

# Superiority of 3 Over 2 Doses of Intermittent Preventive Treatment With Sulfadoxine-Pyrimethamine for the Prevention of Malaria During Pregnancy in Mali: A Randomized Controlled Trial

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(See the article by Harrington et al, on pages 224–230, and editorial commentary by Lake and Taylor, on pages 231–233.)

**Background.** In 2003, Mali introduced intermittent preventive therapy in pregnancy (ITPp) with sulfadoxine-pyrimethamine (SP) for the control of malaria in pregnancy, consisting of 2 doses of SP given in the 2nd and 3rd trimester. This widely used regimen, although very effective, leaves many women unprotected from malaria during the last 4-to-8 weeks of gestation, which is a pivotal period for fetal weight gain. The aim of the study was to compare the efficacy and safety of 3-dose versus 2-dose IPTp-SP for the prevention of placental malaria and associated low birth weight (LBW).

**Methods.** We conducted a parallel-group, open-label, individually randomized controlled superiority trial involving 814 women of all gravidity, enrolled from April 2006 through March 2008. All women were seen at least 3 times and received either 2 ( $n = 401$ ) or 3 ( $n = 413$ ) doses of IPTp-SP. The primary endpoint measured was placental malaria, LBW, preterm births, and maternal anemia were secondary endpoints, and severe maternal skin reactions and neonatal jaundice were safety endpoints.

**Results.** Among the 96% of study subjects who were followed up until delivery, the prevalence of placental malaria was 2-fold lower in the 3-dose group (8.0%) than in the 2-dose group (16.7%); the adjusted prevalence ratio (APR) was 0.48 (95% confidence interval [CI], 0.32–0.71). LBW and preterm births were also reduced; the prevalence of LBW was 6.6% in the 3-dose group versus 13.3% in the 2-dose group (APR, 0.50; 95% CI, 0.32–0.79), and the prevalence of preterm births was 3.2% versus 8.9% (APR, 0.37; 95% CI, 0.19–0.71). No significant reductions in maternal anemia or differences in safety endpoints were observed.

**Conclusions.** Adding a third dose of ITPp-SP halved the risk of placental malaria, LBW, and preterm births in all gravidae, compared with the standard 2-dose regimen, in this area of highly seasonal transmission with low levels of SP resistance.

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Intermittent preventive therapy in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is effective in reducing the risk of placental malaria, low birth weight (LBW), and severe maternal anemia [1, 2], and, together with insecticide-treated nets (ITNs), is the main strategy for the control of malaria in pregnancy in Africa [1]. IPTp consists of the administration of full curative doses of an efficacious antimalarial drug given presumptively in the second and third trimester at least 1 month apart. It provides intermittent clearance or suppression of existing asymptomatic infections from the placenta (treatment effect) and posttreatment prophylaxis by preventing new infections through the maintenance of a suppressive drug level for up to 6 weeks in areas with low levels of parasite resistance to SP [1, 3].

Presently, SP is the only antimalarial recommended for IPTp [1]. The World Health Organization (WHO) recommends at least 2 curative doses of SP in human immunodeficiency virus (HIV)-negative women and 3 doses for HIV-positive women who are not protected by cotrimoxazole [1, 4, 5]. Although SP resistance has increased to high levels in some areas of southern and eastern Africa, resistance in most of western Africa is still low. Furthermore, SP has now been reserved for use as IPT, and the reduced drug pressure may prolong the longevity of this very valuable drug in areas with low-to-moderate resistance [2, 4, 6]. IPTp with SP is thus likely to remain the mainstay of malaria control in pregnancy for several years in these regions.

In Mali, the 2-dose strategy was adopted by the National Malaria Control Program (NMCP) in 2003. Mali has highly seasonal malaria transmission, moderate levels of ITN use, and low levels of SP resistance [7, 8]. A recent survey, conducted from 2005 through 2007 and involving 1696 pregnant women of all parities, showed that placental infection was very common among women who had received the full 2-dose regimen of IPTp (23%; unpublished data), consistent with findings from an earlier trial showing that 2 dose IPTp-SP, although much more effective than chloroquine prophylaxis, was associated with a high risk of placental infection during the transmission season, especially in women who completed the second dose of IPTp-SP early in the third trimester [9]. This suggests that 2 doses may provide insufficient protection against reinfections later in the third trimester, which is a period of rapid in-utero growth.

Three trials have shown that additional doses of SP add significant benefit over the 2 dose regimen among HIV-infected primi- and secundigravidae [2]. In HIV-negative women, the beneficial effect of 3 or more doses is less clear. We conducted an open-label, individually randomized controlled trial that compared the efficacy and safety of the standard 2-dose regimen of IPTp-SP with 3 doses of SP in the prevention of placental malaria, maternal anemia, and low birth weight in Mali.

## METHODS

The study was conducted from April 2006 through March 2008 in 2 health facilities in Bla District, located 320 km east of Bamako in Ségou Region, Mali, which is an area with highly seasonal malaria transmission. The 2 health facilities serve a population of ~39,000.

