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Kidney Function as a Key Driver of the Pharmacokinetic Response to High-Dose L-Carnitine in Septic Shock

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Abstract

Background: Levocarnitine (L-carnitine) has shown promise as a metabolic-therapeutic for septic shock, where mortality approaches 40%. However, high-dose (≥ 6 grams) intravenous supplementation results in a broad range of serum concentrations.

Objectives: We sought to describe the population pharmacokinetics (PK) of high-dose L-carnitine, test various estimates of kidney function, and assess the correlation of PK parameters with pre-treatment metabolites in describing drug response for patients with septic shock.

Methods: We leveraged serum samples and metabolomics data from a phase II trial of L-carnitine in vasopressor-dependent septic shock. Patients were adaptively randomized to receive intravenous L-carnitine (6 grams, 12 grams, or 18 grams) or placebo. Serum was collected at baseline (T0); end-of-infusion (T12); and 24, 48, and 72 hours after treatment initiation. Population PK analysis was done with baseline normalized concentrations using nonlinear mixed effect models in the modeling platform Monolix. Various estimates of kidney function, patient demographics, dose received, and organ dysfunction were tested as population covariates.

Results: The final dataset included 542 serum samples from 130 patients randomized to L-carnitine. A two-compartment model with linear elimination and a fixed volume of distribution

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(17.1 liters) best described the data and served as a base structural model. Kidney function estimates as a covariate on the elimination rate constant (k) reliably improved model fit. Estimated glomerular filtration rate (eGFR), based on the 2021 Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) equation with creatinine and cystatin C, outperformed creatinine clearance (Cockcroft-Gault) and older CKD-EPI equations that use an adjustment for self-identified race.

Conclusions: High-dose L-carnitine supplementation is well-described by a two-compartment population PK model in patients with septic shock. Kidney function estimates that leverage cystatin C provided superior model fit. Future investigations into high-dose L-carnitine supplementation should consider baseline metabolic status and dose adjustments based on renal function over a fixed or weight-based dosing paradigm.

Keywords

critical illness; precision medicine; drug dosing; metabolomics

1.2 Introduction

Sepsis is a clinical syndrome defined by life-threatening organ dysfunction and a dysregulated host response to infection.[1] In 2017, nearly 50 million cases of sepsis were identified worldwide, and mortality in the most severe form, septic shock, approaches 40%.[2] Beyond antimicrobials, treatment for sepsis remains largely non-specific and supportive with a litany of failed clinical trials for more targeted interventions.[3]

Sepsis pathophysiology is complex but is partly characterized by a hypermetabolic state and mitochondrial dysfunction, both of which are associated with greater mortality.[4, 5] Given the lack of targeted metabolic pharmacotherapy, L-carnitine, an endogenous metabolite that serves a key bioenergetic role in the mobilization of fatty acids for mitochondrial beta-oxidation, was recently tested in patients with sepsis. In a phase I, randomized, double-blind trial, high-dose L-carnitine was found to be safe in 31 patients with septic shock and demonstrated a modest, but significant improvement in patient mortality versus placebo.[6] A follow-up phase IIb trial did not find evidence that L-carnitine significantly improved patient mortality or organ dysfunction[7], as measured by the Sequential Organ Failure Assessment (SOFA) score.[8] However, pharmacometabolomic analyses of the phase I trial demonstrated significant interpatient variability in post-treatment L-carnitine concentrations that correlated with mortality.[9, 10] Subsequent work showed that variations in the genetics of the organic cation transporter novel family member 2 (OCTN2), body size, and kidney function may also be important drivers of the observed variability and possibly, therapeutic response.[11] Furthermore, a significant mortality benefit from supplemental L-carnitine was observed in the phase IIb trial in patients with elevated acylcarnitines including acetylcarnitine.[12] Taken together, these findings suggest heterogeneity in the pharmacokinetics (PK) and effectiveness (pharmacodynamics, PD) of high-dose L-carnitine in septic shock.

The overall goal of our study was to construct a population PK model of high-dose, intravenous, L-carnitine in an acutely ill cohort of patients with septic shock to better understand the factors that drive L-carnitine blood concentration variability. Given that

L-carnitine is extensively cleared by kidney elimination[13], we recognized an additional opportunity to leverage trial data and contribute to the ongoing conversation regarding the ideal approach to estimate kidney function in critically ill patients. These patients are prone to acute kidney injury for which serum creatinine is not routinely a reliable measure of renal function.[14] As such, we tested different equations to estimate kidney function based on serum creatinine (S_{cr}), serum cystatin C (S_{cys}), and self-identified race in critically ill patients using high-dose L-carnitine. In addition, we sought to determine if other widely available patient covariates improved the model's predictions. As an exploratory aim, we assessed the relationship between individual patient PK parameters with baseline metabolic status and genomic variability in OCTN2.

