Section 1

Renal physiology in pregnancy

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Chapter 1

Recent advances in renal physiology in pregnancy

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Focus areas for this chapter

Mechanisms of increase in glomerular filtration rate

A robust and maintained renal vasodilation and increased glomerular filtration in pregnancy are good signs for both maternal and fetal outcome. In women with underlying chronic kidney disease (CKD), the level of prepregnancy renal function predicts outcome, with a serum creatinine (SCr) above approximately 120 μmol/l signalling increases in maternal and fetal risks. The presence of hypertension and/or heavy proteinuria increases the risk of accelerated loss of function. If the rise in glomerular filtration rate (GFR) occurs and persists during pregnancy in a woman with underlying CKD, the pregnancy outcome will probably be good and pregnancy is likely to have little long-term impact on maternal kidney function.[1–6] We still do not know the mechanisms that lead to the increased GFR or whether the requisite renal vasodilation is regulated separately from the systemic vasodilation that also occurs early in normal pregnancy.

What determines plasma volume expansion: primary vasodilation (underfill) or primary renal sodium retention and secondary vasodilation?

The systemic vasodilation is critical to accommodate the expanding plasma volume, another major haemodynamic event and prognostic of a successful outcome in both maternal and fetal terms.[7] Does the peripheral vasodilation occur first and drive renal sodium retention and volume expansion,[8] or does primary renal sodium retention begin the sequence of events?

Importance of vascular endothelial growth factor in glomerular health and disease, including pre-eclampsia

The remarkable studies by Karumanchi and colleagues have implicated derangements in vascular endothelial growth factor (VEGF) action in the pathogenesis of pre-eclampsia.[9–11] These observations have also focused attention on the fact that VEGF plays an important role in the maintenance of normal glomerular health in the nonpregnant adult.[12,13] There is a 'dose' effect of VEGF on glomerular structural and functional

integrity and it seems that VEGF can either ameliorate or exacerbate glomerular injury in various models of CKD.[14] We need a better understanding of the role of the VEGF system in the control of normal glomerular function (during normal pregnancy and in the healthy nonpregnant individual) and in various types of renal disease, in order to unravel the role of derangements in VEGF signalling in pre-eclampsia.

Introduction

There are profound adaptations in renal function during pregnancy in normal women, including an early increase in GFR that is first detectable within 3–4 weeks of conception (Figure 1.1).[15] GFR continues to rise to a maximum of 40–50% above nonpregnant values by the end of the first trimester and this increase is maintained throughout most of the rest of the pregnancy, although a late fall in GFR occurs in the few weeks before delivery.[16] An optimal increase in GFR is a good prognosticator for a successful pregnancy outcome. For example, in a serial study of a small group of normal pregnant women, two women who failed to show the early rise in GFR subsequently suffered early miscarriage (Figure 1.1).[15] Also, in women with underlying CKD, those who show a gestational rise in GFR tend to have the best outcomes.[1] There are also marked systemic haemodynamic changes, including increases in plasma volume and cardiac output which, together with large reductions in total peripheral vascular resistance, lead to falls in blood pressure.[17–19]

How the renal and systemic changes are related is unclear. Does the renal vasodilation precede the systemic effects, perhaps driving them, or is there a coordinated, generalised vasodilatory response in early pregnancy that includes the kidney? Is the

