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A Comprehensive Mixed-Method Approach to Characterize the Source of Diurnal Tacrolimus Exposure Variability in Children: Systematic Review, Meta-analysis, and Application to an Existing Data Set

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Abstract

Tacrolimus is widely reported to display diurnal variation in pharmacokinetic parameters with twice-daily dosing. However, the contribution of chronopharmacokinetics versus food intake is unclear, with even less evidence in the pediatric population. The objectives of this study were to summarize the existing literature by meta-analysis and evaluate the impact of food composition on 24-hour pharmacokinetics in pediatric kidney transplant recipients. For the meta-analysis, 10 studies involving 253 individuals were included. The pooled effect sizes demonstrated significant differences in area under the concentration–time curve from time 0 to 12 hours (standardized mean difference [SMD], 0.27; 95% confidence interval [CI], 0.03-0.52) and maximum concentration (SMD, 0.75; 95% CI, 0.35-1.15) between morning and evening dose administration. However, there was significant between-study heterogeneity that was explained by food exposure. The effect size for minimum concentration was not significantly different overall (SMD, −0.09; 95% CI, −0.27 to 0.09) or across the food exposure subgroups. A 2-compartment model with a lag time, linear clearance, and first-order absorption best characterized the tacrolimus pharmacokinetics in pediatric participants. As expected, adding the time of administration and food composition covariates reduced the unexplained within-subject variability for the first-order absorption rate constant, but only caloric composition significantly reduced variability for lag time. The available data suggest food intake is the major driver of diurnal variation in tacrolimus exposure, but the associated changes are not reflected by trough concentrations alone.

Keywords

circadian rhythm, meta-analysis, pharmacokinetics, tacrolimus

Tacrolimus effectively prevents acute rejection in kidney transplantation, but a narrow therapeutic window dictates individualized dosing to balance efficacy and toxicity. Most tacrolimus pharmacokinetic (PK) studies have been conducted in controlled environments with health care staff selecting patients, collecting samples, regulating the time of drug administration, and providing standardized meals. Given the degree of tacrolimus intrapatient variability, there is a need to understand the influence of various factors more likely to be present in the real world.[1] Understanding how these variables influence PK and, in turn, pharmacodynamics is an essential step toward optimizing medication therapy in the transplant population. This knowledge may be particularly vital in the pediatric population as those receiving a transplant between 5 and 18 years of age demonstrate an age-related reduction in long-term graft survival compared to adults.[2,3]

Population PK models have the potential to inform this understanding. However, most models have been developed based on 12-hour measurements after the morning dose in health care settings, including fasted tacrolimus administration and standardized meals.[4-6] While it is widely acknowledged that tacrolimus PK displays diurnal variation with twice-daily dosing, the literature continues to present conflicting data on the

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role of chronopharmacokinetics versus food effects.[7-10] Our objectives were to (1) conduct a detailed literature review to synthesize the knowledge of food and circadian effects on tacrolimus PK and (2) apply the information to characterize diurnal variation in an existing data set using population PK modeling. We hypothesized that the caloric composition within 2 hours of the dose would explain a significant degree of the interoccasion variability (morning vs evening) in pediatric kidney transplant recipients.

Methods

Literature Review and Meta-analysis

Search Strategy. Medline (through PubMed), Embase, and Scopus were searched for articles comparing PK parameters following the morning and evening dose of immediate-release tacrolimus from inception until May 2023. The complete search strategy, including the Preferred Reporting Items for Systematic Review and Meta-Analysis checklist, can be reviewed in the supplemental materials. Abstract and title screening was performed to determine initial study eligibility. Human studies that systematically evaluated PK differences after morning and evening doses of tacrolimus using a noncompartmental PK analysis were included in the meta-analysis. Conference proceedings or abstracts were included if a published full-text article could not be identified. Animal studies and studies without English translations were excluded. Covidence systematic review software (Veritas Health Innovation) was used for screening and data extraction.

Meta-analysis. The mean, standard deviation (SD), and correlation between morning/evening values were extracted for common PK variables (area under the concentration–time curve from time zero to 12 hours [$AUC_{0\text{-}12}$], minimum concentration [C_{min}], and maximum concentration [C_{max}]). If not reported, the authors were first contacted to obtain the information. If no response was received, (1) data presented visually were digitized with the help of Engauge Digitizer Version 12.1 according to recommendations; (2) the median and interquartile range were used to extrapolate means and SDs; or (3) if a mean and 95% confidence interval (CI) were reported, statistical conversion to a mean and SD was performed.[11-13]

