Preeclampsia - maternal risk factors and fetal growth

Rønnaug A. Ødegård

Dr. med. thesis

Institute of Cancer Research and Molecular Biology
Faculty of Medicine
Norwegian University of Science and Technology

CONTENTS

Acknowledgements

This work was supported financially by the Norwegian Medical Research Council, the Norwegian University of Science and Technology, and the Norwegian Cancer Society. Grants from the University Hospital in Trondheim have also contributed.

A number of persons have contributed to the completion of this thesis, and in particular I want to express may gratitude to my mentors who have guided me through these exciting years of preeclampsia studies: Rigmor Austgulen initiated the present study, and introduced me to research in the field of reproduction. Lars Vatten provided invaluable methodological help, Stein Tore Nilsen organised the whole study, and Kjell Å. Salvesen provided important clinical insight in preeclampsia and intrauterine growth.

This work was carried out at the Institute of Cancer Research and Molecular Biology, and Department of Community Medicine and General Practice. Both departments have provided good working facilities, and I have enjoyed the friendly and including atmosphere that characterises both institutes. It has been a pleasure to work with Anita Haugan, and the other collegues in the reproduction group; Shahnaz Rahimipoor, Kristin Rian, Henrik Døllner and Knut Jørgen Arntzen. I also thank Terje Espevik, Nina Liabakk, Egil Lien, Randi Vik and Mari Sørensen for their advices and help during the laboratory work, and Kjartan Egeberg for computer assistance. The secretarial assistance of Dagmar Moholdt and Tove Opdahl is highly appreciated.

This study originates from the birth clinic at the Central Hospital of Rogaland, and I acknowledge the staff at the clinic for their thorough work with blood sampling and registration. I owe particular gratitude to Hege Vefring who organised the blood sampling and processing, and Margrethe Bjelland who collected the clinical data.

Finally, I want to thank my family and my friends who supported and encourraged me during the course of this work. My husband Vidar and my children Ane and Sondre have shown admirable patience and understanding for my absence during busy periods, and my mother has been of invaluable help to us.

Trondheim, January 2002
Rønnaug Astri Ødegård.

Abbreviations

ALAT	Alanin-aminotransferase
ASAT	Aspartat-aminotransferase
BWR	Birth weight ratio (observed through expected birth weight)
FGR	Fetal growth restriction
GH	Growth hormone
HELLP	Hemolysis, elevated liver enzymes and low platelet counts
IL-6	Interleukin-6
IGF-I	Insulin-like growth factor-I
IGFBP-1	Insulin-like growth factor binding protein-1
IGFBP-3	Insulin-like growth factor binding protein-3
PE	Preeclampsia
PL	Placental lactogen
SGA	Small for gestational age
SIDS	Sudden infant death syndrome
MTT	3-(4,5 dimethyl-thiazol-2-yl)-2,5-di-phenyltetrazolium bromide

List of papers included in this study

Paper I: Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KÅ and Austgulen R. Risk factors and clinical manifestations of pre-eclampsia. British Journal of Obstetrics and Gynaecology. 2000; 107:1410-16.

Paper II: Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KÅ and Austgulen R. Preeclampsia and Fetal Growth. Obstetrics & Gynecology. 2000; 96:950-5.

Paper III: Ødegård RA, Vatten LJ, Nilsen ST, Vefring H, Salvesen KÅ and Austgulen R. Umbilical cord interleukin-6 and fetal growth restriction in preeclampsia; a population study in Norway. Obstetrics & Gynecology. 2001; 98:289-94.

Paper IV: Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KÅ and Austgulen R. Umbilical cord plasma leptin is increased in preeclampsia. American Journal of Obstetrics and Gynecology. 2002; 186:427-32.

Paper V: Vatten LJ, Odegard RA, Nilsen ST, Salvesen KA, Austgulen R. Relationship of insulin-like growth factor-I and insulin-like growth factor binding proteins in umbilical cord plasma to preeclampsia and infant birth weight. Obstetrics & Gynecology. 2002; 99:85-90.

INTRODUCTION

1 PREECLAMPSIA

Preeclampsia is a complex and variable maternal disturbance that ranges from a dramatic onset at early gestation to slowly developing symptoms towards term. Hypertension and renal involvement with proteinuria are cardinal signs, which are often accompanied by fluid retention, blood-clotting dysfunction, and reduced organ perfusion. HELLP (haemolysis, elevated liver enzymes, and low platelet count) syndrome is regarded as a variant of preeclampsia, and the fulminante disease, eclampsia, includes convulsions. Preeclampsia is the main cause of maternal and fetal morbidity and mortality in western countries (1, 2), and in Nordic countries, 17 percent of maternal deaths have been ascribed to preeclampsia (2). Antenatal care in Norway includes on average 12 doctor/midwife consultations per pregnancy (3), and since blood pressure monitoring and urinary testing are main aims of the consultations, preeclampsia is a pregnancy complication that also generates substantial societal costs.

1.1 Incidence

The reported incidence of preeclampsia varies between 3-10 % (4, 5-8), and some of this variation may be attributable to differences between study populations. Since nulliparity increases the risk of developing preeclampsia (6, 9), the incidence reported from studies encompassing both nulliparous and parous women will be strongly influenced by the parous state of the participants. However, the reported incidence of preeclampsia in nulliparous women also varies substantially, and (8, 10) some of this variation may depend on the use of different diagnostic criteria for preeclampsia between studies (11).

1.2 Definition of preeclampsia

The definition of preeclampsia varies, but all international classification systems emphasise the need for an increase in blood pressure to at least 140/90 mm Hg after 20 weeks' gestation (6, 12-15). Since blood pressure will increase in most normal pregnancies (6), some authors also argue that there should be an additional increase in

diastolic blood pressure of at least 15 mm Hg (15), whereas others suggest that the diastolic blood pressure increase should be at least 25 mm Hg (6). In all but one of the currently used classification systems, proteinuria is required for the diagnosis of preeclampsia (12), but the cut-off for proteinuria varies. Some suggest that there should be one sample with at least 1+ on semiquantitative dipstick, whereas others suggest at least two separate samples with at least 2+ on the dipstick (13). There is a general agreement, however, that severity of preeclampsia increases with increasing blood pressure and proteinuria, and the definition of severe preeclampsia is practically identical for all international classification systems. The occurrence of preeclampsia at an early gestation may represent a particularly severe form of the disease (16, 17), but there is no consensus on the classification of "early-onset" preeclampsia; the definition ranges from delivery before 30 weeks to delivery before 37 weeks of gestation (16-20).

1.3 Aetiology

Historical perspective

Symptoms of preeclampsia and eclampsia were described already by the ancient Greeks (21), but eclampsia was differentiated from epilepsy first in 1739 by the French obstetrician Sauvages, and later termed *Eclampsia parturentium* (22). In 1843, proteinuria was observed, and led to the view that eclampsia depended on uremic poisoning caused by deficient renal excretion. After the detection of eclamptic hypertension at the end of the 19th century, the disease was regarded a manifestation of essential hypertension, brought to light and peculiarly coloured by pregnancy (22). However, it was recognised that preeclampsia occurred only in the presence of placental tissue, including cases with retained placental tissue and hydatiform mole, where the fetus is absent (23). Therefore, pathology of the placenta was early suspected as a causative factor. In 1967, Robertson and Brosens described specific structural changes of the uteroplacental unit in preeclampsia (24), and until recently, unsuccessful placentation has been regarded a necessary factor for preeclampsia to develop.

Placental factors

In normal pregnancies, the uteroplacental spiral arteries undergo major remodelling by invading cytotrophoblast, and the muscular, intimal and endothelial layers of the spiral arteries are replaced by trophoblast (25). Thereby the arteries are transformed to high flow, low resistance vessels capable of meeting the needs of the fetus, and these physiological changes include the arterial segments of the inner third of the myometrium (Fig.1). Preeclampsia is associated with a failure of trophobıast to transform the spiral arteries; the number of transformed arteries is reduced, and the transformation does not reach the myometrial segments of the arteries (24, 26, 27). Unsuccessful placentation may cause reduced placental blood flow, and subsequent underperfusion of the placenta may induce hypoxic injuries and generate toxic metabolites. The prevailing hypothesis over the last decade has been that toxic metabolites from a hypoxic placenta released to the maternal circulation may cause the generalised syndrome of preeclampsia (23). However, maldeveloped placentas are also observed in cases of fetal growth restriction without preeclampsia (28, 29). Moreover, most infants born after preeclamptic pregnancies have an appropriate birth weight for their gestation, which strongly indicates predominantly normal placentas. Therefore, it has been suggested that the determining factor for the development of preeclampsia may be the maternal response to a normal as well as a maldeveloped feto-placental unit (30).

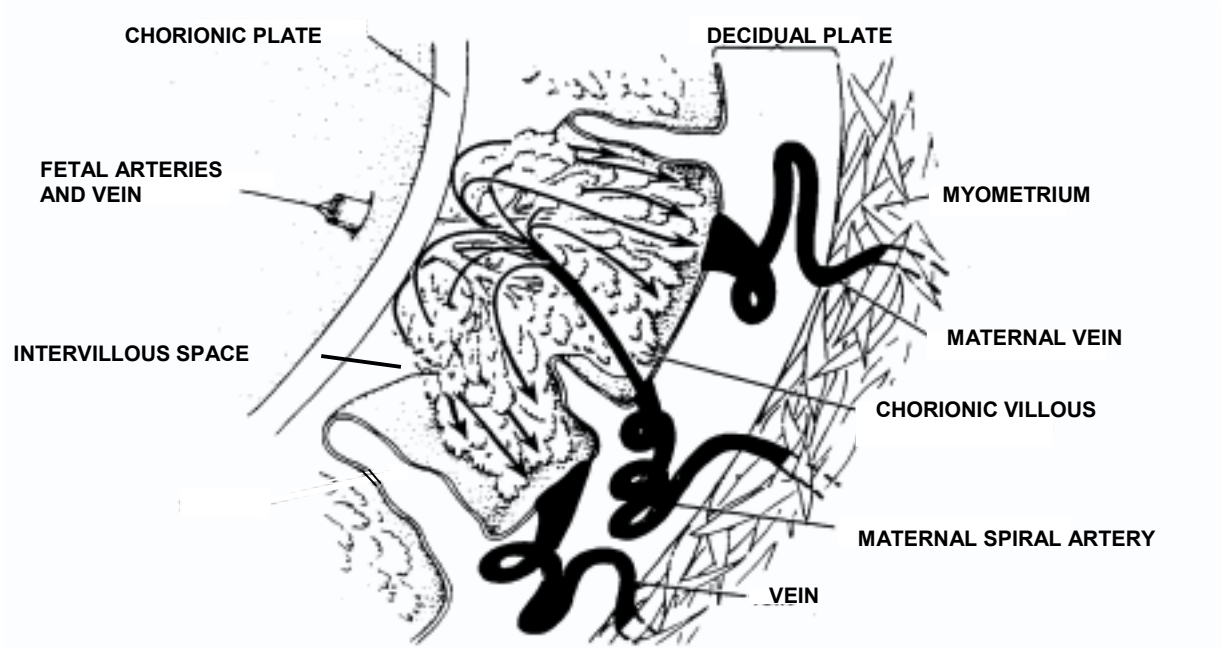

Fig.1. Diagram of maternal/fetal interface showing spiral artery blood flow and villous structure. From: Llewellyn-Jones. Fundamentals of Obstetrics and Gynaecology 1982.

Maternal factors

Cellular and molecular components of the immune system may play an important role in the control of normal trophoblast invasion (31, 32), and aberrant maternal immune response against the invading trophoblast has been considered a causative factor for shallow trophoblast invasion (32). Compared to parous women, primiparous women are at increased risk of preeclampsia (5, 9), and the lower risk among parous women has been interpreted as a consequence of being desensitised to similar fetal antigens during previous pregnancies (33). This hypothesis is supported by studies reporting reduced risk of preeclampsia in pregnancies subsequent to a long period of cohabitation with the father; i.e. long term exposure to paternal antigens (34), and some studies even show that change of partner may increase the risk of preeclampsia in subsequent pregnancies (35). However, a recent study suggests that the risk of preeclampsia among parous women is positively associated with the time interval between the births, regardless of partner-change (Skjærven, R, personal communication). In their study, changing partner between two births increased the interval between births, and they suggest that differences in time interval between births may have confounded the association between change of partner and preeclampsia in previous studies.

Essential hypertension has consistently been associated with increased risk of preeclampsia (36, 37), and as measured at first antenatal visit, increased blood pressure within the normal range, also increases the risk (7, 38-40). Other micro-vascular diseases, including Systemic Lupus Erythematosus (SLE) (41), and metabolic disorders, including obesity, dyslipidemia, insulin resistance and diabetes (38, 40, 42-46) are all associated with preeclampsia. Finally, increased frequency of thrombophilic disorders is also observed in preeclampsia (47). Recently, several authors have reported increased frequency in preeclampsia of certain genotypes involved in vascular disease and remodelling, volume regulation, and blood pressure control. Candidate genes include the T235 allele for angiotensinogen, factor V Leiden mutation, and homozygosity for methylenetetrahydrofolate reductase (46, 48, 49). Although some of these associations have been difficult to reproduce (50-52), it has been suggested that genes involved in circulatory and metabolic regulation may also be involved in the development of preeclampsia (51).

A familial predisposition for preeclampsia has been documented (53), confirming that genetic factors may contribute to its development. The risk of preeclampsia may be two -fold higher among daughters of women who have experienced the disease compared to women with no history of preeclampsia (54). Furthermore, in women with severe preeclampsia, the prevalence of pre-eclampsia and eclampsia among their daughters was significantly higher than among their daughters-in-law (55, 56). Thereby a mother to daughter genetic predisposition is suggested, which fits with a model of mitochondrial inheritance of preeclampsia, as suggested by Folgerø et al. (57).

On the other hand, the single-gene model of inheritance that best explains the overall frequency of preeclampsia is the presence of homozygosity for the same recessive gene both in the mother and the fetus (58). Accordingly, these fetuses would be homozygous for the recessive allele, which they would pass to their male and female offspring. Thus, both male and female offspring would share an increased risk of having a pregnancy complicated by preeclampsia. The studies that failed to identify a paternal contribution included only cases with severe maternal disease. In a study among offspring of cases of preeclampsia that included all degrees of severity, both males and females were more likely than controls to have a child that was the product of a preeclamptic pregnancy (59). A population-based study in Norway among men who had children with more than one woman give further support to the observation of a paternal contribution to the risk of preeclampsia. This study showed that men who fathered a child with a woman whose pregnancy was complicated by preeclampsia were nearly twice as likely to experience preeclampsia with another woman as men without such history (60). Thus, there are indications that the maternal and paternal contribution to the inheritance of preeclampsia may differ according to severity of preeclampsia.

Taken together, a variety of conditions may precede the syndrome of preeclampsia, and most likely preeclampsia results from a combination of modifier genes and environmental factors. Still there is little knowledge about whether these different conditions are associated with specific clinical manifestations of the syndrome. However, if specific patterns of associations exist between maternal risk factors and clinical subtype of preeclampsia, this may help us understand the underlying heterogeneous pathogenesis (61).

Smoking and preeclampsia

A reduced risk of preeclampsia in smoking women has been observed in several studies (38, 62-66). This association is striking, since there is evidence that the chronic metabolic alterations observed in smoking resemble the acute changes observed in preeclampsia, including reduced ratio between levels of prostacyclin and thromboxane A2 (67-70), features of insulin resistance (71), plasma lipoprotein changes (71), and increased lipid peroxidation (72). Furthermore, smoking and preeclampsia share alterations of the maternal, utero-placental and fetal vasculature (73-76), and these vascular factors, as well as the metabolic factors may be closely related to the aetiology of preeclampsia. Therefore, it may appear paradoxical that the incidence of preeclampsia is reduced among smokers. It has, however, been suggested that among smokers, normal endothelial mechanisms may be down-regulated due to chronic, smoking-induced vascular pathology (77). Among non-pregnants, smokers may therefore have lower blood pressure than non-smokers (78), and among pregnant smokers, the incidence of gestational hypertension may be lower (79). In addition, tobacco smoke may reduce blood pressure directly by vasodilating components, and by inducing hypovolemia (80). Thus, if smoking prevents preeclampsia, the effect may be mediated by inhibition of endothelial mechanisms that increase blood pressure, or by direct hypotensive effects of smoking.

There is substantial knowledge regarding pathophysiological mechanisms related to smoking. Therefore, information about smoking and the association with various subgroups of preeclampsia may be useful to gain insight into the heterogeneous pathology underlying preeclampsia. To our knowledge, only one previous study has related smoking to clinical subgroups of preeclampsia (8). That study reported that the risk of both mild and severe preeclampsia was similarly reduced among smokers compared to non-smokers. This relation needs, however, to be studied in more detail, and the effect of smoking in relation to early or late preeclampsia has not yet been studied.

1.4 Pathogenesis

The generalised maternal disturbances in preeclampsia lacked explanation until Roberts et al. in 1989 suggested that activation of the maternal endothelium may be a fundamental process of preeclampsia (81). Implicit in this theory was that injured

endothelium activates the coagulation cascade, increases capillary permeability, and no longer buffers the vasoconstricting effects of circulating pressors. Thus, hypertension, fluid retention, clotting disturbances, and acute underperfusion of the liver and the brain may be attributable to the effects of activated endothelium. The prevailing hypothesis has been that induction of endothelial dysfunction in the maternal circulation may depend on the release of toxic substances from an underperfused and hypoxic placenta (82).

There is evidence that the endothelial function is altered in preeclampsia (83, 84), and placental release of several toxic factors have been suggested, including tumor necrosis factor A and interleukin-1 (85), deported syncytiotrophoblast fragments in the maternal circulation (86), and oxidised lipid products released from local areas with placental ischemia (87). These are all factors that may induce or enhance oxidative stress, and thereby alter the maternal endothelial function (85, 86). Normal pregnancy may constitute a balance between oxidative stress and anti-oxidative defence, and substances from an underperfused placenta may disturb this balance and thereby induce preeclampsia (88). However, some of the established maternal risk factors for preeclampsia, including hypertension and dyslipidemia are also associated with activated endothelium (87-90). Since preeclampsia may occur also in the presence of apparently normal placentas (91), the balance between oxidative stress and anti-oxidative defence may be disturbed by pre-existing systemic oxidative stress in the maternal circulation (30, 88). Thus, endothelial dysfunction in preeclampsia may be induced by both placental and maternal factors (30).

2 FETAL GROWTH RESTRICTION IN PREECLAMPSIA

Preeclampsia increases the risk of fetal growth restriction, but the strength of association varies substantially between studies (8, 92, 93). Some of the variation may be explained by the fact that most studies do not distinguish between clinical manifestations of preeclampsia. However, the few studies that have assessed the association between clinical subtypes of preeclampsia and fetal growth have also shown inconsistent results; some studies suggest that fetal growth is most affected in cases with early onset (16, 19, 94), and in severe preeclampsia (8), whereas other studies demonstrate no clear differences between subtypes of preeclampsia (92).

A number of maternal factors are associated with fetal growth restriction, but whether preeclampsia interferes with these factors is unclear. Maternal smoking reduces birth weight (8, 95), and may have effects on the uteroplacental blood flow in common with preeclampsia. Therefore, a possible synergy between these two growth-inhibiting factors has been suggested, indicating a reduction in birth weight that is stronger than just adding their separate effects (8, 96). However, the results of some investigators do not suggest that preeclampsia and smoking enhance each others' effect on infant birth weight (66).

Nulliparity is another maternal factor associated with low birth weight (9), but one small study suggested that the risk of SGA may be higher if parous women develop preeclampsia than if preeclampsia develops in nulliparous women (97). Furthermore, pre-pregnancy hypertension may reduce (36), and maternal obesity may increase birth weight (98), but how these factors influence fetal growth in preeclampsia is not known. Since all these maternal factors are related to the risk of developing preeclampsia, one should carefully adjust for their effects when assessing the impact of preeclampsia on fetal growth.

2.1 Measurements of fetal growth

The term "fetal growth restriction" (FGR) indicates that the genetic growth potential of the fetus has not been reached, and the term should therefore be restricted to fetuses for whom there is evidence that growth has faltered (99). Several approaches have been used to identify this process, including serial ultrasound measurements to demonstrate deviations from population based weigth standards (100, 101). More recently individualised birth weight standards have been calculated by combining birth weigh estimation based on constitutional factors such as maternal height and weight, with a standard proportionality curve for fetal growth (102). These individualised birth weight standards may better identify adverse neonatal outcome than population based standards (103-105), probably due to improved identification of infants who have not reached their genetic growth potential. Finally, reduced blood flow in the umbilical artery as measured by Doppler ultrasound is also used to identify compromised fetuses (106).

All these methods are time consuming and not routinely performed. Therefore, in most studies, measures of birth size have been used as indicators of impaired fetal

growth. SGA is a commonly used proxy for FGR, and the World Health Organisation (WHO) has defined a cut-off for SGA as birth weight below the 10th percentile of expected birth weight adjusted for gestational age (107). It may seem arbitrary to define the smallest 10 % of any population of fetuses as growth restricted, since some of these infants most likely are constitutionally small (99). However, there is strong evidence for increased fetal and neonatal morbidity and mortality among SGA subjects, and this may indicate an accumulation of infants who have been compromised *in utero* (108-110). Thus, birth size may suffice as a proxy for fetal growth in large populations. By reducing the cut-off for SGA to birth weights less than the 5th percentile, or even lower, e.g. more than 2 SD below expected birth weight, the specificity for true growth restricted infants may be increased.

It has been difficult to estimate appropriate birth weight standards for preterm infants, since these infants' birth weight may be influenced by the factor that caused their preterm birth (111, 112). Therefore, birth weight standards derived from ultrasonographic measurements in healthy gravidae are gaining acceptance, because these standards reflect the weight of healthy fetuses (112, 113).

2.2 Placental morphological changes in fetal growth restriction

In preeclampsia

Being born growth restricted may be the end point of a number of conditions, and the pathogenesis underlying FGR probably differs (114). In preeclampsia, it has been suggested that fetal growth is restricted only when abnormal development of the placenta causes preeclampsia (30). Thus, corresponding to the high frequency of FGR seen in early onset preeclampsia (19), abundant placental changes often characterise cases of preeclampsia delivered remote from term (91).

Restricted fetal growth in preeclampsia is probably due to uteroplacental insufficiency caused by unsuccessful transformation of spiral arteries (115). In preeclampsia a reduced number of arteries undergo the physiological development that enables the low resistance and high flow circulation that characterises normal pregnancy (24, 29, 116). Subsequent reduced blood flow may limit feto- maternal exchange of gases and nutrients, that takes place in the placental terminal villi (Fig.2). Recently, it has become evident that the placental villous response to utero-placental

insufficiency may differ (117, 118). Most cases of FGR show increased development of terminal villi, and thereby an increased placental capillary bed. These changes have been interpreted as an attempt to increase the surface area for substrate exchange (119). In a few cases of FGR, however, no such placental compensation is observed, and the terminal villi show signs of impaired trophoblast differentiation and accelerated ageing of syncytiotrophoblast (118-121). Since these signs of severe placental maladaptation are observed in very preterm deliveries, the notion "early" fetal growth restriction has emerged, usually defined as delivery before 35 weeks of gestation (119). These cases may also be characterised by severely reduced umbilical artery blood flow (118). Since preeclampsia may be associated with fetal growth restriction both in early and late pregnancy (92), these observations may indicate that fetal growth restriction in preeclampsia may be perpetuated by different pathological mechanisms.

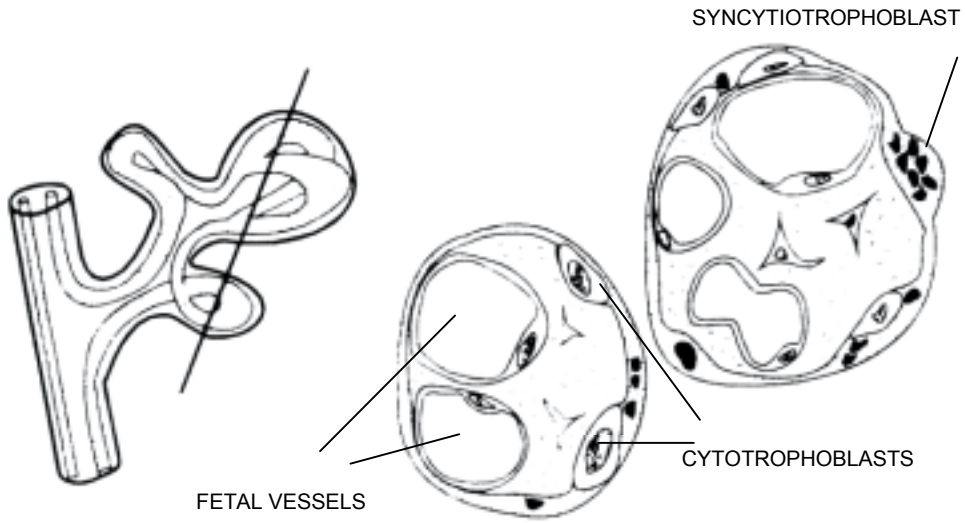

Fig.2. Cross-section through a placental terminal villous. From: Kingdom, J et al. Oxygen and Placental Villous Development: Origins of fetal hypoxia. Placenta 1997.

In maternal smoking

Also smoking-related fetal growth restriction may be associated with uteroplacental insufficiency. *In vitro* studies suggest that maternal smoking, analogous to preeclampsia, may restrict early cytotrophoblast differentiation (73, 74), and thereby inhibit the placentation process. Furthermore, maternal cotinine (a major metabolite of nicotine) levels may be directly related to uteroplacental resistance (75, 122), and reduced intervillous perfusion of the placenta is observed in smoking mothers (76).

Accordingly, syncytial necroses are observed in placentas from smokers (123), and the placental capillary volume fraction may be reduced (124). However, a recent observation suggests that the placental capillary bed increases in response to heavy maternal smoking, and this has been interpreted as a compensation in order to increase the placental surface area for substrate exchange (125).

Tobacco smoke may also restrict fetal growth by direct toxic effects of heavy metals, including cadmium, on fetal and placental cells (126, 127). Moreover, tobacco smoke contains carbonmonoxide, which may induce hypoxia in fetal and placental tissues by replacement of oxygen (128). Taken together, it is evident that the pathogenetic mechanisms that precede and accompany fetal growth restriction in preeclampsia and maternal smoking have common characteristics, but they also differ.

