

UCSF

UC San Francisco Previously Published Works

Title

Metformin Is a Substrate and Inhibitor of the Human Thiamine Transporter, THTR-2 (SLC19A3)

Permalink

<https://escholarship.org/uc/item/4mb4p3rn>

Journal

Molecular Pharmaceutics, 12(12)

ISSN

1543-8384

Authors

Liang, Xiaomin
Chien, Huan-Chieh
Yee, Sook Wah
[et al.](#)

Publication Date

2015-12-07

DOI

10.1021/acs.molpharmaceut.5b00501

Peer reviewed

Metformin Is a Substrate and Inhibitor of the Human Thiamine Transporter, THTR-2 (SLC19A3)

Xiaomin Liang,[†] Huan-Chieh Chien,[†] Sook Wah Yee,[†] Marilyn M. Giacomini,[‡] Eugene C. Chen,^{†,§} Meiling Piao,^{†,||} Jia Hao,[‡] Jolyn Twelves,[‡] Eve-Irene Lepist,[‡] Adrian S. Ray,[‡] and Kathleen M. Giacomini^{*,†}

[†]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, California 94158, United States

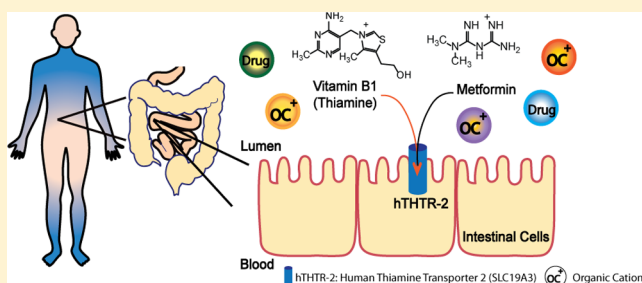
^{||}Department of Pharmacology and Pharmaceutical Sciences, School of Medicine, Tsinghua University, Beijing 100084, China

[‡]Department of Drug Metabolism, Gilead Sciences, Inc., Foster City, California 94404, United States

Supporting Information

ABSTRACT: The biguanide metformin is widely used as first-line therapy for the treatment of type 2 diabetes. Predominately a cation at physiological pH's, metformin is transported by membrane transporters, which play major roles in its absorption and disposition. Recently, our laboratory demonstrated that organic cation transporter 1, OCT1, the major hepatic uptake transporter for metformin, was also the primary hepatic uptake transporter for thiamine, vitamin B1. In this study, we tested the reverse, i.e., that metformin is a substrate of thiamine transporters (THTR-1, SLC19A2, and THTR-2, SLC19A3). Our study demonstrated that human THTR-2 (hTHTR-2), SLC19A3, which is highly expressed in the small intestine, but not hTHTR-1, transports metformin ($K_m = 1.15 \pm 0.2$ mM) and other cationic compounds (MPP⁺ and famotidine). The uptake mechanism for hTHTR-2 was pH and electrochemical gradient sensitive. Furthermore, metformin as well as other drugs including phenformin, chloroquine, verapamil, famotidine, and amprolium inhibited hTHTR-2 mediated uptake of both thiamine and metformin. Species differences in the substrate specificity of THTR-2 between human and mouse orthologues were observed. Taken together, our data suggest that hTHTR-2 may play a role in the intestinal absorption and tissue distribution of metformin and other organic cations and that the transporter may be a target for drug–drug and drug–nutrient interactions.

KEYWORDS: metformin, THTR-2, SLC19A3, drug and vitamin interaction



INTRODUCTION

The biguanide metformin is widely used as first-line therapy for the treatment of type 2 diabetes, and is frequently used off-label for the treatment and prevention of various diseases associated with metabolic syndrome.^{1–3} Although metformin was introduced into clinical practice in the 1950s, the mechanisms involved in its absorption, disposition, toxicity, and pharmacologic action are not fully understood. At physiological pH's, metformin exists primarily as a cation and poorly diffuses across cellular membrane.⁴ Thus, membrane transporters play major roles in the drug's absorption and disposition. Many studies have shown that metformin is a substrate of various polyspecific organic cation transporters including OCT1 (SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3), MATE1 (SLC47A1), MATE2 (SLC47A2), PMAT (SLC29A4), and OCTN1 (SLC22A4).^{5–13} Notably, these transporters are involved in the drug's absorption (OCT3, PMAT, and SERT (SLC6A4)¹⁴), distribution (OCT1 and others), and elimination (OCT2, MATE1, and MATE2).⁴ In fact, genetic polymorphisms in many of these transporters have been associated

with interindividual differences in the disposition and response to metformin.^{4,11,15,16}

Recently, our laboratory demonstrated that OCT1, the major hepatic uptake transporter for metformin, was also the major hepatic uptake transporter for thiamine, vitamin B1.¹⁷ Notably, our study showed that metformin treatment and deletion of *Oct1* in mice had parallel actions in the liver. That is, metformin dosing and *Oct1* deletion were associated with phosphorylation of the energy sensor, AMP-kinase (AMPK), and its downstream target acetyl-coA carboxylase (ACC) in the livers of mice. Further, our study demonstrated that deletion of *Oct1* resulted in reduced levels of both metformin and thiamine in the liver, and that common nonsynonymous polymorphisms in *OCT1* exhibited reduced transport of both compounds.^{17,18}

Based on our OCT1 studies and previous studies in the literature demonstrating that thiamine is a substrate of many

Received: June 26, 2015

Revised: October 24, 2015

Accepted: November 3, 2015

Published: November 3, 2015

polyspecific organic cation transporters that are known metformin transporters, e.g., OCT2, MATE1, and MATE2,^{17,19,20} we hypothesized that the reverse may be true. That is, metformin is a substrate of thiamine transporters, THTR-1 (SLC19A2) and/or THTR-2 (SLC19A3). These high affinity thiamine transporters are expressed in various tissues, such as liver, intestine, kidney, placenta, and muscle.^{21–25} Notably, THTR-2 is the major absorptive transporter for thiamine in the intestine and was recently implicated in the withdrawal of the Janus Kinase 2 inhibitor, fedratinib, from late-stage clinical trials.^{26,27} In brief, a few patients in clinical trials of fedratinib developed Wernicke's encephalopathy, a condition associated with thiamine deficiency. Therefore, inhibition of THTR-2 appears to be the mechanism for this profound adverse event.

In the present study, we tested the hypothesis that THTR-2 is a transporter for metformin and other prescription drugs and that these drugs could also inhibit the transporter resulting in potential drug–vitamin interactions. Our data indicate that metformin, as well as other therapeutic drugs from several pharmacologic classes, is a good substrate and inhibitor of the transporter. The results suggest that THTR-2 may play a role in the oral absorption of metformin and various prescription drugs. Importantly, the transporter may be a target for drug–vitamin and drug–drug interactions.

MATERIALS AND METHODS

Chemicals and Reagents. All radiolabeled chemicals were purchased from American Radiolabeled Chemicals Incorporation (St. Louis, MO, USA), PerkinElmer (Boston, MA, USA), or Moravек Biochemicals (Brea, CA). The specific activity of each radiolabeled chemical was as follows: metformin (1,1-dimethylbiguanide [biguanido-¹⁴C] hydrochloride) (110.2 mCi/mmol), thiamine [³H(G)] hydrochloride (20 Ci/mmol), carnitine hydrochloride, L-[N-methyl-³H] (80 Ci/mmol), histamine (2-(4-imidazolyl)ethylamine dihydrochloride [³H]) (14.2 mCi/mmol), [³H] norepinephrine (44.7 mCi/mmol), [³H] serotonin (hydroxytryptamine, 5-[1,2-³H] creatinine sulfate) (20.3 mCi/mmol), [¹⁴C] TEA (tetraethylammonium) (50 mCi/mmol), [³H] MPP⁺ (1-methyl-4-phenylpyridinium) (85.5 mCi/mmol), choline chloride [1,2-¹⁴C] (50 mCi/mmol), [¹⁴C]-guanidine HCl (53 mCi/mmol), famotidine [³H(G)] (20 Ci/mmol), and [¹⁴C]-aminoguanidine (57.7 mCi/mmol). Unlabeled chemicals were purchased from Sigma (USA). Fedratinib was purchased from Selleck Chemicals (Houston, TX). Cell culture supplies were purchased from the Cell Culture Facility (UCSF, California, USA).

