TITLE: Safety of single dose primaquine in G6PD-deficient and G6PD-normal males in Mali without malaria: an open-label, phase 1, dose-adjustment trial

Authors: Ingrid Chen*, Halimatou Diawara*, Almahamoudou Mahamar, Koualy Sanogo, Sekouba Keita, Daouda Kone, Kalifa Diarra, Moussa Djimde, Mohamed Keita, Joelle Brown, Michelle E. Roh, Jimee Hwang, Helmi Pett, Maxwell Murphy, Mikko Niemi, Bryan Greenhouse, Teun Bousema, Roly Gosling+, Alassane Dicko+

*, + =Equal contributions

Summary:

This phase 1 open-label, non-randomized, dose-adjustment trial showed that single doses of primaquine between 0.40 and 0.50 mg/kg were well-tolerated in G6PD-deficient and G6PD-normal males in Mali, supporting the World Health Organization recommendation on the use of single low-dose primaquine to reduce Plasmodium falciparum malaria transmission.

Institutions:

Malaria Research and Training Centre, Faculty of Pharmacy and Faculty of Medicine and Dentistry, University of Science, Techniques and Technologies of Bamako, Bamako, Mali (Prof A Dicko, MD; H Diawara, MD; A Mahamar, PharmD; K Sanogo, MD; S Keita, MS; D Kone, MD; K Diarra, PharmD; M Djimde, MD)

National University Hospital of Point G, Bamako, Mali (MB Keita)

Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA (J M Brown, PhD; M Roh, MPH; R Gosling, MD)

Global Health Group, Malaria Elimination Initiative, University of California, San Francisco, San Francisco, CA (R Gosling, MD; I Chen, PhD; M Roh, MPH; J Hwang, MD)

School of Medicine, University of California, San Francisco, San Francisco, CA, USA (M Murphy; B Greenhouse, MD)

President's Malaria Initiative, Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA USA (J Hwang, MD)

Department of Medical Microbiology, Radboud university medical center, Nijmegen, the Netherlands (T Bousema, PhD; H Pett, MSc)

Department of Immunology & Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom (T Bousema, PhD)

Department of Clinical Pharmacology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland (M Niemi, MD; H Pett, MSc)

Corresponding author:

Ingrid Chen

550 16th Street, 3rd Floor

San Francisco, CA 94158, USA

Tel: (415) 476-5599

ingrid.chen@ucsf.edu

ABSTRACT

Background: The World Health Organization recommendation on the use of single low-dose primaquine (SLD-PQ) to reduce *Plasmodium falciparum* malaria transmission requires more safety data.

Methods: We conducted an open-label, non-randomized, dose-adjustment trial of the safety of three single doses of primaquine in glucose-6-phosphate dehydrogenase (G6PD)-deficient adult males in Mali, followed by an assessment of safety in G6PD-deficient boys ages 11-17 years, and 5-10 years, including G6PD-normal control groups. The primary outcome was the greatest within-person percentage drop in hemoglobin concentration within 10 days post-treatment.

Results: 51 participants were included in analysis. G6PD-deficient adult males received 0.40, 0.45, or 0.50 mg/kg of SLD-PQ. G6PD-deficient boys received 0.40 mg/kg of SLD-PQ. There was no evidence of symptomatic hemolysis, and adverse events considered related to study drug (n=4) were mild. The mean largest within-person percent drop in hemoglobin between day 0 and 10 was -9.7% (95% CI: -13.5, -5.90) in G6PD-deficient adults receiving 0.50 mg/kg of SLD-PQ, -11.5% (95%CI: -16.1, -6.96) in G6PD-deficient boys aged 11-17, and -9.61% (95%CI: -7.59, -13.9) in G6PD-deficient boys aged 5-10. The lowest hemoglobin concentration at any point during the study was 92 g/L.

Conclusion: SLD-PQ doses between 0.40 and 0.50 mg/kg were well-tolerated in G6PD-d males in Mali.

Trial registration: https://clinicaltrials.gov NCT02535767

Funding: Bill & Melinda Gates Foundation

Key words: primaquine, Plasmodium falciparum, malaria, transmission, G6PD-deficiency, drug safety, hemolysis, mass drug administration

BACKGROUND

The success of malaria control and elimination programs supports global aspirations to eradicate human malaria in the coming decades [1]. In order to achieve these ambitions, new transmission-blocking tools and strategies are needed. In this context, the use of primaquine becomes important, as the only commercially available drug that can stop the transmission of *Plasmodium falciparum* malaria from humans to anopheline mosquitoes.

Primaquine use must be informed by safety considerations, as this drug causes dose-dependent hemolysis in individuals with enzymatic glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most common inherited enzyme deficiency worldwide [2]. Safety depends primarily on primaquine dose, as well as the variant of G6PD-deficiency, which varies across geographies. In Africa most G6PD-deficiency variants are the mild, A minus variant

whilst in Asia, the variants are heterogeneous, with the Mediterranean variant being the most severe [3].

For the treatment of *P. falciparum* gametocytes, the WHO recommends that a 0.25 mg/kg single low dose of primaquine (SLD-PQ) be used without G6PD testing, in conjunction with standard artemisinin-based combination therapy (ACT), in areas approaching malaria elimination and/or facing drug resistance [4, 5]. This WHO recommendation is not backed by systematically collected evidence, and the WHO has expressed a need for additional clinical studies focused on the safety of SLD-PQ in G6PD-deficient (G6PD-d) individuals [5, 6].

