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


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 [Cₘₐₓ]) was found. Females had significantly higher total AUC₀–₂₄ (geometric mean, 59.2 versus 48.2 h · mg/liter; P < 0.02) and higher unbound AUC₀–₂₄ (geometric mean, 6.2 versus 4.8 h · mg/liter; P = 0.02) than males. Overall, a marked 2-fold interindividual variation in the free fraction was observed (7.6 to 15.0%; n = 36). Nutritional status and BMI do not appear to have a major effect on total and protein-unbound pharmacokinetic parameters of rifampin in Indonesian subjects. The large interindividual variability in the free fraction of rifampin suggests that protein-unbound rather than total rifampin concentrations should preferably be used to study exposure–response relationships.

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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 Kima Farma, Bandung, Indonesia), formulated in separate tablets. The bioequivalence of the rifampin tablets and an international reference standard has been established before (28).

A full pharmacokinetic curve for rifampin was recorded between 2 and 6 weeks after the start of TB treatment, when steady state for the TB drugs can be expected (29). Body weight and height (to calculate body mass index [BMI]) and concomitant drug use were also assessed at the pharmacokinetic sampling day. In addition, plasma protein albumin was measured, considering that 30 to 41% of protein-bound rifampin is associated with the serum albumin fraction (30). Informed consent was obtained from all subjects, and the study was approved by the Independent Ethics Committee, Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia.

Blood sampling, bioanalysis, and pharmacokinetic data analysis. Patients refrained from the intake of any food or any drugs (other than study medication) starting from 11:00 p.m. on the day preceding the pharmacokinetic assessment until 4 h after the intake of study medication. TB drugs were taken with 230 ml of still water. Serial blood samples (10 ml) were collected from the antecubital vein just before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after observed TB drug intake. Plasma was immediately separated, frozen at −20°C, and transferred to −80°C within 72 h until transport on dry ice to The Netherlands for bioanalysis.

Bioanalysis. The total (protein-bound plus unbound) plasma concentrations of rifampin were determined with a validated high-performance liquid chromatographic (HPLC) method with UV detection as described previously (27). The lower limit of quantitation for this method was 0.28 mg/liter.

In addition to total plasma concentrations, protein-unbound concentrations of rifampin were measured in all obtained samples. Measurements were based on ultrafiltration to separate bound from unbound rifampin, followed by HPLC. Briefly, 0.5 ml of plasma was added into a CentriFree YM-30 tube (Millipore, Amsterdam, The Netherlands). Plasma was centrifuged for 15 min at 1,650 × g at 25°C with a Rotanta 46 R, rotor 4445 (radius 164, <45°). The clear ultrafiltrate was placed in a thermostated autosampler (4°C), and 50 μl of this solution was injected in the HPLC system. The analytical column was an OmniSpher 5 C18, column (250 by 4.6 mm [inner diameter]; particle size, 5 μm) protected by a Chromguard RP ss 10- by 3-mm column (Varian, Middelburg, The Netherlands). The mobile-phase components were 30% acetonitrile and 70% 10 mM phosphate buffer (pH 5), run during a HPLC gradient with different flow rates. The total run time was 17 min. UV detection was set at 334 nm. The average accuracy of ultrafiltrate spiked with rifampin was 107%. Intraday imprecision in measurement of rifampin in ultrafiltrate varied from 1.4 to 2.4%, interday imprecision varied from 0% (i.e., there was no additional variation upon intraday imprecision as a result of performing the assay on different days) to 3.6%, and overall precision varied from 1.4 to 3.9%, depending on the concentration measured. The range of the method for unbound rifampin plasma concentrations was from 0.06 mg/liter (limit of quantitation) to 13 mg/liter.

Pharmacokinetic analysis. Pharmacokinetic parameters were assessed using standard noncompartmental methods in WinNonLin version 5.3 (Pharsight Corporation) as described before (27). Unbound fractions were calculated by dividing the unbound area under the concentration-time curve from 0 to 24 h (AUC0–24) by the total AUC0–24, for all study subjects.

