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ORIGINAL ARTICLE

Fractal Geometry-Based Decrease in Trimethoprim-Sulfamethoxazole Concentrations in Overweight and Obese People

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Trimethoprim-sulfamethoxazole (TMP-SMX) is one of the most widely drugs on earth. The World Health Organization recommends it as an essential basic drug for all healthcare systems. Dosing is inconsistently based on weight, assuming linear relationships. Given that obesity is now a global "pandemic" it is vital that we evaluate the effect of obesity on trimethoprim-sulfamethoxazole concentrations. We conducted a prospective clinical experiment based on optimized design strategies and artificial intelligence algorithms and found that weight and body mass index (BMI) had a profound effect on drug clearance and volume of distribution, and followed nonlinear fractal geometry-based relationships. The findings were confirmed by demonstrating decreased TMP-SMX peak and area under the concentration-time curves in overweight patients based on standard regression statistics. The nonlinear relationships can now be used to identify new TMP-SMX doses in overweight and obese patients for each of the infections caused by the >60 pathogens for which the drug is indicated.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ There are no data describing the PKs of TMP-SMX in obese people.
WHAT QUESTION DOES THIS STUDY ADDRESS?
☑ The impact of body size on the TMP-SMX concentrations and PK parameters.
WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
☑ This study provides evidence that weight has a profound effect on TMP-SMX clearance and volume of distribution, based on nonlinear fractal geometry-based relationships. Second, it demonstrates the use of smaller clinical study populations based on optimal design and AI algorithms to identify complex clinical predictors.
HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ The use of AI requires much smaller patient sample sizes to identify important PK parameters for drug development and therapeutic studies than currently used in the field. Second, new TMP-SMX doses may need to be increased for treatment of many infections in overweight and obese patients.

Trimethoprim/sulfamethoxazole (TMP-SMX) is commonly used worldwide to treat infections caused by up to 60 different bacterial, protozoal, and fungal species. Consequently, it is perhaps the most widely prescribed antibiotic in the world. As an example, it is recommended by the World Health Organization as one of the essential basic drugs for any healthcare system.[1] The current dose recommendations for TMP-SMX were established based on studies in children and then extrapolated to the adult population.[2,3] Pharmacokinetic (PK) studies using noncompartmental techniques in immunocompromised and critically ill patients have largely supported the same dosing regimen.[4] The recommended dose has been capped at 100 mg/kg for sulfamethoxazole and 20 mg/kg for trimethoprim, based on a patient's total body weight (TBW). The underlying assumptions were that of a linear relationship among ideal body weight (IBW), TBW, and PK parameters, such as drug elimination and volume of distribution.[5,6] These assumptions were never formally tested for either drug in the combination and have been in use for more than 5 decades. The matter is now pressing because the population of the planet is now predominantly overweight and obese.[7,8] As an example, two of every three patients are either overweight or obese in the United States. In Polynesian countries, such as Tonga and Samoa, 90% of adults are obese, whereas in Middle Eastern countries, such as Egypt, 70% of women are obese.[9] TMP-SMX is already being extensively used in these populations. Here, we performed a prospective clinical experiment to identify the effect of obesity on TMP-SMX.

To date, we have examined up to eight antibiotics in carefully planned clinical experiments. In each single case, obesity was associated with changes in clearance and volume, which means that heavier patients achieved lower drug concentrations than leaner ones even when the same dose in milligram/kilogram was administered.[10] The second

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emerging theme has been the relationship between such factors as clearance and volume to the fractal geometry of patients, in contradistinction to the idea of linear relationships between weight and xenobiotic metabolism or volume of distribution.[11–15] In a bid to eliminate bias (i.e., one to find a relationship of weight to xenobiotic metabolism and volume of distribution), we have switched our analyses to agnostic machine learning or artificial intelligence (AI) algorithms. With this approach, all clinical parameters, including clinical chemistry results, demographics, physical examination findings and characteristics, comorbidities, weight measures (including IBW and TBW), height, body mass index (BMI), and body surface area (BSA), are examined as potential predictors of measures of drug elimination and volume in one model. We let the unbiased algorithms select and rank those significant factors that truly drive these PK parameters in order of importance, without the modeler tipping the scale in favor of their favorite covariate. We have previously used multiple adaptive regression splines (MARS) and Random Forests, including their forebear, classification and regression trees, for identifying and ranking covariates in PK and pharmacodynamic (PD) modelling exercises, and each time these approaches have outperformed the previously recommended industry standards based on parametric approaches and frequentist statistics.[16–23] The philosophy underlying all these AI methods is model prediction. Because they are distribution and assumption-free, they test many important but collinear variables in simultaneous models, as are, for example, measures of weight, they also test nonlinear and high-order interactions, not possible with standard PK approaches and software.[24] Importantly, they do not assume what the structure of the covariate should be like.

MARS, which was first introduced by Jerome Friedman[19] in 1991, is an AI algorithm that combines adaptive recursive partitioning and spline-fitting procedures in a manner that retains the positive aspects of both procedures in one algorithm. This makes MARS suitable for simultaneous analysis of linear, nonlinear, and highly dimensional inputs. Here, we used MARS, together with experimental study design, to examine nonlinear relationships between weight and clearance and volume of TMP-SMX in obese subjects.

METHODS

Study design and setting

This study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center (042010-069) and Texas Tech University Health Sciences Center (A10-3592). The study was registered at clinicaltrials.gov on July 20, 2010 (ClinicalTrials.gov identifier: NCT01167452). We recruited healthy people 18 years of age or older without ear infections, urinary tract infections, bronchitis, traveler's diarrhea, *Pneumocystis carinii* pneumonia, or any other bacterial infection to determine study eligibility.

