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Authors

Vora, Bianca
Wen, Anita
Yee, Sook Wah
et al.

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The Effect of Trimethoprim on Thiamine Absorption: A Transporter-Mediated Drug-Nutrient Interaction

Bianca Vora[1], Anita Wen[2], Sook Wah Yee[1], Kim Trinh[3], Mina Azimi[1], Elizabeth A. E. Green[1], Marina Sirota[4], Andrew S. Greenberg[3], John W. Newman[2,5,6] and Kathleen M. Giacomini[1,*]

Trimethoprim is predicted to inhibit several thiamine transporters, including the primary thiamine intestinal absorptive transporter, ThTR-2, and the hepatic and renal organic cation transporters, OCT1, OCT2, and MATEs. To investigate the effect of trimethoprim on thiamine absorption, studies were conducted in cells, mice, and healthy volunteers and supported by use of real-world data. In a randomized, crossover clinical study, seven healthy volunteers were given a single oral dose of thiamine or thiamine plus trimethoprim, followed by blood sampling. The thiamine area under the curve (AUC) increased with trimethoprim co-administration (*P* value = 0.031). Similar results were seen in mice. Trimethoprim appeared to act on thiamine absorption through inhibition of hepatic OCT1 as evidenced from its ability to modulate levels of isobutyrylcarnitine and propionylcarnitine, OCT1 biomarkers identified from metabolomic analyses. Real-world data further supported this finding, showing an association between trimethoprim use and higher levels of triglycerides, LDL cholesterol, and total cholesterol, consistent with OCT1 inhibition (*P* values: 2.2×10^{-16}, 5.75×10^{-7}, and 5.82×10^{-7}, respectively). These findings suggest that trimethoprim increases plasma levels of thiamine by inhibiting hepatic OCT1. Trimethoprim reduced urinary excretion and clearance of biomarkers for OCT2 and MATEs, consistent with inhibition of renal organic cation transporters. This inhibition did not appear to play a role in the observed increases in thiamine levels. This study highlights the potential for drug-nutrient interactions involving transporters, in addition to transporters' established role in drug–drug interactions.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Inhibition of the intestinal thiamine transporter, ThTR-2, can lead to thiamine deficiency. Fedratinib appears to inhibit ThTR-2 clinically resulting in thiamine deficiency. However, it is not known whether other drugs, such as trimethoprim, which are also potent inhibitors of ThTR-2, lead to thiamine deficiency.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Do current prescription drugs, such as trimethoprim, inhibit ThTR-2 and does administration of these drugs result in reduced thiamine levels through ThTR-2 inhibition? This study characterized the effect of trimethoprim on thiamine plasma concentrations using a multifaceted approach involving *in vitro* transporter assays, a prospective human clinical trial, *in vivo* studies in mice, and real-world evidence.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study suggests that despite potent inhibition of intestinal ThTR-2 *in vitro*, trimethoprim appears to increase thiamine plasma levels. Measurement of various biomarkers for OCTs and MATEs, along with plasma levels and renal clearances of thiamine, suggest that the mechanism by which trimethoprim increases thiamine plasma levels is related to its inhibition of hepatic OCT1 and ThTR-2. This study highlights that nutrients are often substrates of multiple transporters and human studies that measure transporter biomarkers are needed to fully comprehend drug-nutrient interactions. Although OCT1 is a well-established transporter for drug–drug interactions (DDIs), this study provides the first investigation into OCT1 being a target for drug-nutrient interactions.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Trimethoprim could serve as an important tool for assessing OCT1-mediated DDI studies, in particular for drugs with limited renal elimination. Secondly, measurement of transporter biomarkers in plasma and urine is useful for understanding the mechanisms involved in transporter-mediated DDIs and drug-nutrient interactions.

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[1]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, California, USA; [2]Department of Nutrition, University of California Davis, Davis, California, USA; [3]Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Medford, Massachusetts, USA; [4]Bakar Computational Health Sciences Institute, University of California San Francisco, San Francisco, California, USA; [5]West Coast Metabolomics Center, Genome Center, University of California Davis, Davis, California, USA; [6]Western Human Nutrition Research CenterUnited States Department of Agriculture, Agriculture Research Service, Davis, California, USA. *Correspondence: Kathleen M. Giacomini (kathy.giacomini@ucsf.edu)

Bianca Vora, Anita Wen, and Sook Wah Yee are joint first authors.

Commonly used prescription drugs can lead to nutrient deficiencies, which can subsequently result in clinically significant adverse events. This is particularly a concern for special patient populations, such as pregnant women, older adults, and those who are more vulnerable to developing nutrient deficiencies due to poor diet or diseases. Drug-induced nutrient deficiencies have previously been reported in the literature, including metformin-induced vitamin B12 deficiency as well as dolutegravir- and sodium valproate-induced folic acid deficiency.[1–3] Although drug-induced vitamin deficiency is not a common adverse event observed in clinical trials for new molecular entities, it represents a potential health risk for vulnerable patient populations.

In 2012, a disastrous clinical trial with fedratinib, a Janus Kinase 2 (JAK2) inhibitor, highlighted the potential importance of pharmaceutical agents as causative of thiamine (vitamin B1) deficiency. Fedratinib was being developed for the treatment of myelofibrosis and was placed on clinical hold by the US Food and Drug Administration (FDA) when several patients developed symptoms similar to Wernicke's encephalopathy (WE), a life-threatening disease caused by thiamine deficiency.[4–7] Although fedratinib was approved by the FDA, its package insert includes a boxed warning about serious and fatal encephalopathy, including WE, and advises assessing thiamine levels prior to and during treatment.[8]

Subsequent studies showed that fedratinib is a potent inhibitor of the thiamine transporter 2 (ThTR-2; SLC19A3), the major intestinal absorptive transporter of thiamine.[9,10] *In vitro* studies have shown that several commonly prescribed drugs can also inhibit ThTR-2.[10–12] Data from our group, using a high-throughput screening assay, showed that 146 prescription drugs (out of 1,360 compounds screened) inhibited ThTR-2 *in vitro*. Several of these were predicted to inhibit ThTR-2 at clinically relevant intestinal concentrations.[12]

One of the drugs which was predicted to be a potent, clinically relevant ThTR-2 inhibitor is trimethoprim. Trimethoprim is an antibiotic, commonly combined with sulfamethoxazole, used in the treatment and prevention of various bacterial infections, including but not limited to urinary tract infections, traveler's diarrhea, pediatric otitis media, and shigellosis.[13–15] Trimethoprim (with sulfamethoxazole) can be taken chronically for certain indications, including prevention of opportunistic infections, such as *Pneumocystis carinii* pneumonia, in patients diagnosed with human immunodeficiency virus (HIV).[13,16–18] Trimethoprim has been shown to reduce creatinine clearance and inhibit several renal transporters, including OCT2, MATE1, and MATE2, when metformin is used as a substrate.[19–25]

The goal of this study was to determine the effect of trimethoprim on thiamine concentrations in healthy volunteers. Based on our *in vitro* studies, we hypothesized that trimethoprim would inhibit ThTR-2 and result in reduced thiamine absorption and systemic plasma levels. We used a multifaceted approach using *in vitro*, clinical trial, *in vivo*, and real-world data to investigate whether trimethoprim modulates thiamine levels through inhibition of ThTR-2 and/or other thiamine transporters in the liver (e.g., OCT1) and/or kidneys (e.g., OCT2, MATE1, and MATE2; **Figure 1**). Because of institutional restrictions on conducting non-coronavirus disease 2019 (COVID-19) related clinical trials that were imposed at the onset of our study, only 7 individuals completed the trial. However, the small clinical trial was complemented with studies in animals, measurement of endogenous transporter substrates, and real-world data, allowing us to make conclusions and present these findings here. Our results strongly support the finding that trimethoprim is a strong organic cation transporter inhibitor, affecting multiple hepatic and renal organic cation transporters clinically. However, its effects on thiamine levels appeared to be due to inhibition of OCT1. To our knowledge, this is the first clinical study investigating the effect of trimethoprim on thiamine concentrations.

