*Chronobiology International*, Early Online: 1–20, (2012)

ISSN 0742-0528 print/1525-6073 online
DOI: 10.3109/07420528.2012.717455

informa
healthcare

# Chronotherapy With Low-Dose Aspirin for Prevention of Complications in Pregnancy

Diana E. Ayala,[1] Rafael Ucieda,[2] and Ramón C. Hermida[1]

[1]*Bioengineering & Chronobiology Laboratories, University of Vigo, Campus Universitario, Vigo, Pontevedra, Spain,* [2]*Obstetric Physiopathology Service, Obstetrics and Gynecology Department, Hospital Clínico Universitario, Santiago de Compostela, A Coruña, Spain*



Preeclampsia and gestational hypertension are major contributors to perinatal morbidity and mortality. Several studies aimed to test the effects of low-dose aspirin (ASA) in the prevention of preeclampsia concluded that the beneficial effects of such treatment outweigh adverse ones. Such benefits have not been fully corroborated by larger randomized trials usually carried out in low-risk women, testing a dose of 60 mg/d ASA presumably ingested in the morning, and including women randomized as late as at 26–32 wks of gestation. The authors conducted a prospective, randomized, double-blind, placebo-controlled, chronotherapy trial on 350 high-risk pregnant women (183 nulliparous), 30.7 ± 5.3 (mean ± SD) yrs of age, and 13.5 ± 1.4 wks of gestation at the time of recruitment. Women were randomly assigned to one of six groups, defined according to treatment (placebo or ASA, 100 mg/d) and time of treatment: upon awakening, 8 h after awakening, or at bedtime. Intervention started at 12–16 wks of gestation and continued until delivery. Blood pressure (BP) was measured by ambulatory monitoring (ABPM) for 48-h at baseline, every 4 wks until the 7th month of gestation, every 2 wks thereafter until delivery, and at puerperium. The effects of ASA on ambulatory BP were markedly dependent on administration time: there was no effect on BP, compared with placebo, when ASA was ingested upon awakening, but the BP reduction was highly statistically significant when low-dose ASA was ingested 8 h after awakening and, to a greater extent, at bedtime ($p < .001$). At puerperium, 6–8 wks after discontinuation of treatment, there was no statistically significant difference in 24-h BP means between the groups of women who ingested ASA at different circadian times. Women ingesting low-dose ASA, compared with placebo, evidenced a significantly lower hazard ratio (HR) of serious adverse outcomes, a composite of preeclampsia, preterm delivery, intrauterine growth retardation (IUGR), and stillbirth (.35, 95% confidence interval [CI]: .22–.56; $p < .001$). The HR of individual outcome variables, i.e., preeclampsia, preterm delivery, IUGR, and gestational hypertension, were also significantly lower with ASA versus placebo ($p$ always $< .041$). There were small and nonsignificant differences in outcomes between placebo and low-dose ASA ingested upon awakening. These four groups combined showed highly significant greater event rate of serious adverse outcomes than women ingesting ASA either in the evening or at bedtime (HR: .19, 95% CI: .10–.39; $p < .001$). There was no increased risk of hemorrhage, either before or after delivery, with low-dose ASA relative to placebo (HR: .57, 95% CI: .25–1.33; $p = .194$). Results indicate that (i) 100 mg/d ASA should be the recommended minimum dose for prevention of complications in pregnancy; (ii) ingestion of low-dose ASA should start at ≤16 wks of gestation; and (iii) low-dose ASA ingested at bedtime, but not upon awakening, significantly regulates ambulatory BP and reduces the incidence of preeclampsia, gestational hypertension, preterm delivery, and IUGR. ABPM evaluation at the first trimester of pregnancy provides sensitive endpoints for identification of women at high risk for preeclampsia who might benefit most from the cost-effective preventive intervention with timed low-dose ASA. (Author correspondence: rhermida@uvigo.es)

**Keywords:** Ambulatory blood pressure monitoring, Aspirin, Intrauterine growth retardation, Gestational hypertension, Preeclampsia, Preterm delivery

## INTRODUCTION

Preeclampsia and gestational hypertension are two major contributors to perinatal morbidity and mortality (Khan et al., 2006). Hypertension complicates ~10% of all pregnancies, increasing the risk of oxidative stress, platelet dysfunction, and endothelial damage (Higashi et al., 2002; Portaluppi et al., 2004; Varani et al., 1999a, 1999b, 2000). Moreover, hypertension in pregnancy is associated with increased risk of adverse fetal, neonatal, and maternal outcomes, including preterm birth,

Submitted July 13, 2012, Returned for revision July 28, 2012, Accepted July 30, 2012

Address correspondence to Prof. Ramón C. Hermida, PhD, Director, Bioengineering and Chronobiology Laboratories, E.I. Telecomunicación, Campus Universitario, Vigo, Pontevedra 36310, Spain. Tel.: 34-986-812148; Fax: 34-986-812116; E-mail: rhermida@uvigo.es

intrauterine growth retardation (IUGR), perinatal death, acute renal or hepatic failure, antepartum hemorrhage, postpartum hemorrhage, and maternal death (Duley, 2009; Roberts et al., 2011; Steegers et al., 2010). Hypertensive complications in pregnancy range from hypertension alone (gestational nonproteinuric hypertension) through proteinuria and multiorgan dysfunction (preeclampsia) to seizures (eclampsia) (Brown et al., 2001). Reported population rates of gestational hypertension vary substantially, ranging from 4% to 10%, including preeclampsia rates of 2% to 5% (Hernández-Díaz et al., 2009; Klemmensen et al., 2007; Lawler et al., 2007; Roberts et al., 2005; Ros et al., 1998; Walker et al., 2009; Wallis et al., 2008), although these values might well underestimate the real prevalence of hypertension in pregnancy (Ayala & Hermida, 2012; Hermida & Ayala, 2002, 2004; Hermida et al., 1998, 2003b, 2004c). At least part of this variation is likely due to under-ascertainment and/or misclassification of gestational hypertension and preeclampsia (Ayala & Hermida, 2012; Hermida & Ayala, 2002; Hermida et al., 1998, 2003b, 2004c; Roberts et al., 2008). Although preeclampsia is among the most severe complications of pregnancy, any form of hypertension during gestation is associated with increased risk of adverse maternal and fetal outcomes (Buchbinder et al., 2002; Roberts et al., 2005). It also appears that history of either gestational hypertension or preeclampsia in a prior pregnancy places the pregnant woman and her offspring at high risk for future, typically within 7 to 12 yrs, development of hypertension (Marín et al., 2000; Svensson et al., 1983).

Many of the physiologic changes of preeclampsia are essentially a reversal of those that accompany a healthy pregnancy, i.e., absence of plasma volume increase, blood pressure (BP) elevation, peripheral vascular resistance increase, and aldosterone insufficiency (Dekker & Sibai, 1993). Although the exact cause of preeclampsia remains unclear, several mechanisms have been suggested, including enhanced sensitivity to vasopressors, abnormal maternal immunologic reaction, and imbalance in the production of vasoactive prostaglandins (thromboxane $A_2$ and prostacyclin), resulting in vasoconstriction of small arteries, platelet activation, and uteroplacental insufficiency (Dekker & Sibai, 1993; Friedman, 1988; Page, 2002; Redman & Sargent, 2003; Sibai et al., 2003; Walsh, 1985). Disordered trophoblast invasion of the maternal spiral arteries in early pregnancy is known to lead to underperfusion of the placenta and, ultimately, placental ischemia and infarction (Redman & Sargent, 2005). The resultant placental damage apparently leads to activation of platelets and the clotting system (Janes et al., 1995; Redman et al., 1978) and to the mentioned imbalance between the vasodilator prostacyclin and thromboxane $A_2$, a vasoconstrictor and stimulant of platelet aggregation (Bussolino et al., 1980; Friedman, 1988; Masotti et al., 1979; Walsh, 1985).

Acetylsalicylic acid (aspirin, ASA) is a nonsteroidal anti-inflammatory drug (NSAID) with demonstrated inhibitory effects on cyclooxygenases (COX), involved in arachidonic acid metabolism and prostaglandin production (Patrono et al., 1985). Previous studies have demonstrated that ASA is a potent antioxidative agent that markedly reduces vascular production of superoxide in normotensive and hypertensive rats (Wu et al., 2002). In addition, ASA was found to prevent angiotensin II–induced hypertension and cardiovascular hypertrophy in rats, mainly through its antioxidative properties in preventing the generation of superoxide (Wu et al., 2004). Moreover, recent results have demonstrated that ASA induces nitric oxide release from vascular endothelium (Grosser & Schroder, 2003; Tauber et al., 2004). This effect appears to be due to a direct acetylation of the endothelial nitric oxide synthase protein. Many studies have demonstrated low-dose ASA (75–150 mg/d) provides marked benefits in the secondary prevention of cardiovascular events (Antiplatelet Trialists' Collaboration, 1994; Antithrombotic Trialists' Collaboration, 2002; Fuster et al., 1993; Hayden et al., 2002; Manson et al., 1991; Willard et al., 1992), although the potential direct effects of ASA on cardiovascular function remain uncertain. Since thromboxane $A_2$ and prostacyclin are derived from arachidonic acid through the action of cyclooxygenase, low-dose ASA (81 mg/d; by acetylation of this enzyme) selectively inhibits the synthesis of platelet thromboxane $A_2$ without affecting the production of endothelium-derived prostacyclin (Walsh, 1985). A higher dose of ASA (325 mg/d), however, significantly reduces prostacyclin levels (Walsh, 1985; Walsh et al., 1992). This selective inhibition has been hypothesized to be the pharmacological basis for the potential beneficial effects of ASA in preventing gestational hypertension and preeclampsia (Beaufils et al., 1985).

Several studies aimed to test the effects of low-dose ASA in the prevention of preeclampsia concluded that the beneficial effects of such treatment outweigh adverse ones (Imperiale & Petrulis, 1991). These controlled trials were usually conducted in small groups of pregnant women selected according to several criteria for establishing high risk of preeclampsia. The benefits shown by these small trials in the prevention of preeclampsia have not been corroborated by larger randomized controlled trials usually carried out in the general obstetric population, testing a dose of 60 mg/d ASA presumably prescribed to be ingested in the morning, and including women randomized in the study as late as at 26–32 wks of gestation (CLASP, 1994; ECPPA, 1996; Golding, 1998; Italian Study of Aspirin in Pregnancy, 1993; Rotchell, 1998; Sibai et al., 1993). These studies concluded that the use of low-dose ASA during pregnancy was safe for the fetus, the newborn, and the mother, but the results did not support the routine prophylactic use of ASA for prevention of preeclampsia. Although participants in these relatively large trials were thought to be at increased risk for preeclampsia, this condition developed in <10% of the women investigated, thus suggesting that the trials indeed included

too many women at low risk. Another study involving only high-risk women concluded that, despite the absence of adverse effects, 60 mg/d ASA compared with placebo did not significantly reduce the incidence of preeclampsia in women with pregestational insulin-treated diabetes mellitus, chronic hypertension, multifetal gestations, or a history of preeclampsia (Caritis et al., 1998).

Some relevant issues, not yet properly and fully addressed, still leave unresolved whether or not low-dose ASA is useful for prophylactic intervention in pregnancy (Askie et al., 2007; Barth, 1998; Bujold et al., 2009, 2010, 2011; Hermida et al., 1997a, 1999, 2003c; Roberge et al., 2012a, 2012b; Ruano et al., 2005; Trivedi, 2011):

(i) *Could a dose >60 mg/d ASA be beneficial?* The effects of ASA on the selective inhibition of thromboxane and prostacyclin in women at risk for preeclampsia are markedly dose dependent (Walsh et al., 1992). It has been documented that doses of 50–60 mg/d ASA commonly tested in multicenter clinical trials with negative results in the prevention of preeclampsia are effective in inhibiting platelet thromboxane but insufficient to inhibit placental thromboxane (Walsh & Wang, 1998). Results from a meta-analysis on the potential benefits of low-dose ASA for prevention of IUGR indicated that the preventive effect was greater at higher doses (100–150 mg/d ASA) as compared with inefficient lower doses (50–80 mg/d ASA) (Leitich et al., 1997). Moreover, a dose-dependent effect of ASA on BP has also been documented in clinically healthy volunteers (Hermida et al., 1997b). A low-dose of 100 mg/d, compared with placebo, significantly reduced ambulatory systolic (SBP) and diastolic BP (DBP) when ingested 8 h after awakening or at bedtime, but not when ingested upon awakening. A higher dose of 500 mg/d ASA, however, increased BP, even when administered at bedtime (Hermida et al., 1997b). Although the highest dose of 500 mg/d ASA tested in this study cannot be recommended in pregnancy, no prospective controlled trial has yet studied if there is a dose-dependent effect in the prevention of preeclampsia with ASA at doses between, e.g., 60 to 150 mg/d. Results from a recent meta-analysis of individual patient data from 32 217 women recruited in 31 randomized trials on prevention of preeclampsia indicated that there is no evidence that using >75 mg/d ASA had more or less effect than a lower dose (Askie et al., 2007), although the author failed to test a higher threshold dose, e.g., 100 mg/d, previously shown to be effective in another meta-analysis (Leitich et al., 1997).

(ii) *Could gestation age for commencing the use of ASA be relevant?* The large trials with negative findings mentioned above included women with gestational age at entry up to 26 or even 32 wks of gestation, too late for any prophylactic intervention in pregnancy (Ayala & Hermida, 2012; Ayala et al., 1997a, 1997b; Hermida & Ayala, 1997, 2001, 2005a; Hermida et al., 2000a, 2001a, 2003b, 2003d, 2004a, 2004c). A retrospective study on women who ingested 100 mg/d ASA concluded that success (no preeclampsia) was associated with use of ASA starting at <17 wks of gestation (Dumont et al., 1999). A meta-analysis on data from >13 000 pregnant women documented a statistically significant reduction in the incidence of IUGR and perinatal mortality among women randomized to ASA treatment before compared with those randomized after the 17th wk of gestation (Leitich et al., 1997). Similar conclusions were obtained in other, although not all, more recent meta-analyses (Askie et al., 2007; Bujold et al., 2009, 2010; Roberge et al., 2012a, 2012b; Ruano et al., 2005). Moreover, with the use of ambulatory BP monitoring (ABPM), differing predictable BP patterns throughout gestation have been identified for clinically healthy and hypertensive pregnant women. In normotensive pregnancies, BP steadily decreases up to the middle of gestation and then increases up to the day of delivery, with final BP values similar to those found early in pregnancy in the same women; the predictable BP variability during pregnancy in normotensive women entails an average increase of 7% in SBP and 9% in DBP between the middle of gestation and delivery. In contrast, women who develop gestational hypertension or preeclampsia show stable BP during the first half of pregnancy and a continuous linear BP increase thereafter until delivery (Ayala & Hermida, 2012; Ayala et al., 1997b; Hermida et al., 2001a). Furthermore, a highly predictable 24-h BP variability characterizes clinically healthy pregnant women as well as women who develop gestational hypertension or preeclampsia (Ayala & Hermida, 2001, 2012; Ayala et al., 1997a; Benedetto et al., 1996; Hermida & Ayala, 2005b; Hermida et al., 2000a, 2003b, 2003d, 2004a, 2004b; Miyamoto et al., 1998). As early as in the first trimester of pregnancy, statistically significant increased 24-h SBP/DBP means by 12/7 mm Hg characterize women complicated with gestational hypertension or preeclampsia compared with women with uncomplicated pregnancies (Ayala & Hermida, 2012; Ayala et al., 1997a; Hermida et al., 2000a, 2003b, 2003d). These collective findings suggest that any potential beneficial effect from the prophylactic use of ASA in pregnancy should be studied by including only women who start using ASA very early in pregnancy, i.e., not later than 16 wks of gestation.

