Population Pharmacokinetic Assessment of the Effect of Food on Piperaquine Bioavailability in Patients with Uncomplicated Malaria

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Previously published literature reports various impacts of food on the oral bioavailability of piperaquine. The aim of this study was to use a population modeling approach to investigate the impact of concomitant intake of a small amount of food on piperaquine pharmacokinetics. This was an open, randomized comparison of piperaquine pharmacokinetics when administered as a fixed oral formulation once daily for 3 days with \((n = 15)\) and without \((n = 15)\) concomitant food to patients with uncomplicated \textit{Plasmodium falciparum} malaria in Thailand. Nonlinear mixed-effects modeling was used to characterize the pharmacokinetics of piperaquine and the influence of concomitant food intake. A modified Monte Carlo mapped power approach was applied to evaluate the relationship between statistical power and various degrees of covariate effect sizes of the given study design. Piperaquine population pharmacokinetics were described well in fasting and fed patients by a three-compartment distribution model with flexible absorption. The final model showed a 25% increase in relative bioavailability per dose occasion during recovery from malaria but demonstrated no clinical impact of concomitant intake of a low-fat meal. Body weight and age were both significant covariates in the final model. The novel power approach concluded that the study was adequately powered to detect a food effect of at least 35%. This modified Monte Carlo mapped power approach may be a useful tool for evaluating the power to detect true covariate effects in mixed-effects modeling and a given study design. A small amount of food does not affect piperaquine absorption significantly in acute malaria.

Malaria is the most important parasitic disease in humans and kills approximately 660,000 people each year (1). Artemisinin-based combination therapy (ACT) is the recommended first-line treatment worldwide. The artemisinin component is a very potent but short-acting drug and kills the majority of parasites, while the longer-acting partner drug is slowly eliminated and kills residual parasites to prevent recrudescent malaria.

The fixed oral combination of dihydroartemisinin and piperaquine is a promising ACT, which has shown excellent cure rates of \(98.7\% (95\%\) confidence interval \(97.6\) to \(99.8\%)\) in a pooled analysis of 3,547 patients with malaria from 12 different sites (2). Dihydroartemisinin-piperaquine is currently recommended as a once-daily dose for 3 days based on patient body weight (daily target dose of 18 mg of piperaquine phosphate/kg of body weight) (3). However, concerns have been raised that small children are underdosed due to the nonlinear relationship between elimination clearance and body weight (4).

Dihydroartemisinin has a very short terminal elimination half-life of approximately 1 h, while piperaquine has a long half-life of approximately 20 to 30 days (4–7). The absolute oral bioavailability of piperaquine has not been reported in humans, since parenteral formulations are unavailable. However, a 50% oral bioavailability compared to an experimental parenteral formulation was reported in rats (8). Piperaquine is a highly lipophilic drug, and its absorption might be facilitated by concomitant intake of fat, as described previously for other lipid-soluble antimalarial drugs such as lumefantrine (9), halofantrine (10), mefloquine (11), and atovaquone (12).

Contradictory results have been reported for the effects of concomitant food intake on piperaquine pharmacokinetics in healthy volunteers and patients with malaria. Sim and colleagues reported a 98% increase in total exposure of piperaquine after a high-fat breakfast (53 g fat) in healthy Caucasian volunteers (\(n = 8\); crossover design) (13). Nguyen et al. reported a more modest 41% increase in total piperaquine exposure after a standardized Vietnamese breakfast (17 g fat) in healthy Vietnamese volunteers (\(n = 14\); parallel design) (14). However, Hai and colleagues reported no significant impact on piperaquine pharmacokinetics after a similar standardized Vietnamese breakfast (17 g fat) in healthy Vietnamese volunteers (\(n = 32\); parallel design) (15). The noncompartmental analysis of data presented here showed no effect of concomitant intake of a small amount of fat (6.4 g fat) on piperaquine exposure in patients with uncomplicated \textit{Plasmodium falciparum} malaria in Thailand (\(n = 30\); parallel design) (16). However, all reported studies used a noncompartmental approach with a low statistical power for detecting concomitant food intake as an influential factor on piperaquine pharmacokinetics, compared to a modeling approach.