Implementation of the WHO recommendation to use 0.25 mg/kg of SLD-PQ requires that each individual be weighed, primaquine tablets be crushed and dissolved in water, and the corresponding amount of primaquine solution for the 0.25 mg/kg dose be carefully measured. This process is labor intensive, introduces the possibility for dosing errors, and can be avoided by the establishment of age-based dosing bands. To develop age-based dosing bands, the therapeutic dose range of SLD-PQ, spanning the lowest efficacious dose to the highest safe dose in vulnerable individuals, must be established. This provides weight-based dose bands, which may then be converted to age-based dosing bands using age-for-weight data [7]. This study aims to contribute to the establishment of the higher bound of therapeutic dose range of SLD-PQ in African settings.

METHODS

Study design and participants

This study was an open-label, non-randomized, dose-adjustment followed age de-escalation trial of the safety of single dose primaquine in G6PD-d and G6PD-normal (G6PD-n) males in Mali without microscopically patent malaria infection. Participants were enrolled sequentially.

In part 1, we investigated three single doses of primaquine in adults: the first was a pre-specified dose of 0.40 mg/kg in seven G6PD-deficient individuals according to a Carestart™ 3 rapid diagnostic test (Access Bio, USA), a dose demonstrated to be safe in a previous study [8]. The remaining two dose groups were determined by a data safety and monitoring board (DSMB), on review of data on adverse events (AEs) and hemoglobin (Hb) concentrations after follow-up of at least 10 days for each prior group. The dose was not escalated if any of the following occurred: two or more participants experienced acute hemolysis resulting in a Hb drop of > 30%, or any participant experienced symptoms requiring blood transfusion or a serious adverse event (SAE) related to the study drug by day 10, including acute renal failure and/or death. The rationale for choosing a 30% drop was based on a prior study showing that Hb concentrations < 70 g/L were associated with fatal anemia; our inclusion criteria ensured that starting concentrations of participant Hb were ≥ 100 g/L, thus drops <30% would not pose risks to fatal anemia [9]). If none of these criteria were met, the DSMB selected the next dose, which could be escalated within a range of 0.05 to 0.2 mg/kg from the last study dose, not to exceed 0.75 mg/kg. This procedure continued until three dose groups were enrolled.

After enrollment of three dose groups, the DSMB reviewed study data and determined the highest dose of primaquine tolerated in part 1, which was then administered to a control group

of seven G6PD-n individuals matched within eight years of age to those enrolled in the highest tolerable dose group. Follow-up for the control group proceeded in the same manner as the intervention groups. G6PD status was validated using semi-quantitative spectrophotometry testing (OSMMR-D G-6-PD test, R&D Diagnostics Ltd ®, Greece).

In part 2, we investigated the safety of single dose primaquine in G6PD-d boys, at a dose 0.1 mg/kg lower than the highest safe dose found in adults, to be conservative. For the first age group (11-17 years inclusive), we enrolled seven G6PD-d boys and seven G6PD-n boys (control group). Following DSMB review of data using criteria described for part 1, if primaquine was safe in this age group, a second age group (5-10 years inclusive) comprised of seven G6PD-d and seven G6PD-n boys would be enrolled.

Participants were recruited from the town of Oulessebougou and its surrounding villages in Mali by the Malaria Research and Training Centre (MRTC) of the University of Bamako, Mali. Prior to recruitment, the study team met with village leaders to arrange for information sessions on the trial for prospective participants in the community, seeking informed consent from those interested in participation. All individuals that provided consent were tested for G6PD deficiency using the Carestart™ 3 test and had their name, address and phone number recorded. Those that tested as G6PD-d scheduled a date to visit the study clinic in Oulessebougou for further screening, while those with a G6PD-n test result were told they might be contacted at a later date for further screening.

Eligible participants were males to reduce the possibility of incorrect classification of G6PD status using qualitative G6PD tests, known to occur more commonly in heterozygote females [10], who provided written informed consent, were able to swallow oral medication, had Hb concentrations ≥ 100 g/L assessed by HemoCue® 301 (AB Leo Diagnostics, Helsingborg, Sweden), did not have malaria parasitemia according to a thick blood smear on enrolment, and agreed to abstain from the ingestion of grapefruit-containing products from 72 hours prior to the start of dosing until completion of follow-up. Eligible participants for part 1 of the study were aged 18-50 years, and for part 2 of the study, were enrolled based on age group.

Participants who reported any of the following conditions were excluded: a known positive HIV and/or hepatitis B test, allergy to primaquine, current use of medication for tuberculosis, HIV, or any drugs that have hemolytic potential in G6PD-d individuals (including sulphonamides, dapsone, nitrofurantoin, nalidixic acid, ciprofloxacin, methylene blue, toluidine blue, phenazopyridine, and co-trimoxazole), use of antimalarial drugs within two weeks before contact with the study team, blood transfusion > 500mL within the last three months, high alcohol intake (> 14 units of 10 g of alcohol per week) within the past six months, and/or the reported use of illicit drugs (marijuana, heroin, cocaine, methamphetamine) within 6 months of study. All eligible participants could be enrolled in the study one time, and any enrolled participant that vomited within one hour of ingesting primaquine was excluded from analysis.

The study was approved by the Ethics Committee of the Malaria Research and Training Centre Faculty of Medicine, Pharmacy and Dentistry of the University of Science, Techniques and Technologies of Bamako (N 2015/89/CE/FMPOS), and the Committee on Human Research at

the University of California, San Francisco (IRB # 14-14495). The study was monitored by an external clinical trials monitor based in Bamako, Mali. Participants were compensated for time and travel. The trial was registered: clinicaltrials.gov NCT02535767.

Procedures

After collection of day 0 samples, each participant received an oral dose of primaquine according to his group assignment, after a fatty snack (biscuits) to minimize gastrointestinal symptoms. The study pharmacist administered primaquine (Sanofi, Laval, QC, Canada) using directly observed therapy of 15 mg tablets crushed and dissolved in 15 mL of drinking water, administered to the nearest 0.1 mL [11].