The standardized mean difference (SMD) was then calculated to accommodate the differences in reported units. As we anticipated between-study heterogeneity, a random-effects model was used to pool effect sizes. The restricted maximum likelihood estimator was used to calculate the heterogeneity variance τ^2.[14] Subgroup analysis was planned a priori to quantify study design–related heterogeneity based on the food exposure described in the study. The subgroups were defined as (1) prolonged fast (8 hours or more since last food intake) for the morning dose with modified fast for evening dose (2-8 hours); (2) fed status (less than 2 hours) in the morning with modified fast for evening dose; and (3) fed status for both morning and evening dose. Sensitivity analyses were planned to evaluate the impact of the study population and subgroup classification on the estimated effect size parameters. The investigation was completed using the R package meta (R Foundation for Statistical Computing).[15,16]

Pharmacokinetic Study Design

The data set was from a single-center, prospective, nonrandomized steady-state PK study in pediatric kidney transplant recipients treated with tacrolimus. The Institutional Review Board at the University of Michigan approved the study. All participants and guardians provided written informed consent before entering the study.

Stable kidney transplant recipients 6-18 years old were eligible for inclusion if they were at least 6 months posttransplant and receiving immediate-release tacrolimus capsules with no dose changes 2 weeks before the PK assessment. Patients were excluded if they were unable to take tacrolimus capsules, had a hematocrit of less than 30%, had significant liver impairment (aspartate aminotransferase, alanine aminotransferase, or total bilirubin greater than 2 times the upper limit of normal), or taking cytochrome P450 (CYP) 3A4/P-glycoprotein inducers or inhibitors (except for prednisone for maintenance immunosuppression).

Participants were admitted to the Clinical Research Unit at the University of Michigan the evening before the 24-hour PK sample collection. Tacrolimus was administered at 8:00 a.m. and 8:00 p.m. Venous whole blood samples were drawn immediately before the morning dose, then at 0.5, 1, 1.5, 2, 4, 6, 9, and 12 hours. After the evening dose, the sampling was continued at 12.5, 13, 13.5, 14, 16, 18, 21, and 24 hours. The blood samples were stored at −80°C until analysis by liquid chromatography–tandem mass spectrometry.[17]

The patients' diet was standardized during the 38-hour study period to assess the effect of food on tacrolimus PK. After arrival at the clinical research unit, patients received their evening tacrolimus dose at 8:00 p.m., followed by an overnight fast. Meals were received at 10:00 a.m., 2:00 p.m., and 6:00 p.m. with snacks at 6:00 a.m., 10:00 p.m., and 2:00 a.m. All subjects were offered meals with consistent calories, protein, fat, and carbohydrates but were not required to consume the entire meal. While previous studies have focused on the impact of high-fat, high-calorie meals, the meals provided were intended to emulate

a balanced diet containing 15% protein, 20% fat, and 65% carbohydrates. Approximately 3000 calories/day were offered to ensure adequate age-appropriate intake for all participants, including active, older adolescent males. The exact caloric composition of the individual intake was calculated and used for analysis. The meal content is provided in the supplemental materials (Table S1). The 6:00 a.m. meal contained no fat, whereas the 6:00 p.m. meal included 7-9.5 g of fat (13%-17% of calories). No food was allowed within 2 hours before or after tacrolimus administration. The meals did not include food with known CYP drug interactions.

Noncompartmental Analysis. PK profiles were first analyzed using standard noncompartmental methods. Maximum concentration (C_{max}) and time to C_{max} (t_{max}) were obtained directly from the observed data. C_{12} was the measured concentration 12 hours after each dose. The area under the concentration–time curve (AUC) was calculated by the linear log trapezoidal method using PKanalix (Version 2021R1; Lixoft SAS).

Continuous data derived from the noncompartmental analysis (NCA) were assumed to be be log-normally distributed and expressed as geometric mean and 95% geometric mean CI. Tacrolimus PK parameters estimated after the morning and evening doses were compared using a 2-sided paired t-test of natural log-transformed values. For the nonparametric variable t_{max}, the Wilcoxon signed-rank test was performed. Statistical significance was set at alpha = 0.05, with analysis performed using RStudio 2022.07.0 (Posit, PBC).

Population PK Model Building. Tacrolimus whole blood concentrations were also analyzed by nonlinear mixed-effects modeling using the stochastic approximation expectation maximization algorithm implemented within the software program Monolix (version 2023R1; Lixoft SAS). A 3-step approach was used to develop the population PK model. First, a base structural model without covariates was selected. Different structural models were explored to define the best base model: 1- and 2-compartment models with first-order absorption and transit compartments or a lag time for describing drug absorption were compared. Additive, proportional, and mixed residual variability models were tested. Parameters were assumed to be log-normally distributed. As bioavailability (F) was not directly quantified, the parameter values for volume of distribution (V) and clearance (CL) correspond to the ratios V/F and CL/F, respectively.

