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Effect of ASA on The Risk of Gestational Hypertension or IUGR and Prostanoid Synthesis in Pregnant Women Screened by Doppler Ultrasound

ACADEMIC DISSERTATION
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II Vainio M, Kujansuu E, Iso-Mustajärvi M and Mäenpää J: Low dose acetylsalicylic acid in prevention of pregnancy-induced hypertension and intrauterine growth retardation in women with bilateral uterine artery notches. BJOG, 109:161-167, 2002.

III Vainio M, Riutta A, Koivisto A-M and Mäenpää J: 9α, 11β-prostaglandin F_2 in pregnancies at high risk for hypertensive disorders of pregnancy, and the effect of acetylsalicylic acid. Prostaglandins Leukot Essent Fatty Acids 69: 79-83, 2003.

IV Vainio M, Riutta A, Koivisto A-M and Mäenpää J: Prostacyclin, thromboxane A2, and the effect of low dose ASA in pregnancies at high risk for hypertensive disorders. Acta Obstet Gynecol Scand, in press.

V Vainio M, Kujansuu E, Koivisto A-M and Mäenpää J: Bilateral notching of uterine arteries at 12 -14 weeks of gestation for prediction of pregnancy induced hypertension and intrauterine growth restriction. Acta Obstet Gynecol Scand, submitted.

ABBREVIATIONS

AA	arachidonic acid
AC ratio	peak systolic velocity/early diastolic velocity
AT III	antithrombin III
ASA	acetylsalicylic acid
β-HCG	beta human chorionic gonadotropin
BP	blood pressure
COX	cyclo-oxygenase
FVW	flow velocity waveform
HDL	high density lipoprotein
ICAM	intercellular adhesion molecule
IL-6	interleukin-6
IUGR	intrauterine growth restriction (retardation)
LDL	low density lipoprotein
NO	nitric oxide
PAI-1, PAI-2	plasminogen activator 1and 2
PECAM	platelet endothelial cell adhesion molecule
PGD_2	prostaglandin D_2
PGE_2	prostaglandin E_2
PGF_2	prostaglandin F_2
PGH_2	prostaglandinH_2
PGI_2	prostacyclin
PIH	pregnancy-induced hypertension
PI	pulsatility index
PlGF	placental growth factor
RI	resistance index
RR	relative risk
SD	standard deviation
S/D ratio	peak systolic velocity/end diastolic velocity ratio S = systolic, D = diastolic
SFlt1	placental soluble fms-like tyrosine kinase 1
SHBG	sex hormone binding globulin
TNFα	tumour necrosis factor-α
TxA_2	tromboxane A_2
9α, 11β– PGF_2, 11-epi-$PGF_{2\alpha}$	9α, 11β-prostaglandin F2
11-dehydro-TxB_2	11-dehydrothromboxane B_2
2, 3-dinor-6-keto-$PGF_{1\alpha}$	2, 3-dinor-6-keto-prostaglandin $F_{1\alpha}$
VCAM-1	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

ABSTRACT

The aim of the study was to evaluate the efficacy of low-dose acetylsalicylic acid (ASA) in the prevention of pregnancy-induced hypertension (PIH) or intrauterine growth restriction (IUGR) in high-risk women screened by Doppler ultrasound at 12-14 weeks of gestation.

It was found that, within a dose range of 0.5-2.0 mg/kg/day, ASA has a favourable effect on the ratio of prostacyclin to thromboxane A_2 in hypertensive pregnant women. Thereafter, 120 women with anamnestic risk factors for hypertensive disorders of pregnancy were screened by Doppler ultrasound at 12-14 weeks of gestation. Ninety women with bilateral notches in the uterine arteries were randomised to ASA (0.5mg/kg/day) (n = 45) and placebo (n = 45) groups. Forty-three in both groups were successfully followed up. Outcome data was also obtained on 29 of the 30 women without bilateral notches.

Five women allocated to ASA had PIH versus 16 of those on placebo (RR 0.31, 95% CI 0.13-0.78). The PIH was proteinuric in two pregnancies in the ASA group as contrasted to ten in the placebo group (RR = 0.20, 95% CI 0.05-0.86). The hypertension developed before 37 gestational weeks in two women randomised to ASA and in nine randomised to placebo (RR = 0.22, 95% CI 0.05-0.97). Two women in the placebo group had pre-eclampsia and one had PIH concomitant with IUGR. In the ASA group there was no IUGR concomitant with PIH.

Studying bilateral notches in the uterine arteries at 12-14 weeks of gestation in high-risk pregnancies turned out to be a sensitive screening test (sensitivity 75-84%) in predicting PIH or IUGR, but it had a rather low specificity (41-50%). With advancing pregnancy, the sensitivity decreased to 35% at 32-34 weeks of gestation, while the specificity and the positive predictive value increased to 94% and 59%, respectively.

The women in the placebo group had significantly lower excretion of the metabolite of PGI_2 at 12-14 weeks in pregnancies destined to develop pre-eclampsia than in other pregnancies. In pregnancies complicated with PIH before 37 weeks of gestation, the balance of PGI_2 and TxA_2 was shifted in favour of TxA_2. High-risk pregnancies with bilateral notches in the uterine arteries were associated with significantly higher urinary levels of 9α, 11β -prostaglandin F_2 than normal pregnancies at both 12-14 and 32-34 weeks of gestation.

In conclusion, ASA given to pregnant women at high risk of gestational hypertension significantly reduces the incidence of PIH and especially early-onset pre-eclampsia, when the treatment is started at 12-14 weeks of gestation. The occurrence of bilateral notching in the uterine arteries at 12-14 weeks of gestation is a very sensitive predictor of hypertensive disorders of pregnancy in high-risk women.

INTRODUCTION

Pregnancy-induced hypertension and particularly pre-eclampsia are leading causes of fetal and maternal morbidity and mortality (Rochat et al. 1988, Saftlas et al. 1990, Onrust et al. 1999) especially in underdeveloped countries (Randeree et al. 1995, Mungra et al. 1999, Lopez-Jaramillo et al. 2001). Hypertensive disorders of pregnancy affect up to 15% of pregnancies (Lyall and Greer 1996). The exact incidence of pre-eclampsia is unknown, but figures between 5 and 8% have been reported (Hauth et al. 2000). Those who suffer early-onset (1%) pre-eclampsia are prone to significant maternal and perinatal morbidity and mortality (Myatt and Miodovnik 1999).

Although the etiology of pre-eclampsia is still obscure there is definitely a genetic component (Roberts and Cooper 2001) and compelling evidence implicates the role of placenta. Inadequate cytotrophoblast invasion and resulting poor placental perfusion (Pijnenborg et al. 1980, Khong et al.1986) may constitute the impetus to endothelial cell dysfunction and decreased production of vasodilator prostaglandins and increased activation of platelets and release of thromboxane A_2, a potent vasoconstrictor (Walsh et al. 1985, Friedman et al. 1988). These findings have led to the use of antiplatelet drugs, mostly low-dose acetylsalicylic acid (ASA) in efforts to prevent the condition. The first trials (Beaufils et al.1985, Wallenburg et al. 1986, Trudinger et al.1988, Hauth et al. 1993, Sibai et al. 1993) recommending ASA treatment concerned only high-risk women presenting a specific indication and suggested reductions of about three-quarter in the incidence of pre-eclampsia. Subsequent trials (CLASP 1993, Italian study 1993, ECPPA 1996) tested ASA for broader indications and could not confirm these results.

The conflicting results concerning ASA treatment may also be a consequence of differences in the time at which treatment began, and the dose of ASA used. The late initiation of treatment is probable one reason for the negative results observed when we remember that trophoblastic invasion is essentially completed by 14-18 weeks (Pijnenborg et al. 1980). Also the optimal dose of ASA in preventing hypertensive disorders of pregnancy is not known, and dosages have varied from 50 to 150mg.

Our studies were conducted to assess the optimal dose of ASA to shift the prostacyclin/ thromboxane A_2 balance in favour of prostacyclin and to evaluate the effect of low-dose ASA in prevention of hypertensive disorders of pregnancy and IUGR with focusing the treatment in high-risk women screened with uterine artery Doppler and starting the treatment before the trophoblast invasion in spiral arteries was completed.

REVIEW OF THE LITERATURE

1. Definition of hypertensive disorders of pregnancy

Confusion still prevails over the terminology and classification of the hypertensive disorders of pregnancy. The latest recommendation from The National High Blood Pressure Education Program Working Group has proposed the term ”gestational hypertension” to replace the term ”pregnancy-induced hypertension” in describing cases in which elevated blood pressure without proteinuria develops in a woman after 20 weeks of gestation and blood pressure levels return to normal postpartum (ACOG 2002). In pregnant women, hypertension is defined as a systolic blood pressure level of 140mmHg or higher, or a diastolic blood pressure level of 90 mmHg or higher, which occurs after 20 weeks of gestation in a woman with previously normal blood pressure (ACOG 2002). Pre-eclampsia is a syndrome defined by hypertension and proteinuria which may also be associated with a myriad of other signs and symptoms such as visual disturbances, headache and epigastric pain. Laboratory abnormalities may include hemolysis, elevated liver enzymes and low platelet counts (HELLP syndrome). Proteinuria is defined as the presence of 0.3g or more of protein in a 24-hour urine specimen (or 1+ or greater in random urine dipstick). Eclampsia is defined as the presence of new-onset grand mal seizures in a woman with pre-eclampsia. The diagnostic criteria for superimposed pre-eclampsia include ”new-onset proteinuria” in a woman evincing hypertension before 20 weeks of gestation, a sudden increase in proteinuria if this is already present in early gestation, a sudden increase in hypertension, or the development of the HELLP syndrome. Women with chronic hypertension who develop headache, visual signs or epigastric pain may also have superimposed pre-eclampsia (ACOG 2002).

2. Pathogenesis of pre-eclampsia

Despite increasing knowledge of the pathophysiology of pre-eclampsia its etiology is still obscure. Several models for its pathogenesis have been proposed and the researches of pre-eclampsia have even an own association ISSP (International Society For The Study Of Hypertension In Pregnancy).

2.1. Placental ischemia

Although the cause of pre-eclampsia remains undefined the condition is now assumed to be a disease related to the placenta (Brosens et al. 1972, Pijnenborg et al. 1991, Meeekins et al. 1994). Pre-eclampsia can develop with abdominal pregnancy (Piering et al. 1993) and the presence of a fetus is not required, as pre-eclampsia can occur with hydatidiform mole (Redman et al. 2001). Pre-eclampsia and associated fetal growth restriction are generally considered to be a consequence of an inadequate uteroplacental circulation, thought to be due to failure of trophoblastic invasion of the spiral arteries (Khong et al. 1986). In early pregnancy trophoblast cells invade the placental bed, leading to remodelling of spiral arteries into maximally dilated low-resistance vascular channels, unable to constrict upon vasoactive stimuli (Pijnenborg et al. 1980, De Wolf et al.1980, Pijnenborg et al.1981). Endovascular trophoblast invasion has been reported to occur in two waves; the first into the decidual segments of the spiral arteries at 8 to 10 weeks of gestation and the second into myometrial segments at 16 to18 weeks of gestation (Pijnenborg et al. 1983).

There is a failure of cytotrophoblasts to undergo transformation of their phenotype to endothelial cell characteristics and this is likely to have a negative effect on the cytotrophoblast endovascular invasion (Zhou et al. 1997). Moreover, the severity of hypertension may be related to the degree of trophoblastic invasion (Madazli et al. 2000). Inadequate blood perfusion in the placenta can also be a consequence of an increased placental mass, as in pregnancies with multiple gestations (Mastrobattista et al. 1997, Coonrod et al. 1995) or hydropic infants (Pridjian and Puschett 2002). Graham and colleagues have established that hypoxia of the placenta in the second half of gestation leads to the aberrant expression of genes encoding cytokines and vasoactive molecyles (one of these has been termed PROXY-1), which may contribute to the pathophysiology of pre-eclampsia (Graham et al. 2000).

2.2. Immunology and genetics

Epidemiological studies strongly suggest that immune maladaptation is involved in the etiology of pre-eclampsia (Taylor 1997, Dekker et al.1998). The disorder develops mainly in first pregnancies (Dekker and Sibai 1999), suggesting that exposure to paternal antigen is protective (Roberts and Cooper 2001). Even a prior abortion may provide protection against the disease (Strickland et al. 1986). The protective effect of multiparity is lost with change of paternity (Robillard et al. 1993, Tubbergen et al. 1999). A previous pregnancy with the same father (Trupin et al. 1996) and a longer period of sexual cohabitation with the father before conception (Robillard et al. 1993) reduce the risk of pre-eclampsia. The conception of an indirect immunologic basis for pre-eclampsia is also supported by the finding that the pre-eclampsia risk is increased in pregnancies with donor insemination (Smith et al. 1997) or with oocyte donation (Söderström-Anttila et al. 1998). There is also a study where the lack of previous exposure to paternal antigens did not predispose to hypertensive pregnancy complications (Laivuori et al. 1998). However, Skjaerven and associates (2002) have provided data supporting the view that the protective effect against pre-eclampsia of a previous pregnancy with the same partner is probably confounded by the time interval between births. The risk of pre-eclampsia in subsequent pregnancies was related to the time elapsed since the prior pregnancy and not to a change of partners (Skjaerven et al. 2002). Candidates for mediators of immune maladaptation in pre-eclampsia include cytokines (especially tissue necrosis factor alpha) and interleukin-2 and -6 (Dekker and Sibai1999).

Epidemiological evidence suggests a familial tendency to pre-eclampsia (Chesley et al. 1968, Chesley and Cooper 1986, Cooper et al. 1988). Chesley and Cooper (1986) found pre-eclampsia in 36% of the sisters, 26% of the daughters and 16% of the granddaughters of eclamptic mothers. Most family data suggest that the maternal genotype is responsible for susceptibility (Roberts and Cooper 2001). A higher incidence of pre-eclampsia is reported in mothers and daughters of affected women (Sutherland et al. 1990, Arngrimsson et al. 1990). Paternal genes in the fetus may contribute substantially to a pregnant woman's risk of pre-eclampsia, and the role of the fetus may be as important as that of mother (Lie et al. 1998).

There is a striking lack of concordance between monozygous twins in regard to pre-eclampsia (Lachmeijer et al. 1998, Thornton and Macdonald 1999, Salonen et al. 2000). A retrospective study of twins from Australia found no clear maternal genetic influences of pre-eclampsia (Treloar et al. 2001). Also O´Shaugnessy and colleagues (2000), who confirmed the monozygosity of twins by DNA fingerprinting, showed concordance of monozygotic twins with pre-eclampsia and that it was as frequent as in discordant pairs.

Although genetic influences have long been regarded as etiologically important in pre-eclampsia (Chesley and Cooper 1986), no single gene has been identified which would explain the inheritance of the disorder. A single recessive gene (Chesley and Cooper 1986) and a single dominant gene (Arngrimsson et al.1995) with incomplete penetrance have been suggested as models. Candidate genes examined have included genes which encode HLA-DR beta (Wilton et al. 1990), HLA-G (Humprhey et al. 1995), angiotensin-converting enzyme (Morgan et al. 1998, Curnow et al. 2000) and tumor necrosis factor α (Lachmeijer et al. 2001). It is most unlikely that there is a single pre-eclampsia gene, and the current majority view is that the condition is under multifactorial control (Broughton-Pipkin et al. 1999).

Chromosomal exclusion mapping and pedigree study suggest a role for genes on chromosomes 1, 3, 4, 9 or 18 (Broughton-Pipkin et al. 1999). Recently, an extensive Icelandic genome-wide scan provided evidence for a maternal susceptibility locus for pre-eclampsia on chromosome 2p13 (Arngrimsson et al. 1999), which was confirmed by a genome scan from Australia and New Zealand (Moses et al. 2000). Harrison and associates (1997) identified a significant linkage between the long arm of chromosome 4 and pre-eclampsia.

Results from a Dutch genome-wide scan indicated that the HELLP syndrome might have a genetic background different from that of pre-eclampsia (Lachmeijer et al. 2001). A recent genome-wide scanning from Finland, again, (Laivuori et al. 2003) found two loci, which exceeded the threshold for significant linkage: chromosome 2p25 and 9p13. In that study the susceptibility locus on chromosome 2p25 was clearly different from the locus 2p12 found in the Iceland study (Arngrimsson et al. 1999) and the locus at 2q23 found in an Australian/New Zealand study (Moses et al. 2000). It is thus obvious that pre-eclampsia has a complex inheritance pattern, similar to that of chronic illnesses such as diabetes, hypertension and asthma involving multiple disease susceptibility loci as well as environmental gene interactions (O´Shaughnessy et al. 2000, Pridjian and Puschett 2002).

2.3. Maternal risk factors

Reduced placental perfusion is an important component in pre-eclampsia, but not sufficient to account for the disorder, since in pregnancies with IUGR without pre-eclampsia (Khong et al.1986) and in preterm births (Arias et al.1993) there are changes in spiral arteries similar to those observed in pregnancies with pre-eclampsia. There are numerous maternal constitutional factors predisposing to the disorder. In patients with pre-existing vascular disease, chronic hypertension and autoimmune disorders such as systemic lupus erythematosus and antiphospholipid syndrome the risk is increased ten times, in chronic renal insufficiency 20 times (ACOG 1996). Women with thrombophilias are more likely to develop pre-eclampsia (Van Pampus et al. 1999, Kupferminc et al.1999).

In a series of women with severe, early-onset pre-eclampsia, 25% had functional protein S deficiency, 18% evinced hyperhomocysteinemia, and 29% had detectable anticardiolipin IgG and IgM antibodies (Dekker et al.1995, van Pampus et al.1999). Predisposing factors also include obesity (Stone et al. 1994, Sibai et al. 1995, Conde-Agueldo et al. 2000), pregestational diabetes

(Sibai et al. 2000) and increased insulin resistance (Kaaja et al.1998, Kaaja et al. 1999a, Laivuori et al. 1999). Women with multiple gestations are more likely to develop pre-eclampsia (Coonrod et al. 1995, Caritis et al. 1998).

The recurrence incidence of pre-eclampsia in the second pregnancy was 19-25.9%, the risk dropping in subsequent pregnancies (Sibai et al.1986). Normal pregnancy is associated with increased levels of cholesterol, triglyceride (Potter et al. 1979) and free fatty acids (Lorentzen et al.1995). Serum triglyceride (Kaaja et al.1995, Hubel et al.1996) and free fatty acid (Lorenzen et al.1995) levels are increased and serum levels of HDL_2 cholesterol are decreased in pre-eclampsia (Kaaja et al.1995). Higher serum cholesterol levels reported before pregnancy (Thadhani et al. 1999) or in the first trimester (van den Elzen et al.1996) have been reported to predict the development of pre-eclampsia.

2.4. Endothelial cell dysfunction

Abnormal placentation and resulting poor placental perfusion may be the impetus for the endothelial changes evidenced in pre-eclampsia (Roberts and Cooper 2001). An immunohistologic study has evidenced morphologic changes in the endothelialisation of uteroplacental vessels (Khong et al. 1992) and an electro microscopic study of the uteroplacental arteries noted gross endothelial damage, massive intramural fibrin deposition, luminal thrombosis and vessel rupture with hemorrhage (De Wolf et al. 1975) in pre-eclampsia. The failure of cytotrophoblasts to mimic a vascular adhesion phenotype is associated with this defect in endovascular invasion (Zhou et al. 1997). The current literature supports the view that angiogenesis is an essential physiologic component of implantation and is associated with various pathological processes in the placenta, including those observed in pre-eclampsia and growth restriction (Sherer and Abulafia 2001). The changes in renal vessels, termed glomerular endotheliosis, provide evidence that the vascular endothelium may be an important target in the disorder (Roberts 1998).

Healthy endothelial cells maintain vascular integrity, prevent platelet adhesion and influence the tone of the underlying vascular smooth muscle. Endothelial cell dysfunction may result from a variety of factors, including physical tear forces, hypoxia, lipid peroxides and other circulating constituents (Branch et al. 1991). When activated by a chronic pathologic process endothelial cell lose these functions and produce procoagulants, vasoconstrictors and mitogens, causing increased capillary permeability, platelet thrombosis and increased vascular tone (Roberts et al. 1991, Roberts et al. 1993). Many markers of endothelial dysfunction have been reported in women who develop pre-eclampsia, suggesting that this is an endothelial cell disorder (Taylor et al. 1998, Roberts et al. 1998).