We designed a clinical experiment in which we recruited and admitted 36 healthy adults distributed equally into the following BMI categories: <25 kg/m^2, 25–40 kg/m^2, and >40 kg/m^2. Twelve volunteers with BMI <25 kg/m^2, or 25–40 kg/m^2, or >40 kg/m^2 were recruited. Each BMI group had 50% women and 50% men. People who were interested in the study were consented at the University of Texas Southwestern Clinical and Translational Research Center. People who were pregnant, had abnormal liver function tests (transaminases >10 times upper limit of normal, alkaline phosphatase >5 times upper limit of normal, total bilirubin >5 times upper limit of normal, or history of allergy to sulfones, sulfonamides, or trimethoprim were excluded from study participation. People with a history of colon resection, gastric bypass, lap band, or any other condition that inhibits gastric absorption were not allowed to participate in the study. People who provided a signed informed consent, and were deemed eligible after a screening visit, were scheduled for admission in the University of Texas Southwestern Clinical and Translational Research Center for an overnight stay. Each person received a single supervised oral dose of TMP-SMX (1600 mg/320 mg) under supervision of both the study nurse and physician in the Pharmacology Unit. Blood was drawn via an intravenous catheter just prior to the dose, and then at 1, 2, 4, 8, 12, and 24 hours after the drug dose. The seven different time points over 24 hours were chosen based on optimal sampling theory.[25,26] The intravenous catheter was removed after the 24-hour blood draw, and the person was discharged from the study.

Drug assay

A robust liquid chromatography tandem mass spectrometry method for the analysis of TMP-SMX, sulfamethazine content in plasma was developed and validated. Each 100 μL plasma sample was spiked with 100 μL of internal standard and protein was precipitated from the mixture using a 500 μL mixture of acetonitrile:methanol (80:20). The supernatant was removed, evaporated to dryness, and reconstituted with 100 uL of methanol:deionized water (50:50). Constituents of a 10-μL injection volume were separated using a gradient elution and Kinetex column (2.6 μ, pentafluorophenyl 150 × 2.1 mm), and eluted using a gradient mobile phase (0.1% formic acid in deionized water:0.1% formic acid in methanol) at 0.25 mL/min. The total sample run time was 23.5 minutes; 10.5 minutes for sample separation and 13 minutes for equilibration. The precursor to product ions were detected using electrospray ionization-tandem mass spectrometry in the positive ion mode with multiple reaction monitoring (trimethoprim, 291.1→230.0 and sulfamethoxazole, 253.9→156.0). The calibration curve was linear ($r = 0.9990$) with a 1 ng/mL lower limit of quantification and 1,500 ng/mL upper limit of quantification. Total analytical variation was $<5\%$ relative SD; intra-day and inter-day precision and accuracy were $<5\%$ relative SD.

PK modeling

We comodeled all 252 concentrations of either TMP or SMX using the ADAPT 5 program of D'Argenio *et al*.,[27] and explored a one, two, and three-compartment model with first-order input and elimination, by implementing the maximum-likelihood solution via the expectation-maximization algorithm.[26,27] The best number of compartments was chosen using Akaike Information Criteria,

Bayesian Information Criteria, and rules of parsimony (Occam's razor).[28,29]

MARS analysis

MARS builds piecewise general additive models that describe nonlinear relationships between the target response and dependent variables allowing for simultaneous interactions between the covariates to change the slope between the target response and each variable using basis functions (BFs) defined within the dataspace. By means of knots or hinges for continuous variables or selected groups for ordinal or categorical variables, the BF essentially uses internal heuristics based on the data to build models. BFs that minimize mean squared errors are retained in a backward step elimination process. Because the MARS heuristic procedures are rooted in information theory, the BF knots define thresholds, which have been found to be physiologically meaningful in some of our prior work. Separate models were explored for each of the following dependent variables: volume, absorption constant, and elimination constant. The covariates included in each model were the following: self-identified race, ethnic group, comorbid conditions, age, gender, height, weight, IBW, TBW, lean body weight, BSA, BMI, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen levels, calculated creatinine clearance, and serum creatinine concentration. Because the MARS algorithms are inherently resistant to collinearity, have the ability to rank variable importance, and can assign surrogates for missing data, we examined models that included the measured height and weight, as well as calculated values from these, such as BMI, BSA, and IBW, in addition to the height and weight variables. The reason was to determine the best predictor for PK parameters, including those commonly used measures of patient's weight and metabolic function: each measure would be chosen in its own merit agnostically if it was good predictor. We used both the V-fold cross-validation procedure and the MARS legacy mode approaches to test selected models. All other options and limits were left in the default setting.

Fractal geometry analyses

If measures of body size were selected as significant predictors by MARS, we log-transformed the values and PK parameter estimate, after Benoit Mandelbrot's foundational work with fractals.[10–15,30,31] In this approach, the slope of the relationship between PK parameter and a measure of body size is examined as fractal dimension. These factors were then reincorporated as potential predictors, and then examined using standard regression models, as described above.

Software

For compartmental PK analyses, we used the ADAPT 5 program of D'Argenio *et al*.[27] MARS was implemented in Salford Systems Data Mining and Predictive Analytics Software (Salford Systems, San Diego, CA). Graphing was performed by importing data into GraphPad Prism 6 (GraphPad Software, La Jolla, CA).

