Malaria Journal

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Plasma folate dynamics in *Plasmodium falciparum*-infected African children treated with artemisinin combination therapy and single low-dose primaquine or placebo

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Abstract

Background Adding single low-dose (0.25 mg/kg) primaquine (SLDPQ) to block *Plasmodium falciparum* transmission is now a WHO recommendation. Whether SLDPQ increases haemolysis in glucose-6-phosphate dehydrogenase deficient (G6PDd) patients, leading to increased folate demand and impaired haemoglobin (Hb) recovery is unknown. This study sought to answer this question.

Methods This randomized, placebo-controlled trial measured serial plasma folate concentrations [Day (D) 0, 3, 7 and 28] in falciparum-infected Ugandan and Congolese children (6 months to 11 years), treated with age-dosed SLDPQ/placebo and artemether-lumefantrine/dihydroartemisinin-piperaquine. Genotyping defined G6PD (G6PD c.202T allele) status. Multiple linear and non-linear, mixed effects, cubic spline regression were fitted to identify factors significantly associated with plasma folate at baseline and over time, respectively.

Results 408 children (3 had missing D0 values) had ≥ 1 plasma folate value. Of these, 66 (16.2%) were G6PD-deficient, 51 (12.5%) heterozygous females, 283 normal and 8 unknown. Mean baseline folate concentrations were 10.83 [standard deviation (SD) 3.58, SLDPQ] vs 10.92 (SD 4.54, placebo) ng/ml, associated independently with baseline Hb [estimate: 0.52 ng/ml (95% CI: 0.26 to 0.79, p = 0.0001)] and baseline parasitaemia [estimate: − 0.18 ng/ml (− 0.32 to − 0.05, p = 0.007)]. For all patients, mean plasma folate concentration paralleled mean haemoglobin concentration with an initial mean fall of 1.65 ng/ml (p < 0.0001 vs. baseline), followed by a sustained rise achieving a mean D28 concentration of 11.04 (SD 4.45) ng/ml. Over time, only age (p = 0.0001), male sex (p = 0.017) and baseline parasitaemia (p = 0.029) were significantly associated with a reduced plasma folate.

Conclusion SLDPQ and G6PD status did not compromise posttreatment plasma folate concentrations in young children with acute uncomplicated falciparum malaria, providing additional evidence of SLDPQ safety and supporting its use without G6PD testing.

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Trial registration The trial is registered, reference number ISRCTN11594437.

Keywords Single low-dose primaquine, Malaria, Glucose-6-phosphate dehydrogenase, Folate

Background

Malaria is a major global public health challenge [1, 2]. The World Health Organization (WHO) reported 263 million cases in 2023, with 94% of cases found in Africa, where *Plasmodium falciparum* is the predominant species [3]. Malaria-induced anaemia is common [4, 5] due to a combination of intra- and extravascular haemolysis of parasitized and non-parasitized red blood cells (RBCs) and bone marrow dyserythropoiesis and suppression [4–6]. Haemoglobinopathies like sickle cell anaemia, thalassaemia, and glucose-6-phosphate dehydrogenase deficiency (G6PDd) confer protection against severe malaria but may exacerbate anaemia during acute malaria [5, 7].

In 2012, with the unrelenting rise of artemisinin resistance in Southeast Asia, the WHO recommended adding single low-dose primaquine (SLDPQ, 0.25 mg/kg body weight) to artemisinin-based combination therapy (ACT), as a gametocytocide to block *P. falciparum* transmission between humans and mosquitoes without testing for G6PDd [8–10]. Although primaquine, an 8-aminoquinoline, is known to cause dose-related acute haemolytic anaemia in G6PDd that is mediated through oxidative metabolites [11–13], the WHO considered SLDPQ to be safe in G6PDd.

G6PD is essential for folate metabolism by producing reduced Nicotinamide Adenine Diphosphate (NADPH) from NADP, as part of the redox reactions in the hexose monophosphate shunt that is coupled to the reduction of glutathione. G6PD deficiency, therefore, yields low NADPH which is the coenzyme needed for the conversion of dietary folate or folic acid supplements into active tetrahydrofolate [14]. Folate is an essential vitamin for normal RBC formation and folate deficiency leads to megaloblastic anaemia. The WHO-defined normal range of plasma folate in all ages is 6–20 ng/ml; a plasma value < 3 ng/ml denotes folate deficiency [15]. The recommended dietary allowance for folate varies with age and physiological demands; children aged 6–12 months require ~ 85 µg daily, gradually increasing to 300 µg/day in early adolescence. Folate body stores, predominantly in the liver, are low, ~ 30 mg, and can be depleted within four months without adequate intake [16, 17]. Poor diet is an important cause of folate deficiency, and in Africa, many staple foods are overcooked and lack adequate folate [18]. Increased folate demand is also seen in chronic haemolysis, such as sickle cell disease, and in pregnancy with or without malaria [19].