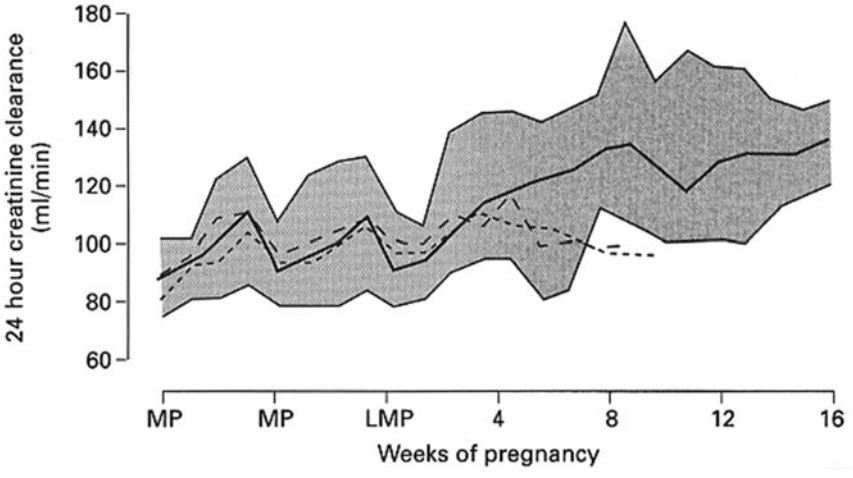

Figure 1.1. Changes in 24 hour creatinine clearance measured weekly before conception and throughout the first 16 weeks of normal pregnancy in nine women with successful obstetric outcome (the solid line represents the mean and the stippled area the range); the broken lines give the course in two women who progressed to miscarriage in the first trimester; MP = menstrual period; LMP = last menstrual period; reproduced with permission from Davison and Noble[15]

vasodilation the primary event that drives the renal sodium retention and plasma volume expansion, or is there a primary renal sodium retention that somehow triggers a vasodilatory vascular reply? One area for targeted research is for combined renal and systemic haemodynamic measurements, particularly during early pregnancy, to establish the relative relationship between systemic and renal vasodilation and the plasma volume expansion. The only study to attempt this, by Chapman *et al.*[20] in normal women, reported that by week 6 of pregnancy both the renal and systemic vasodilation was maximal, the renin–angiotensin system was activated and plasma volume expansion was underway. Unfortunately, there were no measurements earlier in pregnancy and thus the sequence of these events remains unknown.

Rats exhibit systemic and renal haemodynamic changes during normal pregnancy that are similar to those in humans, while the total gestation period is only 22 days. During pregnancy there is a progressive plasma volume expansion, a fall in blood pressure and a rise in GFR of approximately 30% (which falls toward nonpregnant values shortly before delivery).[21]

Mechanism of the gestational rise in GFR

Haemodynamics

Studies in the pregnant rat reveal that the increased GFR is accompanied by a parallel increase in renal plasma flow (RPF), due to renal vasodilation. Glomerular micropuncture studies have shown that both afferent and efferent arteriolar resistances (R_A and R_E, respectively) relax in parallel, which allows a selective increase in glomerular plasma flow to occur.[21] Importantly, parallel reductions in R_A and R_E allow the kidney to vasodilate and permit increases in RPF and GFR without an increase in glomerular blood pressure. Davison and colleagues[22] have used an indirect modelling approach to estimate the impact of normal pregnancy on glomerular haemodynamics. Much like in the rat, the majority of the increased GFR in pregnant women is due to increased RPF, although a small component may be due to falls in plasma protein concentration (reducing colloid osmotic pressure of blood entering the glomerulus). There is no evidence that the glomerular blood pressure increases during normal pregnancy, suggesting that parallel relaxation of R_A and R_E also occurs in normal pregnant women. This finding has important implications for the long-term effects of pregnancy on renal function, since states of chronic renal vasodilation where R_A is selectively relaxed lead to glomerular hypertension and development of injury.[23]

It is known that in the rat maternal factors initiate the gestational renal events since renal vasodilation and increases in GFR and RPF occur in pseudopregnant rats where fetoplacental tissue is absent.[24] In addition, both the peripheral vasodilation and plasma volume expansion are evident in pseudopregnant rats.[24,25] In pregnant women, renal and peripheral vasodilation is well established prior to complete placentation,[20] suggesting that the fetoplacental unit may not be necessary for initiation of the increase in GFR and/or the volume expansion of pregnancy.