Women of all gravidity who were scheduled to receive their first dose of IPTp-SP were eligible for the study if they were 14–45 years of age, at 16–26 weeks gestation (by fundal height), and had no history of antimalarial or cotrimoxazole use within the previous month. Women with a serious illness requiring hospital admission, with severe anemia (hemoglobin level, <7 g/dL), with known HIV infection, or who were planning to deliver elsewhere were excluded.

### Randomization, Masking, and Treatment Allocation

Women were randomly assigned to 1 of the 2 study groups by the study clinicians. Allocation concealment was achieved by keeping 20 allocation slips with preassigned study allocations (10 per arm) in opaque containers. Sequential participants were asked to draw 1 allocation slip from the container without possibility of replacement. The randomization sequence was stratified by clinic and used permuted balanced block randomization (block size of 20). Study drugs were provided by the study clinicians in a dedicated study room, separate from the nurses and midwives who provided antenatal care. Laboratory staff reading the blood smears were kept unaware of the treatment allocation during the trial.

### Endpoints

The primary endpoint was placental malaria infection (asexual stage parasites, any species). Secondary efficacy endpoints included maternal malaria determined by peripheral blood smear; mean hemoglobin, maternal anemia (hemoglobin level, <11 g/dL), and moderate-to-severe anemia (hemoglobin level, <8.0 g/dL); mean birth weight and low birth weight (<2500 g); mean gestational age and preterm delivery (gestational age, <37 weeks); and low placental weight (<500 g). Safety endpoints included severe maternal skin reactions (eg, Stevens-Johnson syndrome and toxic epidermal necrolysis), congenital malformations assessed at birth and at 30 days, and neonatal icterus and vomiting by day 7 and day 30 of life.

### Procedures

A questionnaire was administered at enrollment to collect information on sociodemographic characteristics, maternal height, bed net use, obstetric and clinical histories, and drug use during the pregnancy. A blood sample was obtained by fingerpick for malaria smears and determination of hemoglobin levels (Hemocue 201).

Women were asked to return to the clinic for monthly follow-up visits or between scheduled visits if they were ill. If women did not return for their scheduled visit, they were visited at home. During each visit, women were asked about signs and symptoms and examined for skin rashes. The axillary temperature was measured, and a malaria blood smear taken if a woman had documented fever (temperature,  $\geq 37.5^{\circ}\text{C}$ ).

Within 2 hours after delivery, a finger prick sample was taken from the mother for determination of hemoglobin levels and malaria parasitemia. Placental blood was collected by incision from the maternal side of the placenta for malaria blood smears. The newborns were examined by the study physicians for congenital malformations, and the gestational age was assessed using the Ballard score. Infants were weighed using a digital scale. Mothers and neonates were visited at home by the study staff a week and a month after delivery to examine the newborn. The study was monitored monthly by independent internal clinical monitors on behalf of the Malaria Research and Training Centre in Bamako.

### Interventions

Each SP dose consisted of 1500 mg sulfadoxine and 75 mg pyrimethamine (administered in a total of 3 tablets). Women in the 3-dose group received the first dose between 16 and 24 weeks gestation, the second dose between 20 and 32 weeks, and the third dose at no later than 36 weeks. Women in the 2-dose group also received the first dose between 16 and 24 weeks of gestation and the second dose between 25 and 36 weeks. Women were observed, and the full dose was repeated if vomiting occurred within 30 minutes. Study participants were asked to avoid self-medication of antimalarial drugs until completion of follow-up. All women received ferrous sulphate (200 mg containing 60 mg of iron) and folic acid (0.4 mg) daily. Folic acid was started 2 weeks after each SP dose, as recommended by the Malian Ministry of Health. If clinical malaria occurred during follow-up visits, oral quinine was given at a dosage of 600 mg 3 times daily over 7 days.

### Laboratory Assessment

Thick blood smears were stained with 4% Giemsa for 20 minutes. Asexual parasite densities were counted against 300 white blood cells (WBCs) and expressed per 7500 WBC/ $\mu\text{L}$ . A blood smear was considered to have negative results if no parasites were identified in 100 high-power fields. Slides were read by an experienced microscopist blinded to the treatment allocation. Ten percent of slides were reread by an expert reader blinded to the initial results and treatment allocation.

### Sample Size and Data Analysis

The study was designed to detect an absolute reduction of 7.5% in the prevalence of placental malaria from 15% in the 2-dose group and required 304 women in each treatment arm ( $\alpha = 0.05$ ;

power, 80%). To allow for 5% noncompliance and 20% loss-to-follow-up, 406 individuals were recruited per arm.

Data were double entered and validated using EpiInfo software, version 6.04b (Centers for Disease Control and Prevention), and analyses were conducted using Stata software, version 12, and SPSS software, version 17. The efficacy analysis was on an intention-to-treat basis. The safety analysis for adverse skin reactions was performed on a modified intention-to-treat basis and included only women who took at least 1 dose of study drug. The analysis of safety endpoints measured at or after delivery was done on a per-protocol basis. The impact of the treatment on dichotomous endpoints at delivery was compared using robust stepwise backwards elimination Poisson regression to obtain adjusted prevalence ratios (APRs). We also conducted exploratory analyses of potential effect modification by season, by gravidity, and by ITN use.