Cell Lines and Transfection. Full length cDNAs of human SLC19A2 (NM_006996.2), human SLC19A3 (NM_025243.3), and mouse Slc19a3 (NM_030556.2) were synthesized (GenScript, Inc. NJ) and cloned into the pBabe-puro vector. Human embryonic kidney (HEK) 293 cells were stably transfected with the empty vector and the vector containing the genes of interest using the Phoenix-AMPHO retrovirus system. Puromycin, 10 μ g/mL (Millipore Corporation), was used for selection of stable clones. Single clones were selected and functionally validated. For studies in the transiently transfected cells, full length cDNAs of human SLC22A1 and SLC19A3 were cloned into the pcDNAs/FRT vector and human SLC6A4 (NM_001045.2) was cloned in pCMV6-XL4 vector, which was purchased from OriGene Technologies, Inc. (Rockville, MD). Transfection was performed using Lipofectamine LTX (Life Technologies,

Carlsbad, CA) according to the manufacturer's protocol. Uptake studies were performed as described below.

Transporter Uptake Studies. The stably overexpressing cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM H-21) supplemented with puromycin (5 μ g/mL), penicillin (100 U/mL), streptomycin (100 mg/mL), and 10% fetal bovine serum. Cells were cultured on poly-D-lysine coated 48-well plates for 48 h to reach 95% confluence. Before the uptake experiments, the culture medium was removed and the cells were incubated in Hanks balanced salt solution (HBSS) (Life Technologies) for 15 min at 37 °C. Unless otherwise noted, chemicals, drugs, and radiolabeled compounds were diluted in the HBSS for uptake experiments. The details for drug concentrations and uptake time are described in Results and figure legends. For the pH dependence experiments, HBSS buffer was adjusted to different pHs (5, 6, 6.5, 7.4, 8, and 8.5) by adding hydrochloric acid or sodium hydroxide. For the KCl replacement study, KCl replacement buffer (140 mM KCl, and 5.4 mM NaCl) was identical to the normal HBSS buffer (5.4 mM KCl and 140 mM NaCl) except that the sodium and potassium were iso-osmotically adjusted at pH 7.4. The uptake was performed at 37 °C, and then the cells were washed three times with ice-cold HBSS. After that, the cells were lysed with lysis buffer containing 0.1 N NaOH and 0.1% SDS, and the radioactivity in the lysate was determined by liquid scintillation counting. For the transporter study, the K_m and V_{max} were calculated by fitting the data to a Michaelis–Menten equation using GraphPad Prism software (La Jolla, CA).

Real Time RT-PCR Analysis of mRNA Levels in Cells and Tissues. Total RNA from cell lines and C57BL/6J mouse tissues was isolated using RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Total RNA (2 μ g) from each sample was reverse transcribed into cDNA using SuperScript VILO cDNA Synthesis kit (Life Technologies) according to the manufacturer's protocol. cDNA from human tissues was purchased from Clontech (Human Digestive System MTC panel, Clontech, Mountain View, CA). Quantitative real-time PCR was carried out in 384-well reaction plates using 2 \times Taqman Fast Universal Master Mix (Applied Biosystems, Foster City, CA), 20 \times Taqman specific gene expression probes, and 10 ng of the cDNA template. The reactions were carried out on an Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The relative expression level of each mRNA transcript was calculated by the comparative method ($\Delta\Delta C_t$ method) normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (Gapdh).

Western Blotting and Immunofluorescence Cell Staining. The cells were lysed in RIPA lysis buffer (Thermo Fisher, USA) with cOmplete mini protease inhibitor and PhosSTOP phosphatase inhibitor following the manufacturer's protocol (Roche Diagnostic, Indianapolis, IN). Human kidney tissue lysate was purchased from Abcam, and human duodenum tissue lysate was purchased from GeneTex. The liver samples were collected from patients undergoing liver biopsy at University of California, San Francisco (UCSF), and the normal section of liver was freshly frozen and stored at –80 °C. The committee on Human Research at UCSF (Institutional Review Board (IRB) 10-01717) approved this study. PNGase F enzyme from New England BioLabs was used to deglycosylate the protein according to the manufacturer's protocol. The proteins were separated on 4–20% SDS PAGE gels and

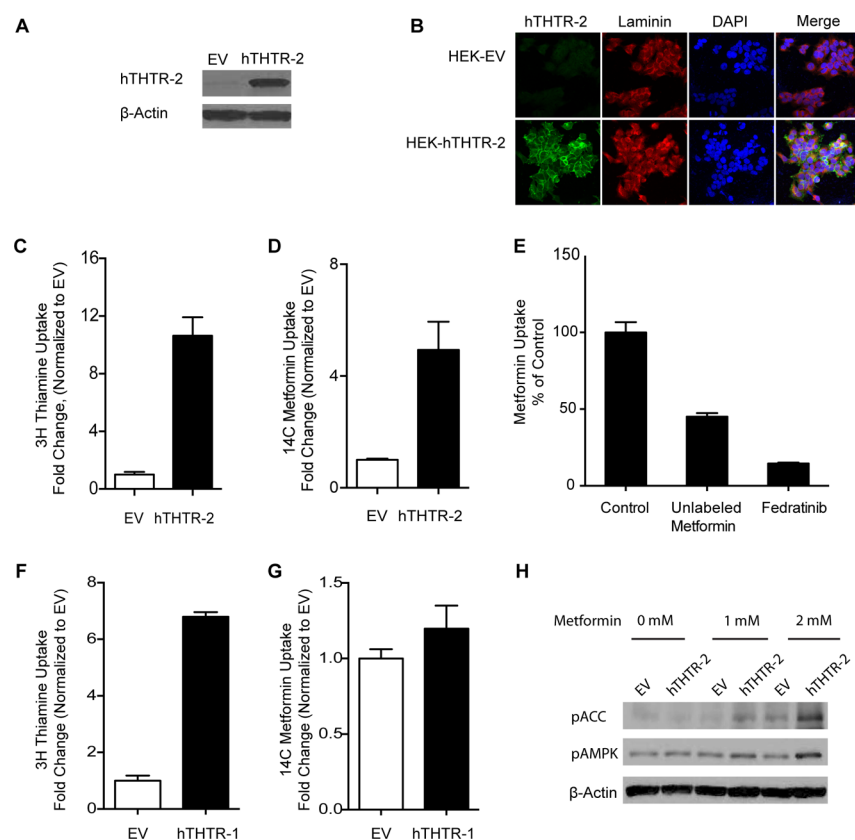


Figure 1. Metformin uptake and effects in cells overexpressing hTHTR-2. (A) Western blotting showed higher hTHTR-2 expression in the HEK-hTHTR-2 overexpressing cells compared with empty vector cells (EV). 12.5 μ g of total protein from cell lysate was deglycosylated by PNGase F. (B) Immunofluorescence cell staining showed that hTHTR-2 is highly expressed in the cell membrane of the hTHTR-2 overexpressing cells. (C) ³H thiamine (25 nM) uptake was significantly higher in HEK-hTHTR-2 overexpressing cells compared to empty vector (EV) transfected cells. Cells were incubated in the uptake buffer for 5 min. (D) ¹⁴C metformin (5 μ M) uptake was significantly higher in HEK-hTHTR-2 overexpressing cells compared to empty vector transfected cells. Cells were incubated in the uptake buffer for 5 min. (E) hTHTR-2 mediated metformin (5 μ M) uptake was inhibited by excess unlabeled metformin (5 mM) and fedratinib (10 μ M). Cells were incubated in the uptake buffer for 5 min. (F) Thiamine and (G) metformin uptake in cells overexpressing hTHTR-1. hTHTR-1 overexpressing cells and empty vector transfected cells were incubated in the uptake buffer containing 25 nM ³H thiamine and 5 μ M ¹⁴C metformin for 5 min. Fold changes are normalized to empty vector control uptake. Results shown are the mean \pm SD for a representative experiment of $n = 3$. (H) Greater phosphorylation of both AMPK and ACC in hTHTR-2 overexpressing cells compared to empty vector cells in cells treated with metformin (1 mM and 2 mM) for 2 h.

transferred to PVDF membrane (Bio-Rad, Hercules, CA). The membranes were blocked with Tris-buffered saline containing 0.05% Tween 20 and 5% nonfat milk for an hour and then incubated with anti-hTHTR-2 antibody (HPA038898, Sigma; 1:1000 dilutions), anti-pAMPK, and anti-pACC (Cell Signaling Technology; 1:1000) overnight at 4 $^{\circ}$ C. Immunoblotting was performed following standard protocol, and signals were detected by ECL chemiluminescence reagent (GE Healthcare, Piscataway, NJ). For immunofluorescence staining, the cells were cultured on poly-D-lysine coverslips (Corning Inc., NY) overnight and fixed in 4% paraformaldehyde (PFA) following standard procedures. The cells were then incubated with the primary antibodies (1:1000 dilutions) overnight at 4 $^{\circ}$ C, washed, and incubated with Alexa Fluor 488 goat anti-rabbit IgG or Alexa Fluor 546 goat anti-mouse IgG (Life Technologies) for 1 h at room temperature. Staining of the DNA in cell nucleus was carried out using DAPI in Prolong Gold Antifade reagent (Life Technologies). The Zeiss LSM 700 with ZEN 2012 software (Zeiss, Germany) was used to analyze.

