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**Preeclampsia and other circulatory diseases  
during pregnancy –  
etiological aspects and impact on female offspring**

**by**

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**Why ?**



## SUMMARY

The aims of this thesis were to investigate and compare environmental effects on the liability of developing preeclampsia and gestational hypertension; to estimate the relative importance of genetic and environmental effects on the liability of developing preeclampsia and gestational hypertension; to assess the effect of childbearing on the risk of circulatory diseases; and to explore the effect of preeclampsia on the female offspring anthropometry in early adulthood.

We used the Swedish Birth Register to compare the effect of maternal diabetes, multiple birth, smoking, place of birth and obesity, on the liability of developing preeclampsia and gestational hypertension among 10,666 primiparous women. Maternal diabetes, multiple birth and obesity were all associated with increased risk of preeclampsia and gestational hypertension. Maternal smoking and non-Nordic maternal place of birth were associated with decreased risk of preeclampsia and gestational hypertension. The similarities in risk factor patterns may indicate similarities in the biological mechanisms underlying the two conditions.

To estimate the relative importance of genetic and environmental effects on the liability of developing preeclampsia and gestational hypertension, we used the Swedish Twin Register linked to the Medical Birth Register and used quantitative genetic analyses of pregnancies of 917 monozygotic and 1,199 dizygotic twin pairs. For preeclampsia, we found that genetic and environmental effects are of about equal importance. For gestational hypertension, the estimates of heritability and non-shared environmental effect were 0.24 and 0.76, respectively. When we considered both diseases together as pregnancy-induced hypertension with different degrees of severity, the estimates of heritability and non-shared environmental effect were 0.47 and 0.53, respectively.

We assessed the effect of childbearing on the risk of circulatory diseases by a cross-linkage of the Swedish Medical Birth Register and the Inpatient Register and analyzed 1,003,489 deliveries among 654,957 women. Compared with non-pregnant and early pregnant period, the risks of venous thromboembolic diseases were increased in third trimester, peaked during three days around delivery (relative risk 80) and declined during the puerperium. For arterial diseases (subarachnoid hemorrhage, intracerebral hemorrhage and cerebral infarction), there was, compared with non-pregnant and early pregnant period, a more than 30-fold increased risk during three days around delivery, which declined during the puerperium. We also studied the effects of maternal characteristics on the risk of stroke and pulmonary embolism during the third trimester of pregnancy, around, and after delivery. Although preeclampsia, multiple birth and Cesarean section were all associated with highly increased risk of both pulmonary embolism and stroke, these complications did not explain the increased risks related to pregnancy.

We explored the effect of intrauterine exposure to preeclampsia on the anthropometry in early adulthood among females. In all 230 young women exposed to preeclampsia in utero and 359 non-exposed women were included. In young adulthood there were no differences in height, body-mass index (BMI), waist to hip ratio or age at menarche between preeclampsia exposed female offspring as compared to non-exposed.

Key words: preeclampsia, gestational hypertension, pregnancy-induced hypertension, risk factors, heritability, twin study, venous thrombosis, pulmonary embolism, subarachnoid hemorrhage, intracerebral hemorrhage, intracerebral infarction, stroke, intrauterine exposure

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**PAPERS I-V**

## ORIGINAL PAPERS

The thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Ros Salonen H, Cnattingius S, Lipworth L. Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. *Am J Epidemiol* 1998; 147: 1062-70.
- II. Ros Salonen H, Lichtenstein P, Lipworth L, Cnattingius S. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. *Am J Med Genet* 2000; 91: 256-60.
- III. Ros Salonen H, Lichtenstein P, Bellocco R, Petersson G, Cnattingius S. Increased risks of circulatory diseases in late pregnancy and puerperium. *In press, Epidemiology*.
- IV. Ros Salonen H, Lichtenstein P, Bellocco R, Petersson G, Cnattingius S. Pulmonary embolism and stroke in relation to pregnancy —how can high-risk women be identified? *Submitted*.
- V. Ros Salonen H, Lichtenstein P, Ekblom A, Cnattingius S. Tall or short —twenty years after preeclampsia exposure in utero? Comparisons of final height, body-mass indexes, waist to hip ratio and age at menarche among girls, exposed and non-exposed to preeclampsia during fetal life. *In press, Pediatric Research*.

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## ABBREVIATIONS

BMI	body-mass index
$c^2$	shared environment
CI	confidence interval
df	degrees of freedom
DZ	dizygotic
$e^2$	nonshared environment
$h^2$	heritability
HLA	human leukocytantigen
ICD	international classification of diseases
IDDM type 1	insulin dependent diabetes mellitus type 1
IRR	incidence rate ratio
MZ	monozygotic
OR	odds ratio
RR	relative risk
SD	standard deviation
SIR	standardized incidence rate
TNF-alpha	tumor necrosis alpha
$\chi^2$	Chi-squared

## INTRODUCTION

Eclampsia (convulsions during pregnancy) has been a feared complication since Hippocrates' time (Chalmers 1989). It was discovered in the 1840s that eclampsia was accomplished by proteinuria and in the late nineteenth century the technique of indirect measurement of arterial blood pressure was developed. Hypertension and proteinuria became accepted as warning signs of eclampsia (pre-eclampsia), and screening of pregnant women was introduced (Chesley 1978). Toxicosis is another term for preeclampsia, commonly used in the past, which expresses a view that toxins were part of the etiology (Chesley 1978).

Hypertension occurring during pregnancy is a major pregnancy complication, with an incidence of 2-10% of all pregnancies (Lindmark 1984, Working group on High Blood Pressure in Pregnancy 2000), and is associated with preterm delivery, fetal growth retardation, abruptio placentae, maternal morbidity and mortality (Chesley 1984, Eskenazi 1993, Williams 1991). The term **pregnancy-induced hypertension** is quite commonly used and encompasses hypertension, either with proteinuria (**preeclampsia**) or without proteinuria (**gestational hypertension**). Unfortunately, different definitions are used, which makes it difficult to evaluate and draw conclusions from the results of different studies. The differences in the definitions and classifications of preeclampsia and gestational hypertension probably originate in how the view and knowledge have changed over time.

## BACKGROUND

### Pathogenic mechanisms

**Preeclampsia** is described as disease with **immune maladaptation** (Dekker 1998) with lower proportion of T-helper cells (Bardeguet 1991), and deposition of immunoglobulins, complement, and fibrin has been noted in the walls of preeclamptic spiral arteries (the terminal branches of the uterine artery) (Dekker 1998). There is a systemic activation of maternal inflammatory cell responses in preeclampsia (Redman 1999), with activation of both granulocytes (Greer 1989) and monocytes (Berge 1988), and increased release of proinflammatory cytokines (Vince 1995).

High blood pressure in preeclampsia is mainly due to a reversal of the vasodilation characteristic of normal pregnancy, replaced by marked increases in peripheral vascular resistance (Visser 1991). It has been shown that the maternal pressor response to **angiotensin-II** is reduced in normal pregnancy, but increased in women who develop preeclampsia (Gant 1973). Recently, agonistic autoantibodies to the angiotensin-I receptor were described (Dechend 2000), but the relevance of this finding remains to be elucidated (Roberts 2000).

The **placenta** is considered the pathogenic focus for all manifestations of preeclampsia, because delivery is the only definitive cure for this disease (Working group on High Blood Pressure in Pregnancy 2000). Early in normal gestation, the spiral arteries are transformed from thick-walled, muscular vessels to sac-like vessels with decreased or lacking vasoconstrictive abilities. This transformation involves invasion of the spiral arteries by endovascular trophoblast cells of the placenta (Pijnenborg 1980, Zhou 1997a, Zhou 1997b). In women who eventually develop preeclampsia, the invasion of the uterine spiral arteries is incomplete, with the vessels remaining thick-walled and muscular (Zhou 1993), and with failure of endothelium-dependent relaxation (Ashworth 1997). The cytotrophoblasts in preeclamptic women seem to fail to express vascular-type adhesion molecules, which impair their ability to form sufficient connections with the uterine vessels (Khong 1992, Zhou 1997b, de Groot 1995, Pijnenborg 1996). However, there is not an all or none invasive phenomenon

in normal and preeclamptic pregnancies (Meekins 1994), and defective placentation may not be present in all preeclamptic women and severe placental ischemia does not necessarily lead to preeclampsia (Khong 1986). The perfusion of the placenta is decreased in preeclampsia, which may lead to early placental hypoxia (Working group on 2000). Moreover, there seems to be an increase in trophoblast apoptosis in placentas from women with preeclampsia, compared with controls (Allaire 2000). Yet another interesting feature in preeclamptic pregnancies is an exaggerated deportation of trophoblasts and syncytiotrophoblast microvillus membranes from the placenta to the maternal venous circulation, compared with normal pregnancies (Chua 1991), which in turn may impair maternal vascular endothelial function (Cockell 1997, Knight 1998). Also the number of fetal blood cells transferred to the maternal circulation is higher in women with preeclampsia, compared with women with normotensive pregnancies (Holzgreve 1998).

Preeclampsia is regarded as an **endothelial disorder** (Roberts 1989, Roberts J 1998, Taylor 1998). Endothelial cells line all blood vessels and regulate thrombosis, vascular tone and the permeability of the vessel wall to cells and molecules by the release of a number of diverse factors. In preeclamptic women, a factor that may cause generalized endothelial dysfunction, has been suggested to be released from the placenta into the maternal circulation, although it has been pointed out that this chronology may not be correct (Dekker 1998). In response to serum/plasma from women with preeclampsia there are increased accumulation of triglycerides in endothelial cells, which may impair their function (de Groot 1995). Increased production of thromboxane- $A_2$  in the preeclamptic placenta (Woodworth 1994) and platelets (Meagher 1993) causing the vasoconstriction and platelet aggregation (Friedman 1988), have been regarded as important, and aspirin has been used to reduce levels of thromboxane in prevention trials (Sibai 1993, Dekker 1993). Elevation of thromboxane is suggested to be a secondary event (Mills 1999), and the results from the trials have generally been disappointing (Sibai 1998), although a recent metaanalysis have reported a moderate (15%) reduction in risk of preeclampsia, among women in trials with antiplatelet drugs (Duley 2001).

It has been hypothesized that in preeclampsia ischemic trophoblast tissue secretes catecholamines as a physiological signal to increase maternal blood flow to the fetoplacental unit, and that the increased levels of norepinephrine causes **oxidative stress** through high levels of lipid peroxides (Manyonda 1998). Oxidative stress has been proposed to cause the endothelial dysfunction (Walsh 1994, Dekker 1996, Staff 1999), and antioxidant levels, such as vitamin E, C and carotenoids are decreased in preeclampsia (Mikhail 1994). Prophylaxis with vitamin E and C have been performed in a trial of women at increased risk of preeclampsia (Chappell 1999). There were indications of a decreased risk of preeclampsia in that selected group, but the beneficial effect may have been overestimated due to a higher proportion of smokers and lower proportion of black women in the treated group (Chappell 1999).

**Factors indicating endothelial dysfunction** are: elevated levels of endothelin (Taylor 1990, Nisell 1991), reduced productions of the vasodilating substances prostaglandin/prostacyclin (de Groot 1995, Fitzgerald 1987, Friedman 1988). Involvement of endothelial nitric oxide in preeclampsia pathogenesis is controversial (Lowe 2000, Anumba 1999), but there are indications of impaired shear stress-mediated release of nitric oxide in preeclamptic uterine circulation (Kublickiene 2000).