### Ethical Approval

The study was approved by the ethical committee of the Faculty of Medicine, Pharmacy and Dentistry of the University of Bamako. Consent forms were in the local language (Bambara). All women gave written informed consent.

## RESULTS

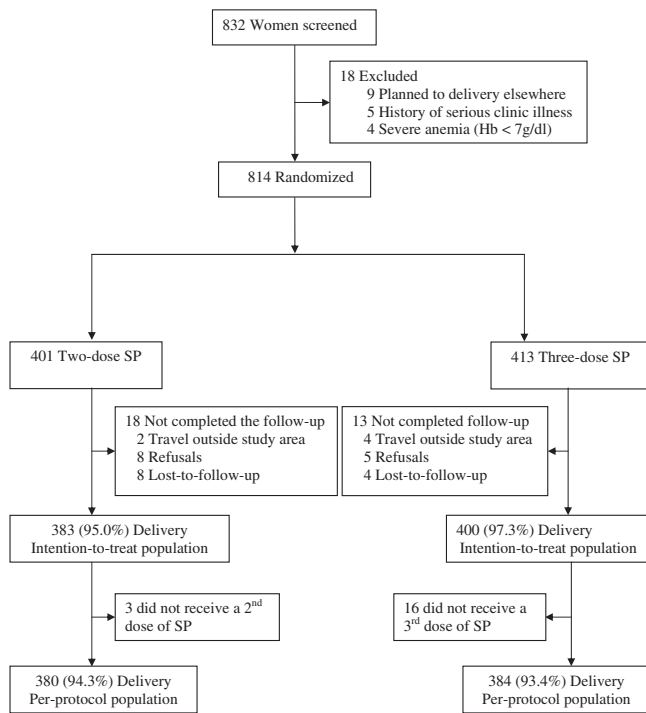
### Study Population Characteristics at Enrollment

A total of 814 pregnant women were enrolled from 21 April 2006 through 19 February 2008; 413 were enrolled in the 3-dose arm, 401 were enrolled in the 2-dose arm, and a total of 783 (96.2%) were followed-up successfully until delivery (Figure 1). The baseline characteristics were similar across the treatment arms (Table 1).

### Efficacy of Regimens

Overall, 4.8% (20 of 413) and 7.2% (29 of 401) of women in the 3-dose and 2-dose group, respectively, had at least 1 episode of clinical malaria (confirmed by microscopic examination) and were treated with quinine (APR, 0.79; 95% confidence interval [CI], 0.56–1.12). At delivery, the prevalence of placental malaria in the 3-dose group was half that in the 2-dose group (8.0% vs 16.7%; APR, 0.48; 95% CI, 0.32–0.71) (Table 2). Interaction models indicated that the beneficial effect was evident in all gravidae groups and in ITN users and nonusers (Table 2). In the 2-dose group, women enrolled before 24 weeks gestation were more likely to have placental malaria than were those enrolled later (APR, 1.21; 95% CI, 1.06–1.37). This was not apparent in the 3-dose group (APR, 1.07; 95% CI, 0.88–1.32).

The risks of LBW and preterm birth were also halved, but the prevalence of moderate and severe anemia was similar in the 2 treatment arms (Table 2 and Figure 3). The impact on LBW was similar across gravidae groups and ITN users and nonusers, but



**Figure 1.** Trial profile. Thirty-one (3.8%) of the 814 enrolled women were lost to follow-up before delivery because of travel (6 [0.7%]), consent withdrawal (13 [1.6%]), and noncompliance with the follow-up schedule (12 [1.5%]). The percentage lost to follow-up was 3.1% (13 subjects) in the 3-dose arm and 4.5% (18 subjects) in the 2-dose arm ( $P = .31$ ). The remaining 783 women (96.2%) were followed up successfully until delivery and contributed to the intention-to-treat analysis; 16 (4.0%) of the 400 women in the 3-dose group did not receive their third dose, and 3 (0.8%) of 383 women in the 2-dose group received only 1 dose and were excluded from the per protocol analysis.

the impact on mean birth weight was greater in primi- and secundigravidae (who experienced an increase in mean birth weight of 99 g) than in multigravidae (who experienced an increase of 50 g) and was greater in non-ITN users (who experienced an increase of 97 g) than in ITN users (who experienced a decrease of 27 g) (Table 2). The observed differences between the 2 treatment arms were greatest during and shortly after the malaria season (Figure 2). There were no differences between treatment groups with respect to fetal loss (Table 2).

The frequency of single-nucleotide polymorphisms (SNPs) was determined in a small number of positive samples at delivery. The frequency of the *dhfr/dhps* quadruple mutant genotype was 85.7% (6 of 7 samples) and 55.6% (5 of 9 samples) in the 2-dose and 3-dose arms, respectively, at delivery ( $P = .31$ ), and no quintuple mutation was observed.