Statistical Analysis. Unless specified, data are expressed as mean \pm standard deviation (SD). Statistical analyses were performed by unpaired Student's *t*-tests to determine significant differences between controls and treatment groups. The data

were analyzed using GraphPad Prism 6.0 (La Jolla, CA). A *p*-value <0.05 was considered statistically significant.

RESULTS

Metformin Uptake in Cells Overexpressing Human Thiamine Transporter 2 (hTHTR-2; hSLC19A3). To examine the interaction of metformin with hTHTR-2, we established a stable cell line overexpressing the transporter as an *in vitro* model for our studies. Stable expression of the transporter was confirmed by Western blotting (Figure 1A), and immunofluorescence cell staining further confirmed that the hTHTR-2 was expressed on the cell membrane (Figure 1B). Importantly, the cell line exhibited robust uptake of thiamine as a positive control (10-fold greater than empty vector transfected cells, Figure 1C). The validated cell line was used in further experiments to determine whether various pharmaceutical agents including metformin were substrates and inhibitors of the transporter. Compared with empty vector transfected cell lines (EV), metformin uptake was about 4.5-fold higher in hTHTR-2 overexpressing cells (Figure 1D). Furthermore, metformin uptake was inhibited by excess unlabeled metformin (5 mM) and fedratinib (10 μ M), a selective THTR-2 inhibitor²⁷ (Figure 1E). In addition, we

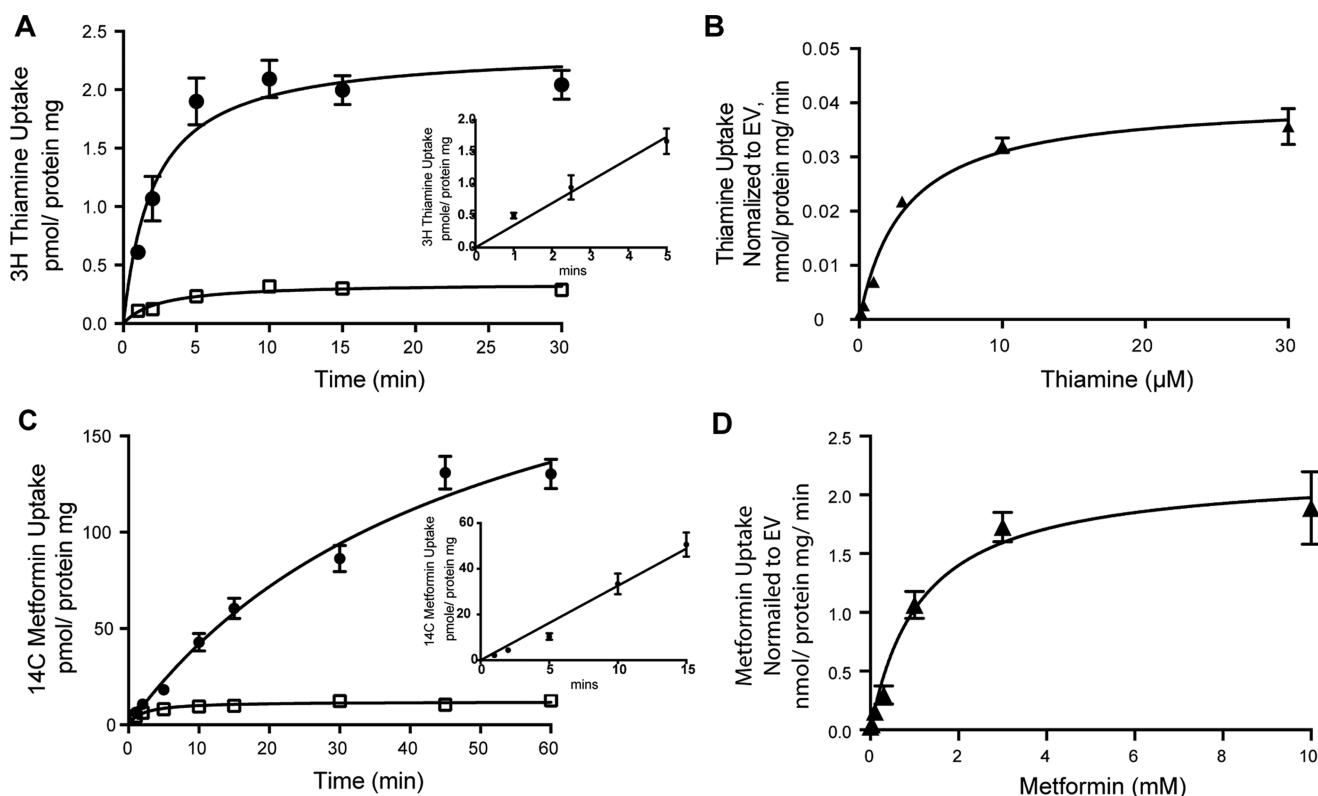


Figure 2. Kinetic characterization of hTHTR-2 mediated thiamine and metformin uptake. (A, C) ^3H thiamine (25 nM) uptake and ^{14}C metformin (5 μM) uptake increased with time in HEK-hTHTR-2. The linear phase of uptake was up to 5 min for thiamine and 15 min for metformin. Data obtained from HEK-hTHTR-2 are represented by close symbols and from empty vector cells by open symbols. (B, D) The initial rate of thiamine and metformin uptake in HEK-hTHTR-2 increased with concentration and was saturable. Cells were incubated with concentrations from 0.1 μM to 30 μM for 3 min for thiamine uptake. For metformin uptake, cells were incubated with concentrations ranging from 0.03 mM to 10 mM for 7 min. Saturable thiamine and metformin uptake was calculated using the difference in accumulation of each compound in hTHTR-2 overexpressing and empty vector cells. The data were fit to a Michaelis–Menten equation. Data shown are the mean \pm SD for a representative experiment of $n = 3$.

tested metformin uptake in a cell line overexpressing THTR-1 (SLC19A2). Our data revealed that THTR-2, but not THTR-1 (Figure 1G), transported metformin. Furthermore, we tested whether metformin treatment in terms of its phosphorylation of the energy sensor, AMP-kinase (AMPK), and its downstream target ACC is modulated by expression of hTHTR-2. Our data show that metformin treatment results in greater phosphorylation of both AMPK and ACC in hTHTR-2 expressing cells compared to empty vector cells (Figure 1H).

Kinetics Studies of hTHTR-2 Mediated Metformin Transport. The uptake kinetics of [^3H] thiamine (Figures 2A and 2B) and [^{14}C] metformin (Figures 2C and 2D) in HEK-hTHTR-2 cells were examined. The uptake of both thiamine (Figure 2A) and metformin (Figure 2C) was time dependent. Thiamine accumulated quickly in the cell line, reaching a plateau at around 5 min (Figure 2A). In contrast, the accumulation of metformin was slower in the stable cell line (Figure 2C). Based on these studies, kinetic parameters were evaluated at 3 min for thiamine and 7 min for metformin. The initial kinetic studies using low thiamine concentrations ranging from 3 nM to 125 nM²⁵ did not result in a saturable uptake of thiamine (Figure SP-1A). However, the uptake kinetics of thiamine exhibited saturable characteristics at higher concentrations of thiamine ($K_m = 3.16 \pm 0.52 \mu\text{M}$, $V_{\max} = 0.0407 \pm 0.00197 \text{ nmol/protein mg/min}$; Figure 2B), and were consistent with previous studies.^{25,27} Like thiamine, metformin also exhibited saturable kinetic properties, however with much lower affinity and a substantially greater maximum transport

rate ($K_m = 1.15 \pm 0.20 \text{ mM}$; $V_{\max} = 2.20 \pm 0.11 \text{ nmol/protein mg/min}$; Figure 2D). Metformin kinetic parameters were confirmed by performing experiments at 37 and 0 $^\circ\text{C}$ (Figure SP-1B) and using the difference in the initial accumulation rates at the two temperatures ($K_m = 0.98 \text{ mM} \pm 0.21 \text{ mM}$; $V_{\max} = 1.91 \pm 0.10 \text{ nmol/protein mg/min}$). Collectively, the kinetic studies revealed that hTHTR-2 is a low affinity and high capacity transporter for metformin.