Statistical analysis. Based on available data for the pharmacokinetics of rifampin in Indonesian patients (27), it was calculated that a minimum of 15 patients per group were required to detect a difference of 25% in the AUC0–24 of total rifampin with a significance level of 0.05 and a power of 80% (two-sided test).

Patients were first divided into three subgroups based on criteria for nutritional status as proposed by the World Health Organization (31). Patients with a BMI of <16.0 kg/m2 were considered severely malnourished, patients with a BMI of >18.5 kg/m2 were regarded as malnourished, 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 (Tmax) 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. AUC0–24 and maximum concentration of drug in serum (Cmax) 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 AUC0–24 and Cmax 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 AUC0–24 and Cmax 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 $\geq 18.5$ kg/m$^2$), and 11 patients were malnourished (BMI of $<18.5$ kg/m$^2$), of which seven patients were severely malnourished (BMI of $<16.0$ kg/m$^2$). 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 AUC$_{0-24}$ of rifampin did not differ between patients with malnutrition (BMI of $<18.5$ kg/m$^2$) and patients with a normal BMI, for both the total AUC$_{0-24}$ (54.8 versus 54.6 h · mg/liter; $P = 0.96$) and the unbound plasma AUC$_{0-24}$ (5.7 versus 5.6 h · mg/liter; $P = 0.95$) (Table 2). Total and unbound geometric mean $C_{\text{max}}$ 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/m$^2$) showed no differences in AUC$_{0-24}$ compared to patients with a normal BMI either; geometric mean values for total and protein-unbound AUC$_{0-24}$ 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 $C_{\text{max}}$ values yielded similar results; geometric mean values for total and protein-unbound $C_{\text{max}}$ 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).

**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$^a$</th>
<th>Value for group$^b$</th>
<th>Total ($n = 36$)</th>
<th>BMI $&lt; 18.5$ ($n = 11$)</th>
<th>BMI $\geq 18.5$ ($n = 25$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total rifampin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-24}$ (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$^d$</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (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$^d$</td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (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$^e$</td>
<td></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$^d$</td>
<td></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$^d$</td>
<td></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$^d$</td>
<td></td>
</tr>
<tr>
<td><strong>Unbound rifampin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-24}$ (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$^d$</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (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$^f$</td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (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$^d$</td>
<td></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$^d$</td>
<td></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$^d$</td>
<td></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$^d$</td>
<td></td>
</tr>
<tr>
<td><strong>Unbound fraction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC ratio (%) (avg)$^c$</td>
<td>10.5 (7.6–15.0)</td>
<td>10.5 (8.9–14.9)</td>
<td>10.5 (7.6–15.0)</td>
<td>0.99$^d$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ CL, clearance; V, volume of distribution.

$^b$ Data are presented as geometric mean (minimum–maximum) unless stated otherwise.

$^c$ The AUC ratio was calculated by dividing unbound AUC$_{0-24}$ by total AUC$_{0-24}$ in all subjects.

$^d$ By independent-sample $t$ test on log-transformed pharmacokinetic parameters between malnourished and well-nourished patients.

$^e$ By Mann-Whitney U test between malnourished and well-nourished patients.

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**Table 1.** Demographic, clinical, and laboratory characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value ($n = 36$)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>35 (18–35)</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>39</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>44 (34–53)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>19.0 (13.5–22.6)</td>
</tr>
<tr>
<td>Severely malnourished (BMI $&lt;16$ kg/m$^2$; $n = 7$)</td>
<td>15.3 (13.5–15.8)</td>
</tr>
<tr>
<td>Malnourished (BMI, 16–18.49 kg/m$^2$; $n = 4$)</td>
<td>16.3 (16.1–16.4)</td>
</tr>
<tr>
<td>Normal BMI (BMI $\geq 18.5$ kg/m$^2$; $n = 25$)</td>
<td>19.6 (18.7–22.6)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8 (2.6–4.2)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.7 (8.9–14.4)</td>
</tr>
<tr>
<td>Rifampin dose (mg/kg)</td>
<td>10.3 (8.5–13.2)</td>
</tr>
<tr>
<td>Severely malnourished (BMI $&lt;16$ kg/m$^2$; $n = 7$)</td>
<td>12.4 (10.5–13.2)</td>
</tr>
<tr>
<td>Malnourished (BMI, 16–18.49 kg/m$^2$; $n = 4$)</td>
<td>11.4 (10.5–13.2)</td>
</tr>
<tr>
<td>Normal BMI (BMI $\geq 18.5$ kg/m$^2$; $n = 25$)</td>
<td>9.6 (8.5–11.5)</td>
</tr>
</tbody>
</table>