2.3 Growth factors

Intrauterine tissues used to be considered solely for their role in the exchange of gas and transfer of nutrients and waste. During the last two decades, however, there is growing evidence that fetal membranes, maternal decidua and the placenta produce hormones, cytokines and growth factors and, when containing hormone receptors, also act as endocrine organs (129, 130, 131). The brain, pituitary, gonadal and adrenocortical steroid and peptide hormones produced by intrauterine tissues are chemically and biologically very similar to their extra-uterine counterparts, and appear to stimulate growth at the feto-maternal interface by autocrine or paracrine actions. In addition, peptide factors may enter the maternal and/or fetal circulation and display endocrine effects on metabolism and fetal growth (31, 132). Accordingly, both preeclampsia and maternal smoking are associated with reduced circulating levels of several pregnancy-associated hormones (127). In the present study we have measured umbilical cord plasma levels of factors known to modify fetal growth among infants exposed to preeclampsia in utero and among controls, and the study had statistical power to analyse the effect of smoking on cord blood levels in both groups.

2.4.1 Interleukin-6

Interleukin-6 (IL-6) is a small cytokine secreted by immunocompetent cells, fibroblasts and endothelial cells (133). The cytokine is highly mitogenic, and main

functions of IL-6 are related to its stimulatory effect on hepatic production of acute phase proteins (134), and to growth and differentiation of haematopoietic stem cells (135). Furthermore, in normal pregnancy, IL-6 is highly expressed and secreted in syncytiotrophoblast (136, 137), whereas in pregnancies complicated by fetal growth restriction, low levels of IL-6 in amniotic fluid (138) and umbilical cord blood (139) have been observed. Several *in vitro* observations have related IL-6 to placental and fetal growth, including a stimulatory effect of IL-6 on trophoblast growth, invasion, and differentiation (136). In addition, IL-6 seems to be increased in tissues that undergo active angiogenesis, which is a crucial feature of successful placentation (140). IL-6 also appears to stimulate the trophoblast release of essential pregnancy hormones in an autocrine or paracrine manner (141-143). In preeclampsia, the syncytiotrophoblast layer is morphologically disturbed (123), and the placental angiogenesis may be severely affected (118). Correspondingly, one small study reported reduced placental expression of IL-6 in preeclampsia, in particular in cases with severe maternal symptoms (144). These observations may indicate that IL-6 may play a role in fetal growth restriction related to placental insufficiency. Main placental synthesis of steroid and protein hormones, including IL-6, takes place in syncytiotrophoblast (141, 143), and since mixed cord blood may reflect the placental compartment, cord blood levels of IL-6 may be an indicator of trophoblast function.

2.4.2 Insulin-like growth factor I, and its binding protein 3 and 1

There is strong evidence that the insulin-like growth factors (IGF-I and IGF-II) and insulin-like growth factors binding proteins (IGFBPs) are essential in regulation of somatic growth (129, 145, 146). In postnatal growth, the IGFs are controlled mainly by Growth Hormone (GH), whereas the fetus appears not to express functional GH-receptors until late in pregnancy (147). The regulation of fetal IGF levels is not well described, but placental lactogen (PL) appears to stimulate fetal IGF-I production by acting via lactogenic receptors and possibly a unique PL receptor in fetal liver cells (147). The IGFs function as part of a complex system that includes specific cell surface receptors, IGFBP proteases and IGFBP-related proteins (148). IGFs have potent mitogenic, differentative, antiapoptotic and metabolic functions, and their actions are determined by the availability of free IGF to react with the IGF receptors. The bioavailability of IGFs is regulated by IGFBPs, and in the circulation, IGFBP-3 is

the major binding protein (149), whereas IGFBP-1 appears to regulate acute changes of serum IGFs (150).

IGF-I is produced by many fetal and intrauterine tissues, and may act within these tissues in an autocrine or paracrine manner (151-153). There is also a consistent strong and positive association between umbilical cord levels of IGF-I and infant birth weight (151), and circulating levels of IGFBP-3 correlate with IGF-I, whereas an inverse relation is observed between circulating IGFBP-1 and birth weight (154-156). Thereby, IGF-I and its binding proteins may also exhibit endocrine effects, and both the placenta, decidua and fetal liver may contribute to the circulating levels of IGF-I and IGFBPs (148, 152, 157). However, experimental studies have shown that placental volume may be a strong determinant of fetal IGF-I levels (158), probably by secretion of placenta lactogen into the fetal circulation, and thereby stimulation of the fetus' own IGF-I production (147).

In pregnancies complicated by severe preeclampsia, some recent studies show increased decidual IGFBP-1 (159, 160), and correspondingly, IGFBP-1 levels may be increased in cord blood from preeclamptic pregnancies (161). Furthermore, cord blood IGF-I levels may be reduced in preeclampsia (162), but there is inconsistency between studies (163, 164). This discrepancy may be attributable to differences between study populations both regarding severity of maternal symptoms and presence of fetal growth restriction. Thus, it remains to be determined whether IGF-IGFBP changes in preeclampsia are attributable to fetal growth restriction accompanying the syndrome, or whether preeclampsia in itself influences the IGF-IGFBP system (161).

2.4.3 Leptin

Leptin, the product of the *obesity* -gene (165), is produced mainly by adipocytes (166). Animal experiments have suggested that the hormone may be active in the feedback loop from adipose tissue stores to centres in the hypothalamus expressing the leptin receptor (*Ob-Rb*). Thereby leptin affects neuroendocrine mechanisms and regulates several hypothalamic-pituitary axes (166-168). The leptin receptor is also expressed in a number of other organs, including kidney, liver, intestines and bones (169-172), and by acting directly, or indirectly by altering the levels of other

hormones, leptin appears to increase energy expenditure, decrease food intake, induce gluconeogenesis and lipolysis (166, 167).

During normal pregnancy, leptin is highly expressed in the placenta (173, 174), and maternal circulating levels of leptin are increased, reaching a peak around 30 weeks of gestation (169). Leptin has been detected in umbilical cord blood from week 18 of gestation, and cord blood levels of leptin increase strongly from the middle part of third trimester towards term (175). This coincides with the development of fetal adipose tissue (176), and the results from several studies indicate that cord blood leptin may reflect fetal adiposity at birth (169, 175, 177-181). However, placenta perfusion studies indicate that some of the placental leptin is secreted to the fetal circulation, and in umbilical cord blood venous levels appear to be higher than arterial levels. Fetal leptin levels fall rapidly after birth (182, 183), and the arteriovenous difference disappears (184). All these observations indicate that the placenta may be an important determinant of umbilical leptin levels.

In preeclampsia, leptin secretion from placental explants is increased (185), and maternal levels of circulating leptin may also be increased compared to normotensive pregnancies (186, 187). Maternal leptin levels may be increased already at 20 weeks of gestational among those who later present with symptoms of preeclampsia (188), and hyperleptinemia is included in the metabolic syndrome that also includes insulin resistance and obesity (189). Leptin may induce metabolic and circulatory changes similar to those observed in preeclampsia (186), and thereby link obesity to preeclampsia. Whether metabolic changes related to preeclampsia are confined to the maternal circulation, or also involve the fetus is not known, but two small studies found no difference in cord blood leptin levels between cases of preeclampsia and controls at term (186, 190).

3 PREECLAMPSIA AND RISK OF LATER DISEASE

Epidemiological observations have shown that low birth weight may be a risk factor for a number of diseases in adulthood, including hypertension (191), non-insulin dependent diabetes (192) and coronary heart disease (193, 194). The basis for these observations is proposed to be that of *in utero* programming (195). That is, an event operating at a critical or sensitive period results in a long-term change in the structure or function of the organism, a biological phenomenon that is well-known

from animal studies. Accordingly, fetal adaptation to inadequate nutrition may induce permanent physiological changes that may favour development of cardiovascular disease in adulthood. However, low birth weight is also associated with an increased risk of cardiac diseases in both parents (196), and preeclampsia is associated with increased risk of maternal deaths related to cardiovascular disease (197, 198). Therefore, confounding factors among parental characteristics such as low socio-economic status, or a specific genotype that causes both conditions may spuriously create the association between low birth weight and coronary heart disease in offspring. Regardless of underlying aetiology, there are several indications of unfavourable patterns of metabolism among subjects born SGA: Cortisol level was increased among 60 – 70 year old men (199), insulin levels were increased at age 20 (200), high cholesterol levels were observed at age 12 (201), and high levels of circulating leptin were observed already at one year of age (202). Most of these studies did not differentiate between underlying causes of low birth weight, and since preeclampsia is a main contributor to low birth weight, both by causing prematurity and by restricting fetal growth, preeclampsia may be linked to risk of cardiovascular disease in offspring. Accordingly, female offspring of preeclamptic pregnancies had slightly increased blood pressure age 17 compared to offspring of normotensive pregnancies (203).

Evidence suggests that immune, hormonal or genetic mechanisms that induce hypertension and preeclampsia during pregnancy may reduce the risk of breast cancer in both the mother (204, 205) female offspring (206-208), and reduced risk is also observed in association with placental pathology (209) and low birth weight (210). There is substantial experimental evidence that the GH-IGF axis is involved in proliferation of both normal breast endothelial cells (211) and breast cancer cells (212), and high circulating levels of IGF-I/II may perpetuate several cancers (213, 214), in particular pre-menopausal breast cancer (215). Birth weight may be a determinant for adult height, and adult height is positively associated with risk of breast cancer (216). Perinatal indicators of fetal growth may therefore be involved in cancer pathogenesis, and the strong association with fetal growth makes the IGF-I system a candidate factor.

AIMS OF THE STUDY

The principal aims of this thesis were

1) to examine the association between maternal risk factors and the risk of preeclampsia, and to explore whether different clinical subtypes of preeclampsia were associated with different risk factor patterns (Paper I)

2) to evaluate the association between different clinical manifestations of preeclampsia and fetal growth restriction. We also wanted to explore whether maternal factors, particularly maternal smoking, could modify the effect of preeclampsia on fetal growth (Paper II)

3) to study the association between cord plasma levels of factors that may modify fetal growth (IL-6, IGF-I, IGFBP-1 and IGFBP-3), and fetal growth restriction in subgroups of preeclampsia, and in controls (Paper III and V). For all groups, we also assessed the effect of maternal smoking on growth factor levels in cord plasma.

4) to compare umbilical cord plasma levels of leptin between preeclampsia and control pregnancies, and to assess the relation between fetal adiposity at birth and leptin levels (Paper IV).

SUBJECTS AND METHODS

All births from January 1993 to December 1995 at the Central Hospital in Rogaland County, Norway constitute the base cohort wherein the present case-control study is nested. In this region of 238,806 inhabitants (per 1995), the Central Hospital is the only delivery unit, and in all, 12,804 deliveries took place during the study period. The primary aim of the cohort was to study sudden infant death syndrome (SIDS), and thus, all infants succumbed by SIDS (9 cases), are excluded from the present study. The study has been approved by the Regional Committee for Ethics in Medical Research, and all study subjects from whom we obtained umbilical cord blood were included after written consent.

1 Cases and controls

Identification of cases

The Norwegian Medical Birth Registry has recorded standardised information from all deliveries since 1967 (217). To identify study subjects, we followed three procedures. First, the Medical Birth Registry was used to identify all women registered with preeclampsia who delivered at Rogaland Central Hospital. Further potential cases were identified from the midwives' consecutive records at the delivery station, and thirdly, we identified all pregnancies with "proteinuria" or "hypertension" from the computerised hospital database. In all, 1300 (10%) women were identified as potential cases. We reviewed the hospital records for each potential case, and included women as cases of preeclampsia if they fulfilled the diagnostic criteria selected for our study.

Definition of preeclampsia

We used a previously described definition of preeclampsia (218). Briefly, persistent diastolic blood pressure of at least 90 mmHg had to develop after 20 weeks of gestation, in addition to an increase in diastolic blood pressure of at least 25 mmHg. Women with a baseline diastolic blood pressure of 90 mmHg or higher were included as cases if diastolic blood pressure had increased by at least 15 mmHg. Proteinuria was defined as 0.3 mg/l (semiquantitative dipstick 1+) in at least one urine sample after 20 weeks of gestation without a simultaneous urinary infection. Twelve women

with no history of hypertension had missing blood pressure data prior to 20 weeks' gestation, but they had a diastolic blood pressure of 105 mmHg or higher at a later visit. It was decided to include these women as cases of preeclampsia, and in all, we identified 323 cases of preeclampsia.

Clinical subgroups of preeclampsia

We first categorised preeclampsia into mild, moderate or severe disease (Paper I and II), as suggested by Redman (219). Mild preeclampsia was defined as diastolic blood pressure increase of at least 25 mmHg and proteinuria 1+ on semiquantitative dipstick. Moderate preeclampsia was defined as an increase in diastolic blood pressure of at least 25 mmHg and proteinuria 2+ on semiquantitative dipstick, and severe preeclampsia as diastolic blood pressure of at least 110 mmHg, increase in diastolic blood pressure of at least 25 mm Hg, and proteinuria 3+ on semiquantitative dipstick, or at least 500 mg/24hours. HELLP syndrome or eclampsia was interchangeable with severe preeclampsia, and eclampsia included seizures in addition to hypertension and proteinuria. HELLP syndrome was defined as elevated serum liver enzyme concentrations (ASAT and/or ALAT > 70) or low platelet counts (≤ 100,000/ml) in addition to having epigastric pain and preeclampsia. Since mild and moderate preeclampsia had similar effects on fetal growth (Paper II), we later merged these two groups into one group (i.e. mild preeclampsia) for the subsequent analyses of growth factor levels in cord blood (Paper III-V).

We used termination of the pregnancy as a proxy variable for gestational age at onset of preeclampsia. No consensus exists regarding the definition of early onset preeclampsia, and initially we used delivery ≤ 32 weeks' gestation as cut-off (Paper 1 and II). This is an arbitrary cut-off reflecting the fact that modern neonatal intensive care rarely have problems by handling infants born after 32 weeks' gestation. However, most obstetricians try to postpone threatening premature labour until 34 weeks. When we reanalysed our data using a cut-off at 34 weeks' gestation, the impact of early onset preeclampsia on fetal growth restriction was similar to using cut-off at 32 weeks' gestation. Therefore, in order to increase the number of subjects in the early onset group, we later used ≤ 34 weeks' gestation as cut-off for early onset preeclampsia.

Selection of controls

As controls, the Medical Birth Registry selected two separate groups of women without preeclampsia who gave birth at Rogaland Central Hospital during the same period as the selection of cases. The one group of controls consisted of randomly selected women that were frequency matched to the cases by maternal age. This procedure was chosen because rates of pregnancy complications and adverse fetal outcomes are higher at young and older maternal ages, and we considered it important to adjust for mother's age. The second control group consisted of mothers who gave birth at this clinic subsequent to case women, in order to ensure identical blood collection between cases and controls. Initially, in the analyses of risk factors for preeclampsia, and fetal outcome (Paper I and II), the data were analysed using the control groups separately, but the results were consistent and very similar for both approaches. We therefore pooled the controls into one group, and have presented all results accordingly. We used the same approach in the analyses of umbilical cord plasma IL-6, and present results according to the pooled control group in Paper III, and in the subsequent Papers (IV and V).

We obtained written consent from 323 cases of preeclampsia and 650 controls, and all were included in the analysis of maternal risk factors (Paper I). All subsequent analyses included only singleton pregnancies with available information about gestational age and birth weight, leaving 307 cases of preeclampsia and 619 controls for the analyses in Paper II. In a small fraction of cases and controls, cord blood was not available, and after excluding subjects with neonatal sepsis, we obtained cord blood from 271 cases and 611 controls for the IL-6 analyses (Paper III). Some of the samples contained insufficient volume for all analyses, and after excluding cases with maternal diabetes, cord blood was available for IFG-I analyses in 258 cases and 609 controls. Furthermore, IGFBP-3 was analysed in cord blood from 255 cases and 601 controls, IGFBP-1 in 256 cases and 604 controls, and finally, leptin was analysed in 256 cases and 607 controls.

2 Study factors

2.1 Maternal risk factors

Most women attend their first antenatal doctor’s visit before 12 weeks of gestation (3). The antenatal maternal data used in this study were based on the clinical

information obtained at this visit. Information on maternal smoking was, however, collected from the records obtained at the routine ultrasound screening visit at 18 weeks. Smoking was initially divided into three categories; non-smokers, smoking 1-4 cigarettes per day, and smoking 5 cigarettes or more per day (Paper I). However, few women reported to smoke more than 10 cigarettes a day, and in the subsequent analyses participants were dichotomised as smokers or non-smokers (Paper II – V). Maternal weight and blood pressure were each divided into four categories: maternal weight in four steps of 10 kg per unit with < 60 kg as reference category, and diastolic and systolic blood pressure were categorised using an increment of 10 mm Hg per unit, starting at 60 mm Hg and 110 mm Hg, respectively. Pre-pregnancy diseases and parity status were also recorded and dichotomised. Pre-pregnancy diabetes is a well-known risk factor for preeclampsia, but this condition was not analysed because it was only present in five cases and one control.

2.2 Standardised birth weight and ponderal index

As a measure of fetal growth, we used birth size expressed as the ratio between the observed and the expected birth weight (birth weight ratio, BWR) (220). The expected birth weight was adjusted for sex and gestational age at birth, and birth weight ratio was used as a measure of fetal growth in Paper II-V. We used birth weight standards derived from weight curves based on ultrasonographic measurements in a healthy population of pregnant Swedish women (113), and gestational age at birth was calculated exclusively from routine ultrasonographic measurements of biparietal diameter at 18 weeks of gestation, according to Norwegian standard curves (221). SGA was defined as a birth weight 2SD or more below the expected birth weight (113). This corresponds to the 2.3 percentile or approximately 840 grams reduction in birth weight for a term infant, or more than 24% lower birth weight than expected (birth weight ratio < 0.76). The standardised birth weight (birth weight ratio) was divided into four clinical categories (Paper III-V): < 0.76 corresponds to SGA, and 0.76 – 0.89 is a broad category of relatively small infants. The category 0.90 – 1.09 includes infants with appropriate weight for their gestation, and the category > 1.09 includes large babies.

We calculated ponderal index by the equation (birth weight x 100)/ (length^3) as a measure of fetal adiposity (Paper IV), and calculated quartile levels of the index based on the distribution among controls.

2.3 Assays for umbilical cord plasma analyses

Interleukin-6. IL- 6 bioactivity was measured by the hybridoma cell line B 13.29 clone 9, which depends on IL-6 for growth (222), and we followed the procedures for colorimetric assays described by Tada et al (223). Details about the analyses are given in Paper 3, but briefly, diluted plasma samples were added to B 13.29 and growth was measured after 64 hrs by using MTT. The intra-assay variation was on average < 7%, and the inter-assay variation 25%. Since a high intra-assay variation was expected, all subjects were analysed in the order they were included in the study, and samples from three cases and six controls were always interspersed on each microtitre plate. Therefore, a relatively high inter-assay variation would only influence the results in a random fashion, and differences between groups would be underestimated.

IGF-I, IGFBP-3 and IGFBP-1. Cord plasma IGF-I and IGFBP-3 were assayed by commercially available radioimmunoassay kits (Mediagnost, Tuebingen, Germany). All samples were run in duplicates, and the procedures were run as suggested by the producer, except that we used half volumes. Cord plasma IFGBP-1 was assayed by a commercially available enzyme immunoassay (Mediagnost, Tuebingen, Germany), and single samples were analysed. The three assays were run in 11 sequences, and for all three, the intra-assay variation was <4%. The inter-assay coefficients of variation for IGF-I, IGFBP-3 and IGFBP-1 were 12%, 10%, and 16% respectively.

Leptin. Cord plasma leptin was measured by a commercially available iodine 125-labeled human leptin radioimmunoassay kit (Linco Research, St.Charles, Minnesota, USA). All samples were run in duplicate, and all procedures were run as suggested by the producer, except that we used half volumes. The intraassay variation was < 10 % on average, and the interassay coefficient of variation was < 13 %.
More details about the assays are given in the respective papers.

3 Statistical analyses

Student *t* test and Mann Whitney *U* test were used to compare group means and medians respectively, and Chi-square statistics were used to compare proportions. We estimated odds ratios for maternal risk factors (Paper I) and SGA (Paper II) as a measure of relative risk between the preeclampsia group and control group, and used unconditional multiple logistic regression analyses to adjust for potentially confounding factors in multivariate analyses (224). Multiple linear regression analyses was applied in the comparison of standardised birth weight between the study groups (Paper II), to adjust for potentially confounding factors, and to test for statistical interactions. We also tested for linear trends in umbilical cord plasma levels of growth factors across categories of standardised birth weight and Ponderal index by multiple regression analyses (Paper IV-V), and Kruskal Wallis *H* test (Paper III). All statistical analyses were calculated using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, Illinois, USA), version 7.5 (Paper I) and version 10.05 (Paper II-V).

MAIN RESULTS

Paper I "Risk factors and clinical manifestations of pre-eclampsia"

Nulliparity and hypertension increased the risk of preeclampsia, with no clear preference for any clinical subtype. High pre-pregnancy weight was related to a higher risk of mild and moderate preeclampsia, whereas previous preeclampsia strongly increased the risk of early onset preeclampsia (OR 42.4; 95% CI 11.9 – 151.6). Maternal smoking was associated with an overall reduced risk of preeclampsia (OR 0.6; 95%CI 0.4 – 0.9), but no effect of smoking was observed in the groups with early onset or repeated preeclampsia.

Paper II "Preeclampsia and fetal growth"

Severe and/or early onset preeclampsia was significantly associated with fetal growth restriction, and the risk of having an SGA infant was substantially higher in women with recurrent preeclampsia. In mild preeclampsia, in contrast, no reduction in birth weight was observed.

Maternal smoking was associated with 4% reduction in birth weight in controls and 7 % birth weight reduction in preeclampsia, and the effect of smoking on birth weight appeared to be added to that of preeclampsia. Furthermore, the combined effect of smoking and preeclampsia on birth weight did not differ between clinical subgroups of preeclampsia.

Paper III "Umbilical cord plasma interleukin-6 and fetal growth restriction in preeclampsia. A population study in Norway"

In severe, as opposed to mild preeclampsia, cord plasma IL-6 was lower than among controls ($P < 0.001$), and in severe preeclampsia there was a sharp decrease in cord plasma IL-6 with decreasing birth weight ratio (P trend < 0.001). By further dividing the preeclampsia group into early and late onset, the strong association between low

IL-6 and low birth weight ratio appeared to be present mainly in cases with early onset disease.

Maternal smoking had no effect on cord plasma levels in preeclampsia and controls.

Paper IV "Umbilical cord plasma leptin is increased in preeclampsia"

Cord plasma leptin increased strongly with gestational age and ponderal index, both in preeclampsia and in controls, and in both groups, females had higher leptin levels in cord plasma than males. However, at each age of gestation, the preeclampsia group had higher leptin levels than controls when we adjusted for differences in ponderal index, and sex between the groups ($P < 0.01$).

Maternal smoking had no influence on cord leptin levels for both groups.

Paper V "Relationship of insulin-like growth factor-I and insulin-like growth factor binding proteins in umbilical cord plasma to preeclampsia and infant birth weight"

Between mild preeclampsia and controls, there were no differences in IGF-I, IGFBP-1 and IGFBP-3. In severe and early onset preeclampsia, umbilical cord plasma IGF-I was approximately 50% lower, IGFBP-1 was more than twice as high as in controls (both $p<0.01$), whereas IGFBP-3 levels did not differ between groups. At each birth weight level, IGF-I was higher and IGFBP-1 was lower in sever and early onset preeclampsia than among controls of similar weight. After adjustment for gestational age, however, birth weight and severe preeclampsia were, independent of each other, associated with IGF-I, whereas birth weight, but not severe preeclampsia, was associated with IGFBP-1.

After adjustment for birth weight, maternal smoking had no influence on cord plasma levels of IGF-I and its binding protein 1 and 3 in preeclampsia or among controls (data not shown in the paper).

DISCUSSION

1 Interpretation of results

Our case-control study was nested within a cohort of nearly 13,000 deliveries, and we identified 323 cases of preeclampsia.

In Norway nearly 100 % of the pregnant women attend the national antenatal care program, and thereby we had an unselected population of pregnant women eligible for our study. However, using informed written consent as a criterion for inclusion introduced the possibility of selecting study subjects that are not representative for the eligible population. In the preeclampsia group, more than 96 % of subjects who met the diagnostic criteria also gave written consent, whereas among controls, the response rate was lower, but still close to 80 %. This is regarded as a highly acceptable response rate, and selection bias is not likely to have had any major influence on our results.

All information regarding pre-pregnancy data and maternal risk factors was collected prospectively on standardised forms before symptoms of preeclampsia occurred. This prospective recording makes it unlikely that information was recorded differently for cases of preeclampsia and controls, and any data inaccuracies or misclassification of exposure is probably distributed randomly among all study subjects. Random misclassification of exposure will result in an underestimation of the true relative risks, and the observed associations are unlikely to be spurious. In our study, maternal smoking during pregnancy represents a variable particularly prone to misclassification. On the one hand, there is always the possibility that the woman may quit smoking in response to information on the adverse effects during pregnancy. On the other hand, an underreporting of maternal smoking during pregnancy has been indicated by studies that compare biochemical tracers of nicotine with maternal self-reporting on smoking (225), although not confirmed by others (226). To meet the first concern, we based our classification of smoking status on information collected at 18 weeks of gestation, since it has been suggested that those who quit smoking will do so early in pregnancy (227). The second concern, underreporting, cannot be excluded as a source of misclassification. As a consequence, the negative association between maternal smoking and preeclampsia (Paper I) is probably not a spurious finding, and may well be stronger than that reported by us. Furthermore, misclassification of

smoking status would underestimate the effect of maternal smoking on birth size (Paper II), and would also dilute possible differences between smokers and non-smokers regarding umbilical cord blood levels of growth factors (Paper III – V). However, a prevalence study regarding maternal smoking during pregnancy was undertaken in Norway during the same period as we performed our study, and the results from that study showed a prevalence of maternal smoking of 22 % (228). In our study 26 % of the control women reported smoking during pregnancy, and we therefore regard underreporting of smoking to be a modest problem in this study.

Gestational age as a potential confounding factor.

All studies in this field meet the problem that differences between groups may be explained by differences in gestational age rather than by the factors that are used as explanatory variables. We used several strategies to avoid confounding by gestational age in our analyses.

Birth weight standards. Most gestational-age adjusted birth weight standards in international use are based on postnatally obtained weights. However, for premature infants these standards have been criticised since the underlying cause of premature delivery also may lead to restricted growth of the fetus (102, 111). Therefore attempts have been made to derive birth weight standards from ultrasound measurements of presumptive healthy fetuses, and as expected, for premature infants these standards are slightly higher that those obtained by postnatal measurement (112, 113). Since premature birth is associated both with preeclampsia and with fetal growth restriction, in analyses of the effect of preeclampsia on fetal growth it is important to use birth weight standards that are properly adjusted for gestational age. Therefore, we chose ultrasound based birth weight standards (Paper II, III and V) (113).