The Effect of pH and Electrochemical Gradient on hTHTR-2 Mediated Metformin Transport. To investigate the effect of pH on the hTHTR-2 transporter uptake, [^3H] thiamine (25 nM) or [^{14}C] metformin (5 μM) was added to uptake buffers, which were adjusted to various pH's ranging between 5 and 8.5. Both thiamine and metformin uptake increased as the buffer pH was raised from 5 to 7.4, and then plateaued between pH 7.4 and 8.5 (Figures 3A and 3B). To investigate the effect of the transmembrane electrochemical gradient on accumulation of metformin in the cell line overexpressing hTHTR-2, sodium was replaced by equimolar concentrations of potassium in the uptake buffer at pH 7.4 and intracellular concentrations of metformin were measured after 20 min incubation. In the normal uptake buffer, the intracellular metformin concentration was 15.7 μM ; while in the KCl replacement buffer, the intracellular metformin concentration was 6.3 μM , which was 60% less in cellular concentration compared to the normal uptake buffer (Figure SP-2A). In addition, the thiamine uptake was reduced in cells preincubated with cardiac glycosides as well as the Na^+/K^+ -ATPase inhibitors

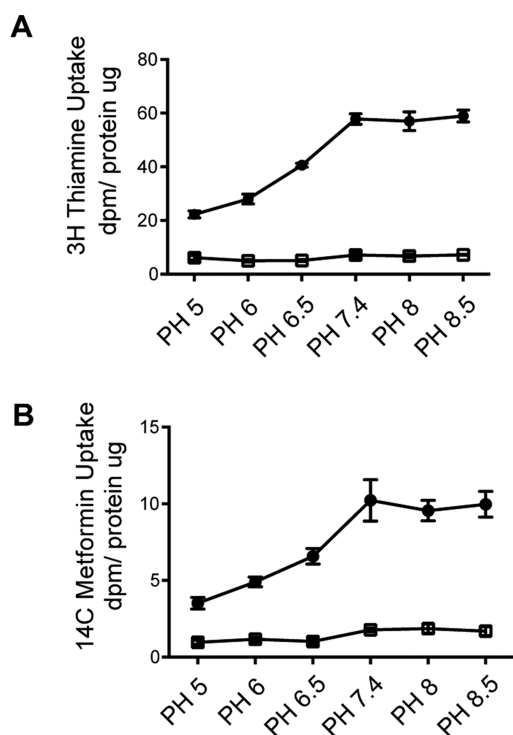


Figure 3. Effect of pH on hTHTR-2 mediated metformin uptake. (A) ³H thiamine (25 nM) or (B) ¹⁴C metformin (5 μ M) was included in the uptake buffer adjusted to pHs 5, 6, 6.5, 7.4, 8, and 8.5, and the cells were incubated for 5 min. Empty squares represent the uptake in HEK-empty vector cells and black circles represent the uptake in HEK-hTHTR-2 cells. Data shown are the mean \pm SD for a representative experiment of $n = 2$.

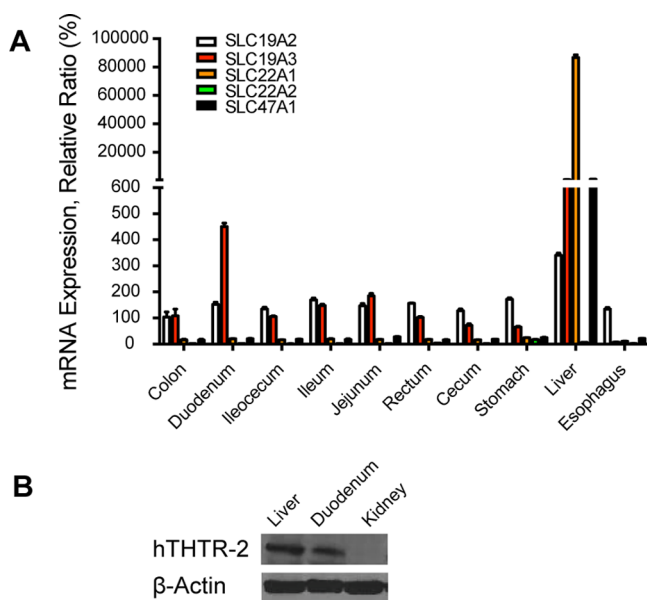


Figure 4. Human THTR-2 mRNA and protein expression in various tissues. (A) The relative mRNA levels of SLC19A2, SLC19A3, SLC22A1, SLC22A2, and SLC47A1 were determined by real-time PCR. The mRNA expression level of SLC19A2 in colon was set to 100%. Data are presented as mean \pm SD, and pooled samples were from 5 to 39 Caucasians. (B) Representative Western blot: hTHTR-2 protein expression in human liver (samples from Giacomini's lab), duodenum (pool samples from GeneTex), and kidney (pool samples from Abcam) was determined by Western blotting.

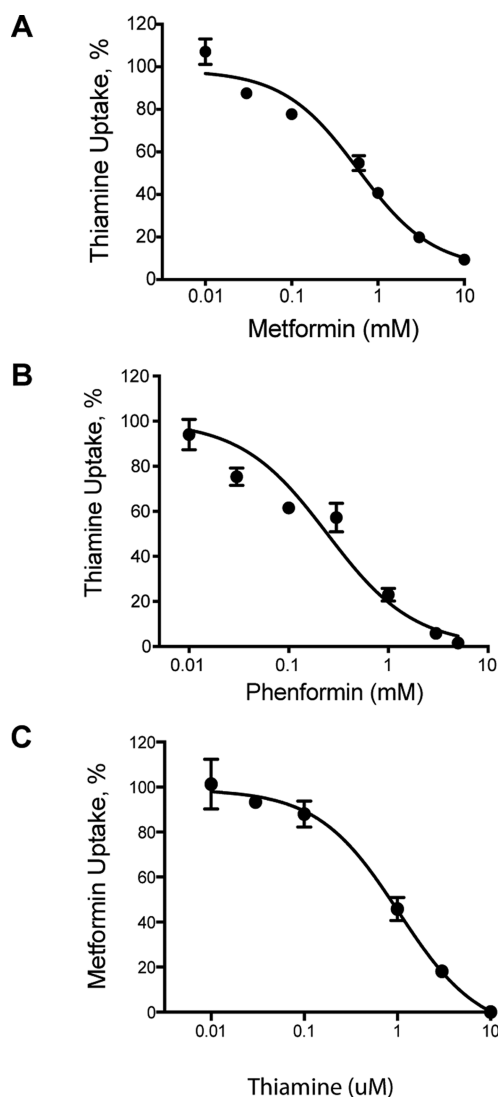


Figure 5. hTHTR-2 mediated interaction between biguanides and thiamine. ³H thiamine (25 nM) was coincubated in uptake buffer containing a range of metformin concentrations (0.01–10 mM) (A) or phenformin concentrations (0.01–5 mM) (B) for 5 min. (C) ¹⁴C metformin was coincubated in uptake buffer containing a range of thiamine concentrations (0.01–10 μ M) for 3 min. The IC_{50} was calculated by fitting the data to nonlinear regression curve. Data shown are the mean \pm SD for a representative experiment of $n = 2$.

digoxin and ouabain,^{28–30} and the inhibition appeared to be time and dose dependent (Figures SP-2B and SP-2C). These data suggest that metformin accumulation in cells over-expressing hTHTR-2 is sensitive to membrane potential.