There are limited data on folate concentrations in malaria. Studies have reported normal or high red cell folate concentrations at disease presentation [20, 21] with the hypothesis that raised concentration is due to de novo folate synthesis by the parasite [22, 23]. Bradley-Moore et al. [22] found a significantly higher mean red cell (but not plasma) folate in children with *P. falciparum* parasitaemia (n = 29) compared to children protected by malaria chemoprophylaxis (n = 35). Similarly, Oppenheimer and Cashin [23] reported significantly higher red cell folate levels in 12-month-old infants (n = 56) with malaria compared to malaria-negative infants (n = 187), while serum folate levels in the same cohort showed no differences at age 2, 6, or 12 months. Abdalla [20] studied 106 falciparum-infected Gambian children aged 6 months to 7 years with a mean haemoglobin (Hb) of 5.3 (2.8 to 11.5) g/dl. Of 77 with measured red cell folate concentrations, 75 had normal/increased concentrations. Two weeks posttreatment with chloroquine, 15 children with paired results had a significant (p = 0.01) rise in the mean red cell folate from 466.2 to 599 µg/l that may have been related to posttreatment reticulocytosis; they also had a non-significant drop in mean serum folate from 13.48 to 9.53 µg/l. All bone marrows (n = 106) showed dyserythropoietic changes (e.g., normoblast multinuclearity, intercytoplasmic bridging, irregularly-shaped nuclei, and karyorrhexis) and megaloblasts were seen in 11 samples but their presence did not correlate with red cell folate concentrations. An earlier study of 7 *P. falciparum*-infected children with Hb ≤ 5.3 g/dl and normal/raised red cell folates using the deoxyuridine suppression test (a sensitive method that provides a measure of the biochemical consequence of vitamin B12 or folate deficiency) showed that their dyserythropoietic marrow changes were unrelated to folate deficiency [24].

The potential benefits of folate supplementation in *P. falciparum*-infected children remain controversial. One study administered folic acid at 1 mg once daily, for 14 days, to children (n = 185) aged 6–119 months with acute falciparum malaria and reported a statistically significant (p = 0.02) but small increase in mean haematocrit on Day (D)14: 27.8 vs 26.4% [adjusted mean Δ = 1.2 (95% CI 0.2 to 2.2)] that was lost by D28 [25]. In 600 children of similar ages with acute *P. falciparum*, folic acid (adjusted by weight at 5 mg for children weighing ~ 15 kg, 7.5 mg for 15–20 kg and 10 mg for > 20 kg) given for 28 days did not increase the mean D28 Hb but iron supplementation [5.5 mg/ml elemental iron, dosed at 5 (< 20 kg) or

7.5 (> 20 kg) ml thrice daily] improved the mean D28 Hb by 0.70 g/dl (95% CI 0.21 to 1.2, p = 0.006) [26]. Moreover, Sazawal et al. [27] concluded that routine iron and folate supplementation increases childhood morbidity and mortality in malaria endemic zones, while Maitland et al. [28] found no effect on the risk of post-discharge readmissions.

There are limited data on the malaria-folate relationship, and no study of SLDPQ has measured serial folate concentrations. This study aimed to assess whether SLDPQ may have an additional haemolytic effect vs. placebo in G6PDd children that would result in lower folate concentrations and, possibly, folate deficiency compared to G6PD normal children that may impede Hb recovery. Evidence from this study could inform recommendations on the use of folic acid.