METHODS

Transporter inhibition studies

Inhibition of thiamine transporters, ThTR-2, OCT1, OCT2, MATE1, and MATE2, by trimethoprim were assessed (**Figure 1**). These stable cell lines (each expressing a respective thiamine transporter) were established previously by our group and were used in the inhibition studies presented here: ThTR-2 (SLC19A3), OCT1 (SLC22A1), OCT2 (SLC22A2), MATE1 (SLC47A1), and MATE2 (SLC47A2).[11,12,26,27] See **Supplementary Information** for more information, including methods to perform transporter inhibition studies.

Prediction of transporter-mediated inhibition

The 2020 FDA Drug–Drug Interaction Guidance was used to evaluate the clinical relevance of a trimethoprim-thiamine interaction for each thiamine transporter: ThTR-2, OCT1, OCT2, MATE1, and MATE2.[28] See **Supplementary Information** for description on the formulas and cutoff values used to predict *in vivo* drug-drug interaction (DDI) potential.

Clinical study design

This study (NCT03746106) was conducted at the Jean Mayer United States Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts University and was approved by the Health Sciences Campus Institutional Review Board at Tufts University. All 7 subjects included in our study were administered (i) 5 mg thiamine orally and (ii) 5 mg thiamine plus 300 mg trimethoprim (orally) with 500 mL of water (**Figure 1**). See **Supplementary Information** for a full description of the randomized, two-arm crossover design.

Animal study

All experiments on mice were performed by MuriGenics, Inc. (Vallejo, CA), a preclinical research and development company. Eleven FVB mice (Charles River), aged 9–11 weeks and weighing ~20–25 g, were used in this study. See **Supplementary Information** for the description of the animal study.

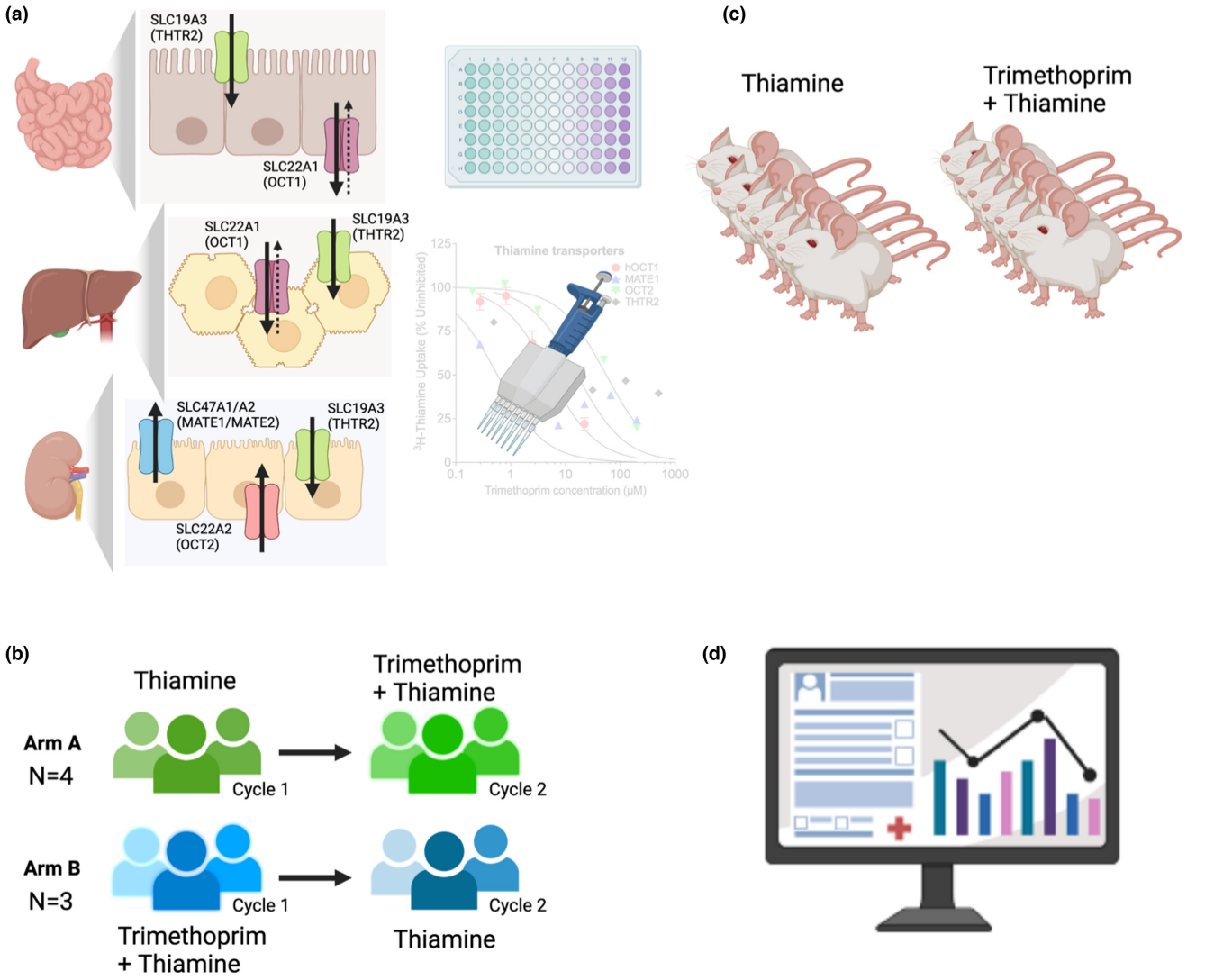

Figure 1 Overview of approach implemented to investigate transporter-mediated trimethoprim-thiamine interactions. (**a**) *In vitro* studies to determine inhibition potencies of trimethoprim for various transporters that interact with thiamine; (**b**) randomized crossover clinical study conducted in healthy volunteers to evaluate the effect of trimethoprim on thiamine plasma concentrations and levels of transporter biomarkers; (**c**) *in vivo* studies conducted in mice to evaluate effect of trimethoprim on thiamine concentrations in the plasma and liver; (**d**) real-world data analysis using electronic health records to investigate the association between trimethoprim use and levels of OCT1 biomarkers.

