Blood pressure, renal functional and structural changes, in normal and preeclamptic pregnancy

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BLOOD PRESSURE, RENAL FUNCTIONAL AND STRUCTURAL CHANGES, IN NORMAL AND PREECLAMPTIC PREGNANCY

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Front cover: Scanning electron micrograph of the glomerular barrier in a glomerulus from a normal rat kidney, depicting its three components: the endothelial cell, the basal membrane and the podocyte foot processes. Reproduced by kind permission from Dr K White, Department of Diabetes and Metabolism, University of Newcastle, Newcastle upon Tyne, United Kingdom.

Back cover: "Children in the Orchard" by John Strevens. Reproduced by kind permission from C.E.O. Idelis, Idelis & Associates Art Gallery, idelis@idelis.com, 709 Richards Avenue, Clearwater, Florida, USA.

John Strevens (1902-1990) was born in London, attended classes at the Regent Street Polytechnic and Heatherley´s Art School, though is largely self-taught. He received initial acclaim in 1947 and since his first success he has exhibited regularly at the Royal Academy and the Paris Salon. Strevens is well known for his colourful presentation of the Edwardian and Victorian eras and he admits that these paintings give expression to his incurable romanticism and devotion to the past. His main interest lies in flower painting and portraiture and he loved painting women and children - his portraits of children are among his most charming and endearing works; his diminutive people are the quintessence of playfulness, innocence and sweetness.

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Success is the ability
To go from failure to failure
Without losing your enthusiasm

Winston Churchill

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time

T. S. Eliot, Four Quartets

With love to "all my famerly"

ABBREVIATIONS

ANOVA	Analysis of variance, *a statistical method*
DF	Degrees of freedom, *a statistical term*
SE	Standard error, *a statistical term describing variability*
SS	Sum of squares, *a statistical term*
BMI	Body mass index, *body weight divided by the square of body length*
CI	Confidence interval, *a statistical term*
C1q, C3, C4	Complement factors 1q, 3 and 4, *serum proteins*
DTPA	Diethylene triamine penta-acetic acid
EDTA	Ethylene diamine tetra-acetic acid
IgA, IgG, IgM	Immunoglobulin A, G and M, *serum proteins with antibody activity*
mRNA	messenger Ribonucleic acid, *play an essential role in the synthesis of proteins*
PAS	Periodic Acid-Schiff reaction, *a histochemical technique for staining carbohydrates*
ROC	Receiving operator characteristic analysis, *a statistical method*
RR	Relative risk, *a statistical term*
w/v	Weight per volume

ABSTRACT

The kidneys play a pivotal role in the adaptive physiology of the pregnant woman, presenting some changes at term similar to the changes found in preeclampsia – a state of increased risk of fetal and maternal morbidity and mortality. The aim of these investigations was to explore possible similarities in blood pressure regulation, renal function and structure in normal term and preeclamptic pregnancy, to ascertain whether both conditions in fact are different degrees of an adaptive process in reaction to pregnancy.

Blood pressure changes were studied in 600 normal pregnancies and in 166 women through their first three pregnancies, revealing an influence of gestational age, ethnicity, parity, baseline BMI and smoking habits - factors similar to known risk factors for preeclampsia. Renal functional changes were studied in 48 pregnant and 12 non-pregnant women, revealing a size- and/or charge-dependant alteration in the filtration process in pregnant women at term compared to non-pregnant women, the glomerular filtration further impaired in preeclampsia. Renal structural changes were studied in 36 hypertensive pregnant women and 12 healthy term pregnant women, and the typical preeclamptic lesion, glomerular endotheliosis, was found not only in all hypertensive patients but also in seven of the twelve controls. Clinically undetected renal disease was not diagnosed in any of the women, leaving very few clinical indications for the renal biopsy in pregnancy.

This opinion could be underscored by the findings that cystatin C levels in pregnancy, not only reliably reflected the glomerular filtration rate, but also correlated significantly with estimated glomerular volume and the degree of endotheliosis. Serum cystatin C consequently seemed to closely reflect the renal functional and structural changes, which are believed to lead to increased blood pressure levels and urinary excretion of albumin, and may thus function as a marker for the stage of the transition between normal adaptive renal changes at term and preeclampsia.

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INTRODUCTION

The kidneys play a pivotal roll, both in the adaptive physiology of normal pregnancy and in the pathophysiology of preeclampsia[1] and some changes in renal function are found to be common to term pregnancy and preeclampsia[2]. This could appear most distressing, as one would wish that a condition such as preeclampsia, being a state of increased risk and one of the leading causes of maternal and foetal morbidity and mortality, could be easily and clearly distinguished from normal term pregnancy.

But if indeed, the opposite is the case, and the transition between normal and seriously complicated pregnancy is gradual, it is essential that this is recognised, as the transition may at times be swift. We would then be in need, not of demarcator of a separate condition, but of a sensitive means of detecting the stage of this transition, to be able to foresee impending complications and perform a timely delivery.

The lack of distinction is also troublesome in research. Efforts to find an effective predictive test early in pregnancy have not been successful in a low-risk population and there is no golden standard diagnostic test to define preeclampsia[3]. The condition is a multisystem disorder, where different aspects of the disease are used in different classifications of hypertensive disorders in pregnancy. This makes it difficult to establish a clear-cut population of women at risk or women with developed preeclampsia for investigation, and also confuses interpretation of the literature in the field where separate classifications are used[4].

The only consistently found pathological lesion in preeclampsia is the renal lesion termed glomerular endotheliosis, which has been regarded as pathognomic for the condition. Renal biopsy has therefore been used as a final means for distinguishing the patient with “pure preeclampsia” from patients with other causes of hypertension and albuminuria, both in research and in the clinical setting.

The investigation of renal changes in term pregnancy and in preeclampsia could verify similarities supporting the concept of similar mechanisms in both conditions. Revealing the nature of these changes could promote a better understanding of the normal physiology in pregnancy and the pathophysiology of preeclampsia, possibly facilitating monitoring of preeclamptic patients.

BACKGROUND

Preeclampsia

Definition and diagnosis

The classifications of hypertensive disorders in pregnancy aim to differentiate preeclampsia, a pregnancy-specific disorder associated with increased fetal and maternal risk, from more benign conditions. Controversies regarding the definition of preeclampsia comprise whether to consider the systolic blood pressure, the absolute blood pressure levels or incremental changes and the necessity of including proteinuria or including systemic changes other than those of renal function[5]. The values used to discriminate normal from abnormal have often been selected arbitrarily and for convenience rather than relevance to the pathophysiology or outcome. It is essential, therefore, that the clinical definition should be wide with a high sensitivity, whereas the research criteria need to be kept stringent[5] with a high specificity and should also reflect the pathophysiology of the condition[4].

The classifications currently most used comprise The Australasian Society for the Study of Hypertension in Pregnancy (ASSHP) classification[6], The USA National High Blood Pressure Education Program Working Group (NHBPEP) classification[7] and Davey and MacGillivray's classification[8], adopted by the International Society for the Study of Hypertension in Pregnancy (ISSHP). The classifications differ in certain aspects and are presented below.

Classification of Hypertensive diseases in pregnancy (ISSHP)

A. *Gestational* (developing after 20 weeks of pregnancy) *hypertension* (diastolic blood pressure >90 mmHg on more than one occasion at least 4 h apart) *and/or proteinuria* (>0.3g/24h or two clean-catch urine specimens at least 4 h apart with 2+ proteinuria by dipstick or protein/creatinine index of 300 or more)
B. *Chronic hypertension and chronic renal disease* (including superimposed preeclampsia)
C. *Unclassified hypertension and/or proteinuria* (where information is insufficient to permit classification)
D. *Eclampsia* (the occurrence of generalized convulsions during pregnancy, delivery or the week after delivery, not caused by epilepsy or convulsive disorders)

The NHBPEP Working Group recommend in their most recent report the elimination of edema from the diagnostic criteria for preeclampsia previously used. The classification of hypertension in pregnancy is now essentially the same as above except for the additional inclusion of patients with a systolic blood pressure>140 mmHg defined as hypertensive and a different grouping:

Classification of Hypertensive diseases in pregnancy (NHBPEP)

- *Chronic hypertension*
- *Preeclampsia-eclampsia*
- *Preeclampsia superimposed on chronic hypertension*
- *Gestational hypertension* (transient or chronic, depending on blood pressure levels at 12 weeks post partum)

However, because of the wide clinical variability in the syndrome of preeclampsia it is possible to have severe disease with all other features of preeclampsia but without proteinuria[5]. The NHBPEP Working Group therefore states that in the absence of proteinuria, pre-eclampsia is highly suspected when increased blood pressure appears accompanied by other features of the condition[7]. A certain increment of blood pressure in pregnancy probably identifies a group of women with increased risk even without the presence of proteinuria[9]. It has therefore been proposed that hypertension or a rise in blood pressure

in combination with proteinuria or other features of the condition should suffice for a diagnosis of preeclampsia to be made[4].

This view has been accepted in the most recent Australasian definition of preeclampsia[6]. The features besides proteinuria indicating preeclampsia include neurological disturbances such as hyperreflexia with severe headache or clonus and convulsions, liver disease with elevated liver enzymes or epigastralgia, thrombocytopenia or hemolysis, renal insufficiency and retardation of fetal growth.

All documents concerning classification of hypertension in pregnancy indicate the need for development of sensitive and specific diagnostic tests for preeclampsia, derived directly from the causative mechanism of the disease. Understanding the pathogenesis and nature of the syndrome is a prerequisite for this.

Epidemiology

Somewhere between 6% and 8% of pregnancies are complicated by hypertension depending on population[10], Swedish figures ranging from 7.2%[11] to 8.8%[12], with preeclampsia developing in 1.5% to 2.0% of pregnancies and eclampsia in 0.2 per 1000 pregnancies[12]. In the US, preeclampsia is reported in 2.6% of pregnancies and eclampsia in 0.56 per 1000 pregnancies[13]; in Great Britain eclampsia occurs in 0.49 per 1000 pregnancies[14]. Hypertensive disorders during pregnancy accounted for 18% of maternal deaths in a Swedish investigation from the seventies[15], still almost 15% more recently in the United States[13]. Risk factors for development of preeclampsia include primiparity, high or low age, heredity, predisposing conditions (such as chronic hypertension, renal disease and diabetes), excessive placental mass (such as in twin pregnancy, hydatiform mole and polyhydramniosis) as well as certain ethnical groups and social factors, whereas smoking may have a protective effect (though preeclamptic smokers have an increased risk of developing serious complications[16]).

Nulliparity is one of the strongest risk factors for the development of preeclampsia[17], possibly suggesting an immunological reaction to the first placentation or paternal antigens, to which protective mechanisms develop in subsequent pregnancies[18]. Supporting this is the increased

risk for preeclampsia with changed paternity[19-21], with pregnancies resulting from sperm and oocytes biologically unrelated to the mother[22] and with the use of barrier methods for contraception[23], whereas previous induced abortion[24-27] and increased sexual activity were found to be protective[28-29].

Both younger women and older women[13] have an increased risk of developing preeclampsia, perhaps due to association with nulliparity in young women and chronic hypertension with older maternal age. The risk of preeclampsia is increased in women with sisters (37%), mothers (26%) and grandmothers (16%) with eclampsia[30] or sisters with preeclampsia[31]. Recently, studies have also found an association between high baseline body mass index and preeclampsia with relative risks of 2.3-5.5[24-25,32-33]. Excessive weight gain during pregnancy does not seem to be associated with preeclampsia[34].

Etiology and pathophysiology

The cause of preeclampsia is unknown. Much research is focused on the placenta, as delivery is the only definitive cure for the condition. Also, signs of poor placentation have been associated with preeclampsia. Placental trophoblast cells have not achieved the full invasion of uterine spiral arteries and the degradation of their muscular component required to transform them to low resistence vessels accommodating increased blood flow[7]. The incomplete placentation has been ascribed to lack of placental expression of HLA-G (human leukocyte antigen G), which is assumed to attenuate maternal immune response to the immunologically foreign placental tissue[35]. Resulting decreased placental perfusion, hypoxia and/or shear stress is believed to increase circulating substances such as inflammatory cytokines, which cause endothelial activation and dysfunction resulting in multiorgan vasospasm and platelet aggregation[36].

