# ASPIRIN IN PREGNANCY

## CLINICAL AND BIOCHEMICAL STUDIES

ASPIRINE IN DE ZWANGERSCHAP

KLINISCHE EN BIOCHEMISCHE ONDERZOEKEN

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*"Aspirine voor je vrouwtje*
*Als er weer een kleintje komt"*

Dirk Witte (1885-1932)

Aan mijn ouders

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Chapter 1

# INTRODUCTION

Aspirin, acetylsalicylic acid, is the most frequently consumed drug in pregnancy,[47] mostly taken without a prescription because of headache or a minor ailment.[226,277] Numerous preparations containing acetylsalicylic acid are freely available over the counter under a variety of proprietary names, and in many cases pregnant women and their doctors may be unaware that aspirin is being taken.

For many years obstetricians have advised against the use of aspirin as a simple analgesic in pregnancy, based on a general tendency to discourage all drug taking in pregnancy as well as on fears of specific complications of aspirin, such as teratogenic effects, maternal and fetal hemorrhage, and premature closure of the ductus arteriosus. But the negative attitude towards the use of aspirin in pregnancy is changing rapidly since evidence has become available that a daily low dose of aspirin (60-80 mg) may markedly reduce the incidence of hypertensive disorders and fetal growth retardation in pregnant women at risk.

Hypertensive disorders are a common complication of the second half of pregnancy. In a recent population-based study of almost 200.000 births in the USA the prevalence of pregnancy-induced hypertensive disorders was 43.1 per 1000 in singleton pregnancies. This large study confirmed earlier observations that nulliparity, advanced maternal age (>35 years of age), and multiple pregnancy are associated with a markedly increased risk.[214]

For the past 40 years, hypertensive disorders of pregnancy, in particular preeclampsia-eclampsia, were the first or the second -after pulmonary embolism- cause of maternal mortality in England and Wales,[56,152] responsible for 15-20% of maternal deaths. In a study of maternal mortality in the Netherlands, 1988-1991, 37% of maternal mortality was attributable to preeclampsia-eclampsia.[221] In developing countries, maternal mortality is 100-200 times higher than in Europe and North America. In these countries deaths from hemorrhage and infection account for a large part of the excess,[61] followed by preeclampsia-eclampsia. Analysis of a large, mainly hospital-based, set of data collected by the World Health Organization indicates that hypertensive disorders of pregnancy may be held responsible for 10-15% of the maternal mortality in various developing countries in Africa, Asia, Latin America, and the Caribbean.[61]

The impact of gestational hypertension on fetal mortality is disputed, due to differences in criteria of selection and diagnosis between various studies. According to the World Health Organization hypertensive disease during pregnancy is the main cause of perinatal mortality and morbidity.[142] Indeed, there seems to be no doubt that pregnancy-induced hypertensive disease associated with proteinuria, that is preeclampsia, is accompanied by a perinatal morbidity and mortality that is substantially higher than in normotensive pregnancies. The main threat to the fetus is an insufficient supply of nutrients through the placenta leading to growth retardation and low birthweight. However, in the absence of proteinuria pregnancy-induced hypertensive disease is not associated with fetal growth retardation and carries a perinatal mortality that is similar to that in normotensive pregnancies.[142] On the other hand, fetal growth retardation caused by an insufficient placenta supply line is not necessarily associated with gestational hypertension.[263]

No other disease in pregnancy has been surrounded for so long by so many uncertainties and controversies, concerning its etiology, pathophysiology, treatment and prevention.[35] The development of high blood pressure during pregnancy signals a disturbance of the physiological adaptation of the maternal circulation, the mechanism of which depend to a large extent on changes in maternal prostaglandin synthesis. In normal pregnancy the synthesis of prostacyclin ($PGI_2$), an endothelium-derived vasodilator and inhibitor of platelet aggregation, increases markedly, which leads to a dominance in the biological balance with platelet derived thromboxane $A_2$ ($TXA_2$), a strong vasoconstrictor and inductor of platelet aggregation. Some women fail to develop or maintain these physiological adaptational responses to pregnancy; in these women a relative dominance of $TXA_2$ over $PGI_2$ results in vasocontriction and platelet aggregation.[260] Hypertension is a frequent, but not obligatory, sign of the circulatory maladaptation that may cause disturbances in maternal systemic and uteroplacental perfusion, responsible for the vascular injury and clinical signs and symptoms of preeclampsia - eclampsia and fetal growth retardation.[263]

The view that diffuse activation and injury of vascular endothelium constitutes a key factor in the pathogenesis of preeclampsia is receiving support from several lines of evidence. Recently, Roberts and Redman[205] suggested that the endothelial dysfunction in preeclamptic patients leads to a loss of normal endothelial depressor functions, which results in increased sensitivity to normally circulating pressor agents. Elevated circulating levels of markers of endothelial cell activation and damage, such as cellular fibronectin,[212] laminin,[9] and Von Willebrand factor[185] were demonstrated in patients with preeclampsia. Elevated levels of plasma endothelin-1 were reported[74] although recent studies failed to demonstrate differences between plasma endothelin-1 concentrations[15] or urine excretion[11]

in normotensive and preeclamptic women. Endothelial damage may affect synthesis of endothelium-derived nitric oxide and cause altered control of arterial tone.[146]

The endothelial activation and injury in preeclampsia may also disturb the finely tuned balance of physiological coagulation, fibrinolysis and platelet activation,[262] as demonstrated by the increased production of thromboxane by platelets[248] and placental tissue,[275] and a reduced placental synthesis of prostacyclin.[275] Elevated platelet thromboxane synthesis and reactivity has been recognized as an important pathophysiologic mechanism in the development of the uteroplacental circulatory insufficiency that may accompany pregnancy-induced hypertensive disorders.[266]

Because of the hypothesis that the maternal and uteroplacental circulatory disturbance in pregnancy-induced hypertensive disease and fetal growth retardation depends at least in part on a functional imbalance between two prostaglandins with opposing physiological effects, thromboxane and prostacyclin, it was attempted to correct the putative imbalance by means of dietary and pharmacologic manipulation of prostaglandin synthesis, in particular with low-dose aspirin, as already suggested by Wallenburg in 1980.[270] Review of early small trials of low-dose aspirin suggested reductions of about three-quarters in the incidence of preeclampsia, but these results were not always confirmed in larger trials.[24] Because of this apparent discrepancy, a large randomized trial of low-dose aspirin for the prevention and treatment of preeclampsia (Collaborative Low-dose Aspirin Study in Pregnancy [CLASP]) was designed. When the CLASP study was conducted, between 1988 and 1992, it became increasingly difficult to randomize patients between active treatment and placebo, possibly due to the results of the smaller trials suggesting a high efficacy of prophylactic treatment with low-dose aspirin,[24] and to information disseminated in the lay press.[191]

Results of previous studies suggest that long-term daily low-dose aspirin has no demonstrable effect on hemostasis in pregnant women as indicated by the bleeding time,[14,281] but no data are available in the accessible literature on the effect on the fibrinolytic system. Prostaglandins, involved in the initiation and maintenance of labor,[116] also have an effect on fibrinolytic variables.[16] An effect of low-dose aspirin, through inhibition of prostaglandin synthesis, can therefore not be excluded; this could be of particular importance after normal or abnormal placental separation, when coagulation in the placental bed is balanced by a marked increase in fibrinolytic capacity.

Based on the considerations presented above the objectives of this thesis can be summarized as follows:

1. to review the literature on the general principles of dietary and pharmacologic manipulation of prostaglandin synthesis in pregnancy.

2. to review the literature on the pharmacology, pharmacokinetics, and prophylactic and therapeutic applications of aspirin in pregnancy.

3. to assess changes in patterns of prescription in the Netherlands of low-dose aspirin for prevention and treatment of pregnancy-induced hypertensive disorders and fetal growth retardation.

4. to present and discuss the CLASP trial.

5. to investigate the effects of labor on variables of the fibrinolytic system.

6. to investigate the effects of low-dose aspirin during pregnancy on prostaglandin and fibrinolytic variables before and after parturition.

7. to formulate guidelines for the application of low-dose aspirin in obstetric practice on the basis of the results of the studies presented and discussed in this thesis.

The results of the studies related to these objectives are described in chapters 2-8 of this thesis.

Chapter 2

# PRINCIPLES OF MANIPULATION OF PROSTAGLANDIN SYNTHESIS IN PREGNANCY*

Manipulation of the prostaglandin synthesis in pregnancy with the aim to correct an unfavorable prostacyclin-thromboxane balance and to prevent or treat pregnancy-induced hypertensive disorders and fetal growth retardation can only be understood against the background of basic prostaglandin biochemistry and of the physiological changes in the prostaglandin system that occur in normal preganancy. Dietary and pharmacologic approaches that are available to manipulate the prostaglandin system will be discussed in this chapter.

## 2.1. General biochemical, physiological and pathophysiological background

Prostaglandins (prostanoids) and other derivatives of eicosanoic (20-carbon) polyunsaturated fatty acids (PUFAs) are collectively termed 'eicosanoids'.[48] They are known to function as autacoids, locally active biochemical mediators released by cells on demand in response to appropriate chemical or physical stimuli.

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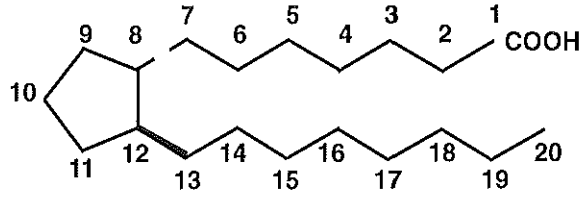


*Figure 2.1 Basic structure of prostanoic acid*

*Biochemistry of eicosanoids*

For the purpose of a discussion of the manipulation of eicosanoid synthesis, we may distinguish two main steps in the formation of these autacoids. The initial step consists of the biosynthesis and storage of arachidonic acid, the immediate fatty acid precursor of eicosanoids with two double bonds (dienoic) in their long chains, which constitute the physiologically most important eicosanoids in man. Eicosanoids with one (monoenoic) or three (trienoic) double bonds are derived from different PUFAs and have biological properties that differ from those of products derived from arachidonic acid; they seem to be not important in man under physiologic conditions, but they may modulate the synthesis of dienoic eicosanoids. Arachidonic acid is either ingested as a dietary constituent or synthesized by desaturation and elongation of 18-carbon (linoleic and gamma-linolenic acid) or 20-carbon (dihomo-gamma-linolenic acid) PUFAs. The enzymes involved in these biochemical processes are not specific and various fatty acids may compete for shared enzymes.[50] Because linoleic acid is an essential fatty acid that cannot be synthesized de novo in human tissues and must be supplied by food, the formation of arachidonic acid and its precursors will depend on the composition of the diet and may be influenced by dietary manipulation.[97]

Much of the arachidonic acid is utilized for energy requirements, but some becomes esterified to the phospholipids of cell membranes and to other lipid pools and may participate in the second step of eicosanoid synthesis, the actual formation of active

prostanoids. To that purpose, arachidonic acid must first be liberated from its ester bonds by the hydrolytic action of phospholipases, activated in the presence of calcium and calmodulin.[55] Once released from its cellular stores, the arachidonic acid is either rapidly reincorporated into phospholipids, or metabolized to a series of oxygenated products through the action of a ubiquitous complex of enzymatic oxygenases, including cyclooxygenase, lipoxygenase, and epoxygenase.[153] Cyclooxygenase - also termed prostaglandin endoperoxide synthase or PGH-synthase - catalyzes the conversion of arachidonic acid into the very unstable cyclic endoperoxides $PGG_2$ en $PGH_2$, which are transformed, enzymatically as well as nonenzymatically, into a variety of active compounds, including the "classical" prostaglandins $E_2$ and $F_{2\alpha}$, prostacyclin ($PGI_2$) and thromboxane ($TXA_2$). The cyclooxygenase products are often referred to as prostanoids, a terminology that will be used interchangeably with that of prostaglandins (Fig.2.1). Through the lipoxygenase pathway arachidonic acid is converted to leukotrienes with 4 double bonds (4-series), potent mediators in inflammatory and hypersensitivity reactions which also interact at various levels with the cyclooxygenase pathway.[190] Also the eicosanoids of the epoxygenase pathway are biologically active and may interact with cyclooxygenase products.[73] Other PUFAs may undergo the same reactions resulting in the formation of monoenoic (1-series) or trienoic (3-series) prostanoids, and of leukotrienes of the 3- and 5-series. Because the capacity of the human body for the synthesis of prostanoids is about 1000 times greater than the amount actually produced, activation of the eicosanoid system is countered with negative feed back reactions of self-catalyzed inactivation.[145]

An exogenous agent that interferes with the availability or functioning of the various enzymes of the arachidonic acid cascade will necessarily inhibit the formation of

prostanoids to some degree. In 1971 Vane discovered that inhibition of the cyclooxygenase enzyme is the mechanism by which acetylsalicylic acid (aspirin) and nearly all nonsteroidal anti-inflammatory agents prevent the formation of the classical prostaglandins, $PGI_2$ and $TXA_2$.[249] This finding not only provided a new tool for the investigation of the physiologic and pathophyiologic effects of endogenous prostanoids in vivo, but it also opened up new pathways for clinical research on prevention and treatment in a variety of diseases, including obstetric disorders, and thus put the clinician in the middle of the field of prostanoid research.

*Prostanoids and maternal adaptation in pregnancy*

Over the past 10 years studies in vivo and in vitro have convincingly shown that the impressive adaptation of the systemic and uteroplacental circulations in pregnancy depends to a large extent on changes in prostanoid synthesis,[261] in particular involving prostacyclin and thromboxane $A_2$.[260] Prostacyclin, with its principal site of synthesis in vascular endothelium and in pregnancy also in trophoblast, brings about relaxation of vascular and uterine smooth muscle leading to vasodilation and inhibition of myometrial contractility. In addition, it is an extremely potent inhibitor of the aggregation of platelets. Thromboxane $A_2$, synthesized mainly by platelets and in pregnancy also in the uterus and some other pregnancy-associated tissues, causes vasoconstriction, platelet aggregation, and stimulates myometrial contraction.[158] The formation of prostacyclin by endothelial cells serves to keep platelet adherence and aggregation on normal vascular endothelium at bay. In normal pregnancy prostacyclin production increases markedly, leading to a dominance of the biologic effects of prostacyclin over those of thromboxane $A_2$.[166,285] It is generally accepted that these pregnancy-induced changes in prostanoid balance determine

the vasodilation and reduced systemic vascular resistance that is characteristic of normal pregnancy.[260,285] Prostacyclin is most likely also responsible for the reduced sensitivity in pregnancy of the maternal vascular system to angiotensin-II and other vasopressors.[264] In the uteroplacental vascular system and in the placenta prostacyclin produced by trophoblast cells serves as a brake on the aggregation of platelets and thus prevents thrombosis and placental infarction.[272]

What induces the activation of prostacyclin synthesis in pregnancy? The answer to this question remains one of the mysteries of reproduction. It is tempting to speculate that the message somehow comes from the fertilized ovum,[195] and immune recognition of the fetal allograft has been postulated as the main stimulus.[260]

*Prostanoids and maternal maladaptation in pregnancy*

Because the cause-and-effect relationhip between putative immunological triggers, biochemical mediators, and physiological adaptational responses is not yet understood, it is also not clear why some apparently healthy young women fail to develop or maintain the physiological adaptational responses to pregnancy. In these women the increase in prostacyclin production does not occur or is not sufficiently maintained, resulting in a relative dominance of the opposing effects of thromboxane $A_2$. Thromboxane dominance appears to be the main mediator of what Wallenburg has called circulatory maladaptation disease,[260] characterized by a relatively increased vascular resistance, increased vascular sensitivity to angiotensin-II and other vasopressors, and the development of thrombosis in the uteroplacental circulation resulting in placental infarction.[76,272] The clinical expression of circulatory maladaptation disease includes pregnancy-induced hypertensive disorders, and uteroplacental circulatory insufficiency with fetal growth retardation.[260] Theoretically,

there could be various causes of maternal circulatory maladaptation : insufficient feto-maternal immuno-stimulation ( e.g. in a first pregnancy ), inadequate response of the target organ due, for instance, to chronic hypertension or diabetes mellitus with vasculopathy, or factors inhibiting the biochemical cascade of prostanoid synthesis.[263] The latter may be caused by lupus anticoagulant or anticardiolipin antibodies, which are known to interfere with the synthesis of prostacyclin from arachidonic acid.[222] Some 10 years ago the recognition of relative thromboxane dominance as an important mechanism in the pathophysiology of circulatory maladaptation disease and the knowledge that aspirin is a potent inhibitor of platelet thromboxane synthesis led to first attempts to use aspirin in the prevention or early treatment of pregnancy-induced hypertensive disorders and fetal growth retardation.[259,270]

## 2.2. Dietary manipulation

The dietary manipulation of prostanoid synthesis is in particular associated with the consumption of fish and fish oil. A small number of observational studies suggest a reduced occurrence of pre-eclampsia and eclampsia, longer gestation, and higher birthweights in populations with a high consumption of marine fish compared with areas with a lower consumption of fish.[3,62,174] A questionnaire study in over 6500 pregnant women in Denmark who did not smoke during pregnancy showed a significant positive association between fish consumption and placental weight, birthweight, and neonatal head circumference, but not gestational age.[175] As Olsen and Secher[176] have recently pointed out, these observations are supported indirectly by the results of a controlled trial conducted by the People's League of Health during 1938-39 in London.[183] In this study in over 5000 pregnant women, allocated alternately to dietary supplements of vitamins,

minerals, and halibut liver oil, or no treatment, a significant reduction in the occurrence of preeclampsia and preterm delivery was observed in women receiving halibut liver oil compared with nontreated women. The usual explanation of the beneficial obstetric effects that are said to be associated with consumption of fish oil is that marine fat has a high content of PUFA precursors of 3-series prostanoids which compete with the formation of arachidonic acid resulting in a shift in prostanoid synthesis in favor of the biological effects of prostacyclin.[3]

*Biochemical, physiological and pathophysiological background*

Two classes of PUFAs are involved in the processes of desaturation and elongation leading to the formation of direct prostanoid precursors (Fig.2.2). One class consists of PUFAs with the first of their 3 to 6 double bonds at the third C-atom counting from the methyl end of the long chain (ω3). Members of this class occur in some vegetable oils (linseed oil), but the main source of the precursor of 3-series prostanoids and 5-series leukotrienes, eicosapentaenoic acid (EPA), is marine fish. EPA is reversibly converted to C22:6,ω3 and both PUFAs are synthesized by algae and phytoplankton, and taken up by man in fish and other sea-foods. In particular fat marine fish such as herring, sardine, mackerel, tuna and cod are a rich source of EPA and other ω3 PUFAs.[10] The second class, that contains the 1- and 2-series prostanoids, counts its first double bond at the 6th C-atom (ω6) and is derived from linoleic and gamma-linolenic acid in various vegetable oils such as corn, sunflower and safflower oil. The immediate precursor of the 2-series prostanoids, arachidonic acid, can also be taken up directly from meat, but since the average diet in developed countries contains less than 0.2% of energy as arachidonic acid, most of the tissue arachidonic acid is derived from linoleate.[144]

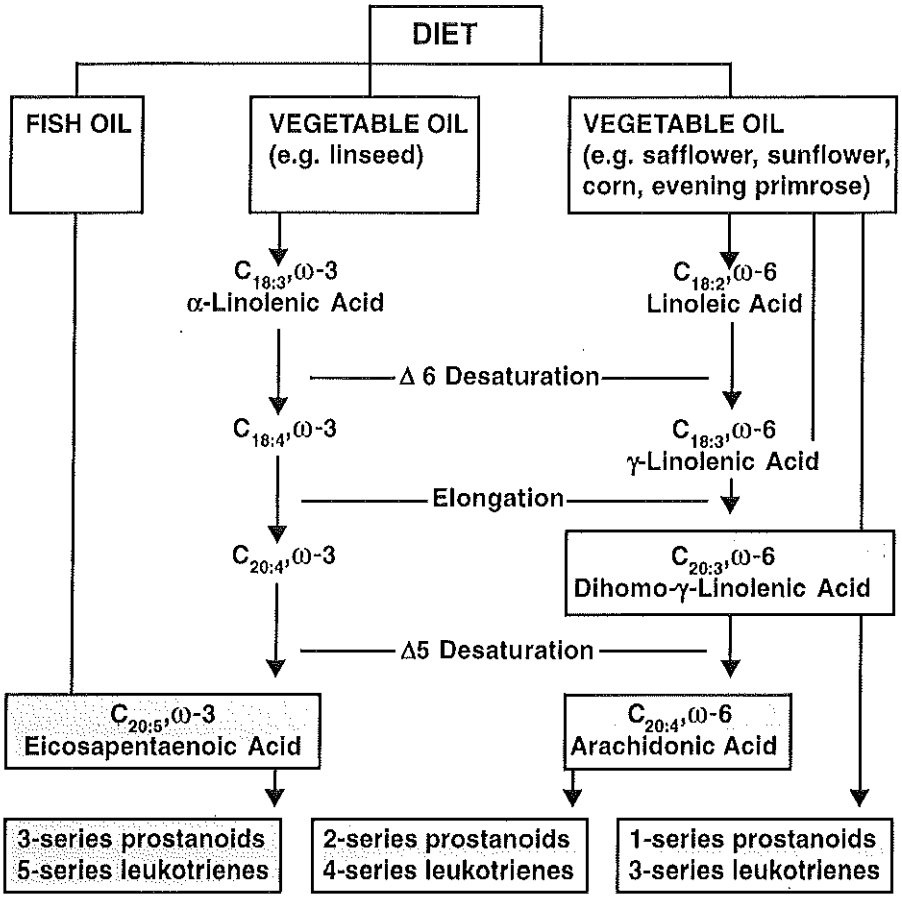


*Figure 2.2 Scheme of the dietary sources and processes of elongation and desaturation of PUFAs resulting in the formation of prostanoids and leukotrienes. PUFAs are indicated by $C_{n:m}$, where n is the number of carbon atoms and m the number of double bonds; ω indicates the number of carbon atoms counted from the methyl end of the long chain to the first double bond.*

Human metabolism is unable to change ω3 to ω6 PUFAs, or vice versa. Although 1- and 3-series prostanoids are not important in man under physiologic conditions, both classes of PUFAs are required for many physiological purposes, such as cell membrane structure and transport and oxidation of cholesterol.

The dietary essential PUFAs in both classes compete with one another and with dietary saturated fats for incorporation in cell membranes and lipid pools, and for the shared enzymes which catalyze elongation and desaturation. In addition, EPA and dihomo-gamma-linolenic acid may compete with arachidonic acid at the level of cyclooxygenase and lipoxygenase.[50,87] For that reason, endogenous synthesis of 2-series prostanoids can be modified quantitatively and qualitatively by manipulating the absolute and relative amounts of ω3 and ω6 PUFAs in the diet, and such dietary manipulation could affect the function of the many physiologic systems in which prostanoids are involved.

A major part of the research on dietary manipulation in nonpregnant individuals concerns attempts to shift the hemostatic balance towards increased vasodilatation and reduced platelet activity with the aim to prevent or treat vascular disease and thrombotic events.[96] In the majority of studies the approach has been to manipulate the ω3 PUFAs, in particular using supplementation of the diet with EPA-rich fish oils or purified EPA. The scientific enthusiasm for this approach is based on the results of epidemiologic studies indicating a low incidence of hypertensive, thrombotic and atherosclerotic disorders among Greenland Eskimos[63] and certain groups in Japan[114], who all have a high consumption of fish and sea mammals. Intake of EPA-rich fish meat, fish oil, or purified EPA has been shown to lead to a reduced formation of dienoic $TXA_2$ by collagen-stimulated platelets and an increased synthesis of trienoic thromboxane ($TXA_3$), which shows only 10% of the biological activity of $TXA_2$.[98,122] In addition, consumption of EPA increases the formation in vivo of trienoic Δ17-prostacyclin ($PGI_3$), biologically equipotent with dienoic $PGI_2$, whereas synthesis of the latter remains unchanged or may even increase slightly.[98,121] These and other experiments indicate that EPA competes with

arachidonic acid for incorporation in tissue and plasma phospholipids as well as at the level of the cyclooxygenase enzyme, resulting in inhibition of $TXA_2$ and its platelet-aggregatory effects while stimulating the synthesis of vasodilator $PGI_3$. There is evidence that EPA may also affect the arachidonic acid cascade in more complex ways, e.g. by inhibition at the level of Δ6-desaturation.

The biochemical changes in eicosanoid synthesis associated with manipulation of the ω3 class of PUFAs explain some, but not all, preventive and therapeutic effects of a diet rich in fish or supplemented with purified EPA with regard to hypertensive, thrombotic and atherosclerotic disease as observed in epidemiologic studies and in a large number of clinical trials.[64,121] It should be realized that the content of various ω3 PUFAs is extremely variable in different species of fish, which makes the extrapolation of fish consumption to marine ω3 fatty acid consumption difficult. For that reason and also because of the marked variations in the design of published studies and the complexity of the mechanisms involved, the putative beneficial effects of dietary supplementation with EPA, although suggestive, remain as yet disputed.[81]

Even more controversial are the effects of dietary manipulation of the ω6 PUFAs, with supplements of linoleic acid and dihomo-gamma-linolenic acid, usually as safflower or sunflower oil. Some studies have used evening primrose oil, which not only has a high content of linoleate (75%), but also contains about 9% of gamma-linolenic acid and various substances regarded as cofactors for prostanoid production. Dietary supplementation with linoleic acid has been shown to stimulate synthesis of PGE and PGF, whereas prostacyclin formation remained unaffected.[121] A diet rich in linoleate appears to reduce platelet $TXA_2$ formation, a biochemically unexpected effect that seems to be based on incorporation of linoleic acid into platelet lipids without bioconversion into

arachidonic acid.[97] A high dietary content of linoleic acid has been linked to reduced blood pressure, an effect that could be mediated by conversion of linoleic acid to arachidonate and subsequent formation of 2-series prostanoids. However, many later studies have failed to confirm such an association.[121] It has been suggested that evening primrose oil could alleviate the symptoms of the premenstrual syndrome through an increased synthesis of $PGE_1$, but a recent double-blind placebo-controlled study failed to support such claims.[118]

The majority of the studies on dietary manipulation of prostanoid synthesis are not properly controlled and they have applied dietary supplementation in various forms from various sources in variable doses for variable periods of time. For that reason, many doubts and uncertainties remain with regard to the biochemical and biological effects and, in particular, the clinical consequences of the manipulation of dietary PUFAs with the aim to prevent or treat hypertensive, thrombotic and atherosclerotic disorders.