THTR-2 mRNA and Protein Expression in the Gastrointestinal Tract. To assess the mRNA expression pattern of THTR-2 in the human gastrointestinal tract, a panel of cDNAs from different segments of the intestinal tract was used (Clontech). The mRNA level of THTR-2 was highest in the liver and duodenum, followed by jejunum, ileum, ileocecum, colon, rectum, cecum, and stomach (Figure 4A). The protein expression of THTR-2 in human liver and duodenum was confirmed by Western blotting (Figure 4B). For comparison, THTR-1 (SLC19A2) and transporters known to interact with metformin, i.e., OCT1 (SLC22A1), OCT2 (SLC22A2), and MATE1 (SLC47A1),^{17,19,20} were included in the study (Figure 4A). The data show that, in the duodenum, mRNA levels of

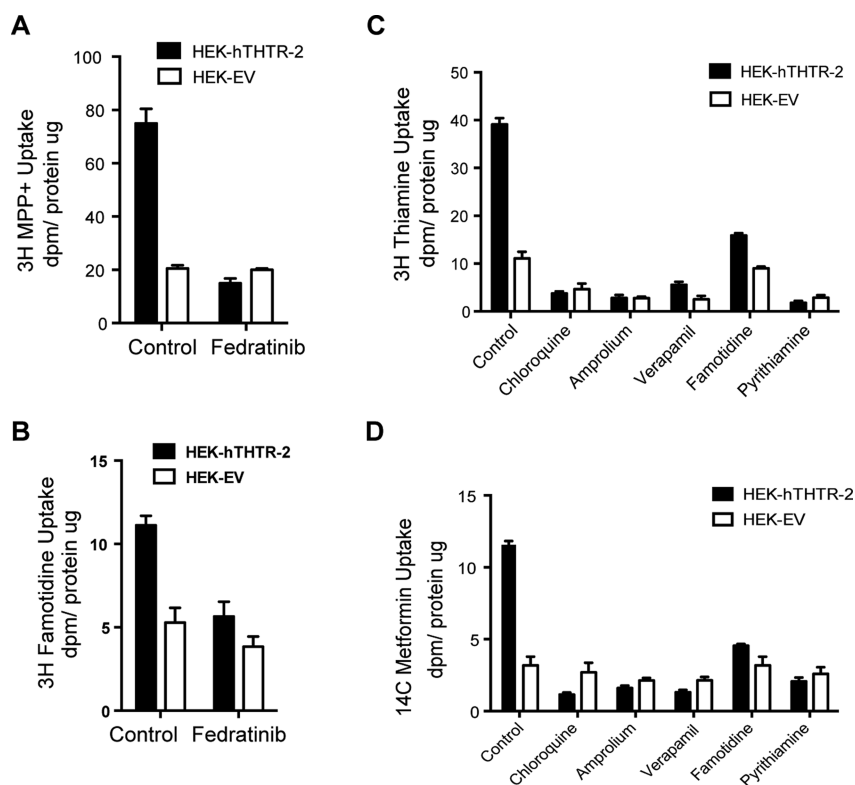


Figure 6. Interaction of various organic cations with human THTR-2. (A) hTHTR-2 overexpressing and empty vector transfected cells were incubated with ³H MPP⁺ in the absence (control) or presence of fedratinib (100 μ M) for 10 min. (B) hTHTR-2 overexpressing and empty vector transfected cells were incubated with ³H famotidine in the absence or presence of fedratinib (100 μ M) for 10 min. (C, D) hTHTR-2 overexpressing and empty vector transfected cells were incubated with ³H thiamine (25 nM) or ¹⁴C metformin (5 μ M) alone (control) or in the presence of chloroquine (1 mM), verapamil (1 mM), famotidine (1 mM), amprolium (1 mM), and pyriithiamine (200 μ M) for 5 min. Data shown are the mean \pm SD for a representative experiment of $n = 2$.

THTR-2 were the most abundant; whereas in the liver, as previously shown,¹⁷ OCT1 mRNA transcripts were most abundant. These data suggest that hTHTR-2 plays a role in intestinal thiamine and metformin absorption.

hTHTR-2 Mediated Interaction Between Biguanides and Thiamine. To further investigate the interaction of metformin with hTHTR-2, we performed an inhibition study of thiamine uptake with metformin and its analogue, phenformin. Various concentrations of metformin (0.01–10 mM) or phenformin (0.01–5 mM) were used in the uptake buffer, which contained 25 nM [³H] thiamine. As shown in Figures 5A and 5B, hTHTR-2 mediated thiamine uptake was inhibited by both metformin ($IC_{50} = 680 \pm 140 \mu$ M) and phenformin ($IC_{50} = 244 \pm 28.4 \mu$ M). To test the reverse inhibition, various concentrations of thiamine (0.01–10 μ M) were included in the uptake buffer, which contained 5 μ M [¹⁴C] metformin. As shown in Figure 5C, hTHTR-2 mediated metformin uptake was potently inhibited by thiamine ($IC_{50} = 1.03 \pm 0.12 \mu$ M).

Interactions between Various Organic Cations and THTR-2. A previous study in our laboratory showed that thiamine was a major endogenous substrate of OCT1 and that the vitamin was also a substrate of other SLC transporters that are well-established transporters for structurally diverse prescription drugs.^{17,19} Therefore, in this study, we hypothesized that the reverse may be true. That is, the thiamine specific transporter, THTR-2, may also interact with various xenobiotics and particularly various prescription drugs. To this end, we tested various organic cations and bases including prescription drugs as substrates and inhibitors of hTHTR-2.

The data demonstrated that the neurotoxin, which is also a canonical substrate of several organic cation transporters, 1-methyl-4-phenylpyridinium (MPP⁺) and the histamine H₂-receptor antagonist famotidine were substrates of hTHTR-2 (Figures 6A and 6B, respectively). Other organic cations including histamine, tetraethylammonium (TEA), serotonin, carnitine, choline, norepinephrine, guanidine, and aminoguanidine were not found to be substrates of hTHTR-2 (Figure SP-3). However, several antithiamine analogues (amprolium and pyriithiamine) and prescription drugs (chloroquine, verapamil, and famotidine) were shown to be inhibitors of hTHTR-2-mediated thiamine and metformin uptake (Figures 6C and 6D).

Uptake Studies in Cells Overexpressing Murine Thiamine Transporter 2 (mTHTR-2; mSlc19a3). To understand whether mouse THTR-2 also plays a role in metformin uptake *in vivo*, we constructed and characterized a stable mTHTR-2 overexpressing cell line (HEK-mTHTR-2). The uptake of [³H] thiamine but not metformin in the overexpressing mTHTR-2 cell line was higher than in the empty vector transfected cells (Figures 7A and 7B). Further, though metformin inhibited thiamine uptake via hTHTR-2, the drug did not significantly inhibit mTHTR-2 mediated thiamine uptake even at concentrations as high as 10 mM (Figure 7C). Similarly, fedratinib was a less potent inhibitor of the mouse THTR-2 orthologue compared with the human orthologue ($22.2 \pm 3.99 \mu$ M vs $1.88 \pm 0.38 \mu$ M) (Figure 7D). Furthermore, both famotidine and MPP⁺ showed substantially less accumulation (over empty vector cell lines) in cell lines

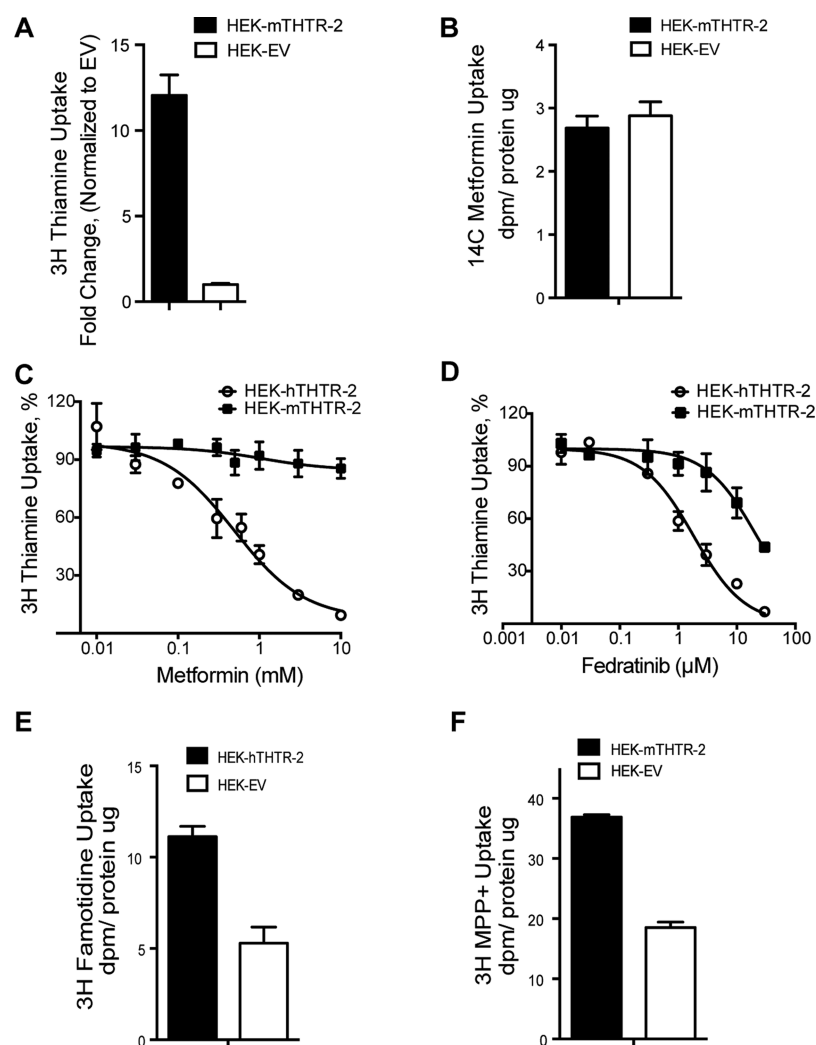


Figure 7. Functional characterization of mouse THTR-2 (mTHTR-2). (A) ^3H thiamine (25 nM) uptake was significantly higher in HEK-mTHTR-2 overexpressing cells compared to empty vector transfected cells. Cells were incubated in the uptake buffer for 10 min. (B) mTHTR-2 overexpressing and empty vector transfected cells were incubated with ^{14}C metformin (5 μM) in the absence or presence of fedratinib (100 μM) for 10 min. (C, D) ^3H thiamine (25 nM) was coincubated with a range of metformin (0.01–10 mM) or fedratinib (0.01–30 μM) concentrations for 5 min. Background uptake in empty vector transfected cells was subtracted from the uptake in the mTHTR-2 cells, and the difference was fit to a nonlinear regression curve. Data shown are the mean \pm SD for a representative experiment of $n = 3$. (E) ^3H famotidine (25 nM) and (F) ^3H MPP $^+$ (6 nM) uptake in mTHTR-2 overexpressing cells. Cells were incubated in the uptake buffer containing ^3H MPP $^+$ or ^3H famotidine for 10 min. Data shown are the mean \pm SD for a representative experiment of $n = 2$.