Ischemic manifestations in the brain, liver, kidneys and placenta can be life threatening and illustrate that this systemic syndrome comprises much more than the hypertension that defines it[37]. Renal manifestations are, however, among the first to arise and appear to have a central role in the pathophysiology.

Apart from a rise in blood pressure and an increased albuminuria, the renal affection is revealed as a decrease in renal blood flow (-20%) and a somewhat greater decrease in glomerular filtration rate (-30%), leading to a reduced filtration fraction[38]. Creatinine levels still often remain normal as glomerular filtration rate is increased by up to 50% in normal pregnancy[39]. However, renal insuffiency does in rare cases progress to acute renal failure. Hypovolemia is typical, even in renal insufficiency and edema, and the renin-angiotensin system is depressed[40]. Sodium excretion may still be impaired in preeclampsia compared to normal pregnancy[41]. Decreased fractional urate clearance is a typical feature and hyperuricemia an important marker[39].

Clinical Picture

The variability in the clinical picture of preeclampsia is wide and patients can present with hypertension, proteinuria and/or a number of other features of the clinical syndrome such as:

- Neurological disturbances: severe headache, visual disturbances, drowsiness, hyperirritability, hyperreflexia, clonus and convulsions
- Liver disease: epigastralgia progressing to hemolysis, elevated liver enzymes, low platelet syndrome (HELLP), rarely rupture of the liver
- Renal insufficiency, edema, hypovolemia, oliguria, rarely anuria
- Retardation of fetal growth, fetal compromise
- Pulmonary edema
- Ischemia of the retina, the adrenal and pituitary glands, rarely necrosis

Management

The principal concern in management is to optimise the timing of delivery. Continuous maternal and fetal surveillance should ensure that delivery may be performed before maternal organ damage arises and before fetal hypoxia develops, but with the greatest possible maturation of the fetus.

In addition to blood pressure measurements and determination of albuminuria, maternal renal function tests, liver enzymes and blood

platelets are monitored. Sensitive markers reflecting the stage of the pathophysiological process are essential for optimal management.

Ultrasonographic evaluation of fetal growth, umbilical blood flow measurements and fetal heart rate tracings ensure fetal well being during expectancy or signal the need for preterm delivery. These methods for fetal monitoring are already developed and of great value in management.

Antihypertensive treatment can lead to impaired blood flow in umbilical vessels, fetal hypoxia, bradycardia and even asystole and has no effect on disease progression. Treatment should be used only to offer maternal protection from cerebrovascular lesions at diastolic blood pressures perhaps above 110 mmHg. Diuretics may be used in small doses in oliguria-anuria, anticonvulsant therapy in hyperreflexia, clonus or convulsions, and freshly frozen plasma in hematological disturbances. Cortisone (Betamethasone) should be used for fetal pulmonary maturation when needed.

Prevention

Low-dose aspirin prophylaxis has not been found to be beneficial in a low risk population[42]. However, the Cochrane Collaboration has shown that aspirin use is associated with a 15% decrease in the risk of preclampsia (32 trials with 29 331 women, RR 0.85; 95% CI 0.78-0.92), which was greater with a higher dose of the drug[43]. Fetal and/or neonatal death was decreased by 14% (30 trials with 30 093 women, RR 0.86; 95% CI 0.75-0.99). This decrease was greatest in women at high risk (4134 women, RR 0.73; 95% CI 0.56-0.96).

The Cochrane Library update on calcium supplementation (9 trials with over 6000 women, RR 0.72; 95% CI 0.60-0.86) shows a moderate decrease in the risk of preeclampsia, which is greatest for women with low baseline calcium intake (RR 0.32; 95% CI 0.21-0.49)[44]. In countries with a low calcium intake the benefits may be substantial[45].

Blood pressure

The long-term regulation of blood pressure is one of the kidneys main functions and blood pressure measurement is an easily accessible method of detecting alterations in renal function. Blood pressure is mentioned separately from renal function only due to the fact that we by renal function often refer to the filtration capacity of the kidneys. There are many short-term mechanisms for arterial blood pressure control, aimed at almost completely correcting abnormal arterial blood pressure within seconds, minutes or hours. But a long-term and complete correction, or the development of hypertension, by necessity involves the kidneys[46].

Short-term mechanisms include nervous mechanisms: the baroreceptor reflexes, the atrial and pulmonary artery reflexes (also dilating renal afferent arterioles and increasing the heart rate, the Bainbridge reflex), the chemoreceptor mechanisms and - in extreme blood flow decrease to the vasomotor center – the CNS ischemic response. There are also hormonal short-term mechanisms: the norepinephrine-epinephrine, the renin-angiotensin and the vasopressin vasoconstrictor mechanisms as well as two intrinsic circulatory mechanisms namely the capillary fluid shift to the interstitial fluid compartment and the stress-relaxation mechanism in blood vessels[47].

Long-term mechanisms of blood pressure regulation are based on the phenomenon of pressure diuresis and pressure natriuresis, that is excretion of water and salt, occurring at a greatly increased rate with increased arterial blood pressure, returning it all the way back to normal. The effectiveness of this renal body fluid system of blood pressure control is increased by the nervous, the renin-angiotensin and the aldosterone systems, all increasing the renal output of water and salt[48].

Development of hypertension is associated with a change in the pressure range of the renal output curve, determining the steady-state blood pressure levels needed to excrete fluid and salt intake fully by pressure diuresis and natriuresis at different rates of net fluid intake. An increased renal arterial resistance, a reduced kidney mass, a

reduced glomerular filtration coefficient or an excessive amount of secreted aldosterone or circulating angiotensin, can shift the pressure range. Even if some forms of hypertension may be initiated by vasoconstriction, the blood pressure would be fully returned to normal by pressure diuresis and natriuresis if the hypertension were not sustained by this shift. Total peripheral resistance is, however, often increased secondarily to hypertension due to an autoregulation mechanism in reaction to increased local tissue blood flow[48].

The mechanisms leading to blood pressure changes during pregnancy are not fully known. Systolic and diastolic blood pressure levels decline in the first trimester until 16 to 20 weeks of gestation, the change in diastolic blood pressure being the greater, but return to initial values near term[49-50]. As profound reductions in renal arterial resistance are found to occur early in pregnancy, leading to an increase in renal plasma flow and glomerular filtration rate[39], it is plausible to suppose that the renal output pressure range is shifted by this change. Proposed mediators of renal vasodilation include progesterone, relaxin, nitric oxide and endothelin, some of these mediators acting through others[39].

In preeclampsia, calculations infer that an increase in total renal vascular resistance is mainly due to an increase in the afferent arteriolar resistance[51]. However, as the compromise in glomerular filtration rate slightly exceeds that of effective renal plasma flow, a reduction in the glomerular ultrafiltration coefficient value is likely[39], which also shifts the pressure range of the renal output curve.

Blood pressure measurement

It is generally accepted that arterial blood pressure cannot be measured with precision by means of sphygmomanometers, as even slight changes in bodily conditions can influence variability[52]. Clinical blood pressure measurements have still proved greatly serviceable in routine health care, provided measures are taken to avoid additional errors due to technique[52]. Recommendations require that the woman should be rested, seated comfortably with her feet supported and her arm at the level of the heart[5]. The right arm should be used for measurement with a cuff of the appropriate size[5].

Controversy has surrounded the decision whether to use Korotkoff phase IV (muffling) or phase V (sound disappearance) for diastolic blood pressure measurement in pregnant women. It has been believed that the hyperdynamic circulation of pregnant women leads to large differences between these sounds, but larger differences have since been proven infrequent[53]. Phase V has been proven to approximate intraarterial diastolic blood pressure levels more closely and is seldom low or zero[53], whereas phase IV is often unreliable or absent[54], leaving phase V as the obvious choice for diastolic blood pressure measurement in pregnancy[5,7]. No clinically significant differences in outcome of pregnancy could be detected when using phase V rather than phase IV[55].

Renal function

By renal function we usually refer to the production of urine through filtration, active and passive reabsorption and secretion, though the kidneys also regulate the fluid balance of the body and blood pressure and are the site of production of renin and erythropoietin. Each kidney is composed of 1-4 million filtering units called nephrons, in which urine is filtrated from a tuft of capillaries, the glomerulus, into the urinary space. Together they form 180 liters of glomerular filtrate daily, or 125 mL/min – a quantity known as the glomerular filtration rate. The rate of total blood flow through both kidneys of a 70 kg man is about 1200 mL/min[56].

There are many methods for determining renal function in pregnancy, renal clearance of para-aminohippurate provides a measure of effective renal plasma flow and clearance of inuline a measure of glomerular filtration rate. Cr 51-EDTA is the golden standard for measurement of glomerular filtration rate, but has been avoided during pregnancy because of its radioactivity[39].

Iohexolclearance has in nonpregnant conditions been found to correspond to clearance of Cr 51-EDTA, when two exact measure points are used after two and three hours since intravenous administration, and therefore represents a suitable alternative for

measurement during pregnancy[57,58]. Clearance of endogenous creatinine is routinely used as an estimate of glomerular filtration rate and is freely filtered in the glomeruli, but is also to some extent reabsorbed and secreted by the tubules with increasing secretion as the glomerular filtration decreases, thus underestimating the severity of renal disease[59]. Circulating levels of creatinine can also be overestimated because of the presence of a chromagen in plasma, which is measured along with true creatinine[39]. Serum creatinine levels can be greatly influenced by muscle mass, by high amounts of cooked meat in diet[60] and sometimes by interference with the tubular secretion as in treatment with trimethoprim-sulfa and cimetidine[61], in ketoacidosis[62] or with high levels of bilirubin[63].

Uric acid is formed as the end product in the degradation of purines, both endogenous and digested, almost exclusively taking the form of urate in plasma[64]. It does to a certain extent reflect renal function and is mainly excreted by the kidney, but roughly a third is excreted through the gastrointestinal tract[64]. Urate is not only freely filtrated through the glomeruli, but also practically totally reabsorbed in the proximal tubuli, to be actively secreted again in the distal tubuli and therefore reflects aspects of renal function other than glomerular filtration rate[64]. Active secretion can be impaired by lactate as in famine, intake of alcohol or certain drugs (such as acetylic acid in low doses or tiazide diuretics)[65]. Decreased serum values are found in hepatic damage and ulcerous colitis, as well as in high concentrations of acetylic acid, due to impaired resorption in the proximal tubuli. In pregnancy serum levels are decreased by approximately 25-35% due to increased glomerular filtration rate and filtered load, reduced tubular reabsorption or both[39,65,66].

In preeclampsia serum urate levels are increased[67,68], with a degree correlated to disease severity[69] and perinatal mortality[70,71], the increase occurring relatively early in the preeclamptic process[72]. The increase is, however, probably not so much due to a decrease in glomerular filtration rate as to an enhancement in renal tubular reabsorption, possibly due to the coupling to sodium reabsorption in the proximal tubule, which may be increased in the hypovolemic state of preeclampsia[39,64].

Glomerular filtration rate and renal plasma flow have in repeated studies been shown to increase markedly in pregnancy by approximately 40-65% and 50-85%, respectively[73-78]. Possible mediators of the reduction in renal vascular resistance have previously been mentioned: progesterone, relaxin, nitric oxide and endothelin, maybe in combined interaction[39].

The normal slight decrease at term in glomerular filtration rate and renal plasma flow is further decreased in preeclampsia, on average by 32% and 24%, confirmed by numerous investigations[39]. Calculations support that the increased renal vascular resistance principally is due to an increase in the afferent arteriolar resistance[51]. There is reason to believe, however, that a reduction in glomerular ultrafiltration is the cause of the greater decrease in glomerular filtration rate than in renal plasma flow[39].

Cystatin C

Cystatin C is a member of the cystatin superfamily, proteins grouped together because of similar amino acid sequences and their cysteine protease inhibitor activity[79]. Proteolytic enzymes are grouped into four major classes, named by the main functional group in their active site: cysteine-, serine-, aspartic- and metallo-peptidases or proteases. Their activity is in turn regulated by protease inhibitors. The first cysteine protease inhibitor, later given the name cystatin by Barrett l981, was found in chicken egg white in 1968[80].

Cystatin C was first found in 1961 in urine from patients with renal failure and in normal cerebrospinal fluid, then called γ-trace, but renamed cystatin C when it was found to inhibit cysteine proteases[81]. The complete amino acid sequence of 120 amino acids was the first to be determined in the cystatin superfamily 1981[82], its calculated molecular weight calculated to 13 359 Da[83]. The coding gene is a so-called housekeeping gene[84] with promoter regions directing the mRNA synthesis at relatively low levels, with little regulation and tissue specificity[85]. Consequently, cystatin C is widely distributed and found in all human biological fluids and tissues examined, but with particularly high concentrations in seminal fluid and cerebrospinal fluid[86-88].