Vasoactive factors

Studies in the rat have implicated nitric oxide (NO) as the renal vasodilatory agent of pregnancy. Both plasma and urinary levels of inorganic nitrite and nitrate (NO_x; oxidation products of NO) and cyclic guanosine monophosphate (cGMP; a major second messenger of NO) increase during pregnancy in rats,[26–28] suggesting an increase in total systemic NO production, which could include kidney. In women, 24 hour

urinary excretions of cGMP and plasma cGMP increase in normal pregnancy[29,30] although the persistence of this increased cGMP excretion long after delivery[20] may reflect the prolonged postpartum rise in plasma atrial natriuretic peptide (ANP), since this agent also signals through cGMP.[31] The findings with plasma and urinary NO_x levels in women have been variable[32] and the only study conducted using a controlled low NO_x intake (a requirement for interpretation of NO_x values in the context of NO activity),[33] reported no elevation in plasma or 24 hour urinary NO_x excretion in late pregnancy compared with the nonpregnant state.[34] It should be noted that renal NO generation represents only a few percent of the total NO_x production[33] and thus a selective local increase in renal NO production would probably be undetected by NO_x measurement in body fluids.

Danielson and Conrad have reported that low-level, acute (nonselective) nitric oxide synthase (NOS) inhibition can reverse the pregnancy-induced rise in GFR without affecting the value of GFR in nonpregnant rats.[35] We reported that chronic nonselective NOS inhibition prevents the gestational rise in GFR and the renal vasodilation,[36] which has been confirmed by Schrier and colleagues.[37] Although *in vivo*, the specificity of 'isoform-specific' inhibitors is questionable, functional studies performed in rats have implicated both the neuronal and inducible NOS isoforms in mediating the renal vasodilation.[38,39] There are no endothelial NOS (eNOS)-specific inhibitors available but the *in vitro* data show midterm decreases in renal cortex total eNOS protein abundance,[40,41] activated phosphorylated (ser 1173) eNOS[41] and membrane fraction NOS activity (predominantly eNOS).[42] According to our *in vivo* and *in vitro* studies, the NOS isoform associated with the midterm renal vasodilation does exhibit functional characteristics of both the neuronal and inducible NOS.[42,43] We observed an increase in the NOS activity, *in vitro*, of renal cortex in the midterm pregnant rat, but only in the soluble fraction which houses predominantly the neuronal and inducible NOS (nNOS and iNOS, respectively). This agrees with a report by Alexander *et al*.[41] of increasing renal nNOS and iNOS protein during pregnancy in rat kidney although we have observed that the renal cortex nNOS protein abundance is unchanged at midterm.[41] However, our laboratories used different nNOS antibodies: one recognising the amino terminal (Baylis and colleagues) and one recognising the carboxy terminal (Alexander and colleagues) of the 160 kDa, nNOS-α protein. There are splice variants of the nNOS gene that encode for fully functional, amino-terminal truncated proteins and using a carboxy-terminal antibody we now report that one of these, the nNOS-β, is upregulated at both the mRNA and protein level in the renal cortex in pregnancy in phase with the renal vasodilation.[44] We are currently exploring the significance and mechanism of the nNOS-β activation in the kidney during pregnancy. It is important to point out that all these data were obtained in rats and that there is no evidence in pregnant women to confirm or exclude a role for NO in the renal response.

Signalling events leading to NO-dependent renal vasodilation

Functional and *in vitro* studies in rats suggest an important role for NO in the gestational renal vasodilation but what is the trigger? It is known that in the rat this is a maternal signal and estrogens have been implicated since they have a widespread stimulatory action on the NO system in nonpregnant women.[45] Estrogens, however, are unlikely to provide the primary stimulus to renal vasodilation since, in the rat, estrogen levels fall throughout pregnancy, increasing only just before term when the renal vasodilation is waning.[46] A novel and exciting possibility was raised by Conrad

et al.,[47,48] who suggested that the ovarian hormone relaxin might provide the trigger. Exogenous relaxin has a powerful NO-dependent renal vasodilatory action in nonpregnant female rats, with and without ovaries, and in male rats. Chronic relaxin administration also dampens the renal vasoconstrictor response to angiotensin II and reduces the myogenic response in resistance-sized renal arteries,[47,49] changes also seen in the kidney during pregnancy.[50,51] The most direct evidence is that removal of relaxin from the pregnant rat, by neutralising antibody (Figure 1.2) or ovariectomy, eliminates the gestational renal vasodilation.[52]