stably expressing mTHTR-2 compared with those expressing the human transporter, hTHTR-2 (Figures 7E and 7F). Collectively, the data suggest that, in comparison to the human THTR-2, the mouse orthologue has a narrower substrate specificity.

DISCUSSION

The major findings of our study are that the human vitamin B1 (thiamine) transporter, hTHTR-2, transports metformin and that metformin and other xenobiotics including phenformin, chloroquine, verapamil, famotidine, amprolium, and pyriithamine inhibit hTHTR-2 mediated uptake of both thiamine and metformin. This is the first study to demonstrate that metformin is a substrate of hTHTR-2, and the transporter also transports famotidine and MPP $^+$. Our findings suggest that the transporter may be a target for drug–drug and drug–nutrient interactions. Notable species differences in the

specificity of THTR-2 between human and mouse orthologues were observed.

Metformin, as an insulin-sensitizer, is among the most widely prescribed drugs for type 2 diabetes. Under physiological conditions, metformin with a pK_a of 11.5 and $\log P$ of -1.43 predominantly exists as a cation (>99.9%) and therefore requires transporters to cross biological membranes.⁴ Many studies have shown that metformin is a substrate of polyspecific organic cation transporters such as OCT1, OCT2, OCT3, PMAT, MATE1, MATE2, and OCTN1 (Table 1),^{7–13} and that these transporters play important roles in the absorption, hepatic uptake, tissue distribution, and renal excretion of the drug.⁴ A summary of apparent K_m values along with tissue distribution of known metformin transporters, including THTR-2 (current study), is presented in Table 1. Notably, the K_m value of metformin for hTHTR-2 is within the range of K_m values for established metformin transporters.

Table 1. Summary of Metformin Transporters

transporter name	K_m (mM)	major tissues	reference
THTR-2 (SLC19A3)	1.15	intestine and liver	current study
OCT1 (SLC22A1)	1.47	liver and kidney	Kimura et al. 2005 ¹³ ; Li et al. 2011 ⁴²
OCT2 (SLC22A2)	1.07	kidney	Choi et al. 2007 ⁴³
OCT3 (SLC22A3)	1.10	multiple tissues (liver, skeletal muscle, fat, and brain)	Chen et al. 2015 ¹⁰
MATE1 (SLC47A1)	0.23	kidney and liver	Chen et al. 2009 ¹¹
MATE2 (SLC47A2)	1.05	kidney	Masuda et al. 2006 ¹²
PMAT (SLC29A4)	1.32	intestine	Zhou et al. 2002 ⁹
OCTN1 (SLC22A4)	NA ^a	GI tract	Nakamichi et al. 2013 ⁸
SERT ^b (SLC6A4)	4	intestine	Han et al. 2015 ¹⁴

^aNo K_m was reported. Significant uptake in overexpressing cells compared to mock cells. ^bData not reproduced in our laboratory (see Figure SP-4B).

Consistent with previous studies, our data show that THTR-2 expression levels were highest in the duodenum.²⁵ Importantly, our study showed that transcript levels of THTR-2 in the duodenum were higher than levels of THTR-1 as well as the metformin transporters, OCT1, OCT2, MATE1, and SERT (Figure 4A and Figure SP-4A). A recently published study demonstrated that the human serotonin transporter (SERT, SLC6A4) transports metformin and suggested that it plays a role in metformin intestinal absorption.¹⁴ However, we did not observe significant metformin uptake in the overexpressing human SERT cell line (gift from Dr. Randy Blakely) though robust uptake of serotonin was observed (Figure SP-4B). We also did not observe significant metformin uptake in HEK cells transiently transfected with the cDNA of SLC6A4 (SERT), however significant metformin uptake was observed in HEK cells transiently transfected with the cDNAs of SLC22A1 and SLC19A3 (Figure SP-4C). Together, our data showed that metformin is a poor substrate of SERT compared with OCT1 and THTR-2. Though both THTR-1 and THTR-2 are involved in dietary absorption of thiamine in the intestine, previous studies suggest that THTR-2 alone can fulfill normal vitamin B1 absorption requirements in conditions associated with THTR-1 dysfunction.²³ Furthermore, THTR-2 deficient but not THTR-1 deficient mice exhibit significantly reduced intestinal thiamine uptake compared to their wild-type littermates.^{23,31} Confocal microscopy of polarized cells shows that THTR-2 is expressed apically, whereas THTR-1 has higher expression levels on the basolateral membrane.³² Collectively, these data suggest that THTR-2 plays a major role in mediating the first step in thiamine absorption, from lumen to enterocyte, whereas THTR-1 plays a more important role in the translocation of thiamine from the enterocyte into the portal circulation. Our data also suggest that THTR-2 can mediate the first step in the intestinal absorption of metformin; however, further studies are needed to understand the contribution of THTR-2 to metformin absorption in the context of other intestinal transporters of metformin.

Though previous studies have indicated that THTR-2 is a thiamine specific transporter,³³ our study provides the first demonstration that synthetic compounds including metformin, famotidine, and MPP⁺ are also substrates of the transporter. Consistent with previous studies demonstrating pH dependence of thiamine uptake,³⁴ our data showed that the uptake of both metformin and thiamine increases with increasing pH (Figure 3). Previous studies suggested that a thiamine/H⁺ antiport mechanism characterized thiamine uptake in intestinal brush border membrane vesicles.^{35,36} However, we did not observe a thiamine/H⁺ antiport uptake mechanism for THTR-2 in the cells stably expressing the transporter though proton antiport mechanisms were observed for MATE1 (Figure SP-5). Our data suggest that the previously observed thiamine/H⁺ antiport mechanism in isolated brush border membrane vesicles is not mediated by THTR-2.

Based on our data showing that THTR-2 exhibited a broader substrate specificity than had been shown in previous studies, we hypothesized that the transporter was a target for drug–drug and drug–vitamin interactions. Our data indicated that metformin and phenformin inhibited thiamine uptake with IC₅₀'s of 680 μ M and 244 μ M, respectively (Figure 5A and 5B). In patients taking 1 g oral doses, metformin concentrations in the gastrointestinal (GI) lumen are estimated to be 24 mM assuming 250 mL of GI fluid. The estimated GI concentrations of metformin and phenformin are >10-fold higher than these IC₅₀ values, suggesting that, at therapeutic doses, the drugs may cause clinically relevant transporter-mediated interaction.^{37,38} Since thiamine deficiency is associated with lactic acidosis,^{39,40} this observation may suggest an alternate mechanism to mitochondrial complex 1 inhibition for metformin and phenformin associated lactic acidosis.⁴¹ That is, metformin may reduce thiamine absorption leading to reduced thiamine levels. In addition, other synthetic analogues including the prescription drugs, chloroquine, verapamil, and famotidine may also lead to reduced thiamine absorption (Figures 6C and 6D). Though vitamin transporters are not on the list of transporters that commonly mediate clinically relevant drug–drug interactions,^{37,38} the recent failure of fedratinib in phase III clinical trials due to occurrences of Wernicke's encephalopathy in a handful of patients suggests that SLC19A3 should be considered a potential new target for drug–vitamin interactions.²⁷ Notably, in some patients Wernicke's syndrome disappeared upon thiamine administration. No information is available about the plasma thiamine levels during fedratinib treatment or following thiamine administration. Therefore, it is not known whether the drug's effects were on thiamine absorption or its uptake into various tissues including the central nervous system (CNS). It is possible that fedratinib inhibits hTHTR-2-mediated uptake of thiamine in the intestine, CNS, and various peripheral tissues. Though other drugs tested as inhibitors of THTR-2 in our study were less potent than fedratinib, several of the drugs including metformin inhibited THTR-2 at concentrations that would be achieved in the GI tract after clinical doses. Interestingly, we also observed the reverse situation. That is, thiamine inhibited hTHTR-2 mediated metformin uptake at low concentrations (IC₅₀ = 1.03 \pm 0.12 μ M) (Figure 5C). Importantly, after thiamine supplementation (25–100 mg), intestinal concentrations of thiamine are predicted to be between 0.3 mM and 1.2 mM.³⁷ Thus, our study suggests that THTR-2 may be a target for drug–vitamin or vitamin–drug interactions, which may depend on the dose of metformin and thiamine.