These standards were derived from a relatively small population (n = 86 women), but the observation that control infant birth weights were practically identical to those expected from the ultrasound-based weight curves was reassuring for the validity of the standards. However, all control infants were born before 231 days of gestation, and among the 37 cases of preeclampsia with infants born before 231 days of gestation (early onset preeclampsia) we cannot exclude that applying ultrasound derived birth weight standards may have slightly overestimated fetal growth restriction. A possible bias in the estimate is,

however, unlikely to explain the severe growth restriction in this subgroup of preeclampsia (Paper II).

On the other hand, there is a positive correlation between maternal weight and offspring's birth weight, and in our study high pre-pregnancy weight was a risk factor for mild and late preeclampsia. Therefore, in these subgroups of preeclampsia increased frequency of birth weights in the upper range of the normal distribution should be expected. By applying population based birth weight standards, we cannot exclude that our study has underestimated fetal growth restriction in mild and late preeclampsia, and in these cases individualised birth weight standards could have been an advantage.

Growth factors. Fetal growth is characterised by different stages; the organs and the skeleton have their most important growth period before third trimester, and the rapid increase in fetal size during third trimester is mainly determined by growth of subcutaneous fat. The expression of a number of growth-associated factors produced by the feto-maternal unit may therefore differ throughout gestation. Accordingly, among controls we found a slightly negative association between IGFBP-1 and gestational age after adjustment for birth weight (Paper V), whereas cord blood leptin was strongly and positively related to gestational age (Paper IV). Therefore, in the analyses of these two factors, comparisons between groups were adjusted for differences in gestational age. For the leptin analyses, an additional attempt was made to avoid confounding by gestational age; because cord blood leptin was so strongly related to gestational age, and only a few controls were born before 34 weeks' gestation, we restricted the leptin analyses to study subjects born at 34 weeks' gestation or later in order to make proper comparisons between groups.

In contrast, the analyses of interleukin-6 showed that control levels of umbilical cord plasma IL-6 were relatively constant throughout gestation (Paper III). This observation is supported by a study of IL-6 in cord blood obtained by chordoscentesis that showed no differences in cord levels of IL-6 within the range of 17 – 42 weeks' gestation (229). Therefore, we could compare IL-6 levels among subjects with early onset preeclampsia (gestational age 34 weeks' or less) with control levels despite the fact that most controls were born after 34 weeks' gestation. The same approach was used in the analyses of IGF-I and IGFBP-3 since no association with gestational age was observed for these two markers.

Incidence of preeclampsia.

The overall incidence of preeclampsia was 2.5% (Paper I), which is a relatively low estimate compared to previous reports (6, 7, 10). In contrast to most previous studies we included both nulliparous and parous women, and since nulliparity is an established risk factor for preeclampsia (8, 39), we expected a relatively low frequency. In addition, we applied a relatively narrow definition of preeclampsia, which increases the specificity of the diagnosis on the costs of sensitivity. The narrow inclusion criteria for preeclampsia in this study also influenced the distribution of clinical subgroups; a high proportion of the preeclampsia group was classified as early onset disease (11 %), and nearly one third was classified as severe preeclampsia.

2 Can the heterogeneous manifestations of preeclampsia be perpetuated by different aetiological conditions?

Maternal risk factors.

Nulliparity and pre-pregnancy hypertension showed no specificity for any clinical subgroup of preeclampsia, whereas the association with recurrent preeclampsia, pre-pregnancy obesity, and maternal smoking varied substantially between the subgroups (Paper I). Accordingly, pre-pregnancy obesity was strongly associated with mild and late preeclampsia, whereas no association with severe preeclampsia was observed. This result is in contrast to a previous study that observed a positive association between obesity and severe preeclampsia (40). Most obese women in that study were, however, substantially heavier than those classified as obese in our study, and this difference complicates direct comparison between the studies.

Recurrent preeclampsia increased the risk for all subgroups of preeclampsia more than ten - fold, but the association with early onset preeclampsia was particularly strong (Adj. OR 42.4). Maternal smoking, in contrast, slightly reduced the risk of all subgroups of preeclampsia, except for the early onset group where smoking had no risk reducing effect. Early onset preeclampsia may be associated with high frequency of pre-existing maternal disorders (17, 230), and the combination with a strong relation to recurrent preeclampsia may indicate that potent pathogenetic mechanisms are activated in this subgroup. Since the overall risk reducing effect of smoking was not very strong, one interpretation of our results could be that a weak vasodilatating or hypovolemic effect of

smoking can not counteract the forceful mechanisms that increase blood pressure in early onset preeclampsia.

There is strong evidence of a genetic predisposition to preeclampsia (53-55, 57-60). However, the variation in patterns of maternal risk factors between clinical subgroups of preeclampsia may indicate that different lines of pathogenesis perpetuate the various clinical manifestations. One may therefor expect that a genetic predisposition to early onset preeclampsia most likely differ from a predisposition to mild and late disease.

Fetal growth restriction.

Mild and late onset preeclampsia had no clear effect on fetal growth, whereas in severe and early onset preeclampsia 21% and 50% of the infants, respectively, were classified as SGA (Paper II). In the early onset group, one third was classified as having mild symptoms, but these infants' birth weight was similar to those with severe symptoms. Furthermore, in the severe preeclampsia group, the majority of SGA infants were born at early gestation, and our results may suggest that gestational age at debut of preeclampsia is a stronger indicator of fetal growth restriction than severity of maternal symptoms.

Corresponding to one previous report (97), we observed that parous women had more than two-fold higher risk of SGA associated with preeclampsia compared to the risk among nulliparous women. In our study, the increased risk among parous women was attributable to a particularly high risk of SGA among women with recurrent preeclampsia.

Thus, in accordance with some (8, 19), but not all previous studies (92), our study showed that the impact of preeclampsia on fetal growth differed strongly between subgroups of preeclampsia. Ness et al. hypothesised that heterogeneous conditions may cause preeclampsia, and a distinction was made between cases where the disease is confined to the maternal compartment, and cases related to placental pathology and thereby include the fetus by restricting its growth (30). According to this hypothesis, our results indicate that placental abnormalities are most likely to be present in cases with severe, recurrent or early onset preeclampsia. Since our control population contained few proper controls for comparison with the recurrent preeclampsia group, in the analyses of growth factors we have focused on severe and

early onset preeclampsia as subgroups likely to be accompanied by severe placenta pathology.

Cord plasma levels of growth factors and subgroups of preeclampsia.

IL-6. Among controls, we found a weak, non-significant association between cord plasma IL-6 and birth weight, whereas in preeclampsia, cord plasma IL-6 levels were strongly and positively associated with birth weight. Subgroup analysis showed that this association could be explained by particularly low IL-6 levels in cases with early-onset disease. In cases with severe preeclampsia, cord plasma IL-6 levels were low only if they belonged to the early-onset group, whereas those included in the late onset group had IL-6 levels no different from controls. These results correspond to previous studies where umbilical venous IL-6 was low in preeclampsia prior to 32 weeks' gestation (231), but equal to control levels in preeclampsia closer to term (131). Since cord levels of IL-6 may reflect the trophoblast production of IL-6, our results may indicate that impaired trophoblast function is most likely to be present if preeclampsia has an early onset.

IGF-I. Between mild preeclampsia and controls, there were no differences in cord blood levels of IGF-I, IGFBP-1 and IGFBP-3. In severe and early onset preeclampsia, in contrast, umbilical cord plasma IGF-I was approximately 50% lower, and IGFBP-1 was more than twice as high as in controls, whereas we observed no difference in IGFBP-3 levels between the groups. However, whereas both birth weight and severe preeclampsia, independent of each other, were associated with IGF-I, birth weight, but not severe preeclampsia, was associated with IGFBP-1, after adjustment for gestational age. Thus, the low levels of cord plasma IFG-I in severe and early preeclampsia were not counteracted by alterations in binding protein levels, and the infants in these subgroups of preeclampsia may have less IGF-I available in the circulation.

One previous study suggested that cord levels of IGF-I were reduced in preeclampsia, beyond the reduction that was attributable to low birth weight (162). In our large study, we could distinguish between clinical subgroups of preeclampsia, and found low levels of cord plasma IGF-I in relation to preeclampsia only in severe or early onset disease. Since the placenta may be a strong determinant of circulating fetal IGF-I levels (147, 158), our results

indicate that the placental function may be impaired mainly in cases of severe and early preeclampsia.

Taken together, in severe and early preeclampsia cord levels of IL-6 and IGF-I were lower than explained by the infants' birth weight. These observations indicate an effect of preeclampsia *per se* on cord plasma level of IL-6 and IGF-I if the disease is severe, or has an early onset. Fetal growth was restricted in these two subgroups of preeclampsia, and if cord levels of IL-6 and IGF-I reflect trophoblast function, our results are in accordance with the hypothesis that placental impairment may perpetuate preeclampsia only in cases where fetal growth is restricted (30). Thus, a major distinction may exist between the aetiology of early and severe preeclampsia, and the causes of mild and late preeclampsia.

3 Cord plasma levels of growth factors related to adult diseases

Leptin. Umbilical cord plasma leptin was higher in preeclampsia than in control pregnancies, after adjustment for gestational age and ponderal index. Our result is in contrast to two previous reports, but these studies were small, and may also have failed to identify differences between groups by not adjusting properly for confounding factors (186, 190).

Fetal hyperleptinemia may result from the pathogenetic processes that lead to preeclampsia, and may reflect the high levels of leptin that is observed in placentas from preeclamptic pregnancies (185). However, also maternal levels of leptin are increased in preeclampsia (44, 186, 188), and hyperleptinemia is associated with several risk factors for preeclampsia, including obesity and insulin resistance (187, 189). Thereby, hyperleptinemia in offspring of women with preeclampsia may be related to a genetic or early environmental pre-disposition.

In adults, hyperleptinemia is associated with increased risk of cardiovascular diseases (232), and low birth weight constitutes another severe risk factor (193, 198, 233-235). Sine cord plasma leptin was strongly related to gestational age, and most controls were born after 34 weeks' gestation, in the leptin analyses we included only cases born within the same range of gestational age as control infants. In this subgrop of preeclampsia (late onset), fetal growth was not materially restricted, nevertheless, the infants were born with a pattern of metabolism that among adults is associated with increased risk of cardiovascular disease. Thus, if newborn leptin levels are

determinant for leptin levels in adulthood, either by *in utero* imprinting of metabolic patterns, or as an indicator of a genetic pre-disposition to hyperleptinemia, our results suggest that preeclampsia may constitute a risk factor for cardiovascular disease in offspring beyond the risk that is attributable to low birth weight.

Taken together, the cord blood analyses in our study suggest that in preeclampsia, offspring is born with altered patterns of growth and metabolism. These alterations appear to differ strongly between subgroups of preeclampsia, and if adult diseases are related to growth alterations *in utero*, one would expect that the impact of preeclampsia on later diseases may differ according to the clinical manifestations of preeclampsia. Accordingly, mild and late preeclampsia may constitute an entity where the close relation to maternal obesity may indicate that the disorder is caused by mechanisms that also cause other diseases in these women, including cardiovascular disease and Type II diabetes. Furthermore, in spite of close to normal fetal growth in these subgroups of preeclampsia, the results from the leptin-study suggest that these infants' metabolism was influenced by the maternal disease in a way that may enhance their risk of cardiovascular disease in adulthood. About two thirds of a population of preeclamptic pregnancies will be characterised by mild and late disease, and these subgroups may therefor have major influence on results related to preeclampsia *in utero* as explanatory variable. Thus, the observation that blood pressure was increased at age 17 among females exposed to preeclampsia may support our speculation that offspring in mild and late preeclampsia will have increased risk of cardiovascular disease and related risk factors as adults (236).

IGF-I. In severe and early preeclampsia the maternal syndrome also includes the fetus by restricting its growth. It has been suggested that mechanisms that restrict fetal growth also may participate in cancer inhibition, as indicated by the reduced risk of breast cancer in subjects with low birth weight, and among women with preeclampsia (204, 205) and their female offspring (206, 207, 208). High levels of IGF-I/II are linked to breast cancer pathogenesis, in experimental as well as clinical studies (211, 212, 215). We observed particularly low levels of cord blood IGF-I in severe and early onset preeclampsia, and if fetal IGF-I levels are important for later cancer development, low frequency of breast cancer should be expected in offspring of pregnancies with severe or early preeclampsia.

The GH-IGF axis is important also for postnatal somatic growth and growth of blood vessels (237). Permanent alterations in IGF-I production and sensitivity by fetal

undernutrition may thereby link fetal growth restriction to catch- up growth and raised blood pressure in adulthood, and high body weight and hypertension may explain the increased risk of Type II diabetes and cardiovascular disease that is observed in adults with low birth weight (195). According to this hypothesis, the very low IFG-I levels in early and severe preeclampsia may indicate that infants in these subgroups have a particularly high risk of developing cardiovascular disease as adults. The maternal risk of cardiovascular disease related to early preeclampsia is not clear; a recent observation links preterm preeclampsia to increased risk of maternal death from coronary heart disease (197), whereas in our study, maternal obesity, which is an established risk factor for cardiovascular disease, did not increase the risk of severe and early preeclampsia. In these subgroups of preeclampsia, umbilical IGF-I levels were lower than indicated by the infants' birth weight, and thereby, the intrauterine influence on their IGF system may differ from the influence on the IGF system in other growth restricted infants. Thus, whether infants with growth restriction related to severe and early preeclampsia experience a similarly increased risk of cardiovascular disease and its risk factors as other growth restricted infants should be addressed in future studies.

4 Maternal smoking and fetal growth restriction

In control infants, maternal smoking was related to a reduction in birth weight of 4%, and birth weight following preeclampsia in non-smokers was 3% lower than expected. Birth weight in smokers with preeclampsia was reduced by 10%, and the combined effect of smoking and preeclampsia on birth weight did not differ between subgroups of preeclampsia. Thus, in contrast to some previous studies that suggested a synergy effect of smoking and preeclampsia on fetal growth (8, 96, 225), our results indicate that the effect of maternal smoking on fetal growth is added to the effect of preeclampsia.

Maternal smoking had no clear influence on cord plasma IL-6, IGF-I and IGFBP-1 in the preeclampsia group or among controls, after adjustment for birth weight. In contrast, in the subgroups of preeclampsia that were associated with fetal growth restriction, cord plasma IL-6 and IGF-I were significantly reduced. Cord plasma IL-6 and IGF-I may reflect trophoblast function, and thereby, our study indicates that trophoblast impairment is less likely to perpetuate fetal growth

restriction in smoking than in preeclampsia. Also, the relatively weak effect of maternal smoking on fetal growth makes the presence of severe placenta pathology less likely among smokers.

CONCLUSIONS

Nulliparity and high pre-pregnancy blood pressure increased the risk for all subgroups of preeclampsia, whereas high pre-pregnancy weight increased the risk of mild and late preeclampsia. The risk of early onset preeclampsia was particularly high in recurrent disease and smoking decreased the risk of all subgroups of preeclampsia, exept the early onset group. Thus, a major distinction may exist between the aetiology underlying early onset preeclampsia, and the cause(s) of mild and late preeclampsia.

Pregnancy outcome in terms of fetal growth differed substantially between subgroups of preeclampsia, and mild and late preeclampsia had little or no impact on fetal growth. Thus, fetal growth restriction was mainly confined to cases with recurrent, severe, or early onset preeclampsia, which indicates that placental impairment is present mainly in these subgroups. Furthermore, debut of preeclampsia at early gestation was a better indicator of fetal growth restriction than severity of maternal symptoms.

In severe and early onset preeclampsia, umbilical cord plasma IL-6 and IGF-I were lower than explained by the infants' birth weight, which indicates an effect of preeclampsia *per se* on cord levels of IL-6 and IGF-I in cases with severe and early disease. Thus, if umbilical levels of IL-6 and IGF-I reflect trophoblast function, our results may support the hypothesis that placental impairment perpetuates preeclampsia only in cases where fetal growth is restricted.

In late onset preeclampsia, cord plasma leptin was higher in the preeclampsia group than in the control group, after adjustment for gestational age and ponderal index. Taken together with the analyses of IL-6 and IGF-I, these results suggest that offspring in preeclamptic pregnancies are born with alterations in patterns of growth and metabolism, and that these alterations differ substantially between subgroups of preeclampsia. If intrauterine growth patterns are important for adult disease, our results suggest that mild and late preeclampsia may be linked to increased risk of cardiovascular disease in offspring, whereas early and severe preeclampsia may reduce offsprings' risk of cancer in adult life.

Maternal smoking added its effect on birth weight to the effect of preeclampsia, indicating no synergy between those two factors on fetal growth restriction. Also the umbilical cord blood analyses of growth factors indicate that

smoking may influence fetal growth by mechanisms that are independent of mechanisms in preeclampsia that also restrict growth.

References

(1) The hypertensive disorders of pregnancy. 1987. World Health Organizatioin, Geneva. Technical Report Series 758.

(2) Augensen K, Bergsjo P. Maternal mortality in the Nordic countries 1970-1979. Acta Obstet Gynecol Scand 1984; 63:115-121.

(3) Backe B. Overutilization of antenatal care in Norway. Scand J Public Health 2001; 29:129-132.

(4) Geographic variation in the incidence of hypertension in pregnancy.World Health Organizational Collaborative Study of Hypertensive Disorders of Pregnanc. Am J Obstet Gynecol 1988; 158:80-83.

(5) Mittendorf R, Lain KY, Williams MA, Walker CK. Preeclampsia. A nested, case-control study of risk factors and their interactions. J Reprod Med 1996; 41:491-496.

(6) Redman CW, Jefferies M. Revised definition of pre-eclampsia. Lancet 1988; 1:809-812.

(7) Sibai BM, Ewell M, Levine RJ, Klebanoff MA, Esterlitz J, Catalano PM et al. Risk factors associated with preeclampsia in healthy nulliparous women. The Calcium for Preeclampsia Prevention (CPEP) Study Group. Am J Obstet Gynecol 1997; 177:1003-1010.

(8) Cnattingius S, Mills JL, Yuen J, Eriksson O, Salonen H. The paradoxical effect of smoking in preeclamptic pregnancies: smoking reduces the incidence but increases the rates of perinatal mortality, abruptio placentae, and intrauterine growth restriction. Am J Obstet Gynecol 1997; 177:156-161.

(9) Conde-Agudelo A, Belizan JM. Risk factors for pre-eclampsia in a large cohort of Latin American and Caribbean women. BJOG 2000; 107:75-83.

(10) Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L et al. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. N Engl J Med 1993; 329:1213-1218.

(11) Brown MA, Buddle ML. What's in a name? Problems with the classification of hypertension in pregnancy. J Hypertens 1997; 15:1049-1054.

(12) Management of hypertension in pregnancy: executive summary. Australasian Society for the Study of Hypertension in Pregnancy. Med J Aust 1993; 158:700-702.

(13) Davey DA, MacGillivray I. The classification and definition of the hypertensive disorders of pregnancy. Am J Obstet Gynecol 1988; 158:892-898.

(14) National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 1990; 163:1691-1712.

(15) Hypertension in Pregnancy. 1996. Wachington DC, American College of Obstetric and Gynecology.

(16) Moore MP, Redman CW. Case-control study of severe pre-eclampsia of early onset. Br Med J Clin Res Ed 1983; 287:580-583.

(17) Dekker GA, de-Vries JI, Doelitzsch PM, Huijgens PC, von-Blomberg BM, Jakobs C et al. Underlying disorders associated with severe early-onset preeclampsia. Am J Obstet Gynecol 1995; 173:1042-1048.

(18) Murphy DJ, Stirrat GM. Mortality and morbidity associated with early-onset preeclampsia. Hypertens Pregnancy 2000; 19:221-231.

(19) Long PA, Abell DA, Beischer NA. Fetal growth retardation and pre-eclampsia. Br J Obstet Gynaecol 1980; 87:13-18.

(20) Brazy JE, Grimm JK, Little VA. Neonatal manifestations of severe maternal hypertension occurring before the thirty-sixth week of pregnancy. J Pediatr 1982; 100:265-271.

(21) Chesley LC. Hypertesinve disorders in pregnancy. 1978. New York, Appleton-Century-Crofts.

(22) Chesley LC. History and epidemiology of preeclampsia-eclampsia. Clin Obstet Gynecol 1984; 27:801-820.

(23) Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. Lancet 1993; 341:1447-1451.

(24) Robertson WB, Brosens I, Dixon HG. The pathological response of the vessels of the placental bed to hypertensive pregnancy. J Pathol Bacteriol 1967; 93:581-592.

(25) Brosens I, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. J Pathol Bacteriol 1967; 93:569-579.

(26) Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Invest 1997; 99:2152-2164.

(27) Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L et al. Placental bed spiral arteries in the hypertensive disorders of pregnancy. Br J Obstet Gynaecol 1991; 98:648-655.

(28) De Wolf F, Brosens I, Renaer M. Fetal growth retardation and the maternal arterial supply of the human placenta in the absence of sustained hypertension. Br J Obstet Gynaecol 1980; 87:678-685.

(29) Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986; 93:1049-1059.

(30) Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. Am J Obstet Gynecol 1996; 175:1365-1370.

(31) Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. The role of cytokines in gestation. Crit Rev Immunol 1994; 14:239-292.

(32) Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999; 180:499-506.

(33) Sibai B. Immunological aspects of pre-eclampsia. Clin Obstet Gynecol 1991; 34:27-33.

(34) Dekker GA, Robillard PY, Hulsey TC. Immune maladaptation in the etiology of preeclampsia: a review of corroborative epidemiologic studies. Obstet Gynecol Surv 1998; 53:377-382.

(35) Trupin LS, Simon LP, Eskenazi B. Change in paternity: a risk factor for preeclampsia in multiparas. Epidemiology 1996; 7:240-244.

(36) Sibai BM, Lindheimer M, Hauth J, Caritis S, VanDorsten P, Klebanoff M et al. Risk factors for preeclampsia, abruptio placentae, and adverse neonatal outcomes among women with chronic hypertension. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. N Engl J Med 1998; 339:667-671.

(37) Sibai BM, Abdella TN, Anderson GD. Pregnancy outcome in 211 patients with mild chronic hypertension. Obstet Gynecol 1983; 61:571-576.

(38) Sibai BM, Gordon T, Thom E, Caritis SN, Klebanoff M, McNellis D et al. Risk factors for preeclampsia in healthy nulliparous women: a prospective multicenter study. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Am J Obstet Gynecol 1995; 172:642-648.

(39) Eskenazi B, Fenster L, Sidney S. A multivariate analysis of risk factors for preeclampsia. JAMA 1991; 266:237-241.

(40) Stone JL, Lockwood CJ, Berkowitz GS, Alvarez M, Lapinski R, Berkowitz RL. Risk factors for severe preeclampsia. Obstet Gynecol 1994; 83 357-361.

(41) Carmona F, Font J, Cervera R, Munoz F, Cararach V, Balasch J. Obstetrical outcome of pregnancy in patients with Systemic Lupus Erythematosus. A study of 60 cases. Eur J Obstet Gynecol Reprod Biol 1999; 83:137-142.

(42) Lorentzen B, Henriksen T. Plasma lipids and vascular dysfunction in preeclampsia. Semin Reprod Endocrinol 1998; 16:33-39.

(43) Nisell H, Erikssen C, Persson B, Carlstrom K. Is carbohydrate metabolism altered among women who have undergone a preeclamptic pregnancy? Gynecol Obstet Invest 1999; 48:241-246.

(44) Kaaja R, Laivuori H, Laakso M, Tikkanen MJ, Ylikorkala O. Evidence of a state of increased insulin resistance in preeclampsia. Metabolism 1999; 48:892-896.

(45) Fuh MT, Yin CS, Pei D, Sheu WH, Jeng CY, Chen YD, et al. Resistance to insulin-mediated glucose uptake and hyperinsulinemia in women who had preeclampsia during preenancy. Am J Hypertens 1995; 8:768-771.

(46) Kupferminc MJ, Fait G, Many A, Gordon D, Eldor A, Lessing JB. Severe preeclampsia and high frequency of genetic thrombophilic mutations. Obstet Gynecol 2000; 96:45-49.

(47) van Pampus MG, Dekker GA, Wolf H, Huijgens PC, Koopman MM, von Blomberg BM et al. High prevalence of hemostatic abnormalities in women with a history of severe preeclampsia. Am J Obstet Gynecol 1999; 180:1146-1150.

(48) O'Shaughnessy KM, Fu B, Ferraro F, Lewis I, Downing S, Morris NH. Factor V Leiden and thermolabile methylenetetrahydrofolate reductase gene variants in an East Anglian preeclampsia cohort. Hypertension 1999; 33:1338-1341.

(49) Dizon-Townson DS, Nelson LM, Easton K, Ward K. The factor V Leiden mutation may predispose women to severe preeclampsia. Am J Obstet Gynecol 1996; 175:902-905.

(50) Livingston JC, Barton JR, Park V, Haddad B, Phillips O, Sibai BM. Maternal and fetal inherited thrombophilias are not related to the development of severe preeclampsia. Am J Obstet Gynecol 2001; 185:153-157.

(51) Morgan T, Ward K. New insights into the genetics of preeclampsia. Semin Perinatol 1999; 23:14-23.

(52) Grandone E, Margaglione M, Colaizzo D, Cappucci G, Paladini D, Martinelli P et al. Factor V Leiden, C > T MTHFR polymorphism and genetic susceptibility to preeclampsia. Thromb Haemost 1997; 77:1052-1054.

(53) Cincotta RB, Brennecke SP. Family history of pre-eclampsia as a predictor for pre-eclampsia in primigravidas. Int J Gynaecol Obstet 1998; 60:23-27.

(54) Mogren I, Hogberg U, Winkvist A, Stenlund H. Familial occurrence of preeclampsia. Epidemiology 1999; 10:518-522.

(55) Arngrimsson R, Bjornsson S, Geirsson RT, Bjornsson H, Walker JJ, Snaedal G. Genetic and familial predisposition to eclampsia and pre-eclampsia in a defined population. Br J Obstet Gynaecol 1990; 97:762-769.

(56) Sutherland A, Cooper DW, Howie PW, Liston WA, MacGillivray I. The indicence of severe pre-eclampsia amongst mothers and mothers-in-law of pre-eclamptics and controls. Br J Obstet Gynaecol 1981; 88:785-791.

(57) Folgero T, Storbakk N, Torbergsen T, Oian P. Mutations in mitochondrial transfer ribonucleic acid genes in preeclampsia. Am J Obstet Gynecol 1996; 174:1626-1630.

(58) Liston WA, Kilpatrick DC. Is genetic susceptibility to pre-eclampsia conferred by homozygosity for the same single recessive gene in mother and fetus? Br J Obstet Gynaecol 1991; 98:1079-1086.

(59) Sean Esplin M, Bardett Fausett M, Fraser A, Kerber R, _Mineau G, Carrillo J et al. Paternal and maternal components of the predisposition to preeclampsia. N Engl J Med 2001; 344:867-872.