Candidate subject-level covariates were initially selected on the basis of biological and mechanistic plausibility for addition to the base covariate model. The subject-level covariates chosen for evaluation were those commonly found to be significant in the existing literature and included age, creatinine clearance (calculated using the Schwartz equation), and total body weight.[4,18] These factors were considered static during the 24-hour study period. Given that the evaluation was focused on intrapatient variability in a single 24-hhour period in patients at the target exposure, the influence of many other commonly reported covariates, such as CYP3A5 and hematocrit, was expected to be minimal. For example, once the target trough is achieved, the concentration–time curve is almost identical for CYP3A5 expressers and nonexpressers.[19] Covariates with a visual relationship between the parameters in the base model were tested as power and exponential functions, where covariate values were centered to the median. The effect of body weight on CL and V was also considered using theory-based allometric scaling (eg, $CL/F = \theta_{CL} * [weight/70]^{0.75}$).[20]

The final model was then developed to evaluate interoccasion (morning vs evening) random effects and covariates. Based on the existing literature, it was hypothesized that the impact would primarily be on parameters related to the absorption (time lag [t_{lag}] and first-order absorption rate constant [k_a]). The effect on CL was also explored. Time of administration (morning or evening) was first evaluated as a categorical predictor. Then, food intake was summarized as total calories, carbohydrate (grams and percentage of the meal), protein (grams and percentage of the meal), and fat (grams and percentage of the meal) at the meal 2 hours before the dose was explored separately. The correlation test based on beta coefficients estimated by linear regression and the Wald test in Monolix were used to guide the inclusion of the food composition variables. Covariate selection was made according to a forward/backward process. In the forward step, including a covariate in the model was based on Pearson's correlation test between each covariate and the random effect on the estimated PK parameter. In the backward step, the Wald test was used to test whether any covariate could be removed from the full covariate model.

Overall model evaluation was based upon the goodness-of-fit plots, the precision of parameter estimates (relative standard error), and changes in the Akaike information criterion. The log-likelihood was computed by importance sampling within Monolix. The initial decision to retain a covariate was based on assessing the Akaike information criterion with and without the covariate. A reduction in the random variability and an improvement in fit were also considered.

Results

Literature Review and Meta-Analysis

Ten studies involving 253 individuals were included in the meta-analysis comparing morning versus

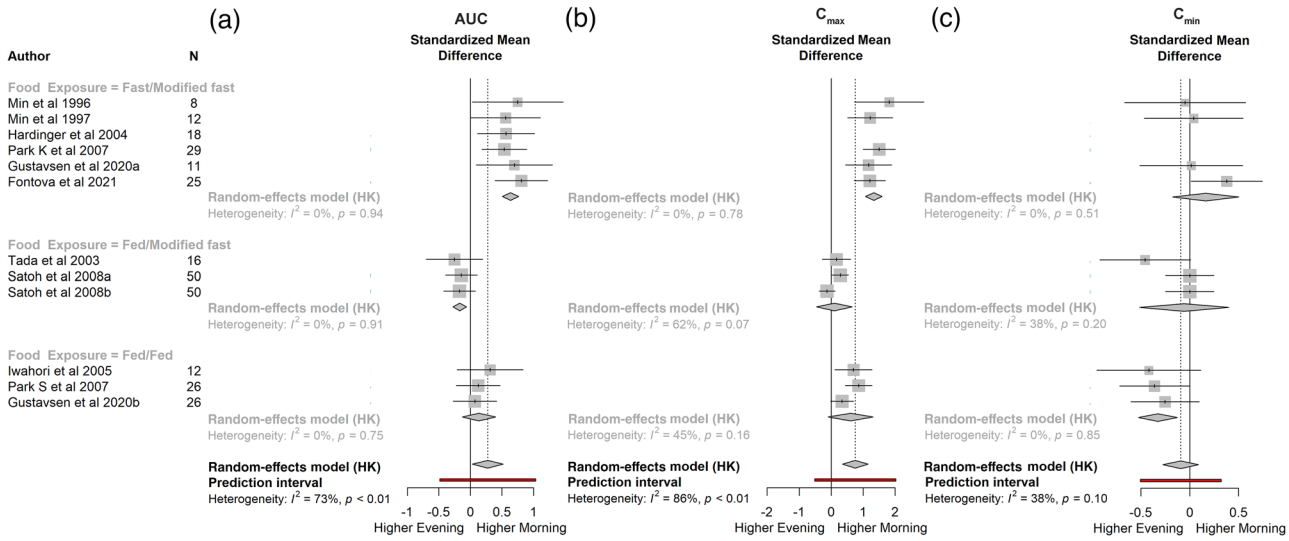


Figure 1. Forest plot of the standardized mean difference for (a) area under the concentration–time curve (AUC) (b) maximum concentration (C_{max}), and (c) minimum concentration (C_{min}). Gustavsen 2020a provides results of the prolonged fast/modified fast scenario, and Gustavsen 2020b presents results of the real-life nonfasting scenario. Satoh 2008a provides results for the 50 participants at 28 days after transplant, and Satoh 2008b provides results for the same population >365 days after transplantation.

evening dosing of immediate-release tacrolimus (Preferred Reporting Items for Systematic Review and Meta-Analysis flow diagram in Figure S1 with study summaries in Table S2).[8,10,21-28] In addition, studies evaluating intravenous administration (n = 1), morning versus evening administration of LCP-tacrolimus (Envarsus XR, Veloxis Pharmaceuticals) (n = 1), and population PK models (n = 4) were identified.[7,9,29-32] All noncompartmental analyses included adult participants with only 2 population PK models evaluating pediatric recipients.