Methods

The study methods have been detailed elsewhere [29]. Briefly, the 'Primaquine in African Children (PAC)' study was a randomized, double-blinded, placebo-controlled, trial of age-dosed SLDPQ combined with either open-label artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DHAPP). The trial was conducted at the Mbale Regional Referral Hospital (MRRH) in Uganda and Kinshasa Mahidol Oxford Research Unit (KIMORU) in the Democratic Republic of Congo (DRC) between July 2017 and December 2019. Enrolled children were aged 6 months to 11 years with acute uncomplicated falciparum malaria, diagnosed by either a positive RDT in MRRH (followed by a blood film to measure the parasitaemia) or a positive malaria slide in KIMORU. Children were excluded if there were signs of severe malaria, haemoglobin concentration < 6 g/dl or any comorbid illness adjudged by the physician to require inpatient care. Other exclusion criteria include concurrent use of drugs known to cause haemolysis in G6PDd, known allergy to primaquine, AL or DHAPP, and previous enrolment into the current study or any other clinical trial. G6PD status was assessed for the most common variant in Africa, the G6PDd A– variant (G6PD c.202T allele) [30]. Genotyping for sickle cell disease and α-thalassaemia were conducted using polymerase chain reaction [31]. A subset of participants were sampled on a first come, first served basis for plasma folate at baseline and days 3, 7 and 28. There was no formal sample size calculation for this observational substudy but 200 patients/arm was deemed reasonable to offer insights into plasma folate changes over time. Plasma folate was measured using chemiluminescent microparticle immunoassay (Abbott Alinity ci-series), and values were interpreted according to the WHO-defined reference range [15].

The PAC study was approved by all relevant ethics committees in Oxford, Uganda and the DRC and written informed consent was obtained from all legal guardians of eligible patients.

Statistical analysis

Patients with at least one plasma folate concentration were included in the analysis. Variables that were normally distributed were summarized by mean and standard deviation, skewed data by median and interquartile range (IQR). Mean differences between two or three groups were analysed using the unpaired *t*-test and Analysis of Variance (ANOVA), respectively. Categorical data were summarized as percentages and compared using chi-squared or Fisher's exact test, as appropriate.

Multiple linear regression was used to assess factors independently associated with baseline folate. Given the complex, non-linear trend of plasma folate over time observed on individual plots, a mixed effects model with a cubic spline was fitted. Cubic splines are piecewise functions that can be used for data smoothing and are useful where the data have sharp changes over time. Univariate and multivariable models were fitted, and outputs were summarised as estimates with respective 95% confidence intervals and p-values. Univariate-determined significant variables were used in the multivariate model. Analysis was according to the available data at each time point and the level of statistical significance was set at 5%. All analyses were done using R software version 4.3.0.

Results

Of the 1137 enrolled children, 408 were sampled for the 4-point (D0, D3, D7 and D28) plasma folate measurements: 320 (78.4%) from MRRH and 88 (21.6%) from KIMORU. Three participants did not have baseline folate measurements but did have follow-up folate measurements. All sampled children had at least one plasma folate measurement over the 4 timepoints; 87.7% (358/408) had complete 4-point measurements while 9.1% (37/408), 3% (12/408), and 0.25% (1/408) had 3, 2 and 1 measurements, respectively.

The baseline characteristics of the 408 children sampled were similar between the SLDPQ and placebo arms (Table 1). Overall, there were more males (54.9%); the median age was 4.9 years (IQR: 2.7 to 7.4), and median (IQR) weight and mid-upper arm circumference (MUAC) were 16.5 kg (IQR 12.0 to 21.2) and 15.5 cm (IQR 14.5 to 16.8), respectively. One participant had moderate acute malnutrition, and another had severe acute malnutrition. The distribution of G6PD status was similar between the groups: ~ 16% (66/408) had G6PDd whilst 12.5% (51/408) were heterozygous females. One participant, 0.2%, (1/408) had sickle cell disease, and 13.5%

