Supplementary files

1. Metformin concentration analyses

Urine and plasma concentration analyses were performed with validated ultra performance liquid chromatography assays at the Radboud university medical center, Nijmegen, the Netherlands. Extraction of metformin from plasma (200 µL) was performed with a 1-butanol-hexane (50:50, v/v) mixture under alkaline conditions followed by back-extraction into diluted phosphoric acid (0.1%). Extraction from urine samples followed the same procedure after dilution (1:10, v/v) in blank human plasma. Chromatography was carried out using an Acquity UPLC HSS T3 1,8 um 2,1 x 100 mm analytical column (Waters, Milford, MA, USA), with an isocratic mobile phase of 100% phosphate buffer (0.02 M), alternated with a wash buffer (combination of phosphate buffer (0.02 M) and acetonitrile (50:50, v/v)) in between samples, pumped at an isocratic flow rate of 0.6 mL/min. The detection wavelength was set at 236 nm. Accuracy across five metformin quality control samples measured in three runs (n=15) ranged from 101-103% in plasma and 98-101% in urine. Intraday precision (n=5) ranged between 1.2-5.8% in plasma and 2.7-8.9% in urine. Interday precision (n=15) varied between 0-2.4% in plasma and 0-3.9% in urine. The lower and higher limit of quantitation (LLOQ and HLOQ) were 0.01 and 5.0 mg/L in the plasma assay, respectively, and 2.1 and 2091 mg/L in the urine assay, respectively. Finally, a stability analysis in plasma and urine confirmed that metformin was stable for a minimum of 7 days at room temperature.

2. Rifampicin concentration analysis and pharmacokinetic evaluation

Rifampicin concentrations were determined to confirm exposure to the perpetrator drug during the first sampling session. For every patient, rifampicin was measured in all eight blood samples, namely at 0 (pre-dose), 1, 2, 3, 4, 5, 6 and 8 hours after witnessed ingestion of metformin. However, only by the first three patients rifampicin was ingested together with metformin at time point zero. Because of the high occurrence of vomiting with this study sequence, it was decided to administer rifampicin three hours after metformin ingestion (see for more details Supplementary file 4).

Analysis of rifampicin concentrations in plasma was performed at the Pharmacokinetic Laboratory of the Faculty of Medicine of Universitas Padjadjaran using a validated ultraperformance liquid chromatography (UPLC) method as used previously [1]. Accuracy was between 95.1% and 102.4% for plasma samples and between 94.5% and 100.7% for CSF samples, depending on the concentration level. The intraday and interday coefficients of variation were <4.2% over the 0.26–30 mg/L concentration range for rifampicin in plasma and <3.4% over the 0.25–30 mg/L range.

A noncompartmental analysis with WinNonLin version 4.1 (Pharsight Corp., Mountain View, CA) was performed to compute the pharmacokinetic parameters of rifampicin. The maximum concentration in plasma was defined as C_{max} and the time to this maximum concentration as T_{max}. Both parameters were directly derived from the plasma concentration-time data. The area under the concentration-time curve from 0 to 5 hours post dose ($AUC_{0\text{-}5h}$) was calculated for all patients using the Linear Up Log Down trapezoidal rule from 0 up to the last concentration.

The Supplementary Table show the results of the pharmacokinetic analysis of rifampicin. The elimination rate constant (calculated from at least three data points in the elimination phase) and elimination half-life could not be calculated due to the lack of data points beyond 5 hours post-dose. Results seem similar or even somewhat higher (peak exposures) compared to previous results in TB-

DM patients [2]. Overall, these data support that there demonstrate significant exposure to the perpetrator drug.

Supplementary Table. Rifampicin pharmacokinetic parameters in TB-DM patients during PK-GC session 1.