1.3 Methods

1.3.1 Study design and participants

Our work was a secondary analysis of the Rapid Administration of Carnitine in Sepsis (RACE) clinical trial (NCT01665092).[7] The RACE study was a multicenter, placebo-controlled, phase IIb clinical trial that adaptively randomized patients with septic shock to saline placebo or one of three dosing arms for intravenous L-carnitine: 6 grams, 12 grams, or 18 grams. The Bayesian adaptive randomization scheme selected the highest dose as the most efficacious.[15] Study drug or an equivalent volume of saline placebo was given as an intravenous bolus (33% of dose) immediately followed by a 12-hour infusion. The trial was conducted in accordance with the Declaration of Helsinki, where all patients or their legal representatives provided informed consent and all sites were approved by their local Institutional Review Board.

Adult patients were eligible for the trial if they were: i) enrolled within 24-hours of the identification of septic shock; ii) required high-dose vasopressors; iii) presented with moderate organ dysfunction (SOFA ≥ 6); and iv) had a blood lactate of at least 18 mg/dL (2 mmol/L). Patients who were pregnant, breastfeeding, immunocompromised, or had a history of seizures were excluded. Serum samples for drug and other metabolomics analysis were collected at baseline (T0), end-of-infusion (T12), and 24 hours (T24), 48 hours (T48), and 72 hours (T72) after treatment initiation. Full inclusion and exclusion criteria, as well as detailed sample collection and processing have been previously described;[7, 12, 16] some additional trial details can be found in the supporting information.

1.3.2 Drug and Metabolite Quantification

Carnitine and acylcarnitines—We used an existing metabolomics data set including time-series measurements of L-carnitine and pre-treatment (baseline) measurements of acylcarnitines. Analytes were measured in serum samples collected in the RACE trial by reverse phase, liquid-chromatography mass-spectrometry (LC-MS) at the Michigan Regional Comprehensive Metabolomics Resource Core at the University of Michigan as previously described.[9, 12] Acylcarnitines are esters formed from the conjugation of L-carnitine and fatty acids of various carbon chain lengths.[17] Absolute quantification for L-carnitine and several acylcarnitines (C2, C3, C4, C5, C8, C14, and C16) was achieved through stable isotope internal standards at a known concentration (NSK-B Cambridge

Isotope Laboratories). An additional eight acylcarnitines were relatively quantified by peak area.

Small polar molecules—We also used an existing metabolomics data set of polar compounds that was acquired from pre-treatment serum samples using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). This assay, which was conducted at the University of Michigan's Biochemical Core, is detailed elsewhere[12, 16, 18] and identified and quantified 27 low-molecular weight metabolites. Metabolites included several amino acids, intermediates of the tricarboxylic acid (TCA) cycle, and other bioenergetic compounds.

1.3.3 Kidney Function Estimates

Quantification of serum creatinine and cystatin C—Serum creatinine was measured clinically as part of the RACE study, and baseline measures were abstracted from the trial's research electronic data capture (REDCap) database.[19] Cystatin C was measured using biobanked residual serum samples using a standard, commercially available enzyme-linked immunoassay (ELISA) assay according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, catalog number DSCTC0).

Equations to estimate kidney function—The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) has established equations to estimate glomerular filtration rate (eGFR) based on S_{cr} and/or S_{cys}, patient age, sex, and race. Given the increasing controversy about inclusion of patient race as a variable[20] and the drawbacks of S_{cr} as a kidney biomarker[21], we estimated eGFR using four iterations of the CKD-EPI equation: the 2009 CKD-EPI equation[22] (includes patient race and S_{cr}); the 2021 CKD-EPI equation[23] (uses S_{cr} but drops patient race); the 2012 CKD-EPI equation [24] (uses S_{cys}); and the 2021 CKD-EPI equation[23] (includes both S_{cr} and S_{cys} without an adjustment for race). All eGFR calculations were calculated according to standard body surface area and are in the units of mL/min per 1.73 m^2. We also tested the estimated creatinine clearance (CrCl) using the Cockcroft-Gault equation.[25]

1.3.4 Transporter Genotyping

L-carnitine is transported into the cell through the OCTN2 transporter, which is also responsible for its renal tubular reabsorption.[13] Given L-carnitine's critical role in metabolic homeostasis, loss of function variants in the gene encoding OCTN2 (SLC22A5) are rare and result in inborn errors of metabolism. Nonetheless, a single nuclear polymorphism (rs2631367, −207C>G) has been associated with lower mRNA levels in previous studies and in the Genotype-Tissue Expression (GTEx) Project.[26–28] We isolated DNA from buffy coat collected in the RACE trial and genotyped patients at the rs2631367 loci using a commercially available TaqMan genotyping assay (ThermoFisher®, assay ID C__26479161_30).