Table 1 Clinical and demographic characteristics of 36 patients enrolled in the study

Characteristics	Median, range; no. of patients (%)
Female gender	18 (50)
Race	
White	26 (72)
Non-white	10 (28)
Ethnicity	
Hispanic	6 (17)
Non-Hispanic	30 (87)
Age, years	37 (18–77)
Weight, kg	89.95 (45.80–232)
IBW, kg	84.26 (51.48–172.13)
Height, m	1.69 (1.55–2.05)
BMI, kg/m^2	30.25 (16.2–59.1)
Serum creatinine, mg/dL	0.78 (0.54–1.26)
Creatinine clearance, nL/min	129.94 (75.18–359.34)
Blood urea nitrogen, mg/dL	12.5 (7–23)
AST, U/L	20.5 (12–59)
ALT, U/L	19.5 (8–76)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; IBW, ideal body weight.

RESULTS

The clinical and demographic characteristics in the subjects we recruited are shown in **Table 1**. The weight ranged from 45–232 kg, a fivefold difference between the minimum and maximum TBW, demonstrating the intended wide weight range accomplished by the study design. The weight was not normally distributed. Forty-four percent were patients with components of metabolic syndrome, with higher rates of 23% in BMI 25–40 kg/m^2 and 100% in the BMI $>$40 kg/m^2 group compared to those with BMI $<$25 kg/m^2, as would be expected. With regard to adverse events, one person fainted during the screening blood draw, which we judged not to be related to the study drug. Three people (8.3%) developed a mild headache postdose, which was deemed possibly related to the study drug. One of the people who had a headache also had elevated blood pressure, skin irritation under the transparent medical dressing over the intravenous line insertion site, as well as a mild rash around the neck and ears; these reactions were not considered to be related to the study drug.

Table 2 shows criteria scores for choosing the best SMX compartment model: by all criteria, the SMX concentrations were best described by a one-compartment model. Specifically, this was not a two or three-compartment model with fat mass and fat-free mass as different compartments. The population PK parameter estimates values were not normally distributed for elimination constant and volume (D'Agostino normality test $P < 0.001$), but the P value was 0.570 for absorption constant. The median (range) and percentage of coefficient of variation for elimination rate constant were: 0.060 (0.015–0.180) hr^{-1} and 54.13%; for volume 9.60 (3.60–41.0) L and 62.84%; and for absorption constant 1.10 (0.46–1.90) hr^{-1} and 31.26%. The observed vs. model predicted concentrations are shown in **Supplementary Figure S1**.

Table 2 Comparison of a one, two, and three PK compartment model for TMP and SMX

	One-compartment	Two-compartment	Three-compartment
SMX			
Akaike Information Criterion	**2,680.91**	2,690.24	2,695.75
Bayesian Information Criterion	**2,709.15**	2,732.59	2,745.16
Negative 2 log likelihood	**2,664.91**	2,666.24	2,667.75
TMP			
Akaike Information Criterion	**1,191.04**	1,200.62	1,212.85
Bayesian Information Criterion	**1,219.27**	1,242.98	1,262.27
Negative 2 log likelihood	**1,175.04**	1,176.62	1,184.85

The figures in bold represent best score.
PK, pharmacokinetic; SMX, sulfamethoxazole; TMP, trimethoprim.

Next, the SMX one-compartment model parameter estimates for each patient were then examined together with all the potential anthropometric, clinical, laboratory, and other physiological predictors *in toto*, using MARS. MARS identifies local relationships in small data regions in a piecewise fashion, which are delineated by hinges, with output as BFs. The only predictor for the SMX elimination rate constant, of all 17 possible covariates, was patient weight in kilograms, which was described by the BF:max (0, WT-45.8). This means that for patients with weight >45.8 kg (i.e., the hinge), the weight was the significant predictor of elimination rate constant, otherwise for weight <45 kg the effect was "0" (zero). It should be noted that the hinge was at the lowest weight in the dataset, thus, we examined for robustness and interpretability, including changes in the error rates. We used both the V-fold cross-validation procedure, in which the algorithm splits the dataset randomly into smaller datasets to identify post-test prediction, and identified a post-test R^2 of 0.51. Next, we examined the log-log transformation of elimination rate constant and weight for the region of data identified by the hinge of weight >45.8 kg, and identified the relationships shown in **Figure 1a**. The slope of that log-log transformation was -0.74 ± 0.19 ($P = 0.0004$), almost exactly three-fourths. This fractal dimension is well known in the relationship between metabolic rates and weight of organisms.[32,33] In **Figure 1a**, the relationship between log-transformed SMX elimination rate constant (and, hence, clearance) and weight explained 98% of the variance. Since the total SMX concentration of the 24-hour dosing interval, defined by the 0–24 hour area under the concentration-time curve (AUC_{0-24}), is given by *f**dose/clearance (where *f* is absorbed fraction), this