#### Adverse Events in Mothers, Neonates, and Infants

No severe skin rashes were observed during the study. One mother died from postpartum hemorrhage in the 2-dose group. There were 4 (0.6%) congenital abnormalities detected

(3 polydactyly and 1 club-foot) from the surface examination at the time of birth; 3 were detected in the 2-dose group, and 1 was detected in the 3-dose group. There were no differences in the prevalence of jaundice, history of fever, or history of vomiting in the neonates at day 7 or at day 30 (Table 3).

## DISCUSSION

Three doses of IPTp with SP was considerably more effective in reducing maternal and placental malaria, low birth weight, and premature delivery than was the standard 2-dose regimen in this area with low SP resistance and highly seasonal malaria. The extra dose of SP was well tolerated. Size at birth and prematurity are important risk factors for infant morbidity and mortality, and both are associated with permanent deficits in childhood growth and neurocognitive development and performance in later life [10–14]. Our findings thus have important public health implications.

The reduction in placental malaria is not surprising, because the 3-dose group received their last dose, on average, 1 month closer to term, clearing existing infections and reducing the susceptibility to new infections at term by providing an extra period of post-treatment prophylaxis of ~4–6 weeks. The latter half of the third trimester is a period of considerable fetal growth, when at least 25% of the total fetal weight gain occurs in healthy pregnancies. This explains the large impact of the third dose on birth weight, despite the fact that the control group had received the standard 2-dose regimen, which itself was highly effective in a previous trial in Mali [9]. The observed difference in mean birth weight of 66 g is comparable to that reported in meta-analyses of previous trials of ITNs (55 g) and of 2-dose IPTp (79 g) [2, 15].

Our data add to the growing body of evidence showing that >2 doses of SP are required to provide adequate protection to pregnant women through the third trimester. This is the first trial that compares the standard 2-dose regimen with more-frequent dosing schedules in western Africa. However, in eastern and southern Africa, there have been 4 previous trials comparing 2-dose IPTp-SP with more-frequent dosing, conducted over a 15-year period in areas representing a wide range of SP resistance; 4 of these involved HIV-infected women (who were not taking cotrimoxazole) [16–19] and 3 involved HIV-negative women [16, 17, 19], 2 of which studies restricted recruitment to primi- and secundigravidae only [16, 17]. We pooled the results from our study with those of the 2 other trials involving HIV-negative women that reported results for primi- and secundigravidae separate from the multigravidae in a meta-analysis. These data show remarkably consistent findings across the studies, with no evidence of heterogeneity ( $I^2 = 0\%$  for the effect on mean birth weight and 20% for LBW) (K. K. Kayentao and F. O. ter Kuile, unpublished data). The other 3 trials all showed that a positive impact on mean birth weight was associated with

**Table 1. Baseline Characteristics at Enrollment, by Intermittent Preventive Therapy in Pregnancy Group**

Characteristics	2-dose SP (n = 401)	3-dose SP (n = 413)	All women (n = 814)
Age, years			
Mean ( $\pm$ SD)	24.5 (6.1)	24.5 (6.0)	24.5 (6.1)
Range	14–43	15–45	14–45
Age <20 years, %	26.2	22.4	24.3
Height <150 cm, %	2.0	1.5	1.7
Weight <50 kg, %	7.9	6.3	7.1
Gestational age in weeks			
Mean ( $\pm$ SD)	21.6 (2.6)	20.8 (2.6)	21.18 (2.6)
Range	16–26	16–26	16–26
Married, %	95.3	95.4	95.3
Gravidity			
Median value (range)	3 (1–14)	3 (1–13)	3 (1–14)
Primi- and secundigravidae	42.1	41.5	41.8
Attended ANC this pregnancy prior to enrollment	128 (32.0)	129 (31.2)	257 (31.7)
Axillary temperature $\geq$ 37.5°C, %	27.7	22.0	24.8
Sleep under bed net	226 (56.4)	225 (54.5)	451 (55.4)
Sleep under ITN	72 (18.0)	88 (21.3)	160 (19.8)
Slept under ITN previous night	59 (14.7)	77 (18.4)	136 (16.7)
Hemoglobin level, g/dL			
Mean ( $\pm$ SD)	10.1 (1.5)	10.2 (1.5)	10.2 (1.5)
Moderate anemia (<11 g/dL), %	72.3	67.3	69.78
Severe anemia (<8 g/dL), %	4.2	2.2	3.2
Positive peripheral malaria smear, %	25.7	27.6	26.7
Clinical malaria, %	12.0	9.2	10.6
Parasite density, geometric mean parasites/ $\mu$ L (95% CI) <sup>a</sup>	1128 (765–1663)	1843 (1351–2514)	1464 (1145–1872)

**NOTE.** Data are no. (%) of enrolled women, unless otherwise indicated. ANC, antenatal clinic; CI, confidence interval; SD, standard deviation; SP, sulfadoxine-pyrimethamine. Clinical malaria defined as positive peripheral smear result in the presence of documented fever ( $\geq$ 37.5°C) or history of fever within the last 24 hours.