The house-keeping gene type indicates a stable production rate of cystatin C by most nucleated cell types and the protein and/or its mRNA is present in virtually all investigated cell types[89]. Cystatin C has shown promise as a marker for glomerular filtration rate, because of its stable production rate, unaffected by any other patho-physiological condition, and because of its almost exclusive elimination from the circulation by glomerular filtration[90-95].

Blood plasma proteins with molecular masses below 15-25 kDa are generally virtually freely filtered through the normal glomerular membrane and then almost completely reabsorbed and degraded by the normal proximal tubular cells, properties that prove true for cystatin C[96,97]. Cystatin C has a molecular mass of 13 kDa, an ellipsoid shape with axes of about 30 and 45Å and has a plasma renal clearance in the rat model virtually identical to ^{51}Cr-EDTA[98,99].

The development of automated particle-enhanced immunoturbidimetric methods, rapid and more precise, substantially improved the possibility of using serum cystatin C as a marker for glomerular filtration rate in clinical routine work[92,93], as did the introduction of a sandwich enzyme immunoassay[100]. Serum cystatin C is a better marker for glomerular filtration rate (determined by "gold standard" procedures such as plasma clearance of ^{51}Cr-EDTA, ^{99m}Tc-DTPA or iohexol) than serum creatinine in many clinical situations, particularly for individuals with small to moderate decreases in glomerular filtration rate[96].

Renal structure

The kidneys consist of together about 2 400 000 filtering units called nephrons, in which urine is filtrated from a tuft of capillaries, the glomerulus, into the urinary space within the surrounding Bowman's capsule. The urine then passes on through the proximal convoluted tubule, the thick and thin portions of the loop of Henle and the distal convoluted tubule to the collecting duct. The filtration barrier between the blood and the urinary space consists of three components:

- **Capillary endothelial cells** perforated by fenestrae up to 100 nm in diameter, and surrounded by a network of filaments and microtubules. The cells have a negative surface charge due to the presence of podocalcyn, a polyanionic glycoprotein[101].

- **Glomerular basement membrane** consisting of type IV collagen, laminin, fibronectin, entactin and heparan sulfate proteoglycan[102]. The latter is a glycosaminoglycan constituting the anionic sites of the membrane, maintaining structural integrity and exerting antiproliferative, antithrombogenic and antilipaemic effects[101].

- **Visceral epithelial cells** also known as podocytes, with long cytoplasmic trabeculae dividing into foot processes, intersecting to form "filtration slits" or "slit pores" between them, bridged by slit diaphragms. The surface of the podocyte foot process is also negatively charged due to the presence of sialic acid[101].

Glomerular mesangial cells are found adjacent to the endothelial cells, providing structural support for capillary loops, but also possessing contractile properties and the ability of phagocytosis[101,102].

The structural components determine the glomerular permeability. Glomerular basement membrane surfaces are blocked by epithelial and endothelial cells, to an extent described by the fraction of the surface area occupied by slits or fenestral openings (0.11 and 0.20, respectively, from morphometric studies in rats)[102]. Slit pore width in humans is similar to that in rats, 43 nm. The slit pore diaphragm

spacings vary in size around 40 Å[102]. The permeability of the slit diaphragm and of a single fenestra can be estimated and multiplied by the fraction of surface area they occupy, to provide an approximation of epithelial permeability and endothelial permeability. The former estimate is 20 times the latter, indicating that the water flow resistance of the fenestrae is negligible[102].

This is assuming, however, that fenestrae are short, water-filled channels, whereas recent electron microscopy studies demonstrate a 300 nm thick filamentous glycocalyx layer coating both fenestral and interfenestral surfaces[103], believed to be composed principally of sulphated proteoglycans[104] and glycoproteins[105]. This condition changes the total barrier hydraulic permeability from $4.1 \cdot 10^{-9}$ to $3.2 \cdot 10^{-9}$ $m \cdot s^{-1} \cdot Pa^{-1}$, the endothelium now accounting for 24% instead of just 2% of the total water flow resistance[97]. Overall hydraulic permeability has been estimated at 3 to $5 \cdot 10^{-9}$ $m \cdot s^{-1} \cdot Pa^{-1}$ in micropuncture measurements[106]. Glomerular basement membrane resistance in vivo is predicted to be 2.3 times that of bare membrane in vitro due to a “channelling phenomenon” with intact cellular layers, thus equalling epithelial resistance in value[102].

Whereas fluid filtration is determined by the hydraulic resistance of the structural components of the glomerular barrier added together (overcome by the net balance of the transcapillary hydraulic pressure and intravascular colloid osmotic pressure to form glomerular filtrate), macromolecule filtration is dependant on the product of the individual layers sieving coefficients which are influenced by one another and by filtrate velocity[102]. The size selectivity in macromolecule filtration is determined mainly by the cellular layers, the glomerular basement membrane contributes to only 13-26% of the diffusional resistance of the barrier[107]. A charge selectivity has also been demonstrated[108], for which the cellular layers are responsible[102], with little or no charge selectivity in the glomerular basement membrane[109-112]. The chemical composition of the filtrate is similar to that of blood plasma, but with very little proteins. Most circulating plasma proteins such as albumin are anionic, passing through the polyanionic capillary wall less readily than neutral or cationic polymers. Orosomucoid may have a role in maintaining and reinforcing the charge barrier [113-116].

Glomerular endotheliosis

Glomerular endotheliosis is defined as glomerular endothelial cell swelling and vacuolisation, obliteration of endothelial fenestrae and encroachment of the capillary space area. It is the characteristic lesion found in renal biopsies from preeclamptic patients, and has been considered pathognomonic for the preeclampsia[117,118].

In preeclampsia, the rim of endothelial cytoplasm is swollen with a reduction in the size and number of fenestrae, drastically reducing the fraction of glomerular basement surface area occupied by fenestrae and making the calculated endothelial contribution to hydraulic resistance of great importance. The glomerular filtration coefficient was estimated be reduced 30% due to these changes[119]. Subendothelial deposits also lengthened the filtration pathway and mesangial interposition reduced filtration surface area despite slight glomerular hypertrophy, but neither gomerular basement thickness nor the filtration slit fraction was altered. The total reduction in the glomerular filtration coefficient, considering also the change in surface area, was estimated at 40% in preeclampsia, similar to the known reduction in glomerular filtration rate[119].

Glomerular endothelial dysfunction is often viewed as a mere reflection of a more widespread dysfunction of all vascular endothelium. The vascular endothelium as a whole may be regarded as a complex regulatory "organ system" with four major regulatory functions[120]:

- **Vasomotor tone** regulated by production of *nitric oxide* (with vasodilating and antiproliferative effects, inhibiting platelet adhesion and aggregation) and *endothelin* (with vasoconstrictor activity and regulatory effects on mesangial and other growth, also produced by mesangial cells). Also, the vasoconstrictor *angiotensin II* is activated by the action of angiotensin converting enzyme within endothelial cells, with a high activity in the glomerulus.

- **Coagulation and fibrinolysis** regulated by production of *prostacyclin* (PGI_2) (exerting a vasodilator effect, inhibiting platelet aggregation, reducing platelet derived growth factor production in both platelets and endothelial cells, inhibiting vascular growth and smooth cell cholesterol ester metabolism and blocking receptors for fibrinogen and von Willebrand factor), *thromboxane B_2* (inducing vasoconstriction and platelet aggregation), *thrombomodulin* (acting as a co-factor in the thrombin-catalysed activation of protein C – a natural anticoagulant), *von Willebrand factor* (part of the factor VIII complex, procoagulatory), *tissue plasminogen activator* (tPA, also involved in PAI-1 production) and *plasminogen activator inhibitor* (PAI-1, also produced in the liver, stored in platelets). *Fibronectin* is also an endothelial glycoprotein reducing erythrocyte deformability, enhancing erythrocyte and platelet adhesion to subendothelial collagen.

- **Growth and repair of endothelium and underlying tissues** by secretion of a variety of growth factors, including *platelet derived growth factor* (PDGF), *fibroblast growth factor* (FGF), *insulin-like growth factor 1* (IGF1) and *transforming growth factor β* (TGF β).

- **Endothelial permeability** involving transcapillary leakage of *albumin* and *fibrinogen* in the glomerulus leading to albuminuria.

Several regulatory mechanisms seem to be able to be activated by a single pathway, as when circulating cytokines induce endothelial synthesis of adhesion molecules, changing the endothelium from a basal state of vasodilation, anti-thrombosis, anti-proliferation and impermeability, to one of vasoconstriction, coagulation, proliferation and permeability [120]. These mechanisms are believed to contribute to the pathophysiology of preeclampsia[36].

AIMS OF THE INVESTIGATIONS

These investigations aimed to reveal possible similarities in the physiology of normal term pregnancy and the pathophysiology of preeclampsia with regard to kidney function. Should similar renal processes be observed, supporting the concept of preeclampsia as the extreme of maternal adaptation to pregnancy, we wished to search for a method of monitoring the development of renal affection.

Specifically, our aim was

- To study blood pressure changes during pregnancy in a normal population of pregnant women in Sweden, with special reference to the impact of parity in term pregnancy.

- To analyse the impact of parity and baseline body mass index on blood pressure levels in normal pregnancy.

- To establish reference values for serum cystatin C in term pregnancy and to compare the use of serum cystatin C as a marker for preeclampsia with the use of serum creatinine or urate.

- To investigate whether serum cystatin C is a reliable method of assessing glomerular filtration rate in pregnant women.

- To investigate, in a controlled study of hypertensive pregnant women, the proportion of women with antepartum renal biopsy findings characteristic for preeclampsia as opposed to renal disease.

- To investigate the relation between serum cystatin C levels and renal structural changes in normal and hypertensive pregnancy in order to evaluate the possible use of serum cystatin C as a marker of the degree of endotheliosis.

MATERIAL AND METHODS

Setting

The present investigations were conducted at the University Hospital, Lund, Sweden. Participants were recruited at the Department of Obstetrics and Gynecology. Renal biopsies were performed at the Department of Diagnostic Imaging and Clinical Physiology. Pathological evaluation was performed at the Department of Pathology, Herlev Hospital, University of Copenhagen, Denmark.

Participants

Blood pressure changes (Papers I and II)
To study blood pressure changes during pregnancy and influencing factors, two historical cohort studies were performed, the advantage of the historical cohort being the lack of loss to follow up and surveillance bias. Six hundred women consecutively giving birth at term (gestational age >37 weeks) at our department were included in the first crossectional study. One hundred and sixty six women, giving birth to their third child at our Department during the year 2000 were included in the second longitudinal study of all the women's three pregnancies. We included only women who had followed the free maternal health care programme in the region during all studied pregnancies.

Patients with essential hypertension, gestational diabetes or diabetes mellitus and chronic renal disease, as well as patients developing gestational hypertension or preeclampsia were primarily excluded from the studies. These women were subsequently included in an additional analysis of the whole cohort.

Renal functional changes (Papers III and IV)

To evaluate renal functional tests in normal pregnancy and preeclampsia, two separate studies were performed. To evaluate serum cystatin C, urate and creatinine as markers for preeclampsia, 100 healthy women at term were recruited prior to delivery from the delivery ward, as well as 45 patients with preeclampsia admitted to the antenatal ward. To evaluate cystatin C as a marker for impaired glomerular filtration rate in pregnancy, we studied 48 previously healthy women admitted to the antenatal ward due to pregnancy complications in their third trimester, as well as 12 healthy non-pregnant women recruited amongst staff and medical students at our Department. We included only previously healthy women with no history of hypertension or renal disease and devoid of drug treatment.

Renal structural changes (Papers V and VI)

To investigate renal structural changes in normal pregnancy and preeclampsia and their relation to potential markers, 36 women with hypertensive disease in pregnancy (26 nulliparae), admitted to the antenatal ward, were enrolled in these studies. During a 20-month period from November 1999 to June 2001, 54 previously healthy women with hypertensive disease were admitted while one of the two involved obstetricians was on duty at the antenatal ward and the involved radiologist was available. These patients had no history of diabetes, essential hypertension or clinically detected renal disease. However, 12 women declined to participate for fear of pain or complications and 6 patients failed to meet the additional inclusion criteria of a diastolic blood pressure measuring no more than 105 mmHg and a blood platelet count of no less than $100 \cdot 10^{-9} \cdot L^{-1}$ at the time of the biopsy.