Bioanalytical methods

Plasma, urine, and liver measurements of thiamine were determined using ultra-performance liquid chromatography tandem mass spectrometry. See **Supplementary Information** for the description of the bioanalytical methods.

Data cleaning and pharmacokinetic analyses

Data cleaning and imputation were performed using JMP Pro 14.1 (SAS Institute, Cary, NC). Outliers identified by the Huber M robust fit procedure were removed. Of the 210 plasma samples planned for collection, four samples were missing. Either due to missing samples or identified as outliers, five thiamine and three trimethoprim concentration values were imputed using multivariate imputation methods in the final data set.

Due to intra- and intersubject variability in baseline thiamine concentrations, thiamine concentrations were adjusted by subtracting thiamine concentrations at time zero from thiamine concentrations at subsequent timepoints, respective to each subject and cycle. Thiamine concentration at $t = 0.25$ hours was used for adjustment for one subject in one cycle because the concentration at time zero was greater than subsequent postdose concentrations and presumably represented a measurement error. Adjusted thiamine concentrations < 0.005 nM were set to 0 nM.

Pharmacokinetic parameters were determined by noncompartmental analysis using Phoenix WinNonlin (Certara, Princeton, NJ). Data are expressed as mean ± standard error unless otherwise noted. Differences in pharmacokinetic parameters were analyzed in R using paired *t*-tests.

Biomarker metabolomics

See **Supplementary Information** for the description of plasma sample extraction and metabolomic methods.

Data processing and analysis

See **Supplementary Information** for data processing and analysis of the metabolomic data.

Real-world data analyses using electronic health records

See **Supplementary Information** for real-world data analyses using electronic health records.

RESULTS

The results described here are obtained from seven individuals who completed the clinical study. The original study design aimed to enroll 21 participants. This enrollment target was based on thiamine pharmacokinetic data from the literature, variability in thiamine levels in the general population, as well as practical considerations. Original recruitment goals could not be met due to the COVID-19 pandemic shutting down clinical research operations at Tufts University. The clinical data were supplemented with *in vitro*, mice, and real-world data, as described below.

Trimethoprim inhibits multiple thiamine transporters

In vitro data demonstrated that trimethoprim is a potent inhibitor of all 5 thiamine transporters evaluated: ThTR-2 (half-maximal inhibitory concentration (IC_{50}): 17 ± 10 μM), OCT1 (IC_{50}: 4.2 ± 0.6 μM), OCT2 (IC_{50}: 44 ± 16 μM), MATE1 (IC_{50}: 14 ± 26 μM), and MATE2 (IC_{50}: 0.03 ± 0.03 μM; **Figure S1**, **Table S1**). Using the FDA guidance for evaluating transporter-mediated drug interactions, trimethoprim was predicted to cause a clinically relevant drug-nutrient interaction (DNI) at ThTR-2 and OCT1 following administration of a single 300 mg dose. Trimethoprim also inhibited OCT2, MATE1, and MATE2 and was predicted to cause a clinically relevant DNI at these transporters for nutrients where the renal route represents a major component of their elimination.[28] Thus, trimethoprim is predicted to modulate thiamine absorption and disposition through inhibition of intestinal (ThTR-2), hepatic (OCT1 and ThTR-2), and renal (OCT2, MATE1, and MATE2) thiamine transporters (**Table S1**).

Trimethoprim increases thiamine levels and reduces thiamine oral clearance in seven healthy volunteers

A randomized, two-arm crossover DNI study was conducted in seven healthy volunteers to determine the effect of trimethoprim on the absorption and disposition of thiamine. All subjects completed the study, and no adverse events were reported. The demographic and baseline characteristics of the healthy volunteers are shown in **Table S2**. Baseline thiamine concentrations (i.e., prior to dosing) were not different between the two arms: thiamine alone (12.3 ± 24.0 nM) vs. thiamine co-administered with trimethoprim (4.5 ± 4.3 nM). Results from these seven subjects showed that thiamine plasma concentrations increased following co-administration with trimethoprim (**Figure 2**). Trimethoprim co-administration with thiamine resulted in a higher thiamine maximum concentration (C_{max}; Wilcoxon-matched paired *t*-test, $P = 0.078$) and area under the curve from 0 to 24 hours ($AUC_{0\text{-}24}$, $P = 0.03$; **Figure 2**, **Table 1**). Additionally, thiamine oral clearance (CL/F; clearance divided by oral bioavailability) was lower with trimethoprim + thiamine coadministration (Wilcoxon-matched paired *t*-test, $P = 0.031$).

Trimethoprim modulates levels of OCT1 biomarkers, isobutyrylcarnitine, and propionylcarnitine

Because trimethoprim was predicted to inhibit OCT1, OCT2, MATE1, and MATE2 at clinically relevant concentrations based on *in vitro* data, we investigated the levels of endogenous biomarkers for each respective transporter after administration of trimethoprim. Plasma isobutyrylcarnitine has previously been measured in DDI studies focusing on OCT1.[29,30] OCT1 acts as a bidirectional liver transporter, capable of facilitating the influx or efflux of compounds in cells based on the electrochemical gradient of the substrates. Previous studies have shown that *in vivo* inhibition of OCT1 and OCT1 polymorphisms with reduced functions are associated with reduced plasma levels of isobutyrylcarnitine and propionylcarnitine.[31] In this study, all seven subjects had reduced isobutyrylcarnitine AUC ($P = 0.0004$) and propionylcarnitine AUC ($P = 0.0005$) in the trimethoprim + thiamine arm compared with the thiamine only arm (**Figure 3**), consistent with the hypothesis that trimethoprim is inhibiting OCT1.[31,32] **Table S3** showed that butyrylcarnitine and carnitine are also significant ($P < 0.05$, q-value < 0.1). For OCT2 and MATE1/MATE2 biomarkers, plasma levels of the established biomarkers N-methylnicotinamide and N^1-methyladenosine were not significantly altered by trimethoprim when comparing the two arms (**Figure 3**, **Figure S2**).[33]

Exploring the impact of trimethoprim on urinary excretion of thiamine and endogenous metabolites of OCT2 and MATEs in five subjects