<sup>a</sup> Among malaria-positive women only.

more-frequent dosing, with increases in mean birth weight ranging from 57 g to 80 g, compared with 91 g among primi- and secundigravidae in our current study (summary estimate across the 3 trials, 80 g; 95% 15–145 g;  $P = 0.02$ ). The summary estimate for LBW gives an APR (95% CI) of 0.64 (0.43–0.95) ( $P = .03$ ), including the 0.47 estimate among the primi- and secundigravidae in our current trial. Thus, it is unlikely that the large beneficial impact observed in our study is a chance finding.

The evidence from the other trials conducted in eastern and southern Africa [16, 17, 19] suggests that the benefits of more-frequent dosing may also apply in areas with higher, albeit moderate, levels of SP resistance. With increasing drug resistance, the minimum inhibitory concentration at which parasite growth is inhibited increases, and the time window for effective drug concentrations that fall below these levels decreases. This results in a progressive shortening of the duration of the suppressive prophylactic effect after treatment [3]. Parasites with triple DHFR mutations have a  $\sim$ 1000-fold reduction in susceptibility to pyrimethamine, which translates into a reduction in the duration of post-treatment prophylaxis

of 1 month, compromising the efficacy of the 2-dose regimen and requiring more-frequent dosing [3].

This study was conducted in an area with low SP resistance and in the same region as our ongoing studies of malaria in pregnancy. In this area, the prevalence of *dhfr/dhps* quadruple mutant genotype among asymptomatic parasitemic pregnant women attending the clinic for antenatal care was only 28% prior to 2009. At this level of molecular resistance, the in vivo response to SP is excellent; weekly follow-up showed that only 2% of 162 women were parasitemic again by day 42 (data not shown). It is likely that repeated dosing with SP as part of IPTp in women may select for a subpopulation of clones and strains with a less sensitive phenotype. Although the number of successfully genotyped samples from parasitemic women in the current study was small (16 samples), there was no suggestion that adding the third dose increased the level of selection.

The previous trials comparing IPTp versus placebo or case management found that women protected by ITNs did not benefit from IPTp to the same degree as women not using ITNs