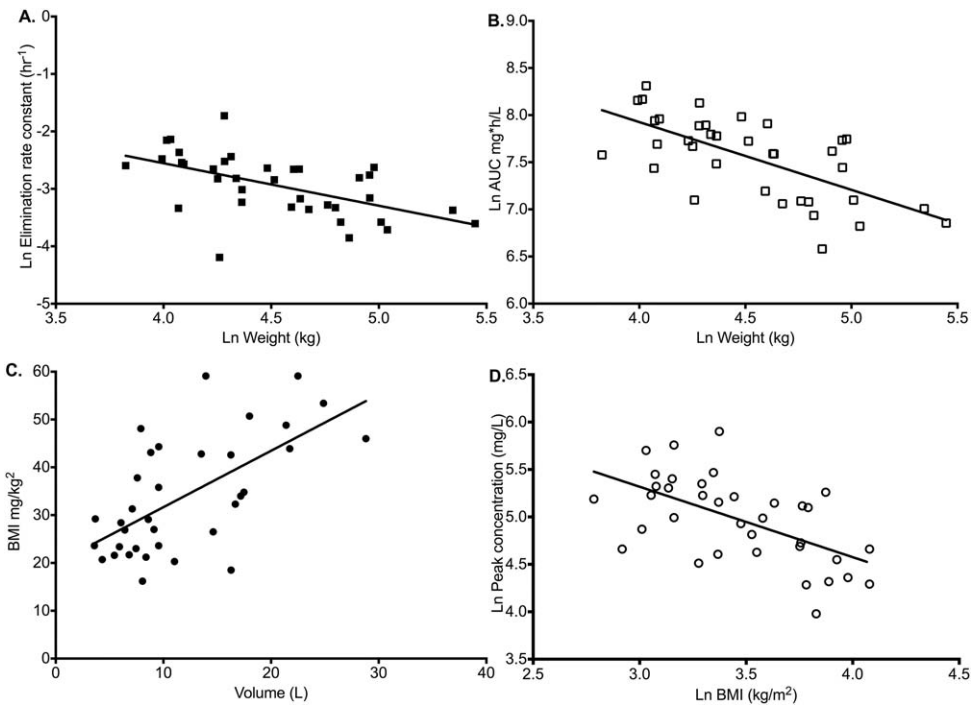

Figure 1 Log-log relationship between weight and sulfamethoxazole pharmacokinetics (PKs). The natural logarithm (Ln) was used for the log-transformation of values. Both weight in kilograms and body mass index (BMI), as well as sulfamethoxazole (SMX) PK parameters and concentrations, were log-transformed following the method of Mandelbroit *et al*.[30,31] (**a**) Shows the relationship between elimination rate (and thus xenobiotic metabolism and excretion) and weight in patients >45.8 kg, which was three-fourths. (**b**) Shows the slope of the relationships between the area under the concentration-time curve (AUC), as derived by trapezoidal rule and weight, for which the log-log slope was also three-fourths. (**c**) Shows the relationship between volume and BMI in patients with BMI >16.2 kg/m^2, that is above the multiple adaptive regression splines-derived hinge. (**d**) Shows the relationship between BMI and peak SMX concentration, which showed a slope of three-fourths on log-log transformation, unlike the volume of distribution.

Table 3 BFs for factors chosen as affecting SMX volume

BF	BF definition	Coefficients	No. (%) of affected patients	Explanation (description of BF)
BF_0		5.24701	36 (100)	
BF_1	Max (0, BMI-16.2)	0.331725	35 (97)	For each increase in BMI above 16.2, the volume of distribution increased by 33%
BF_3	Max (0, ALT-8)		33 (92)	Interacts with BF_5
BF_5	Max (0, 0.78-SCR)*BF_3	2.64024	17 (47)	Volume of distribution increased >2.6 fold increase when ALT >8 U/L and serum creatinine levels <0.78 mg/dL

ALT, alanine aminotransferase; BF, basis function; BMI, body mass index; SCR, serum creatinine; SMX, sulfamethoxazole.

means that if the AI (MARS) findings are correct, then, in clinical terms, the SMX AUC_{0-24} will be lower in heavier patients compared with leaner patients. **Figure 1b** shows that this is indeed the case using the AUC_{0-24} derived trapezoidal method directly on the concentrations measured (not PK model-derived). Uniquely, in **Figure 1b**, the slope of the log-transformed data was -0.72 ± 0.14 ($P < 0.0001$), also close to three-fourths. These results directly confirm the clinical relevance of weight on SMX concentrations achieved, the accuracy of MARS algorithm in predictor selection of weight for elimination rate constant, and the fractal relationships, based on standard regression statistics.

Using similar steps, MARS identified three factors associated with SMX volume of distribution as BMI, serum creatinine, and alkaline phosphatase levels, which were described by BFs in **Table 3**, leading to the relationship:

$$\text{Volume (L)} = 5.24701 + 0.331725 * BF_1 + 2.64024 * BF_5 \quad (1)$$

In **Table 3**, baseline serum creatinine levels and ALT levels were equally and highest ranked at 100%, followed closely by BMI at 69%. The three variables explained 64% of the variance in SMX volume of distribution, which is somehow reassuring. BF_5 is an example of a high-order interaction: ALT levels >8 U/L (i.e., BF_3 with hinge at 8 U/L) affected the effect of serum creatinine on volume, but only in people with serum creatinine <0.78 mg/dL (i.e, hinge at 0.78 mg/dL). On the other hand, for BF_1, the relationship between volume and BMI >16.2 kg/m^2 (and not the BMI >25 mg/m^2 standardly used to define overweight) was linear, and is shown in **Figure 1c**. The slope was 1.18 ± 0.25 ($P < 0.0001$), based on standard frequentist regression. This illustrates the versatility of MARS in simultaneous delineation of linear and nonlinear relationships, as well as higher order interactions at the same time; once hinges are identified, standard regression methods confirmed the relationship. Because the effect of volume of distribution is best seen on peak drug concentration (peak is proportional to dose/volume), we plotted log values of the observed highest concentration (not PK model-derived) vs. log BMI, which revealed the results shown in **Figure 1d**. The slope was -0.74 ± 0.18 ($P = 0.0002$), statistically similar to three-fourths. On the other hand, MARS identified no predictors for the SMX absorption constant.