Overall, the pooled effect size demonstrates the $AUC_{0\text{-}12}$ (SMD, 0.27, 95% CI, 0.03-0.52) and C_{max} (SMD, 0.75; 95% CI, 0.35-1.15) were higher after morning dose administration compared to evening (Figure 1a, b). The effect size for C_{min} was not significantly different overall (SMD, −0.09, 95% CI, −0.27 to 0.09) or across the subgroups without statistical heterogeneity (I^2 = 38.3%; 95% CI, 0%-70.6%; Figure 1c).

However, there was substantial between-study heterogeneity for $AUC_{0\text{-}12}$ (I^2 = 73%; 95% CI, 51.8%-84.8%) and C_{max} (I^2 = 86%; 95% CI, 75.8%-91.3%) suggesting that the subgroups may have a different true effect. Subgroup analysis by food exposure explained the heterogeneity without significant heterogeneity identified within each group. None of the studies were statistical outliers or influential cases. For the subgroup of studies that compared a prolonged fasted state in the morning to a modified fast, there was a significant increase in the mean $AUC_{0\text{-}12}$ (SMD, 0.64; 95% CI, 0.51-0.76; I^2 = 0%; P = .94) and C_{max} (SMD, 1.3; 95% CI, 1.07-1.59; I^2 = 0%; P = .78) in the morning. Among studies in which the fasting period was longer in the evening (fed/modified fast), the inverse was true with lower $AUC_{0\text{-}12}$ in the morning (SMD, −0.17; 95% CI, −0.27 to 0.06; I^2 = 0%; P = .91) but with no significant impact on C_{max} (SMD, 0.09, 95% CI, −0.46 to 0.66]; I^2 = 62%; P = .07). When fed within 2 hours before both morning and evening doses, there was no difference in $AUC_{0\text{-}12}$ (SMD, 0.14; 95% CI, −0.13, 0.40; I^2 = 0%, P = .14) and C_{max} (SMD: 0.61, 95% CI: −0.10, 1.31; I^2 = 45.2%, P = .89) but C_{min} was significantly lower after the morning dose (SMD, −0.33; 95% CI, −0.53 to −0.13; I^2 = 0%; P = .85). Results of the sensitivity analyses are included in the Supplemental material (Figures S6 and S7).

Subject Characteristics

A total of 15 stable pediatric kidney transplant recipients completed PK sample collection. Among them, 10 had complete dietary intake information and were included in the PK assessment. Participants were primarily White adolescents who were a median of 2.64 (0.76-6.44) years after transplant; Table 1 provides the patient characteristics.

Noncompartmental Analysis

All patients underwent 2 successive 12-hour PK investigations (morning and evening doses) in 24 hours. The observed mean tacrolimus concentration–time profiles after morning and evening doses are shown in Figure 2. The AUC and C_{max} were significantly higher after the morning dose compared to the evening dose (Table 2). The geometric mean ratio for C_{max} was 189.3% (95% CI, 154.9%-231.5%) compared to 118.3% (95% CI,

Table 1. Patient Characteristics and Demographics (n = 10)

	n (%)
Male	5 (50)
Female	5 (50)
Race/ethnicity:	
White	9 (90)
Hispanic	1 (10)
Diabetes	1 (10)
	Median (range)
Age (years)	16 (9-18)
Time posttransplant (years)	2.64 (0.76-6.44)
Scr (mg/dL)	1.05 (0.7-1.3)
Height (cm)	156 (132.7-172.5)
Weight (kg)	60.6 (40.1-74.7)
Immunosuppression:	
Tacrolimus dose (mg/kg/dose)	0.037 (0.013-0.069)
Mycophenolate dose (mg/kg/dose)	12.8 (0-18)
Prednisone dose (mg/kg/day)	0.15 (0.0-0.18)

Scr, serum creatinine.