Given that trimethoprim is known to inhibit four thiamine transporters in the kidney, namely OCT2, MATE1, MATE2, and ThTR-2 (as listed in **Table S1**), we examined the levels of thiamine and endogenous substrates of these transporters in urine samples collected from 5 out of 7 subjects at 0–12 hours postdose in both treatment arms. Two subjects were excluded from the analysis due to missing urine data. The results show that the amount of thiamine in the urine was modestly higher in the trimethoprim plus thiamine arm (paired *t*-test, $P = 0.039$; **Table 1**); however, a significant difference was only observed when thiamine concentrations were adjusted by subtracting thiamine concentrations at time zero from thiamine concentrations at subsequent timepoints (**Figure S3**). Additionally, thiamine renal clearance for these five subjects showed a trend toward increased thiamine renal clearance in the trimethoprim plus thiamine arm. Although a significant increase ($P = 0.0056$) in the thiamine renal clearance (in the trimethoprim plus thiamine arm) was observed for the 8–12 hours postdose interval, overall thiamine renal clearance was not significantly different ($P = 0.2$) between the two arms (**Figure S4**). To determine the extent of trimethoprim-mediated inhibition of OCT2 and MATEs, we analyzed the urinary excretion amount and clearance of four endogenous substrates of the transporters: propionylcarnitine, isobutyrylcarnitine, N-methylnicotinamide, and N^1-methyladenosine. In the urinary excretion amount and clearance, N-methylnicotinamide and N^1-methyladenosine showed a significant decrease, particularly in the 0–4-hour urine collection interval in the trimethoprim plus thiamine arm ($P < 0.05$; refer to **Table S4**). Although the trend was not significant at 0–12 hours, the trimethoprim group also exhibited lower urinary excretion amount and clearance of N-methylnicotinamide and N^1-methyladenosine. There were no significant differences

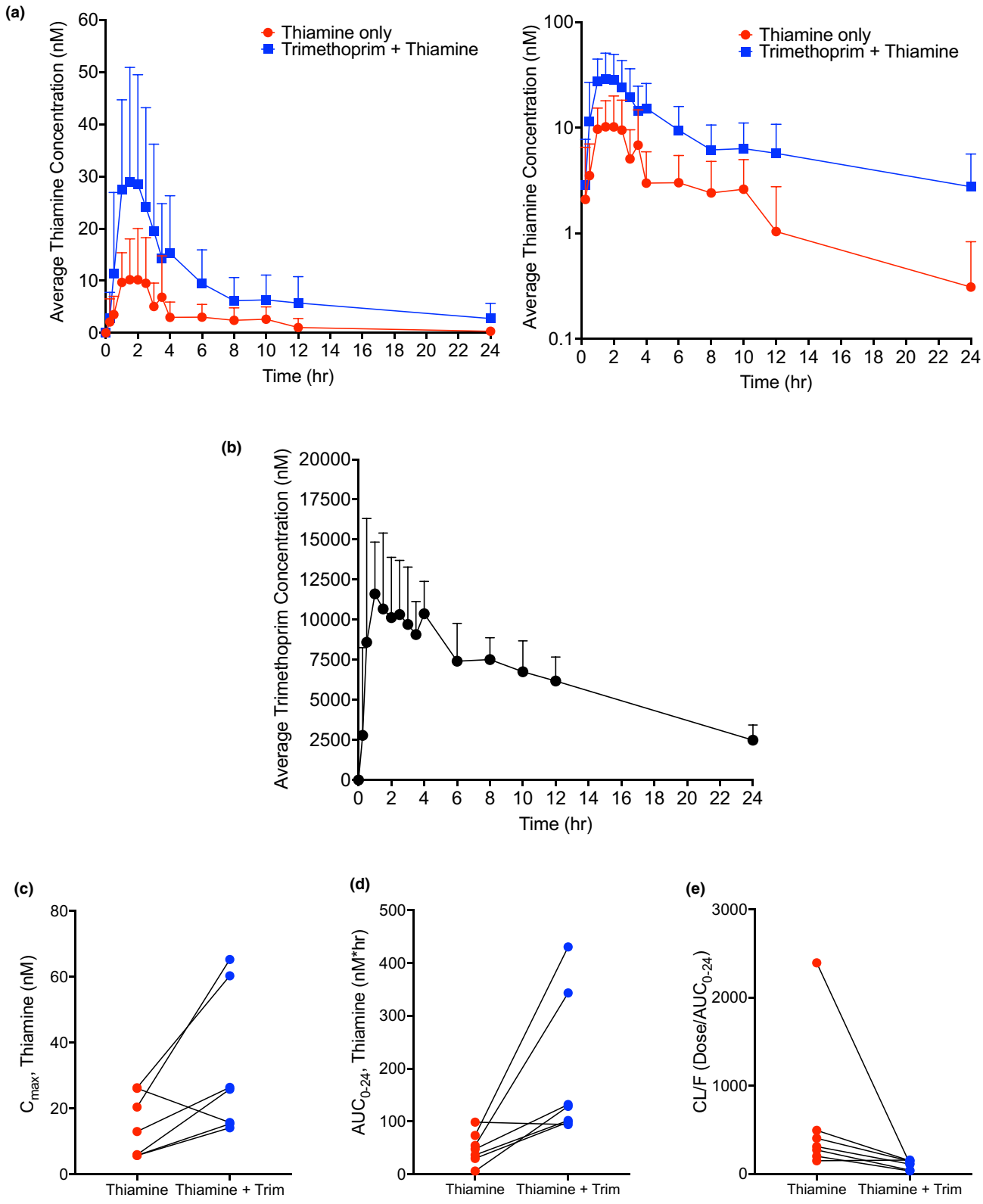

Figure 2 Average thiamine concentrations and pharmacokinetic parameters after administration of thiamine alone or in combination with trimethoprim in seven healthy volunteers. (**a**) Thiamine concentrations were determined following a 5 mg oral dose of thiamine alone or with a 300 mg oral dose of trimethoprim. Data represent mean thiamine concentration ± standard deviation at each timepoint respective to arm. (**b**) Trimethoprim concentrations following 300 mg oral dose of trimethoprim. Data represent mean thiamine concentration ± standard deviation at each timepoint. (**c**) Maximum concentration achieved (C_{max}) and (**d**) area under the concentration-time curve from $t = 0$ h to $t = 24$ h (AUC_{0-24}) were compared between both arms. Each line represents the value from the same individual between both arms. (**e**) Apparent clearance (CL/F), were compared between both arms. Each line represents the value from the same individual between both arms.

Table 1 Summary of pharmacokinetic parameters of thiamine in seven healthy volunteers with or without trimethoprim

Thiamine	Thiamine arm	Thiamine + trimethoprim arm	*P* value (paired *t*-test)	*P* value (Wilcoxon-matched-paired *t*-test)
T_{max} (hours) (Mean, range)	1.8 (1–3)	1.8 (1–3)	0.28	0.34
C_{max} (nM)	15 ± 9.5	32 ± 22	0.046	0.078
$AUC_{0\text{-}24}$ (nM*hr)	50 ± 30	189 ± 138	0.031	0.031
CL/F (dose/$AUC_{0\text{-}24}$; μg/nM*hr)	204 ± 269	37 ± 17	0.15	0.031
V/F (mg/nM)	0.80 ± 0.50 (n = 5)	0.50 ± 0.30 (n = 7)	0.19 (n = 5)	0.31 (n = 5)
$t_{1/2}$ (hr)	8.1 ± 5.3 (n = 5)	12.7 ± 7.2 (n = 7)	0.18 (n = 5)	0.18 (n = 5)
Urinary excretion amount (nmol) (0–12 hr)[a]	1831 ± 1808 (n = 5)	3,111 ± 1,205 (n = 5)	0.039 (n = 5)	0.13 (n = 5)
Renal clearance (L/hr; 0–12 hr)[b]	13.2 ± 5.7 (n = 5)	16.9 ± 6.7 (n = 5)	0.16 (n = 5)	0.19 (n = 5)