*Obstetric applications of dietary manipulation*

There is no evidence to suggest that a dietary deficiency of ω3 or ω6 PUFAs is involved in the pathophysiology of maladaptation disorders of pregnancy. MacGillivray[142] in a small-scale study in Aberdeen found no difference in the amount of linoleic, gamma-linolenic or arachidonic acid between the diets of 15 preeclamptic women and 29 normotensive pregnant controls. The few studies on plasma levels of free and esterified arachidonic acid in women with preeclampsia have not revealed any difference with normotensive pregnant women,[173,274] but plasma levels of total PUFAs, and of EPA, linoleic, and α-linolenic acid were found to be somewhat reduced in term preeclamptic women.[274] The authors suggest that this could represent altered fatty acid metabolism with

altered storage or mobilization from lipid pools. Experience with dietary manipulation of prostanoid synthesis in pregnancy is extremely limited. Worley[283] mentions a study by Gant et al. who are said to have found no difference in the incidence of pregnancy-induced hypertension among pregnant women who received a large daily dietary supplement of dihomo-gamma-linoleic acid when compared with placebo-treated women; however, no data of this study are presented or could be found in the literature. Moodley and Norman[159] report a study in which they randomly allocated 47 primigravid patients with mild to moderate pregnancy-induced hypertension (BP 140/90 mm Hg or more) in the third trimester to supplementation with a daily dose of 4 g of evening primrose oil or matched placebo for a minimum period of two weeks. No differences were found between the two groups with regard to blood pressure, or course and outcome of pregnancy. This is in fact a therapeutic rather than a prophylactic study. Also using a daily dose of 4 g of evening primrose oil during 7 days O'Brien et al[171] investigated the effect of dietary supplementation with ω6 PUFAs on the pressor response to infusion of angiotensin II in 10 normotensive pregnant women accepted for therapeutic termination of pregnancy in the early second trimester, and in 5 healthy nonpregnant female and 5 male volunteers. The diastolic pressor response to angiotensin II was significantly blunted in treated pregnant and nonpregnant subjects as compared to nontreated controls, and the effect was greatest in pregnant women. The systolic response was somewhat reduced in treated pregnant women, but not in nonpregnant subjects. Effects on platelet behavior and prostanoid synthesis were not measured. Accepting that the physiologically reduced sensitivity of the vascular system in pregnancy to the pressor effects of angiotensin II is largely determined by prostacyclin dominance, these results provide indirect evidence that shortterm dietary supplementation with ω6 PUFAs may shift prostanoid synthesis towards an increased

prostacyclin dominance, perhaps by reducing platelet $TXA_2$ formation, as discussed previously.

The effects of supplementation with ω3 PUFAs on pregnancy duration, and on weight and length of the newborn were investigated in 533 healthy women in week 30 of pregnancy who were randomly assigned in a ratio of 2 : 1 : 1 to fish oil (about 2.7g ω3 fatty acids per day), olive oil, or no supplement.[177] Fish oil supplementation was associated with a small but significant increase of, on average, four days in the duration of pregnancy compared with women taking olive oil, but no differences were found with regard to neonatal weight and length adjusted for gestational age. These results are in contrast with those of the earlier epidemiologic study which showed no association between fish consumption and gestational age at delivery.[175] Data on the occurrence of pregnancy - induced hypertension are not presented.

In conclusion, the theoretical basis of dietary manipulation of ω3 and ω6 PUFAs to produce physiologically beneficial changes of prostanoid synthesis remains largely speculative, and the results of the studies discussed above are hypothesis-generating rather than providing evidence that such manipulation could reduce the risk of pregnancy-induced hypertensive disorders, preterm labor, or fetal growth retardation.

*Side-effects of dietary manipulation*

An important aim of dietary PUFA manipulation is to reduce platelet activity, which could cause problems in pregnancy and delivery due to an increased bleeding tendency. Although to the best of our knowledge no toxic effects of ω3 or ω6 PUFAs have been reported in any of the studies in nonpregnant subjects, the highly unsaturated ω3 fatty acids are easily oxidized, which may produce substances that could be toxic in

the mother or the fetus.[223] The longterm effects of dietary manipulation of fatty acids are not known, but the possibility of deleterious effects due to an increased production of peroxides and radicals has been suggested.[126] In 1988 the American Food and Drug Administration banned the use of evening primrose oil as food or drug.[147] Considering the rapidly growing public interest in the preventive and therapeutic use of PUFAs, in particular of EPA in fish oil, and in view of recent medical publications already proposing EPA as a reasonable alternative to aspirin in the prevention and treatment of pregnancy-induced hypertensive disorders,[66,207] further research on its maternal and fetal effects should have a high priority.

## 2.3. Pharmacologic manipulation

Two decades ago Lewis and Schulman[134] published the first retrospective observation that gestation was prolonged by an average of 7 days in 103 women who used 3.25 g or more of aspirin daily during the last 6 months of pregnancy because of musculoskeletal or arthritic disease as compared with women who took no aspirin. In 42% of these women, gestation lasted 42 weeks or more as compared to only 3% in controls who did not take aspirin. Labor lasted 12 h in treated women, and 7 h in untreated controls. These effects were attributed to suppression of endogenous prostanoid synthesis, and one year later this principle was applied therapeutically to the inhibition of preterm labor using indomethacin.[291] The retrospective study by Crandon and Isherwood[49] indicating that preeclampsia occurred less frequently in regular aspirin users than in pregnant women who took no aspirin, forms another landmark in the history of pharmacologic manipulation of prostanoid synthesis to prevent and treat maladaptation disorders of pregnancy.

*Pharmacologic, physiological and pathophysiological background*

Three enzyme systems in the eicosanoid cascade can be pharmacologically inhibited: the phospholipases, the cyclooxygenase, and the specific isomerases that catalyze the conversion of endoperoxides into the specific prostanoids. Finally, end organ receptors may be inhibited by specific antagonists. A few agents have been claimed to stimulate prostacyclin synthesis by complex mechanisms.

*Inhibition of phospholipases*

There are a very large number of drugs and chemical compounds that have been reported to inhibit various phospholipases and thus the availability of arachidonic acid for prostanoid synthesis, including glucocorticoids. The numerous and widespread effects of glucocorticoids and other phospholipase-inhibiting drugs preclude their pharmacologic use for specific inhibition of prostanoid synthesis in pregnancy.

*Inhibition of cyclooxygenase*

A heterogeneous group of pharmacologic agents, many of them chemically unrelated, have antiinflammatory, analgesic, and antipyretic effects and are frequently designated as nonsteroidal antiinflammatory drugs (NSAIDs) or, after their prototype, aspirin-like drugs. Apart from acetylsalicylic acid (aspirin) and sodium salicylate, para-aminophenol derivatives (e.g. acetaminophen), acetic acid (e.g. indomethacin) and propionic acid (e.g. ibuprofen, fenoprofen, naproxen) derivatives are well known representatives. It is generally believed that inhibition of cyclooxygenase, and thus the formation of all prostanoids derived from arachidonic acid, is responsible for many of the beneficial activities and side effects of NSAIDs, although this concept has been

challenged.[1] Individual agents have different mechanisms for inhibition of cyclooxygenase, but their effect is always dependent on reaching the enzyme and, for that reason, the distribution and pharmacokinetic properties of the drugs are important factors determining their activity.

A review of the pharmacology, pharmacokinetics, and potential applications of aspirin in pregnancy will be presented in the chapter 3.

*Inhibition of isomerases*

The evidence implicating platelet $TXA_2$ as a major factor in the pathophysiology of occlusive vascular events has led to the development of selective inhibitors of thromboxane synthase, the isomerase catalyzing the conversion of $PGH_2$ into $TXA_2$. Several compounds (e.g. dazoxiben, dazmagrel, pirmagrel, furegrelate) have undergone clinical testing in coronary and peripheral vascular disease, as yet with disappointing results.[69] Only dazoxiben has been used in a small number of pregnant patients. On theoretical grounds it was expected that selective inhibition of $TXA_2$ synthesis would lead to an accumulation of $PGH_2$ with favorable redirection to formation of $PGI_2$ ("steal mechanism"). However, $PGH_2$ itself has biological effects similar to those of $TXA_2$, and redirection of $PGH_2$ to prostacyclin is apparently not effective enough to antagonize the functional effects of accumulating $PGH_2$.[69]

*Inhibition of thromboxane receptors*

Receptor antagonists do not interfere with eicosanoid synthesis but prevent $TXA_2$ (and $PGH_2$) from activating platelet and vascular receptors. Some of these agents (daltroban, sulotroban, and others) have undergone preliminary clinical testing in

nonpregnant individuals with variable results.[69] A new approach is the combination of $TXA_2$ synthase inhibition with $TXA_2$ receptor antagonism in one drug, such as ridogrel.[181]

*Stimulation of prostacyclin synthesis*

In the past decade mainly five pharmacologic agents have been reported to stimulate $PGI_2$ synthesis in experimental conditions in vitro: nitroglycerin, nafazatrom, dipyridamole, magnesium sulfate, and the experimental drug proadifen. These findings have not been confirmed in vivo.[18,70,172] Of these agents, dipyridamole is the only drug that has been tested in clinical trials,[70] also in pregnant women,[247] the results of which do not support its use as an antiplatelet agent.

## 2.4. Conclusions

There appears to be a sound scientific rationale for attempts to prevent and perhaps treat maladaptation disorders in pregnancy through manipulation of the eicosanoid cascade. Selective modulation of prostanoid formation can be achieved by dietary or pharmacologic interference with various steps in the synthetic cascade. Also, the effects of eicosanoids on end organ receptors can be manipulated. As yet, limited experience with dietary manipulation of prostanoid synthesis has provided no evidence of a beneficial effect on the course and outcome of pregnancy. There is a need for more fundamental research as well as for well-controlled multicenter clinical trials, not only to assess the putative benefits but also the possible side effects and risks of dietary prostanoid manipulation. With regard to pharmacologic manipulation of prostanoid synthesis in pregnancy extensive experience has accumulated with the use of acetylsalicylic acid (aspirin), which will be presented and discussed in chapter 3.

Chapter 3

# ASPIRIN IN PREGNANCY*

Selective inhibition of platelet thromboxane synthesis by an intermittent low oral dose of acetylsalicylic acid (aspirin) will suppress thromboxane dominance and may restore the physiological prostacyclin-thromboxane balance in pregnant women with circulatory maladaptation to relative dominance of the biological effects of prostacyclin. This concept forms the rationale of clinical attempts to prevention or early treatment of pregnancy-induced hypertensive disorders and fetal growth retardation in pregnant women at risk with low oral doses of aspirin. After a brief review of the history, pharmacology, and pharmacokinetics of acetylsalicylic acid, in particular as related to pregnancy, this chapter presents an assessment of the rationale, the potential benefits and possible disadvantages of the use of aspirin in pregnancy.

## 3.1. History

In 1758 an English clergyman in Chipping Norton, Edward Stone, experimented with an extract of the bark of the willow tree (Salix alba) and found it effective in reducing fever.

* *The main substance of this chapter was published in: Bremer HA, Wallenburg HCS. Aspirin in pregnancy. Fetal Mat Med Rev 1992; 4: 37-57.*

In 1763 he reported his discovery to the President of the Royal Society.[279] It took more than 60 years before an active component was isolated from the willow bark and was called salicin; salicylic acid was prepared some years later. At about the same time salicylic acid was also obtained from a distillate of the flowers of the shrub Spiraea ulmaria, the meadow sweet, and in 1860 it was prepared synthetically. Although effective in reducing fever and relieving pain, salicylic acid and its sodium salt appeared to be extremely irritating to the gastric mucosa. In 1893 Felix Hoffmann, a chemist who worked for Bayer in Elberfeld, Germany, became interested in acetylsalicylic acid, developed already 40 years earlier, as a possible alternative to sodium salicylate. Acetylsalicylic acid could be prepared quite easily by combining salicylic acid and acetic acid anhydride and it proved as effective as sodium salicylate in reducing pain and fever without causing gastric irritation. The drug was officially registered in 1899 under the name aspirin: "a" for acetyl and "spirin" derived from one of the natural sources of salicylate, the Spiraea genus of plants.[75] Aspirin has a wide spectrum of effects dependent on a number of variables, including dosage; it is effective as an antiplatelet, analgesic, antipyretic, and anti-inflammatory drug. It was only in 1971 that Vane discovered that aspirin acts by reduction of prostanoid biosynthesis through acetylation and inhibition of cyclooxygenase, a key enzyme in the arachidonic acid cascade.[249]

### 3.2. Pharmacology and pharmacokinetics

Acetylsalicylic acid covalently acetylates the active site of the enzyme cyclooxygenase, thereby irreversibly inhibiting prostanoid synthesis.

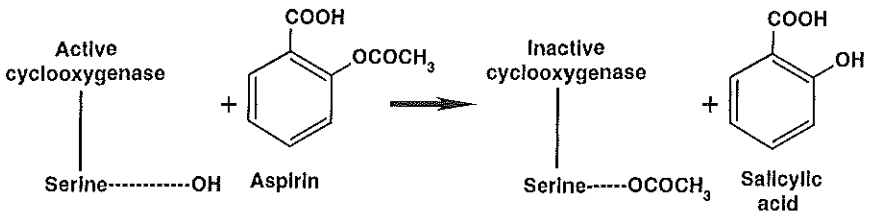


*Figure 3.1. Molecular action of aspirin on cyclooxygenase*

Cyclooxygenase catalyzes the oxygenation and peroxydation of arachidonic acid to the instable prostaglandin endoperoxides, $PGG_2$ and $PGH_2$, which are selectively converted to the primary prostaglandins, prostacyclin and thromboxane $A_2$ by the action of specific enzymes, as discussed in the previous chapter. Prostaglandins are mediators of inflammation, pain, and fever, and the therapeutic effects of aspirin are mainly due to inhibition of prostanoid biosynthesis in varying degrees in cells and organ systems throughout the body.[157] In platelets the principal product of endoperoxyde conversion is thromboxane $A_2$, an important link in the mechanism of platelet aggregation.[272] For that reason, inhibition of thromboxane $A_2$ synthesis by aspirin has a marked effect on platelet aggregation. In vitro platelet aggregation induced by arachidonic acid and other agonists such as ADP and low doses of collagen is inhibited, as is the release reaction.[72] Also adhesion of platelets to collagen under conditions of stasis or low flow is reduced. However, thrombin and high doses of collagen cause a full aggregation response despite the inhibition of thromboxane synthesis caused by aspirin.[170]

Because the reaction between aspirin and the cyclooxygenase enzyme is irreversible, the duration of the inhibitory effect is determined by the rate at which new enzyme is synthesized.[170] Platelets lack nuclei and are therefore unable to synthesize

cyclooxygenase, so that following administration of aspirin platelet aggregation remains impaired for the duration of platelet life span.[170,238] Recovery of thromboxane synthesis and platelet aggregation depends on the formation of new, uninhibited platelets from megakaryocytes. Since circulating platelets are affected on a cohort basis, clearance of irreversibly inhibited platelets depends on platelet lifespan. Indeed, return of platelet function after a single dose of aspirin has been shown to correlate with platelet turnover.[271] This explains the observation that intermittent long-term administration of low doses of aspirin, even as low as 20 mg per day, causes 95 per cent inhibition of thrombin-induced platelet thromboxane production with marked inhibitory effects on platelet aggregation in vitro.[71] In contrast, nucleated cells such as endothelium can easily replace cyclooxygenase and their capacity to synthesize prostanoids will recover rapidly after aspirin exposure.[110,170] Aspirin is easily de-acetylated to salicylic acid (salicylate), a very weak inhibitor of cyclooxygenase, which has no measurable effect on platelet aggregation at concentrations achieved in vivo.[117] It acts mainly on the lipoxygenase pathway that leads to formation of leukotrienes and a variety of other biologically active eicosanoids.[27]

Platelet action contributes to hemostasis in an intricate and dynamic interaction with vascular endothelial reactivity, intravascular flow dynamics, and the coagulation system.[262] Because of the complexity of physiological platelet behavior, the inhibitory effects of aspirin on platelet function observed ex vivo cannot be simply extrapolated to predict effects on hemostasis in vivo. For instance, although aspirin inhibits platelet adhesion to collagen in vitro, adhesion at physiological rates of shear and hematocrit levels is not affected.[245] Nevertheless, aspirin is extensively employed as an antiplatelet, antithrombotic agent in the primary and secondary prevention of arterial thrombotic

disease.[45,188,241]

Other factors that determine the systemic effects of aspirin are its absorption and de-acetylation by the liver. Aspirin is rapidly absorbed after oral administration, partly from the stomach but mainly from the upper small intestine.[104,160] The rate of absorption is determined by many factors, in particular the disintegration and dissolution rates of tablets or capsules, the pH at the mucosal surfaces, and the gastric emptying time.[104] Gastric emptying is known to be slower in pregnant than in nonpregnant women,[101] but the effect of pregnancy on aspirin absorption has not been systematically investigated. Following oral ingestion of a single dose of aspirin peak levels of salicylate in plasma occur after approximately 30 minutes.[117,272]

Elimination after a single therapeutic dose follows a biexponential curve; the half-life of the first exponent is 2-5 min for aspirin and salicylate, whereas the half-life of the second exponent is 13-19 min for aspirin and much longer (3.5-4.5 hr) for salicylate.[208] Salicylate is excreted in the urine, mainly as glycine and glucuronic acid conjugate.[104]

The liver is the main de-esterifier of aspirin; at least 80 % of acetylsalicylic acid is de-acetylated to salicylate during the first pass of blood through the liver.[208,272] The systemic effects of aspirin depend on the amount of acetylsalicylic acid that escapes the rapid hepatic de-acetylation and are therefore strongly dependent on dosage.[170,180] (Fig.3.2). This is most likely also the reason that no aspirin is secreted in breast milk at modest therapeutic doses, in contrast to salicylate.[162]

Salicylates, including acetylsalicylic acid, cross the placenta rapidly because of their high lipid solubility.[109] Fetal plasma concentrations of salicylate reach about 80-90 per cent of maternal levels, 60-90 min after administration of an intravenous dose of aspirin to the mother; fetal aspirin levels reach about one-third of maternal

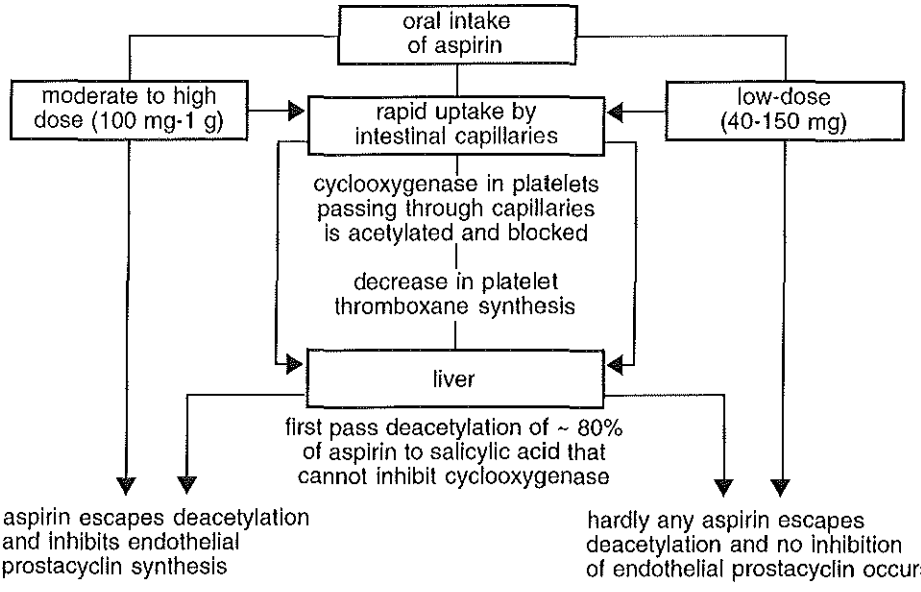


*Figure 3.2. Dose-dependent inhibition of platelet and endothelial cyclooxygenase by aspirin.*

concentrations.[109] Maternal-fetal equilibration is slow because approximately 75 per cent of maternal salicylate is bound to plasma proteins.[209] Considering the marked first-pass hepatic de-acetylation, a very low concentration of acetylated salicylic acid may be expected to reach the placenta after a low dose of aspirin taken by a pregnant woman. Elimination of salicylate from the fetus is slow because of a low capacity of the glycine and glucuronic acid pathways of conjugation.[133]

In view of the problems associated with the measurement of levels of acetylated salicylic acid, determination of thromboxane synthesis by neonatal platelets can provide an indirect but sensitive approach to asses placental transfer of aspirin. Ylikorkala et al.[286] studied the effect of maternal ingestion of a single dose of 100 or 500 mg of aspirin between 30 minutes and 10 hours before birth on thromboxane production by platelets in

umbilical blood. The 100 mg dose caused a small reduction in fetal platelet thromboxane synthesis when compared to a control group without aspirin intake, but the reduction was significantly greater with 500 mg of aspirin. No inhibition of platelet thromboxane synthesis in cord blood was observed with maternal use of a low dose of 20,[229] 37.5,[204] 60,[229] or 80[229] mg of aspirin per day during approximately two weeks before delivery. However, longterm use of a daily dose of 60 mg of aspirin throughout the second and third trimester caused a 63 per cent reduction, as compared with placebo, in platelet thromboxane production in umbilical cord blood.[14] It should be noted that neither a single maternal dose of 100 mg of aspirin during labor[286] nor a daily dose of 80 mg during two weeks before delivery[229] had a noticeable effect on fetal prostacyclin synthesis, as judged by measurement of 6-ketoprostaglandin $F_{1\alpha}$ levels in cord blood[229] or in neonatal urine.[286]

In conclusion, most of the known effects of aspirin are due to acetylation of the enzyme cyclooxygenase with inhibition of prostanoid formation. Because of the marked first-pass hepatic de-acelylation, the half-life of aspirin in the circulation is much shorter than that of salicylic acid. Peak levels of circulating aspirin and placental transfer are highly dose-dependent. The small amount of aspirin that reaches the fetus in case of longterm maternal intake of a low dose of aspirin in the order of magnitude of 60-80 mg per day causes a reduction in thromboxane synthesis by fetal platelets, but it does not affect fetal prostacyclin production.

## 3.3. Obstetric indications for the use of aspirin

*Rationale*

As outlined above, the inhibition of cyclooxygenase by aspirin is irreversible and

recovery of prostanoid synthesis depends on the ability of the cell to synthesize new enzyme. Intermittent administration of a low dose of aspirin of 60-80 mg / day or even less[170] inhibits platelet thromboxane synthesis almost completely but has little or no effect on prostacyclin formation.[14,267] The selective inhibition of platelet thromboxane synthesis by low oral doses of aspirin may be explained by the fact that platelets passing through the gut capillaries are exposed to relatively high concentrations whereas, due to the high first-pass de-acetylation by the liver, the concentration of acetylsalicylic acid in the systemic circulation remains too low to markedly affect the cyclooxygenase enzyme and prostacyclin formation in vascular endothelium.[182] In addition, Walsh has pointed out that there are approximately 1000 times as many vascular endothelial cells capable of forming prostacyclin as platelets producing thromboxane $A_2$.[272] The dose-dependent selectivity of cyclooxygenase inhibition by aspirin is not complete and some suppression of prostacyclin synthesis has been noted, even with daily doses of 60 mg.[14] However, even if vascular prostacylin synthesis is slightly reduced after ingestion of a low dose of aspirin, it will recover rapidly because of the formation of new cyclooxygenase. Alternate-day dosing has been proposed to enhance the selectivity of the inhibitory effect of low doses of aspirin on platelet thromboxane synthesis,[86] but the best pharmacologic selectivity has been obtained with the use of a controlled-release preparation which 'dribbles' aspirin into the presystemic circulation.[37]

There is growing evidence that aspirin modifies the cell-mediated immunologic response and cytokine production,[83] but as yet we do not know how this may fit into the concept of maternal maladaptation to pregnancy.

Prostanoids are also essential in the initiation and maintenance of parturition. Their release into amniotic fluid and maternal serum has been demonstrated in women in active

labor,[116] and exogenous prostaglandins $E_2$ and $F_{2\alpha}$ stimulate uterine activity.[115] For that reason, pharmacologic inhibition of the cyclooxygenase enzyme could present a rational approach to suppress unwanted myometrial activity, and aspirin has been used as a tocolytic agent in cases of threatened or active preterm labor.[193]

*Inhibition of preterm labor*

After the publication in 1973 of the study of Lewis and Schulman as mentioned in the previous chapter, that showed prolongation of pregnancy and labor in women taking aspirin during the last 6 months of pregnancy,[134] aspirin was administered in daily doses from 6 g orally[60] to 10 g intravenously[282] for tocolytic treatment in patients with threatened or active preterm labor. In one report, the investigators claim successful inhibition for more than seven days in six out of 10 cases.[282] Considering the availability of betamimetic adrenergic drugs as effective tocolytic agents and the potential hazards of high doses of aspirin (see below), there seems to be no indication for the use of aspirin in the treatment of premature labor. If the use of a cyclooxygenase inhibitor is indicated, indomethacin appears to be far more effective with less side-effects.[164,165]

*Hypertensive disorders and fetal growth retardation*

In 1979 results of a retrospective study suggested that preeclampsia occurred less frequently in regular aspirin users than in non-aspirin using pregnant women.[49] In two early anecdotal reports on treatment of established preeclampsia, relatively high doses of aspirin (1.5-1.8 g per day) were used.[85,112] Although platelet counts showed improvement, one of the two fetuses died after one week. In a later study, five preeclamptic and thrombocytopenic women received 85 mg of aspirin per day in divided doses.[84] Platelet

Table 3.1 Characteristics of randomized controlled clinical trials of low-dose aspirine to prevent pregnancy-induced hypertensive disorders.

| Reference | Inclusion criteria | Exclusion criteria | Treatment protocol | No. treated | No. controls | Placebo | Clinical end-points | Conclusions |
|---|---|---|---|---|---|---|---|---|
| Beaufils et al (12) | History of complicated pregnancy<br>Vascular risk factors | Secondary hypertension<br>Renal disease | ASA 150 mg/day plus dipyridamole 300 mg/day from 3 months until delivery | 48 | 45 | No | PIH, PE<br>BW | Significant reduction in PE and FGR with ASA |
| Wallenburg et al (265) | Normotensive primigravidae, positive A-II test at 28 weeks | History of hypertension<br>Cardiovascular disease | ASA 60 mg/day from 28 weeks gestation until delivery | 23 | 23 | Yes | PIH, PE<br>BW | Significant reduction of PE and PIH with ASA |
| Benigni et al (14) | History of complicated pregnancy<br>Chronic hypertension | Antiphospholipid antibodies | ASA 60 mg/day from 12th week until delivery | 17 | 16 | Yes | PIH, BW | Significant increase in duration of pregnancy, and fetal weight with ASA |
| Schiff et al (218) | Positive roll-over test in women at risk for pre-eclamptic toxemia | History of thrombocytopenia<br>History of coagulation disorders<br>History of heart failure<br>Chronic renal/pulmonary disease<br>Hepatic disease | ASA 100 mg/day during the third trimester | 34 | 31 | Yes | PIH, PE | Significant reduction in PIH and PE with ASA |
| McParland et al (149) | Nulliparous women with repeated abnormal wave forms on Doppler ultrasound examination | Bleeding disorders<br>Diabetes mellitus<br>Systemic lupus erythematosus | ASA 75 mg/day from 24 weeks until delivery | 48 | 52 | Yes | PIH, PE<br>BW | Significant reduction in PE and hypertension < 37 weeks with ASA |
| Uzan et al (247) | Poor obstetric history | Twin pregnancy<br>Renal disease<br>Cardiovascular disease<br>Diabetes | ASA 150 mg/day or<br>ASA 150 mg/day plus plus dipyridamole 225 mg/day, from 15-18 weeks until delivery | 156 | 73 | Yes | PIH, PE<br>BW | Significant reduction in PE and FGR with ASA |

| Reference | Inclusion criteria | Exclusion criteria | Treatment protocol | No. treated | No. controls | Placebo | Clinical end-points | Conclusions |
|---|---|---|---|---|---|---|---|---|
| Schröcksnadel et al (220) | Positive roll-over test between 28-32 weeks | Preexistent hypertension<br>Impending delivery<br>Renal disease<br>Hepatic disease<br>Heart/pumonary disease<br>Mental disease<br>Diabetes mellitus<br>Neoplastic ilness<br>Abnormal ultrasound<br>Bodyweight > 100 kg<br>Medication with anti-coagulans/analgetics<br>Acetylsalicylic allergy | ASA 80 mg/day from 28-32 weeks until 38 weeks | 22 | 19 | Yes | PIH, PE<br>BW | Significant reduction in PE and FGR with ASA |
| Italian Study (107) | Between 16 and 32 weeks and age under 18 or over 40 or<br>Mild moderate hypertension or<br>Nephropathy or<br>History of PIH/PE in previous pregnancy or<br>History of IUGR or<br>Current twin pregnancy | | ASA 50 mg/day until delivery | 583 | 523 | No | | No difference in PIH/PE, BW |
| Hauth et al (91) | Between 20-22 weeks<br>Nulliparous<br>Healthy<br>Singleton<br>Age under 28 years | Renal disease<br>Collagen vascular disease<br>Diabetes mellitus<br>Multifetal gestation<br>Chronic hypertension | ASA 60 mg/day until delivery | 302 | 302 | Yes | | Significant reduction in PE |
| Sibai et al (227) | Normotensive<br>Nulliparous<br>Between 13-26 weeks | Chronic hypertension<br>Renal disease<br>Diabetes mellitus | ASA 60 mg/day until delivery | 1570 | 1565 | Yes | PE, PIH | Significant reduction in PE |

ASA = aspirin; PIH = pregnancy-induced hypertension; PE = preeclampsia; BW = birthweigt; FGR = fetal growth retardation

counts improved but no improvement in fetal condition was observed, and two fetuses died.