**Table 2. Primary and Secondary Outcomes at Delivery by Intermittent Preventive Therapy in Pregnancy Group**

Characteristics		No. (%) of subjects		Unadjusted absolute risk difference, % (95% CI)	Unadjusted difference in mean or prevalence ratio (95% CI)	Adjusted difference in mean or prevalence ratio (95% CI)	P <sup>a</sup>	
		2-dose SP	3-dose SP					
<b>Primary outcome</b>								
Placental blood smear positive	All gravidae	64/383 (16.7)	32/398 (8.0)	8.7 (4.2–13.3)	0.48 (0.32–0.72)	0.48 <sup>b</sup> (0.32–0.71)	<.001	
	Primi-secundi	32/159 (20.1)	16/165 (9.7)	10.4 (2.7–18.1)	0.48 (0.28–0.84)	0.47 <sup>b,c</sup> (0.27–0.82)	.007	
	Multigravidae	32/284 (14.3)	16/233 (6.8)	7.4 (1.8–13.0)	0.48 (0.27–0.85)	0.48 <sup>b,c</sup> (0.27–0.85)	.012	
By ITN use	ITN user	14/72 (19.4)	5/85 (5.9)	13.6 (3.1–24.0)	0.30 (0.11–0.80)	0.30 <sup>b,d</sup> (0.12–0.80)	.016	
	Non-ITN user	50/311 (16.1)	27/313 (8.6)	7.5 (2.3–12.6)	0.53 (0.36–0.83)	0.53 <sup>b,d</sup> (0.34–0.82)	.005	
<b>Secondary outcome</b>								
Positive peripheral blood smear result	All gravidae	77/383 (20.1)	43/400 (10.8)	9.4 (4.3–14.4)	0.53 (0.37–0.75)	0.54 <sup>b</sup> (0.38–0.77)	.001	
Hemoglobin concentration	All gravidae	Mean g/dL (±SD)	10.9 (1.5)	11.1 (1.5)		0.14 (–0.08 to 0.35)	0.02 <sup>e</sup> (–0.01 to 0.1)	.260
		Hemoglobin level <11 g/dL	188/383 (49.1)	172/400 (43.0)	6.1 (–0.8 to 13.1)	0.88 (0.75–1.01)	0.89 <sup>e</sup> (0.76–1.04)	.144
Hemoglobin level <8 g/dL	All gravidae	8/383 (2.1)	13/400 (3.3)	–1.2 (–7.5 to 1.6)	1.70 (0.72–4.10)	1.81 <sup>e</sup> (0.80–4.11)	.172	
<b>Birth weight<sup>f</sup></b>								
LBW	All gravidae	48/360 (13.3)	25/378 (6.6)	6.7 (2.4–11.0)	0.49 (0.31–0.78)	0.50 <sup>f</sup> (0.32–0.79)	.003	
	Primi-secundi	32/151 (21.2)	15/151 (9.9)	11.3 (3.2–19.3)	0.47 (0.24–0.83)	0.46 <sup>c,f</sup> (0.26–0.82)	.008	
	Multigravidae	16/209 (7.7)	10/227 (4.4)	3.3 (–1.2 to 7.7)	0.57 (0.26–1.24)	0.57 <sup>c,f</sup> (0.27–1.23)	.152	
By ITN use	ITN user	8/68 (11.8)	5/80 (6.3)	5.5 (–3.8 to 14.8)	0.53 (0.18–1.55)	0.53 <sup>d,f</sup> (0.19, 1.46)	.219	
	Non-ITN user	40/292 (13.7)	20/298 (6.7)	6.9 (2.1–11.8)	0.48 (0.29–0.82)	0.50 <sup>d,f</sup> (0.30–0.82)	.006	
Mean (SD), g		2892 (464)	2964 (428)		72.3 (7.8–136.8)	70.4 <sup>f</sup> (8.53–132.2)	.026	
By gravidae group	Primi-secundi	2762 (427)	2853 (457)		91.1 (–9.2 to 191.3)	98.5 <sup>c,f</sup> (1.15–195.9)	.047	
	Multigravidae	2986 (468)	3038 (292)		52.4 (–28.7 to 133.5)	49.9 <sup>c,f</sup> (–30.8 to 130.6)	.219	
By ITN use	ITN user	2937 (515)	2911 (331)		–26.1 (–112.7 to 164.9)	–27.9 <sup>d,f</sup> (–164.3 to 108.5)	.686	
	Non-ITN user	2882 (452)	2979 (450)		97.1 (24.1–170.1)	89.6 <sup>d,f</sup> (19.2–159.9)	.013	
<b>Gestational age</b>								
Preterm	All gravidae	32/359 (8.9)	12/376 (3.2)	5.5 (2.3–9.2)	0.35 (0.18–0.68)	0.37 <sup>f</sup> (0.19–0.71)	.003	
		Mean weeks (±SD)	38.6 (1.6)	38.8 (1.5)		0.2 (–0.03 to 0.4)	0.1 <sup>f</sup> (–0.03 to 0.4)	.101
<b>Placental weight<sup>f</sup></b>								
Placental weight <500 g	All gravidae	113/359 (31.5)	90/375 (24.0)	7.5 (1.0–13.4)	0.76 (0.60–0.97)	0.76 <sup>f</sup> (0.60–0.97)	.024	
Mean g (±SD)	All gravidae	530 (114)	535 (316)		–4.5 (–28.6 to 37.6)	4.9 <sup>f</sup> (–38.0 to 28.3)	.774	
Pregnancy loss <sup>g</sup>	All gravidae	16/383 (4.2)	20/400 (5.0)	–0.8 (–3.7 to 2.1)	1.2 (0.6–2.3)	1.12 <sup>g</sup> (0.59–2.12)	.723	
Perinatal deaths <sup>h</sup>	All gravidae	8/383 (2.1)	10/400 (2.5)	–0.4 (–2.5 to 1.7)	0.84 (0.20–2.40)	0.78 <sup>h</sup> (0.12–2.16)	.342	
Neonatal deaths	All gravidae	6/360 (1.7)	6/376 (1.6)	0.1 (–1.7 to 1.9)	0.95 (0.31–2.9)	0.88 <sup>h</sup> (0.29–2.63)	.817	

**NOTE.** ANC, antenatal clinic; CI, confidence interval; ITN, insecticide-treated nets; LBW, low birth weight; SD, standard deviation.

<sup>a</sup> Bivariate endpoints: Poisson regression with robust variance, continuous endpoints: multiple linear regression.

<sup>b</sup> Adjusted for gravidity, season of delivery, age, malaria at enrollment.

<sup>c</sup> P value for difference of treatment effect by gravidity strata (interaction term): placental malaria, 0.96; LBW, 0.68; mean birth weight, 0.52.