All data are reported as mean ± standard deviation except for T_{max} which is reported as median (range). Some parameters are not normally distributed, therefore we reported *P* values using paired *t*-tests and Wilcoxon-matched-pairs *t*-test.
The pharmacokinetic parameters were calculated using Phoenix WinNonlin NCA.
AUC_{0-24}, area under the concentration-time curve from t = 0 hours to t = 24 hours; CL/F, oral clearance; C_{max}, maximum plasma concentration achieved; T_{max}, time to maximum plasma concentration; $t_{1/2}$, half-life; V/F, volume of distribution.
Note that V/F and $t_{1/2}$ could not be estimated for two subjects in the thiamine arm due to low thiamine concentrations. Therefore, the *P* values reported in the table for these two parameters are from n = 5 subjects only.
[a]Urinary excretion amount of thiamine (nmol): The quantity of thiamine (measured in nmol) present in urine during the 0–12 hour period following adjustments made to thiamine concentrations. These adjustments involved subtracting the initial thiamine concentration at time zero at each timepoint, for each subject and cycle. **Figure S3** displays thiamine amount in the urine.
[b]Thiamine renal clearance (L/hr): To calculate thiamine renal clearance, the urinary excretion amount of thiamine (measured in nmol) during the 0–12 hour period was divided by the thiamine AUC levels for the same period. Thiamine concentrations in plasma were adjusted by subtracting the initial concentration at time zero for each subject and cycle. **Figure S4** displays the thiamine renal clearance values for four-time intervals: 0–4 hours, 4–8 hours, 8–12 hours, and 0–12 hours.

in the renal clearances of isobutyrylcarnitine and propionylcarnitine between the two treatment groups ($P > 0.25$).

Trimethoprim increases plasma thiamine and reduces thiamine partitioning into the liver in mice

In mice, baseline plasma thiamine levels were not different between animals treated with thiamine (group 1: 2.7 ± 0.9 nM) vs. thiamine + trimethoprim (group 2: 1.7 ± 2.2 nM). As in humans, trimethoprim administration was associated with acute increases in plasma thiamine levels in mice. Thiamine levels were significantly different at 15- and 30-minutes postdose between the 2 groups ($P < 0.005$; **Figure 4**). Thiamine AUC from 0 to 1 h ($AUC_{0\text{-}1}$) was higher when thiamine was co-administered with trimethoprim ($P = 0.0043$; **Figure 4**). The AUC from 0 to 2 hours ($AUC_{0\text{-}2}$) was also greater in the combination arm ($P = 0.019$), even after loss of data from one mouse in group 2 that died between hours 1 and 2 of the study (**Figure 4**). Additionally, thiamine levels in the liver appeared lower (3.6-fold) in mice administered both trimethoprim and thiamine compared with mice administered thiamine only ($P = 0.067$). However, when liver levels were normalized to thiamine exposure ($AUC_{0\text{-}1}$ or $AUC_{0\text{-}2}$), the partitioning of thiamine into the liver was significantly lower in the combination arm, consistent with the idea that trimethoprim inhibits thiamine entry (or partitioning) into the liver (**Figure S5**). Notably, the active metabolite of thiamine, thiamine pyrophosphate (TPP or TDP), was not significantly different in the livers of the mice (**Figure S5**). Given that the primary form of thiamine in the body is TPP, it is likely that the amount of TPP produced (via metabolism) from the administered thiamine dose was negligible compared with the amount of endogenous TPP stored in the liver. Together, with the long half-life of TPP, it is unlikely that we would be able to detect differences in the levels of TPP between the two treatment groups.

Real-world data analyses support inhibition of OCT1 by trimethoprim

Missense mutations of OCT1 are associated with increased total cholesterol, LDL cholesterol, and triglyceride plasma levels, as previously reported in different genomewide association studies.[26] Given that trimethoprim appeared to decrease thiamine uptake into the liver via inhibition of OCT1, trimethoprim may be phenocopying the effect of a reduced function allele of OCT1. If true, we hypothesized that individuals on trimethoprim would also have increased lipid levels similar to individuals who harbor reduced function OCT1 alleles.[26] Using electronic health records (EHRs) at University of California – San Francisco (UCSF; access date: October 2020), we identified patients diagnosed with HIV and compared laboratory test results between patients prescribed trimethoprim and patients not prescribed trimethoprim. Based on the criteria described in the methods, we categorized patients into two groups: "on" drug (i.e., prescribed trimethoprim) or "off" drug. We found that triglyceride ($P < 2.2 \times 10^{-16}$, $n = 464$ "on" drug and $n = 928$ "off" drug), LDL cholesterol ($P = 5.75 \times 10^{-7}$, $n = 313$ "on" drug and $n = 1{,}149$ "off" drug), and total cholesterol ($P = 5.82 \times 10^{-7}$, $n = 483$ "on" drug and $n = 966$ "off" drug) levels were significantly higher in patients with HIV prescribed trimethoprim compared with age- and sex-matched patients with HIV not prescribed trimethoprim, when comparing laboratory