(iii) *Could the time of day of ASA administration be relevant?* Previous clinical trials documented effects of ASA upon lipoperoxides, α- and β-adrenergic receptors, and BP in clinically healthy subjects that are dependent on ASA circadian administration time (Cornélissen et al., 1991). Kanzik et al. (1992) showed that ASA produces a >30% inhibition of angiotensin II *only* when ingested at bedtime, in association with the documented administration-time-dependent effects of ASA on plasma renin activity (Wang et al., 1999). Moreover, Snoep et al. (2009) found that low-dose ASA (100 mg/d) when ingested at bedtime, as compared with upon awakening, significantly diminished not only the 24-h level of plasma renin activity but also the excretion in 24-h urine samples of cortisol, dopamine, and

norepinephrine. These authors concluded that the decreased activity of these pressor systems constitutes a biologically plausible explanation for the findings that ASA ingested at bedtime reduces BP, whereas ASA ingested in the morning does not. In fact, an administration-time-dependent influence of low-dose ASA (100 mg/d) on ambulatory BP was demonstrated in randomized prospective trials on normotensive subjects (Hermida et al., 1997b), individuals with prehypertension (Hermida et al., 2009b), and patients with untreated mild hypertension (Ayala & Hermida, 2010; Hermida et al., 2003a, 2005a, 2005b). The findings of these studies are consistent; BP-lowering effect is achieved by low-dose ASA when ingested at bedtime, but not upon awakening. Moreover, results from previous double-blind, randomized, controlled clinical trials on the influence of low-dose ASA on ambulatory BP in pregnant women indicated a highly statistically significant ( $p < .001$) administration-time-dependent effect on BP from ASA (Hermida et al., 1997a, 1999). There was no effect of ASA compared with placebo on BP when both were ingested upon awakening, but the BP reduction was highly statistically significant when ASA was ingested 8 h after awakening and, to a greater extent, when ingested at bedtime (Hermida et al., 1997a, 1999). No other clinical trial, among the many summarized in the meta-analyses mentioned above on the effects of ASA in pregnant women, ever reported if time of ingestion of the medication was controlled and, if so, at what time of the day ASA was ingested by the investigated pregnant women.

In keeping with the contradictory results on the effects of ASA in pregnant women depending on the dose and gestational age at recruitment, the ASEM (ASpirina en EMbarazo, i.e., Aspirin in Pregnancy) study was specifically designed as a prospective, randomized, double-blind, chronotherapy trial to investigate whether bedtime treatment with low-dose ASA (100 mg/d, a dose assumingly affecting both maternal and placental thromboxane; Walsh & Wang, 1998) exerts significantly better BP control during gestation and reduction of the risk of preeclampsia, IUGR, and preterm delivery than ASA upon awakening or placebo in high-risk pregnant women who entered the study protocol at ≤16 wks of gestation. We here extend previous findings (Hermida et al., 2003c) to report the administration-time-dependent effects of low-dose ASA in ambulatory BP and pregnancy outcome on women enrolled in the ASEM trial who were systematically studied by 48-h ABPM from the first obstetric consultation at the hospital until delivery, which marked the termination of treatment with either ASA or placebo, as well as at puerperium, i.e., 6–8 wks after delivery.

## METHODS

### Inclusion and Exclusion Criteria

The sample represents a population of Spanish pregnant women with higher risk for gestational hypertension or preeclampsia than the general obstetric population and who were thus receiving medical care and follow-up at the Obstetric Physiopathology Service (high-risk unit) of the hospital. Reasons for receiving medical care at this unit include familial or personal history of either gestational hypertension or preeclampsia; chronic hypertension; cardiovascular, endocrine, bleeding, or metabolic disease; personal history of spontaneous abortion; multiple pregnancy; obesity; and adolescent or middle-aged nulliparous pregnancy (<18 or >35 yrs). The relative risk of gestational hypertension and preeclampsia in this unit is ~3.5-fold higher than in the general obstetric population in our setting (Hermida & Ayala, 2002; Hermida et al., 1998, 2003b, 2003d, 2004c). Additional inclusion criteria for this trial were gestational age ≤16 wks at randomization and maternal age ≥18 yrs. Exclusion criteria were multiple pregnancy, chronic hypertension or any other condition requiring the use of BP-lowering medication, cardiovascular disorders (unstable angina pectoris, heart failure, life-threatening arrhythmia, atrial fibrillation, kidney failure, and grade III–IV retinopathy), chronic liver disease, any disease requiring the use of anti-inflammatory medication, diabetes or any other endocrine disease such as hyperthyroidism, history of drug/alcohol abuse, night/shiftwork employment, acquired immunodeficiency syndrome (AIDS), intolerance to ABPM, and inability to communicate and comply with all of the study requirements. This prospective single-center study was approved by the Spanish Health Minister and the state Ethics Committee of Clinical Research, and respected the criteria set forth for ethical medical research as outlined in the Helsinki Declaration and instructions to authors for the journal (Portaluppi et al., 2010). All participants gave written informed consent.

### Participants and Diagnostic Criteria

Respecting the inclusion/exclusion criteria for this clinical trial, we recruited 350 high-risk pregnant women (183 nulliparous), 30.7 ± 5.3 (mean ± SD) yrs of age, and 13.5 ± 1.4 wks gestation at the time of inclusion. All participants were adhering to a routine of daytime activity and nighttime sleep. Gestational hypertension was defined as a hyperbaric index (HBI)—total area of BP excess summed over the 24-h period above the upper limit of the time-varying tolerance interval calculated as a function of gestational age (Hermida et al., 2001b)—consistently above the threshold for diagnosis of hypertension in pregnancy (Ayala & Hermida, 2012; Hermida & Ayala, 2002; Hermida et al., 1998, 2003b, 2004c) after the 20th wk of gestation. Preeclampsia was defined as gestational hypertension (following the criteria given above) and proteinuria, ≥300 mg/24-h urine, diagnosed after the 20th wk of gestation in a previously normotensive woman. Preterm delivery was defined as delivery at <37 wks of gestation. Gestational age and fetal growth for diagnosis of IUGR and preterm delivery were determined by monthly echography assessments in all

participants. Body mass index (BMI) was calculated as weight (in kg)/height$^2$ (in m$^2$).

### Study Design

This was a prospective, randomized, double-blind, placebo-controlled clinical trial. Participants were randomly assigned at the time of their first visit to the hospital to one of six groups, defined according to treatment (placebo or ASA, 100 mg/d; Table 1) and to the timing of daily administration of ASA or placebo: upon awakening (Time 1), 8 h after awakening (Time 2), or at bedtime (Time 3). Placebo (microcrystalline cellulose, corn starch, saccharin, and citric acid [included to simulate the flavor of ASA]) and ASA (100 mg uncoated tablets) were prepared in identical presentation and provided monthly to the participants in a box containing 3 blister packs, each with 10 tablets. The boxes, grouped in packs of 7 (to cover medication for the duration of pregnancy) and labeled with the randomization number, were assigned to each woman at the time of recruitment. Randomization of boxes containing either ASA or placebo followed an allocation table constructed by a computerized random-number generator. Treatment of each box (placebo or ASA) was enclosed in serially numbered, opaque, sealed envelopes. Concealed assignment of participants to the six treatment-time regimens was done according to the order of recruitment. Envelopes were open only after conclusion of the trial for every participant. Oral ingestion of ASA or placebo started at 12 to 16 wks of gestation and continued until the day of delivery. The dose of 100 mg/d used in this trial corresponds with the lower ASA dose commercially available in Spain. Adherence to the time-of-day (awakening, 8h after awakening, or bedtime) treatment schedule and prescribed medication (ASA or placebo) was enforced at each follow-up visit. Compliance was measured on the basis of tablet count at the time of each visit to the hospital. Just before commencing each 48-h ABPM session (see below), the same midwife nurse, to avoid examiner bias, obtained 3 to 6 consecutive clinic BP measurements after the woman had rested in a seated position for ≥10 min. Proper cuff size was determined by measurement of upper arm circumference at each study visit.

### ABPM Assessment

At inclusion, as well as at each scheduled visit for ABPM during follow-up, the SBP and DBP of each pregnant woman were automatically measured every 20 min between 07:00 and 23:00 h and every 30 min during the night for 48 consecutive hours with a properly calibrated and validated SpaceLabs 90207 device (SpaceLabs, Issaquah, WA, USA) (Shennan et al., 1993). ABPM was performed at the time of recruitment, always at ≤16 wks of gestation, and then scheduled every 4 wks until the 7th month of gestation and every 2 wks thereafter until delivery. ABPM was also performed at puerperium, i.e., 6–8 wks after delivery. A 48-h, instead of the most common 24-h, monitoring was chosen to improve the

TABLE 1. Baseline characteristics of women investigated according to randomization group

| Variable | Placebo | | | | ASA | | | | $p$ between placebo and ASA |
|---|---|---|---|---|---|---|---|---|---|
| | Time 1 | Time 2 | Time 3 | All | Time 1 | Time 2 | Time 3 | All | |
| Women, n | 59 | 57 | 58 | 174 | 58 | 59 | 59 | 176 | |
| Gestational age at randomization, wks | 13.6 ± 1.6 | 13.6 ± 1.4 | 13.6 ± 1.4 | 13.6 ± 1.5 | 13.6 ± 1.4 | 13.4 ± 1.5 | 13.4 ± 1.4 | 13.5 ± 1.4 | .411 |
| Age, y | 31.5 ± 5.8 | 32.0 ± 4.5 | 30.0 ± 5.2 | 31.1 ± 5.2 | 31.0 ±5.8 | 30.4 ± 5.3 | 29.7 ± 4.8 | 30.3 ± 5.3 | .175 |
| Weight, kg | 65.7 ± 11.1 | 67.3 ± 12.0 | 68.6 ± 13.9 | 67.2 ± 12.4 | 68.1 ± 12.6 | 66.9 ± 15.4 | 67.0 ± 12.8 | 67.3 ± 13.6 | .944 |
| Height, cm | 162.5 ± 5.2 | 162.6 ± 5.6 | 161.3 ± 6.7 | 162.1 ± 5.9 | 162.4 ± 6.2 | 163.2 ± 6.5 | 162.1 ± 6.2 | 162.6 ± 6.3 | .499 |
| BMI, kg/m$^2$ | 24.8 ± 3.9 | 25.5 ± 4.3 | 26.3 ± 4.4 | 25.5 ± 4.2 | 25.8 ± 3.9 | 24.9 ± 4.6 | 25.4 ± 4.3 | 25.4 ± 4.3 | .714 |
| Nulliparous, % | 59.3 | 52.6 | 53.4 | 55.1 | 41.4 | 59.3 | 47.5 | 49.4 | .282 |
| Previous abortion, % | 32.2 | 28.1 | 25.9 | 30.5 | 29.3 | 32.2 | 32.2 | 31.3 | .873 |
| Age at menarche, y | 12.9 ± 1.5 | 12.9 ± 1.6 | 12.5 ± 1.1 | 12.7 ± 1.4 | 12.7 ± 1.4 | 12.9 ± 1.5 | 12.6 ± 1.3 | 12.7 ± 1.4 | .917 |
| Clinic SBP, mm Hg | 120.5 ± 9.7 | 119.2 ± 9.2 | 120.2 ± 9.8 | 120.0 ± 9.6 | 121.4 ± 8.8 | 122.5 ± 10.1 | 121.8 ± 9.8 | 121.9 ± 9.5 | .130 |
| Clinic DBP, mm Hg | 66.6 ± 8.7 | 66.6 ± 7.2 | 67.1 ± 9.0 | 66.8 ± 8.3 | 67.7 ± 8.4 | 68.5 ± 8.5 | 67.7 ± 8.1 | 68.0 ± 8.3 | .182 |
| Clinic PP, mm Hg | 53.9 ± 8.0 | 52.6 ± 6.4 | 53.1 ± 7.7 | 53.2 ± 7.4 | 53.7 ± 6.7 | 54.0 ± 8.2 | 54.1 ± 7.4 | 53.9 ± 7.4 | .372 |
| 48-h SBP mean, mm Hg | 110.5 ± 6.7 | 111.8 ± 7.9 | 109.9 ± 8.5 | 110.7 ± 7.7 | 108.1 ± 8.6 | 113.5 ± 9.9 | 113.4 ± 8.6 | 111.8 ± 9.3 | .251 |
| 48-h DBP mean, mm Hg | 64.5 ± 4.9 | 66.3 ± 5.4 | 64.9 ± 6.5 | 65.2 ± 5.6 | 63.4 ± 6.0 | 66.8 ± 6.8 | 67. 4 ± 6.1 | 66.0 ± 6.5 | .268 |
| 48-h PP mean, mm Hg | 46.0 ± 3.8 | 45.5 ± 4.0 | 45.0 ± 3.0 | 45.5 ± 3.6 | 44.7 ± 4.7 | 46.7 ± 4.6 | 46.0 ± 4.3 | 45.8 ± 4.6 | .402 |

Values are shown as mean ± SD. Clinic BP corresponds to the average of 3 to 6 measurements obtained by a midwife nurse for each woman at the time of their visit to the hospital at the time of randomization. Baseline 48-h BP means were determined by ABPM at randomization before treatment started. Time 1: Women randomized to ingest aspirin or placebo upon awakening. Time 2: Women randomized to ingest aspirin or placebo 8 h after awakening. Time 3: Women randomized to ingest aspirin or placebo at bedtime.

reproducibility of results, as accuracy in the calculation of ABPM characteristics (including mean BP values and the HBI) depends markedly on duration of ABPM (Hermida & Ayala, 2003; Hermida et al., 2002b, 2007b, 2012c). No woman was hospitalized during monitoring. Women were instructed to adhere to their usual activities with minimal restrictions but to keep a similar activity-rest schedule and avoid daytime napping during the two consecutive days of ABPM. They were advised to avoid use of medications other than the assigned aspirin or placebo for the duration of the trial. Participants kept a diary listing the time of retiring to bed at night, awakening in the morning, consumption of meals, participation in exercise, and episodes of atypical physical activity, mood/emotional states, and other events that might affect BP. This individualized information was utilized to edit the ABPM data and to determine the commencement and termination of daytime activity and nighttime sleep spans of each woman. BP series were considered invalid for analysis if ≥30% of the scheduled BP measurements were missing, if data were lacking for >2 consecutive hourly means, if data were obtained while women had an irregular rest-activity schedule during the 2 days of monitoring, or if the nighttime sleep period was <6 or >12 h during ABPM. If invalid according to these criteria, participants were requested to repeat ABPM within the same week. The total number of valid 48-h ABPM profiles provided by the 350 women under investigation fulfilling all mentioned requirements set a priori was 2538.