Until 1993 ten randomized, controlled clinical trials were executed and reported using low-dose aspirin for the prevention of pregnancy-induced hypertensive disease.[12,14,91,107,149,218,220,227,247,265] (Table 3.1). All patients entered were judged to be either at high risk based on obstetric history[12,14,247] or tests and observations in the present pregnancy,[107,149,218,220,265] or at moderate risk.[91,227] The dose of aspirin used varied from 50 mg[107] to 150 mg[12,247] per day and aspirin was combined with dipyridamole in two studies.[12,247] In seven trials aspirin was prescribed during the second and the third trimester of pregnancy,[12,14,91,107,149,227,247] whereas it was used during the third trimester only in the remaining three trials.[218,220,265] All studies, except two,[12,265] were placebo-controlled. Because the study designs are comparable and criteria and end points are well defined, systematic pooling of the results across trials by means of meta-analysis[34] provides more precise estimates of the effects of treatment.[103] Differences for the distinctive items, in treated and non-treated groups, were accumulated and expressed as "odds ratios" with 95% confidence intervals. The results, covering 2803 treated and 2649 untreated patients, are presented graphically in figure 3.3. The 95% confidence intervals for pregnancy-induced hypertension and perinatal mortality cross the line representing an odds ratio equal to 1. This means that only proteinuric hypertension (preeclampsia) and fetal growth retardation occur significantly less often in aspirin-treated pregnant women than in untreated controls. The reported data do not allow to distinguish between perinatal deaths related or unrelated to pregnancy-induced hypertensive disorders. A further analysis of the data shows that aspirin-treated women have an even chance of cesarian section. A striking observation in these controlled studies is the reduction in the incidence

of uteroplacental circulatory insufficiency with fetal growth retardation and a birthweight below the 10th centile in high risk women receiving low-dose aspirin; this finding reaches significance in the meta-analysis.

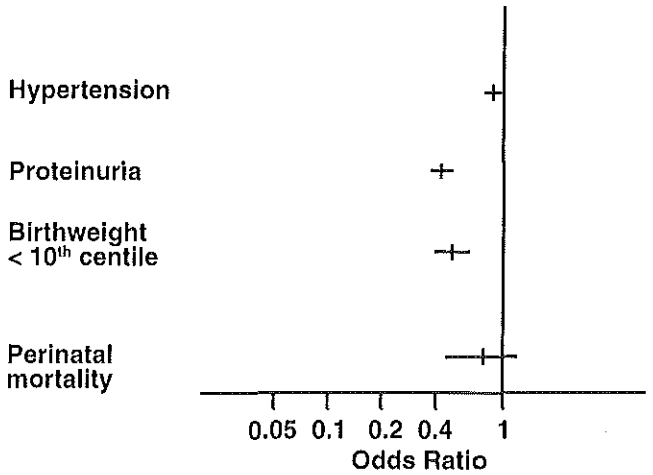


*Figure 3.3. Graphic overview of odds ratios and 95% confidence intervals of effects of low-dose aspirin on the occurrence of hypertension, proteinuric hypertension (preeclampsia), and fetal-neonatal sequelae from 10 randomized controlled clinical trials (see Table 3.1).*

The positive effect of a low dose of aspirin on birthweight corrected for gestational age was also observed in a randomized placebo-controlled, double-blind study in a small group of patients with a clinical diagnosis of fetal growth retardation and an abnormal Doppler-flow pattern in the umbilical arteries.[243] Results of non-randomized, controlled studies in patients with a history of repeated idiopathic severe fetal growth retardation,[267,269] fetal death, lupus anticoagulant, anticardiolipin antibodies or systemic lupus erythematosus[65] support these findings (Table 3.2). On the other hand, prophylactic treatment with a low dose of aspirin appeared to have no effect on fetal growth in a small number of uncomplicated twin pregnancies.[242] In contrast with the prophylactic studies discussed above, the potential benefits of treatment of established circulatory

maladaptation in pregnancy with a low dose of aspirin have hardly been investigated. Pregnancy-induced hypertension often occurs early in the third trimester, and if low dose aspirin treatment would result in even a moderate delay in the progression of the disease, this could have a significant impact on neonatal morbidity and mortality.[255] Unfortunately, results of a recent randomized, double-blind trial in 47 patients with mild pregnancy-induced hypertension using a daily dose of 100 mg of aspirin or placebo did not indicate any improvement in the hypertensive condition or delay in its progression to preeclampsia.[217] There is certainly a need for further prospective trials on the therapeutic use of low dose aspirin in patients with clinical signs of a pregnancy-induced hypertensive disorder or fetal growth retardation.

The evidence that low-dose aspirin restores a disturbed prostacyclin-thromboxane balance in pregnancy to the dominance of the vascular effects of prostacyclin by means of selective inhibition of platelet $TXA_2$ synthesis is supported by the results of three studies, showing that a daily dose of 60-80mg of aspirin for between one and four weeks significantly increases vascular refractoriness to the vasopressor effectsof angiotensin II in pregnant women with an elevated[233,264] or physiological[213] angiotensin sensitivity. In contrast, oral ingestion of 650mg of aspirin twice with an interval of six hours between doses was shown to lead to a marked increase in angiotensin sensitivity,[67] because in this dose aspirin inhibits the biosynthesis not only of $TXA_2$, but also of prostacyclin.

In all clinical trials published no adverse effects attributable to low-dose aspirin were noted, in particular no clinically significant effects on maternal or fetal hemostasis, or on the fetal ductus arteriosus, although one study[227] reported a slightly increased risk of abruptio placentae in aspirin-using pregnant women.

Table 3.2 Characteristics of clinical trials of low-dose aspirine to prevent fetal growth retardation.

| Reference | Inclusion criteria | Exclusion criteria | Treatment protocol | No. treated | No. controls | Randomized | Placebo | End-points | Conclusions |
|---|---|---|---|---|---|---|---|---|---|
| Wallenburg and Rotmans (267) | History of at least previous pregnancies with severe idiopathic FGR | | 1-1.6 mg/kg/day of ASA and 225 mg of dipyridamole daily from 16 weeks until delivery | 24 | 24 | No | No | BW | Reduction in incidence of FGR |
| Elder et al (65) | History of severe FGR and/or hypertension SLE | | ASA 75 mg/day from the first or second trimester until until delivery | 42 | - | No | No | PE, BW | 'Striking' improvement of pregnancy outcome |
| Trudinger et al (243) | Abnormal umbilical artery wave forms on Doppler ultrasound gestational age between 28 and 36 weeks | Severe hypertension | ASA 150 mg/day from 28-36 weeks until delivery | 22 | 24 | Yes | Yes | BW | Significant increase in BW and head circumference |
| Trudinger et al (242) | Uncomplicated twin pregnancy at 28-30 | | ASA 100 mg/day until delivery | 15 | 12 | Yes | Yes | BW | No effect of ASA on BW |

FGR = fetal growth retardation; ASA = aspirine; BW = birthweight, SLE = systemic lupus erythematosus; PIH = pregnancy-induced hypertension

The results of the meta-analysis of published trials indicate that prophylactic treatment with a low dose of aspirin to prevent preeclampsia and fetal growth retardation is effective and safe in women at risk. It should be emphasized that the patients in most of the clinical trials presented above were selected on the basis of a high risk, although some studies contained patients at small or moderate risk.[91,227] A meta-analysis of the results obtained in high-risk pregancies showed more pronounced results.[103]

*Antiphospholipid antibodies*

Lupus anticoagulant and anticardiolipin antibodies to negatively charged phospholipids have been identified in patients with systemic lupus erythematosus and other autoimmmune disorders, and also in patients without apparent disease.[222] There is a strong association between the presence of anti-phospholipid antibodies and an increased risk of spontaneous abortion, stillbirth, fetal growth retardation, preterm birth, and arterial and venous thrombosis.[21,27,59,200]

The lupus anticoagulant and other antiphospholipid antibodies interfere with prostacyclin synthesis by endothelial cells, leading to a relative dominance of platelet thromboxane action,[31] and placental thrombosis and infarction.[26]

Lubbe et al. reported successful pregnancy outcome in 80% of 25 patients after treatment with 40-60 mg prednisone and 75 mg aspirin daily.[138,139] Also other centers have reported good results with this combination,[68,167] but some have not.[21] As yet no controlled studies are available to reliably assess the prophylactic and therapeutic effects of low-dose aspirin in pregnant patients with antiphospholipid antibodies.

## 3.4. Possible side-effects of the use of aspirin in pregnancy

*General*

The general side-effects that can be caused by ingestion of aspirin and other salicylates may also occur in pregnant women. Adverse effects such as gastric irritation, nausea, diarrhea, constipation, bronchospasm, effects on renal function, skin rashes, and angio-edema are usually - but not always - associated with a high aspirin intake or with aspirin hypersensitivity. Aspirin may impair the effect of drugs which act by stimulating the synthesis of arachidonic acid metabolites.[52] Since they are not specific for pregnancy, these potential side-effects will not be reviewed.

The effects of aspirin on hemostasis are not characteristic for pregnancy, they will be discussed in some more detail because of their important obstetric consequences. Pregnancy-specific complications of the use of aspirin may be due to unwanted effects of inhibition of prostanoid synthesis, such as prolongation of pregnancy and labor, and to transfer of the drug across the placenta leading to immediate or delayed disturbances in embryonic or fetal development and functions.[58]

*Effects on maternal hemostasis*

The clinical test used most often to asses effects on hemostasis in vivo, determination of the skin bleeding time, has shown disparate results in aspirin users. Several authors have reported an increase in bleeding time in healthy nonpregnant volunteers after a single dose of 0.65-1.5 g of aspirin,[194,278] but Mielke[150] demonstrated that aspirin increased template bleeding time only if venous congestion was induced and a horizontal incision was made. Frith and Warlow[77] analyzed 70 patients who had taken aspirin for prophylaxis of

thromboembolism for 35 months and could not demonstrate a significant difference in bleeding time between aspirin takers and controls. A daily low dose of 60 mg of aspirin during four months was shown to reduce platelet thromboxane generation to 10% of control values in a small group of pregnant women, but no significant prolongation of bleeding time was observed.[14] Determination of the bleeding time is probably not a reliable test to use as a predictor of clinically significant bleeding in individual patients.[206] The effects of aspirin on hemostasis in various clinical conditions must be determined in properly designed clinical trials.

Large studies on cardiovascular prophylaxis involving long-term use of aspirin in doses between 75 and 500 mg per day have not shown any relevant increase in bleeding tendency.[100,188,235,241] On the other hand, a recent report suggests a significant increase in blood transfusion requirements and in the need for hemostatic products after coronary artery bypass grafting in 101 patients using long-term prophylaxis with a daily dose of 75-300 mg aspirin compared to 101 control patients not taking aspirin.[240] A daily dose of 75 mg of aspirin taken during 12 days was shown to cause an increase in gastric mucosal bleeding in healthy volunteers.[192] In a recent report Baker et al.[8] describe two cases of possible low-dose aspirin-induced gastropathy, one after 6 weeks of 75 mg aspirin daily in a pregnant woman with pregnancy-induced hypertension and one in a healthy woman taking 60 mg aspirin daily for 10 days who participated in a study of cyclooxygenase-dependent platelet reactivity. Only few studies have specifically assessed the risk of maternal bleeding associated with the use of aspirin in pregnancy and peripartum. Lewis and Schulman[134] carried out a retrospective survey of 103 patients who took a daily dose of 3.25 g of aspirin or more during at least the last 6 months of pregnancy. Aspirin users had an average estimated blood loss at delivery of 340 ml, about 100 ml more than in

control groups not taking aspirin. Postpartum hemorrhage of 1000 ml or more occurred once in patients receiving aspirin and not in the control groups.

It is the Australian study by Collins and Turner[43] that is most often cited to emphasize the bleeding risk associated with the regular consumption of aspirin and salicylate in pregnancy. During the 28 months of the study, 144 regular users of "analgesic powders" containing aspirin and salicylate combined with other drugs were identified by testing urine specimens. The user group was divided into 63 constant takers with a reported consumption of 2 to 12 powders per day, and 81 intermittent takers using an aspirin-containing drug at least once a week throughout pregnancy. The rates of anemia (hemoglobin of 10.5 g% or less), antepartum and postpartum hemorrhage, and blood transfusion at delivery were significantly increased in the group of constant takers compared with controls matched for age and parity. Except for the incidence of anemia, the occurrence of these complications was also elevated in intermittent takers, although the difference with the control group was not significant. This study can be criticized on two main points. First, as part of an abusive habit the women not only used high doses of aspirin and salicylates, but also unknown amounts of "other drugs" which may have affected pregnancy outcome. Second, aspirin takers were clearly identified to the attendant obstetricians, which introduces an important potential observer bias, in particular because the amount of antepartum and postpartum hemorrhage appears to have been based on estimate rather than accurate measurement of blood loss.

In 1982 Stuart et al.[237] reported a prospective case-control study in which they evaluated the effects of ingestion of aspirin within 10 days of delivery on maternal and neonatal hemostasis. In the small subgroups of women who took 5 to 10 g of aspirin within five days of delivery (n=10) or immediately postpartum (n=7) they found slightly

lower postpartum hemoglobin levels. In several controlled studies involving pregnant women taking high doses of aspirin in the peripartum period for tocolysis[111,282] or antithrombotic prophylaxis[169] no major bleeding problems were observed during or after delivery. Recently, the possible association between maternal bleeding at delivery and aspirin exposure in the last 10 days of pregnancy has been assessed in a large set of data derived from the Collaborative Perinatal Project.[25] In 2269 women who took aspirin the estimated blood loss at delivery was slightly less than that in 7606 women not exposed to aspirin. There has been no report of increased maternal bleeding tendency in the low dose aspirin studies in pregnancy carried out so far.

A recent editorial[141] discusses the potential risk of epidural hematoma following epidural anesthesia in patients taking aspirin. It supports the earlier recommendation by O'Sullivan[178] that women taking aspirin during pregnancy should stop their medication 7-10 days before delivery and have a bleeding time performed before epidural block is undertaken. Certainly with regard to the use of low dose aspirin this advice appears to be based on theoretical considerations, rather than on reliable data with regard to the risks of aspirin use with extradural anesthesia. As pointed out earlier, the bleeding time test is a poor predictor of clinically significant bleeding.[206]

In conclusion, even a low dose of aspirin affects platelet function but the available clinical evidence does not support the theoretical increase in risk of bleeding in pregnancy, peripartum, or in the puerperium in women taking aspirin in low or analgesic doses.

*Prolongation of pregnancy and labor*

Following the retrospective study by Lewis and Schulman[134] showing a striking

increase in the mean length of gestation and labor in women taking high doses of aspirin during the last 6 months of pregnancy (see chapter 2.3) Waltman et al.[273] demonstrated that 650 mg of aspirin, taken every six hours for ten doses, significantly prolonged the mean instillation-abortion interval in patients undergoing mid-trimester saline-induced termination of pregnancy. A prolongation of gestation in women taking aspirin was also reported by other authors, but in these studies no correction was made for induction of labor.[43,237] On the other hand, intravenous administration of 1 g of aspirin as an analgesic during labor apparently did not cause clinically relevant prolongation of the first stage of labor.[20,154,252]

The few studies available suggest that therapeutic doses of aspirin taken in the last part of pregnancy and before delivery could be associated with some prolongation of pregnancy and labor. Not only the dose of aspirin but also the interval between the last dose and the beginning of labor will be important because, as discussed earlier, prostaglandin synthesis in myometrial cells and decidua may be expected to recover rapidly.

*Teratogenic effects*

Teratogenic effects attributed to salicylates were first reported in the late fifties by Warkany and Takacs,[276] who found that injection of extremely high doses of salicylates in pregnant rats resulted in a variety of fetal malformations, in particular craniorachischisis. Their findings were confirmed in many other studies in rodents. In all animal experiments the dose of aspirin required to achieve even a small proportion of malformed offspring was close to that causing embryonic or maternal death; the extrapolated dose in the human would have to be in the order of magnitude of 30 g per day. Warkany, speaking at

the Ciba Symposium on Congenital Malformations in 1960, emphasized how cautious one should be in applying the results of laboratory experiments to the problem of human malformations, and added: " If we are not careful we may hear very soon that aspirin causes malformations."[5] That was exactly what happened, based on the results of four large case-control studies and some case histories.

Richards[202] examined the drug histories of 833 women with infants with congenital malformations, and compared them with those of matched controls with healthy children. The reported use of aspirin in the study group was 22% as compared with 14% in controls. However, the only defect with a significantly higher occurrence in aspirin users was talipes. In a retrospective study with a similar design Nelson and Forfar[163] also found a small but significant excess of aspirin taking in mothers with malformed infants as compared with control women who delivered children without malformations. In another case-control study Saxen[215] found that 14.9 % of 599 mothers with children with oral clefts had taken salicylates during the first trimester, which was nearly three times more than the 5.6 % reported by control mothers. The results of a retrospective study involving 300 mothers of children with congenital heart disease suggest a small excess of aspirin-taking as compared to controls; however, the difference is only statistically significant if aortic stenosis, coarctation of the aorta, and hypoplastic left heart syndrome are considered together.[183] We found five case reports on a possible association of aspirin taking with congenital malformations in which 12 infants are described with various malformations such as cyclopia, phocomelia, nephro-blastomatosis and others.[2,13,19,148,216]

All these studies indicating a small increase in the risk of teratogenicity associated with aspirin taking in pregnancy were designed as case-control studies "after the fact": data on aspirin exposure were collected after the birth of the (malformed) baby. Such a study

design is known to be liable to a great number of potential biases. Important confounders are drug recall, which may be more complete in women with an abnormal infant than in control women with healthy babies, and observer bias because the interviewers are aware of the outcome of pregnancy.[92] This bias was avoided in the large cohort study by Slone et al.[231] based on data from the Collaborative Perinatal Project. In this project all data concerning drug exposure were collected before birth. After controlling for a wide range of potential confounding factors no differences were demonstrated between the occurrence of congenital malformations in children of 5,128 heavily exposed mothers who took aspirin at least eight times in any of the first four months of pregnancy, in 9,736 children of mothers who took aspirin less frequently, and in 35,418 children whose mothers were not exposed to aspirin during the first four months of pregnancy. No single malformation occurred more frequently in the aspirin groups than in women who had not used aspirin. For example, the relative risk of cardiovascular malformations in the heavily exposed group was 0.75, that of central nervous abnormalities 0.94. The large numbers involved make this a very powerful study. Another large study analyzed prescriptions issued to approximately 10,000 women by general practitioners between 8 weeks before and 20 weeks after conception.[51] The investigators noted a 6 % excess of general prescriptions issued to women who were delivered of a congenitally malformed baby compared to the rest of the population. However, fewer prescriptions for aspirin containing medications than expected were issued to women with a malformed baby, although the difference was not statistically significant. Although additional drug taking without prescription was recorded, the use of "over-the-counter" aspirin may well have been underreported. Turner and Collins[246] reported major congenital abnormalities in 6 of 144 infants of mothers who regularly took several grams of salicylates during pregnancy. This rate did not differ

significantly from that found in a group of matched controls not exposed to aspirin. The possible association between aspirin taking in pregnancy and cardiac abnormalities was recently assessed by Werler et al.[277] The use of aspirin in the first trimester of pregnancy reported by mothers of 1,381 affected children was compared with that among the mothers of a control group of 6,966 infants with other malformations. No excess of aspirin taking was apparent in the group of mothers with offspring with cardiac malformations.

We conclude that evidence obtained in large prospective studies, in particular the Collaborative Perinatal Project, indicating that aspirin in therapeutic doses is not teratogenic outweighs data from retrospective case-control studies, case reports and animal studies suggesting that the use of extremely high doses of aspirin in pregnancy may be associated with a (small) increase in the relative risk of various fetal malformations.

*Effects on fetal and neonatal hemostasis*

The hemostatic mechanisms of the newborn are immature; it takes six to nine months before they have become comparable to those in adult life.[244] In the healthy neonate this does not seem to cause clinical problems,[80] but in the case of transplacental exposure to maternal aspirin the risk of bleeding could be increased, in particular in the ill or premature infant. The first report relating maternal aspirin ingestion and neonatal bleeding was published in 1948[108] and described a case of fatal neonatal cerebral hemorrhage after a maternal suicide attempt with 200 g of aspirin. Two later anecdotal reports[89,90] also relate an excessively high maternal aspirin intake to severe neonatal hemorrhage. In 1970 Bleyer and Breckenridge[17] compared the occurrence of neonatal bleeding in 14 infants

whose mothers took more than 300 mg of aspirin in the week before delivery and in 17 newborns who were not exposed to aspirin in any form during three weeks before birth. In the exposed group three of 14 newborns had minor bleeding problems compared with only one baby in the control group. This is a well-designed study because drug histories were taken before delivery and the neonates were assessed without knowledge of maternal aspirin taking, but the numbers are too small to draw firm conclusions. A larger prospective study was reported by Rumack et al. in 1981.[210] They investigated 108 infants born at or before 34 weeks' gestation or weighing 1500 g or less. The mothers' drug history was taken on the second day after delivery and the mothers of 17 infants appeared to have taken one or more aspirin tablets within one week of delivery; 20 infants had been exposed to acetaminophen; and 71 infants had no antenatal exposure to aspirin or acetaminophen. Between 3-7 days after birth all infants underwent brain scanning. Twelve of the newborns in the aspirin group (71%) showed evidence of cerebral hemorrhage, compared with 50% in the acetaminophen and 44% in the control group. The authors conclude that aspirin taken late in pregnancy causes a significant increase in the risk of intracranial hemorrhage in the newborn and they state that "...the use of aspirin during the last three months of pregnancy is highly questionable and probably inappropriate." The conclusions of this study have been challenged and defended.[46,232] An important point of criticism was that differences were only statistically significant at the 5 per cent level when a one-tailed Fisher test was used, and not when the correct two-tailed test was applied.[232] On the other hand, Corby[46] defended the study in an Editorial comment, stating that the interest of the clinical investigator is not equal to that of the statistician. The results of the study by Rumack et al. were supported by Stuart et al.,[237] who reported neonatal bleeding consisting of petechiae and microscopic hematuria in nine of 10 infants

whose mothers used 5-10 g of aspirin within five days of delivery; also these authors warn against taking aspirin in pregnancy and at delivery. In contrast, several other studies have not shown an association between high maternal aspirin ingestion and neonatal bleeding.[111,169,282] Although longterm maternal use of a low dose of aspirin has been shown to inhibit fetal platelet thromboxane synthesis,[14] no neonatal bleeding was noted in any of the low dose aspirin studies reported.

The most powerful study on this issue so far is that by Brent et al.[25] based on a large data base from the Collaborative Perinatal Project. Term and preterm babies were assessed separately and no differences were found between infants of mothers who had taken aspirin within 10 days of delivery and infants not exposed to aspirin with regard to the incidence of cerebral hemorrhage, neonatal hemoglobin and hematocrit. This study has the statistical power to have an 80 per cent chance of showing an increase in the rate of cerebral hemorrhage from the background 0.2 to 0.6 per cent, a 1 ml blood loss, and a 0.4 per cent fall in hematocrit.[58]

In conclusion, results obtained in one large study in women using analgesic doses of aspirin[25] and clinical experience in trials of low dose aspirin do not support the view that maternal aspirin taking is associated with an increase in fetal or neonatal bleeding risk, except perhaps in case of intoxicating doses.

*Effects on the ductus arteriosus and pulmonary circulation*

The physiology of the regulation and modulation of the patency of the ductus arteriosus during fetal and neonatal life was recently reviewed.[250] Patency of the ductus appears to be maintained by the relaxing effects of prostaglandin $E_2$ and prostacyclin produced in the ductal wall. In the newborn contraction of smooth muscle in the wall of

the ductus due to the combined effects of increasing oxygen tension and decreasing prostanoid synthesis results in functional closure between 10-96 hours after birth. The actual constrictor mechanisms remain to be defined.[39] Prostanoids are also involved in the vascular dilation that takes place in the neonatal lung immediately after birth to allow an increase in blood flow to the expanding lungs at a low perfusion pressure.[32,33,94]

The recognition of the important role of eicosanoid action in keeping the ductus open in fetal life has led to the use of the cyclooxygenase inhibitor indomethacin as an effective pharmacologic tool in the treatment of patent ductus arteriosus in the, usually premature, neonate.[79] For the same reason, fetal cyclooxygenase inhibiton may be expected to carry a risk of premature constriction or perhaps even closure of the ductus, with diversion of right ventricular outflow into the pulmonary vascular bed. Increased pulmonary blood flow may result in pulmonary arterial hypertrophy.[127,129] In addition, cyclooxygenase inhibition at birth could interfere with pulmonary vasodilation and lead to pulmonary hypertension and right heart failure. Indeed, such effects have been shown to occur after high doses of aspirin and indomethacin in experimental animals[95,128,225] and, although rarely, in pregnant women treated with indomethacin for tocolysis.[130,250] High fetal levels of acetylsalicylic acid are needed to produce measurable effects on the fetal circulation. For instance, in their studies in chronically catheterized fetal lambs Heymann and Rudolph had to administer 50-90 mg of aspirin per kg of fetal weight directly into the fetal stomach to obtain reversible constriction of the ductus arteriosus.[95] It is unlikely that such levels can be reached in human pregnancy, even with maternal intake of toxic amounts of aspirin.

Only three reports are available, involving nine infants, to examine the clinical evidence of an association between the use of aspirin in pregnancy and effects on the fetal

ductus and pulmonary circulation. Arcilla et al.[7] describe a baby with severe neonatal cardiorespiratory distress born at 37 weeks' gestation from a mother treated for two weeks with "salicylates" and penicillin for acute polyarthritis; the dose of salicylate is not stated. Hemodynamic studies at four hours of age showed a ductus arteriosus which ended blindly at its aortic end. The authors conclude that this represents a case of premature closure of the fetal ductus. Levin et al.[128] report autopsy findings in an infant whose mother took 1.2-1.6 g of aspirin per day throughout pregnancy. The baby showed all the features of premature closure of the ductus as well as hypertrophy of the pulmonary artery and tricuspid insufficiency. Finally, Perkin et al.[184] relate maternal use of aspirin in pregnancy to persistent pulmonary hypertension in seven newborns with high salicylate levels and no ductus shunt.