Control cases comprising 12 healthy pregnant women in their third trimester (5 nulliparae) were recruited during the study period from the maternal health care centres in the catchment area of the hospital. The women were compensated economically for loss of income for one working day and for possible pain and discomfort experienced during the procedure.

Blood Pressure

Blood pressure measurement routines have been standardised within the region and require the use of the right arm with the patient in a sitting position after at least 10 minutes of rest using a mercury-sphygmomanometer with an appropriate cuff, adjusted to upper arm circumference. The diastolic blood pressure is measured at Korotkoff phase V (when sounds disappear). The measurement is approximated to the nearest 5 mmHg and performed twice at an interval of two minutes. If a discrepancy occurs between the two readings, the measurement is repeated until similar readings are observed, and the mean value of the last two recordings is recorded.

Gestational hypertension was defined as diastolic blood pressure above 90 mmHg determined on two consecutive occasions at least four hours apart, developing after 20 weeks of gestation. Preeclampsia was defined as gestational hypertension with an additional albuminuria of >0.3 g per 24 hours, allowing for the approximation to 0.3 $mg \cdot L^{-1}$ or a qualitative value of 2+ in a random clean-catch-midstream specimen, on two occasions at least four hours apart.

Renal function

Cystatin C

Serum cystatin C was measured by a fully automated particle-enhanced immunoturbidimetric assay for cystatin C in undiluted samples[92]. The reagents were obtained from DAKO A/S, Copenhagen, Denmark. The procedure recommended by the manufacturer was implemented on a Cobas Mira Plus Instrument (Roche, Basel, Switzerland). The total analytical (intra-assay + inter-assay) imprecision of the method, calculated using a control sample with an assigned value of 1.28 $mg \cdot L^{-1}$, was 3.2%.

Creatinine

Serum creatinine was determined by the Kodak Ektachem 700 XR-C system using the enzymes creatinine amidohydrolase and creatinine amidinohydrolase. The total analytical (intra-assay + inter-assay) imprecision of the procedure, calculated using a control sample with an assigned value of 80 $\mu mol \cdot L^{-1}$, was 2.6%.

Urate

Serum urate was determined by the method of Town et al[121] using the enzymes uricase and ascorbate oxidase and the Hitachi 917 analytical system. The total analytical (intra-assay + inter-assay) imprecision of the procedure, calculated using a control sample with an assigned value of 230 $\mu mol \cdot L^{-1} l^{-1}$, was 0.73%.

Iohexol clearance

Serum iohexol levels were determined by high-pressure liquid chromatography[58] in 2 plasma samples drawn 2 and 3 h after intravenous administration of 5 mL of the iohexol solution (Omnipaque, Nycomed Amersham) in an antecubital vein. Iohexol clearance, as a measure of the glomerular filtration rate was calculated and expressed as mL/min, as gestational changes are believed to be unrelated to renal hypertrophy and body surface area[122]. The total analytical (intra-assay + inter-assay) imprecision of the method, calculated using a control sample with an assigned value of 32 $mg \cdot L^{-1}$, was 2.2%.

Renal structure

Renal biopsy safety requirements

The inclusion criteria included requirements of a diastolic blood pressure no more than 105 mmHg and a blood platelet count of no less than $100 \cdot 10^{-9} \cdot L^{-1}$ at the time of the biopsy to ensure the safety of the procedure. The same limits have been used as requirements for the procedure through several years without serious complications at the Department of Diagnostic Imaging and Clinical Physiology, by the same highly experienced physician who performed all biopsies in these investigations. Blood coagulation parameters (plasma activated partial thromboplastin time, plasma prothrombin complex, blood platelets) and blood haemoglobin were checked to be normal within four hours prior to the biopsy and a blood compatibility test was performed on all enrolled women. A dipstick test and an examination of the urine sediment were made to ensure absence of bacteriuria.

Renal biopsy technique

The biopsy sample was taken according to standard procedure with a 1.2 mm (outside diameter) needle using a Bard biopsy device ("biopsy-pistol") together with an ultrasound transducer. Two biopsy samples were taken from each patient. If the extent of cortex material was considered insufficient, which was the case on two occasions, a third biopsy was taken. Each biopsy, approximately 20 mm in length, was immediately submerged in physiological sodium chloride solution and processed and prepared at the Department of Pathology the University Hospital of Lund for further transport to the Department of Pathology at Herlev Hospital, University of Copenhagen, Denmark.

Biopsies were divided into three parts and these were fixed in neutral formaldehyde 4% (w/v) for light microscopy, in glutaraldehyde 2.5% (w/v) for electron microscopy and snap frozen in Tissuetech for immunoflourescence, following standard procedures. The biopsies were examined by at least two experienced pathologists without knowledge of the clinical course.

Light microscopy
To minimise the possibility of observer error all specimens were after the completion of the study referred to an additional senior pathologist for light microscopic evaluation in one and the same session. In the few cases of discrepancy of evaluation that arose, a consensus evaluation was made.

Each specimen for light microscopy was cut into 2 µm sections and stained with PAS-haematoxylin, haematoxylin eosin, trichrome Masson, Congo red for amyloid, and silver methenamin. Changes in tubuli, interstitial tissue, vessels as well as glomeruli were registered. The degree of endotheliosis was evaluated using a semiquantitative scale; 0: no endotheliosis, 1: <20% of the lumina obliterated, 2: 20-80% of the lumina obliterated, 3: >80% of the lumina obliterated. The semiquantitative grading was based upon a general evaluation of all glomeruli in a silver methenamin stained section.

Immunofluorescence
The presence and localisation of IgA, IgG, IgM, properdine, C1q, C3, C4 and fibrin was registered. A semiquantified scale was used for evaluation; (+): not discernible in photographic imaging, +: scarce presence, ++: moderate presence, +++: rich presence.

Electron microscopy
One glomerulus in each biopsy specimen was examined and the occurrence and localisation of dense deposits, mesangial cell interposition around capillary loops and endotheliosis was registered. The degree of endotheliosis was semiquantified; 0: no swelling of endothelial cells with preserved fenestration; 1: at least one capillary loop was normal and 2: all capillary loops showed endothelial swelling.

Morphometric evaluation
The mean glomerular volume was estimated in biopsies containing at least six complete glomerular sections. All glomeruli in one silver methenamin-hematoxylin stained section from the paraffin embedded part of the biopsy were analysed. The cross-sectional area (Aglom) within the outline of each glomerular tuft was computed using a

computer assisted stereologic system (GRID, Interactivision, Denmark). Magnification on the videoscreen was x 1650. Glomerular volume (Vglom) was then calculated from the following formula:

$$Vglom = \beta/d \cdot Aglom^{3/2},$$

where β is a dimensionless "shape coefficient" (= 1.38 for spheres) and d is a "size distribution coefficient", introduced in order to account for variations in glomerular size[119,123].

Statistics

Student's T-test was used for normally distributed paired or unpaired observations as appropriate. Multiple regression analysis of term diastolic blood pressure was performed in Paper I, with the variables parity, previous pregnancies (primi/multigravidae), age, baseline body mass index (BMI), weight gain and smoking with the backward stepwise regression strategy. Analysis of variance (ANOVA) for repeated measurements with Tukey's post-hoc test was used in Paper II for comparison of blood pressure levels between orders of pregnancy in 3-para. Multiple regression analysis of systolic and diastolic blood pressure on BMI, age and smoking was performed for each order of pregnancy and each gestational age in the same paper.

Receiving operator characteristic (ROC) analysis was used for comparing methods of diagnostic testing in Paper III. Calculation of Pearson's correlation coefficient was used for evaluation of correlations in Paper IV and VI. Analysis of covariance was used in Paper IV to confirm differences in reciprocal cystatin C levels between non-pregnant and pregnant women. Also in Paper IV, ANOVA with Dunnet's post-hoc test was used to show differences in renal function tests between the pregnant groups. Fisher's exact test was used in Paper V, to determine differences in degrees of renal structural changes between diagnostic groups. ANOVA was used with Tukey's post-hoc test for comparing means within groups and with orthogonal contrasts to determine linear trends in Paper VI.

ETHICS

Informed consent was received from all women involved in these studies and all prospective studies were approved by the Medical Ethics Committee at the University of Lund. However, special considerations were made regarding the studies involving renal biopsy in normal pregnancy, which are appropriate to relate in this context. Though renal biopsy techniques have been vastly improved in recent years using ultrasonographic guidance, thinner needles and the biopsy pistol-technique, some early renal biopsy studies in pregnancy have been associated with a notable risk for retroperitoneal or perirenal haematoma and haematuria[124]. Most authors have concluded later, that the risks in pregnancy are not greater than in non-pregnant women, where biopsy procedure is routine in renal dysfunction[69,125-126].

The necessity and the safety of renal biopsy during pregnancy on clinical indications have been debated widely[125-127]. Some authors have stressed the importance of a correct diagnosis[126], but recommendations have also been given to limit the procedure to strict indications, such as sudden renal failure or massive nephritic syndrome of unknown origin before the final two months of pregnancy[127]. A small review (n=18) of clinically indicated renal biopsies early in pregnancy in a heterogeneous group of diagnoses was discouraging, resulting in seven cases of renal hematomas of which two required blood transfusion[128]. No description was provided, however, of safety precautions or the renal biopsy techniques used.

A number of larger renal biopsy studies (n=50-90) have been performed on preeclamptic women with modern techniques reporting no renal complications[129-133], providing essential new knowledge in this field. Authors concluded the renal biopsy procedure to be safe and more informative than routine renal function tests[131]. The radiologist involved in our investigation, has had extensive experience of modern renal biopsy techniques, without serious complications or adverse reactions. Thirty preeclamptic patients had been subject to renal biopsy within the study without any complications before the healthy pregnant women were included in the investigation.

It is necessary, when making the ethical decision of whether or not to perform a study, to also consider ethically the consequences of refraining from an investigation. It could be argued, that the vast amount of performed research, involving renal biopsy of preeclamptic women with sometimes substantial cardiovascular and hematological disturbance, based on an unvalidated diagnostic criterion, has indeed been unethical. On finding the typical preeclamptic lesion also in all cases of gestational hypertension without proteinuria in our studies, we had to ask ourselves if the lesion in fact could be present also in normal term pregnancy. Once the question was raised, it would appear unethical to continue to perform an uncontrolled study, using the lesion as diagnostic, and essential to determine the renal histology of normal term pregnancy.

The healthy pregnant women at term who volunteered to participate in this investigation were compensated economically for loss of income for one working day and for possible pain and discomfort experienced during the procedure. The individual woman derived no more gain from her participation than the confirmation of absence of renal disease, but many of these women displayed a great willingness to contribute, having themselves had close friends affected by preeclampsia. Hypertensive women did all receive the diagnosis of preeclampsia, which in most cases did have a significant impact on management of disease and information given regarding their prognosis. The clinical course of disease might, though, well have been the same, whether or not the pathological diagnosis was confirmed by renal biopsy.

In retrospect, we have come to understand that the biopsy might not be of the great value it has previously been ascribed, as seemingly all hypertensive pregnant women and even a substantial proportion of healthy pregnant women present the renal lesion previously believed to be pathognomonous for preeclampsia, albeit to different degrees. If the degree of structural change is linked to changes in the glomerular filtration rate and can be monitored by sensitive renal functional tests, as we suggest, most renal biopsies might well be avoided, but this is information we would not have had without performing these investigations.

Some authors advocate postponing the renal biopsy to the postpartum period, to minimize the risk of haematoma or haematuria due to coagulation disturbances[127]. Regression of some glomerular lesions has been seen, however, as early as the first week postpartum and renal function, which may be paralleled by structural change, is usually restored within a few days[133-137]. Complete resolution has been reported within four weeks[134], while some lesions may arise in the healing stage. Indeed, the healing stage of the renal lesion might well resemble the histopathological findings of renal disease, as it did in the one re-biopsy of our study. The consequences of erroneous diagnoses of renal disease, whether in the clinical or the research context, may not be small. We found no renal disease in our studies.