The BP cuff was worn on the nondominant arm with proper cuff size determined by upper arm circumference measurement at each study visit. ABPM devices were always set to the so-called "blind function," never displaying the actual BP readings after measurement. To ensure proper BP measurement throughout the entire 48 h, we used nickel metal hydride rechargeable batteries. Since these batteries have little memory effect, they can be recharged many times to their maximum capacity, thereby enabling the ABPM device to operate for many consecutive days (Ayala et al., 2012b; Hermida et al., 2004e).

All ABPM profiles obtained during the course of the study were analyzed by comparison with circadian (with reference to the rest-activity cycle) time–specified tolerance intervals of SBP and DBP previously constructed for each trimester of gestation on databases derived from previous assessments of Spanish normotensive pregnant women who also had been evaluated by 48-h ABPM (Hermida et al., 2001b). This procedure, as previously described (Hermida, 1999; Hermida & Fernández, 1996; Hermida et al., 1996, 1997c, 1998, 2000b, 2002d, 2003b, 2004c), enabled calculation of the HBI, as defined above, and also the hypobaric index, i.e., area of BP deficit below the lower limit of the time-of-day-specified tolerance intervals, of each 48-h ABPM profile. The HBI has been shown to be more reproducible than BP mean values in the diagnosis of both essential hypertension (Hermida et al., 2000b, 2002d) and hypertension in pregnancy (Ayala & Hermida, 2012; Hermida & Ayala, 2002; Hermida et al., 1998, 2003b, 2004c). Moreover, use of a lower reference threshold averts hypotension (Hermida et al., 2002d).

### Statistical Methods

The minimum sample size for this trial, 55 women for each of the 6 treatment-time groups, was calculated to show as statistically significant at the two-sided α level of 5% and with a power of 95% a BP difference between ASA and placebo ≥4 mm Hg in the 24-h BP mean at the time of delivery, according to the estimation of interindividual variability provided by previous studies (Hermida et al., 1997a, 1999).

Each individual's clock-hour BP values were first referenced to hours after awakening from nighttime sleep, based on information obtained from each woman's diary. This transformation avoided introduction of bias due to differences among participants in their sleep-activity routine (Hermida et al., 2002c). ABPM profiles were edited according to conventional criteria to correct for measurement errors and outliers; SBP readings >250 or <70 mm Hg, DBP >150 or <40 mm Hg, and pulse pressure (difference between SBP and DBP) >150 or <20 mm Hg were automatically eliminated.

The circadian rhythm of SBP and DBP for every single ABPM profile was objectively assessed by individual multiple-component analysis (Bingham et al., 1982; Fernández & Hermida, 1998; Fernández et al., 2003), a method applicable to nonsinusoidal shaped longitudinal time-series data, i.e., time series of data collected from a single subject, consisting of values distributed at equal or unequal intervals. The method produces estimates of the 24-h rhythm–adjusted time-series mean or MESOR (midline estimating statistic of rhythm, i.e., average value of the rhythmic function fitted to the data), as well as the amplitude (one-half the extent of the temporal variability explainable by rhythmicity) and acrophase (crest time expressed as a lag in time from a designated reference, here the time of awakening from nocturnal sleep) for every fitted component of given period (here 24 and 12 h for SBP and DBP, as currently recommended; Hermida et al., 2002a, 2003d). Since the BP data were obtained at an unequidistant sampling rate, the MESOR provides a better estimation of the true 24-h BP mean than the arithmetic average of all BP values, usually overestimating the true mean due to the denser sampling during the daytime activity compared with nighttime sleep span.

The estimates of 24-h MESOR obtained for all individual ABPM profiles of each pregnant woman were expressed as percentage of the MESOR obtained for that woman at baseline, i.e., the ABPM profile at randomization just before treatment started. This procedure normalizes data and avoids interindividual differences at baseline that might influence the evaluation of changes in BP throughout gestation. The time of sampling for

each ABPM profile was expressed in months from the baseline monitoring, as indication of duration of therapy. The values of 24-h MESOR thus normalized were used to establish their pattern of variation throughout treatment for each of the six groups of pregnant women by polynomial regression analysis. Effects of medication (placebo or ASA) and circadian time of treatment on ambulatory BP during gestation as well as at puerperium were evaluated by analysis of variance (ANOVA). Demographic and clinical characteristics were compared on an intention-to-treat basis among the participants randomized to the six treatment-time regimens—placebo versus ASA ingested at three different times of day according to individual rest-activity cycle—by ANOVA (continuous variables) or nonparametric chi-square test (proportions).

The primary outcome study endpoint was total serious adverse events, which included preeclampsia, preterm delivery, IUGR, and stillbirth. We also used as an additional endpoint the composite of these serious adverse events plus gestational hypertension, as defined above. The Cox proportional-hazard model was used to estimate hazard ratios (HRs), with 95% confidence intervals (CIs), for events associated with treatment-time regimen, with adjustment for significant confounding variables. Maternal age, BMI, parity (nulliparous vs. multiparous), previous abortion (yes vs. no), age at menarche, baseline SBP and DBP, and gestational age at randomization were used as potential confounding variables for adjustment in Cox analyses. For survival analysis, follow-up was established as either the time to the documented event or time to delivery in event-free women. Event rates, with 95% CIs, were calculated as the percent ratio of observed number of events to total number of women per group. A two-sided $p$ value of $< .05$ was considered statistically significant for all comparisons. Statistical analyses were performed using SPSS, version 13.0 (SPSS, Chicago, IL, USA) and KaleidaGraph version 3.6.4 (Synergy Software, Reading, PA, USA).

## RESULTS

### Demographic Characteristics

At baseline, all treatment-time groups were equivalent for gestational age at randomization, maternal age, weight, height, BMI, menarche, and clinic and ambulatory BP. The proportion of nulliparous women and participants with previous abortions was also equivalent in all groups (Table 1). Comparison of BP data obtained at baseline shows the relatively large difference between clinic and ambulatory SBP and DBP.

The median numbers of tablets ingested by the women investigated, to be compared with the maximum average expected number of 175 tablets (medication for 25 wks of gestation), were 171 in the ASA groups and 167 in the placebo groups. Since compliance was very high, we could not detect any difference in the number of missing tablets among groups of women assigned to ingest either ASA or placebo at different times of the day.

### Administration-Time-Dependent Effects of ASA Versus Placebo on Ambulatory BP

Figure 1 shows the variation of the circadian MESOR (expressed as % of the value obtained for each woman at baseline) of SBP (top) and DBP (bottom) during gestation represented in months from randomization in pregnant women ingesting either placebo or 100 mg/d ASA at Time 1 (awakening), starting at 12–16 wks of gestation. The lines in Figure 1 represent the best-fitted waveform model obtained by linear polynomial regression analysis for each treatment group. Results indicate that, first, BP follows a predictable pattern of variation that can be approximated by a second-order model in gestational age (here expressed in months of treatment). There was a steady decrease in SBP and DBP up to the 20th wk of gestation— ~ 1.5 months after randomization—followed by an increase in BP thereafter up to delivery. This pattern of BP variation was fully equivalent for women ingesting placebo or low-dose ASA upon awakening (Figure 1). At the time of delivery, the average 24-h MESOR was equivalent in

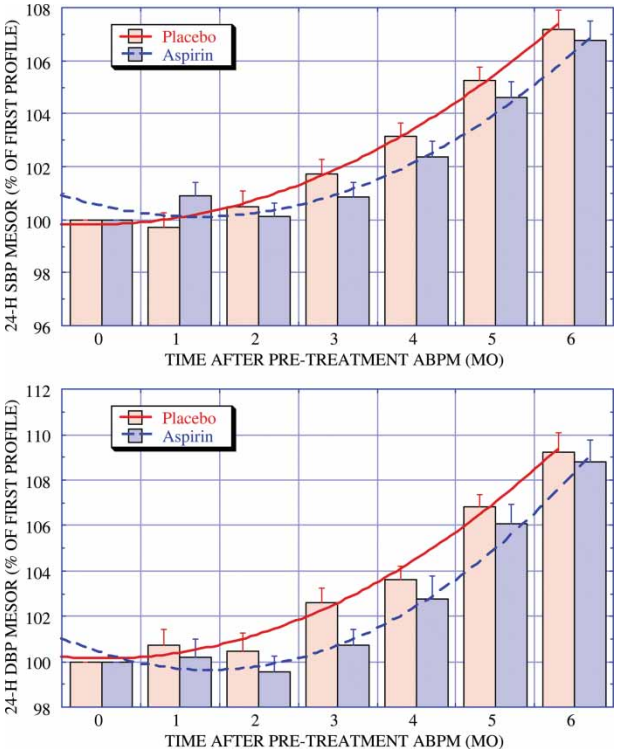


FIGURE 1. Variation of 24-h MESOR of SBP (top) and DBP (bottom) throughout gestation in pregnant women ingesting placebo or low-dose ASA (100 mg/d) upon awakening, starting at 12–16 wks of gestation and until delivery. Data are expressed in % of the baseline 48-h ABPM evaluation per participant. The bars represent means ± SE. The lines represent in each case the best-fitted waveform model obtained by linear polynomial regression analysis.

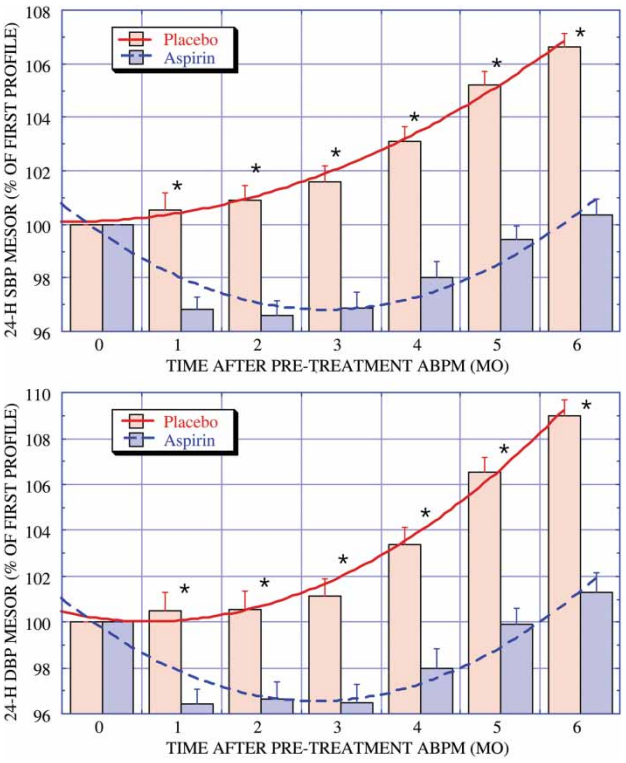


FIGURE 2. Variation of 24-h MESOR of SBP (top) and DBP (bottom) throughout gestation in pregnant women ingesting placebo or low-dose ASA (100 mg/d) 8 h after awakening, starting at 12–16 wks of gestation and until delivery. Data are expressed in % of the baseline 48-h ABPM evaluation per participant. The bars represent means ± SE. The lines represent in each case the best-fitted waveform model obtained by linear polynomial regression analysis. *$p < .001$ between ASA and placebo.

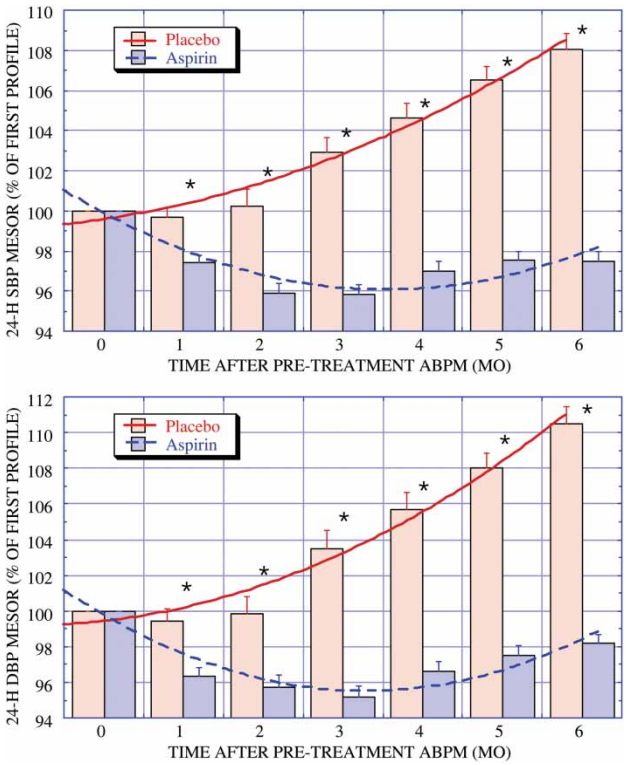


FIGURE 3. Variation of 24-h MESOR of SBP (top) and DBP (bottom) throughout gestation in pregnant women ingesting placebo or low-dose ASA (100 mg/d) at bedtime, starting at 12–16 wks of gestation and until delivery. Data are expressed in % of the baseline 48-h ABPM evaluation per participant. The bars represent means ± SE. The lines represent in each case the best-fitted waveform model obtained by linear polynomial regression analysis. *$p < .001$ between ASA and placebo.



women ingesting either placebo or ASA upon awakening ($p = .719/.726$ for SBP/DBP).

Figure 2 compares the predictable BP variation during gestation in women ingesting either placebo or 100 mg/d ASA at Time 2 (8 h after awakening). The predictable pattern of SBP and DBP variation follows again a second-order model. Contrary to the results illustrated in Figure 1 for women ingesting placebo or ASA at Time 1, there was a significant reduction in SBP (Figure 2, top) and DBP (Figure 2, bottom) when ASA, relative to placebo, was ingested at Time 2 ($p < .001$ between groups at all monthly evaluations after randomization). Differences between treatment groups were even larger and highly statistically significant ($p < .001$) when low-dose ASA and placebo were systematically ingested on a daily basis at bedtime (Time 3; Figure 3).

Comparison of Figures 1–3 indicates, first, lack of differences between the models representing the predictable SBP and DBP variation during gestation for the three groups of women ingesting placebo at different circadian times. Results from ANOVA further indicate lack of differences between these groups in average 24-h SBP and DBP MESOR at all monthly evaluations during gestation ($p$ always $> .201$). Second, results document a highly statistically significant administration-time-dependent effect of low-dose ASA on ambulatory SBP and DBP. There was no effect on BP when ASA, as compared with placebo, was ingested upon awakening; the BP reduction was, however, statistically significant when ASA was ingested 8 h after awakening, and, to a greater extent, when ingested at bedtime. Results from ANOVA indicated differences between treatment groups in the average 24-h SBP and DBP MESOR were statistically significant as soon as at the first month after treatment started ($p < .001$). At the time of delivery, ingesting 100 mg/d ASA at bedtime starting at ≤16 wks of gestation decreased SBP on average 12.4/8.1 mm Hg in 24-h SBP/DBP MESOR compared with ASA upon awakening.