Larger clinical studies in women using therapeutic doses of aspirin have not revealed any evidence of premature constriction or closure of the fetal ductus, although this complication was usually not specifically examined.[42,169] The observation that maternal use of aspirin in the last two weeks of pregnancy is associated with a reduction in the incidence of patent ductus arteriosus in small preterm babies[53] cannot be used to support a putative increase in the risk of closure of the fetal ductus in utero. Doppler echocardiography has not shown any evidence of constriction of the ductus arteriosus in 30 women receiving a low dose of aspirin in the last 2-3 weeks of pregnancy.[229] A recent study on daily maternal low-dose aspirin intake did not show an effect on central or regional circulation in the fetus.[251] No neonatal cardiovascular or pulmonary complications were noted in the low-dose aspirin studies reported so far. Wallenburg et al.[265] specifically mention that autopsy findings in a stillborn baby from a mother taking a daily dose of 60 mg of aspirin from 28 to 41 weeks did not show any abnormalities of the

ductus or the pulmonary vasculature.

In conclusion, although high doses of aspirin can cause constriction of the ductus arteriosus and cause pulmonary hypertension in the fetal lamb, clinical evidence does not support the occurrence of this complication in human pregnancy. If some degree of constriction of the fetal ductus would occur, it could well be reversible after birth following discontinuation of cyclooxygenase inhibition.[82,155] All this makes it extremely unlikely that premature closure of the fetal ductus arteriosus is a risk associated with the use of the low doses of aspirin recommended for the prevention of gestational hypertensive disorders and fetal growth retardation.

*Miscellaneous*

Drugs taken by the pregnant woman and transferred across the placenta could have delayed effects on development and function later in life. Learning impairment in the offspring of rats treated with salicylate was reported by Butcher et al.[30] They state that aspirin may act as a "behavioral teratogen" by inhibiting learning in the offspring at maternal doses too low to produce anatomical malformations. In another study in rats, Voorhees et al.[258] observed developmental delays in offspring produced by a single high dose of aspirin on day 11 of gestation.

In a prospective follow-up study by Streissguth et al.[236] of 1,529 women exposed to drugs in pregnancy, aspirin and acetaminophen were the medications most frequently taken. A cohort of 421 of their children was examined at four years of age and a tenpoint decrement in intelligence quotient (IQ) was observed in children with antenatal aspirin, but not acetaminophen, exposure; girls were more affected than boys. The authors emphasize that the study was not undertaken to examine the possible adverse effects of

aspirin or acetaminophen exposure in utero; the observed relationship was a post hoc finding. In a study that was designed to assess the relationship between maternal aspirin intake during the first 20 weeks of pregnancy and the child's IQ at four years of age, Klebanoff and Berendes[119] examined data relating to a prospective multicenter study on pregnancy and child development as part of the Collaborative Perinatal Project. In these 19,226 pregnancies, 10,159 women were exposed to aspirin during at least the first 20 weeks of pregnancy; the control group consisted of 9,067 women who did not report any use of aspirin at the initial antenatal visit. The mean IQ at four years of age in children exposed to aspirin was 2.1 points higher than that in unexposed children. Although this difference is statistically highly significant, it seems practically irrelevant and the authors emphasize that the results should not be interpreted as claiming that antenatal aspirin exposure has a beneficial effect on child development. However, this prospective and powerful study makes an adverse effect of aspirin exposure on IQ unlikely.

In 1963 Reye et al.[201] described the clinical and pathological features of a group of 21 children with a disease that has become known as Reye's syndrome: acute encephalopathy with fatty degeneration of the viscera. Exogenous toxin exposure was suggested as a likely cause of the syndrome.[239] Epidemiologic evidence has linked the disease to the use of aspirin.[99,168] Although there are two case-reports of possible Reye's syndrome in neonates,[88,179] no association has ever been described between maternal aspirin usage and the syndrome in the newborn.

The occurrence of retrolental fibroplasia has been reported in a neonate whose mother used unspecified doses of aspirin throughout pregnancy; no other cases have been published since.[219]

## 3.5 Conclusions

Aspirin has been around for almost a century and even today it appears to be frequently taken in pregnancy as a mild analgesic, usually without a prescription. Although the use of over-the-counter drugs should be discouraged, in particular in pregnancy, a large body of observational data has provided no scientific evidence that therapeutic doses of aspirin are associated with teratogenicity, increased maternal or fetal bleeding tendency, premature closure of the ductus arteriosus, or any other complication of pregnancy.

Meta-analysis of the results of 10 controlled trials involving over 5400 pregnant women with various risk factors indicates that selective inhibition of platelet thromboxane synthesis with a low daily dose of aspirin in the second and third trimester may significantly reduce the incidence of proteinuric preeclampsia and low birthweight but not pf nonproteinuric gestational hypertension. It should be realized that six of the 10 trials on which this evidence is based comprised fewer than 200 women; that the risk varied between low and very high; that the doses of aspirin used varied from 50 to 150 mg per day, and that prophylactic treatment was started at any time between early in the second and early in the third trimester (Table 3.1). The occurrence of publication bias favoring positive results of low-dose aspirin prophylaxis cannot be excluded.

No reliable data are yet available with regard to the prophylactic effects of low dose aspirin in pregnant women with antiphospholipid antibodies. Also the potential benefits of therapeutic low dose aspirin in women with established pregnancy-induced hypertensive disease or fetal growth retardation remain to be investigated. Although there are no apparent maternal and fetal hazards associated with the use of low dose aspirin in pregnancy, we feel that low dose aspirin prophylaxis should not be adopted into routine

obstetric practice until more reliable risk-benefit ratios have been determined in a large randomized, placebo-controlled clinical trial.[268]

Chapter 4

# LOW-DOSE ASPIRIN IN PREGNANCY : CHANGES IN PATTERNS OF PRESCRIPTION IN THE NETHERLANDS*

## 4.1. Introduction

Aspirin, acetylsalicylic acid, is one of the most frequently consumed drugs, also in pregnancy. It is usually taken without a prescription as an over-the-counter drug for minor ailments. Until recently many gynecologists warned against the use of aspirin in pregnancy, based on a general tendency to advice against all drug taking in pregnancy as well as on fears of specific complications of aspirin, such as teratogenic effects, maternal and fetal hemorrhage, and premature closure of the fetal ductus arteriosus.[24] This attitude may be changing following the publication of reports that a daily low-dose of aspirin may prevent the occurrence of preeclampsia and fetal growth retardation in women at risk, and could reduce the severity of these complications once they have developed.[24,103] In order to assess the effect of these reports on patterns of prescription of low-dose aspirin for preventive and therapeutic use in high-risk pregnancies an anonymous written inquiry was held among practicing gynecologists in the Netherlands in 1989 and repeated in 1991. The results of both inquiries were analyzed and compared.

* *The main substance of this chapter was published in: Bremer HA, Wallenburg HCS. Low-dose aspirin in pregnancy: changes in patterns of prescription in The Netherlands. Eur J Obstet Gynecol Reprod Biol 1993; 52: 29-33.*

## 4.2 Material and Methods

In April 1989 a questionnaire was sent to each of the 38 university and university-affiliated hospitals in the Netherlands with a postgraduate training program in obstetrics and gynecology, and to the 122 hospitals with an obstetric unit but without a postgraduate training program. These hospitals represent 275 and 344 gynecologists, respectively. In May 1991 an identical questionnaire was sent to the then functioning 28 training hospitals (240 gynaecologists) and 120 non-training hospitals with an obstetric unit (378 gynecologists). The inquiry was anonymous. A reminder was sent to all hospitals three weeks after the initial mailing. The questionnaire differentiated between prophylactic prescription of a low-dose of aspirin to prevent pregnancy-induced hypertension (PIH), proteinuric preeclampsia (PE), and fetal growth retardation (FGR), and therapeutic prescription with the intention to treat these complications. Other questions concerned the number of pregnant women that actually had been prescribed low-dose aspirin in the previous four months, indications for prescription, type and dose of aspirin, and the duration of aspirin taking during pregnancy. General questions referred to the number of gynecologists practicing in the obstetric department, to whether or not the reply was given on behalf of other gynecologists in the department, and to the annual number of deliveries.

## 4.3 Results

The response rate to both inquiries was similar; 83 forms (52%) were returned in 1989, and 86 (58%)in 1991. Considering hospitals with postgraduate training in obstetrics and gynecology, and those without a training program, responses were 74% and 43%, respectively, in 1989 and 64% and 57% in 1991. With few exceptions the responding

departments stated that they replied on behalf of all gynecologists practicing in that department. Converting the response rates into numbers of gynecologists, the inquiry comprised 377 (61%) of the practicing 619 gynecologists in the Netherlands in 1989 (training 69%, non-training 54%) and an almost equal number (383 or 62%) in 1991 (60% and 63%, respectively).

In 1989 53% of the respondents had prescribed low-dose aspirin for prevention of PIH-PE and FGR in the previous four months (training 50%, non-training 56%). In 1991 these figures had increased to 79% of the respondents in the same period of time (training 73%, non-training 83%). In 1989 25% of the responding gynecologists had prescribed low-dose aspirin with therapeutic intentions in the previous four months (training 26%, non-training 24%); in 1991 the figures rose to 48% (50% and 45%) in the same period of time. The main indications for prophylactic prescription of low-dose aspirin were a history of PIH-PE in one or more previous pregnancies, FGR in one or more previous pregnancies, or an increased risk of developing PIH-PE in the presence of chronic hypertension, lupus anticoagulant, or systemic lupus erythematosus. In table 4.1 these indications are compared for 1989 and 1991. The differences between 1989 and 1991 are small with regard to the main indications for which Dutch gynecologists prescribed low-dose aspirin in pregnancy, but in 1991 gynecologists involved in postgraduate training show a distinct trend towards prescribing low-dose aspirin for prophylactic purposes in patients with a lower risk as judged from their obstetric history.

Table 4.1. Percentage of respondents in postgraduate training and non-training hospitals in the Netherlands in 1989 and 1991 accepting indications to prescribe low-dose aspirin in pregnancy for prophylaxis of pregnancy-induced hypertension, preeclampsia, and fetal growth retardation.

| Indications | Training hospitals | | Nontraining hospitals | | Total | |
|---|---|---|---|---|---|---|
| | 1989 (n=96) | 1991 (n=106) | 1989 (n=105) | 1991 (n=199) | 1989 (n=201) | 1991 (n=305) |
| History of PIH-PE | 65% | 70% | 64% | 55% | 64% | 63% |
| in 1 pregnancy | 14% | 35% | 26% | 28% | 20% | 32% |
| in ≥ 2 pregnancies | 51% | 35% | 38% | 27% | 44% | 31% |
| History of FGR | 94% | 87% | 99% | 94% | 97% | 91% |
| in 1 pregnancy | 53% | 56% | 68% | 66% | 56% | 61% |
| in ≥ 2 pregnancies | 41% | 31% | 31% | 28% | 41% | 30% |
| History of essential hypertension, renal hypertension SLE, Lupus anticoagulant, antiphospholipid antibodies | 72% | 74% | 56% | 67% | 64% | 71% |
| with obstetric history | 29% | 46% | 14% | 7% | 21% | 27% |
| without obstetric history | 43% | 28% | 42% | 60% | 42% | 44% |

n = number of prescribing respondents; % = percent of number of respondents. PIH = pregnancy-induced hypertension; PE = preeclampsia; FGR = fetal growth retardation; SLE = systemic lupus erythematodes.

The main indications to prescribe low-dose aspirin for therapeutic reasons were PIH-PE and FGR. Additional indications comprised a variety of disorders, including coagulation problems. The therapeutic indications are summarized in Table 4.2 and show marked differences in prescription patterns in 1989 and 1991 between gynecologists involved and not involved in a postgraduate training program. In departments with postgraduate training, prescription of low-dose aspirin for treatment of PIH fell to zero, whereas almost half the number of responding gynecologists not involved in postgraduate training continued to prescribe low-dose aspirin on that indication. On the other hand, the percentage of gynecologists prescribing low-dose aspirin for treatment of FGR rose from 20 to 54 in postgraduate training departments, and fell from 82 to 60 in departments without a training program. The number of gynecologists using low-dose aspirin to treat various other conditions in pregnancy almost tripled between 1989 and 1991.

In 1989 and in 1991 more than 90% of the respondents who prescribed aspirin used tablets or capsules of acetylsalicylic acid in daily doses ranging from 60 to 80 mg. The remaining 10% prescribed calciumcarbasalate in comparable doses. The gestational age at the start of prophylactic low-dose aspirin showed a marked shift between the years of the inquiry. In 1989 24% but in 1991 only 6% of the respondents prescribed low-dose aspirin before 12 weeks'gestation, whereas in 1989 39% and in 1991 63% started between 12 and 14 weeks; the remaining respondents prescribed low-dose aspirin between 15-20 weeks in both years. In 1989 and 1991 40% would stop prophylactic treatment between 36 and 38 weeks'gestation, and 60% would continue until delivery.

Table 4.2. Percentage of respondents in postgraduate training and nontraining hospitals in the Netherlands in 1989 and 1991 accepting indications to prescribe low-dose aspirin for treatment of obstetric complications.

| Indications | Training hospital | | Nontraining hospital | | Total | |
|---|---|---|---|---|---|---|
| | 1989 (n=50) | 1991 (n=72) | 1989 (n=44) | 1991 (n=108) | 1989 (n=94) | 1991 (n=180) |
| Pregnancy-induced hypertension | 52% | 0% | 39% | 48% | 46% | 24% |
| Preeclampsia | 52% | 44% | 39% | 23% | 46% | 34% |
| Fetal growth retardation | 20% | 54% | 82% | 60% | 49% | 57% |
| Other | 24% | 44% | 0% | 27% | 13% | 36% |

n = number of prescribing respondents; % = percent of number of respondents.

The total annual number of deliveries supervised by all respondents was 73,800 in 1991. Of those, 65,610 took place in hospitals were aspirin was being prescribed, and 8190 in hospitals were aspirin was never prescribed for obstetric indications. The hospitals where patients were offered prophylactic aspirin treatment had an annual delivery rate of 58,560; hospitals offering therapeutic treatment had an annual rate of 36,660 deliveries. In the first four months of 1991 the respondents prescribed prophylactic low-dose aspirin in approximately 550 patients, and 171 received therapeutic low-dose aspirin, which results in a calculated preventive treatment rate of 2,8% and a therapeutic treatment rate of 1,4% in the hospitals where aspirin was being prescribed for obstetric reasons. In 1989 these figures were 1,4% and 1,0%, respectively.

### 4.4 Discussion

The response to both inquiries was only 50-60%, comprising approximately 60% of all practicing gynecologists in the Netherlands, and it cannot be accepted that prescription patterns of respondents and nonrespondents are similar. Since the inquiry was anonymous, we do not know whether or not the gynecologists who responded in 1991 were the same as those who returned the questionnaire in 1989. For that reason, conclusions concerning the general attitude of Dutch gynecologists with regard to prescription of low-dose aspirin in pregnancy should be drawn with care. Between 1989 and 1991 a striking increase of about 25% appears to have occurred in the number of gynecologists prepared to prescribe low-dose aspirin in pregnancy for prophylactic or therapeutic reasons. The increase was similar in departments with and those without a postgraduate training program. The rapid increase in popularity of the prophylactic use of low-dose aspirin in pregnancy may be explained by the publication in the period of time covered by the inquiries of the

promising results of three randomized controlled clinical trials.[14,149,218] However, the single controlled study published in the same period of time on the therapeutic use of low-dose aspirin showed no effect on the clinical course of pregnancy-induced hypertension.[217] The trend towards prescribing prophylactic low-dose aspirin in patients at relatively lower risk of PIH-PE and FGR noted in departments with a postgraduate training program cannot be explained on the basis of published reports of controlled trials, which have all involved high risk patients.[24,103]

The rapid increase in the Netherlands in the prescription rate of low-dose aspirin in pregnancy that is apparent from our inquiries could indicate that a growing number of gynecologists are already becoming convinced that such treatment could have beneficial prophylactic and even therapeutic effects, although little more evidence has recently become available. This may, in part, explain the steady reduction in the number of patients recruited for the randomized, double-blind international Collaborative Low-dose Aspirin Study in Pregnancy (CLASP), in which 20 centers in the Netherlands participate. The same phenomenon has been observed in other countries; in New Zealand the CLASP trial could not start at all, because gynecologists and patients were already convinced that low-dose aspirin in pregnancy is effective, and they refused on ethical grounds to participate in a placebo controlled study.[100] It is well possible that recruitment for participation in the CLASP trial among Dutch gynecologists, that started after the first questionnaire was sent out in 1989, has in itself contributed to the increasing belief in the effectivity of prophylactic low-dose aspirin. However, this remains speculative, because we have no information on the participation in the CLASP trial of respondents to the inquiries.

# CLASP: A COLLABORATIVE LOW-DOSE ASPIRIN STUDY IN PREGNANCY FOR THE PREVENTION AND TREATMENT OF PREECLAMPSIA AND FETAL GROWTH RETARDATION*

In chapter three it was concluded that low-dose aspirin should not be adopted into routine obstetric practice until reliable risk-benefit ratios have been determined in large clinical trials.

In March 1986 Doctors Collins, Grant and Redman from Oxford, de Swiet from London, and Wallenburg from Rotterdam, discussed the design of a randomized, double-blind, placebo-controlled, multicenter intervention study on the prophylactic and therapeutic effects of daily low-dose aspirin in pregnancy. Their discussion resulted in a protocol of a Collaborative Low-dose Aspirin Study in Pregnancy (CLASP) that was submitted to the UK Medical Research Council (MRC) for financial support in August 1987.

* *The main substance of this chapter was published in: CLASP Collaborative Group. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. Lancet 1994; 343: 619-29. Parts of the article are quoted verbatim with permission.*

The trial would be organized and coordinated by the National Perinatal Epidemiology Unit and the Clinical Trial Service Unit in Oxford. The Clinical Trial Service Unit and the Radcliffe Infirmary switchboard would provide a 24-hour randomization service. A first meeting of potential collaborators was held at the Royal College of Obstetricians and Gynaecologists in september 1987.

The MRC endorsed the study and agreed to fund it for three years. Additional support was obtained from the Clinical Trial Service Unit (Oxford), Sterling Winthrop, Bayer Europe, and the European Aspirin Foundation. The first woman was recruited into the trial in January 1988.

CLASP became an international study with the participation of Hong Kong and Canadian centers in October and November 1988. Also many obstetric centers in The Netherlands showed an interest to participate in the trial. Because it was thought that the recruitment in the Netherlands would benefit from the appointment of a national study coordinator, an application for funding of a Dutch study coordinator was submitted to the Dutch Preventiefonds, but was rejected. Fortunately, financial support by Bayer, Leverkusen, made it possible in July 1989 to appoint a Duch study coordinator (the author of this thesis) and to establish a Coordinating Center in the Department of Obstetrics and Gynecology of the Erasmus University Hospital-Dijkzigt. The first Dutch centers joined in 1989 and twelve more countries started their participation during the course of the study.

The results of the CLASP trial were reported in an article published by the CLASP Collaborative Group in The Lancet in March 1994;[38] that article forms the basis of this chapter. All parts of this chapter that are quoted from the article are marked with *.

Because of concern expressed by the CLASP Steering Committee that analysis of the

results at a national level might give misleading information, at variance with the whole trial, and because individual countries had been recruiting patients into the trial to provide a global result, it is not possible to present a separate analysis of the results of the patients recruited in the Netherlands. For that reason, the overall results of the trial will be presented and discussed, but data on the Dutch participation in the trial will be included where possible and relevant.

## 5.1 Introduction*

Preeclampsia is a common complication of the second half of pregnancy that can progress to various dangerous maternal crises. It can also cause intrauterine growth retardation (IUGR), fetal death, and, because elective delivery remains the only effective management approach, neonatal death due to complications of prematurity. Preeclampsia originates in the placenta, probably as the result of under-perfusion of the uteroplacental circulation associated with structural and occlusive changes in the spiral arteries.[197] Some cases of fetal growth failure without maternal hypertension show the same spiral artery lesions, which suggests that these cases may differ from preeclampsia only in the maternal response to a common placental pathology.[196] Consequently, if prophylaxis against preeclampsia were effective, it might prevent not only that disorder but also some cases of IUGR without overt preeclampsia.

Preeclampsia is associated with deficient intravascular production of prostacyclin and with excessive production of thromboxane.[29] These findings and the evidence for activation of the clotting system in preeclampsia and IUGR with early involvement of the platelets[198] have led to the use of antiplatelet agents (usually low-dose aspirin) in an

attempt to prevent, or ameliorate, the condition. Even a moderate delay (eg, 1 or 2 weeks) in the development of severe preeclampsia could be important, since neonatal survival at the beginning of the third trimester improves rapidly with increasing maturity.

Low-dose aspirin (60-150 mg per day) is a widely used and well-tolerated antiplatelet treatment, which irreversibly inhibits almost all platelet cyclooxygenase activity, thereby blocking synthesis of the vasoconstrictor and platelet- aggregating agent thromboxane. In preeclampsia, aspirin can to some extent rectify the intravascular imbalance between thromboxane and prostacyclin.[218] The first anecdotal report of the use of aspirin to prevent preeclampsia[85] was followed by several small controlled trials reporting large reductions in the incidence of proteinuric preeclampsia with low-dose aspirin (in some cases with the addition of dipyridamole, another antiplatelet agent). But, similarly large benefits have not been consistently observed in larger randomised controlled trials.[91,107,227,247,257]

The evidence for a better outcome for the fetus with antiplatelet therapy is less clear; reduced rates of IUGR have been reported in only a few trials[12,247] and no randomised study has clearly shown a reduction in perinatal mortality. There are also concerns about possible side-effects of antiplatelet treatment in pregnancy, in particular haemorrhage in the mother, fetus, or newborn baby. Early reports of haemorrhagic complications came from observational studies of women who had taken high doses of aspirin during pregnancy.[42] More recently, a large trial of low-dose aspirin in healthy primigravidae reported a small but significant increase in placental bleeding with low-dose aspirin.[227]

Our randomised, placebo-controlled trial (Collaborative Low-dose Aspirin Study in Pregnancy [CLASP]) was designed to be large enough to provide reliable evidence about the overall safety of low-dose aspirin use in pregnancy and to find out whether treatment

really produces worthwhile effects on morbidity and on fetal and neonatal mortality, either overall or in selected subgroups of pregnancies judged to be at high risk of severe preeclampsia or IUGR.

## 5.2 Patients and methods

9364 women were recruited into the trial from 213 centres in 16 countries over 5 years from January, 1988, to December, 1992. Local research ethics committee approval was required for a hospital to be included as a trial centre.

*Egilibility**

Women were eligible if they were between 12 and 32 weeks of gestation and, in the opinion of the responsible clinician, were at sufficient risk of preeclampsia or IUGR for the use of low-dose aspirin to be contemplated, but without clear indications for or against its use. Women could be considered for:

Prophylactic entry. Women with a history of preeclampsia or IUGR in a previous pregnancy, chronic hypertension, renal disease, or other risk factors, such as maternal age, family history, or multiple pregnancy.

Therapeutic entry. Women with signs or symptoms of preeclampsia or IUGR in the current pregnancy.

Contraindications included an increased risk of bleeding, asthma, allergy to aspirin, or a high likelihood of immediate delivery. The fundamental criterion for entry was that the responsible clinician was uncertain whether or not to recommend aspirin in the individual pregnancy. The range of women studied was expected to be wide, but their

heterogeneity could be defined to some extent by characteristics recorded at entry. In consequence, the effects of aspirin were to be assessed not just overall but also in various categories of women (such as those studied in previous trials with more restricted entry [see below]).

The study was explained to eligible women (with an information booklet to provide further details), who were asked to give consent to take part.

*Randomisation**

Women were enrolled in the study by clinical staff telephoning a central 24-hour service at the Clinical Trial Service Unit in Oxford. Identifying information and baseline details were recorded (Table 5.2) directly on computer before a specific trial treatment pack containing aspirin or placebo tablets was allocated. (Women could be randomised after 8 weeks' gestation but were instructed not to start taking the trial tablets until a date calculated to correspond to 12 weeks' gestation.) A minimisation algorithm[280] was used to limit differences between the treatment groups for certain prognostic baseline variables. After randomisation, no woman was excluded from the trial, irrespective of whether treatment was actually dispensed or taken. For the purposes of analysis women were counted in the treatment group to which they had been originally allocated (i.e., intention-to-treat analyses are reported).

*Treatment**

Women were assigned treatment with either one 60 mg film-coated aspirin tablet daily or a matching placebo tablet (containing microcrystalline cellulose and corn starch). The dose of aspirin was chosen to keep side-effects to a minimum, and yet be sufficient

to inhibit maternal cyclooxygenase-dependent platelet aggregation,[14] and was one that had been reported to prevent preeclampsia.[265] Women were asked to take the study treatment every day until delivery unless advised otherwise by their doctors.

A standard letter was provided to inform the family doctor of the patient's entry into the trial. We recommended that other aspirin-containing preparations be avoided, with paracetamol used, if necessary, for analgesia. Study treatment was not revealed, even after delivery, unless there was a clear medical or personal reason for the treatment to be made known.

*Follow-up**

A single-page follow-up form was completed after hospital discharge of both mother and baby (or at 6 weeks post partum, if either had not been discharged). Brief details were recorded of compliance with study treatment, use of antihypertensive or anticonvulsant drugs, and major events that occurred after randomisation (especially the development of preeclampsia, any fetal loss, or any maternal or neonatal bleeding). The birthweight and vital status of the baby and any neonatal complications were also recorded. Incomplete or inconsistent data were checked and corrected wherever possible. Further information about any report of suspected neonatal intraventricular haemorrhage was requested, and necropsy reports were sought for any fetal losses or neonatal deaths. Liveborn infants in the UK have been flagged through the Office of Population Censuses and Surveys for longer-term follow-up of mortality.

*CLASP in the Netherlands*

In April 1989 a written inquiry was held among practicing gynecologists in the

Netherlands in order to assess the patterns of prescription of low-dose aspirin for preventive and therapeutic use in pregnancies at risk for pregnancy-induced hypertensive disease and fetal growth retardation (chapter 4). The questionnaire was accompanied by an invitation for the introductory meeting of centers interested in participation in the CLASP-trial in the Netherlands. In May 1989 this meeting was held and attended by almost 100 obstetricians-gynecologists. At this meeting a translation of all CLASP material was presented in order to encourage participation. As a result gynecologists in 20 hospitals, 10 training and 10 non-training, subscribed to take part in the study. The national study-coordinator for the Netherlands, the author of this thesis, visited these hospitals for detailed explanation regarding the actual participation in the study. After the final decision to participate in the study, and after consent from local hospital ethics committees had been obtained, the centers were registered by the CLASP Coordinating Center in Oxford, from which they received a hospital number, and an identification number for the 24-hour telephone randomization service. All applications for randomization went through the Dutch Coordinating Center in the Department of Obstetrics and Gynecology of the Erasmus University Hospital-Dijkzigt in Rotterdam, which provided a 24-hours telephone service for participating centers in the Netherlands. After having obtained the necessary patient data from the obstetrician who requested randomization the Dutch coordinating center called the CTSU in Oxford and received a randomization number that was forwarded to the participating center. The first patient in the Netherlands was randomized August 22 1989. During the study the Dutch Coordinating Center and the national study coordinator served as an intermediate between the Coordinating Center in Oxford and the participating obstetricians-gynecologists in the Netherlands. The participating centers were visited by the national study coordinator on a

regular basis to provide them with trial medication and stationery, to solve problems, and to stimulate recruitment.

