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Impact of Fasting Status and Circadian Variation on the Pharmacokinetics of Mycophenolate Mofetil and the Glucuronide Metabolite in Renal Transplant Recipients

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Background. Mycophenolate mofetil (MMF) is an immunosuppressive prodrug often used to prevent allograft rejection following solid organ transplantation. After oral administration, MMF is rapidly hydrolyzed to the active metabolite mycophenolate acid (MPA), which is inactivated by glucuronosyltransferase to the mycophenolic acid glucuronide metabolite (MPAG). The aim was 2-fold: to investigate the impact of circadian variation and fasting versus nonfasting status on MPA and MPAG pharmacokinetics in renal transplant recipients (RTRs). **Methods.** RTRs with stable graft function treated with tacrolimus, prednisolone, and MMF (750 mg BID) were included in this open, nonrandomized study. Two 12-h pharmacokinetic investigations were conducted in succession following morning and evening doses, both in a fasting and in a real-life nonfasting condition. **Results.** A total of 30 (22 men) RTRs performed one 24-h investigation, and 16 repeated the investigation within 1 mo. In a real-life nonfasting state, MPA area under the curve $(AUC)_{0-12}$ and C_0 failed to meet the bioequivalence criteria. Following the evening dose, mean MPA AUC_{12-24} was 16% lower ($P < 0.001$) compared with AUC_{0-12}, and a shorter T_{max} was observed ($P = 0.09$). Under fasting conditions, MPA AUC_{12-24} was 13% lower than AUC_{0-12}, and the absorption rate was slower after the evening dose ($P < 0.05$). MPAG displayed circadian variation only under real-life conditions with lower AUC_{0-12} following the evening dose ($P < 0.001$). **Conclusions.** Both MPA and MPAG showed circadian variation with somewhat lower systemic exposures following the evening dose with limited clinical relevance in the dosing of MMF in RTRs. Fasting status affects MMF absorption rate differently, but with similar results in systemic exposure.

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Following solid organ transplantation, immunosuppressive therapy is essential to prevent organ rejection. Currently, the combination of tacrolimus and mycophenolate mofetil (MMF) is the cornerstone in modern immunosuppression, prescribed to >90% of renal transplant recipients worldwide.[1] MMF is a prodrug of mycophenolic acid (MPA), a potent, noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase. By inhibiting inosine monophosphate dehydrogenase, MPA suppresses cell-mediated immune responses and antibody formation.[2] MMF was initially marketed as a "one-dose-fits-all" drug by the manufacturer.[3] However, it soon became clear that MPA showed considerable intra- and interindividual pharmacokinetic (PK) variability. In fact, it has been shown that dose-normalized systemic exposure of MPA can vary more than 10-fold.[4] Despite this, the role of therapeutic drug monitoring (TDM) is still undergoing continued debate and many transplant centers have yet to implement individualized dosing based on TDM of MMF.[5,6] Although several important factors, including enterohepatic recirculation, protein binding, and alteration in drug absorption have been identified as contributors to the variability of MPA PK, a large part of this variability remains unexplained and unpredictable.[7,8]

Almost all functions of the human body, including those influencing PK processes, such as absorption, distribution,

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metabolism, and excretion of drugs, are affected by circadian rhythm.[9] This time-dependent change in PK due to rhythms in biological function and processes has been defined as chronopharmacokinetic. We have previously shown that tacrolimus displays circadian variation when administered to patients in a fasting state.[10] In contrast, flatter tacrolimus PK profiles and no circadian variation were present in a real-life, nonfasting setting. This was consistent with the tendencies observed in previously published literature.[11–13] When performing area under the curve (AUC)–based TDM, data reflecting the real-life situation are required to develop more clinically applicable population PK models for dose individualization. However, most of these PK models shown in the literature may not perform sufficiently on real-life data as they are developed using data from highly controlled clinical trials in which the participant is informed to have C_0 measured in a fasting condition and that rarely include any data from $AUC_{12–24}$. This is highly relevant for MPA, wherein AUC estimation often is warranted due to the poor correlation between MPA trough concentrations and protection against rejection episodes.[14] Thus, it will be necessary to include real-life data in future dosage models.

