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Exposure to Total and Protein-Unbound Rifampin Is Not Affected by Malnutrition in Indonesian Tuberculosis Patients

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Nutritional status may have a profound impact on the pharmacokinetics of drugs, yet only few data are available for tuberculosis (TB) drugs. As malnutrition occurs frequently among TB patients, we assessed the effect of malnutrition on the steady-state pharmacokinetics of total and protein-unbound rifampin during the intensive phase of TB treatment. In a descriptive pharmacokinetic study in Bandung, Indonesia, patients received a fixed standard rifampin dose of 450 mg once daily during the intensive phase of TB treatment. A full pharmacokinetic curve for rifampin was recorded, and total and unbound concentrations of rifampin were analyzed in all samples. Rifampin pharmacokinetic parameters were compared between severely malnourished (BMI of <16.0 kg/m²), malnourished (BMI of <18.5 kg/m²), and well-nourished (BMI of ≥18.5 kg/m²) individuals. No difference in total and protein-unbound pharmacokinetic parameters between severely malnourished (n = 7), malnourished (n = 11), and well-nourished (n = 25) patients could be demonstrated. In addition, no significant correlation between BMI and exposure (area under the concentration-time curve from 0 to 24 h [AUC₀–₂₄] and maximum concentration of drug in serum [Cₘ₉₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅₅事儿，且营养状态可能显著影响药物的药代动力学特性，而有关数据显示，其中仅有少量数据涉及抗结核药物（anti-TB）。

Inadequate exposure to rifampin and other antituberculosis (anti-TB) drugs may contribute to a suboptimal clinical response in anti-TB treatment. This follows from a recent study performed in a preclinical model, showing that pharmacokinetic variability is an important factor in the emergence of multidrug-resistant TB (1). Furthermore, a meta-analysis of clinical studies showed that pharmacokinetic variability for a single drug (isoniazid) in multidrug TB regimens is associated with therapy failure and acquired drug resistance (2). A number of clinical studies have also reported associations between low concentrations of anti-TB drugs and poor treatment response (3–8), but this association was not found in other studies (9, 10), including one of our studies on plasma rifampin concentrations in Indonesian TB patients (11).

For rifampin and other TB drugs, pharmacokinetic variability and low exposure may be affected by various factors, including gender, comorbidity (HIV/AIDS or diabetes mellitus), genetics, drug formulation, and malnutrition (3, 12–16). Malnutrition occurs frequently among TB patients. A case-control study in Indonesia documented malnutrition in 87% and 33% of cases and controls, respectively (17). A bidirectional interaction exists between malnutrition and TB (18, 19). On the one hand, malnutrition impairs immune function and increases the susceptibility to development of active TB. At the same time, TB leads to severe abnormalities in protein metabolism and loss of lean tissues and fat reserves. It is known that nutritional status can have a profound impact on the pharmacokinetics of drugs (20, 21), yet few data are available for TB drugs, and we are aware of only one publication on the effect of malnutrition on the exposure to rifampin (12).

In pharmacokinetic studies, measurement of rifampin concentrations in plasma or serum usually relates to the total (protein-unbound plus protein-bound) concentration of a drug. An equilibrium between total and protein-unbound concentrations is commonly assumed, yet free rather than total drug concentrations are preferably used in concentration-response evaluations (22), as only protein-unbound drugs are pharmacologically active and diffuse or are being actively transported into tissues and to the sites of action (23, 24). In a previous study among Indonesian TB patients (11), we confined measurements to total concentrations of rifampin, and this may be one of several possible explanations for the absence of a concentration-response relationship in that study. Importantly, malnutrition and associated low concentra-
tions of drug-binding plasma proteins may cause a change in the equilibrium between protein-unbound and bound concentrations, which renders the total drug concentrations misleading (24–26). This means that both total and protein-unbound plasma concentrations should be evaluated when studying the effects of malnutrition on the pharmacokinetics of a drug.

The primary objective of this study was to assess the effect of malnutrition on the steady-state pharmacokinetics of total and protein-unbound rifampin during the intensive phase of TB treatment in Indonesian TB patients. As a secondary objective, we evaluated the interindividual variability in exposure to protein-unbound rifampin, as we feel this may provide relevant information to understand exposure-response relationships for this pivotal TB drug.