2.4.1. Markers of endothelial cell dysfunction

Endothelial cells are the most important source of prostacyclin, which is a potent vasodilator, inhibitor of platelet aggregation and stimulator of renin secretion (Meagher et al. 1993). Prostacyclin production is increased eight to ten fold in normal pregnancy, whereas in pregnancy-induced hypertension the increase is only one to twofold (Fitzgerald et al. 1987a). In the 1980s the hypothesis was advanced that pre-eclampsia occurred secondary to an imbalance of vasodilator and vasoconstrictor prostaglandins (Walsh 1985).

Several clinical studies indicate that plasma concentrations and urinary excretion of prostacyclin are decreased in women with pre-eclampsia (Yamaguchi et al.1985, Ylikorkala et al.1986, Minuz et al. 1988, Kaaja et al.1995, Liu et al.1998, Kaaja et al. 1999a) and the decrease detectable as early as the first trimester of pregnancy (Fitzgerald et al. 1987a). The biosynthesis of thromboxane A_2, a potent vasoconstrictor and platelet-aggregating agent, is also increased in normal pregnancy but is increased further in hypertensive pregnancy and may arise from activated platelets (Wallenburg et al. 1982). The resulting imbalance between prostacyclin and thromboxane is likely to contribute to the enhanced platelet reactivity and vascular damage seen in pre-eclampsia (Lyall and Greer 1996). The subject of prostanoids as mediators of vascular tone is discussed later in this chapter.

Endothelin-1, an endothelium-derived peptide, is a potent vasoconstrictor in the human uterine artery, and the effect is mediated by receptors on the smooth muscle cells (Bodelsson et al. 1992). Most studies have demonstrated an increase in endothelin in the plasma and in placental tissue in pre-eclamptic women (Taylor et al. 1990, Dekker et al. 1991a, Mastrogiannis et al. 1991, Clark et al. 1992, Singh et al. 2001) and especially in women with the HELLP-syndrome (Nova et al. 1991, Bussen et al. 1999). Increased levels of endothelin-1 in early pregnancy have been reported to have predictive value with respect to the later development of pre-eclampsia (Shaarawy et al. 2000)

The production of nitric oxide (NO), a potent vasodilator synthesised by endothelial cells, is elevated in normal pregnancy (Sladek et al. 1997). The data on plasma or urine nitrate, a breakdown product of nitric oxide, in pre-eclampsia are conflicting, with increases (Baker et al.

1995a, Ranta et al. 1999), decreases (Seligman et al. 1994, Davidge et al. 1996), and no changes (Lyall et al. 1995) being reported.

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is reported to be elevated (Baker et al. 1995b, Sharkey et al. 1996, Hunter et al. 2000, Bosio et al. 2001a) or decreased (Lyall et al. 1997, Reuvekamp et al. 1999, Livingston et al. 2000) in the plasma of pregnant women with active pre-eclampsia. VEGF correlated with the severity of hypertension (Kupferminc et al. 1997) and has been hypothesised to be a marker of endothelial cell activation (Cooper et al. 1996). A recent report by Maynard and colleagues (2003) suggests that, the circulating levels of two angiogenic growth factors, VEGF and placental growth factor (PlGF), may play an important role in the pathogenesis of pre-eclampsia. The studies in question have shown deprivation of VEGF and PlGF to be involved in the condition. Soluble fms-like tyrosine kinase 1 (sFlt1) is a variant of the VEGF receptor Flt1 which lacks segments normally binding the protein to a cell membrane and acting as a potent VEGF and PlGF antagonist (Kendall et al. 1996, Shibuya 2001). Increased amounts of sFlt1 reduce free VEGH and PlGF in the blood of patients with pre-eclampsia, and this altered balance causes endothelial dysfunction. As a result, the normal vasculature in the kidney, brain, lungs and other organs is deprived of essential survival and maintenance signals and becomes dysfunctional, resulting in multiorgan disease (Maynard et al. 2003).

Previous studies which reported increased levels of VEGF in pre-eclampsia measured total (bound and unbound) VEGF, whereas those reporting low levels of VEGF in pre-eclampsia measured free VEGF levels, which more accurately reflect effective circulating VEGF (Maynard et al. 2003). However, the studies of Maynard and associates provide no answer as to what upregulates sFlt1 expression in the placenta in pre-eclampsia.

Disorders of coagulation and fibrinolysis occur in pre-eclampsia (Taylor et al. 1998). Low levels of anticoagulant proteins in pre-eclampsia have been shown, these including antithrombin III (Weiner and Brandt 1982), protein C and protein S (Dekker et al. 1995). The prevalence of the inherited factor V Leiden mutation and activated protein C resistance is increased in women with severe pre-eclampsia as compared to those with normal pregnancies (Dizon–Townson et al. 1996, Lindoff et al. 1997). Increased endothelial expression of other procoagulant proteins, including tissue factor (Estelles et al. 1998, Bellart et al. 1999), von Willebrandt factor (Friedman et al. 1995), platelet-activating factor (Rowland 2000), β-thromboglobulin (Socol et al. 1985), cellular fibronectin (Taylor et al. 1991) and thrombomodulin (Hsu et al. 1993) has also been reported. Fibrinolytic activity is normally decreased in pregnancy as a result of increases in plasminogen activator inhibitor 1 and 2 (PAI-1 and PAI-2) activities. PAI-1 concentrations increase progressively in the maternal plasma in normal pregnancy and are even higher in pre-eclampsia (Estelles et al. 1991, Halligan et al. 1994). PAI-2 is synthesised by the placenta; plasma concentrations also increase progressively in normal pregnancy and decrease with reduced placenta function as in pre-eclampsia (Halligan et al. 1994) and in intrauterine growth restriction (Estelles et al. 1991). The ratio of PAI-1 to PAI-2 decreases in normal pregnancy, but increases in pre-eclampsia (Reith et al. 1993). Circulating levels of PAI-1, thrombomodulin and fibronectin have been found to correlate directly with severity of the syndrome (Shaarawy et al.1996).

Soluble adhesion molecules such as the vascular cell adhesion molecule 1 (VCAM-1), are known to be increased in the serum of patients with pre-eclampsia, indicating that these molecules are possible markers of endothelial cell activation (Lyall et al. 1994, Djurovic et al. 1997, Heyl et al. 1999). It has been reported that the intercellular adhesion molecule (ICAM) is increased (Krauss et al.1997, Djurovic et al.1997, Austgulen et al. 1997) or unchanged (Lyall et al.1994, Heyl et al.1999) in pre-eclampsia and the soluble adhesion molecules E-selectin (Heyl et al. 1999) and P-selectin (Halim et al. 1996, Bosio et al. 2001) have been reported to be increased. There are also contrasting results from studies where ICAM, VCAM, P-selectin or E-selectin did not differ between normal and pre-eclamptic pregnancies (Jaakkola et al. 2000, Tziotis et al. 2002). Enhanced platelet activation (Hutt et al. 1994), and increased levels of platelet endothelial cell adhesion molecule-1 (PECAM-1) also occur in women who develop pre-eclampsia (Chaiworapongsa et al. 2002). On the other hand, no statistically significant difference was found in the expression of the adhesion molecule PECAM in the endothelium of normal or pre-eclamptic pregnant woman (Tziotis et al. 2002), nor in placental bed biopsies throughout the period of cytotrophoblast invasion between pre-eclamptic and normal placentas (Lyall et al. 2001).

Cytokines are protein messengers released by immune cells to regulate the function of other immune cells and are produced by macrophages and lymphocytes. Some (Kupferminc et al. 1994, Vince et al. 1995) but not all (Opsjon et al. 1995) studies report higher plasma tumor necrosis factor-α (TNF-α) levels in women with established pre-eclampsia. In pre-eclampsia increased concentrations of the interleukin-6 (IL-6) (Vince et al. 1995), interleukin-1 receptor antagonists (Greer et al. 1994), interleukin-12 (Dudley et al.1996) and interleukin 10 (Rinehart et al. 1999) have been reported.

In conclusion, endothelial dysfunction has been shown to be an early pathogenic feature of pre-eclampsia and many markers of endothelial activation precede clinically evident disease and disappear with resolution of the disease (Roberts 1998). The cause of endothelial dysfunction is not known, but the initiating event has been postulated to be reduced placental perfusion (Harrington and Campbell 1992).

2.5. Oxidative stress

The maternal response to reduced placental perfusion is influenced by maternal constitutional factors –genetic, behavioral or environmental (Roberts and Hubel 1999). The similarities between risk factors in pre-eclampsia and atherosclerosis support the conception that oxidative stress, which is pathogenically important in atherosclerosis (Witztum 1994), could also be the link between reduced placental perfusion and maternal constitutional factors in pre-eclampsia (Roberts and Hubel 1999).

Markers of oxidative stress are present in the blood and tissues of women with pre-eclampsia (Hubel et al.1989) and there is an increase in small dense LDL (Sattar et al. 1997). The reduced placental perfusion in association with the reduction in uterine blood flow known to accompany postural changes and uterine contractions could lead to intermittent intervillous hypoxia (Roberts 1998). Upon reperfusion, free radicals would be generated. The impact of this oxidative stress would be accentuated by maternal constitutional factors (e.g. decreased levels of antioxidants, lipoproteins). It has been shown that malondialdehyde, a marker of lipid peroxidation, is increased in women with pre-eclampsia (Hubel et al. 1989). Activated neutrophils, stable products of oxidative stress (e.g. malondialdehyde), oxidised fragments of syncytiotrophoblast entering the systemic circulation, or cytokines could be the factors transferring oxidative stress from the intervillous space to the systemic circulation (Roberts and Cooper 2001).

2.6. Inflammatory theory

Redman and colleagues (1999) have proposed that endothelial cell dysfunction is one aspect of the generalised systemic maternal inflammatory response. Syncytiotrophoblasts normally shed redundant placental debris into the maternal circulation, and this process depends on apoptosis

(Nelson 1996). Syncytiotrophoblast microfragments are detected in increased amounts in pre-eclampsia (Knight et al. 1998). It has been proposed that increased oxidative stress in the placenta leads to an overload of debris by stimulating apoptosis or necrosis or both (Redman and Sargent 2001). Continual clearance of this debris causes the systemic inflammatory response, which is present in all pregnant women in the third trimester. Pre-eclampsia could ensue when the systemic inflammatory response decompensates. This may occur if the burden of debris is abnormally high, or if the woman's response to the process is excessive (Redman and Sargent 2001). As the alterations in endothelial function present in pre-eclampsia are similar to those seen in atherosclerosis (Roberts and Cooper 2001), the inflammatory response and activation of the mast cells may likewise also be important in the pathogenesis of pre-eclampsia (Kelley et al. 2000).

3. Prostaglandins

3.1. Biosynthetic pathway of prostaglandins

The prostaglandins are a group of 20-carbon unsaturated fatty acids similar in structure but varying in function (Friedman 1988). Arachidonic acid is first cloven from membrane-bound phospholipids by the action of phospholipase A_2. It is then converted to the cyclic endoperoxides PGG_2 and PGH_2 by the complex of enzymes known as prostaglandin synthetase, the first of which is cyclo-oxygenase (COX). PGH_2 is the immediate precursor of PGD_2, PGE, $PGF_{2\alpha}$, prostacyclin (PGI_2) and thromboxane A_2 (TxA_2). PGE is frequently differentiated into PGE_1 and PGE_2, both of which are vasodilatory. PGE_1 is a weak inhibitor of platelet aggregation, PGE_2 a weak platelet aggregator. $PGF_{2\alpha}$ is a venous vasoconstrictor with variable arterial effects. PGI_2 and TxA_2 are substantially more potent than PGE and $PGF_{2\alpha}$ (Friedman 1988).

The major eicosanoid produced by endothelial cells is PGI_2, which it is a potent vasodilator and inhibitor of platelet aggregation. TxA_2 is a potent vasoconstrictor and platelet aggregator produced primarily by platelets (Friedman 1988). Both are unstable chemically and are generally measured *in vivo* as their urinary metabolites 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) or 2, 3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (2,3-dino-6-keto-$PGF_{1\alpha}$) and 11-dehydro-thromboxane B_2 (11-dehydro-TxB_2) or 2, 3-dinor-thromboxaneB_2 (2, 3-dinor-TxB_2) (Friedman 1988).

The measurement of 6-keto-$PGF_{1\alpha}$ in plasma is questionable because of the risk of its artifactual formation during blood collection (Riutta et. al. 1994). In urine, 6-keto-$PGF_{1\alpha}$ is mainly produced by the kidneys rather than filtrated from plasma and does not reflect the total body production of PGI_2. Therefore 2,3-dino-6-keto-$PGF_{1\alpha}$, the major metabolite of prostacyclin in urine should be measured as a noninvasive index of PGI_2 (Riutta et al. 1994).

Because of short half-life of TxA_2 its production has been monitored by measuring thromboxane B_2 (TxB_2), the chemically stabile hydration product of TxA_2 (Riutta et al. 1992). However, urinary TxB_2 originates predominantly from kidney under physiological conditions and does not reflect the total body production of TxA_2. Two major metabolic pathways have been described; β-oxidation to 2, 3-dinor-thromboxane B_2 (2, 3-dinor-TxB_2) and dehydrogenation to 11-dehydro-thromboxane B_2 (11-dehydro-TxB_2), the latter is now established as the most abundant breakdown product of TxB_2, and it is considered the index metabolite of systemic TxA2 production (Riutta et al. 1992).

3.2. Prostaglandins in normal pregnancy

3.2.1. Prostacyclin and thromboxane

The blood volume increases to approximately 50% above the non-pregnant level, the hematocrit falls, and cardiac output increases by an average of 30-40%. Despite these increases in blood volume and cardiac output, blood pressure declines during pregnancy because peripheral vascular resistance is reduced by approximately 40% from normal non-pregnant values. Renal plasma flow and glomerular filtration rate increase and blood urea nitrogen and serum creatinine fall. The renin-angiotensin-aldosterone system is markedly activated during pregnancy (Friedman 1993).

It has been proposed that certain prostaglandins – in particular PGE_2 and prostacyclin are produced in abundance during pregnancy and are responsible for the elevated renin levels and diminished blood pressure (Bay and Ferris 1979, Ylikorkala et al. 1981 b, Pedersen et al. 1983, Walsh et al. 1985).

Studies of maternal plasma prostaglandins or their urine metabolites have yielded conflicting results. Most have reported increased excretion of urine metabolites of PGI_2 and TxA_2 during pregnancy (Minuz et al. 1988, Paarlberg et al. 1998, Delemarre et al. 2000). Plasma PGI_2 (Lewis et al. 1980) or urinary excetion of PGI_2 (Goodman et al. 1982, and Klockenbusch et al. 1994) has been reported to be increased, or plasma PGI_2 has been reported to be unchanged (Ylikorkala and Viinikka 1981a, Koullapis et al. 1982) during normal pregnancy. Plasma TxA_2 has likewise been reported to be increased (Ylikorkala and Viinikka 1980) and urine excretion of TxA_2 has been reported to be unchanged (Ylikorkala et al. 1986, Klockenbusch et al. 1994) in normal pregnancy.

In two longitudinal studies (Paarlberg et al. 1998 and Delemarre et al. 2000) the ratio PGI_2/TxA_2 increased throughout pregnancy.

In conclusion, most studies would indicate that PGI_2 and TxA_2 increase during normal pregnancy. As urinary excretion of TxA_2 metabolites increases two- to five-fold (Ylikorkala et al. 1986, Fitzgerald et. al. 1987a, b 1990) during pregnancy and urinary excretion of PGI_2 metabolites even more (five- to eight fold) than does TxA_2 (Ylikorkala et al. 1986, Fitzgerald et al. 1987a) in normal pregnancy there is a predominance of vasodilatory PGI_2 which contributes to low vascular resistance and to fall in blood pressure (Ylikorkala and Viinikka, 1992).

3.2.2. Prostaglandin D_2

Urinary 9α, 11β-prostaglandin F2 (9α-11β-PGF_2, 11-epi-$PGF_{2\alpha}$) is the primary metabolite of PGD_2 and has long been used in monitoring PGD_2 production in the mast cells (Roberts et al. 1980, Roberts and Sweetman 1985). 9α-11β-PGF_2 is enzymatically formed and produced *in vivo* in humans, and unlike other metabolites of PGD_2, this compound is biologically active and potentially vasoconstrictive (Liston and Roberts 1985). PGD_2 is released by the chorionic tissue of the placenta (Mitchell et al. 1982) and has been shown to constrict the blood vessels of the human placenta and possibly to participate in the local regulation of uteroplacental blood flow (Abramovich and Parkin 1984). Only a few studies have reported on PGD_2 in reproduction (Saito et al. 2002).

3.3. Prostaglandins in pre-eclampsia

3.3.1. Prostacyclin and thrombxane

The role of prostaglandins in the etiology of pre-eclampsia has been investigated since the 1970s. Pre-eclampsia is characterised by increased blood pressure, proteinuria and edema, with general vasoconstriction and platelet hyperactivity (Meagher and Fitzgerald 1993). Theoretically these changes could be caused by deficient production of the vasodilator and antiaggregatory prostacyclin and by an increased synthesis of proaggregatory and vasoconstrictor thromboxane (Ylikorkala and Mäkilä 1985, Friedman 1988, Ylikorkala and Viinikka 1993). It has been suggested that the pathologic findings in pre-eclampsia could be explained by an increase in the thromboxaneA_2/prostacyclin ratio, as a predominance of thromboxane A_2 could account for the vasospasm and activation of intravascular coagulation, whereas a deficiency of prostacyclin could be responsible for endothelial injury through impairment of its cytoprotective function (Friedman 1988).

While most studies so far (Yamaguchi et al. 1985, Ylikorkala et al. 1986, Minuz et al. 1988, Kaaja et al. 1995, Liu et al. 1998, Kaaja et al. 1999a,) report that urine excretion of PGI_2 is lower in pre-eclampsia as compared to normal pregnancy, or that maternal plasma PGI_2 is decreased in pre-eclampsia (Oqino et al. 1986), the only long term prospective study found no difference (Smith et al. 1995). There are also data suggesting that a decrease in prostacyclin production precedes the onset of pre-eclampsia (Fitzgerald et al. 1987a, Mills et al. 1999).

Groups under Fitzgerald (1990) and Klockenbusch (1994) found that the urine metabolite of TxA_2 was higher and Liu and associates (1998) found that maternal plasma TxA_2 was higher in pre-eclampsia as compared to normal pregnancy, whereas in studies by Ylikorkala and colleagues (1986) and groups under Minuz (1988), Kaaja (1995), Paarlberg (1998) and Mills (1999) urine excretion of TxA_2 metabolite and in one study plasmaTxA_2 (Yamaguchi et al. 1985) did not differ between pregnancies involving pre-eclampsia or with normal outcome.

It has been speculated that one possible reason for these conflicting and confusing results is that assessment of eicosanoid formation has been made subsequent to the onset of symptoms (Mills et al. 1999). It is thus difficult to determine whether changes in eicosanoids are the cause or the result of the disease. On the other hand, in prospective studies the number of women who developed pre-eclampsia has been small, limiting the power of their findings.

4. Prediction of pre-eclampsia

Pre-eclampsia is a disorder of unknown etiology with heterogeneous pathophysiological abnormalities, and numerous clinical, biophysical and biochemical tests have been proposed for the prediction or early detection of the disease. The ideal predictive test should be simple, easy to perform early in pregnancy, reproducible, noninvasive and of high sensitivity and high positive predictive value (Dekker and Sibai 1991b). Most tests suffer from poor sensitivity and low positive predictive value, and the majority of them are not suitable for routine use in clinical practice (Caritis et al. 1998).

4.1. Standard methods in antenatal care

Antenatal check-ups involve the measurement of blood pressure, urine protein or albumin and maternal weight. The sensitivity of using an 80-85 mm Hg diastolic blood pressure level in the first half of pregnancy as a test for the subsequent occurrence of pre-eclampsia has been found to be 20-30%, but the predictive value of a negative test was 95% (Moutquin et al. 1985). Villar and Sibai (1990) concluded that neither a mean arterial blood pressure greater than 90 mm Hg in the second trimester nor a threshold increase in systolic or diastolic blood pressure during the third trimester was significantly predictive of the development of pre-eclampsia. Elevated mean arterial pressure in the second half of pregnancy is a good predictor of gestational hypertension but a poor predictor of pre-eclampsia (Conde-Agudelo et al. 1993). Detection of microalbuminuria in predicting the development of proteinuric pre-eclampsia is also of little value (Lopez-Espinoza et al. 1986), but there is also a report of controversial results (Rodriguez et al. 1988). The general conclusion is that weight gain cannot be used to predict the development of pre-eclampsia (Dekker and Sibai 1991).