The aim of this study was to use a potentially more powerful population approach to analyze data from a previously reported study (16) to evaluate the effects of concomitant food intake on...
the pharmacokinetic properties of piperaquine in patients with uncomplicated *P. falciparum* malaria in Thailand.

**MATERIALS AND METHODS**

**Study design.** This was an open, randomized, parallel study of piperaquine pharmacokinetics when administered as a fixed oral formulation once daily for 3 days with and without concomitant fat to patients with uncomplicated *P. falciparum* malaria in Thailand. Clinical and noncompartmental results were reported in full previously (16). Briefly, 30 patients aged 16 to 65 years were enrolled and randomized into one of the two treatment arms. Inclusion criteria were microscopy confirmation of asexual *P. falciparum* or mixed infections, no signs of severe malaria, and willingness to participate in the study (fully informed consent). Exclusion criteria were >4% red blood cell parasitemia, positive urine test for pregnancy, known hypersensitivity to artemisinins or piperaquine, treatment of malaria in the past 4 months, or hematocrit level <30%. Full demographics are given in Table 1. Ethical approval was granted by the Faculty of Tropical Medicine Mahidol University Ethics Committee, Bangkok, Thailand, and the Oxford Tropical Research Ethics Committee (OxTREC), United Kingdom.

**Drug administration.** All patients were given a supervised standard fixed oral formulation of dihydroartemisinin and piperaquine (40 mg dihydroartemisinin and 320 mg piperaquine phosphate per tablet) (Duo-Cotexin; Beijing Holley-Cotec Pharmaceuticals Co., Ltd., China). A weight-based dose regimen, once-daily treatment for 3 days, was employed to achieve a daily target dose of 18 mg of piperaquine phosphate/kg of body weight (Table 1). All patients in the fed group (*n* = 15) received a 200-ml carton of chocolate milk, containing 6.4 g of fat, with each dose. Patients in the fasting group (*n* = 15) were given study medication at enrollment, after at least 2 h of fasting, and consecutive doses after an overnight fast. Patients were asked to continue fasting for 3 h after each dose.

**Pharmacokinetic sampling.** Blood samples during the intensive collection phase (up to 12 h after the last dose) were collected through an indwelling intravenous cannula flushed with 0.5 ml heparinized saline solution after each sample collection. A total of 0.5 ml of blood was discarded immediately before sampling, and blood samples (2 to 5 ml) were collected into lithium heparin tubes predose (0 h); at 0.5, 1, 2, 3, 4, 7, and 24 h after the first dose; at 1, 3, 4, 5, 7, and 24 h after the second dose; and at 1, 2, 3, 4, 5, 6, 8, and 12 h after the third dose. Additional samples were collected by venipuncture on days 4, 5, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and 126 after the first dose. All blood samples were centrifuged at 2,000 × g for 10 min, and plasma was stored in liquid nitrogen within 30 min of collection. Plasma samples were transported on dry ice to the Department of Clinical Pharmacology at the Mahidol-Oxford Tropical Medicine Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Piperaquine plasma samples were quantified by using a previously reported high-throughput assay consisting of liquid chromatography linked with tandem mass spectrometry detection (17). Triplicates of quality control samples at three different concentrations (i.e., 4.5, 20, and 400 ng/ml) were analyzed within each batch of samples to ensure precision and accuracy during drug measurements. The coefficient of variation was below 5% for all quality control samples. The lower limit of quantification was 1.2 ng/ml.

**Population pharmacokinetics.** Piperaquine plasma concentrations were transformed into their natural logarithms and evaluated by using nonlinear mixed-effects modeling in NONMEM v.7.2 (Icon Development Solutions, MD). Automation and visualization of diagnostic results were performed by using Pearl-Speaks-NONMEM (PAN) v.3.6.2 (18, 19), Xpose v.4 (20), R v.2.13.1 (R Foundation for Statistical Computing), and Pirana v.2.6.2 (21).