Compared with normal pregnancies, **endocrinological alterations** are present in preeclamptic pregnancies. Progesterone production is significantly higher in preeclamptic than normal pregnancies, which may contribute to lower prostacyclin production (Walsh 1988). Levels of testosterone are also higher in women with preeclampsia than in normotensive women (Acromite 1999). The presence of estrogen leads to an enhancement of

vascular endothelial growth factor-induced arterial dilatation in normal pregnancy (Storment 2000), and the lower levels of estrogens reported in preeclamptic women (Rosing 1984) may therefore be unfavorable. Moreover, these endocrinological alterations associated with preeclampsia appear to promote oxidative stress (Zhu 1997).

Research concerning pathogenesis of **gestational hypertension** is much less extensive. Gestational hypertension is regarded as latent essential hypertension unmasked by pregnancy (Chesley 1980) and research is commonly focused on difficulties with hemodynamic adaptation (Bosio 1999).

## **Epidemiological factors associated with preeclampsia and gestational hypertension.**

Preeclampsia and gestational hypertension occurs predominately among primiparous women (Campbell 1985). The length of preconceptional sexual cohabitation is inversely related to the incidence of preeclampsia/gestational hypertension (Robillard 1994). The protective effect of parity on risks of preeclampsia and gestational hypertension, disappears if a parous woman changes her partner (Trupin 1996, Tubbergen 1999, Li 2000). Moreover, among women who were normotensive in their first pregnancy, women with a new partner in subsequent pregnancy have increased risk of preeclampsia, as compared to women with the same partner (Li 2000). Induced and spontaneous abortions are reported to be associated with decreased risks of preeclampsia (Seidman 1989, Eras 2000). The recurrence rate of hypertensive disorders in pregnancy, is 20-50% in second pregnancy (Zhang 2000). Women with multiple pregnancies are at increased risk of preeclampsia and higher maternal exposure to paternal antigens has been a suggested explanation (Long 1987, Sibai 1991). These factors all point at an **immunological component** in the etiology of preeclampsia, and possibly also gestational hypertension.

There are also factors indicating that **metabolic predisposition** may be of importance. Maternal obesity, hypercholesterolemia, diabetes and polycystic ovarian syndrome are all reported to be associated with increased risks of preeclampsia (Sibai 1995, Eskenazi 1991, Stone 1994, Thadhani 1999, Garner 1990, Schaffir 1995, Hanson 1993, Siddiqi 1991, Martinez Abundis 1996, de Vries 1998, Kaaja 1995). Interestingly, women with a history of preeclampsia have signs of insulin resistance, hypertriglyceridemia and hyperuricemia even two years after giving birth (Nisell 1999, Fuh 1995). Associations of these metabolic alterations with gestational hypertension are less studied (Thadhani 1999).

**Environmental factors** are also associated with both preeclampsia and gestational hypertension: smoking (Marcoux 1989a) and leisure time physical activity (Marcoux 1989b) are inversely related, while job stress is positively related (Landsbergis 1996, Klonoff-Cohen 1996). Moreover, depression and anxiety in early pregnancy is associated with increased risk of preeclampsia (Kurki 2000).

There are diverging views whether gestational hypertension and preeclampsia are two different entities or different stages of one disease diverges. It has been stated that gestational hypertension is unmasked essential hypertension, while preeclampsia is pregnancy-specific with a different etiology (National high blood pressure education program working group, 1990). More recent data indicate that also preeclampsia is associated with longstanding alteration of the circulatory system (Spaanderman 2000b, Jonsdottir 1995). It has also been proposed that preeclampsia should be considered as the extreme end of the range of maternal adaptation to pregnancy (Redman 1999). By studying risk factor patterns in preeclampsia and gestational hypertension, etiological similarities/differences may be revealed.

## Genetic effects on preeclampsia and gestational hypertension

The degree of genetic influence on pre-eclampsia (proteinuric hypertension) has, to our knowledge, only been estimated from the observed incidence of the disease in relatives, and a familial tendency has been recognized (Sutherland 1981). In a recent study, maternal preeclampsia was associated with 70% excess in risk for preeclampsia in daughters in a geographical area of Sweden (Mogren 1999). Since the diagnostic criteria for preeclampsia were different before 1987, maternal preeclampsia may in fact have included a relatively high proportion of women nowadays defined as having gestational hypertension, which may have influenced the results. Thus, a maternal genetic component of the liability of developing preeclampsia is well-accepted (Cooper 1979, Sutherland 1981, Liston 1991, Arngrimsson 1995), but the magnitude of the genetic effect remains unsolved. An attempt was also made to investigate the genetic influence on gestational hypertension, but as compared to preeclampsia, no familial tendency was found (Sutherland 1981). By comparing pregnancies among twin mothers of different zygosity groups, the relative importance of genetic and environmental effects on the liability of developing pre-eclampsia and gestational hypertension, can be estimated.

Different models of inheritance of preeclampsia, have been tested in family studies, and there are inconsistencies in the findings: a single maternal recessive gene (Sutherland 1981), homozygosity for a single recessive gene shared by mother and fetus (Liston 1991), and either recessive or dominant gene inheritance (Arngrimsson 1990). A meta-analysis on six previous family studies suggested a major dominant gene model, or multifactorial inheritance (Arngrimsson 1995). When studying preeclampsia in 99 female twin pairs, not a single concordant twin pair was identified (Thornton 1991), and it was concluded that a single gene hypothesis should be considered with caution.

Genome-wide scans have been performed in the search for maternal genetic susceptibility for preeclampsia. So far the following loci are reported to be associated with preeclampsia: 4q (Harrison 1997), 7q36, encoding the endothelial nitric oxide synthase (Arngrimsson 1997, Guo 1999), and 2p13 (Arngrimsson 1999, Moses 2000). The renin-angiotensin system is central in blood pressure regulation. In preeclampsia, angiotensin (AII) concentration is decreased, as compared to normotensive pregnancies, but the sensitivity of the vasculature to AII is increased (Baker 1992a, Baker 1992b). An important role for the angiotensinogen locus on chromosome 1q4 was established in essential hypertension (Jeunemaitre 1992). A possible role of a molecular variant of angiotensinogen (T235) was reported for preeclampsia (Ward 1993, Arngrimsson 1993), although a smaller study later was not able to confirm this (Morgan 1995). A variant of the angiotensinogen (Thr235) was also described in association with abnormal physiologic change of the uterine spiral arteries (Morgan T 1999).

Epidemiological studies suggest that immunological mechanisms are involved in the etiology of preeclampsia. Moreover, when the distribution of human leukocyte antigen (HLA) types was compared between preeclamptic and normotensive women, an involvement of the HLA system in the etiology of preeclampsia was suggested (Takakuwa 1997). Hence, the importance of different genotypes of (HLA) for the development of preeclampsia, have been studied repeatedly (Hayward 1992, Wilton 1990), and the HLA-DQB\*04 allele is reported to be associated with increased susceptibility to preeclampsia (Honda 2000). A special type of the so-called non-classical HLA antigen, HLA-G, has been in focus in preeclampsia research. HLA-G is expressed by the extravillous trophoblast at the maternofetal interface, where the classical class I and II antigens are absent, and may therefore contribute to the maternal immune tolerance of the fetus (Shorter 1993, Loke 1997, Hiby 1998). Susceptibility to preeclampsia was investigated in relation to HLA-G deletion polymorphism (Humphrey

1995) and to a null mutation (Aldrich 1999), but no associations were detected. Recently, it was reported that HLA-G expression is defective in preeclamptic placentas, as compared to placentas from normotensive women (Goldman-Wohl 2000a, Goldman-Wohl 2000b). The difficulty of studying the association between HLA genetics and preeclampsia is illustrated by the finding that the association may be dependent of fetomaternal compatibility for HLA-DR genes (Kilpatrick 1989, de Luca Brunori 2000). Likewise, it has been suggested that maternal-fetal transmission of an angiotensinogen allele, associated with low plasma angiotensinogen concentrations, is associated with preeclampsia (Morgan L 1999).

In summary, there seems to be a familial effect on the liability of developing preeclampsia, but there is no simple mode of inheritance. Many genes appear to be involved and preeclampsia should be regarded as a complex disease. Also gestational hypertension may be regarded as a complex disease. To further improve the understanding of preeclampsia and gestational hypertension estimations of the genetic and environmental effects on the liability of developing them would be important.

## **Circulatory diseases in relation to pregnancy**

### **Cardiovascular changes in normal pregnancies**

Heart rate and stroke volume increases already in the first trimester of pregnancy, and is accompanied by a decrease in maternal vascular resistance and increase in peripheral blood flow (Robson 1989a, Clapp 1988). These changes result in a small fall in systolic blood pressure and a marked fall in diastolic blood pressure (Robson 1989a). Also the aortic wall undergoes changes, and aortic distensibility and diameter increases (Eduard 1998), possibly by the action of estrogens (Karas 1994). Experimental data indicates that estrogens also augment uterine artery dilation (Stormont 2000). Moreover, pregnancy-induced alteration in myocardial mechanics and left ventricular hypertrophy are reported (Geva 1997, Gilson 1997, Mabie 1994, Mesa 1999), and during labour there is a considerable increase in cardiac output (Robson 1987). Still twelve weeks postpartum, cardiac output is reported to be elevated as compared to the preconception baseline (Capeless 1991). Interestingly, cardiovascular adaptations seem to be enhanced in a subsequent pregnancy (Clapp 1997).

During pregnancy, the coagulation system is in a prothrombotic state with increases in coagulation factors and inhibition of fibrinolysis (Hellgren 1981, Hellgren 1996, Cerneca 1997), whereas thrombin generation is enhanced (Bremme 1992), and platelet number, function and life span is unchanged (Biswas 1994).

### **Circulatory diseases in relation to pregnancy**

Pregnancy is associated with venous thromboembolism, but the actual magnitude of risk increase of pregnancy on the risk of venous thrombosis remains to be elucidated. It is important since pulmonary embolism is reported to be the leading cause of maternal mortality in the United States, the United Kingdom, and in Sweden (Atrash 1990, Dept of Health 1998, Högberg 1986). When pregnancy-related mortality ratio recently was reported from one center in the United States, pulmonary embolism, together with cardiac disease, accounted for 40% of the 23 maternal deaths per 100,000 live births during 1992-1998 (Panting-Kemp 2000). The postpartum period has been pointed out as a pregnancy-related high-risk period, because 60% of the maternal deaths are reported to occur in the puerperium, and 45% of these deaths occurred within one day of delivery (Li 1996). How much relative risks of venous thrombosis and pulmonary embolism vary through late pregnancy, around delivery and during the puerperium is unknown.

Also, the risk of stroke is influenced by pregnancy status, and again the puerperium is pointed out as the high-risk period (Kittner 1996). From that report it could also be inferred that the increased risk for stroke in the puerperium was mainly confined to the first postpartum days (Kittner 1996). An interesting question is how relative risk of different stroke diagnoses changes according to pregnancy status.

Myocardial infarction in pregnancy is a rare event and the reported incidence rates are 5-7.5/100,000 deliveries (Petitti 1997, Roth 1996). As there is a trend toward childbearing later in life and the risk of ischemic heart disease increases with age, we wanted to estimate the pregnancy-related risk of myocardial infarction.

### **How can high-risk women be identified?**

Considering that pulmonary embolism is one of the leading causes of maternal mortality, and that the puerperium is associated with risk of stroke, it is clearly desirable to improve the ability to identify pregnant women at high risk of these diseases. By identifying women at high risk of pulmonary embolism, thromboprophylaxis can be considered for prevention.