$^a$ Data are presented as median (minimum–maximum) unless stated otherwise.

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**Results.** Thirty-six patients with pulmonary TB were included in the study. Characteristics of the patients are presented in Table 1.
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 AUCₐ₀₋₂₄ 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 AUCₐ₀₋₂₄ 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 Cₘₐₓ 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 pharmacokinetics. Females were found to be exposed to higher concentrations of rifampin than males, as indicated by a significantly higher total AUCₐ₀₋₂₄ (geometric mean, 59.2 versus 48.2 h · mg/liter; P = 0.02) and higher unbound AUCₐ₀₋₂₄ (geometric mean, 6.2 versus 4.8 h · mg/liter; P = 0.02). Total and unbound Cₘₐₓ values did not differ significantly between the two groups (P = 0.16 and P = 0.22, respectively), as was also the case for Tₘₐₓ (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/m² (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 AUCₐ₀₋₂₄ 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 (r²) in both the total and protein-unbound AUCₐ₀₋₂₄.

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 AUCₐ₀₋₂₄ for all 36 subjects was 54.7 h · mg/liter, with a GCV of 26% and a 2.4-fold interindividual variation in total AUCₐ₀₋₂₄ ranging from 32.6 to 88.8 h · mg/liter (Table 2 and Fig. 1). The geometric mean unbound AUCₐ₀₋₂₄ amounted to 5.6 h · mg/liter, with a GCV of 32% and a 3.4-fold interindividual variation in unbound AUCₐ₀₋₂₄, ranging from 2.9 to 9.8 h · mg/liter (Table 2 and Fig. 1). Therefore, interpatient variations in AUCₐ₀₋₂₄ for total and unbound rifampin were considered to be comparable. The same applied for Cₘₐₓ 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 AUCₐ₀₋₂₄ (Spearman’s rho, 0.816; P < 0.001) and between Cₘₐₓ values (Spearman’s rho, 0.696; P < 0.001). The arithmetic mean percent unbound AUCₐ₀₋₂₄ 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 AUCₐ₀₋₂₄ and Cₘₐₓ values in patients who are severely malnourished (BMI of ≈16.0 kg/m²), malnourished (BMI of < 18.5 kg/m²), and well nourished (BMI of > 18.5 kg/m²). In addition, there was no significant correlation between BMI and AUCₐ₀₋₂₄ or Cₘₐₓ 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/m²) than in well-nour-
malnutrition mediated through altered binding to albumin was
patients in our study may be better, suggesting that any effect of
low in these patients as well (42.99%) (12), suggesting a shift in the
Nevertheless, used rifampin for 4 to 6 months, yet the comparison of steady-
interactions (24,25). In this situation, measuring the total con-
are very similar to the situation occurring with drug displacement
changes in plasma protein binding due to protein deficiency
crease in other proteins relevant to binding of drugs) might result
findings might be that malnourished patients in our study had
and total rifampin exposure in our cohort. An explanation for our
correlation between BMI and the steady-state protein-unbound
nourished TB patients. Furthermore, our study did not show a
interindividual variation, the slight increase in the average un-
bound AUC0–24/total AUC0–24), between 7.6 and 15.0%, was ob-
erved 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 interindi-
vidual variability in the free fraction shows that measurement of
solely total concentrations could be misrepresentative of the ac-
tual exposure. This suggests that protein-unbound rifampin con-
centrations should be considered to evaluate along with total
plasma concentrations for assessment of exposure-response rela-
tionships in clinical studies. The same may apply to individualiza-
tion of rifampin dosing based on plasma concentration measure-
ments (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 un-
bound fraction of rifampin found in the lower total concentra-
tions 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 concentra-
tions 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, vari-
ous rifampin 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 lit-
trature. 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