Parameter	**PK-GC session 1**
$AUC_{0\text{-}5h}$ (mg/L*h) (n=23)	46 (15-109)
C_{max} (mg/L) (n=24)	14 (5-34)
T_{max} (h) - median	2 (1-5)

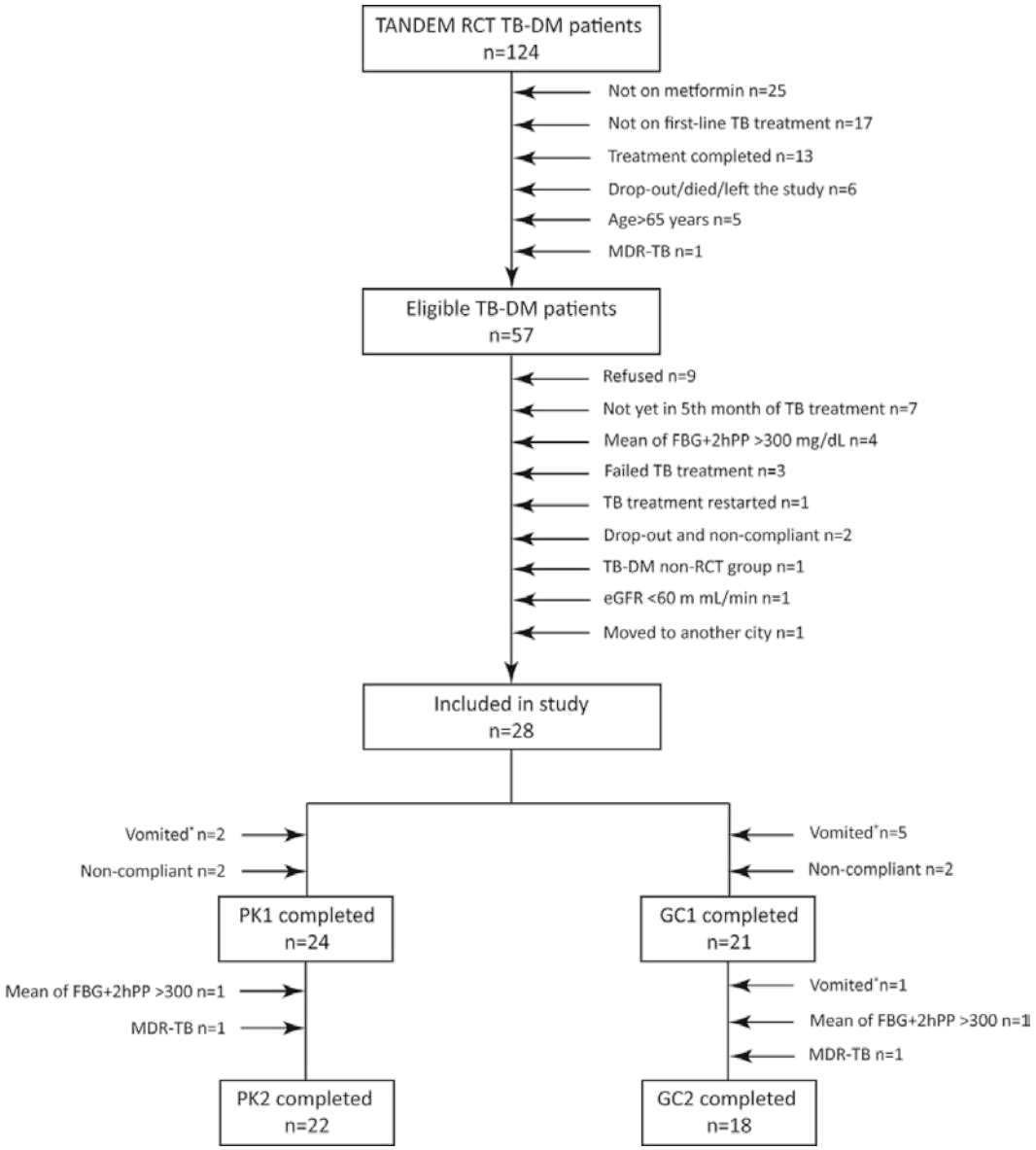
Pharmacokinetic parameters are expressed as geometric mean (range), unless stated otherwise. $AUC_{0\text{-}5h}$, area under the plasma concentration–time curve from time point 0 to 4 fours after drug intake; C_{max}, maximum plasma concentration; PK-GC session 1, pharmacokinetic-glucose curve sampling session 1 during TB treatment; T_{max}, time to maximum plasma concentration