The comparison of histograms shown in Figure 4 (left) for women ingesting placebo at different circadian times indicates no difference between the three groups early before delivery (6 months after initiation of treatment) in either SBP (top; $p = .363$) or DBP (bottom; $p = .410$). The histograms on the right of Figure 4 also indicate lack of differences between these three treatment-time placebo groups in 24-h MESOR of SBP ($p = .358$) and DBP ($p = .573$) at puerperium. Complementing the findings documented in Figures 1–3, the histograms on the left of Figure 5 indicate the highly statistically significant

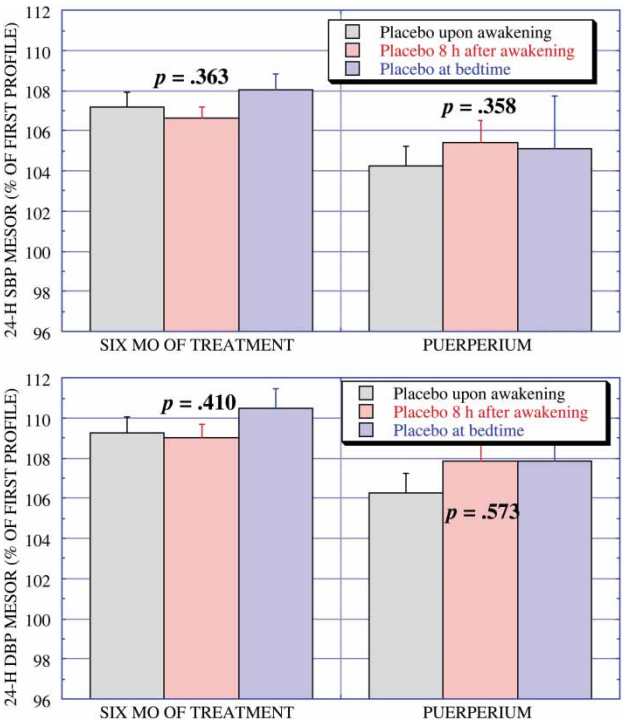


FIGURE 4. 24-h MESOR, expressed in % of the baseline 48-h ABPM evaluation per participant, of SBP (top) and DBP (bottom) at the last evaluation after 6 mo of treatment (left) and at puerperium (right) in pregnant women ingesting placebo at different times of the day, starting at 12–16 wks of gestation. The bars represent means ± SE.

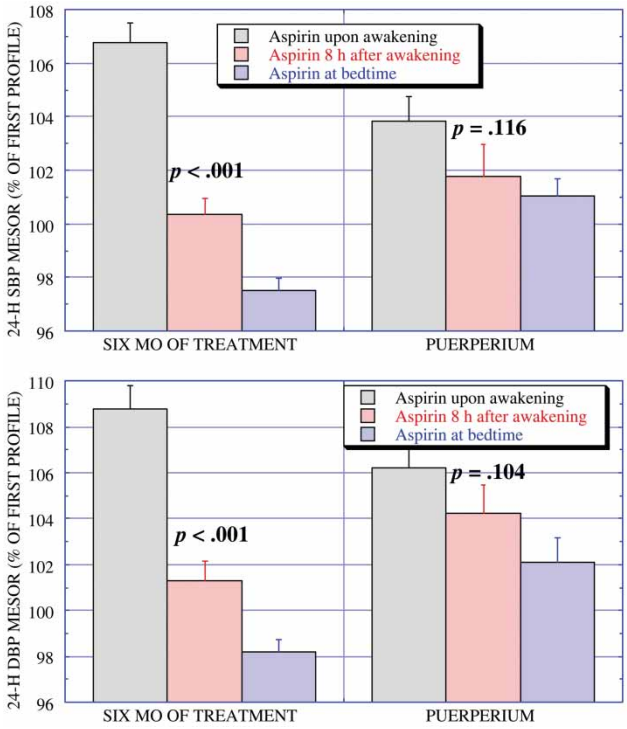


FIGURE 5. 24-h MESOR, expressed in % of the baseline 48-h ABPM evaluation per participant, of SBP (top) and DBP (bottom) at the last evaluation after 6 mo of treatment (left) and at puerperium (right) in pregnant women ingesting low-dose ASA (100 mg/d) at different times of the day, starting at 12–16 wks of gestation. The bars represent means ± SE.

( $p < .001$) administration-time-dependent effect of low-dose ASA on SBP (top) and DBP (bottom) after 6 months of timed treatment. Figure 5 (right) also illustrates that at puerperium, i.e., 6–8 wks after delivery and, therefore, discontinuation of ASA, there was no statistically significant difference in the 24-h MESOR of SBP ( $p = .116$) or DBP ( $p = .104$) between the three groups of women who ingested ASA throughout gestation at different circadian times with respect to their rest-activity cycle. Although comparison of BP at puerperium between the three groups of women who received ASA somehow shows a similar pattern than BP before delivery (Figure 5, left panel), with lower BP in women who ingested ASA in the evening or at bedtime as compared with those treated upon awakening, differences between groups were no longer statistically significant.

### Administration-Time-Dependent Effects of ASA Versus Placebo on Pregnancy Outcome

During the course of this trial, according to the diagnostic criteria stated above, 33 women developed preeclampsia and 75 gestational hypertension; 27 women had preterm deliveries and 48 IUGR; there were 7 stillbirths, 15 cases of antepartum hemorrhage, and 9 cases of postpartum hemorrhage. Event rate of preeclampsia was slightly but significantly lower in women randomized to ingest low-dose ASA than in those ingesting placebo ( $p = .041$; Table 2). The incidence of preterm delivery was significantly lower with ASA ( $p = .008$), mainly because no women ingesting ASA at Time 2 and Time 3 delivered at <37 wks of gestation. Event rates of IUGR and gestational hypertension were also significantly lower in women on ASA than placebo ( $p = .011$ and .002, respectively; Table 2). Accordingly, there was a highly significant reduction in the incidence of total serious adverse outcomes, a composite of preeclampsia, preterm delivery, IUGR, and stillbirth, in women ingesting ASA throughout their pregnancies ( $p < .001$; Table 2). There were no significant differences between low-dose ASA and placebo in the event rates of stillbirth, antepartum hemorrhage, postpartum hemorrhage, and delivery by cesarean section. Gestational age at delivery was slightly, but not significantly ( $p = .067$), higher in women ingesting ASA. Finally, newborn weight was higher with ASA than with placebo ( $p = .040$).

Figure 6 shows the adjusted HRs of evaluated pregnancy outcomes estimated by the Cox proportional-hazard model for the participants of the respective treatment groups (ASA vs. placebo), irrespectively of treatment time. Women in the low-dose ASA treatment group compared with placebo evidenced significantly lower HR of total serious adverse outcomes, either excluding (.35, 95% CI: .22–.56; $p < .001$) or including (.39, 95% CI: .27–.57; $p < .001$) gestational hypertension. Significantly reduced risk with low-dose ASA was also individually documented for preeclampsia, preterm delivery, IUGR, and gestational hypertension (Figure 6). There was no increased risk of hemorrhage, either

TABLE 2. Pregnancy outcomes of women investigated according to randomization group

| Variable | Placebo | | | | ASA | | | | *p* between placebo and ASA |
|---|---|---|---|---|---|---|---|---|---|
| | Time 1 | Time 2 | Time 3 | All | Time 1 | Time 2 | Time 3 | All | |
| Women, n | 59 | 57 | 58 | 174 | 58 | 59 | 59 | 176 | |
| Gestational age at delivery, wks | 39.2 ± 1.5 | 39.1 ± 2.0 | 39.1 ± 2.2 | 39.2 ±1.9 | 39.1 ± 2.1 | 39.8 ± 1.1 | 39.6 ± 1.1 | 39.5 ± 1.6 | .067 |
| Newborn weight, g | 3140 ± 517 | 3183 ± 599 | 3162 ± 624 | 3162 ± 580 | 3156 ± 568 | 3375 ± 453 | 3330 ± 511 | 3286 ± 519 | .040 |
| Newborn Apgar score at | | | | | | | | | |
| 1 min | 9 [9, 9] | 9 [9, 9] | 9 [9, 9] | 9 [9, 9] | 9 [9, 9] | 9 [9, 9] | 9 [9, 9] | 9 [9, 9] | .893 |
| 5 min | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [9, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | .518 |
| 10 min | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | 10 [10, 10] | .419 |
| Event rates | | | | | | | | | |
| Preeclampsia | 11.9 (3.6, 20.1) | 10.5 (2.6, 18.5) | 15.5 (6.1, 24.8) | 12.6 (7.7, 17.6) | 15.5 (6.1, 24.8) | 1.7 (−1.6, 5.0) | 1.7 (−1.6, 5.0) | 6.3 (2.7, 9.8) | .041 |
| Preterm delivery | 6.8 (.4, 13.2) | 10.5 (2.6, 18.5) | 17.2 (7.5, 27.0) | 11.5 (6.8, 16.2) | 12.1 (3.7, 20.5) | 0 | 0 | 4.0 (1.1, 6.8) | .008 |
| IUGR | 20.3 (10.1, 30.6) | 17.5 (7.7, 27.4) | 17.2 (7.5, 27.0) | 18.4 (12.6, 24.2) | 17.2 (7.5, 27.0) | 6.8 (.4, 13.2) | 3.4 (−1.2, 8.0) | 9.1 (4.8, 13.3) | .011 |
| Stillbirth | 5.1 (−.5, 10.7) | 3.5 (−1.3, 8.3) | 0 | 2.9 (.4, 5.4) | 1.7 (−1.6, 5.1) | 1.7 (−1.6, 5.0) | 0 | 1.1 (−.4, 2.7) | .246 |
| *Serious adverse outcomes* | 30.5 (18.8, 42.3) | 35.1 (22.7, 47.5) | 31.0 (19.1, 42.9) | 32.2 (25.2, 39.1) | 29.3 (17.6, 41.0) | 10.2 (2.5, 17.9) | 5.1 (−.5, 10.7) | 14.8 (9.5, 20.0) | <.001 |
| Gestational hypertension | 27.1 (15.8, 38.5) | 29.8 (17.9, 41.7) | 27.6 (16.1, 39.1) | 28.2 (21.5, 34.8) | 25.9 (14.6, 37.1) | 11.9 (3.6, 20.1) | 6.8 (.4, 13.2) | 14.8 (9.5, 20.0) | .002 |
| *Serious adverse outcomes + gestational hypertension* | 47.5 (34.7, 60.2) | 47.4 (34.4, 60.3) | 50.0 (37.1, 62.9) | 48.3 (40.9, 55.7) | 50.0 (37.1, 62.9) | 20.3 (10.1, 30.6) | 10.2 (2.5, 17.9) | 26.7 (20.2, 33.2) | <.001 |
| Cesarean delivery | 27.1 (15.8, 38.5) | 31.6 (19.5, 43.6) | 27.6 (16.1, 39.1) | 28.7 (22.0, 35.5) | 29.3 (17.6, 41.0) | 15.3 (6.1, 24.2) | 25.4 (14.3, 36.5) | 23.3 (17.1, 29.5) | .246 |
| Antepartum hemorrhage | 6.8 (.4, 13.2) | 3.5 (−1.3, 8.3) | 5.2 (−.5, 10.9) | 5.2 (1.9, 8.4) | 3.5 (−1.2, 8.1) | 3.4 (−1.2, 8.0) | 3.4 (−1.2, 8.0) | 3.4 (.7, 6.1) | .415 |
| Postpartum hemorrhage | 3.4 (−1.2, 8.0) | 3.5 (−1.3, 8.3) | 3.5 (−1.2, 8.1) | 3.5 (.7, 6.2) | 1.7 (−1.6, 5.1) | 1.7 (−1.6, 5.0) | 1.7 (−1.6, 5.0) | 1.7 (−.2, 3.6) | .303 |

Values are shown as mean ± SD. Time 1: Women randomized to ingest low-dose ASA or placebo upon awakening. Time 2: Women randomized to ingest low-dose ASA or placebo 8 h after awakening. Time 3: Women randomized to ingest low-dose ASA or placebo at bedtime. The Apgar score (expressed as median [interquartile range]) is determined by evaluating the newborn with five criteria on a scale from 1 to 2, i.e., appearance, pulse, grimace, activity, and respiration. Event rates (95% confidence intervals) are expressed as the percent ratio of observed number of events to total number of women per group. Preterm delivery: Delivery at <37 wks of gestation. IUGR: Intrauterine growth retardation. Gestational age and fetal growth were determined by monthly ecography evaluations. Serious adverse outcomes: Composite endpoint including preeclampsia, preterm delivery, IUGR, and stillbirth.

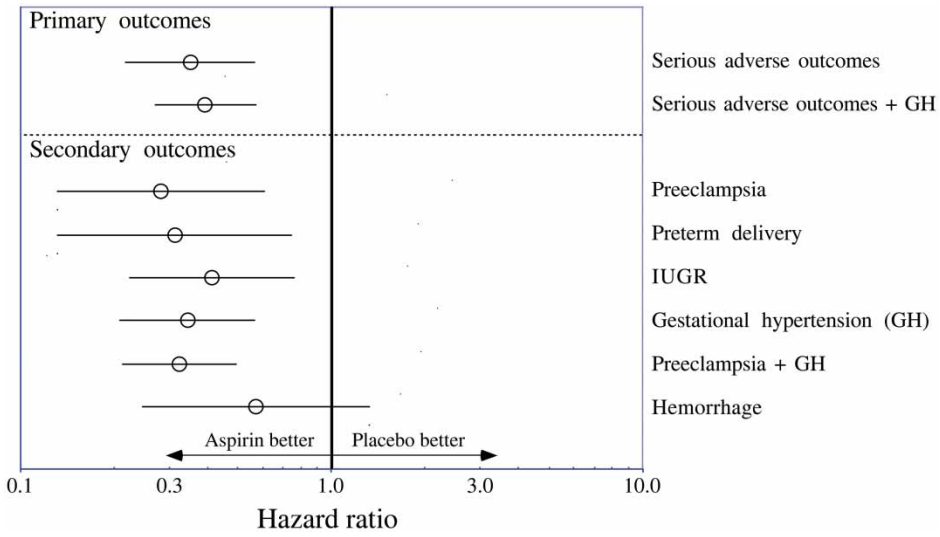


FIGURE 6. Adjusted HRs (with 95% CIs) of adverse pregnancy outcomes as a function of treatment regimen in high-risk pregnant women, i.e., those ingesting either placebo or low-dose ASA (100 mg/d) at different times of the day, starting at 12–16 wks of gestation and until delivery. Serious adverse outcomes include preeclampsia, preterm delivery, intrauterine growth retardation (IUGR), and stillbirth. Hemorrhage includes both antepartum and postpartum hemorrhage.



before or after delivery, with low-dose ASA relative to placebo (HR: .57, 95% CI: .25–1.33; $p = .194$). Advantages of low-dose ASA versus placebo are further reflected in the relatively small numbers needed-to-treat, ranging from 11 for prevention of IUGR to 16 for prevention of preeclampsia (Table 4, left column).

Taking into account the administration-time-dependent effects of ASA, but not placebo, on ambulatory BP regulation throughout gestation documented in Figures 1–3, and the marked differences in event rates between low-dose ASA at Time 2 or Time 3 and ASA at Time 1 (Table 2), we further categorized participants in terms of positive/negative BP response to treatment, i.e., those ingesting ASA 8 h after awakening or at bedtime versus those ingesting ASA upon awakening or placebo at any circadian time (Table 3). Results indicate that women ingesting ASA at Time 2 or Time 3, compared with the other four treatment-time groups evidenced (i) significantly higher gestational age at delivery ($p = .006$); (ii), significantly greater newborn weight by ~200 g ($p = .002$); (iii) significantly improved newborn Apgar score at 5 and 10 min after delivery; and (iv) most important, highly statistically significant reduced event rates of preeclampsia, gestational hypertension, preterm delivery, and IUGR ($p$ always $< .001$).