Preeclampsia is associated with hypercoagulability (Schjetlein 1997). Among women with severe preeclampsia, high frequency of thrombophilic disorders, such as protein S deficiency, activated protein C resistance and factor V Leiden mutation, are observed (Dekker 1995, Lindoff 1997, van Pampus 1999, Kupferminc 2000). Moreover, preeclampsia is described as an endothelial disorder, with exaggerated platelet aggregability (Felfernig-Boehm 2000), altered cerebral blood flow velocity (Williams 1993, Sakai 1994), and a breakdown of cerebral autoregulation with hyperperfusion (Zunker 2000), which may predispose to cerebrovascular events. Also longstanding circulatory alterations in preeclamptic women are reported: altered hemodynamic parameters and volume homeostasis still five months postpartum (Spaanderman 2000a) and failure to increase both venous and arterial compliance in a subsequent pregnancy (Spaanderman 2000b). Moreover, later in life, women who developed preeclampsia during pregnancy are more likely to die from ischemic heart disease (Jonsdottir 1995). Preeclampsia affects 3-8% of all pregnant women (Lindmark 1984), and is thereby likely to influence pregnancy-related risks of other circulatory diseases i.e. pulmonary embolism and stroke.

Risk of preeclampsia is also substantially increased in multiple pregnancy (Coonrod 1995). Multiple pregnancies are associated with higher levels of estrogens than single pregnancies (Duff 1974), which may influence the risk of thromboembolism. Since there is a larger increase in cardiac output in women with multiple as compared to singleton pregnancies (Robson 1989b), multiple pregnancy may also have an effect on risk of other circulatory diseases during pregnancy.

Surgical procedures are associated with risk of thromboembolism (Alexander 1999), and in women delivered with Cesarean section, platelet count is increased as compared to vaginally delivered women (Atalla 2000). Previously, smoking, preeclampsia, and operative delivery are reported to increase pregnancy-related risks of venous thromboembolism (Greer 1999, Lindqvist 1999), but the effects of these risk factors on risk of pulmonary embolism have not been quantified. For stroke during pregnancy and puerperium, Cesarean delivery and hypertension have been identified as potential risk factors (Lanska 2000).

### **Preeclampsia — impact on female offspring**

Environment during fetal life, i.e. access to nutrients and hormonal levels, may possibly have an impact not only on fetal growth, but also on the function of organs throughout life (Barker 1995, Osmond 1993, Forsen 1999). This hypothetical phenomenon is called fetal programming. Birth weight and birth weight for gestational age are reported to be associated

with hypertension (Barker 1990, Barker 1993), coronary heart disease (Leon 1998), body fat distribution (Barker 1997), and insulin resistance in adulthood (Leger 1997). Among other hypothesis it was suggested that there is an association between intrauterine estrogen exposure and subsequent risk of breast cancer (Trichopoulos 1990). High birth weight (Petridou 1990, Kaijser 2000) and twinship (Duff 1974) are associated with high estrogen levels. They are therefore used as proxy variables in epidemiological studies for assessment of the association between intrauterine exposure of estrogens and subsequent risk of breast cancer. The risk of breast cancer is reported to increase with birth weight (Kaijser 2001, Sanderson 1996, Hübinette 2001, Cerhans 2000, Michels 1990), even though the issue is not as yet settled (Ekbom 1997).

In preeclampsia altered placental function is described, and several hormonal factors that may relate preeclampsia to decreased risk of breast cancer have been reported: reduced levels of estrogens (Rosing 1984) and IGF-1 (Halhali 1995), and increased levels of progesterone (Walsh 1988) and androgens (Rosing 1984, Laivuori 1998). Moreover, preeclampsia is associated with increased levels of cytokines, among others TNF-alpha (Hamai 1997). Preeclampsia is also associated with small for gestational age fetuses (Ødegård 2000), and decreased risk of breast cancer was observed among women exposed to preeclampsia in utero (Ekbom 1997). Hypothetically these hormonal alterations also may influence other well-known risk factors for breast cancer such as height (Ziegler 1996), and age at menarche (Lipworth 1995). There are indications of perinatal factors influencing puberty. Girls born small for gestational age have a lower mean age at menarche compared with girls with appropriate weight for gestational age (Persson 1999). As to whether anthropometry and/or puberty onset, are influenced by exposure to preeclampsia in utero is not previously studied.

## AIMS

1. To compare the risk factors for gestational hypertension and preeclampsia. (*paper I*)
2. To estimate the relative importance of genetic and environmental effects on the liability of developing preeclampsia and gestational hypertension. (*paper II*)
3. To estimate the relative importance of genetic and environmental effects on the liability of developing pregnancy-induced hypertension (preeclampsia and gestational hypertension considered as a single disease with different degrees of severity). (*paper II*)
4. To estimate relative risks of venous thromboembolic diseases, stroke and myocardial infarction according to pregnancy status. (*paper III*)
5. To explore whether preeclampsia, multiple pregnancy and Cesarean section explain the pregnancy-related increased risk of venous thromboembolic diseases and stroke. (*paper IV*)
6. To assess the influence of intrauterine exposure of preeclampsia on female offspring's anthropometry and puberty. (*paper V*)

# MATERIAL AND METHODS

## Data sources

**The Swedish Medical Birth Register** is held by the National Board of Health and Welfare, and contains data on more than 99% of all births in Sweden from 1973 and onwards (Cnattingius 1990). Since 1982, data is collected through copies of standardized antenatal, obstetric and pediatric records, which are marked with the mother's unique national registration number, and sent to the National Board of Health and Welfare (Lunde 1980). From the first antenatal visit, information is collected prospectively. Maternal previous reproductive history, smoking habits, height, weight, state of health, and family situation are recorded at the woman's first visit by a midwife who performs an interview and an examination. Information about maternal age, and complications during pregnancy and delivery is collected when the woman is discharged from the hospital. The mother's country of birth is obtained from the Civil Registration, held by Statistics Sweden, through linkage of the personal identification number. Complications during pregnancy and delivery are classified according to the Swedish version of the International Classification of Diseases (ICD). Diagnoses are classified according to the Swedish version of International Classification of Diseases, 8th revision (ICD-8) through 1986, and the 9th revision (ICD-9) from 1987 through 1996. A doctor notes the diagnoses at the time of discharge from the hospital, using a standard form, where definitions of the diagnoses are written in the text beside the ICD-code and a check box.

**The Swedish Twin Register** was established in the late 1950s and is kept by the Karolinska Institutet. The register is a collection of databases, with register-based data as well as data collected through questionnaires or in-person examinations of the twins (FRN 2000). The Swedish Twin Register is the largest twin registry in the world, and contains data on twins born in Sweden, with zygosity determined in pairs born from 1886 through 1958. In 1972, all like-sexed twins born 1926-1958 were sent a questionnaire, from which we have used the female twin pairs with known zygosity.

**The Inpatient Register** is held by the National Board of Health and Welfare, and contains data collected on individual hospital discharges together with the national registration number. From 1987 through 1995, the coverage is approximately 99% (Nyrén, 1998). Each record corresponds to one period of hospitalization and contains up to eight discharge diagnoses coded according to the Swedish version of the International Classification of Diseases currently used.

**The Education Register** was established by Statistics Sweden in 1985. The register covers the Swedish population and is updated annually. Information on the highest formal education attained for each individual, and on education completed from the elementary level to the post-graduates level, is included (Statistics Sweden 1993).

**The Intrauterine preeclampsia exposure cohort** was created by using the Swedish Birth Register, from which women who developed preeclampsia and delivered female offspring from 1973 through 1979, in a geographic area of Sweden, were identified. Only single births were selected. In the registry, ICD-8 codes 637,03, 637,04, 637,09 and 637,99, were used to identify female offspring who possibly were exposed to preeclampsia in utero. Only women whose mothers had symptoms for more than one day before birth were included as being exposed to preeclampsia in utero.

A **cohort unexposed to preeclampsia** was created by a random sample of two female offspring per exposed individual, matched by year and hospital of birth. A structured protocol was used when all individual records were scrutinized, in order to verify exposure status. The diagnostic criteria correspond to the criteria proposed by Davey (Davey 1988).

The Birth Register contains more than 99% of all births in Sweden. All women selected from the register, whose personal records were found (99%), had attended antenatal care and followed the standardized routines. Information on the delivery, including inpatient care, maternal and fetal complications, birth weight, birth length, and health status were also obtained from the individual records. Birth weight and birth length for gestational age were calculated according to Swedish reference standards, based on births from the 1970s, where gestational age is calculated from the date of the last menstruation (Niklasson 1991). Before validation of the individual records, approval from every head of the departments was given.

### **Definition of gestational hypertension and preeclampsia**

During 1987 through 1998 the ICD-9 codes were used: gestational hypertension ICD-9 codes 642D and 642X; mild preeclampsia ICD-9 code 642E; severe preeclampsia ICD-9 codes 642FA and 642FW. These codes correspond to the following criteria: **Gestational hypertension** is defined as blood pressure (BP) of at least 140/90 mm Hg (in at least two readings 6 or more hours apart), without proteinuria, occurring after 20 weeks gestation. **Mild preeclampsia** is defined as a diastolic blood pressure from 90 to 109 mm Hg combined with proteinuria 0.3-4.9 g/day or 1+ or 2+ on a urine dipstick, occurring during pregnancy. **Severe preeclampsia** is defined as preeclampsia with either a diastolic blood pressure of at least 110 mm Hg, or proteinuria of at least 5 grams/24 hours, or both. Essential hypertension corresponds to ICD-9 code 642 A, essential hypertension with superimposed preeclampsia corresponds to ICD-9 code 642H.

During 1973 through 1986 the ICD-8 codes were used: gestational hypertension ICD-8 code 637.01; preeclampsia ICD-8 code 637,03 and 637,04. The definition of gestational hypertension was the same as in ICD-9, but preeclampsia was defined as a rise in blood pressure during pregnancy corresponding to that specified for gestational hypertension, combined with proteinuria or edema.

### **Validation of gestational hypertension and preeclampsia**

The accuracy of the diagnostic recording of gestational hypertension and preeclampsia in the Medical Birth Register was validated in a randomly selected sample of women who delivered in one hospital (University Hospital of Uppsala) during 1987-93, when the ICD-9 codes were used. The diagnoses recorded in the Medical Birth Register were compared with the notes on blood pressure and proteinuria in the individual records according to the criteria for the ICD-9 codes.

Diagnoses in the Medical Birth Register		Diagnoses according to the criteria				642A <sup>§</sup>	642H <sup>  </sup>
		Gestational hypertension	Mild preeclampsia	Severe preeclampsia			
Gestational hypertension <sup>*</sup>	n=114	95	12	1	5	1	
Mild preeclampsia <sup>†</sup>	n=98	3	88	4	3		
Severe preeclampsia <sup>‡</sup>	n=50			50			

\* includes ICD-9 codes 642D and 642X

† includes ICD-9 code 642E

‡ includes ICD-9 codes 642FA and 642FW

§ corresponds to essential hypertension

|| corresponds to essential hypertension with superimposed preeclampsia

Among 114 pregnancies coded as gestational hypertension, 95 had gestational hypertension according to the notes in the individual records (positive predictive value=83%). Among 148 pregnancies diagnosed with preeclampsia, 142 had preeclampsia according to the individual records (positive predictive value =96%).

The preeclampsia recording in the Medical Birth Register from 1973 through 1986, (ICD-8 codes 637,03 and 637,04) was validated according to the criteria proposed by Davey (Davey 1988). Among 216 pregnancies diagnosed with preeclampsia, 107 had preeclampsia according to the individual records (positive predictive value =50%). The lower predictive value is explained by changed criteria, with the addition of proteinuria as a compulsory criterion.

## Validation of normotensive pregnancies

Pregnancies recorded as normotensive i.e. the record had no codes indicating hypertension, in the Medical Birth Register were validated in a randomly selected sample of women who delivered during 1973-78, in a geographically defined area of Sweden. Among 644 pregnancies recorded as normotensive, 6 (1%) had essential hypertension, 21 (3%) had gestational hypertension and 5 (1%) had preeclampsia, yielding a predictive value of 95% for being normotensive.