MATERIALS AND METHODS

Subjects. Study subjects were Indonesian patients with pulmonary TB in the intensive phase of treatment. Diagnosis of pulmonary TB was based on clinical symptoms and chest X-ray examination, confirmed by microscopic detection of acid-fast bacilli. Patients were excluded if they had a body weight (BW) above 55 kg, were below 18 or above 55 years of age, were pregnant or lactating, used any type of comedication that may influence the pharmacokinetics of TB drugs, or had liver or kidney abnormalities (including abnormal liver or renal function parameters) or any known history or medical condition that might affect the pharmacokinetics of TB drugs, such as diabetes mellitus, HIV infection, diarrhea, or vomiting.

Study design. This was a descriptive pharmacokinetic study conducted in an urban outpatient tuberculosis clinic in Bandung, Indonesia. Patients were prospectively and consecutively recruited from the control arm of an intervention study on nutritional supplementation in TB patients. Subsequently, the cohort was completed with data from patients who participated in a clinical trial on high-dose rifampin and who fulfilled the inclusion and exclusion criteria (27). During the intensive phase of TB treatment, all eligible patients received a fixed standard rifampin dose of 450 mg once daily, roughly corresponding to 10 mg/kg in Indonesian people (for people below 55 kg), combined with once-daily isoniazid (300 mg), pyrazinamide (1,500 mg), and ethambutol (750 mg). All patients received TB drugs from the same manufacturer (PT Kimia Farma, Bandung, Indonesia), formulated in separate tablets. The bioequivalence of the rifampin tablets and an international reference standard has been established, and patients with a BMI of 18.5 kg/m² were considered to have no malnutrition. Differences in pharmacokinetic parameters between malnourished patients as well as the severely malnourished subgroup versus well-nourished patients were assessed with independent-sample t tests on logarithmically transformed pharmacokinetic parameters. Time to maximum concentration of drug in serum (T_{max}) values were not transformed and were compared using the Mann-Whitney U test. Apart from categorizing patients in groups with predefined BMI values, BMI was also evaluated as a continuous variable. AUC_{0–24} and maximum concentration of drug in serum (C_{max}) values for both total and unbound rifampin plasma concentrations were correlated with BMI using Spearman’s rho on the untransformed pharmacokinetic parameters.

To evaluate the confounding effect of other possible determinants of exposure to protein-unbound and total rifampin, similar univariate analyses were performed to assess the effects of gender, weight, age, and plasma albumin concentration on the log-transformed AUC_{0–24} and C_{max} values for the total and unbound concentrations of rifampin. After the univariate analyses, a multiple linear regression analysis was performed to assess the variation in log-transformed AUC_{0–24} and C_{max} attributable to the presence of those variables that emerged from the univariate analyses.

To assess the interindividual variability in pharmacokinetics as a secondary objective, all patients were combined in one group. First, the central tendency and spread in each pharmacokinetic parameter (protein-unbound and total rifampin) were described with a geometric mean, geometric coefficient of variation (GCV) (standard deviation [SD]) of In
The majority of the patients were female (61%). Twenty-five patients had a normal BMI (BMI of ≥18.5 kg/m²), and 11 patients were malnourished (BMI of <18.5 kg/m²), of which seven patients were severely malnourished (BMI of <16.0 kg/m²). As all patients received a fixed dose of 450 mg of rifampin; the dosage of rifampin per kilogram of body weight was somewhat higher among malnourished patients than among those with a normal BMI (11.6 mg/kg BW versus 9.7 mg/kg BW [geometric mean]). Albumin concentrations were within the normal range for severely malnourished, malnourished, and well-nourished patients: 3.3, 3.4, and 3.8 g/dl (geometric mean), respectively. Albumin concentrations in severely malnourished and malnourished patients did not differ significantly from those in well-nourished patients (P = 0.12 and P = 0.19, respectively).