*Outcome measures**

The main prespecified endpoints were: development of proteinuric preeclampsia; estimated duration of pregnancy; crude birthweight; birthweight below the third centile for sex and gestational age; stillbirth or neonatal death ascribed to preeclampsia or maternal hypertension or associated with IUGR, ascribed to maternal or neonatal bleeding, or from any cause; death of the baby at any time attributed to preeclampsia or maternal hypertension or associated with IUGR.

The study outcome of proteinuric preeclampsia required the development of hypertension and proteinuria after randomisation. For those with baseline diastolic pressure below 90 mm Hg, hypertension was defined as a rise of at least 25 mm Hg, to 90 mm Hg or higher. For those with an initial diastolic pressure of 90 mm Hg or above, an increment of at least 15 mm Hg was required. Proteinuria was defined as the appearance after randomisation of at least 1+ on protein stick-testing during pregnancy, without evidence of urinary tract infection. IUGR was defined as a birthweight below the third centile for sex and estimated gestational maturity (with values available only for gestational ages of 24 to 42 weeks),[288] and preterm delivery was defined as that before 37 weeks' estimated gestation. Stillbirths included all intrauterine deaths at or after 24 weeks, and neonatal deaths included all deaths after birth up to the age of 28 days. Spontaneous miscarriages and induced abortions before 24 weeks and post-neonatal deaths up to age 1 year were also recorded. All losses after randomisation up to age 1 year are referred to as total mortality. Two senior investigators, unaware of the treatment groups

of the babies' mothers, classified all deaths according to a modification of the systems described by Cole, Hey, and colleagues.[40,93] Deaths attributed to maternal hypertension, preeclampsia, or unexplained IUGR were grouped together in the analyses, as were those attributed to maternal or neonatal bleeding.

The duration of tablet taking was assessed in two ways. First, the approximate date when study treatment stopped was recorded; the proportion of the scheduled treatment duration during which the study tablets were taken could then be estimated. Second, to check these data, we sent a compliance questionnaire at an average of about 3 months after delivery to about 10% of women in the UK whose follow-up data had been received. To avoid distress, this sample was selected from women whose infants were believed to be alive and well. Correct packing and labelling of active and placebo treatment packs and drug stability were also checked throughout the study by destructive testing of a random sample of treatment packs.

*Comparisons and statistical methods**

The protocol specified five main comparisons: the first in the trial as a whole, and the rest with the participants subdivided into four categories according to the principal clinical reason for randomisation (i.e., prophylactic, or therapeutic use of aspirin) and the principal disorder to be prevented or treated (i.e., preeclampsia irrespective of the presence of IUGR, or IUGR alone). In addition, as subsidiary questions, comparisons were to be undertaken of prophylactic use according to parity (0,≥1), prophylactic use according to time of entry to the trial (≤ 20 weeks'or > 20 weeks' gestation), and therapeutic use according to time of entry to the trial (≤ 28 weeks' or > 28 weeks' gestation).

We calculated that in a trial of 4000 women there would be a 95% probability of detecting, at a reasonable level of significance ($2p < 0.02$), a decrease of at least a quarter in the incidence of proteinuric preeclampsia (assuming a control rate of 5-10%), an increase of 100 g in mean birthweight, and an increase of 1 day in mean duration of gestation. A trial of this size could not, however, be relied on to detect differences in rates of stillbirth and neonatal death ascribable to preeclampsia. After the trial started, therefore, the steering committee, unaware of results subdivided by treatment allocation, decided to extend recruitment to about 10.000 women, which would provide a high probability of detecting a decrease of at least a quarter in such deaths (assuming a control rate of 40 per 1000).

Statistical analyses involved simple comparisons of total numbers affected.[189] Standard methods were used to calculate the apparent ratio of the odds of an outcome occurring in the aspirin group compared with the odds in the control group along with its confidence interval -95% for principal analyses and, to take account of the number of comparisons, 99% for subsidiary and subgroup analyses.[6] Alternatively, the reduction in the odds of the event in the aspirin group and its standard deviation (SD) are cited;[6] an odds ratio of 0.8 for example, corresponds to an odds reduction of 20%. (Such odds reductions are slightly larger than risk reductions.)

During recruitment, confidential interim results were reviewed about twice a year by an independent data monitoring committee who were to advise the study chairmen if, in their view, there was proof beyond reasonable doubt that, either for all women or for any category of women, low-dose aspirin was clearly indicated or clearly contraindicated. The steering committee, collaborators, and administrative staff (except those who produced the confidential analyses) remained ignorant of the interim results. No reason to stop the trial

*Table 5.1 Participating countries, centers and numbers of patients recruited into CLASP*

| | No. of women entered | No. of participating centers | Year started |
|---|---|---|---|
| Argentina | 143 | 3 | 1991 |
| Australia | 351 | 13 | 1989 |
| Belgium (Flanders) | 154 | 3 | 1989 |
| Belgium (Franco) | 88 | 7 | 1989 |
| Canada | 819 | 7 | 1988 |
| Germany | 7 | 1 | 1990 |
| Hong Kong | 164 | 1 | 1988 |
| Israel | 159 | 7 | 1990 |
| Malaysia | 135 | 1 | 1989 |
| New Zealand | 12 | 1 | 1990 |
| Russia | 515 | 4 | 1990 |
| Spain | 8 | 2 | 1990 |
| Sweden | 241 | 10 | 1990 |
| The Netherlands | 486 | 20 | 1989 |
| United Arab Emirates | 114 | 1 | 1989 |
| United Kingdom | 5961 | 131 | 1988 |
| United States of America | 7 | 1 | 1990 |
| Total | 9364 | 213 | |

prematurely emerged, so randomisation continued from Jan 1, 1988, to Dec 31, 1992.

### 5.3 Results

9364 women were randomised in 16 countries (Table 5.1), with good balance between the treatment groups for the main prerandomisation prognostic factors recorded (Table 5.2). 74% were entered for prophylaxis of preeclampsia and only 3% for the treatment of IUGR alone. In the Netherlands 486 women were randomized, with a median per hospital of 10 (range 1 - 142). Fifty percent of these patients were randomized by 3 hospitals (Table 5.3). Of these 486 women, 87% were entered for prophylaxis (preeclampsia 57%, fetal growth retardation 30%); 13% were entered for treatment (preeclampsia 7%, fetal growth retardation 6%). In the total group 28% were nulliparous; in the Dutch group 18% were nulliparous at randomization. 62% of the women were enrolled at 20 weeks' gestation or earlier. 2% had already developed preeclampsia, and 28% were primigravidae. The characteristics of the women entered for different reasons varied in the ways that might be expected (Table 5.4). For example, those entered for prophylaxis were less likely to be primigravid, especially if entered for prophylaxis of IUGR, and smoking was more common among those entered for prophylaxis or treatment of IUGR.

Post-delivery follow-up forms were obtained for 9309 (99.4%; 4659 aspirin-allocated and 4650 placebo-allocated) randomised women, and these women had had 9631 (4810 vs 4821) babies or fetal losses. Reported compliance with study treatment was good, with no difference between the groups allocated aspirin or placebo. Of the 8915 randomised women for whom we had information on compliance, 96% started the study medication, and 66% and 88% continued study treatment for at least 95% and 80%, respectively, of the time between randomisation and delivery. Owing to concern among some anaesthetists

*Table 5.2. Prerandomisation characteristics of women studied.*

| | No (%) in allocated treatment group | |
|---|---|---|
| | **Aspirin (n=4683)** | **Placebo (n=4681)** |
| **Main reason for entry** | | |
| Prophylaxis of pre-eclampsia | 3467 (74) | 3460 (74) |
| Prophylaxis of IUGR alone | 546 (12) | 548 (12) |
| Treatment of pre-eclampsia | 538 (11) | 544 (12) |
| Treatment of IUGR alone | 132 (3) | 129 (3) |
| **Woman's age (yr)** | (28.5 SD 5.4)* | (28.5 SD 5.5)* |
| <20 | 186 (4) | 186 (4) |
| 20-29 | 2577 (55) | 2582 (55) |
| 30-39 | 1792 (38) | 1787 (38) |
| ≥40 | 128 (3) | 126 (3) |
| **Estimated duration of gestation (wk)** | (19.4 SD 5.6)* | (19.4 SD 5.6)* |
| <12** | 261 (6) | 241 (5) |
| 12-20 | 2652 (57) | 2685 (57) |
| >20-28 | 1259 (27) | 1239 (26) |
| >28 | 511 (11) | 516 (11) |
| **Diastolic blood pressure (mmHg)** | (76.2 SD 12.2)* | (76.3 SD 12.1)* |
| <90 | 3780 (81) | 3715 (79) |
| 90-109 | 858 (18) | 938 (20) |
| ≥110 | 45 (1) | 28 (1) |
| **Features of current pregnancy** | | |
| Development of pre-eclampsia*** | 110 (2) | 108 (2) |
| Evidence of IUGR | 237 (5) | 234 (5) |
| Proteinuria (1+ or more) | 246 (50) | 245 (5) |
| Cigarette smoker | 970 (21) | 945 (20) |
| **Obstetric history** | | |
| Primigravid | 1310 (28) | 1309 (28) |
| Multiparous, no previous problems | 335 (7) | 331 (7) |
| Multiparous, previous problems**** | 3038 (65) | 3041 (65) |
| **Medical history** | | |
| Chronic hypertension | 933 (20) | 949 (20) |
| Renal disease | 223 (5) | 190 (4) |
| Diabetes | 129 (3) | 146 (3) |

* Mean, SD.
** Women randomised before 12 weeks' gestation were to start study treatment at 12 weeks.
*** Based on changes in diastolic blood pressure between first recorded measurement in current pregnancy and last prerandomisation recorded.
**** Pre-eclampsia, IUGR, or perinatal death in at least one previous pregnancy.

Table 5.3 Recruitment into CLASP in The Netherlands

| | No.of women recruited | | No.of women recruited |
|---|---|---|---|
| Academisch Ziekenhuis Leiden | 45 | Twenteborg Ziekenhuis Almelo | 33 |
| AZR-Dijkzigt Rotterdam | 142 | St.Elisabeth Ziekenhuis Tilburg | 32 |
| Zuiderziekenhuis Rotterdam | 10 | Ziekenhuis Rijnstaete & Velp | 2 |
| Rode Kruis Ziekenhuis Heemskerk | 18 | Hofpoort Ziekenhuis Woerden | 9 |
| AMC Amsterdam | 8 | Academisch Ziekenhuis Nijmegen | 2 |
| Gemini Ziekenhuis Den Helder | 1 | Pasteur Ziekenhuis Oosterhout | 11 |
| Lievensberg Ziekenhuis Bergen op Zoom | 32 | St.Jansdal Ziekenhuis Harderwijk | 15 |
| Ziekenhuiscentrum Apeldoorn | 54 | Ikazia Ziekenhuis Rotterdam | 7 |
| Geertruiden Ziekenhuis Deventer | 47 | Medisch Centrum Alkmaar | 5 |
| Westeinde Ziekenhuis Den Haag | 9 | St.Clara Ziekenhuis Rotterdam | 4 |

*Table 5.4. Comparison of baseline characteristics of women entered for different reasons.*

| | Prophylaxis of pre-eclampsia (n=6927) | Prophylaxis of IUGR alone (n=1094) | Treatment of pre-eclampsia (n=1082) | Treatment of IUGR alone (n=261) |
|---|---|---|---|---|
| Mean (SD) | | | | |
| Women's age (yr) | 28.7 (5.3) | 28.2 (5.0) | 27.8 (6.2) | 26.7 (5.8) |
| Duration of gestation (wk) | 18.6 (5.1) | 18.1 (5.0) | 23.8 (5.6) | 27.6 (4.0) |
| Systolic blood pressure (mmHg) | 124.7 (15.8) | 115.1(12.7) | 137.6 (17.8) | 116.6 (14.5) |
| Diastolic blood pressure (mmHg) | 76.0 (11.0) | 68.8 (9.3) | 87.1 (14.1) | 70.3 (11.0) |
| No. (%) of women | | | | |
| Current pregnancy | | | | |
| Development of pre-eclampsia | 50 (0.7) | 0 | 165 (15.2) | 3 (1.1) |
| Evidence of IUGR | 58 (0.8) | 40 (3.7) | 128 (11.8) | 245 (93.9) |
| Proteinuria | 238 (3.4) | 18 (1.6) | 226 (20.9) | 9 (3.4) |
| Cigarette smoker | 1184 (17.1) | 441 (40.3) | 198 (18.3) | 92 (35.2) |
| Obstetric history | | | | |
| Primigravid | 1819 (26.3) | 29 (2.7) | 643 (59.4) | 128 (49.0) |
| Multiparous, previous problems | 4642 (67.0) | 1048 (95.8) | 307 (28.4) | 82 (31.4) |

that epidural anaesthesia might be contraindicated in women taking aspirin,[141] clinicians were advised that they could stop study treatment a few days before delivery. Even so, 53% of women were reported to have continued study treatment to within 1 day of delivery and 69% to within 1 week. There was excellent agreement between the information provided on the post-delivery form and that obtained from the 1077 (86%) respondents to the compliance questionnaire sent to 1256 women. For example, 92% of those who were reported to have continued study treatment for at least 80% of the scheduled time confirmed that they had done so.

*Incidence of proteinuric preeclampsia**

Overall, 6.7% of aspirin-allocated women developed proteinuric preeclampsia after randomisation compared with 7.6% of those allocated placebo (figure 5.1).

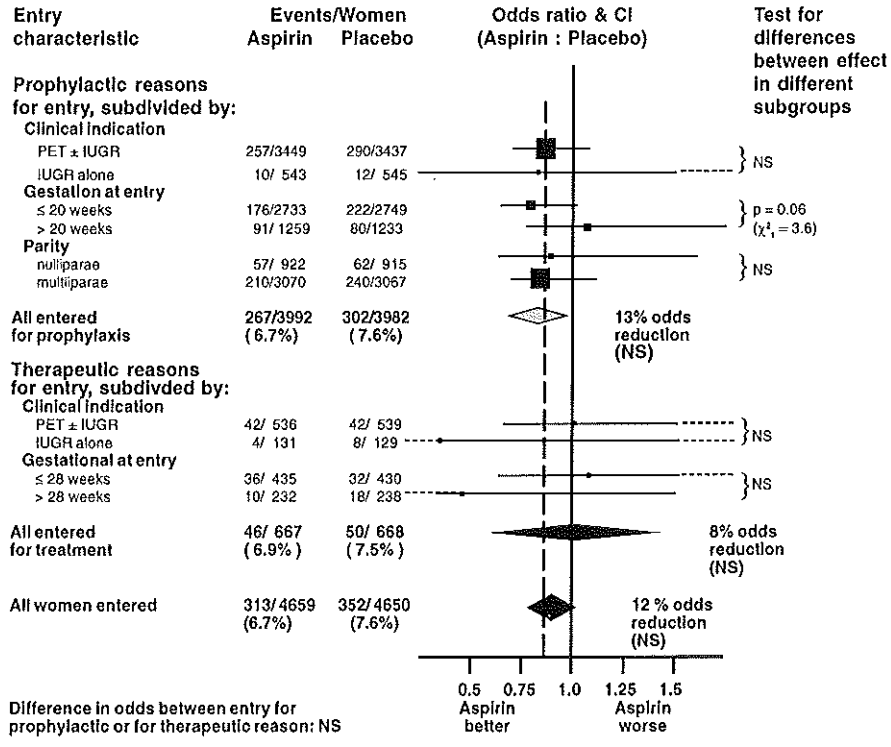


*Figure 5.1. Effects of aspirin on proteinuric preeclampsia developing after randomisation.*

*Odds ratios (black square: area proportional to amount of information contributed[6]), and 99% CI (horizontal line) are plotted for various subgroups of the study population. A black square to the left of the solid vertical line suggests a benefit (but this is significant at $2p < 0.01$ only if the whole CI is to the left of the solid vertical line). $Chi^2$ tests for differences between effects observed in the different subgroups are given to the right of the solid vertical line. The overall results for all women and for those entered for prophylactic and for therapeutic reasons (and 95% CI) are represented by diamonds, with the observed reductions in the odds of preeclampsia developing given to the right of the solid vertical line.*
*PET=preeclampsia; NS=not significant; CI=confidence interval.*

This difference represents a reduction with aspirin in the odds of developing proteinuric preeclampsia of 12%, but is not significant. There was no good evidence that the effect differed between women entered for prophylactic or therapeutic reasons, or for different clinical indications, or between nulliparae and multiparae entered for prophylaxis. The effect seemed to be greater among women entered for prophylaxis at 20 weeks' gestation or earlier (22 % reduction [99% CI 40% reduction to 3% increase]; 2p = 0.02). But the apparent effect in this group was not significantly greater than that among those entered later for prophylaxis, and the opposite pattern was observed among women entered for therapeutic reasons.

Proteinuria without hypertension severe enough to be recognised as preeclampsia was also slightly less common among aspirin-allocated women (11% reduction [99% CI 28% reduction to 11 % increase]; not significant), but new hypertension without associated proteinuria was not (1% increase [99% CI 14% reduction to 19% increase]). Only 7 (0.2%) aspirin- allocated and 7 (0.2%) placebo-allocated cases of eclampsia were reported.

Duration of pregnancy*

The average duration of pregnancy was about 1 day longer among aspirin-allocated than among placebo-allocated women (38.15 [SD 3.82] vs 37.99 [3.93] weeks; 2p=0.05).

The absolute risks of preterm delivery differed substantially between the categories of women studied. Among about 8000 women entered for prophylactic reasons only one-fifth of the controls had preterm delivery and the absolute benefit seemed to be about 2 fewer preterm deliveries per 100 women allocated aspirin (2p = 0.03; figure 5.2), while among

just over 1000 women entered for therapeutic reasons two-fifths of the controls had preterm delivery and the absolute benefit seemed to be about 5 per 100 (2p = 0.03). This difference in mean gestation was small, but aspirin clearly reduced the likelihood of delivery before 37 weeks' estimated gestation (19.7% aspirin-allocated vs 22.2% placebo-allocated; 2p = 0.003); 14% fewer aspirin-allocated women delivered preterm (95% CI 22% to 5% reduction; figure 5.2). The proportional effect was larger (28% reduction [99% CI 46% to 4% reduction]) among women delivering preterm with proteinuric preeclampsia (3.0% aspirin-allocated vs 4.1% placebo- allocated; 2p = 0.003).

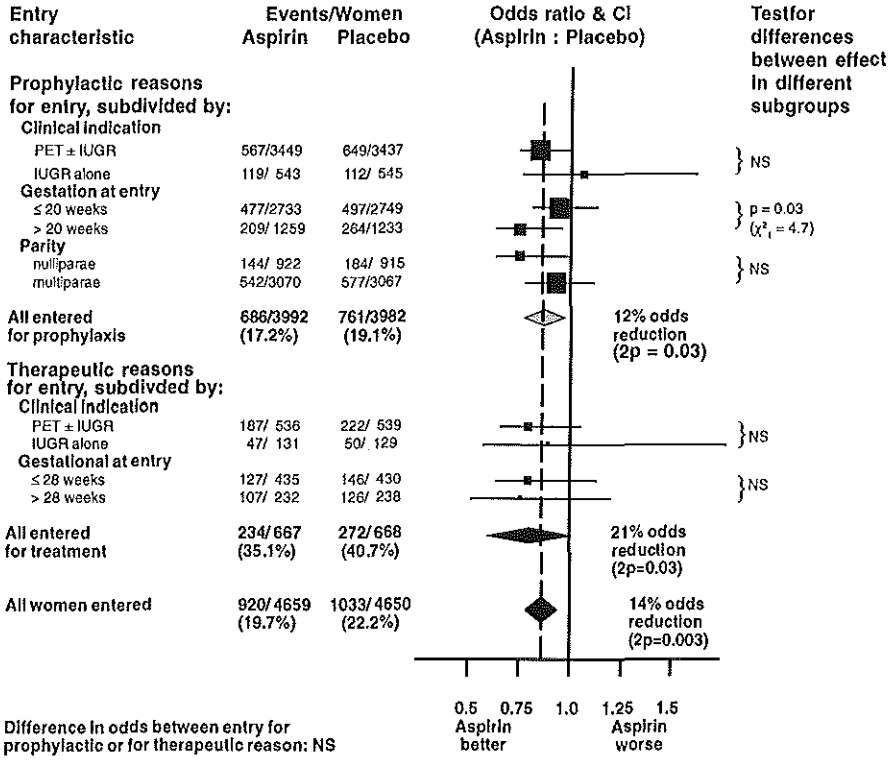


*Figure 5.2. Effects of aspirin on preterm delivery*

*Symbols and conventions as in figure 5.1. Preterm=delivery before 37 weeks' estimated gestation.*

*Birthweight**

The average weight of all babies born to women allocated aspirin was 32 g greater (95% CI 0 to 64 g; 2p = 0.05) than that in the placebo group (3024 [SD 788] vs 2991 [810] g). Aspirin was also associated with a slightly smaller proportion of babies with IUGR, but this difference was not significant (figure 5.3). Nor did the effects differ between those entered for prophylactic reasons and those entered for therapeutic reasons. Among women entered for therapeutic reasons, however, aspirin seemed to have discrepant effects, with an increased incidence of IUGR among those entered at 28 weeks' or earlier, and a decreased incidence among those entered later (p = 0.005 for test of interaction). The adverse trend among those entered for treatment of preeclampsia could largely be accounted for by differences in the duration of gestation at entry.

*Stillbirths and neonatal deaths**

69 pregnancies of women allocated aspirin and 67 of those allocated placebo ended before viability ( < 24 weeks) owing to miscarriage or induced abortion. Among the remainder there were 129 (2.7%) stillbirths or neonatal deaths in the aspirin group and 136 (2.8%) in the placebo group (figure 5.4). This small reduction with aspirin was not statistically significant, but the confidence interval is wide, so neither a reduction nor an increase of about a quarter can be excluded. Among women allocated aspirin for prophylactic reasons, there were slightly fewer stillbirths or neonatal deaths, whereas among those allocated aspirin for therapeutic reasons there were slightly more (p = 0.04 for interaction). However, aspirin was not associated with a significant difference in stillbirths or neonatal deaths either overall or in any subcategory. In particular, although women allocated aspirin had significantly fewer preterm deliveries, this apparent benefit

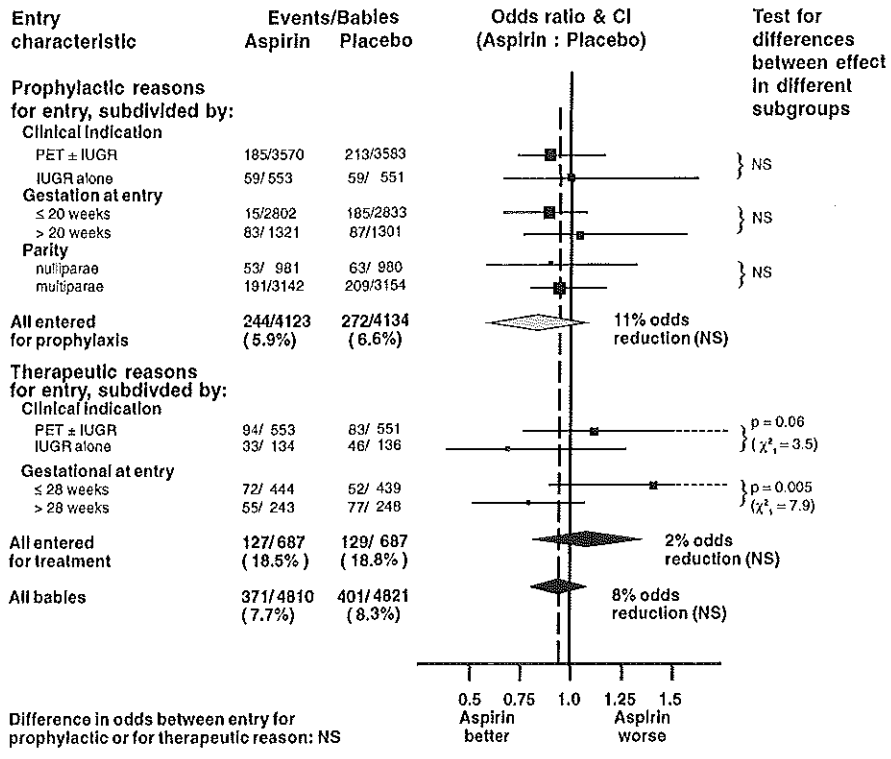


*Figure 5.3. Effects of aspirin on IUGR.*

*Symbols and conventions as in figure 5.1. IUGR=Birthweight below 3rd percentile for sex and gestational age.*

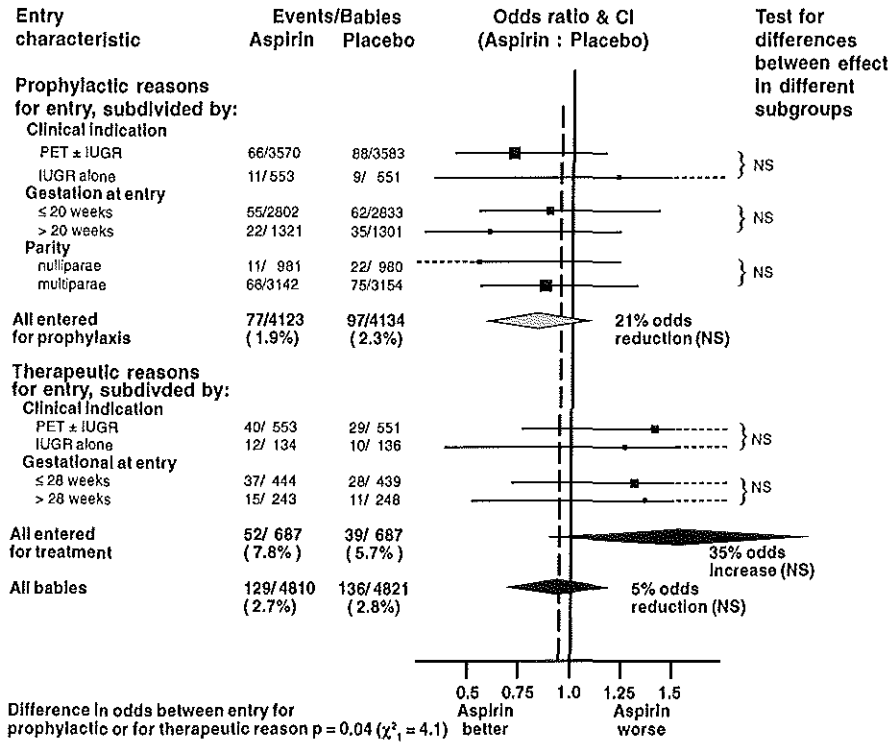


*Figure 5.4. Effects of aspirin on stillbirths and neonatal deaths.*

*Symbols and conventions as in figure 5.1.*

was not reflected in significantly better survival.

There were no significant differences between the aspirin and placebo groups in the numbers of stillbirths and neonatal deaths attributed to preeclampsia, maternal hypertension, or IUGR (74 [1.5%] vs 80 [1.7%]) or in those attributed to maternal or neonatal bleeding (20 [0.4%] vs 31 [0.6%]). Similarly, for total mortality, there were no significant differences in the numbers from any cause (207 [4.3%] vs 226 [4.7%]) or in those attributed to preeclampsia, maternal hypertension, or IUGR (95 [2.0%] vs 114 [2.4%]).