There are limited clinical data, which so far are equivocal. Women who conceive by ovum donation have no circulating relaxin and have blunted increases in creatinine clearance.[53] Healthy women show an elevated GFR during the luteal phase of the menstrual cycle, when circulating relaxin levels are elevated.[20,54] Administration of human chorionic gonadotrophin (hCG), which stimulates further ovarian relaxin release in the late luteal phase, produced no further renal haemodynamic response

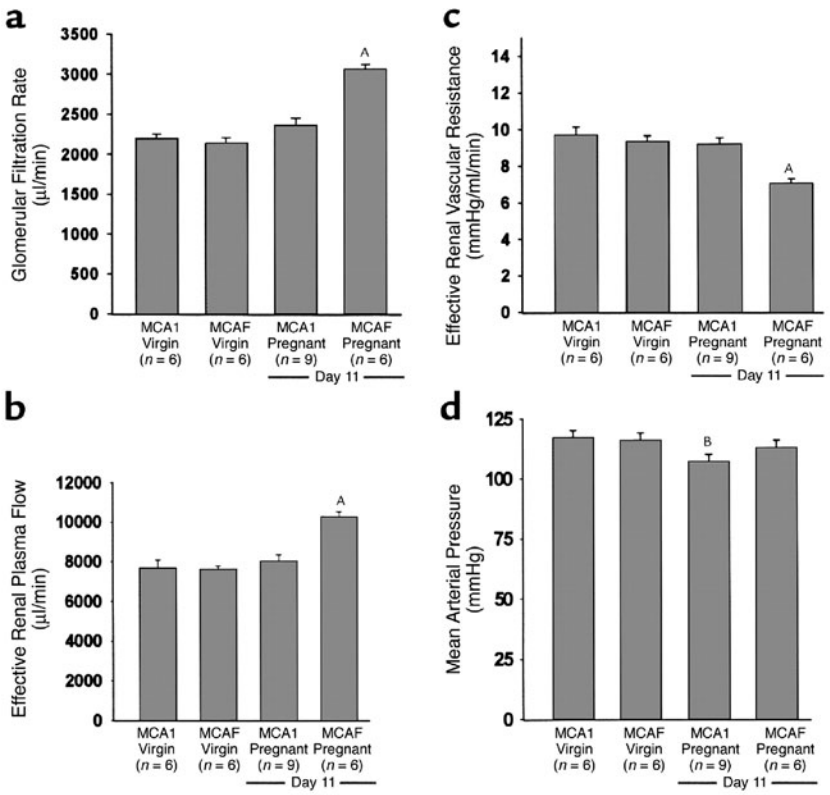

Figure 1.2. Renal function in virgin and 11 day pregnant, conscious rats treated with a relaxin-neutralising antibody (MCA1) or inactive antibody (MCAF); (a) glomerular filtration rate; (b) effective renal plasma flow; (c) effective renal vascular resistance; (d) mean arterial blood pressure; $^{A}P < 0.01$ versus other groups; $^{B}P < 0.05$ versus MCA1 and MCAF virgin; reproduced with permission from Novak *et al.*[52]

in cycling women, possibly because the elevation in relaxin achieved was below the pregnancy values.[54] Smith *et al.*[55] subsequently reported that acutely administered relaxin, to both nonpregnant women and to men, results in a marked increase in RPF, due to renal vasodilation. Unexpectedly, despite the large rise in RPF there was no accompanying increase in GFR, which must mean offsetting changes occurred in the other determinants of GFR.[56] Acutely administered relaxin, in humans, thus does not mimic all the renal haemodynamic responses of the rat, nor of normal pregnancy. Studies with chronic relaxin administration in humans are eagerly awaited.

The relaxin-mediated renal vasodilation in the rat requires NO since NOS inhibition/endothelium removal abrogates the response.[47,49,51] Endothelin also plays a role since the renal haemodynamic responses in normal pregnancy are prevented by blockade of the endothelin type B (ET_B) receptor.[57] ET_B blockade also prevents the relaxin-induced renal haemodynamic responses.[48,49] The link between relaxin and endothelin is probably mediated via relaxin-induced upregulation of the matrix metalloproteinase 2 (MMP2) which functions as an alternative endothelin-converting enzyme to stimulate endothelin production.[58,59] Most probably, endothelial ET_B-mediated NO release provides the vasodilatory stimulus to relaxin, although how this relates to the falls in eNOS abundance/activity reported in midterm pregnancy[40–42] and our recent finding of increased renal nNOS-β in midterm pregnancy are the subject of continuing investigations.