3. Pharmacokinetic evaluation of metformin

A non-compartmental pharmacokinetic analysis was applied to calculate the pharmacokinetic parameters of metformin using Phoenix WinNonlin v.6.3 (Pharsight Corp., Mountain View, California). The maximum concentration in plasma was defined as C_{max} and the time to this maximum concentration as T_{max}. Both parameters were directly derived from the plasma concentration-time data. The area under the concentration-time curve from 0 to 8 hours post dose ($AUC_{0\text{-}8h}$) was calculated using the Linear Up Log Down trapezoidal rule from 0 up to the last concentration. The half-life was calculated by 0.693/β, in which β (first-order elimination rate constant) was derived with linear regression of the last three data points of the log-linear plasma concentration-time curve. Metformin plasma concentration were extrapolated until 12 hours for patients on twice daily metformin with the equation $C_{12h} = C_{8h} * e^{-\beta(t12-t8)}$. The apparent clearance of the drug (CL/F) was calculated using the formula dose/AUC_{0-8h} and the volume of distribution (Vd/F) was calculated by dividing CL/F by β.

Renal clearance of metformin was estimated using the formula $Ae_{0\text{-}\tau}/AUC_{0\text{-}\tau}$. The cumulative amount of metformin excreted in urine during the dosing interval ($Ae_{0\text{-}\tau}$) was obtained by summing the amount excreted in each 2-hour time interval. The net tubular secretion of metformin was calculated by subtracting creatinine clearance from renal clearance of metformin. Creatinine clearance was based on eGFR, as calculated with the CKD-EPI formula [3].

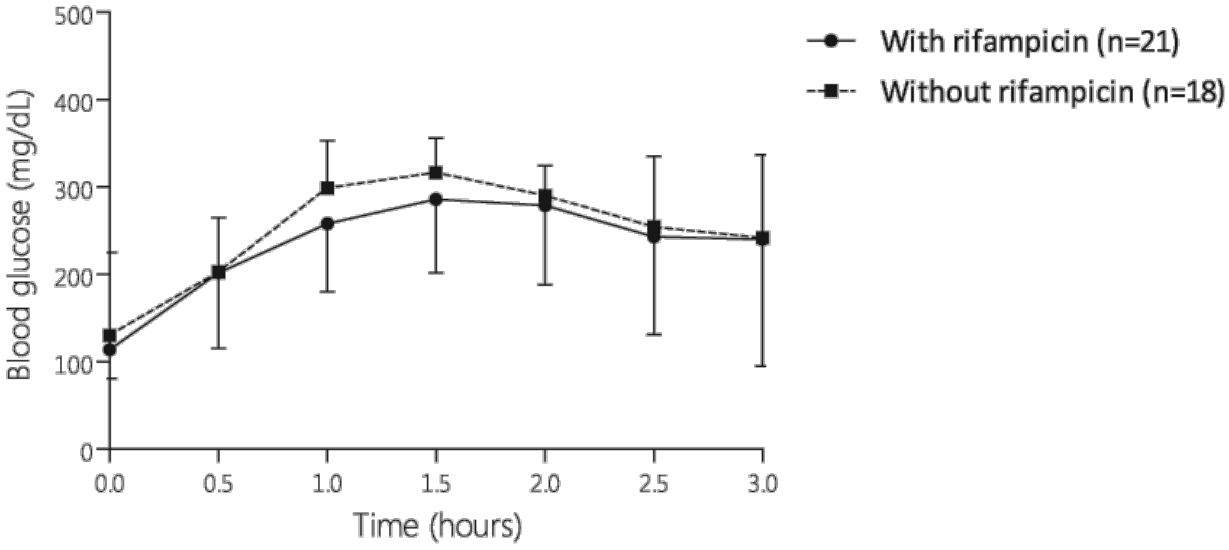
4. Eligibility, inclusion and exclusion of patients