The adjusted HRs with corresponding 95% CIs of pregnancy outcomes for these two comparative groups of pregnant women are depicted in Figure 7. Results indicate significantly reduced HR of total serious adverse outcomes in women ingesting ASA at Time 2 or Time 3 (.19, 95% CI: .10–.39; $p < .001$) and total outcomes plus gestational hypertension (.24, 95% CI: .15–.40; $p < .001$). Women ingesting low-dose ASA in the evening or at bedtime also had significantly lower HRs of preeclampsia, preterm delivery (not shown, as there were no documented cases in these two groups), IUGR, and gestational hypertension (Figure 7). There was no increased risk of hemorrhage, either before or after delivery, with low-dose ASA at these two circadian times compared with ASA upon awakening or placebo (HR: .62, 95% CI: .25–1.59; $p = .321$). The number needed-to-treat by low-dose ASA ingested in the evening or at bedtime to prevent a serious adverse pregnancy outcome is a very low (4 women) and even lower (3 women) when including prevention of gestational hypertension (Table 4; right column). Low-dose ASA administered at this proper circadian time would require numbers needed-to-treat as low as 8 or 9 women for prevention of either preeclampsia, preterm delivery, or IURG (Table 4).

## DISCUSSION

The first major finding from this prospective, randomized, placebo-controlled, double-blind trial on women at high risk for preeclampsia is that low-dose ASA (100 mg/d) starting early in pregnancy (≥16 wks of gestation) has an effect on ambulatory SBP and DBP as a function of the timing of its ingestion in relation to the 24 h rest-activity cycle of each individual woman, i.e., in the evening and, mainly, at bedtime, but not upon awakening. Results indicate that (i) there was no statistically significant difference in BP between women ingesting placebo at different circadian times; (ii) there was a highly statistically significant BP reduction, consistently increasing throughout gestation, in women ingesting 100 mg/d ASA ($p < .001$ for the comparison of BP changes during pregnancy between ASA and placebo independent on treatment time); and (iii) the effects of ASA on BP were markedly dependent on administration time: there was no effect on ambulatory BP, compared with placebo, when ASA was ingested on awakening, but the BP reduction was highly statistically significant

TABLE 3. Pregnancy outcomes of women investigated according to randomization group

| Variable | ASA at Time 1 and placebo at any time | ASA at Time 2 and Time 3 | *p* between groups |
|---|---|---|---|
| Women, n | 232 | 118 | |
| Gestational age at delivery, wks | 39.2 ± 2.0 | 39.7 ± 1.1 | .006 |
| Newborn weight, g | 3160 ± 575 | 3351 ± 482 | .002 |
| Newborn Apgar score at | | | |
| 1 min | 9 [9, 9] | 9 [9, 9] | .080 |
| 5 min | 10 [10, 10] | 10 [10, 10] | .003 |
| 10 min | 10 [10, 10] | 10 [10, 10] | .006 |
| Event rates | | | |
| Preeclampsia | 13.4 (9.0, 17.7) | 1.7 (−.6, 4.0) | <.001 |
| Preterm delivery | 11.6 (7.5, 15.8) | 0 | <.001 |
| IUGR | 18.1 (13.1, 23.1) | 5.1 (1.1, 9.0) | <.001 |
| Stillbirth | 2.6 (.5, 4.6) | .8 (−.8, 2.5) | .272 |
| *Serious adverse outcomes* | 31.5 (25.5, 37.4) | 7.6 (2.8, 12.4) | <.001 |
| Gestational hypertension | 27.6 (21.8, 33.3) | 9.3 (4.1, 14.6) | <.001 |
| *Serious adverse outcomes + gestational hypertension* | 48.7 (42.3, 55.1) | 15.3 (8.8, 21.7) | <.001 |
| Cesarean delivery | 28.9 (23.1, 34.7) | 20.3 (13.1, 27.6) | .085 |
| Antepartum hemorrhage | 4.7 (2.0, 7.5) | 3.4 (.1, 6.7) | .555 |
| Postpartum hemorrhage | 3.0 (.8, 5.2) | 1.7 (−.6, 4.0) | .460 |

Values are shown as mean ± SD. Time 1: Women randomized to ingest low-dose ASA or placebo upon awakening. Time 2: Women randomized to ingest low-dose ASA or placebo 8 h after awakening. Time 3: Women randomized to ingest low-dose ASA or placebo at bedtime. The Apgar score (expressed as median [interquartile range]) is determined by evaluating the newborn with five criteria on a scale from 1 to 2, i.e., appearance, pulse, grimace, activity, and respiration. Event rates (95% confidence intervals) are expressed as the percent ratio of observed number of events to total number of women per group. Preterm delivery: Delivery at <37 wks of gestation. IUGR: Intrauterine growth retardation. Gestational age and fetal growth were determined by monthly ecography evaluations. Serious adverse outcomes: Composite endpoint including preeclampsia, preterm delivery, IUGR, and stillbirth.



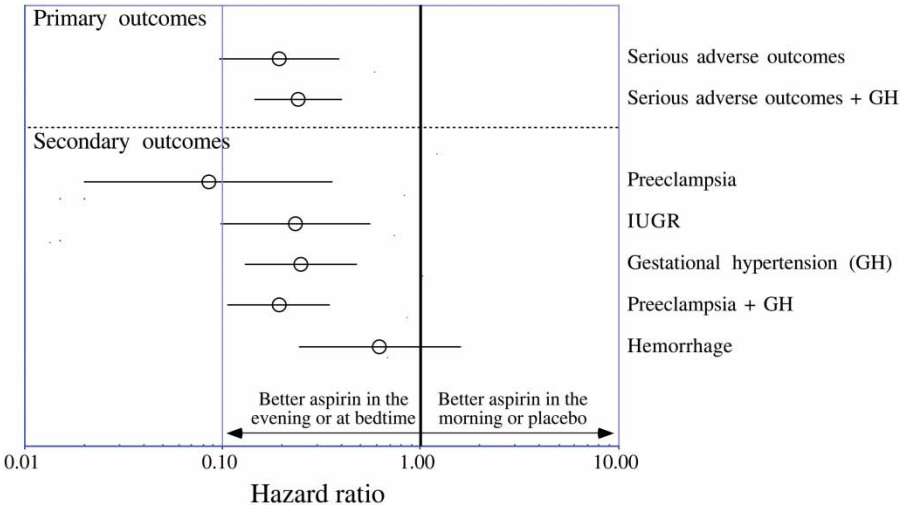


FIGURE 7. Adjusted HRs (with 95% CIs) of adverse pregnancy outcomes as a function of treatment-time regimen in high-risk pregnant women, i.e., those ingesting either placebo or low-dose ASA (100 mg/d) at different times of the day, starting at 12–16 wks of gestation and until delivery. Serious adverse outcomes include preeclampsia, preterm delivery, intrauterine growth retardation (IUGR), and stillbirth. Hemorrhage includes both antepartum and postpartum hemorrhage. For comparison purposes, participants were divided in two groups: those ingesting ASA 8 h after awakening or at bedtime versus those ingesting ASA upon awakening or placebo at any circadian time.

when low-dose ASA was ingested 8 h after awakening (Figure 2) and, to a greater extent, when ingested at bedtime (Figure 3). Moreover, at puerperium, 6–8 wks after discontinuation of treatment, there was no statistically significant difference in 24-h SBP/DBP MESOR between the groups of women who ingested ASA at different circadian times for most of their pregnancies (Figure 5).

These administration-time-dependent effects of low-dose ASA on ambulatory BP are in agreement with conclusions found earlier in clinically healthy subjects with prehypertension (Hermida et al., 2009b) as well as in hypertensive subjects ingesting the same low-dose of 100 mg/d ASA for 3 months (Hermida et al., 2003a, 2005a, 2005b). A higher dose of ASA (500 mg/d), however, showed a pressor effect, even when ingested

TABLE 4. Numbers needed-to-treat (95% CIs) according to circadian time of ASA administration in high-risk pregnant women

| Outcome | ASA at any time vs. placebo | ASA at Time 2 and Time 3 vs. ASA at Time 1 or placebo |
|---|---|---|
| Preeclampsia | 15.6 (8.0–337.2) | 8.6 (6.0–14.9) |
| Preterm delivery | 13.3 (7.6–50.8) | 8.6 (6.3–13.3) |
| IUGR | 10.7 (6.1–46.6) | 7.7 (5.1–15.0) |
| *Serious adverse outcomes* | 5.7 (3.8–11.5) | 4.2 (3.2–6.2) |
| Gestational hypertension | 7.5 (4.6–20.4) | 5.5 (3.8–9.5) |
| *Serious adverse outcomes + gestational hypertension* | 4.6 (3.2–8.6) | 3.0 (2.4–4.1) |

Time 1: Women randomized to ingest low-dose ASA or placebo upon awakening. Time 2: Women randomized to ingest low-dose ASA or placebo 8 h after awakening. Time 3: Women randomized to ingest low-dose ASA or placebo at bedtime. Preterm delivery: Delivery at <37 wks of gestation. IUGR: Intrauterine growth retardation. Gestational age and fetal growth were determined by monthly ecography evaluations. Serious adverse outcomes: Composite endpoint including preeclampsia, preterm delivery, IUGR, and stillbirth.



at bedtime for just 1 week (Hermida et al., 1997b). It has been reported that NSAIDs may increase BP both in normotensive and hypertensive individuals (Johnson et al., 1994; Pope et al., 1993). Whether the administration-time-dependent effects of ASA on BP documented in these studies, performed in Spanish subjects, may be extended to other racial or ethnic populations awaits prospective investigation.

Most important, this prospective trial documents highly significant effects of low-dose ASA on relevant pregnancy outcome variables. Women ingesting low-dose ASA, compared with placebo, evidenced a significantly lower HR of serious adverse outcomes, a composite of preeclampsia, preterm delivery, IUGR, and stillbirth (.35, 95% CI: .22–.56; $p < .001$). The HR of individual outcome variables, i.e., preeclampsia, preterm delivery, IUGR, and gestational hypertension, were also significantly lower with ASA versus placebo. Moreover, these beneficial effects of low-dose ASA on pregnancy outcome were highly dependent on the time of its administration. There were small and nonsignificant differences in outcomes between placebo and low-dose ASA ingested upon awakening. These four groups combined, however, showed a highly significant greater HR of serious adverse outcomes than women ingesting ASA either in the evening or at bedtime (.19, 95% CI: .10–.39; $p < .001$), as well as those of preeclampsia, gestational hypertension, and IUGR analyzed separately. Interestingly, ingestion of ASA in the evening or at bedtime was also characterized by higher gestational age at birth, due to marked prevention of preterm delivery, and significantly greater newborn weight (Table 3). Also clinically relevant, there was no increased risk of hemorrhage, either before or after delivery, with low-dose ASA relative to placebo (HR: .57, 95% CI: .25–1.33; $p = .194$).

Recent meta-analyses have consistently concluded low-dose ASA to be protective against preeclampsia, mainly in high-risk women, although the number-to-treat derived from such analyses seems to be high (Askie et al., 2007; Bujold et al., 2009, 2010, 2011; Roberge et al., 2012a, 2012b; Ruano et al., 2005; Trivedi, 2011). With respect to the potentially most favorable dose of ASA, results in the literature show some controversy. Askey et al. (2007) concluded that there is no evidence that using >75 mg/d ASA had more or less effect than a lower dose. On the contrary, results from a meta-analysis on the potential benefits of low-dose ASA for prevention of IUGR, as reported by Leitich et al. (2007), indicated that the preventive effect was greater at higher doses (100–150 mg/d ASA) as compared with inefficient lower doses (50–80 mg/d ASA). The 100 mg/d ASA dose used in our ASEM trial has been shown to affect both maternal as well as placental thromboxane (Walsh & Wang, 1998) and, until proof might be offered on the contrary, should be the recommended minimum dose of ASA to be used for prevention of hypertensive complications in pregnancy.

Duration of intervention with low-dose ASA during gestation has also been extensively discussed in the literature (Askie et al., 2007; Barth, 1998; Bujold et al., 2009, 2010, 2011; Hermida et al., 1997a, 1999, 2003c; Roberge et al., 2012a, 2012b; Ruano et al., 2005; Trivedi, 2011). As mentioned above, most large trials showing little or no benefit of ASA for prevention of preeclampsia included women with gestational age at entry up to 26–32 wks of gestation, too late for any reasonable or practical prophylactic intervention in pregnancy (Ayala & Hermida, 2012; Ayala et al., 1997a, 1997b; Hermida & Ayala, 1997, 2001, 2005a; Hermida et al., 2000a, 2001a, 2003b, 2003d, 2004a, 2004c). Roberge et al. (2012) recently reported their findings from a meta-analysis aimed to determine whether early ASA administration prevents severe and mild preeclampsia. When compared with controls, ASA starting at ≤16 wks of gestation was associated with a significant reduction in severe (relative risk: .22, 95% CI: .08–.57) but not mild (relative risk: .81, 95% CI: .33–1.96) preeclampsia (Roberge et al., 2012). Bujold et al. (2010) reviewed randomized controlled trials on ASA for prevention of preeclampsia and IUGR. Their meta-analysis concluded that low-dose ASA starting at ≤16 wks of gestation was associated with a

significant reduction in preeclampsia, gestational hypertension, preterm delivery, and IUGR, whereas ASA starting after 16 wks of gestation was not (Bujold et al., 2010). In keeping with the documented findings of the ASEM trial summarized here, ingestion of low-dose ASA for prevention of hypertensive complications in pregnancy should start at ≤16 wks of gestation.

No other previous trial on the potential effects of ASA in pregnancy has ever taken into consideration the most relevant variable of treatment time with respect to the rest-activity cycle of the individual subjects. In fact, the biology of human beings is not constant, as postulated by the concept of homeostasis, but predictably rhythmic over the 24 h and other time scales (Duguay & Cermakian, 2009). In particular, BP exhibits 24-h variation, also in pregnant women (Ayala & Hermida, 2001, 2012; Ayala et al., 1997a; Benedetto et al., 1996; Hermida & Ayala, 2005b; Hermida et al., 2000a, 2003b, 2003d, 2004a, 2004b; Miyamoto et al., 1998), as a result of both cyclic day-night alterations in behavior (e.g., physical activity, mental stress, and posture) and environmental phenomena (e.g., ambient temperature, noise, etc.) plus endogenous circadian (~24-h) rhythms in neural, endocrine, endothelial, and hemodynamic variables (e.g., plasma noradrenaline and adrenaline [autonomic nervous system] and renin, angiotensin, and aldosterone [renin-angiotensin-aldosterone system, RAAS]) (Fabbian et al., 2012; Hermida et al., 2007c; Pinotti et al., 2005; Portaluppi & Smolensky, 2007; Portaluppi et al., 1996, 2012; Smolensky et al., 2012). The high-amplitude circadian rhythm in the RAAS (Angeli et al., 1992; Bartter et al., 1979; Cugini et al., 1980, 1988; Gordon et al., 1966; Katz et al., 1975), showing peak plasma concentrations of renin activity, angiotensin-converting enzyme, angiotensin I and II, and aldosterone just prior to or immediately following the usual time of morning awakening, plays a prominent role in 24-h BP regulation and patterning. The comparatively lower BP during nighttime sleep found in most normotensive individuals, including healthy pregnant women, and some primary hypertensive patients results from withdrawal of sympathetic dominance, predominance of vagal tone, lowered concentration of RAAS constituents, and peak levels of calcitonin gene–related peptide, atrial natriuretic peptide, and nitric oxide as vasodilators (Kanabrocki et al., 2001; Portaluppi et al., 1992; Smolensky et al., 2007; Sothern et al., 1995; Trasforini et al., 1991; Winters et al., 1988). As a consequence, a clear circadian rhythmicity is observed also in the triggering factors of cardiovascular events that may also be altered by disease (Gallerani et al., 1997; Manfredini et al., 1996; Portaluppi et al., 1990, 1994).