In summary, the animal literature suggests that NO plays an important role in mediating the gestational rise in GFR via a relaxin-mediated signalling cascade. While the studies in the rat are quite convincing, studies in women are needed that develop and test these hypotheses. Once the normal signals that evoke renal vasodilation in pregnant women have been established, it will be important to determine whether defects in this signalling system occur where pregnancy adversely impacts maternal renal function.

What determines the plasma volume expansion?

Volume perception in pregnancy

Net sodium accumulation in the extracellular fluid space must drive the maternal plasma volume expansion. Since plasma osmolality falls a little in normal pregnancy there must be excess net free water retention.[18] For a continual sodium retention to coexist in the presence of maintained plasma volume expansion, there must be drastic alterations in the volume sensing and regulatory systems of the body. Normal nonpregnancy physiology would prevent a prolonged rise in plasma volume expansion by activating natriuretic systems and suppressing antinatriuretic systems, to force plasma volume back to a set point considered 'normal'. In contrast, normal pregnancy is a state of continued plasma volume expansion coexisting with continued renal sodium retention. The activity of the primary antinatriuretic system, the renin–angiotensin–aldosterone system (RAAS), is markedly elevated. These findings have led Schrier and colleagues to suggest that normal pregnancy is in fact a chronically 'underfilled' state where the effective circulating volume is always seen as inadequate.[8] According to this hypothesis, there is a primary drive to peripheral vasodilation early in pregnancy that creates the underfill signal and precedes the plasma volume expansion. At present there are unfortunately no serial data early in pregnancy that can separate the cause and effect relationship between peripheral resistance and plasma volume expansion since, as reported by Chapman *et al.*,[20] all keys events are established as early as week 6

of pregnancy. Although difficult, serial studies in earlier pregnancy are required and should be conducted in women because the small size of rats and need for chronic instrumentation in this species will make detection of early haemodynamic changes in an unstressed preparation unlikely.

The underfill theory is persuasive when only the RAAS is considered but other volume regulatory systems do not conform. On the volume conservation side there are complex changes in the sympathetic nervous system in normal pregnancy but the acute sympathetic response to haemorrhage is reset in the pregnant rat so that the expanded blood volume is viewed as normal.[21,60–62] The arginine vasopressin (AVP) osmoregulatory system is markedly altered in normal pregnancy, with resetting of the osmotic threshold for AVP release to sense the lower plasma osmolality as normal.[63] The volume-dependent component of AVP release is also reset to recognise the continually expanding volume as normal,[63] as is the tubuloglomerular feedback system.[64] Thus, the volume conservation systems other than the RAAS apparently adapt throughout pregnancy to sense the expanding volume as 'normal fill'. Of the natriuretic systems that are activated by 'overfill', ANP shows a delayed small rise in pregnant women[20] and little increase until delivery in the rat,[65] more consistent with mild 'overfill' or 'normal fill' than an 'underfill' signal. The increased renal NO production (discussed above) is consistent with an 'overfill' signal, given the significant natriuretic as well as vasodilatory actions of renal NO.[65]

So, different volume perception pathways view the circulating volume in pregnancy quite differently and, while the 'underfill' theory of plasma volume expansion in pregnancy is very attractive, it requires some very sophisticated resetting of the majority of the individual control systems.

Signals influencing renal sodium excretion in pregnancy

There are many conflicting signals that influence renal sodium excretion in normal pregnancy. There is clearly net renal sodium retention throughout most of pregnancy leading to the plasma volume expansion, which suggests a dominant role for the activated RAAS. However, there are also significant natriuretic signals, including the rise in GFR, maintained or mild increases in ANP and marked increases in NO production (at least in the rat).