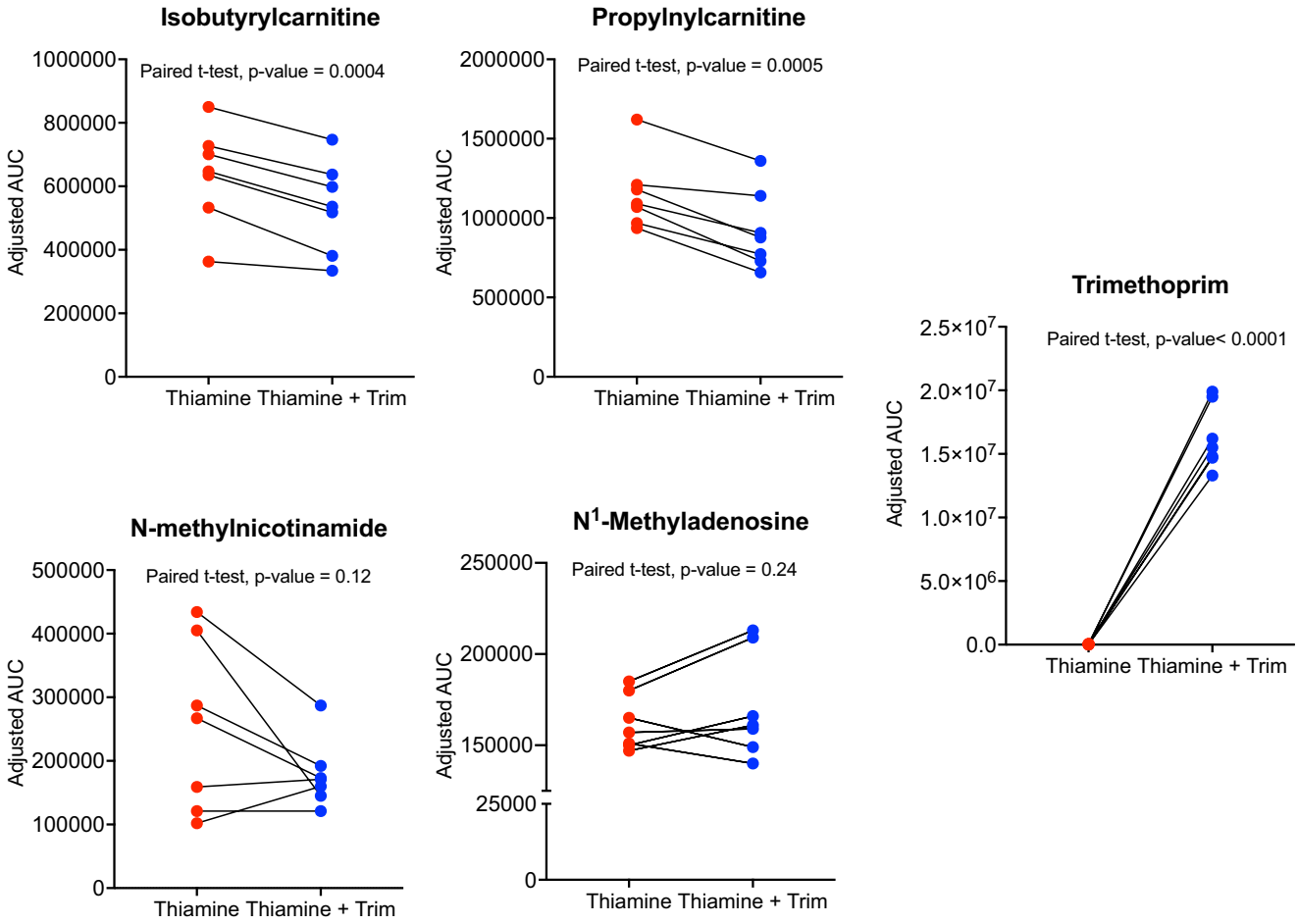

Figure 3 Overall area under the curve (AUC) of plasma endogenous biomarkers for OCT1, OCT2, MATE1, and MATE2 in 7 healthy subjects. The endogenous biomarkers for clinical transporter drug–drug interaction assessment are: isobutyrylcarnitine for OCT1, N-methylnicotinamide for OCT2/MATE1/MATE2, and N^1-methyladenosine for OCT2 and MATE2. Plasma biogenic amines and trimethoprim were measured using an untargeted liquid chromatography-TripleTOF mass spectrometry metabolomics approach. Data was normalized by dividing feature height by the sum of internal standard within each sample, multiplied by the average sum of internal standard in samples and pools. The AUC was calculated in R using the PKNCA package. Group means were compared using paired two-tailed *t*-tests.

test results from samples taken on or after initial HIV diagnosis start date, consistent with inhibition of OCT1 (**Figure 5**, **Table 2**).

Additionally, because statin use can influence lipid levels, we performed subanalyses where we excluded patients who had at least one prescription to drugs classified as antihyperlipidemic-HMG-CoA reductase inhibitors (statins) in their health record. Consistent with our initial analyses, for all three laboratory tests, patients in the "on" drug group had significantly higher triglycerides ($P < 2.2 \times 10^{-16}$, $n = 391$ "on" drug and $n = 782$ "off" drug), LDL cholesterol ($P = 4.79 \times 10^{-3}$, $n = 248$ "on" drug and $n = 959$ "off" drug), and total cholesterol levels ($P = 1.69 \times 10^{-3}$, $n = 402$ "on" drug and $n = 1{,}219$ "off" drug) compared with patients in the "off" drug group, respectively (**Table 2**, **Table S5**).

DISCUSSION

Membrane transporters are known targets for DDIs and are extensively investigated throughout the drug development process. However, transporter-mediated DNIs have been largely ignored during drug development, until 2013, when the fedratinib clinical trial was halted due to several patients displaying symptoms similar to WE, a neurological disorder which often develops due to thiamine deficiency.[34–36] Using various forms of evidence, this study suggests that multiple thiamine transporters, including ThTR-2, OCTs, and MATEs, are important targets for DNIs or DDIs. The effect of drugs on nutrient disposition are complex and may be mediated by various transporters in the liver, kidneys, and intestines.

Although our study was interrupted by the COVID-19 pandemic, and only 7 volunteers completed the clinical study, the data from these individuals, together with our *in vitro* experiments, *in vivo* mouse studies, and real-world data analyses, are consistent with 4 major findings. First, trimethoprim is a potent inhibitor of several thiamine transporters and *in vitro-in vivo* extrapolation of transporter-mediated trimethoprim-thiamine interactions show that trimethoprim is predicted to inhibit the intestinal, liver, and renal transporter ThTR-2, the liver transporter OCT1, and the renal transporters OCT2, MATE1, and MATE2, at clinically relevant concentrations. Second, systemic thiamine plasma concentrations increase when thiamine is co-administered with trimethoprim in both humans

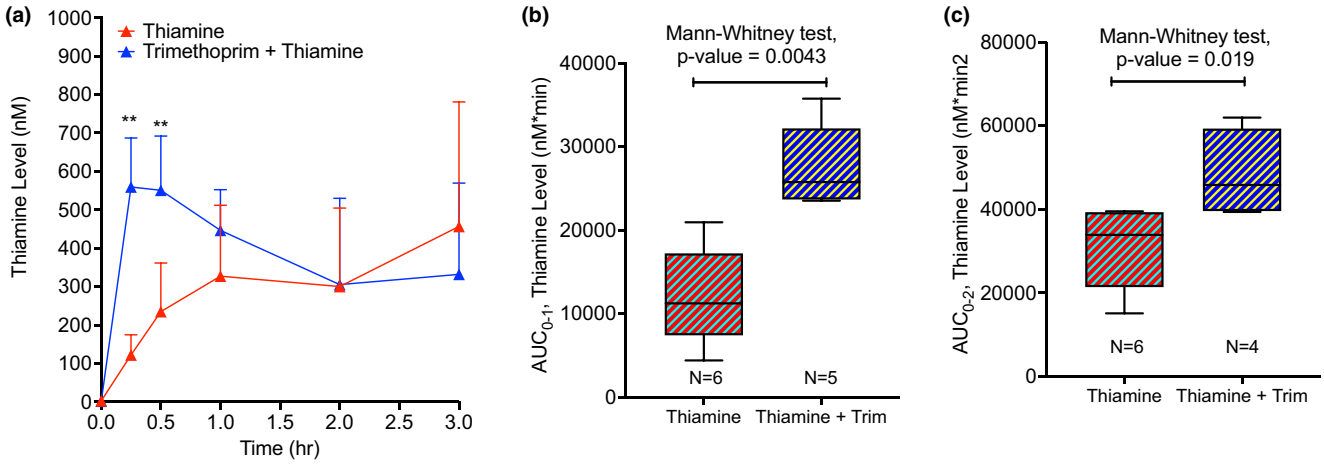

Figure 4 Thiamine plasma concentrations over time and pharmacokinetic parameters in 11 mice. Six mice were given thiamine only and five mice were given thiamine and trimethoprim all by oral gavage. (**a**) Thiamine concentrations were determined following a dose of thiamine alone (2 mg/kg) or with a dose of trimethoprim (61.5 mg/kg). Data represent mean thiamine concentration ± standard deviation at each timepoint respective to arm. $^{**}P < 0.005$ using Mann–Whitney test comparing the 6 mice in the thiamine only group and 5 mice in the thiamine and trimethoprim group. No significance was found at other timepoints. (**b**) AUC from $t = 0$ hours to $t = 1$ hour (AUC_{0-1}) and (**c**) area under the concentration-time curve from $t = 0$ hours to $t = 2$ hours (AUC_{0-2}) were compared between both arms using a Mann–Whitney test.

