TITLE: Intra-individual effects of food upon the pharmacokinetics of rifampicin and isoniazid

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Running title

Rifampicin and isoniazid plasma concentration in tuberculosis patients

ABSTRACT

Poor response to tuberculosis (TB) therapy might be attributable to sub-therapeutic levels in drug-compliant patients. Pharmacokinetic parameters can be affected by co-morbidities or the interaction of drugs with food.

This study aimed to determine the effect of food intake upon pharmacokinetics of rifampicin and isoniazid in a Peruvian TB population. Rifampicin and isoniazid levels were analysed at 2, 4 and 6 hours after drug intake in both fasting and non-fasting state using liquid chromatography mass spectrometric methods.

Sixty patients participated in the study. The median rifampicin Cmax and AUC_{0-6} were higher on fasting days than non-fasting days: 7.02mg/L vs. 6.59mg/L (p:0.054) and 28.64mg·h/l vs. 24.31mg·h/l (p:0.002). There was a statistically significant delay overall of non-fasting Tmax compared to the fasting state Tmax(p=0.005). In the multivariate analysis, besides the effect of fasting, Cmax for females was 20% higher than for males (p=0.03). Concerning isoniazid, there were significant differences in the Cmax on non-fasting day (median 3.51mg/L) compared with the fasting day (4.54mg/L). The isoniazid-dose received had an effect upon the isoniazid-levels (1.26,p:0.038). In the multivariate analysis, isoniazid-exposure on the fasting day was found to be 14% higher than on the non-fasting day (CI:1.02–1.28,p<0.001). Neither radiological extent of the disease nor consumption of food with drug intake nor pharmacokinetics of rifampicin or isoniazid was associated with a poorer treatment outcome.

Rifampicin in particular and also isoniazid pharmacokinetics were significantly affected by the intake of the drug with food between and within individuals.

BAKCGROUND

The current first-line strategy to treat tuberculosis(TB) is based on the standardized short course regimen recommended by WHO of rifampicin(RIF), isoniazid(INH), pyrazinamide(PZA) and ethambutol(ETA) which is usually highly effective[1]. Despite the success of directly observed therapy(DOT) strategies[1,2] in many TB endemic countries, relapse and acquired drug resistance has not been entirely eliminated[3]. Factors such as high baseline bacillary burden or sputum smear-positivity, cavitation, HIV, DM and other underlying diseases have been associated with poorer TB outcome[4-7] and also with impaired pharmacokinetics[8-11] .

Recently, experimental and clinical studies have shown that pharmacokinetic variability expressed in key parameters such as plasma area-under-the-curve(AUC) seems to play an important role in the emergence of acquired multidrug-resistant TB(MDR-TB) in *in vitro* models; inadequate exposure to anti-TB drugs is associated with acquired drug resistance[12,13]. In prospective clinical studies, impaired pharmacokinetic studies have been related to a suboptimal treatment response[14,15] though this is not a universal finding[16-18] and concentrations below the expected range for key drugs in the anti-TB regimen have been frequently found in patients responding well to treatment[19,20].

Several studies suggest that bioavailability of RIF and INH is reduced by dosing the TB drugs with meals[21-23], prompting recommendations that the drugs should be taken on an empty stomach. However, other studies showed no significant difference in the time for which the serum-rifampicin remained above the minimum inhibitory concentration(MIC) for *Mycobacterium tuberculosis,* suggesting that the chemotherapeutic effect is likely to be unaffected[24].

As anti-tuberculosis drugs can cause gastrointestinal upset which may impair adherence to therapy[25-27], an adverse effect heightened by taking medication without food, the current official recommendation of the American Thoracic Society, is to provide TB medication with

meals if gastrointestinal intolerance persists[28]. However, few studies have evaluated if patients dosing the TB drugs with meals are associated with treatment failure or early relapse.

OBJECTIVES

The aim of this study was to determine the frequency and magnitude of any within-person difference in the pharmacokinetics of R and H in a group of patients taking TB treatment on an empty stomach or with food, and to determine the effect upon sputum smear and culture conversion times and end-of-treatment(EOT) and EOT+6 month disease outcomes of taking TB treatment predominantly with food or predominantly fasted during the course of treatment.

METHODS

This observational study was conducted in Lima(Peru), from January-December,2012. People diagnosed with Pulmonary TB commencing supervised treatment under the DOTS programme of the Peruvian National TB programme(PNTP) were invited to participate.