Table 1 Baseline characteristics of study participants

	ACT + SLDPQ (n = 203)	ACT + Placebo (n = 205)	Overall (n = 408*)
Age in years (median, IQR)	5.0 (3.1–7.5)	4.8 (2.6–7.4)	4.9 (2.7–7.4)
Sex n (%)			
Male	115 (56.7)	109 (53.2)	224 (54.9)
Female	88 (43.3)	96 (46.8)	184 (45.1)
Weight (kg)	16.7 (13.0–22.0)	16.3 (11.3–20.4)	16.5 (12.0–21.2)
Temperature °C	37.4 (36.8–38.3)	37.2 (36.6–38.1)	37.2 (36.7–38.2)
MUAC (cm)	15.5 (14.5–16.9)	15.5 (14.4–16.8)	15.5 (14.5–16.8)
Nutritional status n (%)			
Normal nutritional status	201 (99.0)	205 (100.0)	406 (99.5)
Moderate acute malnutrition	1 (0.5)	0 (0.0)	1 (0.2)
Severe acute malnutrition	1 (0.5)	0 (0.0)	1 (0.2)
Splenomegaly n (%)	34 (16.7)	29 (14.1)	63 (15.4)
Plasma folate (ng/ml)	10.85 (8.03–13.78)	10.60 (8.05–13.60)	10.70 (8.0–13.60)
Plasma folate < 3 ng/ml n (%)	0 (0.0)	1 (0.5%)	1 (0.2)
Haemoglobin (g/dl; mean, SD)	10.7 (1.7)	10.6 (1.7)	10.6 (1.7)
G6PD status n (%)			
G6PD normal males	81 (39.9)	84 (40.9)	165 (40.4)
G6PD normal females	61 (30.0)	57 (27.8)	118 (29.1)
G6PD-deficient hemizygous males	31 (15.3)	24 (11.7)	55 (13.5)
G6PD-deficient homozygous females	5 (2.5)	6 (2.9)	11 (2.7)
Heterozygous females (Traits)	20 (9.9)	31 (15.1)	51 (12.5)
Sickle cell status n (%)			
Normal	170 (83.7)	175 (85.4)	345 (84.6)
Trait	29 (14.3)	26 (12.7)	55 (13.5)
Sickle cell disease	1 (0.5)	0 (0.0)	1 (0.2)
Alpha thalassemia status n (%)			
Normal	101 (49.8)	105 (51.2)	206 (50.5)
Silent Carrier	81 (39.9)	81 (39.5)	162 (39.7)
Trait	15 (7.4)	15 (7.3)	30 (7.4)
P. falciparum parasites per µl	48,796 (1668–41,797)	37,213 (2182–114,673)	41,890 (1716–125,243)

MUAC: Mid Upper Arm Circumference; G6PD: Glucose-6-Phosphate Dehydrogenase; IQR: Interquartile Range; SD: Standard Deviation; SLDPQ: Single Low-dose Primaquine

*408 children had at least 1 plasma folate measurement. The plasma folate summaries in this baseline table are for 405 children because 3 children did not have baseline measurements. The rest of the variables are summarised over 408 children WHO had at least one folate measurement during the 4-timepoint (D0, D3, D7, D28)

G6PD status was indeterminate/missing in 8 children (5 SLDPQ, 3 placebo)

Continuous variables are expressed with median (IQR), except haemoglobin (mean, SD)

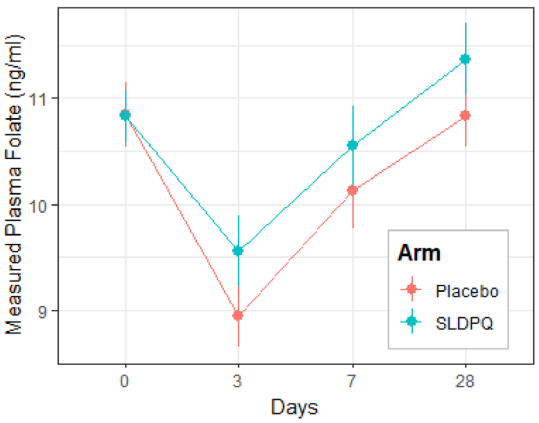
Categorical variables are numbers and percentages, n (%)

Normal nutritional status: MUAC ≥ 12.5 cm; Moderate Acute Malnutrition: MUAC 11.5–< 12.5 cm; Severe Acute Malnutrition: MUAC < 11.5 cm

(55/408) had sickle cell trait. The thalassaemic genotypic status was normal in about half of the population, while almost 40% (162/408) were alpha thalassaemia silent carriers (−α/αα), and 7.4% (30/408) had the thalassaemia trait (−α/−α). Only one (0.2%) child had folate deficiency with a plasma concentration of 2.8 ng/ml.

Combining both arms, there was an initial and significant reduction ($p < 0.0001$) in the mean plasma folate concentrations on D3 vs. baseline: 9.22 vs. 10.87 ng/ml, $\Delta = -1.65$ ng/ml, followed by a gradual increase. By D28, the mean plasma concentration was similar to baseline (Fig. 1). The observed mean plasma folate concentrations at each time point were similar between the arms (Table 2) and not significantly different by G6PD status aside from a trend ($p = 0.054$) for a lower concentration in the G6PDd vs. normal groups (Table 3 and Fig. 2). Changes in mean plasma folate matched those of the mean Hb concentrations (Fig. 3).