Fasting and nonfasting status can be a factor explaining the considerable variation seen in MPA and its main metabolite 7-O-mycophenolic acid glucuronide (MPAG) PK. The most critical phases in the PK of MPA, which can be affected by whether the patient is in a fasting or nonfasting state, are the absorption process, the enterohepatic recycling, and metabolite formation.[15] The few studies conducted in other patient populations have shown a relatively small change in MPA systemic exposure, whereas MPAG, on the other hand, showed greater systemic exposure in the fed condition compared with the fasting state.[16,17] The aim of the current study was 2-fold: to investigate circadian variation and the impact of fasting status on the PK of MPA and MPAG in renal transplant recipients.

MATERIALS AND METHODS

Patients and Study Design

This open, nonrandomized PK study was conducted at the National Transplant Center in Norway, Oslo University Hospital – Rikshospitalet, from December 2015 to May 2017 and has previously been described in detail.[10,18] In brief, renal transplant recipients older than 18 y using twice-daily MMF (Cellcept; F. Hoffmann-La Roche Ltd., Basel, Switzerland) without concomitant drugs known to interact with MPA PK were eligible for inclusion. All patients received a proton pump inhibitor (PPI) according to center protocol. In the early post-transplant phase (2–8 wk after transplantation), two 12-h PK investigations were conducted in succession following morning and evening doses. Approximately half of the patients repeated these PK investigations within 1 mo. The patients were examined either in a fasting state (no food intake 2 h before or after dose), or they administered their immunosuppressive medications as in their everyday life (ie, nonfasting state). Concomitant drugs were administered simultaneously with MMF, also in the fasting state. With this study design, 12-h MPA and MPAG PK were examined following 4 different dosing scenarios: (1) real-life nonfasting morning dose, (2) real-life nonfasting evening dose, (3) fasting morning dose, and (4) fasting evening dose.

The study was performed according to the ethical principles in the Declaration of Helsinki and guidelines for Good Clinical Practice. The study was approved by the Norwegian Medicine Agency (EudraCT number: 2015-004734-10), and the regional ethics committee of Health Region South-East (REK number: 2015/2098). All patients received both written and verbal information and provided their informed consent before inclusion.

Immunosuppressive Treatment

Maintenance therapy consisted of a combination of MMF, tacrolimus, and steroids. MMF was administered at a fixed dose of 750 mg twice a day from the day of transplantation, and dose adjustments were solely conducted in case of side effects. Tacrolimus was initiated on the day of transplantation, given as a starting dose of 0.04 mg/kg for immunological standard-risk patients, and modified to a trough (C_0) target range of 3–7 μg/L. For high-risk immunological patients (presence of donor-specific antibodies at the time of engraftment), tacrolimus starting dose was 0.05 mg/kg and adjusted to a C_0 target of 8–12 μg/L. Prednisolone was given according to a fixed tapering dose regimen beginning at 20 mg/d (80 mg/d in high-risk patients) the day after transplantation and tapered to a maintenance dose of 10 mg/d by weeks 4–8. Induction therapy with basiliximab 20 mg on days 0 and 4 after transplantation was given to all the patients together with intravenous methylprednisolone 250 mg (standard-risk) or 500 mg (high-risk) on day 0. High-risk patients received intravenous human immunoglobulins 0.4 g/kg daily on days 0 and 4 and rituximab 375 mg/m^2 on day 0.

PK Investigation

Administration of oral MMF was performed according to each patients prescribed regimen of twice-daily dosing at 9 AM and 9 PM. Blood samples were collected predose (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h after both morning and evening administration of oral MMF. The precise sample time was recorded. Blood samples were drawn in K2-EDTA vacutainer tubes (4 mL Vacuette K_2EDTA, Greiner Bio-One, Monroe, NC) and centrifuged for 10 min at 4°C (1800*g*). Plasma was separated into Cryovials and stored at −70°C until analysis.