In conclusion, the ethical considerations surrounding investigations and the decision whether or not to perform them are composite. It is essential that the presentation of information to the participator and to the Medical Ethics Committee, regarding benefit and risk, should be as balanced as possible in order to reach an ethical decision from all parties. Much is achieved simply through the process of application, when ethical aspects are brought forward. To refrain from an investigation may not in all instances be the safest path to tread and the most ethical decision, which we believe these investigations illustrate.

RESULTS

Blood pressure changes (Papers I and II)

In the crossectional study, the systolic blood pressure increased slightly during normal pregnancy in both nulliparae and multiparae. The diastolic blood pressure decreased initially in mean by 3.3%, and increased subsequently to term values (at 30-40 weeks), in mean 7.3% above the values at the initial visit.

In nulliparae (*n*=237) the mean increase was significantly greater, 9.9% versus 5.4% in multiparae. The absolute difference in mean values was small, 2.8 mmHg at term, but appeared in every measurement period from 21 gestational weeks and increased in significance with gestational age ($p<0.00l$ at term).

The absolute difference in mean diastolic blood pressure between primigravidae (*n*=179) and multigravidae amounted to 4.5 mmHg at >40 weeks and was also significant ($p<0.01$ at term). The mean diastolic blood pressure in all women during the whole pregnancy was 68.3 mmHg.

Women with a baseline BMI above the mean (23.7 kg/m^2) had a significantly higher systolic and diastolic blood pressure all through pregnancy with a difference in diastolic blood pressure (2.4-4.0 mm Hg) highly significant ($p<0.0001$) up until term ($p<0.05$).

Women smoking at the onset of pregnancy (16.3%) showed a greater mean decrease (2.5 mmHg) in the diastolic blood pressure compared to non-smoking women and also failed to follow the subsequent rise to the same degree, settling at a mean diastolic blood pressure level lower (2.8 mmHg) than non-smokers at term ($p<0.05$).

Multiple regression analysis showed that parity, baseline BMI and smoking all remained as factors significantly influencing term diastolic blood pressure as detailed in Table I. Neither systolic nor diastolic blood pressure could be found to correlate with maternal age or weight gain at any gestational age in this study.

Table I. Multiple regression analysis of term diastolic blood pressure with the ANOVA table for the model.

Variable	Coefficient	SE	t-value	p-value
Intercept	64.117	2.800		
BMI ($kg \cdot m^{-2}$)	0.577	0.105	5.52	<0.001
Multiparity	–3.249	0.821	–3.96	<0.001
Smoking	–3.005	1.078	–2.79	0.006
.	DF SS	Mean square	F-value	p-value
Regression	3 3594.17	1198.06	17.20	<0.0001
Residual	427 29740.44	69.65		
Total	430 33334.62			.

In the longitudinal study, blood pressure patterns were similar to the crossectional study, though both mean systolic and mean diastolic blood pressure levels could be shown to be consistently lower during the second and third pregnancy compared to the first pregnancy at comparable gestational age. The mean differences between the group of nulliparae and the two parous groups were highly significant during the third trimester ($p<0.001$).

The individual reduction in term diastolic blood pressure levels from the first to the second pregnancy varied from >10 mmHg (n=48) to no significant decrease at all (n=60). Further analysis showed that the effect of parity on third trimester diastolic blood pressure levels was greatest in the group of women with a baseline BMI above the 75^{th} percentile, whereas it was non-significant below the 25^{th} percentile.

Multiple regression analysis of systolic and diastolic blood pressure levels on age, smoking and baseline BMI for each order of pregnancy and each gestational age showed a significant influence only of baseline BMI. Diastolic blood pressure levels correlated with baseline BMI only in the first pregnancy.

Change of paternity or a short time space between pregnancies could not be shown to have an impact on BP levels in the second or in the third pregnancy. All results from the two studies remained unchanged also after the subsequent inclusion of women with previous disease or women developing hypertensive disorders during pregnancy.

Renal functional changes (Papers III and IV)

Reference values for cystatin C in term pregnancy were defined after the exclusion of three women with twin pregnancies from the one hundred recruited women, as their cystatin C levels were significantly higher than in singleton pregnancy. The 45 preeclamptic patients displayed a significantly higher mean value of serum cystatin C than the healthy pregnant women at term and twenty-five of the patients had cystatin C levels above the calculated reference interval.

Table II. Mean serum values of cystatin C are given in $mg \cdot L^{-1}$ while mean values of serum urate and serum creatinine are given in $\mu mol \cdot L^{-1}$.

	Healthy			Preeclampsia	
	Mean±SD	Reference interval	95% CI	Mean±SD	95% CI.
Cystatin C	1.05±0.19	0.68-1.42	1.01-1.09	1.55±0.29	1.46-1.63
Creatinine	56±9.7	37-75	54-58	70±23	63-77
Urate	305±6l	186-424	293-317	413±l28	382-456.

Patients with severe preeclampsia (albuminuria >3 g·L) showed higher values of cystatin C (mean 1.61, SD 0.30) than patients with mild preeclampsia (mean 1.47, SD 0.26), though the difference was not statistically significant. Only 10 creatinine values and 17 urate values in the 45 preeclamptic patients were raised above the reference interval calculated for healthy pregnant women at term, though the preeclamptic group also had significantly higher mean values of serum creatinine and urate ($p<0.0001$) than the healthy pregnant women (Table II).

ROC analysis demonstrated that serum cystatin C had a superior diagnostic accuracy for preeclampsia compared to that of serum urate or serum creatinine and that the diagnostic accuracy of serum urate was better than that of serum creatinine. In ROC plots the sensitivity of a marker is plotted against the 1-specificity for each possible cut off level and the analysis is based upon comparing the areas under the curves. Results are detailed in Table III. The optimal cut-off level is that which maximises the sum of the sensitivity and specificity[138].

Table III. ROC analysis of potential markers for preeclampsia, with optimal cut off serum levels given in $mg \cdot L^{-1}$ for cystatin C, in $\mu mol \cdot L^{-1}$ for urate and creatinine.

	Area under curve	95% CI	Optimal cut off	Sensitivity	Specificity
Cystatin C	0.95	0.90-0.98	>1.23	91.1%	86.6%
Creatinine	0.75	0.67-0.82	>57.0	76.7%	63.9%
Urate	0.83	0.76-0.89	>364.0	69.8%	87.6%

Reciprocal values of creatinine correlated significantly with the glomerular filtration rate as determined by iohexol clearance for both non-pregnant and pregnant women (Pearson's correlation coefficient, $r = 0.75$, $p<0.001$), as did reciprocal values of cystatin C. The correlation between reciprocal serum cystatin C levels and glomerular filtration rate was, however, set at different levels for pregnant ($r = 0.76$, $p<0.001$) and non-pregnant women ($r = 0.73$, $p<0.001$), as described by separate regression lines for each group.

Analysis of covariance confirms a significant difference in reciprocal cystatin C levels between the non-pregnant and pregnant women when adjusted for glomerular filtration rate, corresponding to the distance between the fitted regression lines (coefficient, b=-1.03, SE(b)=0.09, $p<0.0001$). The effect of the interaction between pregnancy and glomerular filtration rate was also significant (coefficient, b=0.005, SE(b)=0.001, $p<0.0001$).

The mean serum concentration of cystatin C was also found to be significantly higher in healthy pregnant women than in healthy non-pregnant women ($p<0.001$), while the opposite situation was found for creatinine ($p<0.01$). Preeclamptic women had significantly higher mean serum levels of cystatin C and creatinine than healthy pregnant women (Table IV). The mean cystatin C level was also significantly higher in twin pregnancies than in singleton pregnancies, especially in twin pregnancies with preeclampsia, corresponding to a significantly lower glomerular filtration rate, while the corresponding levels of creatinine did not differ significantly between these groups and the group of healthy pregnant women.

Table IV. Laboratory values of glomerular filtration rate (GFR) in $mL \cdot min^{-1}$, serum cystatin C (CC) in $mg \cdot L^{-1}$ and creatinine (CR) in $\mu mol \cdot L^{-1}$, with data presented as number (*n*), mean±SD.

	Non-pregnant	Pregnant				
	n=12	*n*=48				
		Healthy pregnant	Gestational hypertension	Preeclampsia	Twin pregnant	Twin and preeclampsia
		n=14	*n*=14	*n*=10	*n*=7	*n*=3
GFR	112±13	153±8	130±31	113±20***	136±32	97±30**
CC	0.88±0.11	1.13±0.17	1.36±0.38	1.63±0.38**	1.52±0.37*	2.0±0.30***
CR	61±11	48±9	54±13	68±17**	48±10	68±27

ANOVA with Dunnett's test as a post hoc test showed significant differences between the pregnant groups at the 0.05 level (*), the 0.01 level (**) and the 0.00l level (***), treating the healthy pregnant group as a control.

No significant correlation was found between fetal or placental weight and the serum levels of cystatin C or creatinine; neither between fetal or placental weight and glomerular filtration rate. Glomerular filtration rate, expressed as $mL \cdot min^{-1}$, displayed a moderate correlation to maternal body weight ($r = 0.4$, $p<0.01$).

Renal structural changes (Papers V and VI)

Endotheliosis was present in all patients with preeclampsia, in all patients with gestational hypertension without albuminuria and in seven of the twelve controls. In four controls the endotheliosis was demonstrated either by light microscopy or by electron microscopy, but not by both techniques.

Light microscopy

Hypertensive patients (*n*=35). One biopsy was excluded from evaluation due to lack of glomeruli. The average number of glomeruli in the remaining biopsies was 12 (range 2-27). Endotheliosis was present in all patients with proteinuric hypertension and all except one had this lesion to a severe or moderate degree. Usually all glomeruli in a section showed endotheliosis but the severity could differ from one glomerulus to another. All patients with non-proteinuric hypertension had endotheliosis, but to a milder degree. The endothelial cytoplasm was often finely vacuolated, with a gradual transition towards true foam cells, which were found in some of the biopsies with severe endotheliosis (Table V).

Fine PAS positive deposits in or between the endothelial cells were occasionally observed, but they were sometimes lacking even in glomeruli with marked endotheliosis. PAS positive hyaline globules or small droplets, usually in the podocytes were also occasional findings. Peripheral mesangial interposition, seen as double contours of the peripheral capillary walls in silver stained sections, was present in some glomerular sections, but usually only affecting a few of the capillaries in a glomerulus. Mesangial proliferation and matrix expansion was not a feature of the glomerular lesion and was only present in the one re-biopsy. Focal segmental glomerulosclerosis or hyalinosis was not seen in any biopsies. Global glomerular sclerosis was rare or absent, without difference between groups. A semi-quantitative analysis showed significantly thickened arteriolar walls in hypertensive patients compared with controls (Fisher's Exact Test, $p<0.001$). There was no hyperplasia of juxtaglomerular cells.

The histological picture was markedly uniform in all biopsies in the hypertensive patients. There were no lesions suggesting primary glomerulonephritis.

Controls (*n*=12). The average number of glomeruli was 11 (range 4-23) in each biopsy and showed endotheliosis in five of the women. The degree was slight or moderate.

Table V. Light microscopy; number of women with different degrees of endotheliosis.

	Healthy controls *n*=12	Hypertensive women *n*=35	
Semiquantified score		Non proteinuric hypertension *n*=8	Proteinuric hypertension *n*=27
0	7	0	0
1	4	4	1
2	1	2	12
3	0	2	14

Fisher's Exact Test reveals significant differences between women with proteinuric hypertension and controls ($p<0.00001$), women with non-proteinuric hypertension and controls ($p=0.02$), and between the two hypertensive groups ($p=0.01$).

Immunofluorescence

Hypertensive patients (*n*=32). Sufficient glomeruli for evaluation was obtained in 32 specimens. Scarce presence of IgM, IgA and C3 was found in few glomeruli along the capillary loops and in the mesangium, but never in all glomeruli belonging to one biopsy. Only in one case was IgA nephritis suspected initially, due to IgA deposition in mesangial areas of all glomeruli. The electron microscopic evaluation did not reveal deposits in the mesangium, however, and follow up was normal.

Controls (*n*=12). All specimens contained sufficient glomeruli for evaluation. No controls did show any fluorescence.

Electron microscopy

Hypertensive patients (*n*=33) Electron microscopic evaluation was not possible in three of the biopsy specimens from the hypertensive group due to insufficient material, but the remaining specimens revealed endotheliosis in all biopsies but one (Table VI). Similarly, mesangial cell interposition with double contours and electron dense depositions (primarily subendothelially) were demonstrated in hypertension both with and without proteinuria.