*Early-onset preeclampsia**

A greater proportion of pregnancies continued beyond 37 weeks' gestation in the aspirin-allocated group, and so slightly more aspirin-allocated than placebo-allocated women were at risk of developing preeclampsia in later pregnancy. As a result the comparisons in figure 5.1 may underestimate any effect of aspirin at earlier gestational ages. Figure 5.5 supports this concept, indicating a significant trend ($p = 0.004$) towards progressively greater reductions in proteinuric preeclampsia with aspirin use the earlier the time of delivery. There were similar significant trends to less use of antihypertensive and anticonvulsant therapy among women who delivered early (figure 5.5).

*Other outcomes**

Women allocated aspirin were slightly, though not significantly, less likely than those allocated placebo to go into labor spontaneously, and had greater use of induction and caesarean section before labor. On the other hand, intra- partum caesarean section was less common in the aspirin group ($2p = 0.08$; Table 5.5).

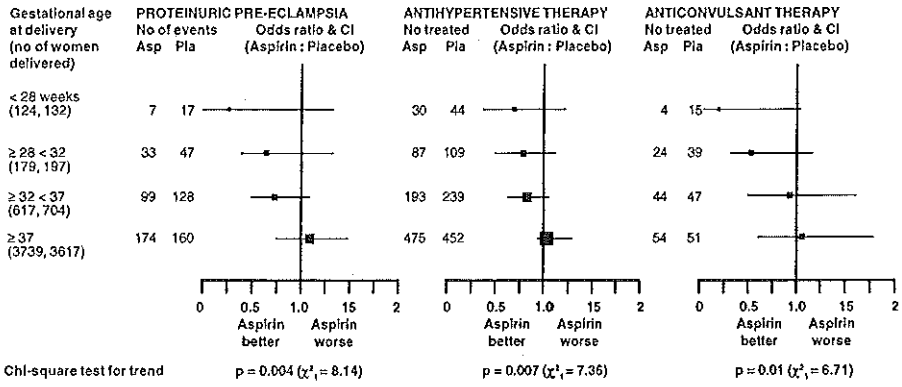


*Figure 5.5. Development of proteinuric preeclampsia and the use of antihypertensive or anticonvulsive therapy according to gestation duration at delivery.*
*Symbols and conventions as in figure 5.1. Ratio of odds of an event in apsirin group to that in placebo group is plotted separately for women delivering at different durations of gestation after randomization. $Chi^2$ test for trend in odds ratios with increasing gestational age at delivery is given below each plot. Asp=aspirin, Pla=placebo group.*

Because of its size CLASP provides especially reliable information about complications of aspirin use in pregnancy. Aspirin-allocated women were reported to have had more placental abruptions and fewer other ante-partum hemorrhages, but these differences were small and not statistically significant (Table 5.5). Moreover, concern about the use of aspirin at the time of epidural anesthesia was not substantiated. Among 2783 women who had epidural anesthesia (1422 aspirin-allocated vs 1361 placebo-allocated), 79 adverse experiences were reported in relation to the epidural (46 [3.2%] vs 33 [2.4%]; not significant). Hemorrhage occurred on only 3 occasions (1 vs 2) and was limited to blood-stained fluid in the cannula. Significantly more women allocated aspirin received blood transfusions after delivery, but this difference was not associated with differences in the occurrence or degree of post-partum hemorrhage. Only 1 maternal death was recorded on the post-delivery follow-up form; this woman, allocated aspirin during pregnancy, died 2 days after delivery from a pulmonary embolus.

*Table 5.5. Effect of aspirin on other outcome measures after randomization.*

| | No (%) in allocated treatment group | |
|---|---|---|
| | Aspirin | Placebo |
| **Total no.** | | |
| Pregnancies with data | 4659 | 4650 |
| Fetal outcomes | 4810 | 4821 |
| **Treatment after randomisation** | | |
| Antihypertensive | 785 (16.8) | 844 (18.2) |
| Anticonvulsant | 126 (2.7) | 152 (3.3) |
| **Labour and delivery** | | |
| Spontaneous onset | 2124 (45.6) | 2190 (47.1) |
| Induced | 1460 (31.3) | 1406 (30.2) |
| Pre-labour caesarean | 1007 (21.6) | 987 (21.2) |
| Intra partum caesarean | 376 (8.1) | 423 (9.1) |
| **Maternal bleeding** | | |
| Placental abruption | 86 (1.8) | 71 (1.5) |
| Other ante partum bleed | 48 (1.0) | 54 (1.2) |
| Post-partum bleed >500 ml | 1200 (25.8) | 1182 (25.4) |
| Transfusion | 188 (4.0) | 147 (3.2) |
| **Fetal/neonatal complications** | | |
| Special care admission | 946 (19.7) | 1016 (21.1) |
| Intraventricular haemorrhage | 33 (0.7) | 45 (0.9) |
| Other neonatal bleeds | 47 (1.0) | 45 (0.9) |

Subsequent maternal follow-up was not done systematically (although it was, in general, blind to treatment allocation); 2 maternal deaths (at 10 and 19 months after delivery) were reported among women allocated aspirin and 1 (at 18 months) among those allocated placebo.

Findings on safety in the babies were reassuring (Table 5.5). 78 intraventricular haemorrhages were reported, of which 88% were diagnosed either by ultrasound or after death. Fewer were reported among babies born to women allocated aspirin (0.7% aspirin-allocated vs 0.9% placebo-allocated), but this difference was not significant. Nor were there significant differences in fetal or neonatal deaths attributed to hemorrhage (20 [0.4%] vs 32 [0,7%]) or in other neonatal hemorrhages. Slightly fewer newborn infants in the aspirin group were admitted to the special care nursery (2p=0.09), but there were no

significant differences in the duration of stay.

## 5.4 Discussion

*Trial design*

The CLASP trial is a typical example of a large and simple trial as advocated in 1984 by Yusuf, Collins and Peto.[289] These authors state that the criteria for a good trial should always be the same with regard to common and serious conditions: a. ask an important question and, b. answer it reliably. These criteria can be used to test the possibility and the desirability of large, simple randomized trials of various widely practicable treatments for common conditions. Yusuf et al.[289] present six main reasons to support their argument for large and simple clinical trials:

1. The identification of effective treatments is likely to be more important if the disease to be studied is common than if it is rare, and studies of common conditions can be large.

2. The identification of effective treatments for common disease is likely to be more important if the treatment is widely practicable than if it is so complex that it can be performed only in specialist centers, and treatment protocols for widely practicable treatments can be simple.

3. Study of the effects of treatment on major endpoints is likely to be more important than study of the effects on minor endpoints; and follow-up protocols for the assessment only of major endpoints can often be simple.

4. No matter what prognostic features are recorded at entry, the duration of survival, etc. among apparently similar patients is likely to be rather unpredictable, so no great increase in statistical sensitivity is likely to be conferred by stratification and/or adjustment for such features. In other words, the reliability of the main treatment comparison is improved little by adjustment for initial imbalances in prognostic features, which suggests that entry protocols too can be simple.

5. The direction -though not necessarily the magnitude- of the net effects of treatment on particular modes of death is likely to be similar in many different subcategories of randomized patients. Therefore, if there is no need to subcategorize patients to decide who needs which treatment, the entry protocol can be simple.

6. If a widely practicable treatment would have a large effect on an important endpoint (e.g., mortality) in a common disease, this would probably already be known, so the true effect is likely to be null or moderate.

These arguments in favor of large and simple trials are challenged by Lubsen and Tijssen.[140] In their view a common condition, a simple treatment, and a simple outcome measure are opportunities rather than design options. Their main criticism regards the fifth argument of Yusuf et al., that the direction of the net treatment effect can be

expected to be similar across patient subgroups. They reject this particular argument because, although attractive from a statistical point of view, it has no a priori biological foundation. It is important in this context to make a distinction between 'quantitative' interactions and 'qualitative' interactions.[187] A qualitative interaction is one whereby the true treatment effects in different subgroups do not even point in the same direction, whereas a quantitative interaction is one whereby the direction of the true effect of treatment is similar, and only the size of the benefit is different. Because their is no reason to suspect a qualitative interaction in the different subgroups involved in the CLASP trial, the objections of Lubsen et al. seem to be not applicable to this trial.

The same authors further object that 'simple' in the context of entry protocols means that very few patient characteristics are recorded at entry, and that data that have not been recorded cannot be involved in the analysis or interpretation of results. Although this is certainly true, it is important to realize that there is a balance between simple entry protocols stimulating the randomization of large numbers and extensive protocols inhibiting the randomization of large numbers; minimal documentation at entry and during follow-up may be expected to increase the willingness of physicians to participate in a clinical trial.[186]

It is the sixth point of Yusuf et al., the moderateness of what can plausibly be hoped for, that has perhaps the most profound implications for trial design. It is chiefly because one should be able to distinguish reliably between moderate and null effects that trials need to be strictly randomized, analyzed, interpreted unbiasedly, and larger than is currently usual. Moderate biases, introduced by sloppy methodology (e.g. non-randomized controls, failure to use an 'intention-to-treat' analysis etc.) may not matter much in those rare instances when large treatment effects await discovery, they matter

enormously when only moderate effects are to be assessed.[289]

*CLASP in the Netherlands*

Because the trial was well designed, and because it was executed with the help of professional collaborators in the Clinical Trial Service Unit, no major problems appeared during the trial. Telephone randomization never took more than a few minutes. There was never a problem with the stocking of collaborating centers with treatment packs. In the majority of cases the follow-up forms were returned, on time and completely filled out, to the national coordinating center within six weeks after delivery of a participating patient. A major problem that appeared during the trial was a steady decrease in recruitment numbers, from an average of 20-30 per month to less than 10 per month during the final stages of the trial, most likely due to the incorporation of low-dose aspirin prophylaxis in clinical obstetric practice in the Netherlands (chapter 4). Comparing Dutch pre-randomization characteristics to overall pre-randomization characteristics, it appears that a markedly larger proportion of women were randomized in the Netherlands with the intention to prevent or treat fetal growth retardation. This trend is in accordance with the results of the inquiry presented in chapter 4.

*Trial results**

The impact of aspirin on proteinuric preeclampsia and its fetal sequelae in CLASP was certainly smaller than in some previous reviews. Moreover, despite the randomisation of more than 9000 high-risk women, there was no statistically significant effect on stillbirths or neonatal deaths, even those specifically attributed to preeclampsia. However, a potentially important effect of aspirin could be discerned on prevention or delay of

delivery with early-onset preeclampsia.

It is important to consider CLASP in the context of previous trials of antiplatelet treatment in pregnancy. Most were of aspirin, but a few were of aspirin with dipyridamole (which in pregnancy[247] or in other clinical settings[6] does not seem to produce any greater clinical benefit than aspirin alone). When the available results from all trials (including CLASP) were taken together (figure 5.6),[44] antiplatelet therapy was associated with a reduction of about a quarter in the incidence of preeclampsia. The results in the different trials are, however, not consistent with each other (test for heterogeneity $p < 0.001$). Review of the earlier small randomised trials (i.e., those with fewer than 200 women) suggested that antiplatelet therapy reduced the incidence of proteinuric preeclampsia by about three-quarters (figure 5.6: $2p < 0.00001$).[44] By contrast, the overall effect on preeclampsia in the larger trials[91,107,227,247,257] was significantly smaller, indicating reductions of only about a quarter before CLASP, or a sixth after the addition of CLASP. In absolute terms, these more modest proportional reductions imply that antiplatelet therapy would on average prevent proteinuric preeclampsia in about 1 woman per 100 treated.

There are several possible explanations for these discrepancies. All the trials that reported large effects of antiplatelet therapy on preeclampsia (and on IUGR) were very small. At least as many women are known to have been randomised in other small but unpublished trials (figure 5.6),[44] and it may be that some small trials with unpromising results have not been published because they were less remarkable. If this were true, the available results from small trials would give a biased estimate of the positive effects of antiplatelet therapy, but large trials would not. This possibility is consistent with the less extreme results observed in the larger trials.

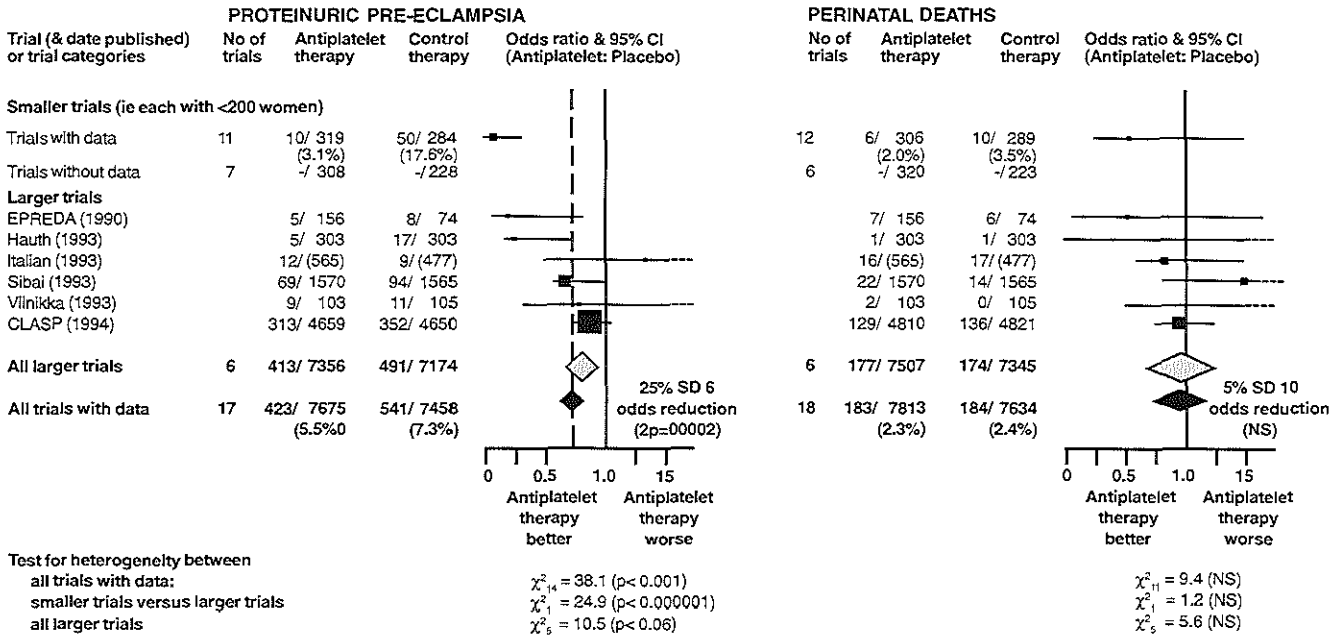


*Figure 5.6. Overview of effects of aspirin reported from all trials of antiplatelet therapy in pregnancy.*

*Symbols and conventions as in figure 5.1. Available results from smaller trials (i.e., those that included fewer than 200 women) were combined and stratified ratio plotted for these trials. Details of the trials included are given in ref.44.*

It is unlikely that differences in the definitions of preeclampsia can explain why the promising results of the early small trials are not replicated in the larger trials. The blood pressure criteria used to define preeclampsia in CLASP were at least as stringent as those used in other trials and select a high proportion of primiparae (which is a characteristic of preeclampsia) independently of proteinuria.[199] The definition of proteinuria used in CLASP of 1+ or more on stick-testing might be thought to be too sensitive in comparison with 24-hour urine protein estimates. But the control rates of proteinuric preeclampsia in CLASP were similar to those in all other trials (7.6% and 6.7%, respectively).

One theoretical possibility is that the benefits of antiplatelet therapy are confined to particular categories of women that were selectively included in the small but not the large trials. In CLASP, various characteristics were recorded at entry, and the protocol specified that the results would be analysed separately in several subgroups. Such analyses did not, however, identify any particular category of women in whom the reduction in proteinuric preeclampsia was as great as that reported in the previous small trials (figure 5.6). Similarly, there were no subgroups in whom clear effects on IUGR, stillbirths, or neonatal deaths could be demonstrated. It is still possible that some special category of women, identified in a way not tested in CLASP, can benefit substantially. If so, such women must comprise a smaller and more selected group than previously thought. Perhaps, antiplatelet therapy moderates not the disease process but some underlying susceptibility factor possessed by a minority of women. The aetiology of preeclampsia is unknown, and there is substantial variability in its presentation. Hence, it is plausible that preeclampsia might be the outcome of several different processes or susceptibilities, such that antiplatelet therapy benefits only a small proportion of women.

With respect to this last possibility, there was evidence that the impact of aspirin on the occurrence of preeclampsia was related to the duration of gestation at delivery, with little effect among women delivering at term but with a significant trend towards a substantially lower incidence among those delivering earlier (figure 5.5). Moreover, in a retrospective analysis of women entered for prophylaxis of preeclampsia, the rates of stillbirth or neonatal death attributed to preeclampsia, hypertension, or IUGR that occurred before 32 weeks of gestation (when the effect of preventing early-onset disease should be greatest) was 5.3% among women allocated aspirin compared with 10.6% among those allocated placebo.

Early-onset preeclampsia differs from that developing at term, not only in the poorer perinatal outcome resulting from the complications of prematurity, but also in higher rates of IUGR,[156] recurrence, maternal morbidity, and mortality,[228] and a marked assocation with underlying maternal disorders, especially chronic maternal hypertension[228] or renal disease.[102] Therefore, susceptibility to early preeclampsia may involve distinct factors amenable to preventive therapies, such as antiplatelet therapy, that have little effect in women who develop preeclampsia later.

The restricted benefits of antiplatelet therapy are important in relation to the possible adverse effects, the most likely of which is bleeding affecting the mother, fetus, or newborn baby. The results of CLASP are reassuring as regards the baby, with no excess of intraventricular hemorrhage, other neonatal bleeds, or mortality ascribable to bleeding. (Further follow-up of the development of the babies is in progress, and will be reported elsewhere.) In the aspirin-allocated mothers there were slightly, but not significantly, more placental abruptions and slightly fewer other ante-partum hemorrhages. About 1 in 100 more women allocated aspirin received blood transfusions after delivery. Although

this difference was significant, its size was uncertain, being consistent with an excess of as little as 1 or 2 per 1000 or as much as about 2 per 100. There was no evidence of an excess of bleeding or other complications with epidural anesthesia.

## 5.5 Conclusions

The results of the trials thus far available do not support the widespread routine prophylactic or therapeutic use of antiplatelet therapy in pregnancy among all women considered to be at increased risk of preeclampsia and/or fetal growth retardation. However, low-dose aspirin appears justified in pregnant women thought to be at high risk of early-onset preeclampsia, that is before 32 weeks of gestation. It is known from the literature that women with early preeclampsia or eclampsia constitute a group at high risk of recurrence of preeclampsia in a next pregnancy. In a study of 159 women with eclampsia in their previous pregnancy Sibai et al. found a recurrence rate of 44% in the next pregnancy if the eclampsia in the previous pregnancy had occurred at a gestational age of less than 30 weeks, and a recurrence rate of 20% when the previous eclampsia had occurred at a gestational age of more than 30 weeks.[217] Therefore, it seems appropriate to start low-dose aspirin early in the second trimester in women with a history of early preeclampsia. Because there is a familial tendency to preeclampsia,[34] and because some medical complications - including chronic hypertension, diabetes ,and renal disease - predispose to preeclampsia,[193] low-dose aspirin also seems justified in these instances.

Finally, in the CLASP study low-dose aspirin was not associated with a significant increase in placental hemorrhages or in bleeding during preparation for epidural anesthesia. Low-dose aspirin was also safe for the fetus and newborn infant, with no

evidence of an increased likelihood of bleeding. A longterm follow-up study of children born to mothers in the CLASP study is currently underway in the United Kingdom, to assess longterm adverse effects due to fetal exposure to aspirin.

Chapter 6

# EFFECTS OF LABOR AND DELIVERY ON FIBRINOLYSIS*

## 6.1 Introduction

In pregnancy impressive changes occur in coagulation and fibrinolysis thought to serve to protect the mother from the increased risk of bleeding imposed by placentation and delivery.[262] Coagulation capacity appears to be increased throughout uncomplicated pregnancy due to elevated concentrations of most plasma procoagulants, except factors XI and XIII, with a further increase in plasma concentrations of factors V, VII, and X in the first days after delivery.[262] The alterations in the fibrinolytic system are characterized by increases in tissue-type plasminogen-activator (t-PA) and in the plasminogen activator inhibitors type 1 (PAI-1) and type 2 (PAI-2) until the end of the 38th gestational week, after which they all decrease except t-PA antigen.[135] According to some authors[136] these changes result in reduction of fibrinolytic activity in pregnancy, but other investigators found no effect on overall fibrinolytic activity,[124] or observed an increase[123]. Although fibrinolysis appears to be enhanced after placental separation,[78,143] information on the effect of labor on fibrinolytic activity is limited. Prostaglandins, involved in the initiation and maintenance of labor,[116] also have an effect on fibrinolytic variables.[16]

* *The main substance of this chapter was published in: Bremer HA, Brommer EJP, Wallenburg HCS. Effects of labor and delivery on fibrinolysis. Eur J Obstet Gynecol Reprod Biol 1994; 55: 163-8.*

One report describes a significant increase in t-PA antigen levels in maternal plasma during the course of the second stage of labor.[287] In another study a significant increase in t-PA antigen levels in maternal plasma was detected immediately following childbirth, before delivery of the placenta.[211] Exact definition of the timing of sampling could be crucial in an investigation of the effects of labor and delivery on fibrinolytic variables. For that reason, we conducted a study of variables of fibrinolysis in plasma of women in whom labor was induced, which allowed standardization of sampling times in relation to the course of labor and delivery.

## 6.2 Material and Methods

*Patient population*

Ten healthy multiparous women who opted for elective induction of labor at term following an uncomplicated singleton gestation participated in the study.[256] None of the women had a history of thrombo-embolic disease. Cervical ripeness was assessed with the Burnhill score.[28] When the cervix was considered favorable, -a score greater than four- the membranes were artificially ruptured and a fluid-filled open-tip catheter was introduced transcervically into the amniotic cavity for the recording of uterine activity. An electrode was attached to the fetal scalp for fetal heart rate monitoring. Uterine contractions were induced with intravenous infusion of an incremental dose of oxytocine starting with 2mU/min, until a uterine activity of 150-200 Montevideo units was obtained.[256] In all cases induction of labor started at 08.15. a.m. Three women with uncomplicated pregnancies with a median gestational age of 38 weeks who underwent repeat elective cesarean section were also included in the study in order to compare levels

of variables of fibrinolysis in peripheral and uterine venous blood. The study protocol as approved by the University and Hospital Ethics Committee was explained, and informed consent was obtained in all cases.

*Blood sampling*

Blood samples were taken 5 minutes before the start of oxytocin infusion, at full cervical dilatation, and within 5 minutes after delivery of the placenta. A sample of 4.5ml was collected without stasis from an antecubital vein through a 19 G needle in CTAD tubes (Becton Dickinson, France, containing 0.5 ml of a solution of 0.11 M Citrate, 15 mMol Theophyllin, 3.7 mMol Adenosin, and 0.198 mMol Dipyridamole, pH 5.2) for determination of tissue-type plasminogen activator (t-PA) antigen, plasminogen activator inhibitor type-1 (PAI-1) antigen and activity, and plasminogen activator inhibitor type-2 (PAI-2) antigen. Another 4.5ml sample was collected in Stabilyte tubes (Biopool, Sweden, containing 0.5ml 0.45 M sodium citrate buffer, pH 4.3) for determination of t-PA activity. A sample of mixed free-flowing cord blood was obtained after delivery with the placenta in situ. In patients undergoing cesarean section, blood was collected simultaneously from an antecubital vein and from both uterine veins before incision of the lower uterine segment. The localization of the placenta was carefully determined and mixed cord blood was obtained immediately after delivery of the placenta. All samples were put on ice, centrifugated for 30 minutes at 2,000 g and 4°C, and plasmas were stored at -70°C until analysis.

*Assay of t-PA antigen*

t-PA antigen was determined using a commercially available enzyme immunoassay (Imulyse$^{R}$, Biopool, Umea, Sweden) that measures both free and complexed t-PA in the range of 1.5 - 30 ng/ml. The maximal sensitivity of the test was 1.5 ng/ml ; intra-assay variation was 8%, inter-assay variation 10%.

*Assay of t-PA activity*

t-PA activity was determined by the conversion of plasminogen to plasmin in the presence of soluble fibrinogen fragments, using a chromogenic substrate according to Verheijen et al.[254] The detection limit of this assay was 50 mIU/ml; intra-assay variation was 5-13%, inter-assay variation 15-20%.

*Assay of PAI-1 antigen*

PAI-1 antigen was measured with a commercially available enzyme immunoassay, using anti-PAI-1 antibodies (TintElize$^{R}$ PAI-1, Biopool, Umea, Sweden). The test measures both free PAI-1 and PAI-1 complexed to activators. The detection limit was 2 ng/ml PAI-1; the intra-assay variation was 2% at 40 ng/ml and 7% at 15 ng/ml.

*Assay of PAI activity*

PAI activity was measured by titration of diluted samples with t-PA according to Verheijen et al.[253] Results are expressed as a percentage of a standard normal plasma pool obtained from 40 healthy volunteers. Intra-assay variation was 2-10%, inter-assay variation 10-15%.

*Assay of PAI-2 antigen*

Plasminogen activator inhibitor type 2 (PAI-2) antigen was determined with a commercially available enzyme immunoassay, using anti-PAI-2 antibodies (TintElize$^{R}$ PAI-2, Biopool, Umea, Sweden. The test detects both the low molecular weight (46.6 kD) form found in placental tissue and the glycosylated high molecular weight (60 kD) form in maternal blood. The detection limit was 6 ng/ml.

*Statistical analysis*

Differences between time-related continuous variables were assessed with the Wilcoxon signed rank test. Relationships between maternal and cord variables were analyzed by Spearman's rank correlation test. Comparisons between maternal and cord samples were made with the Wilcoxon signed rank test. All p-values are two-tailed, and a level of $< 0.05$ is considered to represent statistical significance.

## 6.3 Results

General characteristics of the women who participated in the study are summarized in table 6.1.

*Table 6.1. General characteristics of women studied (n=10), shown as median (range).*

| | | |
|---|---|---|
| Age (yr) | 30 | (20-41) |
| Parity | 1 | ( 1-3 ) |
| Gestation (wk) | 39 4/7 | (38-41) |
| Birthweight (g) | 3245 | (2855-4160) |

All deliveries were uncomplicated and resulted in live, healthy infants. The individual changes in fibrinolytic variables during labor and delivery are presented in figures 6.1-6.5. The only significant change between the beginning of the induction and the end of the first stage of labor was a rise in t-PA antigen ($p= 0.01$). No significant correlation could be demonstrated between the rise in t-PA antigen and the duration of the first stage of labor. All variables, except PAI-2 antigen, showed significant changes after delivery of the placenta compared with the end of the first stage of labor. After delivery of the placenta t-PA antigen and activity showed a significant rise ($p< 0.05$), accompanied by a fall in PAI-1 antigen and PAI activity ($p< 0.01$). PAI-2 antigen concentrations showed no consistent changes throughout labor and delivery. In comparison with concentrations in maternal plasma at the end of the first stage of labor, t-PA activity in fetal plasma was significantly higher ($p< 0.01$), t-PA antigen levels were similar, and PAI-1 antigen, PAI activity and PAI-2 antigen levels were lower in cord plasma ($p< 0.001$). No significant correlations were demonstrated between the levels of fibrinolytic variables in maternal and cord plasma.