(60) Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie NE, Irgens LM. Fetal and maternal contributions to risk of pre-eclampsia: population based study. BMJ 1998; 316:1343-1347.

(61) Zhang J, Zeisler J, Hatch MC, Berkowitz G. Epidemiology of pregnancy-induced hypertension. Epidemiol Rev 1997; 19:218-232.

(62) Conde-Agudelo A, Althabe F, Belizan JM, Kafury-Goeta AC. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. Am J Obstet Gynecol 1999; 181:1026-1035.

(63) Zhang J, Klebanoff MA, Levine RJ, Puri M, Moyer P. The puzzling association between smoking and hypertension during pregnancy. Am J Obstet Gynecol 1999; 181:1407-1413.

(64) Xiong X, Wang FL, Davidge ST, Demianczuk NN, Mayes DC, Olson DM et al. Maternal smoking and preeclampsia. J Reprod Med 2000; 45:727-732.

(65) Spinillo A, Capuzzo E, Egbe TO, Nicola S, Piazzi G, Baltaro F. Cigarette smoking in pregnancy and risk of pre-eclampsia. J Hum Hypertens 1994; 8:771-775.

(66) Marcoux S, Brisson J, Fabia J. The effect of cigarette smoking on the risk of preeclampsia and gestational hypertension. Am J Epidemiol 1989; 130:950-957.

(67) Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol 1988; 71:122-137.

(68) Toivanen J, Ylikorkala O, Viinikka L. Effects of smoking and nicotine on human prostacyclin and thromboxane production in vivo and in vitro. Toxicol Appl Pharmacol 1986; 82:301-306.

(69) Goodfield MJ, Hume A, Rowell NR. The acute effects of cigarette smoking on cutaneous blood flow in smoking and non-smoking subjects with and without Raynaud's phenomenon. Br J Rheumatol 1990; 29:89-91.

(70) Rangemark C, Wennmalm A. Smoke-derived nitric oxide and vascular prostacyclin are unable to counteract the platelet effect of increased thromboxane formation in healthy female smokers. Clin Physiol 1996; 16:301-315.

(71) Eliasson B, Attvall S, Taskinen MR, Smith U. Smoking cessation improves insulin sensitivity in healthy middle-aged men. Eur J Clin Invest 1997; 27:450-456.

(72) Santanam N, Sanchez R, Hendler S, Parthasarathy S. Aqueous extracts of cigarette smoke promote the oxidation of low density lipoprotein by peroxidases. FEBS Lett 1997; 414:549-551.

(73) Genbacev O, Bass KE, Joslin RJ, Fisher SJ. Maternal smoking inhibits early human cytotrophoblast differentiation. Reprod Toxicol 1995; 9:245-255.

(74) Zhang L, Shiverick KT. Benzo(a)pyrene, but not 2,3,7,8-tetrachlorodibenzo-p-dioxin, alters cell proliferation and c-myc and growth factor expression in human placental choriocarcinoma JEG-3 cells. Biochem Biophys Res Commun 1997; 231:117-120.

(75) Andersen KV, Hermann N. Placenta flow reduction in pregnant smokers. Acta Obstet Gynecol Scand 1984; 63:707-709.

(76) Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. Semin Perinatol 1996; 20:115-126.

(77) Salafia C, Shiverick K. Cigarette smoking and pregnancy II: vascular effects. Placenta 1999; 20:273-279.

(78) Friedman GD, Klatsky AL, Siegelaub AB. Alcohol, tobacco, and hypertension. Hypertension 1982; 4:III143-III150.

(79) Ros HS, Cnattingius S, Lipworth L. Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. Am J Epidemiol 1998; 147:1062-1070.

(80) MacGillivray I. The Hypertensive Disease of Pregnancy. Philadelphia: WB Saunders, 1983.

(81) Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989; 161:1200-1204.

(82) Dekker GA, van-Geijn HP. Endothelial dysfunction in preeclampsia. Part I: Primary prevention. Therapeutic perspectives. J Perinat Med 1996; 24:99-117.

(83) Ashworth JR, Warren AY, Johnson IR, Baker PN. Plasma from pre-eclamptic women and functional change in myometrial resistance arteries. Br J Obstet Gynaecol 1998; 105:459-461.

(84) Matteo R, Proverbio T, Cordova K, Proverbio F, Marin R. Preeclampsia, lipid peroxidation, and calcium adenosine triphosphatase activity of red blood cell ghosts. Am J Obstet Gynecol 1998; 178:402-408.

(85) Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. Am J Reprod Immunol 1997; 37:240-249.

(86) Smarason AK, Sargent IL, Starkey PM, Redman CW. The effect of placental syncytiotrophoblast microvillous membranes from normal and pre-eclamptic women on the growth of endothelial cells in vitro. Br J Obstet Gynaecol 1993; 100:943-949.

(87) Henriksen T. The role of lipid oxidation and oxidative lipid derivatives in the development of preeclampsia. Semin Perinatol 2000; 24:29-32.

(88) Walsh SW. Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. Semin Reprod Endocrinol 1998; 16:93-104.

(89) Haynes WG, Sivitz WI, Morgan DA, Walsh SA, Mark AL. Sympathetic and cardiorenal actions of leptin. Hypertension 1997; 30:619-623.

(90) Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A et al. Elevated circulating free fatty acid levels impair endothelium- dependent vasodilation. J Clin Invest 1997; 100:1230-1239.

(91) Ghidini A, Salafia CM, Pezzullo JC. Placental vascular lesions and likelihood of diagnosis of preeclampsia. Obstet Gynecol 1997; 90:542-545.

(92) Xiong X, Mayes D, Demianczuk N, Olson DM, Davidge ST, Newburn CC et al. Impact of pregnancy-induced hypertension on fetal growth. Am J Obstet Gynecol 1999; 180:207-213.

(93) Misra DP. The effect of the pregnancy-induced hypertension on fetal growth: a review of the literature. Paediatr Perinat Epidemiol 1996; 10:244-263.

(94) Brazy JE, Grimm JK, Little VA. Neonatal manifestations of severe maternal hypertension occurring before the thirty-sixth week of pregnancy. J Pediatr 1982; 100:265-271.

(95) Spinillo A, Capuzzo E, Piazzi G, Nicola S, Colonna L, Iasci A. Maternal high-risk factors and severity of growth deficit in small for gestational age infants. Early Hum Dev 1994; 38:35-43.

(96) Duffus GM, MacGillivray I. The incidence of pre-eclamptic toxaemia in smokers and non-smokers. Lancet 1968; 1:994-995.

(97) Eskenazi B, Fenster L, Sidney S, Elkin EP. Fetal growth retardation in infants of multiparous and nulliparous women with preeclampsia. Am J Obstet Gynecol 1993; 169:1112-1118.

(98) Shapiro C, Sutija VG, Bush J. Effect of maternal weight gain on infant birth weight. J Perinat Med 2000; 28:428-431.

(99) Altman DG, Hytten FE. Intrauterine growth retardation: let's be clear about it. Br J Obstet Gynaecol 1989; 96:1127-1132.

(100) Deter RL, Rossavik IK. A simplified method for determining individual growth curve standards. Obstet Gynecol 1987; 70:801-806.

(101) Royston P. Calculation of unconditional and conditional reference intervals for foetal size and growth from longitudinal measurements. Stat Med 1995; 14:1417-1436.

(102) Gardosi J. The application of individualised fetal growth curves. J Perinat Med 1998; 26:333-338.

(103) Clausson B, Gardosi J, Francis A, Cnattingius S. Perinatal outcome in SGA births defined by customised versus population- based birthweight standards. BJOG 2001; 108:830-834.

(104) de-Jong CL, Gardosi J, Dekker GA, Colenbrander GJ, van-Geijn HP. Application of a customised birthweight standard in the assessment of perinatal outcome in a high risk population. Br J Obstet Gynaecol 1998; 105:531-535.

(105) Mongelli M, Gardosi J. Reduction of false-positive diagnosis of fetal growth restriction by application of customized fetal growth standards. Obstet Gynecol 1996; 88:844-848.

(106) Karsdorp VH, van Vugt JM, van Geijn HP, Kostense PJ, Arduini D, Montenegro N et al. Clinical significance of absent or reversed end diastolic velocity waveforms in umbilical artery. Lancet 1994; 344:1664-1668.

(107) Dunn HG. Neurological, physiological and ophtalmological sequeale o flow birth weight. Sequela of low birth weight: The Vancouver Study. Oxford: Blackwell Scientific Publications, 1986: 1-22.

(108) Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford Network. Am J Obstet Gynecol 2000; 182:198-206.

(109) Minior VK, Divon MY. Fetal growth restriction at term: myth or reality? Obstet Gynecol 1998; 92:57-60.

(110) Cnattingius S, Haglund B, Kramer MS. Differences in late fetal death rates in association with determinants of small for gestational age fetuses: population based cohort study. BMJ 1998; 316:1483-1487.

(111) Seeds JW. Impaired fetal growth: definition and clinical diagnosis. Obstet Gynecol 1984; 64:303-310.

(112) Bernstein IM, Mohs G, Rucquoi M, Badger GJ. Case for hybrid "fetal growth curves": a population-based estimation of normal fetal size across gestational age. J Matern Fetal Med 1996; 5:124-127.

(113) Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996; 85: 843-848.

(114) Vik T. Growth, morbidity, ans psychomotor development in infants who were growth retarded *in utero*. Norwegian University of Science and Technology. 1997:9.

(115) Sheppard BL, Bonnar J. The ultrastructure of the arterial supply of the human placenta in pregnancy complicated by fetal growth retardation. Br J Obstet Gynaecol 1976; 83:948-959.

(116) Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A. A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. Br J Obstet Gynaecol 1994; 101:669-674.

(117) Kingdom JC, Kaufmann P. Oxygen and placental villous development: origins of fetal hypoxia. Placenta 1997; 18:613-621.

(118) Todros T, Sciarrone A, Piccoli E, Guiot C, Kaufmann P, Kingdom J. Umbilical Doppler waveforms and placental villous angiogenesis in pregnancies complicated by fetal growth restriction. Obstet Gynecol 1999; 93:499-503.

(119) Kingdom JC, Kaufmann P. Oxygen and placental vascular development. In: Roach RC et al, editor. Hypoxia: Into the next Millenium. New York: Kluwer Academic/Plenum Publishing, 1999: 259-275.

(120) Macara L, Kingdom JC, Kaufmann P, Kohnen G, Hair J, More IA et al. Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. Placenta 1996; 17:37-48.

(121) Huppertz B, Frank HG, Kingdom JC, Reister F, Kaufmann P. Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. Histochem Cell Biol 1998; 110: 495-508.

(122) Morrow RJ, Ritchie JW, Bull SB. Maternal cigarette smoking: the effects on umbilical and uterine blood flow velocity. Am J Obstet Gynecol 1988; 159:1069-1071.

(123) Arnholdt H, Meisel F, Fandrey K, Lohrs U. Proliferation of villous trophoblast of the human placenta in normal and abnormal pregnancies. Virchows Arch B Cell Pathol Incl Mol Pathol 1991; 60:365-372.

(124) Burton GJ, Palmer ME, Dalton KJ. Morphometric differences between the placental vasculature of non- smokers, smokers and ex-smokers. Br J Obstet Gynaecol 1989; 96:907-915.

(125) Pfarrer C, Macara L, Leiser R, Kingdom J. Adaptive angiogenesis in placentas of heavy smokers. Lancet 1999; 354:303.

(126) Bush PG, Mayhew TM, Abramovich DR, Aggett PJ, Burke MD, Page KR. A quantitative study on the effects of maternal smoking on placental morphology and cadmium concentration. Placenta 2000; 21:247-256.

(127) Shiverick KT, Salafia C. Cigarette smoking and pregnancy I: ovarian, uterine and placental effects. Placenta 1999; 20:265-272.

(128) Soothill PW, Morafa W, Ayida GA, Rodeck CH. Maternal smoking and fetal carboxyhaemoglobin and blood gas levels. Br J Obstet Gynaecol 1996; 103:78-82.

(129) Bauer MK, Harding JE, Bassett NS, Breier BH, Oliver MH, Gallaher BH et al. Fetal growth and placental function. Mol Cell Endocrinol 1998; 140:115-120.

(130) Petraglia F, Florio P, Nappi C, Genazzani AR. Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. Endocr Rev 1996; 17:156-186.

(131) Stallmach T, Hebisch G, Joller H, Kolditz P, Engelmann M. Expression pattern of cytokines in the different compartments of the feto-maternal unit under various conditions. Reprod Fertil Dev 1995; 7:1573-1580.

(132) Wegmann TG, Guilbert LJ. Immune signalling at the maternal-fetal interface and trophoblast differentiation. Dev Comp Immunol 1992; 16:425-430.

(133) Hirano T. Interleukin 6 and its receptor: ten years later. Int Rev Immunol 1998; 16:249-284.

(134) Bazan JF. Haemopoietic receptors and helical cytokines. Immunol Today 1990; 11:350-354.

(135) Heinrich PC, Horn F, Graeve L, Dittrich E, Kerr I, Muller-Newen G et al. Interleukin-6 and related cytokines: effect on the acute phase reaction. Z Ernahrungswiss 1998; 37 Suppl 1:43-49.

(136) Stephanou A, Myatt L, Eis AL, Sarlis N, Jikihara H, Handwerger S. Ontogeny of the expression and regulation of interleukin-6 (IL-6) and IL-1 mRNAs by human trophoblast cells during differentiation in vitro. J Endocrinol 1995; 147:487-496.

(137) Kameda T, Matsuzaki N, Sawai K, Okada T, Saji F, Matsuda T et al. Production of interleukin-6 by normal human trophoblast. Placenta 1990; 11:205-213.

(138) Silver RM, Schwinzer B, McGregor JA. Interleukin-6 levels in amniotic fluid in normal and abnormal pregnancies: preeclampsia, small-for-gestational-age fetus, and premature labor. Am J Obstet Gynecol 1993; 169:1101-1105.

(139) Opsjon SL, Austgulen R, Waage A. Interleukin-1, interleukin-6 and tumor necrosis factor at delivery in preeclamptic disorders. Acta Obstet Gynecol Scand 1995; 74:19-26.

(140) Dankbar B, Padro T, Leo R, Feldmann B, Kropff M, Mesters RM et al. Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma. Blood 2000; 95:2630-2636.

(141) Meisser A, Cameo P, Islami D, Campana A, Bischof P. Effects of interleukin-6 (IL-6) on cytotrophoblastic cells. Mol Hum Reprod 1999; 5:1055-1058.

(142) Nishino E, Matsuzaki N, Masuhiro K, Kameda T, Taniguchi T, Takagi T et al. Trophoblast-derived interleukin-6 (IL-6) regulates human chorionic gonadotropin release through IL-6 receptor on human trophoblasts. J Clin Endocrinol Metab 1990; 71:436-441.

(143) Stephanou A, Handwerger S. Interleukin-6 stimulates placental lactogen expression by human trophoblast cells. Endocrinology 1994; 135:719-723.

(144) Kauma SW, Wang Y, Walsh SW. Preeclampsia is associated with decreased placental interleukin-6 production. J Soc Gynecol Investig 1995; 2:614-617.

(145) Spencer JA, Chang TC, Crook D, Proudler A, Felton CV, Robson SC et al. Third trimester fetal growth and measures of carbohydrate and lipid metabolism in umbilical venous blood at term. Arch Dis Child Fetal Neonatal Ed 1997; 76:F21-F25.

(146) Fant M, Salafia C, Baxter RC, Schwander J, Vogel C, Pezzullo J et al. Circulating levels of IGFs and IGF binding proteins in human cord serum: relationships to intrauterine growth. Regul Pept 1993; 48:29-39.

(147) Handwerger S, Freemark M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab 2000; 13:343-356.

(148) Ferry Jr RJ, Cerri RW, Cohen P. Insulin-like growth factor binding proteins: new proteins, new functions. Horm Res 1999; 51:53-67.

(149) Rajaram S, Carlson SE, Koo WW, Rangachari A, Kelly DP. Insulin-like growth factor (IGF)-I and IGF-binding protein 3 during the first year in term and preterm infants. Pediatr Res 1995; 37:581-585.

(150) Wang HS, Chard T. The role of insulin-like growth factor-I and insulin-like growth factor- binding protein-1 in the control of human fetal growth. J Endocrinol 1992; 132:11-19.

(151) Fant M, Munro H, Moses AC. An autocrine/paracrine role for insulin-like growth factors in the regulation of human placental growth. J Clin Endocrinol Metab 1986; 63:499-505.

(152) Han VK, Lund PK, Lee DC, D'Ercole AJ. Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution. J Clin Endocrinol Metab 1988; 66:422-429.

(153) Hill DJ, Clemmons DR, Riley SC, Bassett N, Challis JR. Immunohistochemical localization of insulin-like growth factors (IGFs) and IGF binding proteins -1, -2 and -3 in human placenta and fetal membranes. Placenta 1993; 14:1-12.

(154) Chard T. Insulin-like growth factors and their binding proteins in normal and abnormal human fetal growth. Growth Regul 1994; 4:91-100.

(155) Verhaeghe J, van Bree R, Van Herck E, Laureys J, Bouillon R, Van Assche FA. C-peptide, insulin-like growth factors I and II, and insulin-like growth factor binding protein-1 in umbilical cord serum: correlations with birth weight. Am J Obstet Gynecol 1993; 169:89-97.

(156) Langford K, Blum W, Nicolaides K, Jones J, McGregor A, Miell J. The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by undernutrition? Eur J Clin Invest 1994; 24:851-856.

(157) Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M. Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 1991; 29:219-225.

(158) Owens JA, Kind KL, Carbone F, Robinson JS, Owens PC. Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. J Endocrinol 1994; 140:5-13.

(159) Irwin JC, Suen LF, Martina NA, Mark SP, Giudice LC. Role of the IGF system in trophoblast invasion and pre-eclampsia. Hum Reprod 1999; 14 Suppl 2:90-96.

(160) Giudice LC, Martina NA, Crystal RA, Tazuke S, Druzin M. Insulin-like growth factor binding protein-1 at the maternal-fetal interface and insulin-like growth factor-I, insulin-like growth factor-II, and insulin-like growth factor binding protein-1 in the circulation of women with severe preeclampsia. Am J Obstet Gynecol 1997; 176:751-757.

(161) Wang HS, Lee JD, Cheng BJ, Soong YK. Insulin-like growth factor-binding protein 1 and insulin-like growth factor-binding protein 3 in pre-eclampsia. Br J Obstet Gynaecol 1996; 103:654-659.

(162) Halhali A, Tovar AR, Torres N, Bourges H, Garabedian M, Larrea F. Preeclampsia is associated with low circulating levels of insulin-like growth

factor I and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. J Clin Endocrinol Metab 2000; 85:1828-1833.

(163) Bankowski E, Palka J, Jaworski S. Pre-eclampsia-induced alterations in IGF-I of human umbilical cord. Eur J Clin Invest 2000; 30:389-396.

(164) Lewitt MS, Scott FP, Clarke NM, Baxter RC. Developmental regulation of circulating insulin-like growth factor- binding proteins in normal pregnancies and in pre-eclampsia. Prog Growth Factor Res 1995; 6:475-480.

(165) Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372:425-432.

(166) Rohner-Jeanrenaud F. Neuroendocrine regulation of nutrient partitioning. Ann N Y Acad Sci 1999; 892:261-271.

(167) Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. Ann Intern Med 1999; 130:671-680.

(168) Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. Diabetes 1996; 45:1455-1462.

(169) Dotsch J, Nusken KD, Knerr I, Kirschbaum M, Repp R, Rascher W. Leptin and neuropeptide Y gene expression in human placenta: ontogeny and evidence for similarities to hypothalamic regulation. J Clin Endocrinol Metab 1999; 84:2755-2758.

(170) Gonzalez RR, Simon C, Caballero-Campo P, Norman R, Chardonnens D, Devoto L et al. Leptin and reproduction. Hum Reprod Update 2000; 6:290-300.

(171) Cioffi JA, Shafer AW, Zupancic TJ, Smith-Gbur J, Mikhail A, Platika D et al. Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. Nat Med 1996; 2:585-589.

(172) Houseknecht KL, Portocarrero CP. Leptin and its receptors: regulators of whole-body energy homeostasis. Domest Anim Endocrinol 1998; 15(6):457-475.

(173) Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H et al. Nonadipose tissue production of leptin: leptin as a novel placenta- derived hormone in humans. Nat Med 1997; 3:1029-1033.

(174) Hassink SG, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV et al. Placental leptin: an important new growth factor in intrauterine and neonatal development? Pediatrics 1997; 100:E1.

(175) Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. J Clin Endocrinol Metab 1998; 83:1243-1246.

(176) Gomez L, Carrascosa A, Yeste D, Potau N, Rique S, Ruiz-Cuevas P et al. Leptin values in placental cord blood of human newborns with normal intrauterine growth after 30-42 weeks of gestation. Horm Res 1999; 51:10-14.

(177) Geary M, Herschkovitz R, Pringle PJ, Rodeck CH, Hindmarsh PC. Ontogeny of serum leptin concentrations in the human. Clin Endocrinol (Oxf) 1999; 51:189-192.

(178) Lepercq J, Lahlou N, Timsit J, Girard J, Mouzon SH. Macrosomia revisited: ponderal index and leptin delineate subtypes of fetal overgrowth. Am J Obstet Gynecol 1999; 181:621-625.

(179) Marchini G, Fried G, Ostlund E, Hagenas L. Plasma leptin in infants: relations to birth weight and weight loss. Pediatrics 1998; 101:429-432.

(180) Shekhawat PS, Garland JS, Shivpuri C, Mick GJ, Sasidharan P, Pelz CJ et al. Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. Pediatr Res 1998; 43:338-343.

(181) Geary M, Pringle PJ, Persaud M, Wilshin J, Hindmarsh PC, Rodeck CH et al. Leptin concentrations in maternal serum and cord blood: relationship to maternal anthropometry and fetal growth. Br J Obstet Gynaecol 1999; 106:1054-1060.

(182) Helland IB, Reseland JE, Saugstad OD, Drevon CA. Leptin levels in pregnant women and newborn infants: gender differences and reduction during the neonatal period. Pediatrics 1998; 101:E12.

(183) Schubring C, Siebler T, Kratzsch J, Englaro P, Blum WF, Triep K et al. Leptin serum concentrations in healthy neonates within the first week of life: relation to insulin and growth hormone levels, skinfold thickness, body mass index and weight. Clin Endocrinol (Oxf) 1999; 51:199-204.

(184) Yura S, Sagawa N, Mise H, Mori T, Masuzaki H, Ogawa Y et al. A positive umbilical venous-arterial difference of leptin level and its rapid decline after birth. Am J Obstet Gynecol 1998; 178:926-930.

(185) Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H et al. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. J Clin Endocrinol Metab 1998; 83:3225-3229.

(186) McCarthy JF, Misra DN, Roberts JM. Maternal plasma leptin is increased in preeclampsia and positively correlates with fetal cord concentration. Am J Obstet Gynecol 1999; 180:731-736.

(187) Laivuori H, Kaaja R, Koistinen H, Karonen SL, Andersson S, Koivisto V et al. Leptin during and after preeclamptic or normal pregnancy: its relation to serum insulin and insulin sensitivity. Metabolism 2000; 49:259-263.

(188) Anim-Nyame N, Sooranna SR, Steer PJ, Johnson MR. Longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia. Hum Reprod 2000; 15:2033-2036.

(189) Zimmet P, Boyko EJ, Collier GR, de Courten M. Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. Ann N Y Acad Sci 1999; 892:25-44.

(190) Laml T, Preyer O, Hartmann BW, Huecklinger E, Soeregi G, Wagenbichler P. Decreased maternal serum leptin in pregnancies complicated by preeclampsia. J Soc Gynecol Investig 2001; 8:89-93.

(191) Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens 2000; 18:815-831.

(192) Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C et al. Fetal and infant growth and impaired glucose tolerance at age 64 [see comments]. BMJ 1991; 303:1019-1022.

(193) Forsdahl A. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Br J Prev Soc Med 1977;31:91-95.

(194) Barker DJ. The intrauterine environment and adult cardiovascular disease. Ciba Found Symp 1991; 156:3-10.

(195) Barker DJ. In utero programming of chronic disease. Clin Sci Colch 1998; 95:115-128.

(196) Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. Lancet 2001; 357:2002-2006.

(197) Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. BMJ 2001; 323:1213-1217.

(198) Davey SG, Hart C, Ferrell C, Upton M, Hole D, Hawthorne V et al. Birth weight of offspring and mortality in the Renfrew and Paisley study: prospective observational study. BMJ 1997; 315:1189-1193.

(199) Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB et al. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. J Clin Endocrinol Metab 2001; 86:245-250.

(200) Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C. Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab 2000; 85:1401-1406.

(201) Tenhola S, Martikainen A, Rahiala E, Herrgard E, Halonen P, Voutilainen R. Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. Pediatr Res 2000; 48:623-628.

(202) Jaquet D, Leger J, Tabone MD, Czernichow P, Levy-Marchal C. High serum leptin concentrations during catch-up growth of children born with intrauterine growth retardation. J Clin Endocrinol Metab 1999; 84:1949-1953.

(203) Seidman DS, Laor A, Gale R, Stevenson DK, Mashiach S, Danon YL. Pre-eclampsia and offspring's blood pressure, cognitive ability and physical development at 17-years-of-age. Br J Obstet Gynaecol 1991; 98:1009-1014.

(204) Polednak AP, Janerich DT. Characteristics of first pregnancy in relation to early breast cancer. A case-control study. J Reprod Med 1983; 28:314-318.

(205) Thompson WD, Jacobson HI, Negrini B, Janerich DT. Hypertension, pregnancy, and risk of breast cancer. J Natl Cancer Inst 1989; 81:1571-1574.

(206) Potischman N, Troisi R. In-utero and early life exposures in relation to risk of breast cancer. Cancer Causes Control 1999; 10:561-573.

(207) Ekbom A, Trichopoulos D, Adami HO, Hsieh CC, Lan SJ. Evidence of prenatal influences on breast cancer risk Lancet 1992; 340:1015-1018.

(208) Ekbom A, Hsieh CC, Lipworth L, Adami HQ, Trichopoulos D. Intrauterine environment and breast cancer risk in women: a population- based study. J Natl Cancer Inst 1997; 89:71-76.

(209) Cohn BA, Cirillo PM, Christianson RE, van den Berg BJ, Siiteri PK. Placental characteristics and reduced risk of maternal breast cancer. J Natl Cancer Inst 2001; 93:1133-1140.

(210) Michels KB, Trichopoulos D, Robins JM, Rosner BA, Manson JE, Hunter DJ et al. Birthweight as a risk factor for breast cancer [see comments]. Lancet 1996; 348:1542-1546.

(211) Ng ST, Zhou J, Adesanya OO, Wang J, LeRoith D, Bondy CA. Growth hormone treatment induces mammary gland hyperplasia in aging primates. Nat Med 1997; 3:1141-1144.