and mice, consistent with the potential of trimethoprim to inhibit OCT1- and ThTR2-mediated thiamine uptake. Third, our metabolomics study showed that levels of OCT1 acylcarnitine biomarkers, such as isobutyrylcarnitine, decrease when trimethoprim is administered, consistent with trimethoprim inhibition of hepatic OCT1. In addition, the renal clearances of N-methylnicotinamide and N^1-methyladenosine were reduced when trimethoprim was administered, consistent with inhibition of OCT2 and MATEs. Finally, data from EHRs showed an association between trimethoprim use and increased lipid levels, again consistent with trimethoprim inhibiting OCT1.

Trimethoprim is predicted to inhibit intestinal ThTR-2 at clinically relevant concentrations (**Table S1**). An unexpected finding in this study was that thiamine concentrations increased, rather than decreased, when thiamine was co-administered with trimethoprim. Our initial hypothesis was that trimethoprim would mimic fedratinib and inhibit intestinal ThTR-2, leading to reduced thiamine plasma concentrations. Although time to maximum thiamine concentration (T_{max}) was similar in both arms, thiamine C_{max} and AUC were each higher when thiamine was given in combination with trimethoprim. These data suggest that there was no net inhibition of intestinal ThTR-2-mediated thiamine absorption. However, inhibition of thiamine uptake in the liver via OCT1 and ThTR-2 is consistent with the data. The increase in thiamine AUC in both humans and mice does not exclude inhibition of intestinal ThTR-2 uptake by trimethoprim, which may occur, but does not explain the net effect of trimethoprim on increasing thiamine levels.

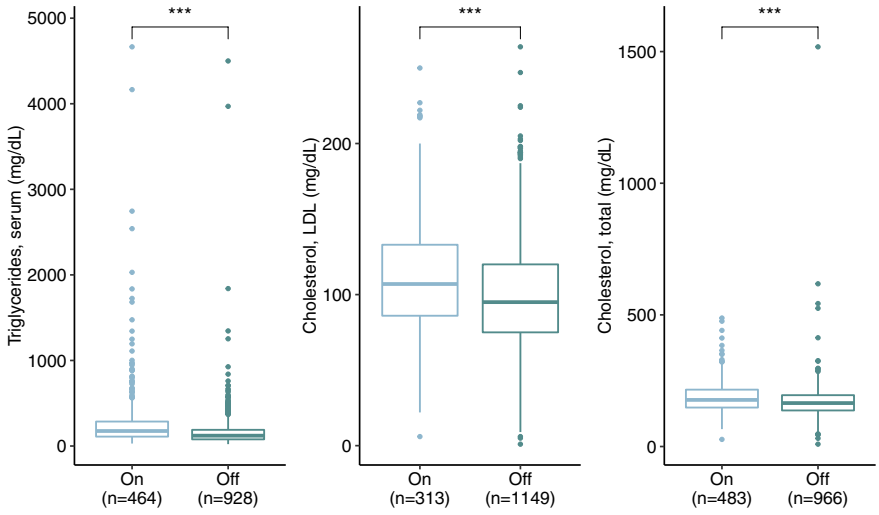

Figure 5 Real-world analyses comparing laboratory test results between patients prescribed trimethoprim vs. patients not prescribed trimethoprim. Boxplots comparing triglycerides, LDL cholesterol, and total cholesterol levels in patients with HIV prescribed trimethoprim vs. patients with HIV not prescribed trimethoprim (*P* value: $<2.2\times10^{-16}$, 5.75×10^{-7}, and 5.82×10^{-7}, respectively). ***P value < 0.001 using two-sample Mann-Whitney *U* tests to compare "on" and "off" drug groups.

Table 2 Summary table of real-world data analyses comparing laboratory test results from patients prescribed trimethoprim vs. patients not prescribed trimethoprim

		Triglycerides	LDL cholesterol	Total cholesterol
Main analyses				
Total patients	On drug (*N*)	464	313	483
	Off drug (*N*)	1,278	1,149	1,410
Matched patients	Ratio	1:2	all	1:2
	On drug (*N*)	464	313	483
	Off drug (*N*)	928	1,149	966
	Average on drug (mg/dL)	275	112	185
	Average off drug (mg/dL)	165	98.7	169
	Median on drug (mg/dL)	176	107	177
	Median off drug (mg/dL)	121	95	165
	P value	$<2.2\times10^{-16}$	5.75×10^{-7}	5.82×10^{-7}
Subanalyses: patients with prescription to statin(s) excluded				
Total patients	On drug (*N*)	391	248	402
	Off drug (*N*)	1,089	959	1,219
Matched patients	Ratio	1:2	all	All
	On drug (*N*)	391	248	402
	Off drug (*N*)	782	959	1,219
	Average on drug (mg/dL)	264	109	178
	Average off drug (mg/dL)	162	99.7	169
	Median on drug (mg/dL)	171	105	172
	Median off drug (mg/dL)	120	97	165
	P value	$<2.2\times10^{-16}$	4.79×10^{-3}	1.69×10^{-3}

Two-sample Mann–Whitney *U* tests were performed to compare laboratory test results for triglyceride, LDL cholesterol, and total cholesterol, respectively, between the "on" and "off" drug groups after patients were age- and sex- matched between both groups. **Table S5** shows the demographics for the "on" and "off" groups used in the real-world data analyses.