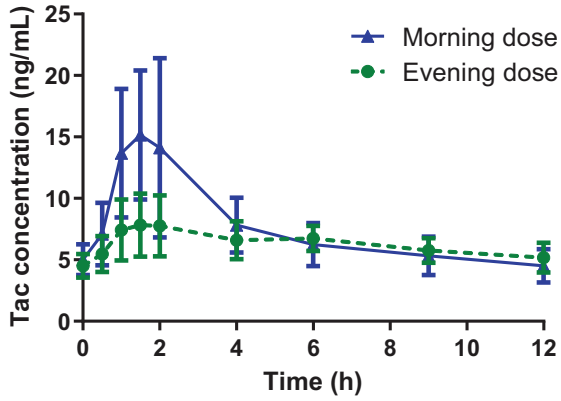


Figure 2. Tacrolimus concentration–time profiles by 12-hour dose interval after the administration of morning (triangles with blue, solid line) and evening (circles with green, dotted line) doses. Each point displays the mean concentration; standard deviations are depicted by error bars.

109.7%-127.5%) for AUC. The results also demonstrated a tendency for a shorter t_{max} after the morning dose (median, 1.5 hours) compared to the evening dose (median, 2 hours) but did not reach statistical significance ($P = .052$). This result appears to be driven by 4 patients who had increases in $t_{max} > 1$ hour for the evening dose compared to the morning dose (Figure 3). For most patients, the concentration 12 hours after the dose was higher after the evening dose ($P = .03$). There was no significant difference in tacrolimus clearance between the two 12-hour PK investigations.

Population PK Modeling

The data were sufficiently described by a 2-compartment model with a lag time as the base structural model, consistent with existing literature for intensive sampling.[4] The unexplained residual variability was best modeled using proportional error. The immediate addition of subject-level covariates resulted in a model that was sensitive to initial estimates, had large relative standard errors, and, in some cases, failed to converge. Therefore, the covariance structure was simplified before adding the covariates. Model complexity was reduced by removing the between-subject random effects that were poorly estimated, including t_{lag}, k_a, intercompartmental CL, and apparent volume of the peripheral compartment, which resolved the issue with adequate model fit. None of the subject-level covariates resulted in biologically plausible improvements in the model (Table S3).

The final estimates for the candidate covariate population models, including time of day and caloric composition covariates, are presented in Table 3. As expected, adding the covariates reduced the unexplained within-subject variability for k_a, but only caloric composition significantly reduced variability for t_{lag} (Table S4). The within-subject variability for CL was low, and the available covariates did not significantly improve the amount of within-subject variability explained by the model (Table S5). As shown by the visual predictive check plots, adding covariates improved the predictions, particularly during the absorption phase, with most of the observed data within the model-predicted range (Figure S3). No significant trends were observed in the goodness-of-fit plots presented in the supplemental materials (Figures S4 and S5).

Discussion

The previous literature evaluating circadian rhythm–dependent changes in tacrolimus PK has been difficult to integrate due to differences in study design. Here, we describe a mixed-effect meta-analysis that helps address this issue by quantifying how food intake influences the observed effect size. When summarizing the effect of food based on the time since the last intake, the importance of food as a contributor to the apparent diurnal variation is evident. The studies most likely to demonstrate the typical diurnal pattern of a significantly higher $AUC_{0\text{-}12}$ and C_{max} with a shorter t_{max} in the morning were those in which the morning dose was received after a longer fast than the evening dose.[8,10,21-24]

The fed/fed conditions were more likely to result in insignificant changes in $AUC_{0\text{-}12}$ but a higher C_{max} and shorter t_{max} in the morning with a smaller overall effect size than the prolonged fast/modified fast studies.[27,28] The fed/fed conditions were the most variable across studies and may explain the conflicting results between studies. For example, in our study, food 2 hours before the morning dose contained no fat, whereas 2 hours

Table 2. Comparison of Tacrolimus Noncompartmental Pharmacokinetic Parameters after Morning and Evening Doses

Parameters	Morning dose (0-12 hours)	Evening dose (12-24 hours)	Geometric mean ratio (%) (95% CI)	*P*-value
	Geometric mean (95% CI)			
AUC_{0-12} (ng • h/mL)	86.2 (68.6-108.2)	72.9 (58.0-91.7)	118.3 (109.7-127.5)	.003
C_{max} (ng/mL)	15.6 (11.4-21.5)	8.3 (6.35-10.7)	189.3 (154.9-231.5)	<.001
C_{trough} (ng/mL)	4.3 (3.3-5.6)	4.9 (3.9-6.3)	86.7 (78.4-95.9)	.03
CL/F (L/h)	23.4 (16.1-33.9)	25.5 (17.5-37.1)	91.8 (83.5-101.0)	.129
k_{el} (hour)	0.052 (0.006-0.098)	0.051 (0.009-0.093)	103.6 (58.54-183.3)	.906
	Median (range)			
t_{max} (hour)	1.5 (1-2)	2.0 (1-6)	47.2 (4.04-90.4)	.052

AUC_{0-12}, area under the concentration–time curve from time zero to 12 hours; C_{max}, maximum concentration; C_{trough}, concentration 12 hours after the dose; CI, confidence interval; CL/F, apparent clearance; k_{el}, elimination rate constant; t_{max}, time to reach C_{max}.