Analytical Assay

Plasma concentrations of MPA and MPAG were determined by a validated ultra-high-performance liquid chromatography (UPLC-MS/MS) method at the Department of Pharmacy, University of Oslo. Protein precipitation was used as sample preparation of the analytes before the UPLC-MS/MS analysis. Plasma samples were thawed at room temperature and briefly mixed on a whirl-mixer (Vortex-Genie 2, Scientific Industries Inc, New York). A sample volume of 20 μL was transferred to a 96-well tray, and 200 μL precipitation solution (95% acetonitrile, 5% methanol) containing internal standards (100 ng/mL MPA-d3, 1 μg/mL MPAG-d3) was added in volumes to each well. The tray was stored at −20°C for 1 h, followed by centrifugation for 10 min at 2862*g* (4°C). Then, 20 μL supernatant was added to 100 μL of mobile phase A (50 nM ammonium acetate with 5% acetonitrile) before 1 μL was injected into the UPLC-MS/MS system.

The UPLC-MS/MS system consisted of a Vanquish UPLC connected to an Altis triple quadrupole mass spectrometer

(Thermo-Fisher, Waltham, MA). Electrospray ionization was in positive mode using selected reaction monitoring as the monitoring technique. A C18-column (Acquity UPLC HSS T3 1.8 µm 2.1 mm × 50 mm, Waters, Milford, MA) with a guard column (Acquity UPLC HSS T3 1.8 µm 2.1 mm × 5 mm guard column, Waters, Milford, MA) was used. Mobile phase A (50 mmol/L aqueous ammonium acetate, 5% acetonitrile) and B (100% acetonitrile) were delivered at a flow rate of 0.4 mL/min, using a gradient. A gradient starting at 30% mobile phase B was used, increasing gradually to 90% from 0.3 to 2 min before returning to 30% at 2.1 min. The total time of analysis was 3 min, and retention times were 1.7 min for MPA and MPA-d3 and 0.4 min for MPAG and MPAG-d3.

Calibrators and quality control samples were prepared in blank plasma and analyzed in each series. Eight calibrators in the range of 0.25–32 and 2.5–320 mg/L were applied to MPA and MPAG, respectively. For both MPA and MPAG, the calibration curve was best fitted by linear regression without a weighting factor and forced origin. No detectable carryover was observed following injection of the highest calibrator level or internal standard of both MPA and MPAG in blank plasma. Between-series and within-series performance of MPA and MPAG were assessed with resulting coefficients of variation <8% and <9%, respectively. The mean accuracy ranged from 92% to 106% for MPA and 92% to 103% for MPAG.

PK Calculations and Statistical Analyses

The maximum concentrations (C_{max}) and time to reach C_{max} (T_{max}) were determined using observed values. The area under the plasma concentration–time curve ($AUC_{0–12}$/$AUC_{12–24}$) was calculated using the trapezoidal method implemented in Pmetrics[19] for R. Two different trough concentrations were assessed, just before the morning (C_0) and evening dose (C_{12}), respectively. All individual PK parameters were logarithmically transformed before statistical analysis. The geometric mean and 90% confidence interval (CI) ratio of evening to morning dose $AUC_{0–12}$, C_{max}, C_0, and T_{max} were calculated and compared using the Student's *t* test. The 90% CI was back-transformed to the original scale and compared with the ranges given in the European Medicines Agency guidelines for bioequivalence.[20] For MMF, the 90% CI should be contained within the acceptance interval of 80%–125%. Associations between C_0/C_{12} and $AUC_{0–12}/AUC_{12–24}$ were estimated using the Pearson correlation coefficient. *P* values below 0.05 were considered statistically significant. Data are presented as mean ± SD unless stated otherwise. All statistical analyses were executed in R version 4.1.1.[21]

RESULTS

Patients

A total of 31 renal transplant recipients with stable graft function were included in the study. One patient was treated with mycophenolate sodium, and not MMF, and was thus excluded from the present analysis. Demographic data and patient characteristics are shown in Table 1 and were considered representative of our kidney transplant population. All patients completed 2 consecutive 12-h PK investigations (morning and evening). Sixteen of the patients repeated these PK investigations within 7–28 d. In the present analysis, a total of 92 (46 morning/46 evening dose) 12-h PK profiles were included. MMF was administered in a fasting state in 11 of the morning-evening dose investigations. In the remaining 35 morning-evening dose investigations, MMF was administered in a real-life, nonfasting state. The patients were told to consume food as in a real-life setting, and the patient-registered time of food consumption for breakfast was approximately 0.5 h before/after the morning dose, dinner 3–4.5 h before the evening dose and supper around 0.5 h before/after the evening dose.