The recommended scheduled by PNTP is a six days/week during the intensive phase(RIF,INH,PZA and ETA) and twice/week(RIF/INH) during the maintenance phase at the time of the study. RIF-dose is 10mg/Kg/day and INH-dose is 5mg/kg/day during the intensive phase of treatment.

Patients who were not sputum smear positive, who were known to have co-morbid HIV disease or DM, or were unwilling or unable to give informed consent were excluded from the study.

A semi-structured questionnaire was given to all participants. Personal data, information about their TB disease, gender, age, height(cm) and weight(Kg) were recorded and the body mass index(BMI) was calculated. A chest radiograph was performed for all participants and the scored developed by Ralph *et al*[29] was used to calculate the severity of pulmonary TB in each case.

All patients were given a diet diary (collected at weekly intervals) where they daily annotated, whether they had eaten and the kind of food in the period beginning 2-hours before, during or within 1-hour after the drug intake.

At day 30 and 60, blood samples were drawn from each patient at the health-centre by dedicated staff into 10ml lithium-heparin tubes at 3 time points -two, four, and six hours after the directly-observed TB drug intake. On one of these days, patients were required to fast at least one hour before and an hour after the drug intake.

Treatment outcome of patients was determined at end of therapy (by personal examination, chest radiography and conventional culture) and 6 months later either by a personal interview or a phone-call.

Laboratory methods

All blood samples were drawn and centrifuged in the health centres (centrifugation at 2000rpm for 10min) and aliquots of the serum was refrigerated and transported to UPCH and stored at -70ºC until batched and transported to the pharmacokinetics laboratory of the Liverpool School of Tropical Medicine. RIF and INH concentrations in each blood sample were determined with validated assays[9,10], described in detail in Annex 1.

Pharmacokinetics outcome measurements

For each patient, the Cmax was defined as the highest of the three concentrations measured at 2, 4 and 6 h, and the Tmax was the time point at which the Cmax occurred. PK parameters were obtained by non-compartmental analysis using the trapezoidal rule and the linear-up-logdown method. MIC data were not available, and no additional analysis of PK-pharmacodynamic(PD) parameters was developed. Although an internationally agreed-upon guideline for therapeutic drug monitoring is lacking, Cmax RIF values were also categorized as normal(>8mg/L), low(4-8mg/L) or very low(<4mg/L) in accordance with previous work[21,30].

Normal INH-Cmax was defined, by comparison with existing pharmacokinetic data, as 3-5mg/litre after a 5mg/kg daily[21]. INH-PK data were categorized according to Pasipanodya *et al* that established that a Cmax level of <2mg/litre after a 300-mg daily dose or a Cmax level of <7mg/litre after a 900-mg biweekly dose were regarded as inadequate[31].

Data analysis

The chi-squared test was used for the comparison of proportions, and the Student t-test or Wilcoxon rank-sum test for paired samples was used for continuous variables, depending on variable distribution. The percentage of treatment days on which treatment was taken in a fasting state was derived for each individual from their diet diary, for use as a continuous exposure variable in the outcome analysis. The data were analysed with Stata-13.

Ethics

The study protocol and consent form were approved by the ethics committee of the London School of Hygiene and Tropical Medicine, Universidad Peruana Cayetano-Heredia and the regional Ministry of Health, Lima, Peru.

RESULTS

Sixty patients were recruited to the study with a median age of 32.7(IQR 23.7-45 years); 34(56.7%) were male. General characteristics of patients are summarized in table 1. The median RIF, INH, ETA and PZA dosages received were consistent with those recommended by the PNTP.

Eight patients withdrew from the study before the first blood sample, two additional subjects were withdrawn after a subsequent new diagnosis of HIV and two more changed treatment regimen (one due to resistance and one due to adverse effects) so were also withdrawn. Thus 48 patients had at least one PK data point and were included in the PK analysis(figure 1).

Eleven patients could not be included in treatment outcome follow-up (six had changed therapy after PK sampling because of drug resistance and five abandoned either the therapy or the study). Thirty-seven participants were included in the follow-up evaluation.

The information on the diet diaries was analysed for the 37 patients with follow-up treatment outcome data. During the intensive phase, patients properly fasted (only drinking water) on a median of 2% of the treatment days (interquartile-range 1-7.5) and they took the TB drugs with water or any other drink (juice, cereal or carbonated beverages) without solid food a median of 4.5% of the treatment days(IQR:1-11.5). The rest of the treatment days, patients consumed some food during drug intake.