1.3.5 Pharmacokinetic modeling

We restricted our secondary analysis to patients who were randomized to receive study drug and who had a baseline and at least one post-treatment serum sample available. For population PK analysis, post-treatment L-carnitine concentrations were baseline normalized

in accordance with United States Food and Drug Administration guidance for modeling endogenous molecules.[29] Baseline normalization was done on the individual level such that each post-treatment L-carnitine concentration was subtracted from the individual's pre-treatment (baseline) measurement. Post-treatment concentrations below baseline were assigned a value of zero.

All data were cleaned in RStudio, and population PK analysis was performed in Monolix modeling platform (Version 2021R1, Lixoft SAS, Antony, France). Given the sparse sampling scheme of the RACE trial in relationship to the drug infusion time, we opted for a fixed population parameter for the volume of distribution (Vd) based on the median weight of the cohort and previous PK reports that Vd for intravenous L-carnitine is 0.2 to 0.3 L/kg.[13]

To determine the optimal structural PK model, we built a series of models with one, two, or three compartments and a linear elimination rate constant (k). We selected the model based on the Akaike information criterion (AIC) and model diagnostic plots. For the best performing structural model, we assessed the impact of different kidney function parameters as a covariate on the elimination rate constant. We tested the performance of eGFR as estimated by the various CKD-EPI equations described above; the CrCl according to Cockcroft-Gault; and S_{cr} and S_{cys} as standalone biomarkers. In addition, we considered the sarcopenia index, calculated as $100*(S_{cr} / S_{cys})$, which is biomarker of muscle mass rather than true kidney function.[30]

Next, we considered additional clinical and demographic patient variables as covariates using the available automated stepwise covariate model (SCM) building algorithm in Monolix. Patient demographics included age, sex, weight, and self-identified black race. We also considered the dose of L-carnitine received, organ dysfunction as measured by the SOFA score (with the kidney function score removed), and the sarcopenia index.

1.3.6 Statistical analysis of individual PK parameters

Once the final covariate model was selected, we explored the relationship between the predicted individual patient PK parameters and baseline metabolites, OCTN2 genotype, and patient mortality. Specifically, we computed the Spearman's coefficient between individual's predicted values for k, the rate constant out of compartment one (k_{12}), and the rate constant out of compartment two (k_{21}) and measured concentration of baseline acylcarnitines and small, polar metabolites measured by NMR. The correlation for comparisons were plotted for relationships with a p-value less than 0.05. We also compared the model predicted individual parameters stratified by OCTN2 genotype and 28-day patient mortality using the Kruskal–Wallis test and the Wilcoxon signed-rank test, respectively. All statistical analyses were performed using R Studio (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA; http://www.rstudio.com/).

1.4 Results

1.4.1 Patients and pharmacokinetic data

Of the 175 patients randomized to receive L-carnitine in the RACE trial, 130 patients had a baseline and follow-up serum sample available for population PK analysis. In these patients, we measured drug concentrations in 542 serum samples. Observations at T12 and T72 were underrepresented (Table 1), as these samples were only collected during the initial 'burn-in' phase of the trial, where the first 40 patients were randomized equally to all trial arms.[15] As such, 60% of the cohort in this secondary analysis was randomized to the 18-g treatment arm, as it was selected as the most efficacious by the Bayesian adaptive design.

Baseline S_{cr} was available for all patients. Four patients did not have a sufficient volume of residual baseline serum to measure S_{cys}, and values were imputed from a simple linear model using S_{cr} and patient age, sex, and weight as predictors (Supplementary Figure 1). Buffy coat for DNA isolation and thus genetic information at the rs2631367 loci of the OCTN2 transporter was only available in a subset of the cohort (N=110, Table 1).

1.4.2 Population pharmacokinetic modeling

The two-compartment and three-compartment structural models provided significant improvements in model fit over the one-compartment model (Table 2, ΔAIC = −204.31 and −206.74 points, respectively). Although the three-compartment model could be considered a superior model based on AIC reduction alone, this model was plagued by high residual standard errors (R.S.E.) for both population parameters and random effects (Table 2). Thus, we opted to proceed with the simpler, more stable two-compartment structural model. Model diagnostic plots, including the observed versus predicted concentrations, the distribution of residuals, and the visual predictive check (VPC), are provided for the 2-compartment structural model in the supplement (Supplementary Figures 2A, 3A, and 4A).