## Design and statistical analyses of the studies

### *Paper I*

We compared risk factors for preeclampsia and gestational hypertension in a population-based cohort, by using data on maternal factors and complications during pregnancy from the Swedish Medical Birth Register and the Education Register. All births of nulliparous women, aged 34 years or less, giving birth at the University Hospital of Uppsala, from 1987 through 1993, were included (n=10,659).

The data were modeled through logistic regression. Crude odds ratios (ORs) and 95 percent confidence intervals (CIs) were calculated to estimate the effects of individual factors on the risk of gestational hypertension or preeclampsia. Multivariate analyses were performed, including those factors that significantly influenced risk of gestational hypertension or preeclampsia in the univariate analyses. Level of significance was set at  $p < 0.05$ .

### *Paper II*

To estimate the relative importance of genetic and environmental effects on the liability of developing preeclampsia and gestational hypertension, we conducted a cross-linkage of the Swedish Twin Register and the Medical Birth Register, by using the individually unique national registration number. Eligible subjects were female twin pairs with known zygosity, both of whom gave birth in Sweden from 1973 through 1993. All pregnancies during the study period were used in order to define whether the women had preeclampsia or gestational hypertension. If a woman had at least one pregnancy with preeclampsia she was classified into that category. The same approach was applied to classify gestational hypertension. The proportion of primiparous women was the same among monozygotic and dizygotic twins.

Gestational hypertension was defined as blood pressure (BP) of at least 140/90 mm Hg (in at least two readings 6 or more hours apart) without proteinuria, occurring after 20 weeks gestation (ICD-8 code 637.01 from 1973 to 1986, and ICD-9 codes 642D and 642X from 1987 to 1993). From 1973 to 1986 preeclampsia was defined as a rise in blood pressure during pregnancy corresponding to that specified for gestational hypertension, combined with proteinuria or edema (ICD-8 code 637,03 and 637,04). From 1987 to 1993 preeclampsia was defined as a rise in blood pressure during pregnancy corresponding to that specified for gestational hypertension, combined with proteinuria (ICD-9 codes 642E and 642F). Pregnancy-induced hypertension was defined as either gestational hypertension or preeclampsia. The final sample size was 916 monozygotic and 1,196 dizygotic twin pairs. Pairs with one individual with gestational hypertension were excluded when preeclampsia was analyzed, and vice versa.

Standard quantitative genetic methods were used to estimate the relative contributions of genetic and environmental factors. Probandwise concordance rates were calculated for both zygosity groups and represent the number of affected twins (pre-eclampsia and gestational hypertension, respectively) in concordant pairs (both affected) divided by the total number of affected twins with the same zygosity (McGue 1992). This rate represents the probability of developing a disease for a woman with an affected co-twin. The phenotypes considered in the present study, pre-eclampsia and gestational hypertension, are categorical (diseased/not diseased), and an underlying normal distribution of liability to the disease is assumed, with a critical threshold exceeded for becoming affected (Neale 1992). The correlation of liability (tetrachoric intraclass correlation) was computed for each zygosity group. Higher correlation of liability among monozygotic compared with dizygotic twins indicate the importance of a genetic component.

By comparing the correlation in liability among twins of different zygosity, we could estimate the proportion of variance accounted for by genetic (heritability =  $h^2$ ), shared environmental ( $c^2$ ) and non-shared environmental ( $e^2$ ) effects, respectively. Monozygotic twins share identical genotypes, whereas dizygotic twins share on average 50 % of their segregating genes. Thus, genetic effects ( $h^2$ ) are indicated when correlation in liability is larger in monozygotic compared with dizygotic twins. Shared environmental effects ( $c^2$ ) reflect twin similarity that is not explained by genetic effects, whereas non-shared environmental effects ( $e^2$ ) are evidenced by within-pair differences. Variance estimates for genetic and environmental effects were obtained by entering two-by-two contingency tables

of disease status in twin pairs (twin 1 versus twin 2), stratified by zygosity status, into a structural model-fitting program (Neale 1994). Calculations were also performed using pregnancy-induced hypertension as an ordinal variable, whereby gestational hypertension was considered a less severe type of pregnancy-induced hypertension than preeclampsia. An underlying normal distribution of liability is still assumed.

### *Paper III*

To estimate relative risks of venous thromboembolic diseases, stroke and myocardial infarction according to pregnancy status, we performed a population-based cohort study through cross-linkage of the Inpatient Register and the Birth Register. Data from 1,014,677 pregnancies delivered from January 1, 1987 to September 30, 1995 were retrieved from the Birth Register, and information regarding maternal age at delivery, parity, date of the first day of the last menstrual period, and date of delivery was collected. In all 1,003,489 deliveries among 654,957 women were analyzed.

For women who gave birth from January 1, 1987 through September, 1995, we obtained data on all discharges from the Inpatient Register from 1987 through 1995. We selected severe circulatory diseases, which usually lead to hospitalization in Sweden: venous thrombosis, pulmonary embolism, subarachnoid hemorrhage, intracerebral hemorrhage, cerebral infarction, and acute myocardial infarction. If a specific diagnosis occurred more than once, only the first event was included. We considered time before pregnancy, starting from January 1, 1987, and the first 27 complete gestational weeks (before the 196th day after the first day of last menstruation), to be time spent in the unexposed period. The date of admittance to hospital was regarded as the date of onset of the disease. Three percent of the cases occurring around delivery were admitted to hospital before the day of delivery.

We divided the exposure time into four different periods: 1) The third trimester of pregnancy (from the 28th gestational week to three days before delivery date); 2) Around delivery (from two days before to one complete day after delivery); 3) Puerperium (from two days to six complete weeks after delivery); and 4) One year postpartum (seven weeks to one year after delivery). Person-time at risk is the sum of the days experienced by all women in each separate exposure period. We excluded person-time before a woman's 12th birthday (one year before the youngest woman's delivery). After the date of onset of disease or death, a woman did not contribute any more person-time.

We calculated incidence rates by dividing the number of cases by the total number of person-years. We used direct standardization to adjust simultaneously for maternal age and parity, which were categorized in the following way: maternal age 12-29, 30-34, and  $\geq 35$  years; parity 1, 2-3, and  $\geq 4$ . The analyses were performed by using STATA. We calculated relative risks (standardized incidence rate ratios) and confidence intervals (CI) by dividing the standardized incidence rates for the different exposure periods by the standardized incidence rate of the unexposed period.

### *Paper IV*

To explore whether preeclampsia, multiple pregnancy and Cesarean section explain the pregnancy-related increased risk of venous thromboembolic diseases and stroke, we used the same population-based cohort as in paper III. Stroke was defined by the following diagnoses: subarachnoid bleeding, intracerebral hemorrhage, unspecified intracranial hemorrhage, occlusion and stenosis of paracerebral arteries, occlusion of cerebral artery, acute unspecified cerebrovascular disease and other unspecified cerebrovascular disease. In addition, the ICD-9 code 674A was used, representing any of the diagnoses above, occurring during pregnancy or puerperium.

Before pregnancy, information about current smoking was not available, and therefore data from the subsequent pregnancy was used to characterize smoking status in the unexposed period. For women with more than one delivery during the study period, exposure data was obtained from each pregnancy.

We calculated incidence rates by dividing the number of cases by the total number of person-days for each period, respectively. Incidence rate ratios were used to estimate relative risks (RR), and were calculated by dividing the incidence rates of the different exposure periods by the incidence rate of the unexposed period. Maternal age, parity, smoking habits, preeclampsia, multiple birth, diabetes, and Cesarean section were all modeled as time-dependent covariates. We performed Poisson regression to study the joint effect of exposure period, and the other independent variables. When analyzing preeclampsia, adjustments were made for: age, parity, smoking, diabetes, and type of birth; when analyzing multiple birth, adjustments were made for: age, parity, smoking, diabetes, and preeclampsia; when analyzing Cesarean section adjustments were made for: age, parity, smoking, and diabetes. Adjusted estimates of the effect of time of pregnancy were obtained by modeling the natural logarithm of the number of cases as a function of the exposure, maternal age, parity, and smoking. We used the natural logarithm of the person-years as offset parameter. Exposures that never occur in non-pregnant state (multiple birth, preeclampsia and Cesarean section) were modeled through creating three levels of exposure: 1) unexposed, 2) exposure period and 3) exposure period combined with either multiple birth, preeclampsia or Cesarean section.

#### *Paper V*

We assessed the influence of intrauterine exposure of preeclampsia on female offspring anthropometry and puberty, by comparing two cohorts of young adult women. The cohorts were created by validation of exposure status in the individual records. We identified 287 women exposed to preeclampsia in utero, and 486 women who were unexposed to preeclampsia. Data on the adult anthropometry of exposed and unexposed women was retrieved from personal telephone interviews, performed by professional female interviewers. Before the interview, a brochure with an invitation to participate in the study was sent by mail. Information about the study, some of the questions, a measuring-tape, and instructions with a picture where and how to measure, were also enclosed. The young women, 20 to 25 years old by that time, were asked to measure their height, weight, and waist circumference at the level of the umbilicus and hip circumference at its widest location while standing. They were also asked about age at menarche, parity and parental heights. The interviewers had no information about exposure status of the individuals or the hypotheses of the study. The response rate was 80% in both groups.

Mean values for the endpoint variables (final height, difference from target height, body-mass index, waist to hip ratio and age at menarche) were compared between the exposed and the unexposed cohorts. Target height was calculated as follows:  $37.85 + 0.75(\text{mid-parental height})$ , proposed by Luo (Luo 1998) We performed ANOVA by Scheffé in order to compare means in more than two groups, and p-values less than 0.05 (two-sided) were considered significant. Provided a power of 80% and 5% level of significance, when we compared the group exposed to severe preeclampsia in utero with the non-exposed group, it was possible to detect the following differences: mean final height of 4.5 cm, mean age at menarche of 0.9 years, BMI 2.5, waist to hip ratio 0.05, or more.

## Outcome measures and independent variables

### Outcome measures

**Gestational hypertension** (*Papers I, II*) was defined as blood pressure (BP) of at least 140/90 mm Hg (in at least two readings 6 or more hours apart) without proteinuria, occurring after 20 weeks gestation. Before 1987, ICD-8 code 637.01 was used (*Paper II*). From 1987 to 1993 ICD-9 codes 642D and 642X were used (*Papers I, II*).

**Preeclampsia** (*Papers I, II*) was defined as hypertension accompanied by proteinuria, two urinary protein dip sticks of at least 1+ or 300 mg of protein, or more in a 24 hour urine collection. Before 1987 ICD-8 codes 637.03 and 637.04 were used (*Paper II*). From 1987 to 1993 ICD-9 codes 642E and 642E were used (*Papers I, II*).

**Venous thrombosis** (*Paper III*) ICD-9 codes: 451, 671D and 671E.

**Pulmonary embolism** (*Paper III and IV*) ICD-9 codes: 415B, and 673C.

**Subarachnoid hemorrhage** (*Paper III*) ICD-9 code: 430.

**Intracerebral hemorrhage** (*Paper III*) ICD-9 codes: 431.

**Intracerebral infarction** (*Paper III*) ICD-9 codes: 434.

**Myocardial infarction** (*Paper III*) ICD-9 codes: 410.

**Stroke** (*Paper IV*) ICD-9 codes: 431-431, 436-437, and 674A.

**Final height** (*Paper V*) obtained from the telephone interviews.

**Weight** (*Paper V*) obtained from the telephone interviews.

**Difference from target height** (*Paper V*) calculated from the parental heights, obtained from the telephone interviews.

**Body-mass index** (*Paper V*) calculated from the women's weights and heights, obtained from the telephone interviews.

**Waist to hip ratio** (*Paper V*) calculated from circumference measures, performed by a standardized measure-tape, obtained from the telephone interviews.