For trimethoprim concentrations, PKs were also found to be best described by a one-compartment model (**Table 2**). The population PK parameter estimates values were not normally distributed for the elimination constant ($P < 0.0001$) and volume ($P = 0.034$), but were normally distributed for absorption constant ($P = 0.481$). The median and percentage of coefficient of variation for the elimination rate constant were: 0.03 (0.02–0.14) hr^{-1} and 51.19%; for volume 52 (16–141) L and 62.84%; and for absorption constant 2.10 (0.63–3.70) hr^{-1} and 30.53%. The observed vs. model-predicted concentrations are shown in **Supplementary Figure S2**.

MARS identified both BMI and weight as the significant predictors of the trimethoprim elimination rate constant, attesting to the ability of the algorithm to break co-linearity (BMI is calculated from weight) and revealing a complex nonlinear interaction. Both weight and BMI predicted 41% of the variance in the elimination rate constant. The relationship between these parameters and trimethoprim elimination rate constant was defined by the equation:

$$\text{Elimination rate constant} = 0.0399 + 0.00036 * BF_4 \quad (2)$$

where BFs are as defined in **Table 4**. As is shown in **Figure 2a**, which is a log-log plot, the relationship between patient's weight and trimethoprim elimination rate constant had a slope of -0.61 ± 0.17; the R^2-value was 0.28. **Figure 2b** shows the relationship between BMI and the trimethoprim elimination rate constant had a slope -0.56 ± 0.20; and the R^2 was 0.18. We also confirmed the effect of weight on the trapezoidal rule-derived AUC_{0-24}, with the log-log plot shown in **Figure 2c**, which had a slope of 0.63 ± 0.14 ($P < 0.0001$), a fractal dimension virtually the same as for ADAPT-derived elimination rate and weight.

The predictors of trimethoprim volume were BSA, serum creatinine, and AST levels. The relationship was described by the equation:

$$\text{volume} = 32.4053 + 29.1674 * BF_1 + 18.7887 * BF_5 \quad (3)$$

where the BFs are as defined in **Table 4**. The most important predictors were AST levels and serum creatinine levels at baseline equally weighted at the apex (100%), and interacting with each other, followed by BSA at 77% variable

Table 4 BFs for TMP elimination rate constant and volume

BF	BF definition	Coefficients	No. (%) of affected patients	Description of BF
Elimination rate constant				
BF_0	Constant/intercept	0.03999123		
BF_2	Max (0, 78.5-weight)		20 (56)	Weight in kg
BF_4	Max (0, BMI-16.2)*BF_2	0.00026024		Interacts with BF_2
Volume				
BF_0	Constant/intercept	32.4053		
BF_1	Max (0, BSA-1.46631)	29.1674	35 (97)	
BF_4	Max (0, 0.74-SCR)		14 (39)	Interacts with BF_5
BF_5	Max (0, AST-12)*BF_4	18.7887	14 (39)	

AST, aspartate aminotransferase; BF, basis function; BMI, body mass index; BSA, body surface area; SCR, serum creatinine; TMP, trimethoprim.

importance. The model shown in Eq. 3 explained 54% of trimethoprim volume of distribution variance. The log-log relationship between BSA and trimethoprim volume had a slope of 1.312 ± 0.357 ($P < 0.001$), as shown in **Figure 3a**. The slope is equivalent to the fractal four-thirds, the inverse of three-fourths. The change in volume of distribution with BSA would best be reflected by changes in peak concentration, which is what was found when peak concentration was plotted against BSA in **Figure 3b** in the range delineated by BF hinges using standard regression analysis. **Figure 3b** is not a log-log slope, but had a slope of -3.73 ± 1.21 (inverse of one-fourth), with a $P = 0.004$.

DISCUSSION

Here, we show that different measures of body size, especially weight and BMI, have a profound effect on the xenobiotic metabolism of TMP-SMX, as well as volume of distribution based on fractal geometry-based relationships manifest as the three-fourths and one-fourth power law. However, none one-fourth fractal signatures were also identified, including 0.63 for the log-log relationship between weight and both trimethoprim elimination rate constant and AUC_{0-24} as well as 1.18 for SMX volume and BMI. We show that the effect of body size on these PK parameters directly affects the concentrations achieved: larger and heavier patients achieved lower peak and AUC_{0-24} concentrations than their leaner counterparts did. This led to a 5.6-fold range difference in SMX AUC and 6.9-fold in peak concentrations, for example. PK/PDs science using preclinical models has revealed that in virtually all antibiotics drug concentrations have a profound influence on microbial effect and resistance suppression: antibiotics have a concentration-effect relationship.[34] Several groups have shown the same on microbial response and resistance suppression in patients on antibacterial compounds, antifungal compounds, and antiparasitic compounds.[22;35–38] We expect to see the same effects in obese patients treated with TMP-SMX, which is used everywhere across the globe. PK/PD-based studies for each of the almost 60 pathogens that this drug is used for are urgently needed in order to identify the optimal exposures to kill each pathogen, and with those results and our PK models, optimal doses designed for treatment of this large patient base, given the global burden of obesity. With this approach, dosing can be individualized by type of infection and patient weight.