4.2. Biochemical and biophysical tests

An isometric exercise test reflecting vascular reactivity in pregnant women has been used to predict pre-eclampsia at 28-32 weeks of gestation (Degani et al. 1985). The test had a sensitivity of 81%, specificity 96% and positive predictive value 81%, the negative predictive value being 93%. The results of rollover test or supine pressor test first described by Gant and colleagues. (1974) yielded highly variable results among different investigators, and had poor reproducibility in the same patient (Dekker and Sibbai 1991b). The angiotensin II sensitivity test (Gant et al. 1973) has come to be regarded as the gold standard among predictive tests for hypertensive disorders of pregnancy. The positive and negative predictive values of the test were 86% and 94%, respectively, but use of the test as a clinical screening procedure is limited by its invasiveness, time-consuming nature and the need for close supervision.

4.3. Hematological and urinary markers

4.3.1. Renal markers

Although mean serum uric acid values are elevated in women with pre-eclampsia, serum uric acid is of limited value in predicting pre-eclampsia (Lim et al. 1998). Hyperuricemia is associated with the severity of pre-eclampsia and high uric acid concentration in pre-eclampsia has been attributed to renal dysfunction (Many et al. 1996). Uric acid is also an antioxidant (Uotila et al. 1992, Uotila et al. 1994) and elevated circulating concentrations of uric acid is proposed to be a marker of free radical generation and hyperuricemia may itself serve a protective role as an antioxidant in pre-eclampsia (Many et al. 1996).

Pre-eclampsia may be related, in part, to a relative Ca intake deficiency, but urinary calcium to creatinine ratios in early pregnancy have only limited clinical value in identifying women with an increased risk of pre-eclampsia (Izumi et al. 1997). Renal kallikrein is thought to play an important paracrine role in the regulation of blood pressure via generation of vasodilatory kinins and stimulation of prostaglandin biosynthesis (Scicli and Carretero 1986). The ratio of the urinary excretion of kallikrein to that of creatinine may be a predictor of pre-eclampsia (Millar et al. 1996). At a ratio of 170 or lower, 83% of pre-eclamptic women could be identified as early as 16-20 weeks of gestation with a positive predictive value of 91%. The same ratios predicted PIH with a sensitivity of 70% and the positive predictive value was 40%.

4.3.2. Placental peptide hormones

Increased beta-human chorionic gonadotropin (β-hCG) plasma concentrations at 14-20 weeks of gestation predicted PIH complicated by proteinuria or IUGR with a positive predictive value of 11-15% (Vaillant et al. 1996). A significant linear association was found between the midtrimester urine beta-core fragment of hCG and pre-eclampsia in a prospective study by Bahado-Singh and associates (1998), but the data is not uniform (Ashour et al. 1997). Determinations of the proposed marker N-terminal proatrial natriuretic peptide as well as serum β-hCG or alpha-fetoprotein are not helpful in predicting pre-eclampsia (Pouta et al. 1998). The combination of the hCG assay and a subsequent Doppler at 24 weeks of gestation has been found to enhance the positive predictive value of the assay from 19 to 75% (Merviel et al. 2001).

Activin and inhibin are dimeric, disulfide-linked glycoproteins produced by the placenta (Qu and Thomas 1995). Maternal serum levels of activin A and inhibin A have been shown to be increased in pre-eclampsia (Muttukrishna et al. 1997, Laivuori et al. 1999). Activin A is increased in pre-eclampsia but not in pregnancies with chronic hypertension or PIH (Petraglia et al. 1995). The value of activin A and inhibin A in predicting pre-eclampsia has been tested in a prospective study (Muttukrishna et al. 2001). Predictive sensitivities were low (16 - 59%), but much better for early onset pre-eclampsia (67- 44% at 15-19 weeks of gestation and 89% at 21-25 weeks of gestation).

4.3.3. Markers from coagulation and fibrinolytic systems

Many markers of endothelial dysfunction, coagulation and fibrinolytic system have been reported in women who develop pre-eclampsia. Fibronectin levels have been significantly higher in pregnancies with pre-eclampsia as compared to control women at 25 to 36 weeks of pregnancy, and fibronectin levels increased 3.6 +/-1.9 weeks earlier than the onset of hypertension and/or proteinuria (Ballegeer et al. 1989). In women in whom clinical pre-eclampsia developed, endothelial cell damage and increased levels of fibronectin seemed to be present as early as at 9 to 12 weeks of gestation (Chavarria et al. 2002). Sensitivity, specificity and positive and negative predictive values at 22 to 26 weeks of gestation were 73%, 87%, 29% and 98%, respectively in healthy nulliparous women (Chavarria et al. 2002).

Even though pre-eclampsia has been associated with lower levels of antithrombin- III, protein C and protein S (Paternoster et al. 1994 and 1996), early antithrombin-III determination has proved to have no value in predicting pre-eclampsia (Paternoster et al. 1999). In one cross-sectional study evaluating six markers of the hemostatic system in pregnancy the thrombin-antithrombin III complexes showed the best sensitivity (70%) in predicting pre-eclampsia (Cadroy et al. 1993). A flow cytometric analysis of whole blood found platelet activation to be increased in pre-eclampsia, but not in other forms of hypertension in pregnancy (Harlow et al. 2002). The results of this latter study did not support those of previous studies suggesting that platelet activation is an early preclinical feature of pre-eclampsia (Janes et al. 1995, Konijnenberg et al. 1997).

4.3.4. Insulin resistance

Metabolic abnormalities linked to the insulin resistance syndrome are also observed in women with PIH to a greater degree than in normotensive pregnant women (Kaaja et al. 1999b, Solomon et al. 2001). Reduced SHBG levels are a marker of hyperinsulinemia and insulin resistance (Haffner et al.1988). First trimester SHBG levels have been significantly reduced in women who developed pre-eclampsia and the SHBG level may be useful biomarker for pre-eclampsia especially among lean women (Wolf et al. 2002). Laivuori and colleagues (1999) found that in women with pre-eclampsia elevated plasma homocysteine levels were inversely related to insulin sensitivity. An elevated plasma homocysteine level in early pregnancy can increase the risk of developing severe pre-eclampsia almost threefold (Cotter et al. 2001). Hypertriglyceridemic dyslipidemia before 20 weeks of gestation was associated with the risk of early but not late onset of pre-eclampsia (Clause et al. 2001). In a prospective case-control study (Chappell et al. 2002a) indices of antioxidant status, oxidative stress, placental and endothelial function and serum lipid concentrations were evaluated from 20 weeks of gestation until delivery. At 20 weeks HDL cholesterol, PAI-1/PAI-2 ratio, leptin and placental growth factor were able to distinguish pre-eclampsia from the low-risk group. The combination of biochemical indices increased the prediction values of the tests.

5. Doppler investigations

5.1. Doppler measurement of uterine arteries

Insufficiency of the uteroplacental circulation due to failure of trophoblastic invasion of the spiral arteries is assumed to be a common etiological factor in both pre-eclampsia (Khong et al. 1986) and intrauterine growth restriction (Brosens 1977). The introduction of color Doppler imaging has made it possible to insonate the uterine artery over its apparent ′crossover′, the external iliac artery (Lees et al. 2003), thus allowing accurate placement of the pulsed Doppler gate over the vessel with good reproducibility (Bower et al. 1993). Using a transvaginal probe, the uterine artery can be identified at the level of the internal cervical os, as it enters the uterus, and as it ascends into the uterine body (Harrington and Campbell 1995). It is possible to examine the uterine artery by the transabdominal approach after 12 week of gestation, when the uterus becomes an abdominal organ. By placement of the transducer in the relevant iliac fossa, color Doppler can follow the course of the uterine artery from the lateral pelvic wall across the external iliac artery onto the cervix and up the lateral wall of the uterus (Harrington and Campbell 1995).

5.2. Doppler indices and diastolic notch of uterine artery

As pregnancy progresses, there is an increase in diastolic flow, as seen in the fall in the resistance index (RI = peak systolic minus end-diastolic Doppler shift over peak systolic Doppler shift) and in the pulsatility index (PI = peak systolic minus end diastolic Doppler shift over mean maximum Doppler shift) and a gradual disappearance of the notch in the uterine arteries. Post-systolic notch, a steep systolic slope in a flow velocity waveform, and a small amount of diastolic flow is typical for non-pregnant state (Harrington and Campbell 1995).

There is also a dramatic rise in the mean velocity of blood flow in the uterine vessels, especially towards the end of the first trimester, at 12-15 weeks (Harrington and Campbell 1995). By the 20th week of pregnancy the majority of patients evince a low-resistance uterine artery flow velocity waveform (FVW) with 16% retaining bilateral notching in the uterine arteries at 18-22 weeks of gestation, and 5.1% (Bower et al. 1993) and 8.9% (Harrington et al .1996) at 24 weeks of gestation. High-resistance patterns assessed by Doppler velocimetry have closely correlated with impaired trophoblastic migration as assessed by examination of placental bed biopsies (Lin et al. 1995).

5.3. Doppler ultrasound of uterine arteries as screening test

The lack of trophoblastic invasion of the decidual and myometrial segments of the spiral arterial vasculature resulting in an increased flow resistance in the uterine arteries (Meekins et al.1994) has provided the possibility of using Doppler velocity waveform analysis in the second trimester as a screening test for pre-eclampsia. Several studies have been published on the subject, with extreme variability in results. This can be partly explained by differences in population selection (low-risk/high-risk), in gestational age at the time of scanning, in scanning techniques (continuous-wave, pulsed-wave Doppler), in the outcome measures and in the different cut-off values utilised. (Valensise 1998, Chien 2000).

5.3.1. Doppler indices

Assessment of impedance in the uteroplacental circulation has usually relied upon basic descriptions of the waveform such as the resistance index and the ratio of peak-systolic to end-diastolic blood flow velocities (S/D ratio). Abnormality has been defined as either an absolute cut-off, for example RI>0.58 (Steel et al. 1990, Frusca et al. 1997) or a measurement greater than a particular centile on the reference range, for example RI>95th centile (Bewley et al. 1991) or RI> 90th centile (Chan et al. 1995). When RI has been used as a predictive test the sensitivity has ranged from 13 to 100 % and specificity from 64 to 94 % in different studies (Table 1).

Peak systolic over early diastolic velocity ratio (AC ratio) was proposed by Irion and colleagues (1998) as a quantitative substitute for the diastolic notch and the predictive values (sensitivities 26% to 34%, positive predictive values 7% to 28%) were similar to those with diastolic notch. North and colleagues (1994) reported similar predictive values of AC and RI for pre-eclampsia and IUGR. Bower and colleagues (1998) compared the pulsatility index (PI) with the AC ratio and a second index of notch (D-C)/B, and found that PI gave the best results, (D = peak of notch, C = nadir of notch, B = end diastolic flow).

5.3.2. Early diastolic notch and bilateral notches of uterine arteries

The presence of an early diastolic notch in the waveform is indicative of increased resistance (Campbell et al. 1983, Adamson et al. 1989). The early diastolic notch has been found to be a significantly better predictor of proteinuric pregnancy-induced hypertension than RI (Harrington et al. 1991, Thaler et al. 1992, Bower et al. 1993, Harrington et al. 1996) or systolic-diastolic ratio (Fleischer et al. 1986) (Tables 1 and 2). In contrast, Aardema and associates (2000a) did not find that the diastolic notch (either bilateral or unilateral) performed better than PI. Also in a recent study in an unselected population (Martin et al. 2001) the uterine artery mean PI > 2.35 at 11-14 weeks of gestation had better predictive value than bilateral notches. Bilateral notching has been found to be superior to unilateral in predicting hypertensive disorders of pregnancy (Harrington et al. 1996, Zimmerman et al. 1997, Antsaklis et al. 2000). Harrington ´s group (1996) found bilateral early diastolic notches in approximately 3.9% of unselected women at 24 weeks, a group that included about 54.5% of women who subsequently developed pre-eclampsia and 21.8% of those who delivered infants with birth weights below the tenth percentile for gestation. The negative predictive value of bilateral notching in predicting pre-eclampsia or IUGR has ranged from 87 to 100% at 16- 24weeks (Papageorghiou et al. 2002), whereas the positive predictive value has varied from 11-17.9 % in an unselected population at 20 weeks (Kurdi et al. 1998) to 75-80% in high-risk women at 22-24 weeks (Venkat-Raman et al. 2001) (Tables 1 and 2). Harrington and colleagues (1996) in a two-stage screening test (at 19-21 and 24weeks) obtained a sensitivity of 78% and a positive predictive value of 31% in an unselected population and the latter increased to 50% for women with bilateral notching. Harrington and co-workers (1997) could improve the specificity of bilateral notching in predicting pre-eclampsia at 12-16 weeks of gestation to 85% by using information derived from multiple parameters, in particular indices of resistance and flow. Chan and associates (1995) concluded that the best criterion for predicting PIH or IUGR is an RI above the 90th percentile with the persistence of bilateral notches. Two recent extensive studies have shown that at 23-24 weeks of gestation bilateral notching and PI >95th centile have similar sensitivities in predicting pre-eclampsia or IUGR (Albaiges et al. 2000, Papageorghiou et al. 2001)

Antsaklis and co-workers (2000) showed the sensitivity of notching to diminish with advancing gestational age while the specificity and positive predictive values of the test increased significantly (Table 2). They concluded that screening is best performed at 24 weeks. At this stage of gestation, using the definition ´any notch` (unilateral or bilateral), the sensitivity for pre-eclampsia was 76% with a specificity of 95%. For pre-eclampsia requiring delivery before 34 weeks, the sensitivity was over 90%. Screening at 20 rather than 24 weeks had a higher sensitivity (81%) and lower specificity (87%) for pre-eclampsia; conversely, by 32 weeks, the sensitivity for pre-eclampsia was just over 70% with 97% specificity.

Aardema and colleagues (2000a) attempted to obviate the subjectivity in defining a notch by using a quantification of the diastolic notch, a notch index, but it did not improve the predictive value of PI. On the other hand Ohkuchi and colleagues (2000) confirmed that the notch index can predict the development of pre-eclampsia and / or small-for-age infants with improved positive predictive value as compared to bilateral notches. Albaiges and colleagues (2000) reported that women at the highest risk are those with bilateral notches and a high mean PI at 23 weeks of gestation. They carry a 40% risk of developing pre-eclampsia and 45% for delivering infants of birth weight less than the tenth percentile. The risk group comprised 2% of the screening population with a relative risk of 50 to 100 for an adverse outcome before 34 weeks of gestation and fetal death.

Chien and colleagues (2000) in their systematic review concluded that the use of the uterine artery flow waveform ratio ± diastolic notch has limited diagnostic accuracy in predicting pre-eclampsia, IUGR and perinatal death. They suggested that future research should focus on Doppler ultrasonic detection of uterine artery diastolic notches alone to predict pre-eclampsia, especially in pregnant women considered to be at high risk of this condition. The conclusion of a recent review of second-trimester uterine artery Doppler screening in an unselected population was that increased impedance to flow in the uterine arteries in pregnant women attending for routine antenatal care identifies about 40% of those who subsequently develop pre-eclampsia and about 20% of those who develop fetal growth restriction. Women with normal impedance to flow in the uterine arteries constitute a group at only low risk of developing obstetric complications related to uteroplacental insufficiency (Papageorghiou et al. 2002).

In conclusion, uterine artery Doppler ultrasound is a noninvasive method detecting the women who are at risk of hypertensive disorders of pregnancy. Doppler is good in predicting a severe pre-eclampsia (Bower et al. 1993, Papageorghiou et al. 2001) and a disease requiring delivery before 37 weeks of gestation or earlier (Harrington et al. 1996, Kurdi et al. 1998, Albaiges et al. 2000, Papageorghiou et al. 2001). The gestational age at screening has moved to 23-24 weeks, as earlier screening has been associated with a higher false-positive rate (Papageorghiou et al. 2002). Uterine artery Doppler flow velocity has limited diagnostic accuracy in predicting hypertensive disorders in low-risk populations (Chien et al. 2000) whereas in high-risk women the pre-test probability of pre-eclampsia of 9.8% is raised to a post-test probability of 23.5% (Chien et al. 2000).

The research should focus on high-risk pregnancies (Chien et al. 2000) and on improvement of the screening efficacy of the earlier uterine artery Doppler (Harrington et al. 2000). Other methods to reduce the high number of false-positive patients resulting from Doppler ultrasound evaluation could be combination of the biochemical markers of pre-eclampsia with Doppler investigation (Aquilina et al. 2001).

Table 1. Description of Doppler ultrasound studies with resistance indices of uterine arteries as a screening method for hypertensive disorders of pregnancy

Trial	n	Subjects	Gestational age at scan	Definition of Doppler abnormality		Predictive values SE (%)	SP (%)	PPV (%)	NPV (%)	Outcome
Campbell et al. 1986	126	Low-risk women	16-18	RI	>2SD	68	69	42	87	PIH, IUGR
Fleischer et al. 1986	71	Hypertensive women	Not defined	S/D	>2SD	81	90	93	91	Adverse outcome related to PIH
Steel et al. 1988	200	Nulliparous women	18-20 24	RI	>0.58	29-45	64-91	9-40	66-93	PE, IUGR
Hanretty et al. 1989	543	Low-risk women	26-30 34	S/D	>95th	-	-	-	-	No difference in outcomes. Lower birth weight if abnormal umbilical waveform.
Steel et al. 1990	1014	Nulliparous women	18 24	RI	>0.58	63-100	89-90	10-13	-	PE, IUGR
Harrington et al. 1991	2437	Low-risk women	20 24	RI	>95th	25	95	-	-	PE, IUGR
Bewley et al. 1991	977	Low-risk women	16-24	RI	>95th	13 21	97 95	67 25	94 94	Any pregnancy complication, severe pregnancy complication
Thaler et al. 1992	140	Hypertensive women	28-40	RI	>2SD Notch	-	-	-	-	Notch in Doppler predicted poor pregnancy outcome better than RI. Adverse outcome in all women with diastolic or systolic notch .

Bower et al. 1993	2058	Low-risk women	18-22 24	RI	>95th	82	87	12	99.5	PE
North et al. 1994	458	Nulliparous women	19-24	RI AC	>95th	40 47	91 90	30 33	94 94	PE or IUGR
Park et al. 1996	2321	Low-risk women	>28	S/D	>2.6 Notch Both	21.4 14.2 11.3	94.4 99.4 99.8	47.5 82.9 92.6	83.5 83 82.7	Adverse outcome
Hoefstaetter et al. 1996	110 421	Uncomplicated pregnancies High-risk pregnancies	PI Notch		>1.2 -	-	-	-	-	Notch was better than PI in predicting an adverse perinatal outcome.
Caruso et al. 1996	54	Hypertensive women	23-24	RI	>90th	100	88-76	69-31	100	Superimposed PE, IUGR
Frusca et al. 1997	456	Nulliparous women	20, 24	RI	>0.58	43-100	92-94	8-36	96-100	PE, IUGR
Zimmerman et al. 1997a	175 172	High- risk and low- risk women	21-24	RI	>0.68	56	80	-	-	PPIH, IUGR
Irion et al. 1998	1311	Nulliparous women	26	RI AC ratio	>0.58	26-29	88-89	7-25	-	PE, IUGR
Aardema et al. 2000b	94	Previous PIH	21-22	PI	>1.3	25-80 83	68-69 71	13-24 29	83-97 97	PIH or IUGR Poor pregnancy outcome
Martin et al. 2001	3324	Singleton pregnancies	11-14	PI	<2.35	27 11.7	95.4 95.6	11 21.9	98.4 91.1	PE IUGR

SE = sensitivity, SP = specificity, PPV = positive predictive value, NPV = negative predictive value, RI = resistance index, S/D = systolic/diastolic ratio, AC ratio = peak systolic/early diastolic ratio, PI = pulsatility index, PE = pre-eclampsia, PIH = pregnancy-induced hypertension, IUGR = intrauterine growth restriction, PPIH= proteinuric pregnancy-induced hypertension

Table 2. Description of Doppler ultrasound studies with notches in the uterine arteries as a screening method in predicting hypertensive disorders of pregnancy.