**Age at menarche** (*Paper V*) obtained from the telephone interviews.

### Independent variables

**Maternal age** (*Paper I, III, IV, and V*) is recorded in the Swedish Birth Register and defined as completed years at the time of delivery.

**Maternal height** (cm) (*Paper I, V*) is collected from the measurement at the antenatal care and recorded in the Swedish Birth Register. In *paper V*, data was collected from the individual records and, if missing in the records, from the telephone interviews.

**Maternal weight** (kg) (*Paper I*) corresponds to early pregnancy weight, is collected at the first visit to antenatal care and recorded in the Swedish Birth Register. In *paper V*, data was collected from the individual records.

**Mother's place of birth** (*Paper I*) is recorded in the Swedish Birth Register and we categorized the place of birth as: born in a Nordic country or born outside a Nordic country.

**Maternal education** (*Paper I*) was obtained from the Education Register, and includes information on the highest formal education obtained for each individual. Education is presented as the number of years.

**Maternal smoking** (*Paper I, IV*) is collected at the first visit to antenatal care. Smoking is categorized as non-daily smoking, 1-9 cigarettes/day or  $\geq 10$  cigarettes/day, and is recorded in the Swedish Birth Register from 1982 and onwards. In *Paper V*, this information was collected from the individual records where the number of cigarettes/day was recorded.

**Diabetes** (*Paper I, V*). Diagnoses were recorded in the Swedish Medical Birth Register. Pregestational and gestational diabetes are coded as ICD-9 codes 648A and 648W, respectively.

**Type of birth** (*Paper I, IV*) is categorized as single or multiple birth, which is recorded in the Swedish Birth Register.

**Zygoty** (*Paper II*) is previously determined through the response to a questionnaire with questions on childhood resemblance. Both individuals were asked the question: "During childhood, were you and your twin partner as like as two peas in a pod or not more alike than siblings in general"? If both individuals of a pair responded 'alike as two peas in a pod' they were classified as monozygotic, if both responded 'not alike' they were classified as dizygotic, else, the zygoty was considered 'not determined' (FRN, 2000). Recently two studies have, by using polymorphic DNA markers, validated the questionnaire-based zygoty diagnoses, and demonstrated high positive predictive value (98%) (FRN, 2000).

**Parity** (*Paper III, IV*) is defined as number of previous live births and stillbirths, including the present birth, as recorded in the Swedish Medical Birth Register. In *Paper V*, the parity variable is collected from the individual records in a similar fashion.

**Preeclampsia** (*Paper IV*). Diagnoses were obtained from the Swedish Birth Register, and were coded as: mild (ICD-9 code 642E), severe (ICD-9 code 642F), and preeclampsia superimposed on essential hypertension (ICD-9 code 642H). Eclampsia corresponds to ICD-9 code 642G. We analyzed preeclampsia with two levels: mild (ICD-9 code 642E) and severe (ICD-9 codes 642F-H). In *Paper V*, we used the Swedish Medical Birth Register to select every female offspring to women with preeclampsia, born during 1973-78 in a defined geographical area of Sweden. Diagnoses of hypertensive diseases during pregnancy were, in the register, classified according to the Swedish version of International Classification of Diseases, 8th revision (ICD-8 codes: 637,03, 637,04, 637,09 and 637,99). Every personal record was scrutinized, to evaluate preeclampsia exposure status according to the criteria proposed by Davey (Davey 1988).

**Cesarean section** (*Paper IV*) data from the Swedish Medical Birth Register.

**Paternal height** (cm) (*Paper V*) was obtained by asking the young women about their fathers' height in the telephone interview.

**Target height** (cm) (*Paper V*) Calculated:  $37.85+0.75(\text{mid-parental height})$ , proposed by Luo (Luo 1998).

## Overview of the papers

Paper	Subjects	Outcome measures	Covariates
I	Nulliparous women, aged 34 years or less, who gave birth at the University Hospital of Uppsala N=10,666	Preeclampsia Gestational hypertension	Maternal age, height, place of birth, education, cohabitation status with infant's father, smoking, history of infertility, diabetes, type of birth, season of birth, infant's sex.
II	Female twin pairs with known zygosity, born 1926-59, both of whom gave birth in Sweden 1973-93. Monozygotic=916 pairs Dizygotic=1,196 pairs	Preeclampsia Gestational hypertension Pregnancy-induced hypertension	Zygosity
III	Women (n=654,957) with at least one delivery (n=1,003,489) in Sweden during 1987-95.	Venous thrombosis Pulmonary embolism Subarachnoid hemorrhage Intracerebral hemorrhage Cerebral infarction Myocardial infarction	Pregnancy status, age, parity
IV	Women (n=654,957) who had had at least one delivery (n=1,003,489) in Sweden during 1987-95.	Pulmonary embolism Stroke	Pregnancy status, age, parity, smoking, preeclampsia, multiple birth and Cesarean section.
V	Women born 1973 through 1978 in a geographically defined area of Sweden, exposed (n=230) and non-exposed (n=359) to preeclampsia in utero.	Final height Age at menarche Body-mass index Waist-to-hip ratio	Maternal age, parity, abortions, height, weight at first antenatal visit, body-mass index, smoking, prepregnancy diseases, gestational age, birth weight, birth weight for gestational age, birth length, birth length for gestational age, and paternal height.

## RESULTS

### Environmental and genetic effects on the liability of developing preeclampsia and gestational hypertension (Papers I, II)

#### Environmental effects

Maternal smoking was associated with decreased risks of developing both gestational hypertension and preeclampsia. Increased risks of preeclampsia were observed for women with multiple pregnancies (OR=4.17), pregestational diabetes (OR=5.58) and gestational diabetes (OR=3.11), while the influence of these factors on risk of gestational hypertension was of a smaller magnitude (Table 2). Compared with Nordic women, women born outside the Nordic countries had decreased risks of both gestational hypertension (OR=0.25) and preeclampsia (OR=0.60).

**Table 1.** Adjusted\* odds ratios (ORs) with 95% confidence intervals (CIs) for gestational hypertension and preeclampsia by maternal and pregnancy characteristics among nulliparous women, 34 years or less, Uppsala County, Sweden, 1987-1993.

	Gestational hypertension		Preeclampsia	
	Adjusted* OR	95% CI	Adjusted* OR	95% CI
Smoking habits				
non-smoker <sup>†</sup>	1.00		1.00	
1-9 cigarettes/day	0.86	0.64-1.14	0.64	0.47-0.85
≥ 10 cigarettes/day	0.48	0.29-0.77	0.55	0.37-0.84
Mother's place of birth				
Nordic countries <sup>†</sup>	1.00		1.00	
Outside Nordic countries	0.25	0.14-0.46	0.60	0.42-0.88
Type of pregnancy				
single <sup>†</sup>	1.00		1.00	
multiple	1.73	0.69-4.35	4.17	2.30-7.55
Diabetes				
no <sup>†</sup>	1.00		1.00	
Pregestational diabetes	2.52	0.89-7.18	5.58	2.72-11.43
Gestational diabetes	1.46	0.53-4.07	3.11	1.61-6.00

\* Adjustments were made for the other variables included in the table and season of birth.

<sup>†</sup> Reference group.

Information about early pregnancy body-mass index (BMI) was available only for the 2,848 women who gave birth from 1992 to 1993. When the analyses were restricted to these women, risks of both gestational hypertension and preeclampsia increased consistently with early pregnancy BMI. Compared to women with the lowest BMI (<19.8), women with the highest BMI (≥29.0) had odds ratios of 4.85 (95% CI 1.97-11.2) and 5.19 (95% CI 2.35-11.48) for gestational hypertension and preeclampsia, respectively. After adjusting for early pregnancy BMI, the directions of the associations between the other studied variables and

preeclampsia or gestational hypertension did not change, although a number of the relations became stronger and the widths of the confidence intervals increased (data not shown).

### Genetic effects

The correlation of liability for pre-eclampsia among monozygotic and dizygotic twins were 0.57 and 0.18, respectively. Among monozygotic twins for whom at least one twin in a pair had pre-eclampsia (n=63), 16 also had a sister who had the disease (probandwise concordance rate = 0.25). Among dizygotic twins for whom at least one twin in a pair was affected (n=63), four had a diseased co-twin (probandwise concordance rate = 0.06). These results suggest a role of genetic factors in the development of pre-eclampsia.

For gestational hypertension the probandwise concordance rates were 0.09 and 0.05 for monozygotic and dizygotic pairs, respectively, while the correlation of liability were 0.28 for monozygotic and 0.05 for dizygotic twins. These results indicate a genetic component also in the etiology of gestational hypertension.

In Table 2, the overall quantitative genetic analyses for preeclampsia, gestational hypertension and pregnancy-induced hypertension are summarized. As a result of small numbers, the confidence intervals are broad, and heritability estimates for preeclampsia and gestational hypertension did not reach statistical significance.

**Table 2.** Estimates of genetic and environmental effects for gestational hypertension, preeclampsia and pregnancy-induced hypertension from structural equation model-fitting, among female twins, born 1958 or earlier, who both gave birth in Sweden during 1973-93.

Measured	Parameter estimates 95% Confidence Interval			Fit of model		
	$h^2$	$c^2$	$e^2$	$\chi^2$	df	p
Preeclampsia	0.54 (0-0.71)	0 (0-0.47)	0.46 (0.29-0.67)	2.42	3	0.49
Gestational hypertension	0.24 (0-0.53)	0 (0-0.33)	0.76 (0.47-1.00)	6.81	3	0.078
Pregnancy-induced hypertension	0.47 (0.13-0.61)	0 (0-0.26)	0.53 (0.39-0.69)	28.50	25	0.28

Notes:  $h^2$ = heritability;  $c^2$ =shared environment;  $e^2$ =non-shared environment;  $\chi^2$ = chi-squared; df=degrees of freedom; p=level of significance.

For preeclampsia, the heritability was estimated at 0.54 and non-shared environmental effect at 0.46, i.e. environmental effects are of about equal importance as genetic effects in the etiology of preeclampsia. For gestational hypertension, the estimates of heritability and non-shared environmental effect were 0.24 and 0.76, respectively. When considering both diseases together as pregnancy-induced hypertension with different degrees of severity, the estimates of heritability and non-shared environment reached statistical significance (0.47 and 0.53, respectively, shown at the bottom of Table 2). There was no indication of shared environmental effects in our data.

## Risks of circulatory diseases in relation to pregnancy

Table 3 presents incidence rate ratios (corresponding to relative risks), standardized for age and parity, for the different diseases. The third trimester of pregnancy was associated with increased risks of venous thromboembolic diseases.

**TABLE 3.** Standardized\* incidence rate ratios (SIR ratios), with 95% confidence interval (CI), of venous thromboembolism, stroke or myocardial infarction, according to a woman's status with respect to pregnancy. Reference group consists of non-pregnant and early pregnant (less than 28th gestational week) women.

Diagnosis	Third trimester of pregnancy (≥ 28 weeks of pregnancy to three days before delivery)		Around delivery (two days before to one day after delivery)		Postpartum			
	SIR ratio	(95% CI)	SIR Ratio	(95% CI)	Puerperium (two days to six weeks)		7 weeks to one year	
					SIR ratio	(95% CI)	SIR ratio	(95% CI)
Venous Thrombosis	6.7	(5.7-7.8)	115.1	(96.4-137.0)	15.2	(13.2-17.6)	0.5	(0.3-0.6)
Pulmonary Embolism	2.7	(1.7-4.2)	80.7	(53.9-117.9)	9.2	(6.5-12.7)	0.7	(0.5-1.1)
Subarachnoid Hemorrhage	0.8	(0.2-2.5)	46.9	(19.3-98.4)	1.8	(0.5-4.9)	1.4	(0.8-2.3)
Intracerebral Hemorrhage	1.3	(0.3-4.1)	95.0	(42.1-194.8)	11.7	(6.1-21.6)	0.5	(0.1-1.2)
Cerebral Infarction	2.2	(0.8-4.8)	33.8	(10.5-84.0)	8.3	(4.4-14.8)	1.5	(0.9-2.5)
Myocardial Infarction	1.2	(0.03-8.2)	27.0	(0.6-180.0)	6.3	(1.1-22.8)	1.9	(0.6-5.0)

\*Standardization was made for maternal age and parity, using the following weights:  
 Maternal age: 12-29 years: (parity=1: 0.571); Parity=2-3: 0.255); (parity≥4: 0.023);  
 Maternal age: 30-34 years: (parity=1: 0.025); Parity=2-3: 0.077); (parity≥4: 0.020);  
 Maternal age: 35-55 years: (parity=1: 0.004); Parity=2-3: 0.017); (parity≥4: 0.009).