Subroutine ADVAN5 and the first-order conditional estimation method with interactions were used throughout modeling. The objective function value (OFV) computed by NONMEM as minus twice the log likelihood of data, goodness-of-fit diagnostics, and simulation-based diagnostics were used to discriminate between models. A total of 91 out of 1,016 samples (9.1%) were quantified below the limit of quantification and omitted during model building. The majority of these omitted samples (82.6%) were in the late terminal phase (>70 days) during the long follow-up period. The impact of omitting these samples was evaluated by using the final model.

One-, two-, three-, and four-compartment distribution models were first evaluated during model building. The best-performing distribution model was assessed together with different absorption models (i.e., first-order absorption with and without lag time and a more flexible transit compartment absorption with a fixed number of transit compartments for the population (22)). Implementation of a fixed relative bioavailability of 100% for the population but allowing for between-subject variability and between-dose occasion variability (i.e., modeled as within-subject variability between doses) in the same parameter was also evaluated. Pharmacokinetic parameters were modeled assuming log-normal distributions, while between-subject variability and between-dose occasion variability were assumed to be normally distributed with a zero mean and Ω² variance. The random residual variability was assumed to be additive, since data were modeled as natural logarithms (i.e., essentially equivalent to an exponential error model for untransformed data).

Body weight was investigated as a covariate by using an allometric function with power values of 3/4 for clearance parameters and 1 for volume parameters. Systematic variability in the relative bioavailability between dose occasions was evaluated as a categorical and a linear covariate for doses 1, 2, and 3. Effects of age, initial parasitemia, hematocrit, temperature upon admission, sex, and concomitant food intake on all parameters were evaluated formally with a stepwise forward-addition (*P* < 0.05) and backward-deletion (*P* < 0.01) covariate approach by using the automated Stepwise Covariate Model (SCM) implemented in PsN. Continuous covariates were tried as linear, piecewise linear, power, and exponential functions, and categorical covariates were tried as linear functions. The final structural model, including body weight as an allometric function on clearance and volume parameters as well as between-subject variability on all parameters and between-dose occasion variability on relative bioavailability and mean absorption time, was also used to investigate the impact of concomitant fat intake on absorption parameters with a full covariate approach (23). Concomitant fat intake was simultaneously

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for group</th>
<th>Fasting patients</th>
<th>Fed patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. of patients</td>
<td></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Total no. of piperaquine</td>
<td></td>
<td>535</td>
<td>541</td>
</tr>
<tr>
<td>Median daily dose of piperaquine phosphate (mg/kg) (range)</td>
<td>17.2 (16.0–18.6)</td>
<td>17.5 (16.0–18.8)</td>
<td></td>
</tr>
<tr>
<td>Median daily dose of dihydroartemisinin (mg/kg) (range)</td>
<td>2.14 (2.00–2.33)</td>
<td>2.19 (2.00–2.35)</td>
<td></td>
</tr>
</tbody>
</table>

**Demographics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for group</th>
<th>Median age (yr) (range)</th>
<th>Median body wt (kg) (range)</th>
<th>No. of males/no. of females</th>
<th>Median auxiliary temp at admission (°C) (range)</th>
<th>Median parasitemia at admission (no. of parasites/μl) (range)</th>
<th>Median diastolic blood pressure (mmHg) (range)</th>
<th>Median systolic blood pressure (mmHg) (range)</th>
<th>Median hematocrit (%) (range)</th>
<th>Median pulse (beats/min) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>38 (18–55)</td>
<td>50 (39–62)</td>
<td>13/2</td>
<td>36.5 (36.2–38.2)</td>
<td>8,000 (448–140,000)</td>
<td>70 (60–80)</td>
<td>110 (90–130)</td>
<td>41 (30–45)</td>
<td>80 (65–96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28 (19–45)</td>
<td>53 (45–73)</td>
<td>13/2</td>
<td>37.1 (35.9–39.2)</td>
<td>6,000 (352–60,000)</td>
<td>70 (60–110)</td>
<td>110 (90–140)</td>
<td>42 (33–47)</td>
<td>84 (72–120)</td>
</tr>
</tbody>
</table>

**TABLE 1 Demographics of patients with uncomplicated *P. falciparum* malaria in Thailand**

Food Effects on Piperaquine Pharmacokinetics

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implemented as a categorical food effect on the relative bioavailability and absorption parameters, followed by bootstrap diagnostics \( (n = 500) \) to evaluate the clinical relevance of such potential covariate effects.