Pregnancy is an interesting haemodynamic state, where the renal tubular actions of the antinatriuretic, vasoconstrictor arm of the volume regulatory control predominate (to allow plasma volume expansion), whereas the vascular actions of the natriuretic, vasodilatory systems must predominate to allow peripheral vasodilation (to accommodate the expanded plasma volume). It is well known that vasoconstrictor responsiveness decreases in a normal pregnancy; the pressor response to angiotensin II is profoundly blunted early in normal pregnancy[66] while the antinatriuretic actions persist. Is it also possible that while the vasodilatory actions of the vasodilators persist, a refractoriness develops to their tubular natriuretic actions?

Some studies in the rat have suggested loss of natriuretic responsiveness to administered ANP during normal pregnancy.[67,68] The acute natriuretic response to volume expansion is dependent on endogenous ANP release and this is also blunted in the pregnant rat.[69] The pressure natriuresis (an NO-dependent event) is also blunted in the pregnant rat.[70,71] Since both ANP and NO signal through cGMP, this suggests that the tubular response to cGMP may become blunted and, indeed, we have reported increased cGMP breakdown in the inner medullary collecting duct of the pregnant rat kidney, a major site of natriuretic action of ANP and NP.[69] This

effect is due to a selective, local increase in abundance/activity of phosphodiesterase 5 (PDE5).[69] We recently reported that the natriuretic response to administered ANP in pregnancy could be restored by local intrarenal PDE5 inhibition (Figure 1.3).[72] This natriuretic refractoriness in pregnancy does seem to be cGMP specific since dopamine-induced natriuresis (which signals via increased cyclic adenosine-3′,5′-monophosphate (cAMP)) is unblunted in the pregnant rat.[73]

The hypothesis of local renal tubular loss of cGMP responsiveness but with maintained systemic vascular responsiveness to cGMP is appealing, but whether this has any relevance to human pregnancy is unclear. The only study to directly address the question, by Irons *et al.*,[74] reported that a low dose of infused ANP produced a minimal natriuresis in normal women in late pregnancy and had no impact on sodium excretion in the same women when studied 4 months postpartum. This study was different in design to the animal studies in that a prolonged 60 minute equilibration time was allowed prior to measurement of sodium excretion. The tubular actions of ANP are very rapid and it is possible that a more robust and earlier natriuretic response was missed in the women when studied postpartum. This is an important area to target for further clinical study.

In summary, the volume expansion of normal pregnancy is critical for an optimal outcome for both mother and baby. An understanding of the basic physiological mechanisms is essential so that situations in which the volume expansion is suboptimal can, perhaps, be corrected.

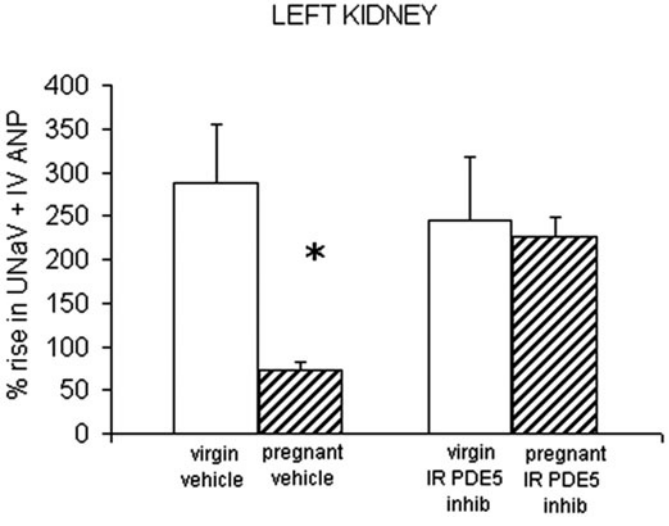

Figure 1.3. The blunted natriuretic response to systemic atrial natriuretic peptide (ANP; 11.6 ng/100 g body weight/minute) is shown in pregnant (versus virgin) rats that received vehicle infusion (2 µl/100 g body weight/minute isotonic NaCl into the left kidney); when the phosphodiesterase 5 (PDE5) inhibitor sildenafil (0.2 µg/100 g body weight/minute) was infused into the left kidney, the natriuretic response to ANP was restored in the pregnant rats; * denotes a significant difference ($P < 0.05$) between virgin and pregnant; IV = intravenous; IR = intrarenal; UNaV = urinary sodium excretion; data derived from Knight *et al.*[72]