classified as having a pregnancy complication.

| | Non-Indigenous | | Indigenous | |
|---|---|---|---|---|
| | N | % | N | % |
| Hypertension | 4 | 2.96 | 4 | 5.8 |
| Preeclampsia | 70 | 51.9 | 5 | 7.24 |
| Diabetes | 38 | 28.2 | 9 | 13.04 |
| Hypertension/PreE + Diabetes | 3 | 2.22 | 2 | 2.89 |
| Proteinuria or Albuminuria | 18 | 13.3 | 23 | 33.3 |
| Preterm | – | – | 8 | 11.6 |
| SGA | 1 | 0.74 | 12 | 17.3 |
| LGA | – | – | 4 | 5.8 |
| Preterm + SGA | 1 | 0.74 | 2 | 1.45 |