Participants were evaluated at the study clinic at hours 1, 2, 4, 6, 8 and days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, and 28 following treatment. On all follow-up days starting approximately 24 hours following primaquine administration, a clinical exam was conducted, and blood samples were taken for Hb measurement and blood slides for malaria and reticulocyte counts (reticulocytosis defined as >2.0%). To assess for oxidative stress, a non-invasive methemoglobin measurement was taken at all follow-up timepoints using a Masimo Rad-57 pulse oximeter (Masimo, Irvine, CA, USA) (methemoglobinemia defined as >3%). Due to technical issues, the pulse oximeter was not functional until the start of enrolment of the second dose group. Urine samples were also collected on each day of follow-up to assess for hemolysis using a urine color chart developed by Hillmen and Hall [12]. If a ≥30% drop in baseline Hb levels was observed by day 7 of follow-up, a blood sample was taken on day 10 for additional assessment of serum bilirubin, urea, and creatinine levels.

AEs were assessed actively during all follow-up visits, and passively through the availability of study clinicians 24 hours/day, 7 days/week. We recorded the duration and severity of AEs (mild: grade 1, moderate: grade 2, severe: grade 3, life threatening: grade 4), relationship to the study drug according to study physician(s) (definite, probable, possible, remotely possible, unrelated, or unclassifiable), actions taken, and the outcome. AEs were further assessed as serious or not serious, according to the treating physician. SAEs were pre-specified in the study protocol and adapted by the DSMB prior to trial initiation, defined as any of the following: development of clinical signs or symptoms of distress including any requirement for hemodialysis, laboratory values of severe hemolytic anemia including a drop in Hb > 40% from baseline levels, a need for blood transfusion, the development of hemoglobinuria identified by black coloration in the urine associated with a rise in creatinine, and any serious adverse event defined as an untoward medical occurrence or effect that at any dose: results in death, is life threatening, requires hospitalization, results in persistent or significant disability or incapacity that is clearly, probably or possibly related to the study drug.

G6PD testing was conducted using three methods: qualitative phenotypic testing, semi-quantitative testing, and genotyping. Qualitative phenotypic rapid testing using the Carestart™ 3 test was conducted for screening throughout the study. Additionally, the field team conducted additional qualitative OSMMR tests (OSMMR-D G-6-PD test, R&D Diagnostics Ltd ®, Greece) in part 1 after enrollment of the first three individuals, excluding individuals with discordant results between the two qualitative tests [13]. Semi-quantitative spectrophotometry testing was conducted on all individuals using cryopreserved blood samples (0.1 mL of whole blood) collected prior to primaquine administration, and was used to determine final G6PD status for

participants for inclusion in analysis. Genotypic testing was conducted for the G6PD G202A single nucleotide polymorphisms (SNPs) in all individuals, in addition to A376G SNPs in adults, both known to be common among G6PD-d individuals in Africa of the *A-* variant at the time of study design [14]. G6PD-d individuals with any SNPs not assessed for, including 986C later identified to be common in Mali, were identified as wildtype in this study [15].

Cytochrome P450 2D6 SNPs were identified for adults, and Polymerase Chain Reaction (PCR) methods were used to detect malaria parasites in all participants, using dried blood spots collected on filter paper prior to primaquine administration (described in Supplemental Material).

Outcomes

The primary safety outcome was the largest within-person percent drop in Hb concentration between baseline levels (day 0) and day 10 following primaquine administration. Pre-specified secondary outcomes consisted of: the incidence of AEs graded by severity and relation to the study drug; the occurrence of acute hemolytic anemia at each primaquine dose, including absolute and fractional changes in Hb concentration on day 7 and day 28 compared to baseline, urine color assessment, reticulocyte count, total and direct bilirubin levels if a within-person Hb concentration drop of ≥30% from baseline levels was seen in an individual between day 0 and 7 (inclusive), methemoglobin concentration, and the development of physical signs or symptoms of hemolytic anemia; a comparison between changes in Hb, the frequency and severity of AEs, and occurrence of markers of acute hemolytic anemia between G6PD-d and G6PD-n participants; and exploratory studies among adults on whether cytochrome P450 2D6 (*CYP2D6*)

SNPs are associated with hemolysis in G6PD-d individuals (described in Supplemental Material).

Statistical analysis

Based on preliminary data, sample size calculations assumed that the average Hb concentration before treatment would be 125 g/L, and that the standard deviation for the within-person change in Hb concentration after treatment would be 17 g/L. Our sample size calculation enabled detection of a 15% or greater within-person drop in Hb concentration after treatment compared to the null hypothesis of no drop with 80% power and one-tailed a significance level of 0.05, requiring 7 individuals per group. This also enabled the detection of a within-person drop in Hb concentration of 20% or more among the G6PD-d participants compared to G6PD-n controls

For the analysis of change in Hb concentrations, the outcomes were the mean largest within-person percent drop in Hb concentration between baseline levels and: day 7, day 10, and day 28 post primaquine treatment. Within age groups (age 5-10, 11-17, and 18+ years), we used a regression model adjusting for baseline Hb concentration to determine whether there was a significant difference in the outcomes between G6PD-d and G6PD-n participants post treatment.

AEs were summarized descriptively for each dosing group, using frequencies of observed events, severity, and proportion of participants experiencing at least one event.

All analyses were conducted using Stata v12 (StataCorp, College Station, Texas).

RESULTS

Part 1: 28 adult males were enrolled between August 13 and December 19, 2015 (Figure 1). The dose groups of primaquine were 0.40 mg/kg, 0.45 mg/kg, 0.50 mg/kg among G6PD-d males, and 0.50 mg/kg in the control group of G6PD-n males (Table 1). The first three individuals enrolled in the 0.40 mg/kg group were incorrectly classified as G6PD-d (Carestart™ screening test), and were G6PD-n according to semi-quantitative spectrophotometry (excluded from analyses, Supplementary Table S1). All G6PD-n individuals were wild-type for both the G6PD G202A and A376G allele, most (15/18) G6PD-d individuals had SNPs for the G202A and A376G allele and had partial G6PD-d, and 3/18 G6PD-d individuals were identified as wild-type but had total G6PD-d according to semi-quantitative spectrophotometry.