Trial	n	Subjects	Gestational age at scan (weeks)	Definition of Doppler abnormality	Predictive values				Outcome
					SE (%)	SP (%)	PPV (%)	NPV (%)	
Fleischer et al. 1986	71	Hypertensive women	Not defined	Unilateral notch	87	95	-	-	PE, abnormal outcome
Harrington et al. 1991	2437	Low-risk women	20 24	Unilateral notch	76	86-97	-	-	PE, IUGR

Bower et al. 1993	2058	Low-risk women	18-22 24	Unilateral notch	82 78	86.9 96	12 28	99.5 99.5	PE
Chan et al. 1995	358	High- risk women	20, 28, 36	Bilateral notch and RI>90^{th} at 20 weeks	21.74 26.67	86.85 97.85	35.71 57.14	93.80 92.54	Severe PIH Severe pregnancy complications
Harrington et al. 1996	1326	Low-risk women	18-21 24	Unilateral notch	22.7 13.7	95.5 95.9	16.1 29.0	97 90.1	PPIH SGA
				Bilateral notches	54.5 21.8	97.9 97.8	50 50	98.3 90.8	PPIH SGA
Harrington et al. 1997	652	Low-risk women	12-16	Bilateral notches	93	69		96-100	PE
				Seven parameters model	92.9 50.9	85.1 84.5	23.6 25.4	99.5 94.3	PE SGA
Zimmerman et al. 1997a	175 172	High-risk and low-risk women	21-24	Bilateral notches	31	87			PPIH, IUGR
Kurdi et al. 1998	1022	Low-risk women	19-21	Bilateral notches	61.9 36.8	88.7 89.2	11.1 17.9	99.0 95.7	PE IUGR
Irion et al. 1998	1311	Nulliparous women	26	Notch	26-30	87-88	7-24		PE, IUGR
Antsaklis et al. 2000	654	Nulliparous women	20 24	Unilateral notch	81 76.1	87.2 95.1	17.3 34	99.3 99.2	PE
Coleman et al. 2000	114	High-risk women	22-24	Bilateral notches	47	53	76	65	PE, SGA, placental abruption, intrauterine death
Ohkuchi et al. 2000	288	Low-risk women	16-24	Bilateral notches NDI	56-17 67-33	92-91 92	18-11 22	99-94 99-95	PE SGA
Aardema et al. 2000	94	Previous PIH	21-24	Unilateral notch	25-50 50	81-81 76	20-22 20	85-90 93	PIH or SGA Poor pregnancy outcome
Albaiges et al. 2000	1757	Low-risk women	23	Bilateral notches and PI>1.45	23 22.6	98.6 98.4	39.4 31.5	97 97.6	PE SGA
Venkat-Raman et al. 2001	170	Women with antiphospholipid antibodies	16-18, 22-14	Bilateral notches	75 80	94 94	75 80	94 94	PE SGA
Papageorghiou et al. 2001	8335	Singleton pregnancies	22-24	Bilateral notches PI>1.63	69 24 13	-	-	-	PE+IUGR PE IUGR

SE = sensitivity, SP = specificity, PPV = positive predictive value, NPV = negative predictive value, PE = pre-eclampsia, PIH = pregnancy-induced hypertension, PPIH = proteinuric pregnancy-induced hypertension, IUGR = intrauterine growth restriction, SGA = small for age, NDI = notch depth index

6. Prevention of pre-eclampsia

During the past two decades, numerous clinical studies and randomised trials have reported various methods to prevent or reduce the incidence of pre-eclampsia. These methods were used in an attempt to correct certain abnormalities such as biochemical imbalance, some pathophysiologic mechanism, or dietary deficiency (Sibai 1998).

6.1. Antihypertensive drugs

Six randomised trials evaluating the use of methyldopa, labetalol and atenelol to reduce the incidence of superimposed pre-eclampsia failed to demonstrate any reduction (Sibai 1996). A meta-analysis of nine randomised trials involving the use of diuretics in pregnancy revealed no decrease in pre-eclampsia (Collins et al. 1985). Easterling and colleagues (1999) identified women at risk of pre-eclampsia by means of measurement of cardiac output in the second trimester, and in this high risk group of women atenolol reduced the incidence of pre-eclampsia. Ketanserin, a selective serotonin-2-receptor antagonist, lowered the rate of pre-eclampsia and severe hypertension in pregnant women with mild to moderate hypertension (Steyn and Odendaal 1997).

6.2. Magnesium supplementation

Prophylactic oral magnesium supplementation was not found to be beneficial in the prevention of gestational hypertension with or without proteinuria in two trials involving a total of 942 women (Spatling et al. 1988, Sibai et al. 1989).

6.3. Zinc supplementation

Plasma, leukocyte and placental zinc levels have been found to be reduced in women with pre-eclampsia as compared with normotensive pregnant women (Lazebnik et al. 1989). Hunt and colleagues (1984) reported a decrease in the incidence of pregnancy-induced hypertension with zinc supplementation, whereas Mahomed with his colleagues (1989) in contrast found a higher incidence of pre-eclampsia among women receiving zinc supplementation. According to a recent review there is insufficient evidence to suggest the use of zinc supplementation for prevention of pre-eclampsia (Mahomed 2002).

6.4. Fish oil supplementation

Fish oil containing the prostaglandin precursors eicosapentaenoic acid and docosa-hexaenoic acid are postulated to prevent pre-eclampsia (Moutquin et al. 1997). Six randomised trials of the preventive efficacy of dietary n-3 fatty acids found no effect on IUGR and PIH (Olsen et al. 2000).

6.5. Antioxidants

Increased markers of lipid peroxidation in the plasma (Hubel et al. 1996, Barden et al.1996) or in the placenta (Poranen et al. 1996) of pre-eclamptic women and the low concentrations of antioxidants in the plasma (Mikhail et al. 1994, Poranen et al. 1996) and placenta (Wang et al. 1996, Poranen et al. 1996) seen in pre-eclamptic women suggest a state of oxidative stress in the disorder. It has been shown that exogenous vitamisn C and E had no effect on lipid peroxidation or antioxidant enzymes in normal placentas, whereas in pre-eclamptic placentas vitamin C decreased peroxidation (Poranen et al 1998). Previous studies of vitamin supplementation in women with established severe pre-eclampsia reported no substantial clinical benefit (Stratta et al. 1994, Gulmezoglu et al. 1997). In a recent randomised placebo-controlled trial (Chappel et al. 2002b) supplementation with vitamins C and E was associated with a 21% decrease in the PAI-1/PAI-2 ratio during gestation, and pre-eclampsia occurred significantly more often in the placebo group (17%) as compared to the vitamin group (8%). Nonetheless, a multicentre clinical trial with large numbers of patients is needed before any decisions can be made regarding clinical usefulness of antioxidants.

6.6. Calcium supplementation

The original epidemiologic observations showed an inverse association between calcium intake and the incidence of pre-eclampsia (Villar et al. 1983). One hypothetical mechanism of action is that calcium supplementation reduces serum parathyroid hormone levels, which in turn reduces the intracellular calcium concentration in vascular smooth muscle cells, diminishing their responsiveness to pressure stimuli (Belizan et al. 1988). A large prospective randomised controlled trial (Belizan et al. 1991) and several other smaller randomised controlled trials, mostly from Latin

America (Villar et al. 1987, Lopez-Jaramillo et al. 1989, Sanchez- Ramos et al. 1994) have demonstrated a trend toward a protective effect of calcium supplementation against pre-eclampsia. This assumption gained support from one meta-analysis (Bucher et al. 1996). However, the trials included in this meta-analysis differed regarding the populations studied, study designs, gestational ages at enrolment, sample sizes and the dose of elemental calcium used, as well as the definitions of end point (Sibai 1998). An extensive placebo-controlled trial among healthy nulliparous women (CPEP) (Levine et al. 1996) found no evidence of a beneficial effect in women due to calcium supplementation. A recent review concluded that calcium supplementation is possible beneficial for women at high risk of gestational hypertension and in communities with low dietary calcium intake (DerSimonian and Levine 1999). Further studies are needed to establish the efficacy of calcium in pre-eclampsia prevention in healthy high-risk populations and the optimum dosage likewise calls for further investigation (Atallah et al. 2002).

6.7. Acetylsalicylic acid

Acetylsalicylic acid (ASA) has been used in several clinical trials to prevent pre-eclampsia (Duley et al. 2001). ASA acetylates the cyclo-oxygenase in the platelets and reduces the formation of TxA_2 (Roth et al. 1975). ASA inhibits irreversibly the cyclo-oxygenase in the platelets for their lifespan. In contras, in endothelial cells, the cyclo-oxygenase enzyme is relatively rapidly resynthesised after being exposed to ASA and prostacyclin synthesis is thus re-established (Viinikka 1990).

The first articles recommending ASA for the prevention of pre-eclampsia or IUGR stressed that their results concerned only patients presenting with a specific indication, so-called high risk patients, including women with previous poor outcome of pregnancy (Beaufils et al. 1985, Benigni et al. 1989, Uzan et al. 1991, Viinikka et al. 1993), or an increased pressor sensitivity to angiotensin II (Wallenburg et al. 1986), or an abnormal umbilical artery flow (Trudinger et al. 1988), or women with positive roll-over test results (Schiff et al. 1989), or abnormal uterine artery flow waveform patterns (McParland et al. 1990), or nulliparae (Sibai et al. 1993, Hauth et al. 1993). Subsequent large trials tested ASA for broader indications and reported negative results (CLASP 1994, ECPPA1996, Rotchell et al. 1998, Golding et al. 1998, Caritis et al. 1998b). A trend towards a reduction in early onset of pre-eclampsia with ASA treatment was shown in CLASP study. It also suggested a more protective effect of ASA the earlier the gestational age at trial entry. A recent Cochrane review of the use of antiplatelet drugs for the prevention of pre-eclampsia showed a 15% decrease in the risk of pre-eclampsia, a14% reduction in the risk of a stillbirth or neonatal death, and an 8% reduction in the risk of preterm birth (Duley et al. 2001). The writers concluded that as the reductions in risk are small to moderate, relatively large numbers of women will need to be treated to prevent a single adverse outcome (baseline risk 8%, the number of patients to be treated to prevent one case of pre-eclampsia was 89). The review left open the questions whether small subgroups of high-risk women might derive greater benefit, and whether earlier treatment, or a higher dose of ASA, would afford additional benefits without an increase in adverse effects. The results of a previous meta-analysis showed that early (started at 12-16 weeks of gestation) ASA treatment (50-80 mg/day) significantly reduced (18 % reduction) the risk of intrauterine growth restriction without increasing perinatal mortality (Leitich et al. 1997). The importance of gestational age at the commencement of prophylaxis is essential as we remember that the second wave of intravascular trophoblast migration is known to occur at 16-18 weeks of gestation (De Wolf et al.1980, Pijnenborg et al. 1983) and the compromised uteroplacental circulation may play an important role in hypertensive disorders of pregnancy (Campbell et al. 1986, Trudinger et al. 1985). This implies that abnormalities leading to pre-eclampsia (Khong et al. 1986) may be established before onset of ASA administration, which would limit the effectiveness of the treatment.

A recent meta-analysis determined the effectiveness of ASA in the prevention of pre-eclampsia in women identified by an abnormal second-trimester uterine artery Doppler examination as at high risk of pre-eclampsia (Coomarasamy et al. 2001) (Table 3).

Table 3. Randomised trials of the effect of acetylsalicylic acid in the prevention of pre-eclampsia in women with abnormal uterine artery Doppler velocimetry.

Trial	Gestation at testing (weeks)	Doppler abnormality	ASA dose	OR (95% CI) for PE	
McParland et al. (1990)	24	RI≥0.58	75mg/day	0.18	(0.05,0.61)
Bower et al. (1996)	24	RI>95%	60mg/day	0.59	(0.20,1.68)
Zimmermann et al. (1997b)	22-24	Bilateral notch	50mg/day	2.3	(0.36,13.77)
Morris et al. (1996)	18	S/D>3.3 or S/D>3 and diastolic notch	100mg/day	0.52	(0.15, 1.82)
Harrington et al. (2000)	17-23	RI>50% and bilateral notch or RI>90% and unilateral notch or RI>95%	100mg/day	0.73	(0.27, 2.03)

Pooling of the results from the five trials showed a significant benefit of ASA in reducing pre-eclampsia. The baseline risk in women with abnormal uterine artery Doppler was 16%, and the number of women needing treatment with ASA to prevent one case of pre-eclampsia was 16 (95% CI 8, 316) (Coomarasamy et al. 2001). On the other hand, in low-risk women another systematic screening study with uterine artery Doppler at 20 and 24 weeks of gestation found no effect of low-dose ASA treatment on the incidence of pre-eclampsia or IUGR (Goffinet et al. 2001). The safety of low-dose ASA treatment in pregnancy has been confirmed by a meta-analysis (Leitich et al. 1997) and a systematic review (Duley et al. 2001).

AIMS OF THE STUDY

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1. To determine the dose of ASA which inhibits the production of the vasoconstrictive, aggregatory thromboxane A_2 while sparing the production of the vasodilatory antiaggregatory prostacyclin (I).

2. To evaluate the efficacy of low-dose ASA in the prevention of hypertensive disorders in high-risk pregnancies screened by transvaginal Doppler ultrasound at 12 to 14 weeks of gestation (II).

3. To assess the value of bilateral notching in the uterine arteries at 12 to 14 weeks of gestation in predicting pregnancy-induced hypertension, pre-eclampsia and intrauterine growth restriction (III).

4. To evaluate the effect of low-dose ASA on thromboxane $A_{2,}$ and prostacyclin throughout pregnancy (IV).

5. To evaluate the urinary excretion of 9α, 11β-prostaglandin F_2 in pregnancy and to compare women carrying a high risk of hypertensive disorders of pregnancy to normotensive pregnant women (V).

PATIENTS AND METHODS

The Ethics Committee of Tampere University Hospital and study II also by the Ethics Committee of the City of Tampere approved the study protocols. All women participating gave their informed written consent. The aims of the studies and study populations are displayed on Table 4.

Table 4. The study populations and the aims of the studies in original communications.

	Aims of the study	**Study population**
Study I	To determine the dose of ASA that shifts the ratio of TxB_2 to PGI_2 in favor of PGI_2	Hypertensive pregnant women (n=7) Non-pregnant women (n=5) Normotensive pregnant women(n=7)
Study II	To evaluate the efficacy of low-dose ASA in the prevention of PIH and IUGR in high-risk women with bilateral notches in the uterine arteries at 12-14 weeks of gestation	High-risk women with bilateral notches in the uterine arteries at 12-14 weeks of gestation (n = 86) randomised to ASA (n = 43) and placebo (n= 43) groups.
Study III	To assess the value of bilateral notches in the uterine arteries at 12-14 weeks of gestation in predicting hypertensive disorders of pregnancy in high-risk women.	The women in the placebo group in study II (n=43) Pregnant women with no bilateral notches in the uterine arteries at 12-14 weeks of gestation (n= 29)
Study IV	To investigate the prostanoid formation in pregnancies at high risk of hypertensive disorders of pregnancy, and the effect of low-dose ASA on prostanoids.	The women in the study II with ASA (n=43) or placebo treatment (n=43).
Study V	To determine $9\alpha,11\beta$-prostaglandin F_2 in pregnancies at high risk of hypertensive disorders of pregnancy and the effect of ASA on $9\alpha,11\beta$-prostaglandin F_2.	The women in study II with ASA (n=43) or placebo treatment (n=43) and 15 normotensive non-pregnant women 17 normotensive pregnant women at 12-14 weeks of gestation 15 normotensive pregnant women at 30-34 weeks of gestation

ASA = acetylsalicylic acid, PIH = pregnancy-induced hypertension, IUGR = intrauterine growth restriction

1. Study I (The dose of ASA)

1.1. Patients

The population of study I consisted of seven hypertensive pregnant women admitted to the maternity clinic of Tampere University Hospital, Tampere, Finland; five voluntary non-pregnant healthy women and seven voluntary normotensive pregnant women served as controls. The inclusion criteria for the group of hypertensive pregnant women were: blood pressure > 140/90 mmHg (or a rise exceeding 30/15 mmHg), proteinuria < 3g/l, duration of gestation 12-35 weeks, and no medical treatment other than antihypertensive therapy. The inclusion criteria for the controls were pregnant or non-pregnant and healthy, age 18-50 years, blood pressure <140/90 mmHg, and no medical treatment. The common exclusion criteria for each group were allergy to ASA, asthma, previous peptic ulcer disease, and use of prostaglandin inhibitors during the past ten days preceding the study. The demographic data of the patients and controls are given in Table 5.

Table 5. Characteristics of the women at trial entry in study I.

Hypertensive pregnant women	Age (yr)	Gestation (wk)	Weight (kg)	Non-pregnant women	Age (yr)	Weight (kg)	Normotensive pregnant women	Age (yr)	Gestation (wk)	Weight (kg)
1	40	16	77	1	27	73	1	29	19	53
2	34	20	68	2	27	51	2	30	29	69
3	40	31	76	3	31	71	3	28	26	60
4	35	32	87	4	36	54	4	26	27	68
5	31	12	82	5	39	66	5	35	33	84
6	34	16	115				6	41	21	72
7	27	20	82				7	26	24	75
Mean	34	21	84		32	63		30.7	25.6	68.7
SD	(4.3)	(7.1)	(13.9)		(4.8)	(8.9)		(5.5)	(4.4)	(10.1)

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1.2. Study design

The objective of this controlled longitudinal study was to determine the dose of ASA that inhibits the production of the vasoconstrictive, aggregatory thromboxane A_2 (TxA_2) while sparing the production of the vasodilatory antiaggregatory prostacyclin (PGI_2). The baseline blood and urine samples were obtained from each subject and serum thromboxane B_2 (s-TxB_2) and the urinary metabolites of thromboxane A_2 and prostacyclin, 11-dehydro-thromboxaneB_2 (11-dehydroTxB_2) and 2, 3-dinor-6-ketoprostaglandin$F_{1\alpha}$ (2, 3-dinor-6-keto-PG$F_{1\alpha}$), respectively, were measured. The hypertensive pregnant and the non-pregnant women received ASA in three periods, each lasting 10-12 days, the periods immediately following each other. The daily dose of ASA during the first period was 0.5mg/kg, during the second 1.0mg/kg, and during the third 2.0mg/kg. The same blood and urine samples were taken after each treatment period as at baseline. The normotensive pregnant women gave only the baseline blood and urine samples, and received no ASA or placebo treatment.

2. Studies II, III and IV

2.1. Patients

Women with risk factors of pre-eclampsia or IUGR were recruited from the population of pregnant women routinely attending antenatal clinics in Tampere and its environs. A total of 120 women were screened by transvaginal Doppler ultrasound at 12 to 14 weeks of gestation. The risk factors included a history of chronic hypertension, familial risk of pre-eclampsia (mother or sister), gestational diabetes, age <20 or > 40 years, previous pre-eclampsia, previous pregnancy with intrauterine growth restriction, or with intrauterine death. The exclusion criteria were gestational weeks <12 or > 14, asthma, allergy to acetylsalicylic acid, previous peptic ulcer, or the use of prostaglandin inhibitors within ten days before investigation. Women with a constant bilateral diastolic notch in the uterine arteries were asked to participate in a randomised placebo-controlled trial. Altogether 90 were found to have bilateral notches and forty-five were allocated to ASA and forty-five to placebo groups. The outcome was also documented in 29 of those 30 women without bilateral notches who were excluded from randomisation. Before ultrasound examination the women gave informed consent. The baseline characteristics of the study population are shown in Table 6.

Table 6. Characteristics of the women at trial entry in studies II and III. Values are given as n (%) or mean [SD].

	ASA (n = 43)	P-value	Placebo (n = 43)	P-value	Control (n = 29)
Maternal age (years)	30.6 [6.3]	NS	30.0 [5.9]	0.001	34.6 [6.3]
Maternal weight (kg)	72.2 [2.5]	NS	72.4 [2.9]	0.001	86.6 [19.1]
Systolic BP (mmHg)	131.0 [15.6]	NS	132.4 [16.4]	0.162	136.7 [6.1]
Diastolic BP (mmHg)	83.8 [10.9]	NS	85.4 [12.1]	0.477	86.4 [10.4]
Primigravid	15 (34.9)	NS	10 (23.3)	0.119	4 (14.3)
Multiparous	27 (62.8)	NS	33 (76.7)	0.321	23 (79.3)
Previous PIH	18 (41.9)	NS	27 (62.8)	0.180	11 (37.9)
Previous pre-eclampsia	14 (32.6)	NS	21(48.8)	0.052	6 (20.7)
Previous IUGR	6 (14.0)	NS	10 (23.3)	0.120	2 (6.7)
Previous stillbirth	3 (7.0)	NS	1(2.3)	0.267	3 (10.3)
Chronic hypertension	16 (37.2)	NS	13 (30.2)	0.790	9 (31.0)
Diabetes	0	NS	4 (9.3)	0.474	2 (6.9)

P-value; differences between ASA and placebo-groups and differences between placebo and control groups. ASA = acetylsalicylic acid; IUGR = intrauterine growth restriction; BP = blood pressure; PIH = pregnancy-induced hypertension; Control = women without bilateral notching in the uterine arteries. NS = non-significance.
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2.2. Study design (Studies II, III, IV)

The prospective double blind randomised placebo-controlled longitudinal trial commenced in October 1997, and the last patient was randomised in November 1999. The ASA prophylaxis was started at 12-14 weeks of gestation. The daily dose of ASA was 0.5mg/kg, adjusted at a follow-up visit if the weight of the woman exceeded the initial weight by at least 10%. The adjusted dose of ASA was calculated according to woman's actual weight. The randomisation and adjustment of ASA-dose took place in the Pharmacy of Tampere University Hospital, and the code was not broken until after the delivery of the last woman randomised.