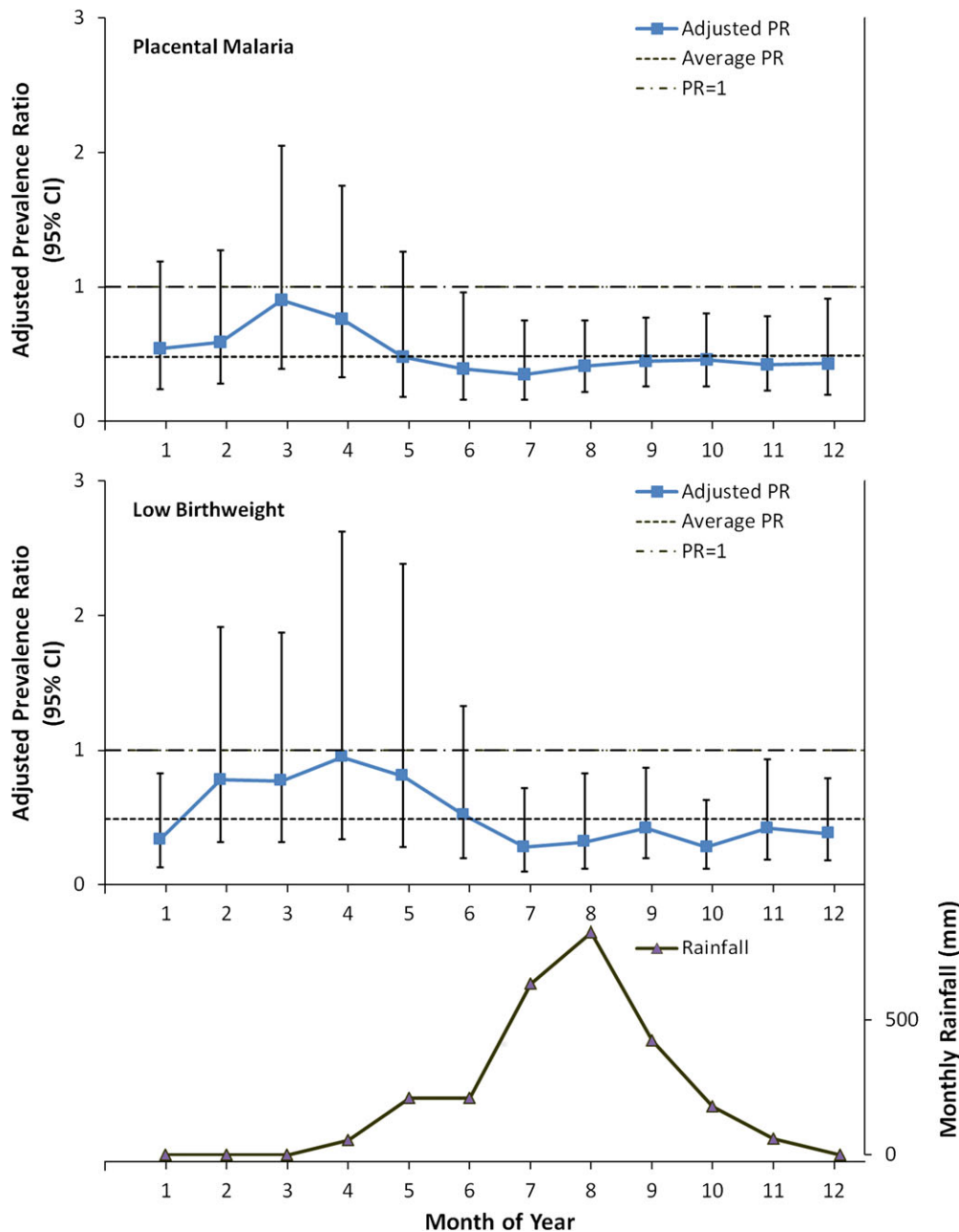
<sup>d</sup> P value for difference of treatment effect by ITN use (interaction term): placental malaria, 0.32; LBW, 0.79; mean birth weight, 0.115.

<sup>e</sup> Adjusted for gravidity, season of delivery and hemoglobin at enrolment.

<sup>f</sup> Adjusted for gravidity, season of delivery and maternal weight at enrolment.

<sup>g</sup> Spontaneous abortion or stillbirth (adjusted for gravidity and season).

<sup>h</sup> Pregnancy loss or early neonatal death in the first week of life (adjusted for gravidity and season).



**Figure 2.** The treatment effect of 3-dose versus 2-dose intermittent preventive therapy in pregnancy (ITPp) with sulfadoxine-pyrimethamine (SP) on placental malaria (*upper graph*) and low birth weight (LBW; *lower graph*) by malaria transmission season. Three-month moving averages of the prevalence ratios (PRs) obtained from Poisson regression models adjusted for gravidity and baseline parasitemia (placental malaria) and maternal weight (LBW). The error bars represent the 95% confidence intervals (CIs). The top horizontal line (—) depicts a prevalence ratio of 1, indicating no difference between treatment groups. Point estimates below this line indicate a beneficial effect in favor of the 3-dose group. The effect is statistically significant if the upper error bar does not cross the PR value of 1. The lower horizontal line (---) at PR values of 0.48 and 0.50 indicates the average adjusted prevalence ratio observed over the duration of the study. Data reflects the average estimates per month of the 2-year period of the study. Malaria transmission is depicted by the average monthly rainfall in the 2 study locations during the study period. Significant beneficial treatment effect on placental malaria was observed during the 7-month period starting in June (1 month after the first rainfall) and ending 1 month into the dry season (December) (adjusted prevalence ratio [APR], 0.43; 95% CI, 0.27–0.69;  $P = .001$ ). A smaller nonsignificant reduction was observed in the dry season from January through May (APR, 0.68; 95% CI, 0.33–1.40;  $P = .29$ ) ( $P$  value interaction term, .33). The corresponding APR values for LBW were 0.37 (95% CI, 0.20–0.68) ( $P = .001$ ) and 0.81 (95% CI, 0.39–1.68) ( $P = .58$ ) ( $P$  value interaction term, .035).