In the multivariable model, two factors were associated significantly with baseline plasma folate; a 1 g/dl increase



SLDPQ: Single low-dose Primaquine

Error bars indicate 95% Confidence Interval

Fig. 1 Mean plasma folate changes over time by treatment arm

in Hb increased the mean plasma folate by 0.52 ng/ml (95% CI: 0.26 to 0.79) whilst each log unit increase in baseline parasitaemia reduced the mean plasma folate by 0.18 ng/ml (95% CI: − 0.32 to − 0.05) (Table 4). In the model of folate dynamics, age, sex, haemoglobin dynamics and baseline parasitaemia were independently associated with folate dynamics over time (Table 5). Older children had lower mean plasma folate concentrations of 0.26 ng/ml for every increase in calendar age, as did males compared to females, mean of 0.98 ng/ml less, and baseline parasitaemia maintained its inverse relationship with changes in plasma folate. By contrast, plasma folate changes over time were positively associated with Hb changes over time, increasing by a mean of 0.41 ng/ml for every 1 g/dl increase in Hb. Of note, G6PD, sickle cell, thalassaemic, and nutritional status and being on SLDPQ were not independently associated with changes in plasma folate.

Discussion

This study has found that essentially all folate concentrations across all time points in these falciparum-infected children were normal. Post treatment, the mean plasma folate concentrations fell initially, progressively rising thereafter to recovery, similar to the mean changes in Hb over time. SLDPQ or G6PD status did not affect the plasma folate dynamics but, relative to G6PD normal children, there was a trend of a lower mean D3 plasma folate in the G6PDd children. These results suggest no clinically meaningful impact on plasma folate by SLDPQ and provide additional safety evidence of SLDPQ in G6PDd children [29, 32, 33].

The initial decline in the mean plasma folate paralleled the well described initial decline of mean Hb [29] and mean reticulocyte count [21, 34]. This period also signals the stimulation of RBC precursors in the bone marrow, leading to a gradual rise in the mean

Table 2 Mean and standard deviation (SD) of plasma folate by treatment group

	SLDPQ (n = 177)	Placebo (n = 181)	Total (n = 358)	p-value
Baseline (SD)	10.83 (3.58)	10.92 (4.54)	10.87 (4.09)	
Day 3	9.36 (4.09)	9.08 (4.15)	9.22 (4.12)	0.530
Day 7	10.16 (4.44)	10.28 (4.90)	10.22 (4.67)	0.805
Day 28	11.24 (4.76)	10.85 (4.12)	11.04 (4.45)	0.412

358/408 participants had complete 4-point folate measurement. Comparison between the two groups done using unpaired t-test

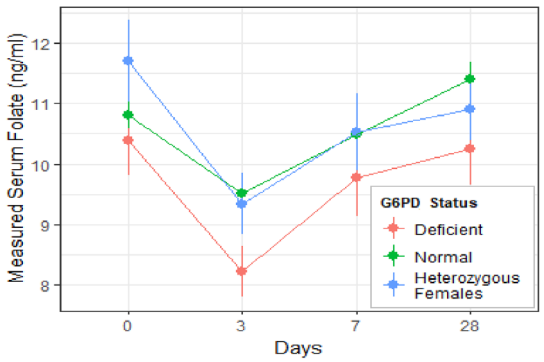
SLDPQ: Single Low-dose Primaquine

Table 3 Mean and standard deviation (SD) of plasma folate by G6PD status across the 4 time points

	Normal (n = 243)	Heterozygous females (n = 47)	Deficient n = 60)	Total (n = 350)	p-value
Baseline	10.82 (3.68)	11.82 (4.93)	10.47 (4.94)	10.90 (4.11)	
Day 3	9.49 (4.40)	9.20 (3.36)	8.31 (3.42)	9.25 (4.13)	0.140
Day 7	10.39 (4.70)	10.23 (4.49)	9.69 (4.86)	10.25 (4.70)	0.588
Day 28	11.39 (4.56)	10.71 (3.48)	10.23 (4.69)	11.10 (4.46)	0.156

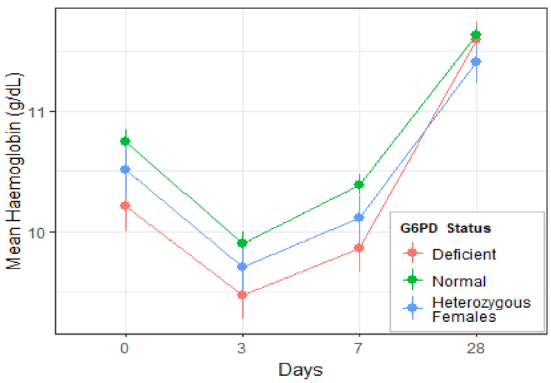
350/408 children with known G6PD status had complete 4-point folate measurement. Comparison between the three groups analysed using Analysis of Variance (ANOVA)

Unpaired t-tests comparing the mean plasma folate between G6PD normal and deficient arms across the four-time points were not significantly different but there was a trend on Day 3: p = 0.534 (D0); 0.054 (D3); 0.306 (D7) and 0.078 (D28), respectively



Error bars indicate 95% Confidence Interval.