Around delivery, there were overwhelming excesses in risks for all assessed diseases. Compared with the unexposed period, the risk of venous thrombosis was more than 100-fold increased around delivery, while the relative risks of pulmonary embolism and intracerebral hemorrhage were 80 and 95, respectively. In the puerperium, the relative risks diminished, but were still increased, except for subarachnoid hemorrhage. After the puerperium, there were no increases in risks, and the risk of venous thrombosis was decreased.

### The effects of preeclampsia, multiple birth and Cesarean section, on risks of pulmonary embolism and stroke related to pregnancy.

Relative risks of pulmonary embolism and stroke related to period, adjusted for maternal age, parity, and smoking, are presented in Table 4.

**Table 4.** Adjusted\* relative risks (RR), with 95% confidence interval (CI), of pulmonary embolism and stroke among women who gave birth in Sweden during 1987-1995.

Period related:	Pulmonary embolism		Stroke	
	RR	(CI)	RR	(CI)
Unexposed <sup>†</sup>	1.0	(ref)	1.0	(ref)
Third trimester <sup>‡</sup>	3.3	(2.2-5.0)	1.9	(1.2-3.0)
Around delivery <sup>§</sup>	77.6	(52.4-114.8)	99.2	(72.1-136.4)
Puerperium <sup>  </sup>	11.0	(8.1-15.1)	7.2	(5.2-10.0)

\* Adjustments were made for maternal age, parity and smoking.

<sup>†</sup> Non-pregnant and pregnant before 28<sup>th</sup> gestational week.

<sup>‡</sup> From the 28th gestational week to three days before delivery date.

<sup>§</sup> From two days before to one complete day after delivery.

<sup>||</sup> From two days to six complete weeks after delivery.

In Table 5 presents adjusted relative risks of pulmonary embolism and stroke in relation to pregnancy, preeclampsia, multiple birth and Cesarean section. The relative risk of **pulmonary embolism** was, during the third trimester, seven times higher among women with preeclampsia (RR=22.6) as compared to non-preeclamptic women (RR=3.0). Around delivery, the relative risks of pulmonary embolism were 211.7 and 75.1 among women with and without preeclampsia, respectively, as compared to unexposed women, but the confidence intervals were wide and overlapping. During the puerperium, the relative effect of preeclampsia was stronger, with eight times higher relative risk of pulmonary embolism, as compared to women without preeclampsia, and almost eighty times higher compared to unexposed women. The risks of pulmonary embolism related to multiple birth showed a similar pattern, but the precision of the estimates was low with overlapping confidence intervals. The effect of Cesarean section on the risk of pulmonary embolism was strong around delivery and in the puerperium, with seven times higher relative risks as compared to vaginally delivered women. When we restricted the analyses to non-preeclamptic women, Cesarean delivery was associated with a four and three fold increase in relative risk of pulmonary embolism around delivery and in the puerperium, respectively (data not shown).

The relative risks of **stroke** were, during the third trimester, 12 times higher in preeclamptic or multiple pregnancies, compared to non-preeclamptic or single pregnancies, respectively (Table 5). Around delivery, women with preeclampsia had a more than 600 times higher risk of stroke than women in the unexposed period, while corresponding risk increase among non-preeclamptic women amounted to a more than 80-fold increase in risk. Around delivery, risks of stroke were also substantially increased among women with multiple and single births (RR=317.3 and 97.5, respectively). During the puerperium, the relative risk of stroke was more than 6 times higher among women with preeclampsia, compared to women without preeclampsia. Among women delivered by Cesarean section, risks of stroke were four times higher compared to vaginally delivered women, both around delivery and in the puerperium. The adjusted relative risks of pulmonary embolism and stroke among non-preeclamptic women, single births and vaginally delivered women, were substantially unchanged as compared to the overall risks.

In spite of highly increased risks of pulmonary embolism and stroke among women with preeclampsia, multiple pregnancy and Cesarean section, these factors could not explain the overall pregnancy-related risks of pulmonary embolism and stroke.

**Table 5.** The adjusted\* relative risks† (RR) of pulmonary embolism and stroke in relation to pregnancy, preeclampsia, multiple birth and Cesarean section among women who gave birth in Sweden during 1987-1993.

Pulmonary embolism												
Period	Preeclampsia				Multiple birth				Cesarean section			
	No (n=105)		Yes (n=20)		No (n=117)		Yes (n=8)		No (n=33)		Yes (n=92)	
	RR	(CI)	RR	(CI)	RR	(CI)	RR	(CI)	RR	(CI)	RR	(CI)
Third trimester	3.0	(1.9-4.6)	22.6	(8.4-61.2)	3.3	(2.2-5.0)	13.5	(1.9-97.1)				n.a.
Around Delivery	75.1	(50.1-112.5)	211.7	(52.4-856.3)	76.5	(51.3-114.0)	215.0	(29.9-1543.8)	42.1	(24.6-71.9)	370.4	(220.3-622.6)
Puer-Perium	9.6	(6.8-13.4)	79.8	(41.9-152.0)	11.4	(8.3-15.6)	15.6	(2.2-112.1)	6.7	(4.5-10.0)	46.7	(30.9-70.8)

  

Stroke												
Period	Preeclampsia				Multiple birth				Cesarean section			
	No (n=102)		Yes (n=28)		No (n=121)		Yes (n=9)		No (n=67)		Yes (n=37)	
	RR	(CI)	RR	(CI)	RR	(CI)	RR	(CI)	RR	(CI)	RR	(CI)
Third trimester	1.5	(0.9-2.6)	18.3	(6.8-49.5)	1.7	(1.0-2.8)	20.9	(5.1-84.5)				n.a.
Around Delivery	85.7	(60.8-120.7)	669.7	(328.9-1363.4)	97.5	(70.5-134.9)	317.3	(78.4-1285.0)	71.2	(48.7-104.6)	309.4	(190.2-503.1)
Puer-Perium	6.3	(4.5-8.9)	39.9	(17.6-90.1)	7.2	(5.2-10.0)	n.a.	(n.a.)	5.4	(3.7-7.9)	19.8	(11.7-33.6)

\* When analyzing preeclampsia, adjustments were made for: age, parity, smoking, diabetes, and type of birth; when analyzing multiple birth, adjustments were made for: age, parity, smoking, diabetes, and preeclampsia; when analyzing Cesarean section adjustments were made for: age, parity, smoking, and diabetes.

† The reference group consists of non-pregnant women and women with pregnancies less than 28 weeks.

n.a. not applicable (not appropriate for period or too few cases)

## The impact of intrauterine preeclampsia exposure on female offspring adult anthropometry

In Table 6, fetal and adult mean anthropometrics are compared between preeclampsia exposed and unexposed groups. Exposure status is analyzed in two levels: mild and severe preeclampsia. Compared with the unexposed group, mean gestational age, mean birth weight and birth length for gestational age were significantly smaller for those being exposed to mild or severe preeclampsia. In early adulthood, there were no significant differences in mean final height, difference from target height, age at menarche, body-mass index, or waist to hip ratio between young women being prenatally exposed or unexposed to preeclampsia.

**Table 6.** Comparison of fetal and adult anthropometry (expressed as mean values) among preeclampsia exposed and non-exposed women, born 1973 through 1978 in Sweden.

	No Preeclampsia n=359		Mild preeclampsia n=135		Severe preeclampsia n=95	
	mean	(SD)	mean	(SD)	mean	(SD)
Gestational age (weeks)	40.2	(1.5)	39.6*	(2.1)	38.2*	(2.3)
Birth weight for gestational age (SD)	-0.1	(1.0)	-0.8*	(1.6)	-1.3*	(1.2)
Birth length for gestational age (SD)	-0.1	(1.1)	-0.5*	(1.4)	-0.8*	(1.4)
Final height (cm)	168.0	(6.3)	167.3	(7.0)	166.9	(6.8)
Difference final height-target height (cm)	-0.5	(5.1)	-0.1	(4.7)	0.4	(5.4)
Age at menarche (years)	13.1	(1.4)	12.8	(1.5)	13.0	(1.4)
Body-mass index	22.8	(3.8)	22.8	(3.7)	22.0	(3.3)
Waist to hip ratio	0.79	(0.08)	0.80	(0.08)	0.80	(0.07)

\* Significantly different from the non-exposed ( $p < 0.05$ ).

Maternal height, birth weight and birth length for gestational age, were significantly associated with final height and difference from target height, and explained approximately 50% of the variance in final height (data not shown). Maternal BMI, maternal smoking and birth weight for gestational age were associated with adult BMI. None of the maternal (height, BMI, and smoking) or fetal (gestational age, birth weight and length for gestational age) characteristics influenced age at menarche, or waist to hip ratio (data not shown).

# DISCUSSION

## Etiological aspects of preeclampsia and gestational hypertension

### Environmental effects

We have found similarities in risk factor patterns for preeclampsia and gestational hypertension, although the magnitude of the observed associations often differed between the two conditions.

#### *Considerations of findings*

Our data confirmed previous reports that maternal smoking is inversely related to the risk of both preeclampsia and gestational hypertension (Marcoux 1989a, Sibai 1995, Misra 1995). This association is studied repeatedly, probably because the result is so unexpected since smoking is associated with cardiovascular diseases, and also because it is difficult to explain the underlying mechanism (Krupski 1991, Zhang 1999, Conde-Agudelo 1999). However, when preeclampsia occurs in smokers, it is associated with higher risk of small for gestational age, perinatal death and abruptio placentae (Cnattingius 1997), indicating that placental perfusion is further impaired, as compared to non-smoking preeclamptic women. Different explanations have been suggested for the protective effect of smoking on risk of preeclampsia and gestational hypertension. The prostacyclin/thromboxane balance may be more advantageous in smokers than in non-smokers (Toivanen 1986), but laboratory data shows that the urinary excretion of thromboxane metabolites is higher in smokers compared to non-smokers (Rångemark 1996). Inhibition of cytokine production among smokers (Madretsma 1996a, Madretsma 1996b) may be advantageous since cytokines play a key role in immunoregulation and serum levels are elevated before the clinical manifestations of preeclampsia (Hamai 1997, Williams 1999). The worse fetal outcome among smokers who develop preeclampsia may be explained by two findings. Increased levels of endothelin-1 are found in both smokers and preeclamptic women (Taylor 1990, Nisell 1991, Florijn 1991a, Florijn 1991b, Haak 1994), and endothelin-1-induced vasoconstriction is enhanced in smokers (Kiowski 1994). Nevertheless, in our data the protective effect of smoking is of the same magnitude on risks of preeclampsia and gestational hypertension.

We showed that, when compared to women born in Nordic countries, women born outside the Nordic countries had a lower risk of developing both gestational hypertension and preeclampsia. This association has not previously been described and the explanations remain to be elucidated. It has previously been described that the relations between insulinemia, insulin resistance, and blood pressure differ among racial groups (Saad 1991). Our findings that both pregestational and gestational diabetes are associated with increased risk of both preeclampsia and gestational hypertension are consistent with other reports (Martinez Abundis 1996, Garner 1990). It has also been reported that gestational hypertension but not preeclampsia is associated with insulin resistance (Roberts R1998, Caruso 1999). It has been hypothesized that the development of preeclampsia starts in early gestation (Zhou 1993), therefore the cause-effect relationship with gestational diabetes is unclear.