TABLE 1.

Demographic data and patient characteristics at first pharmacokinetic investigation (n = 30)

Sex, male/female	22/8
Age, y	62 (22–78)
Height, cm	178 (159–192)
Weight, kg	79 (52–104)
Time since transplantation to PK1, d	28 (13–54)
P-creatinine, µmol/L	122 (70–192)
Albumin, g/L	41 (36–49)
Tacrolimus dose, mg/d	3.0 (1.5–7.0)
Prednisolone dose, mg/d	15 (7.5–20)

Data are presented as numbers or median (range).
PK1, first pharmacokinetic investigation.

Chronopharmacokinetics of MPA and MPAG

The chronopharmacokinetics of MPA and MPAG under both real-life and fasting conditions are summarized in Tables 2 and 3, respectively. Mean plasma concentration–time curves are visualized in Figure 1. In a real-life nonfasting state, $AUC_{0–12}$ and C_0 failed to meet the bioequivalence criteria with ratios and associated 90% CIs of 0.85 (90% CI, 0.79-0.91) and 0.69 (90% CI, 0.61-0.78), respectively (Table 2). Following the evening dose, mean $AUC_{12–24}$ was 16% lower ($P < 0.001$) compared with the morning dose, and a tendency toward a faster absorption, reflected by a shorter (0.79 [90% CI, 0.63-0.99] h) T_{max} was observed ($P = 0.09$). Also under fasting conditions, the bioequivalence criteria were not fulfilled (Table 2). Still, only T_{max} showed a statistically significant difference between morning and evening dose and MPA $AUC_{12–24}$ was 13% lower than MPA $AUC_{0–12}$ (Table 2). For MPA there was also a 4- and 5-fold difference in $AUC_{0–12}$ and $AUC_{12–24}$ between individuals, respectively (min: 14.2, max: 66.9 mg·h/L). MPAG displayed circadian variation only under real-life conditions with significantly lower $AUC_{12–24}$ and C_{max} following the evening dose compared with the morning dose (Table 3).

Correlation C_0/C_{12} and AUC

The mean trough concentration before the morning dose (C_0) was significantly higher compared with the trough concentration before the evening dose (C_{12}) (2.7 versus 1.95 mg/L, $P < 0.01$). No associations between either $AUC_{0–12}$ or $AUC_{12–24}$ and C_0 or C_{12} were observed (Figure 2).

DISCUSSION

The main findings in this study were that MPA and MPAG PK showed circadian variation in the real-life nonfasting setting, with somewhat lower systemic exposure after the evening dose when compared with the morning dose. Fasting

TABLE 2.

Chronopharmacokinetics of MPA under real-life and fasting dose administration

MPA	Day	Night	Ratio	90% CI	*P*
Real-life (n = 35)					
$AUC_{0-12/12-24}$, mg·h/L	39.2 ± 11.0	33.1 ± 8.9	0.85	**0.79-0.91**	**<0.001**
C_{max}, mg/L	7.9 ± 3.0	7.5 ± 2.1	0.98	0.87-1.11	0.80
$C_{0/12}$, mg/L	2.7 ± 1.1	1.9 ± 0.8	0.69	**0.61-0.78**	**<0.001**
T_{max}, h	2.5 ± 2.3	1.7 ± 1.1	0.79	0.63-0.99	0.09
Fasting (n = 11)					
$AUC_{0-12/12-24}$, mg·h/L	38.1 ± 7.5	33.2 ± 9.0	0.86	0.72-1.01	0.11
C_{max}, mg/L	10.9 ± 5.5	7.4 ± 2.7	0.69	0.47-1.01	0.11
$C_{0/12}$, mg/L	2.8 ± 1.2	2.2 ± 0.7	0.79	0.63-0.99	0.09
T_{max}, h	1.2 ± 0.9	2.4 ± 1.6	2.08	**1.10-3.78**	**<0.05**

Data are presented as mean ± SD. Comparison between the morning dose and evening dose were calculated using Student's *t* test. All variables were ln-transformed before the statistical analysis. The bold type indicates a statistically significant difference between the morning and evening doses.
AUC_{0-12}, area under the plasma concentration vs time curve from 0 to 12 h (day); AUC_{12-24}, area under the plasma concentration vs time curve from 12 to 24 h (night); C_0, concentration before the morning dose; C_{12}, concentration before the evening dose; C_{max}, maximum concentration; CI, confidence interval; MPA, mycophenolic acid; T_{max}, time to C_{max}.