Fig. 2 Mean plasma folate changes over time by the glucose-6-phosphate dehydrogenase (G6PD) A⁻ status



Error bars indicate 95% Confidence Interval

Fig. 3 Mean haemoglobin changes over time by glucose-6-phosphate dehydrogenase (G6PD) status

reticulocyte count after D3 and Hb recovery [29, 34, 35]. The erythroid precursor cells have a high affinity for accumulating folate through folate receptors in the early stages of erythropoiesis [36]; therefore, the transient reduction in plasma folate may result from folate uptake into the bone marrow, suggesting that malaria does not inhibit the rapid marrow uptake of folate even though falciparum malaria may result in dyserythropoiesis, poor iron utilization, and bone marrow suppression [20, 21, 24, 37]. However, the possibility cannot be excluded that the fall in plasma folate reflects a fall in the folate pool, assuming a stable equilibrium between plasma and red cell folate, which is ~ 30 fold higher vs. plasma folate [19]. Despite the initial reduction of folate, only one participant was folate deficient on D3 (2.7 ng/ml) and the mean plasma folate of 9.22 ng/ml was well within the 6–20 ng/ml normal range. This is consistent with research showing acute falciparum malaria does not induce folate deficiency in African children [20–22, 24, 25].

Mulenga et al. [25] reported normal, median baseline folate concentrations in falciparum-infected children (6 months to < 10 years) in the folate-supplemented (13.8 ng/ml) and placebo arms (15 ng/ml). Following treatment with folic acid (1 mg/day for 2 weeks), the D14 folate was higher in the supplemented group: 17.7 vs. 13.4 ng/ml and only resulted in a very modest increase in the D14 packed cell volume of ~ 1%. Mulenga's data are consistent with those of van Hensbroek et al. who also reported no benefit of folate supplementation on the D28 Hb concentration in falciparum-infected children [26]. Given the findings of this study, those of others, and the futility of folate supplementation in terms of the effect on Hb in uncomplicated *P. falciparum* and on post-discharge survival in African children with Hb < 6 g/dl [25–28], supplementary folate has not been shown to be beneficial in patients with acute falciparum malaria.

Folate recovery occurred gradually in tandem with a rise in mean Hb after the initial dip. Several mechanisms may be involved, especially the hydrolysis of tissue folate stores to compensate for low plasma levels and maintain homeostasis [38]. This release from tissue stores is supplemented by dietary folate as children recover their appetites and eat more food containing folate like vegetables, nuts, and beans. Folate is reduced to the inactive 5-methyltetrahydrofolate [39], which is taken up by RBCs and tissues and converted to active tetrahydrofolate [40, 41]. Uptake of folate by the bone marrow is by active transport against a concentration gradient, which is a saturable process [36]. Given the continuing rise in mean Hb with rising mean plasma folate in our patients, folate uptake by the marrow was adequate and was probably saturated in some children. Indeed, ~ 4% of our children had exceptionally high posttreatment plasma folate concentrations (> 20 ng/ml) that were probably diet related. Abdalla suggested a rising posttreatment folate may be related to the posttreatment reticulocytosis [20] as reticulocytes have higher folate activity compared to older red cells [42].

This study did not find significant differences in mean plasma folate by G6PD status, nor any apparent additional effect of SLDPQ but there was a trend to a lower D3 plasma folate in the G6PDd group, which may have several explanations. There may have been an initial greater demand for folate in G6PDd patients, a deficiency in NADPH (as suggested by Chen et al. in G6PDd cultured colon cancer cells [14]) or a reduction in the overall folate pool secondary to the initial fall in Hb. An upregulation of the mitochondrial and other cytosolic sources of NADPH is reported with G6PD gene knockout which compensates for the NADPH-dependent activation of

Table 4 Univariate and multivariable analyses of the effect of baseline characteristics on the baseline plasma folate