The women were examined twice during pregnancy, at gestational weeks 24-26 and 32-34, follow-up entailing ultrasound assessment of fetal growth, amniotic fluid volume, umbilical and uterine artery waveform analysis, and non-stressed fetal cardiotocography. Maternal blood pressure and urinary protein excretion were measured at the follow-up visits, during hospitalisation and when women came into the labor. Blood pressure was measured in sitting position after 15 minutes` rest using an automatic cuff.

Pregnancy-induced hypertension was defined as a sustained blood pressure increase to levels 140mmHg systolic and 90mmHg diastolic after 20 weeks of gestation. Chronic hypertension was defined as hypertension developing before 20 weeks of gestation. Pre-eclampsia was defined as blood pressure changes as above together with proteinuria (defined as >300mg/24h or ≥ 1+ dipstick in a random urine sample). Superimposed pre-eclampsia was defined as proteinuria developing during pregnancy in a woman with known chronic hypertension. Delivery before 37 weeks of estimated gestation was defined as preterm. The gestation age was defined by ultrasound assessment. Intrauterine growth restriction was defined as growth under the 10th centile based on postnatal infant weights.

2.2.1. Low-dose ASA in prevention of hypertensive disorders of pregnancy (StudyII)

The objective was to evaluate the efficacy of low-dose ASA in the prevention of pregnancy-induced hypertension and IUGR in high-risk pregnancies as determined by transvaginal Doppler ultrasound study of the uterine arteries at 12 to 14weeks of gestation.

The main outcome measures were pregnancy-induced hypertension, pre-eclampsia and IUGR, duration of pregnancy and birth weight. At delivery the duration of pregnancy, maternal blood pressure and blood loss, maternal hematocrite and urine excretion of protein were registered. Birth weight, Apgar scores, the blood-gas analysis in umbilical artery blood, the thrombocytes and hematocrite were recorded in all offspring.

2.2.2. Bilateral notching in predicting hypertensive disorders of pregnancy (StudyIII)

The objective was to assess the predictive value of Doppler flow velocities in uterine and umbilical arteries in early pregnancy, in identifying pregnant women at risk of subsequently developing PIH, pre-eclampsia or IUGR. The presence or absence of bilateral notches, resistance index (RI), pulsatility index (PI), mean flow and maximum systolic flow velocity in the uterine arteries, and the RI and the PI of the umbilical arteries were investigated. The women with bilateral notches in the uterine arteries at 12-14 weeks were randomised to ASA or placebo groups and ultrasound examination was performed with measuring same parameters at 24-26 and 32-34 weeks. To evaluate the predictive value of bilateral notching for PIH, pre-eclampsia and IUGR we compared the high-risk women with bilateral notching receiving placebo to the women without bilateral notching.

2.2.3. The effect of ASA on prostanoids in normal pregnancy and in hypertensive disorders of pregnancy (Study IV)

The objective was to assess the extent to which prostacyclin and thromboxane A_2 are involved in the pathophysiology of PIH and IUGR. The effect of low-dose ASA (0.5mg/kg/day) on the ratio of prostacyclin /thromboxane A_2 was also investigated. Urine samples were obtained from each randomised woman, with bilateral notching in uterine arteries at 12-14 weeks of gestation, at baseline and at 24-26 and at 32-34 weeks of gestation. In these samples 2, 3-dinor-6-keto-prostaglandin$F_{1\alpha}$ (2, 3-dinor-6-keto-PG$F_{1\alpha}$), 11-dehydrothromboxane B_2 (11-dehydro-TxB_2) were measured.

3. The role of 9a, 11b-prostaglandin F_2 in hypertensive disorders of pregnancy (Study V)

3.1. Patients

The study population here comprised the same women as in studies II, III and IV and to the control groups were recruited 15 voluntary healthy non-pregnant women of reproductive age, 17 pregnant women at 12-14 weeks of gestation and 15 pregnant women at 30-34 weeks of gestation routinely attending antenatal clinics. For the controls the exclusion criteria included hypertension or any other chronic disease and use of prostaglandin inhibitors within ten days before investigation.

3.2. Study design

The purpose of this study was to determine the urinary 9α, 11 -prostaglandin F_2, a primary metabolite of prostaglandin D_2 (PGD_2), in hypertensive disorders of pregnancy and the effect of acetylsalicylic acid on 9α, 11 -prostaglandin F_2. Urinary 9α,11β-prostaglandin F_2 was measured in randomised women (women with bilateral notching) at 12-14, 24-26 and at 32-34 weeks of gestation, in control non-pregnant women and in control pregnant women at 12-14 weeks of gestation and at 30-34 weeks of gestation.

4. Methods

4.1. Assays of prostacyclin, thromboxane A_2 and 9a, 11b-prostaglandin F_2

The biosynthetic capacity of platelets to generate TxA_2 was monitored in study 1 by direct radioimmunological determination of the metabolite TxB_2 in serum. For the measurement of TxB_2 production by platelets during spontaneous clotting blood was taken into a glass tube and incubated for 30 minutes at 37^{o}C. Thereafter, the tubes were placed in an ice to inhibit further TxB_2 production and then immediately centrifuged (1000g, 10 min). The samples were diluted 1:2000 in assay buffer and were measured using direct RIA with double antibody separation (Alanko et al. 1992).

The production of PGI_2 and TxA_2 was monitored by measuring their indicator metabolites, urinary 2, 3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (u- 2, 3-dinor-6-keto-$PGF_{1\alpha}$) and 11-dehydrothromboxane B_2 (11-dehydroTxB_2), respectively, by radioimmonoassays after selective solid-phase extractions (Riutta et al. 1994, Riutta et al. 1992). For analysis of urine metabolites aliquots from urine were frozen immediately after sampling and stored at –70^{o}C until extraction. The measured concentrations of urine prostanoid compounds were related to the urine creatinine concentration to avoid the influence of variations in urine output. Urine creatinine was measured by an enzymatic method (Ektachem 700XR Analyzer, C Series, Kodak, Rochester, NY). The analysis of urine 9α, 11β-prostaglandin F_2 (u-9α, 11β-PGF_2) was carried out as previously described (Mucha and Riutta 2001).

4.2. Doppler studies

At 12-14 weeks of gestation the women were placed in the lithotomy position and transvaginal sonography performed using an Aloka (Aloka ECHO Camera SSD-2000) real-time color Doppler ultrasound system with a 5 MHz vaginal transducer. At 24 to 26 and at 32 to 34 weeks of gestation a 3.5MHz convex transducer was used and the women were in semirecumbent position. An angle of 60 degrees or less was used to obtain waveforms acceptable for analysis. Color Doppler imaging was used to visualise the main uterine artery. Transvaginally the uterine artery was identified at the level of the internal os of the cervix as it approached the uterus laterally and curved upward alongside the uterine body. Transabdominally with the patient semirecumbent the transducer was placed longitudinally on the left and right lower quadrants of the abdominal wall, and the external iliac artery was identified. The transducer was then moved medially until a reproducible uterine artery waveform could be identified. The main uterine artery was located at the uterocervical junction close to the crossover point of the uterine and external iliac artery. The quality of the imaging was considered optimal when repeated (five to six) successive waveforms from each uterine artery were obtained.

The uterine artery waveform analysis checked for the presence or absence of bilateral (right and left waveform) notches. A notch was defined as a decrease in the maximal flow velocity below the maximum diastolic velocity, occurring immediately subsequent to the systolic wave (Thaler 1992) The resistance index (RI), the pulsatility index (PI), mean velocity (cm/s) and maximum (peak) velocity (cm/s) of the uterine arteries and the resistance index and pulsatility index of the umbilical arteries were measured.

4.3. Statistics

In study I differences between groups in quantitative variables were tested using analysis of variance or Student's *t*- test when appropriate. Differences between different ASA doses within a group were tested by paired *t*-test.

In study II the intended sample size of 88 was based on´a priori´ sample size calculation made by Medstadt 2.11 (Astra Gruppen, Copenhagen, Denmark). The incidence of pregnancy-induced hypertension in this highly selected group of pregnant women was assumed to be 20%. The power calculation was based on an 80% power to detect that the incidence of pregnancy-induced hypertension (20%) was almost totally eliminated with ASA prophylaxis (minimal difference between effect rates not to be overlooked: 20%); type 1 error was assumed to be 5%.

In studies II-V differences were assessed by independent samples *t*- test or Mann-Whitney test for quantitative data. For means, 95% confidence interval was also given. Qualitative data were assessed by Pearson's Chi-square or Fisher's exact test.

In studies III-V analysis of variance for repeated measures was used to evaluate changes in flow parameters or prostanoids in proceeding pregnancy using time as within-subject factor and adverse outcome (yes/no) and treatment (ASA/placebo) as between-subject factor. In view of slightly skewed distributions logarithmic transformations were used, but crude values are used in tables and graphs. To evaluate the associations between birth weight, pregnancy duration and flow velocity parameters in the uterine arteries and associations between blood pressure and prostanoids, Pearson's correlation coefficient was calculated.

P-values less than 0.05 were considered statistically significant. Statistical analysis was made by SPSS 9.0 for Windows statistical software.

RESULTS

1. The dose of ASA (Study I)

1.1. Thromboxane A_2

There were no statistically significant differences in baseline *ex vivo* serum TxB_2 concentrations between non-pregnant women, pregnant hypertensive or pregnant normotensive women (Table 7).

Table 7. The serum thromboxane (S-TxB_2, ng/ml) and the urinary metabolites of thromboxane (11-dehydro-TxB_2, pg/µmol creatinine) and prostacyclin (2, 3-dinor-6-keto$PGF_{1\alpha}$, pg/µmol creatinine) and the ratio of urinary metabolites at baseline and after three different daily doses of acetylsalicylic acid (ASA).

	Hypertensive pregnant women (n=7)				Non-pregnant women (n=5)				Normotensive pregnant women (n=7)
ASA (mg/kg)	0	0.5	1	2	0	0.5	1	2	0
S-TxB_2									
Mean	341.2	19.6	2.8	0.8	267.3	10.4	1.2	0.4	244
SD	(109.6)	(9.5)	(1.3)	(0.7)	(135.5)	(11.8)	(1.0)	(0.5)	(98.9)
11-dehydroTxB_2									
Mean	34.9	9.6	5.9	4.0	14.8	5.8	5.4	3.9	39.3
SD	(18.3)	(4.0)	(2.7)	(1.5)	(6.4)	(1.7)	(3.1)	(3.9)	(14.4)
2,3-dinor-6-keto-$PGF_{1\alpha}$									
Mean	44.7	43.1	40.4	41.6	18.2	11.1	11.0	14.0	93.9
SD	(24.2)	(23.9)	(12.7)	(13.6)	(11.3)	(8.1)	(10.8)	(10.6)	(50.9)
2,3-dinor-6-keto-$PGF_{1\alpha}$/ 11-dehydroTxB_2									
Mean	1.6	5.2	9.3	13.3	1.2	1.8	2.3	10.8	2.6
SD	(1.2)	(3.1)	(7.6)	(10.1)	(0.5)	(1.0)	(2.0)	(18.2)	(1.5)

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As compared to baseline, the biosynthetic capacity of platelets to generate TxA_2, as reflected in serum TxB_2, was already inhibited in the hypertensive patients by a dose of 0.5mg/kg of ASA. In non-pregnant women the inhibition of TxA_2 was also significant at all three dose levels of ASA (Fig.1).

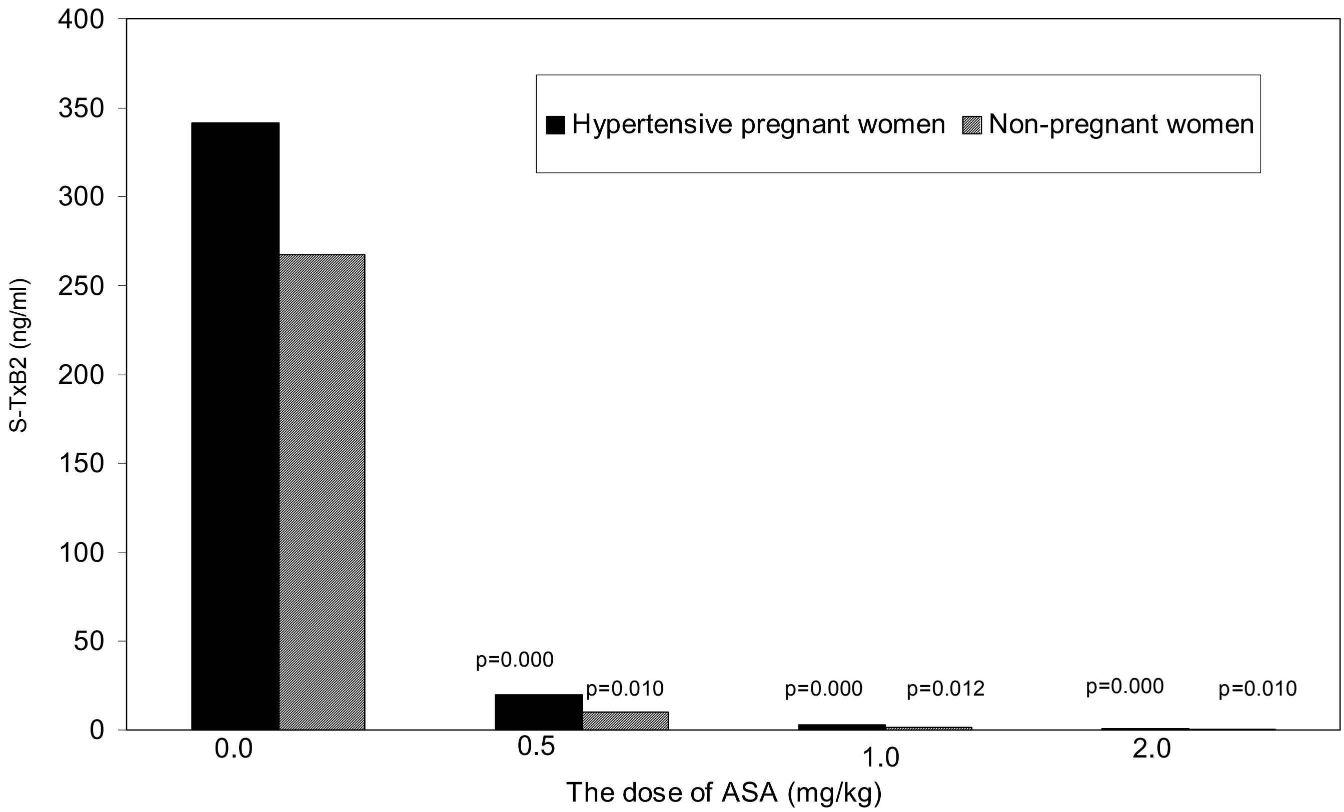

Figure 1. The inhibitory effect of three different doses of acetylsalicylic acid (ASA) on the capacity of platelets to produce TxB_2 (S-TxB_2). P-value expressed as a comparison between TxB_2 concentration at the baseline and TxB_2 concentration after a treatment period with each dose of ASA. Reprinted from: Acta Obstet Gynecol Scand 1999; 78: 82-88:Vainio M et al. In the dose range of 0.5-2.0 mg/kg, acetylsalicylic acid does not affect prostacyclin production in hypertensive pregnancies, with permission from Munksgaard.

At baseline, the mean urinary excretion of 11-dehydro-TxB_2 was in the pregnant women two-fold higher than in non-pregnant women. The difference was significant for both hypertensive ($p = 0.042$) and normotensive groups ($p = 0.005$) (Table 7). As a response to ASA, the urine excretion of 11-dehydro-TxB_2 decreased in a dose-dependent manner in both groups (Fig. 2).

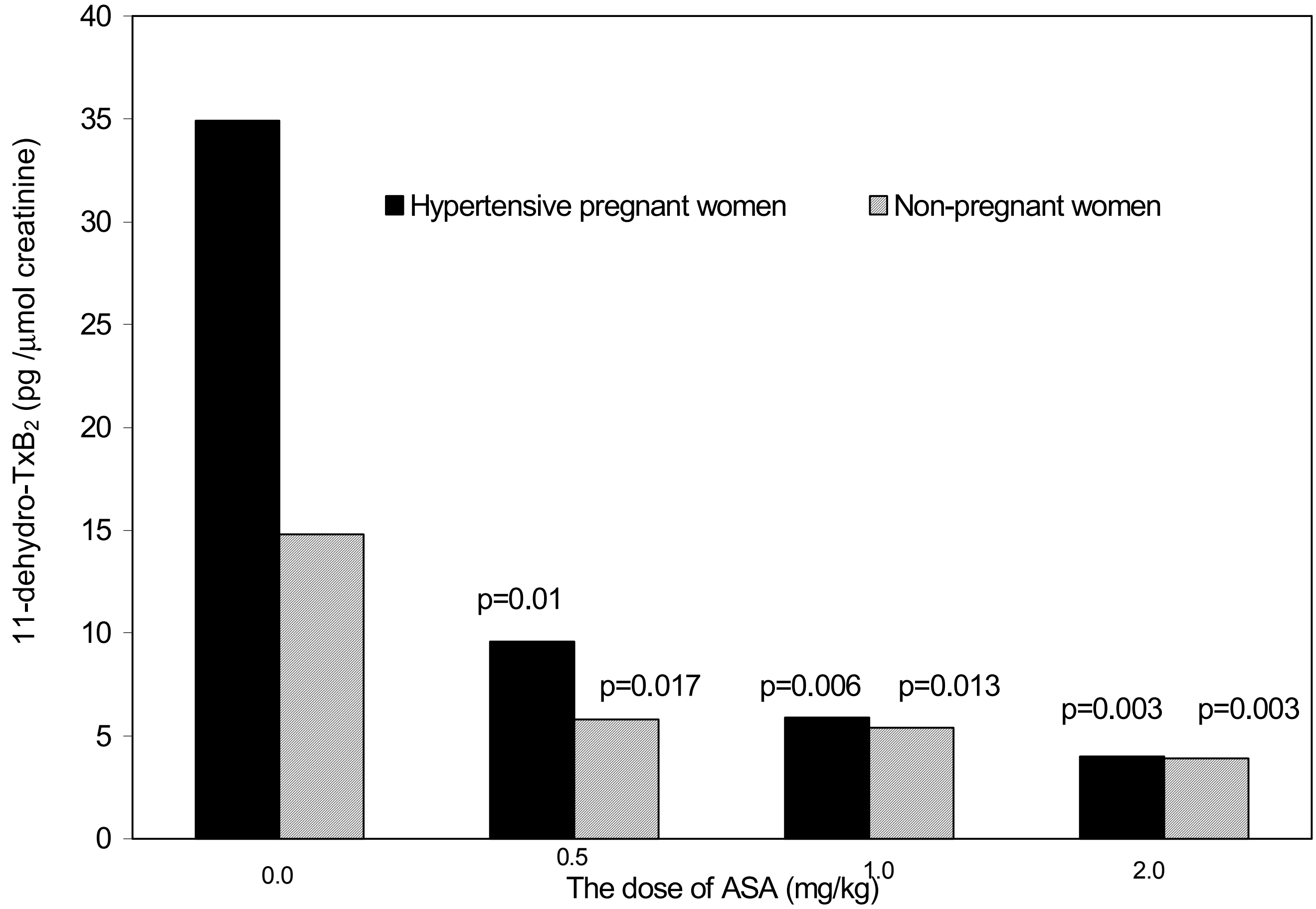

Figure 2. The urinary metabolite of thromboxane, 11-dehydrothromboxane B_2 (11-dehydro-TxB_2) at baseline and after three doses of acetylsalicylic acid (ASA) in hypertensive pregnant and in non-pregnant subjects. P-value expressed as a comparison between 11-dehydro-TxB_2 concentration at the baseline and 11-dehydro-TxB_2 concentration after a treatment period with each dose of ASA. Reprinted from: Acta Obstet Gynecol Scand 1999; 78: 82-88:Vainio M et al. In the dose range of 0.5-2.0 mg/kg, acetylsalicylic acid does not affect prostacyclin production in hypertensive pregnancies, with permission from Munksgaard.