TABLE 3.

Chronopharmacokinetics of MPAG under real-life and fasting dose administration

MPAG	Day	Night	Ratio	90% CI	*P*
Real-life (n = 35)					
$AUC_{0-12/12-24}$, mg·h/L	502 ± 175	452 ± 140	0.90	**0.87-0.95**	**<0.001**
C_{max}, mg/L	60 ± 19	52 ± 14	0.88	**0.84-0.92**	**<0.001**
T_{max}, h	3.6 ± 2.1	3.4 ± 2.2	0.94	0.75-1.18	0.63
Fasting (n = 11)					
$AUC_{0-12/12-24}$, mg·h/L	496 ± 137	473 ± 104	0.96	0.85-1.09	0.62
C_{max}, mg/L	63 ± 20.8	58 ± 11.9	0.95	0.82-1.11	0.56
T_{max}, h	3.1 ± 1.6	3.2 ± 2.4	1.11	0.88-1.39	0.45

Comparison between the morning dose and evening dose were calculated using Student's *t* test. All variables were ln-transformed before the statistical analysis. Data are presented as mean ± SD. The bold type indicates a statistically significant difference between the morning and evening doses.
AUC_{0-12}, area under the plasma concentration vs time curve from 0 to 12 h (day); AUC_{12-24}, area under the plasma concentration vs time curve from 12 to 24 h (night); C_{max}, maximum concentration; CI, confidence interval; MPAG, mycophenolic acid glucuronide; T_{max}, time to C_{max}.

versus nonfasting status did not alter the PKs of either MPA or MPAG to a significant extent.

Several studies have investigated the chronopharmacokinetics of many immunosuppressants, such as tacrolimus[10–13] and cyclosporine A.[12,22,23] However, literature on the chronopharmacokinetics of MPA is sparse,[24–26] and only 1 study has investigated the effect of circadian variation on the PK of MPA in renal transplant recipients treated with MMF.[24] To our knowledge, the present study is the first to explore the chronopharmacokinetics of the primary metabolite MPAG in renal transplant recipients treated with MMF. Similar to our study, Satoh et al[24] performed a study with 30 renal transplant recipients in which they demonstrated that systemic exposure of MPA following the morning dose was greater than that following the evening dose. However, when dose adjusting the systemic exposure, the difference between morning and evening was no longer present. The observed difference in systemic exposure of MPA at daytime versus nighttime is most likely due to the delayed/altered gall bladder contraction during evening/night, which appears to result in an "activation" when the patients wake up. Comparable to the observations in our fasting population, Satoh et al[24] observed significantly higher C_{max} of MPA after the morning dose and shorter T_{max} compared with the evening dose, and no significant difference in the trough concentrations. A limitation with the study of Satoh et al[24] was that only 13 blood samples were collected during a 24-h period, compared with the 26 blood samples collected in our study. Thirteen samples will presumably not be sufficient to describe the complex PK of MPA satisfactory, particularly not the enterohepatic recirculation. Although the bioequivalence criteria were not fulfilled for most of the PK parameters in our study, the differences were not significantly different, most likely due to the considerable interindividual variation.