In all three women who underwent cesarean section the placenta was located centrally on the anterior or posterior uterine wall.No marked differences between left and right uterine vein values were observed. The uterine venous concentrations of the variables studied were not different from the peripheral venous levels, except for t-PA activity, which showed a median value of 0.79 IU/ml (range 0.63 - 1.89) in peripheral plasma compared with a markedly higher median value of 2.19 IU/ml (range 1.37 - 3.59) in uterine venous plasma.

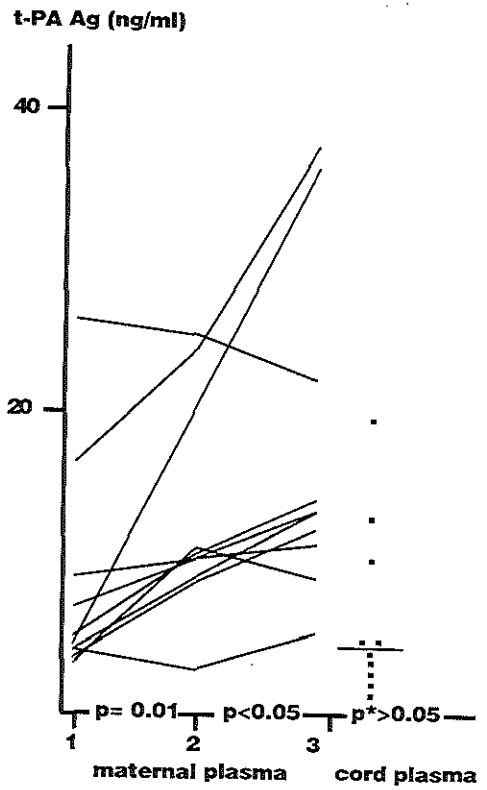


*Fig.6.1. Maternal venous and cord plasma levels of t-PA antigen. 1=5 min before oxytocin infusion; 2=at full cervical dilatation; 3=5 min after delivery of the placenta. *=compared with maternal plasma 2.*

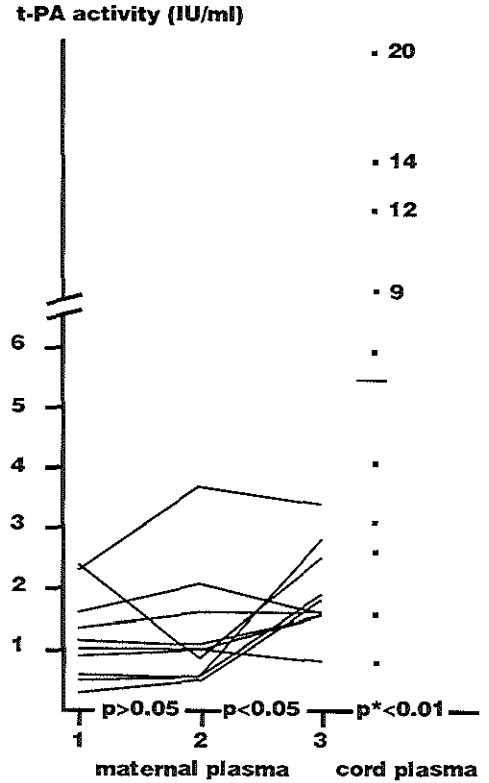


*Fig.6.2. Maternal venous and cord plasma levels of t-PA activity. Further legends as in Fig.6.1.*

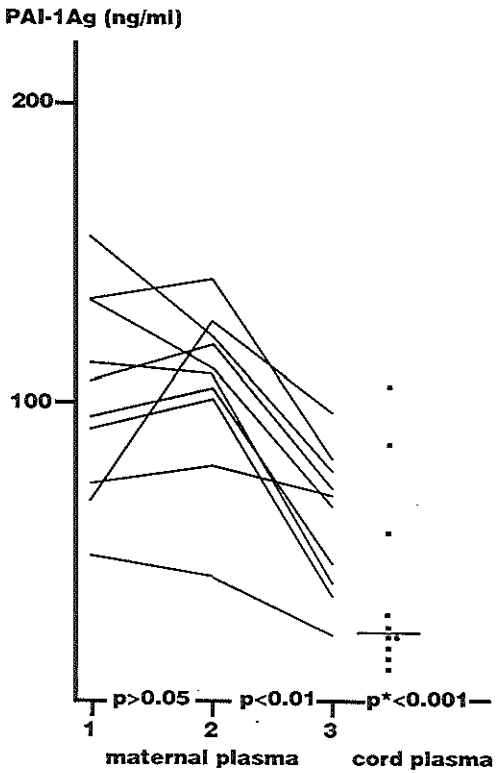


*Fig.6.3 Maternal venous and cord plasma levels of PAI-1 antigen. Further legends as in Fig.6.1.*

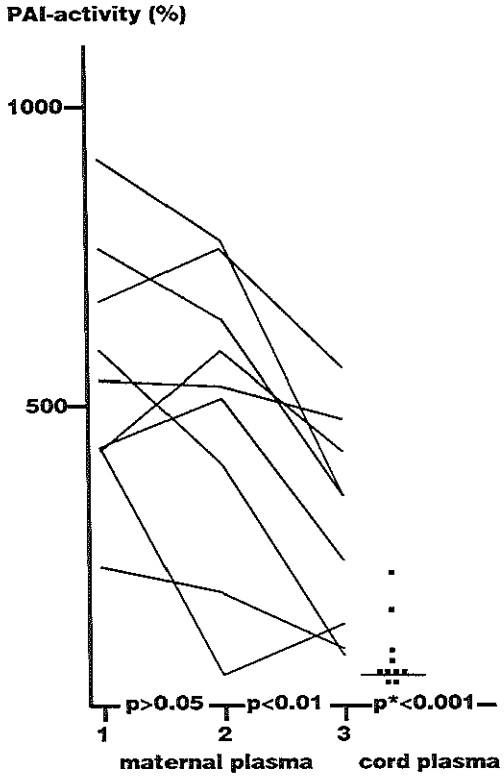


*Fig.6.4. Maternal venous and cord plasma levels of PAI activity. Further legends as in Fig.6.1.*

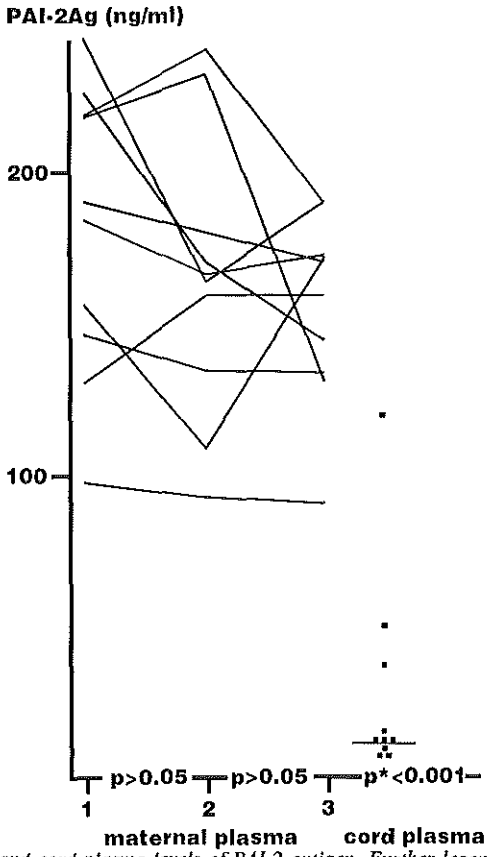


*Fig 6.5. Maternal venous and cord plasma levels of PAI-2 antigen. Further legends as in Fig. 6.1.*

## 6.4 Discussion

Previous studies of hemostasis in uncomplicated childbirth have revealed marked but variable changes in fibrinolysis and fibrinolytic markers.[143,211,287] In these studies samples were obtained from women in spontaneous labor without standardized timing of blood sampling, which may have contributed to the variability of the results. It has been shown that fibrinolytic activity increases after placental separation.[78,143,211,226,287] The results of our study show that t-PA antigen, one of the fibrinolytic variables, increases already during labor, before placental separation. This may be caused by the level of physical

exercise which is known to increase the release of t-PA from vascular endothelium,[203] possibly under neurohumoral control.[41] A positive correlation has been shown to exist between the increase in t-PA and the workload and duration of exercise,[54] but such a relationship could not be demonstrated in our study. The further rise of t-PA antigen and activity following placental separation may be explained by vascular damage in the uterine placental bed,[287] and by release by vessel walls induced by the stress of childbirth[124]. Oxytocin at levels found during active labor fails to stimulate the in vitro activity of t-PA,[151] which makes it unlikely that oxytocin administered for induction of labor is involved in t-PA release.

Because of the standardization of our study, all samples were obtained at approximately the same time of the day, which makes a contribution of circadian variation to the results unlikely. In the non-pregnant state, diurnal variations in blood fibrinolytic activity are caused by changes in the plasma levels of PAI-1, which are highest in the early morning and lowest in late afternoon.[120] However, PAI-1 production is not influenced by diurnal variations in populations with high PAI-1 levels, as in pregnancy.[113] Also, t-PA antigen levels are known to be higher in the morning compared to levels in the evening in non-pregnant individuals.[4]

An explanation for the marked fall of PAI activity following placental separation observed in our study, may be the increase in t-PA, virtually all of which is bound to PAI-1.[234] It is also possible that the rapid decline in PAI-activity after delivery may be explained by the arrest of PAI release from the endothelium of the vascular tree of the placenta after separation of the placenta.[124,135] The absence of marked changes in PAI-2 levels after delivery of the placenta may be explained by its long half-life which appears to be in the order of magnitude of several days.[124] Nevertheless, the observed increase in

t-PA activity after placental separation indicates an excess release of t-PA over PAI and thus an increase in fibrinolytic potential.

The higher levels of t-PA activity in plasma obtained from uterine veins compared to levels in peripheral veins suggest that the uterus and/or placenta are involved in the regulation of t-PA activity, as proposed earlier.[161,226] The low maternal t-PA activity as compared to cord values may be caused by the five (PAI-1) to 20-fold (PAI-2) higher PAI concentrations in maternal plasma, which reduces the amount of free unbound functionally active t-PA.[143]

In conclusion, our study shows that an increase in t-PA antigen can already be detected during labor before placental separation, with a marked further increase in fibrinolytic potential after placental separation.

Chapter 7

# EFFECT OF LOW-DOSE ASPIRIN DURING PREGNANCY ON FIBRINOLYTIC VARIABLES BEFORE AND AFTER PARTURITION*

## 7.1 Introduction

In recent years an increasing number of pregnant women at risk of pregnancy-induced hypertensive disorders and fetal growth retardation are being prescribed low-dose aspirin,[23] although its prophylactic effect remains disputed.[24,107,227] Aspirin in analgetic doses may have an anti-fibrinolytic effect, since it inhibits the endothelial release of tissue-type plasminogen activator (t-PA) after venous occlusion.[131,132] This effect could be mediated by inhibition of prostacyclin synthesis because it is reversed by a synthetic prostacyclin analogue.[16] Low-dose aspirin, 20 mg for 7 days, did not affect fibrinolytic activity after venous occlusion in healthy males.[57] Although low-dose aspirin mainly inhibits platelet thromboxane synthesis, it may also cause a small reduction in prostacyclin synthesis in nonpregnant individuals[71] and in pregnant women.[229] For that reason an effect of long term low-dose aspirin on the fibrinolytic system of the pregnant woman cannot be excluded. Modulation of the fibrinolytic system by low-dose aspirin could be of particular importance after normal placental separation, when coagulation in the placental bed is balanced by a marked increase in fibrinolytic capacity.[211]

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In the present study we compared variables of fibrinolysis and thromboxane and prostacyclin synthesis at the end of pregnancy before and immediately after parturition, in women using low-dose aspirin for prevention of preeclampsia and/or fetal growth retardation, and in women who had not taken aspirin. Because exact definition of the timing of sampling could be important in an investigation on fibrinolytic variables, we conducted this study in plasma of women in whom labor was induced, which allowed standardization of sampling times. In addition, we investigated the effect of low-dose aspirin on fibrinolytic variables in cord blood.

## 7.2 Materials and Methods

*Patients*

Twenty-four healthy pregnant women were enrolled in the study. Of these women eight took a daily dose of 60-80 mg of aspirin (1mg/kg bodyweight) from 12 weeks gestation until delivery because of a history of preeclampsia or fetal growth retardation; 16 women did not use aspirin during pregnancy and served as controls. All women opted for elective induction of labor, an accepted procedure in the Department.[256] Cervical ripeness was assessed with the Burnhill score.[28] When the cervix was considered favorable the membranes were ruptured, and a fluid-filled open-tip catheter was introduced transcervically into the amniotic cavity for the recording of uterine activity. An electrode was attached to the fetal scalp for fetal heart rate monitoring. Uterine contractions were induced with intravenous infusion of an incremental dose of oxytocin starting with 2mU/min, until a uterine activity of 150-200 Montevideo units was obtained.[256] In all cases induction of labor started at 0815 am. Low-dose aspirin users took

their last tablet at 0700 am. The study protocol as approved by the University and Hospital Ethics Committee was explained, and informed consent was obtained in all cases.

*Blood sampling*

Maternal venous blood samples were taken 5 minutes before the start of the oxytocin infusion and within 5 minutes after delivery of the placenta. All samples were obtained from an antecubital vein without occlusion through a 19 G needle.

A sample of 4.5ml was collected in CTAD tubes (Beckton Dickinson, France, containing 0.5ml 0.11 M Citrate, 15 mMol Theophyllin, 3.7 mMol Adenosin, and 0.198 mMol Dipyridamole, pH 5.5) for determination of tissue-type plasminogen activator (t-PA) antigen, plasminogen activator inhibitor type-1 (PAI-1) and type-2 (PAI-2) antigen and PAI-activity. Another 4.5ml sample was collected in Stabilyte tubes (Biopool, Sweden, containing 0.5ml 0.45 M sodium citrate buffer, pH 4.3) for determination of t-PA activity. A 9ml sample was taken in a plastic tube in 1ml disodium-ethylene-diamine-tetra-acetate (EDTA) for determination of synthesis of malondialdehyde (MDA) by stimulated platelets. For determination of 6-keto-prostaglandin $F_{1\alpha}$, ($PGF_{1\alpha}$), a stable metabolite of prostacyclin, a 5ml sample was collected in cooled plastic tubes containing 10$\mu$l heparin and 25$\mu$l indomethacin (0.1% in phosphate buffer, pH 7.4). Finally, a 5ml blood sample was collected in glass tubes and allowed to clot for determination of serum levels of thromboxane $B_2$ ($TXB_2$).

Samples of mixed free flowing cord blood were collected immediately after clamping of the cord with the placenta in situ for determination of the same set of variables.

*Analytical procedures*

Samples for determination of variables of fibrinolysis were transported on melting ice, centrifuged for 30 minutes at 2,000 g at 4°C, and stored at -70°C until analysis. Samples for determination of plasma $PGF_{1\alpha}$ were centrifuged for 10 minutes at 1,500 g at 0°C, and stored at -20°C until analysis. The clotted blood samples for determination of serum $TXB_2$ were incubated for 60 minutes at 37°C to stimulate maximum platelet $TXB_2$ release. After centrifugation for 10 minutes at 2,500 g at 20°C, serum samples were kept at -20°C until analysis.

Tissue-type plasminogen activator (t-PA) antigen was determined using a commercially available enzyme immunoassay (Imulyse®, Biopool, Umea, Sweden) that measures both free and complexed t-PA in the range of 1.5 - 30 ng/ml. The maximal sensitivity of the test was 1.5 ng/ml; intra-assay variation was 8%, inter-assay variation 10%.

t-PA activity was determined by the conversion of plasminogen to plasmin in the presence of soluble fibrinogen fragments, using a chromogenic substrate according to Verheijen et al.[254] The detection-limit of this assay was 50 mIU/ml; intra-assay variation was 5-13%, inter-assay variation was 15-20%.

Plasminogen activator inhibitor type-1 (PAI-1) antigen was measured with a commercially available enzyme immunoassay, using anti-PAI-1 antibodies (TintElize® PAI-1, Biopool, Umea, Sweden). The test measures both free PAI-1 and PAI-1 complexed to activators. The detection limit was 2 ng/ml PAI-1; the intra-assay variation was 2% at 40 ng/ml and 7% at 15 ng/ml.

PAI-activity was measured by titration of diluted samples with t-PA according to Verheijen et al.[253] Results are expressed as a percentage of a standard normal plasma pool

obtained from 40 healthy volunteers. Intra-assay variation was 2-10%, inter-assay variation was 10-15%.

Plasminogen activator inhibitor type-2 (PAI-2) antigen was determined with a commercially available enzyme immunoassay, using anti-PAI-2 antibodies (TintElize$^R$ PAI-2, Biopool, Umea, Sweden). The detection limit was 6 ng/ml.

The capacity of platelets to synthesize $TXA_2$ was determined within 30 minutes after venipuncture by measuring the amount of its stable by-product MDA produced upon stirring platelet-rich plasma with thrombin (1 IU per milliliter final concentration), as described previously.[271] The concentration of MDA was measured by means of the thiobarbituric acid reaction and expressed as nanomoles of MDA produced by $10^9$ platelets.

Plasma concentrations of $PGF_{1\alpha}$ and serum $TXB_2$ levels were determined by radioimmunoassay (E.I. Du Pont de Nemours-NEN Research Products, Boston). A Sep-Pak $C_{18}$ cartridge (Waters, Milford, Mass.) was prewashed with 10 ml of absolute ethanol, 10 ml of distilled water, and 2 ml of air. Two ml of the sample were applied to the column, followed by 2 ml of distilled water and 2 ml of air. The prostaglandin metabolites were eluated with 2 ml of absolute ethanol, followed by 2 ml of air, and the eluate was brought to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in radioimmunoassay buffer, and the assay was performed according to the instruction manual using $^{125}$I-labeled antigen.[105]

*Statistical analysis*

Because the results could not be accepted to follow a normal distribution, data are expressed as medians with 25th and 75th centiles or range. Differences between time-

related continuous variables were assessed with the Wilcoxon signed rank test. Comparisons between maternal and cord samples and between aspirin and control groups were made with the Wilcoxon signed rank test. Relationships between maternal and cord variables were analyzed with Spearman's rank correlation test. All p-values are two-tailed, and a level of $< 0.05$ is considered to represent statistical significance.

### 7.3 Results

General characteristics of the women who participated in the study are summarized in Table 7.1.

None of the aspirin treated women developed a pregnancy-induced hypertensive disorder or fetal growth retardation. All deliveries were uncomplicated and resulted in live, healthy infants.

Effects of labor and delivery on fibrinolytic markers are presented in Table 7.2. Concentrations of t-PA antigen, t-PA activity, PAI-1 antigen, and PAI-2 antigen before labor and after delivery of the placenta were not different between aspirin users and controls. PAI-activity was significantly reduced in the aspirin group as compared with controls, before as well as after parturition ($p=0.02$).

Both t-PA antigen and activity rose whereas PAI-1 antigen and PAI-activity decreased significantly during labor and delivery. These changes were not affected by low-dose aspirin and were not related to the duration of labor. PAI-2 antigen showed a small but significant ($p=0.03$) reduction during parturition in the control group that could not be demonstrated in low-dose aspirin users. No significant correlations were demonstrated between the levels of fibrinolytic variables in maternal and cord plasma. Comparison of fibrinolytic variables in cord blood in the aspirin and control group

*Table 7.1. General characteristics (median, range).*

| | Aspirin (n=8) | Control (n=16) |
|---|---|---|
| Age (yr) | 32 (26-38) | 30 (20-41) |
| Parity | 1 (1-4) | 1 (1-7) |
| Duration of use of low-dose aspirin(wk) | 25.5 (22-28) | - |
| Gestation(wk) | 38.5 (37.4 - 40.2) | 39.4 (36.5 - 42) |
| Birthweight (g) | 3305 (2780 - 3715) | 3235 (2670 - 4160) |
| Estimated bloodloss at delivery (ml) | 200 (100-600) | 200 (100-1100) |

*Table 7.2. Median (interquartile range) concentrations of variables of fibrinolysis in maternal venous plasma.*

| Plasma | Aspirin (n=8) | Control (n=16) | p(*) |
|---|---|---|---|
| t-PA antigen[1] (ng/ml) | 10.8 (6.2-12.6) | 6.5 (4.2-16.4) | n.s |
| t-PA antigen[2] (ng/ml) | 17.6 (14.9-27.2) | 13.9 (10.5-29.3) | n.s |
| p(#) | 0.01 | <0.001 | |
| t-PA activity[1] (IU/ml) | 0.8 (0.6-1.1) | 1.0 (0.4-1.6) | n.s |
| t-PA activity[2] (IU/ml) | 2.8 (1.6-3.8) | 1.7 (1.0-2.7) | n.s |
| p(#) | 0.01 | 0.003 | |
| PAI-1 antigen[1] (ng/ml) | 101.9 (52.6-127.5) | 101.5 (66.9-134) | n.s |
| PAI-1 antigen[2] (ng/ml) | 44.9 (42.9-62.7) | 66.2 (38.2-80.2) | n.s |
| p(#) | 0.01 | <0.001 | |
| PAI-2 antigen[1] (ng/ml) | 168 (146.5-209.8) | 189 (134.4-225.8) | n.s |
| PAI-2 antigen[2] (ng/ml | 160.5 (128.8-193.8) | 165.5 (130.8-192.8) | n.s |
| p(#) | n.s. | 0.03 | |
| PAI activity[1] (%) | 326 (86-428) | 556 (431-829) | 0.02 |
| PAI activity[2] (%) | 108 (70-167) | 339 (100-528) | 0.02 |
| p(#) | 0.01 | <0.001 | |

p(*): Aspirin versus control; p(#): Values obtained 5 min before oxytocin infusion[1] versus values 5 min after delivery of the placenta[2]; n.s= non significant.

*Table 7.3. Median (interquartile range) concentrations of variables of fibrinolysis and prostanoid metabolites in cord blood.*

| | Aspirin (n=8) | Control (n=16) | p* |
|---|---|---|---|
| t-PA antigen (ng/ml) | 4.5 (2.3-26) | 4.5 (3.2-7.5) | n.s |
| t-PA activity (IU/ml) | 7.5 (2.9-8.9) | 3.0 (1.5-11) | n.s |
| PAI-1 antigen (ng/ml) | 14.9 (10.6-77) | 21.2 (17.8-50) | n.s |
| PAI activity (%) | 43 (25-100) | 49 (23-78) | n.s |
| PAI-2 antigen (ng/ml) | 3.7 (0.5-68.2) | 6.6 (3.2-27) | n.s |
| 6-keto-$PGF_{1\alpha}$ (ng/l) | 626 (326-1164) | 862 (471-1182) | n.s |
| $TXB_2$ ($\mu$g/l) | 37.8 (27.7-80) | 217 (123-326) | 0.0002 |
| MDA (nmol/$10^9$platelets) | 2.2 (1.9-2.7) | 5.2 (4.7-5.8) | 0.0001 |

n.s= non significant; * = aspirin versus control

showed no significant differences (table 7.3).

Levels of $PGF_{1\alpha}$ before labor in the aspirin group (median 54.7, range 52-82.8 ng/l) were about 14% lower than in controls (median 63.3, range 50.6-73 ng/l) but the difference was not significant. Fig 7.1 shows the significant increase in $PGF_{1\alpha}$ in maternal plasma during parturition; no significant differences could be demonstrated between the aspirin and control group.

Cord plasma levels of $PGF_{1\alpha}$ were not different between groups and were about 14 times higher than maternal plasma concentrations ($p<0.001$).

Before labor maternal MDA formation in the aspirin group (median 0.52, range 0.48 - 1.00 nmol/$10^9$ platelets) and serum $TXB_2$ concentrations (median 20,8 range 12,0 - 40,2 $\mu$g/l) were significantly lower than those in controls ( MDA median 5.4, range 4.7 - 6.4 nmol/$10^9$ platelets; $TXB_2$ median 281 range 205 - 411 $\mu$g/l; $p< 0.0001$). No significant changes were observed in platelet MDA formation and in serum $TXB_2$ concentrations during parturition in aspirin users and in controls.

In the control group no differences could be demonstrated between platelet MDA synthesis and serum $TXB_2$ concentrations in maternal and cord samples, but in low-dose aspirin users MDA synthesis and serum $TXB_2$ concentrations were significantly reduced in maternal compared with cord samples ($p<0.001$).

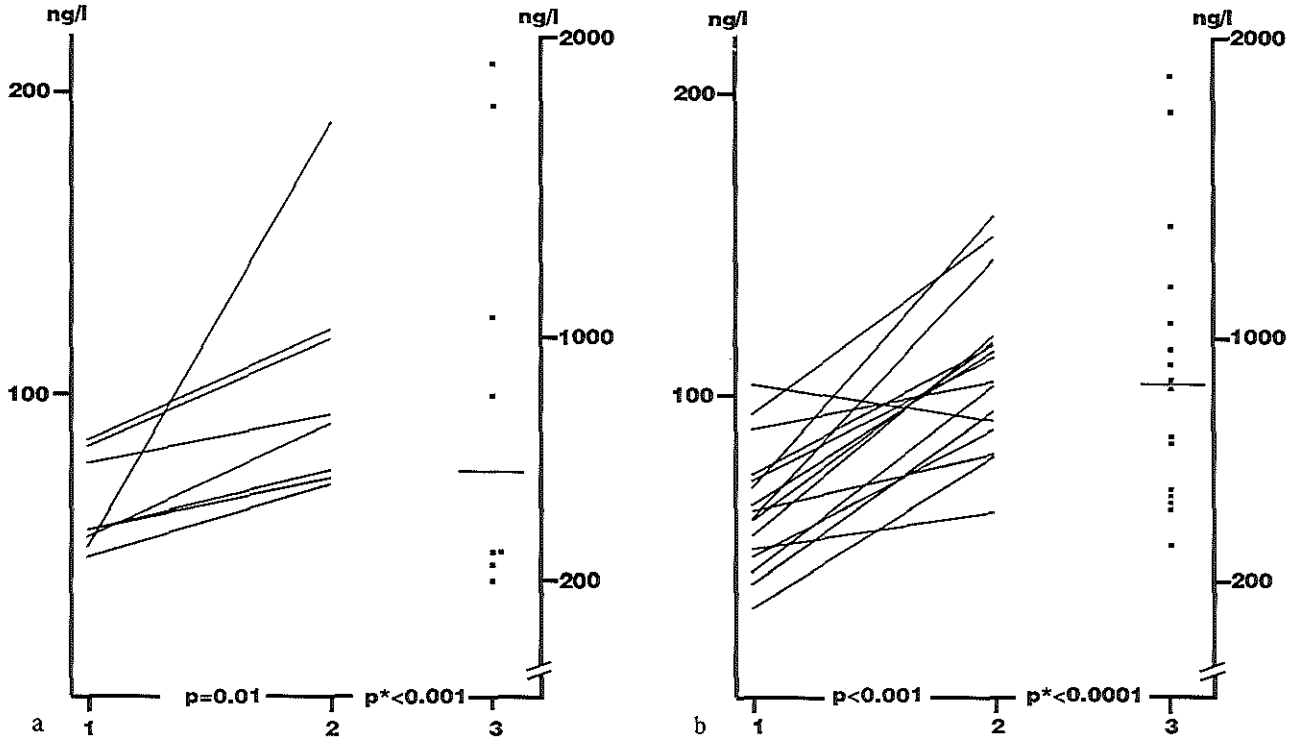


*Figure 7.1. Maternal venous and cord plasma levels of 6-keto-$PGF_{1\alpha}$ in women on long-term low-dose aspirin (a) compared to controls not using aspirin (b). 1= before parturition; 2= after parturition; 3= cord plasma. * = compared with maternal plasma 2.*

## 7.4 Discussion

Results of previous studies suggest that a daily dose of 60-80 mg of aspirin has no demonstrable effect on hemostasis in pregnant women as indicated by the bleeding time,[14,281] but no data could be found in the accessible literature on the effect on the fibrinolytic system. Throughout pregnancy marked alterations occur in the fibrinolytic system, characterized by increases in plasma concentrations of t-PA and its inhibitors PAI-1 and PAI-2 until the end of the 38th week of pregnancy, after which plasma concentrations of all these substances decrease, except those of t-PA antigen.[135] In a recent report on the effects of parturition on fibrinolysis, a significant increase in maternal plasma concentrations of t-PA antigen during labor was demonstrated, with a marked further increase in fibrinolytic potential after placental separation.[22] In previous studies a high dose of aspirin (650 mg) ingested 18 and 2 hours before blood sampling was shown to inhibit the release of t-PA antigen by vascular endothelium induced by venous occlusion.[131,132] A study of the effect of 20 mg aspirin taken for seven consecutive days showed no significant effect on fibrinolytic activity, measured by the euglobulin lysis area and the euglobulin lysis time.[57] In our study no effect of the longterm use of low-dose aspirin on t-PA activity was observed. The only fibrinolytic variable affected by the use of low-dose aspirin during pregnancy was PAI-activity, which was significantly reduced in the aspirin group before as well as after labor. This could lead to a relative dominance of t-PA activity over PAI and hence to an increased fibrinolytic potential in low-dose aspirin users. The question whether or not this causes an increase in effective fibrinolysis in vivo remains to be answered. The observed significant changes in levels of all fibrinolytic markers during parturition, except PAI-2, may be caused by vascular damage in the uterine placental bed and appear to remain unaffected by low-dose aspirin.[287] In the

nonpregnant state, diurnal variations in blood fibrinolytic activity are caused by changes in the plasma levels of PAI-1, which are highest in the early morning and lowest in late afternoon.[120] However, PAI-production is not influenced by diurnal variations in populations with high PAI levels, as in pregnancy.[113] Because of the standardization of our study all samples were obtained at approximately the same time of the day, which makes a contribution of circadian variation to the results unlikely.