<p>| TABLE 3 Mean total and unbound rifampin concentrations, unbound fraction, and interindividual variability at each sampling time point |</p>
<table>
<thead>
<tr>
<th>Time postdose (h)</th>
<th>Conc (mg/liter)</th>
<th>Protein-unbound fraction (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein unbound</td>
<td>Total plasma</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>
<td>8.1 (18; 5.6–11.1)</td>
</tr>
<tr>
<td>1</td>
<td>0.7 (71; 0.08–2.2)</td>
<td>6.5 (102; 0.1–16.6)</td>
<td>9.5 (20; 6.2–14.9)</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>
<td>10.3 (21; 4.4–13.0)</td>
</tr>
<tr>
<td>2</td>
<td>0.8 (62; 0.08–1.7)</td>
<td>7.3 (71; 0.8–15.0)</td>
<td>10.3 (18; 6.3–15.6)</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>
<td>10.2 (20; 6.6–15.0)</td>
</tr>
<tr>
<td>3</td>
<td>0.8 (31; 0.5–1.6)</td>
<td>8.9 (30; 5.1–15.1)</td>
<td>9.8 (25; 5.3–17.4)</td>
</tr>
<tr>
<td>4</td>
<td>0.7 (38; 0.4–1.7)</td>
<td>8.7 (33; 4.2–15.0)</td>
<td>9.7 (26; 5.0–17.0)</td>
</tr>
<tr>
<td>6</td>
<td>0.5 (41; 0.2–1.0)</td>
<td>3.9 (33; 2.1–7.3)</td>
<td>12.4 (21; 8.5–18.4)</td>
</tr>
<tr>
<td>8</td>
<td>0.3 (48; 0.1–0.6)</td>
<td>2.4 (47; 1.0–6.0)</td>
<td>11.0 (21; 6.4–14.7)</td>
</tr>
<tr>
<td>12</td>
<td>0.1 (43; 0.06–0.2)</td>
<td>0.5 (68; 0.1–1.5)</td>
<td>13.3 (23; 7.8–20.6)</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.

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(mean change in free fraction, +1.2%; median change, +1.1%; range, −0.7 to +3.5%, n = 10 rifampin concentrations measured, P = 0.029 [paired t test]) (unpublished data). This effect of temperature on the rifampin free fraction is small and is unlikely to have affected the findings in our study.

This study has its limitations. First, the number of individuals included in the malnourished group seems somewhat low with 11 individuals, as a number of 15 individuals per group was calculated to be required to detect a difference of 25% in rifampin AU_{G−1,24}. 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 AU_{G−1,24} 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-nourished 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 variable, 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 malnutrition on rifampin pharmacokinetics is different in people with another racial or genetic background. A final limitation is that we did not include pharmacogenetic analyses. Pharmacogenetic polymorphisms are proposed to be an important factor in the high variability in rifampin exposure. More specifically, polymorphisms of the SLCO1B1 gene have been associated with lower rifampin exposure (16, 34, 42, 43). To our knowledge, the potential 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 rifampin exposures, is present in only 0 to 3% of the East Asian population (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 difference in total and protein-unbound pharmacokinetic parameters of rifampin during the intensive phase of TB treatment. Similarly, BMI and rifampin (total and unbound) pharmacokinetic parameters did not show any significant correlation. Significantly higher plasma concentrations (total and unbound) were found in females, also when corrected for BMI. Finally, a marked 2-fold interindividual variation in the unbound rifampin fraction was observed. This large interindividual variability in the free fraction of rifampin shows that measurement of solely total concentrations could be misrepresentative of the actual exposure, suggesting that it should be considered to evaluate protein-unbound rifampin concentrations along with total plasma concentrations when studying exposure-response relationships for this pivotal TB drug.

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REFERENCES