Along with the chronopharmacokinetics of MPA and MPAG, the effect of fasting status on MPA and MPAG PK is not well described in the literature.[16,17] Similar to the results in the present study, Bullingham et al[16] reported that with food, the systemic exposure of MPA was equivalent to that following an overnight fast, and found a slower absorption rate in the fed state. For MPAG, on the other hand, our results were in contrast with previously published literature where higher C_{max} and AUC of MPAG in the fed relative to the fasting state has been reported. Even though it has been suggested that more intricate processes involving changes in glucuronidation may occur with food, fasting status does not seem to significantly influence the PK of MPAG. It is necessary to note that the previously published literature on this topic is sparse and includes only 1 study with a small sample size and 1 case report, both performed in different patient populations with significantly higher dosages of MMF.[16,17]

The circadian regulation of the immune system is a factor that could influence the clinical significance of the results from the present study. Nocturnal sleep may also regulate

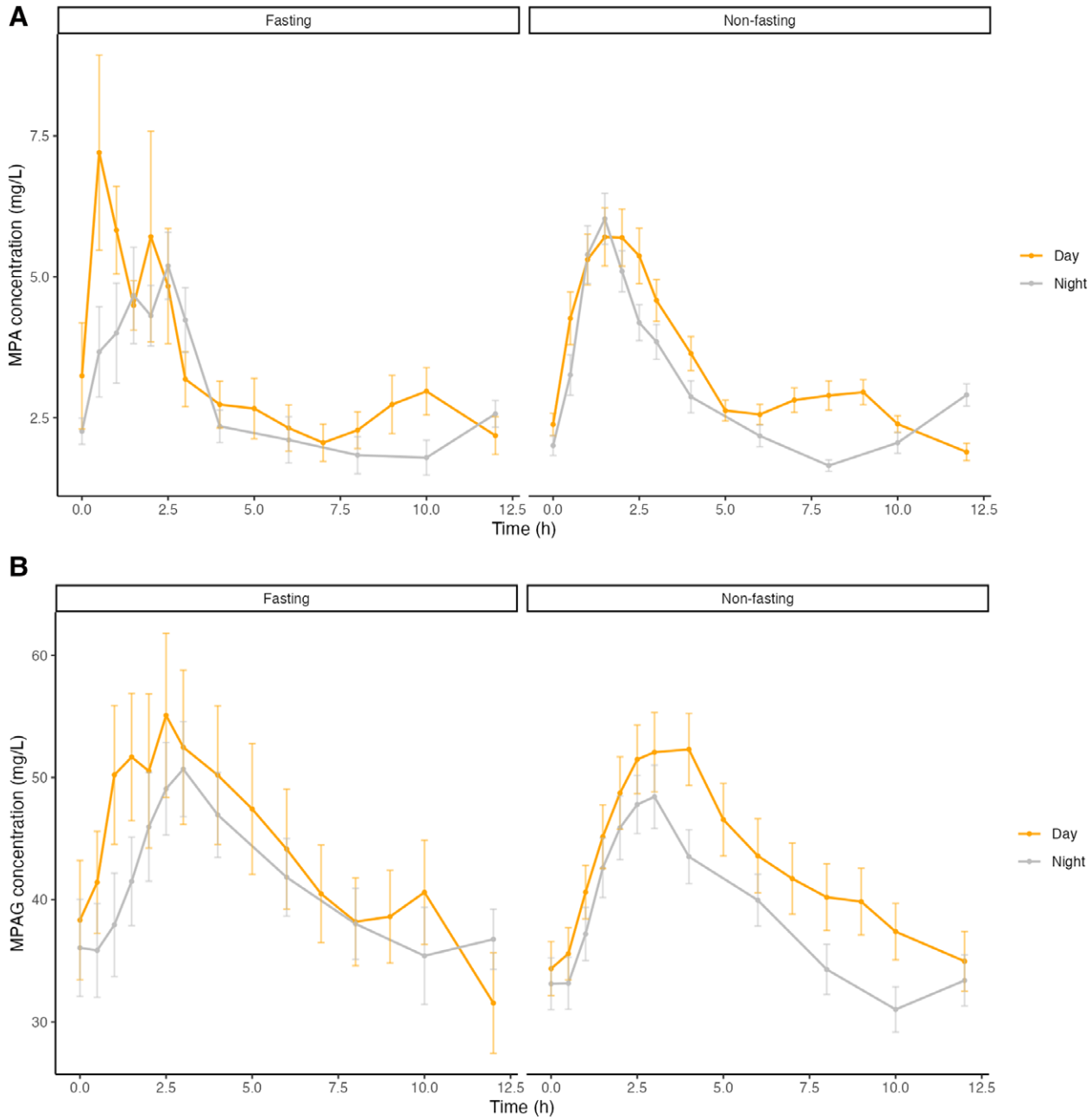

FIGURE 1. Mean (±SD) plasma concentration vs time profiles for (A) MPA and (B) MPAG in a fasting and nonfasting status. Both the pharmacokinetic profiles after the morning dose (orange) and evening dose (gray) are presented in the plot. MPA, mycophenolic acid; MPAG, 7-O-mycophenolic acid glucuronide.