Because the main steps in the mechanisms regulating BP are circadian stage dependent, it is not surprising that BP-lowering medications may display a circadian time dependency in their pharmacokinetics and pharmacodynamics (Hermida et al., 2007d, 2012b; Smolensky & Haus, 2001; Smolensky & Portaluppi, 1999; Smolensky et al., 2010, 2012; Witte & Lemmer, 2003). Many published prospective trials reviewed elsewhere (Hermida & Smolensky, 2004; Hermida et al., 2007d, 2011a, 2012b; Smolensky et al., 2010, 2012) have reported clinically meaningful morning-evening, treatment-time differences in BP-lowering efficacy, duration of action, safety profile, and/or effects on the circadian BP pattern of six classes of hypertension medications and their combinations (Hermida et al., 2010b, 2011e). For instance, the once-daily bedtime, in comparison with upon-awakening, ingestion schedule of angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors results in significantly greater therapeutic effect both on the asleep BP mean and sleep-time relative BP decline towards more of a dipping pattern, independent of the terminal half-life of the medications (Hermida & Ayala, 2009; Hermida et al., 2003e, 2007a, 2009a, 2010a, 2011a). Furthermore, it has been recently documented that the progressive reduction in asleep BP mean, a novel therapeutic target best achieved with bedtime hypertension therapy (Hermida et al., 2011a, 2012b; Portaluppi & Smolensky, 2010; Smolensky et al., 2010), is the most significant predictor of cardiovascular event-free survival (Ayala et al., 2012a; Hermida et al., 2010c, 2011b, 2011c, 2011d, 2012a, 2012d, 2012e, 2012f, 2012g, 2012h).

Despite all these collective information, the potential mechanism(s) of the time-of-day-dependent differences (with reference to the rest-activity cycle) in the BP responsiveness to ASA are as yet unknown. Recent studies have shown statistically significant circadian rhythms in thromboxane and prostacyclin production, circulating platelets, platelet aggregation, clotting and fibrinolytic inhibitors, angiotensin sensitivity in pregnancy, as well as in the inhibition of platelet aggregation produced by ASA (Delemarre et al., 1996; Haus et al., 1990). Another potentially relevant factor to be taken into consideration is the pharmacokinetic observation that ASA exhibits a faster rate of clearance when administered in the morning as compared with the evening (Markiewicz & Semenowicz, 1979). These results complement administration-time-dependent changes that have been described when the pharmacokinetics of NSAIDs were investigated in humans (Labrecque & Reinberg, 1989). It has been also reported that effects of ASA upon α- and β-adrenergic receptors depend on the circadian timing of ASA ingestion (Cornélissen et al., 1991). α-Adrenoceptor blockade reduces peripheral resistance more effectively in the early morning hours than at other times of the day (Panza et al., 1991). A recent study exploring the administration-time-dependent effects of the gastrointestinal therapeutic system formulation of the α-blocker doxazosin (Hermida et al., 2004d) concluded that the daily ingestion of the medication at bedtime resulted in a statistically significant doubling of the daily BP mean reduction as compared with morning dosing. Most important, ASA has been shown to significantly inhibit angiotensin II, an effect

that is dependent on the dose and circadian time of ASA ingestion (Kanzik et al., 1992). Moreover, ASA is known to acetylate a variety of proteins, including COX-2. COX-2 inhibition has also been shown to decrease renin content and to lower BP in a model of renovascular hypertension (Wang et al., 1999). These results may be relevant inasmuch as ASA ingested at the end of the activity cycle, but not upon awakening, could thus target the nocturnal peak of the known circadian rhythm in plasma renin activity (Bartter et al., 1979), while enhancing the nocturnal trough in the production of nitric oxide (Kanabrocki et al., 2001). These hypotheses gain relevancy given the significantly enhanced effect of ASA in reducing the asleep SBP/DBP means in hypertensive patients with a non-dipper BP profile, as previously documented (Hermida et al., 2005b). Patients with an altered non-dipper BP pattern are characterized by attenuated nitric oxide release as compared with dipper patients (Higashi et al., 2002). Finally, Snoep et al. (2009) found that 100 mg/d ASA when ingested at bedtime, as compared with upon awakening, significantly diminished not only the 24-h level of plasma renin activity but also the excretion in 24-h urine samples of cortisol, dopamine, and norepinephrine. Thus, the enhanced reduction in plasma renin activity, beneficial impact on endothelial function, and blocking of α- and β-adrenergic receptors associated with bedtime administration of low-dose ASA could explain its administration-time-dependent effects on BP and pregnancy outcome here documented.

While considering the potential benefits of the cost-effective use of low-dose ASA for prevention of complications in pregnancy (Table 4), one must also discuss potential risks of ASA ingested at different times of the day. In this trial, compliance did not differ between awakening and bedtime dosing. With respect to tolerability, a previous endoscopic trial on volunteers who ingested high-dose ASA (1300 mg) at different times on separate study days showed that evening, in comparison with morning, dosing produced 37% fewer gastric hemorrhagic lesions (Moore et al., 1987). Hence, nighttime administration of ASA appears to be better tolerated than morning administration, and low-dose ASA is more likely associated with lower risks of bleeding than higher doses (Moore et al., 1987). As indicated previously, in our trial there was no increased risk of hemorrhage, either before or after delivery, with low-dose ASA relative to placebo (Table 2).

In conclusion, results from our prospective ASEM trial document highly significant benefits of low-dose ASA on BP regulation and pregnancy outcome in high-risk pregnant women. These beneficial effects are markedly dependent on the circadian time of ASA administration with respect to the rest-activity cycle of each woman, being negligible when ASA is ingested upon awakening. The use of doses of ASA <80 mg/d that do not affect placental thromboxane, commencing therapy with ASA after 16 wks gestation, and, most importantly, lack of proper circadian timing for ASA administration could all explain the lack of positive results in some previous clinical trials (CLASP, 1994; ECPPA, 1996; Golding, 1998; Italian Study of Aspirin in Pregnancy, 1993; Rotchell, 1998; Sibai et al., 1993). Our results indicate that (i) 100 mg/d ASA should be the recommended minimum dose to be used for prevention of complications in pregnancy; (ii) ingestion of low-dose ASA for prevention of complications in pregnancy should start at ≤16 wks of gestation; and (iii) low-dose ASA ingested at bedtime, but not upon awakening, significantly lowers ambulatory BP and reduces the incidence of preeclampsia, gestational hypertension, preterm delivery, and IUGR. For such clinical recommendations to be practical, however, one must be able to properly identify, among the general obstetric population, the women at higher risk for hypertension in pregnancy who might thus benefit most from daily ASA ingestion. Along these lines, we have previously documented the so-called tolerance-hyperbaric test, where diagnosis of hypertension in pregnancy is based on the HBI calculated from 48-h ABPM with reference to a time-specified tolerance limit specified as a function of gestational age, has been shown to provide high sensitivity and specificity for the early identification, at <14 wks of gestation, of subsequent hypertension in pregnancy (Ayala & Hermida, 2012; Hermida et al., 1998, 2004c), and to be a valuable and reproducible approach for the prediction of pregnancy outcome (Ayala & Hermida, 2012; Hermida & Ayala, 2002; Hermida et al., 2003b).

## ACKNOWLEDGMENTS

This work is dedicated to the loving memory of Dr. Manuel Iglesias-Díz and Dr. Inés Silva-Villar.

**Declaration of Interest:** This work was supported by unrestricted grant support from Ministerio de Ciencia e Innovación (SAF2009-7028-FEDER); Consellería de Economía e Industria, Dirección Xeral de Investigación e Desenvolvemento, Xunta de Galicia (INCITE07-PXI-322003ES, INCITE08-E1R-322063ES, INCITE09-E2R-322099ES, IN845B-2010/114, 09CSA018322PR); and Vicerrectorado de Investigación, University of Vigo.

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## REFERENCES

Angeli A, Gatti G, Masera R. (1992). Chronobiology of the hypothalamic-pituitary-adrenal and renin-angiotensin-aldosterone systems. In Touitou Y, Haus E (Eds.). *Biologic rhythms in clinical and laboratory medicine.* Berlin: Springer-Verlag, 292–314.

Antiplatelet Trialists' Collaboration. (1994). Collaborative overview of randomised trials of antiplatelet therapy. I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ* 308:81–106.

Antithrombotic Trialists' Collaboration. (2002). Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 324:71–86.

Askie LM, Duley L, Henderson-Smart DJ, Stewart LA, PARIS Collaborative Group. (2007). Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *Lancet* 369:1791e–1798e.

Ayala DE, Hermida RC. (2001). Influence of parity and age on ambulatory monitored blood pressure during pregnancy. *Hypertension* 38:753–758.

Ayala DE, Hermida RC. (2010). Sex differences in the administration-time-dependent effects of low-dose aspirin on ambulatory blood pressure in hypertensive subjects. *Chronobiol. Int.* 27:345–362.

Ayala DE, Hermida RC. (2012). Ambulatory blood pressure monitoring for the early identification of hypertension in pregnancy. *Chronobiol. Int.* 29:in press.

Ayala DE, Hermida RC, Mojón A, Fernández JR, Iglesias M. (1997a). Circadian blood pressure variability in healthy and complicated pregnancies. *Hypertension* 30:603–610.

Ayala DE, Hermida RC, Mojón A, Fernández JR, Silva I, Ucieda R, Iglesias M. (1997b). Blood pressure variability during gestation in healthy and complicated pregnancies. *Hypertension* 30:611–618.

Ayala DE, Hermida RC, Mojón A, Fernández JR. (2012a). Cardiovascular risk of resistant hypertension: dependence on treatment-time regimen of blood pressure-lowering medications. *Chronobiol. Int.* 29:in press.

Ayala DE, Moyá A, Crespo JJ, Castiñeira C, Domínguez-Sardiña M, Gomara S, Sineiro E, Mojón A, Fontao MJ, Hermida RC, on behalf of the Hygia Project Investigators. (2012b). Circadian pattern of ambulatory blood pressure in hypertensive patients with and without type 2 diabetes. *Chronobiol. Int.* 29:in press.

Barth W. (1998). Low-dose aspirin for preeclampsia. The unresolved question. *N. Engl. J. Med.* 338:756–757.

Bartter FC, Chan JCM, Simpson HW. (1979). Chronobiological aspect of plasma renin activity, plasma aldosterone and urinary electrolytes. In Krieger DT (Ed.). *Endocrine rhythms*. New York: Raven, 49–132.

Beaufils M, Uzan S, Donsimoni R, Colau JC. (1985). Prevention of pre-eclampsia by early antiplatelet therapy. *Lancet* 1:840–842.

Benedetto C, Zonca M, Marozio L, Dolco C, Carandente F, Massobrio M. (1996). Blood pressure patterns in normal pregnancy and in pregnancy-induced hypertension, preeclampsia, and chronic hypertension. *Obstet. Gynecol.* 88:503–510.

Bingham C, Arbogast B, Cornélissen G, Lee JK, Halberg F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia* 9:397–439.

Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. (2001). The classification and diagnosis of the hypertensive disorders of pregnancy: statement from The International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens. Pregnancy* 20:x–xiv.

Buchbinder A, Sibai BM, Caritis S, Macpherson C, Hauth J, Lindheimer MD, Klebanoff M, Vandorsten P, Landon M, Paul R, Miodovnik M, Meis P, Thurnau G, National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. (2002). Adverse perinatal outcomes are significantly higher in severe gestational hypertension than in mild pre-eclampsia. *Am. J. Obstet. Gynecol.* 186:66–71.

Bujold E, Morency AM, Roberge S, Lacasse Y, Forest JC, Giguère Y. (2009). Acetylsalicylic acid for the prevention of preeclampsia and intra-uterine growth restriction in women with abnormal uterine artery Doppler: a systematic review and meta-analysis. *J. Obstet. Gynaecol. Can.* 31:818–826.

Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, Forest JC, Giguère Y. (2010). Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstet. Gynecol.* 116:402–414.

Bujold E, Tapp S, Audibert F, Ferreira E, Forest JC, Rey E, Fraser WD, Chaillet N, Giguère Y. (2011). Prevention of adverse pregnancy outcomes with low-dose ASA in early pregnancy: new perspectives for future randomized trials. *J. Obstet. Gynaecol. Can.* 33:480–483.

Bussolino F, Benedetto C, Massobrio M, Camussi G. (1980). Maternal vascular prostacyclin activity in pre-eclampsia. *Lancet* 2:702.

Caritis S, Sibai B, Hauth J, Lindheimer MD, Klebanoff M, Thom E, Van-Dorsten P, Landon M, Paul R, Miodovnik M, Meis P, Thurnau G. (1998). Low-dose aspirin to prevent preeclampsia in women at high risk. *N. Engl. J. Med.* 338:701–705.

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

Cornélissen G, Halberg F, Prikryl P, Dankova E, Siegelova J, Dusek J, International Womb-To-Tomb Chronome Study Group. (1991). Prophylactic aspirin treatment: the merits of timing. *JAMA* 266:3128–3129.

Cugini P, Manconi E, Serdoz N, Mancini A, Meucci R, Scavo D. (1980). Rhythm characteristics of plasma renin, aldosterone and cortisol in five types of mesor-hypertension. *J. Endocrinol. Invest.* 3:143–149.

Cugini P, Letizia C, Scavo D. (1988). The circadian rhythmicity of serum angiotensin converting enzyme: its phasic relation with the circadian cycle of plasma renin and aldosterone. *Chronobiologia* 15:229–232.

Dekker GA, Sibai BM. (1993). Low-dose aspirin in the prevention of preeclampsia and fetal growth retardation: rationale, mechanisms, and clinical trials. *Am. J. Obstet. Gynecol.* 168:214–227.

Delemarre FM, Didden MA, de Jong PA. (1996). Diurnal variation in angiotensin sensitivity in pregnancy. *Am. J. Obstet. Gynecol.* 174:259–261.

Duguay D, Cermakian N. (2009). The crosstalk between physiology and circadian clock proteins. *Chronobiol. Int.* 26:1479–1513.

Duley L. (2009). The global impact of pre-eclampsia and eclampsia. *Semin. Perinatol.* 33:130–137.

Dumont A, Flahault A, Beaufils M, Verdy E, Uzan S. (1999). Effect of aspirin in pregnant women is dependent on increase in bleeding time. *Am. J. Obstet. Gynecol.* 180:135–140.

ECPPA (Estudo Colaborativo para Prevençao da Pré-eclampsia com Aspirina) Collaborative Group. (1996). ECPPA: a randomised trial of low dose aspirin for the prevention of maternal and fetal complications in high risk pregnant women. *Br. J. Obstet. Gynaecol.* 103:39–47.