1.2. Prostacyclin

The urinary excretion of 2, 3-dinor-6-keto-$PGF_{1\alpha}$ in normotensive pregnancies was two-fold higher than in the hypertensive pregnancies ($p = 0.048$). The urinary excretion of prostacyclin was 2.5 times greater in hypertensive ($p = 0.030$) and five times greater in normotensive pregnancies ($p = 0.007$) than in non-pregnant women, respectively (Table 7). None of the ASA dosages inhibited the excretion of 2, 3-dinor-6-keto- $PGF_{1\alpha}$ in the hypertensive pregnant women. In the control group the inhibition was marginal (Fig. 3).

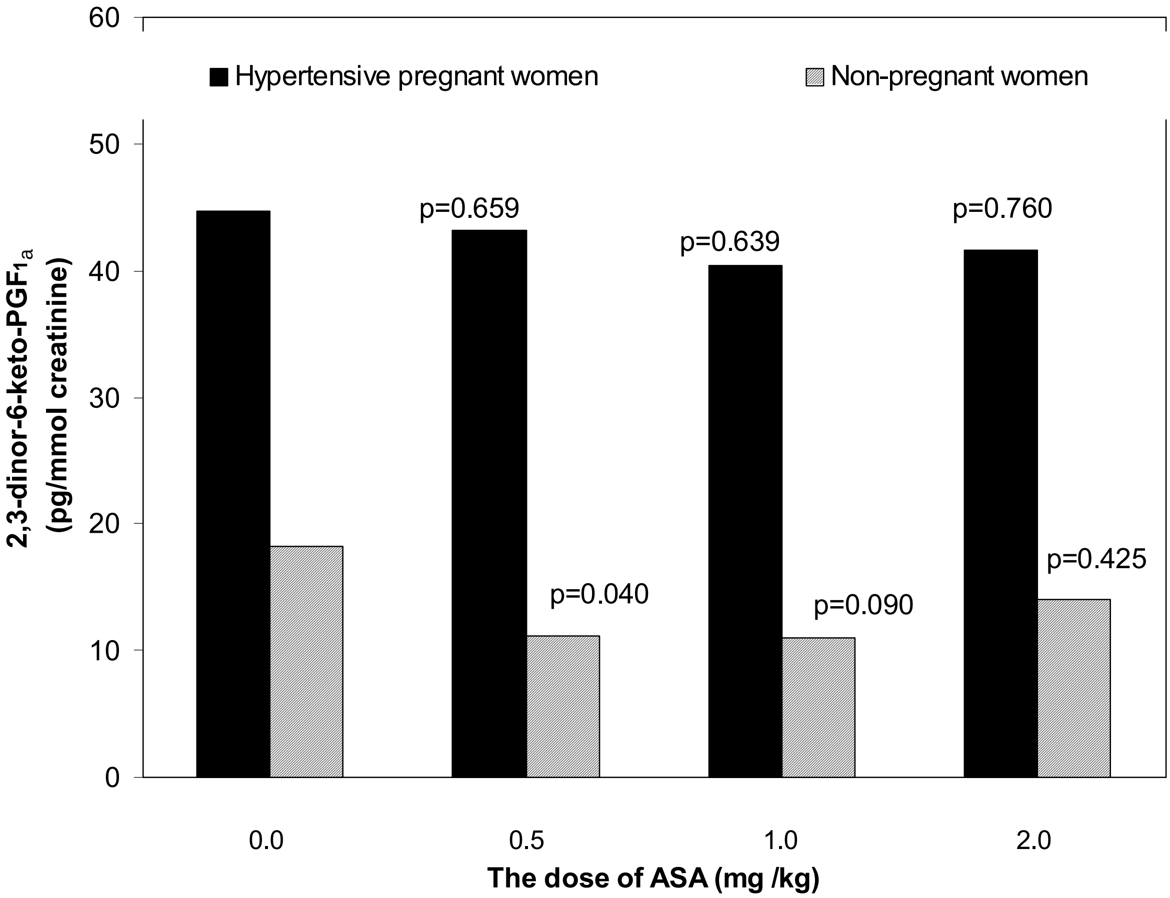

Figure 3. The urinary metabolite of prostacyclin 2, 3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (2, 3-dinor-6-keto-$PGF_{1\alpha}$) at baseline and after three different doses of acetylsalicylic acid (ASA) in hypertensive pregnant and in non-pregnant subjects. P-value expressed as a comparison between 2, 3-dinor-6-keto-$PGF_{1\alpha}$ concentration at the baseline and 2, 3-dinor-6-keto-$PGF_{1\alpha}$ concentration after a treatment period with each dose of ASA. Reprinted from: Acta Obstet Gynecol Scand 1999; 78: 82-88: In the dose range of 0.5-2.0 mg/kg, acetylsalicylic acid does not affect prostacyclin production in hypertensive pregnancies, with permission from Munksgaard

1.3. Prostacyclin/thromboxane A_2 ratio

At baseline the PGI_2/TxA_2 metabolite ratio was almost the same in hypertensive pregnant (1.6) as in non-pregnant women (1.2). Although the ratio was 2.6 in the normotensive pregnant women, the difference did not reach statistical significance (Table 7). In hypertensive pregnancies ASA effectively suppressed the urinary excretion of 11-dehydro-TxB_2 but not that of 2, 3-dinor-6 keto-$PGF_{1\alpha}$. Consequently, in hypertensive pregnancies the ratio PGI_2 to TxA_2 already exceeded that in normotensive pregnancies with the smallest dose of ASA (Fig. 4). In the control non-pregnant women, the ratio of PGI_2 to TxA_2 was not affected by 0.5-2.0mg/kg of ASA (Fig. 4).

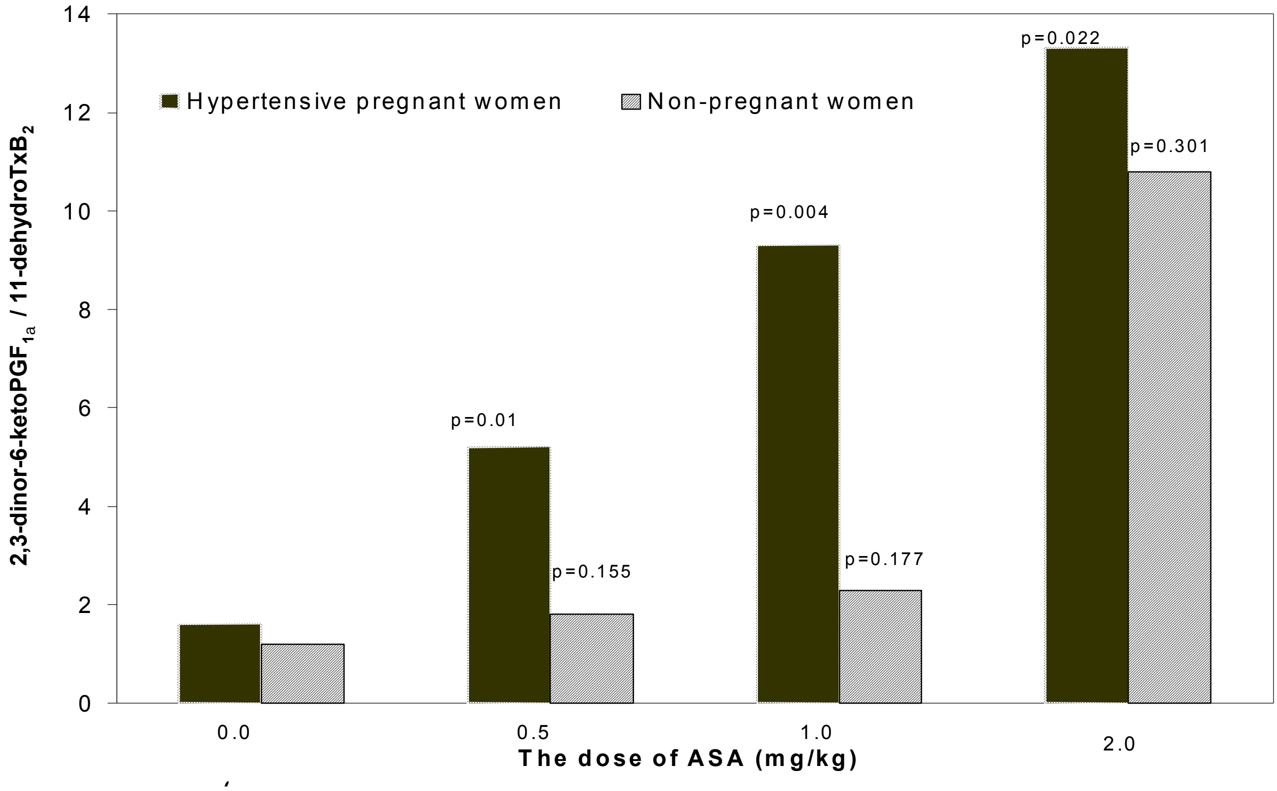

Figure 4. The ratio of the urinary 2, 3-dinor-6-ketoprostaglandin $F_{1\alpha}$ (2, 3-dinor-6-keto-$PGF_{1\alpha}$) to the urinary 11-dehydrothromboxane B_2 (11-dehydro-TxB_2) at baseline and after three doses of acetylsalicylic acid (ASA). P-value expressed as a comparison between the ratio of the 2, 3-dinor-6-keto-$PGF_{1\alpha}$ to the 11-dehydro-TxB_2 at the baseline and after a treatment period with each dose of ASA.

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2. Low-dose ASA in prevention of hypertensive disorders of pregnancy (Study II*)*.

Ninety women with bilateral notches in uterine arteries were randomised and forty-three in both groups were successfully followed up. The characteristics of the women at trial entry are presented in Table 6.

2.1. Hypertensive disorders of pregnancy

Pregnancy-induced hypertension developed in five women (11.6%) allocated to acetylsalicylic acid as compared to 16 (37.2%) of those receiving placebo (relative risk 0.31, 95 % CI 0.13-0.78) (Table 8). Pregnancy-induced hypertension was proteinuric in two pregnancies (4.7%) on acetylsalicylic acid and in 10 (23.3%) on placebo (RR = 0.20, 95 % CI 0.05-0.86). The hypertension set in or was exacerbated before 37 gestational weeks in two (2.3%) women randomised to acetylsalicylic acid and in nine (20.9%) randomised to placebo (RR = 0.22, 95 % CI 0.05-0.97). The recurrence rate of previous pregnancy-induced hypertension was 22.2% in the acetylsalicylic acid and 48.1% in the placebo group (RR = 0.47, 95 % CI 0.19-1.20) and of pre-eclampsia 14.3% and 33.3% (RR = 0.48, 95 % CI 0.13-1.75), respectively. In the acetylsalicylic acid group there was one new case of pregnancy-induced hypertension and no new pre-eclampsia cases, in the placebo group three new cases of pregnancy-induced hypertension and two of pre-eclampsia; the differences between the groups were not statistically significant.

Table 8. Effect of acetylsalicylic acid (ASA) (0.5mg/kg daily) on the rate of pregnancy-induced hypertension (PIH), intrauterine growth restriction (IUGR), and pre-eclampsia. Values are given as n (%)

	ASA (n = 43)	Placebo (n = 43)	RR	95 % CI
PIH	5 (11.6)	16 (37.2)	0.31	(0.13-0.78)
Pre-eclampsia	2 (4.7)	10 (23.3)	0.20	(0.05-0.86)
Hypertension <37 weeks	2 (2.3)	9 (20.9)	0.22	(0.05-0.97)
IUGR	1(2.3)	3(7.0)	0.33	0.04-3.08

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2.2. Birth weight and intrauterine growth restriction

There was no significant difference between the groups in mean birth weight; 3462 g (SD 604) with acetylsalicylic acid and 3553 g (SD 765) with placebo (Table 9). Intrauterine growth restriction (unrelated or related to PIH) occurred slightly more frequent in the placebo group (3/43 *versus* 1/43, respectively), but the difference was not statistically significant (Table 10). There was no significant difference between the ASA and the placebo groups in the number of birth weights < 2500g . The rate of recurrence of previous IUGR was 0% in the ASA group and 10% in the placebo group. Two women in the placebo group had pre-eclampsia and one had PIH concomitant with IUGR. In the ASA group there was no growth restriction concomitant with PIH. The difference between the groups was not statistically significant ($p = 0.241$).

2.3. Duration of pregnancy

There was no difference in the mean duration of pregnancy; it was 39.5 weeks (SD 1.7) among the women on acetylsalicylic acid and 39.3 weeks (SD 1.8) among those on placebo.

2.4. Other maternal outcomes

There were no statistically significant differences between the acetylsalicylic acid and placebo groups in the rates of induction of labour, spontaneous labor or cesarean section. There were also no significant differences in postpartum hemorrhage: 300ml (250-450) among women receiving acetylsalicylic acid and 350ml (250-450) among those on placebo. Post-partum hematocrite was 0.33 and 0.34, respectively (Table 9).

2.5. Other outcomes of the newborn

There were no significant differences between the groups in one- or five- minute Apgar scores, in umbilical artery pH values, hematocrites or in the blood thrombocyte counts of the infants (Table 9). Four neonates in the ASA group and six in the placebo group were treated after birth in the neonatal intensive care unit. There were no stillbirths or neonatal deaths in either group. One neonate without IUGR in the ASA group had hydrocephalus and meningomyelocele; this infant was included in the analysis. In the placebo group one neonate developed pulmonary hypertension.

There was no evidence of cerebroventricular hemorrhage or other hemostatic abnormalities in neonates.

Table 9. Effects of ASA on delivery type, gestational age (GA), birth weight, bleeding and neonatal outcome. Values are given as *n* (%), mean [SD], median {lower and upper quartile} and RR (relative risks) 95 % CI.

	ASA (*n* = 43)	Placebo (*n* = 43)	RR (95 % CI)	P
Spontaneous delivery	25 (58.1)	22 (46.8)	1.15 (0.75-1.78)	
Induced delivery	16 (37.2)	18 (41.9)	0.9 (0.58-1.41)	
Caesarean section	7 (16.3)	4 (9.3)	1.32 (0.80-2.20)	
GA at delivery (weeks)	39.5 [1.7]	39.3 [1.8]		0.381
Blood loss at delivery (ml)	300 {250-450}	350 {250-450}		0.305
Maternal hematocrite	0.33 [0.04]	0.34 [0.03]		0.227
Birth weight (g)	3462 [604]	3553 [767]		0.539
Birth weight < 2500g	3 (7.0)	4 (9.3)	0.85 (0.35-2.05)	
1 min Apgar scores < 5	1 (1.2)	0		
5 min Apgar scores < 5	0	0		
Neonatal hematocrite	0.61 [0.07]	0.61 [0.07]		0.668
Neonatal thrombocytes	271 [69.7]	272 [71.2]		0.940
Umbilical artery pH	7.3 [0.1]	7.3 [0.1]		0.215

3. Bilateral notching in predicting hypertensive disorders of pregnancy (Study III)

We could evaluate the value of flow velocity waveforms in the uterine and umbilical arteries in prediction of PIH or IUGR in 72 pregnancies. The incidences of PIH and pre-eclampsia were significantly higher in women with bilateral notches as compared to control women (p = 0.030 and p = 0.022, respectively), but the incidence of IUGR could not be predicted (Table 10).

Table 10. Bilateral notching in the uterine arteries at 12-14 weeks of gestation in placebo and control groups and pregnancy outcome.

	Bilateral notching		P-*value*	*RR*	*95% CI*
	Present (n = 43)	*Absent* (n = 29)			
PIH	16 (37.2 %)	4 (13.8%)	0.030	2.70	(1.00- 7.25)
Pre-eclampsia	10 (23.3%)	1 (3.4%)	0.022	6.74	(0.91- 49.9)
IUGR	3 (7.0%)	1 (3.4%)	0.469	2.02	(0.22-18.5)

PIH, pregnancy-induced hypertension; IUGR, intrauterine growth restriction < 10^{th} percentile; RR, relative risk; CI, confidence interval.

The predictive values of bilateral notching at 12-14 weeks of gestation for hypertensive disorders of pregnancy are shown in Table 11. No other flow velocity parameters of the uterine and umbilical arteries could predict adverse outcome at 12-14 weeks of gestation.

At 24-26 weeks of gestation 24.4% (28/115) and at 32-34 weeks of gestation 12.2% of the participants (14/115) evinced bilateral notches. The rate of adverse outcome was 64.3% among those who had bilateral notches at 32-34 weeks of gestation and 18.5% among those without (p<0.001). The sensitivity of bilateral notching in predicting adverse pregnancy outcome decreased but specificity and positive predictive value increased with advancing gestation (Table 11).

Table 11. The predictive value of bilateral notching for PIH, pre-eclampsia and IUGR (n = 72)

Gestational week	Outcome of pregnancy	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*
12-14	PIH	84	50	29.6	90.6
	Pre-eclampsia	83	45	27.4	92.8
	IUGR	75	41	24	86.8
24-26	PIH	35	84.6	36.8	84
	Pre-eclampsia	36.4	82	33.3	83.7
	IUGR	50	80.9	38.5	86.5
32-34	PIH	35	94.2	59.3	85.3
	Pre-eclampsia	36.4	90.2	47.4	84.9
	IUGR	50	88.2	51.0	87.6

PPV, positive predictive value; NPV, negative predictive value; IUGR, intrauterine growth restriction; PIH, pregnancy-induced hypertension.

4. The effect of ASA on prostanoids in normal pregnancies and in hypertensive disorders of pregnancy (Study IV).

The output of 2, 3-dinor-6-keto-$PGF_{1\alpha}$ increased in the urine of all women during pregnancy ($p<0.001$). The 11-dehydro-TxB_2 decreased ($p = 0.015$), except where PIH developed before 37 gestational weeks, and the difference between the groups was statistically significant ($p = 0.028$). In pregnancies with PIH before 37 weeks of gestation 11-dehydro-TxB_2 remained at a higher level throughout pregnancy as compared to other pregnancies ($p = 0.017$). Consequently, the 2, 3-dinor-6-keto-$PGF_{1\alpha}$ / 11-dehydro-TxB_2 ratio increased less ($p = 0.028$) in pregnancies with PIH before 37 weeks of gestation as compared to other pregnancies (Fig. 5).

The changes in the urinary output of 2, 3-dinor-6-keto-$PGF_{1\alpha}$, 11-dehydro-TxB_2, or the ratio of 2, 3-dinor-6-keto-$PGF_{1\alpha}$/ 11-dehydro-TxB_2 in the ASA and placebo groups are illustrated in Fig. 6 and Table 12.