During the maintenance phase, patients fasted (only drinking water) with the drug intake a median of 1% of the treatment days(IQR:0-3) and they took the TB drugs either with water or with a drink without a meal a median of 4% of the treatment days(0-10). The rest of the treatment days, patients consumed some food during drug intake.

PHARMACOKINETICS RESULTS

Pharmacokinetics of rifampicin

166 Overall, median serum rifampicin levels at two, four and six hours were 3.25, 6.08 and 4.2mg/L
167 respectively during the non-fasting day and 6.49, 6.08 and 4.23mg/L during the fasting day.
168 The individual difference between RIF PK in the fasting day compared with the non-fasting day
169 was particularly high at 2 hours (figure 2).

170 Comparing PK parameters within individuals, the median Cmax and AUC_{0-6} were higher on
171 fasting days than non-fasting days: 7.02mg/L vs. 6.59mg/L(p=0.054) and 28.64mg·h/l vs.
172 24.31mg·h/l(p=0.002, Wilcoxon signed rank test) respectively.

173 RIF-Cmax, when RIF was taken in a non-fasting state, was significantly lower in male compared
174 with female patients (6 *vs.* 8.3mg/litre;p=0.035); however this effect of gender was not
175 apparent when RIF was taken in a fasting state (6.73 versus 7.55mg/litre;p=0.09).

176 Though not statistically significant there was also a tendency towards lower RIF AUC_{0-6} in male
177 compared with female patients with dosing in both the fasting (27.85 *vs*. 31.75mg·h/l;p=0.09)
178 and non-fasting state (22 *vs*. 27.27mg·h/l;p=0.08).

179 Effect of fasting on Tmax

180 On fasting days, Tmax occurred at 2, 4 or 6 hours in 68.8%, 27.1% and 4.2% of patients
181 respectively. On non-fasting days, Tmax occurred at 2, 4 or 6 hours in 34.8%, 56.5%, and 8.7%
182 respectively, a statistically significant delay overall compared to the fasting state
183 Tmax(p=0.005,Wilcoxon Signed-rank test). Tmax was not associated with gender, age group, or
184 dose received (data not shown).

185 Categorization of Cmax: Adequate *vs.* inadequate levels.

186 When non-fasting, three patients(6.5%) had Cmax values of <4mg/L, 28(60.9%) had rifampicin
187 levels between 4-8mg/L, and 15(32.6%) had values that are regarded as adequate levels for
188 TDM(>8mg/L). When the blood sampling was done during the fasting day, 1(2.1%) had a Cmax
189 value of <4mg/L, 30(62.5%) had levels between 4-8mg/L, and 17 patients(35.4%) had normal
190 levels (>8mg/L)(figure 3). Considering rifampicin AUC_{0-6} values <13mg.h/L as low or inadequate

191 levels of AUC_{0-24}, as suggested elsewhere[14], 13% of patients had a low AUC during the fasting
192 day compared with 2.1% during the non-fasting day.

193 Multivariate analysis

194 A model was constructed to assess the independent effect of fasting during drug intake, RIF-
195 Tmax and gender on the logarithm of RIF-Cmax. RIF-Cmax on the fasting day was found to be
196 15% higher than RIF-Cmax during the non-fasting day(CI:1.01–1.30,p<0.036). Cmax for females
197 was 20% higher than for males(p=0.03). The effect of Tmax did not influence the Cmax (Tmax-
198 4h: 0.98,p=0.9; Tmax 6h: 1.11,p=0.676)(table 2).

199 A further model was constructed to assess the independent effect of fasting during drug
200 intake, the RIF-Tmax and gender on the logarithm of RIF-AUC_{0-6}.

201 RIF-AUC_{0-6} on the fasting day was found to be 14% higher than RIF-AUC_{0-6} during the non-
202 fasting day(CI:1.01–1.28, p<0.02). RIF-AUC_{0-6} for females was 20% higher than for
203 males(p:0.027). When RIF-Tmax occurred at 4h, RIF-AUC_{0-6} was reduced by 20% (p=0.002) and
204 when it occurred at 6h, the RIF-AUC_{0-6} decreased by 50%(p<0.001), as compared with a RIF-
205 Tmax of 2 hours.