**Effect of malnutrition on the pharmacokinetics of rifampin.**

The geometric mean AUC₀–₂₄ of rifampin did not differ between patients with malnutrition (BMI of <18.5 kg/m²) and patients with a normal BMI, for both the total AUC₀–₂₄ (54.8 versus 54.6 h · mg/liter; P = 0.96) and the unbound plasma AUC₀–₂₄ (5.7 versus 5.6 h · mg/liter; P = 0.95) (Table 2). Total and unbound geometric mean Cₘₐₓ and other rifampin pharmacokinetic parameters, especially the primary parameters clearance and volume of distribution, also were similar in the two nutrition groups (Table 2). Severely malnourished patients (BMI of <16.0 kg/m²) showed no differences in AUC₀–₂₄ compared to patients with a normal BMI either; geometric mean values for total and protein-unbound AUC₀–₂₄ were 56.7 and 6.1 h · mg/liter, respectively, among severely malnourished patients, compared to 54.6 and 5.6 h · mg/liter, respectively, for patients with a normal BMI (P = 0.73 and 0.53, respectively). Evaluation of Cₘₐₓ values yielded similar results; geometric mean values for total and protein-unbound Cₘₐₓ were 10.7 and 1.0 mg/liter, respectively, among severely malnourished patients, compared to 10.9 and 1.1 mg/liter, respectively, in patients with a normal BMI (P = 0.91 and P = 0.65, respectively).

**Results.** Thirty-six patients with pulmonary TB were included in the study. Characteristics of the patients are presented in Table 1.

**Table 2** Steady-state pharmacokinetics of total and unbound rifampin based on full pharmacokinetic curves of the total cohort.

<table>
<thead>
<tr>
<th>Parameter in plasma</th>
<th>Total (n = 36)</th>
<th>BMI &lt; 18.5 (n = 11)</th>
<th>BMI ≥ 18.5 (n = 25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total rifampin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC₀–₂₄ (h · mg/liter)</td>
<td>54.7 (32.6–88.8)</td>
<td>54.8 (32.6–85.0)</td>
<td>54.6 (35.0–88.8)</td>
<td>0.96&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cₘₐₓ (mg/liter)</td>
<td>10.9 (6.4–16.6)</td>
<td>10.9 (6.4–16.6)</td>
<td>10.9 (7.1–16.3)</td>
<td>0.95&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tₘₐₓ (h) (median)</td>
<td>2.0 (1.0–4.0)</td>
<td>2.5 (1.0–4.0)</td>
<td>1.5 (1.0–4.0)</td>
<td>0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL/F (liters/h)</td>
<td>8.2 (5.1–13.8)</td>
<td>8.2 (5.3–13.8)</td>
<td>8.2 (5.1–12.9)</td>
<td>0.96&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>V/F (liters)</td>
<td>24.1 (15.1–57.4)</td>
<td>25.1 (16.6–36.0)</td>
<td>23.7 (15.1–57.4)</td>
<td>0.63&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>2.0 (1.4–3.8)</td>
<td>2.1 (1.4–2.8)</td>
<td>2.1 (1.5–3.8)</td>
<td>0.47&lt;sup&lt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Unbound rifampin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC₀–₂₄ (h · mg/liter)</td>
<td>5.6 (2.9–9.8)</td>
<td>5.7 (2.9–8.7)</td>
<td>5.6 (3.2–9.8)</td>
<td>0.95&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cₘₐₓ (mg/liter)</td>
<td>1.1 (0.7–2.2)</td>
<td>1.0 (0.7–2.0)</td>
<td>1.1 (0.7–2.2)</td>
<td>0.45&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tₘₐₓ (h) (median)</td>
<td>2.0 (1.0–4.0)</td>
<td>2.5 (1.0–4.0)</td>
<td>1.5 (1.0–4.0)</td>
<td>0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL/F (liters/h)</td>
<td>79.8 (45.9–156)</td>
<td>79.3 (51.9–156)</td>
<td>80.0 (45.9–142)</td>
<td>0.95&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>V/F (liters)</td>
<td>320 (107–741)</td>
<td>337 (161–654)</td>
<td>313 (108–747)</td>
<td>0.65&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>2.8 (1.2–6.8)</td>
<td>2.9 (2.0–4.0)</td>
<td>2.7 (1.2–6.8)</td>
<td>0.52&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> CL, clearance; V, volume of distribution.
<sup>b</sup> Data are presented as geometric mean (minimum–maximum) unless stated otherwise.
<sup>c</sup> The AUC ratio was calculated by dividing unbound AUC₀–₂₄ by total AUC₀–₂₄ in all subjects.
<sup>d</sup> By independent-sample t test on log-transformed pharmacokinetic parameters between malnourished and well-nourished patients.