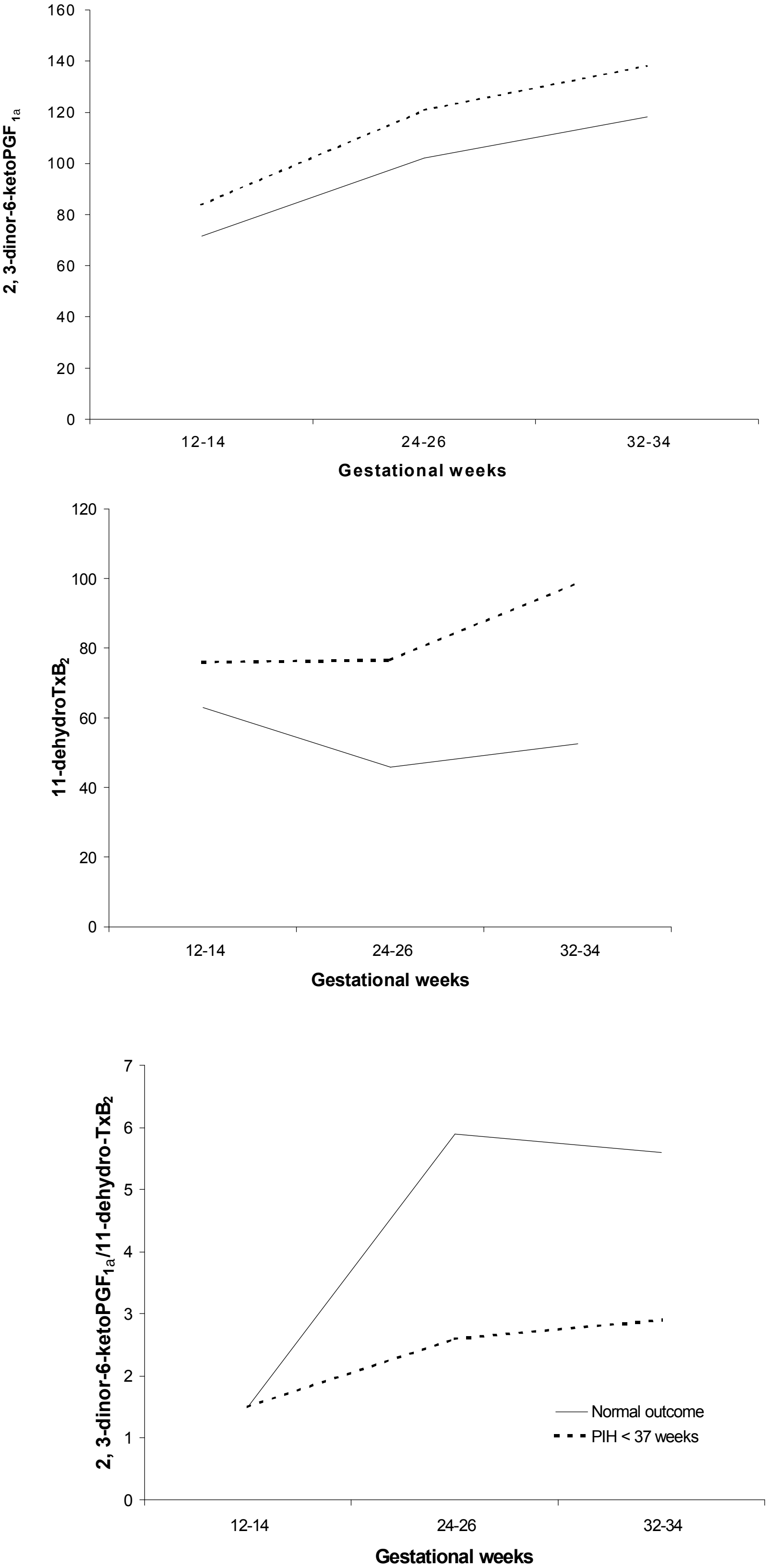

Figure 5. Urinary 2, 3-dinor-6-keto-$PGF_{1\alpha}$ (pg/μmol creat) (mean), urinary 11-dehydro-TxB_2, (pg/μmol creat) (mean), and their ratio at 12-14, 24-26, and 32-34 weeks of gestation in the 86 pregnancies with bilateral notching in the uterine arteries at 12-14 gestational weeks according to pregnancy outcome (PIH before 37 weeks of gestation, n = 9, pregnancies without PIH < 37 weeks of gestation, n = 75). PIH = pregnancy-induced hypertension. Reprinted from: Acta Obstet Gynecol Scand 2003: 82:1-6 Vainio M et al. Prostacyclin, thromboxane A_2 and the effect of low dose ASA in pregnancies at high risk for hypertensive disorders, with permission from Munksgaard.

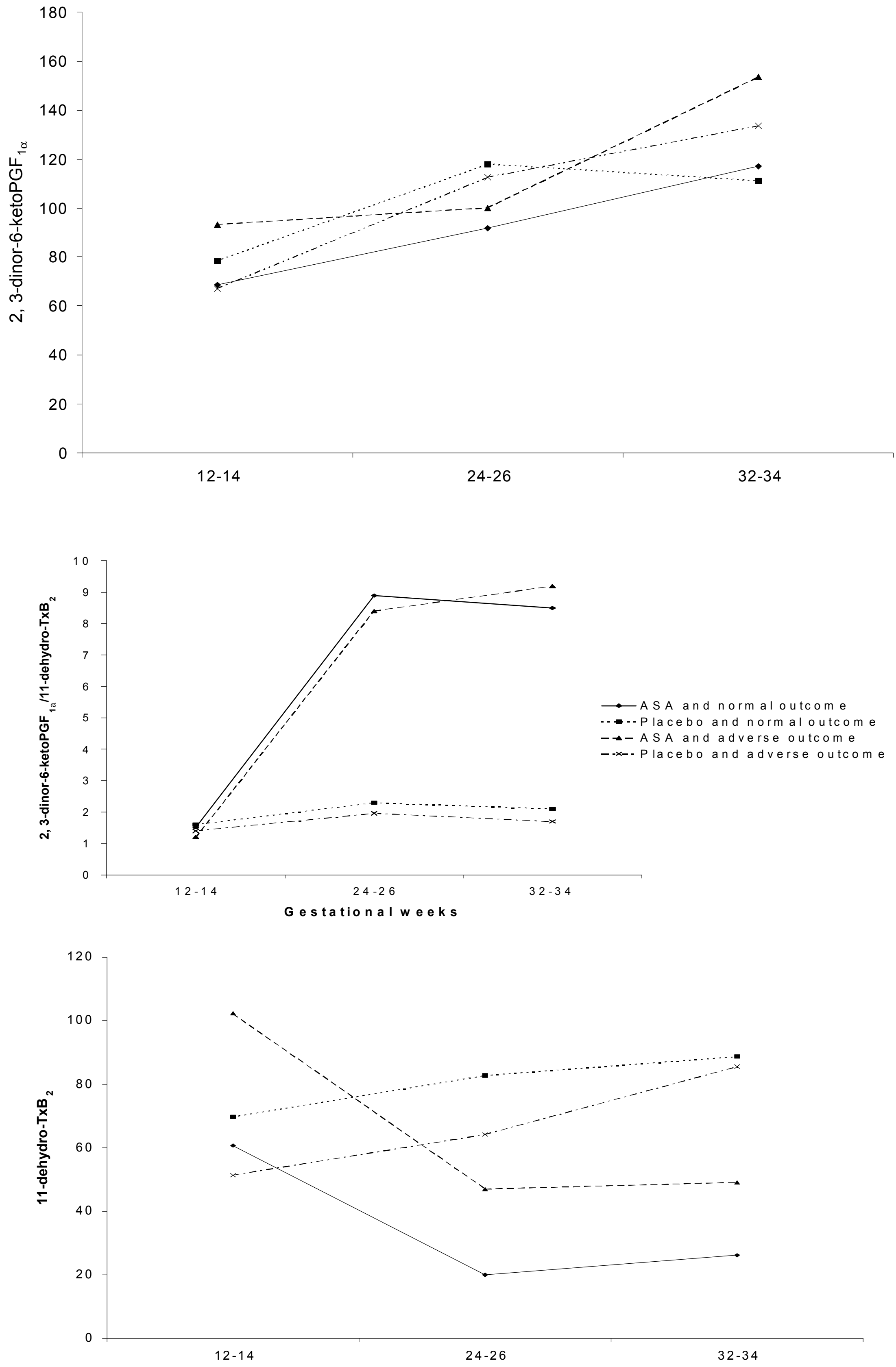

Figure 6. Urinary 2, 3-dinor-6-keto-$PGF_{1\alpha}$ (pg/µmol creat) (mean), urinary 11-dehydro-TxB_2, (pg/µmol creat) (mean), and their ratio at 12-14, 24-26 and 32-34 weeks of gestation in the 86 pregnancies with bilateral notching in the uterine arteries at 12-14 gestational weeks according to pregnancy outcome in acetylsalicylic acid (ASA) and placebo groups. Reprinted from: Acta Obstet Gynecol Scand 2003: 82:1-6 Vainio M et al. Prostacyclin, thromboxane A_2 and the effect of low dose ASA in pregnancies at high risk for hypertensive disorders, with permission from Munksgaard.

Table 12. Levels of 2, 3-dinor-6-keto$PGF_{1\alpha}$, 11-dehydroTxB_2, and the 2, 3-dinor-6-keto$PGF_{1\alpha}$ / 11-dehydroTxB_2 ratio according to the pregnancy outcome in the ASA and placebo groups.

	2, 3-dinor-6-keto$PGF_{1\alpha}$,		11-dehydroTxB_2		2, 3-dinor-6-keto$PGF_{1\alpha}$ / 11-dehydroTxB_2	
	Mean	SD	Mean	SD	Mean	SD
ASA, normal outcome (n = 37)						
12-14	68, 6	34,8	60,7	54,8	1,5	0,9
24-26	91,8	77,6	19,9**	33,5	8,9**	6,8
32-34	117,1	74,1	26,1**	41,0	8,5**	7,8
Placebo, normal outcome (n = 37)						
12-14	78,3	38,2	69,6	68,2	1,6	1,0
24-26	117,9	74,9	82,7**	50,7	2,3**	2,8
32-34	111,1	52,8	88,7**	49,7	2,1**	3,1
ASA, adverse outcome (n = 6)						
12-14	93,2	73,6	102,3$^{¤}$	89,9	1,2	0,8
24-26	100,1	54,6	46,9	86,4	8,4$^{\#}$	5,8
32-34	153,7	94,1	49,1*	88	9,2*	7,6
Placebo, adverse outcome (n = 16)						
12-14	67,1	25,4	51,3$^{¤}$	19,9	1,4	0,7
24-26	112,7	57,1	64,1	22,6	2,0$^{\#}$	1,2
32-34	133,6	124,9	85,5*	36,6	1,7*	1,5

The values (pg/µmol creat) are given as mean with standard deviation (SD). Differences in pregnancies with normal outcomes between ASA and placebo groups ** ($p<0.001$). Differences in pregnancies with adverse outcomes between ASA and placebo groups $^{¤}$ ($p = 0.050$), * ($p = 0.001$), $^{\#}$ ($p = 0.03$). ASA = acetylsalicylic acid. Adverse outcome includes pregnancy-induced hypertension and intrauterine growth restriction. Reprinted from: Acta Obstet Gynecol Scand 2003: 82:1-6 Vainio M et al. Prostacyclin, thromboxane A_2 and the effect of low dose ASA in pregnancies at high risk for hypertensive disorders, with permission from Munksgaard.

The 2, 3-dinor-6-keto-$PGF_{1\alpha}$ increased throughout pregnancy in both ASA and placebo groups ($p<0.001$), and the increase was not different in pregnancies with a normal or an adverse outcome ($p = 0.818$) or between ASA and placebo groups ($p = 0.071$). For 11-dehydro TxB_2 and the 2, 3-dinor-6-keto-$PGF_{1\alpha}$ / 11-dehydro TxB_2 ratio, the interaction between treatment and time proved to be statistically significant ($p<0.001$), meaning that the changes in 11-dehydro TxB_2 and in the 2, 3-dinor-6-keto-$PGF_{1\alpha}$ / 11-dehydro TxB_2 ratio during study period were different in ASA and placebo groups. Therefore analyses were made separately for ASA and placebo groups.

The 11-dehydro-TxB_2 increased ($p<0.001$) and the 2, 3-dinor-6-keto-$PGF_{1\alpha}$ / 11-dehydro TxB_2 ratio was unchanged ($p = 0.093$) in the placebo group regardless of pregnancy outcome. In contrast, in the ASA group 11-dehydro-TxB_2 decreased initially from 12-14 to 24-26 weeks, and thereafter remained fairly constant or increased slightly ($p<0.001$). Again, the pregnancies with normal or adverse outcome did not differ from each other according to pregnancy outcome ($p = 0.647$). In the ASA group, the 2, 3-dinor-6-keto-$PGF_{1\alpha}$ / 11-dehydro TxB_2 ratio increased from 12-14 to 24-26 weeks of gestation, but was thereafter unchanged ($p<0.001$). Pregnancies with a normal or an adverse outcome did not differ from each other ($p = 0.521$).

In the placebo group pregnancies with pre-eclampsia had significantly lower 2, 3-dinor-6-keto-$PGF_{1\alpha}$ ($p= 0.019$) at 12-14 weeks of gestation as compared to other pregnancies.

The outcome in pregnancies with persisting bilateral notches at 24-26 weeks did not differ significantly between the ASA and placebo groups as a whole ($p=0.194$). If bilateral notching had disappeared by 24-26 weeks, the women in the ASA group had a lower incidence of PIH ($p=0.038$) and pre-eclampsia ($p=0.009$) than the women in the placebo group. Likewise, in the pregnancies with persisting bilateral notches at 32-34 weeks of gestation, the outcome of pregnancy did not differ between the ASA and placebo groups ($p=0.580$). Again, women who did not have bilateral notching at 32-34 weeks had fewer cases of PIH ($p=0.026$) and pre-eclampsia ($p=0.007$) in the ASA group as compared to placebo group.

5. 9a, 11b-prostaglandin F_2 in pregnancies at risk of hypertensive disorders of pregnancy, and the effect of ASA (Study V)

Urinary 9α,11β-prostaglandin F_2 was significantly higher ($p<0.001$) in normotensive pregnant women at 12-14 weeks of gestation as compared to non-pregnant women. The women with bilateral notching at 12-14 weeks of gestation had significantly higher urinary 9α, 11β-prostaglandin F_2 levels as compared to normotensive pregnant women at 12-14 weeks of gestation ($p = 0.001$), and at 30-34 weeks of gestation ($p = 0.030$) (Table 13). Urinary 9α, 11 -prostaglandin F_2 increased significantly ($p = 0.018$) throughout pregnancy regardless of outcome, and there was no difference between the ASA and placebo groups ($p = 0.886$) (Fig. 7 and Fig. 8).

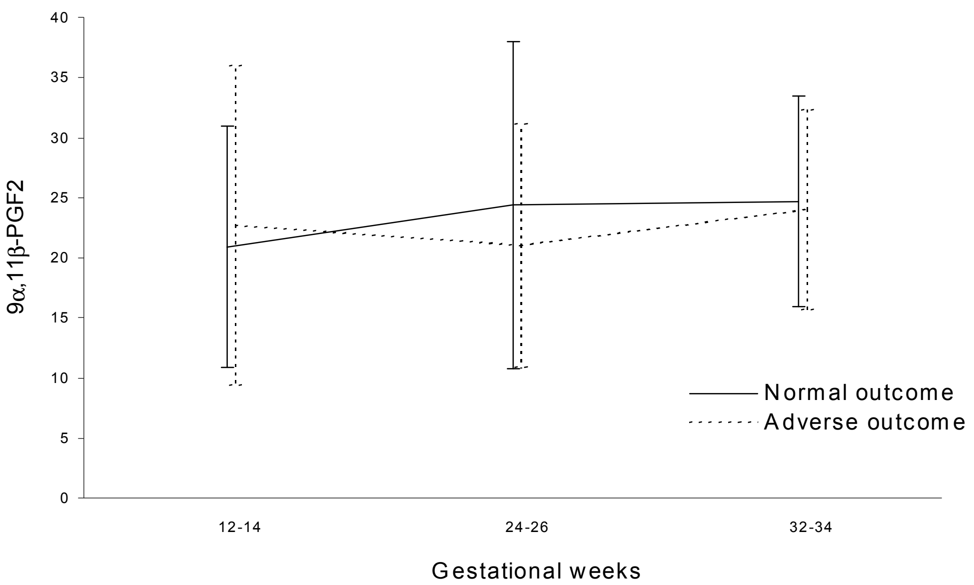

Figure 7.
Urinary 9α, 11β-prostaglandin F_2 (9α, 11β-PGF_2) (pg/μmol creat)(mean) at 12-14, 24-26 and 32-34 gestational weeks in the 86 pregnancies with bilateral notching in the uterine arteries at 12-14 gestational weeks according to pregnancy outcome (normal outcome, n = 64, adverse outcome n = 22). Reprinted from: Acta Obstet Gynecol Scand 2003: 82:1-6 Vainio M. et al. Prostacyclin, thromboxane A_2 and the effect of low dose ASA in pregnancies at high risk for hypertensive disorders, with permission from Munksgaard.

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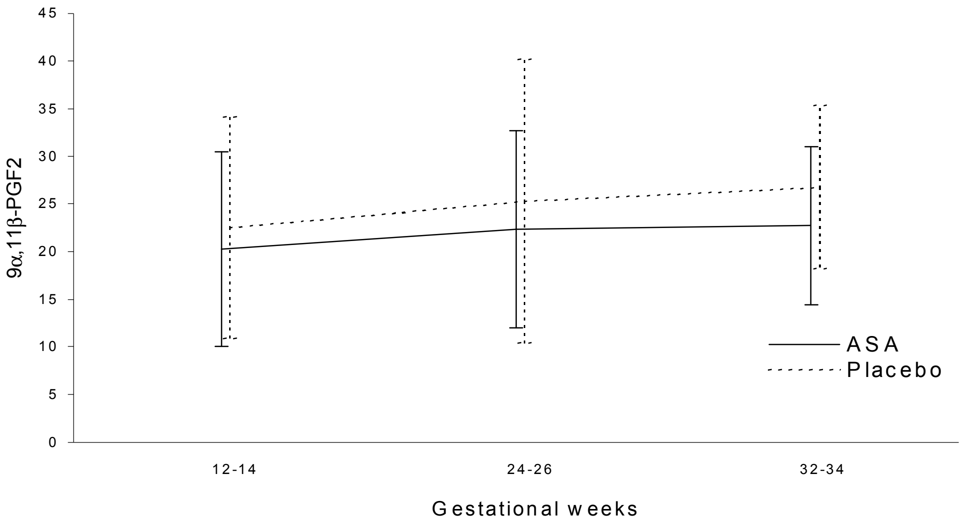

Figure 8.

Urinary 9α, 11β-prostaglandin F_2 (9α, 11β-PGF_2) (pg/μmol creat) (mean) at 12-14, 24-26 and 32-34 gestational weeks in the 86 pregnancies with bilateral notching in the uterine arteries at 12-14 gestational weeks according to the treatment (ASA group n = 43, placebo group, n = 43). ASA = acetylsalicylic acid.

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DISCUSSION

1. Methodology

This was a randomised placebo-controlled trial of ASA prophylaxis commencing as early as at 12-14 weeks of gestation in high-risk women screened by Doppler ultrasound. The size of the study population was based on calculation of the reductive effect of ASA on the incidence of pregnancy-induced hypertension. All the women in this study delivered at term and there were no pregnancies with early severe pre-eclampsia or IUGR necessitating delivery before 32 weeks of gestation. The population should have been much larger to bring out any significant reducing effect of ASA on very early severe pre-eclampsia or IUGR. Also the criteria for women with high-risk of pre-eclampsia could have been more strict. A history of previous intrauterine death without pre-eclampsia, obesities or age under 18 or over 40 years are not so high risk factors for pre-eclampsia as are a history of recurring pre-eclampsia and/or IUGR or chronic hypertension.

Terminology used to describe hypertension in pregnancy is nonuniform. Several overlapping terms are commonly applied to varying clinical manifestations of the same disease process (Davey et MacGillivray 1988, National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy 1990). In this study we used definition according to American College of Obstetricians and Gynecologists (ACOG 1996). The definition is in concordance with the latest recommendations of hypertensive disorders by the National High Blood Pressure Education Program Working Group (ACOG practice bulletin 2002).

The relatively high rate of PIH (37.2%) and pre-eclampsia (23.3%) in the placebo group confirms the high-risk nature of the pregnancies here. The incidence of PIH (13.8%) and pre-eclampsia (3.4%) was much lower in control women without bilateral notching and the difference was statistically significant ($p = 0.003$ and $p = 0.022$).

The randomised women were followed up throughout pregnancy in the same center. This partly explains the good compliance; the dropout rate was only 4.4%. The randomised women visited the research center three times during pregnancy and each time the medication was checked and the dose of ASA adjusted according to increase in body weight.

Using a transvaginal probe at 12-14 weeks of gestation it was possible to identify the uterine artery at the level of the cervical os (Harrington and Campbell 1995). The transabdominal approach was used after 14 weeks of gestation and the uterine artery on each side was identified at the point of apparent crossing over this vessel with the external iliac artery. A notch was defined when repeated (five to six) successive waveforms were obtained. Assessing the presence or absence of a notch is however a qualitative and subjective judgement.

The measured concentrations of the prostanoid compounds were related to the urine creatinine in order to avoid the influence of variations in the urine output. As 6-keto-$PGF_{1\alpha}$ does not reflect total body production of prostacyclin, we measured 2, 3-dinor-6-ketoprostaglandin $F_{1\alpha}$, the major metabolite of prostacyclin in urine. Also we measured 11-dehydro-TxB_2 and not 2,3 dinor-TxB_2, because the former is the most abundant breakdown product of TxA_2 production.

2. Acetylsalicylic acid in preventing hypertensive disorders of pregnancy

The purpose of our study was to establish whether it is possible to prevent pregnancy-induced hypertension or intrauterine growth restriction with low-dose ASA, when the dose is optimised, and prophylaxis initiated before the second wave of trophoblastic invasion of placenta is finished at 16-18 weeks of gestation and focused on a high-risk population screened by Doppler ultrasound.

The results of randomised trials involving acetylsalicylic acid treatment in the prophylaxis of pregnancy-induced hypertensive disorders are confusing and contradictory (Beaufils et al. 1985, Wallenburg et al. 1986, Benigni et al. 1989, Schiff et al. 1989, Uzan et al. 1991, Hauth et al. 1993, Italian study of Aspirin in pregnancy 1993, Sibai et al. 1993, Viinikka et al. 1993, CLASP1994, ECPPA 1996, Caritis 1998, Rotchell et al. 1998, Golding et al. 1998). The discrepancies have been attributed to issues such as whether there is greater benefit for high-risk women, what dose of ASA to use, and when to start treatment. Publication bias has also been suggested as a possible explanation for discrepancy between results (Leitich et al. 1997, Duley 1999).