In conclusion, our study demonstrates that human THTR-2 transports metformin, and may play a role in its absorption and indeed the absorption of other basic drugs after oral dosing. The finding that, at concentrations in the gastrointestinal tract achievable after oral dosing, metformin as well as other drugs inhibited THTR-2 mediated uptake of thiamine and metformin suggests that the transporter may be an important target for drug–drug and drug–vitamin interactions. Future studies investigating the role of THTR-2 in drug safety and efficacy are clearly warranted.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.5b00501.

Five figures including additional data on hTHTR-2 and other metformin transporters mediated uptake (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Kathy.giacomini@ucsf.edu. University of California, San Francisco, 1550 4th Street, Box 2911, San Francisco, CA 94158. Tel: (415) 476-1936 Fax: (415) 514-4361.

Present Address

[§]E.C.C.: Department of Metabolism and Pharmacokinetics, Genentech, South San Francisco, CA 94080.

Author Contributions

X.L., H.-C.C., S.W.Y., M.M.G., E.-I.L., A.S.R., and K.M.G. designed the research; X.L., H.-C.C., S.W.Y., M.M.G. E.C.C., M.P., J.H., and J.T. conducted experiments; X.L., H.-C.C., S.W.Y., M.M.G., E.-I.L., A.S.R., and K.M.G. analyzed data; X.L. and K.M.G. wrote the paper.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a grant from the U.S. Food and Drug Administration (U01 FD004979), grants from the NIH (GM61390, GM117163), and the Burroughs Wellcome Fund (BWF ID 1012485). X.L. and E.C.C. were supported in part by the National Institutes of Health Training Grant [Grant T32 GM007175]. M.P. is supported by China Scholarship Council. We would like to thank Dr. Randy Blakely (Vanderbilt University) for kindly providing the stably transfected HEK-SERT cells. We would like to acknowledge Dr. Hobart Harris, Dr. Richard Castro, and Uk Sok Shin for collecting the human liver tissues.

■ ABBREVIATIONS USED

SLC, solute carrier; OCT, organic cation transporter; MATE, multidrug and toxin extrusion; THTR, thiamine transporter; SERT, serotonin transporter; HEK, human embryonic kidney 293 cell; m, mouse ortholog; h, human ortholog

■ REFERENCES

(1) Boyle, J. G.; Salt, I. P.; McKay, G. A. Metformin Action on AMP-Activated Protein Kinase: a Translational Research Approach to Understanding a Potential New Therapeutic Target. *Diabet. Med.* **2010**, *27*, 1097–1106.

(2) Rojas, L. B. A.; Gomes, M. B. Metformin: an Old but Still the Best Treatment for Type 2 Diabetes. *Diabetol. Metab. Syndr.* **2013**, *5*, 6.

(3) Tock, L.; Dâmaso, A. R.; de Piano, A.; Carnier, J.; Sanches, P. L.; Lederman, H. M.; Ernandes, R. M. Y.; de Mello, M. T.; Tufik, S. Long-Term Effects of Metformin and Lifestyle Modification on Non-alcoholic Fatty Liver Disease Obese Adolescents. *J. Obes.* **2010**, DOI: 10.1155/2010/831901.

(4) Graham, G. G.; Punt, J.; Arora, M.; Day, R. O.; Doogue, M. P.; Duong, J. K.; Furlong, T. J.; Greenfield, J. R.; Greenup, L. C.; Kirkpatrick, C. M.; Ray, J. E.; Timmins, P.; Williams, K. M. Clinical Pharmacokinetics of Metformin. *Clin. Pharmacokinet.* **2011**, *50*, 81–98.

(5) Jonker, J. W.; Wagenaar, E.; Mol, C. A.; Buitelaar, M.; Koepsell, H.; Smit, J. W.; Schinkel, A. H. Reduced Hepatic Uptake and Intestinal Excretion of Organic Cations in Mice with a Targeted Disruption of the Organic Cation Transporter 1 (Oct1 [Slc22a1]) Gene. *Mol. Cell. Biol.* **2001**, *21*, 5471–5477.

(6) Wang, D.-S.; Jonker, J. W.; Kato, Y.; Kusuhara, H.; Schinkel, A. H.; Sugiyama, Y. Involvement of Organic Cation Transporter 1 in Hepatic and Intestinal Distribution of Metformin. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 510–515.

(7) Shu, Y.; Sheardown, S. A.; Brown, C.; Owen, R. P.; Zhang, S.; Castro, R. A.; Ianculescu, A. G.; Yue, L.; Lo, J. C.; Burchard, E. G.; Brett, C. M.; Giacomini, K. M. Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. *J. Clin. Invest.* **2007**, *117*, 1422–1431.

(8) Nakamichi, N.; Shima, H.; Asano, S.; Ishimoto, T.; Sugiura, T.; Matsubara, K.; Kusuhara, H.; Sugiyama, Y.; Sai, Y.; Miyamoto, K.-I.; Tsuji, A.; Kato, Y. Involvement of Carnitine/Organic Cation Transporter OCTN1/SLC22A4 in Gastrointestinal Absorption of Metformin. *J. Pharm. Sci.* **2013**, *102*, 3407–3417.

(9) Zhou, M.; Xia, L.; Wang, J. Metformin Transport by a Newly Cloned Proton-Stimulated Organic Cation Transporter (Plasma Membrane Monoamine Transporter) Expressed in Human Intestine. *Drug Metab. Dispos.* **2007**, *35*, 1956–1962.

(10) Chen, E. C.; Liang, X.; Yee, S. W.; Geier, E. G.; Stocker, S. L.; Chen, L.; Giacomini, K. M. Targeted Disruption of Organic Cation Transporter 3 Attenuates the Pharmacologic Response to Metformin. *Mol. Pharmacol.* **2015**, *88*, 75–83.

(11) Chen, Y.; Teranishi, K.; Li, S.; Yee, S. W.; Hesselson, S.; Stryke, D.; Johns, S. J.; Ferrin, T. E.; Kwok, P.; Giacomini, K. M. Genetic Variants in Multidrug and Toxic Compound Extrusion-1, hMATE1, Alter Transport Function. *Pharmacogenomics J.* **2009**, *9*, 127–136.

(12) Masuda, S.; Terada, T.; Yonezawa, A.; Tanihara, Y.; Kishimoto, K.; Katsura, T.; Ogawa, O.; Inui, K.-I. Identification and Functional Characterization of a New Human Kidney-Specific H⁺/Organic Cation Antiporter, Kidney-Specific Multidrug and Toxin Extrusion 2. *J. Am. Soc. Nephrol.* **2006**, *17*, 2127–2135.

(13) Kimura, N.; Masuda, S.; Tanihara, Y.; Ueo, H.; Okuda, M.; Katsura, T.; Inui, K.-I. Metformin Is a Superior Substrate for Renal Organic Cation Transporter OCT2 Rather Than Hepatic OCT1. *Drug Metab. Pharmacokinet.* **2005**, *20*, 379–386.

(14) Han, T.; Proctor, W. R.; Costales, C. L.; Cai, H.; Everett, R. S.; Thakker, D. R. Four Cation-Selective Transporters Contribute to Apical Uptake and Accumulation of Metformin in Caco-2 Cell Monolayers. *J. Pharmacol. Exp. Ther.* **2015**, *352*, 519–528.

(15) Shu, Y.; Sheardown, S. A.; Brown, C.; Owen, R. P.; Zhang, S.; Castro, R. A.; Ianculescu, A. G.; Yue, L.; Lo, J. C.; Burchard, E. G.; Brett, C. M.; Giacomini, K. M. Effect of Genetic Variation in the Organic Cation Transporter 1 (OCT1) on Metformin Action. *J. Clin. Invest.* **2007**, *117*, 1422–1431.