206 Pharmacokinetics of INH

207 Overall, median serum isoniazid levels at two, four and six hours were 3.27, 1.96 and 0.92mg/L
208 respectively during the non-fasting day and 4.54, 1.19 and 0.75mg/L during the fasting day.
209 The individual difference between INH-PK in the fasting day compared with the non-fasting
210 day was particularly high at 2 hours(figure 4).

211 There were significant differences in the Cmax on non-fasting day(median 3.51mg/L)
212 compared with the fasting day(4.54mg/L)(Wilcoxon Signed-rank test p<0.001). The AUC_{0-6} was
213 12.11mg·h/L on the non-fasting day vs. 13.31mg·h/L during the fasting day(p=0.001).

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INH-Cmax in men was not different to INH-Cmax in women, whether fasted(4.21mg/L v. 4.88mg/L;p=0.21) or not (3.29mg/L v 4.41mg/L respectively,p=0.08). Similarly, INH AUC_{0-6} did not differ between males and females either with fasting(13.21mg·h/L *vs*. 15.13mg·h/L,p=0.28) or non-fasting dosing conditions(12.08mg·h/L *vs*. 12.65mg·h/L,p=0.36). INH-Cmax was not affected by presence of intestinal parasites, age group or BMI (data not shown).

Effect of fasting on INH-Tmax

Tmax occurred at two hours in 80.4% and 95.8% of patients on the non-fasting and fasting day respectively, at 4 hours in 17.4% (non-fasting) and 2.1%(fasting), and at 6 hours in 2.2%(non-fasting day) and 2.1%(fasting day) of patients; a statistically significant delay due to non-fasting (p=0.023,Wilcoxon Signed-rank test).

Tmax was not associated with gender, age group, intestinal parasitic infection, or dose received (data not shown) regardless of fasting condition.

On the non-fasting day, seven patients(15.2%) had Cmax values of <2mg/L, and 39(84.8%) had values that are regarded as adequate levels. When INH was taken in a fasting condition, 3 (6.3%) had Cmax values of <2mg/L, 45(93.8%) had adequate levels(>2mg/L)(figure 5). However, considering isoniazid AUC_{0-6} values <52mg.h/L as low or inadequate levels of AUC_{0-24}, as suggested by Pasipanodya *et al*[14], 100% of patients had a low AUC during the fasting day compared with 95% during the non-fasting day(p=0.162),

A model was constructed to assess the independent effect of gender, INH-dose received and the effect of fasting during drug intake on the logarithm of INH-Cmax(table 3).

The INH-dose received had an effect upon the INH-levels(1.26,p:0.038). Moreover, INH-exposure on the fasting day was found to be 14% higher than on the non-fasting day(CI:1.02–1.28, p<0.001)(Table 3). A further model was constructed to assess the independent effect of gender, INH-Tmax and the effect of fasting during drug intake on the INH-AUC_{0-6}.

239 INH-AUC_{0-6} was found to be 22% higher on the fasting than the non-fasting day(CI: 1.09–1.38,
240 p<0.001. When INH-Tmax occurred at 6h, INH-AUC_{0-6} decreased by 47%(p=0.013).

241 All 37 patients evaluated at the end of therapy were considered cured. 1/37 patients
242 evaluated at six months after the end of the therapy had relapsed two months after having
243 finished the therapy. This 47 year-old male had successfully completed first line TB therapy
244 with clinical and radiological improvement; although a fibrotic right apical scar was seen at end
245 of therapy TB culture at this time was negative. Two months later he restarted TB therapy
246 after an early microbiology confirmed relapse with a positive culture was diagnosed. The Rx-
247 score of this patient had been 42(median Ralph score 22.6). He had fasted 16% of the time
248 during the intensive phase and had not fasted on any occasion in the maintenance phase.

249 For the 37 subjects with treatment outcome data, neither the Rx score nor consumption of
250 food with drug intake nor RIF-PK or INH-PK were associated with a poorer treatment outcome
251 (data not shown).

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DISCUSSION

The results of this study demonstrate that RIF in particular and also INH pharmacokinetics (Cmax, Tmax and AUC_{0-6}) were significantly affected by the intake of the drug with food, as has been shown previously[32]. However, the effect of food was not large and was highly variable between individuals, with some participants achieving higher exposure on non-fasted than fasted days.