Controls (*n*=12) showed endotheliosis in seven cases (Table VI) but only to a slight degree. Mesangial cell interposition and electron dense depositions were not seen.

Table VI. Electron microscopy; number of women with different degrees of endotheliosis.

	Healthy controls *n*=12	Hypertensive women *n*=33	
Semiquantified score		Non proteinuric hypertension *n*=8	Proteinuric hypertension *n*=25
0	7	1	0
1	5	4	6
2	0	3	19

Fisher's Exact Test reveals significant differences between women with proteinuric hypertension and controls ($p<0.00001$), women with non-proteinuric hypertension and controls ($p=0.035$), but not between the two hypertensive groups ($p=0.067$).

Morphometric evaluation

Evaluated biopsies were required to contain at least 6 complete glomerular sections ($n = 34$). Biopsies from both hypertensive patients and healthy controls were evaluated (TableVII). The mean number of glomeruli in the evaluated biopsies was 15 (range 6-33). The results from the stereologic estimation of the glomerular volume are presented in Table VII.

Clinical follow up

Onset of disease in the 36 women with hypertension that participated in the investigation occurred in the third trimester in all cases but one, with onset at 179 days of gestation. Eight women had hypertension without proteinuria at the time of the biopsy and 28 were classified as having preeclampsia with significant proteinuria. The 12 healthy women had uneventful pregnancies and deliveries and no women in this group subsequently developed signs of hypertension during the pregnancy. The healthy controls experienced very little discomfort from the procedure, whereas two hypertensive women developed renal haematoma. They both presented the highest levels of cystatin C and their pregnancies were subsequently complicated by abruption of the placenta. The patient with the most pronounced histopathological findings, earliest onset and a twin pregnancy, required treatment with blood transfusion and embolisation of two millimetre-sized arteries, but experienced full recovery, with the renal heamatoma fully resolved two months postpartum.

One patient with preeclampsia had a mild form of cystic kidney disease noticeable at the ultrasound examination prior to the biopsy, but not discernible in the histopathological evaluation. She was not excluded from the study.

Another patient with preeclampsia had exceptionally high serum levels of cystatin C (2.21mg·L^{-1}), corresponding to a decreased GFR (81 mL·min^{-1} as determined by iohexol-clearance) and was found to have a high level of anti-cardiolipin antibodies (40 IgG Phospholipid Units), for which reason she was excluded from further analysis in this study. The antepartum renal biopsy, though revealing pronounced endotheliosis, did not show any signs of renal disease.

One patient had remaining proteinuria 4 months postpartum and a re-biopsy was then performed, showing moderate mesangial cell proliferation. No immunofluorescence and no deposits in electron microscopy were present, however, and no sign of renal disease could be seen in the antepartum biopsy. The findings were therefore interpreted as a healing stage of the renal lesion. Proteinuria, though persisting 9 months postpartum, resolved within a year.

Correlations with renal functional changes

Glomerular volume and levels of renal function tests increased with rising blood pressure and with additional albuminuria (Table VII). The preeclamptic mean level of serum cystatin C was clearly above the upper reference limit for normal term pregnancy in contrast to the preeclamptic mean serum levels of urate and creatinine. The reference values used for cystatin C, urate and creatinine were those obtained in Paper III. ANOVA showed significant differences between groups and significant linear trends for estimated mean glomerular volume and for mean serum levels of cystatin C, urate and creatinine, with a high significance ($p<0.000001$) for all parameters but creatinine (Table VII).

Table VII. Glomerular volume, serum levels of cystatin C, urate and creatinine, and blood pressure (BP) presented as mean (95% CI), albumin concentrations in urine presented as median (range). Reference intervals are given for renal function tests in normal term pregnancy.

	Healthy controls *n*=12	Hypertensive women *n*=35	
		Non-proteinuric hypertension *n*=7	Proteinuric hypertension *n*=28
Glomerular volume, 10^6 μmol^3	2.88 (2.25-3.52)	3.08 (2.14-4.03)	4.26 (3.63-4.85)
Number of biopsies evaluated, *n*	*n*=8	*n*=5	*n*=21
S-Cystatin C, mg·L^{-1} (reference interval)	1.06 (0.93-1.19) (0.68-1.42)	1.31 (1.11-1.50)	1.58 (1.49-1.67)
S-Urate, μmol·L^{-1} (reference interval)	233 (203-263) (186-424)	353 (277-429)	398 (371-426)
S-Creatinine, μmol·L^{-1} (reference interval)	46 (41-51) (37-75)	59 (48-71)	65 (60-70)
Systolic BP, mmHg	114 (107-122)	144 (134-155)	150 (145-155)
Diastolic BP, mmHg	69 (65-74)	98 (93-102)	101 (98-104)
U-Albumin mg·L^{-1}	<300 (dipstick)	145 (49-259)	910 (316-12112)

ANOVA also showed significant differences in mean serum levels of cystatin C, urate and creatinine between the groups of women with different degrees of endotheliosis as semi-quantified by light microscopy. The linear trends were also found to be significant, with a high significance ($p<0.000001$) for the two first mentioned parameters.

In degree 2 and 3 according to the semi-quantified score, the mean serum cystatin C levels rose above the upper reference limit for normal term pregnancy, which the serum levels for urate and creatinine did not, as illustrated by the box and whisker plots given in Paper VI. Similar patterns were confirmed with significant differences and linear trends in women with different degrees of endotheliosis as semi-quantified by electron microscopy of one glomerulus in each biopsy.

The estimated glomerular volume correlated significantly to serum cystatin C levels ($r=0.60$, $p<0.001$). A poor correlation ($r=0.42$, $p<0.05$) was found to serum urate levels, but no significant correlations were observed between glomerular volume and creatinine ($r=0.27$, ns), systolic ($r=0.34$, $p=0.054$) or diastolic ($r=0.33$, $p=0.064$) blood pressure or log-transformed levels of proteinuria ($r=0.18$, ns).

Placental abruption ($n=2$) only occurred in the preeclamptic group. These patients both had the highest cystatin C levels, clearly above the reference interval for normal term pregnancy (mean 2.18 $mg \cdot L^{-1}$), in contrast to their serum levels of urate (mean 366 $\mu mol \cdot L^{-1}$) and creatinine (mean 58 $\mu mol \cdot L^{-1}$), which were normal for term pregnancy.

DISCUSSION

Renal adaptive physiology of normal term pregnancy showed similarities to the pathophysiology of preeclampsia. The transition between normal and seriously complicated pregnancy seems to be gradual and not exclusive to a certain group of predestined women. The development of preeclampsia may rather be dependant on the extent of maternal adaptation to pregnancy required, as certain risk factors for the condition imply. Shallow placentation in the first pregnancy may necessitate extreme adaptation, as may increasing gestational age or twin pregnancy; metabolic dysfunction may cause an inability to adapt in some aspect; predisposing cardiovascular disease may increase maternal sensitivity to the adaptive mechanisms. Increasing fetal demand for maternal adaptation to ensure supply of oxygen and nutrient may cause the rising blood pressure and renal adaptive mechanisms seen in both normal and preeclamptic pregnancy. Thus, it may be renal adaptive mechanisms at their extreme that lead to maternal pathology.

Blood pressure changes were influenced by the same factors in normal pregnancy as in preeclampsia. Blood pressure levels during normal pregnancy have been suggested to be influenced by gestational age[49,50], ethnicity[49,139], age[49,139-141], parity[140], baseline BMI[142,143], and smoking habits[49,144]. Smaller studies have not been able to support an impact of parity[139,141,145], though one study did show greater changes in cardiac output and stroke volume in parous women than in nulliparae during pregnancy[145].

Gestational age. We found in these investigations blood pressure patterns relating to gestational age in a similar way as previously described[49,50] (Papers I and II). The risk of preeclampsia also increases with gestational age[17].

Ethnicity. We also found higher blood pressure levels in this Swedish population than in other studies with similar procedures, indicating an influence of ethnicity. The mean diastolic blood pressure during pregnancy in our population was 68.3 mmHg to be compared with 60.9 mmHg in MacGillivray's study from the UK[49], in which

differences also were observed between other ethnic groups. A Nigerian study found a higher mean diastolic blood pressure during pregnancy, 80 mmHg[139]. Blood pressure levels have been shown to be high in Sweden also in non-pregnant women[146] suggesting genetic causes. Dietary factors have, however, been shown to have an impact on pregnancy blood pressure levels in other cultures[147]. Ethnicity has been proposed also, to influence the risk of preeclampsia[25], as the incidence of the condition is diverse in different parts of the world[10].

Age. Increases in diastolic blood pressure during pregnancy with increasing maternal age have been shown from 30[49] or from 35[140] years of age and in systolic blood pressure steadily with age. We found no such correlation even when adjusting for parity and BMI, possibly due to a narrow age span with a high mean age (30 years, 28 in nulliparae compared to 27 in the country as a whole, in 1996[148]) in the material, which possibly also might offer an explanation for the higher mean blood pressure in pregnancy in our population.

Parity. Significantly higher term diastolic blood pressure levels were found in nulliparae compared to multiparae, also when adjusting for baseline BMI and smoking (Paper I). Following blood pressure levels through three subsequent pregnancies in 3-parae, with the women acting as their own controls, we found both the systolic and diastolic blood pressure levels significantly higher during the first pregnancy in the third trimester (Paper II), agreeing with a previous study[140]. Nulliparity is one of the strongest risk factors for the development of preeclampsia[17], suggesting a cardiovascular and/or immunological reaction to the first placentation or to paternal antigens, to which protective mechanisms develop in subsequent pregnancies[18]. Similar mechanisms may cause the impact of parity on blood pressure levels during normal pregnancy, offering support for theories on the uniqueness of the first placentation, whether in normal or preeclamptic pregnancy. Also, previous pregnancies terminated at an early stage may lower blood pressure levels in subsequent pregnancies (Paper I) and reduce the risk for preeclampsia[24,26-27,149], implying that protective mechanisms may be initiated early in pregnancy. A long time interval between pregnancies[150] or a change of paternity[19-21,150], though they may or may not be risk factors for preeclampsia, did not show an impact on blood pressure levels in these studies.

Baseline BMI. Significantly higher systolic and diastolic blood pressure levels were found all through pregnancy in women with a baseline BMI above the mean (23.7 $kg \cdot m^{-2}$) as opposed to below the mean (Paper I), which agrees with previous indications[142,143]. The effect of parity on third trimester diastolic blood pressure levels was greatest in the group of women with a baseline BMI above the 75th percentile, whereas it was non-significant below the 25th percentile, suggesting an interesting interrelationship between gestational age, parity and baseline BMI (Paper II). A combination of these three risk factors seemed to have the greatest impact on diastolic blood pressure, also illustrated by the fact that baseline BMI correlated significantly with diastolic blood pressure levels only in the first pregnancy.
The risk for developing hypertensive disease in pregnancy has also been found to be increased in women with a high pre-pregnancy or baseline BMI[24-25,32] or a high BMI postpartum[23], corroborated by recent studies[151,152].

In a recently published study it was suggested that women with a high intake of energy early in the second trimester may have an increased risk of developing preeclampsia[153]. Weight gain during pregnancy has not been associated with preeclampsia[34,152], but recently it has been associated with an increased risk of transient hypertension[152], though no association with blood pressure levels in normal pregnancy could be found in the present studies.

Smoking. The greater initial decrease of diastolic blood pressure in pregnant smokers (Paper I) has been shown previously[49,144]. A similar pattern has been noted in pregnant women with mild hypertension[154]. The vasodilatory effect has been suggested to be mediated through the nicotine receptor[155]. Though moderately smoking pregnant women exhibit lower blood pressure values and a lower incidence of preeclampsia[16,155], the risk for complications in both normal pregnancy and preeclampsia increases considerably with smoking[16].

Renal functional changes in normal term pregnancy resembled those in preeclampsia. Though renal plasma flow and glomerular filtration rate increase markedly in pregnancy by approximately 50-85% and 40-65% respectively[73-78], a slight decrease is detected at term and a further decrease on average by 24% and 32%, respectively, in preeclampsia[39]. Renal vascular resistance increases in preeclampsia due to an increase in the afferent arteriolar resistance[51], though it is believed that a reduced glomerular ultrafiltration causes the greater decrease in glomerular filtration rate than in renal plasma flow[39].