(16) Stocker, S. L.; Morrissey, K. M.; Yee, S. W.; Castro, R. A.; Xu, L.; Dahlin, A.; Ramirez, A. H.; Roden, D. M.; Wilke, R. A.; McCarty, C. A.; Davis, R. L.; Brett, C. M.; Giacomini, K. M. The Effect of Novel Promoter Variants in MATE1 and MATE2 on the Pharmacokinetics and Pharmacodynamics of Metformin. *Clin. Pharmacol. Ther.* **2013**, *93*, 186–194.

(17) Chen, L.; Shu, Y.; Liang, X.; Chen, E. C.; Yee, S. W.; Zur, A. A.; Li, S.; Xu, L.; Keshari, K. R.; Lin, M. J.; Chien, H.-C.; Zhang, Y.

- Morrissey, K. M.; Liu, J.; Ostrem, J.; Younger, N. S.; Kurhanewicz, J.; Shokat, K. M.; Ashrafi, K.; Giacomini, K. M. OCT1 Is a High-Capacity Thiamine Transporter That Regulates Hepatic Steatosis and Is a Target of Metformin. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 9983–9988.
- (18) Wong, R. H. F.; Sul, H. S. Insulin Signaling in Fatty Acid and Fat Synthesis: a Transcriptional Perspective. *Curr. Opin. Pharmacol.* **2010**, *10*, 684–691.
- (19) Kato, K.; Moriyama, C.; Ito, N.; Zhang, X.; Hachiuma, K.; Hagima, N.; Iwata, K.; Yamaguchi, J.-I.; Maeda, K.; Ito, K.; Suzuki, H.; Sugiyama, Y.; Kusuhara, H. Involvement of Organic Cation Transporters in the Clearance and Milk Secretion of Thiamine in Mice. *Pharm. Res.* **2015**, *32*, 2192–2204.
- (20) Kato, K.; Mori, H.; Kito, T.; Yokochi, M.; Ito, S.; Inoue, K.; Yonezawa, A.; Katsura, T.; Kumagai, Y.; Yuasa, H.; Moriyama, Y.; Inui, K.-I.; Kusuhara, H.; Sugiyama, Y. Investigation of Endogenous Compounds for Assessing the Drug Interactions in the Urinary Excretion Involving Multidrug and Toxin Extrusion Proteins. *Pharm. Res.* **2014**, *31*, 136–147.
- (21) Ganapathy, V.; Smith, S. B.; Prasad, P. D. SLC19: the Folate/Thiamine Transporter Family. *Pfluegers Arch.* **2004**, *447*, 641–646.
- (22) Zhao, R.; Goldman, I. D. Molecular Aspects of Medicine. *Mol. Aspects Med.* **2013**, *34*, 373–385.
- (23) Reidling, J. C.; Lambrecht, N.; Kassir, M.; Said, H. M. Impaired Intestinal Vitamin B1 (Thiamin) Uptake in Thiamin Transporter-2-Deficient Mice. *Gastroenterology* **2010**, *138*, 1802–1809.
- (24) Eudy, J. D.; Spiegelstein, O.; Barber, R. C.; Wlodarczyk, B. J.; Talbot, J.; Finnell, R. H. Identification and Characterization of the Human and Mouse SLC19A3 Gene: a Novel Member of the Reduced Folate Family of Micronutrient Transporter Genes. *Mol. Genet. Metab.* **2000**, *71*, 581–590.
- (25) Said, H. M.; Balamurugan, K.; Subramanian, V. S.; Marchant, J. S. Expression and Functional Contribution of hTHTR-2 in Thiamin Absorption in Human Intestine. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2004**, *286*, G491–G498.
- (26) Sanofi Discontinues Clinical Development of Investigational JAK2 Agent Fedratinib (SAR302503). **2013**, 1–2.
- (27) Zhang, Q.; Zhang, Y.; Diamond, S.; Boer, J.; Harris, J. J.; Li, Y.; Rupar, M.; Behshad, E.; Gardiner, C.; Collier, P.; Liu, P.; Burn, T.; Wynn, R.; Hollis, G.; Yeleswaram, S. The Janus Kinase 2 Inhibitor Fedratinib Inhibits Thiamine Uptake: a Putative Mechanism for the Onset of Wernicke's Encephalopathy. *Drug Metab. Dispos.* **2014**, *42*, 1656–1662.
- (28) Nguyen, K. T. D.; Buljan, V.; Else, P. L.; Pow, D. V.; Balcar, V. J. Cardiac Glycosides Ouabain and Digoxin Interfere with the Regulation of Glutamate Transporter GLAST in Astrocytes Cultured From Neonatal Rat Brain. *Neurochem. Res.* **2010**, *35*, 2062–2069.
- (29) Song, H.; Karashima, E.; Hamlyn, J. M.; Blaustein, M. P. Ouabain-Digoxin Antagonism in Rat Arteries and Neurons. *J. Physiol. (Oxford, U. K.)* **2014**, *592*, 941–969.
- (30) Gaskell, E.; Rostron, C. *Therapeutics and Human Physiology*; Oxford University Press: 2013.
- (31) Said, H. M. Intestinal Absorption of Water-Soluble Vitamins in Health and Disease. *Biochem. J.* **2011**, *437*, 357–372.
- (32) Boulware, M. J. Polarized Expression of Members of the Solute Carrier SLC19A Gene Family of Water-Soluble Multivitamin Transporters: Implications for Physiological Function. *Biochem. J.* **2003**, *376*, 43–48.
- (33) Rajgopal, A.; Edmondson, A.; Goldman, I. D.; Zhao, R. SLC19A3 Encodes a Second Thiamine Transporter ThTr2. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2001**, *1537*, 175–178.
- (34) Ashokkumar, B. Thiamin Uptake by the Human-Derived Renal Epithelial (HEK-293) Cells: Cellular and Molecular Mechanisms. *AJP: Renal Physiology* **2006**, *291*, F796–F805.
- (35) Dudeja, P. K.; Tyagi, S.; Kavilaveettil, R. J.; Gill, R.; Said, H. M. Mechanism of Thiamine Uptake by Human Jejunal Brush-Border Membrane Vesicles. *Am. J. Physiol.: Cell Physiol.* **2001**, *281*, C786–C792.
- (36) Laforenza, U.; Orsenigo, M. N.; Rindi, G. A Thiamine/H⁺ Antiport Mechanism for Thiamine Entry Into Brush Border Membrane Vesicles From Rat Small Intestine. *J. Membr. Biol.* **1998**, *161*, 151–161.
- (37) US Food and Drug Administration. *Guidance for Industry*. 2012.
- (38) International Transporter Consortium: Giacomini, K. M.; Huang, S.-M.; Tweedie, D. J.; Benet, L. Z.; Brouwer, K. L. R.; Chu, X.; Dahlin, A.; Evers, R.; Fischer, V.; Hillgren, K. M.; Hoffmaster, K. A.; Ishikawa, T.; Keppler, D.; Kim, R. B.; Lee, C. A.; Niemi, M.; Polli, J. W.; Sugiyama, Y.; Swaan, P. W.; Ware, J. A.; Wright, S. H.; Yee, S. W.; Zamek-Gliszczynski, M. J.; Zhang, L. Membrane Transporters in Drug Development. *Nat. Rev. Drug Discovery* **2010**, *9*, 215–236.
- (39) Romanski, S. A.; McMahon, M. M. Metabolic Acidosis and Thiamine Deficiency. *Mayo Clin. Proc.* **1999**, *74*, 259–263.
- (40) Amrein, K.; Ribitsch, W.; Otto, R.; Worm, H. C.; Stauber, R. E. Severe Lactic Acidosis Reversed by Thiamine Within 24 h. *Crit Care* **2011**, *15*, 457.
- (41) Misbin, R. I. The Phantom of Lactic Acidosis Due to Metformin in Patients with Diabetes. *Diabetes Care* **2004**, *27*, 1791–1793.
- (42) Li, S.; Chen, Y.; Zhang, S.; More, S. S.; Huang, X.; Giacomini, K. M. Role of Organic Cation Transporter 1, OCT1 in the Pharmacokinetics and Toxicity of Cis-Diammine(Pyridine)-Chloroplatinum(II) and Oxaliplatin in Mice. *Pharm. Res.* **2011**, *28*, 610–625.
- (43) Choi, M.-K.; Jin, Q.-R.; Jin, H.-E.; Shim, C.-K.; Cho, D.-Y.; Shin, J.-G.; Song, I.-S. Effects of Tetraalkylammonium Compounds with Different Affinities for Organic Cation Transporters on the Pharmacokinetics of Metformin. *Biopharm. Drug Dispos.* **2007**, *28*, 501–510.