Kidney function as a covariate of the elimination rate constant reliably improved model fit regardless of the equation or biomarker used. Table 3 shows the impact on the AIC after including various kidney function estimates as a covariate on the elimination rate constant. The $eGFR_{cr\text{-}cys}$, estimated according to the 2021 CKD-EPI equation using both S_{cr} and S_{cys}, provided the largest reduction in AIC (*−48.74 points).* The $eGFR_{cr}$ (2021 CKD-EPI using only S_{cr}), $eGFR_{cys}$ (2012 CKD-EPI using only S_{cys}), and CrCl provided a similar improvement over the base structural model (ΔAIC = −39.56, −40.9, and −39.68 points, respectively). A similar AIC reduction (−40.04 points) was seen for $eGFR_{race\text{-}cr}$ (2009 CKD-EPI using both self-identified race and S_{cr}) compared to eGFR estimates without the race factor. Inclusion of S_{cys} concentration as a covariate of the elimination rate (Δ AIC = −37.42 points) outperformed S_{cr} (Δ AIC = −31.71 points) as a single kidney function biomarker. The sarcopenia index, a measure of muscle loss rather than true kidney function, provided a worse model fit compared to the structural base model (Δ AIC = +3.29 points).

With $eGFR_{cr\text{-}cys}$ as a covariate of k, we ran the automated SCM algorithm in Monolix, which considered additional patient factors as covariates of k, k_{12}, and k_{21} in a stepwise fashion. Age as an additional patient covariate of the elimination rate constant was selected

in the final model. Population parameters for this 'best' performing model are shown in Table 4 and model diagnostic plots are shown in Supplementary Figures 2B, 3B, and 4B.

1.4.3 Other patient factors and individual variation in pharmacokinetics

From the final model, we determined the individual population PK parameters for the elimination rate constant (k), the rate constant out of compartment one (k_{12}), and the rate constant out of compartment two (k_{21}). Figure 1A compares individual estimates for the elimination rate to the $eGFR_{cr\text{-}cys}$ stratified by self-identified race. This demonstrated a strong, positive relationship between the elimination rate of L-carnitine and kidney function that is consistent across the two groups. In contrast to kidney function, the relationship between patient weight and the elimination rate was negligible (Supplementary Figure 5, $R^2 = 0.01$).

Individual PK parameters were also compared to OCTN2 genotypes (rs2631367), baseline metabolite concentrations, and patient mortality at 28-days. Twenty-three patients were wildtype (CC) at rs2631367, while 87 patients contained either one (CG, 50 patients) or two (GG, 37 patients) copies of the G allele, which has been associated with greater transporter expression. There was no evidence of a relationship between OCTN2 genotype and any individual PK parameters (by the Kruskal–Wallis rank test, $p>0.05$). Patients who died before 28-days had a lower predicted value for k (Wilcoxon signed rank test, p=5.1e-05, Figure 1B), but similar values for k_{12} and k_{21}. Figure 1C shows the correlation between individual PK parameters and baseline metabolites measured by LC-MS or NMR. Baseline acylcarnitines tended to be negatively correlated to k and positively correlated to $k_{21.}$ Lactate and creatinine were also negatively correlated to k.

1.5 Discussion

The host-response to infection and pharmacotherapy in sepsis is highly heterogeneous.[31] A phase I trial of intravenous L-carnitine in patients with septic shock demonstrated a high degree of interindividual variability in the response to the candidate metabolic-therapeutic.[6, 9] This high degree of variability was also evident in post-treatment blood concentrations of L-carnitine. In this secondary population PK analysis of the subsequent phase IIb trial[7], a two-compartment model with a fixed population parameter for the Vd and eGFR as covariates of the elimination rate constant best fit the observed data. Importantly, we also found that patient mortality and baseline metabolic status, but not transporter genomics, were related to individual drug response. Pre-treatment Scr was the metabolite most strongly associated with L-carnitine elimination (Figure 1C); other metabolites, those attributable to energy metabolism, were also inversely associated with the estimated L-carnitine elimination rate constant. In aggregate, these findings suggest that while renal function is a primary driver of the variability in L-carnitine blood concentrations following high-dose administration in sepsis patients, pre-treatment energy metabolism also contributes.

In addition to the found broad dynamic range of measured L-carnitine blood concentrations, the lack of PK data for L-carnitine given at high-doses in patients with septic shock served as the primary justification for our analysis. We also leveraged prior knowledge

to inform our analysis. In the phase I trial of high-dose L-carnitine in septic shock there was considerable interpatient variability in carnitine and acylcarnitine concentrations post-treatment, with elevated levels associated with mortality.[6, 9] Here, we see a similar broad-dynamic range in concentrations following treatment, with non-survivors characterized by lower individual values for the elimination rate and higher concentrations (Figure 1B). Similarly, all acylcarnitines measured were negatively correlated with individual parameters for the elimination rate as were other energy-related metabolites (Figure 1C). Adverse drug reactions due to L-carnitine were assessed in the phase I[6] and II[7] trials of L-carnitine but known toxicity to the compound, including an increased potential for seizures and gastrointestinal side effects, were not widely reported. This suggests the higher mortality in patients with elevated concentrations is not directly attributable to L-carnitine toxicity, however this cannot be completely ruled out. Rather, we speculate that the patients with elevated concentrations had worse kidney function and greater metabolic dysfunction over the course of the study. Importantly, we and others have shown that elevations in acylcarnitines in patients with sepsis who have not received supplemental L-carnitine are associated with ~~increased~~ disease severity and mortality.[32–34]