A delay in RIF absorption was observed and the median Tmax occurred at 4 hours instead of 2 hours amongst those who took drug with food. By excluding inter-individual variability this intra-individual PK analysis demonstrated the interaction of the food during drug intake in the absorption delay (Tmax) and also in the AUC_{0-6}, which has been corroborated in the multivariate analysis. As has been suggested before and further confirmed by our data here, slower absorption leads to lower plasma concentrations[33].

When RIF was ingested with food, low levels of RIF were observed in more than half of patients although very low levels (<4mg/L) were only observed in less than 10% of patients. Lower levels of RIF-Cmax and lower RIF-AUC_{0-6} were demonstrated among non-fasted men compared with women. Although this was not observed when the drug was taken on an empty stomach, in multivariate analysis gender influenced the pharmacokinetics of RIF regardless of fasting status. These gender differences give cause for consideration of whether dosing recommendations warrant review, though in the absence of a demonstrable impact upon treatment outcome, this is probably premature[34].

INH-Tmax occurred at 2-hours regardless of fasting status although the PK parameters (Cmax) were higher in the fasting blood sampling; other studies have suggested that food causes an absorption delay and also reduces the Cmax[35].

It has been suggested that INH-Tmax may sometimes occurs earlier than 2 hours and we would not have captured this as our earliest sampling time point was 2 hours. The multivariate analysis also demonstrated the effect of fasting on increasing the exposure to INH.

In both univariate and multivariate analysis INH-AUC_{0-6} and INH-Cmax did not differ by gender in either fasted or non-fasted state.

The study was carried out under real-life field conditions and not in the controlled environment of a dedicated PK unit, what could influence the variability of RIF and INH PK. Conversely, this design generates more translatable data since patients are doing what they do every day. Another strength of this study is that we were able to eliminate the confounding effect of inter-individual variability upon interpretation of the effect of food, because patients had blood sampling under both fasting and under non-fasting conditions, and a corresponding matched analysis was performed.

It is usually recommended that RIF and INH are to be given on an empty stomach whenever possible, based on previous PK studies[36]. However, few studies have evaluated if patients dosing the TB drugs with meals are associated with treatment failure or early relapse. In the US Public Health Service TB Trial-22 in which patients received rifapentine/isoniazid, patients receiving medication under fed conditions were significantly associated with treatment failure or relapse although patients were receiving INH and rifapentine instead of RIF[12]. However the exquisite dependence of rifapentine PK is well recognised so these data are not directly relevant.

A secondary aim of our study was to determine if taking TB drugs with meals is associated with a poor response to treatment, an impact that could be hypothesized might result from lower drug exposure. In the event participant diet diaries demonstrated the real world reality in that most patients did not fast on most days around the time of drug intake in either the intensive or maintenance phase. As a result the exposure risk (taking TB drugs in an unfasted state) was

recorded for 98-99% of patients; furthermore there was only one recorded adverse outcome due to an early relapse, resulting in such low power as to render an analysis futile. A limitation of our study design is that follow-up for relapses was short at 6 months, so later relapses would not have been detected. We did not record side-effects and were thus unable to determine whether fasting increased the likelihood of them.

It seems clear that lower serum concentrations, particularly with RIF can still be part of an effective therapy regimen in most patients. However, we could not accurately measure the effect of how the combination therapy might have positively influenced the treatment outcome. Although this synergistic effect can potentially be measured through a microdilution checkerboard assay[37], drug susceptibility testing is done individually for each drug[38]. Thus, any effect of a low RIF or INH concentration might be overcome by the effect of the other agents and thus not directly influence treatment outcome. The study was not designed to definitively prove whether poorer outcome was associated with RIF or INH-exposure. In this under-powered sub-analysis, the treatment outcome was neither influenced by impaired pharmacokinetics (RIF or INH) or by difference in the intake of TB drugs with food. However, the small number of poor treatment outcomes means that this could represent a type-II error. But it could also be that the effect of food on plasma-PK is insufficiently large to impact upon response to treatment either because there are other factors which dominate or because the bit of redundancy in multi drug therapy compensates for this. Moreover, we had hoped to be able to tease out a gradient of different categories of how much patients took their drugs fasting (e.g.: “most”,“some”,“rarely”) and compare outcomes (“treatment outcomes” and “conversion times”) but almost all were “rarely” fasting so this was not possible.