Fabbian F, Smolensky MH, Tiseo R, Pala M, Manfredini R, Portaluppi F. (2012). Dipper and non-dipper blood pressure 24-hour patterns: circadian rhythm-dependent physiologic and pathophysiologic mechanisms. *Chronobiol. Int.* 29:in press.

Fernández JR, Hermida RC. (1998). Inferential statistical method for analysis of nonsinusoidal hybrid time series with unequidistant observations. *Chronobiol. Int.* 15:191–204.

Fernández JR, Mojón A, Hermida RC, Alonso I. (2003). Methods for comparison of parameters from longitudinal rhythmometric models with multiple components. *Chronobiol. Int.* 20:495–513.

Friedman SA. (1988). Preeclampsia: a review of the role of prostaglandins. *Obstet. Gynecol.* 71:122–137.

Fuster V, Dyken ML, Vokonas PS, Hennekens C. (1993). Aspirin as a therapeutic agent in cardiovascular disease. *Circulation* 87:659–675.

Gallerani M, Portaluppi F, Grandi E, Manfredini R. (1997). Circadian rhythmicity in the occurrence of spontaneous acute dissection and rupture of thoracic aorta. *J. Thorac. Cardiovasc. Surg.* 113:603–604.

Golding J. A randomised trial of low dose aspirin for primiparae in pregnancy. (1998). *Br. J. Obstet. Gynaecol.* 105:293–299.

Gordon RD, Wolfe LK, Island DP, Liddle GW. (1966). A diurnal rhythm in plasma renin activity in man. *J. Clin. Invest.* 45:1587–1592.

Grosser N, Schroder H. (2003). Aspirin protects endothelial cells from oxidant damage via the nitric-oxide-cGMP pathway. *Arterioscler. Thromb. Vasc. Biol.* 23:1345–1351.

Haus E, Cusulos M, Sackett-Lundeen L, Swoyer J. (1990). Circadian variations in platelet functions and blood coagulation parameters. In Reinberg A, Smolensky M, Labrecque G (Eds.). *Annual review of chronopharmacology*, Vol. 7. Oxford, UK: Pergamon Press, pp. 153–156.

Hayden M, Pignone M, Phillips C, Mulrow C. (2002). Aspirin for the primary prevention of cardiovascular events: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 136:161–171.

Hermida RC. (1999). Time-qualified reference values for 24 h ambulatory blood pressure monitoring. *Blood Press. Monit.* 4:137–147.

Hermida RC, Ayala DE. (1997). Diagnosing gestational hypertension and preeclampsia with the 24-hour mean of blood pressure. *Hypertension* 30:1531–1537.

Hermida RC, Ayala DE. (2001). Evaluation of the blood pressure load in the diagnosis of hypertension in pregnancy. *Hypertension* 38:723–729.

Hermida RC, Ayala DE. (2002). Prognostic value of office and ambulatory blood pressure measurements in pregnancy. *Hypertension* 40:298–303.

Hermida RC, Ayala DE. (2003). Sampling requirements for ambulatory blood pressure monitoring in the diagnosis of hypertension in pregnancy. *Hypertension* 42:619–624.

Hermida RC, Ayala DE. (2004). Prognostic value of ambulatory blood pressure measurements for the diagnosis of hypertension in pregnancy. *Expert Rev. Cardiovasc. Ther.* 2:89–105.

Hermida RC, Ayala DE. (2005a). Reference thresholds for 24-hour, diurnal, and nocturnal blood pressure mean values in pregnancy. *Blood Press. Monit.* 10:33–41.

Hermida RC, Ayala DE. (2005b). Circadian blood pressure variability in normotensive pregnant women as a function of parity, maternal age, and stage of gestation. *Chronobiol. Int.* 22:321–341.

Hermida RC, Ayala DE. (2009). Chronotherapy with the angiotensin-converting enzyme inhibitor ramipril in essential hypertension: improved blood pressure control with bedtime dosing. *Hypertension* 54:40–46.

Hermida RC, Fernández JR. (1996). Computation of time-specified tolerance intervals for ambulatorily monitored blood pressure. *Biomed. Instrum. Technol.* 30:257–266.

Hermida RC, Smolensky MH. (2004). Chronotherapy of hypertension. *Curr. Opin. Nephrol. Hypertens.* 13:501–505.

Hermida RC, Mojón A, Fernández JR, Ayala DE. (1996). Computer-based medical system for the computation of blood pressure excess in the diagnosis of hypertension. *Biomed. Instrum. Technol.* 30:267–283.

Hermida RC, Ayala DE, Iglesias M, Mojón A, Silva I, Ucieda R, Fernández JR. (1997a). Time-dependent effects of low-dose aspirin administration on blood pressure in pregnant women. *Hypertension* 30:589–595.

Hermida RC, Fernández JR, Ayala DE, Mojón A, Iglesias M. (1997b). Influence of aspirin usage on blood pressure. Dose and administration time-dependencies. *Chronobiol. Int.* 14:619–637.

Hermida RC, Mojón A, Fernández JR, Ayala DE. (1997c). The tolerance-hyperbaric test for hypertension diagnosis. *Biomed. Instrum. Technol.* 31:499–510.

Hermida RC, Ayala DE, Mojón A, Fernández JR, Silva I, Ucieda R, Iglesias M. (1998). Blood pressure excess for the early identification of gestational hypertension and preeclampsia. *Hypertension* 31:83–89.

Hermida RC, Ayala DE, Fernández JR, Mojón A, Alonso I, Silva I, Ucieda R, Codesido J, Iglesias M. (1999). Administration time-dependent effects of aspirin in women at differing risk for preeclampsia. *Hypertension* 34:1016–1023.

Hermida RC, Ayala DE, Mojón A, Fernández JR, Alonso I, Silva I, Ucieda R, Iglesias M. (2000a). Blood pressure patterns in normal pregnancy, gestational hypertension and preeclampsia. *Hypertension* 36:149–158.

Hermida RC, Fernández JR, Mojón A, Ayala DE. (2000b). Reproducibility of the hyperbaric index as a measure of blood pressure excess. *Hypertension* 35:118–125.

Hermida RC, Ayala DE, Iglesias M. (2001a). Predictable blood pressure variability in healthy and complicated pregnancies. *Hypertension* 38:736–741.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2001b). Time-qualified reference values for ambulatory blood pressure monitoring in pregnancy. *Hypertension* 38:746–752.

Hermida RC, Ayala DE, Fernández JR, Mojón A, Alonso I, Calvo C. (2002a). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiol. Int.* 19:461–481.

Hermida RC, Calvo C, Ayala DE, Fernández JR, Ruilope LM, López JE. (2002b). Evaluation of the extent and duration of the "ABPM effect" in hypertensive patients. *J. Am. Coll. Cardiol.* 40:710–717.

Hermida RC, Calvo C, Ayala DE, Mojón A, López JE. (2002c). Relationship between physical activity and blood pressure in dipper and nondipper hypertensive patients. *J. Hypertens.* 20:1097–1104.

Hermida RC, Mojón A, Fernández JR, Alonso I, Ayala DE. (2002d). The tolerance-hyperbaric test: a chronobiologic approach for improved diagnosis of hypertension. *Chronobiol. Int.* 19:1183–1211.

Hermida RC, Ayala DE, Calvo C, López JE, Fernández JR, Mojón A, Domínguez MJ, Covelo M. (2003a). Administration-time dependent effects of aspirin on blood pressure in untreated hypertensive patients. *Hypertension* 41:1259–1267.

Hermida RC, Ayala DE, Iglesias M. (2003b). Circadian rhythm of blood pressure challenges office values as the "gold standard" in the diagnosis of gestational hypertension. *Chronobiol. Int.* 20:135–156.

Hermida RC, Ayala DE, Iglesias M. (2003c). Administration-time dependent influence of aspirin on blood pressure in pregnant women. *Hypertension* 41:651–656.

Hermida RC, Ayala DE, Mojón A, Fernández JR, Alonso I, Aguilar MF, Ucieda R, Iglesias M. (2003d). Differences in circadian blood pressure variability during gestation between healthy and complicated pregnancies. *Am. J. Hypertens.* 16:200–208.

Hermida RC, Calvo C, Ayala DE, Domínguez MJ, Covelo M, Fernández JR, Mojón A, López JE. (2003e). Administration-time-dependent effects of valsartan on ambulatory blood pressure in hypertensive subjects. *Hypertension* 42:283–290.

Hermida RC, Ayala DE, Iglesias M. (2004a). Differences in circadian pattern of ambulatory pulse pressure between healthy and complicated pregnancies. *Hypertension* 44:316–321.

Hermida RC, Ayala DE, Iglesias M. (2004b). Circadian blood pressure variability as a function of parity in normotensive pregnant women. *J. Clin. Hypertens.* 6:126–133.

Hermida RC, Ayala DE, Fernández JR, Mojón A, Iglesias M. (2004c). Reproducibility of the tolerance-hyperbaric test for diagnosing hypertension in pregnancy. *J. Hypertens.* 22:565–572.

Hermida RC, Calvo C, Ayala DE, Domínguez MJ, Covelo M, Fernández JR, Fontao MJ, López JE. (2004d). Administration-time-dependent effects of doxazosin GITS on ambulatory blood pressure of hypertensive subjects. *Chronobiol. Int.* 21:277–296.

Hermida RC, Fernández JR, Ayala DE, Mojón A, Alonso I, Calvo C. (2004e). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiol. Int.* 21:149–170.

Hermida RC, Ayala DE, Calvo C, López JE. (2005a). Aspirin administered at bedtime, but not on awakening, has an effect on ambulatory blood pressure in hypertensive patients. *J. Am. Coll. Cardiol.* 46:975–983.

Hermida RC, Ayala DE, Calvo C, López JE, Mojón A, Rodríguez M, Fernández JR. (2005b). Differing administration time-dependent effects of aspirin on blood pressure in dipper and non-dipper hypertensives. *Hypertension* 46:1060–1068.

Hermida RC, Ayala DE, Fernández JR, Calvo C. (2007a). Comparison of the efficacy of morning versus evening administration of telmisartan in essential hypertension. *Hypertension* 50:715–722.

Hermida RC, Ayala DE. Fernandez JR, Mojón A, Calvo C. (2007b). Influence of measurement duration and frequency on ambulatory blood pressure monitoring. *Rev. Esp. Cardiol.* 60:131–138.

Hermida RC, Ayala DE, Portaluppi F. (2007c). Circadian variation of blood pressure: the basis for the chronotherapy of hypertension. *Adv. Drug Deliv. Rev.* 59:904–922.

Hermida RC, Ayala DE, Calvo C, Portaluppi F, Smolensky MH. (2007d). Chronotherapy of hypertension: administration-time dependent effects of treatment on the circadian pattern of blood pressure. *Adv. Drug Deliv. Rev.* 59:923–939.

Hermida RC, Ayala DE, Chayán L, Mojón A, Fernández JR. (2009a). Administration-time-dependent effects of olmesartan on the ambulatory blood pressure of essential hypertension patients. *Chronobiol. Int.* 26:61–79.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2009b). Ambulatory blood pressure control with bedtime aspirin administration in subjects with prehypertension. *Am. J. Hypertens.* 22:896–903.

Hermida RC, Ayala DE, Fontao MJ, Mojón A, Alonso I, Fernández JR. (2010a). Administration-time-dependent effects of spirapril on ambulatory blood pressure in uncomplicated essential hypertension. *Chronobiol. Int.* 27:560–574.

Hermida RC, Ayala DE, Fontao MJ, Mojón A, Fernández JR. (2010b). Chronotherapy with valsartan/amlodipine combination in essential hypertension: improved blood pressure control with bedtime dosing. *Chronobiol. Int.* 27:1287–1303.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2010c). Influence of circadian time of hypertension treatment on cardiovascular risk: results of the MAPEC study. *Chronobiol. Int.* 27:1629–1651.

Hermida RC, Ayala DE, Fernández JR, Portaluppi F, Fabbian F, Smolensky MH. (2011a). Circadian rhythms in blood pressure regulation and optimization of hypertension treatment with ACE inhibitor and ARB medications. *Am. J. Hypertens.* 24:383–391.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2011b). Decreasing sleep-time blood pressure determined by ambulatory monitoring reduces cardiovascular risk. *J. Am. Coll. Cardiol.* 58:1165–1173.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2011c). Influence of time of day of blood pressure-lowering treatment on cardiovascular risk in hypertensive patients with type 2 diabetes. *Diabetes Care* 34:1270–1276.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2011d). Bedtime dosing of antihypertensive medications reduces cardiovascular risk in CKD. *J. Am. Soc. Nephrol.* 22:2313–2321.

Hermida RC, Ayala DE, Mojón A, Fontao MJ, Fernández JR. (2011e). Chronotherapy with valsartan/hydrochlorothiazide combination in essential hypertension: improved sleep-time blood pressure control with bedtime dosing. *Chronobiol. Int.* 28:601–610.

Hermida RC, Ayala DE, Fernández JR, Mojón A. (2012a). Sleep-time blood pressure: prognostic value and relevance as a therapeutic target for cardiovascular risk reduction. *Chronobiol. Int.* 29:in press.

Hermida RC, Ayala DE, Fernández JR, Mojón A, Smolensky MH, Fabbian F, Portaluppi F. (2012b). Administration-time differences in effects of hypertension medications on ambulatory blood pressure regulation. *Chronobiol. Int.* 29:in press.

Hermida RC, Ayala DE, Fontao MJ, Mojón A, Fernández JR. (2012c). Ambulatory blood pressure monitoring: importance of sampling rate and duration—48 versus 24 hours—on the accurate assessment of cardiovascular risk. *Chronobiol. Int.* 29:in press.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2012d). Sleep-time blood pressure and the prognostic value of isolated-office and masked hypertension. *Am. J. Hypertens.* 25:297–305.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2012e). Sleep-time blood pressure as a therapeutic target for cardiovascular risk reduction in type 2 diabetes. *Am. J. Hypertens.* 25:325–334.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2012f). Blunted sleep-time relative blood pressure decline increases cardiovascular risk independent of blood pressure level—the "normotensive non-dipper" paradox. *Chronobiol Int.* 29:in press.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2012g). Sleep-time blood pressure and the prognostic value of isolated-office and masked hypertension. *Am. J. Hypertens.* 25:297–305.

Hermida RC, Ayala DE, Mojón A, Fernández JR. (2012h). Cardiovascular risk of essential hypertension: influence of class, number, and treatment-time regimen of hypertension medications. *Chronobiol. Int.* 29:in press.

Hernández-Díaz S, Toh S, Cnattingius S. (2009). Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. *BMJ* 338:b2255.

Higashi Y, Nakagawa K, Kimura M, Noma K, Hara K, Sasaki S, Goto C, Oshima T, Chayama K, Yoshizumi M. (2002). Circadian variation of blood pressure and endothelial function in patients with essential hypertension: a comparison of dippers and non-dippers. *J. Am. Coll. Cardiol.* 40:2039–2043.

Imperiale TF, Petrulis A. (1991). A meta-analysis of low-dose aspirin for the prevention of pregnancy-induced hypertensive disease. *JAMA* 266:261–265.