Basic goodness-of-fit diagnostics, visual and numerical predictive checks, as well as bootstrap diagnostics were used to evaluate the appropriateness of the final model. Visual predictive checks were visualized by plotting the 95% confidence intervals of the 5th, 50th, and 95th simulated \( (n = 2,000) \) percentiles against measured piperazine concentrations. The nonparametric bootstrap diagnostics \( (n = 1,000) \) were stratified by drug administration with or without food to maintain an equal distribution of patients in the resampled data.

**Evaluation of population versus noncompartmental approaches.** A traditional power calculation was performed by using the mean results \( (\pm \) standard deviations \( (SD) \) ) of total piperazine exposure from the noncompartmental analysis reported previously \( [16] \). The mean total piperazine exposure in fasting patients was used to calculate the mean exposure in fed patients after a putative food effect, and the SD was based on that observed for fasting patients but was assumed to be proportional to exposure. Standard functionalities in STATA v.12.0 \( (\text{Stata Corp.}, \text{TX, USA}) \) were used to calculate the minimum effect size of food needed for a statistically significant difference between a fasting group \( (n = 15) \) and a fed group \( (n = 15) \) \( (\text{power} = 0.8; \alpha = 0.05) \).

An embedded functionality in \( \text{PaN} \) for rapid sample size calculations of mixed-effects models \( [24] \) was modified to calculate the minimum effect size of food on relative bioavailability needed for a statistically significant covariate relationship \( (\text{modified Monte Carlo mapped power approach}) \). The original study design of 15 fed and 15 fasting patients was extended to include 11,000 simulated patients \( (50\% \text{ in the fed group and 50}\% \text{ in the fasting group}) \). Individual concentration-time data were simulated for these patients with the final model with the addition of a fixed categorical effect size of 5% to 50% of food on relative bioavailability \( (i.e., 1,000 \text{ patients for each effect size with an increment of 5}\% \text{ between simulations}) \). The simulated data were then separately reestimated for each effect size with the final model with and without a food effect on relative bioavailability. The difference in individual objective function values \( (\Delta\text{OFV}) \) was calculated for each patient for the two models. Fifteen \( \Delta\text{OFVs} \) were resampled at random \((\text{bootstrap}; \ n = 10,000)\) from each group \( (i.e., \text{fed and fasting}) \) and effect size \((i.e., 5\% \text{ to 50}\%\) ), and fractions of the 10,000 bootstrap samples above the critical total \( \text{OFV} (\Sigma\Delta\text{OFV}) \) of 3.84 and 6.63 \((i.e., \text{power at alpha values of 0.05 and 0.01, respectively})\) were plotted against the evaluated covariate effect sizes.

**RESULTS**

The study medication was well tolerated, with no severe adverse reactions reported during the study period. There were no statistical differences between the two groups in terms of demographic variables \( (\text{Table 1}) \). A total of 8 patients had recurrent malaria during the 126 days of follow-up, but none of these patients were classified as having recrudescent malaria by PCR genotyping. The median time to a new infection was 42 days \((\text{range, 32 to 87 days})\) in the fasting group \( (n = 5) \) and 70 days \((\text{range, 58 to 125 days})\) in the fed group \( (n = 3) \).

**Population pharmacokinetics.** Piperaquine population pharmacokinetics were well described by a 3-compartment distribution model, with no significant improvement by adding an additional peripheral distribution compartment \( (\Delta\text{OFV} = -5.08) \). A transit compartment \( (n = 3) \) absorption model described the absorption phase well and was superior to all other absorption models \( (\Delta\text{OFV} < -5.19) \). The absorption rate from the last transit compartment could be set as identical to the rate constant between transit compartments without a significant impact on the model \( (\Delta\text{OFV} = 0.41) \). This also provided a more stable absorption model. The final structural model is shown in \( \text{Fig. 1} \).