Part 2: 28 boys were enrolled between May 12, 2016 and January 10, 2017 (Figure 1). Two boys in the G6PD-d, 5-10 age group were misclassified by the Carestart™ screening test and were G6PD-n according to semi-quantitative spectrophotometry (excluded from analyses, Supplementary Table S3). All G6PD-n boys enrolled were wild-type at the G202A allele. Among G6PD-d boys, 7/12 had SNPs at G202A, and 5/12 were identified as wildtype, four of which were characterized with partial G6PD-d, and one with total G6PD-d using semiquantitative testing.

Among men over the age of 18 years (part 1), the mean largest within-person percent drop in Hb concentration among G6PD-d individuals receiving the highest tolerable dose of primaquine (0.50 mg/kg) was -9.7**%** (95%CI: -13.5, -5.90) compared to their G6PD-n counterparts who experienced a drop of -7.89% (95%CI: --11.4, -4.37) (*P*=0.320) (Table 2, Supplementary Figure S1). Among the boys aged 5-10 years (part 2), the mean largest within-person percent drop in Hb concentration among G6PD-d participants was -9.61% (95%CI: -3.08, -16.1), compared to their G6PD-n counterparts who experienced a change of -5.24% (95%CI: -0.252, -10.2%) (*P*=0.199). Similarly, among the boys aged 11-17 years (part 2), the mean largest within-person percent drop in Hb concentration among G6PD-d participants was -11.5% (95%CI: -6.96, -16.1), compared to their G6PD-n counterparts who experienced a drop of -6.89% (95%CI: -2.46, -11.3) (*P*=0.072). There was no significant difference between the mean largest within-person percent drops in Hb concentration among G6PD-d adult men (n=18; -10.5**%** (95% CI: -13.3, -7.6)) and G6PD-d boys aged 5-17 years (n=12; -10.7% (95% CI:-13.9, -7.59, *P*=0.90)).

The mean largest percent change in Hb concentration was a 16.8% drop between day 0 and day 10, which occurred in the group of adult men who received 0.40 mg/kg of SLD-PQ. This group included the individual with the largest within-person percent change in Hb concentration (23% drop in Hb, A- variant of G6PD deficiency): Hb concentration 147 g/L at baseline and 113 g/L 9 days following primaquine administration, positive test for *P. falciparum* malaria at baseline by PCR, positive by blood smear on Days 1, 3, 4, 5, 6, 7, symptomatic malaria diagnosis on Days 6 through 9 following treatment with primaquine. At Day 28, this participant's Hb concentration was 135 g/L. For all participants enrolled, the lowest absolute value of Hb was 92 g/L, observed in a six-year old G6PD-d boy: Hb 112 g/L at baseline, nadir observed on day 10, no malaria detected by blood smear, and Hb 106 g/L by day 28.

During the 28 days of follow-up, no participant experienced a 30% or greater drop in Hb (Figures 2 and 3). There were no events of acute hemolysis, severe or SAEs, nor were there any symptoms or signs of acute renal failure, hemolytic anemia, hemoglobinurea or methemoglobinemia observed. A total of 24 participants (47%) had an AE during follow-up; most (44/46 [96%]) were mild (grade 1), 4 of which were considered related to primaquine (Table 3). All AEs resolved during study follow-up, and none resulted in stopping participation. Thirteen (24%) participants had reticulocytosis, 12 of whom were G6PD-d (Supplementary Figure S2).

At the screening visit, although no participants were symptomatic for malaria and all were smear negative, PCR analysis of stored blood samples revealed that 41% (21/51) participants had asymptomatic malaria parasitemia. During follow-up, a total of 14 participants tested positive for malaria on blood smear, with 4 of these 14 being symptomatic (Supplementary Table S4). There was no evidence that the within-person change in Hb concentration during follow-up was associated with malaria status (Supplementary Figure S3).

DISCUSSION

This study found that a single dose of primaquine as high as 0.50 mg/kg was well-tolerated among G6PD-d adults, and a 0.40 mg/kg dose was well tolerated in G6PD-d adults and children. Our study is the first to systematically investigate the safety of SLD-PQ single at doses ≥ 0.40 mg/kg in G6PD-deficient individuals without malaria, addressing the evidence gap identified by WHO Expert Review Group that issued the recommendation to use SLD-PQ.

Despite consistent measures of reduced Hb in G6PD-d participants exposed to SLD-PQ compared to G6PD normal participants, the reductions in Hb were small and not clinically relevant.

This study also provides data to define the therapeutic dose range of SLD-PQ in West African settings, which will support field implementation of the WHO recommendation, currently based on patient weight and challenged by a requirement to dissolve tablets and carefully measure the correct amount of solution to give to the patient [6, 7]. Age-based dose bands were recently been modeled for African settings, stating a need for clinical studies to ascertain the therapeutic dose range, and preliminarily assuming this to be between 0.1 to 0.4 mg/kg[16]. The lower bound of the therapeutic dose range of SLD-PQ is between 0.125 and 0.25 mg/kg, and our study suggests that the upper bound may be extended to 0.40 mg/kg in west African settings [17-20]. As the severity of G6PD-deficiency can be approximately characterized by geography, these results should not be applied to Asian settings.