Variable	Univariate		Multivariable	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Age	**− 0.14 (− 0.26 to − 0.01)**	**0.033**	− 0.10 (− 0.45 to 0.25)	0.565
Sex				
Female (reference)				
Male	− 0.61 (− 1.39 to 0.16)	0.122		
Weight	**− 0.06 (− 0.116 to − 0.002)**	**0.041**	− 0.07 (− 0.23 to 0.09)	0.379
Temperature	0.16(− 0.22 to 0.54)	0.406		
Length of illness	0.07 (− 0.23 to 0.38)	0.641		
Haemoglobin	**0.39 (0.17 to 0.61)**	**0.001**	**0.52 (0.26 to 0.79)**	**0.0001**
G6PD (n = 397)				
Normal (reference)				
Deficient	− 0.41 (− 1.48 to 0.66)	0.449		
Heterozygous females	0.89 (− 0.31 to 2.09)	0.145		
Splenomegaly				
Normal (reference)				
Splenomegaly	**− 1.45 (− 2.52 to − 0.38)**	**0.008**	− 0.58 (− 1.69 to 0.52)	0.298
Thalassaemia status (n = 395)				
Normal (reference)				
Silent carrier	0.17 (− 0.66 to 0.99)	0.691		
Trait	− 1.00 (− 2.54 to 0.54)	0.201		
Parasitaemia (n = 324)	**− 0.18 (− 0.32 to − 0.04)**	**0.011**	**− 0.18 (− 0.32 to − 0.05)**	**0.007**

Unless otherwise stated, n = 405, the number of children with baseline folate on Day 0

Estimate indicates the regression coefficient

G6PD: Glucose-6-Phosphate Dehydrogenase

folate [14, 43] and this may explain the lack of folate deficiency in our participants, including those with G6PDd. Given the complexity of folate metabolism and the lack of data, more research is needed to tease the mechanisms of RBC folate metabolism in malaria and how that may differ by G6PD status.

Increasing age was associated independently with reduced plasma folate, consistent with previous reports [44, 45]. Folate liver stores rise from birth through adolescence while plasma folate increases until six months of age and declines thereafter [46], associated with weaning from folate-rich breast milk and introducing food [47]. Older children and adolescents have greater physiological needs for folate and their dietary patterns account for the decline in folate. Over time, boys had significantly lower plasma folate concentrations than girls but not at baseline. This may be a chance finding or could be due to possible increased demands during recovery and/or a higher metabolic rate in older boys [48]. Most studies have not found differences in measured folate by sex [47, 49] but Kreusler et al. reported higher folates in girls aged 1 to 5 year-olds [45]. Higher baseline parasitaemia was observed to be associated with reduced plasma folate at baseline and over time, and this may be related to a lower Hb, which is also associated with higher biomass infections [50]. Although malaria parasites can produce their own folate, higher biomass infections may require extrinsic folate for parasite survival.

This study had several limitations. Red cell folate, which is a better indicator of long-term folate status and less affected by dietary intake than plasma folate, was not assessed. Moreover, plasma folate was only measured at four points, which may not have identified the true day of the nadir concentration and the evolution after D28. Most children had folate concentrations within the normal range and were of normal nutrition status; only a minority had sickle cell trait and only one had sickle cell disease. This study did not consider the socioeconomic status; some studies have found a higher status to have an increased relationship with folate [45]. Therefore, findings cannot be extrapolated to groups not represented in this study. As a secondary data analysis, the study was limited to the available data; there was no formal sample size calculation based on a testable hypothesis and missing data at follow-up times reduced statistical power. Despite these limitations, the results of these secondary data analyses reveal important exploratory information

Table 5 Univariate and multivariable analyses of the effect of baseline characteristics on plasma folate over time using mixed effects cubic spline modelling

Variable	Univariate		Multivariable	
	Estimate (95% CI)	**p-value**	**Estimate (95% CI)**	**p-value**
Age	**− 0.14 (− 0.24 to − 0.03)**	**0.011**	**− 0.26 (− 0.39 to − 0.14)**	**0.0001**
Sex				
Female (reference)				
Male	**− 0.73 (− 1.37 to − 0.08)**	**0.028**	**− 0.98 (− 1.76 to − 0.20)**	**0.017**
Length of illness	− 0.01 (− 0.27 to 0.24)	0.912		
Haemoglobin*	**0.41 (0.25 to 0.57)**	**< 0.0001**	**0.41 (0.21 to 0.60)**	**< 0.0001**
G6PD (n = 400)				
Normal (reference)				
Deficient	0.88 (− 1.76 to 0.01)	0.055	− 4.81 (− 11.13 to 1.53)	0.147
Heterozygous females	0.09 (− 0.89 to 1.08)	0.853	− 4.74 (− 11.15 to 1.68)	0.157
Splenomegaly				
Normal (reference)				
Splenomegaly	**− 1.04 (− 1.93 to − 0.15)**	**0.022**	− 0.37 (− 1.32 to 0.56)	0.443
Thalassaemia status (n = 398)				
Normal (reference)				
Silent carrier	0.08 (− 0.61 to 0.77)	0.819	6.92 (− 0.10 to	0.059
Trait	− 1.15 (− 2.44 to 0.13)	0.079	5.62 (− 1.25 to 12.49)	0.117
Parasitaemia (326)	**− 0.14 (− 0.26 to − 0.02)**	**0.021**	**− 0.13 (− 0.25 to − 0.02)**	**0.029**
Treatment Arm				
Placebo (reference)				
SLDPQ	0.44 (− 0.21 to 1.09)	0.182		