(212) Hadsell DL, Greenberg NM, Fligger JM, Baumrucker CR, Rosen JM. Targeted expression of des(1-3) human insulin-like growth factor I in transgenic mice influences mammary gland development and IGF-binding protein expression. Endocrinology 1996; 137:321-330.

(213) Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. J Natl Cancer Inst 1999; 91:620-625.

(214) Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998; 279:563-566.

(215) Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998; 351:1393-1396.

(216) van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000; 152:514-527.

(217) Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. Acta Obstet Gynecol Scand 2000; 79:435-439.

(218) Redman CW. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative Group. Lancet 1994; 343:619-629.

(219) Redman CWE. Hypertension in pregnancy. New York: Perinatology Press 1987:6.

(220) Kramer MS, McLean FH, Olivier M, Willis DM, Usher RH. Body proportionality and head and length 'sparing' in growth-retarded neonates: a critical reappraisal. Pediatrics 1989; 84:717-723.

(221) Eik-Nes SH, Grøttum P, Jørgensen NP, Løkvik B.Normal Range Curves for BPD and MAD. 1983. Drammen, Norway, Scan-Med A/S.

(222) Aarden LA, De Groot ER, Schaap OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. Eur J Immunol 1987; 17:1411-1416.

(223) Tada H, Shiho O, Kuroshima K, Koyama M, Tsukamoto K. An improved colorimetric assay for interleukin 2. J Immunol Methods 1986; 93:157-165.

(224) Kleinbaum David. Logistic Regression. A Self-Learning Text. Springer-Verlag NewYork Berlin Heidelberg. 1998.

(225) Blann AD, McCollum CN. Adverse influence of cigarette smoking on the endothelium. Thromb Haemost 1993; 70:707-711.

(226) Klebanoff MA, Levine RJ, Morris CD, Hauth JC, Sibai BM, Ben Curet L et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol 2001; 15:140-143.

(227) Haug K, Aaro LE, Fugelli P. Smoking habits in early pregnancy and attitudes towards smoking cessation among pregnant women and their partners. Fam Pract 1992; 9:494-499.

(228) Eriksson KM, Haug K, Salvesen KA, Nesheim BI, Nylander G, Rasmussen S et al. Smoking habits among pregnant women in Norway 1994-95. Acta Obstet Gynecol Scand 1998; 77:159-164.

(229) Miyano A, Miyamichi T, Nakayama M, Kitajima H, Shimizu A. Effect of chorioamnionitis on the levels of serum proteins in the cord blood of premature infants. Arch Pathol Lab Med 1996; 120:245-248.

(230) Pampus van MG, Wolf H, Buller HR, Huygens PH, Jacobs C, Dekker GA. Underlying disorders associated with severe preeclampsia and HELLP symdrome. Am J Obstet.Gynecol 176, S26. 2001.

(231) Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T. Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. Pediatr Infect Dis J 2000; 19:238-243.

(232) de Courten M, Zimmet P, Hodge A, Collins V, Nicolson M, Staten M et al. Hyperleptinaemia: the missing link in the, metabolic syndrome? Diabet Med 1997; 14:200-208.

(233) Roseboom TJ, van der Meulen JH, Osmond C, Barker DJ, Ravelli AC, Schroeder-Tanka JM et al. Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45. Heart 2000; 84:595-598.

(234) Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ 1999; 318:427-431.

(235) Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJ. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. BMJ 1999; 319:1403-1407.

(236) Seidman DS, Laor A, Gale R, Stevenson DK, Mashiach S, Danon YL. Pre-eclampsia and offspring's blood pressure, cognitive ability and physical development at 17-years-of-age. Br J Obstet Gynecol 1991; 98:1009-1014

(237) Ferns GA, Motani AS, Anggard EE. The insulin-like growth factors: their putative role in atherogenesis. Artery 1991; 18:197-225.

ERRATA

In thesis:

Page 9, line 14 and page 36, line 11: ”Insulin like growth factor I and its binding protein-1 in umbilical cord plasma in relation to severe preeclampsia and birth weight: a prospective investigation in Norway” is replaced by ”Relationship of insulin-like growth factor-I and insulin-like growth factor binding proteins in umbilical cord plasma to preeclampsia and infant birth weight”.

Page 16, line 9 : “Folgero” is replaced by “Folgerø”.

Page 21: Reference 131 is moved from line 21 to line 17.

Page 30, line 17: “We obtained written consent from 323 cases and 632 controls” is replaced by “We obtained written consent from 323 cases and 650 controls”.

Page 30, line 23: “blood from 270 cases an 611 controls for the analyses (Paper III)” is replaced by “blood from 271 cases and 611 controls for the analyses (Paper III)”.

Page 32, line 21: “intra-assay” is replaced by “inter-assay”.

Page 39, line 23: “after 34 weeks’ gestation” i replaced by “at 34 weeks’ gestation or later”.

Page 43, line 17: Reference 134 is replaced by reference 186.

Page 52, line 4: “systemisk Lupus erythematosus” is replaced by “Systemisk Lupus Erytematosus”.

In Paper II:

Page 952, Table 2: Correct categories of pre-pregnancy weight: < 60, 60-79 and ≥ 80.

In Paper V:

Page 3, line 19: “intra-assay coefficients of variation”. Correction: “inter-assay coefficients of variation”.

Paper I

Paper I is not included due to copyright.

Paper II

Preeclampsia and Fetal Growth

RØNNAUG A. ØDEGÅRD, MD, LARS J. VATTEN, MD, PhD, STEIN TORE NILSEN, MD, PhD, KJELL Å. SALVESEN, MD, PhD, AND RIGMOR AUSTGULEN, MD, PhD

Objective: **To determine if the influence of preeclampsia on birth size varies with clinical manifestations of the disease, and to evaluate whether maternal factors, such as smoking, modify the effect of preeclampsia on fetal growth.**

Methods: **Among 12,804 deliveries in a population of approximately 239,000 over a 3-year period, 307 live singleton infants were born after preeclamptic pregnancies. We compared those with a sample of 619 control infants. Preeclampsia was defined as increased diastolic blood pressure (BP) (increase of at least 25 mmHg to at least 90 mmHg) and proteinuria after 20 weeks' gestation. Clinical manifestations were classified according to BP and proteinuria into subgroups of mild, moderate, or severe (including cases with eclampsia and hemolysis, elevated liver enzymes, low platelets [HELLP] syndrome) preeclampsia, and according to gestational age at onset, as early or late preeclampsia. Birth size was expressed as the ratio between observed and expected birth weights, and infants smaller than two standard deviations from expected birth weights were classified as small for gestational age (SGA).**

Results: **Preeclampsia was associated with a 5% (95% confidence interval [CI] 3%, 6%) reduction in birth weight. In severe preeclampsia, the reduction was 12% (9%, 15%), and in early-onset disease, birth weight was 23% (18%, 29%) lower than expected. The risk of SGA was four times higher (relative risk [RR] = 4.2; 95% CI 2.2, 8.0) in infants born after preeclampsia than in control pregnancies. Among nulliparas, preeclampsia was associated with a nearly threefold higher risk of SGA (RR = 2.8; 1.2, 5.9), and among paras, the risk of SGA was particularly high after recurrent preeclampsia (RR = 12.3; 3.9, 39.2). In relation to preeclampsia and maternal smoking, the results indicated that each factor might contribute to reduced growth in an additive manner.**

Conclusion: **Severe and early-onset preeclampsia were associated with significant fetal growth restriction. The risk of having an SGA infant was dramatically higher in women with recurrent preeclampsia. Birth weight reduction related to maternal smoking appeared to be added to that caused by preeclampsia, suggesting that there is no synergy between smoking and preeclampsia on growth restriction. (Obstet Gynecol 2000;96:950–5. © 2000 by The American College of Obstetricians and Gynecologists.)**

Fetal growth restriction (FGR) is the end point of a number of pregnancy-associated conditions, and the mechanisms that lead to it differ.[1] Fetal growth can be restricted by preeclampsia,[2] but most infants born to women with preeclampsia weigh appropriate for their gestation.[2] Preeclampsia should probably be regarded as a syndrome of heterogeneous origin.[2] Shallow trophoblast invasion of decidual arteries can precipitate preeclampsia,[3] reduce placental perfusion, and cause insufficient transport of nutrients. Placental morphologic changes vary substantially in preeclampsia,[2,4] and it has been hypothesized that FGR might depend on abnormal placental development.[2] In cases in which maternal factors (genetic, metabolic, hemodynamic) are dominant, placental perfusion is not necessarily affected and has little impact on fetal growth.[2]

Clinical manifestations of preeclampsia vary by gestational age at onset (early or late) and by severity of symptoms (mild, moderate, severe). Placental disease has been reported as a consistent characteristic of early preeclampsia,[4,5] and that corresponds to the serious reduction in birth size associated with those cases.[6–8] Reduced birth size has also been seen after clinically severe preeclampsia with later onset,[9,10] but other studies did not show differences in growth between mild and severe preeclampsia.[11]

Among maternal factors, growth restriction caused by smoking during pregnancy is an established risk factor.[9,12] A synergistic effect has been suggested when smoking is combined with preeclampsia, causing lower birth weight than expected by adding their separate

From the Institute of Cancer Research and Molecular Biology, Department of Community Medicine and General Practice, National Center for Fetal Medicine, University Medical Center, Trondheim, Norway, and the Department of Obstetrics and Gynecology, Rogaland Central Hospital, Stavanger, Norway.

The authors thank the Norwegian Medical Birth Registry for assistance. Dr. Ødegård is a Research Fellow supported by the Norwegian Medical Research Council and by the Norwegian University of Science and Technology.

effects.[9] However, the results of others do not support a synergy between smoking and preeclampsia on fetal growth.[13]

In this population-based study, we examined the association between different clinical manifestations of preeclampsia and fetal growth and explored whether a relationship between preeclampsia and fetal growth could be modified by maternal factors, particularly smoking.

Materials and Methods

The study was done between January 1993 and December 1995 at the Central Hospital in Rogaland County, Norway. The birthing clinic at this hospital exclusively serves a region of approximately 239,000 inhabitants, and there were 12,804 deliveries during the study period. The study was considered and approved by the Regional Committee for Ethics in Medical Research.

Since 1967, the Norwegian Medical Birth Registry has used standardized forms to record information on all deliveries.[14] We searched the records of the Birth Registry to identify women with preeclampsia who gave birth at Rogaland Central Hospital during the study period and found approximately 1300 cases with clinical characteristics possibly indicative of preeclampsia. For each potential case we verified and supplemented that information with detailed clinical information from hospital records. After reviewing all relevant records, we found that 323 women fulfilled the diagnostic criteria for preeclampsia. After that, the Medical Birth Registry selected two separate groups of women without preeclampsia who gave birth at the hospital during the same period. One group consisted of the first women who gave birth at the birthing clinic after the women with preeclampsia. The other group was randomly selected by computer among all other births at the hospital, but frequency matched by mother's age to avoid confounding between effect of preeclampsia and maternal age.

Using each control group separately in the analysis yielded almost identical results, so we decided to pool the two groups to increase statistical precision. The results presented are based on those pooled analyses. We excluded women with twin pregnancies and women with unknown gestational ages from analysis, leaving 307 live singleton infants born after preeclamptic pregnancies and 619 controls.

We used a reported definition of preeclampsia,[15] ie, persistent diastolic blood pressure (BP) of at least 90 mmHg had to develop after 20 weeks' gestation and it had to increase by at least 25 mmHg. Nineteen women (four with histories of hypertension) had diastolic BP of 90 mmHg at baseline, and they were included as cases of preeclampsia because their diastolic BP increased further by at least 15 mmHg. Proteinuria also had to be present for preeclampsia; when cutoff was defined as 0.3 mg/L (semiquantitative dipstick 1+) in at least one urine sample after 20 weeks' gestation, without simultaneous urinary infection. Twelve women with no histories of hypertension had no registered baseline BP, but had diastolic pressures of 105 mmHg or higher after 20 weeks' gestation (with proteinuria). They were included as cases of preeclampsia.

Preeclampsia was categorized as mild, moderate, or severe.[16] Mild preeclampsia was defined as diastolic BP increase of at least 25 mmHg and proteinuria of 1+ on semiquantitative dipstick; moderate preeclampsia as an increase in diastolic BP of at least 25 mmHg and proteinuria of 2+ on semiquantitative dipstick; and severe preeclampsia as diastolic BP increased to at least 110 mmHg and proteinuria of 3+ on semiquantitative dipstick, or at least 500 mg/24 hours. Six cases of eclampsia and 16 cases with indications of hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome were classified as severe preeclampsia. Pregnancy termination before or at 32 weeks' gestation was treated as a proxy variable for early-onset preeclampsia.

The primary outcome of this study was expressed as the ratio between observed and the expected birth weight (birth weight ratio),[17] in which the expected birth weight was adjusted for sex and gestational age at birth. Gestational age was calculated exclusively from routine ultrasonographic measurements of biparietal diameter at 18 weeks' gestation according to Norwegian standard curves.[18] Weight curves estimated from ultrasonographic measurements in a population of healthy pregnant Swedish women were used to determine expected birth weights for sex and gestational age.[19]

A small for gestational age (SGA) infant was defined as having a birth weight two standard deviations or more below the expected birth weight, which corresponds to more than 24% lower birth weight than expected (birth weight ratio less than 0.76), or an approximately 840-g reduction in birth weight for a term infant.

In Norway, antenatal care is free, and most women (close to 100%) attend their first antenatal doctors' visits around 12 weeks' gestation. Clinical information is recorded on standardized forms, and the antenatal maternal data analyzed in this study were based on that information. Few women reported smoking more than ten cigarettes per day, so participants were dichotomized as smokers or nonsmokers. Maternal weight was measured at first antenatal visit, classified as prepregnancy weight, divided into the following three categories: under 60, 60–79, and at least 80 kg. In the analysis

of the risk of SGA, maternal weight was dichotomized at 70 kg, and parity as nulliparous or parous.

Student t test was used for comparison of continuous variables between groups. To compare proportions, as indicated by categoric variables, we used χ^2 test. Birth weight ratios of infants whose mothers had preeclampsia were calculated and compared with weight ratios of control infants. That comparison was stratified according to mother's parity, maternal smoking, and the three categories of maternal weight at first antenatal visit, and covariates were included in a multiple linear regression analysis to control for potential confounding. We estimated the odds ratio (OR) for SGA as a measure of relative risk (RR) between infants whose mothers had preeclampsia and control infants and used unconditional logistic regression to adjust for potentially confounding factors in a multivariate analysis.[20] We further explored whether maternal factors (parity, smoking and prepregnancy weight) could modify associations between subgroups of preeclampsia and birth size and tested possible interactions in multivariate models (linear regression for birth weight ratio and logistic regression for SGA). Precision of the estimates of effect (birth weight ratio and OR) were estimated with 95% confidence intervals (CI). All statistical analyses were calculated using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL).

Results

Birth status of infants whose mothers had preeclampsia and controls is shown in Table 1. The mean birth weight was 5% (95% CI 3%, 6%) lower than expected in the preeclampsia group (Table 2). That corresponded to an approximately 175-g lower birth weight than expected for a term infant. Stratified analyses (Table 2) showed that the birth weight was 10% (95% CI 6%, 14%) lower than expected in newborns whose mothers had preeclampsia and reported smoking, compared with 3% (95% CI 1%, 5%) for mothers who had preeclampsia but did not smoke. Among control infants, newborns of smokers also weighed less than expected (4%, 95% CI 3%, 6%). There was no association between maternal baseline BP and birth weight in the preeclampsia group or among control infants (data not shown). We tested for statistically significant interactions between preeclampsia and all the maternal factors listed in Table 2, but found none ($P \geq .10$). We also explored possible statistical interactions between smoking and clinical subtypes of preeclampsia, but there was no interaction with any subtypes (data not shown).

Table 1. Antenatal Maternal Data and Infant Status at Birth

	Preeclampsia (n = 307)	Controls (n = 619)	P
Maternal data			
Age (y)*	26.8 ± 4.7	28.2 ± 4.9	<.001
Prepregnant weight (kg)*	69.9 ± 12.9	65.2 ± 11.0	<.001
Nulliparous	199 (65%)	222 (36%)	<.001
Maternal smoking	57 (19%)	154 (26%)	<.05
Infant data			
Gestational age (d)*	263 ± 24	280 ± 11	<.001
Premature birth†	97 (32%)	25 (4%)	<.001
Small for gestational age	37 (12%)	17 (3%)	<.001
Referred to NICU	107 (35%)	49 (8%)	<.001

NICU = neonatal intensive care unit.
* Mean ± standard deviation.
† Premature birth = delivery before 37 weeks' gestation.

Table 2. Ratios Between Observed and Expected Birth Weights

	Preeclampsia		Controls	
	n*	BWR (95% CI)	n*	BWR (95% CI)
Overall	307	0.95 (0.94, 0.97)	619	1.00 (0.99, 1.01)
Maternal smoking				
No	239	0.97 (0.95, 0.99)	422	1.01 (1.00, 1.02)
Yes	57	0.90 (0.86, 0.94)	154	0.96 (0.94, 0.97)
Nullipara	199	0.95 (0.92, 0.97)	222	0.97 (0.96, 0.99)
Para	108	0.97 (0.93, 1.01)	387	1.01 (1.00, 1.03)
Repeat preeclampsia	48	0.95 (0.90, 1.00)		
No repeat preeclampsia	60	0.99 (0.94, 1.04)		
Prepregnancy weight (kg)				
≤60	67	0.89 (0.85, 0.93)	211	0.97 (0.96, 0.98)
61–79	170	0.95 (0.93, 0.98)	318	1.01 (1.00, 1.03)
≥80	54	1.02 (0.97, 1.06)	48	1.03 (0.98, 1.07)

BWR = birth weight ratio; CI = confidence interval.
* Numbers for some covariates do not total because of missing data.

The risk of having an SGA infant (Table 3) was four times higher (RR = 4.2, 95% CI 2.2, 8.0) in women with preeclampsia than controls. In stratified analyses, we evaluated the association between preeclampsia and risk of SGA for different categories of maternal smoking, parity, and prepregnant weight. The results showed that among paras, the RR of having an SGA infant was 7.9 (95% CI 2.8, 22.2) in women with preeclampsia compared with controls. Among nulliparas the RR was 2.8 (95% CI 1.2, 5.9). The test of statistical interaction between preeclampsia and parity was not significant (P = .08). Women with recurrent preeclampsia were at particularly high risk (RR = 12.3, 95% CI 3.9, 39.2) of having SGA infants compared with controls.

Table 4 shows that birth size was lower with increasing severity of preeclampsia (P trend < .01) and that risk of having an SGA infant increased with disease severity (P trend = .05). For severe preeclampsia, birth weight was 12% (95% CI 9%, 15%) lower than expected, but after mild preeclampsia, birth weight did not differ from the expected weight. The proportion of SGA

Table 3. Relative Risk of Small Size for Gestational Age in Infants Born After Preeclampsia and After Control Pregnancies

	SGA Preeclampsia	SGA Controls	Adjusted RR*	95% CI
Overall	37 of 307†	17 of 619†	4.2	2.2, 8.0
Nonsmokers	23 of 239	10 of 422	4.0	1.8, 9.0
Smokers	12 of 57	7 of 154	4.5	1.9, 9.5
Nullipara	22 of 199	10 of 222	2.8	1.2, 5.9
Para	15 of 108	7 of 387	7.9	2.8, 22.2
Repeat preeclampsia	9 of 48		12.3	3.9, 39.2
No repeat preeclampsia	6 of 60		4.4	1.1, 17.1
Prepregnancy weight (kg)				
≤70	21 of 164	11 of 415	4.2	1.9, 9.4
≥71	14 of 127	5 of 162	4.1	1.3, 12.7

SGA = small for gestational age; RR = relative risk; CI = confidence interval.

* Adjusted for the other variables in the table. For each stratum, controls represent the reference (RR = 1.0).

† Numbers for some covariates do not total because of missing data.

infants born after severe preeclampsia was 21% (95% CI 12%, 29%) compared with 6% (95% CI 1%, 10%) after mild preeclampsia.

Early-onset preeclampsia (Table 5) was strongly associated with low birth weight, 23% (95% CI 18%, 29%) lower than expected, and in early-onset preeclampsia, the frequency of SGA infants was 53% (95% CI 36%, 70%). We also distinguished between severe preeclampsia with late onset and early onset (Table 6), and the results showed that severe preeclampsia relatively late in pregnancy also was related to lower than expected birth weight (9%, 95% CI 6%, 12%).

Discussion

Neonates whose mothers had preeclampsia weighed less than infants born after normotensive pregnancies, and their risk of being born SGA was fourfold higher. Thus, our results agreed with those that reported reduced fetal growth in preeclampsia.[9,12,21] Weight reduction differed strongly between clinical subgroups and was mainly confined to infants whose mothers had early-onset or severe preeclampsia. The most serious

Table 5. Ratios Between Observed and Expected Birth Weights and Percentages of Small for Gestational Age Infants by Early or Late Onset of Preeclampsia

	Early-onset preeclampsia (n = 32)	Late-onset preeclampsia (n = 275)
Birth weight ratio (95% CI)*	0.77 (0.71, 0.82)	0.98 (0.96, 0.99)
SGA % (95 CI)	53.1 (35.7, 70.3)	7.3 (4.2, 10.3)

SGA = small for gestational age; CI = confidence interval.

* $P < .01$ (comparison between early- and late-onset preeclampsia, adjusted for smoking, parity, and prepregnant weight).

growth restriction (23% lower than expected) was in the early-onset group, and more than half of those newborns were SGA.

Ness and Roberts[2] hypothesized that preeclampsia restricts fetal growth when it is caused by placental abnormalities, which result in reduced nutrient supply to the fetus. The serious FGR that accompanies early-onset preeclampsia and the abundant uteroplacental vascular lesions in placental tissues associated with early-onset preeclampsia[4] fit well with that hypothesis. Abnormal observations are less frequent in placental tissue from preeclamptic deliveries at term,[5] and mild and moderate preeclampsia appear to have only negligible effects on birth weight, as reported by others[9] and supported by our data.

Although the association between early-onset preeclampsia and SGA is well established, results have varied substantially, ranging between 18% and 80%.[6–8] However, divergent definitions of early-onset preeclampsia were used, which might account for some variation. To some extent, early-onset and severe preeclampsia are overlapping categories. In our early-onset group, two thirds of women were classified as having severe symptoms. Although less pronounced, there was also lower birth size related to severe preeclampsia with late onset (9% lower than expected), which is in accordance with previous reports. Cnattingius et al[9] found a substantially higher risk of SGA in pregnancies with severe than mild preeclampsia. However, a recent Chinese study reported no difference in risk of SGA infants for women with mild and severe preeclampsia.[11]

Table 4. Ratios Between Observed and Expected Birth Weights and Percentage of Small for Gestational Age Infants by Severity of Preeclampsia

	Mild preeclampsia (n = 103)	Moderate preeclampsia (n = 121)	Severe preeclampsia (n = 83)	P*
Birth weight ratio (95% CI)	1.00 (0.97, 1.03)	0.96 (0.93, 0.99)	0.88 (0.85, 0.91)	<.01
SGA % (95% CI)	5.8 (1.2, 10.0)	11.6 (5.9, 17.3)	20.5 (11.8, 29.2)	.05

CI = confidence interval; SGA = small for gestational age.

* P trend, adjusted for maternal smoking, parity, and prepregnant weight.

Table 6. Ratios Between Observed and Expected Birth Weights by Onset and Severity of Preeclampsia

Severity of preeclampsia	Early-onset preeclampsia (n = 32)	Late-onset preeclampsia (n = 275)
Mild and moderate	0.74 (0.62, 0.86)	0.99 (0.97, 1.02)
Severe	0.78 (0.72, 0.85)	0.91 (0.88, 0.94)

Data are given as birth weight ratio (95% confidence interval).

Ultrasound measurements have become the standard method for pregnancy dating in Scandinavia because ultrasound might predict delivery date more precisely than last menstrual period.[22,23] Gestational ages in the present study were determined by ultrasound. We used fetal growth curves based on ultrasound measurements to estimate expected birth weights for two reasons.[19] First, by using identical methods for pregnancy dating and evaluation of fetal growth,[24] precision and validity of estimated deviations from expected growth (birth weight ratio) can be improved. Second, postnatal measurements to construct birth weight standards in preterm infants have been criticized because the underlying pathogenesis of preterm parturition might restrict fetal growth and cause a lower birth weight than indicated by gestational age.[24,25] Therefore, FGR might be underestimated in premature newborns,[12] and expected birth weights derived from weight curves based on ultrasound will be slightly higher than those from postnatal weight standards.[19,26] In control infants born between 231 and 302 days' gestation, birth weights were practically identical to those expected from ultrasound-based weight curves for them. That might be reassuring for the validity of the method that we used for that range of gestational age. At lower gestational ages, however, we are not provided with similar healthy control infants, so we cannot exclude that growth restriction in 37 infants born before 231 days' gestation was overestimated by ultrasound-based weight curves. Given the magnitude of the growth restriction related to early-onset preeclampsia, it seems unlikely that more than a fraction can be ascribed to possible bias in the estimates.

In control infants, we found that maternal smoking was related to a reduction in birth weight of 4% and that birth weight after preeclampsia in nonsmokers was 3% lower than expected. Birth weight after preeclampsia in smokers was reduced by 10%, which indicated an additive statistical effect of preeclampsia and smoking on birth size. That finding might be an argument against suggested synergy between smoking and preeclampsia.[9,10,27] Our results might suggest that smoking influences fetal growth by mechanisms that are independent of, and not interacting with, mechanisms in preeclampsia that also restrict growth.

Among parous women, the risk of SGA associated with preeclampsia was substantially higher than the risk for nulliparas. Similar observations were reported by Eskenazi et al.[21] We found that women with preeclampsia who had it in previous pregnancies had dramatically higher risk of delivering an SGA infant. The distribution of clinical subtypes (mild, moderate, severe, and late versus early onset) (data not shown) did not differ between nulliparas and paras with preeclampsia, regardless of whether they had it before. The effect on fetal growth of repeated preeclampsia was also present in cases in which clinical severity was only moderate. It remains unknown whether paras with preeclampsia have a separate disease in origin or pathogenesis from preeclampsia in nulliparas. The serious growth restriction associated with recurrent preeclampsia suggests that future studies should focus on placental histopathology associated with various clinical subgroups.