Other studies to date have focused on the safety of the WHO recommended dose of 0.25 mg/kg of SLD-PQ, demonstrated to be safe in Senegal when given with artemether-lumefantrine or dihydroartemisinin-piperaquine or artesunate-amodiaquine to 54 phenotypically G6PD-deficient malaria patients [21], in Tanzania when given with artemether-lumefantrine to 33 phenotypically G6PD-deficient malaria patients [22], and used as mass drug administration at the Thai-Myanmar border with dihydroartemisinin-piperaquine to 124 phenotypically G6PD-deficient individuals [23]. A study investigating the safety of 0.25 and 0.40 mg/kg primaquine in

asymptomatic G6PD-d malaria patients in Burkina Faso is complete (NCT02174900) although results have not yet been reported.

As malaria infection can reduce hemoglobin concentration, this study was intended to examine the isolated effect of primaquine on individuals without malaria. However, many individuals enrolled had asymptomatic malaria (40% during screening and 26% during follow-up), four (8%) of which presented with symptomatic malaria on follow-up. While it is not possible to determine whether Hb drops in these individuals were due to malaria infection or SLD-PQ, the results do suggest that SLD-PQ is well-tolerated in G6PD-d individuals, both with and without malaria.

The main limitations to this study include a small sample size, as well as the exclusion of women, and vulnerable individuals who were already anemic on enrolment, an ethical precaution taken to ensure the safety of study participants. As SLD-PQ is now being rolled out in various countries in Africa, further assessment of its safety should be carried out using pharmacovigilance mechanisms, including emphasis on vulnerable individuals who are anemic, have HIV and/or tuberculosis infection, and/or are unaware of their pregnancy when they take primaquine.

In summary, these results provide evidence towards extending the upper bound of the therapeutic dose range of SLD-PQ to 0.40 mg/kg in sub-Saharan Africa, evidence which may be applied to facilitate the age-based dosing of SLD-PQ in sub-Saharan Africa.

ACKNOWLEDGMENTS

The study team wishes to thank the DSMB (Professor Daniel Chandramohan (chair), Professor Ric Price, Dr Joel Tarning and Dr Sophie Zaloumis), the hard work of the laboratory and field staff, and the participants and members of the community health association in Ouelessebougou for their cooperation throughout the study. We also thank Sanofi for their kind donation of primaquine.

NOTES

The authors state that they do not have associations that might pose a conflict of interest. Activities of the staff from the US Centers for Disease Control and Prevention were considered not to constitute engagement in human subjects research.

The work was supported by the Bill & Melinda Gates Foundation (OPP1089413). Support for AD come from the government of the Mali and different grants including from Bill & Melinda Gates Foundation (OPP1089413). TB is supported by grants of the Bill & Melinda Gates Foundation (AFIRM OPP1034789 and a fellowship from the European Research Council (ERC-2014-StG 639776)). JH receives salary support from the President's Malaria Initiative. All other authors were supported by the Bill and Melinda Gates Foundation (OPP1089413).

The findings of this study have been presented at the American Society of Tropical Medicine and Hygiene 2015 Annual meeting, Philadelphia, PA. Symposium: “Primaquine for falciparum malaria: where are we, what do we know, what do we need to know?” (AD presented, IC was session chair). The findings were also presented at American Society of Tropical Medicine and Hygiene 2016 Annual meeting, Atlanta, GA. Poster No.1536: “Safety of single dose primaquine in G6PD-deficient adult males in Mali without malaria: an open-label phase 2 dose-adjustment trial.” (IC presented).

Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

Figure Legends:

Figure 1. Trial profile. Primaquine was given as a single dose on the day of enrolment. All enrolled individuals completed the study; none were lost to follow-up. G6PD = glucose-6-phosphate dehydrogenase, PQ = primaquine.

Figure 2. Part 1 hemoglobin concentration (g/L) over 28 days of follow-up by primaquine dose group: within-person percent change from baseline (2a), within-person change from baseline hemoglobin concentration (2b), and absolute hemoglobin concentration (2c), Boxplot key: median (line) IQR (box), and range (whisker)

Figure 3. Part 2 hemoglobin concentration (g/L) over 28 days of follow-up by enrollment group (G6PD status and age) : within-person percent change from baseline (3a), within-person change from baseline hemoglobin concentration (3b), and absolute hemoglobin concentration (3c), Boxplot key: median (line) IQR (box), and range (whisker)

References

1. Gates B, Chambers R. From Aspiration to Action. What will it take to end malaria. Available at: http://endmalaria2040.org/assets/Aspiration-to-Action.pdf. Accessed April 25 2016.

2. Ruwende C, Khoo SC, Snow RW, et al. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. Nature **1995**; 376:246-9.

3. Howes RE, Piel FB, Patil AP, et al. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. PLoS Med **2012**; 9:e1001339.

4. WHO. Updated WHO policy recommendation: Single dose primaquine as a gametocytocide in Plasmodium falciparum malaria. Available at: http://www.who.int/malaria/publications/atoz/who_pq_policy_recommendation/en/.

5. WHO. Evidence Review Group on the Safety and Efficacy of Gametocytocidal Doses of Primaquine for Plasmodium falciparum malaria. **2012**.

6. White NJ, Qiao LG, Qi G, Luzzatto L. Rationale for recommending a lower dose of primaquine as a Plasmodium falciparum gametocytocide in populations where G6PD deficiency is common. Malar J **2012**; 11:418.

7. Chen I, Poirot E, Newman M, et al. An assessment of the supply, programmatic use, and regulatory issues of single low-dose primaquine as a Plasmodium falciparum gametocytocide for sub-Saharan Africa. Malar J **2015**; 14:204.

8. Eziefula AC, Pett H, Grignard L, et al. Glucose-6-phosphate dehydrogenase status and risk of hemolysis in Plasmodium falciparum-infected African children receiving single-dose primaquine. Antimicrob Agents Chemother **2014**; 58:4971-3.

9. Douglas N, Lampah D, Kenangalem E, et al. Major burden of severe anemia from non-falciparum malaria species in southern papua: a hospital-based surveillance study. PLoS Medicine **2013**; 10:e1001575.