Previous reports of L-carnitine PK utilized lower doses that are routinely used in the clinic and in patients who are not acutely ill.[13, 35] Administration of radiolabeled L-carnitine demonstrated a renally eliminated drug that can be represented as a 3- compartment model with a central pool (approximating extracellular fluid), a faster equilibrating compartment (likely generalizing to kidney and liver), and a slowly equilibrating compartment (i.e., skeletal muscle).[36] Moreover, endogenous L-carnitine is extensively (>98%) reabsorbed in the renal tubules, and single intravenous doses demonstrate saturation of this process and increased clearance of the compound.[37, 38] Although we tested more complex population PK models with nonlinear elimination and multiple compartments, these models were characterized by a higher AIC and poor predictions compared to the 2-compartment model with linear elimination. However, our work does strongly support the importance of kidney function in the elimination of high-dose exogenous L-carnitine, as each kidney function estimate we considered as a covariate of k dramatically lowered the AIC compared to the base model (Table 3). This strengthens our justification in using L-carnitine to test alternative equations that estimate kidney function in critically ill patients.

Current clinical and drug development standards rely on the measurement of S_{cr} as a biomarker of kidney function. Although wide use and the international standardization of the analytical method to quantify S_{cr} are strengths of this paradigm, there are increasing calls to adopt alternative kidney function biomarkers, particularly in critically ill patients.[14] Another endogenous biomarker, S_{cys}, has demonstrated modest improvement in estimating kidney function for renally eliminated drugs.[39] In our analysis, we found that S_{cys} outperformed S_{cr} as an individual kidney function biomarker and covariate of the elimination rate of a renally cleared compound. We also found eGFR equations that leverage S_{cys} provided superior model performance and that inclusion of race to estimate eGFR weakened model fit. Our work adds to growing calls to reconsider the approach to estimating kidney function in clinical practice and drug development.

Finally, we assessed genetic variability at rs2631367 in the OCTN2 transporter to determine its contribution to L-carnitine blood concentration variability and because we had previously found that it was associated with peak concentrations of L-carnitine.[11] The G allele has been associated with increased mRNA expression of the transporter in eQTL analysis, potentially granting systemic tissue a greater ability to sequester exogenous L-carnitine.[11] Our results here found that the elimination rate and the rates into and out of tissue were not meaningfully related to OCTN2 genotype. Given that OCTN2 is a highly conserved transporter, owing to its critical role in host bioenergetics, it is possible the impact of altered gene transcription was insufficient to impact drug response in a heterogeneous, acutely ill clinical cohort. Moreover, we lacked detailed concomitant medications in the RACE trial and are unable to account for drug-transporter interactions that could impact tissue sequestration of L-carnitine.

Our study has several strengths and limitations that warrant further consideration. We employed rigorous metabolomics and PK methods to build a well-performing population model of high-dose, intravenous L-carnitine in the setting of septic shock. In building the population model, we chose to test the impact of only patient covariates commonly available in the clinical setting. However, as mentioned above, this did not include an accounting of concomitant medication use, in particular those known to adversely impact mitochondrial function such as propofol and valproic acid.[40] Nevertheless, we had a unique opportunity to assess the relationship between less commonly available patient information including OCTN2 transporter genotype and baseline metabolic status. We were also able to assess different approaches for estimating kidney function in critical illness using a therapeutic candidate drug that is extensively cleared by the kidneys. However, we acknowledge since the study was not designed to model L-carnitine PK, the blood sampling scheme for the trial was rather sparse, particularly early during the drug's infusion, which superseded our ability to fit a population parameter for the Vd. In addition, we opted to use baseline normalization when considering drug concentrations post-treatment, as L-carnitine is an endogenous molecule and the investigative product administered was not radio-labeled. Our estimates of kidney function are also indexed to a standard body surface area (i.e., eGFR estimates in mL/min/1.73 m^2). Missingness in patient height data precluded us from individualizing these estimates in absolute units (mL/min). Finally, our measurement of S_{cys} was done using residual, biobanked serum and a commercially available ELISA kit rather than a clinical measurement from a fresh patient sample. As such, our results regarding the optimal method for estimating eGFR must be interpreted as exploratory and requires rigorous further validation using additional cohorts of critically ill patients and probe drug molecules.