Our data suggested an association between multiple pregnancy and both preeclampsia and gestational hypertension, although the latter effect was weaker and had lower precision. More recently these findings have been confirmed (Sibai 2000), and different explanations have been suggested: higher maternal exposure to paternal antigen from the larger placental mass (Sibai 1991), impaired placental perfusion (Roberts 1993), or difficulty with vasodilatation when cardiac output is dramatically increased in late twin gestation (Coonrod 1995). The high

proportion of women developing pregnancy-induced hypertension in multiple pregnancies (approximately 25%) may be of significance when planning antenatal care for these women.

We also found that increasing early pregnancy BMI was associated with increased risks of similar magnitude for both conditions. The collective evidence suggests that there is an association between obesity and both gestational hypertension and preeclampsia (Eskenazi 1991, Sibai 1995). High body fat mass is associated with several lipid disturbances (Van Gaal 1995), which in turn may cause endothelial dysfunction (Endresen 1992). Moreover, obesity may be associated with gestational hypertension and preeclampsia through elevated levels of tumor necrosis factor alpha (TNF-alpha) (Hotamisligil 1995). Tumor necrosis factor alpha in plasma is reported to be significantly higher in preeclamptic compared to non-preeclamptic women, and may give rise to endothelial dysfunction (Vince 1995). Obesity may not be causally related to risk of preeclampsia and gestational hypertension. However, if it is causally related, it is of public health importance, since approximately 18% of all nulliparous women who gave birth in Sweden 1992-97 were overweight or obese (BMI > 26.0) and overweight is an increasing problem among young people worldwide. In our data, in the overweight/obesity group the incidence of gestational hypertension would decrease by 43%, and in the entire population by 10%, if nobody was overweight or obese (calculated by using the crude numbers in our sample). Similarly, in the overweight/obesity group the incidence of preeclampsia would decrease by 60%, and in the entire population by 18%, if nobody was overweight or obese.

### **Genetic effects**

Our results suggest that genetic and environmental effects are of approximately equal importance for liability of developing preeclampsia. The environmental effect seems to be stronger than the genetic effect on the liability of developing gestational hypertension. Moreover, when preeclampsia and gestational hypertension were regarded as a single entity, the results indicated a genetic effect on the liability of pregnancy-induced hypertensive diseases.

### *Considerations of findings*

In the present study, the genetic and environmental effects on the liability of developing preeclampsia and gestational hypertension were obtained by studying pregnancy similarities among more than 2100 twin pairs. In spite of the large number of twin pairs, the estimates were not significant, which may be important in the planning of future twin studies.

For **preeclampsia**, the **probandwise concordance rate** among dizygotic twins was 0.06, i.e. translated to ordinary sisters, 6% of the women with a sister who had preeclampsia will develop preeclampsia. Compared to the overall pregnant population, in whom approximately 3% develop preeclampsia, the risk is two times higher among sisters with the same mother and father. This result is in accordance with a previous study from Norway (Lie 1998). From the Norwegian data the authors also draw the conclusion that paternal genes in the fetus may contribute to a pregnant woman's risk of preeclampsia. In the present study we had no data on paternity. For **preeclampsia** the **maternal genetic effect** on the liability of developing preeclampsia was 54%, while the genetic effect for developing **gestational hypertension** was 24%. Although the hypothesis of a single gene is not completely ruled out (Roberts 2001), data from the present and previous studies suggest that preeclampsia should be considered a complex disease.

To our knowledge, a genetic effect on the development of **gestational hypertension** has not previously been reported. Compared with preeclampsia, the genetic effect seems to be weaker, although the confidence intervals were wide, i.e. the precision was low. Gestational hypertension has been considered as unmasked essential hypertension (Chesley 1980), and is

associated with chronic hypertension later in life (Chesley 1980). The estimated genetic effect on blood pressure variance in the population is approximately 40% (Hong 1994), which is of a similar magnitude as the estimate for gestational hypertension in the present study. Preeclampsia is also associated with hypertensive diseases later in life (Hannaford 1997, Marin 2000), and one may speculate if these conditions have some genetic factors in common. Interestingly, when we modeled preeclampsia and gestational hypertension as a single entity, **pregnancy-induced hypertension**, with different degrees of severity, the estimates of the genetic and environmental effects became significant. Moreover, when data from the genome-wide scan by Arngrimsson was analyzed by combining preeclampsia and gestational hypertension, the results revealed that there may be a genetic effect in common (Arngrimsson 1999). Taking these results together, we suggest that these diseases have some susceptibility genes in common. In our data, the environmental effect on the liability of developing pregnancy-induced hypertension consisted of non-shared environmental effect only. This indicates that intrauterine and childhood environments are not associated with risk of pregnancy-induced hypertension.

In conclusion, maternal genes have a considerable effect on the liability of preeclampsia. According to previous gene hunting experiences described in the “Background”, no major gene should be expected, but rather several genes influencing the liability together with several different environmental factors. To further improve the understanding of preeclampsia and gestational hypertension a larger twin study would be desirable, with information on all pregnancies, more detailed data on disease severity and environmental factors. Gene hunting can also be improved by considering the degree of hypertension and proteinuria in each individual, when analyzing data (Lander 1994).

### *Considerations of methods*

The accuracy of the diagnoses in registries can be a concern. In the study of genetic effects (*Paper II*) different diagnostic criteria were used during the study period. The validation of the preeclampsia diagnosis for the years when ICD-8 was used revealed that introducing proteinuria as a compulsory criterion had substantial influence on the diagnostics of preeclampsia. As the diagnoses are registered independently of zygosity, it is unlikely that underreporting or accuracy of diagnoses differ systematically between monozygotic and dizygotic twin pairs.

For the study of environmental effects (*Paper I*), when ICD-9 codes were used, high predictive values were found for preeclampsia and gestational hypertension in a random sample. Moreover, when the accuracy for normotensive pregnancies was examined in another random sample, with pregnancies prior to the present study (1973-1978), the predictive value was also high, as expected. We considered it unlikely that there were differences between these periods in the accuracy of the normotensive recording. Most of the diagnostic misclassification of outcomes that may have occurred was probably independent of exposure status. Measurements of blood pressure and proteinuria were repeated at every antenatal check, and also measured repeatedly at the delivery clinic. The vast majority of pregnant women in Sweden attend antenatal care, and have their delivery at a delivery clinic, where a doctor notes the diagnoses, at the time of discharge from the hospital. Therefore it is unlikely that misclassification of preeclampsia and gestational hypertension is due to differences in diagnostic routines in different groups of women, e.g. women with multiple pregnancies or diabetes.

Although erroneous measurements of blood pressure and proteinuria are generally independent of exposures, among obese women, falsely high blood pressure values will be noted if a pneumatic cuff of insufficient width is used. Therefore the effect of obesity on the risk of hypertension in pregnancy may have been overestimated.

In the study of environmental effects (*Paper I*), data (e.g. maternal early pregnancy weight and smoking) were collected prospectively during pregnancy, independent of pregnancy outcome. The lower than expected incidence of gestational diabetes (and in turn the low precision of our odds ratios) may be explained by two factors changed over time: the diagnostic criteria for gestational diabetes has changed and the requirements for routine testing. Therefore chance cannot be ruled out completely.

In twin studies, determination of zygosity is crucial. The questionnaire-based determination of zygosity, used in the present study, demonstrated high positive predictive values (98%), when validated by using DNA-markers (FRN 2000).

Twin design makes it possible to separate the familial effect into genetic and shared environmental effects through standard quantitative genetic methods. We consider the assumptions of random mating, equal environments for monozygotic and dizygotic twins and an underlying normal distribution of liability of developing preeclampsia and gestational hypertension (Neale 1992), as reasonable. We studied a large number of twin pairs, but in spite of that, the heritability estimates did not reach statistical significance, and therefore the results should be interpreted with caution.

## **Circulatory diseases in relation to pregnancy status**

During the third trimester of pregnancy, there was an increased risk of venous thromboembolism. Around delivery, risks of all the assessed circulatory diseases were dramatically increased and during the puerperium the risks declined, but were still substantially increased for all diseases, with the exception of subarachnoid hemorrhage. Preeclampsia, multiple birth and Cesarean section are associated with overwhelming increased risks of pulmonary embolism and stroke related to pregnancy, and these risks also increase with maternal age and smoking. However, these factors do not explain the pregnancy-related risks of pulmonary embolism and stroke.

### *Considerations of findings*

Late pregnancy status with a prothrombotic coagulation system and venous stasis, has a strong effect on risk of **venous thromboembolism**. During a few days around delivery, the risk of venous thromboembolism peaked dramatically, which may mirror the physiological alterations taking place during that period. The number of cases around delivery accounted for a large proportion of all cases of venous thromboembolism related to pregnancy. During the puerperium there was a decline, but still substantially increased risk compared to the unexposed period. After the puerperium we observed a decreased risk of venous thrombosis during the first year postpartum. This may well be explained by a smaller proportion of women who use oral contraceptives, or who are pregnant in that period, compared to the unexposed period (Herings 1999, Bloemenkamp 1998, NIH Consensus 1986). Moreover, decreased risk of venous thrombosis may be associated with lower levels of estrogens among lactating women (Diaz 1995). The observed risks can be looked upon as a course, which corresponds to the prothrombotic state of the coagulation system accompanying pregnancy status, with increased risk apparent in the third trimester, the highest risk around delivery, and a decline but still substantially elevated risk during the puerperium.

Pregnancy-related risks of **cerebro-vascular diseases** are mainly confined to a few days around delivery. During the puerperium, again there were declines, but still substantially increased risks of intracerebral hemorrhage and intracerebral infarction, compared to the unexposed period. Pregnancy-related risk of subarachnoid hemorrhage was only confined to a few days around delivery, which probably reflects the effect of labor/delivery on the pressure in the cerebral arteries. The delivery and puerperal periods affected the risk of cerebral

hemorrhage more than the risk of cerebral infarction, which is in agreement with a previous study that presented relative risks of stroke related to pregnancy (Kittner 1996). This may reflect the effect of pregnancy-induced arterial distensibility accompanied by hypertension (Edouard 1998). The pregnancy-related hypercoagulable status (Hellgren 1981, Hellgren 1996, Cerneca 1997), an increased tendency to arterial spasm, and/or hypotension from hypovolemia during delivery, are factors that may be associated with increased risk of cerebral infarction around delivery.

**Myocardial infarction** related to pregnancy and puerperium is rare indeed (0.6 cases/100,000 deliveries). The estimated effect of pregnancy and puerperium on risk of myocardial infarction had low precision, despite the large number of women included in the present study, which further reiterates the rarity of myocardial infarction related to pregnancy.

Our results indicate a strong association between **preeclampsia** and **pulmonary embolism**, which seems to operate from at least the third trimester and throughout the puerperium. The association between preeclampsia and an exaggerated prothrombotic coagulation status, appears to influence risk of pulmonary embolism. Preeclampsia has been reported to be associated with increased risk of venous thrombosis (Lindqvist 1999), but the effect on risk of pulmonary embolism has not previously been reported. Despite the marked effect of preeclampsia on risk of pulmonary embolism, this did not explain the overall risk of pulmonary embolism related to pregnancy.

Our results also indicate a marked effect of **Cesarean section** on risk of **pulmonary embolism**, which also influences the overall risk of pulmonary embolism related to delivery and puerperium.