<sup>e</sup> By Mann-Whitney U test between malnourished and well-nourished patients.
Since patients who were severely malnourished or malnourished had a lower body weight while receiving the same fixed 450-mg dose of rifampin, a similar AUC$_{\text{0–24}}$ is explained by a trend toward a higher clearance per kilogram in both severely malnourished [0.21 liter/(h · kg)] and malnourished [0.21 liter/(h · kg)] patients versus well-nourished patients [0.18 liter/(h · kg)] ($P = 0.09$ and $P = 0.07$, respectively).

Apart from categorizing patients in groups with predefined BMI values, BMI was also evaluated as a continuous variable. There was no significant correlation between BMI and AUC$_{\text{0–24}}$ for both total and unbound rifampin plasma concentrations (Spearman’s rho, 0.035 and −0.055, respectively; $P = 0.84$ and $P = 0.75$, respectively), nor was there a significant correlation between BMI and C$_{\text{max}}$ for total and unbound rifampin plasma levels (Spearman’s rho, 0.000 and 0.095, respectively; $P = 1.0$ and $P = 0.58$, respectively).

Effects of other determinants on the pharmacokinetics of rifampin. In univariate analyses, gender emerged as the only significant determinant of total and unbound plasma rifampin pharmacokinetic parameters. Females were found to be exposed to higher concentrations of rifampin than males, as indicated by a significantly higher total AUC$_{\text{0–24}}$ (geometric mean, 59.2 versus 48.2 h · mg/liter; $P = 0.02$) and higher unbound AUC$_{\text{0–24}}$ (geometric mean, 6.2 versus 4.8 h · mg/liter; $P = 0.02$). Total and unbound C$_{\text{max}}$ values did not differ significantly between the two groups ($P = 0.16$ and $P = 0.22$, respectively), as was also the case for $T_{\text{max}}$ ($P = 0.23$ and $P = 0.22$, respectively). Univariate analyses did not show significant correlations for the determinants body weight and age (data not shown).

As females were overrepresented in the group of patients with a normal BMI (68%) compared to patients with a BMI below 18.5 kg/m$^2$ (45%) ($P = 0.20$), multiple linear regression analyses were performed to disentangle the effects of gender and BMI. These analyses showed that gender was a significant predictor of the AUC$_{\text{0–24}}$ of total and protein-unbound rifampin ($P = 0.02$ and $P = 0.01$, respectively), yet BMI was not ($P = 0.52$ and $P = 0.35$, respectively). Gender alone explained 15% of the variation ($r^2$) in both the total and protein-unbound AUC$_{\text{0–24}}$.

Variability in total and protein-unbound rifampin concentrations. To assess the interindividual variability in pharmacokinetics, all patients were combined in one group. Both total and protein-unbound rifampin concentrations showed considerable variation. The geometric mean total AUC$_{\text{0–24}}$ for all 36 subjects was 54.7 h · mg/liter, with a GCV of 26% and a 2.4-fold interindividual variation in total AUC$_{\text{0–24}}$, ranging from 32.6 to 88.8 h · mg/liter (Table 2 and Fig. 1). The geometric mean unbound AUC$_{\text{0–24}}$ amounted to 5.6 h · mg/liter, with a GCV of 32% and a 3.4-fold interindividual variation in unbound AUC$_{\text{0–24}}$, ranging from 2.9 to 9.8 h · mg/liter (Table 2 and Fig. 1). Therefore, interpatient variabilities in AUC$_{\text{0–24}}$ for total and unbound rifampin were considered to be comparable. The same applied for C$_{\text{max}}$, with GCVs of 27% and 30% for total and protein-unbound rifampin, respectively. Overall, a significant correlation was found between total and unbound plasma rifampin AUC$_{\text{0–24}}$ (Spearman’s rho, 0.816; $P < 0.001$) and between C$_{\text{max}}$ values (Spearman’s rho, 0.696; $P < 0.001$). The arithmetic mean percent unbound AUC$_{\text{0–24}}$ was found to be 10.5%, with a CV of 18% and a marked 2-fold interindividual variation in percent unbound rifampin, ranging from 7.6 to 15.0%.

Figure 2 shows the percent protein-unbound rifampin (free fraction) at the various sampling time points, and Table 3 summarizes the mean total and unbound rifampin concentrations, unbound fractions, and interindividual variabilities at the various sampling time points. The free fraction seemed to be slightly higher in samples taken later in the pharmacokinetic curve, with lower total concentrations (Fig. 2 and Table 3).