In conclusion, we found that high-dose intravenous L-carnitine in patients with septic shock can be reliably modeled at the population level using a two-compartment model with linear elimination. Kidney function as a covariate of the elimination rate dramatically improved model performance, with methods that incorporate $S_{cys,}$ but not patient race, providing the greatest improvement. We also found that patient mortality and baseline metabolites were strongly related to individual patient PK parameters. Future assessment of high-dose L-carnitine as a therapeutic for septic shock could include a more tailored dosing approach that considers renal function and pre-treatment metabolic status. We have previously shown

that pre-treatment acetylcarnitine serum concentration is predictive of therapeutic benefit from L-carnitine treatment.[34] Consideration of these patient features could aid in moving sepsis, a field that presently has few therapeutic options, towards a precision medicine approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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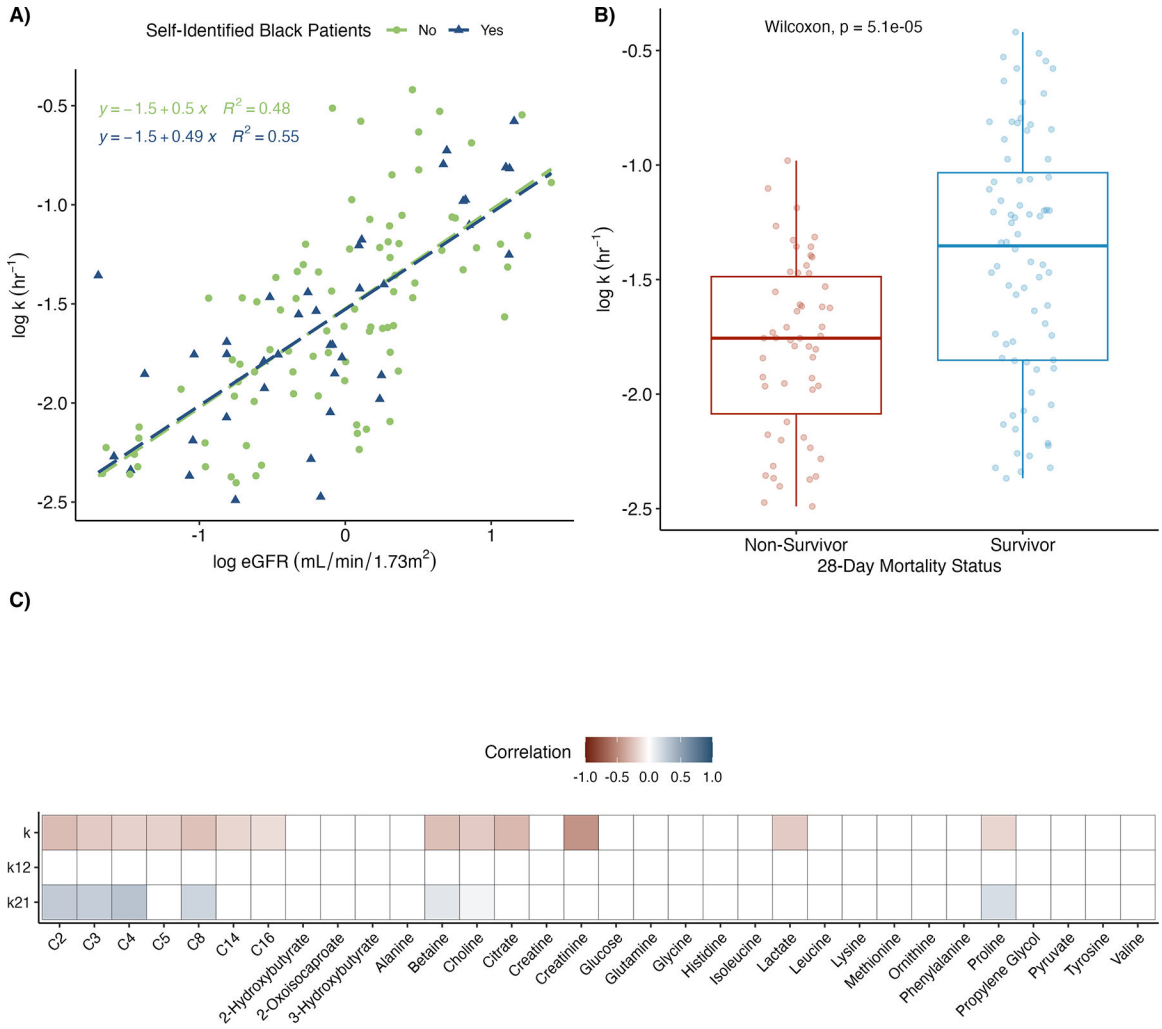

Figure 1. Association of individual patient parameters with patient characteristics.

(A) Scatter plot and line of best fit for the predicted elimination rate constant (k) from the final model versus eGFR. Self-identified black (in blue) and non-black (in green) patients are plotted separately. eGFR was estimated using the 2021CKD-EPI equation with serum creatinine and cystatin c. Patient parameters were log-transformed prior to plotting. (**B**) Boxplots of estimated elimination rate constant (k) stratified by 28-day mortality status. (**C**) Heatmap of Spearman correlation coefficients between conditional mode estimated individual parameters and baseline (pre-treatment) metabolite levels as measured by LC-MS (acylcarnitines, C2-C16) and ^{1}H-NMR spectroscopy. Individual parameters considered were k and rate in-to (k_{12}) and out-of (k_{21}) tissue. eGFR: estimated glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; LC-MS: liquid chromatography-mass spectroscopy; ^{1}H-NMR: proton nuclear magnetic resonance.