**Table 3. Adverse Events Among Newborns, by Study Group**

Adverse events	Proportion (%) of newborns		Absolute risk difference (95% CI)	Prevalence ratio (95% CI)	<i>P</i> <sup>a</sup>
	2-dose SP	3-dose SP			
<b>Icterus</b>					
Day 7	9/365 (2.5)	10/375 (2.7)	-0.2 (-2.4 to 2.1)	1.1 (0.4-2.6)	.90
Day 30	0/361	0/375			
<b>Congenital birth abnormalities</b>					
	3/359 <sup>b</sup>	1/362 <sup>c</sup>			
<b>Fever</b>					
Day 7	10/365 (2.7)	17/375 (4.5)	-1.8 (-4.4 to 0.8)	1.6 (0.76-3.6)	.19
Day 30	9/361 (2.5)	11/375 (2.9)	-0.4 (-2.7 to 1.9)	1.2 (0.49-2.8)	.71
<b>Vomiting</b>					
Day 7	7/365 (1.9)	7/375 (1.9)	0 (-1.2 to 2)	1.0 (0.36-2.9)	.95
Day 30	5/361 (1.4)	9/375 (2.4)	-1 (-2.9 to 0.9)	1.7 (0.6-5.1)	.32

**NOTE.** CI, confidence interval.

<sup>a</sup> *P* values presented here compared percentage of adverse events between treatment arms on day 0 and day 30 separately.

<sup>b</sup> Polydactyly.

<sup>c</sup> Clubfoot.

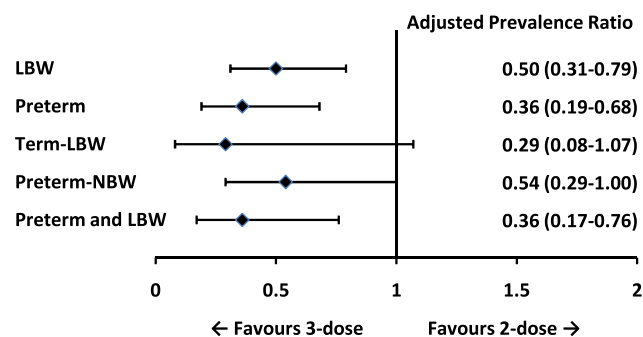
[20–22]. In the current study, there was no conclusive indication that ITN use modified the effect of IPTp. In the current study, there was no conclusive indication that ITN use modified the effect of IPTp; the 15% of women who used ITNs benefited equally from the third dose, compared with women who were not protected by ITNs in terms of reductions in malaria and LBW. However, the third dose appeared to be more effective in increasing mean birth weight in the non-ITN users than in the ITN users (*P* for difference in treatment effect between ITN users and nonusers, .12). Similarly, previous trials have shown IPTp-SP (and other interventions, such as ITNs) to be more effective in first pregnancies [2, 15, 21], yet in this setting, with highly seasonal transmission, gravidity was not found to be a significant effect modifier, and similar reductions in placenta malaria and LBW were observed in the first 2 pregnancies, compared with

multigravidae, although the greatest effect on mean birth weight was observed in the first 2 pregnancies.

In contrast to the large impact on LBW and preterm delivery (Figure 3), the impact on anemia (hemoglobin level, <11 g/dL) was modest and nonsignificant (APR, 0.89; *P* = .14); however, the point estimate is consistent with the summary estimate from the previous trials of 2-dose IPTp and ITNs, which also found an average risk reduction of 10% [2, 15].

Our study is limited by the lack of use of a placebo and provision of study drugs by clinicians hired by the study. The study was partially masked, in that none of the laboratory staff or delivery unit staff were aware of the study assignment, but the lack of use of a placebo makes any study more vulnerable to bias. Both groups otherwise received the same antenatal care, and there was no difference in the number of scheduled visits between the arms.

The policy implications of this study imply that more-frequent dosing would now be appropriate for the control of malaria in pregnancy and its sequelae in areas with low SP resistance in western Africa. Despite the support from many initiatives in sub-Saharan Africa, coverage with 2 doses of SP remains very low, and many women attending ANC receive a single dose of SP only [23]. The experience has been that single-dosing is a common result of unsuccessfully trying to implement a standard 2-dose regimen; extending to 3 doses of IPTp-SP may thus be more effective in an operational context, because it counters the risk underdosing mothers [24]. The other benefit of more-frequent dosing is this allows for a better integration with existing services as part of focused antenatal care, which consists of 4 scheduled visits, including 3 in the second and third trimester.



**Figure 3.** Treatment effect on low birth weight and preterm delivery. LBW, low birth weight (<2500 g); NBW, normal birth weight (≥2500 g); preterm delivery, <37 weeks gestation; term delivery, ≥37 weeks gestation.

These results suggest that malaria in the last few weeks of pregnancy is a major cause of LBW and that the addition of a third dose of SP to the standard 2-dose IPTp regimen used in most countries in western Africa may have a substantial beneficial impact on public health in the Sahel countries in sub-Saharan Africa, most of which currently have low levels of SP resistance.

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**Potential conflicts of interest.** All authors: No reported conflicts.

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