References

1. Arnholdt H, Meisel F, Fandrey K, Lohrs U. Proliferation of villous trophoblast of the human placenta in normal and abnormal pregnancies. Virchows Arch B Cell Pathol Incl Mol Pathol 1991;60: 365–72.
2. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: A hypothesis and its implications. Am J Obstet Gynecol 1996;175:1365–70.
3. Roberts JM, Redman CW. Pre-eclampsia: More than pregnancy-induced hypertension. Lancet 1993;341:1447–51.
4. Ghidini A, Salafia CM, Pezzullo JC. Placental vascular lesions and likelihood of diagnosis of preeclampsia. Obstet Gynecol 1997;90: 542–5.
5. Teasdale F. Histomorphometry of the human placenta in maternal preeclampsia. Am J Obstet Gynecol 1985;152:25–31.
6. Long PA, Abell DA, Beischer NA. Fetal growth retardation and pre-eclampsia. Br J Obstet Gynaecol 1980;87:13–8.
7. Brazy JE, Grimm JK, Little VA. Neonatal manifestations of severe maternal hypertension occurring before the thirty-sixth week of pregnancy. J Pediatr 1982;100:265–71.
8. Moore MP, Redman CW. Case-control study of severe pre-eclampsia of early onset. BMJ 1983;287:580–3.
9. Cnattingius S, Mills JL, Yuen J, Eriksson O, Salonen H. The paradoxical effect of smoking in preeclamptic pregnancies: Smoking reduces the incidence but increases the rates of perinatal mortality, abruptio placentae, and intrauterine growth restriction. Am J Obstet Gynecol 1997;177:156–61.
10. Duffus GM, MacGillivray I. The incidence of pre-eclamptic toxaemia in smokers and non-smokers. Lancet 1968;1:994–5.
11. Xiong X, Mayes D, Demianczuk N, Olson DM, Davidge ST, Newburn CC, et al. Impact of pregnancy-induced hypertension on fetal growth. Am J Obstet Gynecol 1999;180:207–13.
12. Spinillo A, Capuzzo E, Piazzi G, Nicola S, Colonna L, Iasci A. Maternal high-risk factors and severity of growth deficit in small for gestational age infants. Early Hum Dev 1994;38:35–43.
13. Marcoux S, Brisson J, Fabia J. The effect of cigarette smoking on the risk of preeclampsia and gestational hypertension. Am J Epidemiol 1989;130:950–7.
14. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie NE, Irgens

LM. Fetal and maternal contributions to risk of pre-eclampsia: Population based study. BMJ 1998;316:1343–7.
15. CLASP: A randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative Group. Lancet 1994;343:619–29.
16. Redman CWE. Hypertension in pregnancy. New York: Perinatology Press, 1987.
17. Kramer MS, McLean FH, Olivier M, Willis DM, Usher RH. Body proportionality and head and length 'sparing' in growth-retarded neonates: A critical reappraisal. Pediatrics 1989;84:717–23.
18. Eik-Nes SH, Grøttum P, Jørgensen NP, Løkvik B. Normal range curves for BPD and MAD. Drammen, Norway: Scan-Med A/S, 1983.
19. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85:843–8.
20. Kleinbaum D. Logistic regression. A self-learning text. New York: Springer-Verlag, 1998.
21. Eskenazi B, Fenster L, Sidney S, Elkin EP. Fetal growth retardation in infants of multiparous and nulliparous women with preeclampsia. Am J Obstet Gynecol 1993;169:1112–8.
22. Tunon K, Eik-Nes SH, Grøttum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol 1996;8:178–85.
23. Gardosi J, Geirsson RT. Routine ultrasound is the method of choice for dating pregnancy. Br J Obstet Gynaecol 1998;105:933–6.
24. Pollack RN, Divon MY. Intrauterine growth retardation: Definition, classification, and etiology. Clin Obstet Gynecol 1992;35:99–107.
25. Seeds JW. Impaired fetal growth: Definition and clinical diagnosis. Obstet Gynecol 1984;64:303–10.
26. Bernstein IM, Mohs G, Rucquoi M, Badger GJ. Case for hybrid "fetal growth curves": A population-based estimation of normal fetal size across gestational age. J Matern Fetal Med 1996;5:124–7.
27. Salafia C, Shiveric K. Cigarette smoking and pregnancy II: Vascular effects. Placenta 1999;20:273–9.

Address reprint requests to:
Rønnaug A. Ødegård, MD
University Medical Center
Institute of Cancer Research and Molecular Biology
N-7489 Trondheim
Norway
E-mail: ronnaug.odegard@medisin.ntnu.no

Received March 10, 2000.
Received in revised form June 19, 2000.
Accepted July 14, 2000.

Paper III

Umbilical Cord Plasma Interleukin-6 and Fetal Growth Restriction in Preeclampsia: A Prospective Study in Norway

Rønnaug A. Ødegård, MD, Lars J. Vatten, MD, PhD, Stein Tore Nilsen, MD, PhD, Kjell Å. Salvesen, MD, PhD, Hege Vefring, MS, and Rigmor Austgulen, MD, PhD

OBJECTIVE: To study the association between umbilical plasma levels of interleukin-6 (IL-6) in relation to fetal growth in subgroups of preeclampsia, and in control pregnancies.

METHODS: Umbilical cord plasma was collected from 12,804 consecutive births. A total of 271 singleton cases of preeclampsia were identified, and classified as mild or severe, and as disease with early or late onset. As controls, 611 singleton pregnancies without preeclampsia were selected, and the ratio between observed and expected birth weight was used as a measure of fetal growth. In the analysis, we also included maternal smoking during pregnancy. Umbilical cord plasma IL-6 concentration was measured with an IL-6 bioassay. Comparing controls with subgroups of preeclampsia (severe and early onset), this study had a statistical power of 90% to detect a difference in cord IL-6 of 10 pg/mL.

RESULTS: In severe preeclampsia, cord plasma IL-6 concentration was lower than among controls ($P < .001$), and there was a sharp decrease in cord plasma IL-6 with decreasing birth weight ratio (P trend $< .001$). By further dividing the preeclampsia group into early or late onset, the strong association between low IL-6 levels and low birth weight ratio appeared to be present mainly in early-onset disease. These results were not confounded by maternal smoking.

CONCLUSION: Restricted fetal growth related to preeclampsia is associated with reduced umbilical cord plasma IL-6 concentration in cases with early-onset disease. In these cases, fetal growth restriction could be mediated by impaired trophoblast function. (Obstet Gynecol 2001;98: 289–94. © 2001 by the American College of Obstetricians and Gynecologists.)

A number of cytokines have key roles in normal placental and fetal growth.[1–4] Interleukin-6 (IL-6) is a potent mitogen that is secreted by the trophoblast during normal pregnancy,[5] and in vitro observations suggest that IL-6 stimulates growth, invasion, and differentiation of the trophoblast.[6] Interleukin-6 contributes to the regulation of placental hormone production,[7–9] and appears to be involved in angiogenesis.[10,11] Previously, a few small studies have reported reduced IL-6 levels in amniotic fluid (AF)[12] and umbilical cord blood[13] associated with fetal growth restriction (FGR), and these findings support the hypothesis that IL-6 may be related to fetal growth at the fetomaternal interface.

Preeclampsia is a heterogeneous syndrome that is strongly associated with FGR in severe[14] and early-onset disease.[15,16] Fetal growth restriction in preeclampsia is attributed to reduced placental blood flow with subsequent impaired fetomaternal exchange of substrates, and the process may be initiated by unsuccessful transformation of uteroplacental spiral arteries.[17] However, different patterns of adaptation to reduced placental blood flow may take place.[18,19] The frequently observed increased development of placental terminal villi has been interpreted as an attempt to increase the placental surface area in order to enhance substrate transfer.[18] In other cases of restricted fetal growth, placental compensation may be absent, and severe impairment of trophoblast has been described.[20] Typically, these cases are characterized by early delivery.[18]

In the present study we have analyzed the association between IL-6 levels in umbilical cord plasma and fetal growth in preeclampsia (subgrouped according to clinical severity and gestational age at disease onset) and controls in a population of nearly 13,000 consecutive births. Because maternal smoking increases the risk of

From the Institute of Cancer Research and Molecular Biology, the Institute of Community Medicine and General Practice, Norwegian University of Science and Technology, Trondheim; the National Center for Fetal Medicine, University Hospital of Trondheim, Trondheim; and the Departments of Obstetrics and Gynecology, and Clinical Chemistry, Rogaland Central Hospital, Stavanger, Norway.

Rønnaug Ødegård is a research fellow supported by the Norwegian Research Council and the Norwegian University of Science and Technology.

We thank Anita Haugan for laboratorial help, and valuable contribution to the preparation of the paper.

0029-7844/01/$20.00
PII S0029-7844(01)01396-5

FGR, and shares some uteroplacental characteristics with preeclampsia,[21] we also included maternal smoking in the analysis.

MATERIALS AND METHODS

Umbilical cord blood samples were collected in a prospective study of pregnancy outcome that took place from January 1993 to December 1995 at Rogaland Central Hospital in Stavanger, Norway. The birthing clinic at this hospital serves exclusively a region of approximately 239,000 inhabitants, and there were 12,804 deliveries during the study. The Norwegian Medical Birth Registry records information on all deliveries that take place in Norway,[22] and we searched the records to identify potential cases of preeclampsia and to select population controls, as described previously.[15,23]

We initially identified approximately 1300 cases with clinical information possibly indicative of preeclampsia, and verified and supplemented this information with details from hospital records. We identified 307 singleton pregnant women who fulfilled the diagnostic criteria for preeclampsia (see below); umbilical cord blood was available from 271. One case was excluded because of culture-proven neonatal sepsis. The definition of preeclampsia has been reported previously[24]; that is, persistent diastolic blood pressure (BP) of at least 90 mmHg had to develop after 20 weeks' gestation, and diastolic BP had to increase by at least 25 mmHg. In addition, proteinuria had to be present, and cutoff was defined as 0.3 mg/L (semiquantitative dipstick 1+) in at least one urine sample after 20 weeks' gestation without simultaneous urinary infection. Preeclampsia was classified as severe ($n = 70$) if diastolic BP increased to at least 110 mmHg, along with proteinuria 3+ on dipstick, or at least 500 mg/24 hours. Cases with eclampsia and suspected hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome were regarded interchangeable with severe preeclampsia, whereas all other cases of preeclampsia were classified as mild ($n = 200$). We used delivery before or at 34 weeks' gestation as a proxy for early-onset disease,[25] and classified 34 patients as having early-onset preeclampsia and 236 as having late-onset preeclampsia. For comparison, the medical birth registry selected two separate groups of women without preeclampsia who gave birth at the hospital during the same period as described previously.[15,23] One group consisted of the first women who gave birth after the women with preeclampsia. The other group was randomly selected by computer among all other births at the hospital but frequency matched by mother's age to avoid confounding between effect of preeclampsia and maternal age. Using each control group separately in the analyses yielded almost identical results, and we decided to pool the two groups to increase statistical precision. The results presented are based on the pooled analyses. We obtained cord blood from 611 control women, and one was excluded because of culture-proven neonatal sepsis.

Table 1. Descriptive Data in Preeclampsia and in Controls

	Controls ($n = 610$)	Preeclampsia ($n = 270$)
Maternal age (y)*	28 (4.9)	27.0 (4.6)*
Prenatal steroids	1%	14%†
Cesarean delivery	6%	29%†
Maternal smoking	27%	19%†
Gestational age (d)†	280 (11)	265 (23)†
Mean birth weight ratio	1.00 (0.99, 1.01)	0.96 (0.94, 0.98)†
Small for gestational age	2.7%	9.8%†
Interleukin-6‡	16 (16)	16 (20)

Birth weight ratio: observed divided by expected birth weight, with 95% confidence intervals.
* Mean (standard deviation).
† $P < .05$ (compared with controls).
‡ Data expressed as pg/mL [median (interquartile range)].

Information on maternal smoking was obtained at about 18 weeks' gestation, and was available for 259 of the women with preeclampsia and in 570 controls. Because few women reported smoking more than ten cigarettes per day, the participants were dichotomized as smokers or nonsmokers. All other baseline data were obtained at the first maternal visit at about 12 weeks of pregnancy, and the infant data were collected from hospital records after discharge from the hospital. Blood samples were collected passively from the placental side of the umbilical cord after delivery. All blood samples were collected in syringes containing heparin, and chilled to 4C up to 60 hours before being centrifuged at 3000 rpm for 15 minutes. Plasma was stored at −80C until analysis.

As a measure of fetal growth we used the ratio between observed and expected birth weight (birth weight ratio),[26] and the ratio was adjusted for sex and gestational age at birth. Weight curves estimated from ultrasonographic measurements in a population of healthy pregnant Swedish women were used to determine the expected birth weights.[27] Gestational age at birth was calculated from routine ultrasonographic measurements at 18 weeks' gestation. Small-for-gestational age (SGA) was defined as birth weight two standard deviations (SD) or more below the expected birth weight, which corresponds to more than 24% lower birth weight than expected (birth weight ratio less than 0.76),[27] or to approximately 840 g reduction of birth weight for a term infant. This cutoff for SGA corresponds approximately to the 2.3 percentile. Table 1 lists some characteristics of the groups.

Interleukin-6 bioactivity was measured by the hybrid-

Table 2. Umbilical Cord Plasma Interleukin-6, by Levels of Birth Weight Ratio, in Mild or Severe Preeclampsia, and in Controls

	Controls		Mild preeclampsia		Severe preeclampsia	
	n	IL-6, pg/mL (IQR)	*n*	IL-6, pg/mL (IQR)	*n*	IL-6, pg/mL (IQR)
Overall	610	16 (16)	200	18 (19)	70	10 (22)*†
Birth weight ratio						
<0.76	17	11 (12)	15	9 (17)	12	0 (4)‡
0.76–0.89	106	17 (11)	41	15 (17)	27	9 (21)§
0.90–1.09	368	16 (16)	99	18 (20)	25	13 (28)
≥1.10	119	18 (18)	45	20 (23)	6	20 (21)

IL-6 = interleukin-6; IQR = interquartile range.
Birth weight ratios were adjusted for sex and gestational age. Data are expressed as median (interquartile range).
* Compared with controls by Mann–Whitney *U* tests.
† $P < .001$.
‡ $P = .004$.
§ $P = .02$.

oma cell line B 13.29 clone 9, which depends on IL-6 for growth,[28] and we followed the procedures for colorimetric assay described by Tada et al.[29] Briefly, diluted plasma samples were added to B 13.29 and growth was measured after 64 hours by using MTT. Human recombinant IL-6 (Genzyme, Cambridge, MA) was used as a reference standard, and the results were expressed in pg/mL. Each sample was analyzed in duplicate with four dilutions from 1:20 to 1:160 and the assay was run in eight sequences. The detection limit of the assay was 4.9 pg/mL, and IL-6 was detected in umbilical cord plasma from 80% of infants exposed to preeclampsia and in 89% of control infants. All samples below the detection limit were given the value 0. The intra-assay variation was on average less than 7%, and the inter-assay variation 25%. Because a high intra-assay variation was expected, all subjects were analyzed in the order they were included in the study, and samples from three cases and six controls were interspersed on each microtiter plate. Therefore, a relatively high interassay variation would influence the results only in a random fashion, and differences between groups would be underestimated. We examined if the time between blood collection and freezing influenced IL-6 levels, and an interval of 60 hours did not influence the results. We also analyzed whether factors that may inhibit IL-6 activity were present, and found low levels of inhibiting activity in samples with both high and low IL-6 levels, in preeclampsia and control samples (data not shown). Monoclonal anti-IL-6 (R&D Systems, Minneapolis, MN) inhibited the activity of IL-6.

Interleukin-6 had a skewed distribution, and was therefore expressed as the median value [pg/mL (interquartile range)]. Mann–Whitney *U* test and Student *t* test were used to compare continuous variables between groups, and differences between proportions were assessed by χ^2 tests. The standardized birth weight (birth weight ratio) was divided into four clinical categories: less than 0.76 corresponds to a strict definition (−2 SD) of SGA, and 0.76–0.89 is a broad category of relatively small infants. The category 0.90–1.09 includes infants with appropriate weight for their gestation, and the category greater than 1.09 includes large infants. Within the groups, we tested for trend of IL-6 (presented as *P* trend) across ordinal categories of birth weight ratio by Kruskal–Wallis *H* test, and repeated the test after stratifying the groups according to maternal smoking. At each category of standardized birth weight, we compared cord plasma IL-6 concentrations among groups by Mann–Whitney *U* tests. Comparing controls with subgroups of preeclampsia (severe and early onset), this study had a statistical power of 90% to detect a difference in cord IL-6 of 10 pg/mL. All statistical analyses were calculated using the Statistical Package for the Social Science (SPSS) 10.05 (SPSS, Inc., Chicago, IL).

RESULTS

Overall, the concentration of IL-6 in cord plasma did not differ between the preeclampsia group and controls (Table 1). In severe, in contrast to mild, preeclampsia (Table 2), cord plasma IL-6 concentration was, however, lower than among controls (10 compared with 16 pg/mL, $P < .001$). In severe preeclampsia, a sharp decrease was observed in cord plasma IL-6 concentration with decreasing birth weight ratio (*P* trend < .001). Among controls, there was only a slight decrease in cord plasma IL-6 with decreasing birth weight ratio, and in mild preeclampsia, cord plasma IL-6 did not differ from control levels at any category of birth weight ratio (Table 2).

By further dividing the preeclampsia group into early or late onset (Table 3), the results suggest that the strong association between low IL-6 levels and low birth weight ratio was present mainly in early-onset disease. Only eight infants in early preeclampsia had appropriate

Table 3. Umbilical Cord Plasma Interleukin-6, by Level of Birth Weight Ratio, in Early and Late Onset Preeclampsia, and in Controls

	Controls		Late preeclampsia		Early preeclampsia	
	n	IL-6, pg/mL (IQR)	*n*	IL-6, pg/mL (IQR)	*n*	IL-6, pg/mL (IQR)
Overall	610	16 (16)	236	17 (18)	34	0 (9)*†
Birth weight ratio						
<0.76	17	11 (12)	13	10 (13)	14	0 (7)‡
0.76–0.89	106	17 (11)	57	15 (17)	10	0 (0)§
0.90–1.09	368	16 (16)	116	17 (22)	8	3 (17)‡
≥1.10	119	18 (18)	50	22 (17)	1	44

IL-6 = intetrleukin-6; IQR = interquartile range.

Birth weight ratios were adjusted for sex and gestational age. Data are expressed as median (interquartile range).

* Compared with controls by Mann–Whitney *U* tests.

† $P < .001$.

‡ $P < .05$.

§ $P = .001$.

weight for their gestation, and among these infants, cord plasma IL-6 mainly concentration was lower than among controls (3 compared with 16 pg/mL). One third of the early-onset group was clinically classified as having mild preeclampsia (Table 4), but for early-onset disease, cord plasma IL-6 did not differ between mild and severe preeclampsia (2 and 0 pg/mL, respectively).

In late-onset preeclampsia (Table 3), cord plasma IL-6 concentration was no different from control levels at any category of birth weight ratio. Forty-six cases of late onset were classified as severe (Table 4), but in late-onset disease, cord plasma IL-6 concentration did not differ between mild and severe disease (13 and 18 pg/mL respectively, $P = .1$). Thus, the strong association between low IL-6 and low birth weight ratio appeared to be present only in early-onset preeclampsia.

To assess the impact of maternal smoking on the relation between IL-6 and birth weight ratio, we stratified the preeclampsia group and the controls according to maternal smoking during pregnancy. The results showed that the association between cord plasma IL-6 concentration and birth weight ratio was similar in smokers and nonsmokers, both within the preeclampsia group and among controls (data not shown).

DISCUSSION

We observed a strong decrease in cord plasma IL-6 with decreasing birth weight in preeclampsia, and subgroup analysis showed that this association could be attributed to a particular effect in early-onset disease. The subgroup of early-onset preeclampsia included only 34 cases; however, its population base is an obvious advantage. Among controls, we found no clear association between birth weight ratio and cord plasma IL-6 levels. Previously, a study of FGR in preeclampsia found no association with cord blood IL-6 concentration,[30] but failed to account for differences in gestational age between the groups. However, our results may correspond to those of a small study that reported low levels of cord blood IL-6 concentration in preeclampsia before 32 weeks' gestation.[31]

To adjust for differences in gestational age, we calculated a standardized birth weight ratio (observed over expected birth weight) based on ultrasonographic measurements in a population of healthy pregnant women in Sweden.[27] Despite adjustment for gestational age, we cannot exclude the possibility of residual confounding by gestational age related to our main finding.

Usually, IL-6 production increases in gestational tissues before labor,[32] but it is not clear whether labor in itself increases cord blood IL-6 concentration.[13,30] In our study, cesarean delivery was more frequent in early preeclampsia, therefore mode of delivery could have influenced our results. However, a previous study showed no difference in IL-6 between vaginal and cesarean deliveries,[33] and in vitro studies have reported sim-

Table 4. Cord Plasma IL-6 Levels in Subgroups of Preeclampsia

	Late preeclampsia		Early preeclampsia	
	Mild preeclampsia (*n* = 190)	Severe preeclampsia (*n* = 46)	Mild preeclampsia (*n* = 10)	Severe preeclampsia (*n* = 24)
IL-6, pg/mL	18 (19)	13 (19)	2 (25)	0 (7)

IL-6 = interleukin-6.

Late preeclampsia = delivery > 34 weeks' gestation; early preeclampsia = delivery ≤ 34 weeks' gestation. Data are expressed as median (interquartile range).

ilar production of IL-6 in placental tissue explants in labor and in cesarean deliveries.[32,34] Therefore, confounding by mode of delivery may not be a likely explanation for the association between low birth weight and low levels of cord plasma IL-6 concentration related to early preeclampsia. Furthermore, differences in prenatal steroid administration did not influence our results, because cord plasma IL-6 concentration was similar in those who received steroids and those who did not (data not shown).

Maternal smoking is known to reduce fetal growth,[14,35] but we found no association between maternal smoking and cord plasma IL-6 concentration. Moreover, both in preeclampsia and among controls, maternal smoking had no effect on the association between cord plasma IL-6 concentration and birth weight. Therefore, the association between cord plasma IL-6 concentration and low birth weight in early preeclampsia is not likely to be confounded by maternal smoking.

Secretion of placental IL-6 appears to be relatively constant during pregnancy,[5] and venous cord blood levels may be positively correlated with placental secretion.[36] In this study, we used blood that was passively drawn from the umbilical cord. This blood is mainly venous, and cord plasma IL-6 levels may therefore indicate placental production of IL-6. A causal interpretation of our findings may therefore suggest that IL-6 plays a role in reducing fetal growth related to early preeclampsia.

Main placental functions take place in villous trophoblasts, including fetomaternal transfer of substrates and synthesis of proteins and steroid hormones.[7,9] Placental IL-6 is synthesized mainly in villous trophoblasts,[5] and appears to mediate several trophoblast functions by autocrine or paracrine mechanisms.[7,8] The placental secretion of IL-6 is reduced in preeclampsia,[37] indicating impaired trophoblast function and thereby severe placental insufficiency. Therefore, there may be a causal link between low cord plasma IL-6 concentration and low birth weight in early preeclampsia, and impairment of villous trophoblasts. Recent studies have suggested that the placental terminal villi are severely poorly developed in early restriction of fetal growth, both in the presence and absence of preeclampsia,[17–19] showing reduced proliferation of villous trophoblast and accelerated aging of syncytiotrophoblasts.[19]

Fetal growth restriction in late-onset preeclampsia was not associated with a reduction in cord plasma IL-6 compared with control pregnancies. Thus, our results indicate that the underlying pathogenesis of FGR related to preeclampsia may depend on clinical subtype, and that in early-onset preeclampsia trophoblast impairment is more likely to be present than in preeclampsia with late onset.

REFERENCES

1. Stallmach T, Hebisch G, Joller-Jemelka HI, Orban P, Schwaller J, Engelmann M. Cytokine production and visualized effects in the feto-maternal unit. Quantitative and topographic data on cytokines during intrauterine disease. Lab Invest 1995;73:384–92.
2. Lala PK, Hamilton GS. Growth factors, proteases and protease inhibitors in the maternal-fetal dialogue. Placenta 1996;17:545–55.
3. Robertson SA, Seamark RF, Guilbert LJ, Wegmann TG. The role of cytokines in gestation. Crit Rev Immunol 1994;14:239–92.
4. Wegmann TG, Guilbert LJ. Immune signalling at the maternal-fetal interface and trophoblast differentiation. Dev Comp Immunol 1992;16:425–30.
5. Kameda T, Matsuzaki N, Sawai K, Okada T, Saji F, Matsuda T, et al. Production of interleukin-6 by normal human trophoblast. Placenta 1990;11:205–13.
6. Stephanou A, Myatt L, Eis AL, Sarlis N, Jikihara H, Handwerger S. Ontogeny of the expression and regulation of interleukin-6 (IL-6) and IL-1 mRNAs by human trophoblast cells during differentiation in vitro. J Endocrinol 1995;147:487–96.
7. Meisser A, Cameo P, Islami D, Campana A, Bischof P. Effects of interleukin-6 (IL-6) on cytotrophoblastic cells. Mol Hum Reprod 1999;5:1055–8.
8. Nishino E, Matsuzaki N, Masuhiro K, Kameda T, Taniguchi T, Takagi T, et al. Trophoblast-derived interleukin-6 (IL-6) regulates human chorionic gonadotropin release through IL-6 receptor on human trophoblasts. J Clin Endocrinol Metab 1990;71:436–41.
9. Stephanou A, Handwerger S. Interleukin-6 stimulates placental lactogen expression by human trophoblast cells. Endocrinology 1994;135:719–23.
10. Motro B, Itin A, Sachs L, Keshet E. Pattern of interleukin 6 gene expression in vivo suggests a role for this cytokine in angiogenesis. Proc Natl Acad Sci U S A 1990;87:3092–6.
11. Dankbar B, Padro T, Leo R, Feldmann B, Kropff M, Mesters RM, et al. Vascular endothelial growth factor and interleukin-6 in paracrine tumor–stromal cell interactions in multiple myeloma. Blood 2000;95:2630–6.
12. Silver RM, Schwinzer B, McGregor JA. Interleukin-6 levels in amniotic fluid in normal and abnormal pregnancies: Preeclampsia, small-for-gestational-age fetus, and premature labor. Am J Obstet Gynecol 1993;169:1101–5.
13. Opsjon SL, Austgulen R, Waage A. Interleukin-1, interleukin-6 and tumor necrosis factor at delivery in preeclamptic disorders. Acta Obstet Gynecol Scand 1995;74:19–26.
14. Cnattingius S, Mills JL, Yuen J, Eriksson O, Salonen H. The paradoxical effect of smoking in preeclamptic pregnancies: Smoking reduces the incidence but increases the rates of perinatal mortality, abruptio placentae, and intrauterine growth restriction. Am J Obstet Gynecol 1997;177:156–61.