Of the included patients (n=28), 24 patients underwent pharmacokinetic (PK) sampling: 2 patients had vomited in the morning of PK sampling and another 2 patients had been non-compliant during the week prior to the first PK session and were therefore excluded for PK assessment, and not included in the final data analysis. Of these 24 patients, 21 subjects underwent glucose curve (GC) sampling, because another 3 patients had vomited in the morning of GC sampling and were excluded for GC assessment, and thus were also not included in the final data analysis. All other PK/GC curves were included in the data analysis. Of note, after four out of the first six patients (67%) had experienced nausea and vomiting during their first PK-GC session, we adapted the study protocol and separated the administration of the study drugs in time and added the intake of metoclopramide (see Supplementary file 6).

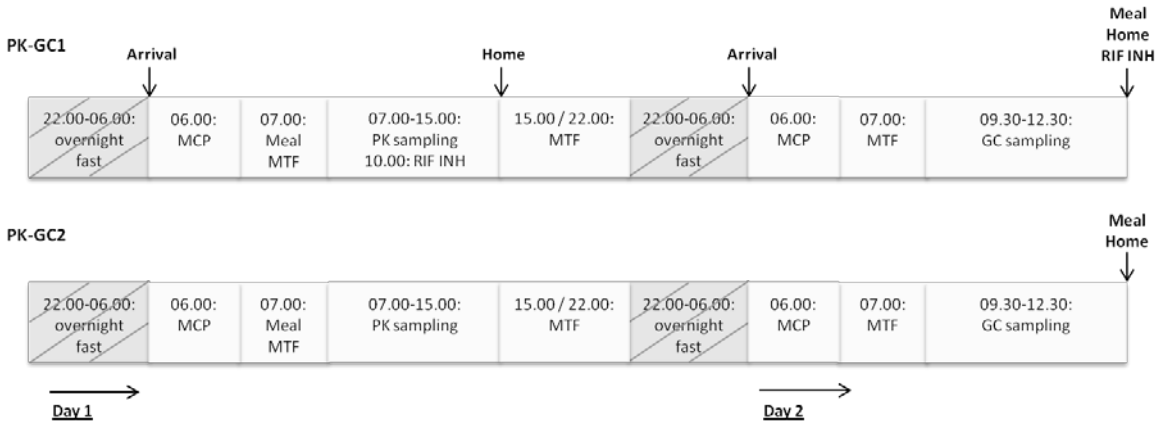
5. Blood glucose curves

Supplementary figure. The blood glucose concentration-time curves following ingestion of 75 g of glucose, 2.5 hours after metformin intake, during TB treatment (with rifampicin, n=21) and after cessation of treatment (without rifampicin, n=18).



Data are presented as median ± interquartile range

6. Schematic overview of the PK-GC sampling days



Overview of the PK-Glucose AUC sampling sessions, in the last month of the continuation phase of TB treatment (PK-GC1) and after completion of TB treatment following a 1-month wash-out period (PK-GC2). Meals were standardized to assure patients received the same meals at each PK-GC sampling session. In the morning of PK sampling metformin was taken with food to alleviate gastro-intestinal adverse effects, while in the morning of GC sampling metformin was taken on an empty stomach to facilitate glucose assessments.

INH= isoniazid, MCP= metoclopramide, MTF= metformin, GC= Glucose curve, PK= pharmacokinetic, RIF= rifampicin

Supplementary references

1. Yunivita V, Dian S, Ganiem AR, Hayati E, Hanggono Achmad T, Purnama Dewi A, et al. Pharmacokinetics and safety/tolerability of higher oral and intravenous doses of rifampicin in adult tuberculous meningitis patients. International journal of antimicrobial agents. **2016**;48(4):415-21.

2. Ruslami R, Nijland HM, Adhiarta IG, Kariadi SH, Alisjahbana B, Aarnoutse RE, et al. Pharmacokinetics of antituberculosis drugs in pulmonary tuberculosis patients with type 2 diabetes. Antimicrobial agents and chemotherapy. **2010**;54(3):1068-74.

3. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. **2009**;150(9):604-12.