Though several investigations have indicated that serum cystatin C is a better marker for glomerular filtration rate than serum creatinine, in particular for individuals with small to moderate decreases in glomerular filtration rate[96], no investigations regarding cystatin C levels in pregnancy have been published except one reporting significantly lower cystatin C levels in healthy pregnant women at term than in their newborns indicating no relationship between the two[156]. The reference interval for serum cystatin C in healthy pregnant women at term (gestational age >37 weeks) obtained in the present investigation (0.68 – 1.42 $mg \cdot L^{-1}$) is close to that previously reported for non-pregnant healthy women below 50 years of age (0.70-1.21 $mg \cdot L^{-1}$), using the same quantitative procedure and calibrator[157], which may facilitate the use of cystatin C as a sensitive marker for glomerular filtration rate also during pregnancy (Paper III).

The fact that serum cystatin C is not decreased in pregnancy due to the increased glomerular filtration rate, though attenuated at term, demands an explanation. The production of cystatin C might be increased during pregnancy due to an increased number of nucleated cells, though cystatin C did not correlate significantly with fetal or placental weight in these investigations (Paper IV).

Serum cystatin C concentrations in healthy pregnant women at term and in their newborns differed significantly and no correlation was detected between them, indicating no transport of cystatin C over the placenta[156]. We have compared serum levels of cystatin C in umbilical vein and umbilical artery samples at delivery, finding no significant differences, which supports the same conclusion (unpublished material).

Serum cystatin C reliably reflected the glomerular filtration rate, in both pregnant and non-pregnant women, though the correlation between reciprocal serum cystatin C levels and glomerular filtration rate was set at different levels for pregnant and non-pregnant women, as described by separate regression lines for each group. The significant difference between the groups after adjustment for glomerular filtration rate and the significant effect of the interaction between pregnancy and glomerular filtration rate indicates an altered filtration process for cystatin C in pregnancy (Paper IV).

Some physiological or pathological renal processes in pregnancy might differently affect the filtration of cystatin C, a strongly positively charged 13 343-Da molecule, and the filtration of iohexol or creatinine, uncharged 821-Da and 113-Da molecules. Indeed, a recent report demonstrated that the glomerular filtration of cystatin C is reduced in certain patients with type I diabetes and albuminuria due to a reduction in mean glomerular filtration slit size, despite a normal glomerular filtration rate determined as the clearance of iothalamate, a 613-Da low molecular mass marker[158].

Cystatin C levels might thus demonstrate a reduced glomerular filtration rate of molecules in the approximate molecular mass range 10-15 kDa, while the glomerular filtration for low molecular mass substances may be normal. Indeed, despite that the clearance of low molecular mass substances like creatinine and iohexol is known to increase in pregnancy, the fractional clearance of dextran particles with a molecular mass of 20 kDa, has been described to decrease in pregnancy[77].

The reduced glomerular filtration of cystatin C in term pregnancy could thus possibly be explained by a reduction in mean glomerular filtration slit size. It is also possible that the charge selectivity of the glomerular barrier is altered in term pregnancy, as filtration of the positively charged cystatin C molecule is restricted, while albumin, a 67 kDa negatively charged molecule, is increasingly excreted in the urine during normal pregnancy[159].

In preeclampsia, a change in both size- and charge-selectivity of the glomerular barrier has been proposed. The swollen rim of endothelial cytoplasm contains fewer fenestrae with a reduction in size[119]. Fewer anionic sites have been demonstrated in the glomerular barrier in renal biopsies from preeclamptic patients two weeks postpartum, compared to kidneys obtained at nephrectomy of non-pregnant trauma patients[160,161]. We speculate that there could be a shift towards a more cationic glomerular barrier with a reduction in mean glomerular slit size in both healthy term pregnancy and in preeclampsia, resulting in higher serum concentrations of cystatin C in both conditions.

Serum cystatin C was a better marker for preeclampsia than serum creatinine or serum urate, as demonstrated by ROC analysis of the data of the present investigation. Serum urate showed a higher diagnostic accuracy than creatinine and has also been shown to be a useful predictor of fetal outcome in preeclampsia[70,71,162], though increasing serum levels in preeclampsia reflect an enhanced reabsorption in the proximal tubuli and not a reduced glomerular filtration rate[39]. Serum creatinine is also of limited use in the assessment of the glomerular filtration rate, which can be reduced by 50% without causing abnormal serum creatinine concentrations[163-165]. Several of the patients with the most severe preeclampsia had normal creatinine levels in this material, whereas all patients with severe preeclampsia had cystatin C levels raised above the upper reference limit for normal term pregnancy (Paper III).

Though preeclampsia can be diagnosed simply through determining hypertension with proteinuria, the diagnosis of the true condition associated with increased risk can still be elusive, as pregnant women can present with hypertension and proteinuria due to other conditions, and a preeclamptic state can be present without raised blood pressure or albuminuria. Also, blood pressure levels and albuminuria are instable markers, often varying within a wide range during the course of the disease. The estimation of serum cystatin C could be helpful in the diagnosis of preeclampsia, reflecting a different feature of the disease as a stable indicator of an altered filtration process. It may also prove valuable for the monitoring of glomerular filtration rate in renal disease in pregnancy and in preeclampsia.

Renal structure changes resembling preeclampsia were also present in healthy term pregnancy. In a systematically performed, controlled antepartum renal biopsy study of patients with hypertensive disease in pregnancy at the time of diagnosis, glomerular endotheliosis was present in all patients with preeclampsia, in all patients with gestational hypertension without albuminuria and in seven of the twelve healthy pregnant controls (Paper V). Clinical follow up did not reveal any signs of subsequently developing preeclampsia in any of the healthy pregnant controls. Electron dense deposits and mesangial cell interposition were only found in hypertensive patients, who also presented a more pronounced degree of endotheliosis. Clinically undetected renal disease was not diagnosed in any of the women.

Earlier renal biopsy studies have avoided the use of healthy pregnant controls, except for occasional specimens, which were evaluated as normal by light microscopy alone[69]. During normal pregnancy the renal blood flow and the glomerular filtration rate increase by at least 40%[73-78] and the kidney volume increases by up to 30 %[166], which suggests that physiological renal morphologic changes might be expected. Autopsy material or necro-kidneys from non-pregnant women have been used as controls with obvious problems of interpretation. In many investigations, renal biopsies have been deferred to the postpartum period, when a preeclamptic lesion may already be resolved or findings may be determined by the healing stage of the lesion. Regression of some glomerular lesions in preeclampsia can be seen as early as the first week postpartum[133-137] and complete resolution has been reported within 4 weeks[134]. Renal function is usually restored within only a few days after delivery.

In the past, glomerular endotheliosis has been regarded as pathognomic for preeclampsia, and has been used to confirm the diagnosis, both in the clinical context and in research. Endotheliosis found in this study, both in gestational hypertension and in healthy pregnant controls, could not constitute a diagnostic criterium for preeclampsia, but might be regarded as the “missing link” in the proposed continuum or gradual transition between normal pregnancy and preeclampsia[2].

Endotheliosis was found to only a mild or moderate degree in normal pregnancies, and since the phenomenon varied from glomerulus to glomerulus, it is not surprising that the semiquantitative assessments by light microscopy differed from those by electron microscopy of one glomerulus. Other general histopathological findings in patients with hypertensive pregnancy were similar to the typical findings described in preeclampsia in previous studies[69,117,133,134,167-171].

Pronounced "foam cells" were scarce, the frequency in previous studies ranging from 4% to 35%[117,133,167] and encountered more often in postpartum biopsies. Neither was mesangial interposition a dominant finding, consistent with most previous studies [117,134,167-169] with few exceptions[170,171] involving severe cases. Focal segmental glomerulosclerosis was not seen in any biopsies, but has also been a variable finding in other investigations, with the highest frequency found in postpartum biopsies in African American women with essential hypertension, arteriosclerosis and interstitial fibrosis[172]. Hyperplasia and degranulation of juxtaglomerular cells have been reported[169], but could not be confirmed in this study.

Fibrin deposition in the glomeruli has been reported by a number of researchers[173-175] in 80-100% of postpartum biopsies, though other investigators only detected fibrin in 44% of cases[176]. We did not find intraglomerular fibrin depositions in this material.

Previous renal biopsy studies have found the diagnosis of preeclampsia by clinical criteria alone to be uncertain. Investigators from Chicago[117,176] found biopsy-proven glomerular capillary endotheliosis in only 84% of the nulliparae with the clinical diagnosis of preeclampsia, and in only 38% of the multiparae. Unsuspected nephrosclerosis, renal disease or both were found in 24% of nulliparae and 65% of multiparae. Consequently, in groups with multiparae, the clinical diagnosis was less accurate[69,125]. It has therefore been suggested, that proteinuric hypertension in multiparae represents an early manifestation of chronic renal disease, rather than preeclampsia. No cases of clinically undetected renal disease were found in primiparae or in multiparae in our material, both groups displaying the same morphologic changes characteristic for preeclampsia.

Thus, undetected renal disease seems to be a rare cause of hypertensive disease during pregnancy in our population. The explanation for the discrepancy with previous findings could be an improved health as well as early detection of chronic renal diseases in fertile women before the childbearing period, together with increased prenatal counselling.

In this study, the healthiest women experienced very little discomfort from the renal biopsy procedure, whereas serious complications arose in the severest case of preeclampsia. Whether subsequent placental abruption could be related to the complications of renal biopsy procedure or rather to the course and severity of disease is open to speculation, but recommendations to avoid renal biopsy in cases of severe early onset preeclampsia seem appropriate. Renal biopsy on clinical indications has been recommended only for cases where the outcome could affect patient management, as in suspected progressive glomerulonephritis with sudden renal failure or massive nephritic syndrome early in pregnancy, when delivery cannot safely be performed. Even in these cases, mere presence of glomerular endotheliosis does not appear to be of great value to ascertain, and as renal disease seems to be rare in our population, very few cases would seem to benefit from a renal biopsy.

It would be a great advantage, however, if we could monitor the degree and progression of glomerular endotheliosis in some fashion, without the renal biopsy having to be performed. As glomerular endotheliosis is paralleled by a reduction in glomerular filtration rate, due to a decrease in number and size of fenestrae impairing hydraulic permeability [119], a sensitive marker of the glomerular filtration rate might fulfil this need.

Serum cystatin C levels correlated significantly with the estimated glomerular volume in these studies, indicating that serum cystatin C might be used as a marker for glomerular swelling, as a feature of renal affection in preeclampsia. The serum levels of cystatin C, urate and creatinine all **increased with the degree of endotheliosis**, clearly indicating increased renal involvement with progression of hypertensive disease (Paper VI).

The mean serum levels of urate and creatinine in preeclampsia were not raised above the corresponding upper reference limits for normal term pregnancy, which reduces the usefulness of these parameters for monitoring hypertensive patients in pregnancy. The mean serum cystatin C levels rose above the upper reference limit for normal term pregnancy in preeclampsia and in women with endotheliosis corresponding to degree 2 and degree 3 of the semi-quantified score, in contrast to serum levels for urate and creatinine.

The "golden standard" for estimating the number-weighted mean glomerular volume from a human kidney biopsy is the Cavalieri principle[177]. We used the method of Weibel-Gomez in our study, which has been shown to result in a slight underestimate of glomerular volume, though with an excellent correlation to the real glomerular volume[178]. It is also well known that glomeruli shrink in paraffin embedded renal tissue compared with plastic embedding[179]. No correction was made for shrinkage in this study, which shows smaller glomerular volumes than in studies using plastic embedded tissue[180]. However, no published data has suggested an effect on the difference in glomerular size between groups or within groups in paraffin embedded material. We therefore believe the presented correlation between glomerular volume and serum cystatin C to be true.

Serum cystatin C levels might thus prove to provide valuable information, not only regarding the glomerular filtration rate, but also regarding the degree of glomerular endotheliosis and increase in glomerular volume in pregnancy and in preeclampsia, explaining its superior performance as a **marker for preeclampsia**, defined as proteinuric hypertension. Preeclamptic patients had a more pronounced degree of endotheliosis than patients with gestational hypertension or normal pregnancy in this study and the degree of endotheliosis has previously been considered to determine the severity of preeclampsia[69,117]. With this view, cystatin C could be expected to provide information also regarding the **severity of preeclampsia**. Indeed, patients with severe preeclampsia defined as albuminuria $>3\ g \cdot L^{-1}$ showed higher values of cystatin C than patients with mild preeclampsia, though the difference was not statistically significant.