Compared to non-preeclamptic women, our results indicated dramatically increased risk (up to 12 times higher) of **stroke** among women with **preeclampsia** in all pregnancy-related periods. Previous studies have also pointed out preeclampsia as a potential risk factor for stroke (Kittner 1996, Lanska 2000), and the risk estimates in the present study demonstrate the severity of the pathophysiological alterations in preeclamptic pregnancies (VanWijk 2000, Sakai 1994, Williams 1993, Bosio 1999, Zunker 2000). Occurrence of headache in women with preeclampsia may be regarded as a warning sign, since it is strongly associated with the presence of abnormal cerebral perfusion pressure (Belfort 1999). Unfortunately we were not able to study the effect of preeclampsia on risks of intracerebral hemorrhage and infarction separately. In preeclampsia, hypertension predisposes to intracerebral hemorrhage, but preeclampsia is also associated with exaggerated platelet aggregability (Felfernig-Boehm 2000), endothelial dysfunction, with increased levels of endothelin (Taylor 1990), which is a vasoconstrictor possibly inducing vasospasm, and may therefore predispose to intracerebral infarction.

Compared with single births, **multiple births** were associated with dramatically increased risk (12 times higher) of **stroke** during late pregnancy. This may reflect the fact that the “normal” maternal cardiovascular adaptations are exceeded or altered in multiple pregnancies (Duff 1974, Duvecot 1994, Veille 1985, Donaldson 1989). Among women delivered by **Cesarean section**, the relative risk for **stroke** from delivery throughout the puerperial period, was 4 times higher compared to vaginally delivered women, which is in agreement with a previous study (Lanska 2000). This association only explained a small fraction of the overall increased risk of stroke related to delivery and puerperium. In connection with the surgical procedure, general anesthesia or an epidural/spinal blockade is necessary, and these methods are associated with blood pressure fluctuations that may contribute to increased risk of stroke related to pregnancy. Therefore it is important to clarify the temporal relationship between Cesarean section and stroke.

In conclusion, we found that there are increased risks of circulatory diseases in late pregnancy, around delivery and during the puerperium. Although preeclampsia, multiple birth

and Cesarean section should be considered as risk indicators for pulmonary embolism and stroke, these factors enhance rather than explain the pregnancy-related risks of pulmonary embolism and stroke.

### *Considerations of methods*

In the comparison of the effect of pregnancy status on the risks of circulatory diseases, our reference group consisted not only of women who were not pregnant, but also pregnant women in the first and second trimesters. The reason we included the first and second trimesters in the unexposed group was that pregnancies terminated before gestational week 28 (stillbirths, induced and spontaneous abortions) are not compulsorily included in the Birth Register. The effects of pregnancy status on risks of circulatory diseases will therefore be underestimated.

We were restricted to women who had at least one birth during the study period, and therefore our reference group only consisted of women who were healthy enough to go through a pregnancy. This may have biased the strength of the effect of pregnancy status on risk of circulatory diseases.

We analyzed data from two registers after removal of the national registration number, and therefore we were not able to evaluate the validity of the assessed diagnoses. The clinical diagnoses of venous thrombosis and pulmonary embolism are difficult. However, we believe that once venous thrombosis is suspected, the diagnostic procedure is the same for pregnant and non-pregnant individuals. General knowledge of increased risk of thromboembolism during pregnancy, and antenatal care may bias the strength of the association, if thrombosis is not suspected to the same extent in the unexposed period (our reference group). On the other hand, there is also a general knowledge of increased risk of thromboembolism associated with the use of oral contraceptives, which in turn is only prevalent in the unexposed period. Moreover, in the unexposed period at least 50% of the cases of thrombosis were in fact early pregnant (before gestational week 28). Consequently, we probably underestimated the effect of pregnancy during all exposure periods.

In the analyzes of the effect of pregnancy status on risks of the different stroke diagnoses, we chose to exclude 34 cases with the imprecise diagnosis with ICD-9 code 674A, representing any of the diagnoses 430-434 and 436-437 during pregnancy or puerperium. When we assessed the effect of preeclampsia, multiple pregnancy and Cesarean section on the risk of stroke, there were too few cases to perform separate analyzes for each diagnosis. Therefore we were not able to distinguish if the effects of preeclampsia, multiple pregnancy or Cesarean section are different for cerebral hemorrhage or infarction.

We regarded the date of admittance to the clinic where the diagnosis was registered as the onset of disease. The symptoms of the assessed conditions are usually so troublesome that it is unlikely that the onset of the disease preceded that date by more than a few days. We were unable to distinguish whether delivery preceded the onset of circulatory diseases or vice versa. Among the women who developed a circulatory disease around delivery, only 3% were admitted before the day of delivery, suggesting that the onset of most cases in this group occurred during or after delivery.

Although the previously performed validation of the accuracy of the preeclampsia diagnosis must be considered as satisfactory, there may be higher level of surveillance among women with preeclampsia, multiple pregnancies and Cesarean section, which may bias the strength of the associations to some degree. The vast majority of the cases were based on the primary discharge diagnosis. Therefore we believe that the majority of cases included are incident. Some women may have died before hospital admittance, and were therefore not covered by the Inpatient Register. These circumstances and migration during the time of follow-up may influence the obtained incidence rates and relative risks, if the proportion of

women who die outside the hospital or migrate during the study period is different in different exposure periods.

When we estimated relative risks of circulatory diseases in relation to pregnancy, we used direct standardization to adjust simultaneously for maternal age and parity. This is feasible when adjustments are made for only two independent variables. When we analyzed risk factors for pulmonary embolism and stroke related to pregnancy, the number of independent variables were greater and we therefore performed Poisson regression. The estimated risks are generally strong, but the confidence intervals are wide and it is therefore important to focus on the dynamics rather than the absolute values.

### **Preeclampsia exposure in utero —impact on female adult anthropometry**

Intrauterine exposure to preeclampsia did not influence final height, age at menarche, body-mass index or waist to hip ratio in young adulthood.

#### *Considerations of findings*

In spite of a smaller birth length for gestational age among women exposed to preeclampsia in utero, there was no difference in final height, as compared to unexposed women. Since it has been reported that birth length for gestational age is a better predictor for final height than birth weight for gestational age (Tuvemo 1999), which is in agreement with our results, there must be catch up growth among the preeclampsia exposed women. We were also able to retrieve data on parental height, and almost 50% of the variance in final height could be explained by birth length for gestational age and parental height. This reflects the strong genetic effect on height, which is reported to explain 80% of the variance in the population (Silventoinen 2000).

In the present study, preeclampsia exposed and unexposed individuals did not differ in age at menarche, BMI, or waist to hip ratio in early adulthood. Neither waist to hip ratio nor age at menarche were affected by any maternal or infant characteristics, which is in contrast to previously reported results of a lower age at menarche among girls born small for gestational age (Persson 1999). For young adult BMI there were weak associations with maternal BMI, maternal smoking, and birth weight for gestational age, which only explained 12% of the variance. In contrast to previous reports (Law 1992), we found no association between low birth weight and adult abdominal obesity. This may be due to low power, or that the effect differs between birth cohorts. Alternatively the underlying cause of low birth weight is important. Since preeclampsia is associated with subsequent hypertension and insulin resistance (Kaaja 1999), it is important to include the underlying causes of low birth weight in studies of adult morbidity, as previously suggested (Churchill 1997, Walker 1998).

Yet another explanation for the lack of association between low birth weight and adult abdominal obesity in our data may be that the associations between fetal and adult anthropometry is explained mainly by genetic factors, as proposed by Hattersley et al. (Hattersley 1999). Preeclampsia is probably influenced by genetic factors (Salonen Ros 2000), and one may only speculate whether there may be genetically determined alterations in both the placental and fetal hormonal synthesis, which in turn protects the female fetus against subsequent risk of breast cancer. Data from two recent studies suggest that genetic factors play an important role in the association between birth weight and blood pressure (Ijzerman 2000, Hübinette, submitted). Genetic effects are reported to explain approximately 50 % of the variations in levels of BMI (Pietiläinen 1999), blood pressure (Hong 1994), and central abdominal fat (Carey 1996) in the population.

In conclusion, the results in the present study suggest that exposure to preeclampsia during fetal life does not influence age at menarche or anthropometric measures in young adults to

the degree that could explain the reduced risk of breast cancer previously reported for that group of women. Thus, prenatal exposure to preeclampsia and adult anthropometry should be regarded as independent risk factors in etiological studies of breast cancer.

### *Considerations of methods*

Information about exposure status was obtained from prospectively collected data in individual antenatal and obstetric records, starting at the women's first visit to antenatal care. Preeclampsia exposure status was validated for each individual according to the diagnostic criteria corresponding to the ICD-9 definition. Birth weight and birth length for gestational age were calculated according to Swedish reference standards, based on births from the 1970s, where gestational age was calculated from the date of the first day of the last menstruation (Niklasson 1991). The proportion of women (mothers) with irregular menses was the same in the exposed and unexposed group. We considered it unlikely that the proportion of erroneous calculations systematically differed between preeclampsia exposed and unexposed women.

Outcome variables were obtained from telephone interviews, performed by professional interviewers who were blinded to exposure status. Possible errors in measuring or recalling height, weight, waist and hip circumferences and parental height were most likely independent of exposure status.

As the response rate was 80% in both the exposed and the unexposed group, and the proportion of women who refused to participate was similar, we regarded the question of selection bias as negligible.

There were no differences in adult anthropometric measurements, and according to the power calculations it would have been possible to detect a difference of at least 4.5 cm in final height, between women exposed to severe preeclampsia and unexposed women. The role of chance cannot be completely excluded. However, the previously reported association between height and risk of breast cancer reveal that considerably large differences in height ( $\geq 10$  cm) had a modest effect on breast cancer risk (Ziegler 1996, Magnusson 1998). Therefore differences in height smaller than 4.5 cm, may not be relevant for the reduced risk of breast cancer among women exposed to preeclampsia in utero. The same applies to mean age at menarche for which 2-3 years difference seems to be relevant for breast cancer risk (Hunter 1997).

## **Implications for the future**

In etiological studies of preeclampsia it would be of interest to explore possible interactions between genes and the environment. Data from genetic linkage or association studies should be combined with individual data on environmental risk factors. This approach should also be applied to sub-groups of women born in non-Nordic countries, as well as second generation immigrants.

To further study pregnancy-related risks of circulatory diseases, data from individual records are necessary to be able to study the course of events (i.e. more precision in the determination of when the diseases occurred in relation to delivery). In addition, coagulation tests are required to be able to discriminate between cases due to thrombophilia and other causes. Study of risk factors for stroke with a subdivision of the different stroke diagnoses is desirable.

## CONCLUSIONS

The similarities in risk factor patterns of gestational hypertension and preeclampsia indicate similarities in underlying biological mechanisms.

For preeclampsia, the genetic and environmental effects are of approximately equal importance, whereas for gestational hypertension the non-shared environmental effect was stronger than the genetic effect.

If gestational hypertension and preeclampsia is considered as one disease with different degrees of severity, the genetic and non-shared environmental effects are of approximately equal importance.

Risks of venous thromboembolic diseases (deep venous thrombosis and pulmonary embolism) and stroke increase in the third trimester, peak during three days around delivery and decline during the puerperium.

Increases in risks of cerebral arterial diseases (subarachnoid hemorrhage, intracerebral hemorrhage and cerebral infarction) and myocardial infarction in relation to pregnancy, are mainly confined to a few days around delivery, and to a smaller extent to the puerperium.

Although preeclampsia, multiple birth and Cesarean section are all associated with highly increased risk of both pulmonary embolism and stroke, these complications do not explain the increased risks related to pregnancy.

We found no evidence that intrauterine preeclampsia exposure influences final height, or age at menarche, and therefore cannot explain the reduced breast cancer risk previously observed for women prenatally exposed to preeclampsia.

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