DISCUSSION

To our knowledge, the present study is the first to obtain both total and protein-unbound full pharmacokinetic curves of rifampin during the intensive phase of TB treatment in malnourished and well-nourished TB patients. The study shows comparable geometric mean total and protein-unbound AUC$_{\text{0–24}}$ and C$_{\text{max}}$ values in patients who are severely malnourished (BMI of <16.0 kg/m$^2$), malnourished (BMI of <18.5 kg/m$^2$), and well nourished (BMI of ≥18.5 kg/m$^2$). In addition, there was no significant correlation between BMI and AUC$_{\text{0–24}}$ or C$_{\text{max}}$ for both total and unbound rifampin plasma levels.

The absence of an effect of malnutrition on rifampin exposure in this study is in contrast with the results by Polasa et al. (12). They found that the AUC of a single dose of rifampin was 29% lower in undernourished (BMI of <18 kg/m$^2$) than in well-nour-
TABLE 3 Mean total and unbound rifampin concentrations, unbound fraction, and interindividual variability at each sampling time point

<table>
<thead>
<tr>
<th>Time postdose (h)</th>
<th>Conc (mg/liter)</th>
<th>Protein unbound fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein unbound</td>
<td>Total plasma</td>
</tr>
<tr>
<td></td>
<td>fraction (%)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.3 (67; 0.1–0.8)</td>
<td>2.7 (103; 0.3–9.1)</td>
</tr>
<tr>
<td>1</td>
<td>0.7 (71; 0.08–2.2)</td>
<td>6.5 (102; 0.1–16.6)</td>
</tr>
<tr>
<td>1.5</td>
<td>0.8 (71; 0.08–1.7)</td>
<td>6.8 (93; 0.2–15.8)</td>
</tr>
<tr>
<td>2</td>
<td>0.8 (62; 0.08–1.7)</td>
<td>7.3 (71; 0.8–15.0)</td>
</tr>
<tr>
<td>2.5</td>
<td>0.9 (46; 0.14–1.8)</td>
<td>8.5 (40; 2.1–15.2)</td>
</tr>
<tr>
<td>3</td>
<td>0.8 (31; 0.5–1.5)</td>
<td>8.9 (30; 5.0–15.1)</td>
</tr>
<tr>
<td>4</td>
<td>0.7 (38; 0.4–1.7)</td>
<td>8.7 (33; 4.2–15.0)</td>
</tr>
<tr>
<td>6</td>
<td>0.5 (41; 0.2–1.0)</td>
<td>3.9 (33; 2.1–7.3)</td>
</tr>
<tr>
<td>8</td>
<td>0.3 (48; 0.1–0.6)</td>
<td>2.4 (47; 1.0–6.0)</td>
</tr>
<tr>
<td>12</td>
<td>0.1 (43; 0.06–0.2)</td>
<td>0.5 (68; 0.1–1.5)</td>
</tr>
</tbody>
</table>

^a Values are geometric means (GCV; range); includes concentrations greater than the limit of quantitation.
^b Values are arithmetic means (CV; range); includes concentrations greater than the limit of quantitation.
^c Number of patients for whom a protein-unbound fraction could be calculated based on quantifiable unbound and total rifampin concentrations.

Also be argued that serum albumin is a suboptimal indicator of nutritional status, especially in marasmic populations, as albumin synthesis can be maintained in such states (32, 33).

Whereas malnutrition did not affect exposure to rifampin, females showed significantly higher total and unbound AUC_{0–24} also when corrected for BMI. In studies with predominantly Caucasian patients, higher serum rifampin concentrations were found in females, and this could not be explained by differences in body weight (30, 34). In Indonesian patients, two of our previous studies found a relationship between gender and rifampin pharmacokinetics (35, 36), but another study did not (11). In African subjects, female patients also showed higher exposures to rifampin (15). An explanation may be that females generally have a lower lean body mass than males. Thus, for drugs that are differentially distributed between water and fat, different drug concentrations can be found in subjects with the same weight (37). However, it should be noted here that gender explained only 15% of the variation (r^2) in both the total and protein-unbound AUC_{0–24}. Thus, other determinants are probably more important in causing the (high) variation in rifampin exposures.