Italian Study of Aspirin in Pregnancy. (1993). Low-dose aspirin in prevention and treatment of intrauterine growth retardation and pregnancy-induced hypertension. *Lancet* 341:396–400.

Janes SL, Kyle PM, Redman C, Goodall AH. (1995). Flow cytometric detection of activated platelets in pregnant women prior to the development of pre-eclampsia. *Thromb. Haemost.* 74:1059–1063.

Johnson AG, Nguyen TV, Day RO. (1994). Do nonsteroidal anti-inflammatory drugs affect blood pressure? A meta-analysis. *Ann. Intern. Med.* 121:289–300.

Kanabrocki EL, George M, Hermida RC, Messmore HL, Ryan MD, Ayala DE, Hoppensteadt DA, Fareed J, Bremmer FW, Third JLHC, Shirazi P, Nemchausky BA. (2001). Day-night variations in blood levels of nitric oxide, T-TFPI and E-selectin. *Clin. Appl. Thromb. Hemostasis* 7:339–345.

Kanzik I, Cakici I, Ersoy S, Ark M, Abacioglu N, Zengil H. (1992). Effects of cyclooxygenase and lipoxygenase inhibitors on digoxin-induced arrhythmias and haemodynamics in guinea-pigs. *Pharmacol. Res.* 26:305–316.

Katz F, Romfh P, Smith JA. (1975). Diurnal variation of plasma aldosterone, cortisol and renin activity in supine man. *J. Clin. Endocrinol. Metab.* 40:125–134.

Khan KS, Wojdyla D, Say L, Gulmezoglu Am, Van Look PF. (2006). WHO analysis of causes of maternal death: a systematic review. *Lancet* 367:1066–1074.

Klemmensen AK, Olsen SF, Osterdal ML, Tabor A. (2007). Validity of pre-eclampsia-related diagnoses recorded in a national hospital registry and in a postpartum interview of the women. *Am. J. Epidemiol.* 166:117–124.

Labrecque G, Reinberg A. (1989). Chronopharmacology of nonsteroid anti-inflammatory drugs. In Lemmer B (Ed.). *Chronopharmacology: cellular and biochemical interactions.* New York: Marcel Dekker, 545–579.

Lawler J, Osman M, Shelton JA, Yeh J. (2007). Population-based analysis of hypertensive disorders in pregnancy. *Hypertens. Pregnancy* 26:67–76.

Leitich H, Egarter C, Husslein P, Kaider A, Schemper M. (1997). A meta-analysis of low dose aspirin for the prevention of intrauterine growth retardation. *Br. J. Obstet. Gynecol.* 104:450–459.

Manfredini R, Gallerani M, Portaluppi F, Fersini C. (1996). Relationships of the circadian rhythms of thrombotic, ischemic, hemorrhagic, and arrhythmic events to blood pressure rhythms. *Ann. N. Y. Acad. Sci.* 783:141–158.

Manson JE, Stampfer MJ, Colditz GA, Willet WC, Rosner B, Speizer FE, Hennekens CH. (1991). A prospective study of aspirin use and primary prevention of cardiovascular disease in women. *JAMA* 266:521–527.

Marín R, Gorostidi M, Portal CG, Sánchez M, Sánchez E, Alvarez J. (2000). Long-term prognosis of hypertension in pregnancy. *Hypertens. Pregnancy* 19:199–209.

Markiewicz A, Semenowicz K. (1979). Time dependent changes in the pharmacokinetics of aspirin. *Int. J. Clin. Pharmacol. Biopharm.* 17:409–411.

Masotti G, Galanti G, Poggesi L, Abbate R, Neri Serneri GG. (1979). Differential inhibition of prostacyclin production and platelet aggregation by aspirin. *Lancet* 2:1213–1217.

Miyamoto S, Shimokawa H, Sumioki H, Touno A, Nakano H. (1998). Circadian rhythm of plasma atrial natriuretic peptide, aldosterone, and blood pressure during the third trimester in normal and preeclamptic pregnancy. *Am. J. Obstet. Gynecol.* 158:393–399.

Moore JG, Goo RH. (1987). Day and night aspirin-induced gastric mucosal damage and protection by ranitidine in man. *Chronobiol. Int.* 4:111–116.

Page NM. (2002). The endocrinology of preeclampsia. *Clin. Endocrinol.* 57:413–423.

Panza JA, Epstein SE, Quyyumi AA. (1991). Circadian variation in vascular tone and its relation to alpha-sympathetic vasoconstrictor activity. *N. Engl. J. Med.* 325:986–990.

Patrono C, Ciabattoni G, Patrignani P, Pugliese F, Filabozzi P, Catella F, Dave G, Forni L. (1985). Clinical pharmacology of platelet cyclooxygenase inhibition. *Circulation* 72:1177–1184.

Pinotti M, Bertolucci C, Portaluppi F, Colognesi I, Frigato E, Foa A, Bernardi F. (2005). Daily and circadian rhythms of tissue factor pathway inhibitor and factor VII activity. *Arterioscler. Thromb. Vasc. Biol.* 25:646–649.

Pope JE, Anderson JJ, Felson DT. (1993). A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. *Arch. Intern. Med.* 153:477–484.

Portaluppi F, Smolensky MH. (2007). Circadian rhythm and environmental determinants of blood pressure regulation in normal and hypertensive conditions. In White WB (Ed.). *Blood pressure monitoring in cardiovascular medicine and therapeutics.* Totowa, NJ: Humana Press, 135–158.

Portaluppi F, Smolensky MH. (2010). Perspectives on the chronotherapy of hypertension based on the results of the MAPEC study. *Chronobiol. Int*. 27:1652–1667.

Portaluppi F, Montanari L, Ferlini M, Gilli P. (1990). Altered circadian rhythms of blood pressure and heart rate in non-hemodialysis chronic renal failure. *Chronobiol. Int.* 7:321–327.

Portaluppi F, Trasforini G, Margutti A, Vergnani L, Ambrosio MR, Rossi R, Bagni B, Pansini R, degli Uberti EC. (1992). Circadian rhythm of calcitonin gene-related peptide in uncomplicated essential hypertension. *J. Hypertens.* 10:1227–1234.

Portaluppi F, Cortelli P, Avoni P, Vergnani L, Contin E, Maltoni P, Pavani A, Sforza E, degli Uberti EC, Gambetti P, Lugaresi E. (1994). Diurnal blood pressure variation and hormonal correlates in fatal familial insomnia. *Hypertension* 23:569–576.

Portaluppi F, Vergnani L, Manfredini R, Fersini C. (1996). Endocrine mechanisms of blood pressure rhythms. *Ann. N. Y. Acad. Sci.* 783:113–131.

Portaluppi F, Boari B, Manfredini R. (2004). Oxidative stress in essential hypertension. *Curr. Pharm. Des.* 10:1695–1698.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27:1911–1929.

Portaluppi F, Tiseo R, Smolensky MH, Hermida RC, Ayala DE, Fabbian F. (2012). Circadian rhythms and cardiovascular health. *Sleep Med. Rev.* 16:151–166.

Redman CW, Bonnar J, Beilin L. (1978). Early platelet consumption in pre-eclampsia. *BMJ* 1:467–469.

Redman CW, Sargent IL. (2003). Pre-eclampsia, the placenta and the maternal systemic inflammatory response—a review. *Placenta* 24: s21–s27.

Redman CW, Sargent IL. (2005). Latest advances in understanding preeclampsia. *Science* 308:1592–1594.

Roberge S, Giguère Y, Villa P, Nicolaides K, Vainio M, Forest JC, von Dadelzen P, Vaiman D, Tapp S, Bujold E. (2012a). Early administration of low-dose aspirin for the prevention of severe and mild preeclampsia: a systematic review and meta-analysis. *Am. J. Perinatol.* 29:551–556.

Roberge S, Villa P, Nicolaides K, Giguère Y, Vainio M, Bakthi A, Ebrashy A, Bujold E. (2012b). Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. *Fetal Diagn. Ther.* 31:141–146.

Roberts CL, Algert CS, Morris JM, Ford JB, Henderson-Smart DJ. (2005). Hypertensive disorders in pregnancy: a population-based study. *Med. J. Aust.* 182:332–335.

Roberts CL, Bell JC, Ford JB, Hadfield RM, Algert CS, Morris JM. (2008). The accuracy of reporting of the hypertensive disorders of pregnancy in population health data. *Hypertens. Pregnancy* 27:285–297.

Roberts CL, Ford JB, Algert CS, Antonsen S, Chalmers J, Cnattingius S, Gokhale M, Kotelchuck M, Melve KK, Langridge A, Morris C, Morris JM, Nassar N, Norman JE, Norrie J, Sorensen HT, Walker R, Weir CJ. (2011). Population-based trends in pregnancy hypertension and pre-eclampsia: an international comparative study. *BMJ Open* 1:e000101. doi: 10.11367bmjopen-2011-00101.

Rotchell YE, Cruickshank JK, Phillips Gay M, Griffiths J, Stewart A, Farrell B, Ayers S, Hennis A, Grant A, Duley L, Collins R. (1998). Barbados Low Dose Aspirin Study in Pregnancy (BLASP): a randomised trial for the prevention of pre-eclampsia and its complications. *Br. J. Obstet. Gynaecol.* 105:286–292.

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

Ruano R, Fontes RS, Zugaib M. (2005). Prevention of preeclampsia with low-dose aspirin—a systematic review and meta-analysis of the main randomized controlled trials. *Clinics (Sao Paulo)* 60:407–414.

Shennan AH, Kissane J, De Swiet M. (1993). Validation of the SpaceLabs 90207 ambulatory blood pressure monitor for use in pregnancy. *Br. J. Obstet. Gynaecol.* 100:904–908.

Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L, Paul RH, Romero R, Witter F, Rosen M, Depp R, and the National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. (1993). Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. *N. Engl. J. Med.* 329:1213–1218.

Sibai BM, Caritis S, Hauth J. (2003). What we have learned about preeclampsia. *Semin. Perinatol*. 27:239–246.

Smolensky MH, Haus E. (2001). Circadian rhythms in clinical medicine with applications to hypertension. *Am. J. Hypertens.* 14(9 Pt 2):280S–290S.

Smolensky MH, Portaluppi F. (1999). Chronopharmacology and chronotherapy of cardiovascular medications: relevance to prevention and treatment of coronary heart disease. *Am. Heart J.* 137(4 Pt 2):S14–S24.

Smolensky MH, Hermida RC, Castriotta RJ, Portaluppi F. (2007). Role of sleep-wake cycle on blood pressure circadian rhythms and hypertension. *Sleep Med.* 8:668–680.

Smolensky MH, Hermida RC, Ayala DE, Tiseo R, Portaluppi F. (2010). Administration-time-dependent effect of blood pressure-lowering medications: basis for the chronotherapy of hypertension. *Blood Press. Monit.* 15:173–180.

Smolensky MH, Siegel RA, Haus E, Hermida R, Portaluppi F. (2012). Biological rhythms, drug delivery, and chronotherapeutics. In Siepmann J, Siegel RA, Rathbone MJ (Eds.). *Fundamentals and applications of controlled release drug delivery*. Heidelberg, Germany: Springer-Verlag, 359–443.

Snoep JD, Hovens MMC, Pasha SM, Frölich M, Pijl H, Tamsma JT, Huisman MV. (2009). Time-dependent effects of low-dose aspirin on plasma renin activity, aldosterone, cortisol, and catecholamines. *Hypertension* 54:1136–1142.

Sothern RB, Vesely DL, Kanabrocki EL, Hermida RC, Bremner FW, Third JLHC, Boles MA, Nemchausky BM, Olwin JH, Scheving LE. (1995). Temporal (circadian) and functional relationship between atrial natriuretic peptides and blood pressure. *Chronobiol. Int.* 12:106–120.

Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. (2010). Pre-eclampsia. *Lancet* 376:631–644.

Svensson A, L Sigström, B Andersch, L Hansson. (1983). Maternal hypertension during pregnancy and high blood pressure in children. *Clin. Exp. Hypertens. B Hypertens. Pregnancy* 2:203–209.

Taubert D, Berkels R, Grosser N, Schroder H, Grundemann D, Schomig E. (2004). Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. *Br. J. Pharmacol.* 143:159–165.

Trasforini G, Margutti A, Portaluppi F, Menegatti M, Ambrosio MR, Bagni B, Pansini R, Degli Uberti EC. (1991). Circadian profile of plasma calcitonin gene-related peptide in healthy man. *J. Clin. Endocrinol. Metab.* 73:945–951.

Trivedi NA. (2011). A meta-analysis of low-dose aspirin for prevention of preeclampsia. *J. Postgrad. Med.* 57:91–95.

Varani K, Gessi S, Caiazza A, Rastelli G, Portaluppi F, Borea PA. (1999a). Platelet alpha2-adrenoceptor alterations in patients with essential hypertension. *Br. J. Clin. Pharmacol.* 47:167–172.

Varani K, Portaluppi F, Merighi S, Ongini E, Belardinelli L, Borea PA. (1999b). Caffeine alters A2A adenosine receptors and their function in human platelets. *Circulation* 99:2499–2502.

Varani K, Portaluppi F, Gessi S, Merighi S, Ongini E, Belardinelli L, Borea PA. (2000). Dose and time effects of caffeine intake on human platelet adenosine A(2A) receptors: functional and biochemical aspects. *Circulation* 102:285–289.

Walker RL, Hemmelgarn B, Quan H. (2009). Incidence of gestational hypertension in the Calgary Health Region from 1995 to 2004. *Can. J. Cardiol.* 25:e284–e287.

Wallis AB, Saftlas AF, Hsia J, Atrash HK. (2008). Secular trends in the rates of pre-eclampsia, eclampsia, and gestational hypertension, United States, 1987–2004. *Am. J. Hypertens.* 21:521–526.

Walsh SW. (1985). Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. *Am. J. Obstet. Gynecol.* 152:335–340.

Walsh SW, Wang Y. (1998). Maternal perfusion with low-dose aspirin preferentially inhibits placental thromboxane while sparing prostacyclin. *Hypertens. Pregnancy* 17:203–215.

Walsh SW, Wang Y, Kay HH, McCoy MC. (1992). Low-dose aspirin inhibits lipid peroxides and thromboxane but not prostacyclin in pregnant women. *Am. J. Obstet. Gynecol.* 167:926–930.

Wang JL, Cheng HF, Harris RC. (1999). Cyclooxygenase-2 inhibition decreases renin content and lowers blood pressure in a model of renovascular hypertension. *Hypertension* 34:96–101.

Willard JE, Lange RA, Hillis LD. (1992). The use of aspirin in ischemic heart disease. *N. Engl. J. Med.* 327:175–181.

Winters CJ, Sallman AL, Vesely DL. (1988). Circadian rhythm of prohormone atrial natriuretic peptides 1–30, 31–67 and 99–126 in man. *Chronobiol. Int.* 5:403–409.

Witte K, Lemmer B. (2003). Rhythms and pharmacodynamics. In Redfern P (Ed.). *Chronotherapeutics.* London: Pharmaceutical Press, 111–126.

Wu R, Lamontagne D, de Champlain J. (2002). Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. *Circulation* 105:387–392.

Wu R, Laplante MA, de Champlain J. (2004). Prevention of angiotensin II-induced hypertension, cardiovascular hypertrophy and oxidative stress by acetylsalicylic acid in rats. *J. Hypertens.* 22:793–801.