15. Oedegard R, Vatten L, Nilsen ST, Salvesen KÅ, Austgulen R. Preeclampsia and fetal growth. Obstet Gynecol 2000;96:950–5.
16. Brazy JE, Grimm JK, Little VA. Neonatal manifestations of severe maternal hypertension occurring before the thirty-sixth week of pregnancy. J Pediatr 1982;100:265–71.
17. Khong TY, DeWolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986;93: 1049–59.
18. Kingdom JC, Kaufmann P. Oxygen and placental vascular development. In: Roach RC, Wagner PD, Hackett PH, eds. Hypoxia: Into the next millenium. 4th ed. New York: Kluwer Academic, 1999:259–75.
19. Todros T, Sciarrone A, Piccoli E, Guiot C, Kaufmann P, Kingdom J. Umbilical Doppler waveforms and placental villous angiogenesis in pregnancies complicated by fetal growth restriction. Obstet Gynecol 1999;93:499–503.
20. Macara L, Kingdom JC, Kaufmann P, Kohnen G, Hair J, More IA, et al. Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. Placenta 1996;17:37–48.
21. Salafia C, Shiverick K. Cigarette smoking and pregnancy II: Vascular effects. Placenta 1999;20:273–9.
22. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie NE, Irgens LM. Fetal and maternal contributions to risk of pre-eclampsia: Population based study. BMJ 1998;316: 1343–7.
23. Oedegard R, Vatten L, Nilsen ST, Salvesen KÅ, Austgulen R. Risk factors and clinical manifestations of pre-eclampsia. Br J Obstet Gynaecol 2000;107:1410–6.
24. CLASP: A randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative Group. Lancet 1994;343:619–29.
25. Moore MP, Redman CW. Case-control study of severe pre-eclampsia of early onset. Br Med J Clin Res Ed 1983; 287:580–3.
26. Cnattingeus S, Haglund B, Kramer MS. Differences in late fetal death rates in association with determinants of small for gestational age fetuses; population based cohort. BMJ 1998;316:1483–7.
27. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85: 843–8.
28. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. Eur J Immunol 1987;17:1411–6.
29. Tada H, Shiho O, Kuroshima K, Koyama M, Tsukamoto K. An improved colorimetric assay for interleukin 2. J Immunol Methods 1986;93:157–65.
30. Stallmach T, Hebisch G, Joller H, Kolditz P, Engelmann M. Expression pattern of cytokines in the different compartments of the feto-maternal unit under various conditions. Reprod Fertil Dev 1995;7:1573–80.
31. Kashlan F, Smulian J, Shen-Schwarz S, Anwar M, Hiatt M, Hegyi T. Umbilical vein interleukin 6 and tumor necrosis factor alpha plasma concentrations in the very preterm infant. Pediatr Infect Dis J 2000;19:238–43.
32. Laham N, Brennecke SP, Bendtzen K, Rice GE. Differential release of interleukin-6 from human gestational tissues in association with labour and in vitro endotoxin treatment. J Endocrinol 1996;149:431–9.
33. Hata T, Kawamura T, Inada K, Fujiwaki R, Ariyuki Y, Hata K, et al. Cord blood cytokines and soluble adhesion molecules in vaginal and cesarean delivered neonates. Gynecol Obstet Invest 1996;42:102–4.
34. Matsuzaki N, Taniguchi T, Shimoya K, Neki R, Okada T, Saji F, et al. Placental interleukin-6 production is enhanced in intrauterine infection but not in labor. Am J Obstet Gynecol 1993;168:94–7.
35. Spinillo A, Capuzzo E, Piazzi G, Nicola S, Colonna L, Iasci A. Maternal high-risk factors and severity of growth deficit in small for gestational age infants. Early Hum Dev 1994; 38:35–43.
36. Miyano A, Miyamichi T, Nakayama M, Kitajima H, Shimizu A. Effect of chorioamnionitis on the levels of serum proteins in the cord blood of premature infants. Arch Pathol Lab Med 1996;120:245–8.
37. Kauma SW, Wang Y, Walsh SW. Preeclampsia is associated with decreased placental interleukin-6 production. J Soc Gynecol Investig 1995;2:614–7.

Address reprint requests to: Rønnaug A. Ødegård, MD, Institute of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, University Medical Center, N-7489 Trondheim, Norway; E-mail: ronnaug.odegard@medisin.ntnu.no.

Received December 4, 2000. Received in revised form March 9, 2001. Accepted March 14, 2001.

Paper IV

Umbilical cord plasma leptin is increased in preeclampsia

Rønnaug A. Ødegård, MD,[a] Lars J. Vatten, MD, PhD,[b] Stein Tore Nilsen, MD, PhD,[c] Kjell Å. Salvesen, MD, PhD,[d] and Rigmor Austgulen, MD, PhD[a]

Trondheim and Stavanger, Norway

OBJECTIVE: The objective of this study was to compare umbilical cord plasma leptin between infants of mothers who experienced preeclampsia and infants of control subjects and to study the relation between cord plasma leptin and infant obesity, as indicated by ponderal index.

STUDY DESIGN: On the basis of a population of approximately 13,000 deliveries, we compared cord plasma leptin from preeclamptic (n = 256 women) and control pregnancies (n = 607 women) after taking the differences in gestational age and ponderal index into account.

RESULTS: Cord plasma leptin increased strongly with gestational age, both in the preeclampsia group and the control subjects ($P < .01$), but at each gestational age the preeclampsia group had higher leptin levels than control subjects ($P < .01$). Adjustment for the higher ponderal index among control subjects ($P < .05$) did not alter the difference in leptin levels between the groups.

CONCLUSION: We found higher levels of umbilical cord plasma leptin in infants of mothers who had preeclampsia (compared with infants of control subjects) after adjusting for differences in gestational age, gender, and infant ponderal index. (Am J Obstet Gynecol 2002;186:427-32.)

Key words: Leptin, preeclampsia, ponderal index, cord blood

Leptin, the product of the obesity gene,[1] is a hormone mainly expressed in adipocytes. Through a negative feedback mechanism between adipose tissue and hypothalamic centers, leptin may contribute to the regulation of obesity by inducing satiety and may stimulate energy expenditure at the expense of storage.[2] The regulation of leptin is not fully understood, but a covariation with insulin is documented.

Circulating leptin levels reflect body fat contents, and the association with insulin has linked leptin to the insulin-resistance syndrome, which includes obesity, glucose intolerance, and dyslipoproteinemia.[3] In addition to its relation to obesity, leptin may stimulate maturation of the reproductive axis. There is a gender difference in leptin, with higher concentrations in females than in males.[4]

In pregnancy, it has been shown that leptin is highly expressed in the placenta.[5] The protein has been detected in umbilical cord blood from week 18 of gestation, followed by increased levels from the middle of the third trimester toward term.[6] This increase coincides with the development of fetal adipose tissue, and the results from some previous studies indicate that cord blood leptin is positively correlated with fetal adiposity at birth.[6-8] Compared with later in life, however, leptin levels are much higher in umbilical cord blood than the weight of the fetus would indicate,[9] and it has been hypothesized that the high leptin concentrations are required to mobilize fat stores to meet the energy demands of the newborn.[9,10]

Preeclampsia increases fetal risk of being born small for gestational age, particularly in cases of early and recurrent disease.[11] The growth-retarded infants exhibit wasting of subcutaneous fat, and one would therefore expect lower leptin levels in umbilical blood from preeclamptic than from normotensive pregnancies. However, 1 previous small study found no difference in cord leptin levels between cases of preeclampsia and controls with delivery at term.[12] Preeclampsia is associated with maternal obesity, and maternal levels of circulating leptin appear to be increased in preeclamptic compared with normotensive pregnancies.[12,13] Because leptin may induce metabolic and circulatory changes that are characteristic of preeclampsia,[14] it has been suggested that leptin may play a role in the pathogenesis of preeclampsia.[12,13]

The main aims of the present study were to compare umbilical cord plasma levels of leptin between pregnancies with preeclampsia and normotensive control pregnancies and to assess the relation between fetal adiposity at birth and leptin levels. The study was based on a population of approximately 13,000 consecutive births; we in-

From the Institute of Cancer Research and Molecular Biology,[a] and the Institute of Community Medicine and General Practice, Norwegian University of Science and Technology[b]; the Department of Obstetrics and Gynecology, Rogaland Central Hospital[c]; and the National Center for Fetal Medicine, University Hospital of Trondheim.[d]

Supported by the Norwegian Research Council, the Norwegian Cancer Society, and the Norwegian University of Science and Technology.

Received for publication February 22, 2001; revised September 7, 2001; accepted September 26, 2001.

Reprints not available from the authors.

0002-9378/2002 $35.00 + 0 ***6/1/120486***

doi:10.1067/mob.2002.120486

Table I. Maternal and fetal characteristics

Characteristic	*With preeclampsia (n = 256)*	*Control subjects (n = 607)*
Maternal age (y)*	26.9 ± 4.6†	28.2 ± 4.9
Nulliparous (%)	64†	36
Smoking (%)	18†	27
Cesarean section (%)	28†	5
Steroids (%)	12†	1
Gestational age (days)*	266 ± 22†	280 ± 11
Female gender (%)	51	50
Ponderal index (birth weight [g] · 100/length [cm]^3)*	2.73 ± 0.32†	2.87 ± 0.27
Birth weight (g)*	3085 ± 848†	3599 ± 491
Cord plasma leptin levels (ng/mL)‡	8.0 (10.0)	7.5 (8.9)

*Values are given as mean ± SD.
†$P < .05$, compared with control subjects.
‡Values are given as median (interquartile range).

cluded 256 cases of preeclampsia and 607 control subjects in the analyses.

Material and methods

Umbilical cord blood samples were collected in a prospective study of pregnancy outcome that took place from January 1993 to December 1995 at Rogaland Central Hospital in Stavanger, Norway. The birthing clinic at this hospital serves exclusively a region of approximately 239,000 inhabitants; there were 12,804 deliveries during the study period.

The Norwegian Medical Birth Registry records information on all deliveries that take place in Norway; we searched the records to identify potential cases of preeclampsia and to select population control subjects, as previously described.[11] Information from the Birth Registry was verified and supplemented with details from hospital records, and we identified 307 singleton pregnant women who fulfilled the diagnostic criteria for preeclampsia. After cases with culture-proven neonatal sepsis (n = 1 case) and maternal diabetes mellitus (n = 4 cases) were excluded, umbilical cord blood was available from 256 cases with preeclampsia. We used a definition of preeclampsia that has been reported previously[15] (ie, persistent diastolic blood pressure of at least 90 mm Hg had to develop after 20 weeks of gestation, and diastolic blood pressure had to increase by at least 25 mm Hg). In addition, proteinuria had to be present, and cut-off was defined as 0.3 mg/L (semiquantitative dipstick, 1+) in at least 1 urine sample after 20 weeks of gestation without simultaneous urinary infection. Preeclampsia was classified as severe if the diastolic blood pressure increased to at least 110 mm Hg, along with proteinuria 3+ on dipstick, or at least 500 mg/24 hours (n = 66 cases). All other cases of preeclampsia were classified as mild (n = 190 cases). For comparison, the Medical Birth Registry selected 2 groups of women without preeclampsia who gave birth at the hospital during the same period, as previously described.[11] In 1 group, control women gave birth subsequent to women with preeclampsia, whereas the other control group was randomly selected, but matched to preeclampsia cases on maternal age. However, the use of each control group yielded nearly identical results, and we therefore pooled the groups to gain statistical power. Among 619 women without preeclampsia who were selected initially, we obtained cord blood from 607 women, after excluding subjects with culture-proven neonatal sepsis (n = 1 subject) and maternal diabetes mellitus (n = 1 subject).

Information on maternal smoking was obtained at ultrasound screening at 18 weeks of gestation, whereas all other baseline data were obtained at the first maternal visit around 12 weeks of pregnancy. Infant data were collected from hospital records after the infant was discharged from the hospital. Blood samples were collected passively from the placental side of the umbilical cord after delivery, in syringes that contained heparin. The samples were chilled to 4°C for as long as 60 hours before being centrifuged at 3000 rpm for 15 minutes; the plasma was stored at –80°C until it was analyzed.

Gestational age at birth was calculated from routine ultrasonographic measurements of biparietal diameter at 18 weeks of gestation, according to Norwegian standard curves. Ponderal index was calculated as a measure of neonatal adiposity, as birth weight divided by the cubed value of birth length by the equation

$$\frac{(\text{birth weight [g]} \cdot 100)}{\text{length (cm)}^3}$$

In Table I, we have described some characteristics of the study groups.

Assay. Leptin level in umbilical cord plasma was measured by a competitive radio immunoassay (Linco Research, St. Charles, Minn) with the use of recombinant ^{125}I-leptin as tracer. All samples were analyzed in duplicate, and the detection limit of the assay was 0.4 ng/mL. Leptin was detected in all but 4 samples. The intra-assay coefficient of variation was always <10%, and the interassay variation was <11% for leptin values in the range between 19.5 and 2.2 ng/mL.

Table II. Cord plasma leptin by infant gender

	With preeclampsia		*Control subjects*		
Gender	*n*	*Leptin level (ng/mL)†*	*n*	*Leptin level (ng/mL)†*	*P value**
Female	115	9.8 (12.2)	299	9.5 (9.8)	.007
Male	111	6.6 (8.0)	302	5.2 (6.9)	.002

*Comparison between infants of mothers with preeclampsia and of control subjects among female and male infants separately, adjusted for gestational age by multiple linear regression analyses.
†Median (interquartile range).

Table III. Cord plasma leptin levels according to fetal ponderal index

	Preeclampsia			*Control subjects*		
*Quartiles of ponderal index**	*n*	*Leptin level (ng/mL)†*	*P trend‡*	*n*	*Leptin level (ng/mL)†*	*P trend‡*
Overall	223§	8.0 (10.0)		601	7.5 (8.9)	
<2.69	73	5.2 (8.7)		146	5.0 (6.2)	
2.69-2.85	58	8.6 (9.6)		152	7.2 (7.3)	
2.86-3.04	56	9.6 (14.7)		153	7.8 (9.4)	
>3.05	36	11.3 (11.9)	<.01	150	10.7 (10.8)	<.01

*Equation: (weight × 100)/length^3.
†Median (interquartile range).
‡Test for linear trend of leptin levels across ordinal categories of ponderal index, adjusted for gestational age and gender by multiple linear regression analyses.
§Numbers do not add up to total because of missing values.

Statistical analyses. Leptin had a skewed distribution and was therefore expressed as the median value in the Tables, and transformed to natural logarithm when included in the statistical analyses. The Student *t* test was used to compare continuous variables between groups; differences between proportions were assessed by chi-square tests. We calculated quartile levels of ponderal index on the basis of the distribution in the control population. Within the groups, we tested the linear association (presented as P for trend) of leptin across quartiles of ponderal index and adjusted for gestational age and gender by multiple linear regression analysis. We calculated the ratio between transformed to natural logarithm leptin and ponderal index and compared this ratio between the groups after adjusting for gestational age and gender by multiple linear regression analyses. All statistical analyses were calculated with the Statistical Package for the Social Science (SPSS), version 10.05 (SPSS, Inc, Chicago, Ill).

Results

Umbilical cord plasma leptin levels increased strongly with increasing gestational age (Fig 1), both in women with preeclampsia and among control subjects ($P < .01$ for both groups). Among control subjects, only 6 infants were born before 34 weeks of gestation, therefore subsequent analyses were restricted to pregnancies with duration B34 weeks.

Before the differences in gestational age were taken into account, there was no clear difference in cord plasma leptin between the preeclampsia group and control subjects (8.0 ng/mL [interquartile range, 10.0 ng/mL] vs 7.5 ng/mL [interquartile range, 8.9 ng/mL]; Table I). Infants in the preeclampsia group were, however, born at earlier gestation than the infants in the control group (266 vs 280 days; Table I). After adjustment for gestational age, umbilical cord leptin levels were higher in the preeclampsia group ($P < .01$), but additional adjustment for maternal smoking, mode of delivery, gender, and prenatal administration of steroids did not influence the results (data not shown). Furthermore, cases with mild and severe preeclampsia showed no clear difference in cord blood leptin levels when we adjusted for differences in ponderal index, gestational age, and gender between groups (data not shown).

Female newborns had higher cord plasma leptin levels than male newborns, both within the preeclampsia group (9.8 vs 6.6 ng/mL; $P < .01$) and among the control group (9.5 vs 5.2 ng/mL; $P < .01$). In Table II, we compare cord plasma leptin levels between the preeclampsia group and the control group for each gender separately; the results show that leptin levels were higher in preeclampsia for both genders ($P < .01$).

The ponderal index was lower in the preeclampsia group than in the control group (2.73 vs 2.87; $P < .05$; Table I). However, in both groups, there was a consistent increase in leptin levels with an increasing ponderal index. This trend was also present after gender and gestational age were taken into account (both *P* trend < .01; Table III). To study the relation between leptin and pon-

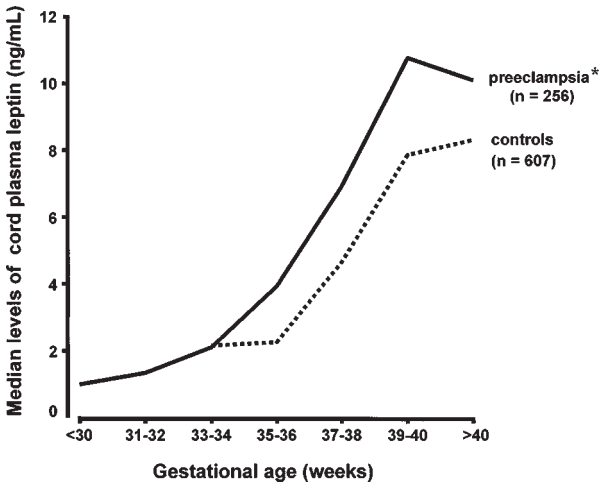

Fig 1. Cord plasma leptin by gestational age in infants of mothers with preeclampsia and among infants of control subjects. *The comparison between groups is adjusted for differences in gestational age by multiple linear regression analyses ($P > .01$).

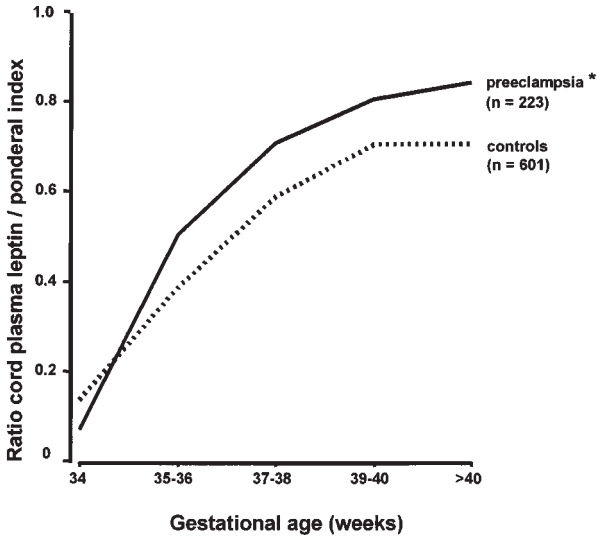

Fig 2. The ratio between cord plasma leptin and ponderal index by gestational age in infants of mothers with preeclampsia and among infants of control subjects. *The comparison between groups is adjusted for differences in gestational age and gender by multiple linear regression analyses ($P > .01$).

deral index further, we calculated the ratio between cord plasma leptin and ponderal index and displayed this relation graphically by gestational age (Fig 2). Fig 2 shows that, at each level of gestational age, the ratio was higher in the preeclampsia group than in the control group ($P < .01$), except at 34 weeks of gestation at which time no clear difference between groups was observed. At this gestational age, however, there was a higher proportion of male fetuses in the preeclampsia group (63%) than in the control group (46%); because cord plasma leptin levels were generally higher in the female fetuses, this disparity could explain the lack of difference in the ratios between leptin and ponderal index at early gestation.

Comment

Our study shows that umbilical cord plasma leptin levels at birth are higher in infants of women with preeclampsia than among control subjects. For both groups, we found a strong increase in cord plasma leptin levels with increasing gestational age at birth, but the preeclampsia group had slightly, but consistently, higher levels of umbilical leptin than the control group at each gestational age. These results are in contrast to 1 previous study that reported no increase in cord blood leptin level in cases with preeclampsia who were born at term, compared with control subjects.[12] The study was, however, small, and the comparison was not adjusted for potential confounding by differences in ponderal index and sex between groups.

In addition to gestational age, we also adjusted for other factors that could potentially disturb the relation with leptin between the preeclampsia and control groups. However, further adjustment for the lower frequency of maternal smoking and vaginal delivery in the preeclampsia group did not materially alter the results. Furthermore, a positive association between the prenatal administration of steroids and cord blood leptin level has been reported.[8] Therefore, we included this factor in the multivariate analyses, but the higher level of cord plasma leptin in preeclampsia was not explained by the more frequent administration of prenatal steroids in this group.

Some previous studies have found substantially higher cord plasma leptin levels among female newborns compared with male newborns,[9,16] whereas other studies have failed to show any difference by gender.[17] Compared with previous studies, our study included a larger number of participants, and our results suggest that the gender dimorphism in circulating leptin is likely to be present already at birth. It has been shown that testosterone may suppress leptin synthesis and release from adipocytes,[18] but at birth, circulating androgens do not vary much by gender.[19] This suggests that factors other than androgens may be responsible for the higher umbilical leptin levels among female offspring.

If fetal adipocytes contribute to cord blood leptin levels, sex differences in fat deposition may constitute 1 such factor. One large study observed no sex differences in ponderal index at birth but reported increased depositions of subcutaneous fat, as measured by subscapular skinfold thickness in female offspring.[20] Therefore, the statistical adjustment for ponderal index in our study may not be sufficient to account for differences in body fat depositions between male and female infants, and this is a shortcoming of our study.

The consistent sex dimorphism in cord plasma leptin may support the hypothesis that the fetus contributes to the production of the leptin that is detected in umbilical cord blood. It is, however, difficult to attribute the higher cord leptin levels in preeclampsia to fetal production, particularly because infants in the preeclampsia group had lower ponderal indices than did the control infants. On the other hand, the expression of leptin is increased in placental explants from preeclamptic pregnancies; in trophoblast cell lines, hypoxia may stimulate the expression of leptin.[21] Preeclampsia may be accompanied by reduced placental perfusion and subsequent placental hypoxia; thereby the higher cord plasma leptin levels that were observed in preeclampsia may be of placental origin. Leptin may induce lipolysis and gluconeogenesis[2]; in preeclampsia, cord plasma hyperleptinemia may provide substrates to maintain threatened cellular functions at the expense of fetal body fat contents.

Maternal circulating levels of leptin appear to be increased in preeclampsia[12,13,22] and may be correlated positively to fetal leptin levels.[12] Placental contribution of leptin to both compartments could explain this correlation, but so could also a disrupted placental barrier. If maternal leptin were transported to the fetal circulation, this could produce a positive correlation between maternal and fetal leptin.[12]

Hyperleptinemia is 1 component of the insulin resistance syndrome, which also includes glucose intolerance, dyslipoproteinemia, and obesity.[3] Because maternal obesity strongly increases the risk of the development of preeclampsia, it has been hypothesized that insulin resistance could be a risk factor for preeclampsia.[12,13] Implicit in this hypothesis was the suggestion that high levels of maternal leptin before pregnancy also may increase the risk of the development of preeclampsia. The results of some studies may support this hypothesis; 1 study showed that maternal leptin levels were higher in pregnant women who later had preeclampsia,[22] and another small study found higher puerperal levels of leptin after preeclamptic pregnancies, although this was statistically insignificant.[13] Our study was not designed to answer this question, but the high umbilical leptin levels that we observed in preeclampsia could be interpreted as a consequence of high maternal leptin levels being an indicator of increased risk of the development of preeclampsia.

Preeclampsia is a strong risk factor for fetal growth restriction, and some observations indicate that infants with intrauterine growth restriction have increased risk of cardiovascular diseases that are related to obesity and insulin resistance later in life.[23] These findings have been interpreted as a consequence of restricted growth in utero that could have imprinted unfavorable metabolic patterns.[24,25] If intrauterine leptin is an important determinant for the set point of leptin later in life, one could speculate that the higher levels of umbilical leptin in preeclampsia might be related to the development of insulin resistance and cardiovascular disease in adulthood.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425-32.
2. Rohner-Jeanrenaud F. Neuroendocrine regulation of nutrient partitioning. Ann N Y Acad Sci 1999;892:261-71.
3. Zimmet P, Boyko EJ, Collier GR, de Courten M. Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. Ann N Y Acad Sci 1999;892:25-44.
4. Hassink SG, Sheslow DV, de Lancey E, Opentanova I, Considine RV, Caro JF. Serum leptin in children with obesity: relationship to gender and development. Pediatrics 1996;98:201-3.
5. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med 1997;3: 1029-33.
6. Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. J Clin Endocrinol Metab 1998;83:1243-6.
7. Geary M, Pringle PJ, Persaud M, Wilshin J, Hindmarsh PC, Rodeck CH, et al. Leptin concentrations in maternal serum and cord blood: relationship to maternal anthropometry and fetal growth. Br J Obstet Gynaecol 1999;106:1054-60.
8. Shekhawat PS, Garland JS, Shivpuri C, Mick GJ, Sasidharan P, Pelz CJ, et al. Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. Pediatr Res 1998;43:338-43.
9. Helland IB, Reseland JE, Saugstad OD, Drevon CA. Leptin levels in pregnant women and newborn infants: gender differences and reduction during the neonatal period. Pediatrics 1998;101:E12.
10. Schubring C, Siebler T, Kratzsch J, Englaro P, Blum WF, Triep K, et al. Leptin serum concentrations in healthy neonates within the first week of life: relation to insulin and growth hormone levels, skinfold thickness, body mass index and weight. Clin Endocrinol 1999;51:199-204.
11. Odegard RA, Vatten LJ, Nilsen ST, Salvesen KA, Austgulen R. Preeclampsia and fetal growth. Obstet Gynecol 2000;96:950-5.
12. McCarthy JF, Misra DN, Roberts JM. Maternal plasma leptin is increased in preeclampsia and positively correlates with fetal cord concentration. Am J Obstet Gynecol 1999;180:731-6.
13. Laivuori H, Kaaja R, Koistinen H, Karonen SL, Andersson S, Koivisto V, et al. Leptin during and after preeclamptic or normal pregnancy: its relation to serum insulin and insulin sensitivity. Metabolism 2000;49:259-63.
14. Haynes WG. Interaction between leptin and sympathetic nervous system in hypertension. Curr Hypertens Rep 2000;2:311-8.
15. Redman CW. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women: CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative. Lancet 1994;343:619-29.
16. Hassink SG, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, et al. Placental leptin: An important new growth factor in intrauterine and neonatal development? Pediatrics 1997;100:E1.
17. Schubring C, Kiess W, Englaro P, Rascher W, Dotsch J, Hanitsch S, et al. Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. J Clin Endocrinol Metab 1997;82:1480-3.
18. Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest 1997;100:808-13.
19. Matsuda J, Yokota I, Iida M, Murakami T, Naito E, Ito M, et al. Serum leptin concentration in cord blood: relationship to birth weight and gender. J Clin Endocrinol Metab 1997;82:1642-4.