Although we have observed “subtherapeutic” Cmax levels of RIF in around 30% of patients, only one patient finally reported a bad treatment outcome. Interestingly when we calculate

low rifampicin exposure through AUC parameters, we observe that only 13% had low AUC exposure. Therefore, further investigation is needed to ascertain whether a review of the purported normal RIF-Cmax >8µg/mL is warranted and if the AUC-threshold proposed from hollow-fibre model studies[14] is a suitable predictor of treatment outcome.

Concerning INH, most patients had normal Cmax values. In contrast, if we consider the threshold of 52mgh/L suggested by Pasipanodya[14], most patients would be classified as having had a low INH-exposure. A main limitation of considering the AUC as a marker of drug exposure is that we had to calculate the AUC_{0-6} instead of AUC_{0-24} as suggested elsewhere[14] due to the limited sample points in our study.

It should be added that it is unclear what is the crucial determinant of drug efficacy at the cellular level and, if so, what the minimum drug exposure (in plasma) needs to be in order to have a high probability of efficacy.

Although therapeutic drug monitoring(TDM) is neither widely used nor recommended during TB treatment, TDM might contribute not only to the identification patients with low levels of RIF or INH but also to a shrinking the time to response and also the duration of treatment[39]. A two and six hour post-dose sampling strategy may facilitate the analysis of both agents and it seems reasonable and practical.

CONCLUSIONS

RIF in particular and also INH pharmacokinetics (both Cmax and AUC_{0-6}) were significantly affected by the intake of the drug with food in a proportion of patients. A clear relationship between the pharmacokinetics parameters and treatment outcome was not demonstrated.

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361 TRANSPARENCY DECLARATION

362 None to declare.

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Table 1 General characteristics of study participants

	TB n (%)
Sex (Male)	34 (56.7)
Age (years)	32.7 (23.7-45)
BMI (Kg/m²)[a]	21.8 (19.8-24.4)
Chronic diarrhoea	2 (3.33)
Intestinal parasite	4 (7.7)
Hospitalization	4 (6.67)
HIV (n=56)	2 (3.57)
TB type	
New	53 (88.3)
Relapse />6 months)	7 (11.67)
Sputum smear +	28 (46.7)
++	17 (28.3)
+++	15 (25)
Symptoms	
Weight loss (n=59)	37 (62.71)
Cough	55 (91.67)
Fever (n=59)	34 (56.67)
Thoracic pain	40 (66.67)
Dyspnoea	28 (46.67)
Haemoptysis	21 (35)
Sweating	37 (61.67)
Anorexia	28 (46.47)
TB diagnosis	
Conventional culture	44 positive (not undertaken in 16)
MODS	41 pos, 7 neg
RIF dosage (mg/Kg/day)	10.2 (9.6-11.2)
INH dosage (mg/Kg/day)	5.1 (4.8-5.6)
EMB dosage ((mg/Kg/day)	20.3 (19.1-22.4)
PZA dosage (mg/Kg/day)	25.4 (23.9-28)
Radiograph (Ralph score)*	22.6 (12.6-40.2)

n= 60 unless specified; a. Numbers are expressed in median and Interquartile range. *Ralph score obtained in the Chest radiography evaluation = % of affected lung + 40 (if cavitation is present)

Table 2. Multilevel linear model of the independent association of variables with rifampicin exposure (Cmax)

Variable	Proportional difference	CI	p-value
Fasting	1.15*	1.01-1.3	**0.036**
Tmax 4h	0.98	0.77-1.26	0.901
Tmax 6h	1.11	0.69-1.78	0.676
Sex	1.2†	1.02- 1.41	**0.027**
Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multilevel linear model. Interpretation of the proportional difference: * Rifampicin Cmax on the fasting day was 15% higher than rifampicin Cmax during the non-fasting day. † rifampicin Cmax in females were 20% higher than rifampicin Cmax in males.			

593 Table 3 . Multivariate regression model of the independent association of variables with
594 isoniazid exposure (Cmax)

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Variable	Proportional difference	CI	p-value
Fasting	1.14*	1.02-1.28	**<0.001**
Sex	1.26	1.08 – 1.48	0.176
Isoniazid dose	1.25	1.01-1.53	**0.038**
Note: The model was considered based on the natural logarithm of the Cmax values. The proportional difference was calculated as the exponential of the coefficient obtained for each variable in the multivariate model. Interpretation of the proportional difference: * Isoniazid Cmax on the fasting day was 14% higher than isoniazid Cmax during the non-fasting day. †			

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607 Annex 1: High-performance liquid chromatography (HPLC) assays.

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