the immune system, and Born et al[27] have shown that sleep considerably enhances the production of interleukin-2 by stimulated T-cells. The number of natural killer cells and lymphocytes is also significantly higher after nocturnal sleep than after nocturnal wakefulness.[27,28] Thus, appropriate nighttime immunosuppression may be essential for transplant recipients to prevent rejection because of the reported augmented immunocompetence during nocturnal sleep. Increased activity of the immune system and the fact that MPA and MPAG show lower systemic exposure after the evening dose compared with the morning dose may influence the clinical relevance of the presented results. However, this must be investigated to further understand the immune system's complex mechanisms.

Several studies have reported that the coadministration of PPIs and MMF may interfere with MMF absorption.[29–33] The interaction is demonstrated by a decrease in MPA C_0, C_{max}, and AUC, and an increase in T_{max}. In the present study, all patients were on proton pump inhibitory treatment. The PPI was given as a once-daily morning dose, which may have influenced the morning/evening MMF absorption differently. Therefore, one can theorize that the use of PPI may have masked an even greater circadian effect on MPA PK.

Although trough-based TDM of MPA is performed in some centers, weak correlations between MPA trough concentrations and MPA systemic exposure (r^2 = 0.003–0.7) have been reported.[34] This agrees well with the present results (Pearson's correlation coefficients ranging from –0.038 to 0.15), and