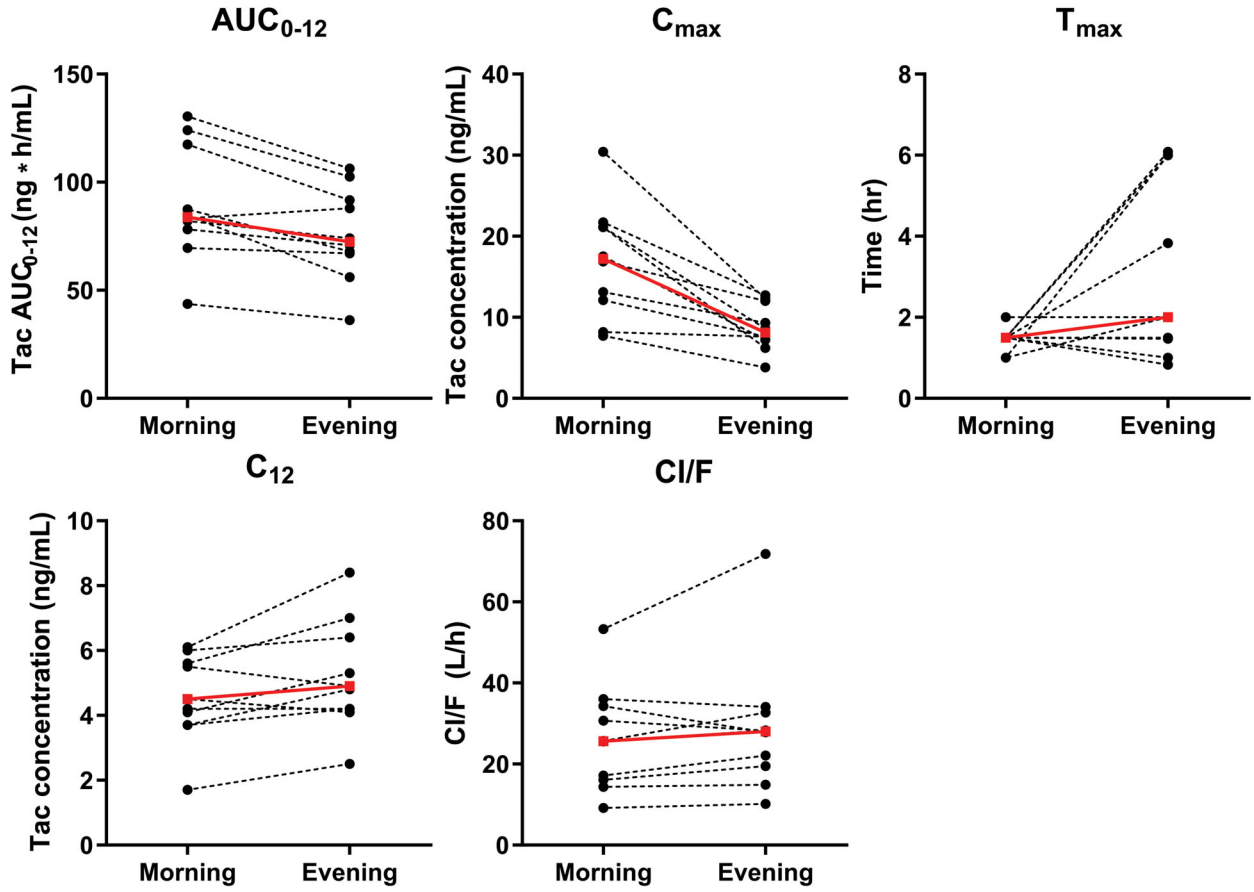


Figure 3. Differences in noncompartmental pharmacokinetic parameters after the morning dose compared to the evening dose for the individual study participants. Differences for individual participants are depicted by black circles connected by the dotted line. The median is displayed by red squares with a solid line. AUC_{0-12}, area under the concentration–time curve from time 0 to 12 hours; C_{12}, concentration at 12 hours; C_{max}, maximum concentration; CL/F, apparent clearance; t_{max}, time to maximum concentration

before the evening dose, fat comprised 10%-84% of the calories consumed. Fat is known to reduce the absorption of tacrolimus and, therefore, may have contributed to the more significant differences observed in our data compared to the studies that did not restrict fat at one of the meals.[33]

While the meta-analysis included only NCA studies, previous population PK models have also provided

Table 3. Comparison of Pharmacokinetic Parameters for the Candidate Covariate Population Models Compared to the Base Model