Table 1.

Patient demographics and clinical characteristics of individuals included in population pharmacokinetic modeling.

Patient characteristics	**Total Patients, N = 130**[1]
L-carnitine dose received	
6 grams	27 (21%)
12 grams	25 (19%)
18 grams	78 (60%)
Sex (N)	
Female	50 (38%)
Male	80 (62%)
Age (years)	62 (53, 70)
Weight (kg)	85 (70, 102)
Self-Identified Race	
Black	41 (32%)
White, Asian, or Other	89 (68%)
Serum Creatinine (mg/dL)	1.93 (1.29, 2.79)
Serum Cystatin C (mg/L)	2.44 (1.57, 3.66)
Baseline SOFA Score (points)	10.0 (8.0, 13.0)
OCTN2 genotype at rs2631367 (~~N~~)	
CC	23 (18%)
CG	50 (38%)
GG	37 (28%)
Unknown	20 (15%)
Serum samples analyzed for pharmacokinetics	
Baseline, T0	130 (100%)
End-of-infusion, T12	23 (18%)
24-hours after treatment initiation, T24	127 (98%)
48-hours after treatment initiation, T48	114 (88%)
72-hours after treatment initiation, T72	18 (14%)

[1]Data shown as n (%); Median (IQR)

SOFA: Sequential Organ Failure Assessment score; OCTN2: Organic cation transporter novel family 2 (gene name: solute carrier family 22 member 5, SLC22A5)

Table 2.
Comparison of structural pharmacokinetic models.

Both two- and three-compartment models provided substantial improvement in model performance based on the reduction in AIC. The simpler, two-compartment model was selected based on model diagnostic plots and the high residual standard errors in the three-compartment model.

Model	1-compartment linear elimination (base)	2-compartment linear elimination	3-compartment linear elimination
Model comparison			
AIC	2685.77	2481.46	2479.03
ΔAIC	-	−204.31	−206.74
Fixed-effect parameters			
V_pop (L)	17.1	17.1	17.1
k_pop (h^{-1})	0.053 (8.18%)	0.22 (6.27%)	0.18 (23.7%)
k12_pop (h^{-1})	-	0.55 (14.4%)	0.84 (16.3%)
k21_pop (h^{-1})	-	0.19 (11.8%)	0.60 (45.6%)
k13_pop (h^{-1})	-	-	0.13 (24.9%)
k31_pop (h^{-1})	-	-	0.087 (77.0%)
Random-effect parameters			
Standard deviation of inter-individual variability (IIV)			
ωV	2.1 (7.28%)	0.48 (24.7%)	0.28 (44.5%)
ωk	0.75 (7.76%)	0.52 (10.9%)	0.57 (19.6%)
ωk12	-	0.24 (69.2%)	0.31 (51.6%)
ωk21	-	0.48 (20.3%)	0.54 (28.5%)
ωk13	-	-	0.49 (315%)
ωk31	-	-	2.25 (34%)
Residual variability (RV)			
b	0.36 (6.16%)	0.36 (7.24%)	0.34 (6.78%)

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate using equations that leverage self-identified black race (race), serum creatinine (cr), and/or serum cystatin C (cys); AIC: Akaike information criteria; V_pop: fixed population parameter for volume of distribution; k_pop: fit population parameter for the elimination rate; k12_pop, k21_pop, k13_pop, k31_pop : fit population parameter for the rate into/out of compartments; ωV, ωk, ωk12, ωk21, ωk13, ωk31: standard deviation of random effects for population parameters; b: estimated value from proportional error model.

Table 3.

Population pharmacokinetic models with different renal function estimates as a covariate of the elimination rate, k.

Results shown represent the population parameter estimate and residual standard error (%).