10. LaRue N, Kahn M, Murray M, et al. Comparison of quantitative and qualitative tests for glucose-6-phosphate dehydrogenase deficiency. Am J Trop Med Hyg **2014**; 91:854-61.

11. Global Health Group UoC, San Francisco, and Malaria Centre at the London School of Hygiene and Tropical Medicine. Single Low-dose Primaquine to Interrupt P. falciparum Transmission in Africa: A Roadmap Update. Meeting Summary. Available at: http://globalhealthsciences.ucsf.edu/sites/default/files/content/ghg/mei-london-pq-summary.pdf. Accessed 22 January 2015.

12. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. The New England journal of medicine **2004**; 350:552-9.

13. Adu-Gyasi D, Asante KP, Newton S, et al. Evaluation of the diagnostic accuracy of CareStart G6PD deficiency Rapid Diagnostic Test (RDT) in a malaria endemic area in Ghana, Africa. PLoS One **2015**; 10:e0125796.

14. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet **2008**; 371:64-74.

15. Maiga B, Dolo A, Campino S, et al. Glucose-6-phosphate dehydrogenase polymorphisms and susceptibility to mild malaria in Dogon and Fulani, Mali. Malar J **2014**; 13:270.

16. Hayes DJ, Banda CG, Chipasula-Teleka A, Terlouw DJ. Modelling the therapeutic dose range of single low dose primaquine to reduce malaria transmission through age-based dosing. BMC Infect Dis **2017**; 17:254.

17. Dicko A, Brown JM, Diawara H, et al. Primaquine to reduce transmission of Plasmodium falciparum malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. Lancet Infect Dis **2016**; 16:674-84.

18. Mwaiswelo R, Ngasala B, Jovel I, et al. Adding a single low-dose of primaquine (0.25 mg/kg) to artemether-lumefantrine did not compromise treatment outcome of uncomplicated Plasmodium falciparum malaria in Tanzania: a randomized, single-blinded clinical trial. Malar J **2016**; 15:435.

19. Okebe J, Bousema T, Affara M, et al. The Gametocytocidal Efficacy of Different Single Doses of Primaquine with Dihydroartemisinin-piperaquine in Asymptomatic Parasite Carriers in The Gambia: A Randomized Controlled Trial. EBioMedicine **2016**; 13:348-55.

20. Lin JT, Lon C, Spring MD, et al. Single dose primaquine to reduce gametocyte carriage and Plasmodium falciparum transmission in Cambodia: An open-label randomized trial. PLoS One **2017**; 12:e0168702.

21. Tine RC, Sylla K, Faye BT, et al. Safety and Efficacy of Adding a Single Low Dose of Primaquine to the Treatment of Adult Patients With Plasmodium falciparum Malaria in Senegal, to Reduce Gametocyte Carriage: A Randomized Controlled Trial. Clin Infect Dis **2017**:ASAP.

22. Mwaiswelo R, Ngasala BE, Jovel I, et al. Safety of a single low-dose of primaquine in addition to standard artemether-lumefantrine regimen for treatment of acute uncomplicated Plasmodium falciparum malaria in Tanzania. Malar J **2016**; 15:316.

23. Bancone G, Chowwiwat N, Somsakchaicharoen R, et al. Single Low Dose Primaquine (0.25mg/kg) Does Not Cause Clinically Significant Haemolysis in G6PD Deficient Subjects. PloS one **2016**; 11:e0151898.

Table 1. **Baseline characteristics of participants overall and by primaquine treatment group**

Part 1: dose adjustment in adult males					
		Primaquine treatment group in G6PD-d males			Control group G6PD-n
Baseline characteristics	Overall	0.40 mg/kg	0.45 mg/kg	0.50 mg/kg	0.50 mg/kg
Number of participants	25	4	7	7	7
Age (years), mean [range]	35 [18,50]	29 [18, 50]	35 [26, 50]	37 [25, 47]	37 [28, 43]
Weight (kg), mean [range]	63.0 [44.2, 83.0]	55.8 [44.2, 70.1]	60.5 [52, 74.4]	62.9 [56.8, 68.5]	69.9 [59.9, 83.0]
Hemoglobin results at baseline (g/L)	149 [120, 175]	138 [120, 151]	152 [144, 175]	151 [136, 162]	150 [136, 165]
Part 2: age de-escalation in boys given 0.40 mg/kg primaquine					
Baseline characteristics	Overall	Age group			
		5-10 years of age		11-17 years of age	
		G6PD-d	G6PD-n	G6PD-d	G6PD-n
Number of participants	26	5	7	7	7
Age (years), mean [range]	10 [5, 17]	7 [5, 8]	7 [5, 9]	13 [11, 16]	13 [11, 17]
Weight (kg), mean [range]	28 [16, 48]	20 [17, 24]	23 [18, 29]	35 [29, 44]	35 [25, 48]
Hemoglobin results at baseline (g/L)	123 [102, 145]	117 [108, 142]	119 [102, 145]	127 [121, 130]	130 [116, 142]

Table 2. **Mean within-person percent change in hemoglobin (Hb) concentration by treatment group: the largest drop between Days 0 and 10 (a), the change between Day 0 and 7 (b), and the change between Day 0 and 28 following treatment with primaquine. And (d) the mean day of study follow-up when the largest Hb drop occurred.**