Model	Base	Morning versus evening	Caloric composition
Model comparison			
AIC	614.2	593.74	580.04
ΔAIC from base	–	−21.46	−34.16
Fixed-effect parameters (RSE, %)			
t_{lag}_pop (hour)	0.56 (25.4)	0.55 (26.6)	0.26 (27.3)
k_a_pop (/hour)	0.46 (28.8)	0.69 (23.2)	0.28 (36.0)
V_1_pop (L)	44.46 (30.3)	42.94 (34.0)	37.71 (21.6)
Q_pop	56.52 (10.4)	56.21 (10.4)	54.57 (6.98)
V_2_pop (L)	1044.24 (18.9)	1211.49 (18.0)	1153.59 (16.3)
CL_pop (L/h)	20.59 (22.0)	19.46 (25.3)	20.05 (22.7)
$\beta_{evening}$ on k_a[a]	–	−1.1 (18.1)	–
$\beta_{protein}$ on t_{lag}[b]	–	–	0.056 (26.5)
$\beta_{\%carbohydrate}$ on k_a[c]	–	–	0.015 (22.6)
$\beta_{\%protein}$ on k_a[d]	–	–	−0.047 (23.8)
Standard deviation of random-effect parameters (RSE, %)			
Between-subject variability			
ωV1	0.4 (153)	0.41 (77.1)	0.31 (66.3)
ωCL	0.64 (27.5)	0.72 (31.0)	0.67 (26.7)
Within-subject variability			
γt_{lag}	0.97 (21.6)	0.97 (20.3)	0.65 (19.8)
γk_a	0.73 (29.4)	0.26 (45.5)	0.21 (40.6)
γCL	0.13 (41.9)	0.14 (29.9)	0.14 (30.9)
Residual variability			
B	0.12 (8.17)	0.12 (7.41)	0.12 (6.86)

Reported as typical value estimate (residual standard error %).

AIC, Akaike information criteria; B, proportional error; CL_pop, typical/population value of clearance; γCL, standard deviation of the within-subject random effect on CL; ωCL, standard deviation of the between-subject random effect on clearance; k_a, first-order absorption rate constant; γk_a, standard deviation of the within-subject random effect on k_a; RSE, relative standard error; γt_{lag}, standard deviation of the within-subject random effect on lag time; V_pop, typical/population value of volume of distribution; ωV1, standard deviation of the between-subject random effect on volume of distribution.

[a] $k_a_pop = k_a_pop \times e^{\beta \times evening}$.

[b] $t_{lag}_pop = t_{lag}_pop \times e^{\beta \times protein\ (g)}$.

[c] $k_a_pop = k_a_pop \times e^{\beta \times \%carbohydrate}$.

[d] $k_a_pop = k_a_pop \times e^{\beta \times \%protein}$.

evidence that the administration time may influence k_a and CL.[7,30-32] Two studies found the time of day to significantly affect the absorption rate constant, with higher values in the morning (2.08-2.18/hour) compared to the evening (0.16-0.687/hour).[30,32] The impact on the absorption rate constant is consistent with the meta-analysis for studies with the same prolonged fast/modified fast food exposure. The models that used a periodic wave function to model the diurnal change also found significant but differing effects on CL.[7,32] Fernández et al[22] reported a lower clearance in the morning, consistent with the NCA results for prolonged fast/modified fast.[10] However, it is important to note that these studies evaluated CL/F; hence, the changes could also be attributed to bioavailability associated with the cyclic nature of food intake.[34] Inversely, the model developed by Prado-Velasco et al,[7] which estimated bioavailability separately, reduced clearance by a factor of 0.16 (physiologically based PK model) and 0.29 (population PK model) in the evening. These results align with the intravenous administration studies discussed below.

After parenteral administration in humans and animals, there has been no clinically meaningful difference in PK parameters between morning and evening administration.[29,35] While the human study found the mean clearance during the infusion was higher in the morning compared to the evening (4.51 L/h [1.29] vs 4.23 L/h [1.21]), this small change in clearance is not likely to be clinically meaningful as there was no

difference in the AUC (273 ng • h/mL [69] vs 292 ng • h/mL [87]).[29] This is consistent with the evaluation of circadian rhythm in hepatic CYP3A4 activity using intravenous midazolam as the probe drug, demonstrating only a 10% fluctuation in CYP3A activity.[36] Similarly, another study evaluating intravenous and oral midazolam found almost no 24-hour variability with intravenous administration (7.2%).[37] However, after oral administration, bioavailability, absorption rate constant, and clearance demonstrated variability with a peak at 2:00 p.m. In the final population PK model, absorption rate constant and clearance were described by a cosine function and k_a by a multiplication factor (1.41/hour).[37] It is important to note that patients only fasted for 2 hours before dosing, and no meal content information is provided in their study. The limited impact of circadian variation related to enzyme or transport rhythms is further demonstrated by intracellular tacrolimus concentrations that mirror that seen in the whole blood.[10] Tacrolimus entrance into peripheral blood mononuclear cells is via P-glycoprotein, which would not be directly influenced by food. There is little clinically relevant diurnal variation in the clearance mechanisms for tacrolimus once it enters the blood.