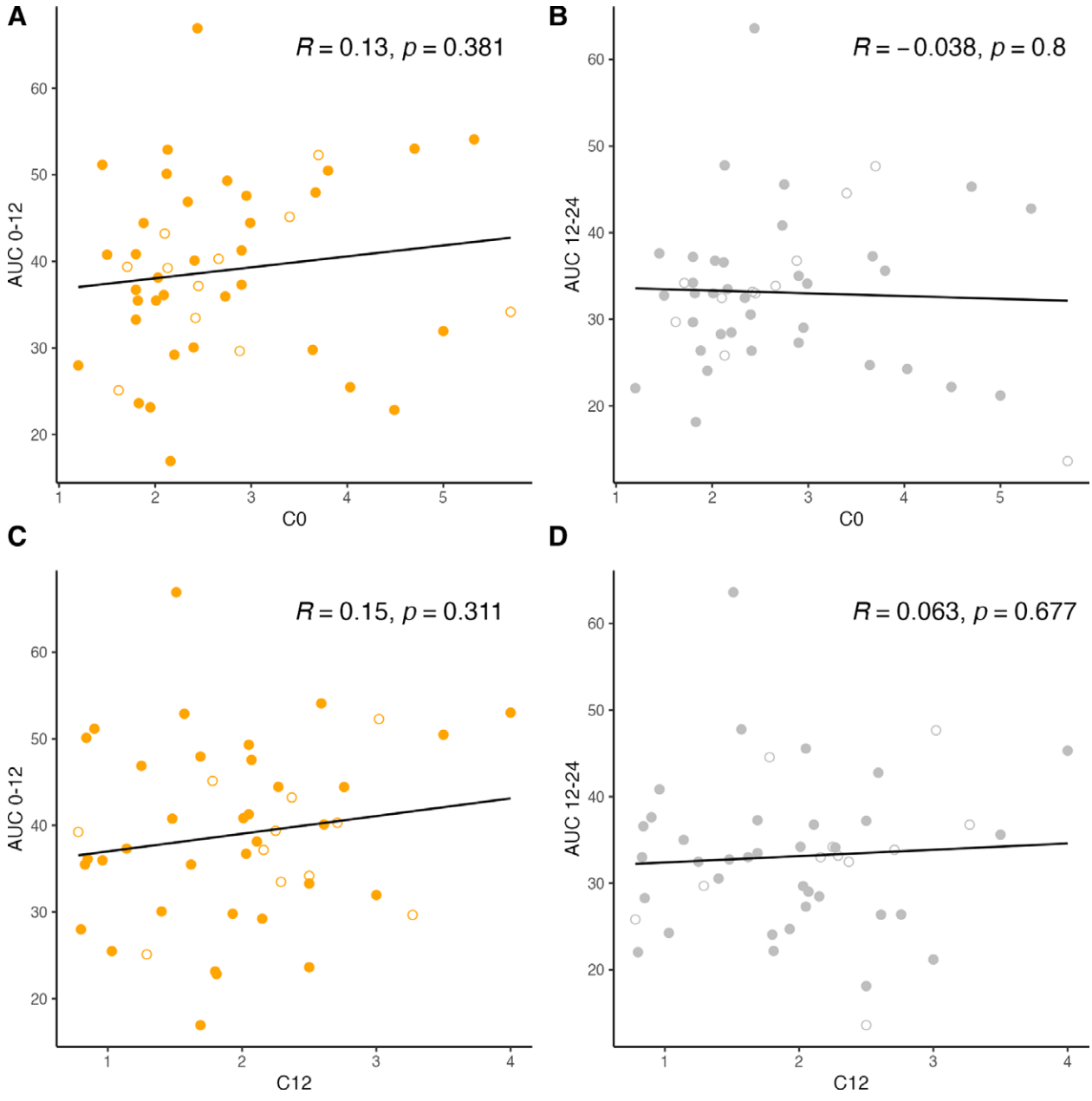

FIGURE 2. Associations between MPA trough concentrations and MPA-AUC. A, Association between C_0 and AUC_{0-12}, (B) C_0 and AUC_{12-24}, (C) C_{12} and AUC_{0-12}, and (D) C_{12} and AUC_{12-24}. The closed dots represent the nonfasting condition, whereas the open dots represent the fasting condition. *R* is the correlation coefficient. AUC, area under the curve; MPA, mycophenolic acid.

further support that MPA trough concentrations do not adequately reflect the MPA systemic exposure. The significant variation observed in MPA trough concentrations is most presumably due to the unpredictable enterohepatic recirculation, accounting for the secondary plasma peaks around 6–12 h after each dosing.[35–37] The mean trough concentration before the morning dose (C_0) was significantly higher than the mean trough concentration before the evening dose (C_{12}) probably due to a delayed enterohepatic recirculation after the evening dose. Therefore, it is essential to be consistent when performing TDM based on trough concentrations (C_0 versus C_{12}) to avoid unnecessary dose adjustments. The poor correlation between MPA trough concentrations and MPA-AUC demonstrates the necessity for new Bayesian forecasting methods based on population PK models for improved individualized dosing of MMF.

The main strength of the present study is the rich sampling obtained following both the morning and the evening dose of MMF, also in the period of the secondary peak brought by enterohepatic recirculation. In total, 1181 MPA samples were included in the present study, on average 26 per 24-h PK investigation. This ensures detailed individual descriptions of MPA and MPAG PK during the entire 24-h interval. Second, the study was performed in a real-life setting, that is, patients took their medications as in their everyday routine. This study obviously also has some limitations. First, this study is conducted in the early posttransplant phase. Because MPA PK changes during the first 6 mo following transplantation, the results from the present study should be extrapolated with caution to the long-term follow-up situation.[38–40] Second, the number of fasting versus real-life patients is unbalanced. Only

11 patients in the fasting group reduce the power of the study and increase the margin of error. Thus, it can be the reason for this group's lack of significant results, even though the main patterns are similar regardless of fasting status. The study was initially intended to be conducted only in real-life patients. However, we reconsidered and included some fasting patients due to the surprisingly flat PK profiles that tacrolimus displayed in the real-life setting.[10]

In conclusion, both MPA and MPAG showed circadian variation with somewhat lower systemic exposures following the evening dose. However, with current knowledge, these results will have limited clinical relevance in the dosing of MMF in renal transplant recipients. Fasting versus nonfasting conditions affect absorption rate differently, but with similar results in systemic exposure, which means that real-life data can be utilized in future clinical trials and when developing new Bayesian forecasting methods based on population PK models.

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