We could not demonstrate an inhibitory effect of the longterm intake of 60-80mg of aspirin on maternal prostacyclin synthesis, which confirms earlier observations by ourselves and by others.[14,267] The observed increased synthesis of prostacyclin during labor confirms earlier observations and appears also to be unaffected by low-dose aspirin.[284]

It is known that in normal pregnancy platelet reactivity increases, not only associated with increasing activity of the cyclooxygenase pathway but also with an elevated sensitivity of the platelet membrane.[137] Previous studies have shown that approximately 90-95% inhibition of maternal platelet reactivity can be achieved with a daily dose of 60 mg of aspirin,[137,264] as is confirmed in the present study. Negatively charged phospholipids are known to stimulate PAI activity[125] and platelets, offering a net negative charge upon activation, may provide a site for activation of PAI.[136] Therefore, the reduced platelet reactivity induced by low-dose aspirin could be responsible for the reduced PAI-activity in the aspirin group.

Chapter 8

# GENERAL CONCLUSIONS AND PRACTICAL IMPLICATIONS

A synthesis will be presented of the results of the studies reported in this thesis in an attempt to formulate guidelines for the application of low-dose aspirin in obstetric practice.

## 8.1 General conclusions.

1. There appears to be a sound scientific rationale for attempts to prevent and treat maladaptation disorders in pregnancy through manipulation of the prostanoid cascade. In theory this can be achieved by selective dietary or pharmacologic inhibition or stimulation of the endogenous synthesis of prostanoids, and by modulation of the effects of prostanoids on end organ receptors. The effects of modulation of prostanoid synthesis in pregnancy by dietary supplementation with polyunsaturated fatty acids, in particular fish oil, on the prevention of hypertensive disorders are as yet completely speculative, but they deserve further investigation. It is concluded that selective pharmacologic inhibition of platelet thromboxane formation appears to yield the most promising clinical results.
2. A large body of observational data has provided no scientific evidence that therapeutic doses of aspirin are associated with teratogenicity, increased maternal or

fetal bleeding tendency, premature closure of the ductus arteriosus, or any other complication of pregnancy. The results of ten controlled trials involving over 5400 pregnant women with various risk factors indicate that selective inhibition of platelet thromboxane synthesis with a low daily dose of aspirin in the second and third trimester may significantly reduce the incidence of preeclampsia and fetal growth retardation. Such evidence is not available with regard to the prophylactic effects of low-dose aspirin in pregnant women with antiphospholipid antibodies. Also, the potential benefits of therapeutic low-dose aspirin in pregnant women with established pregnancy-induced hypertensive disease or fetal growth retardation remain to be established. It is concluded that, although low-dose aspirin appears to be safe in pregnancy, it should not be adopted into routine obstetric practice until reliable risk-benefit ratios have been determined in large clinical trials.

3. Between 1989 and 1991 a striking increase of about 25% appears to have occurred in the number of gynecologists in The Netherlands prepared to prescribe low-dose aspirin in pregnancy for prophylactic or therapeutic reasons. The increase in popularity of the use of low-dose aspirin in pregnancy may be explained by the publication of the promising results of three randomized controlled clinical trials published in the years 1989-1991, and perhaps also by the recruitment for the CLASP trial.

4. In CLASP, a randomized, placebo-controlled, double-blind, clinial trial on the effects of low-dose aspirin in pregnancy on preeclampsia and fetal growth retardation covering 9364 pregnancies, the impact of low-dose aspirin on the prevention of pree-clampsia and its sequelae was smaller than in the earlier reports mentioned in chapter 2. There was no evidence of a therapeutic effect of low-dose aspirin. The use of aspirin was associated with a non-significant reduction of 12% in the incidence of

preeclampsia, and no significant effect on birthweight was detected. However, a significant trend was found towards progressively greater reduction in the development of preeclampsia in aspirin-treated women in early as compared to later pregnancy, suggesting that the prophylactic use of low-dose aspirin moves the occurrence of preeclampsia forward to later stages of pregnancy, at wich time the risk for the fetus and neonate due to prematurity becomes smaller.

A further important contribution of CLASP to our knowledge on the use of aspirin in pregnancy is the reassurence provided as to the safety of aspirin in pregnancy for both the pregnant woman and her baby.

5. Tissue-type plasminogen activator (t-PA), one of the fibrinolytic variables, increases during labor, before placental separation, possibly due to physical exercise, known to increase the release of t-PA from vascular endothelium. After placental separation a marked further increase in fibrinolytic potential can be detected, represented by a further increase in t-PA and a decrease of its inhibitor-type 1 (PAI-1). In conclusion, an increase in t-PA antigen can already be detected during labor before placental separation, with a marked further increase in fibrinolytic potential after placental separation.
6. A study on the effect of a daily oral dose of 60-80 mg of aspirin from 12 weeks gestation until delivery on fibrinolytic variables before and after parturition, showed in the aspirin group a significant reduction of Plasminogen Activator Inhibitor (PAI)-activity before and after parturition. It is concluded that low-dose aspirin reduces PAI-activity and platelet reactivity before and after parturition, and that this reduction in PAI-activity may be caused by the inhibition of platelet reactivity.

## 8.2 Low-dose aspirin in obstetric practice.

Acetylsalicylic acid, registered under the name 'aspirin' since 1899, is the most frequently consumed drug in pregnancy, taken mostly without prescription because of headache or a minor ailment. Until recently many gynecologists advised against the use of aspirin in pregnancy, as a result of a general tendency to discourage all drug-taking in pregnancy as well as due to fears of specific complications of aspirin, such as teratogenic effects, maternal and fetal hemorrhage, and preterm closure of the ductus arteriosus. The negative attitude towards the use of aspirin in pregnancy changed following the publication of reports that a daily low-dose aspirin may prevent the occurrence of preeclampsia and fetal growth retardation in women at risk, and could reduce the severity of these complications once they have developed; a review of small randomized trials suggested that antiplatelet therapy reduced the incidence of preeclampsia by about three-quarters. An anonymous written inquiry held among gynecologists in The Netherlands in 1989 and repeated in 1991 showed an increase from 53% to 79% in the preventive use, and from 25% to 48% with therapeutic intentions (Chapter 4).

However, the results of the CLASP trial did not confirm the benefits of low-dose aspirin in pregnancy as promised earlier by smaller controlled studies. When the available results from all trials, including CLASP, are taken together, the use of antiplatelet therapy is associated with a reduction of about 25% in the incidence of preeclampsia (Chapter 5). The results of available studies do not support the routine prophylactic or therapeutic use of low-dose aspirin in pregnant women considered to be at low or moderate risk of preeclampsia or fetal growth retardation. Women with early preeclampsia or eclampsia constitute a group at high risk of recurrence of preeclampsia in

a next pregnancy. In a study of 159 women with eclampsia in their previous pregnancy Sibai et al. found a recurrence rate of 44% if the eclampsia had occurred at a gestational age of less than 30 weeks, and of 20% if it had occurred after 30 weeks.[230] Wallenburg and Visser found a recurrence rate of 14% if preeclampsia had occurred before 30 weeks of gestation and 9% if it had occurred after 30 weeks (unpublished). Therefore, it seems appropriate to start low-dose aspirin early in the second trimester in women with a history of early preeclampsia. Because of the familial tendency to preeclampsia,[36] and because some medical complications - including chronic hypertension, diabetes, and renal disease - predispose to preeclampsia,[205] the prophylactic use of low-dose aspirin also seems justified in these instances. Thus far, none of the studies reported justifies the therapeutic use of low-dose aspirin in pregnancy.

# SUMMARY

Chapter 1.

In chapter 1 a general introduction is presented. After a summary of the epidemiology and pathophysiology of pregnancy-induced hypertensive disease, the prophylactic and therapeutic use of low-dose aspirin is introduced. The objectives of this thesis are summarized as follows:

1. To review the literature on the general principles of manipulation of prostaglandin synthesis in pregnancy.
2. To review the literature on the prophylactic and therapeutic use of - low-dose - aspirin in pregnancy.
3. To describe changes in patterns of prescription by Dutch gynecologists of low-dose aspirin for prevention and treatment of pregnancy-induced hypertensive disorders and fetal growth retardation.
4. To discuss the design, the execution, the results and the clinical consequences of the Collaborative Low-dose Aspirin Study in Pregnancy (CLASP).
5. To investigate the effects of labor on variables of the fibrinolytic system.
6. To investigate the effects of low-dose aspirin during pregnancy on fibrinolytic and eicosanoid variables before and after parturition.
7. To formulate guidelines for the application of low-dose aspirin in obstetric practice.

Chapter 2.

Based on theoretical considerations, prevention and early treatment of maladaptation disorders in pregnancy could be achieved by dietary or pharmacologic manipulation of prostanoid synthesis. The effects of dietary supplementation with polyunsaturated fatty acids on the prevention of hypertensive disorders and fetal growth retardation are speculative. Pharmacologic inhibition of phospholipases, isomerases, and thromboxane receptors, or stimulation of prostacyclin synthesis, have undergone clinical testing with disappointing results. Selective inhibition of platelet thromboxane formation using low-dose aspirin appears to yield promising clinical results.

Chapter 3.

The results of ten controlled trials involving over 5400 pregnant women with various risk factors indicate that selective inhibition of platelet thromboxane synthesis with daily low-dose aspirin in the second and third trimester of pregnancy may significantly reduce the incidence of preeclampsia and fetal growth retardation. Such evidence is not available with regard to the prophylactic effect of low-dose aspirin in pregnant women with antiphospholipid antibodies. There is no evidence that therapeutic doses of aspirin in pregnancy are associated with teratogenicity, increased maternal or fetal bleeding, premature closure of the ductus arteriosus, or any other complication of pregnancy. The potential benefits of therapeutic low-dose aspirin for pregnancy-induced hypertensive disease or fetal growth retardation remain to be established.

Chapter 4.

With the objective to describe patterns of prescription of low-dose aspirin in pregnancy an anonymous written inquiry was held in 1989 and 1991 among gynecologists in the Netherlands (619 in 1989 and 618 in 1991) practicing in training and non-training hospitals. The response rates were 52% in 1989 and 58% in 1991, covering approximately 61% and 62%, respectively, of the practicing gynecologists in the Netherlands. During the period of the study the use of low-dose aspirin for prevention increased from 53% to 79% and for treatment from 25% to 48%, without increasing evidence for its benefits from the literature published in the same time period.

Chapter 5.

The Collaborative Low-dose Aspirin Study in Pregnancy (CLASP) was performed from 1988-1993 as a randomized, double-blind, placebo-controlled trial of low-dose aspirin for the prevention or treatment of pre-eclampsia and/or fetal growth retardation. Women between 12 and 32 weeks of pregnancy received a daily dose of 60 mg of aspirin or matching placebo until delivery if they were thought to be at higher than average risk of developing severe preeclampsia and/or fetal growth retardation. A total of 9364 women from 213 centers in 16 countries were randomized; among them 486 from the 19 participating Dutch centers. The impact of low-dose aspirin on the prevention of preeclampsia and its sequelae was smaller in CLASP than in the earlier reports discussed in Chapter 2, possibly due to the inclusion of relatively low-risk patients in the CLASP-trial. The prophylactic use of aspirin was associated with a decrease of 13% in the

incidence of preeclampsia, but the reduction was not statistically significant. No significant effect on birthweight was detected. A significant trend was found towards progressively greater reduction in the development of preeclampsia in aspirin treated women in early as compared to later pregnancy. There was no evidence of a therapeutic effect of low-dose aspirin.

Finally, CLASP provided support with regard to the safety of aspirin for the pregnant woman and her baby.

Chapter 6.

A study was conducted on fibrinolytic variables in plasma of ten healthy multiparous women in whom labor was induced, which allowed standardization of sampling times in relation to the course of labor and delivery. Variables determined were tissue-type plasminogen activator (t-PA) and the plasminogen activator inhibitors type 1 (PAI-1) and type 2 (PAI-2). The only significant change between the beginning of the induction of labor and the end of the first stage of labor was a rise in t-PA antigen. All variables, except PAI-2 antigen, changed significantly after delivery of the placenta: t-PA antigen and activity showed an increase, accompanied by a fall in PAI-1 antigen and activity. T-PA activity in cord plasma was higher in comparison with maternal plasma concentrations at the end of the first stage of labor, t-PA antigen levels were similar, and PAI-1 antigen and activity and PAI-2 antigen were lower in cord plasma.

The study shows an activation of maternal fibrinolysis that can already be detected during labor, with a marked further increase after placental separation.

Chapter 7.

The effects were assessed of a daily oral dose of low-dose aspirin from 12 weeks' gestation until delivery on fibrinolytic variables before and after parturition. Labor was electively induced in 24 patients, 8 on low-dose aspirin and 16 controls. Fibrinolytic variables determined in maternal and cord plasma were t-PA antigen and activity, PAI-1 antigen, PAI-activity and PAI-2 antigen. Metabolites of endothelial prostacyclin and platelet thromboxane $A_2$ were also determined. The only maternal fibrinolytic variable affected by low-dose aspirin was PAI-activity, which showed a significant reduction before and after parturition of 40% and 70%, respectively, in low-dose aspirin users compared to controls. Concentrations of thromboxane $B_2$ in women using low-dose aspirin were 7% (maternal serum) and 17% (cord serum) of values in controls, but concentrations of 6-keto-prostaglandin $F_{1\alpha}$ were not affected.

It is concluded that low-dose aspirin reduces PAI activity and platelet reactivity, but not prostacyclin synthesis, before and after parturition. The reduction in PAI activity may be caused by inhibition of platelet reactivity.

Chapter 8.

The conclusions with regard to the objectives presented in Chapter 1 are as follows:

1. Prevention of maladaptation in pregnancy through manipulation of prostanoid synthesis may be achieved by selective inhibition of platelet thromboxane formation.

2. Low-dose aspirin selectively inhibits platelet thromboxane synthesis. Aspirin appears to be safe in pregnancy, but its prophylactic use in obstetric practice should await the establishment of reliable risk - benefit ratios.

3. Between 1989 and 1991 a striking increase occurred in the number of gynecologists in the Netherlands prepared to prescribe low-dose aspirin in pregnancy for prophylactic reasons. This increase was not based on an increase in reported scientific evidence.

4. In CLASP the use of low-dose aspirin was associated with a reduction in the incidence of preeclampsia and its sequelae that did not reach statistical significance. A significant trend was found, however, towards a progressively greater reduction in the incidence of preeclampsia in aspirin treated women in early as compared to later pregnancy. Aspirin appeared to be safe for mother and baby.

5. An increase in tissue-type plasminogen activator (t-PA) antigen was detected during labor before placental separation, with a marked further increase in fibrinolytic potential after placental separation.

6. Low-dose aspirin reduces the activity of plasminogen activator inhibitor (PAI) and platelet reactivity before and after parturition. The reduction in PAI-activity may be caused by inhibition of platelet reactivity.

7. It seems appropriate to start low-dose aspirin (60 mg/day) early in the second trimester in women with a history of early preeclampsia. Because of the familial tendency to preeclampsia, and because some medical complications - including chronic hypertension, diabetes, and renal disease - predispose to preeclampsia, the prophylactic use of low-dose aspirin also seems justified in these instances. There is no evidence to support the therapeutic use of low-dose aspirin in pregnancy.

# SAMENVATTING

Hoofdstuk 1

In hoofdstuk 1 wordt een algemene inleiding tot het proefschrift gegeven. Na een samenvatting van de epidemiologie en pathofysiologie van zwangerschapshypertensie en preëclampsie, wordt het profylactische en therapeutische gebruik van aspirine in de zwangerschap besproken.

De doelstellingen van het proefschrift worden als volgt samengevat:

1. Een overzicht geven van de literatuur met betrekking tot de algemene principes van manipulatie van de prostaglandine synthese in de zwangerschap.
2. Een overzicht geven van de literatuur over het profylactische en therapeutische gebruik van een lage dosis aspirine in de zwangerschap.
3. Het beschrijven van veranderingen in het voorschrijf-gedrag van Nederlandse gynaecologen met betrekking tot aspirine in de zwangerschap ter preventie of behandeling van zwangerschapshypertensieve aandoeningen en foetale groeivertraging.
4. Het bespreken van de opzet, de uitvoering, de resultaten en de klinische consequenties van het CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) onderzoek.
5. Het onderzoeken van het effect van de baring op variabelen van het fibrinolytische systeem.
6. Het onderzoeken van het effect van het dagelijkse gebruik van een lage dosis aspirine gedurende de zwangerschap op het fibrinolytische systeem vóór en na de baring.
7. Het formuleren van richtlijnen met betrekking tot het voorschrijven van een lage dosis aspirine in de verloskundige praktijk.

Hoofdstuk 2

Op theoretische gronden kan preventie en/of vroege behandeling van aandoeningen die een gevolg zijn van maladaptatie in de zwangerschap worden uitgevoerd door manipulatie van het prostaglandine systeem via het dieet of langs farmacologische weg. De resultaten van pogingen om met een dieet rijk aan meervoudig onverzadigde vetzuren zwangerschapshypertensieve aandoeningen en foetale groeivertraging te voorkomen zijn tot heden niet overtuigend. De klinische resultaten van farmacologische interventie door remming van phospholipasen of isomerasen, het gebruik van thromboxaan receptor antagonisten of stimulering van de prostacycline synthese zijn eveneens teleurstellend. Selectieve remming van de vorming van thromboxaan door thrombocyten door middel van een dagelijkse lage dosis aspirine biedt perspectief.

Hoofdstuk 3

Een overzicht van 10 gecontroleerde onderzoeken met ruim 5400 zwangeren laat zien, dat selectieve remming van de thromboxaan produktie door thrombocyten door middel van een dagelijkse lage dosis aspirine gedurende het tweede en derde trimester van de zwangerschap bij vrouwen met verschillende risicofactoren de kans op het ontstaan van preëclampsie en/of foetale groeivertraging kan verkleinen. Een dergelijk effect is niet aangetoond bij zwangeren met antilichamen tegen fosfolipiden. Er zijn geen aanwijzingen, dat het gebruik van aspirine in de zwangerschap in therapeutische doseringen samengaat

met een verhoogde kans op foetale afwijkingen, een verhoogde maternale en/of foetale bloedingsneiging, het prematuur sluiten van de ductus arteriosus, of enige andere complicatie. Het therapeutisch voorschrijven van een lage dosis aspirine bij reeds bestaande preëclampsie of foetale groeivertraging is niet bewezen effectief.

Hoofdstuk 4

In 1989 werd een enquête gehouden onder de Nederlandse gynaecologen naar het voorschrijven van aspirine in de zwangerschap. Deze enquête werd herhaald in 1991. De respons was 52% in 1989 en 58% in 1991. In 1989 schreef 53% van de respondenten aspirine voor ter voorkoming van preëclampsie of foetale groeivertraging; in 1991 was dit gestegen tot 79%. De cijfers voor behandeling van reeds bestaande preëclampsie en groeivertraging waren respectievelijk 25% en 48%. De toeneming van de bereidheid van de gynaecologen om voor de genoemde indicaties een lage dosis aspirine voor te schrijven was niet gebaseerd op in de onderzochte periode verschenen literatuur.

Hoofdstuk 5

Het CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) onderzoek, een gerandomiseerd, dubbel-blind, placebo-gecontroleerd multicenter onderzoek naar het effect van een dagelijkse lage dosis aspirine in de zwangerschap ter voorkoming of behandeling van preëclampsie en/of foetale groeivertraging werd uitgevoerd van 1988 -

1993. Vrouwen met een zwangerschapsduur van 12-32 weken gebruikten dagelijks 60 mg aspirine of placebo tot aan de bevalling, als zij naar de mening van hun gynaecoloog een verhoogd risico liepen op het ontwikkelen van preëclampsie of foetale groeivertraging. In het totaal werden 9364 zwangeren door 213 centra uit 16 landen aangemeld, waaronder 486 uit 19 Nederlandse centra.

Het resultaat van de CLASP met betrekking tot het voorkomen van preëclampsie was minder positief dan in voorgaande onderzoeken. Vergeleken met placebo ging het gebruik van een lage dosis aspirine gepaard met een vermindering van 13% in de incidentie van preeclampsie, een verschil dat statistisch niet significant was. Een significant verschil in geboortegewicht kon evenmin worden aangetoond. Wel werd een significante trend gevonden tot het minder voorkomen van vroege preëclampsie bij aspirine gebruikende zwangeren. Het onderzoek gaf geen aanwijzingen voor een therapeutisch effect van een lage dosis aspirine. Aspirine in de zwangerschap bleek veilig voor moeder en kind.

Hoofdstuk 6

Bij 10 gezonde multipare zwangeren werd het effect bestudeerd van de baring op variabelen van de fibrinolyse. Door de baring electief in te leiden werd het verzamelen van de bloedmonsters gestandaardiseerd. Bepaald werden tissue type plasminogen activator (t-PA) en de plasminogen activator inhibitor type 1 en 2. Tussen het begin van de inleiding en het eind van de ontsluitingsfase trad alleen een significante stijging op van het t-PA antigeen. Alle variabelen, met uitzondering van PAI-2 antigenen, veranderden statistisch significant na de geboorte van de placenta: t-PA antigeen en activiteit stegen,

terwijl PAI-1 antigeen en activiteit significant daalden. De t-PA activiteit in navelstrengplasma was hoger dan in het plasma van de moeder aan het eind van de ontsluitingsfase; de t-PA antigeen spiegels waren vergelijkbaar en PAI-1 antigeen en activiteit en PAI-2 antigeen waren lager in navelstrengplasma.

Dit onderzoek toont aan dat activatie van de fibrinolyse plaatsvindt gedurende de baring met een sterke toeneming na de geboorte van de placenta.

Hoofdstuk 7

Bij 24 zwangeren, 8 met aspirine en 16 controles, werd het effect bestudeerd van een dagelijkse lage dosis aspirine gedurende de zwangerschap op fibrinolytische parameters gedurende de baring. Door de baring electief in te leiden werd het verzamelen van de bloedmonsters gestandaardiseerd. In moederlijk en navelstrengplasma werden t-PA antigeen en activiteit, PAI-1 antigeen, PAI-activiteit en PAI-2 antigeen bepaald. Tevens werden metabolieten van thromboxaan $A_2$ en prostacycline gemeten. De enige maternale variabele van de fibrinolyse die werd beïnvloed door het gebruik van aspirine was de PAI-activiteit, waarin reducties van 40% en 70%, respectievelijk vóór en na de bevalling, werden aangetoond bij aspirine gebruikende zwangeren vergeleken met controles. Bij aspirine gebruikende zwangeren waren de concentraties van thromboxaan $B_2$ in maternaal en navelstrengplasma respectievelijk 7% en 17% van de waarden bij controles, maar de plasmaconcentraties van 6-keto-prostaglandine $F_{1\alpha}$ waren niet verschillend.

Geconcludeerd wordt dat een lage dosis aspirine de PAI-activiteit en thrombocyten reactiviteit onderdrukt, maar de prostacycline synthese intact laat. De vermindering van

de PAI-activiteit wordt mogelijk veroorzaakt door de onderdrukking van de reactiviteit van de thrombocyten.

Hoofdstuk 8

De conclusies betreffende de doelstellingen geformuleerd in hoofdstuk 1 zijn:

1. Preventie van maladaptatie in de zwangerschap door manipulatie van het prostaglandine systeen kan worden verkregen door selectieve remming van de thromboxaan produktie door thrombocyten.
2. Een dagelijkse lage dosis aspirine in de zwangerschap remt de synthese van thromboxaan door thrombocyten. Aspirine lijkt veilig in de zwangerschap, maar het profylactische gebruik gebruik in de obstetrische praktijk dient nader te worden bestudeerd.
3. Tussen 1989 en 1991 was er een opvallende toename van de bereidheid van Nederlandse gynaecologen om in de zwangerschap aspirine voor te schrijven. Deze toename was niet gebaseerd op in die tijd beschikbaar gekomen nieuwe gegevens.
4. Uit het CLASP-onderzoek blijkt een verminderde incidentie van preëclampsie bij het gebruik van een lage dosis aspirine, maar het verschil met placebo is niet statistisch significant. Wel blijkt er bij zwangeren die een lage dosis aspirine gebruiken een significante trend te bestaan tot verminderd optreden van vroege preëclampsie. Aspirine is veilig voor moeder en kind.
5. Tijdens de baring werd een toename van tissue-type plasminogeen activator (t-PA) antigeen gevonden; een duidelijke toename van de fibrinolytische potentie werd gezien

na de geboorte van de placenta.

6. Een dagelijkse lage dosis aspirine verminderde de activiteit van plasminogeen activator inhibitor (PAI) en de thrombocyten reactiviteit voor en na de bevalling. De vermindering van de PAI-activiteit wordt mogelijk verklaard door de verminderde reactiviteit van de thrombocyten.
7. Het voorschrijven van een dagelijkse lage dosis aspirine (60 mg) vanaf het tweede trimester tot aan de baring lijkt geïndiceerd bij zwangeren met een anamnese van vroege preëclampsie. Gezien de familiaire tendens tot het ontwikkelen van preëclampsie en omdat preëclampsie vaker wordt gezien bij chronische hypertensie, diabetes, en nierziekten valt het te overwegen ook in deze gevallen ter profylaxe in de zwangerschap een lage dosis aspirine voor te schrijven. Er is zijn geen aanwijzingen die het therapeutische gebruik van een lage dosis aspirine in de zwangerschap rechtvaardigen.

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