	A. Largest Hb drop between Day 0 and Day 10		B. Hb drop from Day 0 to Day 7		C. Hb drop from Day 0 to Day 28		D. Mean day of study follow-up when largest Hb drop occurred
Primaquine treatment groups	% change*, 95% CI	P**	% change*, 95% CI	P**	% change*, 95% CI	P**	Study day, 95% CI
Part 1: dose adjustment in adult males							
Control group (G6PD normal, 0.50 mg/kg; n=7)	-7.89 (-11.4, -4.37)	--	-2.13 (-8.10, 3.84)	--	-2.49 (-6.88, 1.91)	--	7.9 (5.0, 10.8)
0.40 mg/kg group (G6PD-deficient, n=4)	-16.8 (-24.7, --8.93)	<0.001	-7.53 (-19.2, 4.11)	0.009	-1.00 (-19.4, 17.4)	0.215	7.0 (3.6, 10.4)
0.45 mg/kg group (G6PD-deficient, n=7)	-7.63 (-12.5, -2.75)	0.662	-4.62 (-10.9, 1.68)	0.587	-2.33 (-8.99, 4.32)	0.638	9.4 (1.6, 17.2)
0.50 mg/kg group (G6PD-deficient, n=7)	-9.72 (-13.5, -5.90)	0.320	-3.77 (-9.77, 2.23)	0.606	-0.754 (-6.67, 5.16)	0.481	7.7 (3.9, 11.5)
Part 2: age de-escalation in boys given 0.40 mg/kg primaquine							
0.40 mg/kg G6PD-normal, 11-17 years old (n=7)	-6.86 (-11.3, -2.46)	--	0.314 (-3.69, 4.31)	--	-0.662 (-5.23, 3.90)	--	9.0 (0.9, 17.1)
0.40 mg/kg G6PD-deficient, 11-17 years old (n=7)	-11.5 (-16.1, -6.96)		-6.89 (-10.5, -3.30)	0.006	-6.59 (-13.5, 0.300)	0.111	13.4 (4.0, 22.8)
0.40 mg/kg G6PD-normal, 5-10 years old (n=7)	-5.24 (-10.2, -0.252)	--	4.46 (-3.01, 11.9)	--	1.89 (-6.62, 10.4)	--	7.6 (-1.8, 16.9)
0.40 mg/kg G6PD-deficient, 5-10 years old (n=5)	-9.61 (-16.1, -3.08)	0.199	0.017 (-13.2, 13.3)	0.378	-1.02 (-11.9, 9.89)	0.407	11.4 (4.1, 18.7)

*Negative values indicate drops in hemoglobin.

**P-values adjusted for baseline value of Hemoglobin. Within age groups, we used a regression model adjusting for baseline hemoglobin to compare G6PD-d and G6PD-n participants.

Table 3. Adverse Events

Part 1: dose adjustment in adult males				
	Primaquine treatment group in G6PD-d men			Control group
	0.40 mg/kg (n=4)	0.45 mg/kg (n=7)	0.50 mg/kg (n=7)	0.50 mg/kg G6PD-n (n=7)
Participants with an adverse event, n (%)	4 (100%)	4 (57%)	2 (29%)	2 (29%)
Number of adverse events	4	8	2	2
Number of drug related	0	0	0	0
Cough, Mild	0	3	0	0
Fever, Mild	1	0	0	0
Headache, Mild	1	1	0	1
Headache, Moderate	0	0	1	0
Insect bites, Mild	0	1	0	0
Rhinobronchitis, Mild	0	1	0	1
Skin boil, Mild	0	0	1	0
Symptomatic malaria, Mild	2	2	0	0
Part 2: age de-escalation in boys given 0.40 mg/kg primaquine				
	Age group			
	5-10 years of age		11-17 years of age	
	G6PD-d	G6PD-n	G6PD-d	G6PD-n
Participants with an adverse event, n (%)	2 (40%)	6 (86%)	3 (43%)	1 (14%)
Number of adverse events	8	18	4	1
Number of drug related	1	2	1	0
Abdominal pain, Mild	2	6	0	0
Adenopathy, Mild	0	1	0	0
Back pain, Mild	1	0	0	0
Conjunctivitis, Mild	0	0	0	1
Dental pain, Mild Mild	0	1	0	0
Diarrhea, Mild	0	0	1	0
Ear infection, Mild	0	1	0	0

Fever, Mild	0	1	1	0
Headache, Mild	0	1	1	0
Injury in big toe, Mild	0	2	0	0
Irregular heartbeat, Mild	0	1	0	0
Loss of appetite, Mild	1	0	0	0
Nasopharyngitis, Mild	0	3	0	0
Runny nose, Mild	1	0	0	0
Skin boil, Moderate	0	0	1	0
Sore throat, Mild	0	1	0	0
Vomiting, Mild	3	0	0	0

There were no severe or serious adverse events. There were no adverse events that caused stopping rules. All events were self-reported. Mild events were defined as those causing no or minimal interference with usual social and functional activities. Moderate events as those causing greater than minimal interference. Severe events as those causing inability to perform usual social and functional activities.

Figure 1.