20. Guihard-Costa AM, Grange G, Larroche JC, Papiernik E. Sexual differences in anthropometric measurements in French newborns. Biol Neonate 1997;72:156-64.
21. Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H, et al. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. J Clin Endocrinol Metab 1998;83:3225-9.
22. Anim-Nyame N, Sooranna SR, Steer PJ, Johnson MR. Longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia. Hum Reprod 2000; 15:2033-6.
23. Barker DJ. The intrauterine environment and adult cardiovascular disease. Ciba Foundation Symposium 1991;156:3-10.
24. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C. Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab 2000;85:1401-6.
25. Martyn CN, Hales CN, Barker DJ, Jespersen S. Fetal growth and hyperinsulinemia in adult life. Diabet Med 1998;15:688-94.

Paper V

Relationship of Insulin-Like Growth Factor-I and Insulin-Like Growth Factor Binding Proteins in Umbilical Cord Plasma to Preeclampsia and Infant Birth Weight

Lars J. Vatten, MD, PhD, Rønnaug A. Ødegård, MD, Stein Tore Nilsen, MD, PhD, Kjell Å. Salvesen, MD, PhD, and Rigmor Austgulen, MD, PhD

OBJECTIVE: To determine whether preeclampsia influences insulin-like growth factor-I (IGF-I), insulin-like growth factor binding protein-1 (IGFBP-1), and insulin-like growth factor binding protein-3 (IGFBP-3), independent of its effect on birth weight.

METHODS: Cord blood was collected in 12,804 consecutive deliveries. We identified 258 preeclamptic pregnancies that were subclassified as mild or severe and early or late. For comparison, 609 control pregnancies were selected. Fetal growth was expressed as the ratio between observed and expected birth weight, with adjustment for gestational age at birth. IGF-I, IGFBP-1, and IGFBP-3 were measured in umbilical plasma. The contribution of preeclampsia and birth weight to each measured factor was assessed by multiple linear regression analyses.

RESULTS: Between mild preeclampsia and controls, there were no differences in IGF-I, IGFBP-1, and IGFBP-3. In severe and early onset preeclampsia, umbilical cord plasma IGF-I was approximately 50% lower, and IGFBP-1 was more than twice as high as in controls (both $P < .01$). At each birth weight level, IGF-I was lower and IGFBP-1 was higher in severe or early preeclampsia than among controls of similar weight. Birth weight and preeclampsia were, independent of each other, associated with IGF-I, whereas birth weight, but not preeclampsia, was associated with IGFBP-1, after adjustment for gestational age.

CONCLUSION: Fetal growth restriction caused by severe or early preeclampsia is associated with lower umbilical levels of IGF-I than low birth weight caused by other conditions. Preeclampsia may contribute to the observed IGF-I reduction, either as part of the underlying causes of preeclampsia, or as a consequence of the disease. (Obstet Gynecol 2002;99:85–90. © 2002 by the American College of Obstetricians and Gynecologists.)

Insulin-like growth factor-I (IGF-I) is a mitogenic polypeptide that stimulates cellular proliferation and differentiation.[1] The strong positive correlation between umbilical cord IGF-I and birth weight indicates its importance for fetal growth.[1–4] Thus, IGF-I is expressed by fetal organs,[5] membranes,[6] and by the placenta.[6–9] The function of IGF-I is modulated by six binding proteins with high affinity (IGFBPs). The smaller, such as IGFBP-1, may be responsible for the transfer of IGF-I from the circulation to the extracellular space, whereas the larger IGFBP-3 binds 95% of IGF-I and provides a reservoir for IGF-I in the circulation.[1,9] IGFBP-1 usually inhibits the effects of IGF-I at the cellular level,[9] but is also related to cell growth independent of IGF-I.[9] In pregnancy, the production of IGFBP-1 is strongly increased,[9] and abnormally high levels of IGFBP-1 have been found in umbilical[10–16] blood in conjunction with fetal growth restriction, whereas umbilical IGFBP-3 may be lower in infants born small for their gestational age.[17] It has been hypothesized that inadequate nutrition of the fetus will stimulate production of IGFBP-1 and inhibit the effect of IGF-I.[18] A combination of high umbilical levels of IGFBP-1 and low IGF-I could, therefore, reflect an adaptive response to an intrauterine environment that cannot offer the fetus optimal conditions for growth.[18]

Preeclampsia is a heterogeneous syndrome, with varying effects on fetal growth. In mild cases, fetal growth is usually appropriate,[7] whereas fetal growth restriction is commonly observed in severe preeclampsia or in preeclampsia with early onset.[7] These subtypes of preeclampsia are characterized by abnormally shallow decidual trophoblast invasion, hypoxia, and reduced

From the Department of Community Medicine and General Practice, Department of Cancer Research and Molecular Biology, and Department of Obstetrics and Gynecology, Norwegian University of Science and Technology, Trondheim, Norway; and Department of Obstetrics and Gynecology, Rogaland Central Hospital, Stavanger, Norway.

This study was financially supported by the Norwegian Medical Research Council and the Norwegian Cancer Society.

The authors thank the Norwegian Medical Birth Registry for assistance.

0029-7844/02/$22.00
PII S0029-7844(01)01651-9

uteroplacental blood flow.[7,8,19] A few studies have reported lower IGF-I and IGFBP-3 and higher IGFBP-1 in umbilical cord blood levels from preeclamptic pregnancies complicated by low birth weight.[15,20] One study found that alterations in IGF-I and IGFBP-1 were more pronounced in preeclampsia than could be expected from the smaller size of the offspring,[20] suggesting that preeclampsia may contribute with an effect independent of the relation to birth weight. In this large case-control study, we wanted to find out whether preeclampsia is associated with alterations in IGF-I and its binding proteins IGFBP-1 and IGFBP-3, independent from changes attributed to reduced birth weight.

Table 1. Descriptive Data for Severe and Mild Preeclampsia and for Controls

	Preeclampsia		
	Severe (n = 67)	Mild (n = 191)	Controls (n = 609)
Maternal age (y)*	26.4 (5.0)	27.2 (4.4)	28.3 (4.9)
Nulliparous (n)	70% (47)	63% (125)	36% (216)
Maternal smoking (n)	16% (11)	19% (37)	27% (154)
Cesarean (n)	54% (36)	20% (38)	6% (36)
Gestational age (d)*	249 (28)	270 (19)	280 (11)
Fetal growth restriction (n)	17% (11)	8% (15)	3% (18)

* Mean with standard deviation.

MATERIALS AND METHODS

Umbilical cord blood samples were collected in a prospective study of pregnancy outcome that took place from January 1993 to December 1995 at Rogaland Central Hospital in Stavanger, Norway. The maternity clinic at this hospital serves exclusively a region of approximately 239,000 inhabitants, and in all, 12,804 deliveries took place during the study period. The Norwegian Medical Birth Registry records information on all deliveries that take place in the country,[21] and we used this information to identify potential cases of preeclampsia and to select population controls, as previously described.[22] The study was approved by the regional committee for ethics in medical research and by the Norwegian Data Inspectorate.

From the Medical Birth Registry, we initially identified approximately 1300 cases with clinical information that might indicate preeclampsia. After verifying and supplementing this information with details from the hospital records, we identified 307 singleton pregnant women with certain preeclampsia, and umbilical cord blood was available from 258 of these women. We used a previously described definition of preeclampsia in this study.[23] Briefly, for preeclampsia to be diagnosed, persistent diastolic blood pressure of at least 90 mm Hg had to develop after 20 weeks of gestation, and diastolic blood pressure had to increase by at least 25 mm Hg. In addition, proteinuria had to be present, and cut-off was defined as 0.3 mg/L (semiquantitative dipstick 1+) in at least one urine sample after 20 weeks of gestation without simultaneous urinary infection.

Preeclampsia was classified as severe (n = 67) if diastolic blood pressure increased to at least 110 mm Hg, along with proteinuria 3+ on dipstick, or at least 500 mg per 24 hours. Cases with eclampsia and suspected hemolysis, elevated liver enzymes, low platelets syndrome were also regarded as severe preeclampsia, whereas all other cases of preeclampsia were classified as mild (n = 191).

For comparison, two women without preeclampsia were selected per case of preeclampsia from the cohort of birthing women at the Rogaland Central Hospital, as previously described.[22] Among 619 women without preeclampsia initially selected, cord blood was available from 609. For the whole study population, information on baseline data were obtained at around 12 weeks of pregnancy, at the first maternal visit. All infant data were collected from hospital records. In Table 1, we have described some characteristics of the groups.

Blood samples were collected in syringes from the placental side of the umbilical cord after delivery. The centrifugation syringes contained heparin, and all blood samples were chilled to 4C up to 60 hours before centrifugation at 3000 revolutions per minute for 15 minutes. Plasma was stored at −80C until analyzed.

Birth weight was standardized as the ratio between the observed and expected birth weight, where the expected birth weight was adjusted for offspring gender and gestational age at birth. We used standards of expected birth weights derived from the results of weight curves based on ultrasonographic measurements in a large Scandinavian population.[24] Gestational age at birth was calculated from routine ultrasonographic measurements at 18 weeks' gestation. In tables and text, standardized weight is expressed as the mean value with 95% confidence intervals. Small-for-gestational-age (SGA) was defined as an observed birth weight two standard deviations or more below the expected, which corresponds to a ratio lower than 0.76, or to a birth weight reduction of approximately 840 g for a term infant.

Cord plasma IGF-I and IGFBP-3 were assayed by commercially available radioimmunoassay kits (Mediagnost, Tuebingen, Germany). All samples were run in duplicates, and all procedures were run as suggested by the producer, except that we used half volumes. IGF-I and

IGFBP-3 were detected in all plasma samples, and detection limits were 4.8 ng/mL and 370 ng/mL, respectively. Cord plasma IGFBP-1 was assayed by a commercially available enzyme immunoassay (Mediagnost, Tuebingen, Germany), and single samples were analyzed. The detection limit of the assay was 4.6 ng/mL, and IGFBP-1 was detected in all but one sample. The three assays were run in 11 sequences, and for all three, the intra-assay variation was on average less than 4%. The intra-assay coefficients of variation for IGF-I, IGFBP-3, and IGFBP-1 were 12%, 10%, and 16%, respectively.

For the IGF-I analyses, plasma samples were available from 609 controls and 191 cases of mild and 67 cases of severe preeclampsia. For the IGFBP-1 analyses, plasma samples were available from 604 controls and 190 cases of mild and 66 cases of severe preeclampsia. For the IGFBP-3 analyses, plasma samples were available from 601 controls and 190 cases of mild and 65 cases of severe preeclampsia.

IGFBP-1 had a skewed distribution and was, therefore, expressed as the median value (ng/mL, interquartile range). Student t test and Mann-Whitney U test were used to compare continuous variables between groups. Differences between proportions were assessed by χ^2 tests. The standardized birth weight was divided into four clinical categories: <0.76 corresponded to a strict definition of SGA, and 0.76–0.89 was a broad category of relatively small infants. The category 0.90–1.09 included infants with appropriate weight for their gestational age, and the category >1.09 included large babies. For each level of birth weight, we estimated values of IGF-I, IGFBP-1, and IGFBP-3 between the preeclampsia group and controls, and tested the linear association (yielding a P value for trend) across birth weight categories for each of the three components of the IGF system in multiple regression analyses. We also assessed the independent contribution of birth weight and preeclampsia to levels of IGF-I and IGFBP-1, and adjusted for gestational age, using multiple regression analyses. All statistical analyses were calculated using the Statistical Package for the Social Sciences 10.05 (SPSS, Inc., Chicago, IL).

RESULTS

Overall (Table 2), the severe preeclampsia group had lower levels of IGF-I ($P < .01$) and IGFBP-3 ($P < .05$) in umbilical cord plasma than controls. For IGFBP-1, the severe preeclampsia group had values two times higher than controls ($P < .01$). The measured values varied only modestly with length of gestation. It is, therefore, unlikely that the differences between the groups can be attributed to differences in gestational age at birth. For all three factors, the results for mild preeclampsia did not significantly differ from those of controls (Table 2).

Table 2. Umbilical Cord Plasma (in ng/mL) IGF-I, IGFBP-1, and IGFBP-3 in Cases of Severe and Mild Preeclampsia and in Controls

	Preeclampsia		
	Severe (n = 67)	Mild (n = 191)	Controls (n = 609)
IGF-I (SD)	39* (21)	60 (25)	64 (27)
IGFBP-1 (IQR)	217* (442)	95 (142)	97 (119)
IGFBP-3 (SD)	1241* (395)	1426 (608)	1376 (435)

IGF-I = insulin-like growth factor-I; IGFBP-1 = insulin-like growth factor binding protein-1; IGFBP-3 = insulin-like growth factor binding protein-3; SD = standard deviation; IQR = interquartile range.
* $P < .01$, compared with controls.

Table 3 shows that the most dramatic differences between the groups can be attributed to severe preeclampsia with early onset of symptoms (34 weeks' gestation and earlier). In the "early onset" group, IGFBP-1 (median 611 ng/mL) was more than six times higher than in controls (97 ng/mL). Compared with "late onset" preeclampsia, the median value in the "early onset" group was four times higher ($P < .01$). Nonetheless, in severe preeclampsia with late onset, IGF-I and IGFBP-1 were still significantly different from controls (both $P < .01$).

In Table 4, IGF-I and the binding proteins IGFBP-1 and IGFBP-3 were related to predefined categories of standardized birth weight, and adjusted for gestational age and offspring gender. There was a consistent decrease in IGF-I from the largest to the smallest babies (SGA), both within the severe preeclampsia group and among controls (both P for trend $< .01$), with more than a two-fold difference in IGF-I between the highest and the lowest categories of birth weight. For each level of

Table 3. Umbilical Cord Plasma IGF-I, IGFBP-1, and IGFBP-3 and Standardized Birth Weight in Severe Preeclampsia With Early (34 Weeks' Gestation or Less) or Late Onset and in Controls

	Severe preeclampsia		
	Early onset (<35 wk) (n = 21)	Late onset (n = 46)	Controls (n = 609)
IGF-I (ng/mL)	26*	45*	64
IGFBP-1 (ng/mL)	611*	151*	97
IGFBP-3 (ng/mL)	1216	1253	1376
Standardized birth weight†	0.80	0.94	1.00
95% CI	(0.74, 0.86)	(0.90, 0.97)	(0.99, 1.01)

CI = confidence interval. Other abbreviations as in Table 2.
* $P < .01$, compared with controls.
† Observed over expected birth weight.

Table 4. Umbilical Cord Plasma IGF-I, IGFBP-1, and IGFBP-3 (in ng/mL), by Level of Standardized Birth Weight, Among 67 Cases of Severe Preeclampsia and 609 Controls

Standardized birth weight*	IGF-I		IGFBP-1		IGFBP-3	
	PE	Ctrl	PE	Ctrl	PE	Ctrl
<0.76†	19	32	984‡	193	1101	1124
0.76–0.89	32‡	46	229‡	114	1212	1201
0.90–1.09	51‡	64	181‡	97	1300	1393
≥1.10	60	81	75	68	1380	1523

PE = preeclampsia; Ctrl = controls. Other abbreviations as in Table 2.
* Observed divided by expected birth weight.
† Two standard deviations smaller than the expected value of 1.0; small for gestational age.
‡ $P < .01$, compared with controls.

birth weight, however, IGF-I was significantly lower in the severe preeclampsia group than among controls, after controlling for differences in gestational age.

The results for IGFBP-1 showed an opposite pattern: IGFBP-1 increased strongly with decreasing birth weight, both among controls and in the severe preeclampsia group, but the increase was much stronger in the preeclampsia group. Thus, SGA (birth weight standard less than 0.76) infants in the preeclampsia group had a five-fold higher cord plasma IGFBP-1 (984 ng/mL) compared with babies born with appropriate weight (birth weight standard 0.90–1.09) for their gestation (181 ng/mL). Among controls, the same comparison showed a two-fold difference in IGFBP-1 (193 versus 97 ng/mL).

For IGFBP-3, there was a decrease in birth weight within each study group. By comparing the groups at each level of birth weight, however, there were no significant differences in IGFBP-3 between the severe preeclampsia group and controls.

In the multivariate analyses (Table 5), we found that severe preeclampsia and birth weight were strongly associated with IGF-I levels, after adjustment for gestational age. Table 5 also shows that birth weight, but not severe preeclampsia, was associated with IGFBP-1.

DISCUSSION

In this study, we found that umbilical IGF-I and IGFBP-1 levels in severe or early preeclampsia differed from those of control pregnancies, and these differences were particularly strong when preeclampsia was complicated by very low birth weight. In multivariate analyses, the results suggest that birth weight and preeclampsia, independent of each other, may influence IGF-I. Umbilical IGFBP-1 was also strongly related to birth weight, but the association with preeclampsia was not statistically significant in multivariate analyses. Previously, one study has indicated that IGF-I and the binding proteins may be altered by preeclampsia per se,[20] but compared with our investigation, that study was small. We included a large and representative sample of pregnant women that allowed us to apply a strict definition of preeclampsia.[23] We also distinguished between subtypes (mild or severe; early or late) of preeclampsia with statistical power sufficient to yield precise results. Further, we adjusted for differences in gestational age between the preeclampsia groups and controls, factors that strengthen the validity of our results.[22]

The reason for being small may influence the relation between IGF-I, IGFBPs, and infant birth weight. Previously, two studies have compared different groups of

Table 5. Cord Plasma Levels of IGF-I and IGFBP-1 (Log Transformed) as a Function of Preeclampsia Status (Severe vs Control), Birth Weight, Gestational Age, and Cord Plasma Level of IGF-I/IGFBP-1, and IGFBP-3 in 676 Singleton Pregnancies

Dependent variable	Coefficient of determination (adjusted R^2)	Prediction variables	Regression coefficients	Standard error	P
IGF-I (ng/mL)	0.47	Preeclampsia	−11.0	2.9	<.001
		Birth weight	0.020	0.002	<.001
		Gestational age	−0.57	0.07	<.001
		IGFBP-1 (ng/mL)	−6.6	0.8	<.001
		IGFBP-3 (μg/mL)	−21.6	1.7	<.001
		Constant	154.1	17.5	<.001
IGFBP-1 (ng/mL)	0.26	Preeclampsia	0.17	0.14	.3
		Birth weight	−0.0002	0.000	.04
		Gestational age	−0.011	0.003	.02
		IGF-I (ng/mL)	−0.015	0.002	<.001
		IGFBP-3 (μg/mL)	0.55	0.09	<.001
		Constant	8.4	0.8	<.001

Abbreviations as in Table 2.

neonates who were small for gestation, but for different reasons.[10,18] One group had suffered from intrauterine growth restriction most likely caused by placental disease, whereas the other group was born small for gestational age for a variety of reasons other than placental disease. The results were similar for maternal[10] and umbilical[18] measurements: fetuses with placental insufficiency had the lowest IGF-I and the highest levels of IGFBP-1. In our study, low birth weight in the control group may also have been caused by a variety of reasons, and some infants will simply be constitutionally small. In contrast, infants born after severe and early onset preeclampsia may be a relatively homogeneous group with placental insufficiency.[19] Consequently, the lower values of IGF-I and the very high values of IGFBP-1 associated with the combination of severe preeclampsia and fetal growth restriction may reflect placental disease.

The shallow trophoblast invasion typical for severe or early preeclampsia is associated with highly elevated expression of IGFBP-1 in the decidua,[7,8] and high levels of maternal IGFBP-1 in early pregnancy may be associated with increased risk of severe, but not mild preeclampsia.[25,26] Those results may support the hypothesis that mild and severe preeclampsia may represent separate disease entities, and suggest that IGFBP-1 is involved in initial mechanisms at the maternal-placental interface that may culminate in severe preeclampsia.[7,8] However, our results indicate that IGFBP-1 is more closely linked to birth weight than to preeclampsia, and this could suggest that IGFBP-1 is involved in compensatory or adaptive responses to insufficient fetal nutrition that will accompany severe preeclampsia.[18] On the other hand, the close association between IGF-I and severe preeclampsia may reflect compromised trophoblast function.[27,28] Thus, low IGF-I levels in severe preeclampsia may be the consequence of placental dysfunction rather than the underlying cause.

REFERENCES

1. Chard T. Insulin-like growth factors and their binding proteins in normal and abnormal human fetal growth. Growth Regul 1994;4:91–100.
2. Verhaeghe J, van Bree R, van Herck E, Laureys J, Bouillon R, van Assche A. C-peptide, insulin-like growth factors I and II, and insulin-like growth factor binding protein-1 in umbilical cord serum: Correlations with birth weight. Am J Obstet Gynecol 1993;169:89–97.
3. Giudice LC, deZegner F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, et al. Insulin-like growth factors and their binding proteins in the term and preterm fetus and neonate with normal and extremes of intrauterine growth. J Clin Endocrinol Metab 1995;80:1548–55.
4. Spencer JAD, Chang TC, Jones J, Robeson SC, Preece MA. Third trimester fetal growth and umbilical venous blood concentrations of IGF-I, IGFBP-1, and growth hormone at term. Arch Dis Child 1995;73:F87–F90.
5. Han VKM, D'Ercole AJ, Lund PK. Central localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 1987;236:193–7.
6. Han VKM, Bassett N, Walton J, Challis JRG. The expression of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) genes in the human placenta and membranes: Evidence for IGF-IGFBP interactions at the feto-maternal interface. J Clin Endocrinol Metab 1996;81: 2680–93.
7. Giudice LC, Martina NA, Crystal RA, Tazuke S, Druzin M. Insulin-like growth factor binding protein-1 at the maternal-fetal interface and insulin-like growth factor-I, insulin-like growth factor-II, and insulin-like growth factor binding protein-1 in the circulation of women with severe preeclampsia. Am J Gynecol 1997;176:751–8.
8. Irwin JC, Suen LF, Martina NA, Mark SP, Giudice LC. Role of the IGF system in trophoblast invasion and preeclampsia. Hum Reprod 1999;14(suppl 2):90–6.
9. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: Biological actions. Endocrinol Rev 1995;16:3–34.
10. Holmes R, Montemagno R, Jones J, Preece M, Rodeck C, Soothill P. Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth. Early Hum Dev 1997;49: 7–17.
11. Tazuke SI, Mazure NM, Sugawara J, Carland G, Faessen GH, Suen LF, et al. Hypoxia stimulates insulin-like growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells: A possible model for IGFBP-1 expression in fetal hypoxia. Proc Natl Acad Sci 1998;95:10188–93.
12. Osorio M, Torres J, Moya F, Pezzulo J, Salafia C, Baxter R, et al. Insulin-like growth factors (IGFs) and IGF binding proteins-1, -2, and -3 in newborn serum: Relationships to fetoplacental growth at term. Early Hum Dev 1996;46: 15–26.
13. Leger J, Oury JF, Noel M, Baron S, Benali K, Blot P, et al. Growth factors and intrauterine growth retardation. I. Serum growth hormone, insulin-like growth factor (IGF)-I, IGF-II, and IGF binding protein-3 levels in normally grown and growth-retarded human fetuses during the second half of gestation. Ped Res 1996;40:94–100.
14. Klauwer D, Blum WF, Hanitsch S, Rascher W, Lee PDK, Kiess W. IGF-I, IGF-II, free IGF-I and IGFBP-1, -2 and -3 levels in venous cord blood: Relationship to birthweight, length and gestational age in healthy newborns. Acta Paediatr 1997;86:826–33.
15. Baldwin S, Chung T, Rogers M, Chard T, Wang HS. Insulin-like growth factor-binding protein-1, glucose tolerance and fetal growth in human pregnancy. J Endocrinol 1993;136:319–25.
16. Fant M, Salafia C, Baxter RC, Schwander J, Vogel C,

Pezzulo J, et al. Circulating levels of IGFs and IGF binding proteins in human cord serum: Relationships to intrauterine growth. Reg Pept 1993;48:29–39.
17. Wang HS, Lee JD, Cheng BJ, Soong YK. Insulin-like growth factor-binding protein 1 and insulin-like growth factor-binding protein 3 in pre-eclampsia. Br J Obstet Gynaecol 1996;103:654–9.
18. Langford K, Blum W, Mikolaides K, Jones J, McGregor A, Miell J. The pathophysiology of the insulin-like growth factor axis in fetal growth failure: A basis for programming by undernutrition? Eur J Clin Invest 1994;24:851–6.
19. Ness RB, Roberts JM. Heterogenous causes constituting the single syndrome of preeclampsia: A hypothesis and its implications. Am J Obstet Gynecol 1996;175:1365–70.
20. Halhali A, Tovar AR, Torres N, Bourges H, Garabedian M, Larrea F. Preeclampsia is associated with low circulating levels of insulin-like growth factor I and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. J Clin Endocrinol Metab 2000;85:1828–33.
21. Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie NE, Irgens LM. Foetal and maternal contributions to risk of preeclampsia: Population-based study. BMJ 1998;316: 1343–7.
22. Ødegård R, Vatten LJ, Nilsen ST, Salvesen KÅ, Austgulen R. Preeclampsia and fetal growth. Obstet Gynecol 2000; 96:950–5.
23. CLASP. A randomised trial of low-dose aspirin for the prevention and treatment of preeclampsia among 9364 pregnant women. Collaborative Low-Dose Aspirin Study in Pregnancy Group. Lancet 1994;343:619–29.
24. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85: 843–8.
25. de Groot CJM, O'Brien TH, Taylor RN. Biochemical evidence of impaired trophoblast invasion of decidual stroma in women destined to have preeclampsia. Am J Obstet Gynecol 1996;175:24–9.
26. Grobman WA, Kazer RR. Serum insulin, insulin-like growth factor-I, and insulin-like growth factor binding protein-1 in women who develop preeclampsia. Obstet Gynecol 2001;97:521–6.
27. Ødegård RA, Vatten LJ, Nilsen ST, Salvesen KÅ, Vefring H, Austgulen R. Umbilical cord plasma interleukin-6 and fetal growth restriction in preeclampsia: A prospective study in Norway. Obstet Gynecol 2001;98:289–94.
28. Hill DJ, Clemmons DR, Riley SC, Bassett N, Challis JRG. Immunohistochemical localization of insulin-like growth factors (IGF) and IGF binding proteins, -1, -2, and -3 in human placenta and fetal membranes. Placenta 1993;14: 1–12.

Address reprint requests to: Lars J. Vatten, MD, PhD, Norwegian University of Science and Technology, University Medical Centre, N-7489 Trondheim, Norway; E-mail: lars.vatten @medisin.ntnu.no.

Received June 21, 2001. Received in revised form September 17, 2001. Accepted September 24, 2001.