Second, our study shows the effect of fractal geometry of different measures of human body sizes on both xenobiotic metabolism and volumes of distributions of drugs. We have previously observed a similar impact of weight in kilograms and BMI on clearance and volume of echinocandin antifungals, antituberculous agents, and biologics.[11–15] The current study also sheds light on what aspects of body size matter: there have been decades of old clinical debates as to whether such parameters as IBW (i.e., weight associated with maximum life expectancy), TBW, or lean body weight (TBW minus weight of body fat), BMI, or BSA should be used to dose. In the case of TMP-SMX, the agnostic method MARS did not find IBW or lean body weight as important predictors. Instead, patient weight (and not dry weight) and BMI affected the elimination of both TMP and SMX. Unexpectedly, BSA had an effect on trimethoprim volume and peak concentrations. A literature search revealed that BSA dosing correction has been used with the cephalosporin cefotiam in Sumo wrestlers in Japan, and indeed is commonly used for dosing of cancer chemotherapy agents.[39]

Third, our study has implications on study design for use in PK/PD studies in people, as well as statistical methods used to analyze these results. We demonstrated the versatility of AI analyses in identifying covariates of primary PK parameters. This is important for two reasons. First, these findings were confirmed by standard frequentist statistics. Essentially, MARS identified relationships and hinges at which of these relationships changed or ended, after which standard linear regression methods confirmed the same, with good *P* values. The latter was possible because selection of hinges avoids the problem of averaging out measures of association and slopes across data regions in which relationships change direction. In other words, the question is not about if there are differences in medians or slopes across two samples of populations, but if there are predictive relationships above and below certain covariate thresholds, and if these interact among themselves as well as with the main clinical pharmacology parameter. Second, there were some findings on MARS that while still present when frequentist approaches were used, no longer reached statistical significance. This is because of sample sizes. MARS and other AI methods require much smaller patient sample sizes to identify important clinical correlates, based on our prior work.[21] The

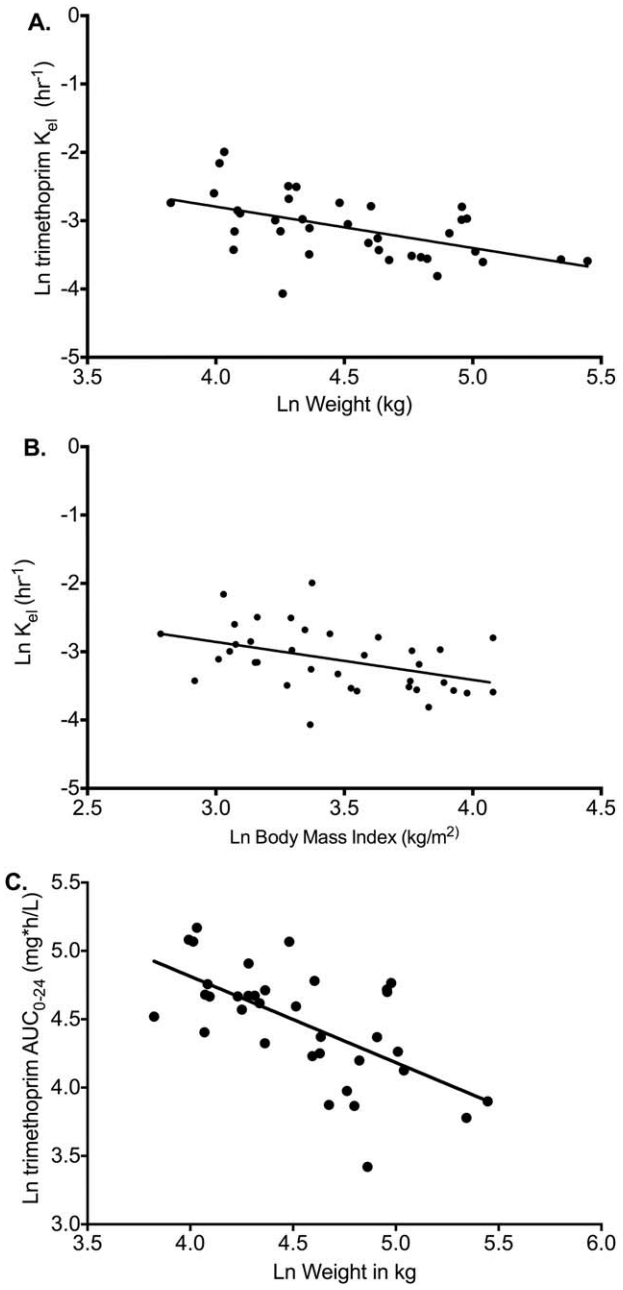

Figure 2 Log-log relationship between weight and trimethoprim metabolism. The natural logarithm (Ln) was used for the log-transformation of values. (**a**) Unlike sulfamethoxazole, the relationship between trimethoprim metabolism and weight in kilograms, did not obey the three-fourths power laws, instead with a dimension of 0.61. (**b**) Similarly, body mass index vs. model-derived elimination rate constant had a log-log slope of 0.56. (**c**) Trapezoidal rule derived 0–24 hour area under the concentration-time curve (AUC_{0-24}) vs. weight regression revealed a log-log slope that was virtually the same as for weight and elimination rate in panel **a**.