Based on the experimental IC_{50} determined in this study, trimethoprim is predicted to cause a clinically relevant DNI at OCT1 in the liver. The organic cation transporter 1, OCT1 (SLC22A1), is a major hepatic uptake transporter for thiamine.[37] To our knowledge, the potential of trimethoprim to inhibit hepatic OCT1-mediated thiamine uptake has not been previously described. The portal vein concentration of thiamine is estimated to be ~0.5 µM, assuming thiamine is not protein bound and has 5% bioavailability.[38] The reported thiamine Km for ThTR-2 varies in the literature (3–125 nM[39] and 1–3 µM[11,40]). The estimated thiamine portal vein concentration of ~0.5 µM is close to saturating ThTR-2 and suggests that both OCT1 and ThTR-2 could play a role in hepatic uptake of thiamine. OCT1 expression levels in the liver are much higher than ThTR-2 levels,[41] consistent with it being a higher capacity thiamine transporter; thus, OCT1 may increasingly play a more predominant role in hepatic thiamine uptake at increasing oral thiamine doses. Our results are not consistent with the results of a study investigating the disposition of a high dose (200 mg) of thiamine in individuals with reduced function OCT1 polymorphisms, which showed no effect of the polymorphisms on thiamine disposition at this dose.[40] Differences between our results and the OCT1 polymorphism study may be related to differences in the study design between studies. First, vastly different doses of thiamine were used in their study (200 mg) and ours (5 mg). Further, in our study, we used a potent and nonspecific transporter inhibitor, trimethoprim, to inhibit OCT1, whereas their study was focused on the effects of reduced function alleles of OCT1, several of which retain some OCT1 function. Studies have shown that broad and potent inhibitors of hepatic transporters, such as rifampicin, have greater effects on the disposition of drugs that are substrates of transporters compared with reduced function polymorphisms of the transporters. For example, a single dose of rifampicin (OATP1B1/1B3 inhibitor) increases plasma levels of glibenclamide, a substrate of OATP1B1/1B3; however, plasma levels of glibenclamide are not increased in individual harboring reduced function OATP1B1 polymorphisms.[42,43]

Because trimethoprim inhibits multiple thiamine transporters in the liver and kidneys, we evaluated the impact of trimethoprim on OCT1, OCT2, and MATE1/MATE2 biomarkers. Many studies have used biomarkers for DDI risk assessments.[29] For example, N-methylnicotinamide, a biomarker for MATE1/MATE2, and N^1-methyladenosine, a biomarker for OCT2 and MATE2, are significantly increased when pyrimethamine, a MATE1/MATE2 *in vivo* inhibitor, is administered.[44] In this study, we found that trimethoprim modulated the plasma levels of OCT1 acylcarnitine biomarkers. Although administration of trimethoprim did not affect plasma levels of OCT2, MATE1, and/or MATE2 biomarkers,

N-methylnicotinamide and N^1-methyladenosine, it did result in lower urinary excretion and renal clearance of these biomarkers (**Table S4**). These results are consistent with a previous study which showed that trimethoprim did not significantly affect AUC levels of N-methylnicotinamide, despite significantly reducing its renal clearance.[24]

To confirm our finding that trimethoprim inhibits OCT1 clinically, we used real-world data to demonstrate that patients prescribed trimethoprim exhibited elevated levels of triglycerides, LDL cholesterol, and total cholesterol, which are known consequences of OCT1 inhibition.[26] Mandal *et al*. previously showed that administration of trimethoprim to rats caused a significant increase in total serum lipid and cholesterol levels as well as modulated lipid and glycogen contents in the liver.[45] Previously, we used EHR data to show that ritonavir and darunavir, both of which are predicted to inhibit OCT1 at clinically relevant concentrations, also increase plasma concentrations of triglycerides, LDL cholesterol, and total cholesterol.[27] The results reported in this study are consistent with the known phenotype of an *Oct1* knockout mice, which exhibit higher triglycerides, LDL cholesterol, and total cholesterol levels.[26] The increase in plasma levels of total cholesterol, triglyceride, and/or LDL cholesterol in individuals with reduced OCT1 function is due to a complicated mechanism in which hepatic energy production is reduced, leading to abnormal lipid levels.[26] The EHR data suggest that patients with HIV prescribed trimethoprim chronically may be at an increased risk for comorbidities associated with elevated clinical lipids.[46–49] Furthermore, drugs which may cause increased lipid levels as a result of off-target effects should be reconsidered in patients taking trimethoprim concomitantly.

There are a number of limitations to this study. First, thiamine was only administered orally. Without a corresponding intravenous dose of thiamine, we were not able to determine the effect of trimethoprim on thiamine bioavailability. Furthermore, our exploratory clinical study and study in mice consist of small sample sizes; thus, the study needs replication. Notably, there is also a limitation in using mice to support the human trimethoprim-thiamine interaction study because mouse *Oct1* has a different kinetic metabolite (K_m) for thiamine compared with human OCT1 (OCT1: $K_m = 1{,}057\,\mu M$; Oct1: $K_m = 143\,\mu M$)[50] and mouse Oct1 is also expressed in the kidneys. Therefore, in mice, the observed increase in thiamine AUC could be due to both hepatic and renal Oct1. In addition, another limitation is that only one dose of thiamine was investigated and thiamine disposition may be dose-dependent as a result of saturable transporter mechanisms. Finally, our EHR analysis was limited by the lack of data on SLC19A3 and OCT1 genotype of the patients, how long patients were on trimethoprim, and patient compliance. As more EHR data becomes available for research purposes, we will be able to account for these variables and covariates and increase the sample size and robustness of our analysis. Controlled, randomized clinical trials in both healthy volunteers and in patients diagnosed with HIV are needed to help address these limitations.

Overall, our study, although limited by the pandemic, demonstrates that trimethoprim administration is associated with increases in thiamine plasma concentrations. The mechanism does not appear to be related to reduced renal secretion of thiamine. Although trimethoprim inhibited secretory transporters for thiamine, including OCT2, MATE1, and/or MATE2, as indicated by reduced renal clearance of biomarkers for these transporters, it did not significantly reduce the renal clearance of thiamine in our study (**Table 1**). If anything, there was a trend toward increased thiamine renal clearance in the trimethoprim arm, consistent with the increase in plasma concentrations and blockade of renal ThTR2- but not ThTR1-dependent resorption. These results suggest that the observed increases in thiamine plasma levels in the presence of trimethoprim are not substantially related to inhibition of its renal clearance. The mechanism likely involves inhibition of thiamine uptake via hepatic OCT1 and ThTR-2 as supported by the *in vitro* studies and the decreased plasma levels of OCT1 acylcarnitine biomarkers. To our knowledge, there is no clinically validated OCT1 inhibitor for use in clinical DDI studies to assess the effect of OCT1 inhibition on the pharmacokinetics of OCT1 substrates. Further studies are needed to determine whether trimethoprim may serve as an important tool for studies of OCT1-mediated DDIs. Our study strongly suggests that transporter-mediated drug-vitamin interactions are complex and may involve multiple transporters.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

The authors declared no competing interests for this work. As Deputy Editor-in-Chief of *Clinical Pharmacology and Therapeutics*, K.M.G. was not involved in the review or decision process for this paper.

AUTHOR CONTRIBUTIONS

B.V., A.W., S.W.Y., J.W.N., and K.M.G. wrote the manuscript. B.V., A.W., S.W.Y., E.A.E.G., M.A., M.S., K.M.G., J.W.N., and A.S.G. designed the research. B.V., A.W., S.W.Y., E.A.E.G., M.A., and K.T. performed the research. B.V., A.W., S.W.Y., E.A.E.G., M.A., K.M.G., and J.W.N. analyzed the data. K.M.G., J.W.N., and A.S.G. contributed new reagents/analytical tools.

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