Implementation of a fixed relative bioavailability of 100% for the population but allowing for between-subject variability and between-dose occasion variability in the same parameter improved the model fit significantly \( (\Delta\text{OFVs of} -4.61 \text{ and -160}, \text{respectively, when implemented sequentially}) \). However, the between-subject variability could be removed after implementation of between-dose occasion variability without a major impact on the model fit \( (\Delta\text{OFV} = 1.94) \). Between-dose occasion variability in the mean absorption time also improved the model significantly \( (\Delta\text{OFV} = -27.6) \).

A fixed allometric function for body weight improved the model fit marginally \( (\Delta\text{OFV} = -3.68) \) but was kept in the final model based on prior strong physiological evidence for such a covariate relationship. Dose-occasion as a categorical covariate effect on relative bioavailability improved the model significantly \( (\Delta\text{OFV} = -11.0) \) and resulted in 21.9% and 50.9% increased bioavailability at dose 2 and dose 3, respectively, compared to dose 1. However, this could be simplified to a linear covariate relationship \( (i.e., 25.3\% \text{ increase in bioavailability per dose}) \) without a significant reduction in model fit \( (\Delta\text{OFV} = -0.062) \). Effects of age and sex on peripheral distribution volume and concomitant food intake on mean absorption time were significant covariates in the forward-addition step \( (P < 0.05) \), but only the effect of age on the peripheral volume of distribution could be retained in the backward-elimination step with a more parsimonious cutoff \( (P < 0.01) \). This covariate relationship resulted in a linear increase in the peripheral volume of distribution of 4.1% for each year of age increase. A separate bootstrap diagnostic from the implementation of a food effect on mean absorption time and relative bioavailability showed a significantly higher absorption rate during concomitant food intake but no effect on total bioavailability \( (\text{Fig. 2}) \).

Basic goodness-of-fit diagnostics resulted in adequate model performance, with no obvious model misspecification \( (\text{Fig. 3}) \). However, a minor trend of data censoring at the lower limit of quantification could be seen, but this resulted in no model misspecification in terms of simulated and observed fractions of censored data \( (\text{data not shown}) \). Furthermore, omitting data below the limit of quantification resulted in almost identical parameter estimates as when these were handled as categorical data \( (i.e., \text{the M3 method resulted in a} < 5.5\% \text{ absolute mean bias compared to when the data were omitted}) \) \( [25] \). The predictive checks demon-
Evaluation of population versus noncompartmental approaches. A noncompartmental analysis and a groupwise statistical comparison of total piperaquine exposure between the fed and fasting groups resulted in 80% power (alpha = 0.05) to detect a minimum difference of 81%. However, the novel power methodology for mixed-effects models resulted in 80% power (alpha = 0.05) for a covariate effect of 35%, thus proving the study design to be adequately powered for a population approach if a putative clinically relevant difference of >35% would be present between fed and fasting patients. The statistical power to detect various degrees of covariate effects is illustrated in Fig. 5.

DISCUSSION

The fixed oral combination of dihydroartemisinin and piperaquine has demonstrated excellent cure rates in adult patients with *P. falciparum* malaria in Thailand (2). In this small study, there was no recrudescent malaria when the combination was administered alone or together with a standardized meal. The relatively high rate of new infections is most likely a consequence of the long follow-up period of 125 days.

Population pharmacokinetics. The population pharmacokinetics of piperaquine were well characterized by the study design and the developed nonlinear mixed-effects model. Piperaquine showed a multiphasic distribution, as described previously (4, 7, 26). A more flexible absorption model improved the model sig-
eraquine concentrations were transformed into their logarithms (base 10). Venous horizontal lines are the lower limit of quantification. **FIG 4** Visual predictive check of the final model describing the population pharmacokinetics of pi 