Model	Structural Model	2009 CKD-EPI $eGFR_{race-cr}$	2021 CKD-EPI $eGFR_{cr}$	2012 CKD-EPI $eGFR_{cys}$	2021 CKD-EPI $eGFR_{cr-cys}$	Creatinine Clearance	Serum Creatinine	Serum Cystatin C	Sarcopenia Index
Model comparison									
AIC	2481.46	2441.42	2441.9	2440.56	2432.72	2441.78	2449.67	2444.04	2484.75
ΔAIC	-	-40.04	-39.56	-40.9	-48.74	-39.68	-31.79	-37.42	3.29
Fixed-effect parameters									
V_pop (L)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
k_pop (h^{-1})	0.22 (6.27%)	0.2 (5.34%)	0.19 (6.71%)	0.23 (5.78%)	0.21 (5.3%)	0.17 (5.92%)	0.22 (5.52%)	0.20 (5.11%)	0.19 (7.93%)
k12_pop (h^{-1})	0.55 (14.4%)	0.56 (12.1%	0.41 (28.7%)	0.39 (17.0%)	0.34 (17.4%)	0.43 (10.8%)	0.55 (15.9%)	0.32 (10.8%)	0.30 (33.8%)
k21_pop (h^{-1})	0.19 (11.8%)	0.2 (12.2%)	0.17 (17.0%)	0.17 (12.2%)	0.16 (13.4%)	0.18 (10.8%)	0.20 (15.5%)	0.15 (9.54%)	0.15 (19.7%)
β_{renal} on k//	-	0.44 (15.9%)	0.45 (16.3%)	0.45 (18.2%)	0.5 (13.1%)	0.48 (14.6%)	-0.50 (15.8%)	-0.54 (15.5%)	-0.08 (156%)
Random-effect parameters									
Standard deviation of inter-individual variability (IIV)									
ωV	0.48 (24.7%)	0.31 (17.6%)	0.23 (38.9%)	0.18 (197%)	0.25 (23.6%)	0.22 (27.8%)	0.43 (18.1%)	0.20 (42.5%)	0.27 (37.4%)
ωk	0.52 (10.9%)	0.45 (10.0%)	0.44 (9.87%)	0.47 (14%)	0.43 (9.62%)	0.46 (8.97%)	0.41 (11.4%)	0.48 (9.26%)	0.55 (9.29%)
ωk12	0.24 (69.2%)	0.27 (29.2%)	0.39 (39.2%)	0.4 (30.5%)	0.24 (63.1%)	0.36 (25.9%)	0.29 (49.1%)	0.41 (28.9%)	0.57 (23.0%)
ωk21	0.48 (20.3%)	0.51 (17.5%)	0.47 (27.8%)	0.54 (26.6%)	0.63 (15.7%)	0.64 (14.6%)	0.56 (18.0%)	0.53 (14.0%)	0.60 (21.7%)
Residual variability (RV)									
b	0.36 (7.24%)	0.36 (6.54%)	0.38 (6.9%)	0.36 (8.43%)	0.36 (6.4%)	0.36 (6.74%)	0.35 (6.32%)	0.37 (6.75%)	0.36 (6.75%)

// k = k_pop × (renal estimate/constant) β. For eGFR and creatinine clearance the constant was 30. For serum creatinine, cystatin C, and the sarcopenia index the constant was set equal to the median observed value (1.9, 2.3, and 81.3, respectively).

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate using equations that leverage self-identified black race (race), serum creatinine (cr), and/or serum cystatin C (cys); AIC: Akaike information criteria; V_pop: fixed population parameter for volume of distribution; k_pop: fit population parameter for the elimination rate; k12_pop and k21_pop: fit population parameter for the rate into/out of second compartment; ωV, ωk, ωk12, ωk21: standard deviation of random effects for population parameters; b: estimated value from proportional error model.

Table 4.

Population pharmacokinetic model selected by the automated stepwise covariate model (SCM) building algorithm in Monolix.

Renal function, estimated as eGFR by the 2021 CKD-EPI equation using both serum cystatin C and serum creatinine, and age were selected as covariates of the elimination rate, k.

Final Model	**2-compartment, linear elimination $eGFR_{cys, Scr}$ & Age as covariates on k**
Model comparison	
AIC	2427.31
ΔAIC vs. structural model [+]	−54.15
Fixed-effect parameters	
V_pop (L)	17.1
k_pop (h^{-1})	0.22 (6.47%)
k12_pop (h^{-1})	0.46 (20.4%)
k21_pop (h^{-1})	0.18 (11.8%)
β_{eGFR} on k[//]	0.45 (16.5%)
β_{Age} on k[//]	−0.52 (39.8%)
Random-effect parameters	
Standard deviation of inter-individual variability (IIV)	
ωV	0.3 (22.8%)
ωk	0.43 (13.7%)
ωk12	0.15 (123%)
ωk21	0.62 (31.6%)
Residual variability (RV)	
b	0.36 (8.06%)

[+]Comparison is to the 2-compartment structural model described in Table 2.

[//]$k = k\_pop \times (eGFR/30)^{\beta eGFR} \times (Age/60)^{\beta Age}$.

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: estimated glomerular filtration rate using equations that leverage self-identified black race (race), serum creatinine (cr), and/or serum cystatin C (cys); AIC: Akaike information criteria; V_pop: fixed population parameter for volume of distribution; k_pop: fit population parameter for the elimination rate; k12_pop and k21_pop: fit population parameter for the rate into/out of second compartment; ωV, ωk, ωk12, ωk21: standard deviation of random effects for population parameters; b: estimated value from proportional error model.