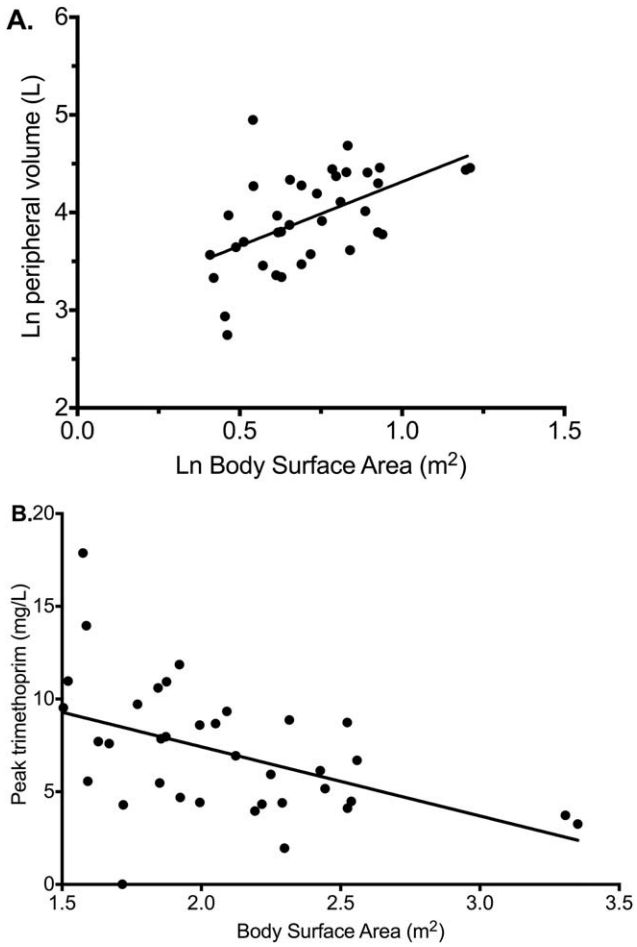

Figure 3 The relationship between body surface area and trimethoprim volume. The natural logarithm (Ln) was used for the log-transformation of values. (**a**) A surprise was that body surface area was a predictor of trimethoprim volume of distribution, with a log-log slope of four-thirds or the inverse of three-fourths. It would have been expected that these two parameters would be related by 1/length or height. (**b**) Interestingly, the observed peak concentration (amount of drug/volume of distribution) revealed a relationship characterized by the inverse of one-fourth. Although these dimensions are unusual, they still, nevertheless, are part of the one-fourth power laws.

cross-validation features of these algorithms allow for automatic creation of learning and test sets from the same data, so that the main outcome is predictive accuracy. Third, our study design was in the form of a clinical experiment. We recruited equal groups of patients targeted and stratified by weight bands, so that all other aspects were equal (i.e., *ceteris paribus*), except the weight and weight-related comorbid conditions. This eliminates noise and bias, allowing for a smaller group of patients to be exposed to the study drug. Fourth, we applied optimal design methods and optimal sampling theory to identify when we would get blood samples, and targeted the most information-rich timepoints. Our approach that incorporates these four factors into design and analyses offers a paradigm to minimize the numbers of patients exposed to drugs in PK studies that will use AI algorithms that can identify predictors from small numbers of samples.

In conclusion, different measures of body size, especially weight and BMI, have a profound effect on the xenobiotic metabolism of TMP-SMX as well as volume of distribution, based on fractal geometry based relationships. PD targets

for TMP-SMX are needed to help guide the dosing regimens for obese patients. The use of MARS and other AI methods require much smaller patient sample sizes to identify important clinical correlates, which is important given the small numbers of patients enrolled in many PK studies.