*Haemoglobin changes over time

G6PD status and Thalassaemia status were not significant when included in the multivariable model

Unless otherwise stated, n = 408

Estimate indicates the regression coefficient

G6PD: Glucose-6-Phosphate Dehydrogenase

SLDPQ: Single Low-dose Primaquine

that can form basis for future statistically powered studies.

Conclusion

This study in falciparum-infected children without folate deficiency showed an initial fall in the mean folate followed by a rise that paralleled the dynamics of Hb. The initial folate fall and subsequent rise were probably related to increased marrow utilisation, followed by marrow saturation and increased folate intake secondary to improved appetite and reticulocytosis. No deleterious effect of G6PD status and SLDPQ on folate dynamics was detected, supporting the safety of SLDPQ in G6PDd and the WHO recommendation to not test for G6PDd.

Abbreviations

ACT	Artemisinin-based combination therapy
AL	Artemether-Lumefantrine
ANOVA	Analysis of variance
DHAPP	Dihydroartemisinin-Piperaquine
DRC	Democratic Republic of Congo
G6PD	Glucose-6-phosphate dehydrogenase
IQR	Interquartile range
KIMORU	Kinshasa Mahidol Oxford Research Unit
MCRI	Mbale Clinical Research Institute
MORU	Mahidol Oxford Tropical Medicine Research unit
MRRH	Mbale Regional Referral Hospital
MUAC	Mid-upper arm circumference
NADP	Nicotinamide adenine diphosphate
NADPH	Reduced nicotinamide adenine diphosphate
PAC	Primaquine in African Children
RBC	Red blood cell
SLDPQ	Single low dose primaquine
UK	United Kingdom
WHO	World Health Organization

Acknowledgements

We thank all patients who participated in this study and the staff members at MRRH and KIMORU for conducting the study. We thank the Data Monitoring and Ethics Committee (Philippe Guerin[chair], Patrice Piola, Michel Van de Vugt, and Charles Opondo) and the Trial Steering Committee (M Boele van Hensbroek [chair], Karen Barnes, Ingrid Chen, and James Tibenderana).

Author contributions
MM and WRJT conceived the study. SA analysed and interpreted the data and wrote up the first draft of the manuscript. MM and WRJT critically reviewed the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the UK Government Department for International Development; UK Medical Research Council; UK National Institute for Health Research; and the Wellcome Trust Joint Global Health Trials Scheme (reference: MR/P006973/1). The funders had no role in study design, data collection, analysis, interpretation, report writing, or decision to publish. MM and WRJT are supported by the Wellcome Trust of Great Britain through its core grant [grant number 220211] to the Mahidol-Oxford Tropical Medicine Research Unit research programme. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Availability of data and materials
Deidentified individual participant data and relevant supplementary data and documents (e.g. data dictionary, protocol, and participant information sheet) will be made available to applicants who provide a sound proposal to the Mahidol Oxford Tropical Medicine Research Unit Data Access Committee ([datasharing@tropmedres.ac] (mailto:datasharing@tropmedres.ac)). A data access agreement will be put in place before sharing.

Declarations

Ethics approval and consent to participate
Ethical approvals were obtained from the following ethics committees: the Oxford University Tropical Research Ethics Committee (reference 53-16), the Mbale Regional Referral Hospital Institutional Review Committee (MRRH-REC OUT—COM 006/2017), the Uganda National Drug Authority (CTA00280), and the Uganda National Council for Science and Technology (HS2205). In the DRC, approvals were granted by the Ministry of Higher and University Education, the University of Kinshasa Public Health School Ethics Committee, and the City of Kinshasa Provincial Government Health Minister (ref 135/MIN.SAN. AFFSOC&ACHUM/CM/JD/2017).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 17 April 2025 Accepted: 5 September 2025
Published online: 15 October 2025

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