In previous trials the dose of ASA has ranged from 50 to 150mg/day for the prevention of hypertensive disorders of pregnancy. The ideal dose, which would inhibit thromboxane A_2 (TxA_2) but not prostacyclin (PGI_2) in pregnant women, is not yet known. Viinikka and Ylikorkala (1981) found that in non-pregnant women dipyridamoli-ASA combinations with ASA doses between 0.5 and 0.8 mg/kg decreased TxB_2 production by 48 to 74% and with ASA doses between 2.6 and 5.7mg/kg by about 90%. Also a daily dose of 50 to 60 mg of ASA for a few months was found to reduce platelet thromboxane generation by about 90% in a small group of pregnant women (Benigni et al. 1989, Hauth et. al. 1993, Viinikka et al. 1993).

In our first study we found that ASA at a dose range of 0.5-2.0mg/kg daily significantly suppressed the excretion of the urinary metabolite of TxA_2 but not the urinary metabolite of PGI_2 and consequently the balance between PGI_2 and TxA_2 was already shifted in favor of PGI_2 at the lowest dose of ASA. The periods with different ASA doses immediately followed each other without a washout period. As the effect of ASA persists for the life span of the platelet (7 to 9 days) and only 10 % of the platelet pool is replenished each day (Viinikka 1991), the cumulative effect of ASA could affect to the results of TxB_2 with ASA doses of 1.0 and 2.0 mg/kg. On the other hand endothelial cells can resynthetise cyclo-oxygenase and thus PGI_2 production recovers rapidly.

The results of CLASP (1994) reassured as regards the baby that no excess of intraventricular hemorrhage or other neonatal bleeds were associated with ASA treatment. At low dosage a great deal of orally ingested ASA is quickly hydrolyzed to the relatively inert salicylate during absorption in the gastrointestinal track and so relatively little ASA reaches the systemic circulation and enters the fetal circulation as an intact compound (Ylikorkala et al. 1986).

ASA is contradicted in patients with ASA allergy and also even when administered at low doses ASA can cause serious gastrointestinal bleeding, as reported in studies using 30 to 50 mg daily (Diener et al 1996, The Dutch TIA Trial Study Group 1991). Also a variety of NSAIDs can inhibit TxA2-dependent platelet function through competitive, reversible inhibition of platelet COX-1 (Patrono et al. 2001). In general these drugs, when used at conventional analgesic dosages, inhibit reversibly platelet COX activity by 70 to 90%. This level of inhibition may be insufficient to block adequately platelet aggregation *in vivo* (Patrono et al. 2001).

ASA resistance has been used to describe a number of different phenomena including the inability of ASA to protect individuals from antithrombotic complications, to cause a prolongation of the bleeding time or to produce an anticipated effect on one or more *in vitro* tests of platelet function, but both the mechanism and clinical relevance of ASA resistant remain to be established (Patrono et al. 2001).

The first articles (Beaufils et al. 1985, Wallenburg et al. 1986, Benigni et al. 1989, Schiff et al.1989, Uzan et al. 1991) recommending ASA for the prevention of pre-eclampsia or IUGR always stressed that their results concerned patients with a specific indication, and subsequent trials tested ASA for broader indications and reported negative results (Sibai et al. 1993, CLASP1994, ECPPA 1996, Rotchell et al. 1998, Golding et al. 1998). In this present study the treatment was focused on a high-risk group of pregnant women with abnormal Doppler blood flow in the uterine arteries, defined as bilateral notches at 12-14 weeks of gestation. The relatively high rate of pregnancy-induced hypertension (37.2%) and pre-eclampsia (23.3%) in the placebo group confirms the high-risk nature of the pregnancies here.

The importance of gestational age at the commencement of ASA treatment is manifest when we remember that trophoblastic invasion of the human placenta develops from as early as eight weeks gestation and is well established by 18 weeks of gestation (Pijnenborg et al.1980). There is substantial evidence that failure of trophoblastic invasion of the maternal spiral arteries is an underlying cause of pre-eclampsia (Brosens et al. 1977, Khong et al. 1986). The late initiation of treatment, sometimes as late as 32 weeks of gestation, reported in several publications is probably one reason for the negative or only partially positive results observed (Uzan 2000). Sullivan and colleagues (1998) found that patients who were treated with aspirin from the first trimester of pregnancy did substantially better than those treated from the second trimester as assessed by the incidence of pre-eclampsia or intrauterine growth restriction, gestational age and birth weight at delivery.

In a meta-analysis by Leitich and colleagues (1997) the preventive effect of ASA on intrauterine growth restriction was greater among women who entered the study before 17^{th} week of gestation. CLASP study (1994) also suggested a trend towards a more protective effect of ASA the earlier the gestational age at trial entry. It is interesting, as we have reported in our study, that in those women by whom ASA worked, the bilateral notching disappeared before 24-26 weeks. After 24 –26 weeks of gestation ASA had no preventive effect on hypertensive disorders of pregnancy in those pregnancies where uteroplacental circulation was so severely compromised that bilateral notching persisted at 24-26 and 32-34 weeks of gestation. This finding suggests that ASA is not effecting only in peripheral circulation but probably also in the formation of placenta. In other words, the results of our study favor an early commencement of the prophylactic ASA treatment

In a recent meta-analysis involving over 30 000 women recruited, antiplatelet therapy was associated with a moderate (15%) reduction in the risk of pre-eclampsia, a 14% reduction in the risk of a stillbirth or neonatal death, and an 8% reduction in the risk of preterm birth (Duley et al. 2001). Remaining questions were whether small subgroups of high-risk women might have greater benefit and whether earlier treatment or a higher dose of ASA would have additional benefits without an increase in adverse events. Subsequently a meta-analysis of five randomised trials of low-dose ASA in pregnant women with abnormal uterine artery Doppler as a screening test showed a significant benefit of ASA in reducing pre-eclampsia (odds ratio [OR] 0.55, 95% confidence interval [CI] 0.32) (Coomarasamy et al. 2001).

Our study showed that ASA at a daily dose of 0.5mg/kg prevents pregnancy-induced hypertension and pre-eclampsia provided treatment is started at 12-14 weeks of gestation, before the trophoblastic invasion is completed, and the prophylaxis is given to high-risk women with abnormal Doppler velocimetry waveform images in the uterine arteries.

3. Bilateral notching in uterine arteries at 12-14 weeks of pregnancy in prediction of subsequent hypertensive disorders of pregnancy .

Campbell and colleagues made the first prospective study of abnormal arcuate artery waveforms at 16-18 weeks of gestation as a predictive test for pre-eclampsia or IUGR in 1986. The sensitivity, specificity, positive and negative predictive values were 68%, 69%, 42% and 87%, respectively, in this unselected population. Thereafter several screening studies by Doppler ultrasound at 16-24 weeks of gestation as a predictor of subsequent hypertensive disorders have been carried out. The studies have varied widely in populations examined, Doppler methodology, cut-off for abnormal values and definitions of the disease, this making for substantial differences in sensitivity (Papageorghiou et al. 2001, Chien et al. 2002).

In the present study bilateral notching in the uterine arteries at 12-14 weeks of gestation proved to be sensitive (75-84%), and it had a high negative predictive value (87-93%), whereas the specificity was only 41-50%. There are two previous studies in a low-risk population using uterine artery Doppler ultrasound at 12-16 weeks of gestation (Harrington et al. 1997) and at 11-14 weeks of gestation (Martin et al. 2001), and one study of 35 years and older pregnant women at 12-13 weeks of gestation (van den Elzen et al. 1995). Harrington and colleagues (1997) succeeded improving the specificity of bilateral notching to 85% by using information derived from multiple parameters, in particular indices of resistance and flow velocity. Martin and associates (2001) found that bilateral notching was not a suitable screening method for pre-eclampsia or fetal growth restriction at 11-14 weeks of gestation in an unselected population. In their study a mean pulsatility index > 2.35 was found in 5% of pregnancies and the sensitivity and specificity of the test were 27% and 11.7% for pre-eclampsia and 95.4 % and 95.6% for fetal growth restriction, respectively.

In the present study the sensitivity of bilateral notching in predicting hypertensive disorders of pregnancy decreased with advancing pregnancy and the specificity and the positive predictive values increased. This is in accord with previous results (Antsaklis et al. 2000). Also the sensitivity and specificity of the uterine artery mean PI > 95^{th} centile in the prediction of pre-eclampsia and fetal growth restriction were lower when the test was carried out at 11-14 weeks rather than 22-24 weeks (Martin et al. 2001). In a low-risk population the increased impedance to flow in the uterine arteries at 23-24 weeks of gestation identifies about 40% of those who subsequently develop pre-eclampsia and about 20% of those who develop fetal growth restriction. Earlier screening is associated with a higher false-positive rate (Papageorghiou et al. 2002).

In a previous review it was found that in a high-risk population the pooled likelihood ratio of diastolic notches in predicting pre-eclampsia was 20.2 and the post-test probability was 55.6, but other flow waveform ratios or screening of low-risk populations had limited predictive values for pre-eclampsia or intrauterine growth restriction (Chien et al. 2000). Their conclusion was that future research should focus on Doppler ultrasonic detection of uterine artery diastolic notches alone to predict pre-eclampsia, especially in pregnant women considered to be at a high risk of this condition (Chien et al. 2000).

In our study the sensitivity of bilateral notching in the uterine arteries in a high-risk population for prediction of PIH and IUGR was high, but the specificity was only 40-50%. In future it should be sought to increase the low positive predictive value of uterine artery Doppler screening by integrating other parameters of placental function or endothelial activation to the test, as done in a study by Aquilina and associates (2001).

4. The changes in prostacyclin and thromboxane production in hypertensive disorders of pregnancy

Endothelial dysfunction has been proposed as a central feature of the pathophysiology of pre-eclampsia, resulting in altered vascular reactivity, activation of a coagulation cascade, and loss of vascular integrity (Roberts et al. 1989, Taylor et al. 1998, Roberts 1998). The primary vasoactive products of endothelial cells are the prostaglandins (Friedman 1988) and the major eicosanoid product produced by endothelial cells is reported to be prostacyclin (PGI_2) (Spector 1988).

During the past two decades numerous studies have evaluated the possibility that pre-eclampsia is causally linked to an imbalance in the formation of PGI_2, a vasodilator, and thromboxane A_2 (TxA_2), a vasoconstrictor. The results of these studies were for a long time conflicting, partly due to the uncertainties of the measurements of maternal PGI_2 production in vivo (Ylikorkala and Viinikka 1993). Other possible explanations were differences in selection of study populations (Yamaguchi et al.1985), in methods of assay (Friedman et al.1988), and in study design (Paarlberg et al.1998). By using the reliable measurement of maternal systemic production of PGI_2 (measurement of urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$).) several studies have reported significantly lower PGI_2 levels (Goodman et al. 1982, Ylikorkala et al. 1986, Minuz et al. 1988, Barden et al. 1994, Kaaja et al. 1995, Kaaja et al.1999a, Mills et al. 1999) in pre-eclampsia, even before 20 weeks of gestation (Fitzgerald 1987b), as compared to normal pregnancies, but in one study no evidence for prostacyclin deficiency in pre-eclampsia could be found (Paarlberg et al. 1998).

It was shown in our first study that the excretion of the urinary metabolite of PGI_2 was two-fold higher in normotensive than in hypertensive pregnancies. Also the urinary excretion of prostacyclin was 2.5 times greater in hypertensive and five times greater in normotensive pregnancies than in non-pregnant women. In the prospective study we found that excretion of the urinary metabolite of PGI_2 increased throughout pregnancy, while in the placebo group pregnancies, which developed pre-eclampsia evinced significantly lower excretion of urinary metabolite of PGI_2 at 12-14 weeks of gestation as compared to other pregnancies. Our findings support the conception of endothelial dysfunction in pre-eclampsia and that the pathophysiological changes occur weeks before clinical disease is evident (Friedman et al. 1991). However, we found no significant difference in the excretion of the urinary metabolite of PGI_2 in the longitudinal study between pregnancies with normal or adverse outcome. It is possible that the sample size calculated to detect clinical differences was not large enough to detect biochemical differences, taking into account the wide variation in individual prostanoid levels.

In the first study pregnant women showed a two-fold higher mean urinary excretion of 11-dehydro-TxB2 as compared to the non-pregnant women, but the difference between normotensive and hypertesive pregnant women was not statistically significant. Also in the longitudinal study the urinary excretion of the TxB_2 metabolite increased significantly and the PGI_2/TxB_2 ratio was unchanged in the placebo group throughout pregnancy, with no significant difference between normal and adverse outcome. However in pregnancies with PIH before 37 weeks of gestation 11-dehydro-TxB_2 was higher throughout pregnancy as compared to other pregnancies.

It has been shown that placenta of pre-eclamptic women produce increased amounts of TxB_2 (Mäkilä et al. 1984), but that the urinary excretion of TxB_2 metabolite does not differ between pregnancies with normal outcome or pre-eclampsia (Ylikorkala et al. 1986, Minuz et al. 1988, Barden et al. 1994, Kaaja et al. 1995). Paarlberg and colleagues (1998) found in severe pre-eclampsia TxB_2 dominance over prostacyclin and in some studies urinary excretion of TxB_2 metabolites were significantly higher in pre-eclamptic women as compared to normal pregnancies (Fitzgerald et al. 1990, Klockenbusch et al. 1994).

By far the largest prospective study of pre-eclampsia and eicosanoids (Mills et al. 1999) suggested that women who developed pre-eclampsia had significantly lower urinary PGI_2 levels even at 13-16 weeks of gestation and thereafter throughout pregnancy, while TxB_2 levels in pre-eclamptic women were not significantly higher overall. We don’t know whether PGI_2 deficiency is a primary change or is secondary development to endothelial cell injury (Roberts 1989), but the resulting low PGI_2/TxB_2 ratio leads to vasoconstriction and increased platelet aggregation with consequent TxA_2 release.

5. 9a, 11b-prostaglandin F2 in normal pregnancies and in pregnancies at high risk of hypertensive disorders of pregnancy and the effect of ASA.

There is abundant evidence that the prostaglandins play a particularly important role in implantation and maintenance of pregnancy, but there are only a few studies reporting on PGD_2 in reproduction (Saito et al. 2002). 9α,11β-prostaglandin F_2 is a primary metabolite of PGD_2 and unlike other primary metabolites it is a bioactive substance and a potent vasoconstrictor (Liston and Roberts 1985). PGD_2 is produced in many organs, for example the endometrium and myometrium (Rees and Kelly 1986) and it is also released from mast cells (Lewis et al. 1982, Matsuoka et al. 2000). It has been assumed that pre-eclampsia reflects an inflammatory-type reaction and that human mast cell activation and overproduction in the myometrium may be involved in the pathogenesis of pre-eclampsia (Purcell 1992, Mitani et al. 2002).

In the present study we were able to show that urinary 9α,11β-prostaglandin F_2 increases throughout pregnancy regardless of outcome. However, pregnancies involving a high resistance in the uteroplacental circulation and bilateral notches in the uterine arteries had significantly higher urinary 9α, 11β-prostaglandin F_2 as compared to normal pregnancies at 12-14 and at 30-34 weeks of gestation. According one theory pre-eclampsia develops when maternal systemic inflammation response decompensates (Redman and Sargent 2001). Also prostaglandin D_2 may play a very important role for the inhibition of fetal-antigen presentation to maternal T cells (Saito et al. 2001). We can hypothesize that increased excretion of urinary 9α, 11β-prostaglandin F_2 could be a consequence of an increased inflammatory reaction in the high-risk pregnancies.
Probably the small number of pregnancies with pregnancy-induced hypertension and especially pre-eclampsia restricted the possibility of establishing statistically significant differences in urinary 9α,11β-prostaglandin F_2 between pregnancies with normal or adverse outcome.

Low-dose ASA reduces lipid peroxides and the formation of prostaglandins by inhibiting cyclo-oxygenase (Walsh et al. 1992). Urinary 9α.11β-prostaglandin F_2 was lower throughout pregnancy in the women treated with ASA, though the difference was not statistically significant. It may be suggested that the dose of ASA was too low to inhibit the production of 9α,11β-prostaglandin F_2.

Further studies are needed to ascertain what is the role of prostaglandin D_2 in the pathogenesis of pre-eclampsia and whether urinary 9α,11β-prostaglandin F_2 could be a predictive marker for pregnancies involving high risk for pre-eclampsia.

SUMMARY AND CONCLUSIONS

The aim of the present series was to evaluate the efficacy of low-dose ASA in the prevention of pregnancy-induced hypertension and intrauterine growth restriction in high-risk pregnancies as determined by transvaginal Doppler ultrasound study of the uterine arteries at 12 to 14 weeks of gestation.

Our first study revealed that within a dose range of 0.5-2.0mg/kg/day ASA has a favorable effect on the ratio of prostacyclin to thromboxaneA_2 in hypertensive pregnancies. In the prospective, randomised placebo-controlled trial 120 pregnant women who in the light of history were considered to carry a high risk of pregnancy-induced hypertension or intrauterine growth restriction were screened by transvaginal sonography at 12 to 14 weeks of gestation. Ninety women with bilateral notching in the uterine artery were randomised and forty-three in both groups were successfully followed up. Outcome data were obtained on 29 of the 30 women without bilateral notches.

The main finding was that low-dose ASA given to women at high risk of gestational hypertension and proteinuric pre-eclampsia significantly reduced the incidence of pregnancy-induced hypertension and especially proteinuric pre-eclampsia. Pregnancy-induced hypertension developed in five women allocated to ASA against 16 of those receiving placebo (relative risk 0.31, 95%CI 0.13-0.78). Pregnancy-induced hypertension was proteinuric in two pregnancies on ASA and in 10 on placebo (RR = 0.20, 95% CI 0.05-0.86). The hypertension set in or was exacerbated before 37 gestational weeks in two women randomised to ASA and in nine randomised to placebo (RR = 0.22, 95% CI 0.05-0.97). Two women in the placebo group had pre-eclampsia and one had pregnancy-induced hypertension concomitant with IUGR. In the ASA group there was no growth restriction concomitant with pregnancy-induced hypertension, but the difference between groups did not reach statistical significance. The small number of women recruited into the study restricted the possibility of conclusions as to the prophylactic effect of ASA on IUGR.

The results indicated that the finding of bilateral notches in the uterine arteries at 12-14 weeks of gestation in high-risk pregnancies is a sensitive screening test (75-84%) in predicting pregnancy-induced hypertension or intrauterine growth restriction, but has rather low specificity (41-50%). The sensitivity of the test diminished with advancing pregnancy to 35% at 32-34 weeks of gestation and the specificity and positive predictive value increased to 94% and 59%, respectively.

In pregnancies complicated by PIH before 37 weeks of gestation, the balance of prostacyclin and thromboxane A_2 shifted in favor of thromboxane A_2. Also in the placebo group 2, 3-dinor-6-keto-$PGF_{1\alpha}$ was at 12-14 weeks of gestation significantly lower in pregnancies, which later developed pre-eclampsia as compared to other pregnancies. The finding supports the theory that endothelial dysfunction and a deficiency in prostacyclin production occurs many months prior to clinical symptoms of pre-eclampsia.

The primary metabolite of PGD_2 is vasoconstrictive 9α-11β-PGF_2 and therefore we evaluated the role of it in hypertensive disorders of pregnancy. Women with bilateral notching (n = 86) were compared with fifteen non-pregnant normotensive women of reproductive age, with 17 healthy normotensive women at 12-14 weeks of gestation and with 15 healthy normotensive women at 32-24 weeks of gestation. Urinary $9\alpha,11\beta$-prostaglandin F_2 was significantly higher in pregnant women at 12-14 weeks of gestation as compared to non-pregnant women. Women with bilateral notching had higher $9\alpha,11\beta$-prostaglandin F_2 as compared to normotensive pregnancies at 12-14, and at 30-34 weeks of gestation. Urinary $9\alpha,11\beta$-prostaglandin F_2 increased throughout pregnancy regardless of outcome of pregnancy or the treatment.

In conclusion, ASA treatment given to women at high risk of gestational hypertension from 12-14 weeks of gestation significantly reduces the incidence of pregnancy-induced hypertension and especially proteinuric pre-eclampsia. Transvaginal Doppler ultrasound and bilateral notching at 12-14 weeks of gestation appear to afford a useful basis in screening for pregnancies involving a high risk of hypertensive disorders of pregnancy.

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