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1. World Health Organization. WHO Model List of Essential Medicine. 19th list. <http://www.who.int/medicines/publications/essentialmedicines/en/>. Accessed 4 June 2016.
2. Hughes, W.T., Feldman, S., Chaudhary, S.C., Ossi, M.J., Cox, F. & Sanyal, S.K. Comparison of pentamidine isethionate and trimethoprim-sulfamethoxazole in the treatment of Pneumocystis carinii pneumonia. *J. Pediatr.* **92**, 285–291 (1978).
3. Furio, M.M., Weidle, P.J., Wordell, C.J. & Liu, H.H. Management of Pneumocystis carinii pneumonia in patients with AIDS and other conditions: experience in a Philadelphia university teaching hospital. *Pharmacotherapy* **8**, 221–234 (1988).
4. Chin, T.W., Vandenbroucke, A. & Fong, I.W. Pharmacokinetics of trimethoprim-sulfamethoxazole in critically ill and non-critically ill AIDS patients. *Antimicrob. Agents Chemother.* **39**, 28–33 (1995).
5. Huxley, J.S. *Problems of Relative Growth* (The Dial Press, New York, 1932).
6. Green, B. & Duffull, S.B. What is the best size descriptor to use for pharmacokinetic studies in the obese? *Br. J. Clin. Pharmacol.* **58**, 119–133 (2004).
7. Kontis, V. *et al.* Regional contributions of six preventable risk factors to achieving the 25 x 25 non-communicable disease mortality reduction target: a modelling study. *Lancet Glob. Health* **3**, e746–e757 (2015).
8. Ogden, C.L., Carroll, M.D., Kit, B.K. & Flegal, K.M. Prevalence of childhood and adult obesity in the United States, 2011–2012. *JAMA* **311**, 806–814 (2014).
9. Swinburn, B.A. *et al.* The global obesity pandemic: shaped by global drivers and local environments. *Lancet* **378**, 804–814 (2011).
10. Hall, R.G. 2nd, Swancutt, M.A., Meek, C., Leff, R.D. & Gumbo, T. Ethambutol pharmacokinetic variability is linked to body mass in overweight, obese, and extremely obese people. *Antimicrob. Agents Chemother.* **56**, 1502–1507 (2012).
11. Hall, R.G., Swancutt, M.A. & Gumbo, T. Fractal geometry and the pharmacometrics of micafungin in overweight, obese, and extremely obese people. *Antimicrob. Agents Chemother.* **55**, 5107–5112 (2011).
12. Pasipanodya, J.G., Hall, R.G. 2nd & Gumbo, T. In silico-derived bedside formula for individualized micafungin dosing for obese patients in the age of deterministic chaos. *Clin. Pharmacol. Ther.* **97**, 292–297 (2015).
13. Hall, R.G. 2nd, Swancutt, M.A., Meek, C., Leff, R. & Gumbo, T. Weight drives caspofungin pharmacokinetic variability in overweight and obese people: fractal power signatures beyond two-thirds or three-fourths. *Antimicrob. Agents Chemother.* **57**, 2259–2264 (2013).
14. Jain, M.K., Pasipanodya, J.G., Alder, L., Lee, W.M. & Gumbo, T. Pegylated interferon fractal pharmacokinetics: individualized dosing for hepatitis C virus infection. *Antimicrob. Agents Chemother.* **57**, 1115–1120 (2013).
15. Hope, W.W. *et al.* Population pharmacokinetics of micafungin in pediatric patients and implications for antifungal dosing. *Antimicrob. Agents Chemother.* **51**, 3714–3719 (2007).
16. Breiman, L. Random forests. *Machine Learning* **45**, 5–32 (2001).
17. Swaminathan, S. *et al.* Drug concentration thresholds predictive of therapy failure and death in children with tuberculosis: bread crumb trails in random forests. *Clin. Infect. Dis.* **63**(suppl. 3), S63–S74 (2016).
18. Breiman, L., Friedman, J., Stone, C.J. & Olshen, R.A. *Classification and Regression Trees* (Chapman and Hall, CRC, Boca Raton, FL, 1984).
19. Friedman, J.H. Multivariate adaptive regression splines. *Ann. Statist.* **19**, 1–67 (1991).
20. Modongo, C., Pasipanodya, J.G., Zetola, N.M., Williams, S.M., Sirugo, G. & Gumbo, T. Amikacin concentrations predictive of ototoxicity in multidrug-resistant tuberculosis patients. *Antimicrob. Agents Chemother.* **59**, 6337–6343 (2015).
21. Chigutsa, E. *et al.* Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. *Antimicrob. Agents Chemother.* **59**, 38–45 (2015).
22. Pasipanodya, J.G., McIlleron, H., Burger, A., Wash, P.A., Smith, P. & Gumbo, T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J. Infect. Dis.* **208**, 1464–1473 (2013).
23. Gumbo, T., Hiemenz, J., Ma, L., Keirns, J.J., Buell, D.N. & Drusano, G.L. Population pharmacokinetics of micafungin in adult patients. *Diagn. Microbiol. Infect. Dis.* **60**, 329–331 (2008).
24. Breiman, L. Statistical modeling: the two cultures. *Statist. Sci.* **16**, 199–231 (2001).
25. Reed, M.D. Optimal sampling theory: an overview of its application to pharmacokinetic studies in infants and children. *Pediatrics* **104**(3 Pt 2), 627–632 (1999).
26. Hiruy, H. *et al.* Subtherapeutic concentrations of first-line anti-TB drugs in South African children treated according to current guidelines: the PHATISA study. *J. Antimicrob. Chemother.* **70**, 1115–1123 (2015).
27. D'Argenio, D.Z., Schumitzky, A. & Wang, X. ADAPT 5 user's guide: pharmacokinetic/pharmacodynamic systems analysis software (Biomedical Simulations Resource, Los Angeles, CA, 2009).
28. Akaike, H. A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* **19**, 716–723 (1974).
29. Ludden, T.M., Beal, S.L. & Sheiner, L.B. Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection. *J. Pharmacokinet. Biopharm.* **22**, 431–445 (1994).
30. Mandelbrot, B. How long is the coast of Britain? Statistical self-similarity and fractional dimension. *Science* **156**, 636–638 (1967).
31. Mandelbrot, B.B. *The Fractal Geometry of Nature* (W.H. Freeman and Company, San Francisco, CA, 1982).
32. West, G.B., Brown, J.H. & Enquist, B.J. A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126 (1997).
33. West, G.B., Savage, V.M., Gillooly, J., Enquist, B.J., Woodruff, W.H. & Brown, J.H. Physiology: why does metabolic rate scale with body size? *Nature* **421**, 713; discussion 714 (2003).
34. Ambrose, P.G. *et al.* Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. *Clin. Infect. Dis.* **44**, 79–86 (2007).
35. Srivastava, S., Pasipanodya, J.G., Meek, C., Leff, R. & Gumbo, T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *J. Infect. Dis.* **204**, 1951–1959 (2011).
36. Dorlo, T.P. *et al.* Failure of miltefosine in visceral leishmaniasis is associated with low drug exposure. *J. Infect. Dis.* **210**, 146–153 (2014).
37. Forrest, A., Nix, D.E., Ballow, C.H., Goss, T.F., Birmingham, M.C. & Schentag, J.J. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob. Agents Chemother.* **37**, 1073–1081 (1993).
38. Gumbo, T., Angulo-Barturen, I. & Ferrer-Bazaga, S. Pharmacokinetic-pharmacodynamic and dose-response relationships of antituberculosis drugs: recommendations and standards for industry and academia. *J. Infect. Dis.* **211**(suppl. 3), S96–S106 (2015).
39. Chiba, K., Tsuchiya, M., Kato, J., Ochi, K., Kawa, Z. & Ishizaki, T. Cefotiam disposition in markedly obese athlete patients, Japanese sumo wrestlers. *Antimicrob. Agents Chemother.* **33**, 1188–1192 (1989).

Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (http://www.wileyonlinelibrary.com/psp4)