With respect to interindividual variability, a marked 2-fold interindividual variation in the unbound rifampin fraction (unbound AUC_{0–24}/total AUC_{0–24}), between 7.6 and 15.0%, was observed. In addition, the variation in the free fraction at the various sampling time points was found to be considerably high (Fig. 2), with CVs ranging from 18 to 26% (Table 3). This large interindividual variability in the free fraction shows that measurement of solely total concentrations could be misrepresentative of the actual exposure. This suggests that protein-unbound rifampin concentrations should be considered to evaluate along with total plasma concentrations for assessment of exposure-response relationships in clinical studies. The same may apply to individualization of rifampin dosing based on plasma concentration measurements (therapeutic drug monitoring) in those settings where this technique can be used in patient care. In light of this observed high interindividual variation, the slight increase in the average unbound fraction of rifampin found in the lower total concentrations later in the pharmacokinetic curve (Fig. 2) might be less relevant, also when considering the relatively small number of patients included in this study. In addition, we cannot exclude that this small effect is an artifact related to ultrafiltration as a means to measure protein binding.

In the current study, we measured free rifampin concentrations using ultrafiltration performed at 25°C, as we also do for other drugs in routine patient care. In ultrafiltration, centrifugal forces are employed as the driving force for the passage of plasma water across a filter membrane (38). Besides ultrafiltration, other methodologies are available to determine plasma protein binding of drugs, such as ultracentrifugation and equilibrium dialysis. For rifampin all these techniques have been used. The review by Kenny and Strates states that in case of equilibrium dialysis, variuos rifamin binding values have been reported based on variable incubation temperatures, protein concentrations, and dialysis times (30). However, the specific effect of temperature on the rifampin-protein complex was not mentioned there or in the literature. For other drugs, an increase in experimental ultrafiltration temperatures has been associated with an increase in the free fraction (39–41), but as far as we know this has not been reported for rifampin. Indeed, we found that measurement of free rifampin at 37°C resulted in a small increase in the rifampin-free fraction.
range, not been investigated. According to Niemi et al., the c.463C
ished group was considerably low (median, 19.6 kg/m²; range,
second limitation, it can be argued that the BMI of the well-nour-
rifampin exposure (16, 34, 42, 43). To our knowledge, the poten-
morphisms of the SLCO1B1 gene have been associated with lower
the high variability in rifampin exposure. More specifically, poly-
were Indonesian, and it cannot be excluded that the effect of mal-
nutrition on rifampin pharmacokinetics is different in people
with another racial or genetic background. A final limitation is
that we did not include pharmacogenetic analyses. Pharmacoge-
genetic polymorphisms are proposed to be an important factor in
the high variability in rifampin exposure. More specifically, poly-
morphisms of the SLCO1B1 gene have been associated with lower
exposure (16, 34, 42, 43). To our knowledge, the poten-
tial impact of such polymorphisms in Indonesian TB patients has
not been investigated. According to Niemi et al., the c.463C>A
polymorphism, which has been associated with decreased rifam-
pin exposures, is present in only 0 to 3% of the East Asian popu-
lation (44). This indicates that at least this specific polymorphism
is of minor importance in our study population and will not affect
differences in rifampin exposures between malnourished and
well-nourished patients.

To summarize, severely malnourished, malnourished, and
well-nourished Indonesian TB patients showed no clear differ-
ence in total and protein-unbound pharmacokinetic parameters
of rifampin during the intensive phase of TB treatment. Similarly,
BMI and rifampin (total and unbound) pharmacokinetic param-
eters did not show any significant correlation. Significantly higher
BMI and rifampin (total and unbound) pharmacokinetic param-
ers were not be calculated to be required to detect a difference of 25% in rifampin
AUC₀–24. However, with a number of 25 individuals included in
the group with a normal BMI, it can be calculated that the present
study could still detect a 24% difference in the AUC₀–24 of total
rifampin with a significance level of 0.05 and a power of 80%. As a
second limitation, it can be argued that the BMI of the well-nour-
ished group was considerably low (median, 19.6 kg/m²; range,
18.7 to 22.6), resulting in a relatively small difference from the
malnourished group (16.3 kg/m²; 16.1 to 16.4). However, also
when comparing the severely malnourished subgroup with well-
nourished patients and when analyzing BMI as a continuous vari-
able, nutritional status and BMI do not appear to have a major
effect on total and protein-unbound pharmacokinetic parameters
of rifampin. Third, it should be considered that all participants
were Indonesian, and it cannot be excluded that the effect of mal-
nutrition on rifampin pharmacokinetics is different in people

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