nificantly and described the variable absorption of pi 

TABLE 2 Population estimates of the final model describing pi 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>95% CI for population estimate</th>
<th>IIV (% CV)</th>
<th>95% CI for IIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CL/F}$ (liters/h)</td>
<td>67.6 (11.6)</td>
<td>54.0–85.5</td>
<td>24.4 (26.0)</td>
<td>17.4–29.6</td>
</tr>
<tr>
<td>$\text{V}_c$ (liters)</td>
<td>3,030 (16.4)</td>
<td>2,160–4,180</td>
<td>51.6 (32.3)</td>
<td>31.2–68.1</td>
</tr>
<tr>
<td>$\text{Q}_1$ (liters/h)</td>
<td>408 (15.0)</td>
<td>309–557</td>
<td>25.8 (48.2)</td>
<td>6.67–37.9</td>
</tr>
<tr>
<td>$\text{V}_p$ (liters)</td>
<td>6,240 (14.6)</td>
<td>4,890–8,530</td>
<td>45.6 (48.8)</td>
<td>18.8–68.4</td>
</tr>
<tr>
<td>$\text{Q}_2$ (liters/h)</td>
<td>109 (13.6)</td>
<td>83.3–143</td>
<td>25.8 (48.2)</td>
<td>6.67–37.9</td>
</tr>
<tr>
<td>$\text{V}_e$ (liters)</td>
<td>24,400 (10.1)</td>
<td>20,000–29,500</td>
<td>24.1 (52.7)/39.4 (22.7)</td>
<td>8.77–35.6/29.2–48.0</td>
</tr>
<tr>
<td>$\text{MTT}$ (h)</td>
<td>2.04 (7.50)</td>
<td>1.80–2.41</td>
<td>24.1 (52.7)/39.4 (22.7)</td>
<td>8.77–35.6/29.2–48.0</td>
</tr>
<tr>
<td>No. of transit comp.</td>
<td>3 (fixed)</td>
<td>1.80–2.41</td>
<td>24.1 (52.7)/39.4 (22.7)</td>
<td>8.77–35.6/29.2–48.0</td>
</tr>
<tr>
<td>$\text{F}$ (%)</td>
<td>100 (fixed)</td>
<td>27.8–33.5</td>
<td>27.8–33.5</td>
<td>38.3–56.0</td>
</tr>
<tr>
<td>$\sigma$ (%)</td>
<td>30.7 (4.42)</td>
<td>27.8–33.5</td>
<td>27.8–33.5</td>
<td>38.3–56.0</td>
</tr>
</tbody>
</table>

**a** Computed population mean values from NONMEM. Interindividual variability (IIV), between-occasion variability, and random residual variability are calculated as $100 \times \sqrt{\text{EXP}(\text{RSE})^2}$.

**b** Assessed by the nonparametric bootstrap method ($n = 1,000$ iterations) for the final pharmacokinetic model. Relative standard errors (RSE) are calculated as $100 \times \sqrt{(\text{standard error/m})}$. Ninety-five-percent confidence intervals are displayed as the 2.5 to 97.5 percentiles of bootstrap estimates.

**c** Between-occasion variability.

**d** CL, elimination clearance; $V_c$, central volume of distribution; $Q_1$, intercompartment clearance; $V_p$, peripheral volume of distribution; $MTT$, mean absorption transit time; No. of transit comp., number of transit compartments; $F$, oral bioavailability; $\sigma$, additive residual error; CV, coefficient of variation.

subjects. The estimated relative bioavailability of pi 

This study demonstrated no clinical impact on concomitant intake of a small amount of fat on the total absorption, day 7 levels, peak concentrations, or time to peak concentrations of pi 

A 26% higher mean absorption rate of pi 

...
(28) and/or the increased dissolution of piperaquine when coadministered with fatty liquids. However, dihydroartemisinin is primarily responsible for the initial parasite-killing effect, and this trend of an increased absorption rate of piperaquine is not likely to have any substantial clinical impact with respect to treatment outcome. Indeed, the prophylactic protective effect of dihydroartemisinin-piperaquine in volunteers (n = 800) at risk of malaria infections in Thailand was not significantly different when administered with or without food (29). Furthermore, the trend of an increased absorption rate associated with concomitant intake of a low-fat meal. The novel methodology for assessing the power of detecting a true covariate relationship presented may be used in mixed-effects modeling to provide a numerical approach to conclude a lack of covariate effects.

**ACKNOWLEDGMENTS**

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We declare that we have no conflicts of interest.

**REFERENCES**