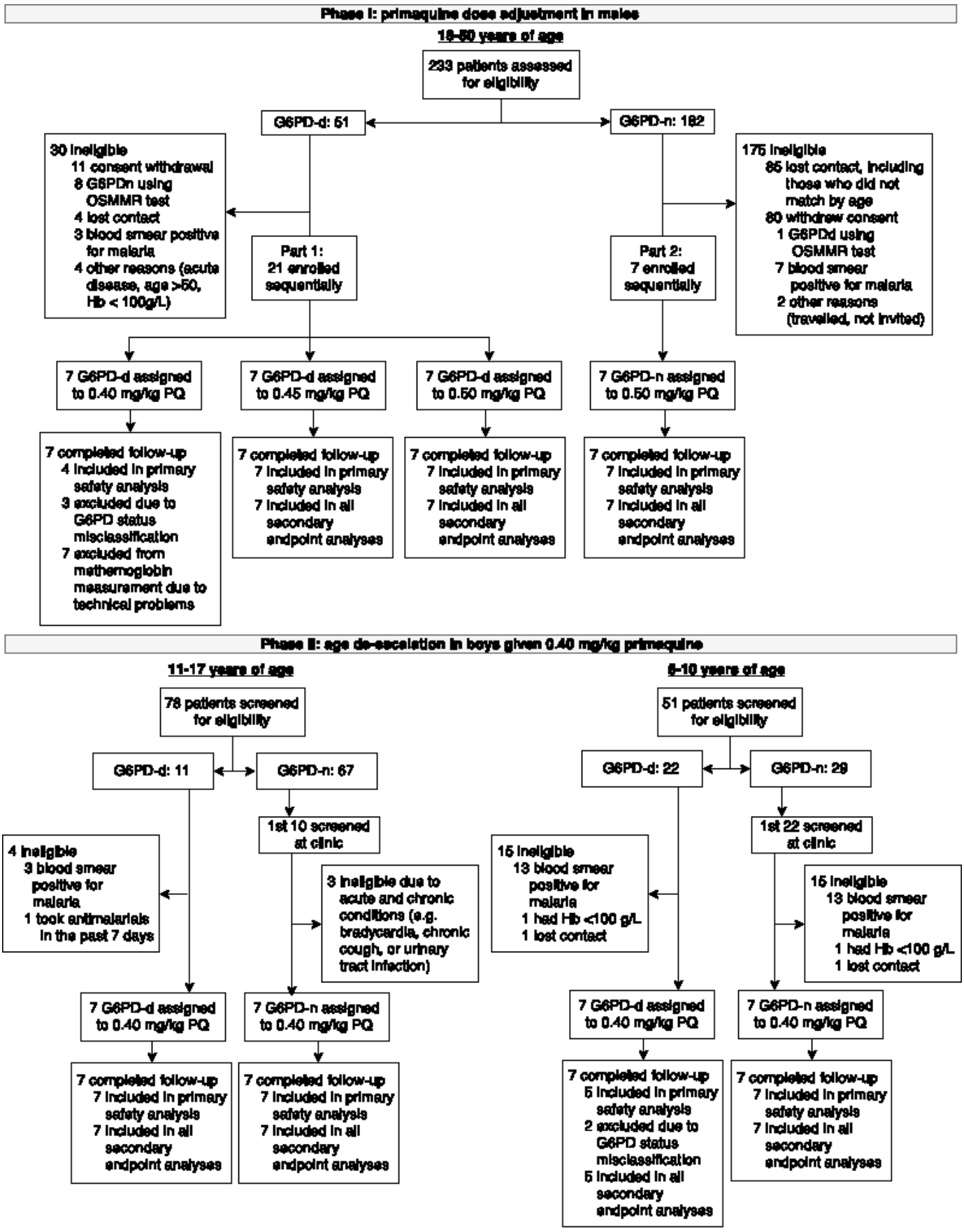

Figure 2.

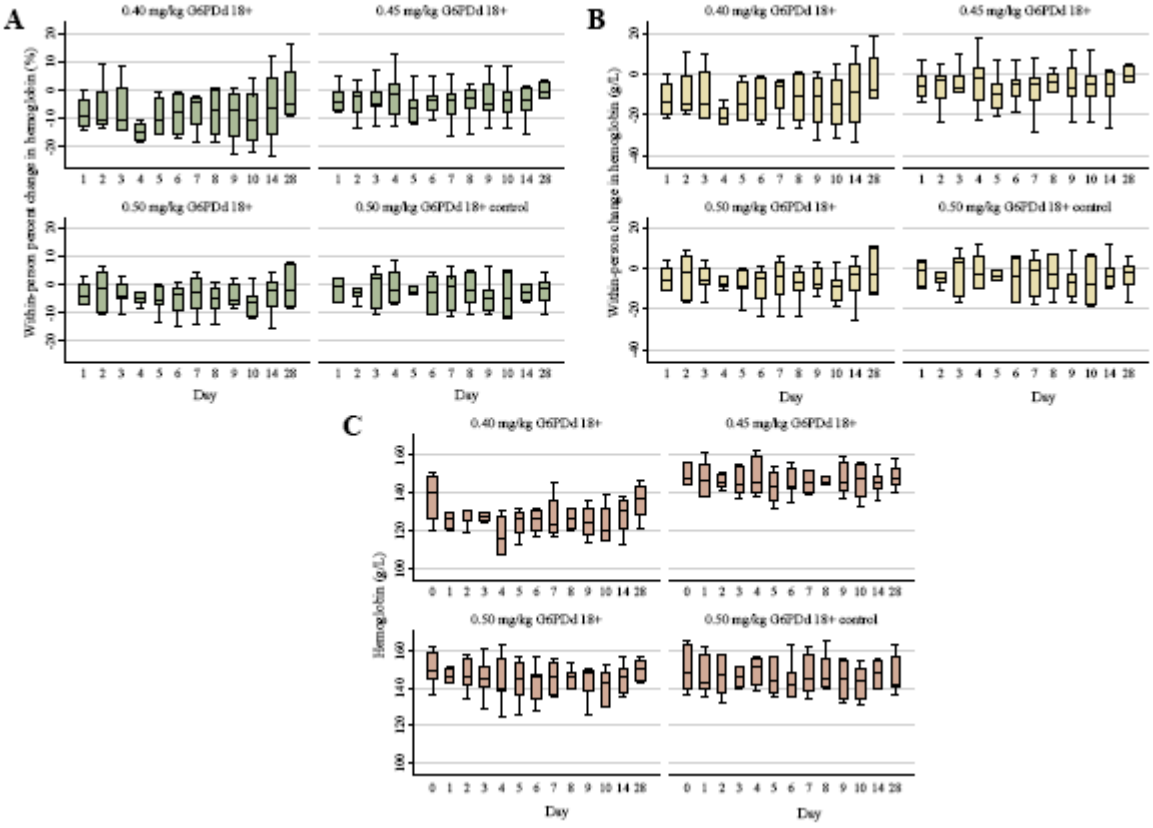

Figure 3.