Even regardless of this, the impairment of renal function in preeclampsia needs to be monitored closely to ensure a timely delivery before impending danger of permanent renal damage. Combined monitoring of glomerular filtration rate and degree of endotheliosis made possible with cystatin C, presents as an attractive alternative and might considerably reduce the need for renal biopsy in preeclampsia, especially considering that glomerulonephritis was not found in any of the hypertensive patients in this series.

Blood pressure levels, both systolic and diastolic, increased with the degree of endotheliosis as expected, showing a highly significant linear trend though not correlating with estimated glomerular volume. Blood pressure levels may be instable, due to hypovolemia, and highly variable within a wide range in the preeclamptic condition and disease progression can be difficult to detect from simply monitoring blood pressure levels, especially as antihypertensive medication is frequently used. However, earlier in the development of the condition, a rise in arterial blood pressure may be the first sign of increased renal arterial resistance and a reduced glomerular ultrafiltration coefficient associated with preeclampsia, as these changes shift the pressure range of the renal output curve. Blood pressure measurement is still an easily accessible method of detecting change in renal function and a good early marker for the development of preeclampsia.

Albuminuria, similarly, may be highly variable during the course of disease progression in preeclampsia, and was not correlated with estimated glomerular volume even when logtransformed values were used or nonparametric tests (calculation of Spearman's correlation coefficient) were performed. Neither did adjustment for collection time or urine concentration (U-creatinine) improve the statistics. However, both a change in charge- and size-selectivity of the glomerular barrier has been proposed in preeclampsia, with fewer anionic sites demonstrated in renal biopsies[160,161], possibly causing a shift towards a more cationic glomerular barrier. If a decrease in negative charge of the glomerular barrier is believed to contribute to the reduced filtration of positively charged cystatin C in preeclampsia and in term pregnancy, it could also increase the excretion rate of negatively charged albumin.

Albumin, an anionic molecule (pI 4.9), has indeed been shown to pass the basement membrane to an increased degree during pregnancy[159]. This does indicate a possible reduction in the negative charge of the glomerular barrier. The increased urinary albumin excretion in preeclampsia could thus, at least in part, be due to a further reduction in anionic sites parallel to a change in pore number or size. It is in fact conceivable, that a loss of negative charge across the endothelial cell membrane might also be a contributing cause of the swelling of endothelial cells. Further research in this area is needed, but should these suppositions prove true, the change in charge-selectivity of the glomerular barrier might be one of the earliest signs of preeclampsia. This would explain, that while it might not be a good marker for the degree of endotheliosis, urinary albumin is an excellent and easily accessible early marker for the development of preeclampsia.

Serum cystatin C consequently seems to closely reflect the renal functional and structural changes, which also give rise to increased blood pressure levels and albuminuria. These parallel changes, probably emanating from the same process, occur **both in healthy term pregnancy and to a greater extent in preeclampsia.** Unpublished data show that cystatin C levels start to rise around 26-28 weeks of gestation, about the time when we can see our earliest cases of preeclampsia. The cystatin C levels then steadily rise until term, indicating a gradual transition between the normal changes in term pregnancy and the extreme changes in preeclampsia.

Why is this of interest? It could be easy to regard markers of renal functional and structural changes as unhelpful in the diagnosis of preeclampsia as they can be altered also in normal pregnancy. We would like to take the opposite view. Understanding preeclampsia as the extreme adaptation to pregnancy, rather than as a distinct separate condition, yields these markers an important role of determining the stage of the transition between normal pregnancy and pregnancy at risk. It also follows, that it may be impossible to define a distinct group of women early in pregnancy that are at an increased risk of developing preeclampsia, for closer monitoring. All pregnant women may develop preeclampsia if adaptive demands are extreme and all women should be monitored by blood pressure measurement and determination of albuminuria, as early markers of the condition.

Also, if preeclampsia were a separate condition that only a certain group of women were prone to, we would have an ill-defined limited number of patients to study and it would be difficult (as it has proved to be) to find any definite answers to our questions. Whereas if the condition were due to an extreme adaptation to pregnancy, the answers would be somewhere to be found in the normal physiology of the pregnant woman. The adaptive mechanisms would be supposed to ensure fetal well-being. The underlying cause could thus be an increased fetal demand of adaptation for various reasons. This concept agrees with the clinical variability of the disease and the multifactorial etiology, the syndrome of preeclampsia. This in turn would mean, that we have to seek for the cause of preeclampsia among a large group of disorders leading to insufficient supply of oxygen and/or nutrients to the fetus. Among these could be found severe anemia, severe hypovolemia, restricted compliance in blood vessels, metabolic dysfunction, placenta dysfunction or insufficiency. The fetus' response to a resulting insufficient supply of oxygen and/or nutrients might forward the maternal adaption to the extreme, thus the "toxemia of pregnancy", an apt description.

We have already quite adequate methods of surveying fetal well-being, and the fetal indications for imminent delivery are well defined. The markers often used to monitor the maternal condition sometimes signal too late for us to be able to intervene in the disease process and prevent serious complications. Greater understanding of the nature of the disease process from normal adaptive mechanisms at term to severe preeclampsia, might help us develop management routines directly based on markers of this process, to in time avoid the serious complications of preeclampsia. The development of serum cystatin C as a marker may aid in this endeavour.

CONCLUSIONS

- **Renal adaptive physiology of normal term pregnancy showed similarities to the pathophysiology of preeclampsia.** The transition between normal and seriously complicated pregnancy seemed to be gradual with preeclampsia at the extreme of adaptive changes to pregnancy.

- **Blood pressure changes** were influenced by the same factors in normal pregnancy as in preeclampsia. Our studies supported the influence of gestational age, ethnicity, parity, baseline BMI, and smoking habits, with an interaction between blood pressure, gestational age, parity and baseline BMI (Paper I and II).

- **Renal functional changes** in normal term pregnancy resembled those in preeclampsia. Though glomerular filtration rate is known to increased markedly in pregnancy, our studies confirmed a slight decrease at term and a further decrease in preeclampsia (Paper III and IV).

- **Renal structural changes** in normal term pregnancy resembled those in preeclampsia as glomerular endotheliosis was found in all patients with preeclampsia and with gestational hypertension without albuminuria, and also in seven of the twelve healthy pregnant controls. Clinically undetected renal disease was not diagnosed in any of the women (Paper V and VI).

and…

- **Serum cystatin C was a better marker for preeclampsia**, defined as proteinuric hypertension, than serum creatinine or serum urate. Reference values for serum cystatin C at term were calculated to 0.68-1.42 mg/L (Paper III).

- **Serum cystatin C reliably reflected the glomerular filtration rate**, in both pregnant and non-pregnant women though the relationship was set at different levels for pregnant and non-pregnant women, indicating an altered filtration process in pregnancy. This could be explained by a change in size- and/or charge-selectivity of the glomerular barrier (Paper IV).

- **Serum cystatin C levels correlated significantly with the estimated glomerular volume and increased with the degree of endotheliosis**. Serum cystatin could as such, function as a marker for the stage of the transition between normal adaptive changes at term and preeclampsia and indicate severity of disease (Paper VI).

- **Serum cystatin C consequently seemed to closely reflect the renal functional and structural changes, which also can give rise to increased blood pressure levels and increased urinary excretion of albumin.** These parallel changes, probably emanating from the same process, occurred **both in healthy term pregnancy and to a greater extent in preeclampsia.** Good markers for the stage of transition in this process may greatly aid us in the management of hypertensive diseases in pregnancy. Indeed, we believe that with this aid, the need of renal biopsy on clinical indications is reduced to very few cases, especially as clinically undetected renal disease seems to be a rare cause of hypertension in pregnancy in our population.

POPULARIZED SUMMARY IN SWEDISH
Populärvetenskaplig sammanfattning

Njurarna spelar en central roll i de förändringsprocesser som anpassar kvinnans kropp till en graviditet. Många av de förändringar som sker mot slutet av en normal graviditet, påminner om de förändringar man ser vid preeklampsi, havandeskapsförgiftning, om än mindre uttalade. Preeklampsi är ett allvarligt risktillstånd som kan uppkomma under graviditet och en av de största orsakerna till mödra- och barndödlighet samt sjuklighet i världen. Benämningen härrör ur termen ”eklampsi”, kramper, och preeklampsi är således tillståndet före vilket man förväntar sig allvarliga graviditetskomplikationer, till exempel graviditetskramper. Men även andra komplikationer riskeras, som till exempel allvarlig njur- och leverpåverkan, blödningsrubbning och svår tillväxthämning av barnet. Diagnosen av risktillståndet ställs genom uppmätning av ett blodtryck >140/90 mmHg och tillkomst av äggvita i urinen.

Oftast kan man förlösa patienten och så rädda både mor och barn om graviditeten är tillräckligt långt framskriden. Svårigheten ligger i att välja optimal tidpunkt för förlossning; innan allvarliga komplikationer har uppträtt, men inte innan fostret har uppnått viss mognadsgrad och goda chanser till överlevnad. Övervakningsmetoder för fostret finns redan väl utvecklade, så att vi inte äventyrar barnets hälsa med att fördröja en förlossning. Till vår hjälp skulle vi också behöva en känslig sjukdomsmarkör för mamman, för att avgöra var i sjukdomsprocessen hon befinner sig, då denna ibland kan förlöpa hastigt.

För att få en ökad förståelse av sjukdomsprocessen har vi återknutit till våra kunskaper om normal sen graviditet, där förändringarna vad gäller njuren kan efterlikna de preeklamptiska. Kanske i själva verket förändringarna i normal sen graviditet och preeklampsi är delar av samma adaptiva förlopp, som gradvis kan bli mer uttalat, och där preeklampsi utgör den mest extrema anpassningen av moderns kropp till graviditeten. Det skulle betyda att alla gravida skulle kunna utveckla preeklampsi om de adaptiva kraven var tillräckligt höga. Alla kvinnor borde då ha en viss övervakning i sen graviditet.

Det skulle också betyda att svar om preeklampsins uppkomst-mekanismer skulle kunna finnas att tillgå i den normalgravida kvinnans graviditetsfysiologi. Vår teori är också att det kan vara ökade krav på syrgas- och näringstillförsel från fostret och graviditeten som driver förloppet att bland annat öka blodtrycket hos mamman för att säkra blodtillförsel till moderkakan. Vid en dåligt fungerande moderkaka, vid ämnesomsättningsrubbningar eller cirkulatoriska rubbningar hos mamman skulle processen kunna drivas in i patologi. Därav begreppet ”havandeskapsförgiftning”, som i själva benämningen innefattar mycket av vad sjukdomen innebär.

Det visade sig i våra studier att funktionella och strukturella njurförändringar i sen graviditet verkligen var påfallande lika de som man finner vid preeklampsi. Blodtycket ökade med nivåer som påverkades av samma faktorer som uppfattats som riskfaktorer för att utveckla preeklampsi. Njurfunktionen inskränks i normal sen graviditet, för att ytterligare försämras vid preeklampsi. Och dessutom hittade vi strukturella förändringar i njurvävnad, som tidigare ansetts exklusiva och diagnostiska för preeklampsi, även hos helt friska gravida kvinnor!

Detta styrker teorin om en successiv övergång från normala anpassningsmekanismer till patologiska sådana i sen graviditet. Det kan då tyckas besvärligt att skilja ut det friska från det sjuka och det har även påståtts att det skulle vara meningslöst att följa njurprover som övervakningsmetod vid preeklampsi, eftersom de kan ändras i normal sen graviditet. Utmaningen är istället att hitta en känsligare markör för de här förändringarna. Cystatin C är ett ämne, vars koncentration i blodet mycket nära speglar njurarnas funktionsnivå. Vi har i dessa studier kunnat visa att cystatin C även hos gravida speglar njurfunktionen tillfredställande. Det visar också på en ändrad filtrationsprocess av blodplasma genom njurkapillärernas vägg i sen graviditet och preeklampsi, vilket troligtvis är anledningen till att det var en känsligare markör för tillståndet preeklampsi än andra vi haft att tillgå. Troligen bidrar denna ändrade filtration även till den ökade äggviteutsöndringen. Cystatin C visade sig också kunna spegla graden av preeklamptiska strukturella förändringar, som också fanns i viss mån hos normalgravida, och bör således kunna vara oss till god hjälp vid övervakning av preeklampsi.

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