The primary limitation of the meta-analysis is that none of the studies compared truly equivalent food exposures in the morning and evening. The study that most closely adhered to the US Food and Drug Administration guidance on fasting (at least 10 hours) for both morning and evening doses evaluated the modified-release formulation, LCP-tacrolimus.[38] There was no difference in AUC, C_{max}, t_{max}, concentration at 24 hours, or total clearance by the time of day when the dose was given after fasting for 8 hours in healthy volunteers.[9] It should be noted that time-dependent P-glycoprotein levels have been reported in the ileum but not the colon. Therefore, LCP-tacrolimus may not demonstrate circadian variation, whereas immediate-release tacrolimus could.[39] Inversely, there was an impact of food. A high-calorie, high-fat meal was associated with a significant reduction in AUC (55%) compared to fasted, which is higher than the approximately 35% reduction in AUC with the IR formulation. C_{max} was also significantly reduced (22%) in the fed state compared to fasted, though to a lesser extent than the 60%-70% reduction seen with the immediate-release formulation.[9,33,40,41] The impact of food before the morning administration of tacrolimus is well established.[41-47] However, the relationship with time after the meal is less studied. The impact of food has only been studied out to 1.5 hours after a meal, at which point a significant reduction in exposure remained.[40]

Clinically, the results presented here are important because current therapeutic drug monitoring practices focused on trough concentrations may underestimate the reduction in exposure related to food.[48] Many transplant centers instruct patients to administer tacrolimus consistently in relation to food, which often includes recommending modified fasting with no food intake 2 hours before and 1-2 hours after the dose, which may not be sufficient to negate the impact.[49] In real life, patients do not consistently adhere to such recommendations, particularly for the evening dose, or cannot tolerate administration in a fasted state due to gastrointestinal side effects. As illustrated by the comparison of C_{min} across studies, changes in trough measures are not proportional to the differences in AUC and are often unreliable estimates of the total drug exposure.[42] In 1 study, a trough of 10 ng/mL was associated with an AUC of 120 ng • h/mL in the fed state but with an AUC of 250 ng • h/mL when given after fasting.[43] To mitigate this problem, some centers monitor AUC using limited sampling strategies. However, as our population PK analysis demonstrates, understanding the impact of food remains important. Including the morning and evening doses as categorical covariates significantly improved the model but not to the extent the more specific food covariates did. Despite offering a limited menu, the caloric composition still represented an important source of within-subject variability, which may be even more remarkable when patients can consume meals of their choice. These findings are consistent with a study by Gustavsen et al demonstrating that a limited sampling strategy validated under fasting conditions did not indicate good agreement with AUC predictions in patients who took tacrolimus without regard to food.[50] Incorporating food characteristics as a continuous covariate rather than the binary fed/fasted typically reported may better describe the expected differential effects on interoccasion PK profiles.

However, the current model is not intended to be implemented in clinical practice but merely to illustrate food composition as a potential covariate. The data for other clinical covariates were limited (eg, no CYP3A5 genotype), and information is from patients who were years posttransplant with stable, normal renal function and hematocrit. The performance of the developed model should be viewed only as a starting point for further evaluation to understand the influence of dietary intake on intrapatient variability. However, understanding the contribution of food is essential to optimize total drug exposure because dietary intake is more modifiable than chronobiology.[51] This is increasingly important as advances in remote monitoring expand the potential for home-based collection of repeated samples.[52-59] While outside the scope of this article, the

potential chronopharmacodynamic effects must also be considered.[10,22,25,60,61]

Conclusions

The combined meta-analysis and pharmacokinetic study in pediatric kidney transplant recipients suggest that when seeking to optimize tacrolimus therapy, greater attention should be paid to the impact of food. Multiple studies have shown large food effects on tacrolimus exposure, with up to 50% reductions in AUC. Yet the focus of the transplant community is most often on factors with less variability (change in generic formulation or assay differences) or that influence exposure only in the first few weeks (genotype).[62-64] These sources of intrapatient variability contribute differences of approximately 10%-15% compared to the 25%-50% change with food. The pronounced food effect suggests improved precision pharmacotherapy could be achieved by adding caloric composition to the assessment. The inability of C_{min} to capture the impact of food is a further argument that AUC is the better marker for exposure. Overall, the data demonstrate that food intake may be a valuable addition to predict tacrolimus PK in adults and children and should be considered when developing and evaluating therapeutic drug monitoring. Further work will be necessary to better characterize the influence of dietary intake on tacrolimus intrapatient variability.

Conflicts of Interest

The authors declare no conflict of interest.

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Data Availability Statement

The data supporting this study's findings are available on request from the corresponding author.

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